Enhanced Production and Thermal Stability of Staphylococcal Enterotoxin A in the Presence of Chitin

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ABSTRACT

The link between the only outbreaks of staphylococcal food poisoning involving canned foods, mushrooms, and lobster bisque soup may be the presence of chitin in these foods. This study was undertaken to evaluate the influence of chitin on production and heat stability of staphylococcal enterotoxin A (SEA). *Staphylococcus aureus* 743 was cultured in brain heart infusion (BHI) broth with and without 0.5% crude chitin for 20 h at 30°C. The *S. aureus* CFU were enumerated in tryptic soy agar cultures. The concentration of SEA produced was estimated with an enzyme-linked immunosorbent assay kit and compared to a standard curve constructed with purified SEA. The concentration of SEA in the broths was adjusted to 100% or 50% of the mushroom weight on a fresh basis. The diluted broths were thermally processed in 211 × 212 cans in a still retort at 121°C for 16.5 or 28 min. Postprocess SEA concentrations were determined. The effect of crude chitin concentration on SEA production was determined by adjusting the chitin level from 0 to 2.0% in BHI broth.

The presence of 0.5% crude chitin in BHI broth increased SEA production by an average of 52%. Numbers of *S. aureus* 743 cells were not affected. Chitin significantly increased the thermal stability of SEA to the 16.5 and 28 min thermal processes, with 10.5% and 7.3% average increases in recovery, respectively. A crude chitin concentration as low as 0.1% enhanced SEA formation. Production of SEA increased at higher concentrations of chitin. Purified chitin powders had similar stimulatory effects on SEA production. These results indicate that chitin-containing canned foods may be prone to cause staphylococcal food poisoning outbreaks due to increased production and postprocess persistence of active enterotoxin.

Key words: Staphylococcal enterotoxin, chitin, heat stability

The heat stability of the serological and biological activities of staphylococcal enterotoxins (SEs) have been well documented (1, 2, 3, 9, 10, 15, 22, 25, 26, 27). In spite of this evidence demonstrating the extreme thermal stability of SEs, it was concluded that commercial retort processes to which most canned foods are subjected would be adequate to destroy SEs present in foods (5, 10). However, there have been five reported outbreaks of staphylococcal food poisoning associated with canned foods (6, 7). One outbreak occurred in 1975 and was ascribed to domestically canned lobster bisque soup (6), and four outbreaks occurred in 1989 that were attributed to canned mushrooms imported from the People’s Republic of China (7).

A common link between the two implicated products may be the presence of chitin in these foods. Chitin is the primary structural component of mushroom tissue, accounting for up to 0.5% of the mushroom weight on a fresh basis (17) and for up to 85% and 26%, respectively, of crustacean and molluscan shells (21). Chitin is a basic helical polymer of repeating N-acetyl-d-glucosamine units with a mass of approximately 1.04 MDa (21). It is reported to bind amino acids and proteins in stable but dissociable complexes, particularly through tyrosine residues, and is used as an anionic exchanger in chromatographic applications and as a solid support for enzyme immobilization (21).

Proteins may be stabilized to heat through immobilization or attachment to supports increasing protein rigidity (14). Hence, staphylococcal enterotoxins may have been stabilized against destruction during thermal processing through the formation of complexes between the SEs and the chitin in mushrooms or in lobster bisque soup, thereby contributing to SE persistence during retorting. This study was undertaken to evaluate the influence of chitin on the production of staphylococcal enterotoxin and on the thermal stability of SE in retort processes.

MATERIALS AND METHODS

*Enterotoxigenic Staphylococcus aureus* growth conditions and SEA detection

Enterotoxigenic *Staphylococcus aureus* 743, a type A enterotoxin producer implicated in a food poisoning outbreak, was obtained from R. Bennett of the Division of Microbiological Studies of the U.S. Food and Drug Administration (FDA). The test organism was maintained on a tryptic soy agar (TSA) (Difco Laboratories, Detroit, MI) slant at 4°C. For the inoculum preparation, a loopful of slant growth was cultured in 10 or 100 ml of brain heart infusion broth (BHI) (Difco) for 18 h at 35°C. An 0.5%
Inoculum was added to 100 ml in a 250-ml flask or to 3 liters in a 6-liter flask of BHI broth and BHI broth containing 0.5% (wt/vol) practical grade chitin flakes (Eastman Kodak Co., Rochester, NY) (see Table 1, product A). The broth cultures were incubated for 20 h at 30°C with shaking at 75 or 125 rpm, with the rotation speed adjusted according to the volume of the culture; the 3-liter volumes were agitated at the lower rate. After 20 h, the S. aureus cells were enumerated through serial dilutions in 0.1% peptone water and pour plated in TSA. Plates were incubated at 35°C for 48 h.

