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■ Keywords

Poultry production, vacuum preservation, storage period, ionizing radiation.

Chicken Meat Submitted to Gamma Radiation and Packed with or without Oxygen

ABSTRACT

The objective of this study was to evaluate the effects on gamma radiation levels on the physical and microbiological characteristics of chicken breast meat. A completely randomized experimental design in a 4x2x3 factorial arrangement was adopted. Treatments consisted of four radiation concentrations (0, 2, 4, or 8kGy), two package sealing methods (with or without vacuum), and three storage times (01, 07, or 14 days), with ten replicates each, totaling 240 chicken breast fillets. Packaging and radiation had no influence ($p>0.05$) on chicken breast meat pH, water retention capacity, or presence of *Salmonella* spp. Breast fillets not submitted to radiation and vacuum packed presented higher water retention capacity ($p<0.05$) than those radiated at 4kGy and vacuum packed. Drip loss in fillets radiated at 8kGy and not vacuum packed was higher ($p<0.05$) than in non-radiated and non-vacuum packed fillets; however, both were not different from the other treatments. Coliform presence increased with storage time in non-radiated samples; however, when these were vacuum-packed, their development was slower. The results of the present experiment suggest that the use of a low radiation dose (2kGy), combined with vacuum packing, may minimize the harmful effects of storage on chicken breast fillets.

INTRODUCTION

Brazil produced 12.7 million tons of chicken meat in 2012, and increased its exports in around one million tons between 2004 and 2012 (Anuário da Avicultura Industrial Brasileira, 2013).

Therefore, products with consistent quality and safety standards should be offered to the consumers to maintain this market position. In order to keep our competitiveness and expansion in the chicken meat market, storage and preservation methods must be improved, supplying meat with adequate quality standards, long shelf life and widely accepted by the consumers.

Microorganism growth and enzyme activity are the main factors that limit chicken meat shelf life, which may be extended, however, if protected by packages and submitted to decontamination.

Radiation is a very interesting technology, as it efficiently reduces most of the problems caused particularly by microbial spoilage and by the use of chemical substances that pose potential health risks, thereby reducing the risks of foodborne disease outbreaks.

In this context, the aim of this study was to evaluate the effects on gamma radiation levels on the physical and microbiological characteristics of chicken breast meat.



THEORETICAL BACKGROUND

Chicken meat quality

Due to the need of supplying safe products to final consumers, technologies that increase food shelf life have become increasingly important, including radiation. Consumers are currently more demanding when choosing food products, and are interested in new technologies. According to Ornellas *et al.* (2006), the lack of accurate information limits the expansion of food radiation.

Radiation

Food preservation using radiation consists in treating food products with electromagnetic energy with the aim of preserving those products by reducing or eliminating their microbial load, particularly of microbes that are harmful to human health.

The advantages of gamma radiation relative to other methods used to destroy bacteria in foods are its high energy content, extensive penetration, and lethality, because it acts at cell level. Its penetration is immediate, uniform, and deep (Baptista *et al.*, 2014). Gamma rays are short-length waves, similar to ultraviolet light (UV) and to microwaves, and are produced by radioactive isotopes, such as cobalt 60 (^{60}Co) and cesium 137 (^{137}Cs) (Spolaore *et al.*, 2001).

When ionizing radiation penetrates the food, part of its energy is absorbed, and the quantity of radiation that penetrates the exposed product mass is called "absorbed dose." Radiation doses are expressed in kiloGrays (kGy) or Grays (Gy), a unit corresponding to the absorption of one Joule of energy per kg of matter (Torgby-Tetteh *et al.*, 2014). Depending on the ionizing radiation dose to which food is submitted, the process is called radurization, radication, or radappertization.

Radurization is considered similar to pasteurization, as it reduces the counts of viable spoilage microorganisms. In this case, other preservation methods also need to be used, such as refrigeration. Radiation doses between 0.4 and 2.5kGy are applied. Radurization can be used to prevent bulb and tuber sprouting, delay fruit maturation, prevent fungal spoilage of fruits and vegetables, and to control insect and mite infestation (Gallas *et al.*, 2009; Franco & Landgraf, 2005).

