Survival of *Staphylococcus aureus* in dried fish products as a function of temperature

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Received: 28 September 2016 / Revised: 23 February 2017 / Accepted: 2 March 2017 / Published online: 15 June 2017
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Abstract We evaluated the survival ability of *Staphylococcus aureus* and the production of Staphylococcal enterotoxin A (SEA) in dried filefishes and julienned squid at 10°, 24°, and 35 °C for 5 months. *S. aureus* survived longer at 10 °C than 24° and 35 °C, and better in dried julienned squid than dried filefishes. At 35 °C, the populations of *S. aureus* were rapidly diminished and undetectable in dried filefishes and julienned squid after 14 and 19 days, respectively. SEA production did not increase during the 5-month storage period, regardless of the temperature and type of dried fish products. Although it is advised to store dried fish products at 10 °C for quality control in retail markets, refrigerated temperature is more likely to facilitate the survival of *S. aureus* in dried fish products. Thus, dried fish products should be produced and stored under hygienic conditions.

Keywords *Staphylococcus aureus* · Staphylococcal enterotoxin A · Dried filefishes · Dried julienned squid · Survival kinetics

Introduction

Staphylococcal food poisoning is one of the most common cause of food-borne illness in many countries [1, 2]. In Korea, *Staphylococcus aureus* is the third most common

pathogen involved in foodborne illness, after *Salmonella* spp. and *V. parahaemolyticus* [2]. After people consume the food containing Staphylococcal enterotoxins (SEs), they have clinical symptoms including vomiting, abdominal pain, and diarrhea within 2–6 h [3, 4]. The minimum amount of SEs that causes clinical symptoms is about 1 ng/g of food [4]. SEs are resistant to extreme environments such as freezing and drying and have heat stability and resistance [5]. So far, nine major serotypes of SEs have been documented, among which staphylococcal enterotoxin A (SEA) is a major cause for staphylococcal food poisoning [6, 7]. SEA is produced nearly under all water activity (Aw) ranges, whereas other toxins are sensitive to low Aw [5].

Dried filefishes and julienned squid are classified as a group of dried fish with and without seasoning according to the Korean Food Standards Codex [8]. Most dried fish products in Korea are imported from Southeast Asia due to their low processing cost. However, imported dried fish products are exposed to a climate of high temperature and humidity and an unsanitary processing environment; thus, dried fish products are easily implicated in contamination by *S. aureus* [9]. Of the 210 dried seasoned fish products collected from the retail markets in Korea, 33.8% were contaminated by *Staphylococcus* spp. [10]. Park et al. [11] indicated that *S. aureus* were detected in 7 (17.95%) of 39 dried seasoned filefishes sold around elementary schools in Korea and the mean contamination level was 1.83 log CFU/g. In our monitoring study [12], 13 dried filefishes and 20 dried julienned squids were purchased from supermarkets. *S. aureus* was detected in one dried filefish (1 log CFU/g) and two dried julienned squid (2–4.9 log CFU/g).

There have been few studies on the effect of temperature on the inactivation of *S. aureus* contaminating dried fish products as well as dried meats such as beef jerky and

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biltong, which is processed similarly to dried seasoned fish products and is consumed considerably in the U.S. [13]. In addition, the survival of *S. aureus* has been reported on vacuum-packaged beef jerky and biltong [14, 15] and dried seasoned biltong [16] stored at ambient temperature. In Korea, the dried fish products are sold at 10 °C or room temperature in convenience stores and traditional markets. However, the behavior and risk of toxin production of *S. aureus* on dried fish products in the retail market have not been assessed.

In this study, we evaluated the survival ability of *S. aureus* and the production of SEA on dried filefishes and julienned squids stored at 10°, 24°, and 35 °C for 5 months, which is the average shelf life of most dried fish products.