Twenty milliliters of each broth culture were also removed and centrifuged at approximately 10,000 × g for 15 min at 4°C. The supernatants from each were evaluated for the presence of SEA with the Tecra Staphylococcal Enterotoxin (SET) Visual Immunoassay (Bioenterprises Pty Ltd, Roseville, Australia). Similarly, uninoculated BHI broth and BHI with 0.5% chitin were evaluated with the Tecra serological assay. Absorbance values were read with a Microplate Autoreader (Bio-Tek Instruments, Wooniski, VT) at dual wavelengths of 405 and 490 nm. Levels of SEA present were estimated from a standard curve prepared with purified SEA (Toxin Technology Inc., Sarasota, FL) at concentrations of 0, 0.25, 0.5, 0.75, 1.0, 1.5, 2.5, 4.0, and 5.0 ng/ml. The sensitivity of the Tecra assay was determined to be 0.5 ng/ml. The remainder of the 3-liter broth cultures were chilled immediately and held overnight at 4°C for subsequent thermal processing studies.

Effect of purified chitin in BHI broth on SEA production

To determine if the crude chitin itself or another component present in the chitin preparation was responsible for the effects on SEA production observed, additional sources of chitin, purified and crude, were evaluated. The product descriptions, sources, and compositional analysis for each chitin test are shown in Table 1. Each chitin product was prepared at 0.5% (wt/vol) in 100 ml of BHI broth in screw-cap flasks. The preparation of the S. aureus inoculum, the culture conditions, and postincubation analysis of the broths were the same as described previously.

Effect of chitin concentration in BHI broth on SEA production

Crude chitin (Table 1, product B) was added to 100 ml of BHI broth in screw-cap flasks at concentrations (wt/vol) of 0%, 0.1%, 0.25%, 0.5%, 0.75%, 1.0%, and 2.0%. The inoculum preparation, incubation conditions, and postincubation analysis of the broths for SEA serological activity and growth of S. aureus were conducted as described previously.

Table 1. Chitin products evaluated

<table>
<thead>
<tr>
<th>Product designation</th>
<th>Manufacturer</th>
<th>Chitin description</th>
<th>Source of chitin</th>
<th>Compositional analysisa</th>
</tr>
</thead>
</table>
| A                   | Eastman Kodak Co., Rochester, NY | Practical grade chitin flakes | Shrimp and crab shells | C: 47.29%  
N: 6.79%  
H: 6.45% |
| B                   | Sigma Chemical Co., St. Louis, MO | Practical grade chitin powder | Crab shells | C: 39.62%  
N: 6.94%  
H: 5.30% |
| C                   | Sigma Chemical Co. | Purified chitin powder  
Preparation method: Hirano and Nagao (12) | Crab shells | C: 44.32%  
N: 6.55%  
H: 6.74% |
| D                   | Sigma Chemical Co. | Purified chitin powder  
Preparation method: Skujins et al. (26) | Crab shells | C: 42.01%  
N: 6.72%  
H: 6.10% |
| F                   | Sigma Chemical Co. | Purified chitin powder  
Preparation method: Skujins et al. (26) | Shrimp shells | C: NA  
N: NA  
H: NA |

a Compositional analysis carbon (C), nitrogen (N), and hydrogen (H) provided by the manufacturers; NA, not available.

b Product B is the starting material for preparation of products C and D.

