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Effect of gamma irradiation (Co60) in the control of *Campylobacter* sp. in chilled chicken (*Gallus gallus*) heart

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The present study aimed to evaluate the efficiency of the irradiation process in the control of *Campylobacter* spp. in chilled chicken heart samples, since this microorganism is related to the contamination of meat and chicken giblets and is responsible for enteritis in humans. The methodology and standards recommended by RDC no. 12 (Brazil, 2001) were applied for the bacteriological analyses. The chilled chicken heart samples were acquired in an industry that undergoes sanitary inspection, located in the West Zone of Rio de Janeiro. Samples were divided into two groups, non-contaminated (NC - originally from the industrial plant) and contaminated (CAMPY– contaminated with *C. jejuni* ATCC 33291/CCAMP/FIOCRUZ 00262 strains by CCAMP/LABZOO/IOC/FIOCRUZ), subsequently separated into four groups: NC and CAMPY control groups and samples irradiated at 1.5, 3.0 and 4.5 kGy. The eight subgroups were analyzed for the presence of *Campylobacter* sp. No statistically significant difference was observed between the four groups namely the non-irradiated controls and the 1.5, 3.0 and 4.5 kGy irradiated samples (p> 0.05). Elimination of *Campylobacter* sp. was observed, with no bacterial growth in any of the irradiated, non-contaminated (CN) and infected (CAMPY) samples. Thus, the efficiency of the Co60 irradiation process of chilled chicken heart for the elimination of the surveyed microorganisms was proven. The lowest dose applied was sufficient to eliminate the enteric pathogen which is of great significance in a public health point of view. However, it should be noted that the Brazil legislation determining the microbiological standards for food does not include a microbiological standard for *Campylobacter* sp. This means that any amount of this enteric pathogen may bring public health risks.

Key words: Food irradiation; chicken heart; *Campylobacter* sp.; Foodborne illness; public health.

INTRODUCTION

Food irradiation is a proven safe storage physical method, considered cold pasteurization, in which the food is exposed to a defined dose of ionizing radiation. Improvements in the microbiological quality of the product

are observed with irradiation, reducing the risk of foodborne illness along with decreased losses in storage and longer shelf life. This method does not influence the appearance and composition of nutrients, and its main objective is food security. However, the great challenge when applying this method is consumer acceptance, often due to confusion of “irradiated” with “radioactive” (Diehl, 1995; Hernandez et al. 2003; Gava, 2006; Miranda; 2012).

Brazil’s poultry industry began the year of 2016 beating several records, including the chickens’ production and exports. Chicken meat, is consolidated as the fourth item of the national export portfolio, which achieved the three best monthly results in the history of the sector's exports in, 2015 (Brazilian Association of Animal Protein, 2015). The importance of studying giblet and chicken meat contamination is highlighted by the fact that, these products are important source of high quality protein rich in essential amino acids, vitamins and minerals, and highly consumed not only in Brazil but throughout the world (Poultry Brazil, 2012). However, the Brazilian health legislation determining the microbiological standards for food, RDC Resolution no.12 (Brasil, 2001) does not state any maximum permissible limit as microbiological criteria regarding the presence of *Campylobacter* sp. in meat and chicken giblets, as it does for other pathogens in Annex II of this standard.

There is the likelihood of these organisms that have been noted during the various stage of animal raising, transport, processing, distribution and marketing, and serve as indicators of the sanitary conditions of the production/handling of raw materials, since they can be responsible for foodborne illness (Clements, 2011; Franco, 2012; Russel, 2009). The present study aimed, to evaluate the effects of gamma radiation (Co60) on the microbiological quality and control of *Campylobacter* sp. in chilled chicken (*Gallus gallus*) heart samples.

