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Combined effects of gamma radiation doses and sodium nitrite content on the lipid oxidation and color of mortadella

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ABSTRACT

The effects of different doses of gamma radiation (0–20 kGy) on the color and lipid oxidation of mortadella prepared with increasing nitrite levels (0–300 ppm) were evaluated using a central composite rotatable design. Higher radiation doses increased the redox potential, promoted the lipid oxidation and elevating the hue color of the mortadellas. Nevertheless, higher addition of sodium nitrite elevated the residual nitrite content, reduced the lipid oxidation and promoted the increase of redness and the reduce of hue color of the mortadellas, regardless of the radiation dose applied. Nitrite addition had a greater effect than irradiation on the quality parameters evaluated, and even at low levels (~75 ppm), its use decreased the deleterious effects of irradiation at doses as high as 20 kGy.

1. Introduction

Nitrite and nitrate salts are added to meat products to confer the characteristic pink color and flavor of cured cooked products. These additives also prevent unwanted alterations caused by the oxidative rancidity of lipids and inhibit the growth of deteriorating and pathogenic microorganisms, acting especially against the growth and production of *Clostridium botulinum* and its toxin (Dutra et al., 2011). However, the use of nitrite in meat products is associated with the formation of N-nitrous compounds, especially N-nitrosamines, which are potentially toxic, mutagenic, and carcinogenic and are linked to the development of stomach, esophagus, liver and brain tumors, as well as the risk of leukemia in children (Eichholzer & Gutzwiller, 2003).

Because of the potential risks of nitrite addition, studies have suggested alternative ways to substitute nitrite or at least to reduce the amount of nitrite applied in the curing process. However, because of the essential role that nitrite plays in cured meat quality and microbiological safety, changes in the products need to be carefully examined (Sebranek & Bacus, 2007). One possible alternative is the use of gamma irradiation, which is recognized as the best technology for the destruction of pathogenic and deteriorating microorganisms in food (Ahn et al., 2003). Recently, Dutra et al. (2016) reported that high doses of gamma radiation (>10 kGy) had a positive effect on the inactivation of *C. botulinum* spores (10^7 spores/g) in mortadella, independent of the sodium nitrite level used.

In addition to the antimicrobial effect, many studies have reported that gamma radiation induces the radiolysis of nitrites (Ahn et al., 2003; Ahn, Kim, Jo, Lee, & Byun, 2002; Ahn et al., 2004a, 2004b; Dutra et al., 2011; Jo, Ahn, Son, Lee, & Byun, 2003) and N-nitrosamines (Ahn et al., 2003, 2002, 2004a, 2004b; Jo et al., 2003), thus reducing the concentrations of these components in the final product.

Many studies have discussed the impacts of applying low doses (<5 kGy) of irradiation to meat products. In the majority of countries, gamma radiation may be applied at any dose, as long as it does not compromise the functional and/or sensorial properties of the product (Dutra et al., 2011). One of the issues associated with the application of high doses of irradiation to meat products is the possibility of undesirable alterations in their quality characteristics (Houser, Sebranek, & Lonergan, 2003). The main effects of radiation on food are indirect and are associated with the action of hydroxyl radicals formed during the radiolysis of the constituent water, resulting in the possibility of color changes and an increased

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lipid oxidation rate (Brewer, 2009), which leads to negative responses by consumers regarding the quality of the product.

Although many studies have evaluated the effect of gamma radiation on the quality of sausages (Ahn et al., 2003, 2004b; Byun, Lee, Yook, Lee, & Kim, 2002), few studies (Dutra et al., 2014, 2011; Jo et al., 2003) have evaluated these effects using different nitrite levels in the formulation. Therefore, the objective of this study was to analyze the effects of gamma radiation applied at different doses on the degree of lipid oxidation and color of mortadella prepared with different nitrite levels.

2. Materials and methods

2.1. Experimental design

Response surface methodology (RSM) was applied to determine optimum levels of two variables having a significant effect on the quality characteristics (lipid oxidation and color) of mortadellas: nitrite content (X_1); and gamma radiation dose (X_2). The range for each variable including X_1 (0–300 ppm) and X_2 (0–20 kGy) was selected. A central composite rotatable design (CCRD) was used and 11 different combinations by 3 central points and 6 axial points to 2^2 full factorial design were carried out in random order (Table 1). Two replicates were performed for this study.

