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Short Communication

Gamma Ray Sterilization Study on a *Perna perna* Mussel Reference Material

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Abstract

In this study the most suitable gamma ray dose for the sterilization of a new *Perna perna* mussel reference material was investigated. Different gamma ray doses were applied and microbiological loads of irradiated subsamples and a non-irradiated control subsample were studied. A 0.90 kGy decimal reduction value was estimated at the microbiological load test conditions and it was concluded that a 5 kGy dose is suitable for the sterilization of the mussel reference material.

Keywords: decimal reduction value, gamma ray sterilization, mussel, *Perna perna*, reference material, security assurance level.

Estudo para a Esterilização por Raios Gama de um Material de Referência de Mexilhão *Perna perna*

Resumo

Neste estudo, a dose de radiação gama mais apropriada para a esterilização de um material de referência de mexilhão *Perna perna* foi avaliada. Diferentes doses de radiação gama foram aplicadas e a carga microbiológica foi investigada nas sub-amostras irradiadas e em uma sub-amostra não irradiada foi usada como controle. Um fator de redução decimal de 0,90 kGy foi estimado nas condições do teste para carga microbiológica e conclui-se que a dose de 5 kGy é a mais adequada para a esterilização do material de referência de mexilhão.

Palavras-chave: esterilização por raios gama, material de referência, mexilhão, nível de garantia de esterilidade, *Perna perna,* valor de redução decimal.

INTRODUCTION

The role of certified reference materials in quality assurance

Materials that are sufficiently homogeneous and stable with respect to one or more specified properties, which have been established to be fit to their intended use in a measurement process, are called reference materials, RMs. When a reference material is characterized by a metrologically valid procedure and accompanied by a certificate that provides the value of the property, its associated uncertainty and a statement of metrological traceability, it is denominated certified reference material, CRM (ISO, 2006).

Due to their very well characterized properties, CRMs are used in diverse aspects of quality assurance systems. They may be used in method validation, uncertainty estimation, instrument calibration and internal quality control procedures (Barwick *et al*., 2001). However, the use as a link in measurement traceability chains is the most important

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application of CRMs, connecting the analytical result of the field laboratory to the realization of the measurement unit of the quantity under consideration. When the traceability chain is completed, it is possible to compare measurement results obtained in different places at different times. Hence, in the context of monitoring programs that use bivalve mollusks as bioindicators, the analysis of appropriate CRMs, along with the unknown samples, is fundamental if reliable and meaningful results are to be obtained.

One of the responsibilities of biological CRM producers is to guarantee the stability of the material, in order to assure that the certified value of the property of interest is constant during its shelf-life. This study deals with the stability of a new *Perna perna* (Linnaeus, 1758) mussel reference material prepared at IPEN – CNEN/SP, which was designed to be used as a metrological tool for biomonitoring programs using bivalve mollusks in Brazil, in the field of trace element determination.

The process of gamma ray sterilization

Gamma ray sterilization has been used world-wide for more than fifty years for food preservation and microbiological control, with economical and health benefits. It is a well established technology whose goal is to greatly reduce or eliminate microorganisms that may cause deterioration of food, without, however, introducing sensorial changes to the products (Farkas, 1998; Molins, 2001). Microorganism inactivation occurs by damage to critical components of cells, often in the genetic material, leading to cell death (Dickson, 2001). The sensitivity to radiation depends on the species involved on a given contamination and on factors such as moisture, temperature and others.

Prior to irradiation of the mussel reference material batch, the literature was revised in order to define the most appropriate radiation dose to ensure the stability of the material during its shelf-life, without degradation of its properties. However, the only information available in the consulted certification reports is that "the material had been irradiated with gamma radiation". Only the report of the oyster reference material NIST SRM 1566b, which was certified for various elements and methyl mercury, reports the dose of 30 kGy (NIST, 2001).

The chemical changes that occur in irradiated foods are a result of the direct action of radiation on carbohydrates, proteins, fats and other compounds and also by the effect of reactive intermediates formed during radiolysis of water (Thayler *et al*., 1996). In the case of minerals present in food, gamma ray irradiation does not affect their content (Stewart, 2001). As methyl mercury is an analyte of interest in the mussel reference material, we considered to use the lowest dose possible to avoid its degradation, without compromising the stability of the matrix material.

