Growth and Production of Enterotoxin A by *Staphylococcus aureus* on “Home-style” French Fries

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**ABSTRACT**

*Staphylococcus aureus* strains were inoculated onto fresh-cut oil-blanched potato strips stored at 21 or 26.7 °C for up to 9 h to determine if the microorganism was capable of growth and staphylococcal enterotoxin (SE) production. Potato strips were assayed for SE using a commercial ELISA kit prior to and following finish frying. *S. aureus* increased by 2.5 to 2.8 log CFU/g over 9 h at 26.7 °C, and SE was detected after 5 h. SE remained serologically detectable following finish frying of the potato strips. It is recommended that oil-blanched potato strips stored at 26.7 °C be finish fried and served, or discarded, within 3 to 4 h to prevent possible production of SE.

Key Words: *Staphylococcus aureus*, enterotoxin A, potatoes, French fries

**INTRODUCTION**

Between 1973 and 1992, there were 417 outbreaks reported in the United States involving more than 18,800 cases of foodborne staphylococcal gastroenteritis (Bean and Griffin, 1990; CDC, 1996). The reported cases probably constitute only a small percentage of the actual number of cases of staphylococcal foodborne disease. Estimates of such occurrence are between 1.2 and 1.5 million cases per year in the United States, resulting in up to 1210 deaths (CAST, 1994). The illness represented 14% of all the cases of foodborne illnesses reported to the Centers for Disease Control and Prevention (CDC) between 1973 and 1987 and ranked as the second most reported cause of foodborne disease behind *Salmonella*.

The potato, in various forms, can support the growth of spoilage microorganisms and pathogenic bacteria and the production of toxin. Foodborne pathogens, including *Clostridium botulinum*, *Bacillus cereus*, *Escherichia coli* O157:H7, *Shigella*, and *Staphylococcus aureus* have been involved in outbreaks associated with the consumption of potato products (Ryan and Cherington, 1971; CDC, 1972, 1975, 1996; Morgan et al., 1988; Seals et al., 1989; Lew et al., 1991).

To produce “home-style” French fries in a foodservice establishment, whole potatoes (peeled or unpeeled) are cut into strips and oil blanched at about 177 °C for 4 min. The potato strips are then held at room temperature (approximately 20 to 27 °C) on sheet pans or in plastic food storage tubs for varying times (up to 8 h) prior to finish frying for 3 to 3.5 min at about 182 to 188 °C (Odiorne, 1996). Fries are generally blanch prior to and following finish frying. *S. aureus* increased by 2.5 to 2.8 log CFU/g over 9 h at 26.7 °C, and SE was detected after 5 h. SE remained serologically detectable following finish frying of the potato strips. It is recommended that oil-blanched potato strips stored at 26.7 °C be finish fried and served, or discarded, within 3 to 4 h to prevent possible production of SE.

Key Words: *Staphylococcus aureus*, enterotoxin A, potatoes, French fries

**MATERIALS & METHODS**

**Cultures, growth conditions, and inoculum preparation**

*S. aureus* ATCC 13565 (FDA 196E) and *S. aureus* ATCC 13566 (FDA S6) were obtained from American Type Culture Collection and maintained on nutrient agar slants at 4 °C. *S. aureus* strain 196E is known to produce staphylococcal enterotoxin Type A (SEA), while strain S6 is known to produce both SEA and staphylococcal enterotoxin Type B (SEB). Cells were grown at 37 °C for 24 h in brain heart infusion broth (BHB; Difco, Detroit, Mich., U.S.A.), centrifuged (12,100 × g at 4 °C for 20 min), washed three times with sterile 0.1% peptone, and adjusted to the desired levels by serial dilution. For growth and visual qualitative SE assay, the two strains were combined in equal proportions prior to inoculation. For the quantitative SEA assay, only *S. aureus* strain 196E was used in order to correlate SEA production with purified SEA.