2.2. Preparation of the mortadella

The products were prepared at the Laboratory of Meat and Derivatives Technology (LabCarnes) at the Department of Food Sciences of the Federal University of Lavras (DCA/UFLA), Brazil. The frozen deboned beef forequarter (beef shoulder) was thawed for 24 h at 4 °C, and the visible fat and superficial connective tissue were trimmed off. The mortadella was produced, according to the following formulation: beef shoulder (57.5%); pork backfat (14.5%); ice (20.0%); sodium chloride (2.0%); cassava starch (5.0%); sodium ascorbate (540 ppm); sodium polyphosphate (0.5%; Fosmax 320, New Max Industrial, Americana, São Paulo, Brazil) and mortadella seasoning (0.5%; New Max Industrial, Americana, São Paulo, Brazil). The meat was obtained in the local market, in sealed packages.

The mortadellas were prepared with different contents of sodium nitrite, as defined by the statistical design (Table 1), and were processed in a KJ-10 cutter (Indústrias Jamar, Tapuã, São Paulo, Brazil). Emulsion batters preparation, stuffing (into 65-mm opaque polyamide casings; Walsroder K plus brand; Walsroder Packaging LLC, Illinois, USA) and cooking in water bath (to an internal temperature of 72 °C, checked in the center of the mortadellas with a flexible thermocouple probe) of each treatment were conducted according to the procedures described by Pereira et al. (2014). The mortadellas (±400 g) were stored (4 °C) for 24 h prior to irradiation.

Table 1
Levels of variables for central composite rotatable design.

Run order	Coded levels		Uncoded levels	
	X_1	X_2	Nitrite (ppm)	Irradiation (kGy)
1	−1	−1	43.6	2.9
2	1	−1	256.4	2.9
3	−1	1	43.6	17.1
4	1	1	256.4	17.1
5	−1,41	0	0.0	10.0
6	1,41	0	300.0	10.0
7	0	−1,41	150.0	0.0
8	0	1,41	150.0	20.0
9 to 11	0	0	150.0	10.0

2.3. Irradiation

The cooked products were placed in styrofoam thermal boxes (Kanauf Isopor ®, São Paulo, Brazil) and subjected to different irradiation doses, as defined by the statistical design (Table 1), in a Gama GB-127 Irradiator (IR-214, MDS Nordion, Ottawa, Canada; with cobalt-60 sources and flow rate of 5 kGy/h) at the Laboratory of Gamma Irradiation of the Center for Nuclear Technology Development, National Commission of Nuclear Energy (CDTN/CENEN). The non-irradiated samples were maintained at the same condition and for similar periods of time as the irradiated samples.

2.4. Technological analysis

The products stored at 4 °C were analyzed in triplicate. The pH values and redox potential (Eh) were obtained by inserting a penetration-type combined Ag/AgCl reference electrode coupled to a DM20 potentiometer (Digimed, São Paulo, São Paulo, Brazil) into five different points of the product.

The residual nitrite content, expressed as sodium nitrite (ppm), was quantified according to official method no. 973.31 of the Association of Official Analytical Chemists (AOAC, 2005).

The total heme pigment (THP) and nitroso heme pigment (NHP) contents were determined by spectrophotometric methods, as proposed by Hornsey (1956), after extraction in acetone/water/acid (40:9:1, v/v) and in acetone/water (40:10, v/v), respectively. The THP was expressed in mg of hematin/kg of sample, whereas the NHP was expressed as the percentage of conversion (%NHP) from heme pigments to nitroso heme pigments in relation to the total heme pigments.

2.5. Lipid oxidation analysis

The lipid oxidation of the samples was assessed by the quantification of the hydroperoxides formed (peroxide index) and the thiobarbituric acid-reactive substances (TBARS index).

The peroxide index was determined by modifications to the PCA-FOX method (perchloric acid ferric-xylenol orange peroxide assay) proposed by Gay and Ebicki (2002). Briefly, 6 g of the sample was homogenized in 25 mL of cold methanol (−18 °C) and centrifuged at 1,400*g* for 3 minutes. One aliquot of the supernatant (enough to contain up to 75 μM of hydroperoxides) was added to 200 μL of analytical solution (2.5 mM xylenol orange tetrasodium and 2.5 mM ammonium ferrous sulfate in 1.10 M perchloric acid solution), and the volume was brought to 2 mL with distilled water. After 30 min of incubation at room temperature away from light, the absorbance was read at 560 nm. The hydroperoxide concentration was determined with the standard calibration curve with cumene hydroperoxide (CHP), and the results were expressed in mg CHP/kg of sample.

