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ARTICLE

Antimicrobial Effect of Nisin against *Bacillus cereus* in Beef Jerky during Storage

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Abstract

The microbial distribution of raw materials and beef jerky, and the effect of nisin on the growth of *Bacillus cereus* inoculated in beef jerky during storage, were studied. Five strains of pathogenic *B. cereus* were detected in beef jerky, and identified with 99.8% agreement using API CHB 50 kit. To evaluate the effect of nisin, beef jerky was inoculated with approximately 3 Log CFU/g of *B. cereus* mixed culture and nisin (100 IU/g and 500 IU/g). During the storage of beef jerky without nisin, the number of mesophilic bacteria and *B. cereus* increased unlikely for beef jerky with nisin. *B. cereus* started to grow after 3 d in 100 IU nisin/g treatment, and after 21 d in 500 IU nisin/g treatment. The results suggest that nisin could be an effective approach to extend the shelf-life, and improve the microbial safety of beef jerky, during storage.

Key words: beef jerky, pathogenic bacteria, *Bacillus cereus*, nisin, microbial safety

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Introduction

From 2000 to 2008, the social cost of food-borne illness in the USA amounted to $9.4 million annually. *Bacillus cereus*, *Clostridium perfringens*, or *Staphylococcus aureus* was charged $1.3 million (Scallan *et al*., 2011). In these pathogens, *B. cereus* is a common food contaminant, and is an etiological agent of two distinct forms of illness, i.e., emetic and diarrheal. *B. cereus* is found in meats, milk, vegetables, and some *B. cereus* are able to grow at 5 or 7°C, acid condition, and heating by sporulation (Dufrenne *et al*., 1994; Simpson *et al*., 1994; van Netten *et al*., 1990). *B. cereus* is not dangerous in low level (< 10^6 CFU/g), however *B. cereus* can multiply to dangerous levels in subsequent time and temperature. The counts of *B. cereus* were reported to be 2.9-4.59 Log CFU/g in meat products and *B. cereus* grow well after cooking (Tewari *et al.*, 2015). Therefore, *B. cereus* in food must be controlled by heat treatment, radiation, and antimicrobials.

Jerky is processed almost everywhere in the world. It is microbiologically safe, easy to prepare, light-weight, has a rich nutrient content, and can be stored without refrigeration (Kim *et al*., 2008b). However, some stressed pathogens included spore-forming bacteria may exhibit lower infectious doses, foodborne disease outbreaks related to jerky products have actually increased (Edison *et al*., 2000; Keene *et al*., 1997). Jerky has been studied for food additives, heating, and irradiation against *Staphylococcus aureus*, *Listeria monocytogenes*, *Bacillus cereus*, *Salmonella* Typhimurium, *Escherichia coli*, etc. for microbial safety, without addressing the quality of jerky during storage (Kim *et al*., 2010).

Nisin is the most commercial bacteriocin produced by *Lactococcus lactis* subsp. *lactis*, which exhibits antimicrobial activity against a wide range of Gram-positive vegetative cells and spores. Nisin have been used for just processed cheese in Korea (Ministry of Food and Drug Safety). Bacteriocin has already been used in more than 50 countries in the food industry as an antagonistic additive (Ray, 1992). In addition, nisin has been permitted in processed meat include limits of 12.5 mg/kg in USA (Food

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and Drug Association), and has mainly been applied to dairy and meat products as a target of Gram positive pathogen (mainly *Listeria monocytogenes*) (Balciunas *et al*., 2013). Meanwhile, *B. cereus* has been investigated in beef gravy, fruit beverage, and cooked chilled foods (Assous *et al*., 2012; Beuchat *et al*., 1997; Choma *et al*., 2000).

There are limited data in the literature describing microbial distribution, particularly pathogens in jerky. However, the hurdle of *L. monocytogenes*, *Salmonella* Typhimurium, and *Salmonella enterica* was studied in jerky for its safety (Boles *et al*., 2007; Calicioglu *et al*., 2003; Yoon *et al*., 2009). Therefore, the purposes of this study were to determine microbial contamination status of the raw materials used for beef jerky, and beef jerky itself, and the antimicrobial effect of nisin on the growth of *B. cereus* inoculated in beef jerky during storage.

Materials and Methods

Preparation of beef jerky

Beef was purchased from a local market for the manufacture of beef jerky. The meat was tempered at 4°C for 24 h and sliced 6 mm thick. The composition of jerky spices was water (10%), soy sauce (9%), starch syrup (5%), sugar (2%), D-sorbitol (6%), pepper (0.5%), ginger powder (0.1%), garlic powder (0.2%), onion powder (0.2%), sodium nitrate (0.007%), sodium citrate (0.01%), potassium sorbate (0.1%), sodium erythorbate (0.036%), and soup stock powder (0.1%). Treated raw meats using jerky spices were phase-dried in a dehydrator at 50°C for 60 min, 60°C for 60 min, and 70°C for 90 min. After drying, the jerky strips were held in the dehydrator overnight, to allow the moisture level in the jerky slices to equilibrate, and then placed into sterile plastic bags.

