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Trial assay: effect of gamma irradiation (Co60) in the control of *Campylobacter* spp. in chilled chicken (*Gallus gallus*) heart

Ensaio experimental: efeito da irradiação gama (Co_{60}) no controle de Campylobacter spp. em coração de frango (Gallus gallus) refrigerado

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ABSTRACT

The food irradiation process has been used since the early twentieth century and is technologically established as an important conservation method by eliminating or reducing pathogenic microorganisms, ensuring food quality and safety. The aim of this research was to approve the effect of a gamma irradiation process (Co60) and identify the presence of *Campylobacter* spp. in chilled chicken hearts, sold in an industry under sanitary inspection, located in the West Zone of Rio de Janeiro/RJ, for three weeks. Irradiation was performed at COPPE/UFRJ, using a Gamma Cell Irradiator with a ^{60}Co gamma source. The hearts of 50 chickens were collected randomly and separated into two groups, one contaminated (CAMPY) on purpose with the intention of observing the efficiency of the gamma irradiation (Co_{60}) process in the control of *Campylobacter* spp., and the other group (NC) naturally derived from the industrial plant. The two groups (contaminated and uncontaminated) were divided into four subgroups, CONTROL samples (non-irradiated), and samples submitted to 1.5 kGy, 3.0 kGy and 4.5 kGy doses, all samples were kept chilled with artificial ice. At the end of the study, the presence of *Campylobacter* spp. was observed in samples CAMPY, and derived from the industry NC, while the irradiation process was efficient in the control of *Campylobacter* spp. at 1.5 kGy, 3.0 kGy and 4.5 kGy in both subgroups analyzed. However, it was also concluded that a lower dose of irradiation than the lowest dose used in this study, ie 1.5 kGy, would be efficient in eliminating microorganisms.

Keywords: Food irradiation. Chicken heart. *Campylobacter* spp. Sanitary inspection. Public health.

RESUMO

O processo da irradiação de alimentos é utilizado desde o início do século XX, e está estabelecido tecnologicamente como um importante método de conservação, eliminando ou reduzindo microrganismos patogênicos, garantindo um a qualidade e a segurança alimentar. Objetivou-se nesta pesquisa analisar o efeito do processo de irradiação gama (Co_{60}) no controle de *Campylobacter* spp. em corações de frango (*Gallus gallus*) refrigerados comercializados numa indústria com inspeção sanitária localizada na Zona Oeste da cidade do Rio de Janeiro/ RJ, durante 3 semanas. A irradiação das amostras foi realizada na COPPE/ UFRJ, com irradiador gama provido de fonte de Cobalto 60. Foram coletados corações de 50 frangos aleatoriamente, separados em dois grupos, um grupo contaminado propositalmente (CAMPY), com a intenção de se observar a eficiência do processo da irradiação gama (Co60) no controle de *Campylobacter* spp., e outro grupo, não contaminado (NC), oriundo naturalmente da planta industrial. Os dois grupos (contaminado e não contaminado) foram separados em quatro subgrupos, amostras CONTROLE (amostra sem irradiar), e amostras submetidas às doses de 1,5 kGy, 3,0 kGy e 4,5 kGy, todos mantidos refrigerados. Ao final do estudo, observou-se a presença de *Campylobacter* spp. nas amostras CAMPY, e oriunda da indústria NC, enquanto o processo de irradiação se mostrou eficiente no controle de *Campylobacter* spp. nas doses de 1,5 kGy, 3,0 kGy e 4,5 kGy nos dois subgrupos analisados. Entretanto, concluiu-se também que uma menor dose de irradiação, do que a menor dose utilizada nesta pesquisa, ou seja 1,5 kGy, seria eficiente na eliminação dos microrganismos.

Palavras-chave: Irradiação de alimentos. Coração de frango. *Campylobacter* spp. Inspeção sanitaria. Saúde pública.

INTRODUCTION

Brazil' poultry industry began 2016 with several records in chicken production and export. Chicken meat, consolidated as the fourth item of the national export portfolio, achieved the three best monthly results in the history of the sector's exports in 2015 (ASSOCIAÇÃO BRASILEIRA DE PROTEÍNA ANIMAL, 2015).

