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Gamma ray irradiation: A new strategy to increase the shelf life of salt-reduced hot dog wieners

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ABSTRACT

This study aimed to evaluate the efficiency of gamma ray irradiation (1.5, 3.0, and 4.5 kGy) on the microbiological safety and shelf life of sodium-reduced hot dog wieners. The effects of this technology on quality parameters (color, lipid oxidation, texture, and sensory acceptance) were studied. The results showed that irradiation had a significant effect on reducing the red color (a *) of the wieners; higher levels of lipid oxidation were also observed at the initial time of storage (ranging from 0.5 to 1.34 mg malonaldehyde per kilogram of sample) when higher doses were applied. Sensory acceptance was not impaired by higher radiation doses. A significant, dose-dependent reduction of the microbial load was observed. Irradiation at a minimal dose provided up to 6 log cycle reductions of lactic acid bacteria. The application of 1.5 kGy is effective at assuring microbial safety without changing the quality of the product.

1. Introduction

Food irradiation consists of processing food products by ionizing radiation in order to control pathogens, reduce the load of deteriorating microorganisms and insect infestation, inhibit plant germination, and extend the shelf life of perishable products (Lberty, Dickson, Achebe, & Salihu, 2013). Irradiation is known as the most effective technology for food sterilization and can deliver food products for specific uses, including in space, by the military, and for elderly and immuno-compromised patients (Yun et al., 2012).

The main purpose of food irradiation is to destroy microorganisms, thus increasing the shelf life of the products (Harder, Arthur, & Arthur, 2016). The inactivation of undesirable microorganisms in food occurs through chemical changes caused by radiation, such as the breakdown of chemical bonds, which are usually covalent in food. Moreover, irradiation leads to the formation of hydroxyl radicals (• OH), which react with the DNA of the microorganisms, causing loss of their reproductive capacity (Moy, 2005).

These characteristics make irradiation a very useful tool to extend the shelf life of foods prone to deterioration. Meat and meat products are very susceptible to contamination due to their high nutritional value (Ayari et a.,l 2016). This is more critical in salt-reduced meat products. The antimicrobial role of salt (NaCl) is related to its ability to reduce water activity (aw), to damage the semipermeable membrane of bacteria, limit oxygen solubility, affect cellular enzyme activities, or force cells to expend energy to exclude sodium ions (Inguglia, Zhang, Tiwari, Kerry, & Burgess, 2017). Therefore, reduction of the salt concentration can increase the perishability of products and for minimizing that collateral effect irradiation can be used.

However, the destruction of microorganisms is not the only consequence of the application of gamma rays. The hydroxyl radical can also react with other food molecules such as vitamins, fat, and pigments, causing nutritional value losses, lipid oxidation, and color loss (Yu, He, Zeng, Zheng, & Chen, 2016). Therefore, the use of this technology must be studied carefully before application in each particular product to ensure the safety and quality of food.

In 1981 the World Health Organization (WHO, 1981) attested to the safety of the use of radiation in food by stating that the use of an average dose of up to 10 kGy in food poses no toxicological hazard and does not introduce important nutritional or microbiological changes (Brewer,

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2009).

Although it is a safe technology, the irradiation process may cause some sensory changes in the food. Specifically in meat products, the major cause of loss of sensory quality is lipid oxidation (Trindade et al., 2009). According to Ham et al. (2017) the radiation dose and source (X-rays, Gamma rays, and E-beam) increase lipid oxidation in various types of meat products. In the case of cooked beef patties, gamma rays led to higher TBARS values, while in cooked pork sausages, X-rays were responsible for higher levels of oxidation. Harder et al. (2016) explained that irradiation can accelerate lipid autoxidation reactions for the following reasons: the formation of free radicals, which combine with oxygen to form hydroperoxides; the breakdown of hydroperoxides, allowing the action of several decomposition products; and the destruction of antioxidant compounds. Regarding aroma, sulfur compounds are known as the key components in the generation of irradiation odor. However, this odor varies greatly depending on the composition of the volatile compounds present in the samples (Feng, Moon, Lee, & Ahn, 2017).

Due to these changes, the combination of irradiation and other conservation methods has been studied. Irradiation in combination with a modified atmosphere, for instance, came almost as a consequence of the need to study packaging for irradiated products (Ehlermann, 2016). The combination of antimicrobial compounds with low doses of radiation (Dussault, Benoit, & Lacroix, 2012), the irradiation of frozen meat before the processing of a meat product (Chouliara et al., 2006), the effect of marinating together with gamma irradiation (Fadhel et al., 2016), the use of vacuum packaging and refrigeration (Fregonesi et al., 2014), and the combination of irradiation with antioxidants (Hwang et al., 2015; Lim, Seol, Jeon, Jo, & Lee, 2008) are examples of processes that have been investigated.