Radication is the treatment of foods with an ionizing energy dose sufficient to reduce viable and non-spore forming pathogenic bacteria to numbers below those detected by bacteriological analysis methods. This dose also inactivates parasites possibly present in foods. The doses used for radication are typically between 2

and 8kGy (IAEA, 2002). This method is applied in fruit juices, fresh meat, and fresh pasta.

Radappertization or sterilization is the treatment of foods with an ionizing energy dose sufficient to prevent decomposition and toxicity of microbial origin, independently of storage time and conditions, provided the food was not previously contaminated. The doses used are generally between 25 and 45kGy (IAEA, 2002). This method is applied by the US space agency NASA in meat products (CNEA, 2011), such as chicken and turkey breasts. Foods submitted to this method do not have an expiration date, even under room temperature, as long as the package is intact. The Laboratory of Food Radiation and Radioentomology of the Brazilian Council of Atomic Energy (CNEA) has researched this technique for the preservation of chicken meat.

According to Mantilla *et al.* (2012a), the application of doses that are considered low, i.e., lower than 10kGy, aim at extending food shelf life by reducing the microbial population initially present in the product and are used for fresh meat, particularly for poultry products.

The radiation doses recommended are 3 to 5kGy for frozen chicken meat and 1.5kGy for refrigerated meat. These doses are effective for the reduction of pathogens, such as *Salmonella* spp (Arzina *et al.*, 2012; Henriques *et al.*, 2013). The *Codex Alimentarius* (2010) states that the radiation dose applied should be sufficient to increase the product shelf life and to eliminate pathogenic microorganisms, especially *Salmonella*. The recommended dose to extend the shelf life and to promote decontamination of chicken meat is 7kGy (Sarwar *et al.*, 2014).

In the USA, minimum and maximum doses of 1.5 and 3 kGy, respectively, are established for chicken meat. Brazilian legislation follows the international recommendations of the United Nations bodies Food and Agriculture Organization (FAO), International Atomic Energy Agency (IAEA), and *Codex Alimentarius*. The current regulations for the application of radiation in foods are described in Resolution n. 21 (BRASIL, 2001). This resolution establishes that any food product may be radiated, provided minimum and maximum dose limits are complied with. The minimum dose must be sufficient to achieve the intended purpose, and the maximum dose must be lower than the dose that may compromise the functional properties or sensorial attributes of that food.

The radiation process is influenced both by external factors (temperature, presence or absence of oxygen, subsequent storage conditions) and by inherent food



factors (physical state, density, moisture content, etc.). This is why there are specific procedures, including different radiation doses, for each product (Cena, 2006; Mahapatra, 2005).

Vacuum packing

In vacuum packing, air is removed from gas-impermeable packages, which are immediately sealed. During storage of vacuum-packed foods, carbon dioxide (CO₂) level increases as a result of tissue and microorganism respiration (Gallas *et al.*, 2009). According to Gallas *et al.* (2009), CO₂ is produced by microorganisms, because tissue anaerobic metabolism produces mainly lactic acid. In this packing technique, carbon dioxide (CO₂) concentration rapidly increases in 10 to 20% during the first four hours, reaching a maximum level of approximately 30%. At the same time, oxygen level is reduced to 1-3% due to the activity of meat enzymes. This modified gas environment hinders the development of rapidly-growing mesophilic heterotrophic aerobes and stimulates the growth of slow-growing lactobacilli. The shelf life of vacuum-packed meats in gas-impermeable packages is much longer compared with meats packed in the presence of atmospheric air (Oluwafemi, 2013).

The lack of oxygen inhibits spoilage, consequently enhancing product quality and shelf life. However, vacuum-packed food will eventually be spoiled by the presence of anaerobic and microaerophilic microorganisms and due to non-oxidative reactions, which may be minimized by cold storage. Also, compression is unavoidable, which makes vacuum-packing inadequate for many products (Novaes, 2009).

MATERIALS AND METHODS

Location

A total number of 240 skinless and deboned chicken breasts were acquired from a processing plant.

Chicken breasts were radiated at Companhia Brasileira de Esterilização, located in Jarinu, SP, Brazil. The meat was then transported in insulated boxes to the Food Analysis Laboratory of Universidade José do Rosário Vellano (UNIFENAS/FETA), Alfenas, MG, Brazil, where it was submitted to physical and microbiological analyses.

Experimental design

A completely randomized (4x2x3) experimental design was adopted. Treatments consisted of four radiation concentrations (0, 2, 4, or 8kGy), two

package sealing methods (with or without vacuum), and three storage times (01, 07, or 14 days), with ten replicates each, totaling 240 chicken breast fillets.

