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Research Note

Microbiological Analysis of Rice Cake Processing in Korea

JUN WANG,[1] JOONG-HYUN PARK,[2] NA-JUNG CHOI,[2] SANG-DO HA,[3] AND DEOG-HWAN OH[2]*

[1]*College of Food Science and Engineering, Qingdao Agricultural University, Qingdao, Shandong, 266109 People's Republic of China;* [2]*Department of Food Science and Biotechnology, Kangwon National University, Chuncheon, Gangwon 200-701, Korea; and* [3]*Department of Food Science and Technology, Chung-Ang University, Anseong 456-756, Korea*

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ABSTRACT

This study was conducted to evaluate the microbial contamination in rice cake materials and products during processing and in the operation environment in nonhazard analysis [and] critical control point factories. Furthermore, the environmental health of the processing facilities and the bacterial and fungal contamination on the workers' hands were investigated. Pour plate methods were used for enumeration of aerobic plate count (APC), yeast and molds (YM), *Bacillus cereus, Staphylococcus aureus,* and *Clostridium perfringens,* whereas Petrifilm count plates were used for enumeration of coliforms and *Escherichia coli*. The respective microbial levels of APC, coliforms, YM, and *B. cereus* were in the range of 2.6 to 4.7, 1.0 to 3.8, not detected (ND) to 2.9, and ND to 2.8 log CFU/g in the raw materials and in the range of 2.3 to 6.2, ND to 3.6, ND to 2.7, and ND to 3.7 log CFU/g during processing of the rice cake products. During the processing of rice cakes, APC, coliforms, YM, and *B. cereus* increased during soaking and smashing treatments and decreased after steaming treatment. *E. coli, S. aureus,* and *C. perfringens* were not detected in any of the raw materials and operating areas or during processing. *B. cereus* was detected on the operators' hands at microbial contamination levels of 1.9 ± 0.19 to 2.0 ± 0.19 log CFU/g. The results showed that *B. cereus* in the end product is presumably the main concern for rice cakes. In addition, the high contamination level of *B. cereus* during manufacturing processes, including soaking, smashing, and molding, and the absence of *B. cereus* from the air sampling plates indicated that the contaminated equipment showed the potential risk to cause cross-contamination.

Rice cakes are one of the most popular foods worldwide, especially in South East Asian countries, including Korea, China, and Japan. They are made from rice flour by steaming followed by subsequent operations to prepare different types of rice cakes. Retrogradation, defined as partial crystallization of amylopectin within the gelatinized starch fraction, is an important physical phenomenon and occurs easily in starch-based foods *(15)*. After steaming, the starch component of rice cakes undergoes retrogradation. Storage temperature and time obviously affect the retrogradation rate of starch-based foods, with a maximum value at 5°C *(12, 15)*. Therefore, rice cakes are not suited to refrigeration and are typically stored at room temperature since low storage temperature would significantly reduce their quality. Consequently, the presence of foodborne pathogens in the cakes may result from cross-contamination at higher temperatures or from their presence in the raw materials and their survival to the steaming process. In addition, *Salmonella* spp., *Staphylococcus aureus,* and *Bacillus cereus* are regarded as the major microbiological hazards for cereal grains and related products *(5)*. From 1998 to 2010, the Centers for Disease Control and Prevention reported that 44 outbreaks and 281 cases of illness in the United States caused by *B. cereus* were related to grains or beans *(3)*. *B. cereus* has been documented worldwide in outbreaks associated with cooked rice *(8, 10)*. It has been reported that 19.3% of the rice cakes with filling in Korea were contaminated with *S. aureus (11)*. Due to the high starch retrogradation rate during storage that shortens their shelf lives, manufacturing rice products, including rice cakes, on an industrial scale is difficult *(17)*. It is also hard to control the food quality. Food safety issues related to traditional Korean foods, especially during the manufacturing processes, need to be addressed and investigated *(9)*. For all of the reasons mentioned above, it is necessary to conduct overall microbiological assessment of rice products in food factories.

Hazard analysis [and] critical control point (HACCP) is designed to identify all hazards (biological, physical, or chemical), critical control points, and critical limits in the production processes, including specific sanitation procedures, cross-contamination, product-formulation controls, employee and environmental hygiene, monitoring, and record-keeping for the procedures *(4)*. The food safety level would improve remarkably if the HACCP system is introduced into factories. Microbial assessment of rice cakes collected from HACCP factories in Korea has been conducted and introduced *(6)*.

