Food Control 87 (2018) 1–8

Contents lists available at ScienceDirect

Food Control

journal homepage: www.elsevier.com/locate/foodcont

Factors that determine the microbiological quality of ready-to-use salted napa cabbage (*Brassica pekinensis*): Season and distribution temperature

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ARTICLE INFO

Article history:
Received 14 September 2017
Received in revised form
6 November 2017
Accepted 4 December 2017
Available online 9 December 2017

Keywords:
Ready-to-use agricultural products
Salted napa cabbage
Microbiological quality

ABSTRACT

A large outbreak of *Escherichia coli* O157:H7 in Japan, caused by salt-pickled napa cabbage, highlighted potential hazards posed by this vegetable, which is a ready-to-use form of *kimchi* or some side dishes that does not undergo heat treatment. Here, microbiological quality of 500 commercial salted napa cabbages were examined (quantitative analysis: Aerobic Plate Count [APC], total coliforms [TC], *Bacillus cereus*, and *E. coli*; qualitative analysis: *E. coli* and seven foodborne pathogens). To identify major factors affecting microbiological quality, we examined the correlation between various production, distribution, and physicochemical factors and the results from quantitative analyses. The overall results revealed that the salinity of salted napa cabbage (average, 3.7%) did not guarantee microbiological quality. Although no pathogenic foodborne bacteria were isolated from the samples, the TC count (an indicator of overall hygiene) reached a maximum of 6.8 log CFU/g. APCs and TC counts were highest in the summer (average, 7.1 and 4.4 log CFU/g, respectively), suggesting that temperature is a significant factor. Indeed, distribution temperature was a major factor (correlation coefficient [r] = 0.7, $P < .05$) for increased bacteria counts; other factors (i.e., salinity, pH, etc.) did not show a strong correlation with bacterial counts. These results highlight the potential hazard of bacterial growth during distribution. Thus, manufacturers should ensure that both the product and the distribution conditions are suitable: salted napa cabbages should be maintained at < 10 °C. Few studies have examined the microbiological quality of salted napa cabbages; therefore, the present study may be useful as a quantitative risk assessment and should help to improve/establish safety regulations.

1. Introduction

Kimchi is a traditional Korean side dish comprising fermented drained vegetables (e.g., Chinese cabbage and radish) mixed with various ingredients (e.g., garlic, ginger, radish, powdered red pepper, and fermented fish sauce) (N. H. Kim, Jang, Kim, Lee, Kim, & Ryu, 2015; Lee, Park, Jung, & Jeon, 2012). It is gaining popularity worldwide due to health-promoting characteristics, which are endowed by its reported anti-cancer (Choi et al., 2013; Kim, Song, Ju, Kang, & Park, 2015), anti-diabetes (Islam & Choi, 2009), anti-obesity (Kim et al., 2011; Sheo, 2004), and anti-oxidative (Hwang, Song, & Cheigh, 2000) effects.

The main ingredient of *kimchi* is a type of napa cabbage (*Brassica rapa* sub sp. *pekinensis*), called *baechu* in Korea, *dà báicài* in China, and *hakusai* in Japan (Codex Alimentarius Commission, 2001). Napa cabbage is salted and supplied as a ready-to-use ingredient for *kimchi* and some side dishes in the Asia-Pacific region (*suan cai* in China and *tsukemono* in Japan) (Solomon, 2014). Preparing salted napa cabbage is both time and labor intensive as it involves the trimming, salting, and washing of a heavy vegetable. Consumption of salted napa cabbage products has increased steadily along with a rising consumer demand for making *kimchi* at home. These days, salted napa cabbage is manufactured and sold not only by large manufacturing plants but also by small farms (N. H. Kim et al., 2015).

The raw material for napa cabbage is a leafy vegetable that

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https://doi.org/10.1016/j.foodcont.2017.12.009
0956-7135/

commonly contains endogenous groups of bacteria. Leafy vegetables grow in soil, which is a source of various microorganisms, and the leaf surface provides a very favorable environment for bacterial attachment and/or aggregation (Berger et al., 2010; Beuchat, 2002). As napa cabbage comprises layer upon layer of leaves, bacterial contamination of the inner leaves or seeds may lead to a deposit of bacteria within the plants. Failure to eliminate harmful bacteria from the raw napa cabbage, therefore, can result in bacterial contamination that potentially persists during production of salted napa cabbage.

The manufacture of salted napa cabbage generally comprises six phases: 1) trimming and cutting, 2) salting, 3) washing, 4) dehydrating (eliminating 2-5% of water content), 5) packaging, and 6) storage or marketing (Fig. S1) (N. H. Kim et al., 2015). There is no heat treatment and only the washing step can potentially eliminate some contaminating microorganisms from the product. Therefore, use of poor quality water or re-use of water is becoming a major concern for microbiological quality (Rhee & Park, 2015). Indeed, several large outbreaks of pathogenic *E. coli* in Korea and Japan have been attributed to *kimchi* or pickled napa cabbage. In Korea in 2012, 1642 people were infected by pathogenic *E. coli* in *kimchi* (Cho et al., 2014). Similarly, in Japan, outbreaks caused by pickled napa cabbage occurred in 2012, with 107 cases (seven of whom died) (Tabuchi et al., 2015). In the latter case, the manufacturing company used inappropriate techniques to rinse and sanitize the vegetables (Tabuchi et al., 2015).

