Effect of the ingredients on the microbial qualities of six varieties of Dasik during storage

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Abstract This study was conducted to evaluate the microbial quality of 6 varieties of brown rice Dasik prepared with roasted bean powder, *Omija* juice, red ginseng, propolis, and a combination of red ginseng and propolis during storage at 5 and 30°C. The changes in moisture content and pH, total plate counts and molds were evaluated. In addition, the survival of artificially inoculated *E. coli* O157:H7 into Dasik was studied during storage. As a result, the moisture content of the samples ranged from 9 to 13% and the pH ranged from 2.7 to 6.7 ($p < 0.05$). In Dasik made with red ginseng, propolis, or a combination of both, total plate count was not increased and the growth of artificially inoculated *E. coli* was inhibited ($p < 0.05$). These results suggest that addition of ingredients such as red ginseng, propolis, and *Omija* juice to Dasik improves the microbial safety and quality during storage.

Keywords: Dasik, food safety, storage, propolis, red ginseng

Introduction

Dasik is a traditional Korean confectionery used since the time of Koryo Dynasty and mainly consumed with Korean tea. Dasik is made from starch (potatoes and sweet potatoes), pine pollen or black sesame powder, flour from rice, etc. (1,2). Dasik is kneaded with honey and sugar syrup, and the dough is pressed into a Dasik mold. Depending on the ingredients, Dasik is white, black, yellow, green, or brown in color.

A trend of globalization has influenced every industry, and as a consequence, the globalization of traditional Korean foods is also flourishing. So far, studies on Dasik have mainly focused on the quality of Dasik made from various ingredients with novel functionalities and sensory evaluations for consumer preference. Published studies include analyses of Dasik with red ginseng powder (3), rice Dasik made with Job's tears flour (4), Dasik prepared with sanghwang mushroom powder (5), Dasik with lotus leaf powder (6), and rice Dasik made with Yam (*Dioscorea japonica*) powder (7).

As mentioned above, Dasik may be composed of various ingredients; of these, brown rice was selected as the main base ingredient in this study. Roasted bean powder, *Omija* juice, red ginseng, and propolis were also selected as other supplemental ingredients. Brown rice is whole grain rice in which only the outermost layer of a grain of rice (the husk) is removed. Brown rice has the advantage of higher fat, protein, vitamin, and dietary fiber contents than white rice; thus, brown rice has beneficial effects on lowering blood pressure. Yet,

since the rough texture of brown rice decreases palatability, studies to improve the texture of brown rice Dasik with modified potato starch powder have been carried out (2). Beans also contain significant amounts of protein, fats, and fiber and therefore are good sources of protein and fat. The soluble fiber in beans helps lower blood cholesterol. Additionally, the isoflavone in beans is effective in preventing osteoporosis and arteriosclerosis. Beans contain saponin, which has an antioxidant effect and inhibits the growth of cancer cells (8,9). *Omija* is known to contain five basic flavors, including salty, sweet, sour, spicy and bitter. It has been used in oriental medicine for coughing and is known for anticancer, antioxidant, and antibacterial effects. Constituents such as ethyl acetate, citronellal, and terpineol, among others, in the crude extract of *Omija* demonstrate antimicrobial activity. (10–13). As a drink, *Omija* is added to boiled water to make a tea or punch as favorite summer drinks. Red ginseng is peeled, heated through steaming at high temperature, and then dried or sun-dried. Red ginseng has effects on cancer, recovery from fatigue, and mental stability. Saponin in red ginseng inhibits the growth of *Lactobacillus* spp. and molds, reinforces immune function, and has detoxification properties. Studies also reported that red ginseng has an antibacterial effect against food poisoning bacteria (14,15). A previous study on Dasik containing red ginseng powder showed lower moisture content and higher ash content than the control group (3). Moreover, addition of red ginseng powder in Dasik showed increased hardness, cohesiveness, stronger color, and flavor than the control group in a mechanical

texture test. Propolis is an antimicrobial substance made by bees and has antimicrobial effects against major foodborne pathogens (16) and oral microorganisms (17).

