Effects of Irradiation on the Survival of Virus in West Coast Oysters

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Gamma irradiation was evaluated as a means of inactivating poliovirus in shucked and whole shellfish. Results indicated that there was a significant survival of virus at all levels of radiation tested.

In recent years the use of ionizing radiation has been proposed as a potential means of eliminating possible food spoilage or pathogenic microorganisms from a variety of foods, such as shellfish (2, 3, 5). However, among these pathogens are viruses which are known to possess a certain degree of resistance to the inactivating effects of gamma radiation (4, 6, 7). Therefore, an investigation was conducted in our laboratories to determine the ability of ionizing radiation to inactivate viruses in shellfish. This report presents our preliminary findings.

Two separate series of experiments were conducted. In the first study, 2-year-old Pacific oysters (Crassostrea gigas) and 3-year-old Olympia oysters (Ostrea lurida), obtained from a Shelton, Washington, oyster grower, were placed in 19-liter stainless-steel aquaria to which was added 3,500 ml of filtered, sterile seawater contaminated with poliovirus 1 (strain Lsc-2ab). Virus titer was approximately 1.0 × 10⁴ plaque-forming units (PFU)/ml. The oysters were allowed to contaminate for 24 hr after which time they were dipped in a 1% hypochlorite solution to inactivate any virus adhering to the shell surfaces, rinsed in distilled water, and dried. The contaminated shellfish were divided into two equal lots. Those to be used in the first experiment were sealed, whole, in polymylar pouches (eight per pouch) and irradiated. The source of the gamma radiation was a Mark II food irradiator having a cobalt 60 source of 40,000 Ci, with a dose rate of 400 krads/hr. All samples were irradiated at an ambient temperature of 20 C. Doses represented 50, 100, 150, 200, 300, and 400 krads of radiation. The samples were allowed to remain at ambient temperature for 1 hr to provide for latent effects of irradiation, and then were assayed for virus content. Control samples consisted of contaminated oysters sealed in polymylar pouches, but not irradiated. In these studies, 100% virus survival was considered to be the virus titer existing in the shellfish after 24 hr of contamination.

Samples were readied for assay by preparing 10% (w/v) homogenates of tissue using nutrient broth as a diluent. All homogenates were blended for 2 min at 6,500 rev/min in a Lourdes homogenizer. After clarification, serial decimal dilutions were prepared in nutrient broth, and the samples were assayed for virus by plating in duplicate monolayer bottles. The assay technique was that of Davis and Dubbecco as modified by Hsiung and Melnick (1). Primary African green monkey kidney tissue was used to prepare monolayers which were grown in 3 oz. (ca. 90 ml) prescription bottles.

In the second study samples of contaminated shellfish were shucked, as aseptically as possible, and then the oysters and fluid were sealed in polymylar pouches (eight per pouch). Samples were then irradiated and assayed as described above.

The results of studies with whole, irradiated oyster samples are presented in Table 1. Although there was a reduction in the total number of viable virus present from all samples, the greatest reduction in virus titer occurred in samples subjected to a dose of 400 krads. However, even after this dose of irradiation 88 virus PFU/g were recovered from C. gigas and 100 PFU/g from O. lurida or, on a per unit weight basis, approximately 13% of the original virus contained per gram of oyster tissues.

The results of irradiation studies with shucked oysters are shown in Table 2. As in the previous experiments, increasing doses of ir-
TABLE 1. Recovery of poliovirus from whole contaminated samples of C. gigas and O. lurida after irradiation

| Sample       | Dose of irradiation (krads) | PFU/g | Per cent survival |
|--------------|-----------------------------|-------|-------------------|
| C. gigas     | 0 (Control)                 | 6.8 x 10^4 | 100              |
|              | 50                           | 5.1 x 10^3  | 75.0             |
|              | 100                          | 4.3 x 10^3  | 63.2             |
|              | 150                          | 3.1 x 10^3  | 45.4             |
|              | 200                          | 1.4 x 10^2  | 20.1             |
|              | 300                          | 1.1 x 10^2  | 16.3             |
|              | 400                          | 8.8 x 10^1  | 13.0             |
| O. lurida    | 0 (Control)                 | 7.7 x 10^4  | 100              |
|              | 50                           | 6.7 x 10^3  | 87.0             |
|              | 100                          | 5.0 x 10^3  | 66.0             |
|              | 150                          | 3.3 x 10^3  | 43.0             |
|              | 200                          | 1.6 x 10^2  | 21.0             |
|              | 300                          | 1.4 x 10^2  | 18.1             |
|              | 400                          | 1.0 x 10^2  | 13.0             |

*Plaque-forming units.

TABLE 2. Recovery of poliovirus from shucked contaminated samples of C. gigas and O. lurida after irradiation

| Sample       | Dose of irradiation (krads) | PFU/g | Per cent survival |
|--------------|-----------------------------|-------|-------------------|
| C. gigas     | 0 (Control)                 | 6.8 x 10^4 | 100              |
|              | 50                           | 4.3 x 10^3  | 63.6             |
|              | 100                          | 2.1 x 10^3  | 31.1             |
|              | 150                          | 1.8 x 10^3  | 26.3             |
|              | 200                          | 1.1 x 10^3  | 16.3             |
|              | 300                          | 1.0 x 10^2  | 15.0             |
|              | 400                          | 5.0 x 10^1  | 7.35             |
| O. lurida    | 0 (Control)                 | 7.7 x 10^4  | 100              |
|              | 50                           | 5.2 x 10^3  | 67.5             |
|              | 100                          | 3.6 x 10^3  | 47.0             |
|              | 150                          | 1.8 x 10^3  | 23.3             |
|              | 200                          | 1.4 x 10^3  | 18.1             |
|              | 300                          | 1.1 x 10^3  | 14.2             |
|              | 400                          | 7.7 x 10^1  | 10.0             |

radiation did lower the total virus count, but none of the test doses were sufficient to inactivate all the virus. The viable virus count in shucked oysters after a dose of 400 krad was still 50 PFU/g for C. gigas, 77 PFU/g for O. lurida or, on a per unit weight basis, 7.3 and 10% of the original virus contained per gram of the respective oyster tissues. Perhaps higher doses of radiation would prove more effective. However, the use of higher levels of irradiation could cause organoleptic changes rendering the oysters unpalatable.

The results reported are preliminary observations. However, they do indicate that viruses in at least one food product, oysters, are able to survive the inactivating effects of gamma radiation. This rate of survival, under experimental conditions, varied from 87 to 7.3%, depending upon the dose of radiation and the nature of the sample. However, due to the mode of pathogenicity of viruses, even a low percent survival of these pathogens cannot be considered satisfactory. In addition, taste testing of noncontaminated oysters subjected to the dose required to inactivate 90% or more of the viruses (400 krad) revealed that they had undergone organoleptic changes which rendered them unpalatable. Thus, it appears that further research is required to determine the ability of viruses in various foods to survive the inactivating effects of irradiation before this method of preservation can be safely applied.

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