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Novel Process of Fermenting Black Soybean [*Glycine max* (L.) Merrill] Yogurt with Dramatically Reduced Flatulence-Causing Oligosaccharides but Enriched Soy Phytoalexins

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Black soybeans [*Glycine max* (L.) Merrill] were germinated under fungal stress with food grade *R. oligosporus* for 3 days and were homogenized and fermented with lactic acid bacteria (LAB) to produce soy yogurt. Fungal stress led to the generation of oxylipins [oxooctadecadienoic acids (KODES) isomers and their respective glyceryl esters] and glyceollins—a type of phytoalexins unique to soybeans. In soy yogurt, the concentrations of total KODES and total glyceollins were 0.678 mg/g (dry matter) and 0.953 mg/g, respectively. The concentrations of other isoflavones (mainly genistein and daidzein and their derivatives) in soy yogurt remained largely unchanged after the processes compared with the control soy yogurt. Germination of black soybean under fungal stress for 3 days was sufficient to reduce stachyose and raffinose (which cause flatulence) by 92 and 80%, respectively. With a pH value of 4.42, a lactic acid content of 0.262%, and a maximum viable cell count of 2.1×10^8 CFU/mL in the final soy yogurt, soy milk from germinated soybeans under fungal stress was concluded to be a suitable medium for yogurt-making. The resulting soy yogurt had significantly altered micronutrient profiles with significantly reduced oligosaccharides and enriched glyceollins.

KEYWORDS: Black soybeans; fermentation; *Rhizopus oligosporus*; lactic acid bacteria; soy yogurt; raffinose; stachyose; phytoalexins; glyceollins

INTRODUCTION

Phytoalexins are small molecule compounds synthesized by plants and contain antimicrobial activity when they are challenged by the natural elements, particularly microbes (*1*). Although toxic to the microbes, many phytoalexins are found to have health benefits for humans and properties of chronic disease prevention. One of the most intensively researched phytoalexins is resveratrol, which is found in fungus-infected grape skins and *Rhizoma Polygoni Cuspidati*—a type of traditional Chinese herbal medicine. Resveratrol has shown bioactivities in the prevention of cancer (*2*) and age-related chronic diseases (*3*) and also acts as a mimic for calorie restriction (*4*). Compared with the well-studied resveratrol, glyceollins (**Figure 1**) (*5*), the fungus-induced soybean phytoalexins, are less known for their bioactivities, but it is possible that these compounds have positive health effects. One animal model study in particular has shown they can inhibit breast cancer cells from proliferating through antiestrogenic effects (*6, 7*). Glyceollins can be produced in the cultivated soybeans through fungal stress (*8*). Our previous study had also observed the generation of glyceollins under the stress of different fermentation starters on black soybeans with the highest amount of >7 mg/g (dried matter) glyceollins formed in *Rhizopus oligosporus* stressed black soybeans [*Glycine max* (L.) Merrill] (*9*). Moreover, a group of oxylipins, oxooctadecadienic acids (KODES), were also generated, which were reported to possess biological properties such as inhibiting acetyl CoA carboxylase activity (*10, 11*). We propose that using food grade fungi to stress germinating black soybeans would be an attractive route to introduce phytoalexins, particularly glyceollins, into black soybean products. Thus, in turn, the production of soy yogurt will be a potential functional food that incorporates health-promoting nutrients such as soy proteins, isoflavones (including glyceollins), polyunsaturated fatty acids, and probiotic strains.

As a probiotic drink, yogurt has been documented to have multifaceted bioactivity including intestinal microbial modulation, alleviation of lactose intolerance, relief of diarrhea, pathogenic bacteria prevention, immune system stimulation, cholesterol control, allergy prevention, irritable bowel syndrome prevention, anticarcinogenic ability, and inhibition activity against *Helicobacter pylori* (*12*). Globally, soy yogurt has a market share of only 1.9% (*13*). Nevertheless, it possesses a number of advantages compared with dairy yogurt. Soy yogurt

