

The effects of ionizing radiation, high energy electron beams, and depuration on shell stock oyster shelflife and *Vibrio vulnificus* content

Effets de l'irradiation ionisante, des faisceaux d'électrons à haute puissance, et de la purification sur la durée de conservation des huîtres et sur la teneur en Vibrio vulnificus

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Abstract

Total bacterial numbers were reduced three to four log cycles upon exposure of shell stock oysters to ionizing radiation (^{60}Co) at 1.0, 2.0 and 5.0 kiloGrays. *Vibrio vulnificus* was detected in the non-irradiated control shell stock oysters, however, it was not detected in any of the shell stock after 1.0, 2.0 and 5.0 kiloGrays of exposure. The shelflife of irradiated shell stock oysters and depurated oysters was also compared. Fifty percent of the irradiated oysters were dead within 12, 10 and 7 days at 1.0, 2.0 and 5.0 kiloGrays of exposure, respectively. In contrast, fifty percent of the depurated oysters were dead after 31 days. Cultures of virulent and avirulent *Vibrio vulnificus* in phosphate buffered saline were quite radiosensitive as no colony forming units could be detected after 0.5 kiloGray exposure.

Résumé

Le dénombrement bactérien a été réduit de trois à quatre cycles logarithmiques après exposition d'huîtres à une irradiation ionisante (^{60}Co) de 1,0 ; 2,0 et 5,0 kiloGrays. *Vibrio vulnificus* est détecté dans les huîtres témoins non irradiées, mais il n'est plus détecté dans les huîtres irradiées après exposition à 1,0 ; 2,0 et 5,0 kiloGrays. La durée de conservation des huîtres irradiées a été comparée avec celle d'huîtres purifiées. 50 % des huîtres irradiées meurent au bout de 12, 10 et 7 jours après exposition respectivement à 1,0 ; 2,0 et 5,0 kiloGrays. Par comparaison, 50 % des huîtres purifiées meurent au bout de 31 jours. Des cultures de *Vibrio vulnificus* virulent et non virulent en solution saline tamponnée phosphatée se sont avérées particulièrement radio-sensibles ; en effet aucune colonie n'a pu être détectée après exposition à 0,5 kiloGray.

INTRODUCTION

The consumption of raw or inadequately cooked shellfish has recently been implicated as a public health risk due to the presence of a variety of pathogenic organisms. Several bacterial agents have been isolated from shellfish such as the American oyster (*Crassostrea virginica*), perhaps most importantly being the *Vibrio* species. *Vibrio vulnificus* is a rod-shaped, halophilic bacterium that is located in warm water estuaries, and is a known human pathogen (M.T. Kelly, 1982).

V. vulnificus is the causative agent of a rapidly induced primary septicaemia that is often fatal in certain individuals who have a history of liver disease, or suffer from some other immunocompromised condition (P.A. Blake, M.M. Merson, R.E. Weaver, D.G. Hollis, P.C. Hueblein, 1979). *V. vulnificus* exhibits a seasonal variation, and seems to pose the most significant health risk in the warm summer months (J.J. Licciardello, L.J. Ronsivalli, 1982). Due to this potential health risk, several methods have been proposed to reduce the numbers of pathogenic bacteria present in shellfish, as well as to increase the shelflife of these shellfish.

The proposed methods include the use of (^{60}Co) gamma irradiation, high energy electrons (linear accelerator) and U.V.-assisted depuration as means of reducing total bacterial numbers present in shellfish, and also increasing the product shelflife. Shell stock oysters were exposed to several different doses of gamma radiation, and were subsequently analysed for the microbiological and shelflife consequences. In 1957, Gardner and Watts irradiated oyster meats (non-shell stock) at several doses and observed a reduction in spoilage at room temperature, however an undesirable "grassy odor" resulted (E.A. Gardner, B.M. Watts, 1957). Shell stock oysters were also exposed to a linear accelerator, and to U.V.-assisted depuration. The linear accelerator data shows the effects in reducing total bacterial numbers, while the depuration data shows the effects of increasing shelflife.

Materials and methods

Source of oysters

Oysters were obtained through Leavin's Seafood of Apalachicola, Florida. Oysters were harvested during the morning hours, and then transported to the University of Florida, Gainesville. The shellfish were stored at 34-36°F, and used as soon as possible for irradiation, or depuration.