MATERIALS AND METHODS

Collection of samples

Chicken heart samples were acquired from a poultry slaughter house that undergoes regular sanitary inspection and has an on-site store for meat sales, located in the western zone of the state of Rio de Janeiro, Brazil. Three samplings were conducted. Giblets were randomly selected taking into account the production date, closest to the beginning of the analysis. The samples were placed in an isothermal container and kept under refrigeration during all stages of the experiment, at a maximum temperature of 7°C (Brasil, 1996). Samples were then distributed in previously identified Zip lock bags (contaminated – CAMPY, non-contaminated - NC, chicken heart - CF, control – non- irradiated samples, and irradiated samples – with 1.5kGy, 3.0kGy and 4.5kGy) with the corresponding date of the analysis. Contaminated aliquots received a prepared homogenized bacterial suspension containing 9.0 mL of 0.1% TPA and mass generated from a *C. jejuni* ATCC 33291/CCAMP 0262 strains seed culture, provided by the *Campylobacter* Bacterial Zoonosis Laboratory, at the Oswaldo Cruz Institute, Oswaldo Cruz Foundation (Rio de Janeiro, RJ, Brazil). Turbidity caused by bacterial growth was at a McFarland scale #1, equivalent to 3.0 x 10^{-8} bacteria mL^{-1} (Bier, 1980).

Irradiation of the samples

The non-contaminated and contaminated samples were then transported to the Nuclear Instrumentation Laboratory at, Alberto Luiz Coimbra Institute of Graduate Studies and Research in Engineering (COPPE), in Federal University of Rio de Janeiro, in an isothermal container, where they were subjected to gamma irradiation process (Co60) at the dosage of 1.5kGy, 3.0kGy and 4.5kGy. The control samples (NC and CAMPY) were not irradiated and remained in the isothermal container throughout the irradiation process of the other samples (Caruso et al., 2011).

Cultivation of *Campylobacter* sp.

For cultivation, identification and maintenance of *Campylobacter* sp. strains, a standard technique was implemented at the Bacterial Zoonoses Laboratory (Filgueiras and Hofer, 1989). The selective medium comprising a nutrient base (4.4 g Columbia agar, 0.4 g activated carbon diluted in 100 mL distilled water) was prepared by adding an FBP supplement as an oxygen-reducing substance (0.5g ferrous sulfate, sodium bisulfite and sodium pyruvate, diluted in 100 mL sterile distilled water) and an antimicrobial mixture (11mg cephalothin, 50mg trimethoprim lactate, 91 mg vancomycin, 20mg actidione, and 22 mg colistin, diluted in 50 mL sterile distilled water). The media was poured into 20 plates (five replicates for each NC and CAMPY sample) and stored in a GasPak jar (in a microaerophilic atmosphere with anaerocult® sachets). The inoculated plates were incubated at 42°C for 48 h.

Campylobacter sp. colonies were isolated after confirming typical morphotinctorial characteristics and subjected to Gram staining. Replating was performed in plates with selective media for mass formation. NC and CAMPY plates were analyzed in control and irradiated samples. The replating was performed to obtain mass formation, to have colonies to realize biochemical tests at the end of the experiment. Both NC and CAMPY plates were analyzed because on the NC plates there was no certainty of finding *Campylobacter sp*. and the samples showed original slaughter house poultry microorganisms, which certainly occured in the CAMPY plates groups. Being a contaminated sample, it is a known fact that microorganisms would be present, and there is need to ascertain the gamma radiation effect on the control of these microorganisms. Therefore, the two groups were evaluated.

Identification of *Campylobacter* sp.

Tests such as the hydrolysis of Na hippurate was performed to be confirm as well as to differentiate 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

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Table 1. *Campylobacter* sp. control in chilled chicken heart.

Group	Doses (kGy)	Week 1					Week 2					Week 3				
		A	B	C	D	E	A	B	C	D	E	A	B	C	D	E
NC	C	+	-	-	+	-	+	+	+	+	-	+	+	-	+	+
	1.5	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	4.5	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
CAMPY	C	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	1.5	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	4.5	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

NC – Non-contaminated samples; CAMPY – Contaminated samples. A, B, C, D and E correspond to the five plates used for each dose.

