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ABSTRACT

The present study aimed to evaluate the efficiency of the irradiation process in the control of *Enterococci* spp. and *Escherichia coli* in chilled chicken heart samples acquired in an industry located in the West Zone of Rio de Janeiro, Brazil, using irradiation doses of 1.5 kGy, 3, 0 kGy and 4.5 kGy. These microorganisms are related to fecal contamination, and are indicators of the sanitary processing conditions of the foodstuffs. The bacteriological analyses were conducted applying the methodologies and standards recommended by Brazilian norms resolution no. 12 (BRASIL, 2001) and instruction no. 62 (BRASIL, 2003) Regarding *Escherichia coli*, no statistically significant difference among the four groups (control, 1.5 kGy, 3.0 kGy and 4.5 kGy) was observed ($p > 0.05$). The Most Probable Number (MPN) for *Enterococci* spp. was not proven in the investigated samples. Thus, the Co60 gamma irradiation process was effective in eliminating *Escherichia coli*, and the lowest dose, of 1.5 kGy, was enough to abolish this enteropathogen from the evaluated samples.

Keywords: Food irradiation. Chicken heart. *Enterococci* spp. *Escherichia coli* Public health.

RESUMO

Objetivou-se na elaboração deste trabalho avaliar da eficiência do processo de irradiação no controle de *Enterococcus* spp. e de *Escherichia coli* em amostras de coração de frango refrigerado, adquiridas numa indústria na Zona Oeste do Rio de Janeiro, empregando-se as doses de 1,5 kGy, 3,0 kGy e 4,5 kGy. Estes microrganismos encontram-se relacionados à contaminação fecal de carne e miúdos de frango como também da avaliação das condições higiênico-sanitárias inerentes ao processamento. Para realização das análises bacteriológicas foi aplicada a metodologia e padrões preconizados pela Resolução RDC n°. 12 (BRASIL, 2001) e da Instrução Normativa n°.62 (BRASIL, 2003). Quanto aos resultados estatísticos obtidos na pesquisa e controle de *Escherichia coli*, não foi evidenciada diferença estatisticamente significativa entre os quatro grupos analisados, controle e irradiados ($p > 0,05$). O Número Mais Provável (NMP) de *Enterococcus* spp. não teve sua presença comprovada nas amostras analisadas. Concluiu-se, com a elaboração desta pesquisa, que o processo da irradiação gama Co60 com que a menor dose empregada, 1,5 KGy foi eficiente na eliminação das bactérias *Escherichia coli* nas amostras analisadas.

Palavras-chave: Irradiação de alimentos. Coração de frango. *Enterococcus* spp. *Escherichia coli*. Saúde coletiva.

INTRODUCTION

Brazil's poultry industry began the year of 2016 breaking several records, including production and exporting of chicken products. Chicken meat, consolidated as the fourth item of the national export portfolio, achieved, in 2015, the three best monthly results in the history of the sector's exports.¹

The importance of studying contamination of chicken giblets and meat is highlighted by the fact that these products are an important source of high quality protein, rich in essential amino acids, vitamins and minerals, and consumed in large scale in Brazil and throughout the world, being the highest preference percentage of the world's slaughter of animals when compared to beef and pork.²⁻⁴

However, it is worth mentioning that the Brazilian health legislation that determines the microbiological standards for food, the Resolution no. 12 (BRASIL),⁵ as stated in item 5 for meat/meat products, sub-clause c, poultry giblets, only follows the standard for coliforms at 45°C g⁻¹ (fecal/thermophilic coliforms), and no maximum permissible value is found for other organisms, such as *Enterococci* spp. in these products. In such cases, Annex II of this same norm is taken into account regarding pathogenic microorganisms, which only states that "products or batches (indicative or representative sample, respectively), unfit for human consumption by presenting [...] a pathogenic microorganism or toxin represent severe hazards to consumer health".

Irradiation of foodstuffs is an effective method does not produce environmental waste and is accepted employed and its use is authorized in more than 50 countries worldwide and for various types of food products.⁶

Food irradiation is being considered as an important technological process, not only in ensuring safety but also in extending the shelf-life of chicken meat and meat products, ready to eat products, including the various ethnic foods from every continent of this planet.^{3,7-10}

In this regard, ionizing irradiation is a safe and proven physical food conservation, used to reduce or eliminate pathogenic microorganisms, in

turn controlling the microbiological quality of several foodstuffs and reducing the risk of foodborne illness.^{4,11-13}