Meat radiation

Samples weighing 100g were collected from all replicates. Samples were individually placed in plastic bags, and half was packed using vacuum and half without vacuum.

Packed samples were maintained in insulated bags with dry ice until radiation. Meat samples were submitted to the following radiation doses: 0kGy (not radiated), 2kGy, 4kGy, and 8kGy. Samples were then maintained in a cold chamber at 2°C until analyses after one, seven, and 14 days of storage.

Evaluated parameters

Before analyses, half (five) of the samples of each replicate was weighed to determine meat physical characteristics and the other half was submitted to the Microbiology Laboratory.

Physical parameters

Drip loss, pH, water holding capacity and water absorption capacity. Drip loss was calculated as the percentage of sample weight loss after storage (Zhuang & Savage, 2012). Meat pH was measured using a pHmeter (Sentron, model 1001) coupled to a fine-tip penetration probe (Sentron type LanceFET, model 1074001).

Water holding capacity was measured according to the method described by Kissel (2009), in which pressure is applied (10kg/5min) on the tissue and the water released is measured. Water absorption capacity was determined by adding 90mL distilled water to 30g of ground meat and collecting 35g of the obtained paste, which was centrifuged for 15min at 3000rpm. The supernatant was discarded and the tube was weighed. Water absorption capacity was calculated as:

$$\%WAC = \frac{[(PW-MW)-SW]}{PC} \times 100$$

where PW = paste weight; MW = meat weight in the paste; and SW = supernatant weight (Roça, 1986).

Microbiological analyses

Bacteriological analyses were carried out at the Microorganism Biology and Physiology Laboratory of Universidade José do Rosário Vellano (Unifenas), Alfenas, MG, according to ANVISA resolution RDC n. 12 and as described by Silva *et al.* (2007).

The presence of *Salmonella* spp was tested by homogenizing 25g of meat from each replicate with



225mL of lactose broth, incubating the tubes at 35°C for 24h, and then transferring 1mL of this solution to 9mL tetrathionate brilliant green broth and 1mL to 9mL selenite cystine broth, followed by incubation at 42°C for 24h. Samples were then seeded on to Salmonella-Shigella agar and Hektoen agar plates. Suspect typical colonies were seeded on triple sugar iron and lysine iron agar slants, which were incubated at 35°C for 24h. *Salmonella*-suspected colonies were submitted to biochemical tests (VM, VP, and indole broth and Simmons citrate agar), according to Silva, Junqueira, and Silveira (2007).

Total coliforms were determined using the most probable number (MPN) index. Samples were homogenized and serially diluted in lactose broth at 10^{-1} to 10^{-3} . One mL of each dilution was inoculated, in triplicate, in lauryl sulfate triptose (LST) broth and incubated at 35°C for 24h. Presumably positive samples, i.e., those producing turbidity and gas in Durham tubes, were plated for confirmation in brilliant green bile broth at 2% and incubated at 35°C for 24h.

Fecal coliform MPN were determined by reseeded LST-positive samples in EC (*E. coli*) broth in triplicate per dilution. Samples were incubated at 45°C for 24h, and then submitted to the following biochemical tests: indole, methyl red, Voges-Proskauer, and citrate.

Statistical analyses

Data were analyzed using the General Linear Model (GLM) procedures of SAS statistical package (SAS Institute, 2001), and means were compared by the test of Tukey-Kramer.

RESULTS

There was no influence ($p>0.05$) of radiation or packing method (with or without vacuum) on drip loss, pH, water holding capacity, or water absorption capacity of the evaluated chicken breast meat samples after one day of storage (Table 1).

There was no influence ($p>0.05$) of radiation or packing method (with or without vacuum) on drip loss, pH, or water holding capacity of the evaluated chicken breast meat after seven days of storage (Table 2). The chicken breast fillets that were not radiated and vacuum-packed absorbed more water ($p<0.05$) than those radiated at 4kGy and vacuum-packed; however, they were not different from the other treatments.

There was no influence ($p>0.05$) of radiation or packing method (with or without vacuum) on pH, water holding capacity, or water absorption capacity of the evaluated chicken breast meat after 14 days of storage (Table 3). Drip loss in fillets radiated with 8kGy and not vacuum packed was lower ($p<0.05$) than in the fillets that were not radiated and not vacuum packed; however, neither were statistically different from the other treatments.

