Microbiological Investigation of Ready-to-cook Pork Bulgogi on Korean Markets

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Abstract

In this study, ready-to-cook (RTC) pork bulgogi was investigated microbiologically to determine contamination levels. The investigation was conducted because of an increasing trend in the consumption of RTC meat products in Korea. Ninety marinated RTC pork bulgogi samples were collected from major retail outlets (M), department stores (D), and local markets (L) in Seoul, Korea from March to June 2011. This study examined total plate counts (TPC), Escherichia coli, and coliform bacterial counts, and the presence of Bacillus cereus, Staphylococcus aureus, Listeria monocytogenes, Salmonella spp., and E. coli O157:H7. The mean TPC values were 5.89, 6.08, and 5.89 Log CFU/g for M, D, and L, respectively. E. coli was not detected in any sample, but coliforms were present in 72 (80%) of the 90 samples collected. B. cereus, E. coli O157:H7, and Salmonella spp. were not detected; however, S. aureus and L. monocytogenes were detected in five (5.5%) and one (1.1%) of the 90 samples. Samples collected from M and D were contaminated with S. aureus and those from L with L. monocytogenes. These results demonstrate that the conditions under which RTC pork bulgogi is handled and processed are unsanitary.

Key words: pork Bulgogi, ready-to-cook food, microbial safety, pathogens, Staphylococcus aureus, Listeria monocytogenes

Introduction

Pork bulgogi is a traditional Korean food. Thin slices of pork, usually arm picnic, shank, or ham, are marinated in a sauce containing soy sauce, onion, garlic, sesame oil and other seasonings. Pork bulgogi products can be contaminated with pathogenic bacteria during the handling or process if the raw materials used are contaminated or unhygienic conditions are applied. The ready-to-cook (RTC) product industry has expanded in Korea because of changes in customer consumption patterns. In Recent years, bulgogi is usually sold in markets as a RTC product. RTC foods, including bulgogi, are displayed open and exposed to contamination by bacterial pathogens; thus, several food-borne disease outbreaks have been associated with the consumption of contaminated RTC foods (Jo et al., 2003). Consumers find RTC products very convenient because these products only require heating to prepare a meal. However, the safety of these products during distribution and sale is frequently not monitored, and RTC bulgogi, in particular, is exposed to a serious risk of contamination or spoilage by bacteria in the ingredients, such as vegetables, soy sauce, and raw meat (Beuchat, 1996; Nguyen-the and Carlin, 1994). The number of cases of food-borne disease caused by the consumption of contaminated RTC food has been increasing. In 2010, 271 cases of food poisoning were reported, and 7,218 cases of food poisoning have been reported in Korea. The most frequent causes of bacterial food poisoning in Korea are contamination with pathogens such as Escherichia coli (28 cases), Salmonella spp. (27 cases) and Staphylococcus aureus (19 cases) (KFDA, 2010a).

E. coli strains rarely cause disease, except certain strains involved in infections of the intestinal and urinary tracts of humans. Pathogenic E. coli can be divided into six pathotypes: enterohemorrhagic (EHEC), enterotoxigenic (ETEC), enteropathogenic (EPEC), enteroaggregative (EAEC), enteroinvasive (EIEC), and diffusely adherent (DAEC). Pathogenic E. coli has become a significant health concern especially E. coli O157:H7, having caused major outbreaks in food (Watterworth et al., 2005).

S. aureus has been recognized as an indicator of food hygiene. S. aureus is a public health concern because of
its ability to produce enterotoxins and grow in highly saline environments. In cured meats, such as cured ham, which have a high pH, the water activity (a_w) should be below 0.90 for preventing the production of staphyloccocal enterotoxin (Gillespie, 2007; Gormley et al., 2010).

Salmonella spp. are the most common pathogens associated with food-borne diseases, typhoid fever in humans, self-limiting enteritis, and fatal infections in animals. Red meat and poultry are good sources of Salmonella.

In addition, improper process and equipment could be associated with increasing the probability of cross contamination with B. cereus and L. monocytogenes. B. cereus, a facultative anaerobic, spore-forming, motile bacterium has been identified as the causative agent of 2 types of gastrointestinal diseases (Ankolekar et al., 2009) and has been isolated from a wide variety of foods including spices (Choo et al., 2007; Konuma et al., 1988), ready-to-serve foods (Harmon and Kautter, 1991), and meat products (Smith et al., 2004).