The TBARS index was determined according to the methodology proposed by Raharjo, Sofos, and Schmidt (1992), with some adaptations. Approximately 10 g of sample was homogenized in 40 mL of 5% trichloroacetic acid (TCA) and 1 mL of 0.15% butylhydroxytoluene (BHT). The extract was centrifuged at 3,000*g* for 2 minutes, and the supernatant was brought to 50 mL with 5% TCA solution. A 5-mL aliquot of the supernatant was added to 5 mL of 0.08 M thiobarbituric acid (TBA) and incubated in a boiling water bath for 5 minutes, and the absorbance was read at 531 nm. The malonaldehyde (MAD) concentration was determined from the standard calibration curve with 1,1,3,3-tetraethoxypropane (TEP), and the results were expressed in mg of MAD/kg of sample.

2.6. Color analysis

The mortadella were sliced in half, and the internal surface was evaluated with a CM-700d colorimeter-spectrophotometer (Konica Minolta Sensing Inc., Osaka, Japan). The CIE color indexes (L^*, a^* and b^*) were obtained with a D65 illuminant at 10° observer angle and with the specular component excluded (SCE mode). The mean value of five readings performed at different surface points was considered (Dutra et al., 2011). The chroma (C^*) and hue angle (h °; graus) were calculated from the chromaticity indexes (a^* and b^*): $C^* = (a^{*2} + b^{*2})^{1/2}$; and $h^\circ = \tan^{-1}(b^*/a^*)$. Samples color were expressed in the CIELCH space: L^* indicates sample lightness; higher C^* values suggests more vivid color; and h° values near 0 are red and near 90° are yellow (Ramos & Gomide, 2007).

2.7. Statistical analysis

The main effects of process variables (nitrite content, X_1; and gamma irradiation, X_2) on quality characteristics (Y) of mortadellas were investigated by using response surface methodology (RSM). The coded (used to fit the data) and uncoded values (real values) of the independent variables are listed in Table 1.

For each experimental factor, in a coded form, the variance was partitioned into components (linear, quadratic and interaction) to assess the adequacy of the following second-order polynomial function and the relative importance of these components:

$$Y = \beta_0 + \sum_{i=1}^{2}\beta_i X_i + \sum_{i=1}^{2}\beta_{ii} X_i^2 + \sum_{i=1}^{2}\sum_{j=1}^{2}\beta_{ij} X_i X_j$$

where Y is the dependent or response variable; β_0, β_i, β_{ii} and β_{ij} are intercept, linear, quadratic and interaction coefficients of the model, respectively; and X_i, X_j and X_{ij} represent the linear, quadratic and interaction effects of independent variables, respectively.

The fitness of the quadratic polynomial models was inspected using model analysis and coefficients of determination (R^2) as outlined by Kim et al. (2013). The significance of the complete mathematical model for each response variable was assessed by the F-test using a sum-of-squares residual for the dependent variable and a confidence level of 95.0% ($P < 0.05$). When the equation for the complete mathematical model was non-significant ($P > 0.05$), the non-significant regression coefficients ($P > 0.10$) were deleted from the second-order polynomial, and a new polynomial was recalculated to obtain a predictive model for each dependent variable. This predicted model was assessed again by the F-test ($P < 0.05$). Once the fitted regression equations were determined, the response surface plots were drawn using Statistica 5.0 (StatSoft, Poland) program.

3. Results and discussion

3.1. Technological characteristics

The regression coefficients and analysis of variance for the pH, redox potential (Eh), residual nitrite content (NO_2R), THP, and NHP values are shown in Table 2.

For the pH values of mortadella, the complete mathematical model was not significant ($P = 0.0561$). Thus, the non-significant ($P > 0.10$) regression coefficients (RC) were deleted from the second-order polynomial, and a new polynomial was recalculated and the fitness assessed again. However, it was also not possible to fit a response surface model ($P = 0.6249$, $R^2 = 0.52$) using the significant coefficients. The mean pH value (6.27 ± 0.09) observed is considered normal for this type of processed food, and although it was not possible to describe a statistical model, the significant RC indicate that pH values were affected by the nitrite levels and the irradiation dose.