Thermal processing of broth cultures and SEA recovery

The concentrations of SEA in the chilled 3-liter 20-h BHI and BHI with 0.5% crude chitin (Table 1, product A) broth cultures were adjusted to target levels of 100 or 50 ng of SEA per ml by the addition of sufficient deionized water to each broth culture. The diluted broths were individually mixed. After dilution the initial preprocess SEA concentrations were higher than the target of 100 ng/ml and slightly lower than the target of 50 ng/ml. The pH values of the broths were approximately 6.5 after dilution. The diluted broth cultures were each dispensed at 180 ml per can into 211 × 212 cans using a Manostat Variastaltic pump (Manostat, New York, NY) and Tygon tubing (Norton Performance Plastics Corp., Akron, OH). Separate tubing was used for each broth. Uninoculated BHI and BHI with 0.5% chitin broths were similarly filled into 211 × 212 cans. This fill volume permitted a can headspace of approximately 7 to 8 mm and average fill weights of 182.3 g for the BHI broth and 190.2 g for the BHI broth with chitin. After being filled, the cans were immediately sealed under vacuum in flowing steam at 40 lb/in² in a Canco 423-1ES-00 closing machine (American Can Co., Greenwich, CT). The sealed cans were chilled at 4°C for approximately 40 min to equilibrate the temperature of the cans before thermal processing.

Filled cans were removed from the cooler immediately before thermal processing in a vertical still retort (Model BRD-962B, Berlin Chapman Co., W. Berlin, WI). The cans were subjected to thermal processes of 16.5 min or 28 min, total process time, at 121°C. For estimation of the pre-thermal-process SEA level in the broths, 2 filled cans of each medium and from each thermal process time were removed prior to processing and further chilled in an ice slurry. The unprocessed cans were opened, the contents were centrifuged at approximately 10,000 × g for 15 min at 4°C, and the supernatants were tested with the Tecra SET assay. The individual
SEA levels determined from the unprocessed cans were used to calculate an average initial SEA concentration for each medium.

The postprocess SEA recovered for each thermal process treatment and medium (BHI alone or BHI with 0.5% chitin) was estimated by evaluation of the contents of 6 individual cans per treatment variable, using the Tecra SET assay as described previously. Concentration or urea pretreatment of the heated samples was not required for SEA detection. The average SEA recovered was calculated for each medium and thermal process and was used to determine the overall destruction of SEA in each medium after each thermal process. An analysis of variance at \( \alpha = 0.05 \) by means of the general linear models procedure of SAS (SAS Institute, Inc., 1985) was used to determine significant differences in SEA destruction between the BHI and BHI with 0.5% chitin.

Native discontinuous polyacrylamide gel electrophoresis (PAGE) and Western blotting (immunoblot) techniques were used to confirm the presence of SEA in the BHI and BHI with 0.5% chitin broths after thermal processing (1).

### RESULTS

Effect of crude chitin on cell growth and SEA production

The presence of 0.5% crude chitin in BHI broth had no effect on cell numbers of \( S. \) aureus 743 under the incubation conditions used (Table 2). The average numbers of staphylococci over four experiments were \( 1.5 \times 10^6 \) CFU/ml in BHI broth without chitin added and \( 1.6 \times 10^6 \) CFU/ml in BHI broth with 0.5% chitin.

The presence of 0.5% crude chitin increased SEA production in the BHI broth overall by an average of approximately 52% (Table 2). The range of enhanced SEA production with chitin present over the five experiments was 18.3 to 84.1%. The largest increases in SEA were detected in the 100-ml volume cultures with higher incubation rotational speed (Table 2, experiments 1 and 2), yielding an increase in SEA of 75.8 to 84.1% (Table 2). There was no serological activity detected in uninoculated BHI or BHI with crude chitin in the Tecra assay. Under the same conditions of inoculation and incubation, the presence of 0.5% cellulose (J. T. Baker, Phillipsburg, NJ) in the BHI resulted in an average 18.2% decrease in the level of SEA produced in comparison to BHI broth alone, even though the average number of \( S. \) aureus cells after the 20-h incubation was similar at \( 2.2 \times 10^9 \) CFU/ml (1).

Effect of purified chitin in BHI broth on SEA production

An additional crude chitin product from a different manufacturer (Table 1, product B) as well as the three purified chitin powders (Table 1, products C, D, and E) were all found to increase SEA production by \( S. \) aureus 743 in BHI broth by an average of 20.5 to 54.6% over the three replications of the experiment (Table 3). The increased levels of SEA produced in the presence of chitin were not significantly different among the chitin products evaluated (Table 3). There was no effect on numbers of the staphylococci by the presence of any of the chitin products in the BHI broth. The numbers of staphylococci present in the broths at the end of the 20-h incubation were similar for each of the chitin products and in BHI alone, and were consistent over the three replications of the experiment (Table 3).
The presence of higher concentrations of 0.25 to 2.0% crude chitin to BHI broth resulted in larger average increases in SEA production, ranging from 72 to 91% (Figure 1). The levels of SEA detected increased as the concentration of chitin increased, but the higher levels of chitin did not result in proportionately larger amounts of SEA (Figure 1). The cell numbers of *S. aureus* 743 were not affected by chitin concentrations of 0 to 1.0%, since the staphylococcal counts were similar at each of those chitin levels (1). The number of staphylococci in the BHI broth with 2.0% chitin were approximately 1.5-fold higher than with the lower chitin concentrations (1).