Listeria spp., particularly Listeria monocytogenes have been recognized as animal pathogens for over 70 years (Suilko et al., 2002; Wesley, 1999). In the 1990s, L. monocytogenes was frequently isolated from all the major food products, such as unfermented dairy products (Ryser, 1999), cheese and meat products (Farber and Peterkin, 1999), and poultry and egg products. L. monocytogenes can survive in vacuum and gas-packed products and grow at refrigerated temperatures; this is a cause of concern for chilled meat products with an extended shelf-life.

The objective of this research was to evaluate the level of contamination of RTC meat products with pathogens on the major Korean markets, such as major retail outlets, department stores, and local markets. Pork bulgogi was chosen as the target RTC meat product and then the microbiological condition was investigated with the food hygiene indicator, such as TPC, E. coli, and coliforms as well as the presence of pathogens, such as B. cereus, E. coli O157:H7, S. aureus, Salmonella spp. and L. monocytogenes.

Materials and Methods

Sample collection

In total, 90 marinated pork bulgogi samples were collected from i.e., 3 samples each from 10 different major retail outlets (M), department stores (D), and local markets (L) located in Seoul, respectively, from March to June 2011 (Table 1). The collected samples were placed in sterile bags, transferred by refrigerated transport to the laboratory, and processed within 24 h of collection.

Food hygiene indicator bacteria

Testing for indicator organisms (total plate counts, E. coli, and coliform bacterial counts) has been introduced as a simple means of assessing the hygiene status of foods and helps ensure food safety (Park, 2004). The experimental procedure in our study was developed on the basis of the Korea Food Code (KFDA, 2010b). A 1:10 dilution sample of pork bulgogi was prepared by adding 25 g of meat sample obtained from a location to 225 mL 0.1% (w/v) peptone water; and the solution was homogenized in a stomacher and mixed thoroughly. Inoculum preparation was added to 225 mL 0.1% peptone water; and then the solution was homogenized in a stomacher (IUL instruments, Germany) for 2 min. Total plated counts, and E. coli and coliform bacterial counts were quantitatively assessed by inoculating the diluted samples on tryptic soy agar (TSA) plates and petrifilms (3M™), respectively, and incubating 35°C for 24 h.

Isolation and characterization of pathogens

B. cereus

Twenty-five grams of meat samples obtained from each location was added to 225 mL 0.1% peptone water and homogenized for 1 min. The mixed samples were inoculated on manitol-egg yolk-polymyxin (MYP, Oxoid) agar and incubated at 35°C for 24 h. We isolated the pink-red manitol-negative colonies with surrounding lecithinase-positive zones of precipitation streak-cultured the isolated colonies on TSA plated plates, and maintained these as pure cultures. The strains on the TSA plate cultures were then, analyzed biochemically by using API 50 CH strips (Biomérieux, France).

E. coli O157:H7

Twenty-five grams of samples obtained from each location was added to 225 mL modified E. coli (mEC) medium with novobiocin as the antimicrobial supplement and incubated at 35°C for 24 h. The mixture was homogenized in a stomacher and mixed thoroughly. Inoculum were streaked on MacConkey sorbitol agar without cefixime and tellurite plates and incubated at 35°C for 16-

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Table 1. Types of samples used in this study

<table>
<thead>
<tr>
<th>Location</th>
<th>Number of places</th>
<th>Number of samples</th>
</tr>
</thead>
<tbody>
<tr>
<td>Major retail outlet</td>
<td>10</td>
<td>30</td>
</tr>
<tr>
<td>Department store</td>
<td>10</td>
<td>30</td>
</tr>
<tr>
<td>Local market</td>
<td>10</td>
<td>30</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>30</strong></td>
<td><strong>90</strong></td>
</tr>
</tbody>
</table>

1/3 samples were collected from each place.
18 h. The sorbitol-negative colonies were then subcultured on eosin methylene blue (EMB) agar plates. Distinct *E. coli* colonies were isolated and streaked onto TSA plates for further characterization. The colonies that showed a green metallic sheen on EMB agar, because of rapid lactose fermentation, were isolated and considered as those of *E. coli*. We performed O157 latex agglutination test (DR0620, Oxoid) (Stampi *et al*., 2004) for confirming the presence of *E. coli* O157 strains.

