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Nutritional and functional evaluation of soy yogurt supplemented with Glasswort (*Salicornia herbacea* L.)

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ABSTRACT

In this study, the effect of glasswort added to soy milk prepared for yogurt-making was evaluated based on the viable cell numbers of lactic acid bacterium in yogurt-fermenting culture, and nutritional ingredients and physiological active compounds in the finished soy yogurt. Contents of crude protein and crude fat were not influenced, but dietary fiber content was proportionally increased by the addition of glasswort to the finished soy yogurt. Viable cell numbers of lactic acid bacterium were a little increased by the glasswort addition. Minerals, including Na, Mg, K, Ca, Fe, Zn, and Al in the finished soy yogurt were significantly increased in proportion to the balance of glasswort powder. Citric and malic acid content were not influenced but lactic acid was increased in proportion to the glasswort content in the finished soy yogurt. Polyphenol and total phenolic compounds contents in the finished soy yogurt were proportional to glasswort content; meanwhile, the DPPH-scavenging and ferric ion reduction activity of soy yogurt was proportional to contents of polyphenol and total phenolic compounds. The yogurt composed of soy fruit (bean) and glasswort body (leaf and stem) may be a reference for development of plant origin drinking yogurt.

KEYWORDS

Soybean milk;
Soybean yoghurt;
Glasswort;
Antioxidant;
Lactic acid fermentation.

INTRODUCTION

Yogurt has been traditionally made with various mammalian's milks by using fermentation metabolism of various lactic acid bacteria and is known to be a good food for health[1,2]. Yogurt contains protein, calcium, riboflavin, vitamin B6 and vitamin B12, which originated from milk or are produced by lactic acid bacteria[3]. Lactose of mammalians' milk is converted to glucose and galactose, and partially fermented to lactic acid by bacterial metabolism, which may be proper food for somebody who is lactose-intolerant[4]. However, it is not suitable for vegans, or ovo-vegetarians.

Contents of protein and lactose contained in mammalians' milk support well the growth of lactic acid bacteria. Lactic acid bacteria ferment lactose to lactic acid for generation of free energy and hydrolyze milk protein to obtain amino acids required for biosynthesis. Lactic acid and some amino acids produced by fermentation metabolism are ingredients of fermentation milk (yogurt) and lactic acid bacteria grown in yogurt are effective at preventing antibiotic-associated diarrhea, in part by aggregating with gastrointestinal pathogens[5,6].

Soy milk contains relatively higher protein than mammalians' milk but lacks lactose or sugar for growth of lactic acid bacteria. Soy milk supplemented with lactose or glucose satisfies the requirement for lactic acid fermentation and is converted to edible yogurt like dairy yogurt by lactic acid fermentation. Soy yogurt contains dietary fiber but does not contain cholesterol; however, dairy yogurt does not contain dietary fiber but contains cholesterol based on the milk composition[7,8].

The soy bean plant is one of the most extensively cultivated crops around the world, whose fruit (soy bean) is mainly composed of protein and lipid, and contains functionally useful compounds for human health, isoflavones, vitamin E, saponin, and anthocyanin[9-11]. Soy bean has been employed as a main material to make various soy sauces: kanjang, doenjang, and gochujang in Asia[12,13]. Glasswort is a halophyte that does not differ markedly from edible vegetables and is quite reminiscent of the seaweed varieties known as brown seaweed and tangle[14,15]. The pharmacological, physiological, and antioxidative effects of glasswort are reported to be useful for cosmetics and foods[16,17]. Especially, glasswort added to nuruk and meju is reported to increase nutritional quality of vinegar and ganjang (Korean soy sauce)[18,19].

In this study, fine powder of glasswort was added to soy milk prepared from fresh soy bean. The soy milk or soy milk with glasswort power was fermented by using a lactic acid bacterium in order to evaluate nutritional variation of soy yogurt supplemented with glasswort. The nutritional ingredients of soy yogurt were analyzed and compared based on contents of nutritional and functional compounds. This study may present a new recipe for preparing nutritionally and functionally improved soy yogurt for human health.

MATERIALS AND METHODS

Preparation of soy milk

Five hundred g of soybean soaked in 5 L of tap water at room temperature for 24 hr was filtered and washed with a strainer. Five L of fresh water was added to the macerated soybean and then ground with an electronic lab blender. The triturated soybean slurry was filtered with 270 mesh sieve. The filtrate was used as soybean milk for yogurt fermentation by properly diluted.

