Effect of retort sterilization on microbial safety and quality characteristics of a rice cake, *songpyeon*

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Abstract Rice cake, *songpyeon*, is a commonly consumed snack food in Korea. Currently, the recommended shelf life of rice cakes is only 1 day at room temperature because of concerns over quality deterioration and microbial safety. In this study, retort sterilization for 15 to 19 min was used to produce microbiologically safe *songpyeon*, and quality characteristics were analyzed. Sterilization of *songpyeon* at 121°C for 15 min resulted in complete eradication of *Bacillus cereus* and *B. subtilis*. Total plate count, yeast, fungi, and coliform counts were below detection. With increasing sterilization time, the average L* and a* values of *songpyeon* decreased from 28.86 to 23.07 and −1.63 to −6.73, respectively, whereas the b* value tended to increase from 4.91 to 9.82 on average, showing low lightness and high yellowness. Texture analysis showed increased gumminess (1240.6 to 2469.63 g) and decreased chewiness (2283.94 to 1366.26 g) and hardness (2933.94 to 1616.26 g) in *songpyeon* with increasing sterilization time. According to sensory evaluation, *songpyeon* sterilized at 121°C for 17 min was preferred by sensory panels based on average appearance (7.65), color (7.15), taste (7.45), and overall acceptance (7.4) scores. Therefore, sterilization at 121°C for 17 min is suggested to be optimal for producing microbiologically safe *songpyeon* with the best quality characteristics.

Keywords: rice cake, shelf-life, retorting, sterilization, physicochemical properties

Introduction

Rice is a major crop consumed as a staple food in Korea. However, intake of rice and processed rice products has been gradually decreasing. This is probably attributable to the influence of Western foods and socio-economic phenomena such as the emergence of small families and changes in food culture (1). As consumers are very concerned with health, consumption of health-oriented or functional foods has become a trend. Accordingly, rice is drawing more attention as a staple food with health benefits such as lowering blood pressure and cholesterol (2,3). More processed rice products are being developed to encourage consumption of rice and rice-based products.

There are several varieties of rice-based products available on the market, such as snacks (4), breads (5), and rice cakes (6), including *songpyeon*. Rice cakes are traditional Korean foods occupying over 60% of the processed rice market (7). They are prepared by steaming rice flour with various fillings. *Songpyeon* in particular is made with various kinds of sweet or semi-sweet fillings, such as sesame seeds, honey, and black-eyed peas. It was traditionally eaten during Chuseok, the autumn harvest festival, but nowadays is eaten all year round.

Rice cakes are often sold at room temperature in local stores and are consumed without further cooking, which renders them susceptible to microbial growth. In addition, the high water content of rice cakes makes them a favorable environment for microorganisms, raising quality and safety concerns (8,9). Choi *et al.* (7) reported that the safety of rice cakes and other convenience foods could be the most affected by climate change among several categories of food studied. Currently, the recommended shelf life of rice cakes is 24 h at room temperature (10). The shelf life and safety of rice cakes are strongly related to the growth of microorganisms (11). Many *Bacillus* spp. commonly found in soil can contaminate cereal grains and vegetables (12). Rice contaminated with *B. cereus* can cause food poisoning (13). As with other rice cakes, there is a concern that *songpyeon* may become contaminated with microorganisms including *Bacillus* spp. *Bacillus* spp. have occasionally been found in rice at a concentration of 10² CFU/g (6). Kim *et al.* (14) reported that less than 1 log CFU/g of *B. cereus* was detected in 5 out of 27 rice samples. Choi *et al.* (7) reported 4.9 log CFU/g total aerobic bacteria, 43 CFU/g molds, 1.29 log CFU/g coliform bacteria, and 1.7 log CFU/g *Staphylococcus aureus* in rice flour. Hyang *et al.* (15) reported that a total bacterial count of 5.0 log CFU/g and a coliform bacteria count > 3.0 log CFU/g

have been detected in rice cakes. This implies that rice flour as well as the rice cake manufacturing process, including conditions in plants, may be microbiologically unsafe (15–17). Inadequate quality control during cooking and distribution may cause hygiene and safety problems in the final food products.