**Recovery of SEA from broth cultures after thermal processing**

The presence of 0.5% chitin in BHI broth significantly enhanced the overall thermal resistance of SEA during retorting with both initial concentrations of SEA and after both the 16.5- and 28-min processes (Table 4). The average increase in recovery of SEA serological activity was 10.5 and 7.3% with crude chitin present in the growth and heating media after the 16.5- and 28-min processes, respectively (Table 4).

Destruction of SEA serological activity was significantly different between the 16.5- and 28-min processes (1). There was no interaction effect between the presence of chitin and the severity of the thermal process (1).

Uninoculated control cans of BHI broth and BHI broth with 0.5% crude chitin were negative for serological activity or other reaction with the Tecra SET assay after the 16.5- and 28-min processes.

The presence of SEA in the thermally processed BHI and BHI with 0.5% chitin broths was confirmed by Western blot (1). A decrease in band intensity with the higher thermal process was evident, indicating a greater degree of SEA destruction as the severity of the process increased (1).

**TABLE 4. Effect of 0.5% crude chitin (practical chitin flakes, Table 1, product A) on recovery of SEA serological activity in BHI broth after thermal processes at 121°C**

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Thermal process (min)</th>
<th>Medium</th>
<th>Avg. preprocess SEA (ng/ml)</th>
<th>Avg. postprocess SEA (ng/ml)</th>
<th>Avg. log decrease SEA with processing</th>
<th>% increase SEA recovered with chitin</th>
</tr>
</thead>
<tbody>
<tr>
<td>3</td>
<td>16.5</td>
<td>BHI</td>
<td>127.3</td>
<td>5.0</td>
<td>1.41 ± 0.05</td>
<td>4.4</td>
</tr>
<tr>
<td></td>
<td></td>
<td>BHI + chitin</td>
<td>131.2</td>
<td>5.9</td>
<td>1.34 ± 0.04</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>16.5</td>
<td>BHI</td>
<td>46.8</td>
<td>1.8</td>
<td>1.43 ± 0.05</td>
<td>13.9</td>
</tr>
<tr>
<td></td>
<td></td>
<td>BHI + chitin</td>
<td>48.2</td>
<td>2.8</td>
<td>1.23 ± 0.03</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>16.5</td>
<td>BHI</td>
<td>38.1</td>
<td>1.6</td>
<td>1.39 ± 0.05</td>
<td>13.1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>BHI + chitin</td>
<td>47.1</td>
<td>3.0</td>
<td>1.21 ± 0.04</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>28</td>
<td>BHI</td>
<td>127.3</td>
<td>2.7</td>
<td>1.69 ± 0.16</td>
<td>7.5</td>
</tr>
<tr>
<td></td>
<td></td>
<td>BHI + chitin</td>
<td>131.2</td>
<td>3.6</td>
<td>1.57 ± 0.09</td>
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<tr>
<td>4</td>
<td>28</td>
<td>BHI</td>
<td>46.8</td>
<td>1.3</td>
<td>1.57 ± 0.03</td>
<td>5.8</td>
</tr>
<tr>
<td></td>
<td></td>
<td>BHI + chitin</td>
<td>48.2</td>
<td>1.7</td>
<td>1.48 ± 0.09</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>28</td>
<td>BHI</td>
<td>38.1</td>
<td>1.2</td>
<td>1.50 ± 0.03</td>
<td>8.7</td>
</tr>
<tr>
<td></td>
<td></td>
<td>BHI + chitin</td>
<td>47.1</td>
<td>2.0</td>
<td>1.37 ± 0.02</td>
<td></td>
</tr>
</tbody>
</table>

* The target pre-thermal-process SEA concentrations were 100 ng/ml for experiment 3 and 50 ng/ml for experiments 4 and 5.