Desalting and grinding of glasswort

Dried glasswort purchased from Buan Hamcho (Buan, Cheonranam-do, Korea) was soaked for 15 min in running tap water for washing and desalting. The desalted glasswort was subsequently dried under sunlight for 48 h and ground using a ceramic ball mill (SW-BM117, 11.5L volume, SW Engineering, Seoul, Korea). The ground glasswort powder was sieved with 325-mesh of stainless sieve. The fine powder of glasswort was added to the freshly prepared soy milk at the ratio of 1, 2, and 3% (w/v).

Pasteurization and inoculation of soy milk

Freshly prepared soy milk containing 2% (w/v) glucose and glasswort powder was heated at 90°C for 45 min to minimize thermochemical reaction between ingredients of soy milk and glasswort. The pasteurized soy milk was cooled down to 38°C and then inoculated with freshly cultivated lactic acid bacterium. 2% (v/v) of *Lactobacillus casei*, which was previously cultivated in soy milk containing 2% glucose at 36°C for 24 hr, was inoculated into the prepared soymilk.

General analysis

Soy yogurt was lyophilized to readily analysis of crude protein, crude fat, and dietary fiber. Analytical values were determined based on the weight ratio of lyophilized yogurt to homogenized fresh yogurt. Crude protein was determined by a manually operated Kjeldahl system based on the total nitrogen content and dry weight[20]. Crude fat was determined based on weigh of extracted oil from the lyophilized yogurt by Soxhlet extraction apparatus. Dietary fiber content was determined using a dietary fiber assay kit (Sigma, St. Louis, MO, USA) after enzymatic removal of starches and proteins[21]. Lipid contained in the dietary fiber was removed by ether extraction.

Determination of organic acid

Soy yogurt was thoroughly mixed with 0.1 volume of 1N-phosphoric acid and centrifuged at 10,000 ×g and 4°C for 40 min. The filtrated supernatant was directly injected into injector of high performance liquid chromatography (Gold apparatus, Beckman, Coulter, Brea, CA, USA) equipped with a Shodex Rspak KC-811 ion exclusion column (Showa Denko, Tokyo, Japan) and a model RI-101 refractive index detector (Showa Denko). The organic acids were calculated based on the peak area obtained with standard materials and dilution ratio.

Determination of minerals

Samples for mineral analysis were prepared from lyophilized yogurt. Mineral content was analyzed using inductively coupled plasma (ICP) optic emission spectrometry (SPECTRO Analytical Instruments, Kleve, Germany). The extract prepared for mineral analysis was directly injected into the ICP injector under specific wavelengths for Mg (279.079 nm), Na (589.592 nm), K (766.491 nm), Ca (396.847 nm), Mn (257.610 nm), Zn (213.856 nm), Al (167.080 nm), Cu (324.745 nm), and Fe (238.204 nm). The mineral concentrations were calculated based on the absorbance obtained with standard materials (AccuTrace Reference Standard; AccuStandard, New Haven, CT, USA) and dilution ratio[22].

Determination of antioxidative compounds and activity

Samples were prepared by same method employed for organic acid analysis. The properly diluted supernatant was used to analyze the antioxidative compounds (polyphenol and total phenolic compounds) and activity. Polyphenol content was assessed using the Prussian blue spectrophotometric method[23]. Content of total phenolic compounds were determined via the Folin–Ciocalteu colorimetric method[24]. DPPH radical scavenging activity of doenjang was determined using a modified version of a method developed previously[25]. Ferric ion reduction activity of doenjang was determined by ferrous ion-ferrozine complex reaction. The supernatant (3.8 mL) was mixed with 50 μL of 10 mM $FeCl_3.4H_2O$ and incubated for 30 min at room temperature. Then, 150 μL of 5 mM Ferrozine was added to the mixture and shaken gently. After 10 min, the amount of Fe^{2+} was monitored by measuring the formation of ferrous ion-ferrozine complex at 562 nm. The concentrations of antioxidative compounds and activity were determined based on absorbance obtained with standard materials and the dilution ratio.

RESULTS

Protein, lipid and dietary fiber in soy yogurt

Crude protein and fat were not influenced by addition of glasswort powder but dietary fiber was increased in proportion to glasswort contents as shown in TABLE 1. It is reasonable based on the contents of protein (less than 2% w/w), lipid (less than 0.5% w/w), and non-hydrolyzed compounds (more than 50%) by protease and alpha-amylase in glasswort powder.

