Risk Assessment of *Staphylococcus aureus* and *Clostridium perfringens* in ready to eat Egg Products

Introduction

Egg products refer to products made by adding other types of food or food additives to eggs or egg contents or products processed by methods, such as separating, drying, freezing, heating, fermentation, or aging eggs. There are nine types of egg products: whole egg liquid, egg yolk liquid, egg white liquid, whole egg powder, egg yolk powder, egg white powder, heat-treated egg product, salted egg, and pidan. It is thought that most egg products are not sensitive to microbial contamination because their consumption is mainly made after heating. However, it is necessary to verify the absence of *Staphylococcus aureus*, with its heat-stable toxin, and *Clostridium perfringens*, which can grow more aggressively under anaerobic conditions, in egg products using scientific experiments. This study aimed to evaluate the risk of *S. aureus* and *C. perfringens* in ready to eat egg products that are widely consumed in South Korea and apply the findings to re-evaluate standards and specifications of Korea Food Code.

Statement of purpose

The purpose of this risk assessment was to present a scientific basis for establishing standards and specifications through the risk characterization of *S. aureus* and *C. perfringens* in egg products, consumption of which has been rapidly increasing due to recent changes in social trends, such as dietary patterns and health concerns. Another purpose was to prepare a microbial risk management strategy to prevent food poisoning and food accidents.
Hazard identification

_S. aureus_

*S. aureus* is a nonmotile, gram-positive (0.5–1.5 µm), facultative anaerobic, asporogenous coccus, which is found in the skin, nasal mucosa, and other site in most animals and humans. *S. aureus* normally grows well under aerobic conditions, but it can survive in anaerobic conditions as well. It is resistant to salt and proliferates well in a medium containing 7.5% NaCl. It is resistant to drought, high temperature (50°C, 30 min), and high salinity (3%–7%). Food poisoning is rarely caused by raw food, but when contamination occurs in cooked food, *S. aureus* grows rapidly to produce enterotoxin, thereby becoming a major cause of food poisoning. Staphylococcal enterotoxins (SEs) are toxins secreted from cells; SEs are thermostable, water-soluble proteins with a low molecular weight of 26–30 kDa and an isoelectric point of 5.7–8.6. They are very resistant to heat; therefore, they may not be completely destroyed even upon heating for 20 min at 120°C. Therefore, preventing enterotoxin production is essential to reduce the risk of food poisoning. Symptoms of food poisoning caused by *S. aureus* include vomiting, diarrhea, and acute gastroenteritis, which causes severe abdominal pain. The latent period is 2–6 h, and clinical symptoms vary according to the intake of enterotoxin and individual susceptibility. The mortality rate is low, and recovery can occur within 24–48 h. On examining the patterns of food poisoning caused by *S. aureus* in South Korea between 2011 and 2015, the numbers of outbreaks and patients were found to show a tendency to decrease. However, with an average of 9.2 outbreaks of food poisoning (average, 161.4 patients), it is reported as a major cause of food poisoning in addition to pathogenic *E. coli*, norovirus, and *Salmonella*.

_C. perfringens_

*C. perfringens* is spore-forming anaerobic gram-positive bacillus. It is widely distributed in natural
environments, such as soil, rivers, and sewage; intestines/feces of humans and animals; and food. The spores can survive for a long time. The optimum temperature range for growth is 43°C–47°C, and little growth occurs at temperatures of ≤15°C or ≥52°C. The optimum temperature for spore and toxin growth is 37°C. *C. perfringens* grows and produces enterotoxin that binds to specific receptors in the small intestine, causing morphological changes, diarrhea, and stomach pain. The latent period is 8–12 h, and recovery usually occurs after mild symptoms, such as diarrhea and abdominal pain. Symptoms disappear after 24 h, but slight symptoms persist for 1–2 weeks. During treatment, it is necessary to drink sufficient water, and in severe cases, water and electrolytes should be provided intravenously. Antibiotic treatment is not recommended. According to food poisoning statistics from the Ministry of Food and Drug Safety, the number of outbreaks and the number of patients gradually increased from 2002 to 2014, and in 2014, the number of outbreaks of food poisoning caused by *C. perfringens* was 28 and the number of patients was 1,689.

**Exposure assessments**

To perform an assessment of the exposure of grilled/boiled eggs, which are heat-treated egg products, to *S. aureus* and *C. perfringens*, analyses were performed on three separate stages, namely retail market, transfer to home, and home storage.

<table>
<thead>
<tr>
<th>Stage</th>
<th>Temperature range (°C)</th>
<th>Storage time (h)</th>
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<tbody>
<tr>
<td>Stage 1</td>
<td>Retail market</td>
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<td>Stage 2</td>
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<td>Home storage</td>
<td>10–25</td>
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</table>
Exposure assessment for *S. aureus* in grilled/boiled eggs

In the investigation of the level of *S. aureus* contamination in 430 grilled/boiled eggs consumed without additional cooking after purchase, contamination was detected in only two samples. The average contamination level was estimated to be $-2.49$ log CFU/g based on the beta distribution of the contamination occurrence level. A growth prediction model was developed for grilled/boiled eggs. The Baranyi model was used as the primary model. A secondary model (square root model) was developed by applying the lag phase duration and growth rate obtained from the primary model. Changes in the bacterial count from the initial contamination level were predicted using a simulation (mean, $-2.49$ log CFU/g; maximum, $-1.48$ log CFU/g). The level of *S. aureus* contamination during the distribution stage was estimated to increase to a relatively high level (mean, $1.19$ log CFU/g; maximum, $8.8$ log CFU/g), and the level at home storage was estimated to be similar to that at the purchase stage (mean, $1.19$ log CFU/g; maximum, $8.8$ log CFU/g). Food intake was calculated using the results of the “Investigation on the Intake and Patterns of 50 Major Livestock Products” (Ministry of Food and Drug Safety, 2015) conducted jointly with Gallup Korea. In addition, the intake of grilled/boiled eggs (70.56 g), which showed the maximum growth rate in the growth prediction model, was applied.