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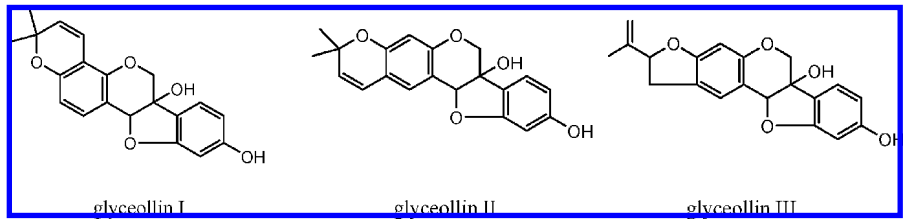

Figure 1. Chemical structures of glyceollins—a group of phytoalexins elicited by fungal stress of black soybeans.

has a high content of essential polyunsaturated fatty acids and isoflavones, which are known to reduce the risk factors of contracting chronic diseases. Black soybean [*Glycine max* (L.) Merrill], used herein, is a soybean cultivar with a black seed coat. This cultivar has been used in traditional Chinese medicine as a functional food due to a number of unspecific health benefits. However, the indigestibility of oligosaccharides, mainly raffinose and stachyose, is blamed as the causative factor for flatulence associated with the human consumption of soy products (*14*). Thus, it would be desirable to remove the flatulence-causing raffinose and stachyose from the soy yogurt to improve the consumers' preferences. The objective of this study was to develop a novel soy yogurt that is enriched with glyceollins and has a reduced content of flatulence-causing oligosaccharides.

MATERIALS AND METHODS

Materials. Black soybeans [*G. max* (L.) Merrill], products of the year 2006, China) were purchased from the local supermarket (Carrefour, Singapore). Raffinose and stachyose were purchased from Fluka Biochemika (St. Louis, MO). Sucrose was purchased from BDH (Auckland, New Zealand). Daidzein and genistein were purchased from Acros (USA). Glyceollin standard was purified according to our previously reported method (*9*). 11-Oxoocatadecadienoic acid (KODE) was purchased from Cayman Chemical Co. (Ann Arbor, MI). MRS agar was purchased from Oxoid Ltd. (Hamsphire, U.K.). Solvents were of spectroscopic or high-performance liquid chromatography (HPLC) grade from commercial sources. The lactic acid bacteria (LAB) starter culture was purchased from Chr. Hansen Company (Hørsholm, Denmark), and it contained blended *Streptococcus thermophilus* and *Lactobacillus delbrueckii* subsp. *bulgaricus* FD-DVS YC-X11. The LAB culture powder was diluted in sterilized cow's milk and inoculated onto the sterilized soy milk according to the guidelines (5 units diluted in 250 mL of milk). The tempeh starter culture, *R. oligosporus*, was bought from PT. Aneka Fermentasi Industri (Bandung, Indonesia). The identity of the fungus was confirmed at the School of Technobiology at Indonesia Catholic University (Jakarta, Indonesia). One gram of *R. oligosporus* culture powder was dissolved in 15.0 mL of sterile deionized water.

Black Soybean Germination under *R. oligosporus* Treatment. The fungal inoculations were carried out on the black soybean cotyledons adapted from a method reported by Boué (*8*) with some modifications. Black soybean seeds (200 g) were surface-sterilized in 400 mL of 70% ethanol for 3 min and then rinsed three times with 500 mL of sterile deionized water to wash away the ethanol. The seeds were soaked in sterile deionized water for 10 h. After draining out the water, each soaked bean was peeled without spoiling the radicals. The prepared fungal culture suspension was inoculated onto the beans and mixed well (15 mL of diluted fungal solution inoculated into 200 g of black soybeans). The inoculated beans were placed on a sterilized container (30 cm × 50 cm) lined with two autoclaved filter papers moistened with 30 mL of sterile deionized water. The containers were sealed with parafilm and incubated for 3 days at 25 °C in the dark. Black spots were observed on the bean when it developed into a sprout. Germinated black soybeans without fungal stress were also prepared with identical procedures. The soaked beans without germination were prepared as a control sample. In total, the following groups were used in this study: ungerminated beans, control (UG); germinated beans (G) and germinated bean sample under *R. oligosporus* stress (GS).