**Processing and storage of potatoes**

Samples of whole, fresh #2 Russet Burbank potatoes were obtained from a fresh pack processor (Nonpareil Corporation, Blackfoot, Idaho, U.S.A.) and transported to the University of Idaho food microbiology laboratory. The potatoes were stored at 7 or 13 °C for a minimum of 2 wk prior to use. Potato strips (about 0.95 cm² × 10 cm long) were oil blanched in partially hydrogenated soybean oil (Holsum Foods, Portland, Oreg., U.S.A.). For noncommercial blanching, strips were heated at 177 °C for 4 min in an electric fry kettle (Sears, Hoffman Estates, Ill., U.S.A.), and finish frying was 3.5 min at 185 °C. For commercial blanching, strips were heated at 177 °C for 2.5 min in a model E12 counter fryer (Pitco Frialator, Concord, N.H., U.S.A.), and finish frying was 2.0...
min at 185 °C. The oil to potato strips ratio (w/w) in the noncommercial kettle was maintained at 8:1 (Kirkpatrick, 1956), while the commercial fryer oil to potato ratio was ca. 26:1 (w/w) (Pitco Fri- alator, 1996). Blanch and finish fry oil temperatures were moni-
tored using a scanning digital thermocouple thermometer (Cole-
Parmer Instrument Co., Niles, Ill., U.S.A.) using type T thermo-
couples (Omega Engineering, Stamford, Conn., U.S.A.).

pH and water activity (a_w) analysis
An Accumet pH meter (Model 15, Fisher Scientific Santa
Clar, Calif., U.S.A.) was used to determine pH of the potato strips. The water activity (a_w) of the potato strips was measured using an Aqua Lab CX-2 (Decagon Devices Inc., Pullman, Wash., U.S.A.) at about 22 to 25 °C.

Growth of Staphylococcus aureus on potato strips
Oil-blanched potato strips were divided into 3 groups: unincu-
勃勃d control and inoculated with S. aureus at about 3 or 5 log CFU/g. Unincubated control and inoculated oil-blanched potato strips were spread onto sterile aluminum trays and incubated at 21 or 26.7 °C. Duplicate samples were analyzed at 0, 0.5, 1, 3, 5, 7, and 9 h. At each time, a sample was placed in a sterile stomacher blender with 0.1% sterile peptone diluent and blended using a stomacher (Tekmar Co., Cincinnati, Ohio, U.S.A.) blender. Samples were then serially diluted in sterile 0.1% peptone and plated using the spread technique on prepared Baird-Parker agar with egg yolk tellurite enrichment (BPA, Difco, Detroit, Mich., U.S.A.) (Bennett and Lancette, 1995).

Staphylococcal enterotoxin detection and quantification
Samples were analyzed for the presence of SE Types A-E using a commercial enzyme-linked immunosorbent assay (ELISA) kit (Tecra; Intl. BioProducts Inc., Redmond, Wash., U.S.A.). The polyvalent ELISA assay had a reported sensitivity of 1 ng SE/mL for any of seven enterotoxins (A, B, C1, C2, C3, D, E). To detect the presence of SE on fresh-cut potatoes, 10 g of oil-blanched potato strip samples were taken at 0, 3, 5, 7, and 9 h. After finish fry-
ing, the samples were blended in a stomacher blender with 0.25 M Tris buffer (pH 8.0) and centrifuged at 3,000 × g for 10 min at 4 °C. The supernatant was filtered through sterile cotton wool (1.0-
cm thick). The filtrate was adjusted to pH 7.0 to 8.0, using sterile 1 N NaOH. The sample was analyzed by ELISA following in-
structions for the kit. The kit was provided with a color card that was used to match the color that developed in the individual ELISA wells. The resulting color matched sample wells were scored 1, 2, 3, 4, or 5. A visual score of ≥ 3 was considered a positive result for presence of SE if test parameters and all control wells were valid. Positive and negative controls were done for all assays.

For all finish fried samples, a native sample was treated as above, except that 100 g of fried potato strips (extracted with 100 mL of 0.25 M Tris buffer, pH 8.0) were analyzed instead of 10 g. From this sample, 100 mL extract was retained for urea treat-
ment. The retained sample was stirred for 4 to 5 h at room temperature, with 36 g urea added. This sample was referred to as the “renau-
ted” sample. The purpose of the urea treated sample was to de-
termines if SE might have been denatured (serologically) by the fying process (Tatini, 1976). After urea treatment, the sample was dialyzed (12,000 to 14,000 MW cutoff) overnight at room temper-
ature against 30% (v/v) polyethylene glycol. The extract was re-
moved from the dialysis tubing and tested for the presence of SE using the commercial ELISA kit.

