GAMMA IRRADIATION IN THE CONTROL OF PATHOGENIC BACTERIA IN REFRIGERATED GROUND CHICKEN MEAT

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ABSTRACT: This work evaluated the effect of gamma radiation on reducting the population of *Staphylococcus aureus*, *Escherichia coli* and *Salmonella typhimurium* in ground chicken breast stored under refrigeration. The experiment included a control and 4 doses of gamma radiation (2.0, 4.0, 6.0 and 8.0 kGy) along with 5 periods of storage under refrigeration (1, 7, 14, 21 and 28 days). Samples of ground chicken breast were inoculated with *Staphylococcus aureus* (ATCC 14458), *Escherichia coli* (ATCC 11105) and *Salmonella typhimurium* (ATCC 0626), irradiated at temperatures between 4 and 8^{o}C and stored under refrigeration (5^{o}C) for 28 days. The increased radiation dose and period of storage under refrigeration caused a reduction of *Staphylococcus aureus*, *Escherichia coli* and *Salmonella typhimurium* populations in the ground chicken breast. Mean radiation D values determined for *Staphylococcus aureus* and *Escherichia coli* were 0.41 and 0.72 kGy, respectively. Gamma irradiation was an effective treatment for chicken meat conservation because the radiation dose of 6.0 kGy kept the ground chicken breast within the microbiological limits established by the Brazilian legislation, for up to 28 days under refrigeration.
Key words: gamma irradiation, pathogenic bacteria, chicken meat, refrigeration

IRRADIAÇÃO GAMA NO CONTROLE DE BACTÉRIAS PATOGÊNICAS EM CARNE DE FRANGO REFRIGERADA

RESUMO: Este trabalho avaliou a capacidade da radiação gama em reduzir a população das bactérias *Staphylococcus aureus*, *Escherichia coli* e *Salmonella typhimurium* em peito de frango moído armazenado sob refrigeração. O experimento consistiu do controle e 4 doses de radiação gama (2,0; 4,0; 6,0 e 8,0 kGy), durante 5 períodos de armazenamento sob refrigeração (1, 7, 14, 21 e 28 dias). As amostras de peito de frango moído foram inoculadas com *Staphylococcus aureus* (ATCC 14458), *Escherichia coli* (ATCC 11105) e *Salmonella typhimurium* (ATCC 0626), irradiadas em temperaturas entre 4 e 8^{o}C e armazenadas sob refrigeração (5^{o}C) por 28 dias. As maiores doses de radiação e os períodos mais longos de armazenamento sob refrigeração causaram uma redução nas populações de *Staphylococcus aureus*, *Escherichia coli* e *Salmonella typhimurium* do peito de frango. A média dos valores D de radiação determinados para *Staphylococcus aureus* e *Escherichia coli* foram 0,41 e 0,72 kGy, respectivamente. A irradiação gama foi um eficiente tratamento para a conservação da carne de frango porque a dose de radiação de 6,0 kGy manteve o peito de frango moído dentro dos limites microbiológicos estabelecidos pela legislação brasileira por até 28 dias sob refrigeração.
Palavras-chave: irradiação gama, bactéria patogênica, carne de frango, refrigeração

INTRODUCTION

Despite substantial efforts to avoid contamination, an upward trend in the number of outbreaks of foodborne diseases caused by nonsporeforming pathogenic bacteria are reported in many countries.

Authough several decontamination methods exist, one of the most versatile treatment among them is the processing of foods with ionizing radiation. Decontamination of food by ionizing radiation is a safe, efficient, environmentally clean and energy efficient process.

Radiation treatment with doses of 2.0-7.0 kGy, depending on the condition of irradiation, initial contamination and the type of food, can effectively eliminate potentially pathogenic nonsporeforming bacteria including both long-time recognized pathogens such as *Salmonella* and *Staphylococcus aureus* as well as emerging or "new" pathogens such as *Campylobacter, Listeria monocytogenes* or *Escherichia coli* O157:H7 from suspected food products .

Candidates to radiation decontamination are mainly poultry and red meat, egg products, and fishery products. With today's demand for high-quality convenience foods, irradiation in combination with other processes holds a promise for enhancing the safety of many minimally processed foods (Farkas, 1998). These products are still incipients in Brazilian market but in a near future they might fill a considerable part of the market because of the advantages of the low-processed foods.