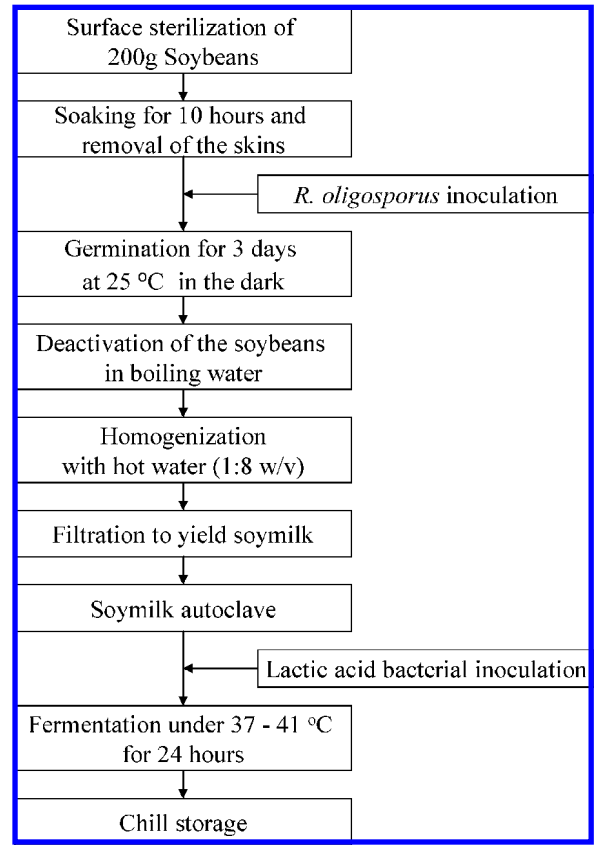

Figure 2. Flowchart of fermentation processes of the soy yogurt. *R. oligosporus* culture powder (1.0 g) was dissolved in 15 mL of sterile deionized water and applied to the soybeans (15 mL of fungal solution inoculated into 200 g of black soybeans). The LAB culture powder was diluted in sterilized cow's milk and inoculated onto the sterilized soy milk according to the guidelines (5 units diluted in 250 mL of milk). The soy milk was inoculated with 1.0 mL of the prepared LAB starter culture solution (0.02 unit per 100 mL) and incubated at 37−41 °C for 24 h.

Soy Yogurt Fermentation. The soy yogurt making procedures are illustrated in **Figure 2**. Briefly, the beans were dehulled, mixed with water (1:8 w/v), and blended in a Braun MX2050 blender for 5 min. The resulting slurry was filtered through a double layer of cheesecloth to yield soy milk. The soy milk (100 mL) was dispensed into a 250 mL glass container with a metal lid and was autoclaved at 121 °C for 15 min. After it had been cooled to room temperature, the soy milk was inoculated with 1.0 mL of the freshly prepared LAB starter culture solution (0.02 unit inoculated into 100 mL) and incubated at 37−41 °C for 24 h. After fermentation, the soy yogurt was stored in a chiller for chemical analysis.

Isoflavones and KODES Analysis. Sterilized soy milk and soy yogurt were freeze-dried to a constant weight before extractions. For

Table 1. Sucrose and Oligosaccharide Concentrations in Black Soybeans, Sterilized Soy Milk, and Soy Yogurt

	concentration (mg/g of dry matter)								
	control (UG)			germinated (G)			Germinated under *R. oligosporus* stress (GS)		
	soybean[a]	soy milk[b]	soy yogurt	soybean[a]	soy milk[b]	soy yogurt	soybean[a]	soy milk[b]	soy yogurt
stachyose	37.2 ± 0.4	27.2 ± 1.7	24.3 ± 0.5	1.1 ± 0.0	0.9 ± 0.1	0.7 ± 0.0	2.9 ± 1.0	2.8 ± 0.8	1.8 ± 0.2
raffinose	8.7 ± 1.1	7.6 ± 0.2	7.3 ± 0.4	1.6 ± 0.1	1.0 ± 0.1	1.4 ± 0.1	1.8 ± 0.1	1.2 ± 0.3	1.7 ± 0.4
sucrose	82.4 ± 2.4	59.7 ± 1.0	2.1 ± 0.1	61.5 ± 0.1	54.7 ± 2.9	17.5 ± 0.8	41.4 ± 0.7	38 ± 4.7	4.2 ± 0.7

[a] To analyze the soluble sugar in black soybeans, the beans were first homogenized with a suitable amount of water. The solid material was discarded. The yield slurry was freeze-dried for analysis. [b] Soy milk was sterilized at 121 °C for 15 min.