Another challenge regarding rice cakes is to retard retrogradation of starch during storage. As the surface of the product hardens, the texture, appearance, and quality of the product decrease, and the product becomes unappetizing to consumers. To minimize retrogradation, rapid distribution of *songpyeon* to consumers is necessary.

Sterilization is recommended as a means to maintain the quality and safety of *songpyeon* and allow for consumption more than one day after purchase. High-pressure sterilization by retort has advantages, as it can cook and sterilize products in a short time with little nutrient loss, increasing shelf life. It has been widely used in the manufacture of many instant food products, such as retorted rice cakes (17), retorted salmon (18), ready-to-eat black clams (19), fish curry (20), ready-to-eat “Fish Peera” from anchovies (*Stolephorous commersoni*) (19), and mushroom curry (21).

Therefore, the purposes of this study were to investigate the optimum retorting conditions for cooking and sterilizing *songpyeon* filled with black-eyed peas and to evaluate the quality characteristics of sterilized *songpyeon*.

Materials and Methods

Sample preparation *Songpyeon* made with non-glutinous rice flour with fillings of chopped black-eyed peas (CBP) or unchopped black eyed peas (UCBP) were purchased from a local rice cake manufacturing plant in Yeonggwang, Jeonnam, Korea in 2012. The composition of the *songpyeon* was as follows: 50% rice flour, 23% ramie leaves, 22% black eyed peas, 4% sugar, and 1% salt. The non-glutinous rice cultivar *Ohdae* was used; it was purchased from a farm in Haenam, Jeonnam, Korea. To make *songpyeon*, the rice was washed four times and soaked in water at a 1:2 ratio of rice to water (w/v) for 4 h. After the water was discarded, the rice was ground with a grinder (YJ-308; Youngjin Food Co., Hwansung, Korea). After the frozen ramie leaves were thawed in water, they were mixed with rice flour four times during the milling process (SJ-450; Samjin Food Machine, Daegu, Korea). The mixture was kneaded with a mixer (YJ-304; Youngjin Food Co.) for 20 min. Finally, the dough was placed into a *songpyeon*-molding machine (SM2100; Sami General Food Machine, Anyang, Korea) and the *songpyeon* was kept in a freezer until further treatment. The frozen *songpyeon* was kept cool in an icebox during delivery to the laboratory and kept in a freezer until use.

In the following experiments, *songpyeon* samples were divided into the outer shell and the filling. Four parts of *songpyeon* were analyzed, which are designated UCBP OUT (outer shell of *songpyeon* filled with unchopped black-eyed peas), UCBP (unchopped black-eyed peas used for filling), CBP OUT (outer shell of *songpyeon* filled

with chopped black-eyed peas), and CBP (chopped black-eyed peas used for filling).

Microorganisms *Bacillus cereus* (KCCM 11204) and *B. subtilis* (KCCM 11314) were obtained from the Korean Culture Center of Microorganisms (South Korea). The strains were grown in trypticase soy broth (TSB; Difco Laboratories, Detroit, MI, USA) at 37°C for 24 h. Working cultures were maintained on trypticase soy agar slants (Difco) and stored at 4°C. Stock cultures of each strain were maintained in TSB with 20% glycerol and stored at –80°C.

Sterilization conditions The frozen *songpyeon* was thawed at room temperature (21±3°C) for 2 h and was packaged in a retort pouch (polyethylene terephthalate/aluminum/nylon/cast polypropylene, 14 cm wide and 22 cm high), vacuum-packed, and subjected to retort sterilization (STERI-ACE KRS-300/Touch Sc type; Kyunghan Co. Ltd., Seoul, Korea) at 121°C for 15, 16, 17, 18, and 19 min based on preliminary experiments.

Determination of microbiological quality Ten grams of the outer shell or the filling of *songpyeon* was aseptically weighed, mixed with 90 mL of 0.1% peptone water in a sterile plastic bag, and stomached (Stomacher Lab Blender 400; Seward Ltd., Worthing, UK) for 2 min. Then, 10-fold serial dilutions were prepared for each sample in 0.1% peptone water. One milliliter of the appropriate sample dilution was pour-plated with PCA media (Difco). After incubation at 35°C for 48 h, total aerobic plate counts and coliform counts were determined on PCA media and violet red bile glucose agar (Difco), respectively. Concentrations of yeasts and molds were determined using 3M Yeast and Mold Petrifilm (3M, Maplewood, MN, USA) after a 30°C incubation for 72 h.

