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ARTICLE

Microbial Risk Assessment of Non-Enterohemorrhagic *Escherichia coli* in Natural and Processed Cheeses in Korea

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Abstract

This study assessed the quantitative microbial risk of non-enterohemorrhagic *Escherichia coli* (EHEC). For hazard identification, hazards of non-EHEC *E. coli* in natural and processed cheeses were identified by research papers. Regarding exposure assessment, non-EHEC *E. coli* cell counts in cheese were enumerated, and the developed predictive models were used to describe the fates of non-EHEC *E. coli* strains in cheese during distribution and storage. In addition, data on the amounts and frequency of cheese consumption were collected from the research report of the Ministry of Food and Drug Safety. For hazard characterization, a dose-response model for non-EHEC *E. coli* was used. Using the collected data, simulation models were constructed, using software @RISK to calculate the risk of illness per person per day. Non-EHEC *E. coli* cells in natural- (n=90) and processed-cheese samples (n=308) from factories and markets were not detected. Thus, we estimated the initial levels of contamination by Uniform distribution × Beta distribution, and the levels were -2.35 and -2.73 Log CFU/g for natural and processed cheese, respectively. The proposed predictive models described properly the fates of non-EHEC *E. coli* during distribution and storage of cheese. For hazard characterization, we used the Beta-Poisson model ($\alpha=2.21\times10^{-1}$, $N_{50}=6.85\times10^{7}$). The results of risk characterization for non-EHEC *E. coli* in natural and processed cheese were 1.36×10^{-7} and 2.12×10^{-10} (the mean probability of illness per person per day), respectively. These results indicate that the risk of non-EHEC *E. coli* foodborne illness can be considered low in present conditions.

Keywords microbial risk assessment, *Escherichia coli*, cheese, exposure assessment

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Introduction

Cheese consumption has been increasing gradually in Korea since the 1990s (KDC, 2016), but the cases of contamination with *Listeria monocytogenes*, *Staphylococcus aureus*, and *Escherichia coli* have been reported (Jo *et al*., 2007; Tekinsen and Özdemir, 2006; Thayer *et al*., 1998). Especially, *E. coli* has been isolated from various cheeses in many countries (Haran *et al*., 2011; Zinke *et al*., 2012).

E. coli, a facultative anaerobic Gram-negative bacillus, is commonly found in the intestinal flora of humans and animals, and certain strains are pathogenic (MFDS, 2010; Olsvik *et al*., 1991). According to infection symptoms and pathogenesis, pathogenic *E. coli* strains are classified e.g., enteropathogenic *E. coli* (EPEC), enteroinvasive *E. coli* (EIEC), enterotoxigenic *E. coli* (ETEC), enterohemorrhagic *E. coli* (EHEC), and enteroaggregative *E. coli* (EAEC) (Nataro and

Kaper, 1998; Yoon, 2009). Among the pathogenic *E. coli* strains, *E. coli* O157:H7 is one of the major concerns in the dairy industry, and the survival of the pathogens in various cheeses has been well documented (Griffin and Tauxe, 1991; Reitsma and Henning, 1996). Thus, several countries (EU, USA, and Canada) have a quantitative standard or "zero tolerance" policy for control of the pathogens in cheese (EC, 2005; FDA, 2009; Health Canada, 2008); several microbiological risk assessments for *E. coli* O157:H7 in cheese have also been conducted (FSANZ, 2009; Perrin *et al*., 2015). However, microbial risk assessment for non-EHEC *E. coli* in cheese has not been conducted. Hence, there is a lack of scientific evidence to determine microbial risk of non-EHEC *E. coli*.

EPA (2012) recommends microbiological risk assessment to evaluate the risk posed by bacteria, to prevent foodborne illnesses, and to identify environmental factors influencing microbial growth. The microbiological risk assessment should include hazard identification, exposure assessment, hazard characterization, and risk characterization (Codex, 1999).

The objective of this study was to conduct microbial risk assessment for non-EHEC *E. coli* in natural cheese which is manufactured from milk fermentation by adding start culture enzyme, and salt and processed cheeses which are manufactured from natural cheese using emulsifiers in Korea.

Materials and Methods

Hazard identification

To identify the hazards of *E. coli*, the general characteristics and foodborne-illness outbreaks linked to *E. coli* in cheese were collected from other studies.

Exposure assessment

Prevalence of *E. coli*

To evaluate non-EHEC *E. coli* prevalence and the contamination level, natural- (n=90) and processed-cheese samples (n=308) were collected from various cheese factories and markets. At two factories, samples were collected throughout the manufacturing process from raw milk to packaged cheese. Natural-cheese samples were collected from raw milk, pasteurized milk, cheese before ripening, cheese after packaging, cheese before shipping, and markets. Processed-cheese samples were also collected after packaging, before shipping, and in markets. In addition, distributed cheeses were collected from local markets in five cities in Korea. Cheese samples were evaluated in both summer and winter to reduce the effect of external environmental factors such as temperature, humidity and contamination levels of the pathogen. The collected samples were placed in an ice cooler and were transported to a laboratory. One-milliliter samples of raw milk and pasteurized milk were serially diluted with 0.1% buffered peptone water (BPW; Becton, Dickinson, and Company, USA). The diluents were then surface-plated on tryptic soy agar (TSA; Becto, Dickinson, and Company) and *E. coli* /Coliform Count petrifilm (3M™, USA) to quantify total bacteria, and non-EHEC *E. coli* and coliform counts, respectively. In addition, 25 g or 1 slice of cheese was aseptically transferred into a sample bag (3M™), and 25 mL of BPW was added, and the mixture was homogenized for 120 s with a pummeler (BagMixer®, Interscience, France). One milliliter of the homogenate was serially diluted with BPW, and 0.1-mL diluents for TSA and 1-mL diluents for non-EHEC *E. coli* / Coliform Count petrifilm (3M™) were then surface-plated, respectively. The plates and petrifilms were incubated at 35°C for 24 h, and then the colonies were manually counted.

