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Microbiological Contamination of Fresh-Red Pepper and Packaged-Red Pepper Powder in South Korea

A-Ram Jeong[1], Mi Jin Jo[1], MinSeon Koo[1], Se-Wook Oh[2], Kyung-Hyung Ku[1], Jae-Bok Park[1], and Hyun Jung Kim[1†]

[1]*Korea Food Research Institute, Gyeonggi 463-746, Korea*
[2]*Department of Food and Nutrition, Kookmin University, Seoul 136-702, Korea*

Abstract

The aim of this study was to determine microbiological contamination of fresh-red pepper and packaged-red pepper powder commercially available in South Korea. Thirty-seven fresh-red peppers were collected from 5 farms and 31 packaged-red pepper powders were purchased from retail markets in South Korea. Foodborne pathogens (*Escherichia coli*, *Salmonella* spp., *Listeria monocytogenes*, *Staphylococcus aureus* and *Bacillus cereus*), total viable counts, *Enterobacteriaceae*, coliforms, yeast and mold, and *Aspergillus flavus* were determined. Detection percentage of contamination of *Bacillus cereus* in fresh-red pepper was 8.1%, which was lower than the 39% of detection rate in packaged-red pepper powder. The contamination level of *Bacillus cereus* was 1～3 log CFU/g in packaged-red pepper powder. *Escherichia coli* was detected in 5.4% of fresh-red pepper samples and was not detected in packaged-red pepper powder. *Enterobacteriaceae* and coliforms were detected in both of fresh-red pepper and packaged-red pepper powders. Foodborne pathogens, except *Bacillus cereus* and *Escherichia coli*, were not detected.

Key words: red pepper, red pepper powder, microbiological contamination

INTRODUCTION

Red pepper is used worldwide as powders, pastes, pickles, oleoresin, and sauces because of its unique taste and flavor. It also has been used for various medical applications (1,2). Most red peppers are consumed as red pepper powder with the annual consumption amount of 2.5～3.5 kg per capita in South Korea. Its retail market scale is estimated at one billion dollars per year (3). Consumer demands for packaged-red pepper powder, compared to traditional home-made red pepper powder, have sharply increased.

As consumers' interest in food safety have recently increased, the microbiological quality of agriculture products has gained recognition as an important issue for food safety in South Korea. Physicochemical hazards, such as chip (ferrous particle) and pesticides that endanger the safety of red pepper powder have been studied (3-6). Hazard Analysis and Critical Control Points (HACCP) guidelines on packaged-red pepper powder have recently recommended so that no foodborne pathogens, including *Bacillus cereus*, should be contaminats in raw materials (fresh or dried red pepper). However, little information is available on microbiological contamination of packaged-red pepper powder. In the case of red pepper powder, the quality of raw material (fresh-red pepper), as well as processing conditions such as drying, storage and grinding, can influence the quality of the final product (7,8). Therefore, a microbiological survey of fresh-red peppers which were cultivated in different areas could provide meaningful information. The aim of this research was to determine the microbiological contamination of packaged-red pepper powder, as well as the fresh-red pepper in Korea. We examined the prevalence and concentration of total viable counts, *Enterobacteriaceae*, coliforms, yeast, and mold as indicator microorganisms, as well as foodborne pathogens such as *Escherichia coli* (*E. coli*), *Salmonella* spp., *Listeria monocytogenes* (*L. monocytogenes*), *Staphylococcus aureus* (*S. aureus*), *Bacillus cereus* (*B. cereus*), and *Aspergillus flavus* (*A. flavus*) in fresh-red pepper and packaged-red pepper powder commercially available in South Korea.

MATERIALS AND METHODS

Materials

Media and supplements for microbiological determination were purchased from Merck (Darmstadt, Germany), Difco (Becton Dickinson, Sparks, USA) and

†Corresponding author. E-mail: hjkim@kfri.re.kr
Phone: +82-31-780-9271, Fax: +82-31-709-9876

Oxoid (Hampshire, UK). Butterfield's phosphate buffered dilution water was obtained from Sigma Chemical Co. (St. Louis, MO, USA).

