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FINAL TECHNICAL REPORT

DEPURATION OF SHELLFISH BY IRRADIATION

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SUMMARY

The potential benefits of food irradiation technology can be extended presently to provide solutions to unique problems of shellfish sanitation. The present threat of bacterially and virally induced illness resulting from the consumption of raw or partially cooked shellfish can be significantly reduced through the application of food irradiation techniques.

Previous studies by the University of Lowell Radiation Laboratory and the U.S. National Marine Fisheries Service N.E. Laboratory in Gloucester, MA on softshelled clams (Mya arenaria) demonstrated the effectiveness of low to medium doses of Cobalt 60 source gamma irradiation in the inactivation of Escherichia coli, Salmonella typhimurium, Staphylococcus aureus and Streptococcus faecalis. Log decrement (D 10) values were determined at 0.37, 0.51, 0.42 and 0.97 kiloGray (kGy), respectively. Although bacterial survival was greatly decreased, post-irradiation survivorship of live clams receiving 0.10 kGy indicated no significant increases in mortality rate at six days post exposure. Organoleptic evaluation of fried and steamed softshelled clams showed no significant deterioration in appearance, aroma, taste and texture at doses up to 2.0 kGy. Clams exposed to 3.0 kGy underwent a decline in textural quality when fried but were still ranked as acceptable to consumers.

Post-irradiation survival and organoleptic studies when extended to hardshelled clams (Mercinaria mercenaria) and American oysters (Crassostrea virginica) showed no significant decline in consumer qualities or 6 day post irradiation survival in oysters at doses of up to 3.0 kGy. However 5 day post-irradiational survival of hardshelled clams exposed to 2.0 kGy and 3.0 kGy was significantly lower than non-irradiated controls.

The capacities of the American oyster to sustain relatively high doses of gamma irradiation were demonstrated by 6 day post-exposure survivorship values of greater than 90% for samples receiving 3.0, 5.0 and 7.0 kGy. This exceptional radioresistance is most probably related to the physiological adaptations of facultative anaerobiasis which incidentally include radioprotective mechanisms. Among these are: 1.) low body fat reserves; 2.) facultative anaerobic metabolism with concomitant tissue anoxia; 3.) low cell division rate; and 4.) accumulation of succinate, a naturally occurring radioprotective agent as a metabolic by-product of anaerobic glycolysis.

Investigations of inactivation of selected viruses were undertaken with Baylor College of Medicine's Department of Virology and Epidemiology. Initial studies of inactivation of Polio I virus and a simian rotavirus (SA-11) was conducted in both hardshelled clams and oysters. Buffalo Green Monkey cell culture was used to monitor poliovirus activity - expressed as

plaque forming units (PFUs). Polio D 10 values of 2.7 and 3.3 kGy were recorded for viruses recovered from Mercinaria and Crassostrea respectively. SA-11 activity was determined directly by plaque formation in Fetal Rhesus monkey kidney (FRhK) cell culture. The D 10 values for SA-11 in Mercenaria and Crassostrea were 1.37 and 2.4 kGy respectively.

Of greatest interest was the behavior of Hepatitis A virus (HAV), in live, irradiated shellfish. As detection of HAV from environmental samples is quite problematical, several approaches to the problem of quantifying viral titers were implemented. Radioimmunofocus analysis, (RIFA), immunoflourescent focus (IFF), in-situ viral RNA detection and direct cytopathic effect (CPE) techniques were employed to evaluate the effectiveness of gamma irradiation in reducing viral activity. The resultant D 10 determinations ranged from 1.2kGy as determined in hardshelled clams by immunoflourescent focus analysis to 3.2 as determined in oysters by in-situ RNA hybridization. The average log decrement value for HAV in oysters was calculated at 2.0 kGy.

From these data it appears that doses of up to 2 kGy can be applied to: 1.) reduce or eliminate bacterial pathogens, 2.) reduce the infectivity of human viral pathogens by one or more orders of magnitude, and 3.) preserve market qualities of longevity, appearance, odor, taste and texture.

These studies indicate that food irradiation technology has a unique application in alleviation of the public health difficulties presented by consumption of raw shellfish. Food irradiation is the sole technique presently available which can effectively reduce infectious viral burdens and concomitantly retain those qualities which consumers desire in the uncooked shellfish.

GAMMA IRRADIATION OF MOLLUSCAN SHELLFISH

INTRODUCTION

Food processing by ionizing irradiation has been shown to be safe, effective and efficient for the purposes of food preservation, sanitation and reduction of post-harvest storage losses. Irradiation is effective in the disinfestation of insect pests, inactivation of food-borne parasites and destruction of microbial pathogens. The wholesomeness and safety of foods, exposed to doses of up-to 10 kiloGray (kGy) of ionizing irradiation, have been endorsed by the World Health Organization's Food and Agriculture Organization (WHO/FAO), the International Union of Microbiological Societies, the American Medical Society and others. The joint FAO/International Atomic Energy Agency Division of Isotope and Radiation Applications (1988) lists 32 nations which presently accept irradiated foods as safe for general consumption; over 40 commodities in all have been approved for trade and consumption. The technology is presently applied to storage crops such as onions and potatoes, and dried commodities such as grains and spices and seasonings. Fresh and dried fruits as well as processed meats, froglegs, poultry, fin-fish and frozen shrimp are being successfully irradated and marketed.

PROBLEMS ASSOCIATED WITH RAW SHELLFISH

Food irradiation processing holds additional promise as a solution to the particularly difficult and quite serious public health problems

which result from the public's consumption of live or inadequately cooked shellfish. Shellfish species most frequently implicated as sources of foodborne infection are Mya arenaria, the softshelld clam (which is also known as the steamer clam), Mercenaria mercenaria, the hardshelled clam (which is also referred to as the quahog or the cherrystone clam) and Crassostrea virginica (the American oyster). Contaminated softshelled clams (consumed as steamed clams and fried clams) may pose a disease problem as the cooking times are generally brief and may be of insufficient duration to provide the high internal temperatures required for effective sterilization of microbial pathogens. The popular practice of ingesting raw marine mollusks "on-the-half-shell" (as oysters and hardshelled clams) has been linked repeatedly to serious disease outbreaks and is clearly documented as an increasing health threat to the consuming public.

Unsanitary molluscan and crustacean shellfish recently have been identified as being responsible for several epidemics of enteric illnesses (U.S. GAO 1984, Verber, 1984). Molluscan shellfish become contaminated from bioaccumulation of microbial pathogens concentrated by filterfeeding from overlying waters recieving wastewater contaminated with enteric wastes.

Metcalf (1985) reported enteric pathogens isolated from contaminated shellfish and from waters overlying shellfish harvesting beds. Levin, (1978) lists bacterial agents which have been recovered from shellfish as Bacillus cereus, Clostridium perfringens, enteropathic Escherichia coli, Salmonella spp., Staphylococcus aureus, Vibrio colerea and Vibrio parahaemolyticus. Viral pathogens responsible for shellfish borne disease outbreaks include Coxsackie virus, echo virus, hepatitis A virus, Norwalk virus and polio viruses .

It is estimated by the U.S. National Marine Fisheries Service (1985) that within a five year period, over 4,500 cases of viral diseases have been incurred in the U. S. as a direct result of consuming contaminated shellfish stock. Thus, typhoid fever, viral hepatitis, cholera and acute diarrheal disease are attributed to ingestion of shellfish-borne microbial pathogens. Although thousands of cases of direct shellfish related illnesses are diagnosed and reported annually, a significantly larger number of cases may escape proper diagnosis and/or reportage. Additional incidences of disease may be contracted secondarily by non-consumers who have had contagious personal contact with infected shellfish consumers. These subsequent incidences of transmission of illness from consumers to the general population have not been satisfactorily documented, although incidences of secondary transmission and infection are speculated to be high.

Internationally, many coastal countries persistently report frequent, extensive outbreaks of molluscan borne diseases. A single outbreak of oyster-borne viral disease resulted in morbidity estimated as high as 2,000 individuals in Australia (Rao and Melnick 1986). The Shanghai Public Health Bureau reported the most intensive hepatitis-A outbreak in recent history; an epidemic in which more than 16,000 persons were afflicted was caused by contaminated clams harvested from heavily polluted coastal waters.(Anon. 1988).

In addition to the direct, adverse health effects of compromised shellfish sanitation, there are significant direct and indirect economic impacts on coastal shellfish harvesting states resulting from a broad public perception of a dangerous or uncertain risk due to questionable practices related to shellfish sanitation. A 12-million dollar industry

is found in Massachusetts alone; other states maintain even larger fiscal interests in a sound and healthy shellfish industry. Increased certainty in shellfish sanitation is critical to the general public health as well as local economies of many producing states.

Although the 24 coastal states of the Northeast, Central Atlantic, Gulf Coast and Pacific regions are most directly effected by present shellfish sanitation conditions, inland states share the burden of public health impacts as a result of inadvertant distribution and marketing of contaminated seafoods. In recent years disease outbreaks attributed to microbially unsanitary shellfish have resulted in proposed state legislation to ban the sale of raw clams in all restaurants within the state jurisdiction.

APPLICATION OF IRRADIATION TECHNOLOGY TO BIVALVE SHELLFISH

Investigations of the application of food irradiation science to fisheries and seafood products were initiated over two decades ago. Nickerson (1963) exposed softshelled clams and clam meats to irradiation doses equivalent to 8 kGy and detected no significant differences in organoleptic qualities between irradiated and control samples after 40 days storage at 6 ^{o} degrees C. Slavin et al.(1963), Slavin and Ronsivalli (1963), and Ronsivalli et al. (1965) reported that clam meats exposed to 4.5 kGy were found to be of equal quality to unirradiated controls held at similar temperatures. Connors and Steinberg (1964) employed a professional taste panel to evaluate organoleptic differences between non-irradiated control clam meats and samples irradiated at doses of 2.5, 3.5, 4.5 and 5.5 kGy; the panel found no statistically significant differences attributed to the irradiation

treatment.

The above referenced studies were undertaken to evaluate the effects of irradiation treatment in extending the storage life of shucked clam meats and products. The objectives of the present study however were to assess the applicability of food irradiation technology to live shellfish and to discern the potential of the process in alleviating health threats associated with consumption of live or undercooked molluscan shellfish.

OBJECTIVES OF STUDY

Specifically, the purposes of the present study were to examine the feasibility of employing low dose gamma irradiation to ameliorate some of the persistent health problems linked to uncooked or undercooked whole clams, raw quahogs and raw oysters.

The specific objectives of the study were to evaluate the effects of low dose and/or mid dose irradiation exposures on four general areas of concern. The specific tasks addressed were:

1. To determine the effects of low dose irradiation exposure on the post-irradiation survival of various shellfish species.

2. To quantify the effects of gamma irradiation on the organoleptic marketability of shellfish as expressed in the the qualities of appearance, aroma, taste and texture.

