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Microbial Contamination by *Bacillus cereus, Clostridium perfringens,* and *Enterobacter sakazakii* in *Sunsik*

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Abstract The powdered cereal *sunsik* is a partially thermal-processed product that required safety evaluations for food-borne pathogens. Thirty-six *sunsik* products from Korean markets were collected and analyzed for contamination by total viable cell counts, coliforms, *Escherichia coli*, and the spore-forming *Clostridium perfringens* and *Bacillus cereus*. *Enterobacter sakazakii*, as a newly emerging pathogen, was also analyzed. Approximately 28% of *sunsik* were contaminated at 5 log CFU/g for total viable counts. Coliforms and *E. coli* were detected in 33 and 4% of the samples, respectively. The spore-forming *B. cereus* was found in 42% of the samples at a maximal level of 3 log CFU/g on average. About 6% the samples were contaminated with *Cl. perfringens* at an average level of 15 CFU/g. Forty-five % of *sunsik* contained *E. sakazakii*, at levels from 0.007 to over 1.1 cell/g by MPN method. In addition, one *sunsik* product for infants and children showed over 3 log CFU/g for both *B. cereus* and *E. sakazaki*. Therefore, concern should be placed on controlling for microbial hazards such as *B. cereus* and *E. sakazakii* in *sunsik*, particularly for the products fed to infants under 6 months of age.

Keywords: *sunsik*, ready-to-eat, microbial contamination, *Bacillus cereus, Clostridium perfringens, Enterobacter sakazakii*

Introduction

Our changing society as well as eating habits may be playing roles in pathogen emergence, and may be contributing to current food safety concerns (1). Recently, the market for minimally processed and ready-to-eat (RTE) food has been increasing. The growth of this market, rather than that of other thermally processed foods, may be due to the desire for eating convenience with a nutritional advantage, as well as consumer interest in health and well-being (2-4). The consumption of *sunsik* and *saengsik* products, the original RTE foods in Korea, has increased rapidly. *Saengsik* means eating uncooked food and *saengsik* products are powdery foods, usually made of unheated and dried agricultural or marine products. *Sunsik* products, also called '*misutgaru*', are powdery as well, and made of the same materials as *saengsik*; however, their ingredients go through partial thermal processing, particularly for the grains. Generally, these kinds of foods are not sterilized completely, so there is a possibility of acquiring food-borne illness from contaminating pathogens such as *Escherichia coli* O157:H7, *Clostridium (Cl.) perfringens*, *Bacillus cereus*, *Staphylococcus aureus*, and *Listetria monocytogenes* (5-8). Furthermore, such powdered foods can intermix with various microorganisms during the manufacturing process, so it is necessary to monitor and analyze the microbial safety of final products (9-11). There are some researches on the microbial hazard of raw materials used in *seangsik* (3, 12-14). *Bacillus* spp. were shown to be distributed at 4-7 log CFU/g, and coliforms were at 1-4 log CFU/g (15). Chang *et al.* (3, 12) reported that the final *saengsik* products were contaminated with *B. cereus* and *Cl. perfringens*. The pathogens were not detected at high enough levels to cause food poisoning, but this indicates there are certain hazards with the raw materials and during processing. Other powdered foods such as garlic and onion powders and whole black pepper powder were also contaminated at 6 log CFU/g for microorganism, and 5 log CFU/g for spore-forming bacteria (10). In the case of *sunsik*, 3.1 and 3.8 log CFU/g were found for aerobic bacteria, and yeast and mold (11), respectively. However, only a few cases have reported no data for food-borne pathogen contamination. Generally, food manufacturing companies have a microbial standard of less than 4.5 log CFU/g for viable counts of bacteria. The Korean Food Code prescribes the standard that *Salmonella* spp., *S. aureus*, *Vibrio parahaemolyticus*, *Cl. perfringens*, *L. monocytogenes*, *E. coli* O157:H7, *Campylobacter jejuni*, *B. cereus*, and *Yersinia enterocolitica* can not be detected in commercial foods that do not require additional preparation or heating before eating (16). Thus, we believed there was a need for analyzing the levels of contaminating microorganisms in *sunsik*.