Initial level of contamination with non-EHEC *E. coli*

Beta distribution is a continuous probability distribution parametrized by two shape parameters (α_1 and α_2), and the interval of the distribution is zero to one (Johnson *et al*., 1995). When the number of positive samples is low, beta distribution can be used to estimate bacterial prevalence. The data on non-EHEC *E. coli* prevalence in cheese were fitted to a Beta distribution (α_1, α_2), where α_1 is the number of positive samples + 1, and α_2 is the number of all tested samples - positive samples + 1 (Vose, 1998). Uniform distribution is also a continuous probability distribution defined by the two parameters (*a* and *b*), and the distribution indicates equal probability in the range of two parameters. Because non-EHEC *E. coli* were detected under detection limit, initial concentration was assumed in the range of zero to detection limit. Thus, the data on the non-EHEC *E. coli* contamination level in cheese from cheese factory storage were fitted to a Uniform distribution (*a*, *b*), where *a* is the minimal contamination level, and *b* is the maximal contamination level. Finally, the initial contamination level (Log CFU/g) was calculated by prevalence × contamination level using the @RISK software, version 5.7 (Palisade Corp., USA).

Non-EHEC *E. coli* growth during distribution and storage

To calculate non-EHEC *E. coli* growth during distribution and storage, predictive models for natural and processed cheeses from a study by MFDS (2013) were used as follows.

<Natural cheese>

$$\mu_{max} = [0.00268 \times (T - 5.4235)]^2 \quad (1)$$

$$LPD = \left[\frac{1}{(-0.0522 + 0.0142 \times T)}\right]^2 \quad (2)$$

<Processed cheese>

$$\mu_{max} = 0.0036 - 0.0030 \times T + 0.0004 \times T^2 \quad (3)$$

$$LPD = \left[\frac{1}{-0.0826 + 0.0275 \times T}\right]^2 \quad (4)$$

The μ_{max} (Log CFU/g) is the maximum specific growth rate, *LPD* (h) is lag phase duration, and *T* (°C) is temperature. In addition, to simulate non-EHEC *E. coli* growth under changing temperature and time, probabilistic distributions for temperature and time from a study by Lee *et al*. (2015) were used.

Cheese consumption

Data on cheese consumption and intake frequency of cheese were taken from the study of Lee *et al*. (2015) to calculate the non-EHEC *E. coli* risk as a result of cheese consumption in Korea. According to a study by Lee *et al*. (2015), the mean consumption amounts of natural cheese and processed cheese are 12.40±19.43 g/d (95% confidence interval: 0.915-34.90 g/d) and 19.46±14.39 g/d (95% confidence interval: 2.6-40.0 g/d), respectively, and the consumption frequencies of cheese are 0.0389 and 0.0232 for natural and processed cheese, respectively. The ratios were fitted to the Discrete distribution{(0,1), [1 – (daily frequency of consumption), daily frequency of consumption]} (Lee *et al*., 2015). Finally, ingested *E. coli* cell counts were calculated as a result of consuming natural or processed cheese from the final concentration at the time of consumption taking into account the consumption amount and frequency.

The dose-response model

Twenty-eight dose-response models for *E. coli* infection were surveyed from other studies. Because about 90% of *E. coli* foodborne illness in Korea occurred by EPEC (Hong *et al*., 2005), the following dose-response model developed by Powell *et al*. (2000) for EPEC was used in this study.

$$P = 1 - \left\{1 + \left(\frac{D \times [(2^{1/\alpha} - 1)]}{N_{50}}\right)\right\}^{-\alpha} \quad (5)$$

Where *P* is the probability of illness, *D* is the ingested *E. coli* cell number (CFU/serving), N_{50} is the dose infecting 50% of the population with *E. coli*, and α is a coefficient.

Risk characterization

The results of the exposure assessment, dose-response model, and cheese consumption amount and frequency were used to estimate the risk of non-EHEC *E. coli* in cheese by means of a simulation in software @RISK according to the scheme of the simulation model in Fig. 1. In the simulation for risk characterization, the sampling type was Median Latin Hypercube, and the generator seed was random with settings for 10,000 iterations. Tables 1 and 2 show simulation models and formulas for calculating the risk of non-EHEC *E. coli* in natural and processed cheeses by means of @RISK. Sensitivity analysis to determine factors influencing the risk was also conducted in @RISK.

Results and Discussion

Hazard identification of *E. coli* in cheese

Pathogenic *E. coli* causes diarrhea in infants or acute enteritis in adults (MFDS, 2010). Although ground beef and fresh vegetables are considered major vectors for pathogenic *E. coli* (MFDS, 2010), there are several reports about *E. coli* isolated from various cheeses in many countries. The most frequently isolated *E. coli* serotype in cheese is *E. coli* O157:H7 in many countries (BCCDC, 2013; CDC, 2010; Honish *et al*., 2005), but other pathotypes such as EPEC, ETEC, and EAEC were also isolated from various cheeses (Baranceli *et al*., 2014; Bonyadian *et al*., 2014; Najand and Ghanbarpour, 2006). In addition, the most frequently isolated pathotype in Korea in various foods is EPEC (Hong *et al*., 2015). Thus, after non-EHEC *E. coli* was identified as a hazard in cheese, subsequent quantitative microbial risk assessment for natural and processed cheeses was conducted.

Initial level of non-EHEC *E. coli*

Non-EHEC *E. coli* cell counts were found to be below the detection limit (natural cheese: 2 CFU/g; processed