Materials and methods

Bacterial culture

The strain of *S. aureus* (ATCC 13565) that produced SEA was purchased from the Korean Culture Center of Microorganisms (KCCM, Seoul, Korea) and maintained at -80 °C in tryptic soy broth (TSB) (BD, Sparks, MD, USA) containing 20% glycerol. Ten microliters of thawed stock culture was inoculated into 10 mL of sterile TSB, which was then sealed with a silistopper and incubated at 35 °C for 24 h on a rotary shaker (VS-8480SR, Vision, Korea) at 140 rpm. After incubation, 1 mL of the culture was serially diluted using 9 mL of 0.1% sterilized peptone water (BD, Sparks, MD, USA) to obtain an initial population of approximately 6.0 log CFU/g in the dried filefish and julienned squid.

Preparation of sample and inoculation

We purchased the domestic dried filefish and julienned squid from the supermarket to investigate the survival of *S. aureus* in dried fish products. The Aw of samples was measured using Aqualab Lite (Decagon Devices, Inc. Pullman, WA, USA). Five grams of dried filefish or julienned squid was aseptically weighed and placed in a petri dish. Dried filefish and julienned squid were inoculated with 50 µL of the diluted culture of *S. aureus* at a concentration of ~6.0 log CFU/g, respectively. After inoculation, they were packed into a polyethylene bag and stored at 10°, 24°, and 35 °C. At select times, the samples were homogenized (BagMixer, Interscience, Paris, France) and diluted, and plated onto Baird-Parker agar (BD, Sparks, MD, USA) in duplicate and incubated at 36 °C for 48 h. The colonies on the plates were counted with an automated colony counter (Scan 1200, Interscience, Saint Nom,

France). The mean of the duplicate plates was graphed at each sampling period to generate a survival primary model of *S. aureus*. We repeated the same experiment twice.

Quantification of SE in dried fish products

The SEA strain (cat no. AT 101) was purchased from Toxin Technology (Sarasota, FL, USA) and maintained at -80 °C in 1 mg/mL sterile deionized water. The presence of SEA was analyzed using a method described previously [17]. The standard curve for SEA was produced with OD values of known concentrations of SEA (0.1–3.0 ng/mL) at 414 nm using an ELISA reader (Power wave XS, Biotek, USA). To detect the presence of SEA in dried filefish and julienned squid, five grams of dried filefish or julienned squid inoculated with 50 µL of the diluted culture of *S. aureus* were kept in a polyethylene bag and stored at 10 and 24 °C. After select intervals, the dried filefish and julienned squid were blended in a stomacher blender (BagMixer, Interscience, Paris, France) with 5 mL of 0.1% sterilized peptone water, and 1 mL was centrifuged (Centrifuge, VS-550, Vision Scientific, Daejeon, Korea) at 3000 rpm for 15 min at 4 °C. Two hundred microliters of the supernatant from each sample was analyzed for the quantification of SEA using a TECRA SE visual immunoassay (3 M, USA) kit and an ELISA reader according to the manufacturer's procedures.

Effect of storage temperature on survival of *S. aureus*

Survival curves of *S. aureus* were iteratively fitted to the Weibull model using a Gina FiT V 1.5 Program (Geeraerd and Van Impe Inactivation Model Fitting Tool). The Weibull equation is represented as follows:

$$\text{Log}_{10}(N) = \log_{10}(N_0) - ((t/\Delta) \times P) \quad (1)$$

Delta (δ): time for the first decimal reduction, P: shape, N₀: initial log number of cells, t: time.

The Weibull model has two parameters: delta (δ) and P. Delta is referred to as the scale parameter and represents the time of the first decimal reduction concentration for a part of the population. P is a shape parameter and accounts for the Weibull distribution. If $P < 1$, the model shape is upward concavity, and if $P > 1$, the model shape is downward concavity. If $P = 1$, the distribution is a linear survival curve, which corresponds to a first-order decay reaction [18, 19]. The fitness of the model was assessed using the coefficient of determination (R^2) provided by the Gina FiT V 1.5 Program.

After developing primary survival curves, the delta and P values were applied to the second-order polynomial model by using Microsoft Excel 2010 (Microsoft Corp.,

Redmond, WA, USA) to assess the effect of temperature on the kinetic parameters of the primary survival curve. The second-order polynomial model used the following equation [20].

$$Y = a + (b \times T) + (c \times T^2) \quad (2)$$

Y: delta, a, b, c: constant, T: temperature.