Inoculation of *songpyeon* with vegetative cells of *Bacillus* spp.

One milliliter of an overnight culture of *B. cereus* and *B. subtilis* at a final concentration of 10⁶ CFU/ml was introduced with a syringe into the UCBP OUT, UCBP, CBP OUT, and CBP *songpyeon* samples. The inoculated *songpyeon* was vacuum-packed and subjected to retort sterilization at 121°C for 15 min. After sterilization, samples were taken every 12 h to detect *Bacillus* spp. growth during storage at room temperature. The samples were serially diluted and appropriate dilutions were pour-plated on MYP (Difco) for *B. cereus* or PDA (Difco) and LB (Difco) for *B. subtilis*. Pink colonies on MYP were counted as *B. cereus* (22). This experiment was performed three times.

Determination of moisture content Two grams of *songpyeon* that had previously been blended for 15 s in a laboratory blender according to an official method were weighed in aluminum dishes and completely dried in a vacuum oven at 105°C. The differences in the weight of the samples before and after drying were calculated to determine moisture content according to the AOAC method (23).

This experiment was performed three times.

Color analysis The color of *songpyeon* was measured with a colorimeter (CM-3500d; Minolta Co., Ltd., Tokyo, Japan). The measured color was represented by Hunter's color values of L* = lightness (0 = black, 100 = white), a* (a* = greenness, +a* = redness) and b* (b* = blueness, +b* = yellowness). This experiment was performed three times.

Textural analysis *Songpyeon* was cut into pieces (2.0×2.0×0.5 cm), and five replicates were subjected to compression testing on a Stable Microsystems TAX-T2 texture analyzer (TAXT2; Stable Micro System Ltd., Godalming, UK) using a 35 mm diameter cylindrical probe according to the manufacturer's instructions. The force (kg) required for compression to a thickness of 10 mm was measured. The pre-test speed, test speed, and post-test speed were 2.0, 5.0, and 5.0 mm/s, respectively. Hardness, chewiness, and gumminess were calculated from a texture profile analysis graph.

Sensory evaluation Sensory evaluation measurements were performed by 20 trained sensory panelists, except for overall acceptance, which was measured by 50 panelists. The panels were composed of undergraduate and graduate students from the Department of Food Science and Technology, Chonnam National University (Gwangju, Korea). *Songpyeon* samples were compared in terms of appearance, color, flavor, taste, moistness, cohesiveness, and overall acceptance on a 7-point hedonic scale from 1 (dislike very much) to 7 (like very much), using previously published methods (24,25). This experiment was performed three times.

Statistical analysis All measurements were performed three times, except texture analysis, which was repeated five times. SPSS for Windows ver. 10.0 was employed to analyze the results obtained from the replicates (version 2008; SPSS Inc., Chicago, IL, USA). Analysis of variance and Duncan's multiple range test were used to detect differences among samples. $p < 0.05$ was considered statistically significant.

Results and Discussion

Characteristics of microorganisms A preliminary study showed that natural microflora (non-inoculated microorganisms) were detected in the raw ingredients of *songpyeon* after retorting at 121°C for less than 10 min, but not after retorting for more than 10 min. Retorting *songpyeon* at 121°C for 10 min completely inactivated *Bacillus* spores and other microorganisms, including aerobic bacteria, yeast, and molds (data not shown). Sterilization at 121°C for 10, 12, and 14 min resulted in inactivation of microorganisms as assessed by total plate count and yeast, mold, and coliform counts. In addition, as *Bacillus* spp. are spore-forming, sterilization for less than 15 min might be insufficient to ensure safety of the product from the potential growth

Table 1. *Bacillus cereus* and *Bacillus subtilis* counts in *songpyeon* retort-sterilized for 121°C for 15 min after storage at 37°C for up to 72 h