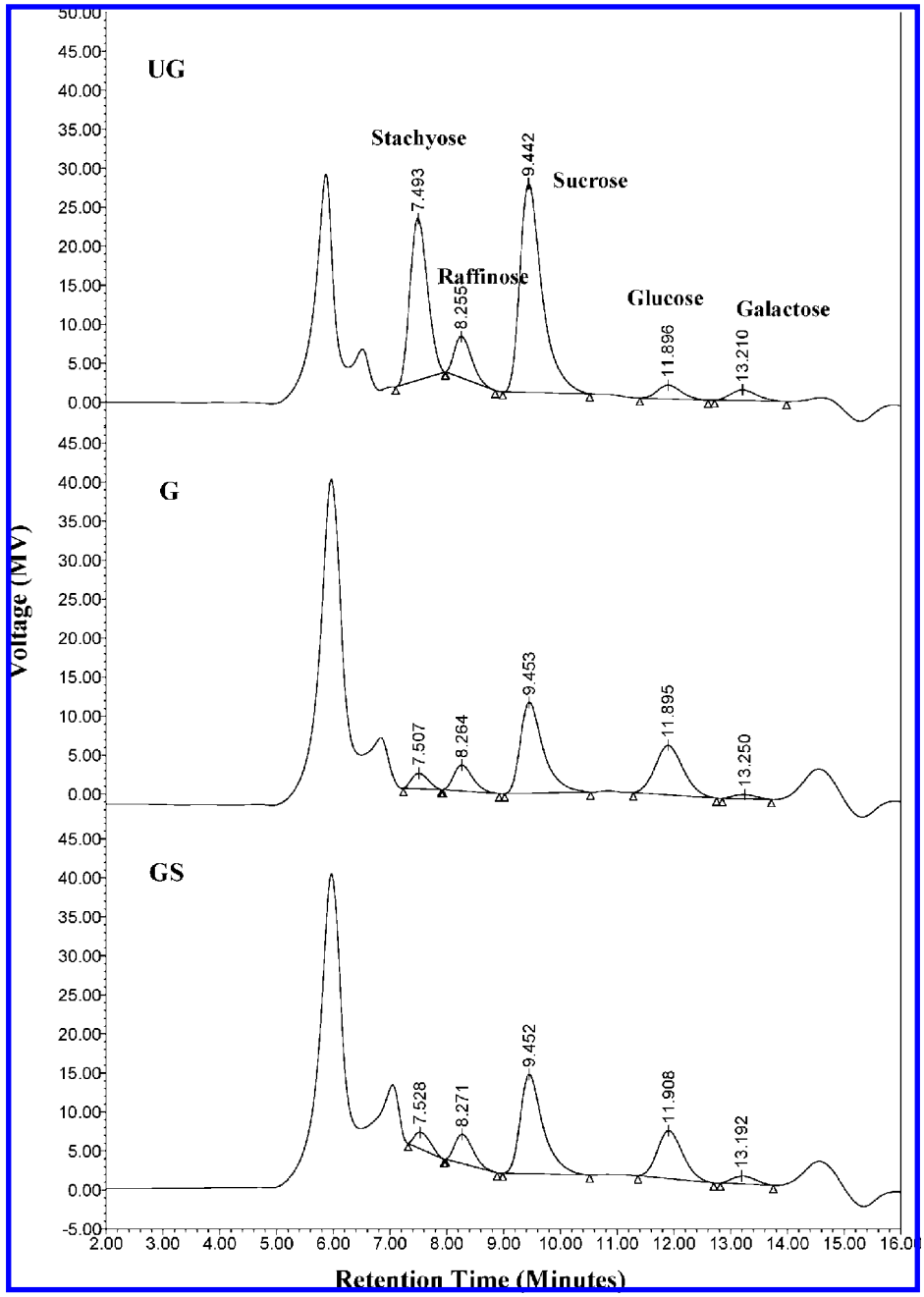

Figure 3. HPLC chromatogram of saccharides in ungerminated black soybeans (UG), germinated without stress (G), and germinated with *R. oligosporus* stress (GS). The sugar identities were verified by spiking the samples with standards, respectively.