The data were also analyzed by SAS version 9.3 (SAS institute Inc., Cary, NC, USA). We analyzed significant differences in delta and *P* values between the dried filefishes and julienned squid at the same temperature by using the *T* test. The significant differences in delta and *P* values among the temperature were also determined by one-way ANOVA followed by Duncan's multiple range test at $p < 0.05$.

Results and discussion

Survival model of *S. aureus* in dried filefishes and dried julienned squid as a function of temperature

The survival curves and kinetic parameters for *S. aureus* in dried filefishes and julienned squid at 10, 24, and 35 °C for 5 months of storage are shown in Fig. 1. The kinetic data for the survival of *S. aureus* in dried fish products were well fitted to the Weibull equation ($R^2 > 0.95$). After 5 months of storage, the populations of *S. aureus* exhibited reductions of 1.60 (filefish) and 0.98 (julienned squid) at 10 °C and 4.45 (filefish) and 5.43 (julienned squid) log CFU/g at 24 °C. *S. aureus* was undetectable in both samples with over 6 log CFU/g reduction after 14 and 19 days at 35 °C, respectively.

The delta values of *S. aureus* in dried julienned squid is significantly higher than those in dried filefish at all temperatures ($p < 0.05$) (Table 1; Fig. 1). The populations of *S. aureus* in dried filefishes declined by 1 log CFU/g twice as fast as that in dried julienned squid. In addition, a significantly higher delta value was observed at the lower temperature, regardless of the sample type tested ($p < 0.05$). The results indicate that *S. aureus* can survive better in dried julienned squid and lower temperatures than that in dried filefish and higher temperatures. The shape parameters (*P*) of the two products at 35 °C were significantly ($p < 0.05$) higher than those at other temperatures, and the shape parameter with $P > 1$ corresponded to downward concavity. This indicated that *S. aureus* exhibited a long lag phase in the beginning and rapid death at the end of the survival curve at 35 °C. As shown in Fig. 1, *S. aureus* in dried filefishes and julienned squid rapidly decreased after 5 and 11 days at 35 °C, respectively. On the other hand, a significant difference in *P* values was not

observed between 10 °C and 24 °C (Table 1). The *P* values were close to 1 and the survival model showed a linear survival curve.

We also developed a secondary model of delta and *P* values as a function of temperature (Fig. 2). The effect of temperature on the delta and *P* values of the survival curve at both samples are well explained by the polynomial second-order model, as shown in the coefficient of determination values ($R^2 = 1$). As a result, *S. aureus* in dried fish products survived longer at 10 °C than at 24 and 35 °C and the shape of the survival curve changed from linear to a concave downward as the temperature increased. This result is similar to that of another study [21], which reported that *S. aureus* in dried seasoned fishes at 35 °C decreased much faster than that at 7 or 18 °C. It was also reported that the survival period of *S. aureus* was longer at lower temperatures than higher temperatures [22]. In addition, *S. aureus* inoculated in the bacon could survive at −22 °C even after 30 days of storage [23]. Although the mechanism and evidence concerning the higher survival probability of *S. aureus* at lower temperature are not clarified, some studies indicate that *S. aureus* increased in glycolytic enzyme production and changed the lipid components of its membrane to acclimate to cold environment [24, 25]. On the other hand, no growth or death of *S. aureus* was observed in Ready to eat (RTE) dried meat such as biltongs, beef jerkins, dried seasoned beef strips, and sausages [14–16]. In addition, the growth of *S. aureus* in processed marine products was reported in other studies [26–28]. This difference in these studies is attributed to the difference in water activity (*A_w*). Actually, the rich proteins in seafood support the growth of *S. aureus* [29]. However, the *A_w*s of dried filefishes and julienned squids in the present study were 0.48 and 0.76, respectively, which were lower than the minimum level for *S. aureus* growth. *S. aureus* do not grow aerobically at *A_w* of ≤ 0.85 or anaerobically at *A_w* of 0.88 [14]. The finding that *S. aureus* survived better in dried julienned squid than in filefishes is also explained by the different *A_w*.