**S. aureus**

Twenty-five grams of samples, obtained from each location was added to 225 mL tryptic soy broth (TSB) with 10% NaCl and enriched at 35°C for 24 h and then inoculated on Baird-Parker agar plates enriched with egg yolk (EY) tellurite. The plates were incubated under aerobic conditions at 35°C for 24 h. Typical black and shiny convex-shaped colonies of *S. aureus* surrounded by clear zones of approximately 2-5 mm were isolated and cultured on TSA plates for further identification (Normanno *et al*., 2005). The presumed *S. aureus* colonies were examined by Gram staining and catalase test. Gram- and catalase-positive isolates were further identified using a latex agglutination kit (Pastorex™ Staph-Plus; Bio-Rad) and API Staph System (Biomérieux).

**Salmonella spp.**

Twenty-five grams of meat samples obtained from each location was added to 225 mL buffered peptone water (BPW), incubated at 35°C for 24 h, and homogenized for 1 min in a stomacher. After pre-enrichment, 0.1 mL of the culture solution was added to 10 mL Rappaport-Vassiliadis (RV) broth. The RV broth was then incubated at 42°C for 18 h. Loopfuls of the broth were streaked on Salmonella-Shigella (SS) agar and xylose lysine deoxycholate (XLD) agar plates. Red colonies with black centers on XLD agar were presumed as colonies of *Salmonella* spp. and were isolated and inoculated on TSA plates. Subsequently, the colonies were inoculated and streaked on triple sugar iron (TSI) slant. Because the inoculums could not ferment glucose and lactose, further study for gram staining, urease test and lysine decarboxylase test were not conducted.

**L. monocytogenes**

Twenty-five grams of meat samples obtained from each location was added to 225 mL Listeria enrichment broth (LEB) and incubated at 35°C for 18-24 h. After incubation, the broth was homogenized and inocula were streaked on modified Oxford (MOX) agar plates with antimicrobial supplements and incubated at 37°C for 48 h. The presumed colonies of *L. monocytogenes* were further confirmed and characterized. Morphologically typical colonies of *L. monocytogenes* were verified by Gram staining, catalase reaction, motility test, hemolysis on 5% sheep blood agar, CAMP test with *S. aureus* ATCC 25923, and fermentation of sugars by API Listeria (Biomérieux) (Chaudhari *et al*., 2004).

**Statistical analysis**

Analysis of variance (ANOVA) was performed on all data with the general linear model (GLM) procedure by using the program Statistical Analysis System (SAS version 9.1). The bacterial total plate counts in colony forming units (CFU)/g were transformed to logarithmic values (Log CFU/g) for statistical analyses. Mean values were determined using Tukey’s studentized range (HSD) tests.

**Results and Discussion**

**Distribution of food hygiene indicators in RTC pork bulgogi**

Detecting pathogenic bacteria in food is very important, but this involves a relatively complex procedure. Therefore, testing for indicator organism has been introduced as a simple means of assessing the hygiene status of food samples and helps ensure food safety. We assessed the hygiene status of pork bulgogi from major retail outlets, department stores, and local markets by collecting and analyzing 90 bulgogi samples (30 samples from each type of location).

Table 2 shows the microbial distribution in samples obtained from major retail outlets. The mean value of total plate counts for 3 samples obtained at a particular place (total, 10 places) in each of the location groups ranged from 4.59 to 7.07 Log CFU/g. Total coliforms detected in samples obtained from 6 out of 10 major retail outlets ranged from 1.00 to 4.09 Log CFU/g. We observed similar coliform detection rates in samples obtained from department stores and local markets. Samples obtained from M3 (major retail outlets, place 3) showed the lowest total plate count and coliforms were not detected. However, samples obtained from M6 and M10 showed high total plate counts, even though the coliforms detected were 1.00 and 2.84 Log CFU/g, respectively. Samples obtained from the 10 department stores showed total plate counts and coliform counts ranging from 4.47 to 7.58 Log CFU/g and 2.23 to 4.25 Log CFU/g, respectively.
Samples obtained from L1 to L10 showed the mean values of total plate counts and coliforms ranging from 4.57 to 7.34 and 2.13 to 4.15 Log CFU/g, respectively. E. coli were not detected in any samples. The total plate counts for most of the samples in our study were higher than that for raw meat. In the previous study, the counts of mesophilic microorganisms in raw pork meat ranged from 3.9×10^5 to 3.9×10^6 CFU/g, and coliforms were not detected. The average number of aerobic mesophilic bacteria of marinated meat product was 2.3×10^6 CFU/g, and the highest bacterial average was 3.1×10^7 (Björkroth, 2005). However, the study by Kwak et al. (1998) on pork bulgogi seasoning showed a high mesophilic total plate count of 5.97 Log CFU/g and a mean value of coliforms (logarithmic value for most probable number [MPN]/g) of 2.65 Log MPN/g. This result suggest that garlic, onion, soy sauce, and other ingredients in the seasoning used for pork bulgogi should have been the source of contamination and increased bacterial count.

