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Irradiated vacuum-packed lamb meat stored under refrigeration: Microbiology, physicochemical stability and sensory acceptance

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ABSTRACT

Reducing spoilage and indicator bacteria is important for microbiological stability in meat and meat products. The objective was to evaluate the effect of different doses of gamma radiation on the shelf-life of lamb meat, vacuum-packed and stored under refrigeration, by assessing the microbiological safety, physicochemical stability and sensory quality. Lamb loin cuts (*Longissimus dorsi*) were irradiated with 1.5 kGy and 3.0 kGy. The samples, including control, were stored at 1 ± 1 °C during 56 days. Samples were analyzed on zero, 14, 28, 42 and 56 days by their microbiological and physicochemical characteristics. Sensory quality was carried out on day zero. The results showed a reduction ($p < 0.05$) in the microbial load of the irradiated samples. The acceptance of lamb loins was not affected ($p > 0.05$) by the radiation doses. Thus gamma irradiation at 3.0 kGy was effective in reducing the content of microorganisms, without harming the physicochemical characteristics evaluated.

1. Introduction

The Brazilian agribusiness has great potential to develop lamb production due to the increasing demand for lamb meat (Leão et al., 2012). Despite a progressive increase in consumption, lamb meat is marketed mainly as frozen cuts, especially in the state of São Paulo, Brazil. To meet the increasing demand for convenience products, an alternative would be to offer the lamb meat as chilled cuts (Fernandes et al., 2012).

However, chilled meat has a much shorter shelf life than frozen meat, which could represent serious problems in product distribution. There are three main factors that reduce the shelf life of meat, including lamb. The most important is microbial growth, which can affect not only the color, but also the safety of the meat. The other two factors are the oxidative stress effects on myoglobin, which cause color deterioration and lipid oxidation, leading to rancidity. All these factors contribute to additional side effects, such as the formation of undesirable odors and flavors (Duong et al., 2008).

As an attempt to avoid those problems, gamma radiation can be applied to reduce the count of spoilage microorganisms and extend the shelf life of meat and meat products during refrigerated storage (Hui, 2001). Treating food using ionizing energy is a well-known process that focuses primarily on improvement of safety for a wide range of products, extending its useful life (Arvanitoyannis, 2010; Diehl, 2002; Farkas, 1998, 2006; Stefanova, Toshkov, Vasilev, Vassilev, & Marekov, 2011). When biological materials are exposed to gamma irradiation, the atoms/molecules of the material eject electrons, producing ions and free radicals. Free radicals are produced when a molecule is split into two atoms each, retaining its respective electrons. They can damage DNA in fast growing cells (bacteria, fungi, insect eggs, parasite larvae and sprouting vegetables) causing defects in the genetic instructions. The effects of ionizing radiation on living organisms depend on the total dose absorbed, the rate of absorption and the environmental conditions (temperature, atmospheric gases) during irradiation (Brewer, 2004).

The advantages of ionizing radiation for food preservation include the high efficiency on bacterial inactivation, the unaltered chemical composition of the product and the significant thickness of the material, which can be treated after packing in containers (Lawrie & Ledward, 2006; Zhou, Xu, & Liu, 2010). According to the International Commission on Microbiological Specifications for Foods (ICMSF, 1986), the limit for total microbial count for cuts of meat is 10^7 CFU·g^{-1}, since

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higher microbial loads lead to sensory loss due to off-flavors, off-odors and slime. Thus, the application of this technique in vacuum packed meats has been reported by several authors (Ahn et al., 2004; Houser, Sebranek, & Lonergan, 2003; Jo, Lee, & Ahn, 1999; Krízek, Matejková, Vácha, & Dadáková, 2012; Lacroix, Smoragiewicz, Jobin, Latreille, & Krzystyniak, 2000, Zhu, Mendonca, & Ahn, 2004) using doses ranging from zero to 20 kGy in meat and meat products.