The objective of this study was to evaluate the microbial contamination levels of aerobic plate count (APC), coliforms, yeasts and molds (YM), *Escherichia coli, B. cereus,*

* Author for correspondence. Tel: 82-33-250-6457; Fax: 82-33-241-0508; E-mail: deoghwa@kangwon.ac.kr.

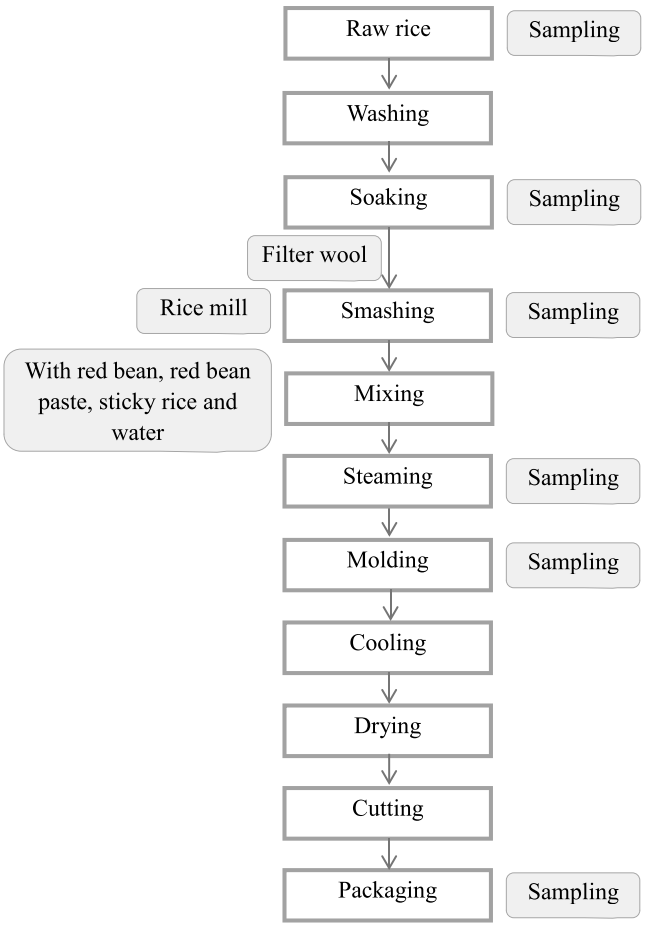

FIGURE 1. *Technical routes for the production of rice cake products (garaetteok).*

S. aureus, and *Clostridium perfringens* in the rice cake raw materials and products during processing and in the operation environment in non-HACCP factories. We aimed to provide a basis and recommendations for government departments to make decisions for food safety management.

MATERIALS AND METHODS

Sampling plan according to processing of rice cakes. Figure 1 shows the processing routes for rice cakes (garaetteok). The raw materials, including rice, sticky rice, and red bean, for rice cakes from different operation units according to the technical routes (Fig. 1) were purchased randomly from two non-HACCP factories that produced rice cakes from July 2011 to August 2011. In addition, the contamination source from equipment surfaces, operators, and work spaces were sampled to detect the microbiological contamination levels. Detailed information for the sampling plan is shown in Table 1.

Pretreatment of samples. For consistent surface sampling, equipment surfaces (10 by 10 cm areas) and the technical routes were swabbed using a Swab kit (3M China Ltd., Shanghai, China). The operation was performed according to the manufacturer's instructions. For the evaluation of workers' hands, the glove juice method was used to collect bacteria from the hands of workers at processing plants in rice cake factories; this method was codified by the American Society for Testing and Materials and further adapted by the U.S. Food and Drug Administration (FDA) *(14)*. All samples were transported to the laboratory at Kangwon National University for microbial analysis in an ice-box to maintain the temperature at 4 ± 1°C. The microbial analysis was conducted in the laboratory within 2 h of sample collection.

Microbiological analysis. (i) Rice cake samples. Twenty-five grams of raw materials or rice cake products was mixed with 225 ml of 0.1% sterile peptone water in a sterilized stomacher bag and pummeled for 2 min (Lab Blender 400, Seward, London, UK) at 200 rpm at room temperature (23 ± 2°C). Next, 1-ml aliquots of the homogenates were serially diluted in 9 ml of the 0.1% sterile peptone water used for microbiological analysis. Pour plate methods were used for enumeration of APC, YM, *B. cereus, S. aureus,* and *C. perfringens,* whereas Petrifilm count plates (3M Company, St. Paul, MN) were used for enumeration of coliforms and *E. coli*.