The hygienic and sanitation level of 2 major retail outlets (M6 and M10), one department store (D6), and one local market was found to be low and should be improved. The hygiene status of marinated meat as observed by monitoring the indicator microorganisms, was significantly different among individual stores in each group (p<0.05), even though the total plate counts and coliform counts were almost similar among the groups (p>0.05).

### Isolation and characterization of pathogens

Table 2. Distribution of total plate counts, coliform, and *E. coli* counts in marinated meat from major retail outlets

<table>
<thead>
<tr>
<th>Samples</th>
<th>TPC</th>
<th>Coliform</th>
<th>E. coli</th>
</tr>
</thead>
<tbody>
<tr>
<td>M1</td>
<td>5.57±0.04bc</td>
<td>2.51±0.30b</td>
<td>ND4</td>
</tr>
<tr>
<td>M2</td>
<td>5.19±0.16c</td>
<td>1.38±1.08c</td>
<td>ND</td>
</tr>
<tr>
<td>M3</td>
<td>4.59±0.27d</td>
<td>ND4</td>
<td>ND</td>
</tr>
<tr>
<td>M4</td>
<td>5.97±0.10b</td>
<td>1.30±1.42c</td>
<td>ND</td>
</tr>
<tr>
<td>M5</td>
<td>5.80±0.11b</td>
<td>4.09±0.05a</td>
<td>ND</td>
</tr>
<tr>
<td>M6</td>
<td>7.07±0.54a</td>
<td>2.84±0.24b</td>
<td>ND</td>
</tr>
<tr>
<td>M7</td>
<td>6.84±0.09a</td>
<td>ND4</td>
<td>ND</td>
</tr>
<tr>
<td>M8</td>
<td>5.28±0.15c</td>
<td>ND4</td>
<td>ND</td>
</tr>
<tr>
<td>M9</td>
<td>5.57±0.23bc</td>
<td>ND4</td>
<td>ND</td>
</tr>
<tr>
<td>M10</td>
<td>7.00±0.16a</td>
<td>2.84±0.24b</td>
<td>ND</td>
</tr>
</tbody>
</table>

1*M*: major retail outlet  
2*Mean±SD* (Log CFU/g) of samples  
3TPC: total plate counts  
4ND: not detected  

*Means with different superscript within same row are significantly different (p<0.05).

Table 3. Distribution of total plate counts, coliform, and *E. coli* counts in marinated meat from department stores

<table>
<thead>
<tr>
<th>Samples</th>
<th>TPC</th>
<th>Coliform</th>
<th>E. coli</th>
</tr>
</thead>
<tbody>
<tr>
<td>D1</td>
<td>6.90±0.05b</td>
<td>3.62±0.05ab4</td>
<td>ND4</td>
</tr>
<tr>
<td>D2</td>
<td>5.83±0.15c</td>
<td>ND4</td>
<td>ND</td>
</tr>
<tr>
<td>D3</td>
<td>5.59±0.20e</td>
<td>3.25±1.02d</td>
<td>ND</td>
</tr>
<tr>
<td>D4</td>
<td>6.50±0.32b</td>
<td>3.44±0.40bc4</td>
<td>ND</td>
</tr>
<tr>
<td>D5</td>
<td>4.64±0.43d</td>
<td>3.87±0.20bc4</td>
<td>ND</td>
</tr>
<tr>
<td>D6</td>
<td>7.58±0.08a</td>
<td>3.13±0.21d</td>
<td>ND</td>
</tr>
<tr>
<td>D7</td>
<td>4.47±0.07d</td>
<td>4.25±0.07d</td>
<td>ND</td>
</tr>
<tr>
<td>D8</td>
<td>5.91±0.24a</td>
<td>4.13±0.06d</td>
<td>ND</td>
</tr>
<tr>
<td>D9</td>
<td>6.58±0.10b</td>
<td>2.28±0.17d</td>
<td>ND</td>
</tr>
<tr>
<td>D10</td>
<td>6.83±0.06b</td>
<td>3.30±0.13d</td>
<td>ND</td>
</tr>
</tbody>
</table>