the analysis of isoflavones and KODES, the sample extraction and analysis method was based on our previous paper (*9*). In a typical run, the accurately weighed freeze-dried sample was mixed with 80% ethanol (7.0 mL/g of dry matter) and extracted for 1 h at 50 °C with continuous shaking. After the mixture had been cooled, it was centrifuged at 14000*g* for 15 min. The supernatant was analyzed in a Waters HPLC system with a 2996 PDA detector and a wavelength range set at 200−400 nm. The HPLC column was a Shimadzu ODS-

VP (4.6 × 250 mm, 5 μm particle size). The mobile phase consisted of water (A) and acetonitrile (B). The following gradient was used in the sample analysis: 0−1 min, 100% A; 1−17 min, 55% A; 17−27 min, 10% A; 27−33 min, 10% A; 33−35 min, 100% A; 35−40 min, 100% A. Total genisteins (genistein, genistin, and malonyl-genistin) and total daidzeins (daidzein, daidzin, and malonyl-daidzin) were used to express the total concentration of each aglycone, glucoside, and malonyl glucoside isoflavone form. The concentration of glucoside and malonyl glucoside isoflavones were calculated using the aglycone isoflavone standard calibration curve and expressed as the equivalent concentration of their respective aglycone. Glyceollins and KODES were also calculated according to the standard calibration curves (*9*). Total glyceollins were used to represent the concentration of glyceollin varieties. Total KODES were used to represent the concentration of KODES and KODE glyceryl esters.

Oligosaccharides Analysis. Oligosaccharides in black soybeans, sterilized soy milk, and soy yogurt were analyzed. To compare the soybean soluble sugars (sucrose and oligosaccharides) with soy milk and soy yogurt, the beans were initially homogenized with a suitable amount of water. The obtained slurry was filtered through cheesecloth to remove the solid materials. The solution was freeze-dried for subsequent analyses. Sterilized soy milk and soy yogurt were directly freeze-dried for subsequent analyses. One gram of each freeze-dried sample was weighed accurately and dissolved in 1.0 mL of deionized water. After the addition of 30 mL of ethyl acetate, the samples were sonicated for 30 min at 4 °C and then centrifuged at 6500*g* for 15 min. The supernatant containing fats and hydrophobic substances was discarded. The pellet was dried in a vacuum oven for 2 h at room temperature. The dried pellet was suspended in 20 mL of deionized water and sonicated for 30 min at 4 °C before acetonitrile (1:2, v/v) was added to precipitate the proteins. The mixture was centrifuged at 18000*g* for 15 min, and the supernatant was filtered through a 0.45 μm PTFE membrane. The analysis was carried out on a Waters HPLC system with a Waters 2414 refraction index detector and Waters Sugar-Pak I cation exchange and size exclusion column (300 mm × 6.5 mm) with a Waters Guard-Pak guard column. The mobile phase was deionized water. An isocratic flow rate of 0.4 mL/min was applied. Serial concentrations of sucrose, stachyose, and raffinose standards were also prepared and analyzed using the same HPLC condition. The sample concentrations were calculated from the standard calibration curve.

Viable Bacterial Counting. Briefly, for viable bacterial counting in yogurt, 1.0 mL of the sample was dissolved in 9.0 mL of 0.9% sterilized sodium chloride solution. After serial dilution (e.g., 10^{-1}, 10^{-2}, 10^{-3}, 10^{-4}, 10^{-5}), 1.0 mL of the diluted solution was dispensed to the plate and poured with MRS medium. Each dilution has duplicate plates. The MRS plates were incubated in a 37 °C incubator for 24 h. The number of viable bacteria was counted after incubation.

Titratable Acidity. The method was adapted from AOAC (1984) (*15*). One gram (or 1 mL) of sample was diluted in 10 mL of deionized water. After adding three to four drops of phenolphthalein, the sample solution was titrated with a 0.05 M NaOH solution. Titratable acidity was calculated as percentage of lactic acid.

Statistical Analysis. Comparison of results among the samples was done by SPSS software (version 12.0, SPSS Inc., Chicago, IL). All of the results were statistically analyzed with a 95% confidence level.