3. To confirm the gamma radiation doses necessary to effect log decrement reductions in selected bacterial flora in softshelled clams.

and,

4. To determine the inactivation responses of selected viral pathogens to a range of radiation exposures.

METHODS AND MATERIALS

POST-IRRADIATION SURVIVAL OF BIVALVE MOLLUSKS

SOFTSHELLED CLAMS

Bivalve mollusk survival studies were conducted at the University of Lowell Radiation Laboratory / D.O.E. 1.2 megaCurie ^{60}Co irradiation facility. Investigations were made of the ability of Mya arenaria, the softshelled clam, to survive various irradiation doses and antecedent holding conditions. Clam survivorship studies involved thirty-three batches (consisting of approximately 50 individuals each) in which clams were packaged in two-liter polyethylene containers and irradiated over a dose range from 0.24 to 3.30 kGy. The ^{60}Co irradiation of small batches of live molluscan shellfish was undertaken to determine dose effects on continued survival of softshelled clams, hardshell quahogs, and oysters. Exposures were administered in small batches of 50 to 100 individuals packaged in groups of 25 to 50 in small polyethylene containers (measuring 12 x 12 x 17 centimeters). Small container sizes were selected to minimize the bulk density and provide relatively as uniform doses as possible throughout the batches.

Post-irradiation longevity of irradiated and control samples of softshell clams was tested across the range of exposures. Siphon withdrawal and valve closure reflexes were used as response indicators of viability. Each individual was tested at 24 hr intervals by physically stimulating the extended siphon by a gentle probing. This resulted in siphon withdrawal in live specimens. Testing persisted for each individual until response failure. All cohorts were followed until the last survivor failed to respond. Survival curves were plotted for irradiated batches and their corresponding controls. Direct comparisons

of survivorship at day 6 (post irradiation) for treated samples and non-irradiated controls were made by the non-parametric Mann-Whitney U-test for ordered events (Sokol and Rolf, 1981). Mortality curves were plotted for clam populations exposed to various doses.

HARDSHELLED CLAMS

Survival of the quahog (M. Mercenaria) was tested in preliminary trials. Cohorts of 30 specimens each were exposed to doses of 0.5, 1.0, 3.0, 5.0 and 7.0 kGy. The studies indicated critical exposure range between the 1.0 and 3.0 kGy doses. Additional cohorts of 50 hardshell clams each were exposed to doses of 0 .5, 1.0, 2.0 and 3.0 kGy. The endpoint used to judge the occurence of death in quahogs was the failure of reflexive shell closure upon physical stimulation of mantle tissues. Testing was continued until the last survivor failed to respond. Survival curves were plotted for each of the cohorts.

OYSTER SURVIVAL

The oyster, C. virginica, also was tested for lethal response to acute irradiation doses. Sample sizes of 50 oysters each were exposed to doses of 1.0, 1.5, 2.0, 2.5, 3.0, 5.0, and 7.0 kGy in two trials. Viability of each specimen was tested daily by challenging adductor muscle tension. The endpoint used to determine mortality in the oysters was the judged to be the point at which sustained tension on the adductor muscle as no longer met by resistence to forced shell opening. Cohort survival schedules were recorded until the death of the last survivor and survival curves were plotted. Irradiation doses were calculated in accord with gamma cave geometry and were measured directly by an array of radiochromic thin film dosimeters located within each container. The functional relationship between the dose calculated by

geometry and exposure time and the exposure indicated by dosimetry was determined by correlation analysis.

ORGANOLEPTIC STUDIES

Subjective tests were made of the relative sensory appeal and palatability various food items prepared from uncontaminated, irradiated shellfish. Organoleptic evaluations were made of irradiated and non-irradiated samples of the the three principle species used in this study.

Softshelled clams (M. arenaria) were prepared and presented to organoleptic evaluation panels as steamed clams and fried clams; quahogs were prepared as raw cherrystone clams on the half-shell and as baked quahogs. Oysters were evaluated raw (as oysters on the half-shell), and as baked oysters.

Whole shellfish samples were irradiated at the Lowell Radiation Laboratory at doses of 0.50, 1.0, 2.0 and 3.0 kGy, and transported in plastic bags on ice to the U.S. Department of Commerce National Marine Fisheries Service (NMFS) Northeast Fisheries Center Laboratory at Gloucester, MA where they were prepared for testing as the above dishes and presented to professional taste panels comprised of six to ten panelists. Each panelist evaluated three servings each at each of the radiation dose levels and compared these treated portions directly to a known, non-irradiated, reference control sample.

The each sample was judged sequentially for the properties of appearance, odor, flavor and texture. Each property was judged qualitatively on a nine point scale ranging from "Inedible" (at a score

of one point), through "Very Poor", "Poor", "Slightly Poor", "Borderline", "Fair", "Good", "Very Good" and "Excellent" (with the last category having the highest assigned value of nine points).

Possible organoleptic differences attributable to irradiation dose effects were evaluated for statistically significant differences in the appearance, aroma, taste and texture categories by a one-way model analysis of variance (ANOVA). Frequency histogram plots were constructed to display organoleptic responses to gamma dose for each shellfish preparation type.

BACTERIACIDAL EFFECTS OF GAMMA IRRADIATION IN MYA ARENARIA:

This portion of the study was undertaken to determine the effects of gama exposure on reduction of bacterial populations in the softshell clam, Mya arenaria.

Clams used in this study were harvested directly from Massachusetts coastal waters or purchased from local wholesale seafood distributors. Shellfish were transported to the Lowell Radiation Laboratory facility where they were held in air at 4 ^{o}C for less than 24 hours prior to experimentation.

Certain samples, used in studies of indigenous bacterial populations, were harvested under special permit from closed, polluted clam flats. Bacteriological analyses of environmentally contaminated clams taken from these polluted waters were conducted on 47 two-liter batches which were irradiated over the 0.26 to 3.30 kGy dose range and 13 non-irradiated control batches of approximately 50 clams each. Samples in this study were held for 62 hrs. post-irradiation and transported to the Gloucester NMFS laboratory for bacteriological

analyses.

In preparation for analysis, the shells were washed externally, the clams were shucked, the meats homogenized and 100 gram aliquots were serially diluted for aerobic plate count, coliforms and fecal streptococcal counts. Replicate determinations were made for each procedure in accord with standard methods : aerobic plate counts were made onto pour plates of Standard Methods Agar reinforced with 0.5% bacto-peptone and 0.5% NaCl and incubated at 20°C for 5 days or 37°C for 2 days before counting colonies. Fecal streptococcal counts were determined in K.F. Streptococcus - agar incubated 46 hrs at 35°C . Fecal coliforms and E. coli were estimated by Most Probable Number (MPN) methods: dilutions were incubated in lauryl sulfate tryptose broth at 35°C for 25 hrs, innoculates from positive tubes were transferred to E.C. broth at 44.5°C for 24 hrs; confirmation of presence of E. coli from positive E.C. tubes was made by IMViC tests.

INNOCULATION STUDIES:

Uncontaminated, freshly shucked clam meats obtained from a local supplier were used as the substrate to quantitatively assess irradiation induced inactivation of three, commonly encountered, shellfish - borne bacterial pathogens. Quantification of D_{10} inactivation doses on known population densities of the selected bacterial pathogens was determined after inoculation of clam meats with specific cultures. Samples of fresh, uncontaminated, shucked, homogenized clams were separately innoculated with washed cells of E. coli, Salmonella typhimurium, Staphylococcus aureus or Shigella flexneri at densities in excess of 10 cells per gram of meat and irradiated over a dose range of 0 to 3.60 kGy in the Marine Products Development Irradiator at the Northeast Fisheries

Center Gloucester Laboratory. Serial dilutions were made and bacterial inactivation was determined in Staphylococcus by counting typical colonies after 48 hrs at 35°C. Salmonella counts were estimated by a 3 tube MPN procedure with pre-enrichment in lactose broth for 24 hrs at 35°C, followed by inoculation in selenite cystine broth for an additional 24 hrs and confirmation of positive tubes by plating out on agar. Data were plotted as log bacterial survivors vs. exposure dose. The inverse of the regression slope was calculated as D_{10} value for each bacterial species.

VIRAL INACTIVAION STUDIES

The critical issues in shellfish sanitation practices relate to problems of viral carriage by contaminated bivalve shellfish. The major viral pathogens transmitted to consumers by molluscan shellfish appear to be RNA viruses of the human enteric picornavirus and rotavirus types. Shellfish borne hepatitis is caused by ingestion of shellfish containing hepatitis A virus (HAV) which is a naked, icosahedral, single stranded RNA virus. Poliovirus, a picornavirus, similarly may be harbored in shellfish from polluted waters (although risk of disease transmission to consumers presently is regarded as minimal due to the success of immunization programs). Quantities of poliovirus, presumably originating from inoculated infants, have been recovered from coastal waters.

Poliovirus and the simian rotavirus SA-11, viruses which are not present health threats, were used as experimental models in determining such factors as viral survival and longevity in shellfish, dynamics of viral carriage, and efficiency of viral isolation and recovery from infected mollusks.

Viral pathogen models are usefull as indicators the effectiveness of gamma treatments in reducing population numbers and/or infectivity of viral agents. Poliovirus, simian SAll virus and hepatitis A virus were employed as models in these studies to determine survival of viral populations in bivalves under various conditions of inoculation and irradiation. Hardshell clams and oysters were used for viral carriage with the viruses being introduced into the shellfish by inoculation. Simian rotavirus, SAll, used in this study was cultured in fetal rhesus monkey kidney (FRhK) cell culture (MA-104) and poliovirus 1 (Pl) was derived from buffalo green monkey (BGM) cell culture in accord with procedures cited by Lewis and Metcalf (1988). HAV used in this study was harvested from a persistently infected culture in the African green monkey kidney cell line developed by Simmonds et al. (1985).

Viral cultures were maintained at Baylor College of Medicine and shipped as harvested, concentrated frozen cultures to the University of Lowell where viral titers were thawed, diluted where necessary and inoculated into clams and oysters prior to irradiation. Subsequent to gamma irradiation processing, the shellfish were individually shucked and frozen, and shipped to Baylor for viral recovery, isolation, assay and enumeration.

Preliminary studies of viral recovery from quahogs and oysters were undertaken with the simian rotavirus SAll and poliovirus Pl as test models. An electric drill with circular saw was used to gain access to the viscera by a one-inch (2.5 cm) diameter hole cut through the shell to expose the cardiac area. Virus suspensions were intoduced either into the hemolymph fluid through the pericardial sac or the suspensions were inoculated into the hepatopancreas visceral mass. Rotavirus SAll and poliovirus Pl were recovered from mantle fluids and tissues

separately and assayed from each fraction separately.

In these trials detection of SA11 through cytopathic effect (CPE) methods was used to quantify viral presence as plaque forming units (PFU) in in RhMK (MA-104) cell culture. Polivirus P1 assayed as PFUs in BGM cell culture. Recovery and assay procedures are reviewed by Rao and Melnick (1986). Results of these trials showed slight differences in viral recoverability associated with injection route, with the visceral mass injection route being more conservative; subsequent HAV trials were conducted via visceral mass injections.

