

# QUANTITATIVE MICROBIAL RISK ASSESSMENT OF FERMENTED BEAN PASTES CONTAMINATED WITH CLOSTRIDIUM PERFRINGENS

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ARTICLE INFO	ABSTRACT
Received 17. 10. 2020 Revised 8. 9. 2021 Accepted 14. 9. 2021 Published 1. 4. 2023	This study evaluated <i>Clostridium perfringens</i> risk in fermented bean pastes. The prevalence in fermented bean pastes, development of the predictive model, temperature, time, and consumption data were investigated. A simulation model was prepared in @RISK with the obtained data to calculate the probability of foodborne <i>C. perfringens</i> foodborne illness from fermented bean paste consumption. Only 74 of the 1,097 samples were positive for <i>C. perfringens</i> . The initial contamination level was estimated to be 0.7 log CFU/g. It showed the probability of foodborne <i>C. perfringens</i> infection per person per day upon consumption of fermented bean pastes was $8.0 \times 10^{-12}$ , which is considered low risk.
	Keywords: foodborne illness, quantitative microbial risk assessment, <i>Clostridium perfringens</i> , fermented bean pastes, predicted model
INTRODUCTION	t could be grouped together based on similar physiochemical properties to conduc

*Clostridium perfringens* is the fourth most prevalent cause of foodborne illness in Korea, and one of the most common cause of foodborne diseases in the United States of America (CDC, 2018; MFDS, 2020). *C. perfringens* is widely distributed in the environment, food, and the excrement of humans and animals (Han et al., 2007; Heo et al., 2018; Juneja et al., 2006; Songer, 1996). It is a gram-positive anaerobic spore-forming bacterium (FDA, 2017). Vegetative cells of *C. perfringens* mostly die during food preparation, but *C. perfringens* spores can survive extreme conditions. Clinical symptoms of *C. perfringens* infection include diarrhea and abdominal pain. Foodborne disease resulting from *C. perfringens* infection is caused by the production of *Clostridium perfringens* enterotoxin (CPE), which affects the intestines when more than 10<sup>6</sup> vegetative cells or spores of *C. perfringens* are consumed (McDonel, 1980; MFDS, 2016).

In Korea, fermented bean pastes have been consumed for many years. In addition, the export of these pastes has recently increased (KAT, 2016). Thus, to meet the safety requirements for consumption and export, studies, especially those investigating fungal toxins and biogenic amines present in these pastes, have been conducted (Han et al., 2007; Lee et al., 2009; Sivamaruthi et al., 2018). Park (2013) detected *C. perfringens* in three traditional bean-based sauce samples (20%) and suggested that there is a possibility for *C. perfringens* contamination in fermented bean pastes. Thus, we decided to evaluate the risk of *C. perfringens* contamination in fermented bean pastes.

To evaluate the risk of *C. perfringens* contamination in foods, quantitative microb ial risk assessment (QMRA) is most appropriate (Jeong et al., 2018; WHO, 2002). QMRA involves hazard identification, exposure assessment, hazard characteriz ation, and risk characterization (CAC, 2015; Lee et al., 2019). However, complet ing QMRA for one pathogen with one food is both time-consuming and expensiv e. There are four fermented bean pastes [*Doenjang* (Korean fermented soy paste), *Cheonggukjang* (Korean short-fermented bean paste), *Gochujang* (Korean red pe pper paste), and *Ssamjang* (mixed paste containing *Gochujang*, *Doenjang*, and ot her ingredients)] in Korea. Thus, if a set of various fermented bean pastes with si milar physiochemical properties undergoes QMRA assuming a worst case scenari o with regard to *C. perfringens* contamination, it can be assumed that the risk of c on suming the entire set of fermented ban pastes; this can save both time and expense es. Therefore, the objective of this study was to identify fermented bean pastes tha t could be grouped together based on similar physiochemical properties to conduc t QMRA assuming a worst case scenario with regard to *C. perfringens* contaminat ion and to identify the fermented bean paste with the lowest death rate of *C. perfringens* during storage and cooking and the one with the highest consumption amo unt and frequency.