Chouliara *et al.* (2008) mentioned that chicken meat radiated at doses higher than 4kGy present higher drip loss. However, in the present study it was observed that drip loss was lower in the samples treated at higher radiation doses after 14 days of storage. Xiao *et al.* (2011) obtained lower water holding capacity when chicken thighs were treated with radiation. Damage to the structural integrity of the muscle fiber membrane causes meat to reduce its water holding

Table 1 – Effect of gamma radiation and package-sealing method (with or without vacuum) on the physical characteristics of chicken breast meat after one day of storage.

Treatment	DL	WAC	pH	WHC
0kGy radiation + vacuum packing	3.43	29.32	5.75	83.54
2kGy radiation + vacuum packing	3.39	29.68	5.83	86.55
4kGy radiation + vacuum packing	3.41	29.60	5.68	83.84
8kGy radiation + vacuum packing	3.40	30.67	5.72	85.76
0kGy radiation + packing with no vacuum	3.37	30.80	5.79	87.08
2kGy radiation + packing with no vacuum	3.37	30.90	5.70	85.14
4kGy radiation + packing with no vacuum	3.35	28.20	5.79	84.85
8kGy radiation + packing with no vacuum	3.36	29.10	5.83	87.00
Mean	3.43	29.78	5.76	85.45
CV	1.33	4.63	1.35	4.72

Gray (Gy) or kiloGray (kGy): measurement unit in which one Gray is equivalent to one Joule of energy per kilogram of radiated food. Means followed by different letters in the same column are significantly different ($p<0.05$). DL = drip loss; WAC = water absorption capacity; WHC = water holding capacity.



Table 2 – Effect of gamma radiation and package-sealing method (with or without vacuum) on the physical characteristics of chicken breast meat after seven days of storage.

Treatment	DL	WAC	pH	WHC
0kGy radiation + vacuum packing	11.34	31.02a	5.90	95.12
2kGy radiation + vacuum packing	11.78	30.26ab	5.87	93.34
4kGy radiation + vacuum packing	13.80	28.94b	5.79	99.80
8kGy radiation + vacuum packing	12.40	30.58ab	5.94	97.60
0kGy radiation + packing with no vacuum	16.62	30.74ab	5.89	95.42
2kGy radiation + packing with no vacuum	13.34	30.72ab	5.88	97.90
4kGy radiation + packing with no vacuum	15.62	30.28ab	5.84	97.86
8kGy radiation + packing with no vacuum	11.52	29.92ab	5.80	92.56
Mean	13.30	30.31	5.87	95.95
CV	4.88	3.19	1.94	5.48

Gray (Gy) or kiloGray (kGy): measurement unit in which one Gray is equivalent to one Joule of energy per kilogram of radiated food. Means followed by different letters in the same column are significantly different ($p < 0.05$). DL = drip loss; WAC = water absorption capacity; WHC = water holding capacity.

capacity (Petracci & Cavani, 2012; Ahn, 2013). For instance, Leonel (2008) radiated chicken meat with doses up to 3kGy and did not observe any effect on meat water holding capacity; however, it was reduced up to four months of storage and increased again after six months. Damage to the structural integrity of muscle fibers may also reduce meat water absorption capacity, as observed in the vacuum-packed chicken breast meat radiated with 4kGy and stored for seven days compared with the non-radiated and vacuum-packed samples after the same storage period.

Meat pH was not different among treatments, probably because the period of *rigor mortis* was already completed when samples were collected.

Meat pH lowers *post mortem* due to the breakdown of glycogen, producing lactic acid, which accumulates in the muscle, thereby reducing its pH. Leonel (2008) also did not observe any effect of radiation on chicken meat pH.

The presence of *Salmonella* spp was not detected in none of the 12g samples after 14 days of storage. The lack of detection of *Salmonella* in the present study is probably due to the absence of *Salmonella* in the meat batches collected in the processing plant. In the study of Al-Bachir *et al.* (2010), 4kGy radiation was sufficient to achieve microbiological level accepted by the official authorities. However, in another study with ground chicken breast meat, the best microbiological

Table 3 – Effect of gamma radiation and package-sealing method (with or without vacuum) on the physical characteristics of chicken breast meat after 14 days of storage.

