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 $N = \text{dose}$] 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.

Key words: microbial risk assessment, *Staphylococcus aureus*, cheese, exposure assessment, risk characterization

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

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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). There-

fore, 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.*

Figure 1. Flow diagram of quantitative microbial risk assessment of *Staphylococcus aureus* in natural and processed cheeses. IC = initial concentration; C_i = concentration of *S. aureus* in cheese at that point; G_i = growth of *S. aureus* in cheese at that point; f = function of; T_{tr} = temperature during transport to market; t_{tr} = time for transportation to market; T_s = temperature of storage/display; t_s = time of storage/display; T_t = temperature during transportation to consumer homes; t_t = time for transportation to consumer's home; T_r = temperature held at home refrigerators; t_r = time held at home refrigerators; SS = serving size; D = dose per serving; F = consumption frequency; D_{Fre} = dose per day. Color version available online.

aureus with primers of 27F (5'-AGA GTT TGA TCM TGG CTC AG-3') and 1492R (5'-TAC GGY TAC CTT GTT ACG ACT T-3'). The PCR mixture (30 μ L) was formulated with 3 μ L of 10 $\times$ reaction buffer, 1 μ L of 10 mM deoxynucleotide triphosphates, 1 μ L of 5 $\times$ Band Doctor (Applied Biosystems, Foster City, CA), 1 μ L of each 10 pM primer, 1 μ L of template DNA, 21.5 μ L of sterilized distilled water, and 0.2 μ L of 2.5 U EF-Taq polymerase (Solgent, Daejeon, Korea). Activation of Taq polymerase was carried out at 95°C for 2 min, followed by 35 cycles of 95°C, 55°C, and 72°C for 1 min each, finishing with a 10-min step at 72°C. A multiscreen filter plate (Millipore Corp., Bedford, MA) was used for purification of the amplified samples, after which the sequencing reaction was conducted by a Prism BigDye Terminator v3.1 Cycle sequencing Kit (Applied Biosystems). The DNA samples containing

the extension products were placed in Hi-Di formamide (Applied Biosystems). The mixture was then exposed to 95°C for 5 min, followed by 5 min on ice, and then analyzed by using a DNA analyzer (ABI Prism 3730XL DNA analyzer, Applied Biosystems).

Initial Contamination Level. Appropriate data distributions were identified through a literature search, and the collected prevalence data on *S. aureus* were fitted to β -distribution (α_1 = the number of positive samples+1, α_2 = tested total samples – positive samples + 1). Contamination levels for the final product at cheese factories were fitted to a uniform distribution (a = minimum contamination level, b = maximum contamination level), because uniform distribution can be defended as being the maximally noncommittal distribution for an estimate of probability given that no information is available about the probability (Vose,

1998). Ultimately, the initial concentrations (log cfu/g) of *S. aureus* on natural and processed cheese were calculated by prevalence $\times$ contamination level.

Contamination Levels at Consumption. To predict *S. aureus* growth patterns during storage and transportation of the cheese, the mathematical models developed by the MFDS (2013) were used for both natural and processed cheese, with consideration of the worst-case scenario. Among those examined, Brie cheese (natural) and Mozzarella slices (processed) displayed the highest maximum specific growth rate ($\mu_{\max}$). The equations were as follows:

$$\text{Brie cheese: } \sqrt{\mu_{\max}} = 0.0268 \times (T - 5.2687), \quad [1]$$

$$\lambda = 18864.1 \times e^{-0.6432T}, \quad [2]$$

$$\text{Mozzarella slices: } \sqrt{\mu_{\max}} = 0.0265 \times (T - 6.3547), \quad [3]$$

$$\lambda = 4946.4 \times e^{-0.3085T}, \quad [4]$$

where T = temperature; and λ = lag phase duration. The equations were used to calculate the kinetic parameters ($\mu_{\max}$ and λ) to estimate the changes in *S. aureus* cell counts during storage and transportation.

Temperature Profile. Temperature information during transportation from the factory to the markets was collected by personal communication. Temperatures for cheese storage at the markets were measured using an infrared ray thermometer (HS33CT, Hansung, Seoul, Korea) every 30 min for 6 to 9 h. Home refrigerators were monitored using a data logger (Testo 174H, Testo, Lenzkirch, Germany), and the storage temperatures of home refrigerators were measured every 10 min for 3 to 4 d. These temperature data were fitted to the @RISK program, version 5.7 (Palisade Corp., Ithaca, NY), to obtain appropriate data distributions.

