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RESEARCH NOTE

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Distribution of Indicator Organisms and Incidence of Pathogenic Bacteria in Raw Beef Used for Korean Beef Jerky

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Abstract The objective of this study was to evaluate the microbial safety of raw beef used to produce Korean beef jerky. The raw beef samples harbored large populations of microorganisms. In particular, psychrophilic bacteria were found to be most numerous (9.2×10^3-1.0×10^5 CFU/g) in the samples. Mesophilic bacteria and anaerobic bacteria were present in average numbers (10^3-10^5 CFU/g). Spore-forming bacteria and coliforms were not detected below detection limit. Yeast and molds were detected at 2.2×10^1-7.8×10^2 CFU/g in the raw beef. Ten samples of raw beef were analyzed for the presence of pathogenic bacteria. *Bacillus cereus* was isolated from sample B, G, and H. The *B. cereus* isolates from raw beef samples were identified with 99.8% agreement according to the API CHB 50 kit.

Keywords: Korean beef jerky, indicator organism, pathogenic bacteria, microbiological safety

Introduction

The incidence of food-borne illness continues to be a significant public health concern worldwide. In the United States, estimates of the number of illnesses each year from microorganisms in food range from 6.5 to 33 million people, while deaths could be as high as 9,000 (1).

Jerky is a food known to have been produced at least since ancient Egypt. Humans have traditionally made jerky from animal meat that was too large to be consumed all at once such as bear, buffalo, or whale. North American Indians mixed ground dried meat with dried fruit or suet to make 'pemmican'. 'biltong' is dried meat or game used in many African countries. This product is a nutrient-dense meat that has been made lightweight by drying. A pound of meat or poultry weights about 4 oz after being made into jerky. Because most of the moisture is removed, it is shelf stable – able to be stored without refrigeration – making it a handy food for backpackers and others who don't have access to refrigeration (2).

Jerky meats are generally processed by subjecting the final package to high heat treatment, and stored at refrigeration temperatures to achieve adequate shelf life. These products offer safety and excellent quality to the consumer. However, though mild heat treatment kills vegetative forms of microorganisms, it is inadequate to kill bacterial spores. Among the spore-forming bacteria, members of *Bacillus* spp. are widely distributed in the environment and in raw and processed food products such as rice, milk and dairy products, spices, vegetables, meat products, farinaceous foods (3), and cooked or chilled vegetables (4,5). Some strains of *Bacillus cereus* are able to grow at 5 or 7°C (6,7). This organism is ubiquitous in the environment and is found in the intestinal tracts of animals and humans (8,9).

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The objective of this work was to evaluate the microbiological safety of raw beef used to produce beef jerky.

Materials and Methods

Sample preparation Raw beef round was purchased from a supermarket and meat shop in Seoul, Korea. Ten shops were selected at random for market groupings. The raw beef was transported to the laboratory at low temperature (<7°C), stored at 4°C, and analyzed within 24 hr.

Enumeration of indicator organisms To measure the microbial quality of the stored product, 10 g samples of meat from duplicate packs of each sample were aseptically transferred into a sterile stomacher bag with 90 mL of sterile 0.1% peptone water (Difco Laboratories, Detroit, MI, USA) and macerated for 2 min. Ten-fold serial dilutions in 0.1% peptone water were then prepared. Mesophilic microorganism counts were determined on plate count agar (PCA, Difco) at 35°C for 48 hr. Psychrotrophic microorganisms were incubated at 21°C for 72 hr using PCA. Anaerobic microorganism counts were determined by spread-plating on PCA and incubating in a BBL anaerobic jar (Difco) at 35°C for 48 hr. Spore-forming bacteria were treated at 80°C for 30 min, spread on PCA plates and incubated at 35°C for 48 hr. Yeast and molds were counted after incubation at 25°C for 5 to 7 days on potato dextrose agar (PDA, Difco) adjusted to pH 3.5 with tartaric acid. Coliform counts were determined on Violet Red Vile agar with MUG (Difco) at 35°C for 48 hr. Microbial colonies were counted and expressed as colony forming units (CFU)/g of beef.