Sunsik is much tastier than *saengsik* because the cereal ingredients are roasted, and it is usually cheaper than *saengsik*. As a result, *sunsik* is much more popular than *saengsik* for the elderly. Sometimes it is available as a baby food for the weaning stage. However, the ingredients, except the cereals, are mixed after undergoing only washing, drying, and pulverizing processes, without a heat process. This may pose some risk for pathogen contamination from unsanitary manufacturing processes and from contaminated raw materials (9). Therefore, it is necessary

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to monitor for microbial content and analyze its risk. In addition, *sunsik* is usually manufactured for small-sized retailers, department stores, and wholesale marts, which may have greater concerns for strict safety analysis (18). In particular, non-heated or partially-heated powdered foods and agricultural products must be evaluated for poisoning from spore-forming bacteria and coliforms for market growth in those industries. Also, there are some *sunsik* products made specifically for infants, which demand safety analysis.

Therefore, to monitor and evaluate the microbial risk of *sunsik*, we detected and enumerated contaminating spore-forming *B. cereus*, *Cl. perfringens* (23-27), and *E. sakazakii* (19-22) microbes, which have not been examined in *sunsik* and are widely spread in nature.

Materials and Methods

Collected samples and pre-treatments Thirty-six powdered *sunsik* products from 18 companies were purchased at markets and from on-line cyber-markets in Korea. All the products were tested within 1 year from the date of purchase. All instruments and culture media used in the experiments were sterilized at 121°C for 20 min. Every sample was handled on a clean sterilized bench. Twenty-five g of sample were mixed with 225 mL of sterilized saline. After homogenizing for 120 sec in a sterile stomacher bag (IUL, Barcelona, Spain), 1 mL of homogenized sample was used for analysis.

Total plate counts One mL of rehydrated sample was serially diluted with 9 mL of sterilized saline and spread onto plate count agar (PCA, Oxoid Ltd., Hampshire, England). The colony counts were performed after incubation for 24 hr at 37°C.

Coliform and *E. coli* detection One mL of rehydrated sample was serially diluted with 9 mL of sterilized saline and spread onto deoxycholate lactose agar (DLA, Difco Lab., Detroit, MI, USA) and incubated for 24 hr at 37°C. The quantitative MPN method for coliforms was performed with the positive samples. Enrichment incubation was done by LB (Lactose broth, Difco Lab.) for the negative samples, and they were then identified. The dark red colonies on DLA were counted in the total plate counts for counting coliforms. For *E. coli* detection, serially diluted sample was spread on eosin methylene blue agar (EMB, Difco Lab.) and incubated for 24 hr at 37°C. The green metallic-colored colonies on EMB agar were selected and spread again on chromogenic *E. coli* coliform medium (Oxoid Ltd.). After incubating for 24 hr at 37°C, IMViC (indole production, methyl red, Voges-Proskauer, citrate utilization test) was done for the identification of *E.coli*.

Detection of spore-forming bacteria *B. cereus* and *C. perfringens* One mL of rehydrated sample was serially diluted with 9 mL of sterilized saline and spread onto MYP agar (Oxoid Ltd.) and incubate for 24 hr at 37°C. On MYP agar, the colonies with eosin pink opaque halos were counted. They were then underwent Gram-positive, nitrate reduction, and β-hemolysis tests. Two primers of BC1 (5'-ATTGGTGACACCGATCAAACA-3') and BC2 (5'-TCA TACGTATGGATGTTATTC-3') from the *gyrB* gene (28) were used to confirm *B. cereus* by PCR. One mL of rehydrated sample was spread on a cooked meat medium, and incubated for 24 hr at 37°C. If the samples needed enrichment, they were inoculated on added egg-yolk reinforced clostridial medium (Difco Lab.) and incubated under anaerobic conditions for 24 hr at 37°C. Colonies that were light yellow, showing a white opaque halo, and an approximate 2 mm diameter were selected and counted. Gram-positive testing was performed, and PCR was completed with the primers α1 (5'-TGCTAATGTTACTGCC GTTGATAG-3') and α2 (5'-ATAARCCCAATCATCCCAA CTATG-3') to confirm *Cl. perfrigens* producing α-toxin (33).