This experimental work evaluated the sensitivity of *Staphylococcus aureus* (ATCC 14458), *Escherichia coli*

(ATCC 11105) and *Salmonella typhimurium* (ATCC 0626) to gamma radiation in ground chicken breast stored under refrigeration. This work also established the radiation D values for reducing *Staphylococcus aureus* and *Escherichia coli* in the food.

MATERIAL AND METHODS

The lyophilized cultures of *Staphylococcus aureus* (ATCC 14458), *Escherichia coli* (ATCC 11105) and *Salmonella typhimurium* (ATCC 0626) were originally obtained from ATCC by the Fundação Oswaldo Cruz of the Instituto Nacional de Controle de Qualidade em Saúde, located in São Paulo-SP, Brazil. The cultures of *Staphylococcus aureus* and *Escherichia coli* were reactived separately in BHI (Brain Heart Infusion) and the culture of *Salmonella typhimurium* was reactived in nutrient broth, all held at 35-37°C for 24 hours. Following, the cultures were inoculated again into the respective medium and incubated at 35-37°C for 24 hours. These culture suspensions containing approximmately 10^6 CFU mL^{-1} of *Staphylococcus aureus*, 10^6 CFU mL^{-1} of *Escherichia coli* and 10^7 CFU mL^{-1} of *Salmonella typhimurium* were used to contaminate the samples.

The chicken breasts were obtained from chicken slaughtered in the city of Rio Claro/SP at the same day of the irradiation. The chicken breasts were kept refrigerated until they were ground in a food-processor. Each 500g of ground chicken breast were mixtured in the food-processor with 10 mL of each culture suspension. Samples of 50g of ground chicken breast were accomodated in petri plates, packed with parafilm and irradiated.

The samples were irradiated with doses of 2.0, 4.0, 6.0 and 8.0 kGy at a rate of 0.929 kGy h^{-1}. The gamma rays came from a panoramic source of ^{60}Cobalt, with activity of 2,000 Ci, located in the Centro de Energia Nuclear na Agricultura – Universidade de São Paulo in Piracicaba-SP. During the irradiation the temperature of the samples was kept between 4 and 8°C using dry ice. The control of the dose of radiation absorbed by the samples was made using polimethylmetacrilat dosimeters. After irradiation the samples were stored under refrigeration (5°C) for 1, 7, 14, 21 and 28 days of storage. Individual petri plates of chicken meat were withdrawn at each sampling period and 25 grams of the sample were assayed for each microbiological analysis. The mimimum detection level for the plate counts was 1 CFU (colony forming unit) per plate.

The count of *Staphylococcus aureus* was accomplished in selective medium BPA (Baird-Parker-Agar) and BHI, incubated at 35°C for 48 hours (Vanderzant & Splittstroesser, 1992). The count of *Escherichia coli* population was accomplished in petrifilm plates incubated at 42°C for 48 hours. The detection of *Salmonella typhimurium* was accomplished, after enrichment in nutrient broth at 35-37°C for 24 hours, using the kit Salmonella Rapid Test (Oxoid) held at 41°C for 24 hours.

The experimental design was a 5x5 factorial scheme, including a control and 4 doses of gamma radiation (2.0; 4.0; 6.0 and 8.0 kGy) along with 5 periods of storage under refrigeration (1, 7, 14, 21 e 28 days), with 3 replicates in each treatment.

Results were submitted to Variance analysis, Hartley test, F test ($p<0.05$) and polinomial regression analyses ($\alpha=0.05$) using the software SAS-Statistic.

RESULTS AND DISCUSSION

Staphylococcus aureus

At all periods of storage under refrigeration, the increased of the dose of radiation caused reduction on *Staphylococcus aureus* population in ground chicken breast (Figure 1). The dose of 2.0 kGy reduced *Staphylococcus aureus* population in aproximmately 4 logarithmic cycles (from 10^7 to 10^3 CFU g^{-1}). The dose of 4.0 kGy reduced *Staphylococcus aureus* population in aproximmately 6 logarithmic cycles (from 10^7 to 10^1 CFU g^{-1}). Colonies of *Staphylococcus aureus* were not detected in the samples irradiated with 6.0 and 8.0 kGy.