Storage time (h)	Parts ¹⁾	<i>B. cereus</i>	<i>B. subtilis</i>
0	CBP OUT	ND ²⁾	ND
	CBP	ND	ND
	UCBP OUT	ND	ND
	UCBP	ND	ND
12	CBP OUT	ND	ND
	CBP	ND	ND
	UCBP OUT	ND	ND
	UCBP	ND	ND
24	CBP OUT	ND	ND
	CBP	ND	ND
	UCBP OUT	ND	ND
	UCBP	ND	ND
36	CBP OUT	ND	ND
	CBP	ND	ND
	UCBP OUT	ND	ND
	UCBP	ND	ND
48	CBP OUT	ND	ND
	CBP	ND	ND
	UCBP OUT	ND	ND
	UCBP	ND	ND
72	CBP OUT	ND	ND
	CBP	ND	ND
	UCBP OUT	ND	ND
	UCBP	ND	ND

¹⁾CBP, inside of *songpyeon* filled with chopped black-eyed peas; CBP OUT, outer shell of *songpyeon* filled with chopped black-eyed peas; UCBP, inside of *songpyeon* filled with unchopped black-eyed peas; UCBP OUT, outer shell of *songpyeon* filled with unchopped black-eyed peas

²⁾ND, not detected.

of *Bacillus* spp. during storage. Furthermore, preliminary sensory evaluation showed that product sterilized for less than 15 min had undesirable hardness levels and an unappetizing pale greenish color due to incomplete cooking. Therefore, based on the preliminary results, sterilization at 121°C for 15–19 min was chosen as the appropriate time to achieve complete inactivation of spores and proper cooking of the product. The effects of sterilization at 121°C for 15 min on the four parts of *songpyeon* inoculated with *B. cereus* and *B. subtilis* are shown in Table 1. Microbial analysis was performed after samples were stored at 37°C for 0, 12, 24, 48, and 72 h. As a result, no *Bacillus* spp. were detected in CBP, CBP OUT, UCBP, or UCBP OUT after 72 h of storage. In addition, total plate counts, yeasts, molds, and coliforms were negative in all *songpyeon* samples sterilized for 15, 17, or 19 min and stored for 72 h at 30°C (Table 2).

Bacillus cereus spores present in the raw ingredients used to make *songpyeon* may survive the general steaming process involved in *songpyeon* manufacturing, germinate, and multiply during storage at room temperature (26). *Songpyeon* may also be contaminated after manufacturing, and during handling, packaging, and transportation (27). In our preliminary study, retorting at 121°C for over 10 min successfully inactivated vegetative cells and spores of *Bacillus* spp.

Table 2. Microbial characteristics of *songpyeon* retort sterilized at 121°C for 15, 17, and 19 min

Sterilization times (min)	Parts ¹⁾		Total plate count	Yeast	Mold	Coliforms
15	CBP	Outer shell	ND ²⁾	ND	ND	ND
	CBP OUT	Filling	ND	ND	ND	ND
	UCBP	Outer shell	ND	ND	ND	ND
	UCBP OUT	Filling	ND	ND	ND	ND
17	CBP	Outer shell	ND	ND	ND	ND
	CBP OUT	Filling	ND	ND	ND	ND
	UCBP	Outer shell	ND	ND	ND	ND
	UCBP OUT	Filling	ND	ND	ND	ND
19	CBP	Outer shell	ND	ND	ND	ND
	CBP OUT	Filling	ND	ND	ND	ND
	UCBP	Outer shell	ND	ND	ND	ND
	UCBP OUT	Filling	ND	ND	ND	ND

¹⁾CBP, inside of *songpyeon* filled with chopped black-eyed peas; CBP OUT, outer shell of *songpyeon* filled with chopped black-eyed peas; UCBP, inside of *songpyeon* filled with unchopped black-eyed peas; UCBP OUT, outer shell of *songpyeon* filled with unchopped black-eyed peas

²⁾ND, not detected.

Table 3. Moisture content and color values of *songpyeon* retort sterilized at 121°C for different treatment times