***E. sakazakii* detection and quantitative analysis** Three preparations of 1, 10, and 100 g each of sample were added to 9, 90, and 900 mL of buffered peptone water (BPW, Difco Lab.), respectively. The samples were stomached for 120 sec and incubated for 24 hr at 37°C. Ten mL of each incubated sample was added to 90 mL of EE (*Enterobacter sakazakii* Enrichment Broth Mossel, Difco Lab.) broth and again incubated for 24 hr at 37°C. The samples were spread and incubated on 5 plates of violet red bile glucose agar (VRBGA, Oxoid). Then the purple-halo colonies were selected, and again incubated for 48 hr at 25°C on tryptic soy agar (TSA, Difco Lab.). Quantitative analysis was done by the MPN method of the U.S. FDA (34, 35) with the green colonies on CESA (chromogenic *Enterobacter sakazakii* agar DFI formulation, Oxoid Ltd.). Identification was completed using an API 20E kit and an ID32 kit (bioMerieux, SA, Etoile, France). Confirmation was done by PCR with the primers ompA-1 (5'-GGATTTAACCGTTTCC-3') and ompA-2 (5'-CGCC AGCGATGTTAGAAGA-3'), and VITEC (Automatic Microbial Identification System; bioMerieux).

Results and Discussion

Collection distribution of *sunsik* samples and ingredient analysis Thirty-six *sunsik* samples were collected nationally. The manufacturing companies were located in Deagu (22%), Gyeonggi (19%), Chungbuk (19%), and other regions of Korea, except for Seoul and Jeju Island. The main raw ingredients were cereals, and the most common was brown rice at 83% (30/36); black beans were the next most common ingredient at 80% (29/36). Sea alga such as sea tangles and seaweeds; carrots and lotus roots; cabbage and sesame leaves; nuts such as peanuts, walnuts, and pine nuts; and fruits like bananas and apples were mixed into the products; the other ingredients were milk powder, glucose, salt, calcium, and vitamins (Table 1). On average, 26 raw materials were combined, but some samples had over 50 raw materials. There were 3 *sunsik* products (10%) made for infants and children.

Contamination of total bacteria, coliforms, and *E. coli* in *sunsik* Detection and enumeration analyses were performed by the standard method of the Korean Food Code. According to the results, there were 2-5 log CFU/g for total plate counts of the *sunsik* samples. About 28% of them showed high levels of contamination, at over 5 log

Table1. Total ingredient frequencies for *sunsik* products analyzed in this study

Ranking	Raw ingredients	No.	%	Ranking	Raw ingredients	No.	%	Ranking	Raw ingredients	No.	%
1	Brown rice	30	83.3	33	Green perilla	7	19.4	66	Soybean powder	2	5.6
2	Black bean	29	80.6	34	Onion	7	19.4	67	Sweet persimmon	2	5.6
3	Black sesame	26	72.2	35	Angelica keiskei	6	16.7	68	Pak-choi	2	5.6
4	Glutinous rice	25	69.4	36	Soybean	6	13.9	69	Wheat corn	2	5.6
5	Corn	21	58.3	37	Calcium	5	13.9	70	Almond	1	2.8
6	Millet	21	58.3	38	Laver	5	13.9	71	Buckwheat	1	2.8
7	Barley	20	55.6	39	Glucose	5	13.9	72	Burdock	1	2.8
8	Sea tangle	20	55.6	40	Sweet potato	5	13.9	73	Chestnut derma	1	2.8
9	Black rice	19	52.8	41	Wheat	5	13.9	74	Chlorella	1	2.8
10	White bean	18	50	42	Wormwood	5	13.9	75	Cocoa	1	2.8
11	Adlay	17	47.2	43	Banana	4	11.1	76	Crowndasy	1	2.8
12	Glutinous millet	17	47.2	44	Green tea	4	11.1	77	Dropwort	1	2.8
13	Carrot	15	41.7	45	Non-glutinous millet	4	11.1	78	Egg	1	2.8
14	Nonglutei	14	38.9	46	Pine nuts	4	11.1	79	Eucommia ulmoides	1	2.8
15	Sesame	14	38.9	47	Rice	4	11.1	80	Garlic	1	2.8
16	Cabbage	13	36.1	48	Walnut	4	11.1	81	Ginger	2	2.9
17	Spinach	12	33.3	49	Fructose	3	8.3	82	Gingko nut	1	2.8
18	Peanut	12	33.3	50	Radish	3	8.3	83	Oligosaccharide	1	2.8
19	Salt	11	30.6	51	DHA	3	8.3	84	Jujube	1	2.8
20	Hemp	11	30.6	52	Mulberry leaves	3	8.3	85	Jujube seed	1	2.8
21	Potato	11	30.6	53	Safflower seed	3	8.3	86	Korean leek	1	2.8
22	Barley corn	10	27.8	54	Acanthopanax	2	5.6	87	Lettuce	3	2.10
23	Chestnut	10	27.8	55	Arrowroot	2	5.6	88	Malt	1	2.8
24	Squash	10	27.8	56	Cordyceps	2	5.6	89	Papaya	1	2.8
25	Apple	9	25	57	Balloonflower	2	5.6	90	Pea	1	2.8
26	Brown seaweed	9	25	58	Codonopsis	2	5.6	91	Persimmon leaves	1	2.8
27	*Pyogo* mushroom	9	25	59	Cordyceps	2	5.6	92	Pumpkin seed	1	2.8
28	Anchovy	8	22.2	60	Dandelion	2	5.6	93	Seasoning	4	2.11
29	Kale	8	22.2	61	Milk	2	5.6	94	Sunflower seed	1	2.8
30	Millet	8	22.2	62	Leek	2	5.6	95	Tea leave	1	2.8
31	Pine needles	8	22.2	63	Nelumbinis Semen	2	5.6	96	Vitamin	1	2.8
32	Pleuropterus multiflorum	8	22.2	64	Lotus root	2	5.6	97	*Yongji* mushroom	1	2.8
				65	Sea lettus	2	5.6				