RESULTS

Soy Yogurt Fermentation. As shown in the flowchart (**Figure 2**), black soybeans were first treated with *R. oligosporus* for 3 days. On the first day after fungal inoculation, the surface of soybean cotyledon turned dark brown, which was most likely due to the necrosis of the surface tissue correlated with the presence of phytoalexin glyceollin production (*16*). During the following days, the necrotic surface was slowly covered with *R. oligosporus* mycelia. The most suitable condition for fungal growth, soybean germination, and glyceollin production was a spore suspension inoculation at 1×10^8 mL^{-1} incubated at 25 °C for 3 days. Soybean sprouts formed from germination were usually discarded as they mainly contained insoluble matters. Therefore, during germination, the length of soybean sprout was required to be controlled as short as possible. In this study, the length of sprouts was <1 cm after 3 days when the glyceollin level peaked; therefore, germination was stopped at day 3 to minimize the loss of nutrients.

After 6 h of LAB fermentation, the soy milk turned into a gel form in all three samples. After 24 h, a set and soft product with a yogurt-like flavor was obtained. The fermentation was terminated, and the product was chilled. The yogurt from stress germination treated beans had a slight tempeh-like odor and was a bit darker, most likely due to the pigments formed due to fungal stress. Yogurt flavor is highly dependent on the characteristics of LAB strains. Different LAB strains as well as flavor additives can be applied to improve the yogurt sensory properties. Properly designed sensory evaluations remain to be carried out.

Oligosaccharide Contents. The oligosaccharide contents are shown in **Table 1**. Representative HPLC chromatograms of saccharides in the three sample types are shown in **Figure 3**. It was observed that germination alone dramatically reduced the black soybean oligosaccharides (97% for stachyose and 82% for raffinose) compared with the ungerminated one (UG) ($p < 0.05$). In the stress-germinated counterpart, the oligosaccharide content was also dramatically reduced by a slightly lesser amount ($p < 0.05$). About 25 and 50% of sucrose had been reduced, respectively, in the samples with germination and with germination plus stress ($p < 0.05$). The results were in agreement with an earlier study on yellow soybean (*17*). Addition of fungal stress had only negligible effects as the fungi did not noticeably hinder the bean growth. When the germinated beans were processed into soy milk, some raffinose and sucrose loss was observed, most likely during filtration. After LAB fermentation, the soy yogurt of ungerminated beans contained 65% of stachyose and 84% of raffinose compared with the soybeans themselves on a dry weight basis ($p < 0.05$), whereas a very low amount of oligosaccharides remained in both samples G and GS ($p > 0.05$).

Isoflavones and Total KODES. Two major groups of isoflavones, including genisteins (genistin, malonyl-genistin, and genistein) and daidzeins (malonyl-daidzin and daidzein) were detected in all samples (**Table 3**). Trace levels of glyciteins (glycitein and malonyl-glycitein) were also detected, but the values were not significant compared to other isoflavones. Similar trends in the isoflavone contents were obtained in both soy milk and soy yogurt. Compared with the control, the total genisteins had increased significantly in both germinated beans (G and GS) and in the corresponding soy yogurt ($p < 0.05$). Furthermore, the total genisteins in fungus-stressed soy milk was significantly higher than in nonstressed counterparts ($p < 0.05$), whereas the total genisteins between fungus-stressed soy yogurt and the nonstressed counterparts were not significantly different ($p > 0.05$). On the other hand, the total daidzeins in both soy milk and soy yogurt remained unchanged after germination under fungal stress ($p > 0.05$). However, it increased by ~18% with germination treatment alone ($p < 0.05$). As expected, no glyceollins were detected in the control soy milk or in the soy yogurt. In contrast, the glyceollin content reached about 0.1% in stress-germinated soy milk and soy yogurt (1.09 and 0.95 mg/g, respectively). Lesser but significant amounts of glyceollins (0.10 and 0.20 mg/g, respectively) were also found in the soy milk and soy yogurt made from germinated beans ($p < 0.05$). The total isoflavones (the sum of total daidzein + total genistein + total glyceollins) for the GS yogurt reached 3.24 mg/g (dry matter) and is the highest among the three types

