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NOTE

Antibacterial Effects against Various Foodborne Pathogens and Sensory Properties of Yogurt Supplemented with *Panax ginseng* Marc Extract

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Abstract

Panax ginseng marc is produced from fresh ginseng roots during processing and is generally treated as industrial waste. The primary aim of this study was to improve its utilization in the dairy industry as a potential high-value resource. Yogurt was prepared from 11% skim milk powder, 0.1% pectin, 10% sucrose, and ginseng marc ethanol extract (GME, 0.5% and 1.0%) in milk, and was inoculated with a 0.02% yogurt culture (*Lactobacillus acidophilus*, *Bifidobacterium longum*, and *Streptococcus thermophilus*). After fermentation at 40°C for 6-8 h, the physicochemical properties of samples were analyzed by the AOAC, Kjeldahl, and Soxhlet methods. Sensory evaluation was performed based on consumer acceptability scores with a 7-point scale, and antimicrobial effects were measured by the agar plate method. The moisture, crude protein, crude fat, and ash contents of yogurt supplemented with 1% GME were 85.06±0.06%, 4.41±0.01%, 4.30±0.05%, and 0.81±0.03%, respectively, with no significant changes noted from those of yogurt without GME (control), except for an increase in the crude fat content. The sensory scores of color, flavor, texture, overall taste, and overall acceptance of yogurt supplemented with below 1% GME did not differ significantly ($p<0.05$) to those of the control yogurt. In addition, the growths of *Staphylococcus aureus*, *Bacillus cereus*, *Listeria monocytogenes*, *Escherichia coli*, and *Enterobacter sakazakii* were inhibited during fermentation and storage. These results suggest that GME could be used in dairy products as a supplement and in the food industry as an antimicrobial material.

Keywords *Panax ginseng* marc, yogurt, sensory evaluation, antibacterial effect

Introduction

Panax ginseng has been used as a traditional medicinal herb for thousands of years and is known to have functional value such as reducing the risk of cancers and chronic diseases, and improving the intestinal microflora (Cimo *et al*., 2013; Lee *et al*., 2013b). Ginseng marc is a by-product obtained during commercial ginseng extract manufacturing from fresh ginseng roots, but is generally discarded as waste or fed to poultry (Chung *et al*., 2015). Several studies on ginseng marc have reported that it contains polysaccharides, and exhibited anti-tumorigenic activity against melanoma cells (Seo *et al*., 2015), a cholesterol-lowering effect in broiler chicken (Kim *et al*., 2014), and showed applicability for dairy and bakery products

(Choi *et al*., 2014; Jung *et al*., 2010).

Yogurt is widely consumed in various countries because of its good taste as well as its nutritional value owing to the presence of bioactive peptides and essential minerals. Intake of the recommended level of yogurt has been shown to reduce the incidence of cardiovascular diseases, cognitive diseases, musculoskeletal diseases, dermatological conditions, obesity, and nervous system diseases through clinical trials (Yildiz, 2016). Recent studies related to yogurt have been performed to attract consumers or seek value-added ingredients (e.g., grape pomace extract and high-fiber food) (Karnopp *et al*., 2017). These materials have been linked to antioxidant activity and can be added to dairy products for producing functional foods (O'Sullivan *et al*., 2014).

Although some supplements may contribute to enhancing a product's functionalities, there are limitations of their antibacterial effects during the fermentation and storage periods (Sah *et al*., 2016). Therefore, the objective of this study was to produce yogurt supplemented with ginseng marc that meets consumer acceptance, and to detect its antimicrobial activity against foodborne pathogens during the yogurt fermentation and storage periods.

Materials and Methods

Strains

Yogurt culture (ABT-B: a mixture of *Lactobacillus acidophilus*, *Bifidobacterium longum*, and *Streptococcus thermophilus*) was purchased from Culture Systems, Inc. (Mishawaka, USA). *Bacillus cereus* KCCM11341, *Enterobacter sakazakii* ATCC51329, *Staphylococcus aureus* 1573, *Salmonella* Typhimurium (National Veterinary Research and Quarantine Service, Korea), *Listeria monocytogenes* H7962 (Centers for Disease Control and Prevention, USA), and *Escherichia coli* O157:H4 FRIK125 (Wisconsin Food Research Institute, USA) were used for evaluation of the antibacterial effect. All pathogenic strains were activated in trypticase soy broth (Difco Labs, USA).

