Low Dose Gamma Irradiation to Reduce Pathogenic *Vibrios* in Live Oysters (*Crassostrea virginica*)

Linda Andrews  
Michael Jahncke  
Kumar Mallikarjunan

**ABSTRACT.** Pathogenic strains of *Vibrio* (*Vibrio vulnificus* and *V. parahaemolyticus*), natural inhabitants of estuarine and ocean environments, can cause serious illness and death in susceptible persons from consumption of raw half-shell oysters. Objectives of this study were (1) to establish the irradiation dose needed to reduce pathogenic *vibrios* to nondetectable levels and (2) to determine consumer’s ability to differentiate between irradiated and control oysters. Live oysters, *Crassostrea virginica*, with naturally incurred and artificially inoculated pathogenic *vibrios*, were exposed to 0-3 kGy dose Cobalt-60 gamma radiation. *Vibrio vulnificus* (MO-624) was reduced from $10^6$ cfu/g oyster meat to

---

Linda Andrews is affiliated with the Experimental Seafood Processing Laboratory, Coastal Research and Extension Center, MSU, 2710 Beach Boulevard, Biloxi, MS 39531.

Michael Jahncke is affiliated with the Virginia Seafood Agricultural Research and Extension Center, VPI, 102 South King Street, Hampton, VA 23669.

Kumar Mallikarjunan is affiliated with Biological Systems Engineering, Seitz Hall, Virginia Tech, Blacksburg, VA 24060.

This work is a result of research sponsored in part by the National Oceanic and Atmospheric Administration, Department of Commerce under Grant #NA860039 GMO9920, the Mississippi-Alabama Sea Grant Consortium, Virginia Sea Grant Consortium, Mississippi State University, and Virginia Polytechnical Institute. The views expressed herein are those of the authors and do not necessarily reflect the views of NOAA or its sub agencies.

This work is MAFES #J10335.

Journal of Aquatic Food Product Technology, Vol. 12(3) 2003  
© 2003 by The Haworth Press, Inc. All rights reserved.  
10.1300/J030v12n3_07  
71
non-detectable levels (< 3 mpn/g oyster meat) with 0.75-1.0 kGy irradiation exposure. *Vibrio parahaemolyticus*, 03:K6 (TX-2103), required 1.0-1.5 kGy for reduction to non-detectable levels. Using triangle difference testing, sensory panelists were asked to identify differences between treated (1 kGy) and untreated oysters. Sensory difference tests, triangle method, by 146 volunteers confirmed that panelists, many of whom worked in the seafood industry, were unable to distinguish non-irradiated from irradiated oysters (p < 0.001).

**KEYWORDS.** Oysters, irradiation, vibrios

**INTRODUCTION**

*Vibrios* are natural inhabitants of estuarine waters. Some strains of *Vibrio vulnificus* and *Vibrio parahaemolyticus* have been proven to cause illness in humans. Hlad (1997) reported on *Vibrio* related illnesses in Florida during the years 1981-1994. As reported, 69% percent of *Vibrio* illnesses were associated with gastroenteritis, 31% with primary septicemia, with several deaths each year from primary septicemia. There are approximately 15-30 fatalities each year from *Vibrio vulnificus* infections. Deaths occurred in immunocompromised individuals many of whom had underlying liver disease. The Centers for Disease Control (1999) reported that of the 218 reported *Vibrio* associated illnesses, 67% (146 cases) were linked to oysters with 25 deaths (Cook, 2001). Curlane and Vestegaard (2001) suggested annual estimates of *Vibrio* illnesses of approximately 8,000 cases with 31 deaths. *Vibrio* numbers in oysters follow a consistent pattern of concentration from year to year. Oyster samples have been collected weekly, over the last 5 years, from Plaquemines Parish, Louisiana. As a reference for *Vibrio* coincidence in Gulf oysters, Figure 1 represents the highest counts recorded in each month during the sampling period. *Vibrio vulnificus* numbers have been consistent during the past 5 years. A deviation in *Vibrio vulnificus* numbers occurred in June 2001, due to tropical storm “Allison” dropping 20 inches of rainfall over the harvesting areas in a few days and *Vibrio vulnificus* numbers were reduced to winter levels. During the five year survey period, there were only 4 weeks during which *Vibrio vulnificus* numbers were at non-detectable concentra-
tions. Currently, there is increasing regulatory pressure for the oyster industry to implement post harvest treatments (PHT) to reduce *Vibrio vulnificus* numbers in raw oysters. Irradiation processing of live oysters has been investigated for over 15 years. Use of irradiation to remediate environmental strains of *Vibrio* spp. has proven successful using low doses of gamma irradiation (< 1.0 kGy) from Cobalt-60 (Grodner and Hinton, 1988; Grodner and Watson, 1989; Kilgen, 1995). Previous irradiation studies used naturally incurred environmental strains of *Vibrio vulnificus* and *Vibrio parahaemolyticus*. However, highly infective strains such as *V. parahaemolyticus* 03:K6 are more resistant to PHTs (Cook, 2001). Thus, in this study, two clinical isolates of *V. vulnificus* (MO-624) and *V. parahaemolyticus* (O3:K6) were artificially inoculated into whole oysters. This study was designed to determine the effect of irradiation on reduction of *Vibrio vulnificus* (MO-624) and *V. parahaemolyticus* (03:K6) numbers in raw oysters. In addition, the ability of consumers to differentiate between irradiated oysters and non-irradiated oysters was evaluated.