Isolation and identification of pathogenic microorganisms Beef samples were screened for the presence of 7 pathogenic bacteria by culturing in differential media. Each sample (25 g) was weighed and aseptically transferred into a sterile stomacher bag containing 225 mL of 0.1% sterile

peptone water and macerated for 2 min. Samples were plated onto appropriate isolation medium and incubated as described in the previous section. Serial dilutions were performed if required. For *B. cereus*, the samples was prepared by heating to 80°C for 30 min, cooling immediately in cold water, spreading on Cereus selective agar (Merck Laboratories, Damstadt, Germany), and incubating for 24 hr at 30°C. The pink colonies on the Cereus selective agar were isolated and grown on tryptic soy agar (TSA) for 24 hr at 30°C. *Clostridium* numbers were determined using STP agar (Oxoid, Basingstoke, UK) incubated in a BBL anaerobic jar at 35°C for 24 hr. *Staphylococcus aureus*, after incubation at 36°C for 24 hr on TSB agar (Difco) with 10% NaCl, was plated on mannitol salt agar (Difco) with egg yolk enrichment and Baird-Parker agar (Difco) with EY Tellurite enrichment, and incubated at 36°C for 24 to 48 hr. *Clostridium botulinum, Listeria monocytogenes, Shigella* spp., and *Yersinia enterocolitica* counts were determined as described in the FDA's Bacteriological Analytical Manual (10).

Results and Discussion

Enumeration of indicator organisms The frequencies at which the indicator organisms were observed in each sample of raw beef used for Korean jerky are shown in Table 1. The raw beef samples harbored large populations of microorganisms. Psychrophilic bacteria were found to be more numerous (9.2×10^3-1.0×10^5 CFU/g) than the other strains in raw beef. Mesophillic bacteria and anaerobic bacteria were present in average numbers (10^3-10^5 CFU/g). The findings of this study are in agreement with similar studies in that the total number of viable organisms in raw beef were approximately 10^3-10^4/g. Spore-forming bacteria and coliforms were not detected in these samples. Yeast and molds were detected at 2.2×10^1-7.8×10^2 CFU/g in the raw beef samples.

As previously reported, the shelf-life of beef depends on the initial cell number, storage time and temperature, and packaging (11). The total cell number at putrefaction have been reported to be 10^7/cm^2 (12), 10^8/cm^2 (13,14), and 10^8-10^9/g (15). The total bacterial population identified in this study is in agreement with other studies (16-21).

Isolation and identification of pathogenic microorganisms The incidence of various pathogenic bacteria in 10 raw beef samples is shown in Table 2. *B. cereus* was isolated from samples B, G, and H. These raw beef samples have a good track record in terms of food safety. However, food-borne pathogens may be present, and several outbreaks of food-borne disease have been traced to raw beef. The pathogens most frequently associated with raw beef are *Escherichia coli* O157:H7, *B. cereus, C. botulinum, Clostridium perfringens, Slamonella* spp., *Shigella* spp., *L. monocytogenes, St. aureus,* and *Y. enterocolitica*. The presence of certain microorganisms can be used as an

Table 1. Distribution of microbial groups in raw beef (unit: CFU/g)

Sample	Mesophilic bacteria	Psychrotrophic bacteria	Anaerobic bacteria	Spore-forming bacteria	Yeast & molds	Coliforms
A	4.0×10^3	5.5×10^4	6.1×10^3	ND[1)]	1.9×10^2	ND
B	3.4×10^4	3.1×10^4	2.0×10^4	ND	2.4×10^1	ND
C	3.8×10^3	9.2×10^3	4.2×10^3	ND	5.0×10^1	ND
D	3.1×10^4	2.2×10^4	1.1×10^4	ND	1.7×10^2	ND
E	8.6×10^4	3.6×10^4	6.1×10^4	ND	2.2×10^1	ND
F	4.1×10^4	1.7×10^4	2.4×10^4	ND	2.8×10^2	ND
G	6.9×10^4	9.4×10^3	5.1×10^4	ND	1.4×10^2	ND
H	1.4×10^5	1.1×10^4	1.4×10^4	ND	1.2×10^2	ND
I	7.7×10^4	1.0×10^5	7.1×10^4	ND	3.8×10^2	ND
J	6.4×10^3	5.3×10^4	6.8×10^3	ND	7.8×10^2	ND

[1)]Not detected.

