APPLIED AND ENVIRONMENTAL MICROBIOLOGY, Sept. 1994, p. 3483–3484
0099-2240/94/$04.00+0

Vol. 60, No. 9

Effect of Time and Temperature on Multiplication of *Vibrio vulnificus* in Postharvest Gulf Coast Shellstock Oysters

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Received 25 March 1994/Accepted 28 June 1994

After harvest, shellstock oysters stored under controlled temperatures of 10, 13, and 18°C and at ambient outside air temperature (23 to 34°C) were sampled after 12 and 30 h for *Vibrio vulnificus*. At 13°C and below, *V. vulnificus* failed to multiply in the oysters. In oysters held at 18°C for 30 h and under ambient conditions for 12 and 30 h, *V. vulnificus* numbers were statistically greater ($P < 0.05$) than those in oysters at harvest. These data indicate that endogenous *V. vulnificus* can multiply in unchilled shellstock oysters.

It is well established that *Vibrio vulnificus* can cause primary septicemia in individuals with certain underlying diseases and that oysters serve as a vector in transferring the bacterium from estuarine waters to humans (1, 9). Although the infective dose of *V. vulnificus* has not been established, there is a relationship between the incidence of infection and the time of year when high numbers of *V. vulnificus* are present in the environment and in oysters (6, 7, 10). Minimizing the number of *V. vulnificus* in oysters that reach consumers may help to reduce *V. vulnificus* infections among at-risk individuals.

Two studies have reported that endogenous *V. vulnificus* can multiply in postharvest shellfish (2, 4); however, Murphy and Oliver (8), who introduced a transposon-containing strain of *V. vulnificus* into oysters by feeding, recorded no increase in *V. vulnificus* in shellstock stored at temperatures as high as 22°C for up to 10 days. They attributed the difference from the earlier findings (2, 4) to better technique for enumerating *V. vulnificus*. More recently, Kaspar and Tamplin (5) documented multiplication of endogenous *V. vulnificus* in oysters held at 30°C and suggested that the strain of *V. vulnificus* used by Murphy and Oliver (8) failed to reproduce because of an inability to compete with endogenous microbial flora.

The present research was undertaken to define the effect of time and temperature on the multiplication of *V. vulnificus* in shellstock oysters during postharvest storage and to determine if existing federal guidelines are adequate to prevent *V. vulnificus* multiplication.

Oysters (*Crassostrea virginica*) were harvested from Buoy Reef in Mobile Bay, Alabama, with an oyster dredge. For each study, 250 commercial-size oysters were harvested and culled between 9 and 11 a.m. on each of five occasions. One-third of the oysters were cooled with chilled forced air to an internal temperature of 7 to 10°C within 60 min of harvest on board the boat. The remaining oysters were held at ambient temperature in an insulated box to protect them from solar heating during return to the laboratory. Oysters cooled on the boat were stored under mechanical refrigeration at 10°C. The remaining oysters were divided into three groups which were stored under controlled temperature at 13 or 18°C or placed in a burlap bag and held outside in the shade at ambient air temperature (AAT). All oysters were under storage within 2 h of harvest. Two of the oysters in each group held at AAT were fitted with thermocouples, and their internal temperatures were continuously recorded (OM160 Data Logger; Omega Technologies Inc., Stamford, Conn.).

Duplicate samples, each consisting of 12 oysters, were collected for bacteriological analysis from each treatment at 12 ± 1 and 30 ± 1 h after harvest. Oysters for the 0-h *V. vulnificus* counts were taken from those chilled on the boat and were analyzed within 3 h of harvest. Shellstock oysters were scrubbed with a brush under running tap water, drained on absorbent paper for 10 min, and opened with a sterile shucking knife. The shell contents were then transferred to a sterile blender jar. An equal weight of phosphate-buffered saline (PBS) was added, and the shucked oysters were homogenized for 90 s. A 20-g portion of the homogenate was placed in a jar and diluted to 100 g with PBS. Subsequent dilutions were made on a volume basis in PBS.