One milliliter of the suitably diluted sample suspensions was placed in an empty sterile plate. Fifteen milliliters of each melted agar cooled to approximately 45°C was added to the plate. The solution was covered and mixed thoroughly by gently tilting and swirling the dish, and then the plate was placed on a flat surface and left undisturbed for about 10 min to allow the agar to completely gel. Plate count agar (PCA; Difco, BD, Sparks, MD) was used for enumeration of APC, potato dextrose agar supplemented with sterile 10% tartaric acid (PDA; Difco, BD) (pH 3.5 ± 0.1) was used for enumeration of YM, mannitol egg yolk polymyxin agar (MYP; Difco, BD) with 50% egg yolk enrichment and Antimicrobic Vial P (Difco, BD) was used for enumeration of *B. cereus,* Baird-Parker agar (BP; Difco, BD) with egg yolk tellurite enrichment was used for enumeration of *S. aureus,* and tryptose-sulfite-cycloserine agar (TSC; Oxoid, Basingstoke, UK) with kanamycin sulfate and polymyxin B sulfate was used for enumeration of *C. perfringens*. In addition, the TSC plates should be overlaid with 10 ml of TSC agar without egg yolk emulsion.

(ii) Falling bacteria. Enumerations of APC, coliforms, and YM in the environment of processing plants were performed using

TABLE 1. *Sampling plan for monitoring microbial levels of rice cake products in non-HACCP factories*

	First trial		Second trial
Sampling plan	Factory A	Factory B	Factory A
Date	7 July 2011	18 Aug. 2011	30 Aug. 2011
Target samples	Garaetteok, sirutteok, gyeongdan	Garaetteok	Garaetteok, sirutteok, gyeongdan
Materials	22	7	22
Equipment surfaces	8	8	8
Operators	1	1	1
Falling bacteria	3 areas	3 areas	3 areas

TABLE 2. *Microbial content of raw materials for rice cake products in non-HACCP factories*[a]

Sample	Aerobic plate count	Coliforms	Yeasts and molds	*E. coli*	*B. cereus*	*S. aureus*	*C. perfringens*
Rice	3.9 ± 0.87 B[b]	1.1 ± 0.43 A	1.8 ± 0.93 A	ND[c]	1.8 ± 1.09 A	ND	ND
Water	2.6 ± 0.80 A	1.0 ± 1.39 A	ND	ND	ND	ND	ND
Sticky rice	4.7 ± 0.37 B	2.1 ± 0.00 B	2.3 ± 0.00 A	ND	2.8 ± 0.08 C	ND	ND
Red bean	4.2 ± 2.25 B	3.8 ± 0.15 D	2.4 ± 0.03 A	ND	2.1 ± 0.45 B	ND	ND
Red bean paste	4.5 ± 0.04 B	2.9 ± 0.80 C	2.9 ± 0.19 B	ND	1.4 ± 1.42 A	ND	ND

[a] Data are expressed as mean ± SD in log CFU per gram.
[b] Values followed by different letters in a column are significantly different ($P < 0.05$).
[c] ND, not detected (<1.0 log CFU/g).

PCA, eosin methylene blue agar (EMB; Difco, BD), and acidified PDA agar (pH 3.5 ± 0.1), respectively. The petri dishes with different agars were exposed to the environment for 5 min to detect the falling bacteria from air. The data are expressed as CFU per plate·5 min, indicating the contamination level of the processing spaces of rice cakes.

The incubation of PCA, PDA, EMB, MYP, BP, and TSC followed the conditions described by the Animal, Plant and Fisheries Quarantine and Inspection Agency *(1)*, Yen and Lin *(18)*, Wang et al. *(16)*, Park et al. *(13)*, and Juneja et al. *(7)*, respectively. The colony on each plate was recognized as each target microorganism as described online for each medium (https://www.bd.com/ds/technicalCenter/misc/difcobblmanual_2nded_lowres.pdf). The confirmation and identification of *B. cereus, S. aureus,* and *C. perfringens* at the species level were conducted using the API rapid test kit (bioMérieux, Inc., Marcy l'Etoile, France).

Statistical analysis. Each time interval consisted of two plates per replicate. The means of cell populations from each treatment were calculated from three replications of each experiment. Analysis of statistical tests was performed using SPSS 20 (IBM Corporation, New York, NY). The data are expressed as the mean ± standard deviation (SD). All data were analyzed by analysis of variance. Mean values were compared by Tukey's test at a significance level of 0.05.