b % increase SEA recovered was calculated from the difference in average log decrease SEA between BHI and BHI with chitin for each experiment and thermal process.
DISCUSSION

Chitin is the second most abundant polysaccharide in nature, next to cellulose (16), and is a common structural component of the cell walls of fungi, including mushrooms, and of the cuticles of crustaceans and molluscs (21). In living systems, the long polymeric chains of chitin are usually associated with proteins and/or glucans (21). That chitin was present in the only two canned food products implicated in staphylococcal food poisoning (mushrooms and lobster bisque soup) suggested that chitin might play some role in production of enterotoxins or affect their heat stability.

The presence of 0.5% crude chitin in BHI broth (a concentration representative of chitin levels present in fresh mushrooms) increased the production of SEA. Higher levels of SEA were produced in the 100-ml culture volumes than in the 3-liter cultures. This effect may be the result of increased agitation in the smaller volumes with the higher speed of rotation. Alternatively, the surface-to-volume ratio in the flasks of 100 ml of medium may be sufficiently different from that of the 3-liter cultures to affect SEA formation. The amplified SEA production occurred independently of the growth of S. aureus 743, since the numbers of staphylococci present after the 20-h incubation period were comparable in medium with or without added chitin. In contrast, when 0.5% cellulose was added to the BHI broth, SEA production was reduced, independently of growth, by an average of about 18%. Cellulose and chitin share many functional properties and physical characteristics. These results suggest the effect of chitin on SEA production was probably not due solely to the presence of particulates or a polysaccharide in the medium, but might be unique to chitin.

Woodburn and others (30) reported increased SEA formation in unshaken colloidal dispersions if corn starch or carrageenan was present, but not agar or low-methoxyl pectin, suggesting that the effect on SE production may be dependent on the colloid present. Similarly, Tieno and Hanna (28) demonstrated that the production of toxic shock syndrome toxin 1 (TSST-1), a staphylococcal exoprotein similar in molecular weight, isoelectric point, and secondary structure to SEs (13, 18), was stimulated by the presence of certain fibers in BHI broth supplemented with yeast extract. A combination of polyester and carboxymethylcellulose had the largest stimulatory effect on TSST-1 formation, with a 100- to 300-fold increase over the control broth without fibers. Cotton, which is almost pure cellulose, and cellulosic materials, however, had no effect on TSST-1 production (28), indicating that the effect was fiber specific.

It has been shown that the cell surface of S. aureus is important in the production of SEB (4). Tweten and Iandolo (29) and Christianson and coworkers (8) demonstrated that mature SEB and SEA, respectively, are transiently associated with or sequestered in specialized regions at the cell wall before being released to the extracellular environment. S. aureus is reported to have a moderately hydrophobic cell surface (23). Microbial cells with hydrophobic character are attracted to hydrocarbon films (24), suggesting that hydrophobic interactions as well as electrostatic attraction and Van der Waals forces may be important in bacterial attraction and attachment to these surfaces or to fibers (19). Reid and coworkers (23) found that a clinical isolate and TSST-1-producing strain of S. aureus adhered to diaphragm fibers. Such interactions between the hydrophobic cell surface of S. aureus and chitin in the BHI broth may have occurred and influenced the release of SEA from the cell wall area, to partially account for the stimulatory effect on SEA observed in this study.

The three purified chitin products (Table 1, products C, D, and E) had stimulatory effects on SEA production similar to those of the crude (practical grade) chitins (products A and B), without affecting growth of S. aureus 743. Two of the three purified chitins (products C and D) were derived from crab shells, while shrimp shells were the source for the third purified material (E), suggesting the effect on SEA formation was not unique to crab chitin. The crude chitin product B is the starting material for preparation of purified colloidal chitins C and D. Chitin C is prepared by the method of Hirano and Nagao (12) using aqueous 56% methanesulfonic acid hydrolysis and precipitation in ice-water, whereas chitin D is purified using the method of Skujins et al. (25) with concentrated hydrochloric acid digestion and precipitation in a 50% aqueous ethanol solution. The purification procedures may alter the physical state of the chitin starting material in the acid treatments, resulting in an opening of the chitin chain structure to form colloidal chitin (12, 21). This information suggests that the increased SEA production by purified chitins was not related to the purification method used. It is unlikely that a contaminant in the crude chitin was carried through both purification methods, was present in the purified products, and was responsible for the stimulatory effect, although this possibility cannot be entirely dismissed.

