Heat Processing of Oysters Naturally Contaminated with *Vibrio cholerae* Serotype 01

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**ABSTRACT**

Pathogenic *Vibrio cholerae* 0-Group 1 survived for more than 3 weeks in artificial sea water with little loss in viability. Live oysters placed in such contaminated, artificial sea water took up but did not concentrate *V. cholerae*. Heat treatments provided by an in-can pasteurization process and by preparation of naturally contaminated oysters according to common recipes effectively reduced the numbers of *V. cholerae* by 5 logs/g.

The foodborne pathogen, *Vibrio cholerae* serotype 01 Inaba, biotype El Tor, has been identified as the causative agent in recent cases of severe gastroenteritis among residents of southern U.S. coastal regions (2). These outbreaks plus the increasingly frequent isolation of *V. cholerae* 01 organisms from estuarine waters and shellfish (3,5) have raised concern that these organisms may have established endemic foci in U.S. coastal waters.

The potential contamination of oysters taken from approved harvesting areas with *V. cholerae* 01 organisms poses an obvious hazard to public health. One possible remedy for such a hazard would be heat processing of oysters. The feasibility of using an in-can pasteurization process to eliminate hepatitis A virus from shucked oysters has been investigated (6). In that study, thermal processing at 60.8 C for up to 42 min was insufficient to inactivate hepatitis A in size 300 x 206 cans of inoculated, shucked oysters.

The objective of this study was to determine whether heat treatment by the proposed pasteurization process (6) or by preparation according to common oyster cooking recipes would effectively eliminate the pathogen in shucked oysters naturally inoculated with *V. cholerae* 01.

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Survival of *V. cholerae* 01 in canned, heat-treated oysters at 57.2°C

Shellstock oysters naturally inoculated with *V. cholerae* 01 were shucked and prepared according to recipes for oyster stew and oysters Rockefeller (1). Two batches of oyster stew were prepared in the following manner: (a) butter was melted in a pan; (b) flour, seasonings, and undrained oysters were added to melted butter; (c) the oyster mixture was simmered and stirred for 3-4 min until the edges of the oysters curled; (d) scalded milk was added and the mixture was removed from heat. Internal oyster temperatures were measured at the center of the pot.

Four batches of oysters Rockefeller were prepared in the following manner: (a) a shucked oyster naturally inoculated with *V. cholerae* 01 was placed on a half-shell; (b) a mixture of chopped spinach, onion, parsley, bread crumbs, butter and spices was placed over and around the oyster; (c) the oyster was impaled on a thermocouple to monitor the internal temperature during baking; (d) oysters were baked for 10 min in a 232.2°C oven on a bed of hot rock salt.

Our analyses of the canned- and recipe-prepared oysters were restricted to bacteriological quality.

**RESULTS AND DISCUSSION**

Public Health authorities faced with recent outbreaks of cholera (2) have been concerned that these incidents might indicate the establishment of an endemic focus of *V. cholerae* 01 in the coastal regions of the United States. Long-term persistence of the pathogen could be accomplished by a life-cycle mechanism involving sequential contaminations from human, to water, to shellfish and back to human. The ability of *V. cholerae* 01 to survive sea water exposure sufficiently for natural inoculation of shellfish to occur was critical to this study.

Figure 1 illustrates the survival of *V. cholerae* 01 in artificial sea water at ambient room temperature. From an initial concentration of \(7.5 \times 10^4/\text{ml}\), the pathogen declined gradually (approximately 2 logs in about 3 weeks). It must be realized that this long-term survival reflects the absence of competitive microflora that would be present in the marine environment. However, this result does suggest that no strongly vibriocidal activity could be attributed to sea water constituents *per se*.

When *V. cholerae* 01 in artificial sea water is ingested by living oysters, there appears to be neither a concentration nor an inhibitory effect. The simultaneous uptake of bacteria from artificial sea water and their appearance in shellfish at 2 h are then followed by a slow parallel decline in each substrate (Fig. 2).

With an initial inoculum of \(2.47 \times 10^5\) *V. cholerae* 01/g in a 300 × 206 can of shucked oysters, a heat treatment of 48.9°C for 60 min reduced the count of *V. cholerae* 01 to \(5.42 \times 10^3/\text{g}\). Duplicate thermal treatment trials were conducted at water bath temperatures of 54.4 and 57.2°C.
C. In the first trial at 54.4 C, the V. cholerae 01 count was reduced from 9.45 x 10^5/g to 5.5/g, with a processing time of 60 min. In the second trial at 54.4 C, no V. cholerae 01 was recovered with a processing time of 60 min from an initial inoculum of 3.2 x 10^4/g. Neither trial conducted at 57.2 C yielded any time of 60 min. In the second trial at 54.4 C, no processing times were prepared according to published recipes for C.

Data for heat processing at 57.2 C are shown in Table 1. Data from the study of Peterson et al. (6) indicate that hepatitis A is substantially more heat resistant than V. cholerae 01 in shucked oysters. In the light of present evidence, hepatitis A would be the controlling microorganism of public health significance in the design of any pasteurization process for shucked oysters.

The internal oyster temperatures in the 300 x 206 cans were measured at the point of minimum heating for all tests. However, the relatively large sample (200 g) with the resulting large temperature gradient within the can precluded the calculation of accurate D-values from these data.

Shucked oysters naturally inoculated with V. cholerae 01 were prepared according to published recipes for oyster stew and oysters Rockefeller (1). V. cholerae 01 was not recovered from oyster samples taken after the simmering phase and from the final stew. Internal oyster temperatures were approximately 80 C after simmering for 3 min. The oysters Rockefeller were baked in a 232.2 C oven for 10 min on a bed of hot rock salt. V. cholerae 01 was not recovered from samples of oysters Rockefeller. Internal oyster temperature at the end of baking was approximately 94 C, but this final temperature was variable because of thermocouple slippage. Accordingly, the thermal treatment that a consumer would give shucked oysters in typical recipe preparations would eliminate V. cholera 01 at levels expected from natural contamination.

We conclude from our studies that V. cholerae 01 can survive for at least 3 weeks in artificial sea water with little loss of viability. These pathogens can be taken up but not concentrated by live oysters. Our heat studies indicate that V. cholerae 01 is significantly more sensitive to a stringent pasteurization process that is nevertheless insufficient to eliminate the well-recognized health hazard of hepatitis A virus.

Traditional recipes such as oyster stew and broiled oysters used in home and restaurant cooking are entirely sufficient to eliminate V. cholerae 01.

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TABLE 1. Recovery of V. cholerae 01 and indigenous microflora from naturally inoculated oysters heated at 57.2 C for various times.

| Duration of heat (min) | Trial 1 | | | | Trial 2 |
|------------------------|--------|---|---|---|
|                        | SPC/g  | V. cholerae 01/g | SPC/g | V. cholerae 01/g |
| 0                      | 4.90 x 10^3 | 1.20 x 10^3 | 2.07 x 10^5 | 1.68 x 10^4 |
| 15                     | 1.07 x 10^3 | 1.03 x 10^4 | 2.00 x 10^5 | 1.06 x 10^4 |
| 30                     | 2.91 x 10^3 | 0          | 3.65 x 10^4 | 0          |
| 45                     | 1.99 x 10^4 | 0          | 8.90 x 10^3 | 0          |
| 60                     | 1.81 x 10^1 | 0          | 1.10 x 10^3 | 0          |

^aSPC = total plate count on standard plate count agar.