Post irradiation viral recoveries employed a polyethylene glycol (PEG) concentration procedure developed during the course of these studies by Lewis and Metcalf (1988). Viral culturing and enumeration assays utilized immunoflourescent focus methods as a direct assay.

In the IFF assay, monolayers of cultured cells were allowed to become confluent on coversips and were then inoculated with dilutions of shellfish homogenate and incubated 1.5 to 2 hrs at 36 ^{o}C. Coverslips were overlayed with agarose and incubated for approximately one week. The agarose overlay was removed and the cells washed three times with PBS before air drying and fixing with acetone at -20 ^{o}C. Cells were washed again with PBS and allowed to react with antiHAV IgG for 40 to 60 minutes. Cells were stained with a flourescent-antiglobulin conjugate and examined under UV microscope for flourescent foci indicating the activity of infectious particles.

Quantal methods of viral detection involved radioimunoassay, (RIA), nucleic acid probe (RNA probe) hybridization assay and a recently

developed cytopathic effect (CPE) assay for viral survival.

The RIA procedures require inoculation of 60 mm petri dishes cultures of AGM kidney cells with serially diluted fractions of shellfish homogenate.

RESULTS

RESULTS OF POST-IRRADIATION SURVIVORSHIP STUDIES

Actual gamma doses received, as determined by dosimetry within the sample containers, showed moderate variation in exposure across the volume of the containers. The minimum/maximum differences were determined to be less-than 15% in the softshelled clam, Mya, but as high 20% in the oyster, Crassostrea.

Correlation analysis between dose estimates (based upon source activity, gamma cave geometry, and exposure times) and received-dose determinations from Fricke tube and/or radiochromatic film dosimeter packs placed in the samples yielded a correlation coefficient of 0.96.

POST-IRRADIATION SURVIVAL OF SOFTSHELLED CLAM (MYA ARENARIA)

Clam survival studies were designed to investigate dose effects on live products over a short time span (equivalent to the greatest expected shelf life of clams sold on the retail market). Live softshell clams are held in the marketplace, on ice, exposed to air, for not more than five days. A six day post-treatment cumulative mortality study was undertaken to determine maximum levels possible for marketing purposes. Comparison of survival schedules between irradiated and control live clams showed no adverse effects of irradiation at doses below 1.00 kGy (Figure 1). Mortality of non-irradiated samples averaged 2.6 % after six days of storage at 4 ^{o}C ; this is in agreement with expected mortality under marketing conditions.

Mann-Whitney U-tests for unpaired comparisons of 18 irradiated batches and their respective controls at six days post treatment were evaluated by data comparisons to critical value, p= 0.05 and 0.01, for the two tailed t distribution. No significant differences were observed

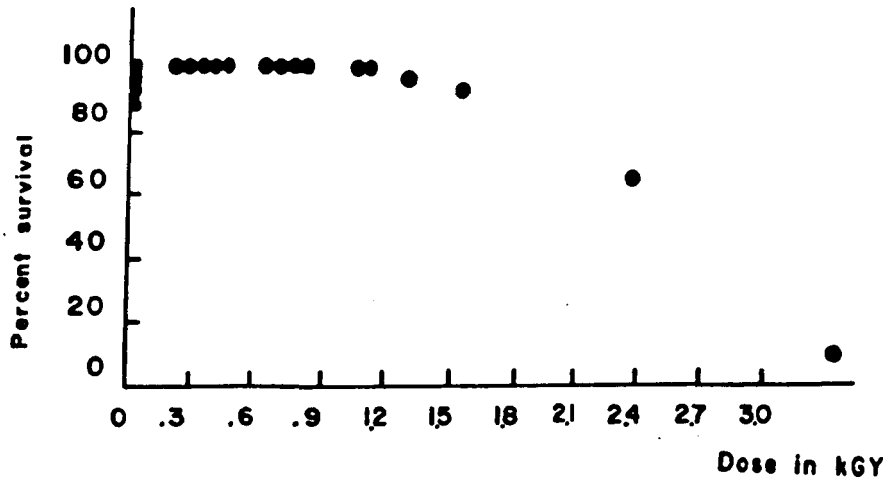


Figure 1

Survival of irradiated Mya arenaria
at day 5 post treatment

at doses below 0.60 kGy. Over the range of 0.006 to 1.00 kGy, irradiated clams showed a slight but significant, prolongation of shelf-life under the test conditions. At doses in excess of 1.00 kGy however, a significant acceleration in mortality was observed.

POST-IRRADIATION SURVIVAL OF QUAHOG (VENUS MERCENARIA)

The hardshell clam or quahog showed a similar dose response as the softshelled bivalve. Batches of 50 clams each were exposed to doses of 0.5, 1.0, 2.0 and 3.0 kGy and cohort mortalities were compared to those of controls. On the sixth day post exposure, no increases in mortality attributable to irradiation were observed between the non-irradiated control group and the 0.5 kGy and 1.0 kGy exposure groups. The 2.0 kGy and 3.0 kGy groups experienced accelerated mortality schedules as illustrated in Figure 2. Matched pair t-test comparisons of daily mortality of exposure vs. control groups showed significant differences between the morality schedules of the control sample and those exposed to 2.0 and 3.0 kGy. No significant differences were demonstrated between controls and either the 0.5 or 1.0 kGy exposure doses. The lower exposure dose samples survived comparably to controls for 15 days post exposure. Median survival times for these three cohorts appear comparable. Both the 0.5 and the 1.0 kGy exposure groups achieved median survival times of 15 days post exposure when held at 4 ^{o}C. in air whereas the non-irradiated control group achieved median survival of 17 days under similar holding conditions. It appears that doses of 1.0 kGy or less have no adverse effects upon the survival and marketability of the hardshell clam. Low dose exposures may be somewhat effective in reducing populations of bacterial species which accelerate spoilage and thus may prolong marketlife.

Mercenaria mercenaria

Survivorship Post Irradiation

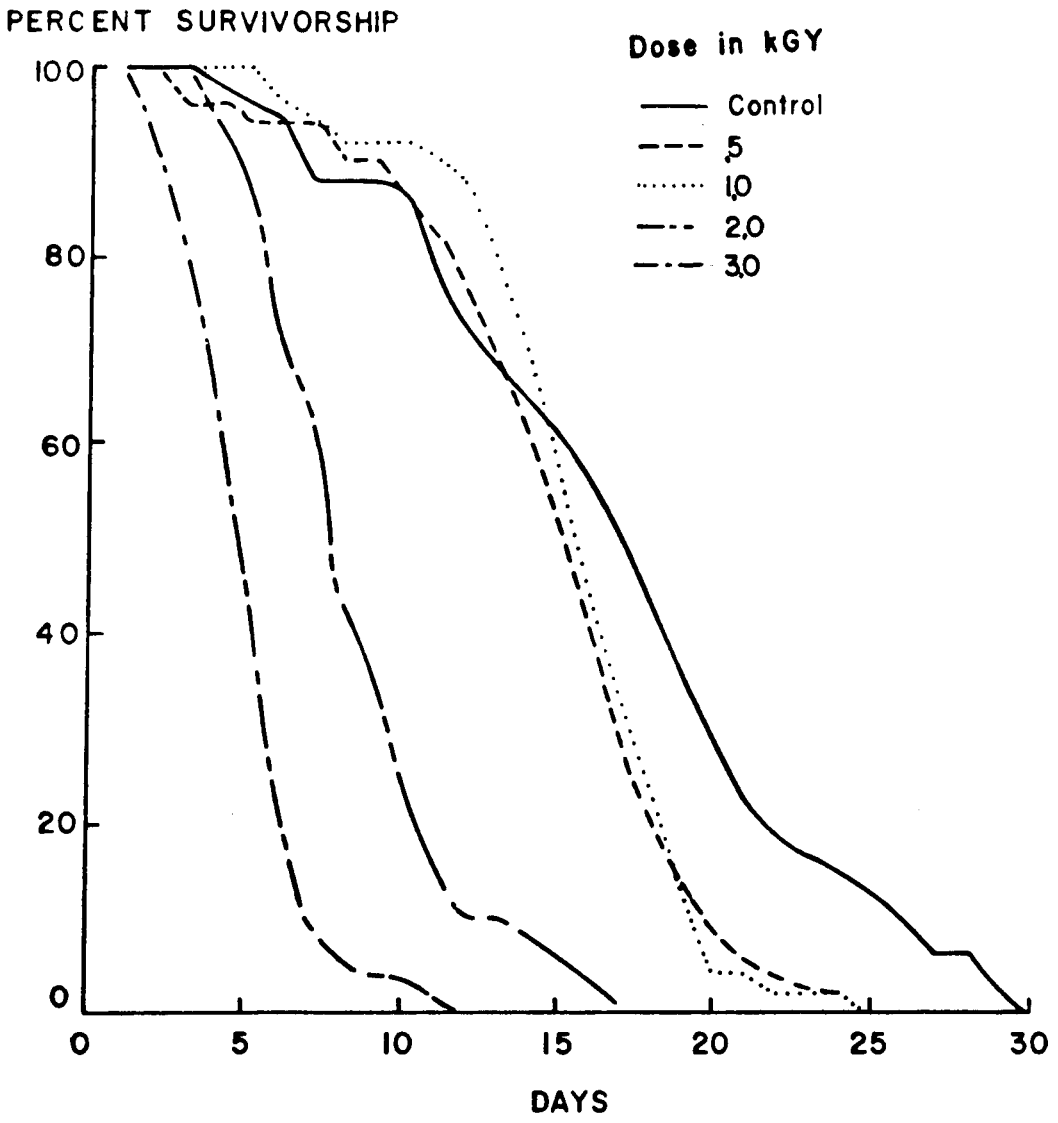


Figure 2.

