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Growth of *Staphylococcus aureus* and enterotoxin production in pre-cooked tuna meat

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ABSTRACT

This study investigated growth of enterotoxin-producing *Staphylococcus aureus* and time–temperature combinations needed for enterotoxin production in pre-cooked tuna meat. Frozen samples (50 ± 5 g) of pre-cooked albacore tuna (loin, chunk and flake) and skipjack tuna (chunk and flake) were thawed overnight at 5–7 °C, inoculated with five strains of *S. aureus* (2–4 log CFU/g), and incubated at 37 and 27 °C for up to 36 h. Changes of *S. aureus* populations in samples during incubation were determined by plating on Baird–Parker media. Results indicate that increases in *S. aureus* populations varied among the 5 types of tuna samples. Incubation at 37 °C for at least 6 or 8 h was required to allow an increase in *S. aureus* populations by >3 log CFU/g in inoculated albacore or skipjack tuna meat. A similar increase of *S. aureus* counts (>3 log CFU/g) in albacore and skipjack samples required more than 8 and 10 h, respectively, when samples were incubated at 27 °C. No enterotoxin was produced in albacore or skipjack tuna meat inoculated with five strains of enterotoxin-producing *S. aureus* and exposed to 37 °C for 12 h or 27 °C for 16 h. All the samples showed clear sign of spoilage before enterotoxin was detected.

1. Introduction

The United States imported about 91% of seafood consumed in 2011 and became the second only to China in seafood consumption (National Marine Fisheries Service, 2012b). According to the National Marine Fisheries Service (NMFS) of the National Oceanic and Atmospheric Administration (NOAA), Americans consumed 4.7 billion pounds (2.1 billion kg) of seafood in 2011 with the average American consuming 15.0 pounds (6.8 kg) of edible meat per person (National Marine Fisheries Service, 2012b). Of the 15.0 pounds of seafood consumed per person in 2011 in the U.S., about 2.25 pounds (1.02 kg) were canned tuna, which was the second most consumed seafood in the U.S. after shrimp (National Marine Fisheries Service, 2012a). Tuna fish are widely consumed around the world due to high nutritional values and good taste (USDA, 2005).

In 2011, the U.S. produced 385 million pounds (174.6 million kg) of canned tuna valued at $768.7 million (National Marine Fisheries Service, 2012a). Canned tuna is commonly produced from pre-cooked tuna mixed with edible oils, brine, water, or sauces in cans. Typically, eviscerated tuna are pre-cooked to facilitate "cleaning" – the manual separation of the meat from the skin and bones. There is a potential for the pre-cooked fish to be contaminated with *Staphylococcus aureus* during the cleaning process because humans are the main reservoir of *S. aureus* (FDA, 2011). If recontaminated, there is a potential for *S. aureus* to multiply in pre-cooked tuna meat to significant numbers to produce heat-stable enterotoxins in the canned products before the products are commercially sterilized. While outbreaks of staphylococcal food poisoning have not been associated with commercially canned tuna, they have been linked to canned, smoked and salted products, boiled paste and sausages where the growth of competing organisms are inhibited (Bryan, 1980; Nakano et al., 2004; Simon & Sanjeev, 2007). Although cells of *S. aureus* can be inactivated by thermal sterilization processes, the enterotoxins they produced may not be eliminated by the process. Therefore, *S. aureus* has the potential to be a pathogen of safety concern in canned tuna products.

S. aureus can produce heat-stable enterotoxins capable of causing gastroenteritis in humans. The enterotoxins are single-chain proteins (MW 26,000–29,000), which are resistant to proteolytic enzymes and heat (Center for Food Safety and Applied Nutrition, 2013). There are five main enterotoxin designated SEA, SEB, SEC (subtypes 1, 2, 3), SED, and SEE, though additional serotypes have recently been described as SEG, SEH, SEI, SEJ, and SEK (Balaban & Rasooly, 2000). It has been reported that the

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populations of *S. aureus* usually need to reach 5–6 log CFU/g, either at the end of the exponential phase or during the stationary stage of its growth, before detectable amount of staphylococcal enterotoxins would be produced (Castillejo-Rodriguez, Gimeno, Cosano, Alcala, & Perez, 2002; Fujikawa & Morozumi, 2006; Le Loir, Baron, & Gautier, 2003; Sutherland, Bayliss, & Roberts, 1994). The amounts of enterotoxin required to produce food poisoning in humans differ among persons due to a wide variation in the sensitivity among individuals. It was reported that a dose of 1 μg of SEA might be sufficient to cause illness in an adult (Gilbert, 1974). An investigation of a staphylococcal gastroenteritis outbreak among school children associated with chocolate milk consumption estimated that as little as 184 ng of SEA in the milk could cause the illness in school children (Bergdoll & Wong, 2006; Evenson, Ward Hinds, Bernstein, & Bergdoll, 1988).

Although several studies have investigated growth of *S. aureus* in foods (Dengremont & Membré, 1995; Eifert, Gennings, Carter, Duncan, & Hackney, 1996; Ross & McMeekin, 1994), no study has been performed to examine growth of *S. aureus* in pre-cooked tuna meat. The temperature in a canned tuna production plant may range from around 20 °C in the processing room >30 °C in the sterilization room. Therefore, the objective of this study was to investigate growth of enterotoxin-producing *S. aureus* in pre-cooked albacore and skipjack tuna meat as well as the time–temperature combinations needed for the bacteria to increase populations in pre-cooked tuna meat by >3 log CFU/g and to produce enterotoxins. Tuna samples were inoculated with *S. aureus* and held at 27 °C to represent the worse condition that might occur in the processing room during canned tuna production and at the optimal temperature (37 °C) for *S. aureus* to grow and produce enterotoxins (Center for Food Safety and Applied Nutrition, 2013; Notermans & Heuvelman, 1983; Vandenbosch, Fung, & Widomski, 1973).