Dried fish products are known as risk foods for *S. aureus* because they are exposed to a climate of high temperature and humidity with a complex manual manufacturing process [9]. Currently, dried fish products are advised to be stored at 10 °C for quality maintenance at retail markets in Korea. However, Simon and Sanjeev [29] reported that *S. aureus* was not detected in dried fish products, in contrast to other results [30, 31]. This was attributed to the improved processing environment and the adaptation of GMP and HACCP. Therefore, we should not only improve the manufacturing environment for domestic dry fish products but also pay special attention to the imported dried fish products.

Fig. 1 Survival curves of *S. aureus* in dried filefish (A) and dried julienned squid (B) at 10, 24, and 35 °C

Table 1 Delta and *P* value of *S. aureus* in dried filefish and dried julienned squid at various temperatures

Parameter	Sample	10 °C	24 °C	35 °C
Delta ¹	Dried filefish	79.55* ^a	15.99* ^b	5.33* ^c
	Dried julienned squid	154.60 ^a	34.64 ^b	10.39 ^c
P ²	Dried filefish	0.94	0.81*	1.76*
	Dried julienned squid	1.06	1.18	2.71

Delta, The time of the first decimal reduction concentration for a part of the population (day); P, Shape parameter

* Mean values (*n* = 4) between the samples are significantly different by *t* test at *p* < 0.05

^{a-c} Mean values (*n* = 4) within each row are significantly different by Duncan's multiple range test at *p* < 0.05

Toxin production of *S. aureus* in dried filefishes and dried julienned squid

SEs are resistant to drying and SEA is produced in a wider range of water activity values than other toxins. Actually, SEA is detected more often in dried cured beef ham samples than in other dried food products [32]. In this study, we measured the quantity of SEA along with the populations of *S. aureus* in dried filefishes and dried julienned squids stored at 10 and 24 °C for 5 months. Figure 3 shows the survival populations of *S. aureus* and the presence of SEA in dry fish products at 10 and 24 °C. Overall, the toxin was detected in both dried filefishes and julienned squids inoculated with over 6 log CFU/g of *S. aureus*. SEs were

Fig. 2 Second-order polynomial models for the effect of temperature on the delta (A) and P (B) of *S. aureus* in dried filefish and dried julienned squid. (filled circle), dried filefish; (filled square), dried julienned squid; T, Temperature

produced when the number of *S. aureus* populations was over 5–6 log CFU/g [7, 33]. Tango et al. [28] also reported that SEA production occurs in RTE-cooked fish paste containing more than 6.3 log CFU/g of *S. aureus*. In this study, the range of toxin production in dried filefishes and dried julienned squids did not reach the minimum amount that causes clinical symptoms. Similarly, the amount of

toxin produced by *S. aureus* isolated from dried seasoned fishes was -0.23–0.71 ng/mL [21]. However, the presence of enterotoxins in dried fish products should be carefully monitored and controlled in retail markets because very small concentrations of SEs can lead to food poisoning. A large outbreak of staphylococcal food poisoning was reported due to 0.5 ng/mL of SEs in chocolate milk [4], which was under the minimum level. In this study, the level of SEA in both dried filefishes and dried julienned squids did not change for nearly 5 months, regardless of the storage temperature and product type, although the populations of *S. aureus* decreased throughout 5 months of storage. The results of this study show that dried fish products may have the potential to cause *S. aureus* food-borne illness because the toxin can survive well in dried fish products regardless of the temperature and population of *S. aureus*. Therefore, dried fish products should be produced and stored under hygienic conditions and the *S. aureus* population should be controlled at the beginning of the storage period in the retail market to prevent the production of enterotoxins in dried fish products, including dried filefishes and dried julienned squids. Furthermore, more studies are needed to control the production and survival of SEs in low water activity products such as dried fish products.

Fig. 3 Survival curve and toxin production of *S. aureus* in (A) dried filefish and (B) dried julienned squid at 10 and 24 °C. (filled circle), *S. aureus* (log CFU/g); (filled triangle), staphylococcal enterotoxins A (ng/g)

Acknowledgement This research was supported by the Ministry of Food and Drug Safety (MFDS) Research Grant (11162-044).

Compliance with ethical standards

Conflict of interest The authors declare no conflict of interest.

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