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Quantitative microbial risk assessment for *Staphylococcus aureus* in natural and processed cheese in Korea

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ABSTRACT

This study quantitatively assessed the microbial risk of *Staphylococcus aureus* in cheese in Korea. The quantitative microbial risk assessment was carried out for natural and processed cheese from factory to consumption. Hazards for *S. aureus* in cheese were identified through the literature. For exposure assessment, the levels of *S. aureus* contamination in cheeses were evaluated, and the growth of *S. aureus* was predicted by predictive models at the surveyed temperatures, and at the time of cheese processing and distribution. For hazard characterization, a dose–response model for *S. aureus* was found, and the model was used to estimate the risk of illness. With these data, simulation models were prepared with @RISK (Palisade Corp., Ithaca, NY) to estimate the risk of illness per person per day in risk characterization. *Staphylococcus aureus* cell counts on cheese samples from factories and markets were below detection limits (0.30–0.45 log cfu/g), and pert distribution showed that the mean temperature at markets was 6.63°C. Exponential model [$P = 1 - \exp(7.64 \times 10^{-8} \times N)$, where N = dose] for dose–response was deemed appropriate for hazard characterization. Mean temperature of home storage was 4.02°C (log-logistic distribution). The results of risk characterization for *S. aureus* in natural and processed cheese showed that the mean values for the probability of illness per person per day were higher in processed cheese (mean: 2.24×10^{-9}; maximum: 7.97×10^{-6}) than in natural cheese (mean: 7.84×10^{-10}; maximum: 2.32×10^{-6}). These results indicate that the risk of *S. aureus*-related foodborne illness due to cheese consumption can be considered low under the present conditions in Korea. In addition, the developed stochastic risk assessment model in this study can be useful in establishing microbial criteria for *S. aureus* in cheese.

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INTRODUCTION

Microbial risk assessment is a part of microbial risk analysis, which is composed of risk assessment, risk management, and risk communication (FAO/WHO, 1997). It is a valuable assessment tool for understanding the risks posed by microorganisms and the prevention of foodborne illness. By conducting microbial risk assessment, the probability of experiencing illness due to pathogenic and environmental factors, influencing microbial growth, can be assessed (EPA, 2012). This assessment consists of hazard identification, exposure assessment, hazard characterization, and risk characterization (Codex Alimentarius Commission, 1999). Hazard identification is the identification of biological, chemical, and physical agents that may cause adverse health effects, and may be present in a particular food or group of foods. Exposure assessment is the qualitative or quantitative evaluation of the likely intake of biological, chemical, and physical agents via food. Hazard characterization refers to the qualitative or quantitative evaluation of the adverse health effects associated with the hazard, whereas risk characterization is the process of determining the qualitative or quantitative estimation, including attendant uncertainties, of the probability of occurrence of known or potential adverse health effects, as well as severity, in a given population based on hazard identification, hazard characterization, and exposure assessment (Codex Alimentarius Commission, 1999).

In 1997, the United States emphasized the importance of quantitative microbial risk assessment for achieving food safety objectives at the National Food Safety Initiative (Washington, DC). In addition, the interagency risk assessment consortium was established for food safety management, and the development of predictive models and other quantitative risk assessment tools has also been promoted (NFSI, 1997). The European Union

has developed quantitative risk assessment methods at scientific steering committees, whereas Canada manages a decision-making framework and risk analysis framework at the Canadian Food Inspection Agency (Ottawa, ON, Canada). Besides these examples, Australia, Japan, and Korea employ the quantitative risk assessment tool of the Codex Alimentarius Commission.

Staphylococcus aureus is one of major pathogens related to outbreaks of foodborne illness, and this pathogen has been the cause of 171 outbreaks of foodborne illness in the past decade in Korea (MFDS, 2014b). *Staphylococcus aureus* has been isolated from various foods, especially meat and chicken (Jablonski and Bohach, 2001; Tamarapu et al., 2001; Jørgensen et al., 2005; Colombari et al., 2007). The Centers for Disease Control and Prevention (Atlanta, GA) reported 32 cheese-related foodborne outbreaks between 1973 and 1992, 11 of which may have been caused by raw milk cheese (Altekruse et al., 1998). In addition, *S. aureus* was isolated from cheese on sale in California in 2010 (FDA, 2010).