Sample collection

Samples of fresh-red pepper (n=37) were collected from five different farms located in Goesan, Yeongyang, Andong, Imsil, and Gochang in South Korea (Fig. 1). All the samples were collected from September to November in 2009. Microbiological analyses were initiated within 24 hr after sample collection. For packaged-red pepper powder, a total of 31 samples (14 HACCP and 17 non-HACCP products) were purchased from retail markets in Gyeong-gi province (South Korea), using a stratified sampling method to reflect the provincial production amounts of packaged-red pepper powders (Fig. 1). Samples were stored at 4°C before analyses.

Microbiological analyses

Fresh-red pepper and packaged-red pepper powder (25 g) were added to 225 mL of Butterfield's phosphate buffered dilution water and homogenized using a BagMixer Blander (Interscience, St. Nom, France) for 2 min.

Enterobacteriaceae were enumerated in violet red bile glucose agar and incubated at 37°C for 24 hr. The results were expressed as Colony Forming Unit per gram of sample (CFU/g) according to the International Organization for Standardization (9). Detected *Enterobacteriaceae* was identified by the Vitek-2 compact system (bioMéreux, Marcy l'Etoile, France).

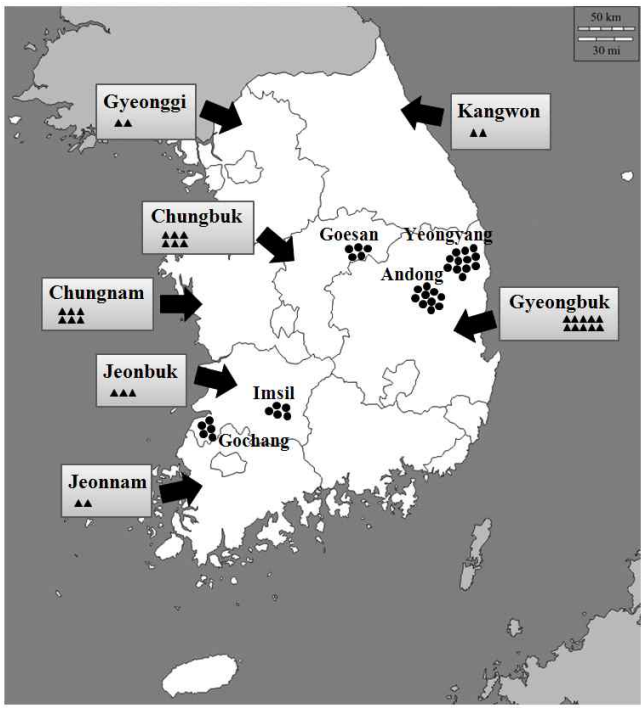

Fig. 1. Sampling sites of fresh-red peppers and origins of packaged-red pepper powders used to determine the microbiological contamination of red pepper and packaged-red pepper powder in South Korea. Closed circle, sampling site of fresh-red pepper powder; closed triangle, origin of packaged-red pepper powder. Number of symbols represents the number of samples.

Coliforms and *E. coli* counts were determined with the three-tube most probable number (MPN) system using three 10-fold dilutions in 5 mL brilliant green bile lactose broth (BGLB) tubes that each contained a Durham's tube (10). The BGLB tubes were incubated for 24 hr and 48 hr at 37°C for selective enrichment of coliforms. Broth cultures from the tubes showing both growth and gas production were streaked on coliform agar plates. The coliform agar plates were incubated for 24 hr at 37°C and then examined for characteristic *E. coli* colonies of a dark-blue to violet. Selected colonies were identified using the Vitek-2 compact system.

Fungi (yeast and mold) and *A. flavus* were enumerated in Potato dextrose agar and AFPA Base incubated at 25°C and 30°C for 5～7 days and 24～48 hr, respectively. The results were expressed as CFU/g.

Pathogen analyses

B. cereus, *Salmonella* spp., *S. aureus* and *L. monocytogenes* were detected according to the Korea Food additives code standard (10). For *B. cereus* detection, 25 g of the sample was added to 225 mL of Butterfield's phosphate buffered dilution water and homogenized using a BagMixer Blender for 2 min. After decimal dilution, 100 μL samples were placed on the surface of Mannitol Egg Yolk Polymyxin agar including egg yolk emulsion and *B. cereus* selective supplement in triplicate. Following the incubation at 30°C for 24 hr, pink colonies surrounded by a zone of precipitation were counted and *B. cereus* was identified using the Vitek-2 compact system.