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, 15 confirmed strains were deposited in the *Campylobacter* collection/ Bacterial Zoonosis Laboratory (IOC/ FIOCRUZ/ RJ/ Brazil).

Statistical analysis

Statistical differences between groups were evaluated by applying Friedman's test, with a significance level set at 0.05.

RESULTS AND DISCUSSION

No significant difference was observed between the three samplings, regardless of group (NC or CAMPY) and dosage subgroup (control, 1.5, 3.0 and 4.5 kGy), indicating no effect on the positive results for the presence of *Campylobacter* sp. (p=0.444). This is probably caused by the fact that the positive samples for *Campylobacter* sp. were the control samples, not subjected to the irradiation process, confirming the occurrence of *Campylobacter* sp. in meat and chicken giblets, as reported by Azeredo et al. (2010), Campos et al. (2015), Freitas and Noronha (2007).

The results of Co60 gamma radiation process in chilled chicken heart samples exposed to different radiation doses are displayed in Table 1.

During the first week, of the five control plates seeded with the *in natura* samples (NC), two (A and D) were positive (40%), whereas the contaminated samples (CAMPY) were all positive (100%). For the samples irradiated at 1.5kGy, 3.0kGy and 4.5kGy, both the non-contaminated (NC) and contaminated (CAMPY) samples showed no cell growth (100%), demonstrating the efficiency of the Co60 gamma irradiation process in the control of *Campylobacter* sp. The result corroborates with the reports of Ahn et al. (2013) which states that irradiation is an effective technological process to eliminate pathogens in poultry meat. In addition, an advantage of ionizing Co60 radiation includes high penetration and uniform dosage. In concordance with the results of this study, Mendonça (2002), Olson (1998) and Raut et al. (2012) reported that the majority of the mundane enteric pathogens such as *Campylobacter jejuni*, can be significantly reduced or eliminated with low dose (<3.0 kGy) irradiation.

During the second week, control plates A, B, C and D were positive (80%). However, both NC and CAMPY samples irradiated with all three doses did not demonstrate colony growth, and the process was thus effective in eliminating *Campylobacter* sp. Raut et al. (2012) which evaluate the effectiveness of the irradiation process in the elimination of *Campylobacter* sp. by, testing the sensitivity of *Campylobacter jejuni* and *Campylobacter coli* in chicken meat samples at doses ranging from 0.110 to 0.190 kGy. These authors demonstrated that, treatment with a dosage of 1kGy can achieve complete elimination of *Campylobacter* sp. in poultry meat samples and the results completely justifies the elimination of microorganism using 1.5kGy irradiation process in both NC and CAMPY samples of this study.

In the third and final week of the experiment, control samples A, B, D and E were positive (80%), while all irradiated samples showed no development of colonies (100%). Kudra et al. (2012) compared the efficacy of irradiation process in the control of *Campylobacter jejuni* in chicken breasts to that of modified atmosphere packaging (MAP). The D_{10} sensitivity value regarding irradiation of this enteric pathogen ranged from 0.31+0.01 kGy in vacuum packaging, and 0.29+0.03kGy in MAP, respectively. Irradiation was effective in eliminating *C. jejuni* from chicken breast packed both in vacuum or MAP, thereby reducing the possibility of cross-

contamination in retail shops or in domestic kitchens. The doses used in the present study were higher and reached their goal regarding the control of *Campylobacter* sp. Therefore, additional means to mitigate quality changes appear to be required for these products.

Comparable to the results obtained in the present study, Chun et al. (2010) and Haughton et al. (2012) investigated the applicability of UV-C irradiation in the inactivation of *Campylobacter jejuni* in ready-to-eat poultry and chicken fillets, respectively, and their results demonstrated the control of this microorganism during storage using this type of irradiation. The presence of *Campylobacter* sp. regardless of the dosage subgroup (control, 1.5, 3.0 and 4.5 kGy) were compared among the NC groups, and no significant difference between the three samplings was observed, with no effect regarding positive results for the presence of this microorganism *(*p=0.444). The presence of *Campylobacter* sp. in chilled chicken heart samples was evidenced in the present study, regardless of the type of treatment, similar to other studies that observed the presence of this microorganism derived from feces and meat and chicken giblets (Bognar, 2012; Trassi, 2012). Also, in regardless of the dosage subgroup (control, 1.5, 3.0 and 4.5 kGy) was also compared among the CAMPY groups, and again no significant difference between the three samplings was observed, with no effect regarding positive results for the presence of this microorganism *(*p=1.000).