CFU/g for bacteria (Fig. 1). Coliforms were detected in all the samples except for three. Eleven % of the samples were contaminated with *E. coli* (Fig. 2). Ko *et al.* (11) reported that *sunsik* was contaminated with 3.1 log CFU/g for the total plate counts on average. This is below the data we acquired. Kim *et al.* (29) reported on the microbial analyses of unprocessed agricultural and marine products, where bacteria were found at 5 log CFU/g in brown rice, carrots, and seaweeds, and 6 log CFU/g in lettuce. They also reported *E. coli* contaminations of 6.25% (2/32) and 5.4% (2/37) in sweet potatoes and sesame leaves, respectively. Generally, this seems to indicate that the major ingredients of *sunsik* are highly contaminated with bacteria.

Sunsik undergoes a heat treatment step such as roasting or steaming only for the cereal ingredients; the other ingredients are only washed, dried, and pulverized. The principal means of contamination for *sunsik* seems to come from the raw materials. Therefore, pre-treatments should be required, such as washing with sanitizing agents and increasing the washing time, in order to reduce contamination by bacteria from the raw ingredients. However, for our data, a level of 2-5 log CFU/g for total plate counts of the *sunsik* samples does not seem high in terms of contamination. The only concern comes from the *E. coli* contamination of *sunsik* (Fig. 1 and 2).

Analyses of spore-forming *B. cereus* and *Cl. perfringens* in *sunsik* Recently, various toxigenic *Bacillus* species were detected in food (29-32), and *B. cereus* is a new

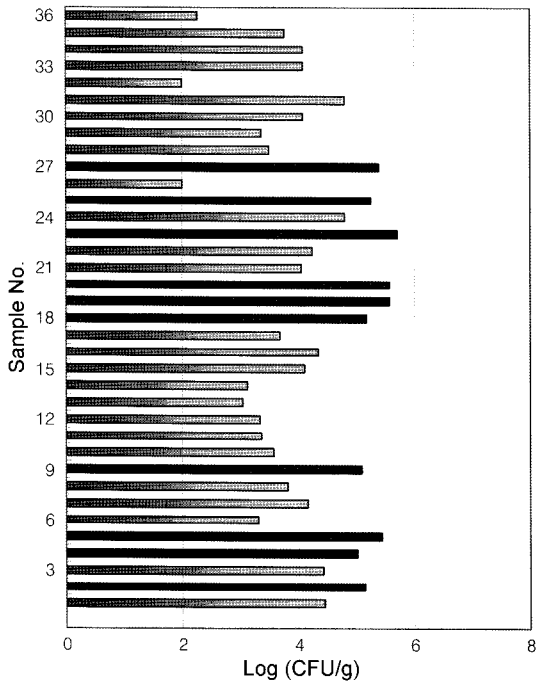

Fig. 1. Total aerobic plate counts for *sunsik*.