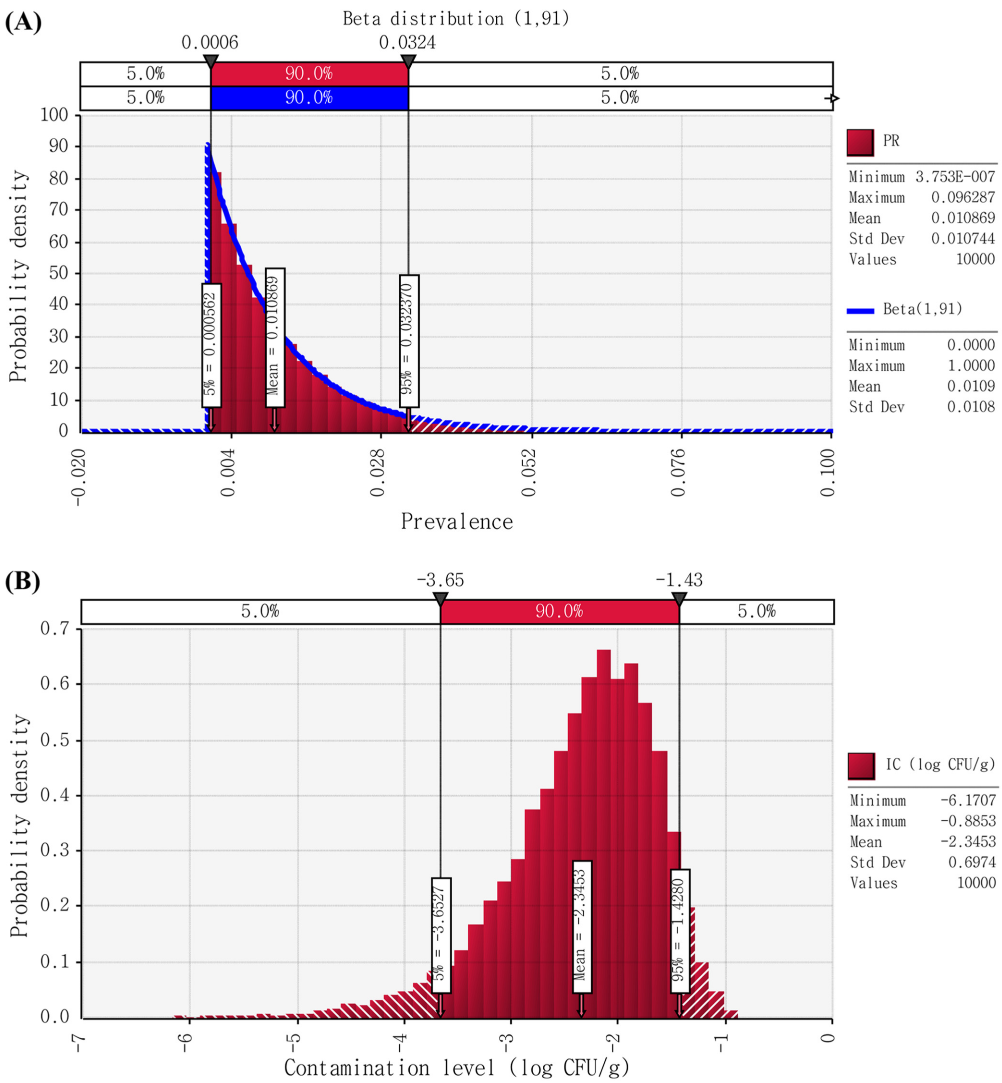

Fig. 1. Fitted Beta distribution (A) and probability density (B) of the simulated initial level of contamination with *Escherichia coli* in natural cheese.

Table 1. The simulation model and formulas in a Microsoft Excel® spreadsheet used to calculate the risk of illness of *Escherichia coli* in natural cheese by means of the @RISK software

Input Model	Unit	Code	Formula	References
		PRODUCT		
Product				
Pathogen Contamination level				
Non-EHEC *E. coli* prevalence		PR	=RiskBeta(1,91)	Vose (1998)
Concentration	CFU/g	C	=RiskUniform(0,2)	Vose (1998)
Initial contamination level	CFU/g	IC	=PR×C	Vose (1998)
	Log CFU/g	log(IC)	=log(PR×C)	
		TRANSPORTATION		
Transportation time	h	$time_{trans}$	=RiskPert(1,3,6)	Personal communication[a]
Food temperature during transportation	°C	$Temp_{trans}$	=RiskPert(0,4,10)	Personal communication[a]

Table 1. The simulation model and formulas in a Microsoft Excel® spreadsheet used to calculate the risk of illness of *Escherichia coli* in natural cheese by means of the @RISK software (Continued I)

Input Model	Unit	Code	Formula	References
Growth				
h_0	Log CFU/g	h_0	=average(growth rate×lag time), Fixed 2.26	MFDS (2013), Baranyi and Roberts (1994)
Y_0	Log CFU/g	Y_0	=average($Y_0 i$), Fixed 3.36	MFDS (2013), Baranyi and Roberts (1994)
Y_{end}	Log CFU/g	Y_{end}	=average($Y_{end} i$), Fixed 9.04	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	$Trans_{Lt}$	=IF{$Temp_{trans}$>4, $[1/(-0.0522+0.0142\times Temp_{trans})]^2$, 1320}	MFDS (2013)
Growth rate	Log CFU/g/h	$Trans_{Gr}$	=IF{$Temp_{trans}$>5.4235, $[0.0268\times(Temp_{trans}-5.4235)]^2$, 0}	MFDS (2013), Ratkowsky *et al*. (1982)
Non-EHEC *E. coli* growth	Log CFU/g	C1	=IC+1/{1+EXP[-ln(q)]}× [1-$10^{-\lvert Y0-Yend\rvert}$/LN(10)]×$Trans_{Gr}$×$time_{trans}$	MFDS (2013), Baranyi and Roberts (1994)
MARKET				
Market storage				
Storage time	h	$Mark\text{-}time_{st}$	=RiskPert(0,2,48)	Personal communication[b]
Food temperature during storage	ºC	$Mark\text{-}Temp_{st}$	=RiskUniform(2,4)	Personal communication[b]
Growth				
h_0	Log CFU/g	h_0	=average(growth rate×lag time), Fixed 2.26	MFDS (2013), Baranyi and Roberts (1994)
Y_0	Log CFU/g	Y_0	=average($Y_0 i$), Fixed 3.36	MFDS (2013), Baranyi and Roberts (1994)
Y_{end}	Log CFU/g	Y_{end}	=average($Y_{end} i$), Fixed 9.04	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	$Mark_{st}\text{-}Time_{Lt}$	=IF{Mark–$Temp_{st}$>4, [1/(-0.0522+0.0142×Mark–$Temp_{st}$)]^2,1320}	MFDS (2013)
Growth rate	Log CFU/g/h	$Mark_{st}\text{-}R_{Gr}$	=IF{Mark–$Temp_{st}$>5.4235, [0.0268×(Mark–$Temp_{st}$-5.4235)]^2,0}	MFDS (2013), Ratkowsky *et al*. (1982)
Non-EHEC *E. coli* growth	Log CFU/g	C2–1	=C1+1/{1+EXP[-ln(q)]}×[1-$10^{-\lvert Y0-end\rvert}$/ LN(10)]×$Mark_{st}$ –R_{Gr}×Mark–$time_{st}$	MFDS (2013), Baranyi and Roberts (1994)
Market display				
Storage time	h	$Mark\text{-}time_{dis}$	=RiskPert(0,48,168)	Personal communication[b]
Food temperature during storage	ºC	$Mark\text{-}Temp_{dis}$	=RiskTriang(0.60703,4.1000,15.18)	Lee *et al*. (2015)
Growth				
h_0	Log CFU/g	h_0	=average(growth rate×lag time), Fixed 2.26	MFDS (2013), Baranyi and Roberts (1994)
Y_0	Log CFU/g	Y_0	=average($Y_0 i$), Fixed 3.36	MFDS (2013), Baranyi and Roberts (1994)
Y_{end}	Log CFU/g	Y_{end}	=average($Y_{end} i$), Fixed 9.04	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	$Mark_{dis}\text{-}Time_{Lt}$	=IF{Mark–$Temp_{dis}$>4, [1/(-0.0522+0.0142×Mark–$Temp_{dis}$)]^2,1320}	MFDS (2013)
Growth rate	Log CFU/g/h	$Mark_{dis}\text{-}R_{Gr}$	=IF{Mark–$Temp_{dis}$>5.4235, [0.0268×(Mark–$Temp_{dis}$-5.4235)]^2,0}	MFDS (2013), Ratkowsky *et al*. (1982)
Non-EHEC *E. coli* growth	Log CFU/g	C2	=(C2–1)+1/{1+EXP[-ln(q)]}×[1-$10^{-\lvert Y0-end\rvert}$/ LN(10)]×$Mark_{dis}$–$R_{Gr}$×Mark–$time_{dis}$	MFDS (2013), Baranyi and Roberts (1994)