Microbiological analysis

Each sample (25 g) was taken aseptically using a sterile stomacher bag containing 225 mL of 0.1% sterile peptone water, and macerated for 2 min. Decimal serial dilution in 0.1% peptone water was prepared. The number of mesophilic bacteria counts were determined using plate count agar (PCA, Difco Laboratories, USA), at 37°C for 48 h. *B. cereus* numbers were determined using cereus selective agar (Merck, Germany), at 30°C for 24 h. Microbial colonies were counted, and expressed as log colony forming units per gram (Log CFU/g). Pathogenic microorganisms of each sample were isolated, and identified as described in Table 1.

Preparation of *B. cereus* strains and addition to beef jerky

B. cereus strains isolated in raw meat, spices, and spiced meat were used for hurdle technology. *B. cereus* was grown on PCA (Difco) overnight at 30°C, and then left at ambient temperature for one week, to sporulate. When spores were detected microscopically, spore suspensions were created in sterile 0.1% peptone water, and heat treated (80°C for 10 min) to kill vegetative cells. Spores were enumerated by viable counts, and the suspensions were adjusted to 10^6 spore/mL. Mixed inocula were prepared, by combining spore suspensions in equal concentrations. Spores were inoculated to the beef jerky, to give a predicted level of 10^3 CFU/g.

Preparation of nisin and addition to beef jerky

Nisin (Sigma-Aldrich, USA) was used as a form of stock solution. A standard stock solution of nisin containing 1×10^5 IU/mL was prepared, by dissolving 100 mg of nisin in 0.02 M HCl (1 mL), and adding 9 mL of distilled water.

Table 1. Conditions for the isolation, growth, and identification of pathogenic bacteria in raw meats

Pathogenic bacteria	Isolation culture condition	Growth culture condition	Identification
Escherichia coli O157:H7	Sorbitol MacConkey agar, 35°C, 24 h	Modified EC medium, 35°C, 24 h	Gram stain, API 32E, serotypes
Bacillus cereus	Cereus selective agar, 30°C, 24 h	Tryptic soy agar, 30°C, 24 h	Gram stain, API CHB 50
Clostridium perfringens	Clostridium perfringens agar, 35°C, 24 h	Cook Meat medium, 35°C, 24 h	Gram stain, API 20A
Salmonella spp.	Hektoen enteric agar, 35°C, 24 h	Selentie F broth, 35°C, 24 h	Gram stain, Triple sugar iron agar (TSI), MIL, API 32E
Listeria monocytogenes	Oxford agar, 30°C, 48 h	Listeria enrichment broth, 30°C, 24 h	CAMP test, hemolysis, API Listeria, serotypes
Staphylococcus aureus	Mannitol salt agar with egg yolk, 35°C, 48 h	Tryptic soy broth with 10% sodium chloride, 35°C, 24 h	Gram stain, catalase, coagulase, API staph
Yersinia enterocolitica	Yersinia selective agar with cefsulodin, irgasan, novobiocin, 35°C, 24 h	Peptone sorbitol bile broth, 10°C, 10 days	Gram stain, urea, citrate, motility test, API 32E

Nisin was added at concentrations of 100 IU/g and 500 IU/g, respectively to the beef jerky.

Package and storage of beef jerky

A coextruded, multilayered film (C5045, nylon/PE/nylon/PE/nylon/LLDPE, Cryovac Division, Sealed Air Corporation, USA) was used for packaging and the pouches were heat-sealed under vacuum. Beef jerky samples were then stored at room temperature (25°C) for 60 d, and samples were taken at regular intervals throughout the storage period for quality measurements.

Results and Discussion

The pathogens most frequently associated with raw meats are *E. coli* O157:H7, *B. cereus, Salmonella* spp., *L. monocytogenes*, and *S. aureus* (Edison *et al*., 2000; Kim *et al*., 2008b). For the determination of microbial contamination, the incidences of pathogenic bacteria in raw meat, spices, spiced meats, and jerky products are summarized in Table 2. Five strains of *B. cereus* were isolated from raw meat, spices, and spiced meat, while no pathogens were detected in the final products. In addition, no other pathogens were detected. These results may be a drying process using dehydrator.

Five isolated strains using cereus selective agar were Gram positive, rod shaped, spore forming bacteria, and catalase-positive. These strains did not grow on Simmon's citrate, produced NO_2, and need to take arginine for growth. Therefore, these isolates were identified as *B. cereus* by ATB automated identification system, with 99.8% identity.

The antimicrobial effect of nisin against mesophilic bacteria in beef jerky during storage is shown in Fig. 1(a). The number of mesophilic bacteria in control samples (not inoculated with *B. cereus* and without nisin) steadily increased, and reached more than 3.5 Log CFU/g after 28 d. In samples inoculated with *B. cereus*, the number of mesophilic bacteria was 3.2 Log CFU/g after 7 d of storage, and remained around 3 Log CFU/g, during 60 d storage. Detection time of mesophilic bacteria was delayed by added nisin. The number of mesophilic bacteria in beef jerky samples with 100 IU nisin/g meat was detected at 2 d, and reached more than 2.5 Log CFU/g after 28 d. With 500 IU nisin/g, the number of mesophilic bacteria was detected at 28 d, and remained below 2.5 Log CFU/g during 60 d storage. Some stressed microorganisms including spore-forming bacteria can survive the pasteurization process and cause outbreak at storage temperature (Carlin *et al*., 2000; Paik *et al*., 2006).

