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REFRIGERATED POULTRY BREAST FILLETS PACKED IN MODIFIED ATMOSPHERE AND IRRADIATED: BACTERIOLOGICAL EVALUATION, SHELF LIFE AND SENSORY ACCEPTANCE

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ABSTRACT

In the present study the effects on shelf life and sensory acceptance of gamma-irradiated refrigerated poultry breast fillets subjected to modified atmosphere packaging (80% CO_2/20% N_2 or vacuum) were investigated. After irradiation with 2 kGy, sensory acceptance tests and monitoring of bacterial growth were performed in order to determine the sanitary quality of the samples. It has been found that irradiation, used in combination with modified atmosphere packaging, can double the shelf life of refrigerated poultry breast fillets by reducing the populations of aerobic mesophilic and psychrotrophic bacteria, enterobacteria, coliforms, *Listeria* spp. and *Aeromonas* spp., without significantly modifying its color or its overall appearance, the lactic acid bacteria being the most resistant to exposure to radiation and carbon dioxide.

Key words: Irradiation, poultry breast, modified atmosphere, sensory acceptance.

INTRODUCTION

As an important source of proteins, poultry meat has a high biological value and it has been frequently recommended for its nutritious low fat content. However, it is also a highly perishable product that has a relatively short shelf life even when it is kept in refrigeration. Thus, developing more appropriate technologies for conservation of poultry meat still remains a goal that the scientific community has been eagerly pursuing.

Modified atmosphere packaging (MAP) is a method that consists of the removal of air followed by its replacement with a gas or gas mixture, depending on the type of product (23). Three gases are commonly used in food packaging: O_2, N_2 and CO_2. Each has an specific function: O_2 generally stimulates the growth of aerobic bacteria while inhibiting the growth of anaerobic ones; CO_2 is an inhibitor of bacterial growth and fungus; whereas N_2 is used as a filling gas, replacing O_2 as an alternative to vacuum packaging when the product is fragile or when there is a need to prevent the collapse of the package due to the absorption of CO_2 by the product (6, 7).

Researchers in food microbiology have demonstrated that MAP is able to increase the shelf life of various foods although usually not sufficing to effectively eliminate spoilage microorganisms, including the pathogenic species when not used in combination with other processes. On the other hand,

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according to the scientific literature (5, 17), gamma irradiation used in combination with MAP has been efficiently used in the production of safe food.

Irradiation can reduce the populations of spoilage pathogenic microorganisms and sterilize survivors, while MAP suppresses the growth of survivors during storage. Irradiation together with MAP could also act synergistically to eliminate bacteria. In addition, since many lactic acid bacteria are known to produce antimicrobicrobial compounds that have inhibitory effect on the pathogenic microorganisms, their growth in irradiated MAP and vacuum packs during storage could render an additional protection to such products (18, 28).

Gamma radiation is a type of ionizing electromagnetic radiation emitted from radionuclides such as ^{60}Co and ^{137}Cs and exposure to it is one of the cheapest ways food preserve foods (9). The intensity of the effects of ionizing radiation become larger proportionally to the radiation dose, also depending on the radioresistance of the microorganisms (10, 31). Its combination with other processes can turn smaller doses effective to ensure the microbiological stability of the product during distribution, marketing and consumption with lower chances of changes in nutritional and/or sensory characteristics (27).

The aim of this work was to evaluate the combined effect of MAP and low dose gamma radiation on the quality of fresh poultry breast fillet using bacteriological and sensory analyses.

MATERIALS AND METHODS

Packing and irradiation treatments

The experiments were performed in two phases. On the first day (day zero) of the first phase, 5 kg of refrigerated poultry breast fillets were purchased in a market in Niterói, RJ and transported in insulated polystyrene boxes with ice to the Laboratory of Microbiological Control of Animal Products of the Fluminense Federal University.