Cheese Consumption in Korea. Information on the amount of consumption and frequency data for natural and processed cheeses were cited from a study by the MFDS (2013) to estimate the risk of *S. aureus* exposure through consumption of natural and processed cheese in Korea.

Dose-Response Model

The probability of foodborne illness from the intake of *S. aureus* cells was estimated by the following dose-response model, developed by Rose and Haas (1999):

$$P = 1 - e^{-r \times N}, \quad [5]$$

where P is the probability of illness, r is the probability of *S. aureus* single cells causing foodborne illness, and N is the number of *S. aureus* cells ingested (cfu/serving).

Risk Characterization

To estimate the probability of experiencing *S. aureus* foodborne illness per person per day due to cheese consumption, simulation models were prepared with the exposure assessment data, including prevalence, contamination level, temperature distribution, amount and frequency of cheese consumption, and dose-response model, in the @RISK program (Palisade Corp.) with settings for 10,000 iterations of Latin Hypercube sampling (Tables 1 and 2).

RESULTS AND DISCUSSION

Prevalence and Contamination Level of *S. aureus*

For all cheese samples (90 natural cheese and 308 processed cheese samples), *S. aureus* was below the detection limit (natural cheese: 0.30 log cfu/g; processed cheese: 0.45 log cfu/g). Such low levels of *S. aureus* contamination may be due to the “zero-tolerance” policy of Korea. In contrast, the European Union has quantitative microbial criteria for *S. aureus* on soft cheese ($n = 5$, $c = 2$, $m = 100$, $M = 1,000$, where n = number of sample units to be chosen independently and randomly from the lot, c = maximum allowable number of sample units yielding results between m and M , m = a microbiological limit that separates good quality from marginally acceptable quality, and M = a microbiological limit above which sampling results are unacceptable or defective) and fresh cheese ($n = 5$, $c = 2$, $m = 100$, $M = 100$), and Canada, Australia, New Zealand, and Hungary also have quantitative microbial criteria. This implies that the quantitative microbial criteria of *S. aureus* in cheese in Korea could be considered if the microbial risk of *S. aureus* in cheese is not increased. Thus, quantitative microbial risk assessment is needed for quantitative evaluation of the risk of *S. aureus* in cheese.

Because the *S. aureus* contamination was below the detection level in all samples, initial levels of *S. aureus* contamination were calculated with the β distribution $\times$ uniform distribution. The β distribution describes the probability (P) of an event occurring, and is a result of applying Bayes' Theorem to the observed events. In our research, the prevalence data were fitted to β distribution because of the lack of information about P before the observed events (Vose, 1998). According to

Table 1. Simulation model and formulas in Excel spreadsheet (Microsoft Corp., Redmond, WA) used to calculate the risk of *Staphylococcus aureus* in natural cheese with @RISK (Palisade Corp., Ithaca, NY)

