Effect of ionizing radiation on *Vibrio* bacteria in *Crassostrea virginica* (American oyster)

*Effet d'une irradiation ionisante sur les bactéries Vibrio chez l'huître Crassostrea virginica (huître américaine)*

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Abstract

The presence of *Vibrio* bacteria in raw shellfish poses a significant health hazard to the consuming public. These human pathogens are natural, seasonal components of the microbial flora of shellfish which can result in primary septicemic or diarrheal fatal infections. Existing shellfish depuration neither targets nor eliminates the *Vibrio* bacteria. For this reason, additional purification processes are necessary. In this study, the effect of low dose gamma radiation on the inactivation of *Vibrio cholerae* (01 and non 01) and *Vibrio vulnificus* is investigated. Log decrement ($D_{10}$) values ranging only to a high of 0.09 kGy in the *Vibrio* strains support the hypothesis that food irradiation technology can provide an additional effective and efficient shellfish sanitation method.

Keywords: *Vibrio*, shellfish, depuration, gamma irradiation, food irradiation.

INTRODUCTION

Incidences of shellfish-borne *Vibrio* infection have been reported with increasing frequency in the American hemisphere and throughout the world (Stahr et al., 1989; Nip-Sakamoto and Pien, 1989; Farmer et al., 1991; Molero, 1989; Garay et al., 1985; CDC, 1991). Currently employed depuration practices are of questionable effectiveness in the elimination of naturally occurring *Vibrios* from contaminated shellfish stock (O’Neill et al., 1991). Clearly, a more reliable and efficient method is needed, specifically to resolve *Vibrio* sanitation problems impacting commercial shellfish harvests.
The objective of this study was to investigate the effectiveness of low-dose gamma food irradiation applications on the survival of several Vibrio strains experimentally inoculated into mantle fluid from the American oyster (*Crassostrea virginica*) and to investigate the inactivation of Vibrios found naturally colonising shellfish stock.

**Materials and methods**

The UMass-Lowell/U.S.D.O.E. 0.5 Megacurie $^{60}$Co source was used to irradiate samples in a dry environment and dosimetry was monitored with a Far West Technology Inc. (Galeta, Ca.) opti-chromic dosimetry system.

Commercially obtained oysters were shucked, and the mantle fluid was collected, pooled, and centrifuged at $1,000 \times g$ for 10 minutes. The supernatant was divided into 50 ml aliquots, frozen, and stored at $-70^\circ C$ for later use.

Cultures of *Vibrio cholerae* non-01 (ATCC # 35971), *V. cholerae* 01 (ATCC # 14035), *V. vulnificus* (ATCC # 33817) and *V. vulnificus* (Jackson Estuarine Laboratory, University of New Hampshire, Durham N.H.) were maintained on thiosulfate citrate bile salts sucrose agar (TCBS) and grown overnight in nutrient broth at $37.5^\circ C$ (supplemented with 1% NaCl for cultures of *V. vulnificus*). One ml of an overnight culture was inoculated into 100 ml of nutrient broth and grown for 7 hours at $37.5^\circ C$ in a shaking water bath (100 rpm). A 1:100 dilution of this culture was made with mantle fluid (4°C), dispensed into 19 screw capped test tubes in 5 ml aliquots, irradiated at selected doses, serially diluted into 0.85% NaCl, and plated in triplicate onto TCBS agar. All plates were incubated at $35^\circ C$ overnight, colonies were counted, and log decrement ($D_{10}$) values were calculated by least squares regression analysis.

The radiation doses, in kiloGrays (kGy), administered to each culture were:

- *V. cholerae* non-01: 0.00, 0.05, 0.09, 0.22, 0.41, 0.62 kGy
- *V. cholerae* 01: 0.00, 0.05, 0.10, 0.24, 0.37, 0.61 kGy
- *V. vulnificus* (ATCC): 0.00, 0.02, 0.04, 0.07, 0.09, 0.14 kGy
- *V. vulnificus* (N.H.): 0.00, 0.02, 0.05, 0.07, 0.11, 0.15 kGy

After determination of the $D_{10}$ range for the four Vibrio strains, two dozen commercially obtained oysters were divided into control and irradiated groups which received a dose of 0.85 kGy. Following irradiation, both groups were separately homogenized and plated onto TCBS plates. The plates were incubated overnight at $35^\circ C$ and examined for colony formation.

**Result and discussion**

Log decrement ($D_{10}$) values for *V. cholerae* non 01, 01 and the two *V. vulnificus* were determined to be 0.06 kGy, 0.04 kGy, and 0.07 kGy, and 0.04 kGy, respectively (figures 1-4). In figure 5, the data obtained in all *V. vulnificus* studies are combined, and the $D_{10}$ value obtained is 0.08 kGy. These inactivation values are extremely low when compared to $D_{10}$ values cited for other bacterial species. (Bandekar et al., 1987), determined the $D_{10}$ value for *V. parahaemolyticus* to be 0.10 kGy. Comparative inactivation values stated for *E. coli*,

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**Note:**

The content appears to be cut off or incomplete, possibly due to pagination or formatting issues. The provided text includes a full discussion on experimental methods, results, and conclusions related to the study of Vibrio strains using gamma food irradiation. The text is scientifically detailed, focusing on methods, results, and discussion related to the survival and inactivation of these bacteria.
Table I: Radiation inactivation of microbial contaminants in foods