In the literature, there are two studies on analyte degradation in reference materials by gamma radiation for elements in the form of organometallic compounds. The producers of the human hair reference material NIES CRM No. 13 reported that the material was not irradiated to avoid degradation of methyl mercury, main analyte of interest in this material. The producers recommended that the CRM should be stored at -20 ^{o}C in the dark (Yoshinaga *et al*., 1997). This option is acceptable for hair, rather stable biological matrix. As for the marine sediment reference material NRCC CRM PACS-2, certified for tin organic species, it was observed that while methanolic solutions of tin species suffered strong degradation to inorganic tin, the degradation was much lower for sediments spiked with these species. It was suggested that the sediment matrix acted as a barrier to degradation by gamma radiation (Yang *et al.*, 2003).

The purpose of this study was to define the most suitable gamma ray dose for the sterilization of a new *Perna perna* reference material prepared at IPEN - CNEN/SP, bearing in mind that such dose should not damage the properties of interest in the material, in particular, the content of methyl mercury.

MATERIALS AND METHODS

The preparation procedures for the mussel reference material were presented in a previous contribution to this journal (Moreira *et al*., 2007).

Subsamples of the mussel reference material were irradiated with the following gamma ray doses: 1, 2, 3, 4, 5, 15, 25, 30 and 50 kGy, using the ^{60}Co source of the Multipurpose Irradiator of the Radiation Technology Center, CTR, at IPEN – CNEN/SP. All subsequent material handling was performed in a laminar flow hood to prevent bacterial contamination from the environment.

About 3 g of material irradiated in different doses and non-irradiated material (used as control) were rehydrated with 20 mL of saline solution (sodium chloride 0.9 % (w / v), Baxter) in sterile sampling bags (Whirl-Pak). Afterwards 100 mL saline suspensions were prepared. Suspensions were filtered through 0.45 μm pore size cellulose nitrate membranes (Sartorius AG), which were placed on Petri dishes with culture medium (Tripcase Soy Agar, Biomérieux). These were then incubated in an oven (Eletrolab) at 20 ^{o}C. Counting of colony forming units, cfu, was performed after a 1-week incubation period with a binocular stereoscope (Askania GSZ).

The irradiation of the mussel reference material batch was performed with the radiation dose of 5 kGy after the bottling of the material. Dosimeters were placed on both sides of the bottles to verify the occurrence of possible gradients on the flux of gamma rays that could somehow influence the homogeneity of the material. In addition, samples were rotated 180 degrees in the middle of the irradiation to minimize this effect. According to the Certificate of Dosimetry, the mussel reference material batch was sterilized with an average gamma ray dose of 5.1 ± 0.3 kGy (CTR, 2007).

RESULTS AND DISCUSSION

By observing the Petri dishes in binocular stereoscope, bacterial growth was observed only for the non-irradiated

subsample and subsamples irradiated up to 3 kGy. It shows clearly the suitability of gamma ray irradiation for biological material sterilization purposes. Figure 1 presents the aspect of the Petri dish obtained with a non-irradiated subsample. The growth of colonies of two different microorganisms was observed: salmon-colored colonies and yellow colonies. Due to its characteristic intense yellow color, it is possible that the second type of colony is formed by *Staphylococcus aureus* (Rosenbach, 1884). However, only with specific tests it would be possible to affirm precisely which microorganism developed on the Petri dishes.

From the colony counting of the Petri dishes, the microbiological load of the non-irradiated material was estimated as 3.3 x 10^3 cfu g^{-1}, for the freeze-dried material. Considering the moisture of 85% obtained during the freeze-drying process, the colony counting was calculated as 495 cfu g^{-1} for the fresh mussel material. This microbiological load may be considered low as it is inferior to 10^3 cfu g^{-1}, tolerance level acceptable for fresh, chilled or frozen shellfish for human consumption, according to Brazilian legislation (ANVISA, 2001). For a 3 kGy dose, it was observed the formation of 9 cfu g^{-1} for the freeze-dried material, which indicates the efficiency of gamma ray irradiation for the sterilization or disinfection of the mussel reference material, at least for the microorganisms that developed in the used culture medium. The irradiation process will then contribute to the enhancement of the shelf-life of the material.