Input model	Unit	Code	Formula	Reference
Product				
Pathogen contamination level				
<i>S. aureus</i> prevalence	cfu/g	PR	=RiskBeta(1,91)	Vose (1998)
Concentration	cfu/g	C	=RiskUniform(0,2)	Vose (1998)
Initial contamination level	log cfu/g	IC	=PR×C	Vose (1998)
Transportation				
Transportation time	h	time _{trans}	=log(PR×C)	
			=RiskPert(1,3,6)	Latorre et al. (2011); personal communication ¹
Food temperature during transportation	°C	Temp _{trans}	=RiskPert(0,4,10)	Personal communication ¹
Growth				
h ₀	log cfu/g	h ₀	=average(growth rate×lag time), Fixed 0.68	MFDS (2013); Baranyi and Roberts (1994)
Y ₀	log cfu/g	Y ₀	=average(Y ₀), Fixed 3.35	MFDS (2013); Baranyi and Roberts (1994)
Y _{end}	log cfu/g	Y _{end}	=average(Y _{end}), Fixed 9.07	MFDS (2013); Baranyi and Roberts (1994)
ln(q)		ln(q)	=LN{1/[EXP(h ₀)-1]}	MFDS (2013); Baranyi and Roberts (1994)
Lag time	h	Trans _{lt}	=18864.1×exp(-0.6432×Temp _{trans})	MFDS (2013)
Growth rate	log cfu/g/h	Trans _{Gr}	=IF{Temp _{trans} > 5.2687, [0.0268×(Temp _{trans} -5.2687)] ² , 0}	MFDS (2013); Ratkowsky et al. (1982)
<i>S. aureus</i> growth	log cfu/g	C1	=IC+1/{1+EXP[-ln(q)]}×[1-10 ^{-Y₀-Y_{end}}]/LN(10)]×Trans _{Gr} ×time _{trans}	MFDS (2013); Baranyi and Roberts (1994)
Market				
Market storage				
Storage time	h	Mark-time _{st}	=RiskPert(0,2,48)	Personal communication ²
Food temperature during storage	°C	Mark-Temp _{st}	=RiskUniform(2,4)	Personal communication ²
Growth				
h ₀	log cfu/g	h ₀	=average(growth rate×lag time), Fixed 0.68	MFDS (2013); Baranyi and Roberts (1994)
Y ₀	log cfu/g	Y ₀	=average(Y ₀), Fixed 3.35	MFDS (2013); Baranyi and Roberts (1994)
Y _{end}	log cfu/g	Y _{end}	=average(Y _{end}), Fixed 9.07	MFDS (2013); Baranyi and Roberts (1994)
ln(q)		ln(q)	=LN{1/[EXP(h ₀)-1]}	MFDS (2013); Baranyi and Roberts (1994)
Lag time	h	Mark _{st} -Time _{lt}	=18864.1×exp(-0.6432×Mark-Temp _{st})	MFDS (2013)
Growth rate	log cfu/g/h	Mark _{st} -R _{Gr}	=IF{Mark-Temp _{st} > 5.2687, [0.0268×(Mark-Temp _{trans} -5.2687)] ² , 0}	MFDS (2013); Ratkowsky et al. (1982)
<i>S. aureus</i> growth	log cfu/g	C2-1	=C1+1/{1+EXP[-ln(q)]}×[1-10 ^{-Y₀-Y_{end}}]/LN(10)]×Mark _{st} -R _{Gr} ×Mark-time _{st}	MFDS (2013); Baranyi and Roberts (1994)
Market display				
Storage time	h	Mark-time _{dis}	=RiskPert(0,48,168)	Personal communication ²

Continued

Table 1 (Continued). Simulation model and formulas in Excel spreadsheet (Microsoft Corp., Redmond, WA) used to calculate the risk of *Staphylococcus aureus* in natural cheese with @RISK (Palisade Corp., Ithaca, NY)

Input model	Unit	Code	Formula	Reference
Food temperature during storage	°C	Mark-Temp _{dis}	=RiskTriang(0.60703,4.1000,15.18)	This research
Growth				
h ₀	log cfu/g	h ₀	=average(growth rate×lag time), Fixed 0.68	MFDS (2013); Baranyi and Roberts (1994)
Y ₀	log cfu/g	Y ₀	=average(Y ₀), Fixed 3.35	MFDS (2013); Baranyi and Roberts (1994)
Y _{end}	log cfu/g	Y _{end}	= average(Y _{end}), Fixed 9.07	MFDS (2013); Baranyi and Roberts (1994)
ln(q)		ln(q)	=LN{1/[EXP(h ₀)-1]}	MFDS (2013); Baranyi and Roberts (1994)
Lag time	h	Mark _{dis} -Time _{Lt}	=18864.1×exp(-0.6432×Mark-Temp _{dis})	MFDS (2013)
Growth rate	log cfu/g/h	Mark _{dis} -R _{Gr}	=IF{Mark-Temp _{dis} >5.2687,[0.0268×(Mark-Temp _{dis} -5.2687)] ² ,0}	MFDS (2013); Ratkowsky et al. (1982)
<i>S. aureus</i> growth	log cfu/g	C2	=(C2-1)+1/{1+EXP[-ln(q)]}×[1-10 ^{Y₀-end} /LN(10)]×Mark _{dis} -R _{Gr} ×Mark-time _{dis}	MFDS (2013); Baranyi and Roberts (1994)
Transportation (car)				
Transportation (car) storage				
Transportation time	h	time _{car}	=RiskPert(0.325,0.984,1.643)	Jung (2011)
Food temperature during transportation	°C	Temp _{car}	=RiskPert(10,18,25)	Jung (2011)
Growth				
h ₀	log cfu/g	h ₀	=average(growth rate×lag time), Fixed 0.68	MFDS (2013); Baranyi and Roberts (1994)
Y ₀	log cfu/g	Y ₀	=average(Y ₀), Fixed 3.35	MFDS (2013); Baranyi and Roberts (1994)
Y _{end}	log cfu/g	Y _{end}	=average(Y _{end}), Fixed 9.07	MFDS (2013); Baranyi and Roberts (1994)
ln(q)		ln(q)	=LN{1/[EXP(h ₀)-1]}	MFDS (2013); Baranyi and Roberts (1994)
Lag time	h	Car-Time _{Lt}	=18864.1×exp(-0.6432×Temp _{car})	MFDS (2013)
Growth rate	log cfu/g/h	Car-R _{Gr}	=IF{Temp _{car} >5.2687,[0.02×(Temp _{car} -5.2687)] ² ,0}	MFDS (2013); Ratkowsky et al. (1982)
<i>S. aureus</i> growth	log cfu/g	C3	=C2+1/{1+EXP[-ln(q)]}×[1-10 ^{Y₀-Y_{end}} /LN(10)]×Car-R _{Gr} ×time _{car}	MFDS (2013); Baranyi and Roberts (1994)
Home				
Home storage				
Storage time	h	Home-time _{st}	=RiskNormal[250,1742,176.0175,RiskTruncate(0,4320)]	This research
Food temperature during storage	°C	Home-Temp _{st}	=RiskLogLogistic[-29.283,33.227,26.666,RiskTruncate(-5,20)]	This research
Growth				
h ₀	log cfu/g	h ₀	=average(growth rate×lag time), Fixed 0.68	MFDS (2013); Baranyi and Roberts(1994)
Y ₀	log cfu/g	Y ₀	=average(Y ₀), Fixed 3.35	MFDS (2013); Baranyi and Roberts (1994)
Y _{end}	log cfu/g	Y _{end}	=average(Y _{end}), Fixed 9.07	MFDS (2013); Baranyi and Roberts (1994)
ln(q)		ln(q)	=LN{1/[EXP(h ₀)-1]}	MFDS (2013); Baranyi and Roberts (1994)
Lag time	h	Home-T _{Lt}	=18864.1×exp(-0.6432×Home-Temp _{st})	MFDS (2013)
Growth rate	log cfu/g/h	Home-R _{Gr}	=IF{Home-Temp _{st} >5.2687[0.0268×(Home-Temp _{st} -5.2687)] ² ,0}	MFDS (2013); Ratkowsky et al. (1982)