Poultry samples were aseptically divided in 40 pieces of 18g each and were individually packed in nylon multilayered pouches having a low permeability to oxygen (60 cm^3/ m^2.day). Four groups of samples were tested: 1) control (Air/0kGy), 2) vacuum-packed (Vacuum/0kGy), 3) air-packed and irradiated with 2kGy (Air/2kGy) and 4) vacuum-packed and irradiated with 2 kGy (Vacuum/2kGy). The irradiation process was performed at Centro Tecnológico do Exército by exposing the samples to a ^{137}Cs gamma source to a 2 kGy dose.

In the second phase of experiments, fillet samples were obtained in the same conditions as in phase 1 out of 2 kg of fresh poultry breast. The samples were aseptically divided in 20 pieces of 18g and were individually packed in nylon-polibarrier pouches. However different packing atmosphere was tested, yielding two sets of samples: 1) modified atmosphere packed (MAP /0kGy) and 2) modified atmosphere packed and irradiated with 2 kGy (MAP /2kGy). The gas mixture used was 80% CO_2 and 20% N_2.

Bacteriological analyses

The samples were stored at 1°C ± 1°C during the experiments and bacterial tests were performed on days 1, 3, 5, 7, 9, 12 and 18 of storage. The following growth media and analytical procedures were included: plate count agar (PCA; Merck) for counting of heterotrophic aerobic mesophilic bacteria (HAMB) and heterotrophic aerobic psychrotrophic bacteria (HAPB); Violet Red Bile Glucose Agar (VRBG; Himedia) for counting of enterobacteria; Man, Rogosa and Sharpe Agar (MRS; Himedia) on double layer for lactic acid bacteria (LAB); Oxford *Listeria* Base (Himedia) with *Listeria* Selective Supplement (Oxford Formulation; Oxoid) (SR0140) for counting of *Listeria* spp.; Mac Conkey Agar with *Yersinia* Selective Supplement (SR109; Oxoid) for *Yersinia* spp. and Starch-ampicillin Agar (SA; Himedia) enriched with 1% ampicilin for A*eromonas* spp..

In addition, Merck`s miniaturized methodology (16), as modified by Franco and Mantilla (8), was used for coliform enumeration. It consisted of employing automatic pippetors connected to sterilized pointers for preparation and inoculation of 0.1 mL (100µL) from different dilutions into 1 mL (1000µL)

of Fluorocult selective broth.

Sample preparation required 162 mL of peptonized saline solution at 0.1% for dilution to 10^{-1} followed by homogenization in a stomacher. Serial dilutions to 10^{-6} were then performed and poured onto plates that were then incubated at 35-37°C for 24 to 48 hours, excepting those prepared for counting of PAHB, that were kept in refrigerators at 4° C for 7 to 10 days.

A Quebec-type colony counter provided readings of counts and by inspection of morphological and tinctorial characteristics the identification of the species of bacteria were performed. Such data were then expressed as log CFU/g.

In addition, enumeration readings of coliforms were obtained by using ultraviolet light inside a dark room. The presence of thermotolerant coliforms was confirmed by adding the Kovacs reagent for the indol test. The Most Probable Number (MPN) was determined by using Mac Crady`s table and multiplying the result by 10 in order to account for the fact that inoculation was 10 times smaller than the standard. The data were then converted into log units of the Most Probable Number/g (log MPN/g).

Sensory evaluation

Acceptance tests were used in the sensory evaluations. Samples from the different treatments were randomly submitted to the appreciation of 33 untrained judges for evaluation of color and overall impression according to a nine-point hedonic scale, 9 corresponding to ‘‘disliked extremely’’ and 1 ‘‘liked extremely’’. Scores from 1-5 were considered acceptable.

Statistical analysis

Due to the fact that the bacterial populations in the beginning of the two phases were different, normalization to the initial reading of each phase was applied to all data of the corresponding phase so that the bacterial growth during both phases could be compared and described according to the modified Gompertz`s equation (11) by using an specific computer program, DMFIT, based on predictive microbiology (4). The shelf life of meat was then considered equal to the time needed for the counting of heterotrophic aerobic mesophilic bacteria to reach 7 log CFU/g.