TABLE 1 : General character of soybean yogurt supplemented with glasswort

Nutrients	Composition (%, w/w)			
	Soymilk	Soymilk + 1% GP	Soymilk + 2% GP	Soymilk + 3% GP
Crude protein	4.81±0.4	4.82±0.4	4.82±0.5	4.81±0.3
Crude fat	2.85±0.1	2.85±0.1	2.86±0.2	2.86±0.1
Dietary fiber	0.11±0.02	0.61±0.02	1.13±0.05	1.66±0.04

Influence of glasswort on bacterial growth

Content of glasswort powder added to soy yogurt was maximally 3% (w/w), which may be enough to influence on bacterial growth based on the contents of Mg, Mn, and Fe. Practically, growth of *L. casei* was a little increased by addition of glasswort powder to soy milk prepared for cultivation of lactic acid bacterium as shown in TABLE 2.

Minerals in soy yogurt

Glasswort contains Na, K, Mn, Mg, Ca, Fe, Zn, Al and Cu, at levels significantly higher than in soybean[14,26]. Accordingly, the minerals contained in the soy yogurt may be proportional to the mineral balance of glasswort because minerals contained in glasswort become ingredients of soy yogurt. A variety of minerals analyzed for soy yogurt were increased in proportion to content of glasswort powder in soy yogurt as shown in TABLE 3.

TABLE 2 : Viable cell numbers (CFU) of lactic acid bacteria grown in soymilk or soymilk with glasswort powder (GP), which was determined based on the mean values obtained from triple tests.

Incubation time (hr)	Medium Compositions			
	Soymilk	Soymilk + 1% GP	Soymilk + 2% GP	Soymilk + 3% GP
12	6.1×10^8	6.3×10^8	4.8×10^8	5.3×10^8
24	4.3×10^8	7.0×10^8	6.1×10^8	4.6×10^8
36	5.6×10^8	8.6×10^8	9.5×10^8	7.8×10^8
48	6.2×10^7	6.8×10^7	8.4×10^7	8.2×10^7

TABLE 3 : Mineral contents in fermented soy yogurt or soy yogurt with glasswort powder (GP) that were cultivated for 48 hr.

Mineral contents (mg/kg)	Medium Compositions (ND, not detected)			
	Soymilk	Soymilk + 1% GP	Soymilk + 2% GP	Soymilk + 3% GP
Na	58.46±3.4	593.1±14.4	1,064.9±33.8	1,445.5±42.6
Mg	186.9±6.7	240.9±9.5	282.4±12.8	334.1±13.6
K	1,159±48.2	1,180±35.9	1,232±44.1	1,250±42.5
Ca	171.5±6.6	198.4±5.8	219.5±8.2	260.7±7.4
Fe	5.49±0.18	9.39±0.43	14.25±0.47	16.90±0.63
Mn	ND	0.23±0.01	0.48±0.01	0.71±0.01
Zn	ND	1.25±0.02	1.73±0.03	2.06±0.02
Al	ND	4.55±0.2	11.26±0.8	16.29±1.6

Organic acids in soy yogurt

The organic acids contained in soy yogurt may be generated from sugars contained in soy milk by fermentation metabolism of lactic acid bacterium or intrinsically contained in soy bean or glasswort. Content of lactic acid in soy yogurt was increased in proportion to percentage balance of glasswort as shown in TABLE 4. Greatly lower concentration of citric and malic acid than lactic acid was detected in soy yogurt but succinic and acetic acids were not detected.

Antioxidative compound and activity in soy yogurt

Soybean contains various antioxidative compounds including polyphenols, total phenolic compounds, tannin, and proanthocyanidin[27]. Phenolic compounds are ubiquitous chemicals found in most plants but those contents are different depending on part (leaf, stem, or root) of plant and plant species[28]. According to the analytical data for glasswort, total phenolic contents and polyphenol were higher than those in soybean[29]. Polyphenol and total phenolic compounds were increased in proportion to percentage balance of glasswort powder. Activity of soy yogurt for ferric ion reduction and DPPH-scavenging was proportional to polyphenol and total phenolic compound as shown in TABLE 5.

TABLE 4 : Organic acid contents in fermented soy yogurt or soy yogurt with glasswort powder (GP) that were cultivated for 48 hr.

Organic acids (mg/kg)	Medium Compositions (ND, not detected)			
	Soymilk	Soymilk + 1% GP	Soymilk + 2% GP	Soymilk + 3% GP
Citric acid	245±21.3	211±8.3	289±7.2	201±4.9
Malic acid	460±18.4	403±21.5	485±23.1	463±16.6
Lactic acid	9,756±333.6	10,979±425.4	11,270±389.2	11,791±369.7
Succinic acid	ND	ND	ND	ND
Acetic acid	ND	ND	ND	ND

TABLE 5 : Antioxidative compounds in fermented soy yogurt or soy yogurt with glasswort powder (GP) that were cultivated for 48 hr.