According to Aymerich, Picouet, and Monfort (2008), radiation pasteurization (radurization), which refers to the inactivation of non-spore bacteria, with a low absorbed dose requirement (1–10 kGy) is appropriate for foods, including meats. A maximum dosage of 10 kGy represents a low amount of energy (equivalent to that needed to raise water temperature by 2.4 °C); this is why the technology is considered non-thermal, thus preserving the freshness and the nutritional quality of the meat and meat products when compared with thermal methods (Ahn et al., 2004; Aymerich et al., 2008).

The aim of this study was to investigate how different low-doses of gamma radiation affected the shelf life of the vacuum-packed lamb meat when stored under refrigeration, assessing its microbiological and physicochemical stability and sensory quality.

2. Material and methods

The lamb loin samples (*Longissimus dorsi*), weighing from 150 to 250 g, were obtained from a local slaughterhouse, following animal welfare standards and good manufacturing practices, established by the Ministry of Agriculture, Livestock and Food Supply, Brazil (Brasil, 2003). The meat was acquired from various animals of the same breed, race and conditions of breeding and the samples were brought to the laboratory in coolers, packed with ice and took approximately 10 min.

All samples were individually vacuum-packed using 180 × 370 mm multilayer EVA/PVDC plastic bags; 48 to 62 μm thickness; O_2 permeability of 30 $cm^3 \cdot m^{-2} \cdot day$ (1 atm/23 °C/0% RU) and water steam permeability of 10 $g \cdot H_2O \cdot m^{-2} \cdot day$ (1 atm/38 °C/90% RU) (model BB494, CRYOVAC, Jaguariuna, Brazil). The packed meats from all treatments were kept at 1 ± 1 °C for 56 days.

2.1. Irradiation process

The irradiation process was accomplished by a Cobalt-60 irradiator, multipurpose commercial compact, located at the Institute of Nuclear Energy Research (IPEN), in the city of São Paulo, Brazil. A dose rate of 12 $kGy \cdot h^{-1}$ in static mode was used. Samples were packed side by side in coolers to minimize changes in temperature, during the process dosimeters were fixed in the front and back of the coolers. To ensure uniformity of irradiation, an inversion of 0°–180° was carried out. The treatments were zero (control), 1.5 and 3.0 kGy. These low doses were chosen in an attempt to eliminate/decrease microbial proliferation and cause less impact on the physicochemical characteristics and sensory acceptance as a goal at that moment.

2.2. Evaluation of the lamb loins

Microbiological, physicochemical and sensory parameters were evaluated, the three irradiation treatments were assessed at five storage intervals: zero, 14, 28, 42 and 56 days, except the control treatment, which was analyzed only until the 28th day of storage because of microbiological spoilage. Sensory analysis of the three treatments was evaluated only at the beginning of storage (day zero).

2.2.1. Microbiological analysis

The total count of anaerobic psychotropic microorganisms was performed according to Johnston and Tompkin (1992). The presence of *Salmonella* was identified using a rapid pre-enrichment method (AOAC 2003.09). *Staphylococcus aureus* was determined using the AOAC 2003.11 method. Coliforms at 45 °C was determined using the AOAC 998.08 method (Horwitz, Latimer, & Association of Official Analytical Chemistry — AOAC, 2007). Lactic acid bacteria were analyzed as described by Hall, Ledenbach, and Flowers (2001), Kennedy, Buckley, and Kerry (2004) and Lauzurica et al. (2005). Anaerobic mesophilic bacteria were determined as described by Brasil (2003).

2.2.2. Physical and chemical analyses

A portable colorimeter (HunterLab, MiniScan XE, Reston, USA) was used for measuring objective color using the L*, a* and b* scales of the CIELab system. A D65 illuminant was used at an observation angle of 10° and a cell opening of 30 mm. The readings were obtained at three different points, 30 min after the exposure of the samples to the atmosphere.

The pH was measured, in triplicate, using a pH meter (model HI-99163, Hanna Instruments, São Paulo, Brazil) with a combined electrode for perforation of meat. The samples used for both color and pH analyses were assessed for lipid oxidation using the thiobarbituric acid reactive substances (TBARS) assay, according to Vyncke (1970). The results were expressed as milligrams of malonaldehyde (MDA) per kilogram of sample ($mg \cdot kg^{-1}$).