The significance of differences between sensory parameters was determined by using the one-way ANOVA test along with Tukey’s post test using the SAS program. Differences at $p < 0,05$ were considered to be statistically significant.

RESULTS AND DISCUSSION

The results are summarized in Table 1, 2, and Figure 1. The initial value of HAMB (day 0) was 5.5 log CFU/g (for samples analyzed on first phase) and 5.8 log CFU/g (for samples packed in MAP). Regarding shelf life extension, the poultry fillets packed in modified atmosphere and irradiated were those that had their shelf life doubled to 10 days, followed by those that were air and vaccum packed that were also irradiated (9 days). Then came the unirradiated samples (MAP and vacuum) (7 days) and finally the unirradiated samples packed in atmospheric air, that had a shelf life of only 5 days at 1 °C ± 1°C. Such results indicate that gamma irradiation potentialized the conservation effects of MAP and efficiently extended the shelf life of the product.

Patsias *et al.* (24) also noted that poultry fillets refrigerated at 4°C MAP-packaged (70% N_2-30% CO_2) reached such limit after 10-12 days. Similarly, Chouliara *et al.* (5) reported an increase in the shelf life of irradiated poultry meat packed in modified atmosphere and, as the count of total bacteria in samples packed in air reached 7 log CFU /g on the 5^{th} and 6^{th} days of storage at 4°C, in agreement with the findings in this work. However, the researchers also found a longer shelf life, equal to 25 days, for fillets in MAP (CO_2/30% 70% N_2) irradiated with 2 kGy. A lower initial microbial load (4.3 log CFU/g) could possibly explain the longer shelf life relatively to the findings in this work since it is

known that the greater the initial population is, the higher the dose required to achieve the same final population of bacteria is (9). In addition, the greater the amount of bacteria present is, the higher the concentration of CO_2 required for reduction of that microbiota becomes (23).

Table 1. Shelf-life and bacterial growth parameters of poultry breast fillets wrapped in air, vacuum and modified atmosphere and irradiated (2 kGy) or not irradiated (0 kGy) and kept at 1 ° C ± 1°C for 18 days.

Treatment (Packing/(Dose)	Shelf Life (days) Based on Limit: 7log CFU/g	Bacterial Growth Parameters	HAMB[a]	HAPB	Ent	CT	CTer	La	Lis	Aero
		Initial count normalized to 1.0 log cycle								
Air / 0 kGy	5	g[b]	0,6	0,5	-	0,4	1,1	1,6	0,5	-
		Lag [c]	1,9	2,2	1,2	2,4	8,9	-	1,4	-
		CF [d]	4	3,9	3	1,1	1,7	2,1	2,4	nd
Vacuum / 0 kGy	7									
		g	0,7	1,2	0,4	0,7	-	5,3	0,2	-
		Lag	3,8	-	5,1	2,8	-	8,7	4,2	-
		CF	3,8	3,7	2	1,0	nd[e]	1,6	1,7	nd
MAP/ 0 kGy	7									
		g	0,5	1,5	1,4	0,4	-	0,5	-	-
		Lag	4,8	4,2	4,7	5,3	-	6,3	-	-
		CF	2	2,6	1,2	0,9	nd	1,4	nd	nd
Air / 2 kGy	9									
		g	0,7	6,5	2,3	1,7	-	0,6	-	-
		Lag	5	-	7,8	-	-	12,4	-	7,7
		CF	3,3	1,6	0,7	1,1	nd	1,8	nd	0,8
Vacuum / 2 kGy	9									
		g	1	1,7	1,7	1,7	-	0,9	-	0,8
		Lag	4,4	1,8	3,8	6,9	-	2,3	-	7,7
		CF	2,7	1	0,5	0,7	nd	2,4	nd	0,7
MAP/ 2 kGy	10									
		g	0,6	0,6	-	-	-	0,4	-	-
		Lag	3,3	9,4	-	-	-	4,1	-	-
		CF	1,7	2,6	nd	nd	nd	2,6	nd	nd

[a] HAMB: Mesophilics; HAPB: Psicrotrophic;Ent:*Enterobacteriaceae;* CT: Totals coliforms; CTer: Thermotolerant Coliforms; La:Lactic acid bacteria; Lis: *Listeria* spp.; Aero: *Aeromonas* spp.
[b] g : Doubling time (days).
[c] Lag: Adaptation phase (days).
[d] CF: Final Count (log CFU/g and log MPN/g for coliforms
[e] nd: Not detected count.