The sensitivity of microorganisms subjected to some stress, such as radiation, can be estimated by the decimal reduction value, D_{10}. This value is the amount of radiation necessary to reduce 90% of the initial population of a specific bacterium under specified conditions (Molins, 2001).

The D_{10} value can be determined graphically, applying Equation 1 to the slope of the plot of colony forming units (in logarithms to base 10) versus absorbed dose, as shown in Figure 2 for the data in this study. In this case, from the slope a = - 1.1124, a D_{10} value of 0.90 kGy was calculated.

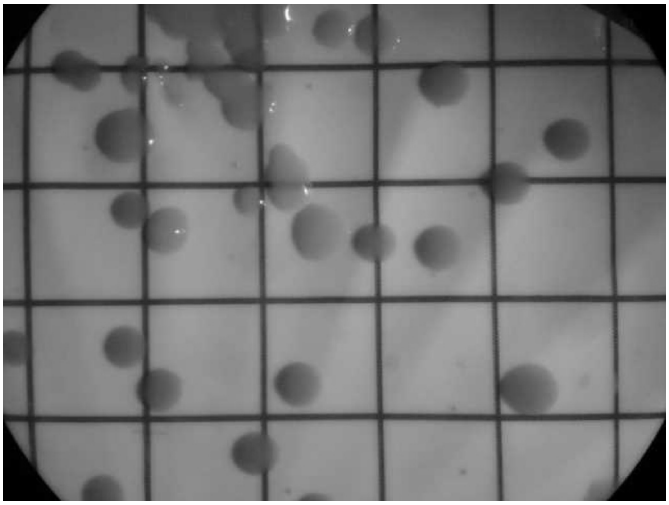

Figure 1 – Types of Colony Forming Units at non-irradiated samples (control).

$$D_{10} = -1\left(\frac{1}{a}\right) \quad (1)$$

where:

a is the slope of the graph of bacterial population versus irradiation dose.

From the plot on Figure 2, it is observed that a gamma ray irradiation with a 4 kGy dose allows the decontamination of the candidate reference material, at least for the microorganisms that have developed in the culture conditions used in the test. Due to the uncertainty of the method used for the determination of the microbiological load, we considered to add a 1 kGy dose for the sterilization of the mussel material batch, in order to obtain a 1-log security assurance level, SAL. The efficacy of antimicrobial processes can be considered in terms of the number of reductions in log 10 (number of D_{10} values) necessary to reach certain level of security. In the United States of America, a reduction level security policy is five cycles of log 10, in order to obtain sterilization that is considered effective and safe in terms of food safety (Molins, 2001). In this study a SAL of five cycles would be unnecessary since the material is not intended for human consumption.

In this study, the effective gamma ray dose for the sterilization of a mussel reference material prepared at IPEN- CNEN/SP was established by studying the effect of the irradiation on microbiological growth on the material. It is expected that the 5 kGy gamma ray dose used will not cause degradation on the trace element content of the material (Stewart, 2001). However, subsequent studies are on the way to verify if the methyl mercury content suffered any degradation with the irradiation.

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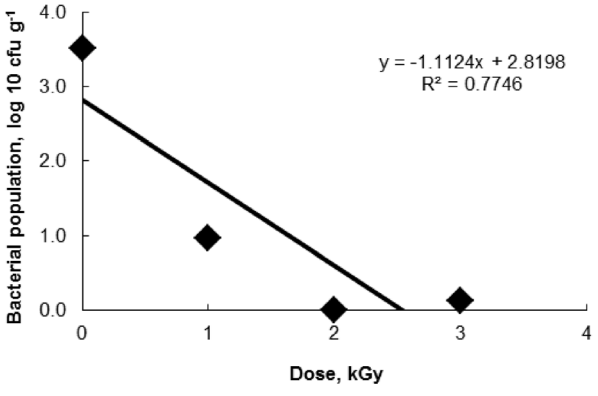

Figure 2 – Microbiological reduction versus gamma ray dose.

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