Staphylococcus aureus population remained between 10^7 and 10^8 CFU g^{-1} during the period of storage of the control samples. The samples irradiated with 2.0 kGy showed *Staphylococcus aureus* population between 10^3 and 10^4 CFU g^{-1} during the period of storage. *Staphylococcus aureus* populations between 10^1 and 10^2 CFU g^{-1} were observed during the period of storage of the ground chicken breast irradiated with 4.0 kGy (Figure 2).

The results of this research concerned the reduction of *Staphylococcus aureus* population in irradiated chicken meat are similar to those found by some authors. Thayer & Boyd (1992) didn't find viable CFU of *Staphylococcus aureus* in mechanically deboned chicken meat inoculated with 8.0×10^3 CFU g^{-1} of *Staphylococcus aureus* ATCC 13565 and irradiated with 1.50 kGy. However, these authors refers to the dose of 3.0 kGy as

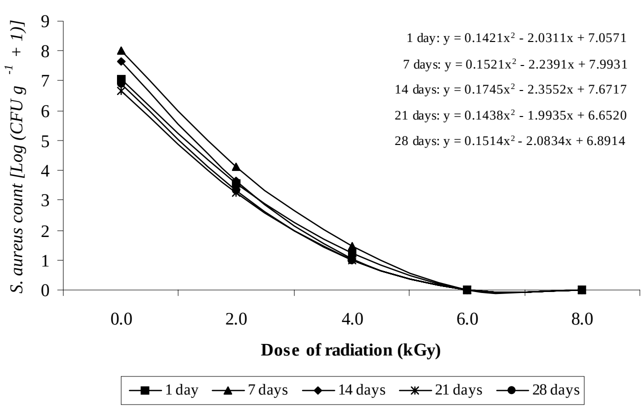

Figure 1 - Effect of gamma radiation on the reduction of *Staphylococcus aureus* population in ground chicken breast stored under refrigeration.

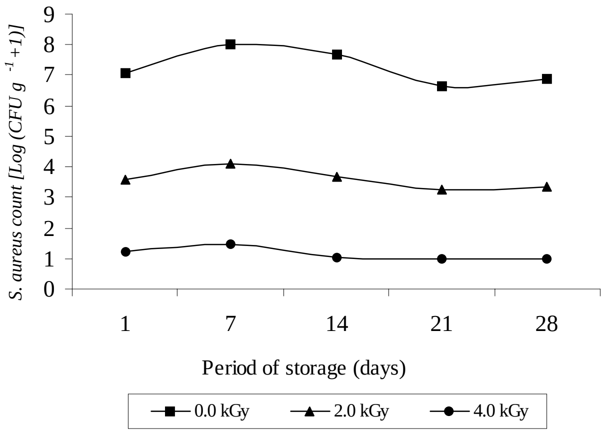

Figure 2 - Variation of *Staphylococcus aureus* population during the period of storage under refrigeration of irradiated ground chicken breast.

minimum for consumer's protection against foodborne disease caused by *Staphylococcus aureus* in that product.

Nouchpramool et al. (1985) observed that the dose of radiation of 3.0 kGy was able to eliminate *Staphylococcus aureus* in frozen shrimp. The dose of 2.5 kGy was able to eliminate *Staphylococcus aureus* in smoked fish (Research, 1978). However, Kolsarici & Kirimca (1995) verified that *Staphylococcus* were resistant to doses of radiation up to 3.0 kGy in chicken meat.

Klinger et al. (1986) observed reduction of 2 logarithmic cycles in *Salmonella, Staphylococcus* and coliforms populations in chicken carcasses irradiated with 4.5 kGy. The authors mentioned the dose of 7.0 kGy as efficient in the decontamination of the product.

Idziak & Incze (1968) observed that doses of radiation from 5.0 to 7.0 kGy promoted reduction of 11 logarithmic cycles in *Staphylococcus aureus* count in chicken carcasses.

Thayer et al. (1997) concluded that *Staphylococcus aureus* can be eliminated or greatly reduced in number in bison, ostrich, alligator and caiman meats by doses of gamma radiation between 1.5 and 3.0 kGy and storage at 5°C.