Table 1. The simulation model and formulas in a Microsoft Excel® spreadsheet used to calculate the risk of illness of *Escherichia coli* in natural cheese by means of the @RISK software (Continued II)

Input Model	Unit	Code	Formula	References
			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 2.26	MFDS (2013), Baranyi and Roberts (1994)
Y_0	Log CFU/g	Y_0	=average($Y_0 i$), Fixed 3.36	MFDS (2013), Baranyi and Roberts (1994)
Y_{end}	Log CFU/g	Y_{end}	=average($Y_{end} i$), Fixed 9.04	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-$Time_{Lt}$	=IF{$Temp_{car}$>4, [1/(-0.0522+0.0142×$Temp_{car}$)]^2,1320}	MFDS (2013)
Growth rate	Log CFU/g/h	Car-R_{Gr}	=IF{$Temp_{car}$>5.4235, [0.0268×($Temp_{car}$-5.4235)]^2,0}	MFDS (2013), Ratkowsky *et al.* (1982)
Non-EHEC *E. coli* growth	Log CFU/g	C3	=C2+1/{1+EXP[-ln(q)]}×[1-$10^{-\|Y0-Yend\|}$/ 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)]	Lee *et al.* (2015)
Food temperature during storage	°C	Home-$Temp_{st}$	=RiskLogLogistic[-29.283, 33.227, 26.666, RiskTruncate(-5,20)]	Lee *et al.* (2015)
Growth				
h_0	Log CFU/g	h_0	=average(growth rate×lag time), Fixed 2.26	MFDS (2013), Baranyi and Roberts (1994)
Y_0	Log CFU/g	Y_0	=average($Y_0 i$), Fixed 3.36	MFDS (2013), Baranyi and Roberts (1994)
Y_{end}	Log CFU/g	Y_{end}	=average($Y_{end} i$), Fixed 9.04	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}	=IF{Home–$Temp_{st}$>4, [1/(-0.0522+0.0142×Home–$Temp_{st}$)]^2,1320}	MFDS (2013)
Growth rate	Log CFU/g/h	Home-R_{Gr}	=IF{Home–$Temp_{st}$>5.4235, [0.0268×(Home–$Temp_{st}$-5.4235)]^2,0}	MFDS (2013), Ratkowsky *et al.* (1982)
Non-EHEC *E. coli* growth	Log CFU/g	C4	=C3+1/{1+EXP[-ln(q)]}×[1-$10^{-\|Y0-Yend\|}$/ LN(10)]×Home–R_{Gr}×Home–$time_{st}$	MFDS (2013), Baranyi and Roberts (1994)
			CONSUMPTION	
Daily consumption average amount	g	Consump	=RiskPearson5[2.6488, 25.81, RiskTruncate(0,100),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	=RiskDiscrete[{0,1},{CF(0),CF(1)}]	MFDS (2013)
		ConFre	=IF(CF=0,0,Consump)	MFDS (2013)
			DOSE-RESPONSE	
Non-EHEC *E. coli* amount		D	=10^{C4}×ConFre	
Parameter of α		α	=Fixed 2.21×10^{-1}	Powell (2000)
Parameter of N_{50}		N_{50}	=Fixed 6.85×10^7	Powell (2000)
RISK				
Probability of illness/person/day		Risk	=1-(1+{D×[($2^{1/\alpha}$)-1]/N_{50}})$^{-\alpha}$	Powell (2000)

[a]With a supervisor of a cheese manufacturing plant
[b]With a manager in charge of cheese products at markets

Table 2. The simulation model and formulas in a Microsoft Excel® spreadsheet used to calculate the risk of *Escherichia coli* in processed cheese by means of the @RISK software