Also, the radiation dose has a great impact on the characteristics of the final product. Feng et al. (2017) reported that increasing the dose from 0.5 to 4.5 increased lipid oxidation in raw turkey breast. Kim et al. (2012) observed a reduction in red color intensity (CIE a *) in vacuum-packaged dry fermented irradiated sausages with 2 and 4 kGy, when compared with those irradiated with 0.5 and 1 kGy. However, sausages irradiated with 4 kGy showed lower microbial growth and no increase in lipid oxidation during 90 days of storage. Thus, the chosen dose should strike a good balance between effectiveness in controlling microbial development and detrimental effects on sensory and lipid oxidation attributes.

Considering all these facts, and the specificity of the application of radiation in foods in order to find the correct balance between preservation and damage effects, it is clear that there are few studies on the use of this technology in meat products with sodium reduction. This study is a step forward in the search for the development of nutritious, safe and healthy meat foods. Thus, the objective of this study was to evaluate the efficacy of irradiation with different doses of gamma rays on the inactivation of microorganisms in vacuum-packaged hot dog sausages with reduced sodium content, and its impact on physical and chemical stability and sensory acceptance of the product.

2. Materials and methods

2.1. Sausage preparation

The sausage formulations studied in this work are described in Table 1. It is important to consider that in this formulation, sausages also have a 50% reduction in the level of phosphate. Although the focus of this study is the reduction of sodium, we consider that the reduction of phosphate is also a trend that should be consolidated in the coming years, so in this study this reduction has already been considered in the formulation. However, as all formulas had the same phosphate level (0.25 g/100g) this was not a variable in the study.

The sodium reduction formulation studied in this work was previously optimized, in order to find the least amount of salt that can be added to the product without compromising its physical-chemical and sensory qualities. The results of this optimization study are demonstrated by Rodrigues et al. (2020).

Table 1
Formulations of irradiated and non-irradiated hot dog wieners.

Ingredients	F1.25	F2
Ground beef (*Supraspinatus*)	60	60
Pork backfat	10	10
Cassava starch (Yoki, Brazil)	2	2
Salt (NaCl) (Cisne, Brazil)	*1.25*	*2*
Sodium Nitrite (Cori, Brazil)	0,015	0.015
Sodium erythorbate (Cori, Brazil)	0.05	0.05
Sodium tripolyphosphate (Cori, Brazil)	0.25	0.25
Sausage seasoning (New max, Brazil)	0.5	0.5
Carmine	0.05	0.05
Water/Ice	25.89	25.14
Total	**100**	**100**

The formulation F1.25 was used for F0, F1.5, F3.0, and F4.5 treatments.

For sausage production, the ingredients listed in Table 1 were weighed separately and homogenized in a bowl cutter (Trademark Tecmafrig, Sao Paulo, Brazil) in the following order: The meat and salts were initially comminuted for 1 min. Then the pork fat and half of the ice were added, followed by 1 min of chopping. The remaining ice was added, and chopping was continued. Finally, cassava starch was added and mixed for a few more minutes until a homogeneous batter was obtained. Throughout the process the temperature was maintained below 14 °C. The batter was stuffed into cellulosic casings (Viscofan do Brasil, São Paulo, Brazil) with a pneumatic filler (V25 Sirman) and cooked in a smokehouse (SL 218 Arprotec, Valinhos, Brazil) at 60 °C for 30 min (first with dry air and after 15 min steam was added), then 70 °C for 40 min and finally 80 °C until an internal temperature of 72 °C was reached. The sausages were cooled with running water and stored in cold storage for 16 h. The casings were then removed, and the sausages were vacuum packed (High barrier packaging for frozen and chilled meats, Composition: Polyethylene (PE) + Polyvinyl chloride (PVDC) + Ethylene vinyl acetate (EVA), thickness: 39–60 mm, oxygen permeability (23 °C, 0% RH): max 25 $cm^3/m^2/24$ h, Brand: Sealed air, code BB2470, size 120 × 220, Brazil.) and stored under refrigeration (4 °C) for 75 days.

2.2. Sausage treatments and irradiation

After processing and packaging, the reduced-sodium (1.25% NaCl) hot dog sausages were irradiated with three different doses: 1.5, 3.0, and 4.5 kGy, designated as treatments F1.5, F3.0, and F4.5, respectively. Treatments were analyzed over 75 days of refrigerated storage together with two control formulations: sodium-reduced, non-irradiated sausages (F0) and not sodium reduced (2% NaCl), non-irradiated sausages (F2).

Irradiation was performed the day after processing and packaging of the sausages by using the Cobalto-60 irradiator located at the Nuclear Energy Research Institute (IPEN), in Sao Paulo, Brazil, at a rate of 5 kGy h^{-1} in static mode. The whole study was repeated three times.