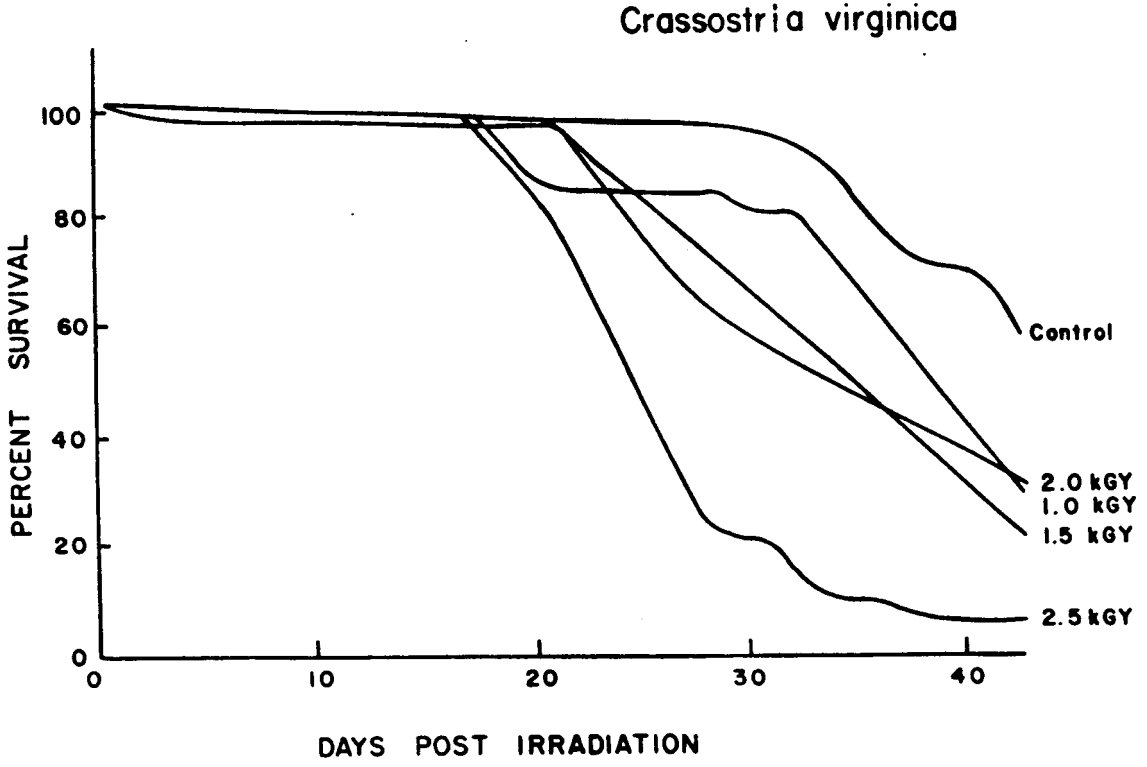


Figure 3. Post-irradiation survival of the American Oyster, Crassostria virginica

POST-IRRADIATION SURVIVAL OF OYSTERS (CRASSOSTREA VIRGINICA)

Oysters appear to exceptionally resistent to acute radiation effects; no significant differences in six-day survival times were observed at doses up-to 3.0 kGy. Median post-irradiation survival times were in excess of 25 days for doses of 2.5 kGy and lower (Figures 3). Batches exposed to 3.0, 5.0 and 7.0 kGy doses displayed earlier onset of radiation effects with median response failure occuring around the eleveth day post exposure. The response for each of these three higher doses appear so similar as to suggest simultaneous mortality as a result of acute dose effects at exposures beyond 3.0 kGy.

ORGANOLEPTIC RESPONSED OF BILVALVES TO IRRADIATION

SOFTSHELL CLAMS

The soft shelled clam is not eaten raw, consumed exclusively as a cooked seafood product, these items are prepared as steamed clams, deep fried (in batter until the outer batter is firm and golden in color), or in a milk-based chowder with potatoes. Organoleptic trials were conducted on steamed and fried clams as these preparations present the greatest risk of sustained microbial presence.

Organoleptic qualitative evaluations of the softshelled clam dishes of interest, steamed and fried clams, treated over the 0.5 to 3.0 kGy dose range. Control samples were identified as such to the taste pannel and were ranked as being of very good quality in appearance, aroma, tast and texture with average evaluation scores of 8.0, 8.5, 8.0 and 8.2 in the respective catagories (Table 1, Fig.4). The irradiated products displayed a slight but consistent, progressive, decrease in perceived qualities over the 0.5 to 3.0 kGy radiation exposure range. The fried clams scored the lowest averages in the category of textural

Table 1.

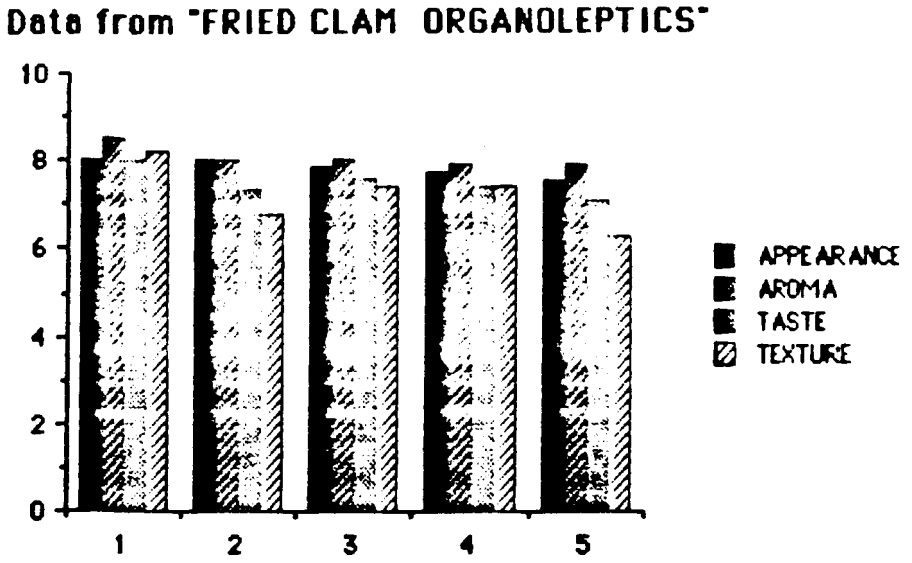
Organoleptic Evaluation of Irradiated Mya arenaria

FRIED CLAMS

	CONTROL Mean	S.D.	0.5 kGy Mean	S.D.	1.0 kGy Mean	S.D.	2.0 kGy Mean	S.D.	3.0 kGy Mean	S.D.
APPEARANCE	8.0	(0.94)	8.0	(0.47)	7.8	(0.42)	7.7	(0.67)	7.5	(0.97)
ODOR	8.5	(0.53)	8.0	(0.67)	8.0	(0.67)	7.9	(0.74)	7.9	(0.74)
TASTE	8.0	(0.94)	7.3	(1.06)	7.6	(1.17)	7.4	(1.26)	7.1	(1.37)
TEXTURE	8.2	(0.92)	6.8	(1.23)	7.4	(0.97)	7.4	(1.07)	6.3	(1.83)

STEAMED CLAMS

	CONTROL Mean	S.D.	0.5 kGy Mean	S.D.	1.0 kGy Mean	S.D.	2.0 kGy Mean	S.D.	3.0 kGy Mean	S.D.
APPEARANCE	8.8	(0.41)	8.7	(0.82)	8.8	(0.41)	8.5	(0.55)	8.5	(0.55)
ODOR	9.0	(0.00)	8.5	(0.84)	8.8	(0.41)	8.5	(0.55)	8.3	(0.52)
TASTE	9.0	(0.00)	8.3	(0.52)	8.2	(0.98)	8.2	(1.33)	7.3	(1.86)
TEXTURE	8.3	(0.41)	8.5	(0.55)	8.3	(1.21)	7.3	(2.25)	7.0	(2.45)



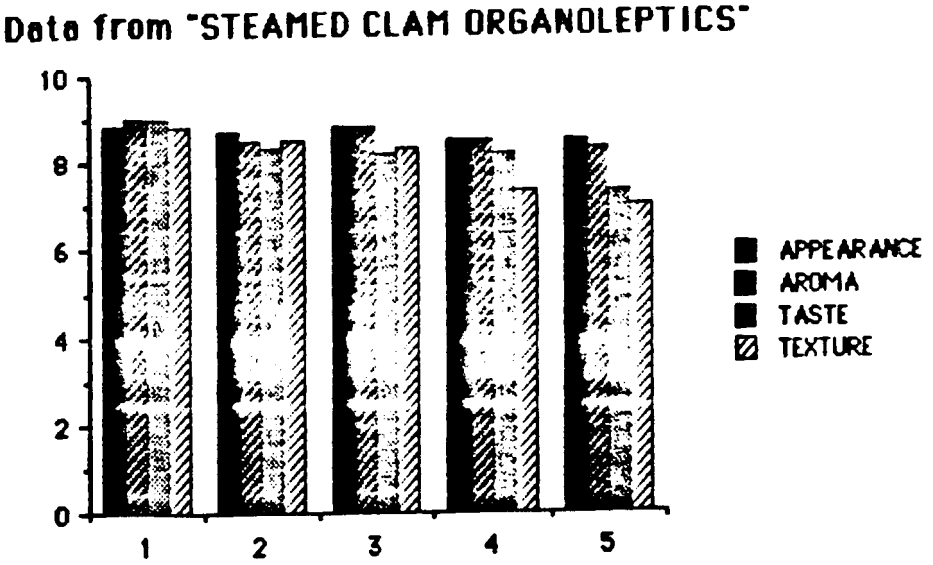


Figure 4. Organoleptic ratins of irradiated Mya arenaria

1 = Control

2 = 0.5 kGy

3 = 1.0 kGy

4 = 2.0 kGy

5 = 3.0 kGy

alterations. Average scores of 6.8 and 6.3 were recorded for the 0.5 and 3.0 kGy exposure groups as compared to a score of 8.2 for the non-treated controls. Interestingly, mean scores of 7.4 were recorded for both the 1.0 and 2.0 sample batches. Scores in the range of 6 points are regarded as fair quality. Thus the highest dose exposure group suffered a decline in textural quality which dropped the rating from the "very good" range to the "fair" range. All other qualities of irradiated, fried clams averaged to be in the "good" to "very good" range, (with the exception of the texture of the 0.5 kGy batch which was awarded an average of 6.8 points, in the "fair" range).

Irradiated clams prepared as steamed clams were ranked as "good" to "very good" across the dose range with the greatest effects of treatment being perceived in the textural category where clams exposed to the 300 kGy dose scored in low range of "good", averaging 7.0 .

QUAHOG ORGANOLEPTIC RESPONSES

Quahogs were evaluated as baked and raw preparations on the half-shell. The baked irradiated cherrystones scored well with the batch which received the highest dose tested (3.0 kGy) recieving scores of 8.3 for appearance; 7.8 for odor; 7.3 for tast and 8.0 for texture. The raw cherrystones on-the-half-shell recieving the highest dose treatment were awarded average scores of 7.3, 7.8, 7.0 and 7.8 respectively for the same qualities (Table 2, Fig. 5).

OYSTER ORGANOLEPTIC RESPONSES

Oysters similarly proved to be quite resistent to changes resulting from treatment doses. Baked oysters were graded exclusivley with averages of high sevens and eights for categories and all doses. Raw oysters-on-the-half-shell were awarded somewhat lower scores; the least

Table 2.

