Oyster Irradiation: Pathogenic *Vibrio* Response and Consumer Difference Testing

Linda Andrews and Benedict Posadas, Mississippi State University

Technical Report

Sea Grant
Mississippi-Alabama

MASGP-02-016
Mississippi-Alabama Sea Grant Consortium

Project Number: GMO-99-20

Program Year: 2002

Grant Number: NA86RG0039

This publication was supported by the National Sea Grant College Program of the U.S. Department of Commerce’s National Oceanic and Atmospheric Administration under NOAA Grant # NA86RG0039, the Mississippi-Alabama Sea Grant Consortium, and Mississippi State University. The views expressed herein do not necessarily reflect the views of any of those organizations.
OYSTER IRRADIATION: PATHOGENIC VIBRIO RESPONSE AND CONSUMER DIFFERENCE TESTING

Linda Andrews and Benedict Posadas

Experimental Seafood Processing Laboratory
Coastal Research and Extension Center
MSU, 2710 Beach Blvd., Biloxi, MS 39531

key words: oysters, irradiation, vibrios, sensory, consumer survey

This manuscript has been approved for publication MAFES # D10302

Sea Grant Report #MASGP-02-016
Introduction

*Vibrios* are natural inhabitants of estuarine waters throughout the world. A few strains of *Vibrio vulnificus* and *Vibrio parahaemolyticus* have been proven to cause illness in humans. Hlady (1997) published a review of *Vibrio*-related illnesses in Florida from 1981-1994. Sixty-nine percent of these illnesses were from gastroenteritis and 31% from primary septicemia; several deaths occurred each year mainly from primary septicemia. On average, there are 15-30 fatalities from *Vibrio vulnificus* infections each year. The deaths occurred in immunocompromised individuals most of whom had liver disease. In 1999, the Center for Disease Control reported 218 cases, 93% which were from seafood and 67% from oysters, resulting in 25 deaths (Cook, 2001). Curlale and Vestegaard (2001) suggested annual estimates of *Vibrio* illnesses to be approximately 8,000 cases with 31 deaths. Most cases presented mild symptoms and did not require a physician’s care and therefore were not officially reported.

Several post-harvest processes for remediation of *Vibrios* in oysters are under investigation, among these is irradiation processing. Irradiation processing of live oysters has been investigated for over 15 years. Use of irradiation to remediate environmental strains of *Vibrio* has proven successful with low doses of gamma irradiation (<1.0 kGy) from Cobalt-60 (Grodner and Hinton, 1988; Grodner and Watson, 1989; Kilgen et al., 1995).

In previous irradiation studies, researchers used naturally-incurred environmental strains of *Vibrio vulnificus* and *Vibrio parahaemolyticus*. In this study, two highly infective strains of *Vibrio* isolated from patients stricken with the diseases were used. It has been shown (Cook, 2001) that highly infective strains such as *V. parahaemolyticus* 03:K6 are among the most resistant *Vibrios* to processing. In order to determine the true effectiveness of irradiation processing, to prevent food-borne disease, it was necessary to test these strains against the process. One objective of this study was to determine the effect of irradiation on pathogenic strains of *Vibrio* isolates obtained from known illnesses attributed to oyster consumption. In addition, the ability of consumers to differentiate irradiated oysters from untreated control oysters was examined and a survey of consumer attitudes toward food irradiation and irradiated oysters was conducted.

Methods

**Bacterial Inoculation**

*Vibrio* spp were tested in two different studies. 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 about 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 1-2 ml per gal water. This concentration achieved an approximate 10^7-10^8 concentration of the bacterium in water. The oysters were then allowed to filter feed in the water for 18-20 hours. Oysters were then removed from the aquarium and the shells washed in 50 ppm chlorine water. This achieved an approximate 10^6-7 *Vibrio*/g oyster meat.

Oysters were processed using a research-size irradiator (Shepherd Model 484) located at the Nuclear Science Center, Louisiana State University. This irradiator contains a radioactive source of Cobalt-60 with the current dose rate of approximately 900 rads per min. The dose range in the circulating chamber was 747-1057 rad/min. A dosimetry study was performed previously to determine the best location in the irradiation chamber for oysters in the 4 L container to achieve the most consistent dose rate. During the process, the oysters did not become radioactive, they were not killed by the irradiation at the levels used (<3 kGy), and exhibited little or no alteration in temperature. Processing consisted of absorbed doses of 0-3 kGy gamma irradiation. Oysters were stored under refrigeration for up to 21 days. During storage, oysters were tested for microbial quality by standard methods recommended by the FDA. Pure broth cultures (18-20 h) of both pathogens were also irradiated at 0-3 kGy.