2.3. Physical and chemical analyses

Sausages were tested for objective color, pH, lipid oxidation, texture, and sensory acceptance, and a microbiological analysis was performed (*Salmonella* sp, coliform thermotolerant, Coagulase-positive staphylococci, sulfite-reducing Clostridia, anaerobic psychrotrophs, and lactic acid bacteria). Descriptions of the methods are as follows:

2.3.1. Color

Six samples were cut in half and kept at room temperature for internal color evaluation. The parameters L * (brightness), a * (green-red), and b * (yellow-blue) of the CIELAB system were measured using a

spectrophotometer (MiniScan XE, HunterLab brand) using D65 with an observation angle of 10°.

2.3.2. pH value

A pH meter (Model HI 99163, Mark HANNA) was used. Two points in each sausage were read in three sausages per treatment (6 replicates).

2.3.3. Lipid oxidation

For lipid oxidation analysis, the TBARS index was evaluated according to the methodology described by Vincke (1970). The sausages were ground in a multiprocessor for 2 min. Then, 5 g were weighed into 50 mL centrifuge tubes, and 25 mL of trichloroacetic acid (7.5 g/100 mL) was added. This mixture was homogenized in an ultra turrex (Turratec TE-102, TECNAL, Brazil) for 2 min and filtered. Then, 5 mL of the filtered sample was placed in the test tubes and mixed with 5 mL thiobarbituric acid (0.02 M). The samples were placed in a thermal bath (Marconi, Brazil) at 98 °C for 40 min. The concentration of thiobarbituric acid reactive substances (TBARS) was calculated from spectrophotometric readings at 538 nm (Biospectro, SP -22, Brazil). The calibration curve was constructed by using a solution of tetraethoxypropane. The results were expressed in milligrams of malonaldehyde per kilogram of sample.

2.3.4. Texture profile analysis

Instrumental texture profiling was performed by using the TAXT2i Texture Analyzer (Stable Micro Systems, Godalming, UK). Sausages were cooked in boiling water for 5 min and cut into cylinders 2 cm high for texture evaluation. Eight samples for each formulation were compressed twice to 50% of their height (strain mode) with an SMS P/20 probe, at a test speed of 2.0 mm/s, with 2 s between compressions. The studied parameters were hardness (g), elasticity (dimensionless), and chewiness (g.mm).

2.4. Microbiological analyses

Counts of lactic acid bacteria (LAB), anaerobic psychrotrophic bacteria, thermotolerant coliforms, coagulase-positive Staphylococci, and sulfite-reducing Clostridia and the presence of *Salmonella* were determined. For LAB plating, DeMan, Rogosa and Sharpe (MRS) (Acumedia, Neogen Corporation) agar was used; plates were incubated at 37 °C for 48 h. For anaerobic psychrotrophic bacteria, the Aerobic Count Plate, 6400 (Petrifilm™ 3M Health Care, St. Paul, MN, USA), was used; plates were incubated at 21 °C for 72 h. Anaerobiosis was maintained by keeping the plates in an anaerobic jar (Probac do Brazil, Sao Paulo, Brazil) with an anaerobiosis generator (Anaerobac, Probac do Brazil, Sao Paulo, Brazil). For thermotolerant coliforms, Coliform count plates (6410, Petrifilm™ 3M Health Care, St. Paul, MN, USA) were utilized. Plates were incubated at 45 °C for 24 h. Coagulase-positive Staphylococci were counted using STX count plates (6490, Petrifilm™ 3M Health Care, St. Paul, MN, USA) and 24 h of incubation at 37 °C. For sulfite-reducing Clostridia, Tryptose Sulphite Cycloserine (TSC) (Sigma) agar was used; plates were incubated at 45 °C for 24 h under anaerobic conditions. For *Salmonella* analysis, the previous enrichment procedure was performed by diluting 25 g of the sample in 225 ml of enriched peptone water (Merck KGaA, Darmstadt, Germany) followed by incubation at 37 °C per 24 h before performing the readings using DuPont Qualicon BAX System equipment. The Bax Kit for Salmonella (DuPont Nutrition and Health, Wilmington, USA) was used to determine the presence of Salmonella by polymerase chain reaction (PCR).

2.5. Sensory tests

An acceptance test was performed by using a nine-point hedonic scale (1, dislike extremely; 2, dislike very much; 3, dislike moderately; 4, dislike slightly; 5, neither like nor dislike; 6, like slightly; 7, like moderately; 8, like very much; and 9, like extremely) to evaluate appearance, aroma, texture, flavor, and overall quality (Meilgaard, Civille, & Carr, 1991). For the sensory test, the sausages were boiled for 5 min, cut into 2-cm pieces, and served monadically to 119 consumers. Each consumer evaluated all five treatments, which were served in completely randomized blocks. Sensory analysis was conducted in the Faculty of Animal Science and Food Engineering at the University of São Paulo, Pirassununga, Brazil. The tests were approved by the local Research Ethics Committee (N° 2.078.895).