Input Model	Unit	Code	Formula	References
			PRODUCT	
Product				
Pathogen Contamination level				
Non-EHEC *E. coli* prevalence		PR	=RiskBeta(1,309)	Vose (1998)
Concentration	CFU/g	C	=RiskUniform(0,2.8)	Vose (1998)
Initial contamination level	CFU/g	IC	=PR×C	Vose (1998)
	Log CFU/g	log(IC)	=log(PR×C)	
			TRANSPORTATION	
Transportation time	h	$time_{trans}$	=RiskPert(1,3,6)	Personal communication[a]
Food temperature during transportation	ºC	$Temp_{trans}$	=RiskPert(0,4,10)	Personal communication[a]
Growth				
h_0	Log CFU/g	h_0	=average(growth rate×lag time), Fixed 0.65	MFDS (2013), Baranyi and Roberts (1994)
Y_0	Log CFU/g	Y_0	=average($Y_0 i$), Fixed 3.11	MFDS (2013), Baranyi and Roberts (1994)
Y_{end}	Log CFU/g	Y_{end}	=average($Y_{end} i$), Fixed 7.32	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	$Trans_{Lt}$	=IF{$Temp_{trans}$>4, [1/(-0.0826+0.0275×Tempcar)]^2,1320}	MFDS (2013)
Growth rate	Log CFU/g/h	$Trans_{Gr}$	=IF($Temp_{trans}$>8.6,0.0036-0.0030×$Temp_{trans}$+ 0.0004×$Temp_{trans}^2$,0)	MFDS (2013), Ratkowsky *et al*. (1982)
Non-EHEC *E. coli* growth	Log CFU/g	C1	=IC+1/{1+EXP[-ln(q)]}×[1-$10^{-\lvert Y0-Yend\rvert}$/ LN(10)]×$Trans_{Gr}$×$time_{trans}$	MFDS (2013), Baranyi and Roberts (1994)
			MARKET	
Market storage				
Storage time	h	$Mark\text{-}time_{st}$	=RiskPert(0,2,48)	Personal communication[b]
Food temperature during storage	ºC	$Mark\text{-}Temp_{st}$	=RiskUniform(2,4)	Personal communication[b]
Growth				
h_0	Log CFU/g	h_0	=average(growth rate×lag time), Fixed 0.65	MFDS (2013), Baranyi and Roberts (1994)
Y_0	Log CFU/g	Y_0	=average($Y_0 i$), Fixed 3.11	MFDS (2013), Baranyi and Roberts (1994)
Y_{end}	Log CFU/g	Y_{end}	=average($Y_{end} i$), Fixed 7.32	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	$Mark_{st}\text{-}Time_{Lt}$	=IF{Mark–$Temp_{st}$>4, [1/(-0.0826+0.0275×Mark–$Temp_{st}$)]^2,1320}	MFDS (2013)
Growth rate	Log CFU/g/h	$Mark_{st}\text{-}R_{Gr}$	=IF(Mark–$Temp_{st}$>8.6,0.0036-0.0030× Mark–$Temp_{st}$+0.0004×Mark–$Temp_{st}^2$,0)	MFDS (2013), Ratkowsky *et al*. (1982)
Non-EHEC *E. coli* growth	Log CFU/g	C2–1	=C1+1/{1+EXP[-ln(q)]}×[1-$10^{-\lvert Y0-Yend\rvert}$/ LN(10)]×Mark–$R_{Gr}$×Mark–$time_{st}$	MFDS (2013), Baranyi and Roberts (1994)
Market display				
Storage time	h	$Mark\text{-}time_{dis}$	=RiskPert(0,48,168)	Personal communication[b]
Food temperature during storage	ºC	$Mark\text{-}Temp_{dis}$	=RiskTriang(0.60703,4.1000,15.18)	Lee *et al*. (2015)
Growth				
h_0	Log CFU/g	h_0	=average(growth rate×lag time), Fixed 0.65	MFDS (2013), Baranyi and Roberts (1994)

Table 2. The simulation model and formulas in a Microsoft Excel® spreadsheet used to calculate the risk of *Escherichia coli* in processed cheese by means of the @RISK software (Continued I)

Input Model	Unit	Code	Formula	References
Y_0	Log CFU/g	Y_0	=average($Y_0 i$), Fixed 3.11	MFDS (2013), Baranyi and Roberts (1994)
Y_{end}	Log CFU/g	Y_{end}	=average($Y_{end} i$), Fixed 7.32	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	$Mark_{dis}$-$Time_{Lt}$	=IF{Mark-$Temp_{dis}$>4, [1/(-0.0826+0.0275×Mark-$Temp_{dis}$)]^2,1320}	MFDS (2013)
Growth rate	Log CFU/g/h	$Mark_{dis}$-R_{Gr}	=IF(Mark-$Temp_{dis}$>8.6,0.0036-0.0030× Mark-$Temp_{dis}$+0.0004×Mark-$Temp_{dis}{}^2$,0)	MFDS (2013), Ratkowsky *et al*. (1982)
Non-EHEC *E. coli* growth	Log CFU/g	C2	=(C2–1)+1/{1+EXP[-ln(q)]}×[1-10$^{-\lvert Y0\text{-}Yend\rvert}$/ 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	ºC	$Temp_{car}$	=RiskPert(10,18,25)	Jung (2011)
Growth				
h_0	Log CFU/g	h_0	=average(growth rate×lag time), Fixed 0.65	MFDS (2013), Baranyi and Roberts (1994)
Y_0	Log CFU/g	Y_0	=average($Y_0 i$), Fixed 3.11	MFDS (2013), Baranyi and Roberts (1994)
Y_{end}	Log CFU/g	Y_{end}	=average($Y_{end} i$), Fixed 7.32	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-$Time_{Lt}$	=IF{$Temp_{car}$>4, [1/(-0.0826+0.0275×$Temp_{car}$)]^2,1320}	MFDS (2013)
Growth rate	Log CFU/g/h	Car-R_{Gr}	=IF($Temp_{car}$>8.6, 0.0036-0.0030×$Temp_{car}$+0.0004×$Temp_{car}{}^2$,0)	MFDS (2013), Ratkowsky *et al*. (1982)
Non-EHEC *E. coli* growth	Log CFU/g	C3	=C2+1/{1+EXP[-ln(q)]}×[1-10$^{-\lvert Y0\text{-}Yend\rvert}$/ 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)]	Lee *et al*. (2015)
Food temperature during storage	ºC	Home-$Temp_{st}$	=RiskLogLogistic[-29.283, 33.227, 26.666, RiskTruncate(-5,20)]	Lee *et al*. (2015)
Growth				
h_0	Log CFU/g	h_0	=average(growth rate×lag time), Fixed 0.65	MFDS (2013), Baranyi and Roberts (1994)
Y_0	Log CFU/g	Y_0	=average($Y_0 i$), Fixed 3.11	MFDS (2013), Baranyi and Roberts (1994)
Y_{end}	Log CFU/g	Y_{end}	=average($Y_{end} i$), Fixed 7.32	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}	=IF{Home–$Temp_{st}$>4, [1/(-0.0826+0.0275×Home–$Temp_{st}$)]^2,1320}	MFDS (2013)
Growth rate	Log CFU/g/h	Home-R_{Gr}	=IF(Home–$Temp_{st}$>8.6,0.0036-0.0030× Home–$Temp_{st}$+0.0004×Home–$Temp_{st}{}^2$,0)	MFDS (2013), Ratkowsky *et al*. (1982)
Non-EHEC *E. coli* growth	Log CFU/g	C4	=C3+1/{1+EXP[-ln(q)]}×[1-10$^{-\lvert Y0\text{-}Yend\rvert}$/ LN(10)]×Home–$R_{Gr}$×Home–$time_{st}$	MFDS (2013), Baranyi and Roberts (1994)