For quantification of SE, samples were treated and extracted as described prior to ELISA testing. A standard curve was generated for quantification by adding purified SEA (Toxin Technology, Inc., Sarasota, Fla., U.S.A.) at various concentrations (0.25 to 5.0 ng/mL) into an extract of fresh-cut, oil-blanched potato strips. The SEA containing extracts were tested using the commercial ELISA kit. After the extracts were subjected to ELISA testing and the stop solution was added to all test wells, the contents were immediately transferred to a sterile 96 well microtiter plate. A Titertek Multi-
skran MC (Flow Laboratories Inc., McLean, Va., U.S.A.) was used to measure optical density at two wavelengths (414 and 540 nm). The resulting optical density data were plotted against the concentra-
tion in each sample to generate standard curves. To determine recovery percentages of SEA from the extract procedure, 10, 15, 20, 30, 40, or 50 ng/g SEA were added in triplicate (3 samples for each concentration) to oil-blanched potato strips prior to extraction as described. The SEA-containing samples for each concentration were then extracted with 15, 20, or 30 mL of Tris buffer, as de-
scribed, prior to ELISA assay. These treatment combinations were assayed to determine if increasing SEA concentration or total ex-
tract volume increased toxin recovery.

Statistical analysis
The experimental design for the growth study was a completely randomized design (CRD) of duplicate samples with two repli-
cations of the experiment. Due to the lack of growth of the bacterium on potato strips at 21 °C only the 7 °C whole potato storage temperature was evaluated. Each treatment combination was fit to a quadratic model of the form:

$$
\text{log CFU}_{ij} = \beta_0 + \beta_1 i + \beta_2 i^2 + \epsilon_{ij}
$$

where log CFU = log CFU/g for treatment i and replication j, \( \beta_0 \) = initial estimated log CFU/g of treatment i, \( \beta_1 \) = linear rate of change over time of treatment i, \( \beta_2 \) = quadratic rate change over time of treatment i, \( \chi \) = time after inoculation (h) and, \( \epsilon_{ij} \) = error term (normally distributed) for treatment i and replication j. Each statistical model was examined for adequate fit by residual analy-
sis. Multiple degree of freedom contrasts were then used to test pre-planned comparisons of the quadratic relationships. All com-
putations were carried out using SAS 6.12 (SAS Institute Inc., 1996). Generation times (GT) during exponential growth on potato strips were determined using:

$$
g = (\log t_0) - (\log t_f)/\log 2t
$$

where, g = generation time, \( t_0 \) = log CFU/g at 9 h, \( t_f \) = log CFU/g at 3 h, t = time between \( t_0 \) and \( t_f \) (Prescott et al., 1993).

The SEA recovery data results were analyzed using a one-way analysis of variance to compare SEA amounts added to those re-
covered and to compare total extract volume with amounts reco-

dered (StatMost Ver. 2.01, DataMost Corp., Salt Lake City, Utah, U.S.A.).

RESULTS & DISCUSSION
The pH range of raw potato strips was 6.03 to 6.13 for strips produced from potatoes stored at 7 or 13 °C. The pH range of stored oil-blanched and finish-fried potato strips was 5.71 to 6.17. The \( a_w \) range of stored oil-blanched and finish fried potato strips was 0.933 to 0.989 (at about 25 °C). The pH and \( a_w \) of the raw, oil-blanched, and finish fried potato strips were within the range for growth of S. aureus (Notermans and Hauvelman, 1983; Smith et al., 1983; Genigeorgis, 1989; Halpin-Dohnalek and Marth, 1989).