According to Thayer (1995) low doses of ionizing radiation (<3.0 kGy) may eliminate or significantly decrease the population of the most common enteric pathogens such as *Campylobacter jejuni, Escherichia coli*, *Staphylococcus aureus, Salmonella* spp., *Listeria monocytogenes* and *Aeromonas hydrophila*. Ionizing radiation can be an effective step in a program to kill enteric pathogens associated with meat and poultry products.

Mean radiation D value (dose of radiation to decrease the microorganism population in 1 logarithmic cycle) determined for *Staphylococcus aureus* in refrigerated ground chicken breast was 0.41 kGy (TABLE 1). This result was similar to those reported by some researchers. Thayer et al. (1997) obtained radiation D value of 0.37 kGy for *Staphylococcus aureus* in high-value meats irradiated at 5°C. Hau et al. (1992) found radiation D value of 0.29 kGy for *Staphylococcus aureus* in frozen shrimp. Grant & Patterson (1992) reported radiation D values for *Staphylococcus aureus* between 0.25-0.43 kGy in several kinds of food. Josephson (1983) reported radiation D value of 0.46 kGy for *Staphylococcus aureus* in nutrient broth, but in seafood this author reported values between 0.8 and 1.9 kGy for the bacteria.

TABLE 1 - Linear equations for *Staphylococcus aureus* reduction in irradiated ground chicken breast and the radiation D value for each period of storage under refrigeration.

Period of storage	Linear equation (y = log (CFU g^{-1} + 1) ; x = kGy)	Radiation D value
(days)		(kGy)
1	y = -2.353x + 8.84	0.42
7	y = -2.662x + 10.05	0.38
14	y = -2.563x + 9.50	0.39
21	y = -2.221x + 8.27	0.45
28	y = -2.302x + 8.56	0.43
Average		0.41

Escherichia coli

The number of *Escherichia coli* colonies decreased with the increased of the dose of radiation and the increased of the period of storage under refrigeration.

The dose of 6.0 kGy was required to inhibit *Escherichia coli* growth in the samples at 1 and 7 days of refrigerated storage. At 14, 21 and 28 days of storage, the dose of 4.0 was enough to inhibit bacteria growth in the samples (Figure 3).

The samples irradiated with 2.0 kGy showed an approximated reduction of 3 logarithmic cycles on *Escherichia coli* population in relation to the control. The dose of 4.0 kGy reduced approximately 6 logarithmic cycles the *Escherichia coli* count. The doses of 6.0 and 8.0 kGy reduced *Escherichia coli* population below the detectable level (Figure 3).

Escherichia coli population decreased approximately 2 logarithmic cycles during the period of storage of the control samples (from 10^8 to 10^6 CFU g^{-1}). The samples irradiated with 2.0 kGy also showed reduction of 2 logarithmic cycles on *Escherichia coli* population during the period of storage (from 10^4 to 10^2 CFU g^{-1}). The samples irradiated with 4.0 kGy showed *Escherichia coli* population of 10^1 CFU g^{-1} at 1 day of storage and colonies were not detected just at 14 days of refrigerated storage of the ground chicken breast (Figure 4).

The results found in this work concerned the reduction of *Escherichia coli* count in irradiated chicken meat are similar to those obtained by Bánáti *et al.* (1993) who reported that dose of 2.0 kGy reduced 4 logarithmic cycles the *Escherichia coli* population in irradiated chicken meat. Still according to these authors, at low levels of

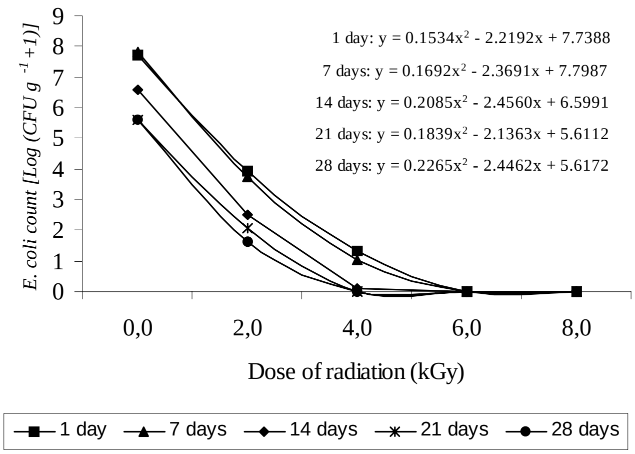

Figure 3 - Effect of gamma radiation on the reduction of *Escherichia coli* population in ground chicken breast stored under refrigeration.