1*D*: department store  
2*Mean±SD* (Log CFU/g) of samples  
3TPC: total plate counts  
4ND: not detected  

*Means with different superscript within same row are significantly different (p<0.05).

Table 4. Distribution of total plate counts, coliform, and *E. coli* counts in marinated meat from local markets

<table>
<thead>
<tr>
<th>Samples</th>
<th>TPC</th>
<th>Coliform</th>
<th>E. coli</th>
</tr>
</thead>
<tbody>
<tr>
<td>L1</td>
<td>6.12±0.17bc</td>
<td>3.53±0.08d</td>
<td>ND4</td>
</tr>
<tr>
<td>L2</td>
<td>5.62±0.31d</td>
<td>3.90±0.13abc</td>
<td>ND</td>
</tr>
<tr>
<td>L3</td>
<td>4.57±0.13c</td>
<td>4.15±0.08a</td>
<td>ND</td>
</tr>
<tr>
<td>L4</td>
<td>4.87±0.09a</td>
<td>2.13±0.32f</td>
<td>ND</td>
</tr>
<tr>
<td>L5</td>
<td>6.14±0.17bc</td>
<td>ND3</td>
<td>ND</td>
</tr>
<tr>
<td>L6</td>
<td>5.69±0.09a</td>
<td>2.26±0.22f</td>
<td>ND</td>
</tr>
<tr>
<td>L7</td>
<td>6.09±0.04e</td>
<td>3.03±0.26c</td>
<td>ND</td>
</tr>
<tr>
<td>L8</td>
<td>6.49±0.11b</td>
<td>4.08±0.05ab</td>
<td>ND</td>
</tr>
<tr>
<td>L9</td>
<td>7.34±0.32e</td>
<td>3.79±0.22ab4</td>
<td>ND</td>
</tr>
<tr>
<td>L10</td>
<td>6.16±0.02bc</td>
<td>3.61±0.14ad</td>
<td>ND</td>
</tr>
</tbody>
</table>

1*L*: local market  
2*Mean±SD* (Log CFU/g) of samples  
3TPC: total plate counts  
4ND: not detected  

*Means with different superscript within same row are significantly different (p<0.05).

We evaluated the prevalence of selected bacterial pathogens (*B. cereus*, *E. coli* O157:H7, *S. aureus*, *Salmonella* spp., and *L. monocytogenes*) in marinated pork bulgogi collected from different types of retail shops located in Seoul, Korea. *B. cereus*, *E. coli* O157:H7, and *Salmonella* spp. were not detected in any samples (Table 5). We had two pink-red colonies with lecithinase-positive zones on the MYP agar, which were guessed as *B. cereus*, however, they were finally identified as *B. subtilis* according to their biochemical characterization found using API 50 CH strips.
Three colonies that showed a green metallic sheen on EMB agar were isolated and investigated for their O157 latex agglutination ability for the purpose of evaluating the presence of \(E. \) coli O157 strains. According to their incompetence of agglutination, the isolates were finally concluded not to be \(E. \) coli O157:H7.

The presumed colonies on XLD agar were isolated and inoculated on triple sugar iron (TSI) slants. After incubation, it was confirmed that these bacteria could metabolize glucose and lactose. The presence of these characteristics confirmed that the inoculums were not \(Salmonella\) spp. A previous study had reported a high prevalence rate (20%) of \(Salmonella\) spp. in red meat from butcher shops in Gaborone, Botswana, which was attributed to inadequate temperature control, improper handling of raw meat, and inadequate environmental conditions (Mrema et al., 2006). However, no \(Salmonella\) spp. was isolated from pork bulgogi in our study.