Table 2. Isoflavones and Total KODES Concentrations (Milligrams per Gram, Dry Matter) in Soy Milk and Soy Yogurt[a]

	total daidzeins		total genisteins		total glyceollins		sum	total KODES	
	soy milk	soy yogurt	soy milk	soy yogurt	soy milk	soy yogurt	soy yogurt	soy milk	soy yogurt
UG	1.384 ± 0.113	1.441 ± 0.147	0.458 ± 0.053	0.439 ± 0.067	ND	ND	1.88	ND	ND
G	1.572 ± 0.108	1.698 ± 0.155	0.866 ± 0.112	0.830 ± 0.093	0.100 ± 0.029	0.198 ± 0.187	2.726	0.117 ± 0.27	0.111 ± 0.024
GS	1.371 ± 0.136	1.411 ± 0.121	0.919 ± 0.103	0.876 ± 0.083	1.090 ± 0.035	0.953 ± 0.015	3.24	0.779 ± 0.056	0.678 ± 0.08

[a] Analysis of soy milk and soy yogurt was based on their respective freeze-dried samples. Total daidzeins are the sum of daidzein, daidzin, and malonyl-daidzin; total genisteins are the sum of genistein, genistin, and malonyl-genistin, total glyceollins are the sum of glyceollin I, II, and III isomers; total KODES are the sum of KODES and respective glyceryl esters. UG, ungerminated beans; G, germinated beans without stress; GS, germinated beans under fungal stress; ND, not detectable. The unit is mg/g of dry matter. All runs were in triplicate, and the results are expressed as mean ± RSD. A representative HPLC trace for the analysis was published in our previous paper and is not included here (*9*).

Table 3. Comparison of pH Value, Titratable Acidity, and Viable Lactic Acid Bacteria in the Samples with Three Different Treatment Methods[a]

	control soybeans (UG)		germinated soybeans (G)		germinated soybeans *under R. oligosporus* stress (GS)	
	in soy milk	in soy yogurt	in soy milk	in soy yogurt	in soy milk	in soy yogurt
pH value	6.50 ± 0.02	4.15 ± 0.01	6.44 ± 0.02	4.39 ± 0.01	6.01 ± 0.01	4.42 ± 0.01
titratable acidity (%)	0.091 ± 0.003	0.253 ± 0.005	0.115 ± 0.007	0.247 ± 0.003	0.135 ± 0.005	0.262 ± 0.012
viable LAB count (CFU/mL)		$(4.3 \pm 0.5) \times 10^8$		$(3.2 \pm 0.3) \times 10^8$		$(2.1 \pm 0.1) \times 10^8$

[a] The percentage of lactic acid was used as a representative of titratable acidity. Calculation: % lactic acid = [(volume of 0.1 M NaOH) × 0.9]/sample volume. Values represent the mean ± RSD; $n = 3$. Soy milk was sterilized at 121 °C for 15 min.

of yogurts. Similarly, no KODES were detected in the control soy milk, but a significant amount (0.78 mg/g) was detected in the stress-germinated counterpart ($p < 0.05$) (**Table 2**). The amount was reduced to 0.68 mg/g in the corresponding soy yogurt ($p < 0.05$). Trace amount of KODES were also detected in the soy milk and soy yogurt from germinated beans, probably due to minor contamination of microbes during the germination ($p < 0.05$).

The pH value and titratable acidity in the autoclaved soymilk and fermented soy yogurts and viable LAB counts of final soy yogurt are shown in **Table 3**. A slightly acidic pH value (6.44) was obtained for soy milk from germinated beans, which was very close to the control soy milk (6.50). The pH value of stress-germinated soy milk was lower (6.01). Apparently, the stress may lead to generation of free fatty acids, such as the observed KODES. After LAB fermentation, all yogurts had a pH value below 4.50 with comparable titratable acidity contents (0.25%). This value is comparable to the vales found in the literature (*18*). It was reported recently that inoculation of LAB on soy milk at 40−45 °C for a few hours decreased the pH value to 4.4−4.8 accompanied with total titratable acidity increase of 0.5−0.26% (*19*). All three soy yogurts contained viable LAB numbers above 10^8 CFU/mL, thus meeting the criteria of probiotic foods. The fungal stress germination treatment thus has no adverse effects on the resulting soy yogurt fermentation. The purported antimicrobial activity of glyceollins did not seem to cause significant inhibition of LAB growth. This could be due to the dilution effect, resulting in a much lower concentration of glyceollins in the soy milk compared to that in the soybean. It is also likely that they are more selective in inhibiting the invasion of the fungi.