**Microbial Analysis**

Control oysters and processed oysters were shucked and cultured for *Vibrio vulnificus* or *Vibrio parahaemolyticus* using the 3 tube MPN Method. Oyster homogenate was 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 mCPC and TCBS medium and incubated another 18-22 h at 35°C. Actual counts were determined from 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 (10^-11^-10^-12_) 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 and Consumer Survey**

For the sensory study, fresh oysters were collected in the months of December (2001) and early March (2002) and were tested for bacterial quality prior to being presented for human consumption. Oysters (200 each for both the control and test oysters for each of the two panel trials) were irradiated with 1 kGy gamma irradiation (controls were not irradiated) and were stored under refrigeration at 3-5°C. A standard sensory “triangle difference test” was conducted within 1 week of the irradiation process. Panelists were presented, in random order, 2 like oysters and 1 different and were then asked to select
the one they thought was the odd sample. Comments regarding the sensory appearance and overall quality of the oysters were accepted but not demanded.

The consumer survey was conducted at the Mississippi State University Coastal Aquaculture Unit in December, 2001 and the Boston International Seafood Show in March, 2002. Respondents were asked their attitudes and preferences toward raw oysters, in general and irradiated oysters, in particular. Respondents were asked whether they eat raw oysters or not, and if not, to indicate the main reasons for not eating raw oysters. They were also asked about their primary food safety bacteriological concerns about raw oysters, frequency of eating raw oysters, and source of raw oysters. A series of questions was asked regarding their attitude toward radiation and irradiated oysters, interest in buying irradiated raw oysters, and willingness to pay for a dozen irradiated raw oysters if purchased in the supermarket. Respondents' characteristics including sex, marital status, age, household income, and educational attainment were also asked. Statistical analysis was conducted to compare attitudes and preferences toward raw and irradiated oysters by consumers and non-consumers of raw oysters. This project was approved for use of human subjects by the Internal Review Board of Mississippi State University #01-362

Telephone interviews with a simple random sample of adults living in the Baltimore and Houston Metropolitan Statistical Areas (MSAs) in households with telephones were done by the Social Science Research Center at Mississippi State University in June of 2002. Households were selected using random digit dialing procedures; within a household the adult to be interviewed was selected by asking to speak with the person in the household who is 18 years of age or older and who will have the next birthday.

Results and Discussion

Microbial Sensitivity to Irradiation Processing

Results of the initial irradiation response of pure broth cultures are shown in Figure 1. *V. vulnificus* MO-624 (log 7/g oyster meat) proved to be more sensitive to the irradiation exposure than the *V. parahaemolyticus* (log 7/g oyster meat) requiring 1.5 kGy and 2.0 kGy, respectively, to reduce each to non-detectable levels. That the *V. parahaemolyticus* 03:K6 was less sensitive to irradiation processing than *Vibrio vulnificus* was not surprising. Cook (2001) reported that the *V. parahaemolyticus* 03:K6 was the most resistant to processing of any of the *Vibrios* tested in his laboratory.

Naturally-incurred *V. vulnificus* (10^3/g oyster meat) was reduced to non-detectable levels with 0.75 kGy irradiation. Figure 2 represents the response of environmental *V. vulnificus* following processing and during refrigerated storage. Note that even in the control oyster, stored under refrigeration (<4°C), *V. vulnificus* counts were reduced over time as previously reported (Andrews et al., 2000; Cook, 1997; Cook and Ruple, 1992).
In Figure 3, the response of pathogenic *Vibrio vulnificus* MO-624 (10^7/g oyster meat) is presented. This pathogenic strain of *V. vulnificus*, at this concentration, was more resistant to irradiation processing than the naturally-incurred strains and was reduced to non-detectable levels with 1 kGy absorbed dose. This pathogen was also reduced over refrigerated storage. Figure 4 demonstrates the response of artificially-inoculated *Vibrio parahaemolyticus* 03:K6 TX-2103(log 4/g oyster meat). This particular strain of *V. p.* again proved to be somewhat more resistant than the pathogenic *V. vulnificus* and required up to 1.5 kGy to reduce to non-detectable levels. The *V. parahaemolyticus* were less sensitive to reduction during refrigeration than the *V. vulnificus*.