Continued

Table 1 (Continued). Simulation model and formulas in Excel spreadsheet (Microsoft Corp., Redmond, WA) used to calculate the risk of *Staphylococcus aureus* in natural cheese with @RISK (Palisade Corp., Ithaca, NY)

Input model	Unit	Code	Formula	Reference
<i>S. aureus</i> growth	log cfu/g	C4	$=C3+1/\{1+\text{EXP}[-\ln(q)]\} \times [1-10^{(Y_0-Y_{\text{end}})/\text{LN}(10)}] \times \text{Home}-R_{\text{G}} \times \text{Home}-\text{time}_{\text{st}}$	MFDS (2013); Baranyi and Roberts (1994)
Consumption				
Daily consumption average amount	g	Consump	$=\text{RiskPearson}[2.6488, 25.81, \text{RiskTruncate}(0, 100), \text{RiskShift}(-3.2572)]$	MFDS (2013)
Daily consumption frequency	%	ConFre	Fixed 3.894	MFDS (2013)
		CF(0)	$=1-3.894/100$	MFDS (2013)
		CF(1)	$=3.894/100$	MFDS (2013)
		CF	$=\text{RiskDiscrete}\{[0, 1], [CF(0), CF(1)]\}$	MFDS (2013)
		ConFre	$=\text{IF}(CF = 0, 0, \text{Consump})$	MFDS (2013)
Dose-response				
<i>S. aureus</i> amount		D	$=10^{C4} \times \text{ConFre}$	Rose and Haas (1999)
Parameter of r		r	Fixed 7.64×10^{-8}	
Risk				
Probability of illness/person/day		Risk	$=1-\exp(-r \times D)$	Rose and Haas (1999)

¹Personal communication with supervisor of a cheese manufacturing plant.²Personal communication with manager in charge of cheese products at markets.

the distribution, the initial contamination levels of *S. aureus* in cheese were estimated to be -2.35 and -2.73 log cfu/g for natural and processed cheeses, respectively (Tables 1 and 2 and Figure 2).