Preparation of yogurt

Ginseng marc was obtained from Il-Hwa Research Institute (Korea). Ginseng marc was extracted three times with 4 volumes of 75% ethanol at 60°C for 12 h, and then lyophilized using a freeze-drying method. To prepare the yogurt, 11% skim milk (Seoul Milk Co., Korea), 0.1% pectin (Buy Chem, Korea), and 10% sucrose (Cheil Jedang, Korea) were added to 0.2 L of milk (Seoul Milk Co., Korea), with or without ginseng marc extract (GME) at various concentrations (0.5% and 1.0%), and heated at 85°C for 10 min (Karnopp *et al*., 2017; Shah *et al*., 2000). After cooling, the 0.02% yogurt culture was inoculated into each yogurt sample and then incubated at 40°C in a water bath (Lab Companion, Korea) until the pH decreased to 4.5. After fermentation was complete, the yogurt samples were stored at 4°C for 21 d.

Physicochemical analysis

The moisture and ash contents were measured by the AOAC method (2006). Crude protein and crude fat contents were determined by the Kjeldahl method and Soxhlet extraction, respectively (Jung *et al*., 2010). All tests were performed in five times and the results are expressed as means ± standard deviation (SD).

Antibacterial effect

Yogurt samples with and without GME supplementation were collected every 2 h during fermentation and then every 7 d during the storage period for analysis of the antibacterial activity. Cell numbers of each sample were determined by the standard plate-count method. The media of agar plates used in this study were bromoceresol purple agar for LAB (Choi *et al*., 2014), *Salmonella Shigella* agar for *S.* Typhimurium, mannitol salt agar for *S. aureus* 1573, MacConkey sorbitol agar for *E. coli* O157:H4 FRIK125, tryptic soy agar for *B. cereus* KCCM11341 and *E. sakazakii* ATCC51329, and Oxford agar for *L. monocytogenes* H7962 (Chung *et al*., 2015; Sah *et al*., 2016). A 100-μL diluted yogurt sample was spread on agar plates. After incubation at 37°C for 2 d, the cell colonies were counted.

Sensory evaluation

The sensory evaluation for consumer acceptability was conducted by 49 untrained panelists (28 female, 21 male, 21 to 60 years old) and approved by the Institutional Biosafety Committee (approval number: 2017-003) (Korea). Completely fermented yogurt samples were ripened for 1 d at 4°C. Two concentrations of GME were used for this evaluation: 0.5% and 1.0%. The color, flavor, texture, overall taste and acceptability were evaluated on a 7-point scale as follows: 1 = extremely dislike, 2 = very dislike, 3 = slightly dislike, 4 = neither like nor dislike, 5 = slightly like, 6 = very like, 7 = extremely like.

Statistical analysis

All results are expressed as means±SD. For statistical

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comparisons, the proximate composition was analyzed using one-way analysis of variance with Duncan's post-hoc test ($p<0.05$), and sensory evaluation was analyzed using the non-parametric Kruskal-Wallis/Mann-Whitney test ($p<0.05$). All statistical tests were performed using the SPSS 21.0 program (SPSS Inc., USA).

Results and Discussion

Proximate composition of yogurt supplemented with ginseng marc

The compositions of the GME, yogurt, and yogurt supplemented with GME at 1% are presented in Table 1. According to Lee *et al*. (2013b), carbohydrates have the largest contribution in *Panax ginseng* at 67.3±0.0%, followed by crude protein (12.5±0.3%), moisture (5.9±0.6%), ash (3.7±0.2%), and crude fat (1.3±0.1%). Water is traditionally used as the main extracting solvent for ginseng concentrate. However, the general components of ginseng marc were found to differ when using alcohol as the extract solvent, according to the alcohol concentration (Kang *et al*., 2004; Kim, 2007). In this study, the crude fat and protein compositions of the GME were increased by 31.8% and 13.6%, respectively.

