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ARTICLE

Thermal Inactivation of Sodium-Habituated *Staphylococcus aureus* in Ready-to-Heat Sauces

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Abstract

The objective of this study was to evaluate the effect of sodium habituation on thermal resistance of *Staphylococcus aureus* in various ready-to-heat (RTH) sauces. The strain mixture of *S. aureus* strains KACC10768, KACC10778, KACC11596, KACC13236 and NCCP10862 was habituated up to 9% of NaCl. The inocula of NaCl-habituated and non-habituated *S. aureus* were inoculated in 5 g portions of pork cutlet, meat and *Carbonara* sauces at 7 Log CFU/g, and the samples were vortexed vigorously. The inoculated samples were then exposed to 60 and 70°C in a water-bath, and survivals of total bacteria and *S. aureus* were enumerated on tryptic soy agar and mannitol salt agar, respectively, every 30 min for 120 min. At 60°C, the cell counts of total bacteria and the significant difference in survivals between sodium-habituated and non-habituated *S. aureus* were observed only in the *Carbonara* sauce; the tailing effect, which is the period of no reduction of bacterial cell counts, was observed in pork cutlet, meat and *Carbonara* sauces subjected to 60°C. At 70°C, total bacterial populations and sodium-habituated and non-habituated *S. aureus* cell counts in meat and *Carbonara* sauce also significantly decreased ($p<0.05$) after 30 min of heat treatment, followed by the obvious tailing effect. Sodium-habituated *S. aureus* cell counts in meat and *Carbonara* sauces were higher ($p<0.05$) than those of non-habituated *S. aureus* at 70°C. The results indicate that sodium habituation of *S. aureus* cells may increase the thermal resistance of the pathogen in RTH sauces; moreover, heating RTH sauces for a short time before serving may not sufficiently decrease the cell counts of *S. aureus*, particularly for sodium-habituated strain.

Key words: *Staphylococcus aureus*, ready-to-heat sauces, thermal inactivation, NaCl-habituation

Introduction

Staphylococcus aureus has been related to the foodborne disease in various foods, such as pork, chicken and ready-to-eat (RTE) salad in the United States (CDC, 2012). The pathogen grows in foods and produces enterotoxins, and *S. aureus* also is heat stable bacteria in cooking process (Bang *et al*., 2008). In addition, the pathogen is a major cause of bacteremia, and it is closely related to higher mortality and morbidity, compared to the bacteremia caused by other pathogens (Naber, 2009). Since lifestyle has been changed, convenient foods such as RTE and ready-to-heat (RTH) foods have become popular. Because of this consumption pattern in the United States 968 cases of foodborne disease were caused by fast foods in 2010 (CDC, 2012). Especially, there were 55 reported outbreaks with 1,875 cases caused by various sauces in the United States from 1990 to 2003 (Dewaal *et al*., 2006).

Optimum growth conditions for *S. aureus* are at 35-41°C of growth temperature, 6-7 of pH, 0.990 of a_w, and 0% of NaCl (Schelin *et al*., 2011). However, *S. aureus* can grow even at pH 4.2 and up to 15% of NaCl, which is the condition found in various sauces (Ingham *et al*., 2005; USDHHS/FDA, 2011).

When foodborne pathogenic bacteria are exposed to sublethal stresses such as acid, heat, salt and various preservatives, the pathogen may become more tolerant to the subsequent other stresses called cross-protection or cross-resistance response (Archer, 1996; Beales, 2004; Hill *et al*., 2002). Moreover, continuous exposure of pathogenic bacteria to sublethal stresses may also increase the resistance of bacteria to the same or other stresses (Koutsoumanis *et al*., 2003). For instance, the acid (pH 3.5) chal-

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lenge, followed by osmotic treatment (a_w 0.9) at 25°C increased *Escherichia coli* stress tolerance than the reverse order exposure (Shadbolt *et al*., 2001). In a case of *Listeria monocytogenes* the pathogen of heat shock at 45°C for 1 h increased the tolerance to NaCl (25%), crystal violet (0.01%), and ethanol (18%) (Lin and Chou, 2004). Exposure of *S. aureus* to heat, hydrogen peroxide, and alkaline media also developed homologous stress responses of the pathogen (Cebrian *et al*., 2010). Thus, exposure of *S. aureus* to sodium in food-related conditions may increase thermal resistance of the bacteria by habituation.