2. Materials and methods

2.1. Bacteria culture preparation

Three *S. aureus* strains (ATCC13565, ATCC 13566 and ATCC 25923) obtained from ATCC (Manassas, Virginia) and 2 strains (N-5014 and N-5018) obtained from the Grocery Manufacturers Association (Washington, DC) were used in this study. Strains ATCC 13566, ATCC 25923, and N-5018 produce enterotoxins A (SEA) and B (SEB) while strains N-5014 and ATCC 13565 produce SEA and enterotoxin D (SED). Each strain was individually grown in 10 ml tryptic soy broth (TSB, Bacto, Becton Dickinson, Sparks, MD) overnight at 37 °C, streaked onto a Baird–Parker (EMD Millipore, Gibbstown, NJ) plate containing 5% egg-yolk tellurite emulsion (Difco, Becton Dickinson) and then incubated at 37 °C for 48 h. One typical colony of *S. aureus* that is smooth, convex, and gray to jet-black surrounded with an outer clear zone was transferred to 10 ml fresh TSB and incubated overnight at 37 °C. After overnight incubation at 37 °C, a volume of 100 μl of enriched culture was transferred into 150 ml fresh TSB and incubated at 37 °C. Growth of *S. aureus* in the medium was measured every 2 h by plating on trypticase soy agar (TSA, BBL, Becton Dickinson) to determine the increases of bacterial cells and enterotoxin production as a result of the growth of *S. aureus*. Each enriched culture in TSB (0.05 ml) was tested for coagulase activity (Coagulase Plasmas Rabbit with EDTA, BBL, Becton Dickinson) as according to procedures described in the FDA's Bacteriological Analytical Manual (FDA, 1998). The ability of each *S. aureus* strain to produce enterotoxin was verified using a commercial test kit (SET-RPLA Staphylococcal Enterotoxin Test Kit TD0900, Thermo Scientific, Sunnyvale, CA) with a detection limit of 0.75 ng/ml (Park & Szabo, 1986).

To prepare a cocktail of *S. aureus* cells, the five *S. aureus* strains were individually grown in 10 ml TSB at 35–37 °C for 20–24 h. Each enriched culture (10 μl) was transferred to 10 ml fresh TSB and incubated at 35–37 °C for 12–16 h to prepare an overnight culture. The TSB cultures of the five strains were then pooled into a 50 ml sterile centrifuge tube and cells were harvested after centrifugation (3000 × *g*, 15 min, 5 °C; Beckman J6-MI, Beckman Coulter, Brea, CA). The cell pellet was resuspended in 50 ml sterile phosphate-buffered saline to produce a multi-strain cocktail suspension of 10^{7-8} CFU/ml.

2.2. Tuna samples

Frozen pre-cooked albacore (loin, chunk, and flake) and skipjack (chunk and flake) samples were provided by a canned tuna producer (San Diego, CA). All samples were cut into pieces (5.0 cm × 5.0 cm × 2.5 cm) of 50 ± 5 g under sanitary conditions, individually sealed in a vacuum plastic bag and kept in a −80 °C freezer to minimize changes in quality and prevent growth of microorganisms until use. The frozen samples were used for studies within 4 months.

2.2.1. Temperature profiles of samples during incubation

Frozen tuna samples were thawed overnight (12–14 h) in a refrigerator (5–7 °C). Thawed samples were placed individually in 400-ml sterile plastic cups with lids loosely covered (Gosselin North America, Tewksbury, MA). The cups were held at room temperature for 10 min to mimic the inoculation process and then incubated at 27 or 37 °C. Change of temperature in a sample during incubation was monitored and recorded at 1.3 cm below surface with thermocouples and data loggers (TrackSense® Pro Mini, Ellab, Centennial, CO) at 2 min intervals. Additional thermocouples, which had been stored at room temperature, were used to monitor temperature change inside the incubators.

2.2.2. Sample characterization

The moisture content of each type of tuna samples was determined following the Method (934.01) of the Association of Official Analytical Chemists Method (AOAC International, 2005) by measuring the overnight weight loss in an oven (1330 GM Gravity Oven, VWR, Radnor, PA) at 105 °C. The water activity of each sample was measured using an AquaLab water activity meter (Series 3, Decagon, Pullman, WA).

The salt (NaCl) content of a sample was measured according to Method 937.09 (AOAC International, 2005). Briefly, finely comminuted tuna sample (5 g) was mixed with 25 ml of 0.1 N $AgNO_3$ (Alfa Aesar, Ward Hill, MA) and 15 ml of 68% HNO_3 (VWR, Radnor, PA). The sample mixture was boiled until the color disappeared followed by addition of 5 ml of ferric alum indicator (RICCA Chemical Company, Arlington, TX). The sample mixture was then titrated with 0.1 N ammonium thiocyanate solution NH_4SCN (Alfa Aesar) to a permanent, salmon-colored endpoint. The NaCl content in the sample was calculated by using Eq. (1).

$$\text{NaCl}\ (\%) = \frac{\frac{(25.0\ \text{ml} - \text{ml}\ NH_4SCN)}{1000\ \text{ml/l}}(0.1\ \text{mol/l})(58.5\ \text{g/mol})}{g\ \text{Sample Weight}} \times 100 \quad (1)$$

Sample pH was determined by measuring the pH of a sample suspension prepared from homogenizing 10 g of sample in 90 ml of deionized water using a Stomacher (400C, Brinkmann, Northbrook, IL) set at 275 rpm for 30 s with a pH meter (Orion, Thermo Scientific, Beverly, MA).