Today, consumers seek to optimize their time, combining practicality, technology and price in products offered in large specialized supermarket chains. The chicken heart is the noblest part of the chicken, and a slaughter of about 80 birds is necessary to obtain 1.0 kg of the packaged product. It is one of the gastronomic gems offered at steakhouses. Due to its location, physiological functionality and composition, the passage and installation of various circulating microorganisms through the bird circulatory system is favored. Because of this, any fault in handling/food processing good practice programs can interfere with product quality, as well as harm consumer health (XAVIER et al., 2016).

Outbreaks of waterborne and foodborne diseases affect 2 million people each year in all countries, according to the World Health Organization (World Health Organization, 2015). These diseases may be associated with the consumption of almost any kind of food product such as meat, eggs, dairy products, fruits, vegetables and seafood, due to infections caused by *Escherichia coli* O157:H7 and pathogenic species of *Campylobacter* spp., *Listeria* spp., *Salmonella* spp., *Shigella* spp. and *Staphylococcus* spp. which can all be fatal (ALVES; OLIVEIRA, 2013; MASTRO, 2015).

Food irradiation is a safe physical storage method, considered cold pasteurization, in which the food is exposed to a defined dose of ionizing radiation. This results in improvements in the microbiological quality of the product, reduced risks of foodborne illness, less losses in storage and longer shelf life, with no influence on food appearance and composition and maintenance of the nutritional value of macroconstituents and microcomponents. The main aim of this process is food security, although consumer acceptance is still the biggest challenge (URBAIN, 1986, KILCAST, 1994, FARKAS, 2007, ALAM KHAN; ABRAHEM, 2010, IBARRA et al., 2010, TESFAI et al., 2014).

Campylobacteriosis is a worldwide-distributed zoonosis. *Campylobacter* species are associated with warm-blooded animals, commensal to the gastrointestinal tract of cattle, pigs, sheep, cats, dogs, wild and domestic rodents, poultry and birds. It has been shown that a large percentage of meat-cut animals releases this microorganism in stool. Birds are the main reservoir of *C. jejuni*, which can be found in feces and freshly slaughtered poultry carcasses. Many cases of human enteritis are associated with the consumption of contaminated water and/or food of animal origin, or resulting from contact with contaminated animals (BRASIL, 2011; GONSALVES et al., 2016; SILVA et al., 2014). The main causes of campylobacteriosis are associated not only to the consumption of contaminated and improperly processed meat, but also incorrect food handling practices, generating cross-contamination with other raw foods, such as salads. The importance of studying chicken giblets and meat contamination is paramount, because these are an important source of high quality protein, rich in essential amino acids, vitamins and minerals and are largely consumed in Brazil, as well as and worldwide (CAMPOS et al., 2015, FRANCO, 2012; GONÇALVES et. al., 2012).

In this context, the present study aimed to evaluate the effects of gamma radiation (Co60) on the microbiological quality and control of *Campylobacter* spp. in chilled chicken (*Gallus gallus*) heart.

MATERIAL AND METHODS

Chilled chicken hearts were purchased, randomly referring to 50 birds, in an industry located in the West Zone of Rio de Janeiro, under regular Sanitary Inspection. They were transported in an isothermal container with chemical ice to the CCAMP/ Bacterial Zoonosis Laboratory/IOC/ FIOCRUZ, with chilling temperature maintained (BRASIL, 1996). At the laboratory, following the RDC Resolution no. 12 criteria (BRASIL, 2001), 200g were weighed, and aliquoted into two separate groups, classified as NC (non-contaminated) and CAMPY (contaminated) and then separated into 4 subgroups, in plastic zip -lock bags, each containing 25g, identified as CONTROLS (not subjected to the irradiation process), 1.5 kGy, 3.0 kGy and 4.5 kGy.

The group composed of the contaminated samples received a prepared bacterial suspension with 9.0 mL of 0.1 % buffered peptone water (BPW) and mass generated from a seed culture of the *C. jejuni* ATCC 33291/ CCAMP 0262 strains by CCAMP/ LABZOO/ IOC/ Oswaldo Cruz Foundation (Rio de Janeiro, RJ, Brazil) and vortexed in order to homogenize the inoculum. A visual comparison of the suspension clouding caused by the bacterial growth at a McFarland # 1 scale was conducted, equivalent to 3.0 X 10^{-8} bacteria/mL (BIER, 1980).