Therefore, the objective of this study was to evaluate if sodium habituation of *S. aureus* in various foods increases its thermal resistance in various RTH sauces.

Materials and Methods

Habituation of *S. aureus* to sodium

The isolated colonies of *S. aureus* strains (KACC10768, KACC10778, KACC11596, KACC13236 and NCCP10862) on mannitol salt agar (MSA; Difco™, Becton Dickinson and Company, USA) were cultured in brain heart infusion broth (BHI; Difco™) at 35°C for 24 h. The 0.1 mL of the culture was then transferred into 10 mL of BHI broth for subculture at 35°C for 24 h. To habituate *S. aureus* to sodium, 0.1 mL of the culture was inoculated in 10 mL of BHI broth supplemented with 3% NaCl, following incubation at 35°C for 24 h, and 0.1 mL of the culture was transferred to 10 mL BHI broth supplemented with 6% NaCl and the culture was incubated at 35°C for 24 h. Eventually, 0.1 mL of this culture was transferred to 10 mL of BHI broth supplemented with 9% of NaCl, which could be found in fermented sea foods (Kim *et al*., 2006). It was then incubated at 35°C for 24 h.

Non-habituated *S. aureus* was prepared by consecutive incubation only in BHI broth. The 10 mL of sodium-habituated and non-habituated *S. aureus* were centrifuged at 1,912 *g* and 4°C for 15 min. The supernatants were then discarded, and the cell pellets were washed twice with phosphate buffered saline (PBS, pH 7.4; 0.2 g of KH_2PO_4, 1.5 g of $Na_2HPO_4 \cdot 7H_2O$, 8.0 g of NaCl, and 0.2 g of KCl in 1 L of distilled water), and the pellets were resuspended in PBS to prepare sodium-habituated and non-habituated *S. aureus* inocula at 8-9 Log CFU/mL.

Inoculation and heat challenge

Pork cutlet, meat, and *Carbonara* sauces were purchased from a local grocery store. Five gram portions of each sauce were transferred into tubes, and the sauce samples were immersed in a water bath until internal temperatures of the sauce samples reached at 60 and 70°C. The 0.1 mL portions of the inocula were inoculated into the sauce samples at 6-7 Log CFU/g, and the samples were immersed again in a water bath at 60 and 70°C after vortexing samples. The samples were microbiologically analyzed at 0, 30, 60, 90, and 120 min.

Microbiological analysis

To enumerate survivals of total bacteria and *S. aureus*, 5 mL of 0.1% buffered peptone water (BPW, Difco™) was added to the samples followed by vortexing, and each sample was then transferred to a filter bag (BagFilter®, Interscience, France). The samples were pummeled for 30 s by a pummeler (BagMixer®) and serially diluted with BPW. The 0.1 mL portions of the diluents were spread-plated on tryptic soy agar (TSA; Difco™) and MSA for total bacteria and *S. aureus*, respectively. The agar plates were incubated at 35°C for 24 h, and colonies were manually counted.

Statistical analysis

The experiment was repeated twice with two samples per repeat. Microbiological data (CFU/g) were transferred to Log CFU/g, and analyzed by the mixed model procedure of SAS® version 9.2 (SAS Institute Inc., USA). Pairwise *t*-test was used to compare least significant means of the fixed effects (heating time×inocula) to determine significant differences at alpha=0.05.

Results and Discussion

The heat treatment at 60°C significantly decreased ($p<0.05$) bacterial populations of total bacteria in pork cutlet sauce (Data not shown in a tabular form). *S. aureus* in the pork cutlet sauce also rapidly decreased ($p<0.05$) by more than 5 Log CFU/g after 30-min heat challenge, and then gradually decreased ($p\geq0.05$) to below detection limit (0.3 Log CFU/g) after heat challenge for 120 min (Fig. 1). Hence, the pork cutlet sauce was not further subjected to 70°C and sodium-habituated strain was not tested for the sauce.