From a microbiological point of view, as Dasik is not processed through a chemical or thermal process to control contaminant microorganisms during manufacturing, the microbial quality of Dasik during short-term storage is of concern to consumers. To our knowledge, there have been no studies on the microbial quality of Dasik based on the ingredients. In this respect, a study on the microbial quality of Dasik during storage is much needed. In addition, as Dasik is usually consumed after a meal as a dessert, it would be beneficial to make Dasik with natural antimicrobial agents, such as propolis, that have an inhibitory effect on oral microorganisms to prevent cavities.

Therefore, the purpose of this study was to evaluate the changes in moisture content and pH of brown rice Dasik made with 6 different ingredients and to evaluate the effect of these ingredients on the microbial quality of Dasik with the aim to improve its quality and safety during storage at two different temperatures.

Materials and Methods

Materials Brown rice was purchased from Seulsung Nonghyup (Imgeumnimpyo, Incheon, Korea) and oligosaccharide was obtained from CJ Cheiljedang Co. (Seoul, Korea) as the main base ingredients of Dasik. Ginseng, propolis, *Omija* juice (*Schizandra chinensis*), and roasted bean powder were purchased from Korea Ginseng Co. (Daejeon, Korea), Withealth propolis Co. (Kyungnam, Korea), Moonkyung farm Co. (Moonkyung, Korea), and CFEA Co. (Chulwon, Korea), respectively.

Microorganisms *E. coli* O157:H7 ATCC 35150 was obtained from the department of Food and Nutrition at Yeungnam University. A stock culture of the pathogen was maintained in trypticase soy broth (TSB, Difco Laboratories, Detroit, MI, USA) with 20% glycerol and stored at -80°C . Working cultures were maintained on a slant of

trypticase soy agar (TSA, Difco) and stored at 4°C . The pathogen was activated and incubated aerobically in TSB at 37°C for 24 h. After three consecutive subcultures, stationary phase *E. coli* O157:H7 ($8-9 \log \text{CFU/mL}$) were used in this study.

Dasik preparation Dasik was prepared according to previous research (18,19). Dasik samples (5.0 g) made with each ingredient were cut out using a Dasik mold, and the samples were stored at either refrigeration temperature of 5°C for 15 days or in an incubator at 30°C for 7 days. Dasik made with brown rice and oligosaccharide was used as a control. The ingredients and the mixing ratio of 6 Dasik samples used in this study are listed in Table 1.

Moisture content and pH measurement Samples stored at 5 and 30°C were taken out for measurement of moisture content and pH on days 0, 5, and 10 and on days 0, 3, 5, and 7, respectively. Moisture content of the Dasik sample (1 g) was measured three times using a moisture analyzer (FD-720; Kett, Tokyo, Japan). For pH measurement, 5 g of Dasik samples were homogenized with 25 mL of distilled water, and pH was measured using a pH meter (420A; Orion Research Inc., Boston, MA, USA) on days 0, 5, and 10 for Dasik samples stored at 5°C and on days 0, 3, 5, and 7 for Dasik stored at 30°C .

Microbiological analysis of 6 varieties of Dasik During storage at 5°C and 30°C , 5 g of each Dasik sample were mixed with 45 mL of sterile peptone water (Bacto Peptone, Becton Dickinson Co., Sparks, MD, USA) and homogenized using a stomacher (Bag mixer 400; Interscience, Auvergne, France). One milliliter of the homogenized sample was mixed with 9 mL of 0.1% sterile peptone water, and serial dilutions were made using 0.1% peptone water. Next, an appropriately diluted sample was pour plated onto Plate count agar (PCA, Merck, Darmstadt, Germany) and incubated at 35°C for 24 h. For mold counts, appropriate dilutions were surface plated onto pH adjusted potato dextrose agar (PDA, Merck) and incubated at 25°C for 5 days. After incubation, colonies were enumerated.