Staphylococcus aureus began to grow after 1 to 3 h at 26.7 °C at both low and high inoculation levels and at both whole potato storage temperatures (Fig. 1 and 2). The pathogen increased from 2.4 to 3.2 log CFU/g and 5.4 to 5.9 log CFU/g at both low and high inoculation levels, respectively (Fig. 3). At 26.7 °C, S. aureus grew faster (P < 0.05) than at 21 °C at both inoculation levels. The high (about 5.0 log CFU/g) initial inoculation level of S.
aureus/g of oil-blanced potato strips yielded slower (P < 0.05) growth than the low (about 3.0 log CFU/g) initial inoculation level (Fig. 1 and 2), but the shapes of these growth curves were not different (P > 0.05). These differences may have been due to competitive inhibition at the higher inoculation level, which has been reported for S. aureus by others (Smith et al., 1983).

A whole potato storage temperature of 13 °C yielded faster (P < 0.05) growth at 26.7 °C of S. aureus than did the 7 °C at both inoculation levels (Fig. 1 and 2). A whole potato storage temperature of 13 °C resulted in shorter generation times for Staphylococcus aureus at both inoculation levels. There were no obvious trends between pH or aw and growth rate that might suggest these factors contributed to the differences. It is recognized that whole potato storage temperature influences the reducing sugar concentration that may have affected growth of the organisms (Pavlista and Ojala, 1996). While the differences were statistically significant, they were not of practical importance, as there was less than one log cycle difference after 9 h at 26.7 °C.

No viable S. aureus cells were recovered from the frying processes either from the fry oil or the finish fried potato strips (data not shown). Populations of S. aureus were reduced by about 6.0 to 7.5 log CFU/g by the finish frying processes.

Using the visual ELISA assay, S. aureus strains 196E and S6 produced detectable SE at both inoculum levels after 5 h storage at 26.7 °C on the fresh-cut, oil-blanced potato strips (Table 1). No SE was detected on potato strips held at 21 °C during the 9 h storage at either inoculation level (data not shown). Visual ELISA analysis indicated that SE was produced at 26.7 °C after 5 h on potato strips when S. aureus populations reached 5.0 and 6.7 log CFU/g, for the low and high inoculation levels, respectively. The scores for the low inoculation levels remained at the same SE positive visual score of 3 for the remainder of the 9 h storage, while the high inoculation level samples increased to a score of 5 (Table 1). The visual ELISA score for the high inoculation samples after frying was reduced from 5 to the baseline positive score of 3. Pretreatment of samples with 6 M urea prior to concentration and serological assay did not increase visual scores for the finish fried potato strip samples.

A standard curve was prepared by adding various concentrations of purified SEA to an extract of fresh-cut, oil-blanced potato strips followed by ELISA (Fig. 4). Based on that curve, recovery of SEA ranged from 9.2% to 40.7% (data not shown). Similar SEA recovery rates of 18% to 26% from sausage extracts containing 100 ng had been reported by Schonwalder et al. (1988) using a polyclonal antibody ELISA. Relationship of the recovery of SEA and the total extract volume was not significant (p = 0.06). More importantly, there was no relationship between amount of toxin added and concentrations recovered (p = 0.20).

Enterotoxin recovery from food is dependent upon the efficiency of the extraction method, type of food, proteinaceous substances, and the sensitivity and reliability of the detection method (Schonwalder et al., 1988). Freed et al. (1982) recognized that...
blank extracts of the same food origin, and same dilution should be used in an ELISA to prevent errors due to the food matrix. Although blank (negative control) extracts (potato strips extracted with Tris buffer) were used, under normal circumstances, identical negative control food samples would rarely be available. In our study, SEA may have been bound to various components of the oil-blanched potato strips or the frying oil within the potato strips. As this is likely, the first report on SEA extraction of oil-blanched and finish fried potato strips, the results could not be reliably compared to previous research. Note that the manufacturer made no claim to quantifiable results or recovery of staphylococcal enterotoxins from foods and that the ELISA kit was not necessarily intended to be used for quantitative data (Tecra; International Bioproducts Inc., Redmond, Wash., U.S.A.).