The chemical composition of the samples was measured using the methodology of Horwitz et al. (2007) to measure moisture (950.46), mineral residue (ash) (920.153) and protein (981.10). The lipid content was determined as described by Bligh and Dyer (1959).

The cook loss (CL) was evaluated as described by Koohmaraie (1996). The samples were cooked using an electric oven at 180 °C until the internal (geometric center) temperature reached 72 °C. CL, as a percentage, was determined using the following equation:

$$CL = \left[\frac{\text{Initial weight} - \text{final weight}}{\text{Initial weight}}\right] \cdot 100.$$

After cooking, the samples were cut parallel to the muscle fibers into ten pieces measuring 2 × 1 × 1 cm. The forces required to shear these cuts, in kilograms, were determined using a Warner Bratzler texturometer.

2.2.3. Sensory analyses

Sixty-three consumers were recruited, students, professors and employees of the Faculty of Animal Sciences and Food Engineering, an enjoyment of lamb meat was the only selection criterion. The consumers read and signed a consent form before they performed the tests. An acceptance test using a 9-point hedonic scale for the consumption of cooked samples was performed to evaluate the sensory quality of the samples at time zero.

Tests were conducted in individual booths illuminated by white light, as described by Meilgaard, Civille, and Carr (1991). Samples were cooked, as described in Section 2.2.2, and were stored in an oven at 60 °C for up to 30 min. A randomized complete block design was used and the samples were served to the participants individually, inside disposable plastic cups coded with three-digit numbers. The panelists assessed the aroma, texture, juiciness, flavor and overall quality.

2.3. Statistical analyses

Statistical analyses were performed using Statistical Analysis Software SAS Institute Inc., (2006). The studies were analyzed by contrast means in order to compare treatment groups. A classification of the following contrasts was performed:

Contrast 1

Y_1 = (control) vs (irradiated samples), zero day until 28 days.

Contrast 2

Y_2 = (1.5 kGy) vs (3.0 kGy), zero day until 28 days.

Contrast 3

Y_3 = (1.5 kGy) vs (3.0 kGy), 42 days until 56 days.

With this classification, it was possible to analyze the effect of time, treatment, as well as the interaction between them. After that, the analysis of variance (ANOVA) was performed for each contrast of each variable, in order to distinguish the samples. Three replicates were performed for each treatment at each storage time.

3. Results and discussion

3.1. Chemical composition

The chemical composition of the samples is shown in Fig. 1. The values are close to those found in several studies, which reported values ranging from 74.05% to 76.2% for moisture, 18.85% to 20.6% for protein, 1.08% to 1.15% for ash and 2.1% to 2.4% for lipids (Bonagurio, Pérez, Furusho-Garcia, Santos, & Lima, 2004; Pinheiro et al., 2008; Zapata et al., 2011; Zeola, Silva-Sobrinho, Neto, & Marques, 2004).

3.2. Microbiological stability

Salmonella sp. was not detected in any sample, indicating agreement with the Brazilian legislation (Brazil, 2001). The limits for *S. aureus* and coliforms at 45 °C, established by the Brazilian legislation, are below 10^4 CFU·g^{-1} and 5×10^3 CFU·g^{-1}, respectively. Therefore, samples from all treatments met the limits, since the counts during storage were always <10 est. CFU·g^{-1} of sample. Similar results were found by Fernandes et al. (2012), in vacuum packed lamb loin during storage at 4 ± 1 °C.

Results for lactic acid bacteria, mesophiles and psychotropics are presented in Table 1. For all microorganisms there were decreased initial counts for irradiated samples compared with control. This was expected because gamma radiation is widely known to reduce initial microbial counts. The differences between the control and 3.0 kGy samples was 3.74 log cycles for lactic acid bacteria, 4.0 log cycles for mesophilic anaerobes and 3.58 log cycles for psychotropic anaerobes. Regarding mesophilic anaerobes, a difference ($p < 0.0001$) for treatment effect in contrast 1 (control versus irradiated samples) was observed. There was a significant difference ($p = 0.039$) for contrast 2 as the 1.5 kGy and 3.0 kGy samples presented differences in microbial growth during the 28 days of storage. However, these differences weren't noticed in contrast 3, between the irradiated samples.