Table 2. Incidence of pathogenic bacteria presented in raw beef

Pathogenic bacteria	Sample[1)]									
	A	B	C	D	E	F	G	H	I	J
Escherichia coli O157:H7	-	-	-	-	-	-	-	-	-	-
Bacillus cereus	-	+	-	-	-	-	+	+	-	-
Clostridium botulinum	-	-	-	-	-	-	-	-	-	-
Clostridium perfringens	-	-	-	-	-	-	-	-	-	-
Slamonella spp.	-	-	-	-	-	-	-	-	-	-
Shigella spp.	-	-	-	-	-	-	-	-	-	-
Listeria monocytogenes	-	-	-	-	-	-	-	-	-	-
Staphylococcus aureus	-	-	-	-	-	-	-	-	-	-
Yersinia enterocolitica	-	-	-	-	-	-	-	-	-	-

[1)]-, Not detected; +, detected at less than 10.

Table 3. Tentative identification of the 3 isolates from raw beef using Cereus selective agar

Characteristics	Result[1)]	Characteristics	Result
Gram-stain	+	Cellobiose	+
Shape	Rod	Maltose	+
Spore formation	+	Lactose	-
Cell diameter>1.0 μm	+	Melibiose	-
Sporangium swollen	-	Saccharose	-
Spore shape	Ellipsoidal	Trehalose	-
Spore position	Central	Inulin	+
Catalase	+	Melezitose	-
Anaerobic growth	+	Raffinose	-
Egg-yolk lecithinase	+	Starch	+
Glycerol	+	Glycogen	+
Erythritol	-	Xylose	-
D-Arabionse	-	Gentiobiose	-
L-Arabionse		D-Turanose	-
Ribose	+	D-Lyxose	-
D-Xylose	-	D-Tagatose	-
L-Xylose	-	D-Fucose	-
Adonitol	-	L-Fucose	-
β-Methyl-D-xyloside	-	D-Arabitol	-
Galactose	-	L-Arabitol	-
D-Glucose	+	Gluconate	-
D-Fructose	+	2-Keto gluconate	-
D-Mannose	+	5-Keto gluconate	-
L-Sorbose	-	Ortho-nitro-phenyl-galactoside	-
Rhamnose	-		
Dulcitol	-	Arginine	+
Inositol	-	Lysine	-
Mannitol	-	Ornithine	-
Sorbitol	-	Simmon's sitrate	-
α-Methyl-D-mannoside	-	Hydrogen sulfate	-
α-Methyl-D-glucoside	-	Urea	-
N-Acetyl glucosamine	+	Tryptophane	-
Amygdalin	+	Indole	-
Arbumin	+	Voges-Proskauer	+
Esculin	+	NO_2 production	+
Salicin	+		

[1)]+, Positive; -, negative.

indicator of food safety or to detect incorrect processing.

B. cereus is the dominant aerobic bacterium in cooked, pasteurized, and chilled products, because of the probable survival of its spores during the pasteurization step after packing. *Bacillus* spp. have also been detected in other cooked chilled foods (4,22). Many *Bacillus* spp. are able to grow in anaerobic conditions, thus the oxygen depletion created by packing under vacuum does not prevent their growth (4). *B. cereus* produces diverse extracellular toxins and enzymes, such as lecithinase, proteases, β-lactamase, sphinogomyelinase, cerolysin, and hemolysin BL. There are 2 (emetic and diarrhea) types of *B. cereus* food poisoning (23). *B. cereus* intake of 10^7-10^8 cells/g food is needed to induce diarrhea and 10^6-10^7 cells/g to induce emesis (24).

Bacillus isolates (HJ 801, 802, and 803) that can cause spoilage and are used as indicators of food safety, were isolated from raw beef and identified by morphological and biochemical methods as well as with the API identification kit.

The isolates obtained from Cereus selective agar were Gram-positive, rod shaped, anaerobic, and formed endospores. *Bacillus* isolates put out a same physiological quality indolently. Biochemical and cultural tests on this isolate reveal it to be catalase-positive, to not grow on Simmon's citrate, to produce NO_2, and to require arginine for growth; the ATB automated identification system resulted in 99.8% agreement for the identification of *B. cereus* at the species level (Table 3). Thus, 3 isolates were tentatively identified as *B. cereus* which could be used for inoculated pack studies to confirm microbiological safety.

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