Although there was a lack of precision in the quantification of SEA using the ELISA, we considered it as a semi-quantitative measurement of the toxin produced during growth of S. aureus on the potato strips. At the low inoculation level (about 3.0 log CFU/g), S. aureus strain 196E produced 0.15 to 0.17 ng/g and 0.23 to 0.26 ng/g SEA on fresh-cut, oil-blanched potato strips after 7 and 9 h storage at 26.7 °C, respectively (Fig. 5). Enterotoxin production at the high inoculation level was only detected in one of the replications at 5 h when the S. aureus population reached 6.68 log CFU/g. Other researchers had reported estimated levels of SEA in contaminated chocolate milk and canned mushrooms ranging from 0.4 to 79 ng/g (Evenson et al., 1988, Anderson, 1996). The rapid production of SE under the conditions studied may be due to the lack of competing microflora (Noleto et al., 1987). The blanching treatments essentially eliminated the background microflora to < 10 CFU/g (data not shown).

### Table 1—Staphylococcal enterotoxin production by Staphylococcus aureus Strains 196E and S6 on fresh cut, oil-blanched potato strips held at 26.7 °C

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Visual ELISA Score&lt;sup&gt;a&lt;/sup&gt; at Holding Time After inoculation (h)</th>
<th>Negative control</th>
<th>Positive control</th>
<th>Fried Potato Strips (2 to 3.5 min at 185 °C)</th>
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<td></td>
<td>0 3 5 7</td>
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<td>Native sample</td>
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<td>Noncommercial frying vat</td>
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<td>Low&lt;sup&gt;b&lt;/sup&gt;</td>
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<td>High&lt;sup&gt;c&lt;/sup&gt;</td>
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<tr>
<td>Commercial frying vat</td>
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<td>Low</td>
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<tr>
<td>High</td>
<td>1–2 1–2 3 4 5</td>
<td>1–2 3 3</td>
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<sup>a</sup>Staphylococcus aureus Strains 196E and S6 combined in equal numbers.
<sup>b</sup>Low initial inoculation level of about 3 log CFU/g.
<sup>c</sup>High initial inoculation level of about 5 log CFU/g.
<sup>d</sup>Visual ELISA scores 1-2 = Negative; ≥ 3 = Positive.

Fig. 4—Standard curve created with varying concentrations of purified staphylococcal enterotoxin Type A in an extract of fresh-cut, oil-blanched potato strips.

Fig. 5—Production of staphylococcal enterotoxin Type A by Staphylococcus aureus 196E on fresh-cut oil-blanched potato strips stored at 26.7 °C from a low inoculation level (about 3 log CFU/g).
Staphylococcal enterotoxins were detected on finished fried potato strips in both the native and renatured samples from both commercial and non-commercial finish frying processes. The detection of viable SE serological activity after finish frying at 185 °C for 2 to 3.5 min was not surprising. The toxin can remain serologically and biologically active after exposure to 121 °C for 19 to 30 min in buffer or beef bouillon (Denny et al., 1971, Humber et al., 1975, Tibana et al., 1987, Anderson, 1996). Denny et al. (1971) and Lee et al. (1977) demonstrated that using food as the heating medium was shown to increase the thermal stability of staphylococcal enterotoxin 2- to 5-fold. They suggested that the food components provided some protective effect possibly through protein-protein interactions (Denny et al., 1971). It has been reported that SEA remained serologically and biologically active after heat treatment in canned mushrooms at 121 and 127 °C for 16.5 to 28 min and 11 to 15 min, respectively (Anderson, 1996).

CONCLUSION

THE POTENTIAL OCCURRENCE OF S. AUREUS ENTEROTOXIN ON finished home-style French fries is a food safety concern. Ingestion of as little as 100 to 200 ng of SE can produce symptoms of staphylococcal intoxication (Everson et al., 1988), and several factors contribute to susceptibility of persons to the enterotoxins. To determine a normal serving size, large-sized French-fry orders were purchased from 6 local area restaurants. The weight of a large order of French fries ranged from 96 to 156 g with a mean of 135 ± 23 g. If this serving size was applied to the estimated consumption of French fries produced under abuse conditions could contain approximately 31 to >124 ng of SEA. Therefore, it is recommended that oil-blanced potato strips stored at room temperature should be finished fried and served or discarded within 3 to 4 h after Blanching and that this practice should be strictly adhered to in order to prevent the production of SEA.

REFERENCES


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