2.6. Statistical analysis

Three independent repetitions were conducted in a completely randomized balanced design. For statistical purposes, each repetition was considered a block and the effect of blocks was tested by analysis of variance (ANOVA) in order to verify differences among the replicates. Results were evaluated by ANOVA and Tukey's test, at the 5% level of significance. To perform the data analysis, the plot mixed procedure (SAS 2014. SAS/STAT® 13.2) was used. Both treatments and storage time were considered fixed-effect factors when analyzing physicochemical and microbiological parameters. For sensory analysis, treatments were considered as fixed-effect factors and consumers as random-effect factors (Stanley, Bower, & Sullivan, 2017).

3. Results and discussion

3.1. Lipid oxidation

Irradiated sausages had higher levels of oxidation at the beginning of the storage period (Table 2) than non-irradiated ones. The effect of irradiation on the acceleration of lipid oxidation has been reported in several studies and is related to the generation of hydroxyl radicals, which catalyze oxidation reactions (Feng et al., 2017; Kim et al., 2012; Park et al., 2010). Damage to muscle structure by radicals and the exposure of fatty acids to oxygen has also been related to the increase of TBARS in irradiated products (Cheng et al., 2011). Generally, irradiation causes both the induction of oxidation and its progression during storage; however, this behavior may vary depending on the characteristics of the meat and its products, such as lipid content, fatty acid composition, antioxidants, heme pigments, and oxidation initiators (Cava, Tárrega, Ramírez, & Carrasco, 2009).

In the second week of analysis, a significant decrease in TBARS values was observed, and from then on, the values remained below the maximum acceptable level (1 MDA.kg^{-1}) that could cause a perception of rancidity (Amaral et al., 2015; Das, Anjaneyulu, Gadekar, Singh, & Pragati, 2008). In this study, the fact that TBARS were the highest at initial time can be attributed to the higher oxidation potential of the radiation energy in this period, which results in the formation of a large amount of free radicals in the sausage (Chouliara et al., 2006). After this time, the lipid oxidation rate declined, and the compounds formed previously decreased as a result of the interaction of products reactive to TBA with other constituents of muscle tissue or to the destruction of malonaldehyde by the surviving microflora (Cheng et al., 2011). The increase in the value of TBARS at the beginning of the storage period and the decrease at the end of shelf life is an unusual behavior, but it has also been reported by Kim et al. (2012), in their study on effects of low-level gamma irradiation on fermented pork sausage.

3.2. pH value

Irradiated sausages showed a small pH change at the initial storage time (Table 2). Reduction in pH due to irradiation was also observed by Ham et al. (2017). However, in this case, the reduction occurred on a very small scale, which would not change the quality characteristics of the products. Over the 75 days of storage, a reduction of pH was observed for non-irradiated sausages (F0). For the F2 treatment, a significant drop in pH was observed at the end of the storage period. The

Table 2
Concentration of thiobarbituric acid reactive substances (TBARS) and variation of pH values during 75 days of refrigerated storage for sausages with 1.25% or 2% NaCl submitted to different doses of radiation.

	Time	F0	F1.5	F3.0	F4.5	F2%
pH	Initial	6.18^{aA}	6.03^{bA}	6.03^{bBC}	6.13^{abA}	6.10^{abA}
	15 days	5.76^{bC}	5.97^{aA}	6.00^{aBC}	6.01^{aA}	6.01^{aA}
	30 days	5.64^{bC}	6.01^{aA}	6.06^{aAB}	6.10^{aA}	6.07^{aA}
	45 days	5.59^{aC}	5.92^{aA}	5.80^{aC}	5.92^{aA}	5.92^{aA}
	60 days	5.64^{bC}	6.02^{aA}	6.02^{aBC}	6.10^{aA}	6.05^{aA}
	75 days	5.93^{cB}	6.11^{abA}	6.16^{aA}	6.12^{abA}	5.99^{bcA}
TBARS	Initial	0.6849^{bABC}	0.7937^{abA}	1.0847^{abA}	1.3450^{aA}	0.5196^{bA}
	15 days	0.3976^{cC}	0.5289^{bA}	0.6976^{aAB}	0.6852^{aB}	0.3030^{cA}
	30 days	0.7273^{aAB}	0.5281^{abA}	0.6279^{abABC}	0.7078^{abB}	0.4226^{bA}
	45 days	0.5366^{aBC}	0.4829^{aA}	0.4814^{aC}	0.4747^{aB}	0.2389^{bA}
	60 days	0.5246^{abBC}	0.5549^{abA}	0.6270^{aABC}	0.5000^{abB}	0.3219^{bA}
	75 days	1.0059^{aA}	0.6455^{bA}	0.5720^{bBC}	0.6724^{bB}	0.4173^{bA}