Exposure assessment for *C. perfringens* in grilled/boiled eggs

The investigation of the level of *C. perfringens* contamination in 430 grilled/boiled eggs revealed no contamination in any product. The average contamination level was estimated to be $-3.23$ log CFU/g based on the beta distribution of the contamination occurrence level. A growth prediction model was developed and the growth patterns of *C. perfringens* were confirmed in grilled/boiled eggs. The initial level of *C. perfringens* contamination in grilled/boiled eggs that was obtained by a simulation was low (mean, $-3.23$ log CFU/g; maximum, $-1.54$ log CFU/g). This level is expected to increase slightly during the distribution stage (mean, $-3.12$ log CFU/g; maximum,
−1.31 log CFU/g). The contamination level at the time of consumption after home storage was estimated to be similar to that at the purchase stage (mean, −3.12 log CFU/g; maximum −1.31 log CFU/g). According to the intake and consumption frequency survey (1,500 people), approximately 2.2% of the 1,500 people in South Korea consumed grilled/boiled eggs more than once per day, and the average intake was 70.56 g per day.

Hazard characterization

**S. aureus**

The minimum tolerable dose of SEs is 100 ng, and the concentration of *S. aureus* producing SEs at this concentration was found to be 4–8 log CFU/g in various studies. However, because 5 log CFU/g was the most frequently reported in the literature, it was considered desirable to establish this level as the minimum tolerable dose for humans.

**C. perfringens**

Park (2014) conducted a meta-analysis of the minimum infectious dose of bacteria that causes food poisoning and found the minimum infectious dose of *C. perfringens* to be $10^2$ cells/g; maximum, $10^8$ cells/g; median, $10^7$ cells/g; fifth percentile, $10^2$ cells/g; 25th to 75th percentiles, $10^6$–$10^8$ cells/g; and 95th percentile $10^8$ cells/g. In this study, the minimum infectious dose was established to be $10^7$ cells/g, which had the highest relative frequency for the minimum dose. This is similar to the result of approximately $10^5$–$10^8$ cells/g reported by Leggett et al. (2012).
Risk characterization

*Staphylococcus aureus* in grilled/boiled eggs

Using a simulation to estimate the risk level of food poisoning caused by *S. aureus* due to the consumption of egg products, it was shown that the probability of food poisoning caused by *S. aureus* was 0% in the 0%–95% range and $2.48 \times 10^{-4}$ in the cumulative range of up to 99% when egg products are consumed by one person per day in the current distribution environment and at the current consumption level. In other words, the probability of food poisoning is close to zero for most consumers that correspond to 95%, but there is a $2.48 \times 10^{-4}$ probability of food poisoning in some consumers with extreme consumption patterns.

*C. perfringens* in grilled/boiled eggs

The intake of *C. perfringens* on consumption of grilled/boiled eggs was estimated based on the contamination level, intake, consumption frequency, etc. in the consumption stage, and the final results were obtained through more than 10,000 iterations using @RISK. According to the estimated results obtained by simulating the risk of food poisoning caused by *C. perfringens* due to the consumption of egg products, the probability that food poisoning caused by *C. perfringens* can occur in one person per day when *C. perfringens* is consumed in the current distribution environment and at the current consumption level was estimated to be maximum $4.75 \times 10^{-14} \pm 5.31 \times 10^{-13}$. Consequently, the likelihood of food poisoning was estimated to be extremely low.

Impact of establishing microbial standards in Korea Food Code

To establish new standards and re-evaluate the current standards, the changes in *S. aureus* and *C. perfringens* concentrations were predicted according to scenarios of extreme conditions. Estimation of the contamination level at the consumption stage in the current distribution environment under the assumption
of initial contamination levels of 1, 2, and 3 log CFU/g revealed the levels of *S. aureus* contamination to be 5.19, 6.03, and 6.81 log CFU/g, respectively, which exceed the minimum tolerable dose of 5 log CFU/g. Therefore, it is considered appropriate to maintain the current standards as zero tolerance (n = 5, c = 0, m = 0/25 g). In particular, it is considered necessary to conduct careful management of ready to eat egg products (grilled/boiled eggs) that are commercially distributed at room temperature.

For *C. perfringens*, estimation of the contamination level at the consumption stage in the current distribution environment under the assumption of initial contamination levels of 1, 2, and 3 log CFU/g revealed the level of *C. perfringens* contamination to be less than the minimum tolerable dose of 8 log CFU/g for humans. In addition, the probability of the occurrence of food poisoning per person per day was low at $1.46 \times 10^{-12}$. Thus, the risk of food poisoning caused by *C. perfringens* under the current standards of zero-tolerance is very low. However, when considering the risk and harmonization of specifications, it is deemed appropriate to consider establishing quantitative specifications.

**Gaps in the data**

This risk assessment used results obtained upon establishing certain assumptions using limited data; thus, re-evaluation may be warranted if additional data become available. It is also necessary to continuously perform risk assessment to establish reasonable scientific standards and specifications by considering the risk, exposure frequency, and other factors.

**Reference**