Thus, in this setting, the present study aimed to evaluate the efficiency of gamma radiation (Co60) with respect to bacteriological parameters in chilled chicken heart (*Gallus gallus*) samples, to evaluate the presence of *Escherichia coli* and *Enterococci* spp., microorganisms indicative of the production/handling sanitary conditions of raw materials that are responsible for several foodborne illnesses.¹⁴⁻¹⁶

MATERIAL AND METHODS

Chilled chicken hearts were purchased from an industry located in the West Zone of Rio de Janeiro. The giblets were randomly selected taking into account the production date closest to the beginning of the analyses. The samples were then transported and maintained chilled at a maximum of 7°C in an isothermal container during all steps of the experiments.¹⁷

They were subsequently placed in plastic zip-lock bags and transported on ice to the Nuclear Instrumentation Laboratory/Federal University of Rio de Janeiro (LIN/UFRJ) where three groups were submitted to the gamma irradiation process (Co60) at 1.5 kGy, 3.0 kGy and 4.5 kGy. Control samples were not irradiated and remained on ice during this step. After irradiation, the control and irradiated samples were then transported to the Bacterial Zoonosis Laboratory at the Oswaldo Cruz Institute (Oswaldo Cruz Foundation) for the bacteriological analyses regarding *E. coli* and *Enterococci* spp.

The Most Probable Number (MPN) miniaturization technique of total and fecal coliforms for *E. coli* was performed using the Fluorocult LMX[®] Quick Method,¹⁸ while the Most Probable Number (MPN) for *Enterococci* spp. was performed using the Quick ChromoCult[®] Broth diagnostic assay¹⁹ for each chilled chicken heart sample, both irradiated at the three doses and controls, by serial dilutions from 10⁻¹ to 10⁻⁸.

The statistical analyses were conducted using the SPSS version 18 software package. The non-parametric Friedman test was applied to compare the four groups (controls and three doses) regarding research technique and

bacteriological enumeration techniques, with a significance level of 0.05 (5%).

RESULTS AND DISCUSSION:

No significant difference between samples regarding positivity in the control group were observed for *E. coli* ($p = 1.000$). In addition, no significant difference between the samples subjected to the irradiation process, at 1.5 kGy, 3.0 kGy, 4.5 kGy regarding the MPN for *E. coli* were observed ($p = 1.000$).

Concerning the midline enumeration of *Escherichia coli* from the four groups, a four log cycle decrease on the days the analyses were performed was observed during the first week, while during the second week elimination of the microbiota occurred and, finally, on the third week, a three logarithmic cycle decreases was observed, demonstrating the efficiency of the irradiation process, with MPN values smaller than <3 in the irradiated groups, being considered zero for the statistical analyses (Figure 1).

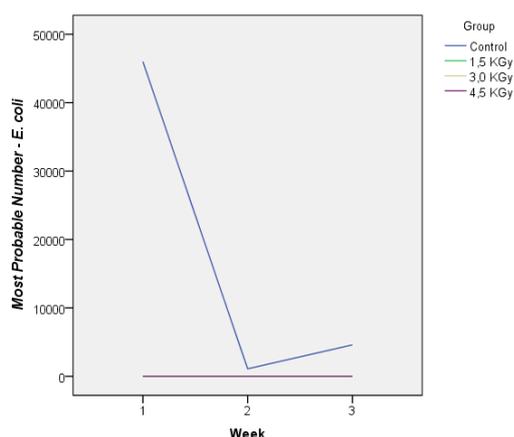


Figure 1. Results observed in *E. coli* NMP control during the three weeks of the experiment, at CCAMP / LABZOO / IOC / FIOCRUZ, on January 2016.

Similarly, to the results observed herein when comparing the control group to the irradiated samples, Bánáti et al.²⁰ using two *E. coli* control methods in chicken breast, of irradiation with an electron beam associated to pH, reported that a 2.0 kGy dose reduced the *Escherichia coli* population from three to four log cycles in the irradiated chicken meat samples.

Another study, conducted by Xavier et al.,²¹ on chilled chicken heart samples regarding the efficiency of another gamma irradiation process (Cs137) with the same doses applied herein albeit associated with cooling observed logarithmic decreases in the bacterial count of the samples, as well as increases in product shelf-life.

In the study conducted by Adu-Gyamfi et al.,²² was also reported the microbiological quality of chicken at different retail outlets (supermarkets, local markets and farms) in Accra was investigated, and D_{10} -values of *E. coli* was determined when the samples were treated with irradiation doses rates of between 1.151 - 1.089 kGy h^{-1} using a Cobalt-60 source. This study has revealed the D_{10} -values of *Escherichia coli* were 0.22 and 0.32 kGy in refrigerated and frozen chicken respectively. In this present study, it was also revealed that *E. coli* had a significantly lower value, even to its total elimination, when irradiated at 1.0 kGy.