# MATERIALS AND METHODS

### Evaluation of prevalence and initial contamination

To evaluate the prevalence of *C. perfringens* in fermented bean pastes. 1,097 ferm ented bean pastes samples [*Doenjang* (n=324), *Ssamjang* (n=299), *Gochujang* (n=359), and *Hansik-doenjang* (n=115)] were purchased from supermarkets and traditional markets in Korea. Twenty-five-gram portions of the fermented bean pastes were placed in filter bags (Circulator 400 standard bags; Seward, Worthing, UK) and 225 mL of sterile 0.85% saline was added to the filter bag. The samples were then homogenized for 2 min using a pummeler (Seward, Worthing, UK). Homoge nate (1 mL) and diluent (100  $\mu$ L) aliquots were dispersed on tryptose sulphite cyc loserine agar (TSC; Difco, Sparks, MD, USA) plates and incubated at 37 °C for 1 8–24 h in an aerobic jar with an anaerobic pack (Oxoid, Basingstoke, Hampshire, UK). The colonies were then analyzed using the VITEK\*2 ANC ID card (BioMér ieux, Marcy l'Etoile, France). It was concluded that the result was positive for *C. perfringens* if it was acceptable or higher. @RISK (Palisade Corp., Ithac, NY, USA) was used to estimate the appropriate probabilistic distribution from the prevalen ce data.

### Determination of distribution and storage temperature and time

Storage temperature and time data for the distribution of fermented bean pastes to the market, market storage, and market display were collected through personal c ommunication with managers.

#### Survey of consumption pattern

According to the results from the Korea National Health and Nutrition Examinati on Survey (**KCDC**, 2017), *Doenjang* was the most consumed fermented bean pas te. Thus, the data regarding the amount of *Doenjang* consumption were analyzed using the @RISK to determine an appropriate probabilistic distribution. The cons umption frequency for *Doenjang* was estimated by dividing the number of total re spondents (6,628 people) in the survey by the number of respondents (2,743 peop le) who consumed *Doenjang*.

## Evaluation of kinetic behavior of C. perfringens in fermented bean pastes

For C. perfringens strains, KCCM12098, KCCM40946, KCCM40947, and KCTC 5101 with toxin-producing genes were used. Four C. perfringens strains were cult ured in 10 mL cooked meat medium (Oxoid, Basingstoke, Hampshire, UK) at 37° C for 24 h under anaerobic conditions created using an anaerobic pack, and 3 mL aliquots of the culture were inoculated in 30 mL Duncan and strong medium (15.0 g proteose peptone, 10.0 g sodium phosphate, 4.0 g raffinose, 4.0 g yeast extract, 1.0 g sodium thioglycolate, and 50.0 mL 0.51 mM caffeine in 1 L distilled water) and anaerobically incubated at 37°C for 48 h. The C. perfringens strains were har vested by centrifugation at 1,912× g and 4°C for 15 min. The supernatant was disc arded and the cells were washed twice with phosphate buffered saline (PBS; pH 7 .4, 8.0 g NaCl, 1.5 g NaHPO<sub>4</sub>, 0.2 g KH<sub>2</sub>PO<sub>4</sub>, and 0.2 g KCl in 1 L distilled water ). To make the inoculum, the cell pellet was resuspended in 3 mL PBS and the str ains were mixed together (Seo et al., 2021). C. perfringens inoculum (4 mL) was added to 400 g Cheonggukjang to achieve a final concentration of 3.0 log CFU/g; the inoculated samples were placed in sample bags (3M, St. Paul, MN, USA) and stored at 7, 15, 25, and 35°C for up to 10 days. The physicochemical properties (p H and Aw) of Cheonggukjang (pH=6.96, Aw=0.934) offered better growth condit ions for C. perfringens than those of other fermented bean pastes [Doeniang (pH= 5.20, Aw=0.827), Gochujang (pH=4.79 Aw=0.79), and Ssamjang (pH=5.29-5.78) (Kim and Lee, 2001; Kim et al., 2016)]. Thus, C. perfringens in Cheonggukjang s urvived longer than in Doenjang. During storage, 10 g portions of the Cheongguk jang were placed into sample bags with 20 mL buffered peptone water (Dickinson and Company, Sparks, MD, USA). The homogenates were spread on TSC agar, w hich were then incubated anaerobically at 37°C for 24 h, and typical black colonie s with halos sére counted. The kinetic behavior of *C. perfringens* was evaluated vi a development of a primary model with the Weibull model parameters  $[\log(N) = 1 \log(N_0) - (time/\delta)^{\circ}$ ; N, cell counts; N<sub>0</sub>, initial cell counts;  $\delta$ , treatment time for the fi rst decimal reduction;  $\rho$ , curve shape], and a secondary model with an exponentia l model [ $\delta = a \times exp(k \times T)$ ; a, constant; k, the rate constant; T, storage temperature] was developed as a function of temperature. To evaluate the model performance, *C. perfringens* was inoculated in *Cheonggukjang*, as described above, and the sam ples were stored at 20 and 30°C. During storage, *C. perfringens* cells were counte d as described above to obtain the observed values. The observed values were co mpared to the values derived using the predictive models. The observed values w ere compared to the values derived using the prediction models, and the differenc es between the observed and predicted data were indicated by the root mean squar e error (*RMSE*) values (**Ross, 1996**).