Table 1. Formula for 6 kinds of Dasik containing various ingredients (unit: g)

Sample ¹⁾	Ingredients						
	Brown rice flour	Roasted bean powder	Oligosaccharide	Syrup	Red ginseng extract	Propolis	<i>Omija</i> juice
a	70	-	20	-	-	-	-
b	70	-	20	-	7	-	-
c	70	-	20	-	-	0.05	-
d	70	-	20	-	7	0.05	-
e	70	20	20	-	-	-	-
f	70	-	20	-	-	-	14

¹⁾a: Brown rice flour + oligosaccharide

b: Brown rice flour + oligosaccharide + red ginseng extract

c: Brown rice flour + oligosaccharide + propolis

d: Brown rice flour + oligosaccharide + red ginseng extract + propolis

e: Brown rice flour + roasted bean powder + oligosaccharide

f: Brown rice flour + oligosaccharide + *Omija* juice

Inoculation study of Dasik with *Escherichia coli* O157:H7 Two hundred microliter of *E. coli* O157:H7 overnight culture was mixed with 5 g of Dasik ingredients, mixed and made into Dasik to make a final concentration of 3 log CFU/g. After inoculation, Dasik samples were dried for 30 min for bacterial attachment and incubated at 30°C for 7 days. Samples of Dasik were taken at the predetermined time intervals (0, 3, 5, or 7 days) and homogenized with 45 mL of sterile 0.1% peptone water, after which serial dilutions were made in 9 mL of sterile 0.1% peptone water. An appropriate dilution was plated into Eosin methylene blue agar (EMB, Merck) and incubated at 37°C for 24 h. After incubation, survivors of the pathogen were enumerated.

Statistical analysis of the data The experiment was performed in triplicate, and the results were presented as an average. The data obtained were subjected to statistical analysis using analysis of variance, and the level of significance of means was determined by applying Duncan's Multiple Range Test. Statistical analysis was performed using SAS program (Version 9.2, SAS Institute Inc., Cary, NC, USA). Tukey's test was used for comparison of the same data. Evaluations were based on a significance level of $p < 0.05$.

Results and Discussion

Measurement of moisture content The changes in moisture content of Dasik samples during storage are shown in Fig. 1. The moisture content of 6 kinds of Dasik samples stored at 5 and 30°C was in the range between 9 and 14%. Dasik with *Omija* juice had significantly higher ($p < 0.05$) moisture content (12-14%) compared to the control Dasik without other ingredients, which showed relatively low moisture content (9%). Dasik made with propolis had low

moisture content (9%). Six Dasik samples showed no significant changes in moisture content during storage ($p > 0.05$).

Particularly, with the addition of *Omija* juice, the moisture content increased when Dasik was made with syrup rather than with honey (20). Yun and Kim (3) studied the characteristics of Dasik made with sweet potato starch and wheat flour by adding different concentrations of red ginseng powder. The authors reported that the moisture content of Dasik made with sweet potato starch was higher than that of wheat flour Dasik and that addition of a higher concentration of red ginseng powder resulted in Dasik with considerably lower moisture content compared to other ingredients. Lee and Kim (21) demonstrated that the moisture content of rice Dasik containing red ginseng powder was lower than control samples, but there were no significant differences. Kim *et al.* (14) investigated the quality characteristics of brown rice Dasik containing three different varieties of Korean ginseng powder (white, red, and black) and reported that the moisture content of the Dasik was highest in Dasik with white ginseng, followed by red ginseng and black ginseng. Depending on the ingredient added, the moisture content of Dasik may be affected due to the absorptive capacity of the ingredient *per se* (22-25).