Source of Gamma radiation

The source of low-dose gamma radiation was a ^{60}Co unit named a Gamma-Cell 220. This was a 30 kilocuries source that is housed on the campus of the University of Florida in the USDA Entomology Research Center. The opening of the unit was 8" tall, with a diameter of 6" in which oysters could be placed for irradiation. The subsequent flow chart details how the shell stock oysters were irradiated and monitored.

Source of a linear accelerator

The preliminary data presented was obtained by Dr Rodrick from a linear accelerator located at the Mevex Corporation, in Ontario, Canada. The University of Florida has obtained its own linear accelerator from France, which should be operational March 1, 1992, so that these experiments may be repeated. Shell stock oysters were exposed to several different doses in the linear accelerator, and were analysed in the same fashion as the oysters irradiated in the ^{60}Co source.

Depuration source

Depuration tanks were 55 gallons or larger, and were filled with artificial sea water at approximately 2.5% NaCl, or a specific gravity of 1.022. The tanks are recirculating systems, equipped with a pump and filter, as well as a disinfecting ultraviolet lamp. The oysters are placed in the tanks for 48 hours @ 60-72°F, and allowed to purge themselves of bio-accumulated material. At the end of 48 hours, oysters are removed, stored at 34-36°F, and monitored for death.

Methodology flow chart

The flow chart of figure 1 demonstrates the protocol for the gamma irradiation of oysters. Approximately 12-15 oysters were stacked in a 6" by 8" Plexiglas container, that was packed with ice both above and below the oysters. Oysters were irradiated at 1, 2 and 5 kiloGrays. A separate set oysters were irradiated for the microbiological and shelflife studies. The microbiological analysis consisted of carrying out a dilution series (10^{-1} to 10^{-8}), after preparing an oyster homogenate of approximately 50 grams of irradiated oyster meat in 450 ml of peptone water in a Waring Blender. Most Probable Number (MPN) tubes of alkaline peptone water @ 2.5% NaCl were prepared out of this serial dilution, and incubated for 18-24 hours @ 37°C. All positive MPN tubes were transferred to thiosulfate citrate bile salts (TCBS) agar for enumeration of "vibrio-like" organisms, and to cellobiose polymixin colistin (CPC) agar for enumeration of *V. vulnificus*, and further biochemically tested (C.A. Kaysner, M.L. Tamplin, 1988). The total number of bacteria was determined using the MPN technique (Bam, 1984). All bacteriological media were obtained from Difco Corp.

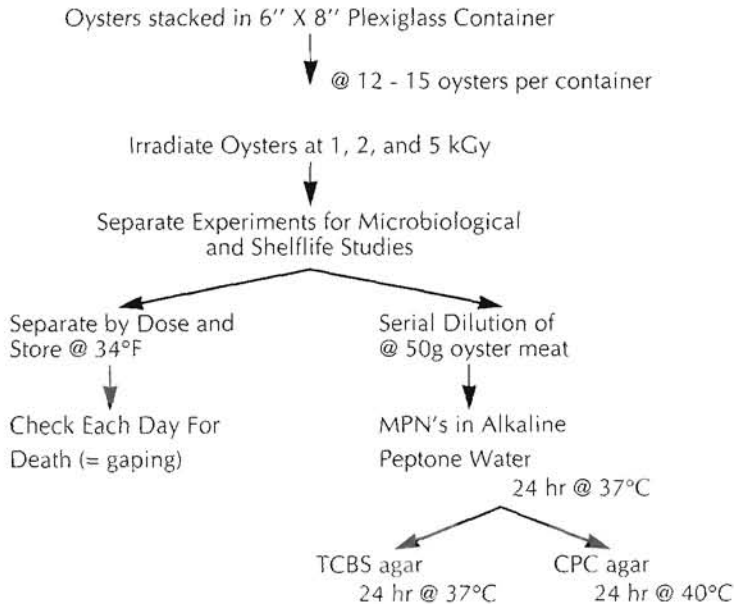


Figure 1: Methodology flow chart

Cultures of both virulent and avirulent *V. vulnificus* were grown separately in 250 ml of Trypticase Soy Broth (TSB) @ 2.5% NaCl for a 8 hours. This broth was centrifuged at 10,000 x g for 10 minutes, thus leaving a pellet. The resulting supernatant was discarded, and the pellet was washed in Phosphate Buffered Saline (PBS) @ pH 7.6. This resuspension was recentrifuged under the same parameters. A new pellet was obtained, and then again resuspended in PBS, and diluted to an optical density of 0.64 @ 420 nm in a Beckman -25 Spectrophotometer. This optical density yields approximately 2×10^8 bacteria per ml. This suspension was subsequently irradiated @ 1.0, 2.0, and 5.0 kGy, and the numbers of *V. vulnificus* were determined by plating on either TCBS or CPC agar after an appropriate serial dilution in peptone.