Organoleptic Evaluation of Irradiated Mercenaria mercenaria

BAKED CHERRYSTONE CLAMS

	CONTROL Mean	S.D.	0.5 kGy Mean	S.D.	1.0 kGy Mean	S.D.	2.0 kGy Mean	S.D.	3.0 kGy Mean	S.D.
APPEARANCE	8.8	(0.44)	8.4	(0.73)	8.6	(0.53)	8.6	(0.73	8.3	(0.87)
ODOR	8.6	(0.73)	8.4	(0.73)	8.3	(0.71)	8.3	(0.71)	7.8	(0.97)
TASTE	8.3	(0.71)	8.1	(1.05)	7.8	(1.39)	8.3	(0.87)	7.3	(1.32)
TEXTURE	8.2	(0.67)	7.6	(1.13)	7.9	(0.78)	8.1	(0.78)	8.0	(0.87)

RAW CHERRYSTONES

	CONTROL Mean	S.D.	0.5 kGy Mean	S.D.	1.0 kGy Mean	S.D.	2.0 kGy Mean	S.D.	3.0 kGy Mean	S.D.
APPEARANCE	8.3	(0.82)	7.5	(1.22)	8.0	(0.89)	7.8	(1.17)	7.3	(0.82)
ODOR	8.7	(0.82)	8.0	(0.89)	8.2	(0.75)	7.7	(1.51)	7.8	(0.75)
TASTE	8.5	(0.84)	7.5	(0.84)	7.7	(0.82)	7.0	(1.55)	7.0	(1.26)
TEXTURE	8.8	(0.41)	7.8	(1.17)	8.8	(0.89)	7.5	(1.22)	7.8	(1.17)

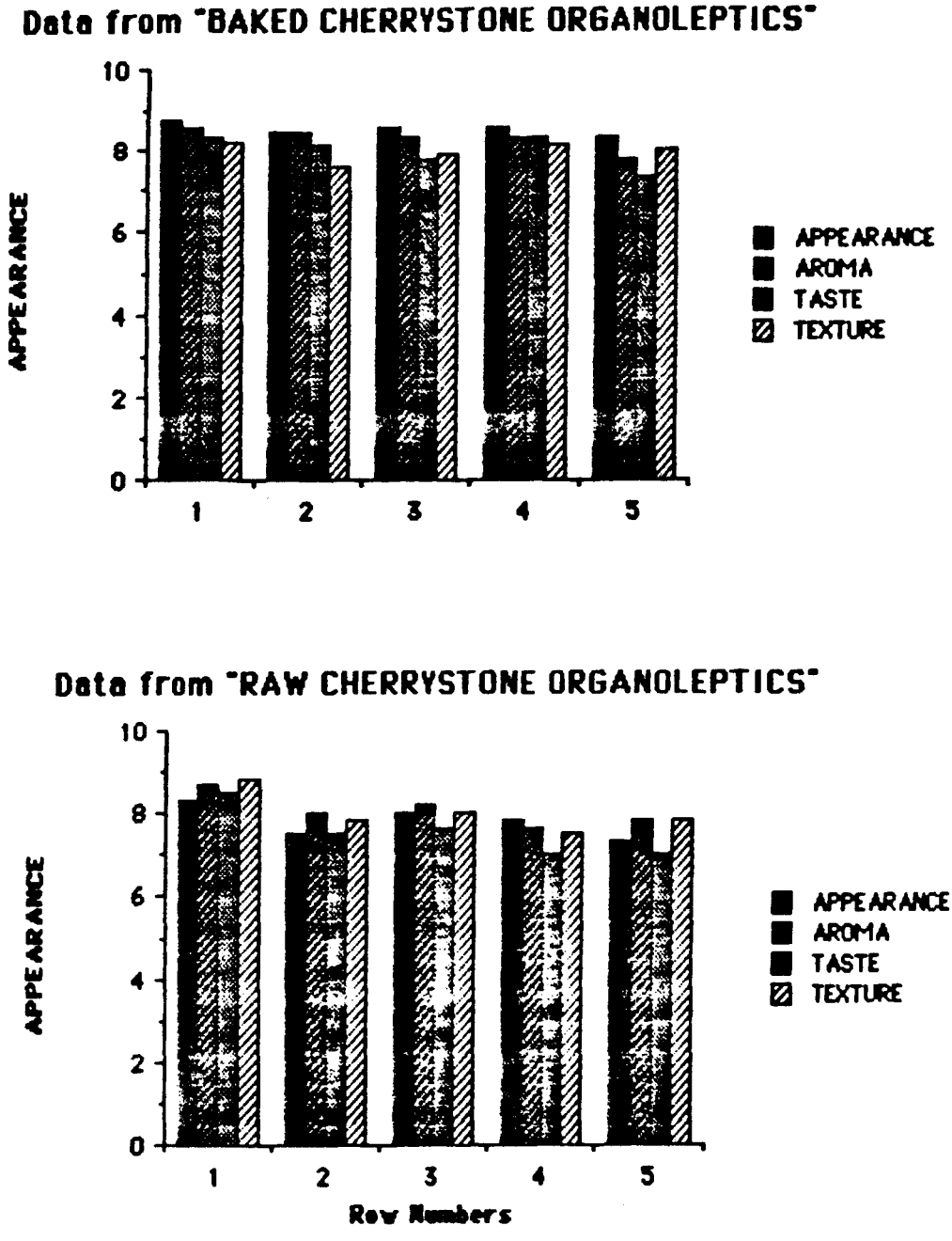


Figure 5. Organoleptic Ratings of irradiated Mercenaria.

1 = Control
2 = 0.5 kGy
3 = 1.0 kGy
4 = 2.0 kGy
5 = 3.0 kGy

the 3.0 kGy exposure. It should be noted that scores of this magnitude are none-the-less regarded as being of fair, but acceptable, quality (Table 3, Fig. 6)

BACTERIAL INACTIVATION RESULTS

Attempts at quantifying bacterial reductions in clams taken from environmentally polluted local waters did not show consistently clear results. These clams were harvested from comercially closed, polluted waters which recieved untreated sewage effluent. They showed a very high degree of variability for all bacterial indicators. High variability was noted from shellfish harvested from the same beds, located a few meters apart. Extreme variability in distributions of pathogenic organisms makes direct assessment of the effectiveness of irradiation difficult. Statistical measures of variability of bacterial concentrations in clams from the most severely polluted waters in Boston Harbor and near-by regions were so great and presented problems in analysis.

Visual estimates of lines of best fit describing the decrement doses due to irradiation treatment appeared to be uncharacteristically high. Total plate counts incubated at 20^{o} and at 30^{o} C showed exceptionally high deactivation doses (in the range of 1.8 to 1.6 kGy). E. coli and Streptococcus appeared to have D_{10} doses of 1.6 and 1.0 (two to three times higher than reported determinations from othr sources). The values cited from other reoports in the literature were more in keeping with those which these studies produced from bacterial inoculations of single species. It is possible that initial contamination in clams of Boston Harbor origen presented such a great range of variability in bacterial burdens of both treatment and control

Table 3.

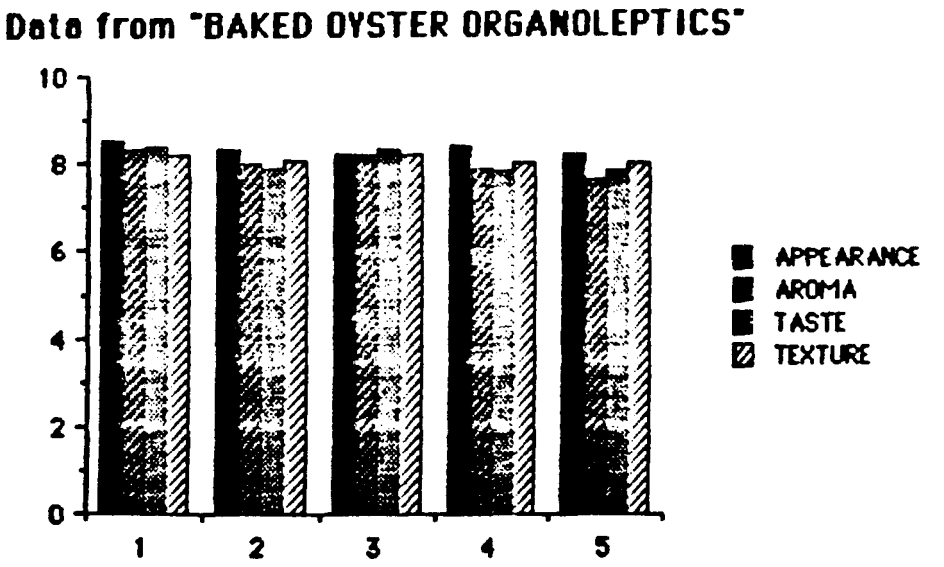
Organoleptic Evaluation of Irradiated Crassostrea virginica

BAKED OYSTERS

	CONTROL Mean	S.D.	0.5 kGy Mean	S.D.	1.0 kGy Mean	S.D.	2.0 kGy Mean	S.D.	3.0 kGy Mean	S.D.
APPEARANCE	8.5	(0.71)	8.3	(0.95)	8.2	(0.63)	8.4	(0.70)	8.2	(0.63)
ODOR	8.3	(0.82)	8.0	(1.05)	8.2	(0.79)	7.9	(1.10)	7.7	(0.94)
TASTE	8.4	(0.70)	7.9	(1.29)	8.3	(0.67)	7.8	(1.14)	7.8	(0.92)
TEXTURE	8.2	(0.79)	8.18	(1.10)	8.2	(0.79)	8.0	(0.94)	8.0	(0.82)

RAW OYSTERS

	CONTROL Mean	S.D.	0.5 kGy Mean	S.D.	1.0 kGy Mean	S.D.	2.0 kGy Mean	S.D.	3.0 kGy Mean	S.D.
APPEARANCE	8.0	(0.70)	8.2	(0.83)	7.6	(0.98)	7.9	(1.17)	7.9	(1.12)
ODOR	8.3	(0.87)	7.4	(1.86)	7.8	(1.12)	7.7	(1.05)	7.2	(1.12)
TASTE	8.0	(1.04)	7.1	(0.99)	7.3	(0.64)	7.0	(1.77)	6.6	(1.30)
TEXTURE	8.2	(0.83)	7.6	(0.98)	7.6	(1.04)	7.5	(1.12)	7.3	(1.45)



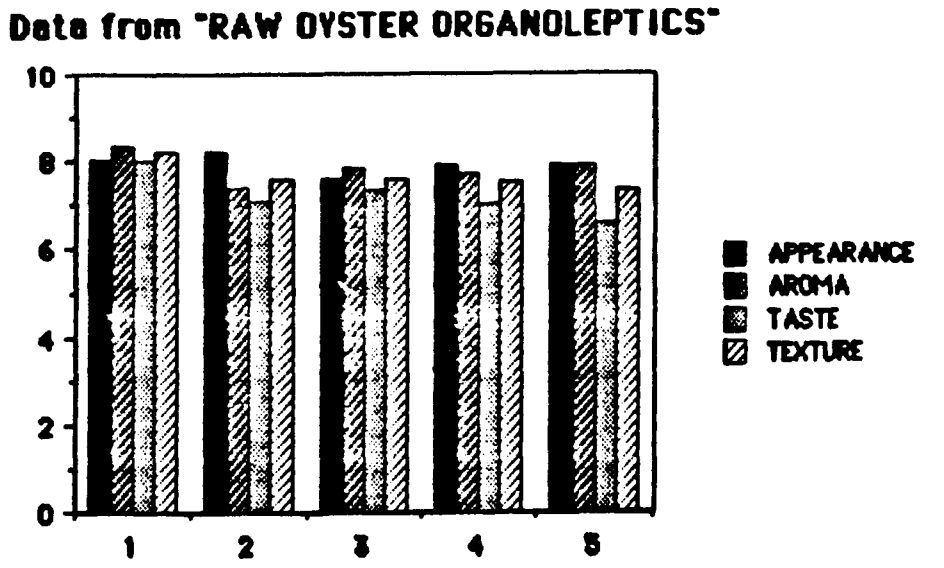


Figure 6. Organoleptic ratings of irradiated Crassostrea.

