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Risk Assessment of *Staphylococcus aureus* and *Clostridium perfringens* in cheese

Introduction

In Korea, the amount of cheese consumption, as well as its importation, is steadily increasing. Previous reports have described the isolation of bacteria from cheese and food poisoning that has been associated with cheese consumption in foreign countries. However, there are no incidents of food poisoning that are related to cheese consumption in Korea. Furthermore, despite the extremely low possibility of growth of bacteria that cause food poisoning in cheese, due to the fermentation and refrigeration, Korean authorities apply zero-tolerance standards regarding *Staphylococcus aureus* and *Clostridium perfringens* contamination in cheese. As the current standards have insufficient scientific basis, it is necessary to assess the risks of these bacteria in cheese products through microbial risk assessment and to utilize these results in the establishment of new standards.

Statement of purpose

The present study aimed to conduct a microbial risk assessment for *S. aureus* and *C. perfringens* in natural and processed cheese, and utilize the results as fundamental data in the establishment of standards for the management of these bacteria.

Hazard identification

S. aureus

S. aureus is non-motile, gram-positive (0.5–1.5 µm), facultative anaerobic and asporogenous coccus that exists on the skin, nasal mucosa, and other soft tissues of most animals and human beings. It usually grows well in aerobic conditions but can also survive in anaerobic conditions, and its salt tolerance enables it to rapidly proliferate in a medium containing 7.5% NaCl. It is resistant to dryness, heat (50℃, 30 min), and sodium (3%–7%). It rarely causes food poisoning via food materials, but is a main source of food poisoning through contaminated cooked food where it rapidly proliferates and generates enterotoxins. Staphylococcal enterotoxins (SEs) are toxins secreted by *S. aureus* that are heat-stable and water-soluble proteins, with a low molecular weight of 26–30 kDa and an isoelectric point of 5.7–8.6. Enterotoxins generated by *S. aureus* have extremely high heat resistance and are not completely destroyed even after heating at 120℃ for 20 min.

To reduce the risk of food poisoning, it is essential to block the generation of enterotoxins in advance. *S. aureus* exists in various foodstuffs such as dairy food, including milk, cream, and cheese; processed meat products, including ham and meats; and other protein foodstuffs, including fish meat products in the U.S. and Europe. In Korea, also, it is discovered from a wide variety of food items. Symptoms of food poisoning by *S. aureus* include nausea, diarrhea, and severe abdominal pain due to acute gastroenteritis. The incubation period is 2–6 h, and clinical symptoms differ according to the amount of enterotoxins that have entered the body and individual sensitivity. Its fatality rate is relatively low, and most patients recover within 24–28 h. The number of outbreaks and patients that developed food poisoning due to *S. aureus* in Korea from 2011 to 2015 has decreased. Nevertheless, it is continuing to be reported as a key material that causes food poisoning by contributing to an average of 9.2 outbreaks (an average of 161.4 patients per outbreak), along with enteropathogenic

Escherichia coli, Norovirus, and *Salmonella*.

C. perfringens

C. perfringens is an anaerobic, gram-positive, and spore-forming bacterium. It is extensively distributed in natural environments, including soil, streams and sewage, intestinal tracts, and feces of human beings and animals (major mammalian species), as well as food items. The spores of *C. perfringens* generally remain viable for an extended period of time. *C. perfringens* produces toxins that cause food poisoning, and in the process, nursing cells form spores. The toxins are divided into five types, namely A, B, C, D, and E, of which the A and C types are mainly involved in food poisoning in humans. The optimal temperature range for growth is 43℃–47℃, and it nearly stops proliferating at temperatures below 15℃ or above 52℃. The optimal temperature for the formation of spores and toxins is 37℃. After generating enterotoxins during the proliferation of *C. perfringens* and binding with specific receptors in the small intestine, morphological changes, diarrhea, and abdominal pain may develop. The incubation period is around 8–12 h, and patients usually recover after light symptoms of diarrhea and abdominal pain. The major symptoms disappear within 24 h, but the minor ones often remain for another one to two weeks. For treatment, sufficient intake of fluid and electrolytes is recommended, possibly via intravenous administration in severe cases. The administration of antibiotics is not encouraged. According to the food poisoning statistics of the Ministry of Food and Drug Safety (MFDS), the number of outbreaks and patients gradually increased from 2002 to 2014; the reported number of *C. perfringens* food poisoning outbreaks and patients was 28 and 1,689, respectively, in 2014 alone.

Exposure assessments

Exposure assessments of *S. aureus* and *C. perfringens* were conducted by developing a scenario that was divided into three stages, namely transportation stage, sale/purchase stage, and consumption stage.

Exposure assessment for *S. aureus* in cheese

No *S. aureus* was detected in 90 natural cheese samples and 308 processed cheese samples. According to the results of the estimation of the initial contamination levels using the Beta distribution and the Uniform distribution, the average contamination level of natural cheese samples was -2.4 log CFU/g and that of processed cheese samples was -2.7 log CFU/g. The growth model for *S. aureus* in natural and processed cheese samples was developed using the Baranyi model, exponential equation, and polynomial equation.

Changes in bacterial counts were predicted through the simulation of risk assessment. In the case of natural cheese samples, the bacterial count apparently increased during the transportation and sale/purchase stages, reaching -2.2 log CFU/g in the final consumption stage. For processed cheese samples, the bacterial count increased during the transportation and sale/purchase stages, reaching -2.3 log CFU/g in the consumption stage,. However, the final bacterial counts in neither natural cheese nor processed cheese appeared to pose major health risks. According to the Korean National Health and Nutritional Examination Survey, the average natural cheese consumption was 12.40 ± 19.43 g and the frequency of consumption per day was 3.89%. The average consumption of processed cheese was 19.46 ± 14.39 g and the frequency of consumption per day was 2.32%.