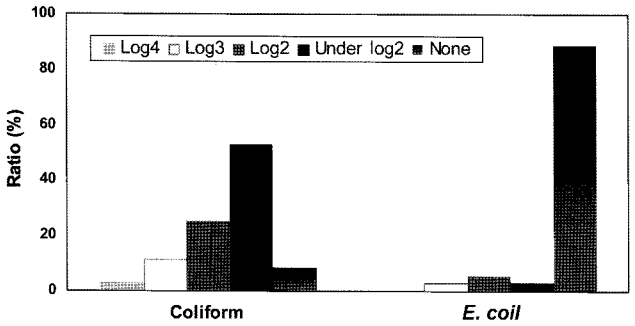

Fig. 2. Detection and enumeration of coliforms and *E.coli* in *sunsik* (CFU/g).

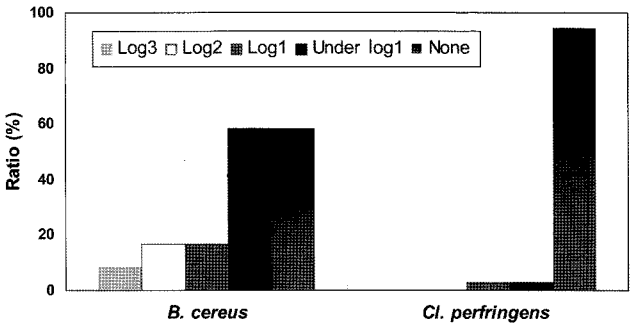

Fig 3. Detection and enumeration of *B. cereus* and *Cl. perfringens* in *sunsik* (CFU/g).

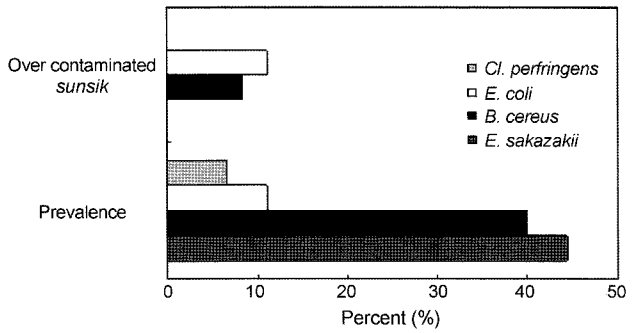

Fig. 4. Over-contaminated *sunsik* and prevalence of *Cl. perfringens*, *E. coli*, *B. cereus*, and *E. sakazakii* in *sunsik*. Over-contaminated *sunsik* by the standard for *saengsik* regulation indicating contamination of over 3 log CFU/g for *B.cereus* and *E.coli* detection.

pathogen of concern for some Korean foods. *B. cereus* was detected in 42% of the total samples and amounted to approximately 3 log CFU/g on average; in some foods, such levels would seem to require regulation by the Korean Food Code. The minimal and maximal levels were 1.5 and 3.9 log CFU/g, respectively (Fig. 3). Kim *et al.* (29) reported that *B. cereus* was distributed in brown rice, carrots, sweet potatoes, and sesame leaves at prevalence levels of 52, 34.1, 53.1, and 48.6%, respectively, in a total of 327 unprocessed agricultural and marine products. This indicates that unprocessed raw materials are contaminated with *B. cereus,* which would probably survive heat treatments because of its spore-forming activity. However, *B. cereus* might cause food poisoning when it is at levels over 5 or 6 log CFU/g in foods, according to the Food Safety and Inspection Service of the United States Department of Agriculture and U.S. FDA (35). Therefore, *sunsik* would not be a food safety hazard upon immediate intake after rehydration; however, spore-forming *B. cereus* could survive and multiply during storage of the product after rehydrating with water or milk (23). Again, some *sunsik* containing over 3 log CFU/g could be regulated by the Food Code standard for *saengsik* (Fig. 4). However, *saengsik* is totally non-heated, and *sunsik* is partially-heated, so *sunsik* might have to be regulated differently, such as by the detection of *B. cereus* below 3 log CFU/g and so on.