**METHODS**

**Bacterial Inoculation**

Oysters, *Crassostrea virginica*, alive and in the whole shell, containing naturally incurred *Vibrio vulnificus*, were tested in the warmer months, June-September 2001. In addition, oysters were inoculated with pathogenic strains of *Vibrio vulnificus*, MO624 and *Vibrio parahaemolyticus* 03:K6, TX2103. Batches of 48-50 oysters were placed into a 10 gal. aquarium with brackish water (2% sea salt) at 29-30°C and allowed to acclimate for approximately 4 hours. A fresh culture (18-24 hr) of either *Vibrio vulnificus*, MO624 or *Vibrio parahaemolyticus* 03:K6 TX2103 in T1N1 broth (1% tryptone, 1% NaCl) was prepared and used to inoculate the aquarium at approximately 10-20 ml per gal. water. The oysters were then allowed to filter feed in the water for 18-20 hours. Oysters were removed from the aquarium and the shells washed in 50 ppm chlorine water. This achieved an approximate 10⁶⁷ *Vibrio* oyster meat. Oysters were processed using a research size irradiator (Shepherd Model 484) located at the Nuclear Science Center, Louisiana State University. The irradiator contains a radioactive source of Cobalt-60 with a dose rate (at the time of these experiments) of approximately 900 rads per min. The dose range in the circulating chamber was

- TS Allison (June 2001)
- June/July 2002

Log mpn/g oyster meat

- ■ *Vibrio* sp
- □ *V. vulnif*
747-1057 rad/min. A dosimetry study was performed to determine the best location in the irradiation chamber for oysters in the 4 L container to achieve the most consistent dose rate. During irradiation the oysters were not killed at the levels used (< 3 kGY), and exhibited little or no change in temperature. Processing consisted of absorbed doses of 0-3 kGy gamma irradiation. Afterwards, oysters were stored under refrigeration for up to 21 days at 3-5°C. During refrigerated storage, oysters were tested for microbial quality by standard methods recommended by the USDA (Gulf Coast Seafood Laboratory, Dauphine Island, AL, USA). Pure broth cultures (18-20 h) of both pathogens were also irradiated 0-3 kGy. Irradiation processing was performed in quadruplicate for broth cultures and sextuplet for oyster samples. Results are reported as means of duplicate values for each replication.

**Microbial Analysis**

Control oysters (non-irradiated) and irradiation processed oysters were shucked and cultured for *Vibrio vulnificus* or *Vibrio parahaemolyticus* using the 3 tube MPN Method. Oyster homogenates were prepared in duplicate using 1 dozen oysters each in a 1:10 dilution in phosphate buffered saline (PBS), blended for 90 sec. in sterile stomacher bags then diluted to 8 serial dilutions. One ml of each dilution of PBS was inoculated into 3 tubes each of alkaline peptone water. After 18-22 h incubation at 35°C, each tube was subcultured to Modified Colistin/Polymyxin B/Cellobiose Agar (mCPC) and Thiosulfate-Citrate-Bile Salts-Sucrose Agar (TCBS) and incubated another 18-22 h at 40°C and 35°C, respectively. Actual counts were determined using MPN Tables (BAM, 1995). Aquarium water samples and pure cultures of 18-22 h *V.p.* in T1N1 were also inoculated in serial dilutions to extinction, followed by subculture to TCBS. Presence of green colonies on TCBS was considered presumptively positive for *V.p.* 03:K6 as inoculated. These were then further identified using appropriate biochemical tests, i.e., trypticase soy agar with 8% salt and API-Test Strips 20E (API:Table 6B and BAM:Table 3 Section 9:10, "Biochemical characteristics of several Vibrionaceae").