Growth of *S. aureus*

Because we assumed that the initial concentration of *S. aureus* on cheese would increase during transportation and display, the *S. aureus* contamination levels at each step were predicted with the μ_{max} and λ values calculated by mathematical models, which are presented in Equations [1] to [4]. The predicted amounts of *S. aureus* in natural cheese at arrival at the market, after storage at the market, at the moment of purchase, at the time of home arrival, and at the time of consumption were -2.34 , -2.34 , -2.25 , -2.22 , and -2.17 log cfu/g, respectively, whereas the values for processed cheese were -2.73 , -2.73 , -2.65 , -2.62 , and -2.32 log cfu/g, respectively (data not shown). During transportation and display at markets, significant *S. aureus* growth on the natural and processed cheese samples was not observed, due to the well-managed cold chain system. However, if the cheeses were placed at maximum temperature at the market (15.18°C), which was estimated from examination of the temperature distribution (*triang* distribution) at markets (Figure 3), the μ_{max} values of natural and processed cheese would be 0.27 and 0.23 log cfu/g per hour, respectively, indicating that the *S. aureus* cell counts on the cheese would significantly increase under such conditions.

Temperature

Data on temperature during transportation were collected through personal communication with the supervisor of a cheese manufacturing plant; it was not necessary to collect many samples because the temperature variation was minimal. Thus, a pert distribution was used to obtain the temperature distribution during transportation, with minimum (0°C), mean (4°C), and maximum (10°C) data, and the values were fitted to a pert distribution (minimum = 0°C , mean = 4°C , maximum = 10°C) to be used for prediction of *S. aureus* growth on the natural and processed cheeses. At the markets, the observed temperature of the cheese ranged from 0.90°C to 14.30°C , with a mean of 6.65°C , and the appropriate data distribution was determined to be the *triang* distribution (Figure 3). This fitted *triang* distribution indicated that the minimum, mean, and maximum temperatures were 0.61°C , 6.63°C , and 15.18°C . In Korea, cold storage temperature is legally defined as 0°C to 10°C (MFDS, 2014a), thus temperatures above 10°C , which were found in the collected temperature

Table 2. Simulation model and formulas in Excel spreadsheet (Microsoft Corp., Redmond, WA) used to calculate the risk of *Staphylococcus aureus* in processed cheese with @RISK (Palisade Corp., Ithaca, NY)

Input model	Unit	Code	Formula	Reference
Product				
Pathogen contamination level				
<i>S. aureus</i> prevalence	cfu/g	PR	=RiskBeta(1,309)	Vose (1998)
Concentration	cfu/g	C	=RiskUniform(0,2.8)	Vose (1998)
Initial contamination level	log cfu/g	IC	=PR×C	Vose (1998)
			=log(PR×C)	
Transportation				
Transportation time	h	time _{trans}	=RiskPert(1,3,6)	Latorre et al. (2011); Personal communication ¹
Food temperature during transportation	°C	Temp _{trans}	=RiskPert(0,4,10)	Personal communication ¹
Growth				
<i>h</i> ₀	log cfu/g	<i>h</i> ₀	=average(growth rate×lag time), Fixed 0.75	MFDS (2013); Baranyi and Roberts (1994)
<i>Y</i> ₀	log cfu/g	<i>Y</i> ₀	=average(<i>Y</i> _{0<i>t</i>}), Fixed 3.12	MFDS (2013); Baranyi and Roberts (1994)
<i>Y</i> _{end}	log cfu/g	<i>Y</i> _{end}	=average(<i>Y</i> _{end<i>t</i>}), Fixed 8.19	MFDS (2013); Baranyi and Roberts (1994)
ln(<i>q</i>)		ln(<i>q</i>)	=LN{1/[EXP(<i>h</i> ₀)-1]}	MFDS (2013); Baranyi and Roberts (1994)
Lag time	h	Trans _{Lt}	=6208.0×exp(−0.3871×Temp _{trans})	MFDS (2013)
Growth rate	log cfu/g/h	Trans _{G_r}	=IF{Temp _{trans} > 5.4538[0.0260×(Temp _{trans} −5.4538)] ² , 0}	MFDS (2013); Ratkowsky et al. (1982)
<i>S. aureus</i> growth	log cfu/g	C1	=IC+1/{1+EXP[−ln(<i>q</i>)]}×[1−10 ^[(<i>Y</i>₀−<i>Y</i>_{end})/LN(10)] ×Trans _{G_r} ×time _{trans}	MFDS (2013); Baranyi and Roberts (1994)
Market				
Market storage				
Storage time	h	Mark−time _{st}	=RiskPert(0,2,48)	Personal communication ²
Food temperature during storage	°C	Mark−Temp _{st}	=RiskUniform(2,4)	Personal communication ²
Growth				
<i>h</i> ₀	log cfu/g	<i>h</i> ₀	=average(growth rate×lag time), Fixed 0.75	MFDS (2013); Baranyi and Roberts (1994)
<i>Y</i> ₀	log cfu/g	<i>Y</i> ₀	=average(<i>Y</i> _{0<i>t</i>}), Fixed 3.12	MFDS (2013); Baranyi and Roberts (1994)
<i>Y</i> _{end}	log cfu/g	<i>Y</i> _{end}	=average(<i>Y</i> _{end<i>t</i>}), Fixed 8.19	MFDS (2013); Baranyi and Roberts (1994)
ln(<i>q</i>)		ln(<i>q</i>)	=LN{1/[EXP(<i>h</i> ₀)-1]}	MFDS (2013); Baranyi and Roberts (1994)
Lag time	h	Mark _{st} −Time _{Lt}	=6208.0×exp(−0.3871×Mark−Temp _{st})	MFDS (2013)
Growth rate	log cfu/g/h	Mark _{st} −R _{G_r}	=IF{Mark−Temp _{st} > 5.4538[0.0260×(Mark−Temp _{trans} −5.4538)] ² , 0}	MFDS (2013); Ratkowsky et al. (1982)
<i>S. aureus</i> growth	log cfu/g	C2−1	=C1+1/{1+EXP[−ln(<i>q</i>)]}×[1−10 ^[(<i>Y</i>₀−<i>Y</i>_{end})/LN(10)] ×Mark−R _{G_r} ×Mark−time _{st}	MFDS (2013); Baranyi and Roberts (1994)
Market display				
Storage time	h	Mark−time _{dis}	=RiskPert(0,48,168)	Personal communication ²
Food temperature during storage	°C	Mark−Temp _{dis}	=RiskTriang(0.60703,4.1000,15.18)	This research
Growth				