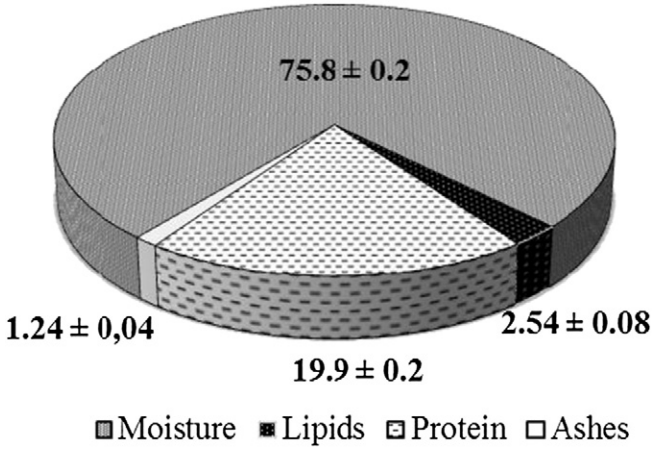

Fig. 1. Composition of meat. Means are expressed as percentages, followed by standard error for the different components (moisture, lipids, protein and ashes) of meat.

Table 1
Microbial load for control and irradiated meat.

Microorganism	Time (days)	Irradiation doses		
		Control	1.5 kGy	3.0 kGy
Lactic acid bacteria (log(CFU·g^{-1}))	0	5.82 ± 1.12^{aA}	2.42 ± 1.98^{bA}	2.08 ± 1.71^{bA}
	14	7.80 ± 0.84^{aB}	4.42 ± 1.84^{bB}	2.67 ± 2.32^{bB}
	28	8.50 ± 0.76^{aC}	6.78 ± 1.11^{bC}	5.43 ± 1.14^{bC}
	42	–	6.69 ± 1.60^{b}	6.52 ± 0.77^{b}
	56	–	6.51 ± 1.62^{b}	5.95 ± 1.22^{b}
Anaerobic mesophilic (log(CFU·g^{-1}))	0	6.33 ± 0.92^{aA}	4.12 ± 1.05^{bA}	2.33 ± 1.90^{cA}
	14	8.06 ± 0.95^{aB}	5.69 ± 0.64^{bB}	2.68 ± 1.41^{cB}
	28	9.05 ± 0.74^{aC}	7.01 ± 1.00^{bC}	5.74 ± 1.05^{cC}
	42	–	6.99 ± 1.42^{b}	6.78 ± 0.92^{b}
	56	–	6.77 ± 1.67^{b}	6.18 ± 1.08^{b}
Anaerobic psychrotrophic (log(CFU·g^{-1}))	0	5.50 ± 1.62^{aA}	2.39 ± 1.95^{bA}	1.92 ± 1.81^{cA}
	14	7.48 ± 0.76^{aB}	5.85 ± 0.38^{bB}	4.05 ± 1.17^{cB}
	28	8.24 ± 0.62^{aC}	6.42 ± 0.73^{bC}	5.45 ± 0.82^{cC}
	42	–	7.33 ± 0.21^{b}	6.36 ± 0.80^{c}
	56	–	7.43 ± 0.31^{b}	6.33 ± 0.57^{c}

Values expressed as means ± standard error. CFU·g^{-1} = colony forming units per gram. Different lowercase letters on the same row indicate significant differences ($p < 0.05$) in treatment and capital letters within the same column indicate significant differences ($p < 0.05$) in time relative to the microorganism.

Similarly, lactic acid bacteria presented a difference ($p < 0.0001$) for treatment effect in contrast 1. However, no significant difference ($p > 0.05$) between irradiated samples throughout storage was found. Considering the time of storage, there was a significant effect ($p = 0.0019$), with microbial growth for all treatments between zero and 28 days. Also, the mesophilic anaerobic, limit of 10^7 CFU·g^{-1} was not reached. For anaerobic psychotropics, contrast 1 presented a significant difference ($p < 0.0001$) between control and irradiated samples. Contrast 2 was also significant ($p = 0.0262$). Moreover, there was a significant effect of time ($p < 0.0001$) as counts increased over time.