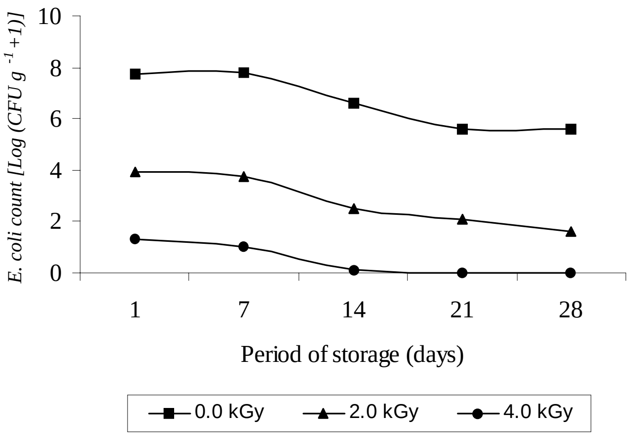

Figure 4 - Variation of *Escherichia coli* population during the period of storage under refrigeration of irradiated ground breast chicken.

contamination, the dose of 2.0 kGy is enough to inactivate most of the nonsporeforming bacteria in meats but, when the contamination exceeds 10^6 CFU g^{-1}, higher doses of radiation are required to reduce the bacteria to acceptable counts.

Lescano et al. (1991) did not detect *Escherichia coli* in chicken meat irradiated with 2.5 kGy. However, the dose of 4.0 kGy was necessary to inactivate *Escherichia coli* in extracts of refrigerated fish (Viana, 1993).

Mean radiation D value determined for *Escherichia coli* in refrigerated ground chicken breast was 0.72 kGy (TABLE 2). This value was higher than those reported by some authors. According to Urbain (1986) the radiation D value for *Escherichia coli* varies from 0.20 to 0.40 kGy in phosphate solution. The same values were found in irradiated red meats and poultry stored under refrigeration (Farkas, 1987; Patterson, 1988; Thayer, 1993 and Tsuji, 1983). In frozen meats the value reported is 0.55 kGy (Farkas, 1987).

TABLE 2 - Linear equations for *Escherichia coli* reduction in irradiated ground chicken breast and the radiation D value for each period of storage under refrigeration.

Period of storage	Linear equation ($y = \log(\text{CFU g}^{-1} + 1)$; x = kGy)	Radiation D value
(days)		(kGy)
1	y = -1.296x + 7.11	0.77
7	y = -1.306x + 7.06	0.77
14	y = -1.623x + 6.32	0.62
21	y = -1.403x + 5.37	0.71
28	y = -1.405x + 5.23	0.71
Average		0.72

Salmonella typhimurium

The resistance of *Salmonella typhimurium* to gamma radiation decreased with the increased of the dose of radiation and the increased of the period of storage under refrigeration (TABLE 3). The control samples presented bacterial activity at all the periods of storage under refrigeration and the irradiated samples showed interaction between gamma radiation and refrigeration on the reduction of *Salmonella typhimurium* population. The dose of 2.0 kGy inhibited bacterial activity from 21 days of storage. The dose of 4.0 kGy was able to inhibited bacterial activity from 7 days of storage. Bacterial activity was not detected in the samples irradiated with 6.0 and 8.0 kGy just at 1 day of storage. During all the period of storage under refrigeration, bacterial activity was not observed in the samples irradiated with 6.0 and 8.0 kGy.

The control samples and the samples irradiated with 2.0 kGy presented bad odour from 7 days of storage. From 21 days of storage, these samples had a rotten appearance and brownish coloration. The samples irradiated with 4.0, 6.0 and 8.0 kGy did not present bad smell or rotten appearance in any of the periods of storage.