Measurement of pH The pH measurements of 6 types of Dasik samples over a period of 15 days at 5°C and 7 days at 30°C are shown in Fig. 2. The pH levels of most Dasik samples ranged between 6-6.5, except for Dasik made with *Omija* juice (pH 2.7-3.1). The pH of Dasik containing roasted bean powder was the highest at both temperatures (pH 6.5-6.8), and the pH of Dasik containing *Omija* juice was the lowest (pH 2.7-3.1) among 6 Dasik samples, probably due to the low pH of *Omija* extract (3.39-3.62) (10) ($p < 0.05$). During storage, the pH levels of 6 Dasik samples were decreased slightly at both temperatures. For all Dasik samples, at 5°C there was a tendency for pH to decrease

Fig. 1. Changes in moisture content of 6 kinds of Dasik during storage at 5°C (A) and 30°C (B). a: Brown rice + Oligosaccharide, b: Brown rice + Oligosaccharide + Red Ginseng, c: Brown rice + Oligosaccharide + Propolis, d: Brown rice + Oligosaccharide + Propolis + Red Ginseng, e: Brown rice + Oligosaccharide + Bean powder, f: Brown rice + Oligosaccharide + *Omija*. Means with different letters are significantly different at $p < 0.05$ by Duncan's multiple range test.

Fig. 2. Changes in pH levels of 6 kinds of Dasik during storage at 5°C (A) and 30°C (B). a: Brown rice + Oligosaccharide, b: Brown rice + Oligosaccharide + Red Ginseng, c: Brown rice + Oligosaccharide + Propolis, d: Brown rice + Propolis + Red Ginseng, e: Bean powder, f: Omija. Means with different letters are significantly different at $p < 0.05$ by Duncan's multiple range test.

Fig. 3. Total plate counts of 6 kinds of Dasik during storage at 5°C for 15 days (A) and at 30°C for 7 days (B).

during storage, whereas at 30°C, the pH increased slightly up to 3 days and then decreased during storage.

Microbiological quality of Dasik samples Total plate counts of 6 Dasik samples stored at 5°C for 15 days (A) and 30°C for 7 days (B) are shown in Fig. 3. In general, 1-3 Log CFU/mL of bacteria were detected during storage at both temperatures. For Dasik samples stored at 5°C for 15 days, total plate counts were highest in those made with roasted bean powder or *Omija* juice, followed by red ginseng, control, propolis, or a combination of red ginseng and propolis, on day 5. Total plate counts in Dasik made with roasted bean powder or *Omija* juice increased significantly over the control during 15 days of storage at 5°C ($p < 0.05$). Dasik made with red ginseng inhibits the increase of total plate counts, and Dasik made with either propolis or a combination of propolis and red ginseng showed the lowest total plate counts among the 6 Dasik samples

(Fig. 3A).

For Dasik samples stored at 30°C for 7 days, the results were similar to those stored at 5°C, yet more pronounced. The total plate counts were highest in Dasik made with roasted bean powder, followed by *Omija* juice, propolis, control, red ginseng, and a combination of propolis and red ginseng. The lowest total plate counts were observed in the Dasik made with red ginseng or with a combination of red ginseng and propolis (Fig. 3B). It seems that Dasik made with red ginseng was more effective at a higher temperature than at a low temperature.

Overall, among Dasik samples stored at both temperatures, Dasik made with roasted bean powder had the highest total plate counts among the 6 types of Dasik ($p < 0.05$). In contrast, Dasik containing a mixture of propolis and red ginseng had the lowest total plate counts during storage ($p > 0.05$). Lee *et al.* (18) studied bacterial growth in nutrient broth supplemented with 1% *Omija* extracts and reported

Table 2. Changes in mold and yeast counts (CFU/g) of 6 Dasiks during 7 days of storage at 30°C