Examining 90 pork bulgogi samples, 5 samples (5.6%) tested were found to be positive for \(S. \) aureus. Four (13.3% of 30) of them were obtained from major retail outlets, and the remaining one (3.3% of 30) was obtained from a department store; none were detected in samples from local markets. In the previous studies, \(S. \) aureus has been isolated from several foods, including meat and meat products, chicken, milk and dairy products, fermented food items, vegetables, and fish products (Wieneke et al., 1993; Tamarapu et al., 2001). Salted food products, such as ham, have been reported to be responsible for about 24% of all cases of \(S. \) aureus food poisoning (Qi and Miller, 2000). Comparing the results of \(S. \) aureus in this study with those of other studies, the prevalence of \(S. \) aureus detected is very highly regarded. Normanno et al. (2005) suggested that coagulase-positive staphylococci and \(S. \) aureus were the most predominantly detected bacteria in various kind food products, e.g., 26.1% from fresh meat and 48.1% from other meat products.

Characterization of isolated pathogens

Tables 6 and 7 show the characteristics of isolated \(S. \) aureus and \(L. \) monocytogenes, respectively. Samples showing the presence of \(S. \) aureus were positive for coagulase,
Therefore, microbial risk assessment should be strictly for detecting food-borne pathogens in marinated meat. However, there are no specific standards for detecting food-borne pathogens in marinated meat. According to the Korea Livestock Products Sanitary Control Act, E. coli O157:H7 should not be detected in marinated meat. However, there are no specific standards for detecting food-borne pathogens in marinated meat. Therefore, microbial risk assessment should be strictly regulated and performed by evaluating the data scientifically.

### Acknowledgments

This study was supported by Technology Development Program for Agriculture and Forestry (Project: 111018-3), Ministry for Food, Agriculture, Forestry and Fisheries, Republic of Korea. This work was also supported by the Priority Research Centers Program through the National Research Foundation of Korea funded by the Ministry of Education, Science and Technology (2009-0093824).

### Table 7. Morphological and biochemical characteristics of Listeria monocytogenes isolated from marinated pork bulgogi

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gram stain</td>
<td>+1) Rod</td>
</tr>
<tr>
<td>Shape</td>
<td>Rod</td>
</tr>
<tr>
<td>Catalase</td>
<td>+</td>
</tr>
<tr>
<td>Motility</td>
<td>+</td>
</tr>
<tr>
<td>β-Hemolysis</td>
<td>+</td>
</tr>
<tr>
<td>Differentiation L. innocua/L. monocytogenes</td>
<td>+2)</td>
</tr>
<tr>
<td>Esculin hydrolysis</td>
<td>+</td>
</tr>
<tr>
<td>α-Mannosidase</td>
<td>+</td>
</tr>
<tr>
<td>Acidification</td>
<td>-</td>
</tr>
<tr>
<td>D-Arabinol</td>
<td>-</td>
</tr>
<tr>
<td>D-Xylose</td>
<td>-</td>
</tr>
<tr>
<td>L-Rhamnose</td>
<td>-</td>
</tr>
<tr>
<td>Methyl-α-D-glucopyranoside</td>
<td>+</td>
</tr>
<tr>
<td>D-Ribose</td>
<td>-</td>
</tr>
<tr>
<td>Flucose-1-phosphate</td>
<td>-</td>
</tr>
<tr>
<td>D-Tagatose</td>
<td>-</td>
</tr>
</tbody>
</table>

1) Positive  
2) Negative

catalase, and hemolysis tests and negative for fermentation of xylitol, D-melibiose, D-raffinose, D-xylose, and methyl-α-D-glucopyranoside (Table 6). L. monocytogenes isolates hydrolyzed esculin on MOX agar and were positive for motility, α-hemolysis, and CAMP tests. The isolates were identified with 98.6% accuracy on the basis of the results of the fermentation of sugars detected by using the API kit (Table 7). The pathogens were finally confirmed by 16S rRNA gene sequencing and polymerase chain reaction (data not shown).

According to the Korea Livestock Products Sanitary Control Act, E. coli O157:H7 should not be detected in marinated meat. However, there are no specific standards for detecting food-borne pathogens in marinated meat. Therefore, microbial risk assessment should be strictly regulated and performed by evaluating the data scientifically.

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(Received 2011.12.10/Revised 2012.7.16/Accepted 2012.8.14)