Most irradiated samples did not exceed 10^7 CFU·g^{-1}, which is the limit set by ICMSF (1986) as the total microbial count on meat. However, the dose of 1.5 kGy was not enough to fully inhibit the growth of the microorganisms as this limit was reached at 28 days of storage for mesophilic anaerobes and at 42 days for psychotropic anaerobic bacteria.

It can be observed that the non-irradiated lamb loin had a maximum shelf-life of 14 days under chill conditions, presenting counts above 10^7 CFU·g^{-1}. On the other hand, samples irradiated with 3.0 kGy had counts below 10^7 CFU·g^{-1} even after 56 days These results are similar with those found by Park et al. (2010), who concluded that gamma radiation may be more effective for reducing bacterial counts compared to electron beam irradiation for beef burgers.

Table 2
Objective color (L*, a*, b*) for control and irradiated meats.

Color	Time (days)	Irradiation doses		
		Control	1.5 kGy	3.0 kGy
L*	0	41.31 ± 2.67	38.52 ± 2.67	37.00 ± 2.67
	14	37.73 ± 2.18	37.19 ± 2.18	33.38 ± 2.18
	28	37.67 ± 2.18	36.61 ± 2.18	35.53 ± 2.18
	42	–	38.88 ± 2.18	37.95 ± 2.18
	56	–	38.43 ± 2.18	38.56 ± 2.18
a*	0	5.58 ± 2.12	6.96 ± 2.12	6.11 ± 2.12
	14	10.16 ± 1.73	9.35 ± 1.73	9.04 ± 1.73
	28	7.14 ± 1.73	8.20 ± 1.73	8.78 ± 1.73
	42	–	10.46 ± 1.73	8.64 ± 1.73
	56	–	13.19 ± 1.73	9.97 ± 1.73
b*	0	8.90 ± 1.19^{a}	9.79 ± 1.19^{a}	9.22 ± 1.19^{a}
	14	13.25 ± 0.97^{b}	12.21 ± 0.97^{b}	10.17 ± 0.97^{b}
	28	10.48 ± 0.97^{c}	11.66 ± 0.97^{c}	11.28 ± 0.97^{c}
	42	–	13.24 ± 0.97	11.77 ± 0.97
	56	–	12.78 ± 0.97	13.61 ± 0.97

Values expressed as means ± standard error. Different lowercase letters on the same column indicate significant differences ($p < 0.05$) in time of storage.

Table 3
pH, shear-force, cook loss and TBARS[a] for control and irradiated meat.

Analysis	Time (days)	Irradiation doses		
		Control	1.5 kGy	3.0 kGy
pH	0	5.57 ± 0.1[a]	5.56 ± 0.1[a]	5.68 ± 0.1[a]
	14	5.3 ± 0.1[b]	5.35 ± 0.1[b]	5.51 ± 0.1[b]
	28	5.2 ± 0.1[c]	5.13 ± 0.1[c]	5.25 ± 0.1[c]
	42	–	5.13 ± 0.1[c]	5.32 ± 0.1[c]
	56	–	5.15 ± 0.1[c]	5.07 ± 0.1[c]
Shear-force (kg)	0	2.58 ± 0.62	2.34 ± 0.62	2.46 ± 0.62
	14	2.01 ± 0.62	3.22 ± 0.62	2.98 ± 0.62
	28	2.42 ± 0.62	2.31 ± 0.62	2.88 ± 0.62
	42	–	2.47 ± 0.62	3.24 ± 0.62
	56	–	2.37 ± 0.62	2.42 ± 0.62
Cook loss (%)	0	22.6 ± 3.55	25.77 ± 3.55	22.64 ± 3.55
	14	25.65 ± 3.55	27.17 ± 3.55	25.57 ± 3.55
	28	28.77 ± 3.55	23.52 ± 3.55	25.84 ± 3.55
	42	–	28.29 ± 3.55	23.01 ± 3.55
	56	–	24.72 ± 3.55	29.30 ± 3.55
TBARS[a] (mg MDA[b]/ kg sample)	0	0.15 ± 0.05	0.09 ± 0.05	0.09 ± 0.05
	14	0.04 ± 0.05	0.06 ± 0.05	0.03 ± 0.05
	28	0.19 ± 0.05	0.17 ± 0.05	0.17 ± 0.05
	42	–	0.08 ± 0.05	0.05 ± 0.05
	56	–	0.06 ± 0.05	0.04 ± 0.05