Results and discussion

The MPN results of both irradiated and non-irradiated oysters, obtained from Apalachicola, are found in table I. A 3-4 log reduction in total bacterial numbers was observed at every exposure dose, with the exception of the 2 kGy exposure, when compared to the non-irradiated controls. The numbers of bacteria observed on TCBS and CPC agar is also shown. On TCBS, the numbers of "Vibrio-like" organisms were reduced 8 log cycles at 1 kGy, 1 log cycle at 2 kGy, and 5 log cycles at 5 kGy. There was no *V. vulnificus* found on any of the CPC agar plates streaked from positive MPN tubes post-irradiation. This would indicate that *V. vulnificus* is quite radiosensitive, and this correlates well with the work of Grodner (R.M. Grodner, L.S. Andrews, 1991).

Figures 1 and 3 show the effect on shelflife of irradiating oysters at 1, 2 and 5 kGy. At the 1 kGy exposure, 50% of the oysters were dead within 12 days. At the 2 kGy exposure, 50% of the oysters were dead within 10 days. Finally, at the 5 kGy exposure, 50% of the oysters were dead within 7 days. Figure 2 also shows the shelflife of deperured oysters. On day 20 post-deperuration, only 33% of the oysters had died. The data in figure 4 shows deperuration results obtained by Leavin's Seafood in an independent deperuration experiment. Leavin's found that 50% of the oysters were dead within 31 days post-deperuration.

Figure 5 shows the results obtained using the linear accelerator in Ontario, Canada. As exposure dose was increased, the colony forming units of bacteria

Table I: Microbiological consequences of oyster irradiation

	1 kGy		2 kGy		5 kGy	
	con	exp	con	exp	con	exp
MPN's Alk. Peptone	4.3E8	2.3E5	9.3E7	1.5E7	4.3E8	9.3E4
TCBS confirmed	4.3E8	—	4.3E7	2.3E6	2.3E8	9.0E3
CPC agar	4.0E7	—	9.0E6	—	2.3E8	—

con = conservative/non exposed
exp = exposed

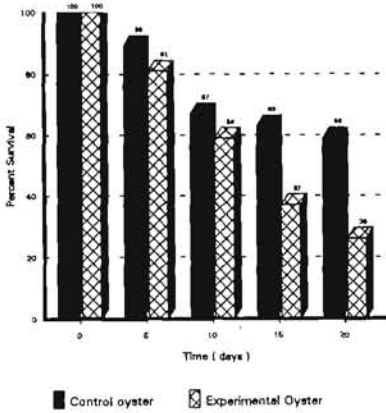


Figure 1: Shelflife data of irradiated oysters (Avg. observed @ 1.0 kGy)

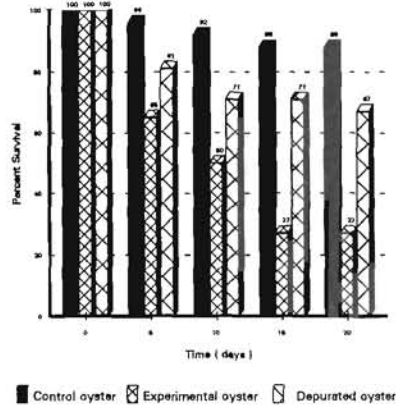


Figure 2: Shelflife data of irradiated and deputed oysters (Avg. observed @ 2.0 kGy)

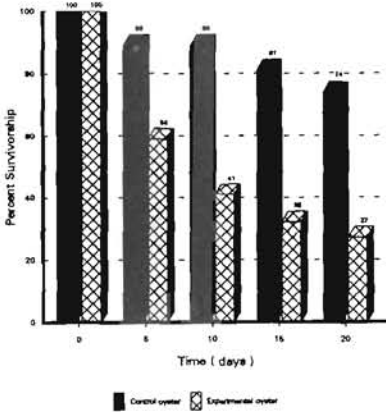


Figure 3: Shelflife data of irradiated oysters (Avg. observed @ 5.0 kGy)