Although consumption of salted napa cabbage is increasing and bacterial contamination is a potential hazard, few studies have examined the microbiological quality of salted napa cabbage distributed in the market. As most consumers tend not to wash commercial salted napa cabbage at home (Rhee & Park, 2015), collecting microbiological data is important if we are to ensure its hygienic status. Thus, the aim of this study was to examine factors linked to production/distribution and their correlation with the microbiological/physicochemical properties of the commercial product itself, and to identify factors that may compromise the microbiological quality of the product.

2. Materials and methods

2.1. Sample collection

A total of 500 commercial salted napa cabbages were collected from retail markets in South Korea (seven cities and eight provinces) from February to November 2015. Samples were collected in winter (February, n = 40), spring (March to May, n = 196), summer (June to August, n = 184), and autumn (September to November, n = 80) and transported to the laboratory via the delivery service provided by each market within 12 h of manufacture. Products were then subjected to immediate physicochemical and microbiological analyses.

2.2. Status of collected salted napa cabbages

The baseline characteristics of the collected products are presented in Fig. S2. The manufacturer, storage of napa cabbage, and the classification of the final product were surveyed by telephone, fax, and the internet. Characteristics associated with distribution including packaging type (styrofoam or paper box), the number of ice packs enclosed, the core temperature of the products, the temperature inside the packaging, and the atmospheric temperature were all checked immediately after receiving the samples. Temperature was measured using a probe thermometer (Pocket digital thermometer A1. T9233C, DAIHAN Scientific, Seoul, Korea). For more details, see Fig. S3.

2.3. Physicochemical analysis

Physicochemical properties were analyzed as previously described, with some modifications (Heu, Kim, & Shahidi, 2003). Briefly, salted napa cabbage samples were cut and shredded using a flame-sterilized knife and transferred to a blender for homogenization. Samples for physicochemical analysis were prepared by adding 10 g of each blended sample to 10 vol of deionized water, followed by homogenization at 230 rpm for 2 min. The pH was measured using a pH meter (MP 200, Mettler-Toledo, Columbus, OH, USA). Salinity and Brix were measured using a salt meter (ES-421, Atago Co. Ltd., Tokyo, Japan) and a Brix electronic refractometer (PAL-1, Atago Co. Ltd.), respectively.

2.4. Microbial counts in salted napa cabbages

All microbiological analyses were performed according to the US Food and Drug Administration Bacteriological Analytical Manual (USFDA., 2011) and the Korea Food Code (Ministry of Food and Drug Safety, 2011), with the modifications described previously (Choi et al., 2014; Jeon et al., 2015).

Briefly, 25 g of each sample was cut into small pieces and aseptically placed into stomacher bags (Circulator 400 standard bags, Seward, Worthing, UK) containing 225 ml of sterile 0.85% saline. Samples were homogenized using a stomacher at 230 rpm for 2 min (Circulator 400, Seward). Homogenized samples were then serially diluted (10-fold) in 9 ml of 0.85% sterile saline, and 100 μl of diluent was spread-plated onto selective agar plates (in duplicate). Also, 1 ml of sample was spread-plated onto five plates to reduce the detection limit to 10 CFU/g. The selective agar plates and relevant incubation conditions were as follows: Plate Count Agar (PCA, Difco, Becton Dickinson, Sparks, MD, USA) for Aerobic Plate Counts ([APCs], 35 °C, 48 h); Violet Red Bile Agar (VRBA, Difco) for total coliforms ([TC], 37 °C, 24 h); Tryptone Bile X-glucuronide Agar (TBX, Difco) for *E. coli*; and Mannitol Egg Yolk Polymyxin agar (MYP, Difco) supplemented with 50% egg yolk and antimicrobial vial P (Difco) for *Bacillus cereus* (30 °C, 24 h). In the latter case, typical colonies were selected and identified by VITEK 2 (bioMerieux, Marcy-L'Etoile, France) with a BCL card (bioMerieux). The identified colonies were calculated by multiplication of the ratio of counts.

2.5. Detection of Escherichia coli and foodborne pathogens

To examine the pathogenic microorganisms in salted napa cabbages, 25 g of each sample was transferred to a stomacher bag and mixed with 225 ml of appropriate buffer solution or medium. The mixture was then homogenized for 2 min at 230 rpm. The following target microorganisms were enriched in the appropriate buffer solution or medium: *B. cereus* (Tryptic soy polymyxin broth [Difco] at 30 °C for 24 h); *Escherichia coli* and pathogenic *E. coli* (modified EC broth [Difco] at 37 °C for 24 h); *Listeria monocytogenes* (UVM-modified Listeria enrichment broth [Difco] at 30 °C for 24 h); *Salmonella* spp. (buffered peptone water [Difco] at 37 °C for 18 h); *Staphylococcus aureus* (Brain Heart Infusion broth [Difco] at 35 °C for 24 h); and *Vibrio parahaemolyticus* (in alkaline peptone water [Difco] at 36 °C for 24 h).

To enrich *Clostridium perfringens*, 1 ml of sample (diluted 10-fold in sterile saline) was added to cooked meat broth (10 ml) and incubated under anaerobic conditions at 30 °C for 24 h in an anaerobic pack (Oxoid, Basingstoke, Hampshire, UK). Enriched UVM-modified Listeria enrichment broth (0.1 ml) was inoculated into 10 ml of Fraser Listeria broth (Difco) supplemented with Fraser Listeria broth supplement and incubated at 30 °C for 24 h. Pre-enriched buffered peptone water (0.1 ml) was transferred to