**Irradiated Oyster Difference Test**

Two consumer panels were conducted; one in December, 2001, at the Mississippi State University Coastal Aquaculture Unit Open House (Gulfport, MS) and the other at the Boston Seafood Show, March, 2002 (Boston, MA). There were 80 tests performed at the Open House and 66 tests performed at the Seafood Show. Out of 146 triangle tests conducted, 56 trials resulted in correct answers. By Chi-square statistical analysis, this number was determined to be less than would be expected by random chance selection (Stone and Sidell, 1985). Therefore, there was no significant difference observed between the irradiated oysters and the control samples (p<0.001).

An expert panel within the Mississippi State University community also tasted the oysters and reported that the irradiated oysters maintained a "raw like" quality, as was commented by the consumer panelists. There were no flavor or visual changes noted. Oysters for sensory testing were irradiated within a dose range of 1-1.5 kGy gamma rays.

**Consumer Survey**

Consumer attitudes and preferences toward raw oysters in general, and irradiated oysters, in particular, were evaluated from results of consumer surveys conducted through personal and telephone interviews. Seventy-five personal interviews were conducted at the Mississippi State University Coastal Aquaculture Unit Open House in Gulfport, Mississippi on December 6, 2001. Another survey was conducted among 140 participants of the 2002 International Boston Seafood Show in Boston, Massachusetts on March 12-14, 2002.

Telephone interviews of adults living in the Baltimore and Houston areas were conducted in June, 2002. Of the eligible respondents contacted in the Baltimore MSA, 610 completed the interview and 85 refused to participate. Of the eligible respondents contacted in the Houston MSA, 606 completed the interview and 67 refused to participate. The sampling error for both the surveys is no larger than ± 4.0%. A higher proportion of the respondents interviewed personally than those interviewed by telephone reported eating raw oysters in 2001. More than 60 percent of the respondents at the Boston Seafood Show and Gulfport Aquaculture Open House ate raw oysters, while 28 percent of the respondents in the Baltimore and Houston telephone interviews reported
eating raw oysters in 2001 (Figure 5). More of the male respondents tend to consume raw oysters than female respondents. Among male respondents from Baltimore and Houston MSA’s, about 40 percent stated that they ate raw oysters in 2001. A lower percentage of female respondents (20%) from the two MSAs reported eating raw oysters. Among male respondents at the Boston seafood show, 69 percent reported eating raw oysters in 2001. A lower percentage of female respondents (49%) at the seafood show indicated eating raw oysters.

Summary and Conclusions

Irradiation of 1-1.5 kGy was effective in reducing 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 good quality shelf-life for >15 days. Results of telephone interviews showed that 28 percent of adults in Maryland and Houston ate raw oysters. Personal interviews in Boston and Gulfport revealed that more than 60 percent of the participants ate raw oysters. More of the male respondents tend to consume raw oysters than female respondents.

References


**Acknowledgements**

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 and Mississippi State University. The U. S. Government and the Mississippi-Alabama Sea Grant Consortium are authorized to produce and distribute reprints for Governmental purposes notwithstanding any copyright notation that may appear hereon. The views expressed herein are those of the authors and do not necessarily reflect the views of NOAA or its sub-agencies.
Figure 1: Irradiation of *Vibrios* in pure broth culture

![Graph showing log-10/ml levels of Vibrio vulnificus and V. parahaemolyticus with Co-60 irradiation doses.](image)

Co-60
Mean values of 4 replications (Std.Dev. <0.5 log<sub>10</sub>/ml)

Figure 2: Irradiation of naturally-occurring *Vibrio vulnificus*

![Graph showing log-10 V.v./g oyster meat levels over days of storage with various irradiation doses.](image)

Mean values of 6 replications (Std.Dev. < 0.5 log<sub>10</sub> oyster meat)
Figure 3: Irradiation of artificially-inoculated *Vibrio vulnificus*

![Graph showing the effect of irradiation on *Vibrio vulnificus*](image)

Mean values for 6 replications (Std.Dev.<0.5 $\log_{10}$ oyster meat)

Figure 4: Irradiation of artificially-inoculated *V. parahaem. 03:K6*

![Graph showing the effect of irradiation on *V. parahaem. 03:K6*](image)

Mean value for 6 replications (Std.Dev.<0.5 $\log_{10}$ oyster meat)
Figure 5: Percent of respondents who ate raw oysters