Table 2. Results of poultry fillets color and overall impression in the different treatments (Tukey analysis)

Color		Overall impression	
Treatments	Mean*	Treatments	Mean*
MAP/0kGy	5,24[a]	Vacuum/0kGy	5,76[a]
Vacuum/0kGy	5,0 [ab]	MAP/0kGy	5,24 [ab]
Air/0kGy	4,7 [abc]	Air/0kGy	4,81 [ab]
Vacuum/2kGy	3,67 [cd]	Vacuum/2kGy	4,57 [ab]
MAP/2kGy	3,45 [d]	MAP/2kGy	3,91 [b]
Air/2kGy	3,36 [d]	Air/2kGy	3,67 [b]

*Means followed by same letters do not differ by Tukey test at 5% probability.

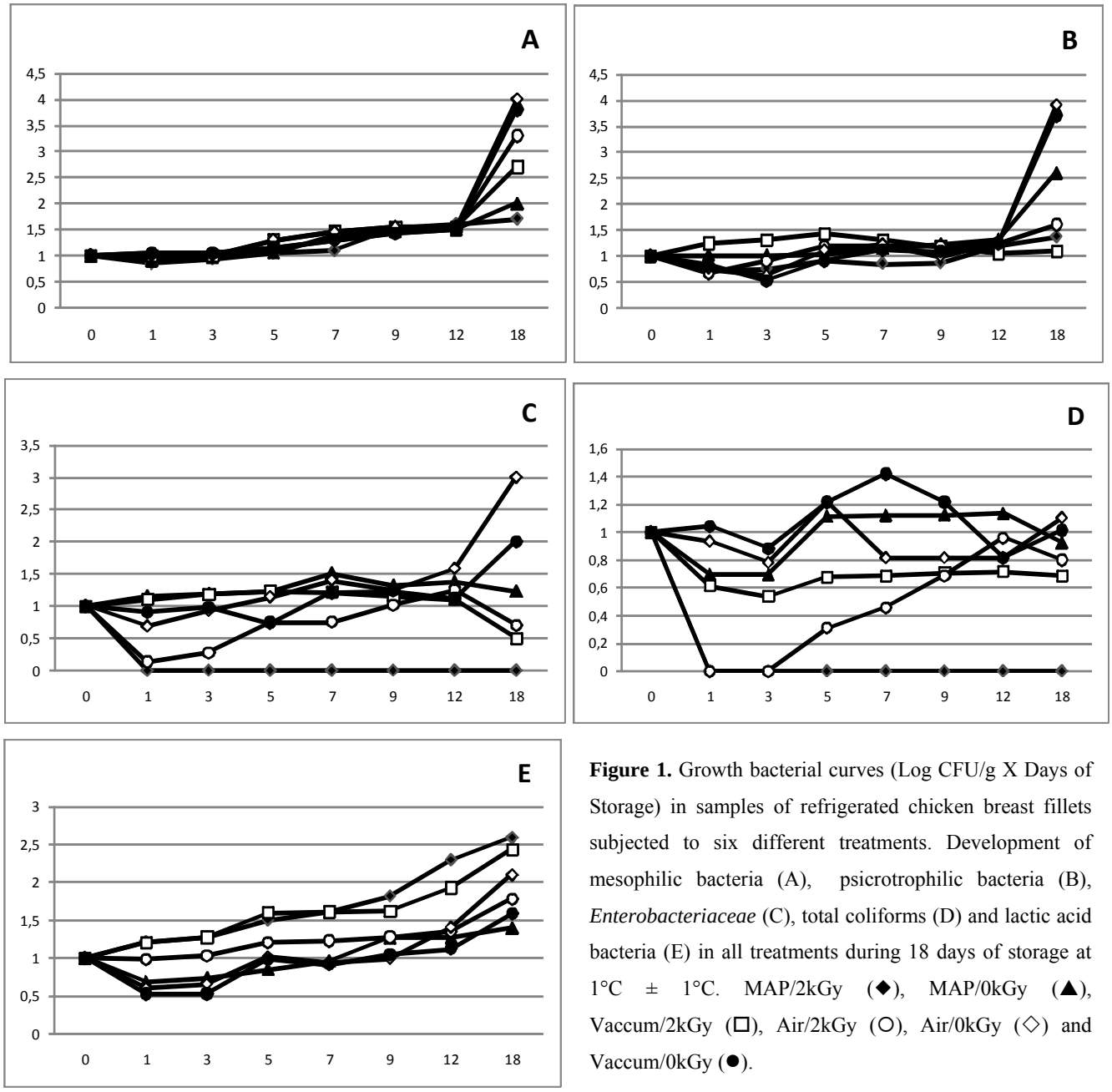

Figure 1. Growth bacterial curves (Log CFU/g X Days of Storage) in samples of refrigerated chicken breast fillets subjected to six different treatments. Development of mesophilic bacteria (A), psicrotrophilic bacteria (B), *Enterobacteriaceae* (C), total coliforms (D) and lactic acid bacteria (E) in all treatments during 18 days of storage at 1°C ± 1°C. MAP/2kGy (◆), MAP/0kGy (▲), Vaccum/2kGy (□), Air/2kGy (○), Air/0kGy (◇) and Vaccum/0kGy (●).

According to the data from this work, the yielded the longest lag phase for mesophilic bacteria was found for air packed samples treated with 2 kGy that had its population reduced by 2.3 log cycles. That finding is in agreement with those from similar works, made by Lescano (13) and Thayer (30).

Although extending the shelf life, such treatment yielded a high population of mesophilic bacteria at the end of the experiment, possibly due to the growth of more radioresistant species, such as the lactic acid bacteria that are also more resistant to CO_2 when compared with other bacterial groups.

The two methods used together have considerably