Continued

Table 2 (Continued). Simulation model and formulas in Excel spreadsheet (Microsoft Corp., Redmond, WA) used to calculate the risk of *Staphylococcus aureus* in processed cheese with @RISK (Palisade Corp., Ithaca, NY)

Input model	Unit	Code	Formula	Reference
<i>h</i> ₀	log cfu/g	<i>h</i> ₀	=average(growth rate×lag time), Fixed 0.75	MFDS (2013); Baranyi and Roberts (1994)
<i>Y</i> ₀	log cfu/g	<i>Y</i> ₀	=average(<i>Y</i> ₀ ²), Fixed 3.12	MFDS (2013); Baranyi and Roberts (1994)
<i>Y</i> _{end}	log cfu/g	<i>Y</i> _{end}	=average(<i>Y</i> _{end} ²), Fixed 8.19	MFDS (2013); Baranyi and Roberts (1994)
ln(<i>q</i>)		ln(<i>q</i>)	=LN(1/[EXP(<i>h</i> ₀)-1])	MFDS (2013); Baranyi and Roberts (1994)
Lag time	h	Mark _{dis} -Time _{Lt}	=6208.0×exp(-0.3871×Mark-Temp _{dis})	MFDS (2013)
Growth rate	log cfu/g/h	Mark _{dis} -R _{Gr}	=IF{Mark-Temp _{dis} > 5.4538[0.0260×(Mark-Temp _{dis} -5.4538)] ² ,0}	Ratkowsky et al. (1982)
<i>S. aureus</i> growth	log cfu/g	C2	=(C2-1)+1/{1+EXP[-ln(<i>q</i>)]}×[1-10 ^{Y₀-Y_{end}} /LN(10)]×Mark _{dis} -R _{Gr} ×Mark-time _{dis}	MFDS (2013); Baranyi and Roberts (1994)
Transportation (car)				
Transportation time	h	time _{car}	=RiskPert(0.325,0.984,1.643)	Jung (2011)
Food temperature during transportation	°C	Temp _{car}	=RiskPert(10,18,25)	Jung (2011)
Growth				
<i>h</i> ₀	log cfu/g	<i>h</i> ₀	=average(growth rate×lag time), Fixed 0.75	MFDS (2013); Baranyi and Roberts (1994)
<i>Y</i> ₀	log cfu/g	<i>Y</i> ₀	=average(<i>Y</i> ₀ ²), Fixed 3.12	MFDS (2013); Baranyi and Roberts (1994)
<i>Y</i> _{end}	log cfu/g	<i>Y</i> _{end}	=average(<i>Y</i> _{end} ²), Fixed 8.19	MFDS (2013); Baranyi and Roberts (1994)
ln(<i>q</i>)		ln(<i>q</i>)	=LN(1/[EXP(<i>h</i> ₀)-1])	MFDS (2013); Baranyi and Roberts (1994)
Lag time	h	Car-Time _{Lt}	=6208.0×exp(-0.3871×Temp _{car})	MFDS (2013)
Growth rate	log cfu/g/h	Car-R _{Gr}	=IF{Temp _{car} > 5.2687[0.0260×(Temp _{car} -5.4538)] ² ,0}	MFDS (2013); Ratkowsky et al. (1982)
<i>S. aureus</i> growth	log cfu/g	C3	=C2+1/{1+EXP[-ln(<i>q</i>)]}×[1-10 ^{Y₀-Y_{end}} /LN(10)]×Car-R _{Gr} ×time _{car}	MFDS (2013); Baranyi and Roberts (1994)
Home				
Home storage				
Storage time	h	Home-time _{st}	=RiskNormal[250.1742,176.0175,RiskTruncate(0.4320)]	This research
Food temperature during storage	°C	Home-Temp _{st}	=RiskLogLogistic[-29.283,33.227,26.666,RiskTruncate(-5,20)]	This research
Growth				
<i>h</i> ₀	log cfu/g	<i>h</i> ₀	=average(growth rate×lag time), Fixed 0.75	MFDS (2013); Baranyi and Roberts (1994)
<i>Y</i> ₀	log cfu/g	<i>Y</i> ₀	=average(<i>Y</i> ₀ ²), Fixed 3.12	MFDS (2013); Baranyi and Roberts (1994)
<i>Y</i> _{end}	log cfu/g	<i>Y</i> _{end}	=average(<i>Y</i> _{end} ²), Fixed 8.19	MFDS (2013); Baranyi and Roberts (1994)
ln(<i>q</i>)		ln(<i>q</i>)	=LN(1/[EXP(<i>h</i> ₀)-1])	MFDS (2013); Baranyi and Roberts (1994)
Lag time	h	Home-T _{Lt}	=6208.0×exp(-0.3871×Home-Temp _{st})	MFDS (2013)
Growth rate	log cfu/g/h	Home-R _{Gr}	=IF{Home-Temp _{st} > 5.4538[0.0260×(Home-Temp _{st} -5.4538)] ² ,0}	Ratkowsky et al. (1982)
<i>S. aureus</i> growth	log cfu/g	C4	=C3+1/{1+EXP[-ln(<i>q</i>)]}×[1-10 ^{Y₀-Y_{end}} /LN(10)]×Home-R _{Gr} ×Home-time _{st}	MFDS (2013); Baranyi and Roberts (1994)