^{abc} - Different lowercase letters on the same line indicate that the samples presented significant differences among treatments at the same study time point ($P \leq 0.05$).
^{ABC} - Different upper case letters in the same column indicate that the samples presented significant differences during the study time ($P \leq 0.05$).
pH and TBARS values are averages of six samples in three replicates (18 measurements)/TBARS: Concentration of thiobarbituric acid reactive substances (mg TBA/kg sample).
F0: Sausage with 1.25% NaCl without irradiation/F1.5: Sausage with 1.25% NaCl irradiated with a dose of 1.5 kGy/F3.0: Sausage with 1.25% NaCl irradiated with 3.0 kGy/F4.5: Sausage with 1.25% NaCl irradiated with a dose of 4.5 kGy/F2: sausage with 2% NaCl, without irradiation.

pH decrease during the storage period can be attributed to the generation of lactic acid during the growth of lactic acid bacteria (Laranjo et al., 2016). These results indicate that sausages that had a reduced salt level or those that were not irradiated showed higher lactic acid generation, due to more rapid growth of lactic acid bacteria. It is known that irradiation and salt addition (NaCl) act to control bacterial growth, contributing to lower lactic acid generation and therefore lower acidity in samples irradiated and without sodium reduction (Fadhel et al., 2016).

3.3. Objective color

The effect of storage time and radiation on sausage color can be seen in Fig. 1 (L Brightness, a * red-green and b * yellow-blue). Although we observed slightly lower values in the F2 curve, statistical analysis did not reveal a significant ($P > 0.05$) change in the luminosity of the samples over time or among treatments. In contrast, the application of 1.5, 3.0, or 4.5 kGy caused a reduction ($P < 0.05$) in the red color intensity of the wieners. This difference was maintained until the 30th day of storage, at which time the values of a* among the treatments began to converge. It should be noted that the values of a* for the irradiated samples differed (were lower) from those of the non-irradiated F0 treatment but were similar to those of F2. As for the parameter b*, it was observed that the F2 treatment presented lower values than the other treatments.

According to Brewer (2004) the irradiation of meat leads to the formation of green pigments, which causes a decrease in the values of a* (red-green). According to the author, the characteristic absorption peak of irradiated meat is 615 nm. In addition, the green pigments formed tend to disappear over time, while the red pigments (540 nm) remain stable. The irradiation of meats (and products) also leads to the formation of metmyoglobin, corroborating the changes in the color of samples from red to brownish (Galán, García, & Selgas, 2011). In the case of cured meat products (with addition of nitrite), Ham et al. (2017) suggested that the reduction of red color is due to the decomposition of nitrosyl hemochrome as well as to the destruction of myoglobin molecules by free radicals formed. In contrast, some authors observed increased red color intensity in irradiated meat products, such as fermented wieners (Kim et al., 2012) and cured dried ham (Cava et al., 2009). This increase was explained as resulting from the formation of carboxymyoglobin, which is more stable than oxymyoglobin (Chouliara et al., 2006).

3.4. Texture profile analysis

Analysis of the texture profile revealed a significant difference ($P < 0.05$) only between treatments and not over time. The difference found was between F2 and the other samples (Fig. 2) for the parameters hardness and chewiness. That is, there was a difference only in relation to the concentration of NaCl and not in relation to the application of radiation. A reduction in hardness caused by the reduction of NaCl has already been observed in other studies (Horita, Messias, Morgano, Hayakawa, & Pollonio et al., 2014; Marchetti, Argel, Andrés, & Califano, 2015), and chewiness is directly related to hardness (Chewiness = hardness x cohesiveness x springiness). This reduction is due to the lower ionic strength, lower protein extraction, and lower gel strength in meat products emulsified with NaCl reduction or substitution (Yotsuyanagi et al., 2016). Different doses of radiation did not affect the texture of wieners ($P > 0.05$). Park et al. (2010) also demonstrated that irradiation (0, 5, 10, 15, and 20 kGy) did not affect the hardness of beef sausage patties. Likewise, Ham et al. (2017) found that irradiation (gamma-ray, electron-beam, and X-ray-m 0, 2.5, 5, 7.5, and 10 kGy) did not affect the hardness of pork wieners.

3.5. Microbiological analyses

In the microbiological analyses, the following pathogens were studied: presence of *Salmonella* sp. and counts of thermotolerant coliforms, coagulase-positive Staphylococci, and sulfite-reducing *Clostridium*. No quantifiable presence of any of the pathogens evaluated was verified. The growth of spoilage bacteria (anaerobic psychrotrophic and lactic acid bacteria) is described in Table 3.

Effective reduction of bacterial growth was observed in irradiated samples, especially treatments exposed to 3.0 and 4.5 kGy. The degree of reduction of the microbial load increased with the dose applied (dose-dependent effect). Samples irradiated with 3.0 and 4.5 kGy had almost two log cycle reductions of lactic acid bacteria compared to F2% and more than 2 log cycle reductions compared to F0 at the initial time of storage. The reduction of NaCl and increase in moisture and water activity of the product favors microbial growth. This can be verified by comparing the growth of lactic bacteria in F0 and F2. Sodium reduction makes the product more susceptible to microbial spoilage, which justifies the use of technologies such as irradiation to ensure its safety. Microbial growth was significantly slower ($P < 0.05$) in irradiated wieners over the storage time.