Sample ¹⁾	Sterilization and Cooking time (min)	Moisture content (%)	Color value		
			L*	a*	b*
CBP	15	47.33±0.16 ^{NS2)}	28.25±1.07 ^{a3)}	-1.46±0.59 ^a	5.44±0.68 ^d
	16	48.12±0.94	26.29±1.80 ^b	2.22±0.60 ^{ab}	6.55±0.73 ^{cd}
	17	47.09±0.90	25.48±0.46 ^{bc}	-3.60±1.03 ^{bc}	7.33±0.90 ^{bc}
	18	47.33±0.64	24.4±0.51 ^{bc}	-4.59±0.81 ^c	8.37±0.76 ^{ab}
	19	47.12±0.24	23.6±0.44 ^c	-6.54±1.06 ^d	9.28±0.63 ^a
UCBP	15	44.44±0.23 ^{NS}	29.46±0.71 ^a	-1.80±0.20 ^a	4.38±0.57 ^c
	16	45.75±0.39	28.84±1.10 ^b	-2.38±0.53 ^a	5.45±0.60 ^{bc}
	17	45.67±0.38	24.62±0.52 ^{bc}	-3.16±0.68 ^a	6.55±0.68 ^{bc}
	18	46.07±0.90	23.42±0.50 ^{bc}	-5.19±0.86 ^b	9.31±0.65 ^{ab}
	19	45.02±0.64	22.53±1.103 ^c	-6.92±1.63 ^c	10.35±0.79 ^a

¹⁾CBP, inside of *songpyeon* filled with chopped black-eyed peas; UCBP, inside of *songpyeon* filled with unchopped black-eyed peas

²⁾NS, not significant at $p < 0.05$.

³⁾Different letters within the same row are significantly different ($p < 0.05$).

(data not shown). Therefore, we confirm that sterilization for 15 min at 121°C inactivates spore-forming *Bacillus* spp. and increases the microbial safety of *songpyeon*.

Quality characteristics of *songpyeon*

Moisture content: The moisture content of *songpyeon* sterilized at 121°C for different times is shown in Table 3. The moisture content of UCBP and CBP *songpyeon* sterilized for 15 min was 47.33 and 44.44%, respectively. No significant differences in moisture content were observed when *songpyeon* was sterilized for 15–19 min between samples and among sterilization durations. This result agreed with the result reported by Yoon (17), in which the moisture content of retorted rice cakes was 43.17–44.04%. The results show that longer sterilization times did not affect the moisture content of *songpyeon*, probably because of the impermeability of the retort pouch.

Color analysis: The Hunter's color values of *songpyeon* are shown in Table 3. L* values of UCBP and CBP *songpyeon* sterilized at 121°C for

15–19 min decreased gradually from 29.46 to 22.53 (UCBP) and 28.25 to 23.60 (CBP), whereas the a* values decreased from 1.80 to 6.92 (UCBP) and from 1.46 to 6.54 (CBP). b* values increased from 4.38 to 10.35 (UCBP) and from 5.44 to 9.28 (CBP) with increasing retort sterilization time. This was caused by α -amylase activity, which breaks down starch and produces reducing sugars that play a role in the browning reaction (17). Lee *et al.* (28) reported that *songpyeon* containing chlorophyll becomes darker as temperature increases. In agreement with this result, ramie leaves in rice cakes, along with high temperatures, influence chromaticity. In this study, *songpyeon* developed low lightness and high yellowness. These results are attributable to disintegration of starch, dextrin, or reducing sugars generated during heat sterilization (17,25,29).

Texture analysis: Texture analysis is an important means of measuring the physical characteristics of a product. Figure 1 shows the results of hardness, gumminess, and chewiness analysis of sterilized *songpyeon*. The hardness values of *songpyeon* sterilized for

Fig. 1. Texture analysis of *songpyeon* exposed to sterilization at 121°C for different treatment times. CBP, *songpyeon* filled with chopped black-eyed peas; UCBP, *songpyeon* filled with unchopped black-eyed peas.

15-19 min decreased with increasing sterilization time, from 2948.24 to 1440.92 g (UCBP) and from 2919.63 to 1790.60 g (CBP). Additionally, when swelling starch is heated, the degree of degradation differs

depending on particle size; the longer the sterilization time, the lower the hardness of the rice cake, owing to gelatinization (28,30). Gumminess increased with increasing sterilization time, from 1190.60 to 2519.63 g (UCBP) and from 1290.60 to 2419.63 g (CBP). This result agreed with the results of a previous study that reported increased gumminess with increasing dextrinization time. Chewiness decreased from 2448.24 to 1441.92 g (UCBP) and from 2119.63 to 1290.60 g (CBP) with increasing sterilization time owing to dextrinization (17). In sum, gumminess of *songpyeon* increased, whereas hardness and chewiness decreased, during sterilization because of the effects of gelatinization (Fig. 1).