1 = Control
2 = 0.5 kGy
3 = 1.0 kGy
4 = 2.0 kGy
5 = 3.0 kGy

batches so as to present difficulties in quantifying reductions accomplished by radiation treatment. (Underestimates of population densities in control batches would result in overestimation of doses required to knock down the bacteria. As a result of those difficulties a decision was made to utilize more precise, quantified inoculation doses of monospecific pathogen cultures in the remainder of the studies.

Bacterial inactivation in samples of homogenized, spiked clam meats was estimated by regression analysis in the calculation of D_{10} decrement curves. In these analyses E. coli was reduced by a factor of 10 for each 0.37 kGy dose received ; Salmonella typhimurium populations were decremented by a factor of 10 in response to dosages of 0.51 kGy and Staphylococcus aureus was calculated to be reduced by an order of magnitude with an exposure of 0.42 kGy. Streptococcus faecalis was decremented by a factor of ten in response to a dose equivalent to 0.97 kGy (Figure 7). These results indicate effective bacterial inactivation is possible with relatively low dose radiation treatment.

VIRAL INACTIVATION STUDIES:

Preliminary screening results of viral inactivation by gamma exposure appear in Table 4. Poliovirus 1 and SA11 virus recovered seperately from tissues and fluids of quahogs and oysters following inoculation by either tissue or hemolymph routes showed varied responses and efficiencies of recovery. The D_{10} determinations for P1 recovered from shellfish subjected to the different administration routes and recovery fractions ranged from 1.32 to 6.62 kGy in these preliminary trials. Average polio log inactivation doses were 2.7 kGy in the quahog and 3.3 in the oyster. The range of spread in these log inactivation

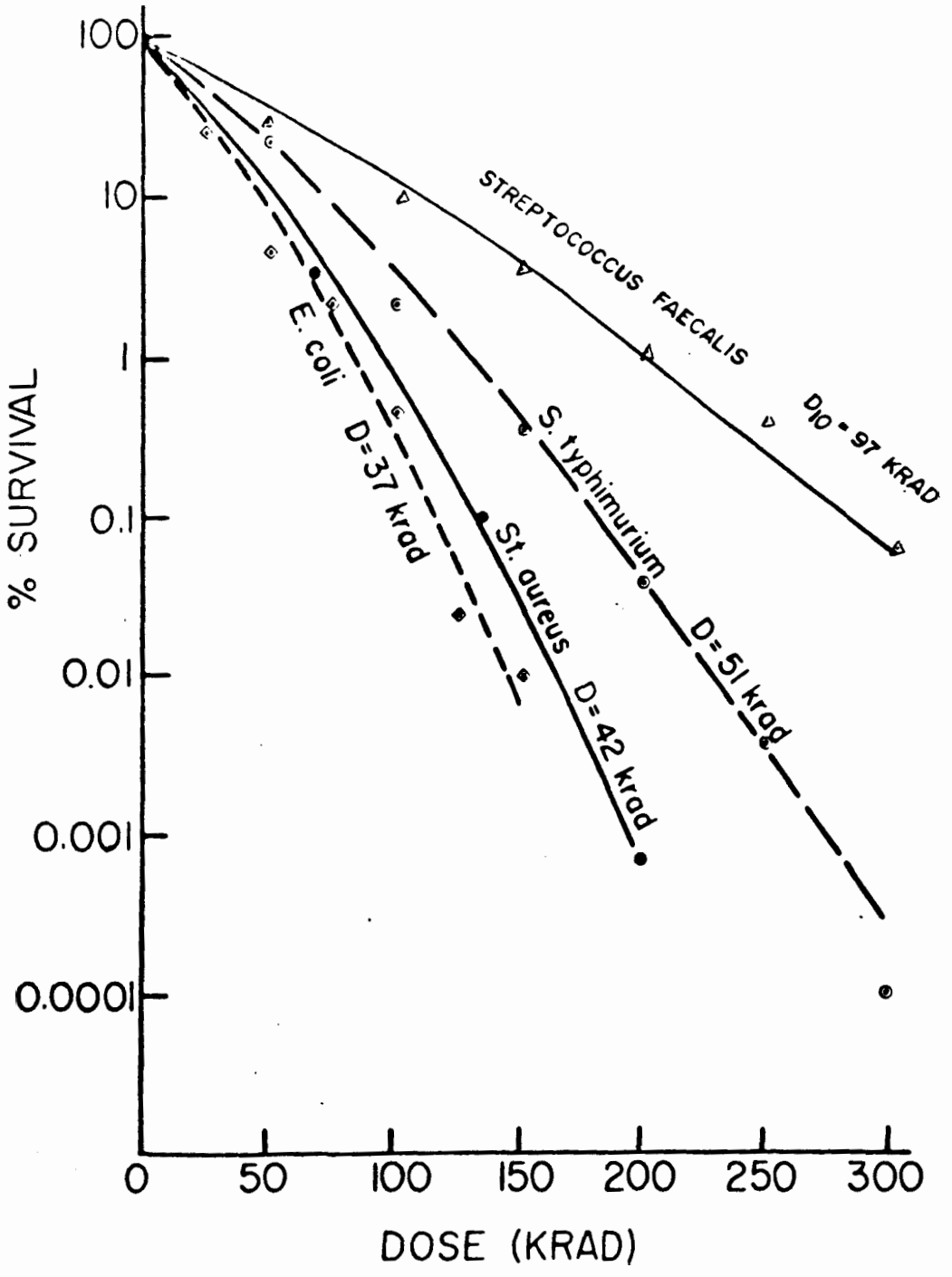


Figure 7. Bacterial inactivation by gamma irradiation. Kilorad equals 0.01 kiloGray.

Table 4. Preliminary studies of viral inactivation.

^{60}Co Radiation Inactivation of Enteric Viruses in Shellfish

Virus	Shellfish	Injection Route	Virus Recovery at Radiation Dose (Krad) Total PFU/% (Not Inactivated) Control	50	100	300	500	D_{10}
Rotavirus SA11	M. mercenaria	Mantle fluid	9.1×10^4	$\frac{5.4\times10^4}{59}$	$\frac{6.45\times10^4}{71}$	$\frac{1.8\times10^2}{0.2}$	$\frac{5.0\times10^1}{0.05}$	136.2
Poliovirus 1	C. virginica	Mantle fluid	3.4×10^4	$\frac{1.9\times10^4}{56}$	$\frac{1.72\times10^4}{50}$	$\frac{9.4\times10^1}{0.3}$	$\frac{11}{0.03}$	132.2
Poliovirus 1	C. virginica	Tissues	2.55×10^5	$\frac{7.4\times10^4}{29}$	$\frac{6.38\times10^4}{25}$	$\frac{3.0\times10^4}{12}$	$\frac{4.4\times10^3}{1.7}$	333.3
Poliovirus 1	M. mercenaria	Mantle fluid	4.37×10^4	$\frac{1.92\times10^4}{44}$	$\frac{1.81\times10^4}{41.4}$	$\frac{2.23\times10^3}{5.1}$	$\frac{6.19\times10^2}{1.4}$	270.2
Poliovirus 1	C. virginica	Tissues* Tissues**	3.6×10^5	$\frac{7.4\times10^4}{20.6}$	$\frac{7.0\times10^4}{19.4}$	$\frac{3.0\times10^4}{8.3}$	$\frac{5.1\times10^3}{1.4}$	322.6
		Mantle fluid**	8.4×10^4	$\frac{5.4\times10^4}{64.3}$	$\frac{1.1\times10^4}{13.1}$	$\frac{2.7\times10^4}{32}$	$\frac{8.3\times10^3}{9.9}$	662.3
		Totals	4.44×10^5	$\frac{1.3\times10^5}{29.5}$	$\frac{8.1\times10^4}{18.4}$	$\frac{5.7\times10^4}{13}$	$\frac{1.34\times10^4}{3.0}$	401.6
Rotavirus SA11	C. virginica	Mantle fluid* Mantle fluid**	8.7×10^4	$\frac{7.76\times10^4}{89}$	$\frac{3.55\times10^4}{41}$	$\frac{1.27\times10^2}{0.15}$	$\frac{2.7\times10^1}{0.03}$	132.3
		Tissues**	8.68×10^4	$\frac{5.04\times10^4}{58}$	$\frac{2.43\times10^4}{28}$	$\frac{2.41\times10^3}{2.8}$	$\frac{3.0\times10^1}{0.02}$	148.1
		Totals	2.37×10^5	$\frac{1.25\times10^5}{53}$	$\frac{8.88\times10^4}{37}$	$\frac{2.68\times10^3}{1.1}$	$\frac{5.7\times10^1}{0.02}$	136.8
Rotavirus SA11	C. virginica	Tissues* Tissues**	1.73×10^6	$\frac{4.34\times10^5}{25}$	$\frac{2.38\times10^5}{14}$	$\frac{1.15\times10^4}{0.66}$	$\frac{3.53\times10^3}{0.2}$	216.9
		Mantle fluid**	1.72×10^5	$\frac{2.86\times10^5}{166}$	$\frac{1.16\times10^5}{67}$	$\frac{1.43\times10^4}{8.3}$	$\frac{5.21\times10^3}{3.0}$	313.5
		Totals	1.9×10^6	$\frac{7.20\times10^5}{38}$	$\frac{3.54\times10^5}{19}$	$\frac{2.58\times10^4}{1.3}$	$\frac{8.74\times10^3}{0.5}$	247.5

Rotavirus SA11 assayed in Fetal Rhesus Monkey Kidney (MA-104) Cell Cultures.
Poliovirus 1 assayed in Buffalo Green Monkey (BGM) cell Cultures.

* Injection route

** Recovery Site

doses is very large and reflects the methodological problems inherent in conducting trial runs. The average log decrement values however are more in keeping with expectations derived from literature values.

Rotavirus SAll D_{10} reductions ranged from 1.32 to 3.13 kGy in the oysters and quahogs with the average value in quahogs being 1.4 kGy. This simian rotavrus in oysters showed an average inactivation value of 2.2 kGy under the preliminary testing (Fig. 8).

The tissue injection route and whole body recovery procedures appeared to result in greater and more consistent retention and/or recovery of viral plaque forming units (PFUs) when compared with the mantle fluid injection route and separate recovery procedures. It therefor was regarded as the more conservative and preferred inoculation and recovery method for working with hepatitis A virus procedures.

HAV IN QUAHOGS AND OYSTERS

The inactivation dose required for a ten-fold reduction of HAV carried in quahogs, as determined by immunoflourescent focus (IFF) techniques, was calculated as 1.66 kGy (Fig. 9) A similar IFF assay of HAV inactivation in oysters determined the D_{10} value to be 1.48 kGy (Fig. 10).