Table 2. The simulation model and formulas in a Microsoft Excel® spreadsheet used to calculate the risk of *Escherichia coli* in processed cheese by means of the @RISK software (Continued II)

Input Model	Unit	Code	Formula	References
			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	
Non-EHEC *E. coli* amount		D	$=10^{C4}\times$ConFre	
Parameter of α		α	$=$Fixed 2.21×10^{-1}	Powell (2000)
Parameter of N_{50}		N_{50}	$=$Fixed 6.85×10^{7}	Powell (2000)
RISK				
Probability of illness/person/day		Risk	$=1-(1+\{D\times[(2^{1/\alpha})-1]/N_{50}\})^{-\alpha}$	Powell (2000)

[a]With a supervisor of a cheese manufacturing plant
[b]With a manager in charge of cheese products at markets

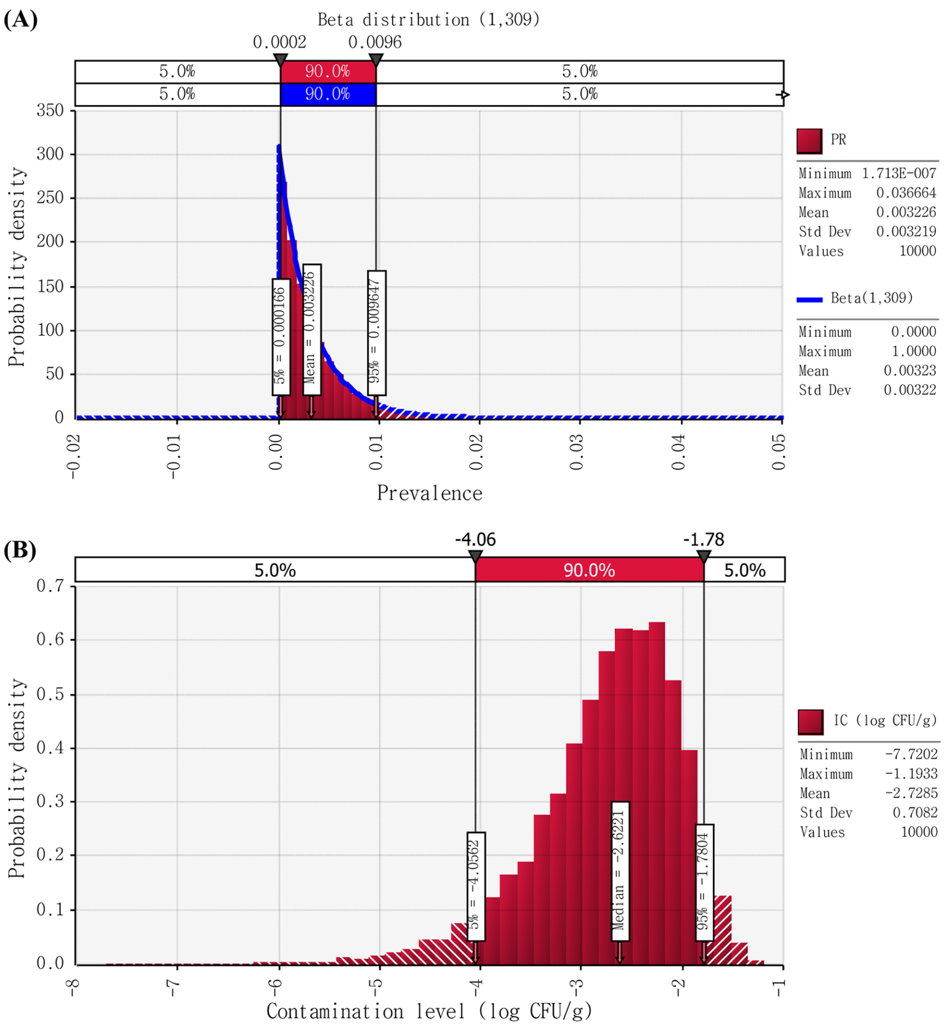

Fig. 2. Fitted Beta distribution (A) and probability density (B) of the simulated initial level of contamination with *Escherichia coli* in processed cheese.

cheese: 2.8 CFU/g) in all samples. Thus, it was assumed that non-EHEC *E. coli* cell counts in cheese to be above 0 CFU/g, but below the detection limit (2 CFU/g), and then we described contamination levels of the pathogen with Uniform distribution (0,2) and Uniform distribution (0,2.8) for natural and processed cheese, respectively (Figs. 2 and 3). Therefore, using the @RISK software, the initial contamination level of non-EHEC *E. coli* were calculated by Beta distribution(1,91) × Uniform distribution(0, 2), and Beta distribution(1,309) × Uniform distribution(0, 2.8) for natural and processed cheese, respectively. As a result of the simulation, the initial level of contamination with non-EHEC *E. coli* in cheese was 2.35 and -2.73 Log CFU/g for natural and processed cheese, respectively (Figs. 2 and 3).

Non-EHEC *E. coli* growth and cheese consumption

The cumulative distributions of non-EHEC *E. coli* growth during distribution and storage (initial concentration, concentration after transportation, concentration after storage in a market, concentration at the time of purchase, concentration when at home, and concentration at the time of consumption) were analyzed. As a result of the simulation, in natural cheese, the initial concentration was -2.35 Log CFU/g, and concentration at the final stage (at the time of consumption) was -2.31 Log CFU/g (data not shown). This result indicates that non-EHEC *E. coli* in natural cheese may not grow during distribution and storage under the conditions in Korea. In addition, non-EHEC *E. coli* growth probability in processed cheese was simi-