V. vulnificus was enumerated by a three-tube, most-probable-number technique, using alkaline peptone water enrichment (3). After incubation at 35°C for 12 to 18 h, the contents of alkaline peptone water tubes exhibiting turbidity were streaked onto modified colistin-polymyxin B-cellobiose agar plates. *V. vulnificus*-like colonies which developed on such plates after incubation at 39°C for 18 to 20 h were confirmed by an enzyme immunoassay procedure (11) and a monoclonal antibody specific for *V. vulnificus* (Aquatic Diagnostics, Gainesville, Fla.). Selected isolates were verified as *V. vulnificus* by API 20E strips (Analytab Products, Plainview, N.Y.).

Differences among treatments were tested with the general linear model analysis of variance procedure, using Duncan's post-hoc test (version 5.01; Number Cruncher Statistical System, Kaysville, Utah).

The temperatures of oysters harvested from Gulf Coast waters during summer months approached 30°C (Table 1). If stored out of direct sunlight, the oysters usually cooled 1 to 2°C below the AAT as a result of evaporative cooling and remained slightly below AAT during storage. The temperatures measured in the oysters during storage in these five studies ranged from 22.8 to 31.1°C.

The effect of storage temperature on numbers of *V. vulnificus* in oysters is presented in Fig. 1 as the mean $\log_{10}$ change in *V. vulnificus* count from the 0-h count for each time period and test temperature. The numbers of *V. vulnificus* in oysters held at 18°C for 30 h and at AAT for 12 and 30 h were statistically greater ($P < 0.05$) than those in oysters at harvest. Under the

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TABLE 1. Environmental conditions and numbers of *V. vulnificus* in oysters at harvest and internal temperature of oysters during storage at AAT for 30 h

Date of harvest	Harvest area[a]		*V. vulnificus* mean count (MPN/g)[b]	Storage temp (°C)[c]		
	Temp (°C)	Salinity (ppt)		Maximum	Minimum	Mean
28 June 1993	28.0	12	3,300	28.9	25.8	26.7
12 July 1993	28.7	12	1,800	29.3	22.8	24.0
10 Aug. 1993	31.0	15	330	30.6	26.7	28.3
16 Aug. 1993	29.0	16	5,100	31.1	24.7	27.2
9 Sept. 1993	27.8	16	2,300	28.9	25.6	27.1

[a] Water characteristics on reef when oysters were harvested.
[b] Number of *V. vulnificus* organisms in oysters at harvest. Mean of 2 to 12 oyster composites. MPN, most probable number.
[c] Internal temperature of oysters during 30 h of storage under ambient conditions.

other study conditions, the *V. vulnificus* counts did not change statistically from the initial count during storage.

These findings support previous reports of *V. vulnificus* multiplication in postharvest shellstock oysters (2, 4, 5) and provide some insight into the temperatures required for growth of the bacterium in shell oysters. National Shellfish Sanitation Program guidelines (12) recommend that shellstock be stored at a temperature of 7.2°C or less. Storage at that temperature is adequate to suppress *V. vulnificus* reproduction.

The multiplication of *V. vulnificus* in oysters held at summer AAT was quite rapid and appeared to peak before 12 h. This finding suggests a need to cool oysters immediately after harvest to prevent increases in *V. vulnificus* numbers. Even at moderate temperatures (18°C), limits should be placed on the length of time shellstock oysters may be held without refrigeration.

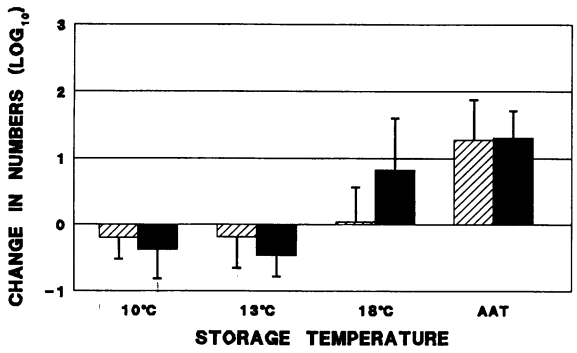

FIG. 1. Change in numbers of *V. vulnificus* in oysters held at various temperatures for 12 (▨) and 30 (■) h. Bar heights represent the mean (n = 5) change from the 0-h count expressed as $\log_{10}$ of the number of *V. vulnificus* organisms. Error bars indicate standard deviation for five trials combined. AAT indicates oysters stored at outside AAT.

I thank Mable L. Carter and Tony K. Previto for technical assistance.

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