Determinations of hepatitis A virus deactivation doses in oysters by insitu single stranded RNA (ssRNA) hybridization resulted in a D_{10} estimate of 2.68 kGy. However a subsequent ssRNA hybridization trial yielded an even higher average value of 3.65 kGy. A newly developed cytopathic effect (CPE) analysis utilizing a rapidly growing strain of HAV provided a D_{10} estimate of 2.06.

An average estimate of HAV inactivation derived from analysis of pooled data from all assays resulted in an overall estimate of 2.02 kGy

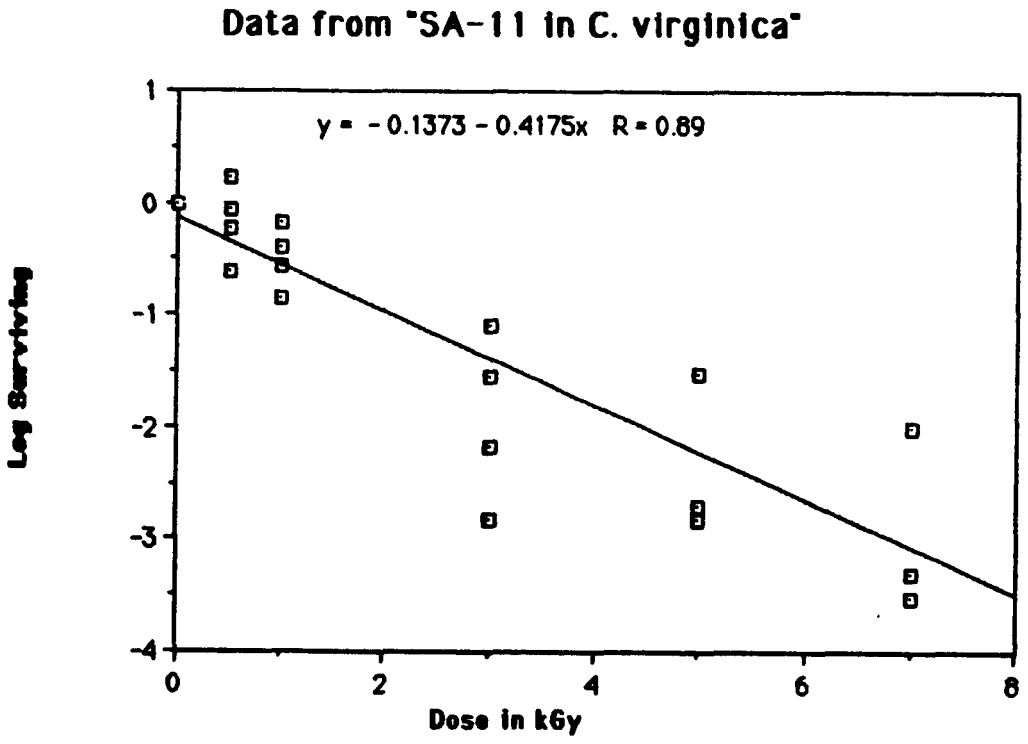


Figure 8. Inactivation of Simian rotavirus, SA11 in Crassostrea virginica. D_{10} equals 2.2 kGy.

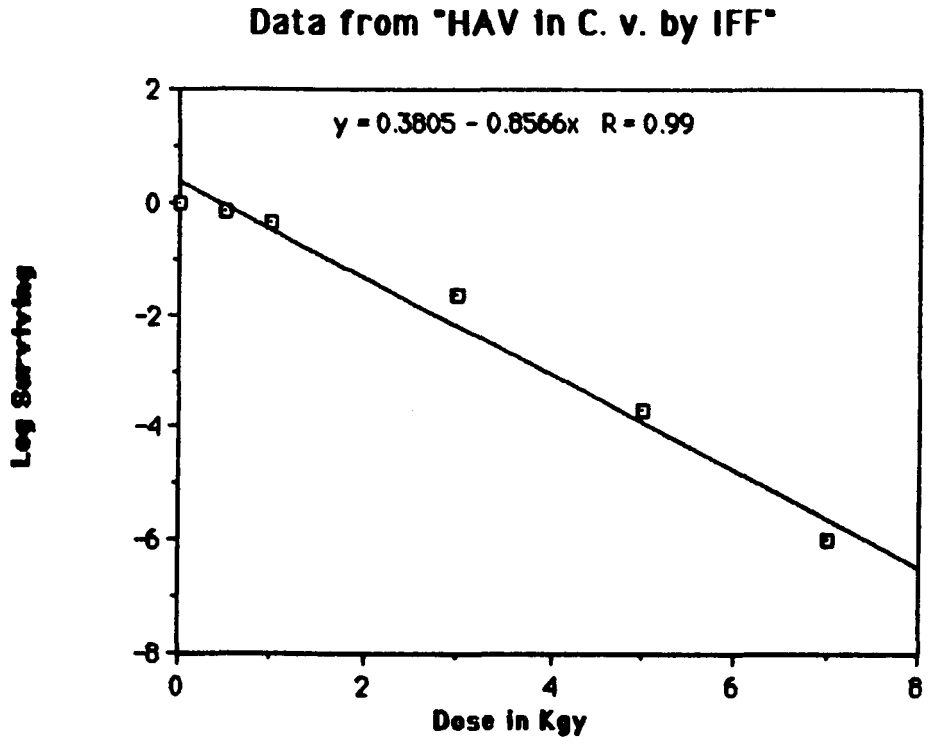


Figure 9. Estimaton of hepatitis A virus inactivation by irradiation. Viral detection by immunoflourescent focus. D_{10} estimate is 1.66 kGy.

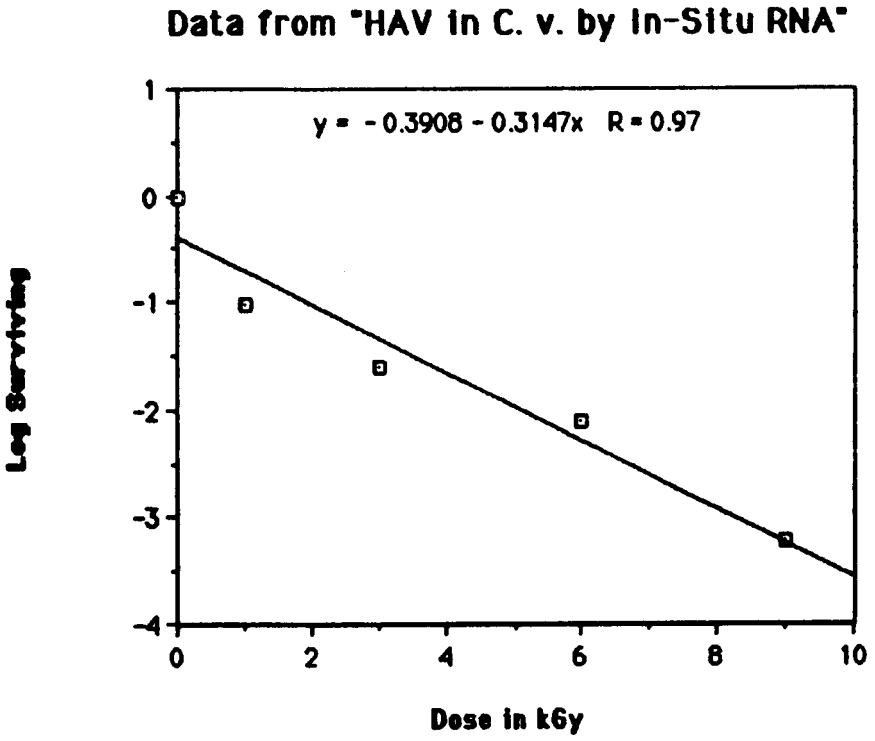


Figure 10. Estimation of hepatitis A inactivation in Crassostrea by gamma irradiation. Viral detection by in-situ RNA hybridization. D_{10} estimate is 2.68 kGy.

for the virus (Fig. 11).

DISCUSSION

Application of food irradiation technology to the shellfish industry has a high potential for allevating some portion of the public health risks from ingestion of enteropathogenic viruses and bacteria as a result of raw modes of consumption. Obvious benefits are to be derived from radurization processing of marine products.
improved shellfish sanitation snd a greater degree of certainty that the success of the treatment is not coupled to the physiological condition of the individual shellfish being treated. A recognized variability exists in the efficiency and performance of individual clams in self-depuration in traditional systems which employ ultraviolet systems (Piel et al.,1974).

DISCUSSION

RADIORESISTENCE IN BIVALVES

The effects of irradiation on market shelf life of live products were found to be negligible at low doses. Softshelled clams had no adverse effects of exposure to doses upto 1.5 kGy whereas oysters sustained doses of upto 2.5 kGy without significant deterioriation of shelf-life. The remarkable capacity of intertidal bivalve mollusks to sustain such intense radiation exposure is unparalleled.

It is speculated that several, incidental, physiological and metabolic adaptations to life in the intertidal zone predispose these bivalves to sustain exceptional levels of acute radiation exposure. Possible adaptations which contribute to this unique radioresistence are:

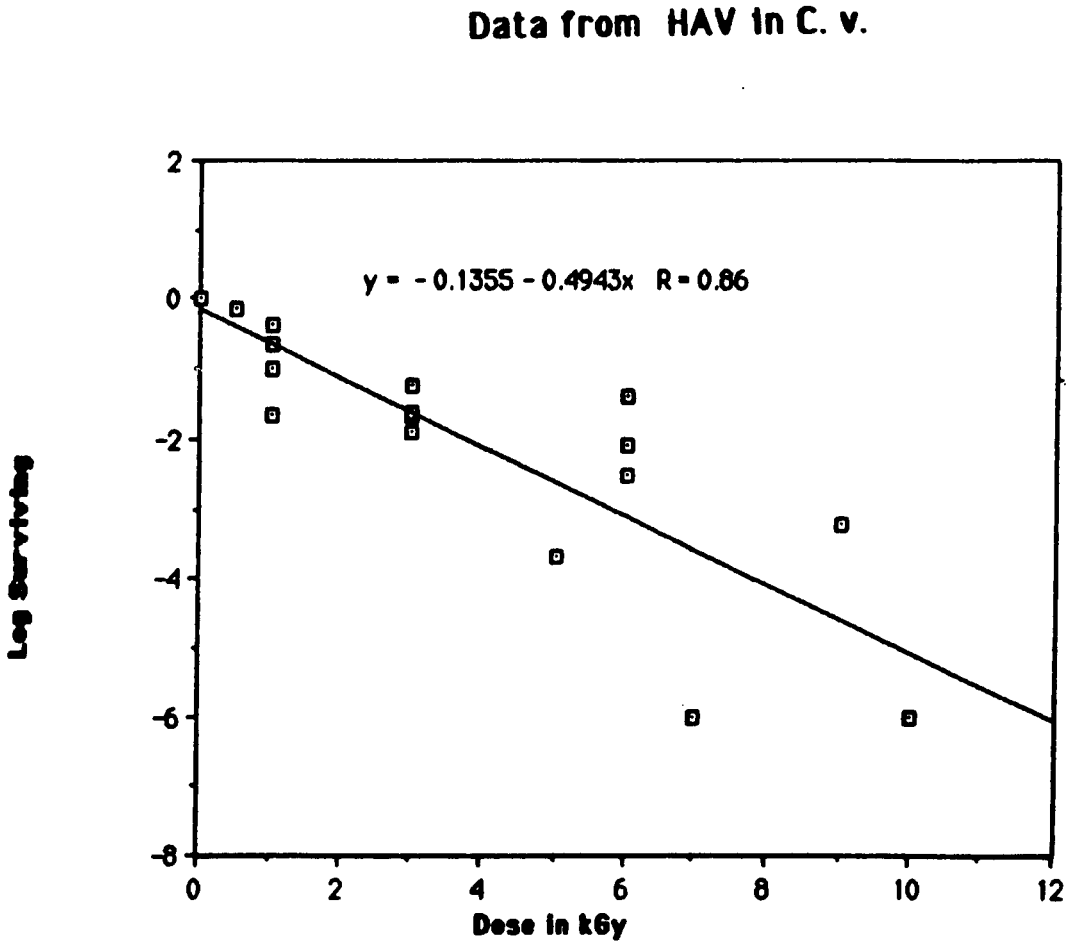


Figure 11. D_{10} estimate of HAV in Crassostrea by gamma irradiation. Pooled data from all techniques.