## Determination of dose-response model

Dose response models were searched through published literatures to estimate the probability of foodborne illness after *C. perfringens* consumption.

## Preparation of simulation model

A simulation model was prepared in Excel<sup>®</sup> (Microsoft Corp., Redmond, WA, US A) spreadsheet with the initial concentration of *C. perfringens*, predictive models, probabilistic distributions for time and temperature used in the predictive models t o describe the bacterial growth, a probabilistic distribution of consumption amoun t of *Doenjang*, the most consumed fermented bean paste, and consumption freque ncy, followed by the dose-response model. By Monte Carlo simulation in @RISK , the probability of foodborne *C. perfringens* foodborne illness by fermented bean paste consumption per person per day and the correlation coefficient were calcula ted (Tab 1).

Input model	Unit	Variable	Formula	References
PRODUCT				
Pathogens contamination level				
C. perfringens concentration	CFU/g	PR	=RiskExpon(0.95716,RiskShift(0.99913), RiskTruncate(0,1000))	This research
	CFU/g	С	=IF(<10, RiskUniform(1,10), PR)	This research
	log CFU/g	IC	=log(C)	This research
MARKET TRANSPORTATION				
Transportation time	h	Time <sub>trans</sub>	=RiskPert(0.5,3,5)	This research
Food temperature during transportation	°C	Temp <sub>trans</sub>	=RiskWeibull(1.3219,2.8404,RiskShift(3.1093),Risktruncate(1,40))	This research
Death				
Delta		δ	$=1/(0.007+0.001\times exp(0.1108\times Temp_{trans}))$	This research
ρ		ρ	Fixed 0.7310	This research
C. perfringens survival model		C1	=IC-(Time <sub>trans</sub> / $\delta$ ) <sup><math>\rho</math></sup>	Mafart et al., 2002
Market storage				
Storage time	h	Time <sub>st</sub>	=RiskUniform(0.1,48)	This research
Food temperature during storage	°C	Temp <sub>st</sub>	=RiskPert(22,25,26)	This research
Death				
Delta		δ	$=1/(0.007+0.001\times exp(0.1108\times Temp_{st}))$	This research
ρ		ρ	Fixed 0.7310	This research
C. perfringens growth model		C2	=C1-(Time <sub>st</sub> $\delta)^{\rho}$	Mafart et al., 2002
MARKET DISPLAY				
Display time	h	Time <sub>dis</sub>	=RiskUniform(0,336)	This research
Food temperature during display	°C	Temp <sub>dis</sub>	=RiskLogLogistic(16.608,5.6198,14.584,Risk Truncate(1,40))	This research
Death				
Delta		δ	$=1/(0.007+0.001\times exp(0.1108\times Temp_{dis}))$	This research
ρ		ρ	Fixed 0.7310	This research
C. perfringens growth model		C3	=C2-(Time <sub>dis</sub> / $\delta$ ) <sup><math>\rho</math></sup>	Mafart et al., 2002
COMSUMPTION				
Daily consumption average amount	g	Consump	=RiskExpon(12.114, RiskShift(0.0055837), RiskTruncate(0,350))	KCDC, 2017
Daily consumption frequency	%	ConFre	Fixed 41.4	KCDC, 2017
		CF(0)	=1-41.4/100	KCDC, 2017
		CF(1)	=41.4/100	KCDC, 2017
		CF	=RiskDiscrete({0,1},{CF(0),CF(1)})	KCDC, 2017
		Amount	=IF(CF=0,0,Consump)	KCDC, 2017
DOSE-RESPONSE				
C. perfringens amount	CFU	Ν	=10 <sup>C3</sup> ×Amount	
Parameter of r		r	=Fixed 1.82×10 <sup>-11</sup>	Golden et al., 2009
RISK				
Probability of illness/person/day		Risk	$=1-\exp(-r\times N)$	Golden et al., 2009