For *Salmonella* spp. detection, 25 g samples were mixed with 225 mL of buffered peptone water and enriched for 18～24 hr at 37°C. One hundred μL of mixture was inoculated into the Rapapport-Vassiliadis broth and incubated at 42°C for 24 hr. Selective growth was conducted in Xylose-Lisine-Deoxycholate agar and Rambach agar at 37°C for 24 hr, respectively. Identification of red colonies showing a black center and typical red colonies were conducted using the Vitek-2 compact system.

For *S. aureus* detection, 25 g samples were added to 225 mL of Tryptic Soy Broth containing 10% NaCl and incubated at 37°C for 18～24 hr. Enriched samples were inoculated on Baired-Parker agar including egg yolk tellurite emulsion and incubated at 37°C for 24 hr. Convex black and shiny colonies with a narrow white entire margin and surrounded by clear zone into opaque medium

Table 1. Occurrence of *Enterobacteriaceae*, coliforms and foodborne pathogens in fresh-red pepper

Microorganisms	Prevalence (No. of positive samples/ No. of total samples)							
	Total	Harvesting area						
		A-1	A-2	B-1	B-2	C	D	E
Enterobacteriaceae	37/37	5/5	5/5	6/6	6/6	5/5	5/5	5/5
Coliforms	37/37	5/5	5/5	6/6	6/6	5/5	5/5	5/5
Escherichia coil	2/37	2/5	0/5	0/6	0/6	0/5	0/5	0/5
Bacillus cereus	3/37	0/5	0/5	1/6	0/6	0/5	2/5	0/5
Staphylococcus aureus	0/37	NA[1)]	0/5	NA	0/6	0/5	0/5	0/5
Listeria monocytogenes	0/37	NA	0/5	NA	0/6	0/5	0/5	0/5
Salmonella spp.	0/37	NA	0/5	NA	0/6	0/5	0/5	0/5

[1)]NA: Not available.

were selected. Detected *S. aureus* was identified using the Vitek-2 compact system.

For *L. monocytogenes* detection, 25 g samples were incubated in *Listeria* enrichment broth at 30°C for 24 hr. Enriched samples were isolated using PALCAM agar, which included PALCAM *Listeria* selective supplement and incubated at 30°C for 24～48 hr. Identification of typical green colonies surrounded by a black zone was conducted using the Vitek-2 compact system.

RESULTS AND DISCUSSION

Microbiological contamination in fresh-red pepper

Epidemiological studies related to foodborne disease outbreaks due to the consumption of fresh produce indicate that most pathogens of concern are derived from direct and indirect fecal contamination (11). In this respect, coliforms and *E. coli*, as well as *Enterobacteriaceae* counts, can be considered a good hygiene indicator, especially for fecal contamination. Coliforms and *Enterobacteriaceae* were detected in all samples (37/37, Table 1). *E. coli* was detected in 5.4% (2/37) of the samples. Previously reported levels of coliforms ranged from 2 to 5～7 log CFU/g for red pepper and mixed vegetables including pepper (12-14). Generally, *Enterobacteriacea* were detected with a high prevalence in vegetables. *Enterobacteriaceae* were detected in 73% (43/60) of mixed vegetables, including chili Fresno, with levels of 1.6 log CFU/g to 6.9 log CFU/g (15). For whole lettuces, the detection rate of *Enterobacteriaceae* was 68% (103/151), and 18% of the samples contaminated with level over 4 log CFU/g (16). *E. coli* was also reported as contaminating vegetable products; to be precise, there was a 7.1% detection rate in whole vegetables and an 11.4% contamination in fresh cut vegetables (17). The lower contamination rate of *E. coli* than coliforms and *Enterobacteriacea* is coincident with our result. The isolated *Enterobacteriaceae* were identified as *Enterobacter* spp., *Klebsiella* spp., *Leclercia* spp., *Pantoea* spp., *Pseudomonas* spp. and *Serratia* spp. (Table 2). *Enterobacteriaceae* such as *Enterobacter* spp., *Erwinia* spp. and *Serratia* spp., which are known as the major species of epiphytic microflora, which were detected in many unprocessed vegetables. Reports on *Enterobacteriaceae* contamination suggested that 80～90% of mesophilic bacteria isolated from minimally processed fresh vegetables consist of *Enterobacter* spp., *Erwinia* spp. and *Pseudomonas* spp., where counts range from 3 to 6 log CFU/g (13,18).