Many countries have quantitative microbial criteria for *S. aureus* in various foods, especially cheese; however, Korea applies a "zero tolerance" policy for *S. aureus* in cheese (MFDS, 2014c). Hence, the *S. aureus* risk in cheese in Korea needs to be evaluated. Accordingly, the objective of this study was to conduct a microbial risk assessment for *S. aureus* in natural and processed cheese in Korea.

MATERIALS AND METHODS

Hazard Identification

Staphylococcus aureus is a gram-positive, facultative anaerobic foodborne pathogen that is capable of growth from 7°C to 48.5°C, pH 4.2 to 9.3, and NaCl concentrations of 0 to 15% (Bergdoll, 1989; Le Loir et al., 2003; Schmitt et al., 1990). Because of the wide ranges of these extrinsic factors, *S. aureus* is readily isolated from a variety of foods, such as milk and dairy products, meat, and chicken (Jablonski and Bohach, 2001; Tamarapu et al., 2001; Jørgensen et al., 2005; Colombari et al., 2007). *Staphylococcus aureus* was first identified in 1880 in Aberdeen, United Kingdom, and the first recorded food-related *S. aureus* outbreak was due to Cheddar cheese in 1884 (Anonymous, 1984). Although foodborne illness related to cheese has not yet occurred in Korea, occasional occurrence has been observed in countries that have high annual consumption of cheese, such as the United States and European countries, especially related to consumption of natural cheeses (Barrett, 1986; FDA, 2010; NPR, 2010). Therefore, acceptable limits and microbial criteria for contamination of cheese by *S. aureus* have been established in many countries.

In Korea, the criteria for cheese were divided into those for natural cheese and those for processed cheese. Natural cheese is defined as that manufactured by removing the whey after coagulation of raw milk or milk product through addition of lactic acid bacteria, protein curd enzymes, and organic acid, whereas processed cheese refers to that containing more than 50% milk solids derived from natural cheese (MFDS, 2014c). After hazard identification of *S. aureus* in cheese, quantitative microbial risk assessment was performed in natural and processed cheese, as presented in Figure 1.

Exposure Assessment

Prevalence. To evaluate the prevalence and contamination levels of *S. aureus*, natural and processed cheese samples were collected from 2 cheese factories in Korea, and analyzed during summer and winter to allow comparison of the effects of season. For natural cheese (Brie and Camembert), the following samples were collected for analysis: raw milk, pasteurized milk, cheese before ripening, cheese after packaging, and cheese before shipping. For processed cheese (Cheddar slices, Mozzarella slices, and Gouda slices), the following samples were collected: cheese base, cheese after packaging, and cheese before shipping. Upon collection, the samples were placed in a cooler and quickly transported to our laboratory for evaluation of the prevalence and contamination levels of *S. aureus*. The milk samples were plated on tryptic soy agar (**TSA**; Becton, Dickinson and Co., Sparks, MD) and mannitol salt agar (**MSA**; Becton, Dickinson and Co.) for enumeration of total bacteria and *S. aureus* cell counts, respectively. The cheese samples (25 g or 1 slice) were aseptically transferred into a sample bag (3M, St. Paul, MN), and 50 mL of 0.1% buffered peptone water (Becton, Dickinson and Co.) was added, after which the samples were pummeled for 120 s (BagMixer, Interscience, St. Nom, France). Aliquots of 1 mL of the homogenates were then plated on TSA and MSA. To evaluate contamination levels at the markets, natural cheese (Brie, Camembert, and Mozzarella cheese) and processed cheese (Cheddar slices, Gouda slices, and Mozzarella slices) samples were collected from markets during the summer and winter for analysis. Natural cheese (25 g) and processed cheese (1 slice) were also analyzed according to the methods described above. The colonies were counted manually after incubation at 35°C for 24 h. Presumptive colonies of *S. aureus* were further analyzed by 16s rDNA analysis for confirmation of *S.*