The results of this work are similar to those reported by some authors who have observed that 2.5 kGy is an efficient dose of radiation to inhibit the growth of *Salmonella* and increase the shelflife of refrigerated chicken meat (Thayer & Boyd, 1992; Lescano et al., 1991; Lamuka et al., 1992). Similar results were observed by Hanis et al. (1989) who concluded that, despite this dose of radiation is not enough to promote pasteurization of the product, the microorganisms are greatly affected by the interaction between radiation and refrigeration.

In the literature there is not a consent about the dose of radiation to control *Salmonella* in chicken meats. According to USDA (1992) and Morrison et al. (1992) doses of 1.5 to 3.0 kGy can eliminate 99,5 to 99,9% of *Salmonella* population in poultry. Other authors affirm that higher doses of radiation are necessary to inhibit *Salmonella* and assure a product stable and healthy in a sanitary and microbiological point of view. Farkas (1987), Döllstädt et al. (1990) and Viana (1993) reported that doses

TABLE 3 - Detection of *Salmonella typhimurium* in irradiated ground chicken breast stored under refrigeration.

Period of storage (days)	Dose of radiation (kGy)				
	0.0	2.0	4.0	6.0	8.0
1	+	+	+	-	-
7	+	+	-	-	-
14	+	+	-	-	-
21	+	-	-	-	-
28	+	-	-	-	-

+: bacterial activity detected in at least one of the replicates
-: bacterial activity detected in none replicate

between 3.0 and 5.0 kGy could inactivate the nonsporeforming bacteria from meats, poultry and fish. Patterson (1988) affirmed that doses between 5.0 to 7.0 kGy are effective to inactivate *Salmonella* in several kinds of meat.

Idziak & Incze (1968) observed that the dose of 5.0 kGy reduced the number of viable colonies of *Salmonella* in chicken carcasses, which could be conserved for 20 days under refrigeration. However, Hanis et al. (1989) observed that the dose of 5.0 kGy was not enough to eliminate *Salmonella typhimurium* in refrigerated chicken but no bacteria of that species was detected in the meat irradiated with 10.0 kGy.

According to Silva et al. (1997), the anexx I of the Edict n. 01/1987 of the Divisão Nacional de Vigilância Sanitária de Alimentos of the Secretaria Nacional de Vigilância Sanitária establishes the absence of *Salmonella* in 25 g of the product as the microbiological specific pattern for chicken meat. Nevertheless, this Edict does not present microbiological specific patterns or limits to any other microorganism.

The anexx II of the same Edict, concerning the microbiological limits for the analysis of foods for which specific patterns do not exist, establishes as acceptable for human consumption the product that presents up to 10^3 of *Staphylococcus aureus* for g or mL, up to 10^2 of faecal coliforms for g or mL and the absence of *Salmonella* in 25 g or mL.

The samples irradiated with 4.0 kGy presented counts of 10^1 CFU g^{-1} of *Staphylococcus aureus* (Figure 1) and counts between 10^0 and 10^1 CFU g^{-1} of *Escherichia coli* (Figure 3), what placed these samples within the microbiological limits established by the Brazilian legislation. Besides, these bacteria are mesophilic pathogens that do not usually grow in temperature of refrigeration, therefore, even if presenting few viable CFU, the infected product (presence of the pathogen) would not become infectant (pathogen in number to cause the disease) because these bacteria have to reach counts of 10^6 CFU g^{-1} to cause alimentary toxinfection.

Salmonella typhimurium was not detected in the samples irradiated with 6.0 kGy in any period of storage under refrigeration, placing these samples within the microbiological limits established by the Brazilian legislation. The dose of 4.0 kGy only was able to inhibited this bacteria activity from 7 days of storage.

However, as negligences and inadequate refrigeration conditions and manipulation are often present in the preparation of foods, it should be considered the dose of 6.0 kGy as a safety dose of radiation for the treatment of the product aiming its conservation. The dose of 6.0 kGy was the minimum dose of radiation required for the complete inactivation of *Staphylococcus aureus*, *Escherichia coli* and *Salmonella typhimurium* in ground chicken breast stored under refrigeration.

CONCLUSION

Gamma irradiation was an effective treatment for chicken meat conservation because the radiation dose of 6.0 kGy kept the ground chicken breast within the microbiological limits established by the Brazilian legislation, for up to 28 days under refrigeration.

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