Generally, a low number of *Cl. perfringens* exist in raw meat, poultry, vegetables, and spices (33). These bacteria cause necrotizing enterocolostomy and necrotizing enteritis in infants (37). From this study, 2 samples were contaminated with *Cl. Perfringens,* and its average level was 15 CFU/g (Fig. 3). Chang *et al.* (3) reported that *Cl. perfringens* was detected in brown rice, barley, sorghum, and glutinous rice under 2 log CFU/g, approximately, and had a 75% detection rate. Symptoms are generally caused by large numbers (10^8 cells) of vegetative cells. So, the risk of *Cl. perfringens* does not seem to be high based on our analysis of *sunsik*.

Prevalence and frequency of *E. sakazakii* in *sunsik* We need to ensure that foods are safe from *E. sakazakii* contamination, especially for non-sterilized foods, since it is widely spread in nature and severely deadly to infants. There is a possibility for *E. sakazakii* infection from unheated or cross-contaminated foods, especially in infant formulas or baby foods, in infants as well as elderly persons with weak immune systems (38). Forty-five % of *sunsik* samples were contaminated with *E. sakazaki*. MPN

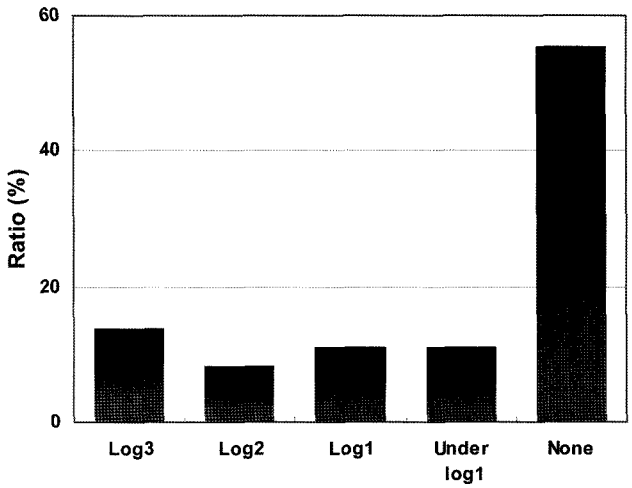

Fig. 5. Detection and enumeration of *Enterobacter sakazakii* in *sunsik* (MPN/100 g).

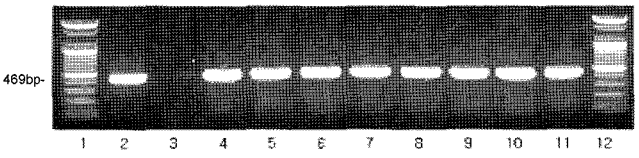

Fig. 6. Detection of *E. sakazakii* by amplification of the *ompA* gene. Lane 1 and 12, 100 bp ladder; 1, *E. sakazakii* NCTC 11467 (positive control); 2, *E. cloacea* KCCM 12178 (negative control); 3-11, *E. sakazakii* wild strains from *sunsik*.

analysis for enumeration (34, 35) was performed for 16 samples where *E. sakazakii* was detected. According to the results, the level of *E. sakazakii* contamination was determined as 0.7 to 2.24×10^3 MPN/100 g (Fig. 5). Iversen and Forsythe (39) detected 67 wild strains of *E. sakazakii* from 468 agriculture and marine products. Thus, *E. sakazakii* could widely contaminate unheated food materials. Also, Friedemann (40) stated that foods other than infant formula have rarely been examined for *E. sakazakii*. In addition, the results of 2 *sunsik* products for infants and children showed 4 log CFU/g for total plate counts, over 3 log CFU/g, on average, for *B. cereus*, as well as *E. sakazaki* contamination (Fig. 3 and 5). Therefore, those *sunsik* products would not be suitable for infant feeding, especially infants under 6 months of age. Such products need stricter microbial control to ensure their safety. Jung and Park (22) suggested a rehydration liquid temperature of 60°C, with a 20 min waiting period at room temperature, to reduce *E. sakazakii* up to 2 log for powdered infant formula (22). Therefore, a proper rehydration temperature may also control the number of *E. sakazakii* in powdered *sunsik*.