followed by MAP unirradiated samples. The final count was higher in the control samples followed by the vacuum-packed ones. The bacterial counts of irradiated samples remained low throughout the storage period when compared with the ones subjected to MAP. Similarly, Miyagusku (17) found that samples of poultry meat packed in vacuum or modified atmosphere (30%N_2/70% CO_2), irradiated with 3.0, 5.0 and 7.0 kGy had a longer lag phase and lower counts for psychrotrophic bacteria throughout the storage period when compared with air-packed unirradiated samples.

There was no detectable growth of enterobacteria in irradiated samples treated with MAP. In addition, their longest lag phase was found for irradiated air-packaged fillets. Such result was expected because enterobacteria are very sensitive to irradiation according to several authors (2, 17, 5). In addition, the present results are also in agreement with those reportef by Chouliara et al. (5) that enterobacteria grew more slowly under conditions of MAP (30% CO_2/70% N_2 and 70% CO_2/30% N_2) than in aerobiosis.

As no growth of total coliform bacteria was observed in samples packed in modified atmosphere and irradiated, it can be concluded that the combination of both processes did not allow any growth of that bacterial group. Abu-Tarboush *et al.* (1) also found that poultry meat irradiated with 0.5 kGy and stored at 4°C had no coliforms. Irradiated samples packed in air and vaccum were found to display the longest adaptation phase, leading to very low populations at the end of the experiment.

Growth of thermotolerant coliforms was only observed in unirradiated samples packed in air, their countings remaining very low when compared with those for the other bacteria studied. Miyagusku (17) also informed the elimination of *E. coli* in irradiated samples of thigh and breast poultry meat packed in vacuum and modified atmosphere.