Sample	Storage time (days)			
	0	3	5	7
a	2.0x10±0.51 ^{1a}	2.0x10±0.18 ^{3b}	2.1x10±0.52 ^{3b}	2.1x10±0.11 ^{3b}
b	2.1x10±0.01 ^{1b}	4.2x10±0.02 ^{1b}	4.0x10±0.05 ^{1b}	2.4x10±0.01 ^{1b}
c	2.3x10±0.01 ^{1BC1)B2)}	5.2x10±0.02 ^{1Ab}	1.0x10±0.01 ^{1Cb}	3.8x10±0.01 ^{1ABb}
d	1.8x10±0.01 ^{1Bb}	4.3x10±0.02 ^{1Ab}	1.0x10±0.01 ^{1Bb}	1.2x10±0.01 ^{1Bb}
e	3.3x10±0.03 ^{1Ab}	2.8x10±0.01 ^{1Ab}	2.2x10±0.01 ^{1Ab}	4.0x10±0.01 ^{1Bb}
f	3.9x10±0.06 ^{1b}	4.3x10±0.02 ^{1b}	4.4x10±0.54 ^{1b}	5.5x10±0.01 ^{1b}

¹⁾Means with same capital letter in a row are not significantly different at $p<0.05$ by Duncan's multiple range test.

²⁾Means with same small letter in a column are not significantly different at $p<0.05$ by Duncan's multiple range test.

Fig. 4. Growth inhibition of *E. coli* in 6 kinds of Dasik during storage at 30°C for 7 days.

antibacterial activity against *Bacillus subtilis*, *Staphylococcus aureus*, *E. coli*, and *Pseudomonas aeruginosa* (24).

The growth of molds in 6 Dasik samples stored at 30°C for 7 days is shown in Table 2. During storage at 30°C, the counts of the molds in 6 Dasik samples increased initially, but Dasik samples made with either propolis or red ginseng, or the combination of both, showed lower mold growth than control. Dasiks made with roasted bean powder and *Omija* juice had higher counts of molds ($p<0.05$).

Inoculation study of Dasik with *Escherichia coli* O157:H7 In order to simulate the worst scenario of pathogen contamination during Dasik manufacturing, *E. coli* O157:H7 (~3 log CFU/g) was artificially inoculated into 6 kinds of Dasik samples, which were stored at 30°C for 7 days (Fig. 4). The inoculated pathogen grew better in Dasik made with roasted bean powder than the control and showed the highest growth of the 6 Dasik samples ($p<0.05$). This was in agreement with the microbial quality evaluation result, in which the highest total plate counts were observed in Dasik made with roasted bean powder.

Soybean is one of the most important legumes consumed worldwide and is a high source of protein, fiber, vitamins, and minerals.

Compared to other ingredients added in this study, nutrition rich roasted soybean powder supported total plate counts as well as the growth of artificially inoculated *E. coli* O157:H7. Shori (26) reported that inclusion of soybean in yogurt increased lactic acid bacterial counts, with two-fold higher total plate counts compared to plain yogurt, supporting the results of this study.

In Dasiks made with propolis, red ginseng, or the combination of both, the growth of *E. coli* O157:H7 was inhibited during storage at 30°C. The highest inhibition of *E. coli* populations was observed in Dasik made with *Omija* juice during storage ($p<0.05$). This may be due to the low pH of *Omija* juice, in the range of pH 2.7-3.1, as well as the presence of organic acids in *Omija* juice. Chung *et al.* (12) reported inhibition of the growth rate of bacteria in the presence of *Omija* ethanol extract in nutrient broth, suggesting the antimicrobial effect of *Omija* ethanol extract.

In summary, Dasik is a Korean traditional confectionery, and Dasik may be made of various ingredients. However, as Dasik is not processed through a chemical or thermal process to control contaminant microorganisms during manufacturing, it is important to evaluate the microbial quality of Dasik made with different ingredients during short-term storage for safety. The results from this study indicate that addition of ingredients that have antimicrobial activity, such as red ginseng, propolis, or a combination of both, or *Omija* juice to Dasik may improve the microbial quality and safety of Dasik.

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