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References

1. Vanne L, Karwoski M, Karppinen S, Sjoberg AM. HACCP-based food quality control and rapid detection methods for microorganisms. Food Control 7: 263-327 (1996)
2. Brackett RE. Microbiological safety of chilled foods: Current issues. Trends Food Sci. Tech. 3: 81-85 (1992)
3. Chang TE, Moon SY, Lee W, Park JM, Han JS, Song OJ, Shin IS. Microflora of manufacturing process and final products of *seangsik*. Korean J. Food Sci. Technol. 36: 501-506 (2004)
4. Cristopher H, Sommers A, Boyd G. Variation in radiation sensitivity of foodborn pathogens associated with complex ready-to-eat food products. Radiat. Phys. Chem. 75: 773-778 (2006)
5. Melderum RJ, Smith RMM, Ellis P, Garside J. Microbiological quality of random selected ready-to-eat foods sampled between 2003 and 2005 in Wales, UK. Int. J. Food Microbiol. 108: 397-400 (2006)
6. Bean NH, Griffin PM. Foodborn disease outbreaks in the united stages, 1973-1987: Pathgens, vehicles, and trends. J. Food Protect. 53: 804-817 (1990)
7. Notermans S, Borgdorf M. A global perspective of foodborne disease. J. Food Protect. 60: 1395-1399 (1997)
8. Aycicek H, Sarimehmetoglu B, Cakiroglu K. Assessment of the microbiological quality of meals sampled at the meal serving units of a military hospital in Ankarka, Turky. Food Control 15: 379-384 (2004)
9. Watada AE, Kim SD, Kim KS, Harris TC. Quality of green beans, bell pepper, and spinach stored in polyethylen bag. J. Food Sci. 52: 1637-1640 (1987)
10. Kwon JH, Byung MW, Cho HO. Quality evaluation of ground garlic and onions treated with chemical fumigants and ionizing radiation. Korean J. Food Sci. Technol. 19: 107-112 (1987)
11. Ko JK, Ma YH, Song KB. Effect of electron beam irradiation on microbial qualities of whole black pepper powder and commercial *sunsik*. Korean J. Food Sci. Technol. 37: 308-312 (2005)
12. Chang TE, Han JS, Song OJ, Chung DH, Shin IS. Study on reducing methods of natural food-borne pathogenic microorganisms originated from *seangsik*. Korean J. Food Sci. Technol. 36: 501-506 (2004)
13. Kim DJ, Ha SD, Ryu K, Park KH. Hazard analysis and determination of CCPs for powdered raw grains and vegetables, *seangsik*. Korean J. Food Sci. Technol. 36: 1032-1040 (2004)
14. Kim SH, Kim JS, Park JH. Antibiotic resistance of *Enterococcus* isolated from the processed grains foods, *saengsik* and *sunsik*. Food Sci. Biotechnol. 16: 470-476 (2007)
15. Kim DH, Song HP, Yook HS, Chung YJ, Kim YJ, Byun MW. Distibution of microflora in powdered raw grains and vegetables and improvement of hygienic quality by gamma irradiation. J. Korean Soc. Food Sci. Nutr. 31: 589-593 (2002)
16. KFDA. Food Code. p. 64. Korea Food and Drug Administration, Seoul, Korea (2005)
17. Jang JH, Jang JS, Lee SY, Kim HS, Kang SM, Park JH. Growth inhibition effects of ethanol and sodium chloride on *Bacillus cereus*. Korean J. Food Sci. Technol. 35: 998-1002 (2003)
18. Gabis D, Faust RE. Controling microbial growth in food processing. Food Technol.-Chicago 12: 81-82 (1988)
19. Guillaume-Gentil O, Sonnard V, Kandhai MC, Marugg JD, Joosten H. A simple and rapid cultural method for detection of *Enterobacter sakazakii* in environmental samples. J. Food Protect. 68: 64-69 (2005)
20. Arad I, Baras M, Gofin R, Bar-Oz B, Peleg O. Dose parity affect the neonatal outcome of very-low-birth-weight infants? Eur. J. Obstet. Gyn. R. B. 94: 283-289 (2001)
21. Farmer JJ, Asbury MA, Hickman FW, Brenner DJ. The *Enterobacteriaceae* study group. *Enterobacter sakazakii*: A new species of '*Enterobacteriaceae*' isolated from clinical specimens. Int. J. Syst. Bacteriol. 30: 569-584 (1980)
22. Jung MK, Park JH. Prevalence and thermal stability of *Enterobacter sakazakii* from unprocessed ready-to-eat agricultural products and powdered infant formulas. Food Sci. Biotechnol. 15: 152-157 (2006)
23. Becker H, Schaller W, von Wiese W, Terplan G. *Bacillus cereus* in