Values expressed as means ± standard error. Different letters in the same column indicate significant differences ($p < 0.05$) in time storage.
[a] TBARS = Thiobarbituric acid reactive substances.
[b] MDA = Malondialdehyde.

3.3. Physicochemical stability

The physicochemical parameters are presented in Tables 2 and 3. No significant difference ($p > 0.05$) was observed in most cases indicating that gamma radiation did not cause undesirable changes to the physicochemical characteristics of lamb loin.

The objective colors (L*, a* and b*) were not different ($p > 0.05$) among treatments, indicating that the irradiation did not cause pigment oxidation (Table 2) although b* (yellow) showed an increase over time ($p < 0.05$), up to 28 days, for all samples (Fig. 2).

These results are similar to those obtained by Yang et al. (2011), in which samples of beef hamburgers un-irradiated or irradiated with 2.5 kGy prior to storage at 4 °C for 7 days were evaluated. For the irradiated samples, they reported no significant difference in L*, while a* and b* showed an initial decrease on day zero, and then remained constant. These authors did not detect significant changes in b* during storage, differing from the present study, but reported significant increases in

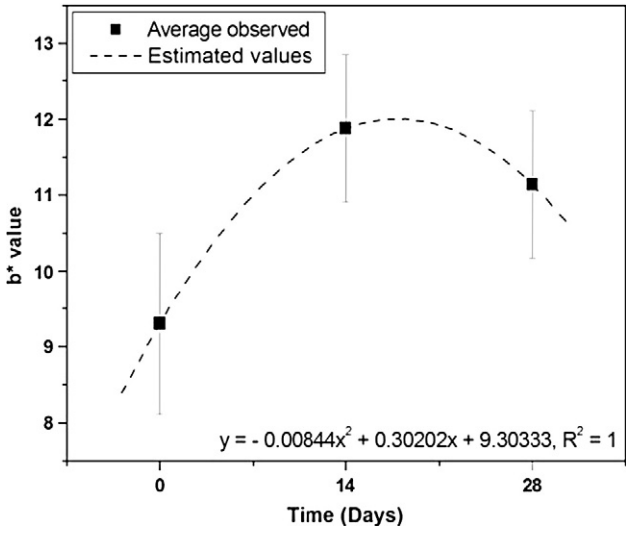

Fig. 2. Evolution of b* value during the 28 days of storage. The dotted line indicates a square regression with the equation in the bottom of the figure with $R^2 = 1$.

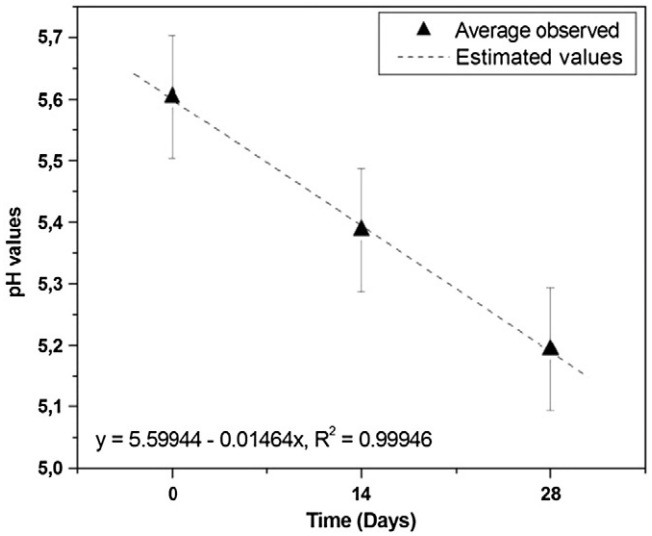

Fig. 3. Evolution of pH meat during the 28 days of storage. The dotted line indicates a linear regression with the equation in the bottom of the figure with $R^2 = 0.99946$.