The dose-dependent effect of radiation for reducing the microbial load of meat products is well known (Fadhel et al., 2016; Fregonesi et al.,

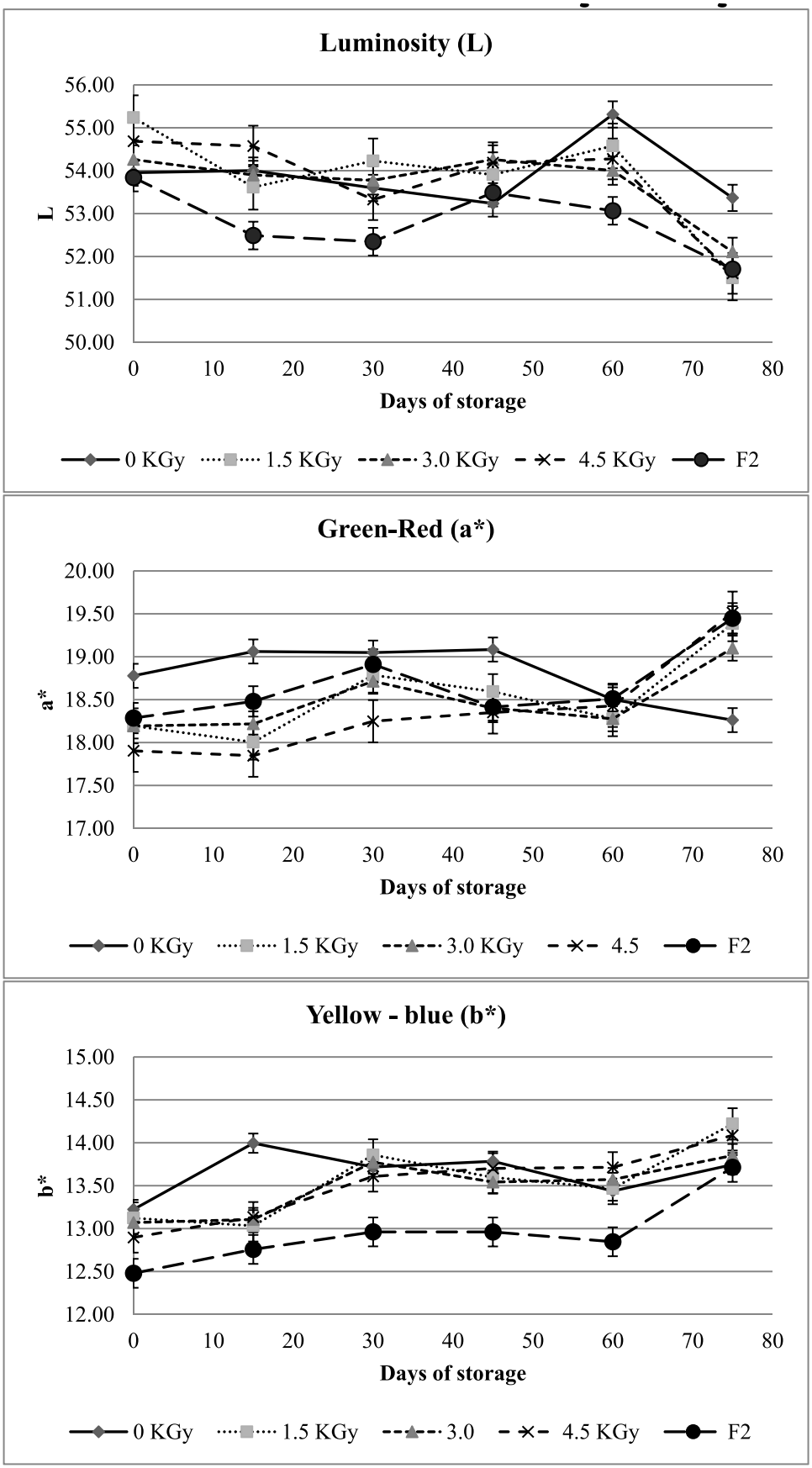

Fig. 1. CIE Laboratory color measurements for sodium-reduced wieners irradiated at doses of 1.5, 3.0, and 4.5 kGy, and non-irradiated wieners with sodium reduction (F0) and without sodium reduction (F2) for 75 days in refrigerated storage (4 °C).