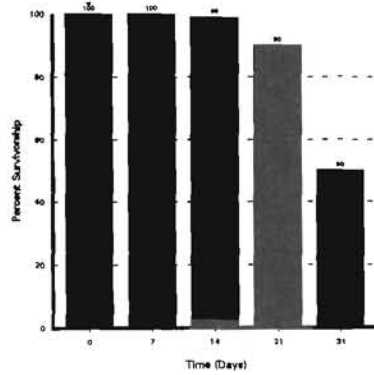


Figure 4: Oyster depuration data (summer 1990)

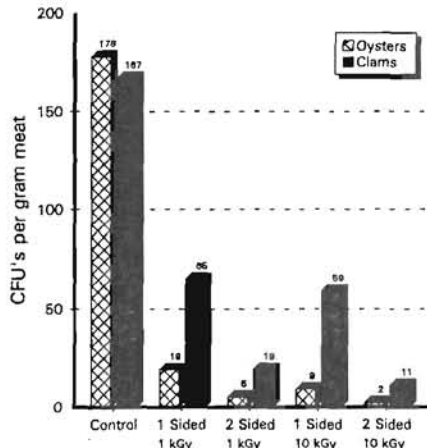


Figure 5: Linear accelerator data on oysters and clams

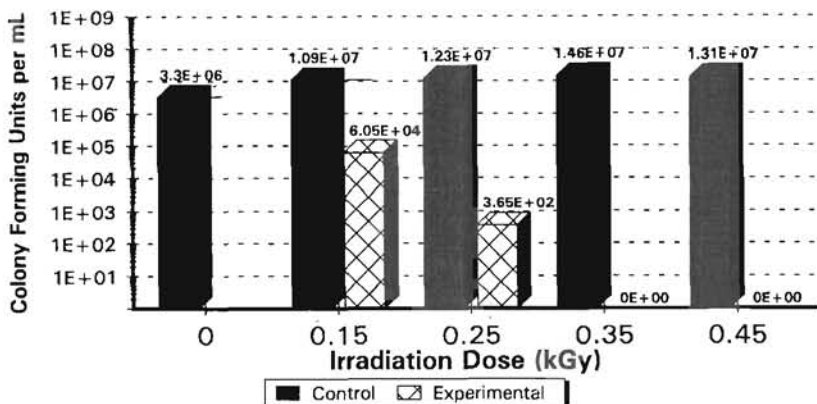


Figure 6: *Vibrio vulnificus* (O's) on CPC agar post-ionizing radiation (0.641 Abs.@ 420nm in PBS 6/13/91)

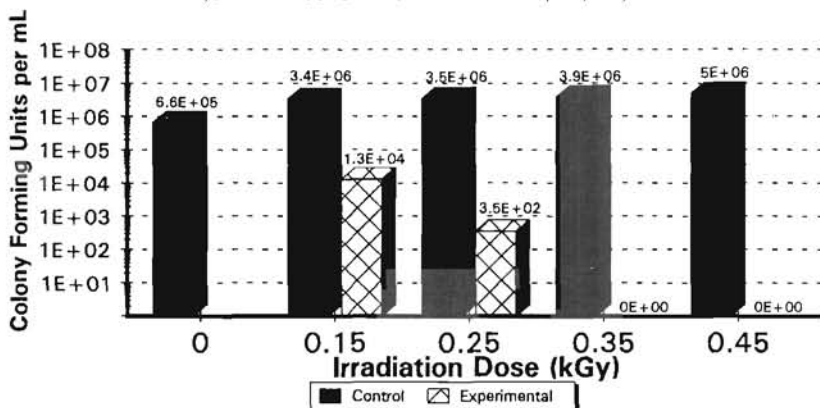


Figure 7: *Vibrio vulnificus* (O's) on TCBS agar post-ionizing radiation (0.641 Abs.@ 420nm in PBS 6/13/91)