Continued

Table 2 (Continued). Simulation model and formulas in Excel spreadsheet (Microsoft Corp., Redmond, WA) used to calculate the risk of *Staphylococcus aureus* in processed cheese with @RISK (Palisade Corp., Ithaca, NY)

Input model	Unit	Code	Formula	Reference
Consumption				
Daily consumption average amount	g %	Consump	=RiskWeibull[1.3482,20.932,RiskShift(0.26384),RiskTruncate(0,100)]	MFDS (2013)
Daily consumption frequency		ConFre	Fixed 2.323	MFDS (2013)
		CF(0)	=1-2.323/100	MFDS (2013)
		CF(1)	=2.323/100	MFDS (2013)
		CF	=RiskDiscrete{[0,1],[CF(0),CF(1)]}	MFDS (2013)
		ConFre	=IF(CF = 0,0,Consump)	MFDS (2013)
Dose-response				
<i>S. aureus</i> amount		D	=10 ^{C4} × ConFre	Rose and Haas (1999)
Parameter of r		r	=Fixed 7.64 × 10 ⁻⁸	
Risk		Risk	=1-exp(-r × D)	Rose and Haas (1999)
Probability of illness/person/day				

¹Personal communication with supervisor of a cheese manufacturing plant.

²Personal communication with manager in charge of cheese products at markets.

data, can be considered as temperature abuse. Hence, it is possible that *S. aureus* present on natural and processed cheese may occasionally be exposed to such abusive temperatures. However, the simulation for *S. aureus* growth with the observed temperature distribution did not show significant growth of the pathogen, because the 95th percentile for temperature at markets was 12.65°C (Figures 3 and 4). Lee et al. (2008) also surveyed the storage temperature of major chilled foods in refrigerators at markets in Korea, during which the mean temperature was determined to be 8.2°C, showing a similar value to the results presented herein. In addition, Choi et al. (2011) also measured the surface temperatures of food in cold storage, finding it to range from 4.6°C to 10.6°C, with a mean of 6.6°C.