## **RESULTS AND DISCUSSION**

# Initial contamination level of C. perfringens

Of the 1,097 samples tested, only 74 samples were *C. perfringens* positive, and 93 .3% of samples had a bacterial load below the detection limit (1 log CFU/g). Thus, only cell counts for the positive samples were analyzed by @RISK, and the expo nential distribution [Riskexpon (Riskexpon (0.95716, RiskShift (0.99913))] was d etermined as an appropriate probabilistic distribution. To estimate the initial conta mination levels, for *C. perfringens* positive samples, the exponential distribution was determined, otherwise data were extracted between 0 and 1 log CFU/g becau se "below detection" means the cell counts were between 0 and 1 log CFU/g. Wit h this simulation, the mean of the initial *C. perfringens* contamination level in fer mented bean pastes was estimated to be 0.7 log CFU/g (Fig 1).

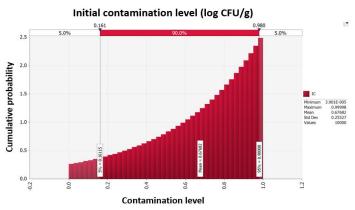


Figure 1 Probability density for simulated initial contamination level of *Clostridium perfringens* in fermented bean pastes.

# Temperature and time for transportation and storage

Transportation and storage time and temperature for fermented bean pastes were d etermined through survey or interview with market managers. The probabilistic di stribution for the temperature of the transport vehicle was appropriate with the W eibull distribution [RiskWeibull(1.3219,2.8404,Riskshift(3.1093),RiskTruncate(1, 40))] and the Pert distribution was appropriate for transportation time. because the transportation time was 0.5–5 h. According to the probabilistic distribution, when

the fermented bean pastes were transported from the site of production to the site of sale, the mean temperature was  $5.7^{\circ}$ C. According to market staff, the products were stored at  $22-26^{\circ}$ C using the automatic control system for 0.1-48 h. Hence, t he temperatures were fitted to the Pert distribution [RiskPert (22,25,26)] because t he minimum, mode, and maximum temperatures were 22, 25, and  $26^{\circ}$ C, respectiv ely. And, the storage time was fitted to the uniform distribution [RiskUniform(0.1,48)]. The probabilistic distribution for the display temperature was adequate with loglogistic distribution [RiskLogLogistic(16.608,5.6198,14.584,RiskTruncate(1,40))] and the uniform distribution [RiskUniform(0,336)] was appropriate for display time distribution because the display time was 0-336 h.

#### Consumption of fermented bean pastes

Of the fermented bean pastes consumed widely in Korea, *Doenjang* was the most consumed. Hence, the raw data for daily consumption amounts of *Doenjang* deriv ed from KNHANES (**KCDC**, 2017) were fitted with @RISK. "Exponential distribution (12.114, Riskshift (0.0055837))" was selected to be the appropriate probab ilistic distribution to describe the consumption of fermented bean pastes and the p robabilistic distribution showed that the average daily consumption of *Doenjang* was 12.12 g, with the consumption frequency being 41.4% in Korea.