Among foodborne pathogens, *B. cereus* was detected in 8.1% (3/37) of the samples, while *S. aureus*, *L. monocytogenes*, and *Salmonella* spp. were not detected in fresh-red peppers (Table 1). Contaminations of *B. cereus* in spices have been previously reported (19,20). Pathogenic detection other than *B. cereus* has been reported but was not found to be common. Abadias et al. (17) isolated *Salmonella* spp. from 4 of 236 samples (1.7%) of fresh-cut vegetables. Reports on the lack of contamination by *L. monocytogenes*, *S. aureus*, and *Salmonella* spp. supported our results (13,19,21-23).

Microbial contamination in packaged-red pepper powders

Enterobacteriaceae were detected in 45% (14/31) of packaged-red pepper powders with the maximum contamination of 4.76 log CFU/g (Table 3). Detected *Enterobacteriaceae* were identified as *Enterobacter* spp., *Klebsiella* spp., *Leclercia* spp., *Pantoea* spp. and *Pseudomonas* spp. (Table 2). Banerjee and Sarkar (19) reported that *Enterobacteriaceae* was detected in 50% of red chili powder samples collected from retail markets in India, and the contamination level was 3.1 to 5 log CFU/g. Coliform contamination was found in 55% (17/31) of the samples, but the levels in most of the positive samples (16/17) were below 4 log CFU/g. In a former report, coliforms were detected at the level of 2.4 log CFU/g in red pepper powders (24). While *E. coli* was not detected in packaged-red pepper powder, 5.4% of fresh-red pepper was contaminated with *E. coli*, as described a cvt Yeast and mold were detected in 32%

Table 2. Identification of *Enterobactericeae* isolated from fresh-red pepper and packaged-red pepper powder

Isolated species	No. of positive samples/ No. of total samples (Detection %)	
	Fresh-red peppers	Packaged-red pepper powders
Enterobacter spp.	3/37 (8.1)	2/31 (6.5)
Klebsiella spp.	10/37 (27.0)	4/31 (12.9)
Leclercia spp.	1/37 (2.7)	1/31 (3.2)
Pantoea spp.	18/37 (48.6)	5/31 (16.1)
Pseudomonas spp.	2/37 (5.4)	1/31 (3.2)
Serratia spp.	3/37 (8.1)	0/31 (0.0)

(10/31) and 42% (13/31) of the samples, respectively. The contamination level of yeast ranged from 1 to 4 log CFU/g and that of mold ranged from 1 to 3 log CFU/g, while *A. flavus* was not detected in any analyzed samples (Table 3). Contamination levels of yeast and mold have been reported as 3.1～3.7 log CFU/g in red pepper powders (24,25).

The foodborne pathogens, *Salmonella* spp., *L. monocytogenes* and *S. aureus* were not detected in the analyzed samples. *B. cereus* was the most frequently detected pathogen, with a detection rate of 39% and contamination levels of 1 to 3 log CFU/g (Table 3). The International Commission on Microbiological Specifications for Foods (26) established maximum limits of 4, 4 and 3 log CFU/g for yeast and mold, coliforms, and *E. coli*, respectively, in spices. In Germany, however, the standard values for *B. cereus* and *S. aureus* are 4 and 2 log CFU/g in spice, respectively. *E. coli* should be absent, and the *Salmonella* count should be zero in a 25 g sample (27). Choo et al. (20) reported that *B. cereus* was detected at 84.3% in 140 dried red peppers, and the average count was 4.3 log CFU/g, which was higher than the prevalence and contamination level in this study. *B. cereus* was also found in red chili powder with the contamination level of 2 to 4 log CFU/g (19). Alternatively, *Salmonella* and *S. aureus* were not detected among any of the samples. In this study, the detection rate of *B. cereus* in fed peppe pepper was 8% (3/37), which was lower than that in packaged-red pepper powder (39%, 12/31). Because red pepper farms are usually operated on a small scale, and the consumers show a preference for a bright red color in the red pepper powder, fresh-red peppers are typically dried in ambient temperatures outdoors, and not dried under controlled hot air. *B. cereus* is one of the soil-borne microorganisms widely distributed in the natural environment in earth, water, and dust, and it has a high likelihood of infecting food products through original food ingredients (28). *B. cereus* may often contaminate dried red pepper powder during the outdoor drying processes. Future research is needed on the molecular epidemiology of the *B. cereus* that contaminates red pepper powder.