Antioxidant (mg/L)	Medium Compositions			
	Soymilk	Soymilk + 1% GP	Soymilk + 2% GP	Soymilk + 3% GP
Polyphenol	2.43±0.3	2.68±0.1	2.89±0.2	2.91±0.2
Total phenolic compounds	0.201±0.01	0.247±0.01	0.262±0.01	0.289±0.01
Fe^{3+} reduction	45.99±1.2	46.46±2.3	47.65±1.6	49.36±1.8
DPPH-scavenging activity	27.47±2.1	45.99±1.9	58.49±3.8	62.88±4.6

DISCUSSION

Soy yogurt is a less common fermentation food than dairy one, which may be a result of tradition or historical culture of the yogurt. Dairy milk can be naturally fermented by lactic acid bacteria originated from intestine or mammary gland of mammalians because dairy milk contains nutritional ingredients including protein and sugar (lactose) required for growth of lactic acid bacteria. Soy bean contains relatively higher protein, minerals, and vitamins than dairy milk but doesn’t contain proper sugar for growth of lactic acid bacteria[7]. Dairy milk produced by mammals is a natural food obtained without artificial process but soy milk is a processed food obtained by artificial operations. It is the reason why soy yogurt is an uncommon fermentation food. Practically, soybean has been used for preparation of various soy sauces and paste by processes of cooking, fermenting, and salted ripening because the cooked and hydrous soy bean is proper for growth of bacteria belonging to *Bacillus* genus or fungi belonging to *Aspergillus* genus[30,31]. Soy yogurt is greatly different from the soy sauce based on the kinds of microbial catalyst, fermentation metabolites, and preparation process. Salt is required for soy sauce after fermentation but sugar (glucose or lactose) is required for soy yogurt before fermentation.

The flavor of soy milk has to be different from dairy milk, a primary cause for soy yogurt to be different from dairy yogurt. Nevertheless, soy milk is often used as a substituted beverage for dairy milk in various countries. People who drink soy milk may adapt to the taste and flavor of soy milk by frequent drinking or those who are curious about unfamiliar food may try soy yogurt. Accordingly, soy yogurt produced by sugar-added lactic acid fermentation of soy milk may be accepted by people who drink soy milk or who have adapted to soy flavor. Those who can't digest lactose and have milk allergy may prefer to drink soy yogurt as a substitute for dairy yogurt.

The chemical ingredients of glasswort powder added to the soy milk may be completely extracted to be nutritional ingredients of soy yogurt during pasteurization and fermentation. Fine powder of glasswort may be completely mixed with aggregated soy protein induced by extracellular polysaccharide and acidity of lactic acid which was increased in proportion of glasswort content[32]. Mn, Fe, and Mg may activate lactic acid fermentation because the minerals function as cofactors absolutely required for activity of superoxide dismutase and specific enzymes catalyzing glycolysis pathway or TCA cycle[33-35].

The viscosity of soy yogurt induced by acidity may be effective to mask the feeling irritation caused by fine particle of glasswort powder and may be increased in proportion to glasswort content. The weakness of soy yogurt supplemented with glasswort powder may be compensated by the reinforcement of dietary fibers mineral and antioxidative effect. The increase of mineral, antioxidative fiber and antioxidative compound in soy yogurt may enhance the nutritional and physiological function but may not improve flavor. The nature of yogurt is determined by base materials that are mammalians' milk or soy milk but the appetizing flavor of yogurt is controlled by additives such as fruits, aromas, or sweeteners. Accordingly, the nutritional function of soy yogurt is improved by addition of glasswort and the flavor controlled by additives. From the nutritional standpoint, mineral, polyphenol and total phenolic compound contained in soy yogurt may be more helpful for health than dairy yogurt.

CONCLUSION

Glasswort may be useful as a supplement for soy yogurt when evaluated on the basis of bacterial growth, organic acid production, mineral contents, and antioxidative compounds. Especially, DPPH scavenging activity and ferric ion reduction activity of soy yogurt is a strong point in comparison with dairy yogurt. Soy milk is good but soy yogurt supplemented with glasswort is preferable for people who don't want to have food of animal origin. The environmental condition of soy milk for growth of lactic acid bacteria is improved by glasswort addition. Accordingly, glasswort is helpful for fermentation bacteria, nutritional improvement, and functional compound increased in soy yogurt. This research may be a basic reference for development of the soy yogurt supplemented with functional plants that are useful for human health.

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