Antibacterial effect of yogurt supplemented with ginseng marc

After fermentation, LABs number of yogurt (control) and yogurt supplemented with 1% GME were 9.2±0.3 and 9.1±0.2 Log CFU/mL, respectively. pH of yogurt (control) and yogurt supplemented with 1% GME were 4.5±0.0. Six strains of foodborne pathogens (3 gram-positive and 3 gram-negative strains) were inoculated into yogurt (control) and yogurt supplemented with 1% GME to determine the antibacterial effects. During the yogurt fermentation and storage periods, yogurt supplemented with 1% GME showed a lower cell number of pathogens than observed for the control yogurt sample for *S. aureus* 1573, *B. cereus* KCCM11341, *L. monocytogenes* H7962, *E. coli* O157:H4 FRIK125, and *E. sakazakii* ATCC51329, but not for *S.* Typhimurium 15 (Fig. 1). *B. cereus* KCCM11341 and *E. sakazakii* ATCC51329 were found to be the most sensitive pathogens tested, as their viable cell numbers decreased most rapidly when the yogurt sample supplemented with 1% GME was stored in a refrigerator for 1 d. Although almost pathogens are affected by the acidic pH, low temperature, and inhibitory peptides derived from milk (Beales, 2004; Hashemi *et al*., 2016), these antibacterial effects might be enhanced by GME supplementation. GME contains acidic polysaccharides, oligosaccharides, and polyacetylene (Kang *et al*., 2004; Seo *et al*., 2015). The addition of plant polyphenol such as ginseng, olive, and peanut sprouts in yogurt production have been reported to enhance antioxidant and antimicrobial effect (Cimo *et al*., 2013; O'Sullivan *et al*., 2014). In addition, polyacetylene, a lipid-soluble compound of ginseng marc, was previously reported to exert antimicrobial effects against *S. aureus*, *Bacillus subtilis*, *Cryptococcus neoformans*, and *Aspergillus fumigatus* (Fukuyama *et al*., 2012). Kim *et al*. (2014) reported that supplementation of red ginseng marc enhanced antibacterial activity to improve the meat quality of broiler chicken. Chung *et al*. (2015) also reported that the growth of *Salmonella enterica* and *E. coli* was inhibited in poultry litters treated with red ginseng marc.

Table 1. Proximate analysis of yogurt supplemented with *Panax ginseng* marc extract[1]

	Yogurt	Yogurt + 0.5% GME	Yogurt + 1% GME
Moisture (%)	84.95 ± 0.07[a]	85.00 ± 0.11[a]	85.06 ± 0.06[a]
Crude fat (%)	4.11 ± 0.03[a]	4.20 ± 0.08[ab]	4.30 ± 0.05[b]
Crude protein (%)	4.69 ± 0.01[a]	4.55 ± 0.03[ab]	4.41 ± 0.01[b]
Ash (%)	0.81 ± 0.00[a]	0.81 ± 0.01[a]	0.81 ± 0.03[a]

[1]Unit: %. All dates are the means±standard deviation (n=5).

[a-d]Means in the same row with different letters are significantly different ($p<0.05$)

GME: *Panax ginseng* marc extract (Moisture content: 2.87±0.05%; Crude fat: 33.10±0.44%; Crude protein: 26.70±0.21%; Ash: 6.12±0.02%)

Sensory evaluation of yogurt supplemented with ginseng marc

A sensory evaluation was conducted to compare the consumer acceptability between control yogurt and yogurt supplemented with GME. As shown in Table 2, there was no significant difference in any of the sensory parameters ($p<0.05$) between yogurt and yogurt supplemented with 0.5% GME. The control yogurt showed the highest scores with respect to flavor (5.2), overall taste (5.6), and overall acceptance (5.5). The yogurt supplemented with 0.5% GME had the highest scores with respect to color (5.5), which was due to the color of the ginseng marc itself (yellow-brown). The yogurt supplemented with 1% GME showed low scores in the sensory evaluation, which might be related to the presence of triterpenoid peptides or propylene glycol in GME, which are known to cause the bitter taste of ginseng extract (Szente and Szejtli, 2004; Tamamoto *et al*., 2010). Various masking agents have been inv-