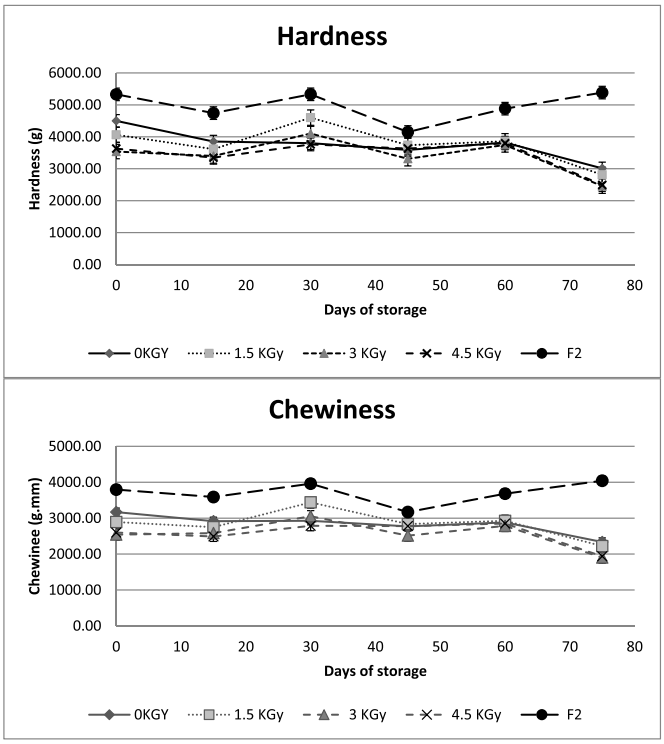

Fig. 2. Hardness and Chewiness measurements for sodium-reduced wieners irradiated at doses of 1.5, 3.0, and 4.5 kGy, and non-irradiated wieners with sodium reduction (F0) and without sodium reduction (F2) during 75 days of refrigerated storage (4 °C).·
F0: Sausage with 1.25% NaCl without irradiation/F1.5: Sausage with 1.25% NaCl irradiated with a dose of 1.5 kGy/F3.0: Sausage with 1.25% NaCl irradiated with 3.0 kGy/F4.5: Sausage with 1.25% NaCl irradiated with a dose of 4.5 kGy/F2: sausage with 2% NaCl, without irradiation.

2014; Jouki et al., 2013; Kim, Chun, Song, & Song, 2010). However, this study demonstrates that it is possible to produce healthier meat products (by reducing sodium) without compromising food safety, since salt (NaCl) is known to be a bacteriostatic agent. In addition, a large increase in shelf life can be obtained with irradiation of sausage at any dose.

The results also showed that lactic acid bacteria are more sensitive to the presence of salt than anaerobic psychrotrophic bacteria, since the former showed a lower growth rate when more salt was added to the formulation, i.e., the F2 growth rate was lower than the F0 (not significantly). In this way, lactic acid bacteria are the main cause of deterioration in wieners with reduced sodium. The growth of these bacteria with consequent generation of lactic acid contributes to the acidification of the product. Likewise, Fadhel et al. (2016) found that the addition of marinade (mango, curry, water, fructose, glucose, onions, garlic, salt, canola oil, and vinegar) to meat products also reduced the growth of lactic acid bacteria. The subsequent combination of marinade with 1.5 kGy of gamma radiation reduced the count of these bacteria to levels below the limit of detection.

3.6. Sensory analysis

The sensory test showed differences in the acceptance of wieners mainly in relation to the amount of salt in the formulations (Table 4). That is, F2 samples differed from the other samples in most attributes. Differences in acceptance indicated that consumers did not like the salty taste of the F2 formulation. These results may signal the changing dietary habits of the population: when seeking to reduce sodium intake in their diet, consumers re-educate their palate and become more critical about this attribute.

In general, the irradiation of foods leads to the development of

Table 3
Growth of lactic acid bacteria and psychrotrophic anaerobic bacteria during 75 days of refrigerated storage of hot dog wieners with 1.25% or 2% NaCl submitted to different doses of radiation.

	Time	F0	F1.5	F3.0	F4.5	F2
Lactic acid bacteria (Log CFU/g)	Initial	2.41^{A}	2.11^{A}	ND*	ND	1.96^{A}
	15 days	3.67^{A}	1.64^{B}	ND	ND	3.61^{A}
	30 days	5.81^{A}	1.00^{B}	ND	ND	4.14^{A}
	45 days	7.80^{A}	3.78^{BC}	0.52^{C}	0.92^{C}	5.46^{AB}
	60 days	7.94^{A}	4.64^{BC}	2.01^{C}	0.92^{C}	5.35^{AB}
	75 days	7.57^{A}	2.24^{B}	ND	ND	7.30^{A}
Psychrotrophics (Log CFU/g)	Initial	2.80^{A}	1.75^{A}	1.12^{A}	0.82^{A}	2.48^{A}
	15 days	4.27^{A}	2.17^{AB}	ND	ND	4.31^{A}
	30 days	5.00^{A}	4.85^{A}	ND	ND	5.10^{A}
	45 days	5.83^{A}	2.72^{B}	2.65^{B}	2.55^{A}	5.71^{A}
	60 days	6.90^{A}	3.12^{B}	2.89^{B}	2.64^{B}	6.66^{A}
	75 days	5.74^{A}	ND	ND	ND	6.03^{A}

CFU = colony forming units/F0: Sausage with 1.25% NaCl without irradiation/ F1.5: Sausage with 1.25% NaCl irradiated with a dose of 1.5 kGy/F3.0: Sausage with 1.25% NaCl irradiated with 3.0 kGy/F4.5: Sausage with 1.25% NaCl irradiated with a dose of 4.5 kGy/F2: sausage with 2% NaCl, without irradiation.
*ND, Not detected.
^{ABC} Different upper case letters in the same row indicate that the samples presented significant differences according to the radiation dose ($P \leq 0.05$).