SEA production was increased at crude chitin concentrations as low as 0.1%. This concentration of chitin is approximately fivefold less than chitin levels found in fresh mushrooms. No information is available regarding residual levels of chitin that may be present in processed products from crustaceans, like lobster bisque soup. The stimulatory effect on SEA was related to chitin concentration, but higher concentrations up to 2.0% chitin did not result in additional proportionate increases in SEA levels. Growth of S. aureus 743 was not affected by chitin concentrations up to 1.0%, since the numbers of staphylococci after 20 h were comparable. At 2.0% chitin however, the numbers of staphylococci were approximately five times higher after the 20-h incubation. The additional chitin present may have been sufficient to provide increased surface adsorbing area.

The presence of crude chitin (Table 1, product A) in the growth medium and during thermal processing significantly increased the persistence of two different initial concentrations of active SEA after the 16.5- and 28-min retort processes at 121°C. Approximately 10% more SEA was recovered with chitin present after the 16.5-min process, on the basis of the difference in the average log decrease of SEA activity with and without chitin. The equivalent lethality or sterilizing value (F0) of the 16.5-min process for broth in
211 × 212 cans was not determined; however, 16.5 min at 121°C with an initial product temperature of 41°C was found previously to deliver an F₀ value of 7 to whole mushrooms in brine in the same size can (1). Chitin similarly increased the persistence of SEA activity by approximately 8% after a severe thermal process of 28 min at 121°C. These results may be important since the enterotoxin levels that may be found in contaminated foods linked to foodborne illness are usually low, ranging from 0.4 to 10 ng/g or ml (11, 26) and chitin appears to be effective in enhancing thermal stability at low SE concentrations. Although the F₀ value delivered with the 28-min process was not determined for the broth in 211 × 212 cans, it was previously shown to be equivalent to an F₀ of 18 for whole mushrooms in brine, with an initial product temperature of 41°C (1).

The serological and biological activities of a comparable concentration of SEA (58 ng/g) produced in situ in blanched mushrooms were previously found to survive retort processes equivalent to those administered to the broths in this study (1, 2). It has been shown by others that food components in the heating menstrua increase the thermal stability of SEs approximately two- to fivefold by providing an unknown protective effect against heat inactivation (9, 15). Chitin is commonly found in nature in association with proteins and is known to bind proteins in stable but dissociable complexes upon pH changes (21). As a basic polymer, chitin is used in chromatographic applications as an anionic exchanger and as a support for affinity chromatography (21).

Similarly, the use of chitin as a support for enzyme immobilization further substantiates its ability to bind proteins in stable complexes (21). Proteins may be stabilized to heat through immobilization or attachment to such supports by increasing the rigidity of the protein and maintaining an active protein conformation (14). Complex formation between SEA and chitin in the growth and heating media may have occurred to further stabilize an already resistant protein against denaturation during thermal processing.

The results of this study suggest that foods containing even low concentrations of chitin may pose an increased risk to the population from staphylococcal food poisoning, if the foods become contaminated with S. aureus and are mishandled to permit growth and SE production. The effect of 0.5% crude chitin in stimulating SEA production by up to 52 to 88% (Table 2 and Figure 1) may have a more significant impact on the potential for food poisoning than its ability to further enhance the thermal stability of SEA by 8 to 10%, since it is established that enterotoxins are not destroyed by retort processes. When these two effects are combined, however, it could result in approximately a 1.5- to 2-fold increase in SEA present in a thermally processed food. As an example of such a calculation: an expected SE concentration in foods of approximately 10 ng/g and a 52 to 88% increase in production with chitin results in 15.2 to 18.8 ng/g; reduced an average of 2.5 log in thermal processing of whole mushrooms (1, 2), and 10% increased recovery leaves 0.08 to 0.10 ng of SE per g postprocess. Without chitin, 10 ng/g reduced 2.5 log with processing results in 0.05 ng/g. This combination could have been a factor in the outbreaks of staphylococcal food poisoning with imported canned mushrooms and canned lobster bisque soup. The nature of the interactions that may occur between SEA and chitin and the role that chitin plays in SE production should be investigated further.

REFERENCES