L*, while a* values remained unchanged over the 7 days. Luciano et al. (2009) concluded that b* increased over 14 days of refrigerated storage in high oxygen atmospheres in the meat of lambs fed a concentrate diet and a diet with added tannins.

There was no significant difference ($p > 0.05$) in pH among treatments, with a mean value of 5.33 (Table 3). All samples showed a significant decrease ($p = 0.0002$) in pH over time (Fig. 3), presumably due the growth of lactic acid bacteria, as their metabolism consumes cellular glycogen and lactic acid is released, causing acidification (Cayré et al., 2003). According to Gonçalves, Zapata, Rodrigues, and Borges (2004), the desirable pH for lamb meat is in the range of 5.4 and 5.9, close to the values found in this experiment.

No differences were observed among treatments ($p > 0.05$) or over storage time ($p > 0.05$) for cook loss and shear force (Table 3), demonstrating that irradiation did not affect those parameters. The average cook loss was 25.60%, close to values found by Fernandes et al. (2012), who obtained a mean value of 24.33% and also found no difference in lamb loin stored at 4 °C under vacuum over 28 days. Similar results were found by Gonçalves et al. (2004), who evaluated the effect of sex and maturation time on lamb *Longissimus dorsi* and *Semimembranosus* muscles, from intact males and castrated males and females, when stored at 2 °C for 14 days. This study reported no significant effect of time, with values ranging from 17.67 to 24.59%. The shear force of the lamb meat did not differ ($p > 0.05$), with mean values close to those reported by Gularte, Treptow, Pouey, and Osório (2000), 2.6 kgf and 2.3 kgf for lamb males and females, respectively.

There were no significant differences ($p > 0.05$) among treatments or throughout storage regarding lipid oxidation (Table 3), showing a mean value of 0.094 mg of MDA per kg of sample. Although gamma radiation is a pro-oxidant factor, the low doses used in this experiment did not cause significant changes which might be related to the low fat content (2.54%) of the samples.

3.4. Sensory acceptance

The results of the acceptance test with 63 panelists are shown in Table 4. No significant difference ($p > 0.05$) was found among samples, thus gamma radiation did not damage the sensory characteristics of the meat.

Park et al. (2010) conducted sensory evaluation of vacuum-packed hamburgers irradiated with doses of 0, 5, 10, 15 and 20 kGy followed by accelerated storage at 30 °C for 10 days. They found no difference ($p > 0.05$) in color, chewiness and flavor for samples irradiated with

Table 4
Sensory acceptance of control and irradiated meat.

Attribute	Irradiation doses		
	Control	1.5 kGy	3.0 kGy
Aroma	6.78 ± 1.34	6.71 ± 1.52	6.89 ± 1.36
Texture	7.40 ± 1.47	7.16 ± 1.55	7.44 ± 1.25
Juiciness	7.43 ± 1.33	7.34 ± 1.41	7.40 ± 1.26
Flavor	7.27 ± 1.37	7.20 ± 1.20	7.20 ± 1.30
Overall quality	7.25 ± 1.29	7.19 ± 1.23	7.25 ± 1.15

Values of the respective attribute, expressed as means ± standard error. No statistically significant differences ($p > 0.05$) were found in these attributes.

gamma rays at 10.0 kGy and overall quality when using 5 kGy. Similarly, Miyagusku, Chen, Leitão, and Baffa (2003) evaluated slices of chicken breast without skin or bone and concluded that 3.0 kGy irradiation increases shelf life from 5 to 22 days without modifying the sensory attributes.

4. Conclusions

A dose of 3.0 kGy extended the shelf-life, from 14 to 56 days, of lamb loin stored at 1 °C, by reducing microorganisms without harming the physicochemical characteristics or consumer acceptance of the meat.

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