<table>
<thead>
<tr>
<th>Microbial species</th>
<th>Medium</th>
<th>(D_{10})</th>
<th>Author</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Aeromonas hydrophila</em></td>
<td>Chilled Fish</td>
<td>0.16 kGy</td>
<td>Palumbo et al., 1985</td>
</tr>
<tr>
<td><em>Campylobacter jejuni</em></td>
<td>Raw Beef</td>
<td>0.15 kGy</td>
<td>Tarkowski et al., 1984</td>
</tr>
<tr>
<td><em>Clostridium perfringens</em></td>
<td>Minced Clam Meat</td>
<td>0.37 kGy</td>
<td>Gombas and Gomez, 1985</td>
</tr>
<tr>
<td><em>Escherichia coli</em></td>
<td>Minced Clam Meat</td>
<td>0.37 kGy</td>
<td>Mallett et al., 1985</td>
</tr>
<tr>
<td><em>Salmonella typhimurium</em></td>
<td>Frozen Shrimp</td>
<td>0.22 kGy</td>
<td>Mallett et al., 1985</td>
</tr>
<tr>
<td><em>Shigella dysenteriae</em></td>
<td>Frozen Shrimp</td>
<td>0.41 kGy</td>
<td>Mossel, 1985</td>
</tr>
<tr>
<td><em>Shigella flexneri</em></td>
<td>Minced Clam Meat</td>
<td>0.42 kGy</td>
<td>Mossel, 1985</td>
</tr>
<tr>
<td><em>Staphylococcus aureus</em></td>
<td>Minced Clam Meat</td>
<td>0.97 kGy</td>
<td>Mallett et al., 1985</td>
</tr>
<tr>
<td><em>Streptococcus faecalis</em></td>
<td>Oyster Liquor</td>
<td>0.04 kGy</td>
<td>Jonsson, 1986</td>
</tr>
<tr>
<td><em>Vibrio cholerae (El Tor)</em></td>
<td>Oyster Liquor</td>
<td>0.06 kGy</td>
<td>This Study</td>
</tr>
<tr>
<td><em>Vibrio cholerae (Non-01)</em></td>
<td>Frozen Shrimp</td>
<td>0.10 kGy</td>
<td>Bandekar et al., 1987</td>
</tr>
<tr>
<td><em>Vibrio parahaemolyticus</em></td>
<td>Oyster Liquor</td>
<td>0.07 kGy</td>
<td>This Study</td>
</tr>
<tr>
<td><em>Vibrio vulnificus</em></td>
<td>Oyster Liquor</td>
<td>0.10 kGy</td>
<td>El-Zawahry and Grez, 1981</td>
</tr>
<tr>
<td><em>Yersinia enterocolitica</em></td>
<td>Phosphate Buffer</td>
<td>0.10 kGy</td>
<td>Mallett et al., in press</td>
</tr>
<tr>
<td>Hepatitis A Virus</td>
<td>Live Oyster</td>
<td>2.02 kGy</td>
<td>Mallett et al., in press</td>
</tr>
<tr>
<td>Poliovirus 1</td>
<td>Live Quahog</td>
<td>3.30 kGy</td>
<td></td>
</tr>
</tbody>
</table>

*S. typhimurium, S. aureus, and S. faecalis* are 0.37, 0.51, 0.42 and 0.97 kGy, respectively table I.

These preliminary studies with commercially obtained shellfish confirmed that a dose of 0.85 K Gy was sufficient to completely eliminate *Vibrio* bacteria from naturally colonized oysters. Homogenates of unirradiated oysters were found to have 10⁵ CFU/ml, after irradiation, whole oysters were found to have non-detectable level of *Vibrio*. Previous studies performed, have demonstrated that doses of up to 2 kGy can be administered to American oysters (*Crassostrea virginica*) while preserving the market qualities of shelflife, appearance, odor, taste, and texture (Mallett et al., 1991). Therefore, the application of food irradiation techniques would be a very welcomed addition to *Vibrio* shellfish sanitation. Due to the extreme sensitivity of *Vibrio* sp., low doses of ionizing gamma irradiation could provide a very effective additional method of resolving the *Vibrio* problem. Doses as low as 1.0 kGy could produce as much as a 10 log decrease in numbers of *Vibrio*, virtually eliminating the presence of the pathogen from shellfish. Irradiation of shellfish should not be considered as an exclusive method of sanitation to replace current practices, nor should it be used to effect a “clean-up” of substandard or improperly handled products. Shellfish to be irradiated should be harvested from certified clean waters and should meet guidelines of good manufacturing practice (GMP) as exemplified by the U.S. National Shellfish Sanitation Program (NSSP, 1990). Radiation should be regarded as an additional purification procedure to provide an increased measure of safety in the elimination of *Vibrio* sp. (which are not removed by existing procedures). *Vibrio* are ideal targets for food irradiation due to their extreme sensitivity to ionizing radiation and low level gamma application may ultimately prove to be an ideal solution to the vexing problem of *Vibrio* contamination in shellfish.
Figure 1: Demonstrates the log decrement ($D_{10}$) plot on an irradiated culture of *Vibrio cholerae* non-01

Figure 2: Demonstrates the log decrement ($D_{10}$) plot on an irradiated culture of *Vibrio cholerae* 01 El tor
Figure 3: Demonstrates the log decrement ($D_{10}$) plot on an irradiated culture of *Vibrio vulnificus* (ATCC - 33817)

\[ y = 2.0001 - 22.578x \quad R^2 = 0.772 \]

Figure 4: Demonstrates the log decrement ($D_{10}$) plot on an irradiated culture of *Vibrio vulnificus* (UNH)

\[ y = 2.0014 - 14.120x \quad R^2 = 0.991 \]
Figure 5: Demonstrates the log decrement ($D_{10}$) plot on an irradiated culture of *Vibrio vulnificus* composite

\[ y = 1.8401 - 12.785x \quad R^2 = 0.774 \]

REFERENCES