Table 2. Isolation of pathogenic bacteria presented in raw meat, spices, spiced meat, and jerky

Pathogenic bacteria	Raw meat	Spices	Spiced meat	Jerky
Escherichia coli O157:H7	−	−	−	−
Bacillus cereus	+	+	+	−
Clostridium botulinum	−	−	−	−
Clostridium perfringens	−	−	−	−
Salmonella spp.	−	−	−	−
Shigella spp.	−	−	−	−
Listeria monocytogenes	−	−	−	−
Staphylococcus aureus	−	−	−	−
Yersinia enterocolitica	−	−	−	−

−, negative; +, positive.

B. cereus can be detected in jerky, because of the strong survival of spores in the processing. Therefore, the antimicrobial effect of nisin was investigated against *B. cereus* during storage (Fig. 1(b)). The number of *B. cereus* in beef jerky without nisin was 2.4 Log CFU/g at 0 d, and remained around 3 Log CFU/g during storage. The number of *B. cereus* with 100 IU nisin/g meat was not detected at 2 d, but was detected at 4 d. These values were around 3 Log CFU/g during storage. With 500 IU nisin/g, *B. cereus* was detected at 28 d, and remained around 2.2 Log CFU/g during storage.

The inactivation of nisin is known by the presence of proteolytic enzymes produced by *B. cereus* (Beuchat *et al*., 1997). In control samples (without inoculated *B. cereus* and nisin), no major changes in *B. cereus* growth during storage were observed. The remaining of the number of *B. cereus* in beef jerky without nisin may be depend on low water activity of jerky. Nisin was reported as biopreservative against *B. cereus* in cook-chill foods of soybean sprout, cooked rice, and milk (Kim *et al*., 2008a; Penna *et al*., 2002; Vessoni *et al*., 2002). In addition, nisin was applied as incorporating film against *B. cereus* (Alrabadi, 2012).

The increase in the numbers of microorganisms depends on the initial numbers of microorganisms and the storage temperature (Carlin *et al*., 2000; Paik *et al*., 2006). Nisin reduced initial numbers of microorganism and delayed the occurrence as inoculum in this study, and these results indicate that by supplementing it to the beef jerky, high risk of illness can be avoided.

In conclusion, the incidences of pathogenic bacteria in raw meat, spices, spiced meats, and jerky products were studied. Just five strains of *B. cereus* were isolated from raw meat, spices, and spiced meat, while no pathogens were detected in the final products. The effect of nisin on

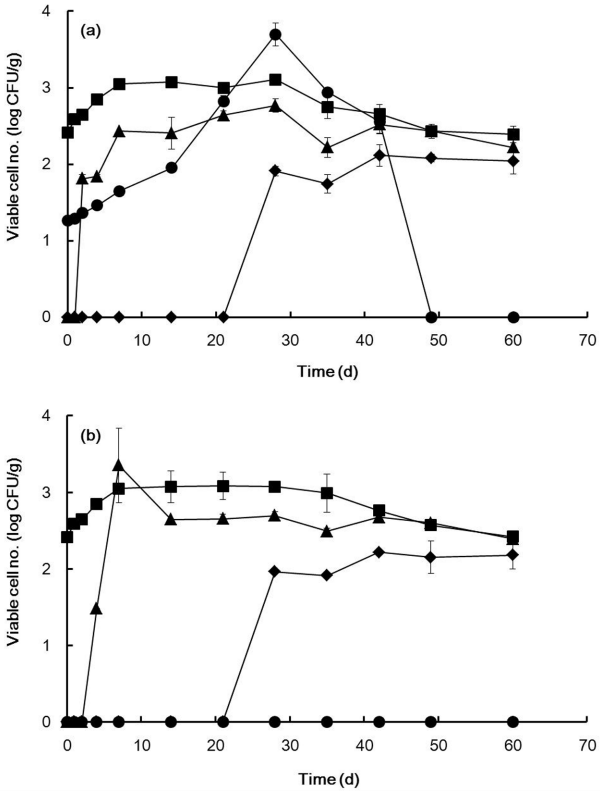

Fig. 1. The number of (a) mesophilic bacteria, and (b) *Bacillus cereus*, in the absence or presence of nisin in beef jerky products during storage at 25°C. Control packages (●), packages inoculated with *Bacillus cereus* (■), packages inoculated with *Bacillus cereus* and nisin 100 IU (▲), and packages inoculated with *Bacillus cereus* and nisin 500 IU (◆).

the growth of *B. cereus* inoculated in beef jerky during storage was demonstrated. The addition of nisin can decrease the initial cell count of mesophilic bacteria and *B. cereus* in beef jerky. The results suggest that nisin could be an effective approach to extend the shelf life, and improve the microbial safety of beef jerky, during storage.

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