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# Post-harvest treatments defeat Vibrio vulnificus

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## Bacteria account for 95 percent of all U.S. seafood-related deaths

*Vibrio* contamination of mussels and other shellfish can be effectively eliminated through several varied treatments.

*Vibrio vulnificus* is currently considered the most infectious and lethal of all human pathogenic vibrios. In the United States, it accounts for 95 percent of all seafood-related deaths. The organism is naturally ubiquitous in marine coastal waters and shellfish, and requires at least 0.5 percent sodium chloride for growth.

With fatality rates as high as 60 percent, septicemic infections caused by the organism usually result from the consumption of raw shellfish. The incubation period varies from several hours to several days, with a median of 26 hours. The most frequent symptoms include fever, chills, nausea, hypotension, and endotoxic shock. Liver damage and cirrhosis arising from chronic alcoholism, chronic renal disease, diabetes, and immunocompromising diseases are considered major factors in susceptibility. Secondary necrotic lesions of the extremities often necessitate surgical debridement or limb amputation.

Levels of *V. vulnificus* decrease in oysters stored at temperatures ranging 0.5 to 22 degrees-C for 10 days, which suggests that temperature abuse of oysters may not be a factor in increasing the public health risk of *V. vulnificus* through raw oyster consumption. However, the data also suggests that even with proper temperature control, indigenous levels of *V. vulnificus* can remain sufficiently high in oysters to produce infection in compromised individuals.

## Presence in shellfish

Although *V. vulnificus* is widely distributed, its density can vary widely. In a 1998-1999 study, for example, shell oysters (*Crassostrea virginica)* were sampled at 275 restaurants or seafood markets throughout the United States. The oysters were harvested from the Gulf of Mexico, Pacific, Mid-Atlantic, and North Atlantic coasts of the U.S. and Canada.

*V. vulnificus* densities in oysters harvested during summer months from the Gulf coast and mid-Atlantic states sometimes exceeded 10,000 most probable number (MPN) per gram. The majority (78 percent) of lots harvested on the North Atlantic, Pacific and Canadian coasts contained densities below the detectable level of 0.2 MPN per gram. None exceeded 100.0 MPN per gram.

In analyses of edible bivalves at sites on the coast of Valencia, Spain, only two samples were confirmed as *V. vulnificus* because the organism can not grow in the high salinity of the Mediterranean Sea or compete with other predominant *Vibrio* species. In Italy, a survey of 600 mussel samples obtained from retailers in the Puglia region found *V. vulnificus* in 2.8 percent of the samples.

Along the southern coast of Brazil, *Crassostrea gigas* oysters were found to contain *V. vulnificus* organisms ranging 3 to 30 MPN per gram in 12 percent of the samples, with 25 isolates pathogenic. Seasonal sampling of oysters at two sites in the tropical waters of India detected the vibrio in 85 percent of the samples. Counts ranged from 10 per gram during the summer months to 1,000 per gram in the monsoon season. The density was controlled more by salinity than the relatively constant water temperature above 25 degrees-C.

Oysters from the U.S. Gulf of Mexico exhibited a similar seasonal distribution of *V. vulnificus*. The median concentration of 2,300 MPN/g of oyster meat from May through October was followed by a gradual reduction during November and December to below 10 per gram, where it remained from January through mid-March. A sharp increase to much higher summer levels in late March and April resulted with water temperatures up to 26 degrees-C.

In a year-long study of shrimp imported to Denmark, the microorganism was detected in three of 46 frozen raw shrimp samples. However, the organism was not recovered from any of 61 frozen cooked products tested, suggesting that the products were adequately heat treated and cross-contamination

did not occur during processing. Imported shrimp products should not constitute a hazard to public health, provided the shrimp are consumed with adequate cooking and headed with sufficient care to prevent puncture wounds that could provide an opportunity for infection.

# Processing effects

Tests have shown that postharvest treatments such as low-temperature pasteurization, rapid chilling, freezing, high-pressure processing, irradiation, and heat shock are capable of achieving reductions of *V. vulnificus*. However, most of these processes require either significant monetary investments or major efforts in personnel training. In addition, oysters are often killed during the process.

## *High pressure*

A 6-log (99.9999 percent) reduction of *V. vulnificus* in phosphate-buffered saline (PBS) at 241 megapascals (mpa) required five minutes, which included a three-minute pressure come-up time. More than a 5.4-log reduction in *C. virginica* oysters at 345 mpa occurred during a six-minute pressure come-up time. The organism in both PBS and oysters was reduced to nondetectable levels at 586 mpa during a seven-minute pressure come-up time. In addition to reducing the pathogen, the oysters had an extended shelf life and required reduced hand-shucking time.

## *Electrolyzed oxidizing water*

In another study, Pacific oysters (*Crassostrea gigas)* were inoculated with a five-strain cocktail of *V. vulnificus* at levels of $10^6$ and treated with electrolyzed oxidizing water containing 1 percent sodium chloride at room temperature. Reductions were determined at two, four, six, and eight hours. A significant 1.05-log reduction of the organism occurred between four and six hours. Treatments exceeding six hours should be avoided to prevent the oysters' death.

## *Ice immersion*

Unwashed *C. virginica* oysters were rapidly chilled by immersion in ice for three hours and examined for their total plate count, fecal colifiorm, and *V. vulnificus* contents several times during a seven-day post-treatment, refrigerated storage period at 7.2 degrees-C. The numbers of *V. vulnificus* generally declined when compared to the controls, but some increases in total heterotrophic bacteria and fecal coliforms were observed in the treated samples. The study did not support the use of ice immersion as a postharvest treatment because of the relatively small declines in the pathogen, usually less than 1 log (90 percent).

## *Irradiation*

Live oysters, *C. virginica*, with naturally and artificially inoculated pathogenic *V. vulnificus* were exposed to 0-3 kGy of gamma irradiation. The organism was reduced from $10^6$ colony-forming units per gram to nondetectable levels below 3 MPN per gram with 0.75-1.0 kGy irradiation exposure. Sensory panels indicated the participants were unable to distinguish the irradiated oysters from nonirradiated oysters.

## *Heat shock process*

Further work with *C. virginica* oysters harvested from the Gulf of Mexico containing $10^2$-$10^4$ MPN per gram of *V. vulnificus* were subjected to a heat-shock process of one to four minutes at internal meat temperatures ranging 45 to 53 degrees-C. *V. vulnificus* and total bacterial levels were significantly reduced 1-4 log in the finished product.

If the temperature was elevated to 50 degrees-C for 10 minutes, the levels were reduced to non-detectable, with the greatest reductions in oysters with the highest initial levels. The process, however, was not able to eliminate the organism in oysters with MPNs greater than $10^3$. The time required to reduce the microbial population by one decimal at 47 degrees-C for 52 strains averaged 78 seconds.

## *Cold storage*

The time required for normal levels of *V. vulnificus* in *C. virginica* oysters from the Gulf of Mexico stored at 0 and 4 degrees C to reach undetectable levels normally exceeded the usual storage life of 14 days for shucked oyster meats and 21 days for shellstock oysters. Oysters stored at abuse temperatures of 7, 13, and 21 degrees-C over a 10-day period had *V. vulnificus* populations reaching $10^5$-$10^6$/g, a 2- to 4-log increase.

Freezing and storing the oysters for 12 weeks at minus-20 degrees-C did not result in complete destruction of the organism. However, other studies have shown that storage periods extended past 12 weeks result in a continuing decrease in *V. vulnificus* populations.

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