For meat and *Carbonara* sauces, total bacterial populations decreased ($p<0.05$) by 1.5-3.2 Log CFU/g (Data not shown in a tabular form). *S. aureus* also significantly decreased ($p<0.05$) to 1.9-3.5 Log CFU/g during heat challenge at 60°C for 120 min, regardless of sodium-habituation (Fig. 2). There was no difference in *S. aureus* cell counts between sodium-habituated and non-habitu-

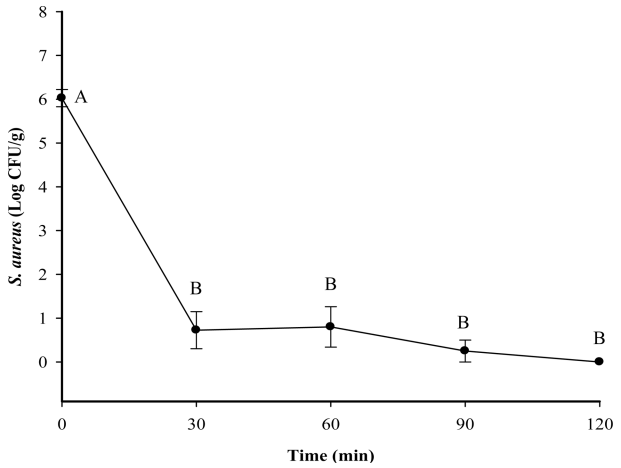

Fig. 1. Survival of *Staphylococcus aureus* in pork cutlet sauce during heat challenge at 60°C for 120 min. A-B, means with different letters are different (p<0.05).

ated *S. aureus* in meat sauce during heat challenge, but sodium-habituated *S. aureus* cell counts were higher (p<0.05) than non-habituated *S. aureus* cell counts in *Carbonara* sauce (Fig. 2).

During heat challenge at 60°C, more reduction of *S. aureus* cell counts was observed in pork cutlet sauce than those in meat and *Carbonara* sauces (Figs. 1 and 2). The combined effect of heat with lower pH value (pH 3.5) of pork cutlet sauce compared to meat (pH 4.5) and *Carbonara* sauces (pH 5.7) may cause rapid decrease of *S. aureus* cell counts to the below detection limit because low pH of samples may sensitize the bacterial cells than other cells to thermal stress (Yoon *et al*., 2011).

After 30-min heat challenge at 70°C, *S. aureus* populations in meat and *Carbonara* sauces decreased (p<0.05)

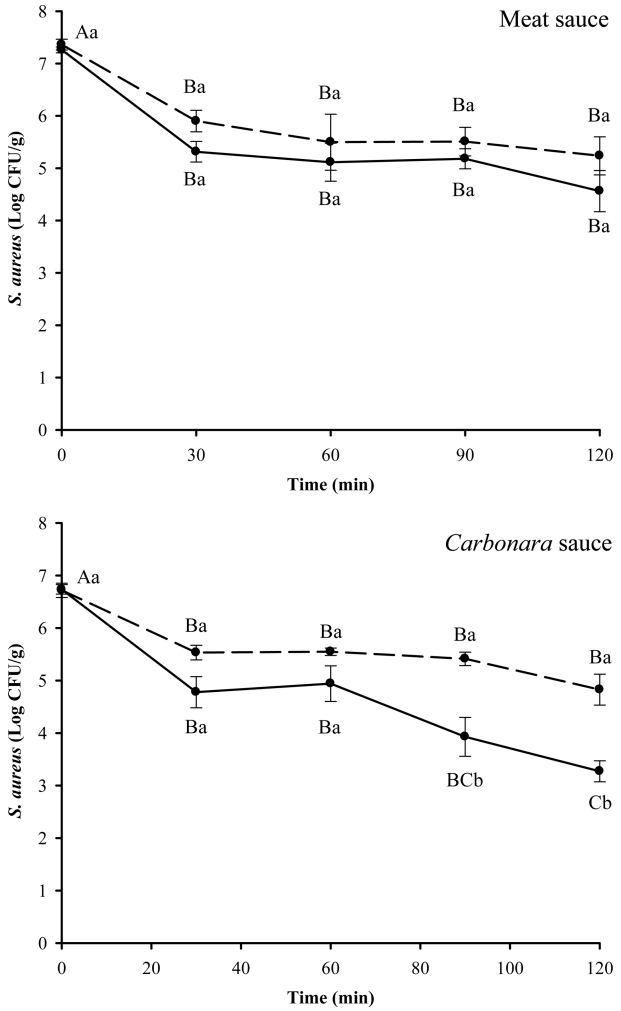

Fig. 2. Survival of sodium-habituated (dashed line) and non-habituated (solid line) *Staphylococcus aureus* in meat and *Carbonara* sauces during heat challenge at 60°C for 120 min. A-C, means within dashed line or solid line with different letters are different (p<0.05); a-b, means within time with different letters are different (p<0.05).