LAB prevailed in irradiated samples, while heterotrophic aerobic mesophilic bacteria prevailed in unirradiated samples. However, Ntzimani *et al.* (21) showed that lactic acid bacteria were dominant throughout the storage period, regardless of the packaging of smoked turkey breast stored in air, vacuum and modified atmospheres (30% CO_2/70% N_2) and (50% CO_2/50% N_2) kept at 4 ± 0,5°C for up to 30 days. In this work, a long lag phase was observed for samples packaged in air and irradiated (12.4 days). In contrast, a short lag phase (4.1 days) was found for the irradiated MAP samples, leading to final count of lactic acid bacteria relatively higher. Such results suggest that the combined treatment did not interfere with bacterial adaptation and was not able to eliminate lactic acid bacteria as effectively as for the other bacteria studied. A possible explanation for that is that Gram positive bacteria are generally much more resistant to inhibition by CO_2 and to irradiation than Gram-negative ones (9). Other authors also reported that MAP had a small effect on the population of lactic acid bacteria due to the ability of these facultative anaerobic bacteria to grow under high concentration of CO_2 (24, 3, 5).

A similar result was informed by Miyagusku (17), who found that microaerophilic conditions led to a fast adaptation of lactic acid bacteria due to the vacuum and modified atmosphere packaging that fostered their development. However, Patterson (25) found that the sensitivity of *Lactobacillus* sp. to irradiation was significantly higher when poultry samples were irradiated in CO_2.

Listeria spp. was only detected in unirradiated air- and vacuum-packaged samples. Likewise, Zhu *et al.* (32) showed that irradiation (1.0 to 2.5 kGy) effectively reduced the number of *L. monocytogenes* in vacuum-packaged turkey hams and breast rolls. Samelis *et al.* (26) found that the dose of 4 kGy sufficed to completely eliminate them in frozen beef.

The combination of irradiation and modified atmosphere packaging was effective in the reduction of the population of *Listeria* spp. in refrigerated poultry fillets. The longer adaptation phase in samples packed in vacuum led to a higher number of counts at the end of the experiments, as compared with those packed in air.

No detection of *Yersinia* spp. occurred in the experiments performed in this work. According to Jay (5), pork is the most common source of those pathogens.

Sensory properties (color and overall impression) of raw poultry breast meat are given in Table 2. In relation to the color attribute, it was observed that the treatment Air/2kGy was the one preferred by the judges, but differences were not significant at the 5% level relatively to the MAP/2kGy and Vacuum/ 2kGy treatments. Thus, it can be concluded that packaging in modified atmosphere, used in combination with irradiation, did not significantly affect the acceptance of color. In contrast, the color of the MAP/0kGy samples was rejected, possibly due to the fact that high concentrations of CO_2 can cause poultry meat to become paler, as quoted by Parry (23).

Irradiation of samples packaged in modified atmosphere or in air can cause the color of fillets to become more attractive, probably due to the intensification of a reddish coloration, as cited also by Nanke *et al*. (20), Lewis *et al.* (14), Nam and Ahn (19) and Kim *et al.* (12).

Regarding the scores for the overall impression (Table 2), it was found that the Vacuum/0kGy and MAP/0kGy samples were rejected. The use of CO_2 in high concentrations can cause an increase in the drip of fresh meat according to Church (7), which may have contributed to the rejection by the judges. All the other treatments were accepted, with the highest scores being assigned to the Air/2kGy and MAP/2kGy samples which had similar results.

CONCLUSION

This study shows that the process of irradiation can turn the coloration of the poultry fillets more attractive. Also, a higher radioresistance was observed for the lactic acid bacteria when compared to the other bacterial groups analyzed. In addition, enterobacteria and coliforms were efficiently eliminated by using a combination of packaging in high concentrations of CO_2 (80%) and irradiation. Thus it can be concluded that the combined use of modified atmosphere packaging (80% CO_2 and 20% N_2) and gamma irradiation at a dose of 2 kGy can significantly improve the microbiological safety of refrigerated poultry fillet, doubling its shelf life, without changing its sensory properties. Further studies should be conducted in order to determine the optimum dose and the ideal mixture of gases that would render the poultry fillets the most attractive quality and safety characteristics possible, as well as the longest shelf life achievable.

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