DISCUSSION

Soy-based products have great potentials because of soy proteins, isoflavones, and unsaturated fatty acids. However, soy yogurts are often made with ingredients from animal milk or its derivatives to provide desirable texture and flavor (*20*). Introduction of the accompanying saturated fats and lactose in animal milk may compromise the positive health image of soy yogurt. Our two-step stress germination and fermentation approach dramatically altered the nutritional profile of the resulting soy yogurt by naturally reducing the level of undesired oligosaccharides while enriching the glyceollins, as well as maintaining the level of soy isoflavones. Compared with the control, raffinose and stachyose had been degraded to significantly low levels in the nonstressed germinating and fungus-stressed germinating beans. The carbohydrates were utilized as energy for the seeds' germination (*21*). The activity of endoenzymes from the soybean itself and exoenzymes from foreign environment can initiate such carbohydrate utilization (*22*). Because a large amount of exoenzymes can be secreted during fungus growth (*23*), it is possible that oligosaccharide reduction in the fungus-stressed samples would have been more significant than that in nonstressed germinating beans due to a synergistic action of exoenzymes and endoenzymes. The necrosis of the soybean cells contacting the fungus conidia may reduce the activity of the endoenzymes in the soybean (*5*). The exoenzymes from *R. oligosporus* have been known to degrade stachyose and (or) raffinose (*24, 25*). LAB strains used in this fermentation did not show much effect on the oligosaccharide concentration, although some LAB strains can produce α-galactosidase and have the ability to hydrolyze stachyose and raffinose in fermented soy milk (*26*).

Glyceollins are derived from daidzeins; hence, one might expect that the increment of glyceollins would lead to a drop in the daidzein concentration. However, the total amount of daidzeins had only a slight decrease, whereas 1.09 mg/g of glyceollins was accumulated in the stressed-germinated beans. This was most likely due to an increased biosynthesis of isoflavones, particularly the malonyl-glucoside forms of daidzein and genistein during seed germination (*27, 28*). Fungal stress may have further stimulated the isoflavone biosynthesis for defense purposes. Large increases of genistein in both germinated bean samples were observed. From the conversion of soy milk to soy yogurt, the isoflavones showed minor changes with only slight fluctuations in their different forms. After LAB fermentation, glyceollins were largely retained in soy yogurt for potential health benefits of the final product. The amount of the glyceollins generated by fungus-stressed germination treatment was expected to be dependent on the cultivar of the bean. However, this requires further investigation to optimize the glyceollin contents in the final product.

During fermentation, a number of antimicrobial metabolites may be produced to inhibit microorganism growth (*29*). Therefore, a relatively lower viable LAB count in the fungus-stressed soy yogurt may result due to a particularly higher content of antimicrobial compounds, that is, glyceollins, in retarding the growth of LAB. Although the minimum dose required for a probiotic product to elicit health effects remains unclear, some papers have suggested that the viable cell count for a satisfactory fermented probiotic is required to be above 10^6 CFU/mL (or gram), and this amount may supply a sufficient "daily dose" (*30*). In the present study, a viable LAB number above 10^8 CFU/mL was observed, which fulfilled the stated criteria. Whether important probiotic strains such as bifidobacteria can also grow to the same level warrants further study.

In summary, we have demonstrated for the first time that germinated black soybeans under fungal stress can be fermented into a soy yogurt which features a low amount of flatulence-causing oligosaccharides but with a significant level of isoflavones including glyceollins. Artificial additives were not used in the overall processing of this soy yogurt, which may have compromised the potential health benefits and claims of the final product. This is only the prototype of a potential product, and much future work remains to be done for scaling up the production processes and optimizing flavor attributes. In a broader perspective regarding the health benefits, we have a long way to go in terms of garnering scientific evidence on the chronic disease prevention properties of the soy yogurt described herein.

ABBREVIATIONS USED

LAB, lactic acid bacteria; KODES, oxooctadecadienoic acids; UG, ungerminated bean sample; G, germinated bean sample; GS, germinated bean sample under *R. oligosporus* stress.

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