Treatment	DL	WAC	pH	WHC
0kGy radiation + vacuum packing	14.70ab	30.40	5.79	96.40
2kGy radiation + vacuum packing	13.02ab	29.48	5.74	95.26
4kGy radiation + vacuum packing	13.06ab	29.38	5.70	99.86
8kGy radiation + vacuum packing	12.01ab	30.00	5.71	95.88
0kGy radiation + packing with no vacuum	17.54a	30.88	5.70	97.54
2kGy radiation + packing with no vacuum	14.42ab	31.06	5.89	95.30
4kGy radiation + packing with no vacuum	15.42ab	30.58	5.76	97.92
8kGy radiation + packing with no vacuum	9.08b	29.70	5.87	93.80
Mean	13.68	30.19	5.77	96.51
CV	3.20	4.48	2.84	3.69

Gray (Gy) or kiloGray (kGy): measurement unit in which one Gray is equivalent to one Joule of energy per kilogram of radiated food. Means followed by different letters in the same column are significantly different ($p < 0.05$). DL = drip loss; WAC = water absorption capacity; WHC = water holding capacity.



Table 4 – Effect of gamma radiation and package-sealing method (with or without vacuum) on total and fecal coliform counts (MPN) in chicken breast meat after 14 days of storage.

Treatment	Total coliforms (MPN/g)	Fecal coliforms (MPN/g)
1 day of storage		
0kGy radiation + vacuum packing	8.1	8.1
2kGy radiation + vacuum packing	0	0
4kGy radiation + vacuum packing	0	0
8kGy radiation + vacuum packing	0	0
0kGy radiation + packing with no vacuum	7.8	7.8
2kGy radiation + packing with no vacuum	0	0
4kGy radiation + packing with no vacuum	0	0
8kGy radiation + packing with no vacuum	0	0
7 days of storage		
0kGy radiation + vacuum packing	14.3	14.3
2kGy radiation + vacuum packing	0	0
4kGy radiation + vacuum packing	0	0
8kGy radiation + vacuum packing	0	0
0kGy radiation + packing with no vacuum	15.2	15.2
2kGy radiation + packing with no vacuum	0	0
4kGy radiation + packing with no vacuum	0	0
8kGy radiation + packing with no vacuum	0	0
14 days of storage		
0kGy radiation + vacuum packing	16.8	16.8
2kGy radiation + vacuum packing	0	0
4kGy radiation + vacuum packing	0	0
8kGy radiation + vacuum packing	0	0
0kGy radiation + packing with no vacuum	18.5	18.5
2kGy radiation + packing with no vacuum	0	0
4kGy radiation + packing with no vacuum	0	0
8kGy radiation + packing with no vacuum	0	0

MPN = most probable number.

results were obtained with 6kGy (Al-Bachir, 2013). These results show that low radiation doses can be efficiently used to control pathogens in chicken meat. The recommended radiation doses for frozen chicken meat are 3-5kGy, and 1.5-2.5kGy for refrigerated meat that will be consumed within a short period of time (Arzina *et al.*, 2012; Henriques *et al.*, 2013)

The results demonstrate the presence of total and fecal coliforms only in the samples that were not radiated, independently of packing method and storage time (Table 4). Therefore all the evaluated radiation doses (2, 4, and 8kGy) were sufficient to eliminate total and fecal coliforms in the meat samples. Some authors (Torgby-Tetteh *et al.*, 2014) state that ionizing radiation destroys microorganisms by damaging their DNA.

Despite being found in non-radiated samples, total and fecal coliform counts were reduced with storage time when samples were vacuum packed (with no oxygen). Microorganism growth and replication are influenced by pH, humidity, temperature, and oxygen, which is required for coliform growth (Mantilla *et al.*, 2012b).

Finally, it should be mentioned that, although all samples belonged to the same batch, those that were not submitted to radiation presented total and fecal coliform contamination. Also, the lowest radiation dose (2kGy) eliminated those microorganisms without any effect on meat physical characteristics. It was also found that vacuum packing in the absence of radiation did not favor total and fecal coliform development due to the absence of oxygen.

CONCLUSION

The results of the present experiment suggest that the use of a low radiation dose (2kGy), combined with vacuum packing, may minimize the harmful effects of storage on chicken breast fillets.

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