Pelicia et al.,²³ on the other hand, evaluated the ability of gamma radiation to reduce *Escherichia coli* populations in chicken breast meat, and observed that a 2,0 kGy, 4,0 kGy and 8.0 kGy were sufficient to eliminate fecal coliforms in the meat samples, while the results obtained herein, with lower doses (1.5 kGy, 3.0 kGy and 4.5 kGy), were more efficient in reducing/eliminating microorganism counts. Some authors, as Al-Bachir and Othman²⁴ and Torgby-Tetth et al.⁴ state that ionizing irradiation destroyed these microorganisms in meat chicken. Gamma irradiation is effective in reducing viable bacteria and eliminating potential pathogens such as *Escherichia coli* from chicken, these results corroborate those obtained in this experiment.

Regarding the presence of *Enterococci* spp., all samples (controls, 1.5 kGy, 3.0 kGy and 4.5 kGy) were negative for this microorganism, regardless of the irradiation dose (Figure 2). In addition, no significant differences between the experiment weeks, samplings and sample analyses were observed regarding for presence of *Enterococci* spp. using a 0.05 significance level ($p = 1.000$).

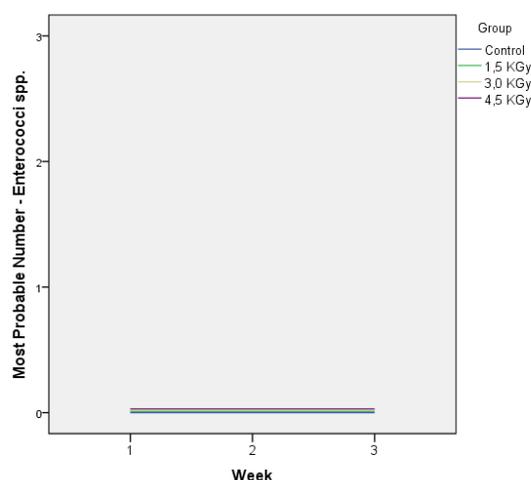


Figure 2. Results observed in the control *Enterococci* spp. NMP during the three weeks of the experiment, at CCAMP / LABZOO / IOC / FIOCRUZ, on January 2016.

On the other hand, considering the analyses carried out by Tabatabaei Yazdi and Jouki,²⁵ *Enterococci* spp. counts in meat ostrich irradiated with 3.0 kGy, were shown to increase with product storage time. The same was reported by Henry et al.²⁶ for turkey meat, who noted that, although the irradiation was effective in reducing microorganisms, observed increases in *Enterococci* spp. in the foodstuffs during storage. This was also observed by Xavier et al.²¹ while analyzing irradiated chilled chicken hearts with the same doses applied herein, of decreases/elimination in microorganism counts, which increased during storage. However, at the 4.5 kGy dose, the count value was < 3, or equal to zero, thus considered as zero for the statistical analyses.

Another study carried out by Soares et al.²⁷ with raw scallop muscle and gonads irradiated with Cs137 at 2.0 and 5.0 kGy observed no positive counts for this microorganism either in the control group or the irradiated samples, similarly to what was observed herein, in which all the chilled chicken heart samples irradiated with Co60 were negative for *Enterococci* spp., regardless of the irradiation dose, as well as the controls.

CONCLUSIONS

Gamma Co60 irradiation when applied to chilled chicken heart was effective in eliminating the *E. coli* population initially present in the analyzed samples. Comparisons to the literature indicated that a 1.5 kGy dose would be sufficient in this regard. The presence of *E. coli* in the evaluated samples indicates the need to improve the hygienic-sanitary standards in the processing line of chicken giblets, as well as continued health education for handlers, employees and consumers on the dangers and risks to which they are submitted. *Enterococci* spp. were absent from all investigated samples, controls (non-irradiated) and irradiated samples at all three doses.

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AUTHOR CONTRIBUTION

The Professor Dr. MCLS accompanied the studies of nuclear radiation, irradiation of samples and participated in the sequence alignment and drafted the manuscript. The researchers from CCAMP / LABZOO / IOC / FIOCRUZ, Dr. SSD e Dr. WTCE, for using this laboratory, and supporting of samples analysis, as well contributed to the suggestion of scientific references. Professor Dr. ABMF was responsible for the statistical analysis of the results of the present study. elaboration of the graphics with statistical results. The author, MMBBSX for carrying out the article, the review and approval of the final manuscript proof. Professor Dr. RMF as project advisor and reviewer activities.

COMPETING INTERESTS

The authors declare there are no competing interests.

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