Irradiation of the samples

The NC and CAMPY samples were transported to the Nuclear Instrumentation Laboratory (LIN) at the Alberto Luiz Coimbra Institute of Graduate Studies and Research in Engineering (COPPE)/ Federal University of Rio de Janeiro (UFRJ) in the isothermal container, where they were submitted to the gamma irradiation, in a Gammacell 220 irradiator with cobalt 60 source (E = 1.25 MeV) with activity of 12,000 Ci process, at 1.5 kGy, 3.0 kGy and 4.5 kGy doses (Caruso et al., 2011). The treatment of the aliquots for each dose, 1.5 kGy, lasted for 101 minutes. The process was based on conditioning the samples in the irradiator compartment, programming it, waiting while the aliquots were subjected to the irradiation process, finishing the process, controlling the temperature, and starting a new process, of the lowest dose, 1.5 kGy , for a higher dose, 4.5 kGy. The control samples (NC and CAMPY) were not irradiated, they remained in the isothermal with container throughout the irradiation process of the other samples. During the opening of the isothermal container for further irradiation, the temperature was also measured, oscillating at $\pm$ 4°C.

Cultivation of Campylobacter spp.

After sample irradiation they were returned to the lab at CCAMP/LABZOO/ IOC/ FIOCRUZ, where they were washed in 225 mL of sterile BPW, with friction of the entire surface during one minute, and subsequently subjected to a distinct standard methodology (FILGUEIRAS, HOFER, 1989).This was conducted in a flow chamber, where they were sown obeying the T form, on plates identified also containing a selective medium comprising a nutrient base (4,4g of Difco Columbia Agar, 0.4g of activated charcoal and 100mL of distilled water), an oxygen reducer (5,0 mL of the FBP supplement composed of 0,5g of Ferrous Sulphate, Sodium Bisulphite and Sodium Pyruvate diluted in 100mL sterile distilled water) and 0.5 mL of an antimicrobial solution consisting of 11mg Cephalothin (Sigma), 50mg Trimethoprim lactate (Roche), 91mg Vancomycin (Sigma), 20mg Acti-dione (Upjohn), and 22mg Colistin (Sigma), diluted in 50mL sterile distilled water. The seeded plates were then placed in GasPak jars, in a microaerophilic atmosphere with anaerocult® sachets, incubated in an oven at 42°C

(identified by sowing date and opening date). After 48 hours the typical *Campylobacter* spp. cell morphology of the suspect colonies was confirmed by Gram staining. Some colonies were isolated and plated on selective and identified (A, B, C, D, E) media. These plates were then returned to the GasPak jars (again in a microaerophilic atmosphere with anaerocult® sachets), and were incubated in a stove at 42°C/48h.

Identification of Campylobacter spp.

Tests such as the hydrolysis of Na hippurate was performed to confirm as well as to differentiate of genus/species (LIOR, 1982). Characterization of *Campylobacter* genus was also conducted, since there are differences between *Campylobacter jejuni* and Campylobacter coli (the former produces glycine and forms a purple halo on the tube surface while the latter does not produce glycine and produces a colorless halo on the surface of the tube). Indoxil acetate hydrolysis was also verified: the results were interpreted as negative when no change in disk coloring occurred, and as positive when the disk became blue-green/dark blue, indicating the presence of C. jejuni and C. coli. To complete the biotyping, the presence of deoxyribonuclease enzyme (DNAse) was also determined, using the methyl green agar DNAse test, evidence of enzyme activity on the substrate was obtained by observing the presence of a pink/salmon halo. At the end of the experiment, and confirming the morpho-coloring characteristics and the results of the biochemical tests, the samples identified as containing *Campylobacter coli* were grown in a stock solution in Brucella Agar were deposited in the *Campylobacter* Collection/ Bacterial Zoonosis Laboratory/IOC/ FIOCRUZ/ RJ/ Brazil.

RESULTS AND DISCUSSION

The results regarding the presence of *Campylobacter* spp. in chilled chicken heart and the efficiency of the gamma irradiation (Co_{60}) process are displayed in Table 1.

Table 1: Results regarding the presence of *Campylobacter* spp. in chilled chicken heart and the efficiency of the gamma irradiation (Co_{60}) process.