RESULTS AND DISCUSSION

Microbial content of rice cake raw materials. In this study, 51 materials in total used for rice cakes were obtained from two non-HACCP factories and analyzed. The levels of APC, coliforms, YM, *E. coli, B. cereus, S. aureus,* and *C. perfringens* in the materials, including rice, glutinous rice, red bean, red bean paste, and water, are shown in Table 2. All of the materials were contaminated by APC and coliforms (Table 2). The contamination level of APC in all of the materials was not significantly different ($P < 0.05$), except for water contamination, with an APC at 2.6 ± 0.80 log CFU/g. Red bean had the highest coliforms contamination level, whereas red bean paste had the highest YM contamination level. All the rice cake materials were also contaminated by YM and *B. cereus,* except for water. These results were consistent with previous research indicating that rice is a significant vehicle for *B. cereus (2). E. coli, S. aureus,* and *C. perfringens* were not detected (<1.0 log CFU/g) in any of the materials. In HACCP factories, all materials were contaminated by APC, coliforms, YM, and *B. cereus,* with the exception of red bean paste, cinnamon powder, and black sesame, which were not contaminated by coliforms *(6)*.

Microbiological contamination levels in processing units of rice cakes. According to the processing routes of rice cakes shown in Figure 1, the contamination levels of rice cakes (garaetteok) from different processing units in two non-HACCP factories were analyzed. The processing parameters for smashing, steaming, and molding for rice cakes in the two factories are the same, except for the soaking time: 2 h in factory A and 4 h in factory B. The microbial levels of APC, coliforms, YM, *E. coli, B. cereus, S. aureus,* and *C. perfringens* in garaetteok after different manufacturing procedures, including soaking, smashing, steaming, and molding, and in the final product in factories A and B, are shown in Tables 3 and 4, respectively. APC increased from 2.8 to 4.5 log CFU/g to 6.0 to 6.2 log CFU/g after 2 to 4 h of soaking and two smashing treatments, whereas coliforms, YM, and *B. cereus* increased from 1.0 to 1.3 log CFU/g, 1.7 to 1.9 log CFU/g, and 1.6 to 1.9 log

TABLE 3. *Microbial levels of manufacturing processes for rice cake products (garaetteok) in non-HACCP factory A*[a]

Process	Aerobic plate count	Coliforms	Yeasts and molds	*E. coli*	*B. cereus*	*S. aureus*	*C. perfringens*
Rice	4.5 ± 0.44 C[b]	1.0 ± 0.50 B	1.9 ± 1.13 B	ND[c]	1.9 ± 1.32 A	ND	ND
Soaking	6.0 ± 0.43 D	2.7 ± 0.34 C	2.1 ± 0.24 C	ND	3.7 ± 1.44 C	ND	ND
Smashing	6.2 ± 0.94 D	2.4 ± 0.37 C	2.1 ± 0.44 C	ND	3.7 ± 1.53 C	ND	ND
Steaming	2.9 ± 0.10 A	ND	ND	ND	2.1 ± 0.20 A	ND	ND
Molding	3.9 ± 0.80 C	1.0 ± 0.98 B	0.2 ± 0.17 A	ND	2.8 ± 1.04 B	ND	ND
Product	3.4 ± 0.18 B	0.3 ± 0.29 A	ND	ND	2.0 ± 0.27 A	ND	ND

[a] Data are expressed as mean ± SD in log CFU per gram.
[b] Values followed by different letters within a column are significantly different ($P < 0.05$).
[c] ND, not detected (<1.0 log CFU/g).

TABLE 4. *Microbial levels of manufacturing processes for rice cake products (garaetteok) in non-HACCP factory B*[a]

Process	Aerobic plate count	Coliforms	Yeasts and molds	*B. cereus*	*S. aureus*	*C. perfringens*
Rice	2.8 ± 0.34 A[b]	1.3 ± 1.88 A	1.7 ± 0.00 A	1.6 ± 0.04 A	ND[c]	ND
Soaking	6.1 ± 0.08 D	3.6 ± 0.23 C	2.2 ± 0.64 C	2.9 ± 0.33 D	ND	ND
Smashing	6.1 ± 0.14 D	3.3 ± 0.17 C	2.7 ± 0.30 C	3.1 ± 0.11 D	ND	ND
Steaming	2.3 ± 0.03 B	ND	2.5 ± 0.05 B	2.0 ± 0.16 B	ND	ND
Molding	2.6 ± 0.02 B	ND	2.4 ± 0.27 B	2.8 ± 0.33 C	ND	ND
Product	3.0 ± 0.01 C	0.8 ± 0.07 B	1.6 ± 0.04 A	2.8 ± 0.05 C	ND	ND

[a] Data are expressed as mean ± SD in log CFU per gram.
[b] Values followed by different letters within a column are significantly different ($P < 0.05$).
[c] ND, not detected (<1.0 log CFU/g).