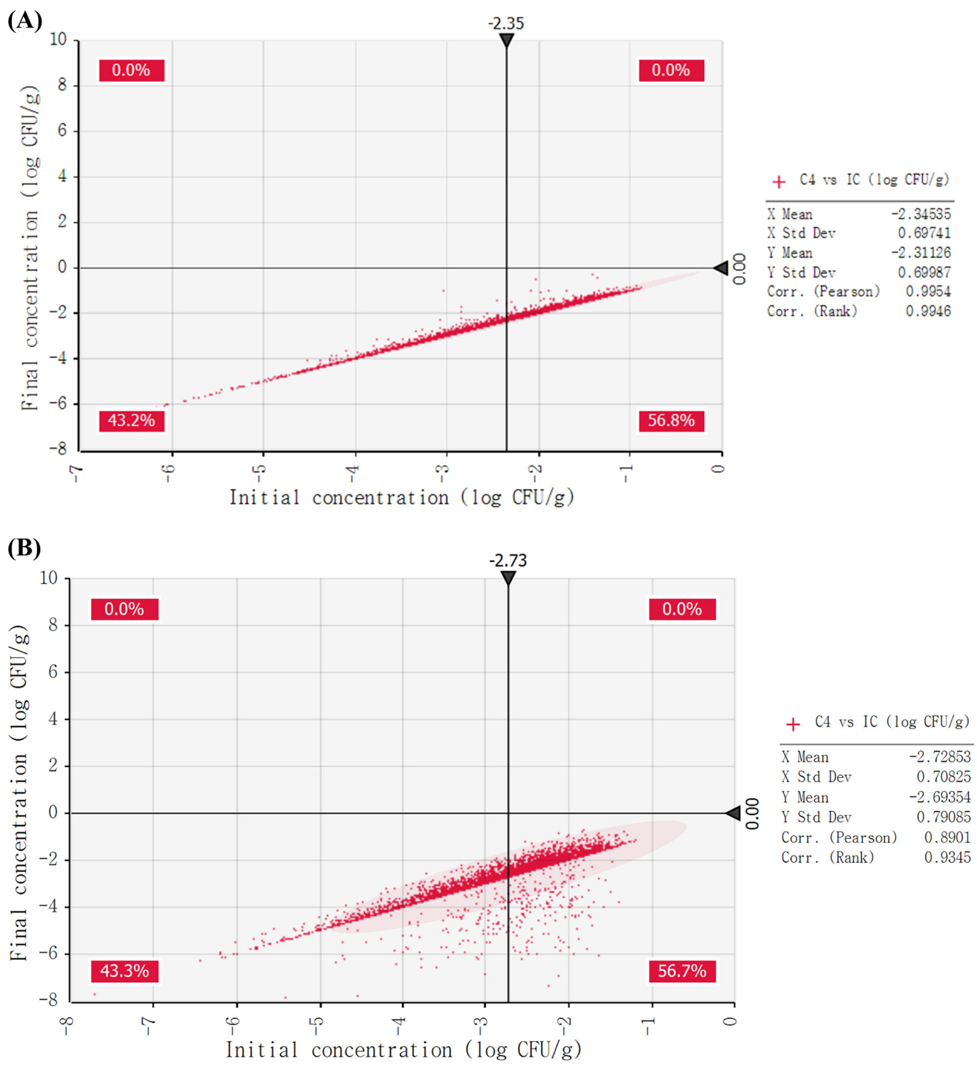

Fig. 3. The scatter plots of the initial concentration level versus the home consumption level in terms of *Escherichia coli* in natural (A) and processed cheese (B).

lar to that in natural cheese (data not shown). Moreover, the results of comparison of the initial concentration with final concentration indicate that none of the 10,000 iterations could yield more than 0 Log CFU/g at the point of final concentration (Fig. 4).

The dose-response model and risk characterization

After cheese consumption, to estimate the probability of non-EHEC *E. coli* foodborne illness, the Beta-Poisson model ($\alpha = 2.21\times10^{-1}$, $N_{50} = 6.85\times10^{7}$) was used (Powell *et al*., 2000). Subsequently, the simulation model was prepared with the values of input variables such as non-EHEC *E. coli* prevalence, temperature, and time for distribution and display in markets, and home storage, the amount of cheese consumption, and intake frequency as presented Tables 1 and 2. The simulations were conducted by random sampling from the distribution described above for 10,000 iterations, and the mean probabilities of a non-EHEC *E. coli* outbreak as a result of cheese consumption per person per day in Korea were 1.36×10^{-7} and 2.12×10^{-10} for natural and processed cheese, respectively (Table 3), which are higher than the risk (7.84×10^{10}) of *S. aureus* foodborne illness per person per day as a result of natural cheese consumption and the risk (3.64×10^{-9} to 1.30×10^{-7}) of listeriosis per person per day as a result of eating lettuce at a restaurant in Korea (Ding *et al*., 2013; Lee *et al*., 2015). These results indicate that natural cheese poses a high risk of a non-EHEC *E. coli* outbreak as compared to processed-cheese-related and *S. aureus*-related