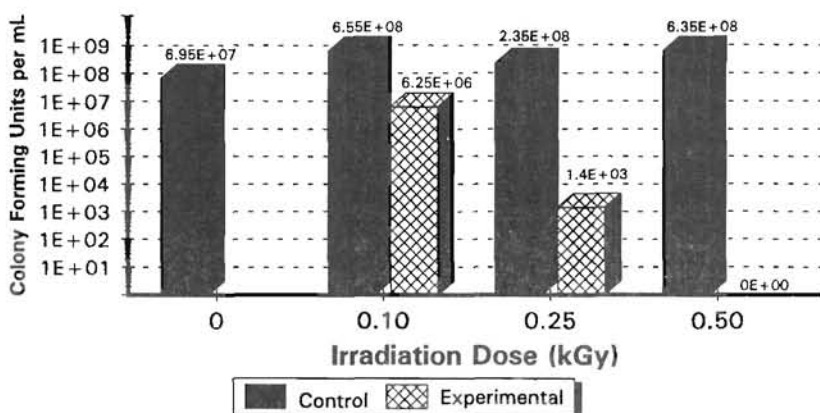


Figure 8: *Vibrio vulnificus* (T's) on CPC agar post-ionizing radiation (0.638 Abs.@ 420nm in PBS)

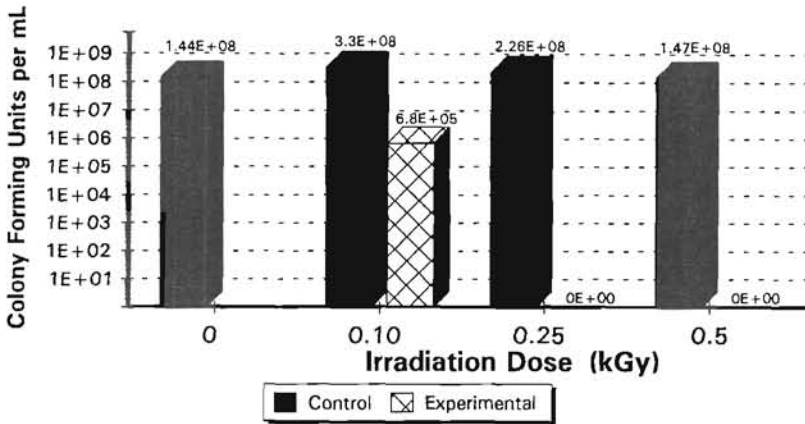


Figure 9: *Vibrio vulnificus* (T's) on TCBS agar post-ionizing radiation (0.638 Abs.@ 420nm in PBS)

per gram of meat were reduced. Furthermore, the reduction in bacterial numbers was even greater when the shellfish were flipped over during exposure, thus providing each side of the bivalve with equal doses of irradiation.

Figures 6 and 7 show the effects of low-dose gamma irradiation on a culture of the virulent/encapsulated/opaque (O) form of *V. vulnificus*. *V. vulnificus* was suspended in phosphate buffered saline, irradiated and plated on TCBS and CPC agars. The radio sensitivity of *V. vulnificus* was determined using an endpoint method, and the opaque form was undetected on either agar after 0.35 kGy of exposure. Figure 8 and 9 are identical to 6 and 7, except that the avirulent/non encapsulated/translucent (T) form of *V. vulnificus* was used. The T form was not detected on TCBS after 0.25 kGy, however, it was detected after 0.25 kGy on CPC agar. These data would indicate that the radio sensitivity of *V. vulnificus* falls between 0.25-0.35 kGy of gamma irradiation. It also correlates well with the shell stock data, as no *V. vulnificus* was detected in any of the shell stock irradiated at 1, 2 and 5 kGy.

Low-dose gamma irradiation proved effective in reducing the numbers of *V. vulnificus* in shell stock oysters. *V. vulnificus* demonstrates some radiosensitivity, as it was not detected in any of the irradiated shell stock samples (negative CPC results), as compared to the non-irradiated control. Total bacterial numbers are reduced 3-4 log cycles. However, the product shelflife of the irradiated shell stock is lower than the non-irradiated control, and the depurated oyster.

CONCLUSION

Gamma irradiation, high energy electrons and U.V.-assisted depuration may prove to be effective processes in reducing the risk of *Vibrio* infections associated with the ingestion of raw shellfish. Treatment of shell stock oysters with gamma irradiation and high energy electrons leads to a reduction in total bacterial numbers, and a reduction in the numbers of *V. vulnificus*. However, the

shelflife of the irradiated product is compromised when compared to the non-irradiated control or the deperated product. It is the goal of irradiation processing to yield products that are safer to eat in terms of reduced pathogens, as well as products that have an extended shelflife. It appears that low-dose gamma irradiation is effective microbiologically, but does not extend the shelflife of the live oyster. Specifically, the shelflife of the irradiated oysters was reduced by approximately 20 days, when compared to the non-irradiated controls. Further research is being performed to address the plausibility of high energy electrons beams as means for reducing bacterial numbers, as well as increasing product shelflife.

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