Many reports have suggested that foodborne diseases can be caused when *B. cereus* exists in food at levels greater than 3～6 log CFU/g (28-31). The level of *B. cereus* in this study was lower than the reference level mentioned above, suggesting a low probability of foodborne disease from the consumption of red pepper powder itself. However, red pepper powder is frequently used as condiment in a wide range of foods that are seasoned with red pepper powder and usually held before cooking or serving, for example, stir fried pork dishes and Korean style vegetable salads. If these foods are stored improperly, the *B. cereus* that contaminates the red pepper powder might multiply in the seasoned food and a potential health risk associated with the contamination of packaged-red pepper powders by *B. cereus* could not be avoided. Generally, *B. cereus* forms heat-stable spores that can survive heating at 135°C up to 4 hr; therefore, in food products or that are already contaminated by this pathogen, the spores will not be destroyed by the normal heating procedure (28).

Recently, HACCP has been used as a guideline in red pepper powder production to ensure that quality and

Table 3. Prevalence and contamination level of microbiological contamination packaged-red pepper powder

Microorganisms	Prevalence (No. of positive samples/ No. of total samples)	Level of contamination (log CFU/g)				
		1～2	2～3	3～4	4～5	5～6
Enterobacteriaceae	14/31	7[1)]	4	2	1	0
Coliforms	17/31	5	6	5	1	0
Escherichia coil	0/31	0	0	0	0	0
Bacillus cereus	12/31	6	6	0	0	0
Salmonella spp.	0/31	0	0	0	0	0
Listeria monocytogenes	0/31	0	0	0	0	0
Staphylococcus aureus	0/31	0	0	0	0	0
Yeast	10/31	2	2	6	0	0
Mold	13/31	5	8	0	0	0
Aspergillus flavus	0/31	0	0	0	0	0

[1)]Number of samples that contaminated with the indicated level of microorganisms.

safety measures have been applied to the products in Korea (10). The absence of foodborne pathogens, such as *B. cereus*, was recommended in HACCP guidelines for red pepper powder production; however, there have been no microbiological standards for packaged-red pepper powders in Korea. Prevalence and contamination level of *B. cereus* were slightly lower in HACCP products than in non-HACCP products, while those of indicator microorganisms, including coliforms, *Enterobacteriaceae*, and mold, were higher in HACCP products than in non-HACCP products with no statistical significance (data not shown). This result suggested that more consideration need to be given to prevention or control of microbiological contamination during red pepper powder processing to insure a good hygienic products, including HACCP products. However, the number of collected samples is limited, so more studies are needed to adequately evaluate HACCP products.

CONCLUSION

Red pepper powder is one of the major spices consumed in South Korea. Guidelines on the microbiological quality of fresh-red pepper and red pepper powder have not previously been available, despite the fact that red pepper powder is a major condiment in South Korea. Little information is available on microbiological contamination of packaged-red pepper powder. Therefore we determined microbiological contamination of fresh-red pepper collected from farms located in different areas and packaged-red pepper powder commercially available in South Korea. Qualitative and quantitative determination of foodborne pathogens (*E. coli*, *Salmonella* spp., *L. monocytogenes*, *S. aureus* and *B. cereus*), total viable counts, *Enterobacteriaceae*, coliforms, yeast and mold, and *A. flavus* showed no contamination of foodborne pathogens except *B. cereus* and *E. coli*. *B. cereus* and *E. coli* were detected in 8.1% and 5.4% of fresh-red pepper samples, respectively. *B. cereus* was detected in 39% of packaged-red pepper powders at levels of 1～3 log CFU/g. *Enterobacteriaceae* and coliforms were detected in both fresh-red pepper and packaged-red pepper powder. The microbial monitoring results from this study provide information on the microbiological contamination of red pepper powder and will be useful in making guidelines for the microbiological quality of packaged-red pepper powders.

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