Exposure assessment for *C. perfringens* in cheese

No *C. perfringens* was detected in 90 natural cheese samples and 308 processed cheese samples. Estimation of the initial contamination levels using the Beta distribution and the uniform distribution indicated that the average contamination level of natural cheese was -2.4 log CFU/g and that for processed cheese was -2.7 log CFU/g. *C. perfringens* exists as spores in nature, and when the temperature and other conditions reach the level that allows their growth, they germinate to become vegetative cells and grow. In the present study, cheese, which is a medium where microbial proliferation rapidly occurs, was used to observe the growth of *C. perfringens* spores; however, no growth of spores was observed. Therefore, risk assessment for *C. perfringens* was conducted under the assumption that the initial contamination level immediately after the production would be the same as that at the time of final consumption, without using a growth model. According to the Korean National Health and Nutritional Examination Survey, the average intake amount of natural cheese is 12.40 ± 19.43 g and the frequency of consumption per day is 3.89%, whereas the average consumption of processed cheese is 19.46 ± 14.39 g and the frequency of consumption per day is 2.32%.

Hazard characterization

S. aureus

The minimum concentration of *S. aureus* for human health risk is 100 ng, and its cell concentration in forming enterotoxins at the minimum concentration, as stated in most relevant research papers, is 10^5–10^6 cfu/g. In the present study, the exponential model developed by Rose and Haas (1999) was used as the dose-response model for *S. aureus*.

C. perfringens

Park (2014) conducted a meta-analysis of the minimum infective dose of foodborne pathogens in an effort to examine the minimum infective dose needed for the study of quantitative microbiological risk assessment for major bacteria that cause food poisoning in Korea. The distribution of the minimum infective dose of *C. perfringens* was 10^2 spores/g, the maximum was 10^8 spores/g, the median was 10^7 spores/g, 10^2 spores/g was at the 5^{th} percentile, 10^6–10^8 spores/g was between the 25^{th} and 75^{th} percentiles, and 10^8 spores/g was at the 95^{th} percentile. Meta-analysis indicated that the minimum infective dose of *C. perfringens* was 10^7 spores/g, which was the level that showed the highest relative frequency. The study used the exponential model (Golden et al., 2009), which is the only dose-response model for *C. perfringens*.

Risk characterization

S. aureus in cheese

The amount of intake of *S. aureus* through the consumption of cheese was estimated based on the contamination level of *S. aureus* in the consumption stage, consumption amount of cheese, and frequency of consumption, and the final results were drawn through 10,000 iterations using @RISK.

The risk of food poisoning by *S. aureus* through the consumption of natural cheese was estimated using simulation, and the probability of occurrence of food poisoning by *S. aureus* when natural cheese was eaten in the present distribution environment and consumption level was predicted to be low, at an average of 7.84×10^{-10} per person. Risk estimation for food poisoning by *S. aureus* through the consumption of processed cheese in the current distribution environment and consumption level using simulation indicated that the probability of food poisoning by the bacterium was 2.24×10^{-9} per person, which is also considered relatively low.

C. perfringens in cheese

The intake of *C. perfringens* in cheese was estimated based on the contamination level of *C. perfringens* in the consumption stage, consumption amount of cheese, and frequency of consumption, and the final results were derived through 10,000 iterations using @RISK.

Risk estimation for food poisoning by *C. perfringens* through the consumption of natural cheese using simulation predicted that the probability of food poisoning by *C. perfringens* through the consumption of natural cheese in the current distribution environment and consumption level was 9.57×10^{-14} per person, which is extremely low. Risk estimation for food poisoning by *C. perfringens* through the consumption of processed cheese in the current distribution environment and consumption level using simulation showed that the probability of food poisoning by the bacterium was 3.58×10^{-14} per person, which is also considered low.

Impact of establishing microbial standards in the Korean Food Code

Contamination levels of *S. aureus* at the consumption stage were estimated under the extreme assumption that the initial contamination levels of the bacterium were 10, 100, and 1,000 cells (1, 2, and 3 log CFU/g) per 1 g of food. Based on the results, the contamination level in both natural and processed cheese at the consumption stage was 15,000 cells (4.2 log CFU/g, 99% confidence level) when the initial contamination level of *S. aureus* was 1,000 cells/g, showing that the level fell short of the minimum concentration for human health risk of 50,000 cells (5 log CFU/g).

The result of the contamination levels of *C. perfringens* at the consumption stage under the extreme assumption that its initial contamination levels were 10, 100, and 1,000 cells (1, 2, and 3 log CFU/g) per 1 g of food also confirmed that the contamination levels were much lower than the minimum concentration for human health risk (1,000,000 cells/g).

The standard of *S. aureus* in cheese was established at n = 5, c = 1, m = 10, and M = 100 in 2014 (Notification No. 2014-15, June 30, 2014) and those of *C. perfringens* in cheese was modified from n = 5, c = 0, and m = 0/25 g to n = 5, c = 2, m = 10, and M = 100 in 2015 (Notification No. 2015-55, August 25, 2015).

Key words: Cheese, Risk Assessment, *Clostridium perfringens*, *Staphylococcus aureus*, Pathogen