Facultative anaerobic metabolic capacities

Minimalization of body lipid stores

Anatomically simplified nervous system

Natural production of radioprotective agents

Low metabolic rate and low cell turnover (division rate)

The relationship of the above to physiological resistence to the effects of acute radiation doses are the result of evolutionary adaptations to sustain life in the intertial zone with its periodic submergence and exposure to air. Bivalves are not capable of respiration in the absence of volumes of water, consequently exposure of the shellfish flats to air during the low tide cycle presents problems of functional anaerobiasis.

Common adaptations to the rigors of life in the littoral zone include the maintenance of alternate glycolytic pathways which enable stored energy reserves to be either oxidatively metabolized during periods of adequate oxygen availability, or the food substrate can be chemically oxidized in the absence of molecular oxygen during prolonged periods tidal recession.

Total, whole-organism anaerobiasis would presumably result in the elimination of any "oxygen enhancement effect" which might otherwise magnify the degree of radiation damage incurred at the tissue level.

A physiological correlative to anaerobic metabolic strategies is a minimization of body stores of lipids. Energy reserves are preferentially stored as glycogen, as opposed to lipids or oils which are the preferred energy storage molecules of many obligately aerobic marine organisms. Whole oysters tissues have total lipid contents of only 2.4%, and clams maintain only 1.2% of tissue weight as lipid

only 2.4%, and clams maintain only 1.2% of tissue weight as lipid (Cataldo et al., 1989).

The absence of molecular oxygen would preclude the metabolism of fats as an energy source because a necessary first step in lipid utilization would be the oxidation of the highly reduced lipids to a suitable carbohydrate substrate. In the absence of significant quantities of lipids, metabolic (and organoleptic !) problems associated with radiation induced lipid peroxidation are lessened.

In anaerobic glycolysis and fermentation of pyruvate, marine mollusks utilize alternate, anaerobic pathways related to the tricarboxcylic acid cycle. Phosphoenolpyruvate (PEP) from the glycolitic sequence is converted to oxaloacetate (OXA) which is subsequently reduced through malate and fumarate to succinate (Hoar, 1975). Succinate acts as a hydrogen acceptor in this scheme and is accumulated in anaerobic tissues as a stored metabolic end product. Succinate has been reported in other organisms to serve as a radioprotective agent, presumably as an inhibitor of membrane lipid peroxidation (Ronai et.al.1987). Thus it is possible that the intertidal bivalves are synthesizing quantities of radioprotective chemical agents as normal by-products of metabolism.

A lesser degree of protection could be expected from aerobically respiring specimens and specimens spent a relatively short time in the anaerobic mode.

The relatively simple nervous system of the bivalves is of probable advantage in sustaining high radiation insult. The primative, non-centralized distribution of nerve trunks and ganglia is less likely to incur damage. This neurological simplicity may confir a certain

advantage of radioinsensitivity upon these species.

An interesting capacity for recovery of temporarily impaired reflex responses has been observed in irradiated clams. It was not uncommon to note temporary failure of the siphon withdrawal response in the first 24 to 48 hrs. post-irradiation, only to observe restoration of the reflex over the subsequent days. Homeostatic integration and survival of these mollusks apparently does not demand a constantly functional and active nervous system at all times.

It is also speculated that the low metabolic rate and low cell turnover rate provide additional capacity to temporarily sustain very high radiation doses. Radiation induced damage to nucleic acids and mechanisms of cell division is no as likely to immediately incapacitate the less mitotically active mollusk cells. Microbial agents exhibit a more accelerated cell division or replication schedule if they are to impart pathogenic effects. Potentially infective agents may sustain radiation damage which becomes manifest immediately upon entry into cell division or viral particle replication. Thus a radiation dose absorbed by an intertidal mollusk may result in rapid decimation of it indigenous microbial populations without manifestation of acute effects to the shellfish.

PRESERVATION OF ORGANOLEPTIC QUALITIES

The preservation of market qualities of irradiated shucked clam meats has been reported by several previous workers: Slavin and Ronsivalli (1963) stated the optimum dose for preservation of soft-shelled clams is 4.50 kGy. Chowder prepared from clams irradiated at

this dose and stored at 0.6 - 1.7 °C for 15 days was well accepted by expert and nonprofessional taste pannels. Ronsivalli et al. (1965) state the preferred dose for preservation of clam meats packed in air and stored at 0.6 °C for up to 28 days is 4.5 kGy. Nickerson (1963) irradiated soft shelled clam meats at doses up to 8.0 kGy and reported no detectable differences in taste after deep fat-frying of samples which had been stored for 40 days at 6.1 - 7.2 °C.

The absence of significant quantities of lipids combined with the possible prophylactic effects of elevated succinate levels in reducing lipid peroxidation may preclude the accumulation of peroxidation products and may contribute to the maintenance of desired taste and aroma subsequent to irradiation.

BACTERIAL ELIMINATION

Irradiation presents some distinct advantages as a means of improved bacterial elimination. The traditional self depuration systems currently in service are limited in abilities to handle large volumes of clams and are time-consuming due to the required 48 hr. depuration schedule. Direct comparisons of the effectiveness of irradiation versus flow-through UV depuration have not been made, however previous studies of bacterial clearance in traditional systems indicate bacterial reduction after 48 hrs. is approximately equal to that obtained after irradiation at 1.00 kGy. Work of Hartland and Timoney (1979) indicates 20 hrs of flow-through depuration are necessary for a factor of 10 reduction of E. coli and S. typhimurium in hard shelled clams and American oysters held at 6 °C in sea water . Piel et al. (1974) analyzed UV depuration of fecal coliforms from soft shelled clams and showed that

treatment. The present study shows irradiation, even at low doses to compares quite favorably with the traditional method of depuration. The gamma irradiaton exposures necessary to reduce bacterial populations in clam meats by a factor of 10 (D_{10}) are: 0.37 kGy for E. coli, 0.42 kGy for Staph. aureus, 0.51 kGy for S. typhimurium and 0.97 kGy for Strept. faecalis. These inactivation values compare well with the bacterial reductions obtained after 48 hours of treatment in conventional recirculating U.V. depuration plants.

Gamma irradiation of shellfish appears to have several advantages over the more traditional methods of microbial depuration which depend upon the mollusk's ability to irrigate large volumes of recirculating, U.V. treated water through the gill and mantle cavity regions. This rate of bacterial clearance in this process is dependent upon the pumping rate of the shellfish and requires a high state of physiological well-being and intact shell conditions of each individual shellfish processed. These prerequisites to effective depuration are not always met in practice. Those shellfish which do not actively irrigate the gill cavities and which fail to evacuate their gut contents cannot effectively depurate or self-purge potential pathogens.

Under ideal conditions, softshell clams depurate bacterial populations by about two logs in a 48 hour period, but the responses of hardshelled clams and oysters have not been as extensively investigated. Doses in the range of 2 kGy can achieve bacterial reductions in the range of 10^{-4} for Staphylococcus and 10^{-2} for Streptococcus pathogens. Lower dose irradiations kGy may be beneficial as a supplementary process to traditional treatment. As the two methods presumably would be multiplicative in effect, bacterial reductions by factors of 10^{4} may be

possible in marketable live clams treated at doses of 1.00 kGy.

VIRAL PROBLEMS

The public health problems associated with viruses from shellfish harvested in waters which are subject to discharges of hunan enteric wastes must be recognized as presenting serious , albeit ill- defined , health risks to consumers . Metcalf (1982) and others have stated the dangers from Hepatitis A virus and Norwalk virus (a suspected etiological agent of non-bacterial gastroenteritis). These viral pathogens are only presumed to be adequately depurated by traditional techniques. Mechanisms of viral clearance from contaminated mollusks are not as well understood.

The ability of shellfish to bioaccumulate viruses and translocate viral particles from the feeding apparatus to internal tissues has been documented by Metcalf et al. (1979). Molluscan amoebocytes are capable of ingesting intact virions and transferring such pathogens to internal tissues where the viruses may remain sequestered for several weeks. This capacity of shellfish to retain virues for periods of several weeks has been well documented and has special significance when considering the mollusk's lack of ability to self-purge pathogens from within its own tissues. The effectiveness of traditional depuration treatment in ridding harvested shellfish of viral health threats is open to some question. Sanitation of raw, live clams and oysters by whole organism gamma irradiation of shellfish will apparently eliminate the many problems associated with current treatment techniques.

Inactivation of microbial pathogens in bivalves by radiation treatement is obviously independent of the physical state of the molluscan carrier.

VIRAL RELATIONSHIPS

The viral pathogens, hepatitis A and Norwalk virus, pose the more serious threats to shellfish sanitation. As Norwalk virus has not been successfully cultured to-date, it is not possible to speculate on the effectiveness of gamma irradiation in addressing that segment of the problem. It appears however that HAV populations in shellfish can be reduced approximately by a factor of 10 following treatment with 2 kGy. Whether-or-not reductions in infectivity of this order would be achieved as a result of the treatment remains to be observed. If reductions in infectivity were to directly parallel reductions in infectious viral particles, then this process could find significant application in reducing incidences of viral hepatitis, possibly by a factor of ten.

A potential difficultiy with irradiation of shellfish might be encountered if the bacterial indicators of pollution were destroyed by radiation doses which were insufficient to effectively deactivate viral infectivity. Such a situation might be encountered if, by chance or design, shellfish harvests which did not meet minimum sanitary standards were to be processed in an irradiatator and the bacterial indicators of inferior quality were to be eliminated, possibly leaving still dangerously elevated viral densities.

While it is strongly believed that food irradiation technology is capapable of providing more sanitary shellfish supplies and increasing the microbial quality of harvested stock, it should be clearly and unequivocable stated that irradiation processing must not be used in an attempt to market stocks of inferior microbial quality. Benefits are to be derived in providing increased consumer safety from infection by

those supplies which are capable of meeting present standards yet still present a discrete, albeit low, probability of infection.

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