## Kinetic behavior of C. perfringens during distribution

The prediction model was developed to describe the kinetic behavior of C. perfrin gens in fermented bean pastes during transportation, storage, and display. To sele ct products for predictive model development, survival of C. perfringens contami nants was compared among fermented bean pastes during storage, and Cheonggu kjang had the slowest decrease in cell counts. Thus, the predictive model was dev eloped with Cheonggukjang. Cell counts of C. perfringens were enumerated at 7, 15, 25, and 35°C. The Weibull model was then used to fit the data and calculate  $\delta$ and  $\rho$  values.  $\delta$  decreased as temperature increased.  $R^2$  values were 0.833-0.944, i ndicating that the developed model was appropriate. The p values ranged from 0.4 7 to 1.20 (Tab 2). To evaluate the effect of temperature on the  $\delta$  values, a seconda ry model was developed using the exponential growth model. The model was [ $\delta$  =  $1/(0.007)+(0.001 \times exp (0.1108 \times T))]$ , with 0.981 of  $R^2$ , indicating that the seconda ry model was developed properly. Validation of model performance showed that t he RMSE values were 0.723 and 0.238 at 20 and 30°C, respectively. This indicate s that the developed predictive models were appropriate to predict the number of C. perfringens cells in Cheonggukjang during transportation, storage, and display. In addition, the extrapolation verification for Hansik-doenjang showed an RMSE value of 0.505, implying that the predictive model developed using Cheonggukjan g can be applied to other fermented bean pastes.

**Table 2** Parameters calculated by the Weibull model for *Clostridium perfringens* in fermented be an pastes during storage at 7°C, 15°C, 25°C, and 35°C

Parameters		Tempera	ature (°C)	
Parameters	7	15	25	35
δ	$145.50\pm64.35$	$66.52\pm4.94$	$45.22\pm9.46$	$17.34\pm0.33$
ρ	$1.20\pm0.76$	$0.54\pm0.12$	$0.71\pm0.10$	$0.47\pm0.10$
$R^2$	$0.882\pm0.076$	$\boldsymbol{0.869 \pm 0.011}$	$0.944\pm0.003$	$0.833\pm0.006$

#### Dose response model

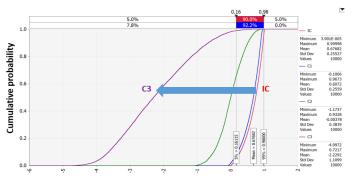
There was only one dose-response model for *C. perfringens* infection, and it was t he exponential model  $[P = 1 - \exp(-r \times N), r = 1.82 \times 10^{-11}]$  developed by Golden et al. (2009), where P is the probability of illness, *r* is the probability of *C. perfringens* cells causing foodborne illness, and *N* is the *C. perfringens* cell number (CFU/serving) ingested. As a result, this model was contained in the simulation model.

## **Risk characterization**

The simulation model was prepared as shown in Table 2 with the data for *C. perfr* ingens contamination level, predictive models used to simulate the kinetic behavi or of *C. perfringens* with the probabilistic distributions of temperature and time, p robabilistic distribution of consumption amounts, consumption frequency, and do se response model. According to the simulation, the probability of *C. perfringens* infection per person per day from consuming fermented bean paste was  $8.0 \times 10^{-12}$  (Tab 3). The cumulative density determined using this simulation showed that est imated the number of *C. perfringens* cells decreased gradually from initial contam ination (IC) to display (C3) (Fig 2). In addition, the correlation coefficient determ ined using this simulation showed that the consumption amount and contaminatio n level had positive correlations with the risk, and that market storage time, market display time, market storage temperature, transportation time, and market displa y temperature were negatively correlated with the risk.

Table 3 Probability of foodborne illness by Clostridium perfringens per person per day with consumption of fermented bean paste

Probability of illness/person/day	5%	25%	50%	95%	99%	Mean
	0	0	0	2.5×10 <sup>-11</sup>	1.7×10 <sup>-10</sup>	8.0×10 <sup>-12</sup>



Contamination level

**Figure 2** Changes of *Clostridium perfringens* contamination level, predicted by distributions in fermented bean pastes during transportation; initial concentration (IC), concentration after market transportation (C1), concentration after market storage (C2) and concentration after market display (C3).

## CONCLUSION

The risk for *C. perfringens* foodborne illness from the intake of the assessed ferm ented bean pastes produced in Korea can be considered low, and it might be cause d by low initial concentration of *C. perfringens* in the samples and decreased *C. p erfringens* cell counts during transport and storage.

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