CFU/g to 2.4 to 3.6 log CFU/g, 2.1 to 2.7 log CFU/g, and 2.9 to 3.7 log CFU/g, respectively. *E. coli, S. aureus,* and *C. perfringens* were not detected (<1.0 log CFU/g) in any of the processing units. After soaking and smashing, the microbial levels increased significantly compared to those of rice. There was no significant difference between the soaking and smashing processes. After steaming treatment, the microbial levels decreased significantly to the point where coliforms were not detected, whereas APC, YM, and *B. cereus* decreased to 2.9 to 3.3 log CFU/g, not detected (ND) to 2.5 log CFU/g, and 2.0 to 2.1 log CFU/g, respectively. The microbial levels were slightly increased after the molding operation, indicating that molding might cause cross-contamination. A previous study reported that the coliforms, YM, and *B. cereus* levels of rice cake end products in HACCP factories were 1.8, 1.5, and 1.3 log CFU/g, respectively *(6). S. aureus* and *C. perfringens* were not detected in any of the processing units in HACCP factories.

Comparing factories A and B shown in Tables 3 and 4, respectively, the contamination levels of rice in factory A were higher than in factory B, but the commination levels were similar after soaking and smashing operations. The main reason may be that the soaking time in factory B (4 h) was longer than in factory A (2 h). In addition, the contamination levels after steaming treatment and the final contamination levels of rice cake products in factory A were lower than those in factory B, including APC, coliforms, YM, and *B. cereus*. These results indicated that factory A applied more effective control measures than factory B, i.e., there were no uniform measures among non-HACCP factories. Therefore, HACCP certification is necessary for rice cake factories to ensure food safety standards since the HACCP system provided more efficient prevention of foodborne diseases after application in factories involved in all processes from the primary producer to the final products.

Microbiological levels of equipment for rice cakes. The microbial levels of equipment for rice cakes (garaetteok), including molding inlet, cooling bath, drying plate, knife, filter wool, and rice mill in the processing, are shown in Table 5. The results showed that most of the equipment for rice cake processing was contaminated with high levels of APC, coliforms, YM, and *B. cereus*, but not with *E. coli, S. aureus,* or *C. perfringens*. The contamination levels for APC, coliforms, YM, and *B. cereus* in the molding inlet were 5.4 ± 0.06, 3.9 ± 0.10, 3.0 ± 0.22, and 3.8 ± 0.00 log CFU/100 cm^2, respectively, levels that were the highest contamination levels among all of the tested equipment. This could well explain why the contamination levels of rice cakes increased (Tables 3 and 4) after molding. In addition, the contamination levels for APC, coliforms, YM, and *B. cereus* of rice mill were 4.0 ± 0.76, 1.5 ± 0.00, 1.2 ± 0.00, and 2.4 ± 0.04 log CFU/100 cm^2, which were rather high. This is another reason that could explain why the contamination levels were similar after soaking and smashing operations with different microbial levels in rice from factories A and B. The processing equipment presented the main potential risk for rice cake products due to the high probability of cross-contamination. Effective control measures for non-HACCP factories to reduce the contamination

TABLE 5. *Microbial contamination of equipment for rice cake products (garaetteok) in non-HACCP factories*[a]