Table 4
Results of the sensory analysis of wieners with 1.25% or 2% NaCl submitted to different doses of radiation.

Treatment	Appearance	Aroma	Texture	Taste	Overall quality
F0	6.04^{AB}	6.13^{A}	6.23^{AB}	6.75^{AB}	6.43^{AB}
F1.5	6.04^{AB}	6.12^{A}	5.70^{B}	6.21^{B}	6.07^{B}
F3.0	6.40^{A}	6.54^{A}	6.58^{A}	6.94^{A}	6.82^{A}
F4.5	6.28^{A}	6.50^{A}	6.50^{A}	6.81^{AB}	6.56^{AB}
F2	5.58^{B}	6.06^{A}	4.38^{C}	5.55^{C}	5.34^{C}

^{ABC} - Equal letters in the same column indicate that there was no significant difference between the samples. Scale: 1-I have greatly disliked it; 2-I disliked very much; 3-I disliked moderately; 4-I slightly disliked; 5-Neither liked nor disliked; 6-I liked it slightly; 7-I liked moderately; 8-I liked it very much; 9-I liked it greatly·
F0: Sausage with 1.25% NaCl without irradiation/F1.5: Sausage with 1.25% NaCl irradiated with a dose of 1.5 kGy/F3.0: Sausage with 1.25% NaCl irradiated with 3.0 kGy/F4.5: Sausage with 1.25% NaCl irradiated with a dose of 4.5 kGy/F2: sausage with 2% NaCl, without irradiation.

strange flavors and aromas. These aromas are due to the generation of several sulfurous compounds, which are highly volatile (Galán, Garcia & Selgas 2011). Those flavors are generated by the degradation of amino acids and have been described as sweet, old, sulfurous, or pungent (Ahn, Jo, & Olson, 2000). According to Brewer (2009), irradiation also leads to the formation of alkanes and alkenes by the decomposition of unsaturated fatty acids and amino acids. This decomposition results in volatile compounds such as pentanal, hexanal, heptanal, (E) -2-heptanal, octanal, 1-octene, (Z) -octenal, (E) -octenal, and (E, Z) −2,4- decadienal, which can produce pungent, rancid, and moldy odors.

In this study, no difference in acceptance was found between irradiated and control samples (F0). Al-Bachir and Zeinou (2009) explain that the use of a sealed package is a very effective method to reduce the generation of flavors and aromas, as they are generated by the oxidation of compounds. However, since the level of lipid oxidation of the wieners

irradiated at the initial time was higher than that of non-irradiated wieners, it is likely that the off-flavors resulting from irradiation were masked by sausage seasoning. Galán et al. (2011), however, found that bologna wieners irradiated with a dose of 4 kGy were less preferred by consumers than non-irradiated ones and those irradiated with 2 kGy or 3 kGy. A semi-trained panel recognized sweet, bloody, and sulfide odors in fermented sausages irradiated with a dose of 2 kGy (Lim et al., 2008).

4. Conclusion

Irradiation, at the doses studied, was an effective method for reducing the microbial load and ensuring the safety of sausages with reduced sodium content. This technology has produced excellent results in microbial tests, even at the lowest dose applied (1.5 kGy). Irradiation at this dose was capable of reducing microbiological growth without the negative effects of lipid oxidation. As for the other doses (3.0 and 4.5 kGy), an even higher reduction of the microbial load was achieved. In light of these results, it would be interesting to conduct further studies in order to evaluate the effect of irradiation on the conservation of unrefrigerated meat products.

CRediT authorship contribution statement

Isabela Rodrigues: Conceptualization, Investigation, Resources, Writing - original draft, Writing - review & editing, Visualization, Funding acquisition. **Aline Baldini:** Investigation, Resources, Visualization. **Manoela Pires:** Writing - review & editing, Resources. **Julliane Carvalho Barros:** Writing - review & editing, Resources. **Raul Fregonesi:** Conceptualization, Writing - original draft. **César Gonçalves de Lima:** Formal analysis, Statistical analyses. **Marco Antonio Trindade:** Conceptualization, Visualization, Funding acquisition, Supervision.

Declaration of competing interest

The authors concur with the submission of the manuscript "**EFFECT OF GAMMA RAY IRRADIATION ON THE SHELF LIFE OF SALT-REDUCED HOT DOG WIENERS"** to this Journal and declare no conflicts of interest.

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