Dose	Non-contaminated samples (NC)	Contaminated samples (CAMPY)
CONTROL	+	+
1.5 kGy	-	-
3.0 kGy	-	-
4.5 kGy	-	-

Clavero et al. (1994) studied the inactivation of *Campylobacter jejuni* in beef by gamma irradiation, and found that the D_{10} value for *Campylobacter* spp. in ground meat ranged from 0.175kGy to 0.235kGy, confirming that low doses are sufficient, as reported herein, to eliminate *Campylobacter* spp. present in the heart of chilled and irradiated chicken.

According to Thayer (1995) low doses of ionizing radiation (<3.0 kGy) can eliminate or significantly reduce the population of the most common enteric pathogens, such as *Campylobacter jejuni,* associated with meat and poultry and products thereof.

According to Molins (2004) the D_{10} dosages for *Campylobacter jejuni* are 0.27 kGy, i.e., the dose required to kill 90 % of the bacterial population present . Using D_{10} as a parameter equivalent to 1.0 kGy, i.e the lowest dose adopted in this study, the efficiency of the process used for the elimination of microorganisms and the maintenance of the sanitary quality of the product was proven.

In a study by Caruso (2011) in a study on irradiated chicken livers with doses of 0.20 kGy, 0.27 kGy, 0.30 kGy and 0.35 kGy, in raw and *in vitro* chicken livers contaminated with *Campylobacter jejuni* to inactivate these microorganisms, results showed that almost all of the analyzed livers, both chilled and frozen, showed the presence of this bacteria.

In a study conducted by Clements (2011) the contamination process by *Campylobacter* spp. was related to the processing of slaughtered chickens, considering food irradiation as a physical method for microorganism control contributing to food security, a relevant goal for the achievement of this study, demonstrating that *Campylobacter* spp. contamination in poultry is indeed present, even if said research did not include chicken carcasses samples.

In a study conducted by Kudra et al. (2012), *Campylobacter jejuni* control in chicken breast meat was evaluated associating the irradiation process to modified atmosphere packaging (MAP). Only irradiation was effective in eliminating *C. jejuni* from packaged meat or poultry in MAP, with doses of 0,29 + 0,03 kGy in MAP and 0,31 + 0,01 kGy in vacuum, for the purpose of extending product validity, converging with the data obtained herein.

Confirming the importance of the irradiation process, according to the study by Ahn et al. (2013) populations of the most common enteric pathogens, such as *Campylobacter jejuni*, can be significantly diminished or eliminated by low-dose irradiation (<1.0 kGy), which corroborates the results obtained herein.

Xavier et al. (2016) researched the microbiological quality of the chilled chicken heart using gamma irradiation with 1.5 kGy, 3.0 kGy and 4.5 kGy doses, found similar results, corroborated the results obtained and emphasizing that lower doses than those used in the experiment would be sufficient to reduce and to eliminate the *Campylobacter* spp. present in the uncontaminated and intentionally contaminated samples.

CONCLUSIONS

The need to improve sanitary conditions of poultry production, preventing the risks of contamination reaching the consumer is required, in addition to the continual encouragement of good hygiene practices and appropriate heat treatment of poultry by the food manipulators, traders and consumers. In this context, in the present study, *Campylobacter* spp. Was isolated from chilled chicken heart and gamma irradiation (Co60) was proven as an effective method for the elimination of microbial contaminants in the final product, observed at the lower radiation doses applied of 1.0 kGy, maintaining efficiency until the end the experiment.

The Brazilian health legislation determining the microbiological standards for food does not include *Campylobacter* spp. as microbiological criteria, especially in meat and chicken giblets. This should, be reviewed in light of several studies demonstrating that the microorganism is present in the whole poultry production and product development chain. In addition, it is recommended to strengthen inspection and control systems and establish greater microbiological control during exposure of these products in the formal trade, as well as also improve the sanitary quality of the product processing and, therefore, prevent public health risks.

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AUTHOR CONTRIBUITION

The Professor Dr. Mauro Carlos Lopes Souza 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. Sheila da Silva Duque and Dr. Wagner Thadeu Cardoso Esteves, for using this laboratory, and supporting of samples analysis, as well contributed to the suggestion of scientific references. The author, Marta Maria Braga Baptista Soares Xavier for carrying out the manuscript. Professor Dr. Robson Maia Franco as project advisor and reviewer activities.

COMPETING INTERESTS

The authors declare there are no competing interests.

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