For home refrigerator temperatures, log-logistic distribution (−26.084, 30.149, 24.258) was determined to have the best fit, and the minimum and maximum temperatures were determined to be −3.5 and 10.8°C, respectively, with mean and standard deviation of 4.02°C and 2.27 (Figure 4). The study by Bahk (2010) also showed that log-logistic distribution was appropriate for home refrigerator temperatures, with 3.52°C as the mean temperature.

Cheese Consumption in Korea

According to the report by MFDS (2013), the mean value for natural cheese consumption was estimated to be 12.40 ± 19.43 g/d (95% CI: 0.915–34.90 g/d) with a frequency of 0.0389, whereas the mean consumption of processed cheese was 19.46 ± 14.39 g/d (95% CI: 2.6–40.0 g/d) with a frequency of 0.0232.

Dose–Response Model

Rose and Haas (1999) suggested that the exponential model for the dose of *S. aureus* translated into likely occurrence of foodborne illness. The exponential model [$P = 1 - \exp(7.64 \times 10^{-8} \times N)$] was used to calculate the risk of foodborne illness of *S. aureus* on natural and processed cheese.

Risk Characterization

To estimate the risk of foodborne illness due to *S. aureus* per serving of cheese with the simulation model, the values of input variables such as prevalence, temperature, and time of market storage, display at markets, home storage, amount of consumption, and intake frequency were randomly sampled from the appropriate distributions presented above for 10,000 iterations. The estimated probabilities of *S. aureus*-related foodborne illness per person per day were found to be 7.84 ×

Figure 2. Probability density of simulated initial contamination (IC) level of *Staphylococcus aureus* in (A) natural and (B) processed cheese. Color version available online.

10^{-10} and 2.24×10^{-9} for natural and processed cheese, respectively, with low values of 1.22×10^{-8} and 9.45×10^{-9} , even for the 99th percentile (Table 3). These

results suggest that the probabilities of *S. aureus*-related foodborne illness occurring per 1 million people per year are 0.286 and 0.818 for natural and processed

Figure 3. Temperature distribution at markets. Histogram is the graph of raw data and line is the best-fitted distribution (*triang*). Color version available online.

cheese, respectively. In a quantitative risk assessment of leafy green vegetables consumed at a salad bar, Franz et al. (2010) showed the mean probability of foodborne illness for *Escherichia coli* O157:H7, *Salmonella*, and *Listeria monocytogenes* to be 6.04×10^{-6} , 6.83×10^{-6} , and 1.23×10^{-8} , respectively. In addition, the probabilities of listeriosis per person per day from eating lettuce at a restaurant or at home in Korea were 3.64×10^{-9} to 1.30×10^{-7} and 4.71×10^{-8} to 1.68×10^{-6} , respectively (Ding et al., 2013). Compared with these studies, the estimated risk of *S. aureus* on natural and processed cheeses determined herein can be considered as very low.

The low risk of *S. aureus* foodborne illness through cheese consumption may be caused by low initial levels of *S. aureus*, low consumption of cheese, low intake frequency, and low temperatures for product transpor-

tation and storage in Korea. In Greece, the amount of cheese consumed annually per person is 32.57 kg (89.23 g/d; Wisconsin Cheese, 2014), twice that in Korea. Thus, the comparatively low cheese consumption in Korea may result in a low risk of *S. aureus* in cheese.

Even though processed cheese had lesser growth of *S. aureus* compared with natural cheese, the annual risk estimate of processed cheese was higher, which was likely because of the higher consumption of this type of cheese. The regression coefficient values in Figure 5 show that the factors influencing *S. aureus* risk for natural and processed cheese in Korea were intake frequency and home storage temperature.

In conclusion, the risk of *S. aureus*-related foodborne illness through cheese consumption is low in Korea, and the developed stochastic risk assessment model in this study could be useful for quantitative risk assessment.

Figure 4. Temperature profile and distribution of home refrigerators. Histogram is the graph of raw data, and line is the best-fitted distribution (loglogistic). Color version available online.

Table 3. Probability of foodborne illness from *Staphylococcus aureus* per person per day due to the consumption of natural and processed cheeses

Cheese type	Probability of illness/person per day					Maximum	Mean
	5%	25%	50%	95%	99%		
Natural cheese	0	0	0	0	1.22×10^{-8}	2.32×10^{-6}	7.84×10^{-10}
Processed cheese	0	0	0	0	9.45×10^{-9}	7.97×10^{-6}	2.24×10^{-9}

(A)

(B)

Figure 5. Regression coefficient values for sensitivity risk factors affecting probability of illness per person per day due to the consumption of natural cheese (A) and processed cheese (B). Color version available online.

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