Swab point	Aerobic plate count	Coliforms	Yeasts and molds	*E. coli*	*B. cereus*	*S. aureus*	*C. perfringens*
Inlet of molding	5.4 ± 0.06 E[b]	3.9 ± 0.10 C	3.0 ± 0.22 A	ND[c]	3.8 ± 0.00 D	ND	ND
Cooling bath	3.0 ± 0.03 C	ND	1.6 ± 0.27 A	ND	1.7 ± 0.21 B	ND	ND
Drying plate	4.0 ± 0.02 D	1.5 ± 0.34 B	2.2 ± 0.14 A	ND	2.6 ± 0.00 C	ND	ND
Knife	3.4 ± 0.56 C	1.2 ± 1.20 B	3.4 ± 0.65 A	ND	1.1 ± 1.10 A	ND	ND
Filter wool	1.4 ± 0.00 A	ND	3.3 ± 0.08 A	ND	0.5 ± 0.50 A	ND	ND
Rice mill	4.0 ± 0.76 D	1.5 ± 0.00 B	1.2 ± 0.00 A	ND	2.4 ± 0.04 C	ND	ND
Workbench	1.8 ± 0.00 B	ND	1.2 ± 0.00 A	ND	ND	ND	ND
Inner packaging	2.7 ± 0.65 C	0.3 ± 0.49 A	ND	ND	2.2 ± 0.21 A	ND	ND

[a] Data are expressed as mean ± SD in log CFU per 100 cm^2.
[b] Values followed by different letters within a column are significantly different ($P < 0.05$).
[c] ND, not detected (<1.0 log CFU/100 cm^2).

TABLE 6. *Microbial contamination of operators (hands) in non-HACCP factories*[a]

Factory	Sample	Aerobic plate count	Coliforms	Yeasts and molds	*E. coli*	*B. cereus*	*S. aureus*	*C. perfringens*
A	Operator 1	3.3 ± 0.20 A[b]	ND[c]	1.5 ± 0.50 A	ND	1.9 ± 0.60 A	ND	ND
B	Operator 1	2.8 ± 0.71 A	ND	1.3 ± 0.55 A	ND	2.0 ± 0.16 A	ND	ND

[a] Data are expressed as mean ± SD in log CFU per hand.
[b] Values followed by different letters within a column are significantly different ($P < 0.05$).
[c] ND, not detected (<1.0 log CFU per hand).

TABLE 7. *Contamination levels of air in different manufacturing areas in non-HACCP factories*[a]

Sample	Aerobic plate count	Coliforms	Yeasts and molds
Preparation areas	31 ± 0.06 B[b]	ND[c]	18 ± 0.00 B
Processing areas	34 ± 0.00 B	ND	27 ± 0.09 C
Packaging areas	13 ± 0.05 A	ND	10 ± 0.15 A

[a] Data are expressed as mean ± SD in CFU per plate·5 min.
[b] Values followed by different letters within a column are significantly different ($P < 0.05$).
[c] ND, not detected (<1.0 CFU per plate·5 min).

levels in the final products would involve ensuring the sanitation of the processing equipment for rice cakes.

Microbiological contamination levels of workers' hands and plant environments. The hands of workers at processing plants in rice cake factories were evaluated for contamination by using the glove juice method, a method that was codified by American Society for Testing and Materials and further adapted by the FDA *(14)*. The microbial contamination levels of operators' hands and the air in different processing areas were determined and are shown in Tables 6 and 7. The APC, YM, and *B. cereus* contamination levels of hands were 2.8 to 3.3, 1.3 to 1.5, and 1.9 to 2.0 log CFU/g, respectively. APC levels of 1.4 to 2.3 log CFU/g and no coliforms, YM, or *B. cereus* were detected in HACCP factories *(6)*. Coliforms, *E. coli, S. aureus,* and *C. perfringens* were also not detected in non-HACCP factories. As for the contamination levels of falling bacteria in different manufacturing areas, the APC contamination levels in preparation, processing, and packaging areas were 31 ± 0.06, 34 ± 0.00, and 13 ± 0.05 CFU/plate·5 min, whereas the YM levels were 18 ± 0.00, 27 ± 0.09, and 10 ± 0.15 CFU/plate·5 min, respectively. Coliforms were not detected. The results indicated that sanitation control for the processing point was more important than environmental air since *B. cereus* were detected on operators' hands and on the surface of some equipment.

In conclusion, the prevalence of microbiological organisms in the raw materials, processes, and equipment used for rice cake production were determined. In all raw materials, processes, and operating areas, *E. coli, S. aureus,* and *C. perfringens* were not detected. *B. cereus* in the end product is presumably the main concern for rice cakes. In addition, the high contamination level of *B. cereus* during manufacturing processes, including soaking, smashing, and molding, and the absence of *B. cereus* from the air sampling plates, indicated the contaminated equipment showed the potential risk to cause cross-contamination. Periodic monitoring for microorganism contamination in raw materials, processes, and equipment and effective measures such as education on personal and environmental hygiene for employees, prevention of cross-contamination, ingredient control, and step-by-step process control are needed to reduce the microbial risk of food poisoning.

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