As in the present study, Azeredo (2007), in his experiment on irradiated chicken livers with doses of 0.20kGy, 0.27, 0.30 and 0.35, also used two groups, one non-contaminated and one contaminated with *Campylobacter* sp. and concluded that there was no significant difference between the contaminated samples and those with other treatments. Clavero et al. (1994) observed ground beef samples contaminated with *Campylobacter jejuni* which were subjected to irradiation treatment with Co60 gamma doses ranging from 0 to 2.52kGy, and observed significant values depending on the combination performed in the experiment, of temperature and fat content. The authors concluded that, regardless of the selected treatment, pathogens were highly sensitive to gamma irradiation, and a D_{10} value for *Campylobacter jejuni* was determined ranging from 0.175 to 0.235kGy.

The authors also observed that, a 2.5kGy dose would be sufficient to eliminate $10^{10.6}$ *Campylobacter jejuni*, resulting in a high probability of complete inactivation of much higher populations than those occasionally present in preparations with ground meat. Patterson (2008) investigated the sensitivity of different *Campylobacter* species to irradiation in poultry samples and reported D_{10} values ranging from 0.12 and 0.25 kGy. This could be attributed from the results of this study that use of gamma irradiation (Co60) could be beneficial in the control of *Campylobacter* sp. in chilled chicken heart samples.

Though not considered the best indicators of fecal contamination in meat and chicken giblets and also these microorganisms not being part of the food composition, the presence of *Campylobacter* points out flaws in sample handling/processing or poor sanitary hygienic conditions, which allows the proliferation of these and other enteropathogens (Franco, 2012; Franco and Landgraf, 2008). Thus, the contaminated samples analyzed herein are a public health concern.

Keener et al. (2004) have reported that the Food and Drug Administration (FDA) and United States Department of Agriculture (USDA) approved the irradiation of poultry meat at a maximum dose of 3.0 kGy to control causative pathogens of foodborne illness, such as *Campylobacter* sp. No microbiological standard regarding the presence of *Campylobacter* sp. exists in the current Brazilian health legislation RDC no. 12 (Brasil, 2001). Thus it is not possible to assume harm from any random value of *Campylobacter* sp. in samples in this regard, and the samples analyzed herein are therefore contaminated, thus unfit for consumption and pose public health risks.

Conclusions

Co_{60} gamma irradiation, when applied to chilled chicken heart at doses of 1.5, 3.0 and 4.5 kGy, was effective in the elimination of *Campylobacter sp*. that was initially present in the samples. Comparisons with the literature indicated that, 1.5 kGy dose would be sufficient in eliminating this microorganism, as it shows sensitivity to low gamma radiation doses.

However, statistically significant differences were not observed among the four groups (control, 1.5kGy, 3.0kGy and 4.5kGy) in any of the analyses carried out. No microbiological standard regarding the presence of *Campylobacter* sp. exists in the current Brazilian health legislation RDC no. 12. Thus, the samples evaluated in the present study were contaminated, therefore unfit for consumption. The presence of these microorganisms in the analyzed samples indicates the need to improve the hygienic-sanitary standards in the production line and preparation of chicken giblets, together with repeated health education for handlers, employees and consumers about the dangers and risks to which they are subjected to.

The presence of high bacterial load in the control samples were observed in the present study. Inspection is therefore imperative with regard to the production, transport and slaughter of poultry. Compliance with regulatory standards and Good Handling Practices also ensure the quality and safety of this type of food and thereby prevents risks to public health.

Conflict of Interests

The authors have not declared any conflict of interests.

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