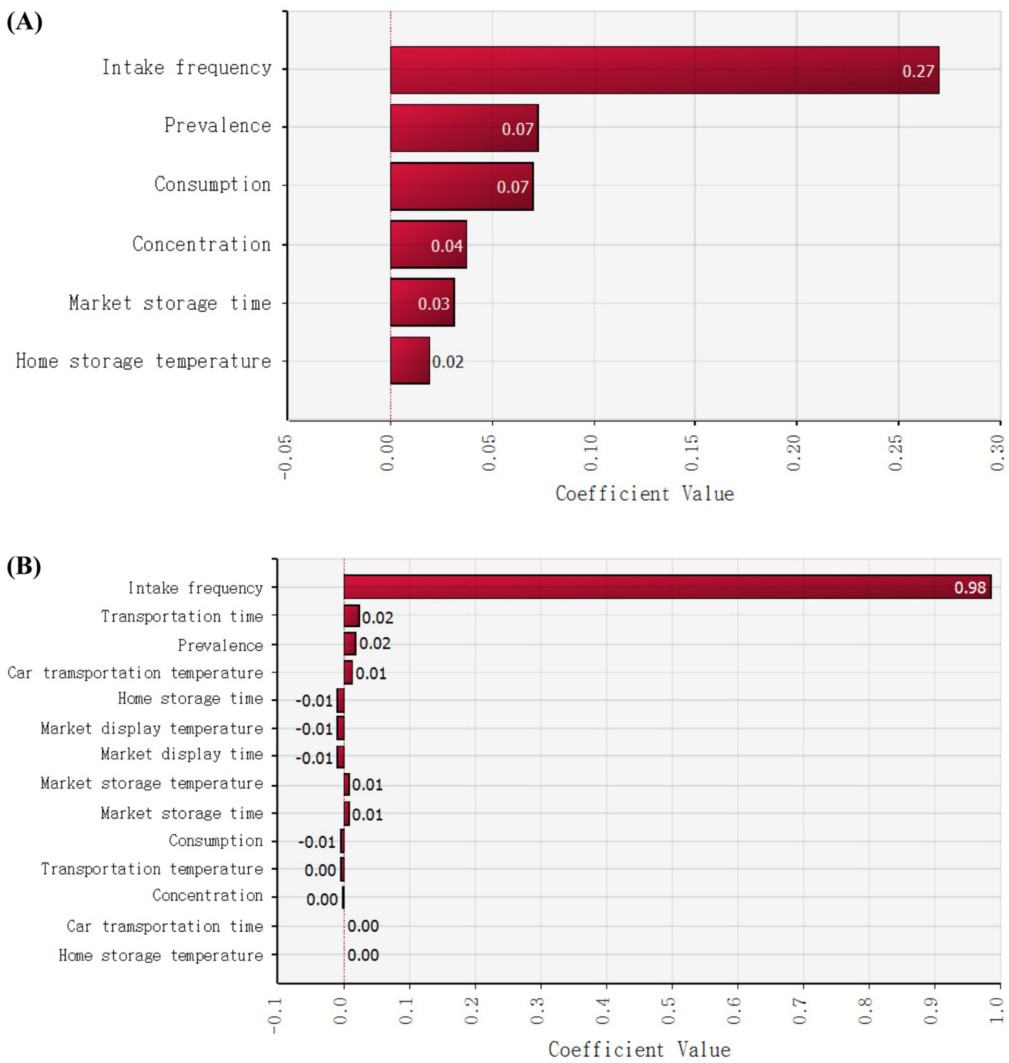

Fig. 4. The regression coefficient (A) and the correlation coefficient (B) values for the sensitivity risk factor affecting the probability of illness per person per day as a result of consumption of natural cheese.

Table 3. Probability of foodborne illness caused by *Escherichia coli* per person per day as a result of consumption of natural and processed cheeses

Probability of illness/ (person · d)	5%	25%	50%	95%	99%	Maximum	Mean
Natural cheese	0	0	0	0	3.34×10^{-6}	2.26×10^{-4}	1.36×10^{-7}
Processed cheese	0	0	0	0	4.59×10^{-9}	1.20×10^{-7}	2.12×10^{-10}

foodborne illnesses as a result of natural cheese consumption and listeriosis as a result of lettuce consumption in Korea. In addition, sensitivity analysis revealed that intake frequency was the most influential factor for this risk, whereas the other factors such as storage temperature and time were not obviously related (Fig. 5).

Thus, our results indicate that non-EHEC *E. coli* cannot grow in natural and processed cheeses under the present distribution and storage conditions, and that a different factor is more important for the risk of illness. Consumption frequency of processed cheese is lower than that of natural cheese, if we assume that the consumption amount of natural and processed cheese is similar. Accordingly, processed cheese poses a lower risk than natural cheese for non-EHEC *E. coli*.

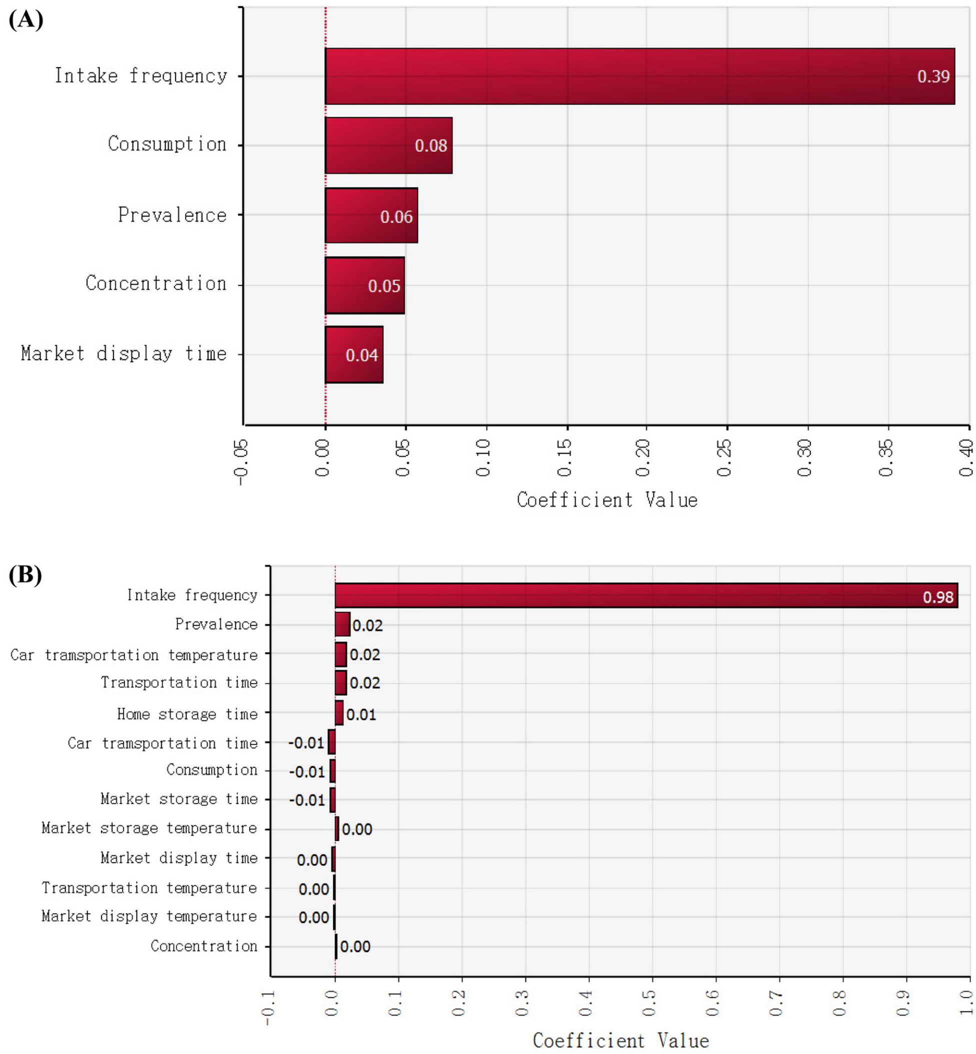

Fig. 5. The regression coefficient (A) and the correlation coefficient (B) values for the sensitivity risk factor affecting the probability of illness per person per day as a result of consumption of processed cheese.

Conclusion

The risk of a non-EHEC *E. coli* outbreak via cheese consumption seems to be low for natural and processed cheese in Korea, and the intake frequency of cheese is the most influential factor for this risk. In addition, the microbial risk assessment model that we developed in this study can be useful for quantitative risk assessment.

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