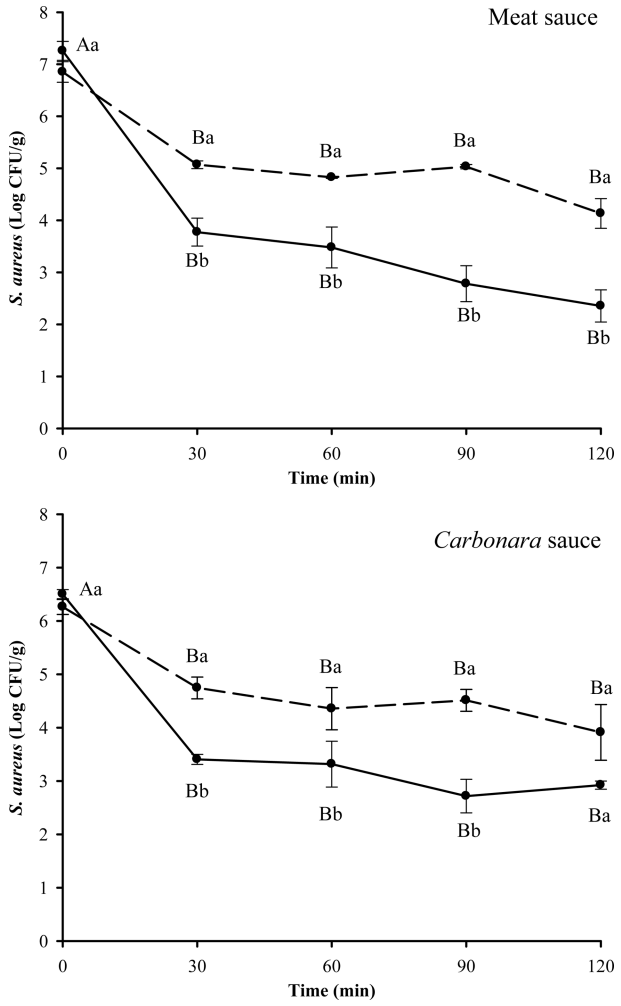

Fig. 3. Survival of sodium-habituated (dashed line) and non-habituated (solid line) *Staphylococcus aureus* in meat and *Carbonara* sauces during heat challenge at 70°C for 120 min. A-B, means within dashed line or solid line with different letters are different (p<0.05); a-b, means within time with different letters are different (p<0.05).

by 1.8-3.5 Log CFU/g and 1.5-3.1 Log CFU/g, respectively, regardless of sodium habituation (Fig. 3). This result suggests that *S. aureus* contamination in RTH sauces, which are usually heated only for short time before serving, may not decrease sufficiently bacterial contamination, resulting in foodborne illness. After 30-min heat challenge, tailing effect, which is the period for no reduction of bacterial cells counts, was observed in survival curves (Fig. 3). There were total *S. aureus* cell count reductions of 2.7-4.9 Log CFU/g and 2.4-3.6 Log CFU/g after 120 min of heat challenge for meat and *Carbonara* sauces, respectively, which were close to the survival counts after 30-min heat challenge (Fig. 3). The tailing effect in inactivation curves is attributed to a proportion of the survivals with higher stress resistance or to damage repair of injured bacterial cells on media (Chen, 2007; San Martin *et al*., 2002).

During heat challenge at 70°C, sodium-habituated *S. aureus* cell counts were higher ($p<0.05$) than those of non-habituated *S. aureus* in meat and *Carbonara* sauces (Fig. 3). A study by Yoon *et al*. (2009, 2011) showed that *Escherichia coli* O157:H7 had higher thermal resistance in non-intact beef formulated with NaCl after cooking at 65°C. Sequential exposure of *L. monocytogenes* to sublethal stresses including sodium also increased *L. monocytogenes* survival under subsequent stresses (Skandamis *et al*., 2009). Taken together, sodium habituation of *S. aureus* may increase its thermal resistance in RTH sauces.

In conclusion, exposure of *S. aureus* to sodium under food-related conditions may increase its thermal resistance in RTH sauces, and short-time heating RTH sauces before serving may not decrease sufficiently cell counts of *S. aureus*, especially for sodium-habituated strain.

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