infant foods and dried milk products. Int. J. Food Microbiol. 12: 1-15 (1994)
24. Notermans S, Dufrenne J, Teunis P, Beumer R, Giffel M, Peeters-Weem P. A risk assessment study of *Bacillus cereus* present in pasteurized milk. Food Microbiol. 14: 143-151 (1997)
25. Reyes JE, Bastias JM, Gurierrez MR, Rodriguez MO. Prevalence of *Bacillus cereus* in dried milk products used by children school feeding program. Food Microbiol. 24: 1-6 (2007)
26. Ahmed AH, Moustafa K, Marth EH. Incidence of *Bacillus cereus* in milk and some milk products. J. Food Protect. 46: 126-128 (1983)
27. Byrne B, Dunne G, Bolton DJ. Thermal inactivation of *Bacillus cereus* and *Clostridium perfringens* vegetative cells and spore in pork luncheon roll. Food Microbiol. 23: 803-808 (2006)
28. Rosenquista H, Smidtbl S, Andersena R, Jensenb GB, Wilcksa A. Occurrence and significance of *Bacillus cereus* and *Bacillus thuringiensis* in ready-to-eat food. FEMS Microbiol. Lett. 250: 129-136 (2005)
29. Kim SH, Kim JS, Choi JP, Park JH. Prevalence and frequency of food-born pathogens on unprocessed agricultural and marine products. Korean J. Food Sci. Technol. 38: 594-598 (2006)
30. Park SY, Choi JW, Yeon JY, Lee JM, Lee DH, Kim KS, Park KH, Ha SD. Assessment of contamination levels of foodborne pathogens isolated in major RTE foods marketed in convenience stores. Korean J. Food Sci. Technol. 37: 274-278 (2005)
31. Lim JH, Kim YH, Ahn YT, Kim HU. Studies on the contamination and inhibition of *Bacillus cereus* in domestic raw milk and milk products. J. Anim. Sci. Technol. 42: 215-222 (2000)
32. Todd E, Park C, Clecner B, Fabricius A, Edwards D, Ewan P. Two outbreaks of *Bacillus cereus* food poisoning in Canada. Can J. Public Health 65: 109-113 (1974)
33. Yoo HS, Lee SU, Park KY, Park YH. Molecular typing and epidemiological survey of prevalence of *Clostridium perfringens* types by multiplex PCR. J. Clin. Microbiol. 35: 228-232 (1997)
34. Center for Food Safety and Applied Nutrition. Isolation and enumeration of *Enterobacter sakazakii* from dehydrated powdered infant formula. U.S. Food and Drug Administration. http://www.cfsan.fda.gov. Accessed May 2, 2002.
35. Feng P. Most probable number from serial dilutions. Appendix 2. In: Bacteriological Analytical Manual *online*. U.S. Food and Drug Administration. http://www.cfsan.fda.gov. Accessed May 2, 2006.
36. Park SY, Choi JW, Yoen JH, LEE MJ, Oh DH, Hong CH, Bahk GJ, Woo GJ, Park JS, Ha SD. Assessment of contamination level of foodborne pathogens in the main ingredients of *kimbab* during the preparing process. Korean J. Food Sci. Technol. 37: 122-128 (2005)
37. Park JH, Han NS, Yoo JY, Kwon DJ, Shin HK, Koo YJ. Screening of the foodstuffs influencing the growth of *Bifidobacterium* spp. and *Clostridium perfringens*. Korean J. Food Sci. Technol. 25: 582-588 (1993)
38. Park JH, Jung MK. Food safety by *Enterobacter sakazakii*: Newly emergent pathogen from infant formula foods. Trends Agr. Life Sci. 3: 44-53 (2005)
39. Iversen C, Forsythe S. Risk profile of *Enterobacter sakazakii*, and emergent pathogen associated with infant milk formula. Trends Food Sci. Tech. 14: 443-454 (2003)
40. Friedemann M. *Enterobacter sakazakii* in food and beverages (other than infant formula and milk powder). Int. J. Food Microbiol. 116: 1-10 (2007)