Sensory evaluation: Table 4 shows sensory evaluation measurements of *songpyeon* filled with CBP and UCBP performed after sterilization at 121°C for 15-19 min. The appearance scores for *songpyeon* sterilized for 19 min were low, 3.5 for CBP and 3.6 for UCBP. The highest appearance score was found in *songpyeon* sterilized for 17 min (7.5 for CBP and 7.8 for UCBP). These results are consistent with measurements taken with a colorimeter, in which the highest color acceptance value was found in *songpyeon* sterilized for 17 min. Accordingly, the highest color score as determined by the panelists was also given to *songpyeon* sterilized for 17 min (6.9 for CBP and 7.4 for UCBP). Scores for taste were also the highest in *songpyeon* sterilized for 17 min, with scores of 7.5 for CBP and 7.4 for UCBP. The lowest scores for taste were given to *songpyeon* sterilized for 19 min. Moreover, *songpyeon* sterilized for 19 min received low scores for overall acceptance owing to the increased gumminess. For these reasons, *songpyeon* that had been retort sterilized for 19 min was not preferred by the panelists. Generally, sterilization times greater or less than 17 min resulted in lower scores because of softer textures and darker colors. Yoon (17) reported that at least 16 min is needed for dextrinization to improve the color and flavor of rice products. That study reported that rice cakes dextrinized for 16 min showed the highest acceptance scores in sensory evaluation. Based on the sensory evaluation measurements, 17 min was the sterilization time that maximized the sensory qualities and palatability of *songpyeon*.

Table 4. Sensory evaluation of *songpyeon* retort sterilized at 121°C for different treatment times

Sterilization time (min)	Parts ¹⁾	Appearance	Color	Taste	Overall acceptance
15	CBP	4.9±1.0 ^{b,2)}	4.0±0.7 ^d	3.3±0.7 ^c	4.5±1.1 ^d
	UCBP	4.7±0.8 ^b	3.5±0.9 ^d	3.0±1.3 ^c	4.1±1.7 ^d
16	CBP	5.4±1.2 ^b	4.6±0.7 ^c	3.5±0.7 ^c	5.3±1.1 ^c
	UCBP	5.1±0.9 ^b	4.9±1.0 ^c	3.2±0.8 ^c	5.5±1.4 ^c
17	CBP	7.5±0.2 ^a	6.9±0.4 ^a	7.5±0.8 ^a	7.3±0.7 ^a
	UCBP	7.8±0.4 ^a	7.4±0.6 ^a	7.4±0.5 ^a	7.5±0.8 ^a
18	CBP	7.1±0.6 ^a	5.9±0.8 ^b	5.1±0.7 ^b	6.1±0.8 ^b
	UCBP	6.9±0.8 ^a	5.4±0.9 ^b	4.3±0.6 ^b	6.3±0.7 ^b
19	CBP	3.5±1.0 ^c	3.5±0.8 ^d	3.0±1.2 ^c	4.5±0.8 ^d
	UCBP	3.6±0.8 ^c	3.4±0.9 ^d	2.7±1.3 ^c	4.1±1.7 ^d

¹⁾CBP, inside of *songpyeon* filled with chopped black-eyed peas; UCBP, inside of *songpyeon* filled with unchopped black-eyed peas

²⁾Different letters within the same row are significantly different ($p < 0.05$).

In summary, retort sterilization for 15-19 min produced micro-biologically safe *songpyeon*. Our results showed that 15 min was sufficient to sterilize *songpyeon* and rid it of *B. cereus* and *B. subtilis*. Total bacterial counts, yeast and mold, and coliforms were not detected in the samples after 15 min of sterilization. The moisture content of CBP and UCBP parts sterilized for different times did not differ between the treatments. As the sterilization time of *songpyeon* increased, the L* value and the a* values decreased and the b* values increased. The gumminess of *songpyeon* increased and the hardness and chewiness decreased with increasing sterilization time. Sensory evaluation showed that *songpyeon* sterilized for 17 min was preferred by the panelists. It can be concluded that retort sterilization, which uses less energy than conventional cooking methods, produces safe *songpyeon* with extended shelf life at room temperature without affecting quality and physicochemical properties. Therefore, it is expected that retort technology will improve the safety and quality of *songpyeon* during distribution and home consumption.

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