**Sensory Analysis**

For the sensory study, fresh oysters were collected from approved harvest areas during December (2001) and early March (2002), and were tested for bacterial quality. Oysters (200 control and 200 for each
RESULTS AND DISCUSSION

Microbial Sensitivity to Irradiation Processing

Results of the initial irradiation response of pure broth cultures are shown in Figure 2. *V. vulnificus* MO-624 (log 7/g oyster meat) were more sensitive to irradiation compared with *V. parahaemolyticus* 03:K6 (log 7/g oyster meat). In the broth cultures, dosages of 1.5 kGy and 2.0 kGy were required to reduce the populations of *V. vulnificus* MO-624 and *V. parahaemolyticus* 03:K6 to non-detectable levels, respectively. Cook (2001) reported that the *V. parahaemolyticus* 03:K6 was more resistant to PHT processing compared with any other *Vibrio* spp. tested in his laboratory. Figure 3 represents the response of environmental *V. vulnificus* following processing and during refrigerated storage. Naturally incurred *V. vulnificus* (10^3/g oyster) was reduced to non-detectable levels with 0.75 kGy dose irradiation. For the control oysters, stored under refrigeration (< 4°C), *V. vulnificus* counts were reduced during refrigeration as previously reported (Andrews et al., 2000; Cook, 1997; Cook and Ruple, 1992). In Figure 4, the response of pathogenic *Vibrio vulnificus* MO-624 (log 7/g oyster) is presented. This pathogenic strain, at this concentration, was reduced by 7 log to non-detectable levels with a 1 kGy absorbed dose. Figure 5 demonstrates the response of artificially inoculated *Vibrio parahaemolyticus* 03:K6 TX-2103 (log 4/g oyster). This strain exhibited greater resistance when compared with the naturally incurred *V. vulnificus* and *V. vulnificus* MO-624, requiring up to 1.5 kGy for reduction of 4 log to non-detectable levels. Refrigerated storage also reduced *V. parahaemolyticus* concentrations but to a lesser extent compared with *V. vulnificus*.
FIGURE 2. Irradiation of *V. vulnificus* MO-624 and *V. parahaemolyticus* 03:K6 TX-2103 in pure broth culture.

- Black bars: *V. vulnificus*
- Gray bars: *V. parahaemolyticus*

<table>
<thead>
<tr>
<th>Gy</th>
<th>Log-10/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>7</td>
</tr>
<tr>
<td>0.5</td>
<td>5.5</td>
</tr>
<tr>
<td>1.0</td>
<td>3</td>
</tr>
<tr>
<td>1.5</td>
<td>1.5</td>
</tr>
<tr>
<td>2.0</td>
<td>&lt;1</td>
</tr>
</tbody>
</table>

Mean values of 4 replications (Std. Dev. < 0.5 log_{10}/ml)
FIGURE 3. Irradiation of naturally incurred *Vibrio vulnificus*.
FIGURE 4. Irradiation of artificially inoculated Vibrio vulnificus (MO-624).

Mean values for 6 replications (Std. Dev. < 0.5 log_{10}/g oyster meat)
FIGURE 5. Irradiation of artificially inoculated *V. parahaem* 03:K6 (TX-2103).

Mean values for 6 replications (Std. Dev. < 0.5 log<sub>10</sub>/g oyster meat)
Sensory Evaluation

Consumer panels were conducted December, 2001, at the Coastal Aquaculture Unit (CAU) Open House (Gulfport, MS) and at the Boston Seafood Show, March, 2002. Eighty tests were conducted at the CAU Open House and 66 tests were conducted at the Seafood Show. Triangle difference tests were conducted. Panelists were presented with three (3) randomly coded oysters. Two were similar one was different. Panelists were asked to identify the odd sample. Out of 146 trials there were 56 correct answers, indicating no significant difference between irradiated and non-irradiated oysters ($p < 0.001$).

SUMMARY AND CONCLUSIONS

Irradiation of $\approx 1.5$ kGy was effective in reducing large concentrations of both pathogenic and non pathogenic *Vibrios* to non-detectable levels. Oysters treated with $< 1.5$ kGy irradiation did not develop significant sensory changes and maintained a good quality shelf-life for $> 15$ days ($p < 0.001$).

REFERENCES