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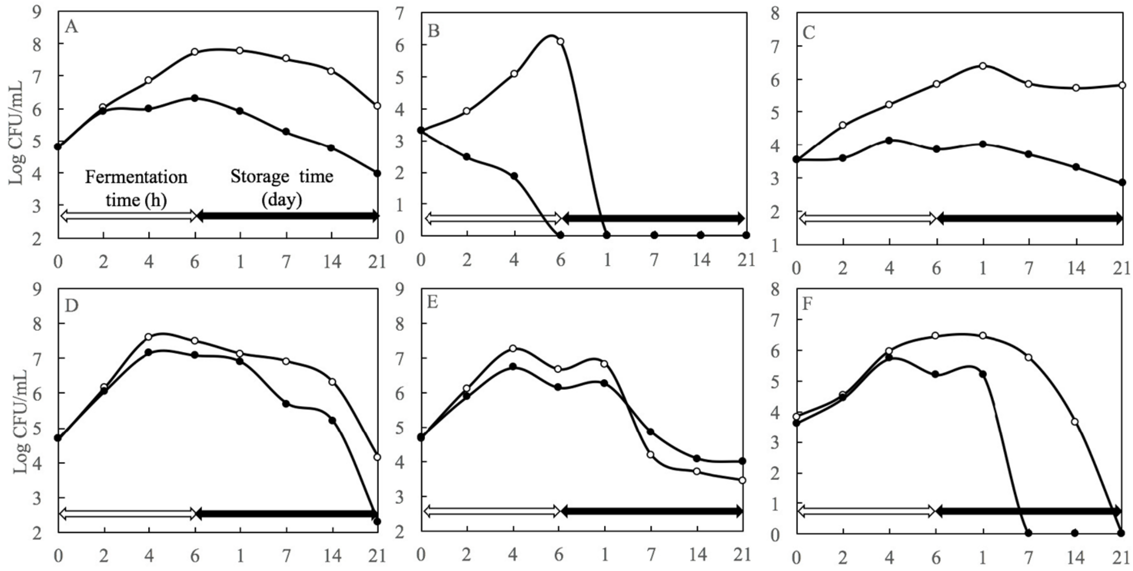

Fig. 1. Anti-bacterial effects of yogurt supplemented with 1% *Panax ginseng* marc extract during fermentation and storage. A, *Staphylcoccus aureus* 1573; B, *Bacillus cereus* KCCM11341; C, *Listeria monocytogenes* H7962; D, *Escherichia coli* O157:H4 FRIK125; E, *Salmonella* Typhimurium 15; F, *Enterobacter sakazakii* ATCC51329; ○ , yogurt; ● , yogurt added ginseng marc extract.

estigated to minimize this bitter taste. Many carbohydrate products such as α-, β-, and γ-cyclodextrin, glucose, lactose, honey, corn starch, oligosaccharide, sucrose, and xylose have been used to improve the taste and flavor of red ginseng extract (Chung *et al*., 2011; Lee *et al*., 2009). In this study, when 0.54% β-cyclodextrin and 4.7% corn syrup were added to reduce or mask the bitter taste of the GME, there was no significant difference (p<0.05) between samples supplemented with 0.5% GME and 1% GME (data not shown).

In conclusion, these results indicate that ginseng marc is a suitable yogurt supplement based on evaluations of consumer acceptance and the fact that yogurt supplemented with ginseng marc could effectively inhibit common foodborne pathogens such as *S. aureus*, *B. cereus*, *L. monocytogenes*, *E. coli*, *S.* Typhimurium, and *E. sakazakii*. The present study demonstrates that ginseng marc fermented with lactic acid bacteria can be a beneficial material for dairy products and can be used widely in the food industry as a supplement and natural antibacterial extract.

Table 2. Sensory evaluation of yogurt supplemented with *Panax ginseng* marc extract

	Yogurt	Yogurt + 0.5% GME	Yogurt + 1% GME
Color	5.35 ± 0.94[a]	5.48 ± 1.00[a]	4.92 ± 1.11[a]
Flavor	5.24 ± 0.97[a]	5.16 ± 1.03[a]	4.92 ± 1.39[a]
Texture	5.28 ± 0.94[a]	5.28 ± 0.89[a]	4.68 ± 1.18[a]
Overall taste	5.56 ± 0.87[a]	5.16 ± 0.75[a]	4.85 ± 1.57[b]
Overall acceptance	5.48 ± 0.82[a]	4.85 ± 1.57[a]	3.80 ± 1.53[b]

1 = extremely dislike, 4 = neither like nor dislike, 7 = extremely like. 49 panelists were involved. All dates are the means ± SD.
[a,b]Means in the same row with different letters are significantly different (p<0.05).

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