The redox potential (Eh) is a parameter that can be used as indicator of the microbial stability and could be related with the sensorial quality of meat products. The Eh values constantly changes during storage, caused as well by intrinsic factors (like microbiologic and enzymatic activities) as by extrinsic factors (like atmospheric oxygen and irradiation) (Houser et al., 2005; Rödel & Scheuer, 1999a). Consequently, in meat products conclusions can be drawn from the change in the Eh values, if the redox potential of the product has been measured directly after processing (Rödel & Scheuer, 1999a).

Regarding the Eh values, although the complete model was not significant ($P = 0.1333$), it was possible to fit a mathematical model ($Eh = 38.6591 + 2.3627X_2$; $P = 0.0122$; $R^2 = 0.52$) as a function of the linear irradiation component (coded level X_2) alone. According to the model, higher radiation doses produced higher Eh values. Irradiation promotes the radiolysis of water, leading to the formation of highly oxidizing free radicals (Brewer, 2009) and raising the Eh values. Moreover, the primary effects of irradiation on the nitrate or nitrite ions are its ionization and decomposition into O_2 (Gesi & Takagi, 1964), which contributes to the increase of Eh.

The raising in Eh values with irradiation contradicts the observation of other authors, who observed lower Eh values when higher irradiation doses was applied to cooked ham (Houser et al., 2005). However, this difference may be explained by the difference in the products evaluated. According to Rödel and Scheuer (1999b), due the variety of meat products (fermented, cooked, raw etc) and their varying compositions (ingredients, additives etc.) and methods of production, differences in redox potentials have to be expected. Mortadella is an emulsion-type product and, during emulsification, the meat is finely ground, which induces higher

Table 2
Regression coefficients (RC) and analysis of variance[1] of the regression models for pH, redox potential (Eh), residual nitrite (NO_2R), total heme pigment (THP) and nitrosoheme pigment (NHP) of the experimental sausages.

	pH		Eh (mV)		NO_2R (ppm)		THP (ppm haematin)		NHP (% of THP)	
	RC	*p-value*	RC	*p-value*	RC	*p-value*	RC	*p-value*	RC	*p-value*
Constant (β_0)	6.1930	***<0.0001***	39.7599	***<0.0001***	82.9602	***0.0208***	93.5860	***<0.0001***	41.7228	***0.0027***
NO_2	−0.0086	*0.6882*	−1.1058	0.1984	55.3293	***0.0152***	11.0521	***0.0047***	4.8283	*0.3460*
$NO_2 \times NO_2$	0.0570	***0.0649***	−1.0106	0.3078	−2.9353	*0.8784*	−2.8038	*0.3508*	0.0418	*0.9943*
Irrad	−0.0634	***0.0260***	2.3627	**0.0249**	−11.6114	*0.4815*	0.8238	*0.7331*	−0.3105	*0.9493*
Irrad × Irrad	0.0451	*0.1214*	−0.5076	0.5932	−6.2759	*0.7446*	1.0440	*0.7174*	10.4265	*0.1185*
$NO_2 \times$ Irrad	0.0625	***0.0807***	−0.8750	0.4440	−22.2727	*0.3492*	1.2750	*0.7089*	−0.0630	*0.9927*
R^2	0.89		0.75		0.75		0.83		0.47	
F-test (regression)		*0.0561*		*0.1333*		*0.1279*		*0.0514*		*0.5327*

The abbreviations are as follows: NO_2 = level of sodium nitrite (ppm); and Irrad = level of gamma irradiation (kGy).
[1] Significative values ($P < 0.10$ for regression coefficients; $P < 0.05$ for F-test) are shown in bold.

amounts of atmosphere oxygen come into the sausage mixture (Rödel & Scheuer, 2000), leading to a higher lipid peroxidation. The mean Eh value (38.67 ± 2.92 mV) observed in the mortadellas was within the range of values (0–60 mV) described by Rödel and Scheuer (1999b) for different kinds of cooked sausages.

The complete mathematical model was not significant ($P = 0.1279$) for residual nitrite values, but it was possible to fit a model ($NO_2R = 76.2811 + 55.3293X_1$; $P = 0.0024$; $R^2 = 0.66$) from the linear nitrite component (coded level X_1). Higher additions of nitrite implied to higher residual nitrite concentrations. This is in accordance with the values observed in other studies (Dutra et al., 2011; Pérez-Rodríguez, Bosch-Bosch, & Garciá-Mata, 1996). The added nitrite derivatives react with myoglobin and other compounds present in meat (sulfhydryl groups of amino acids, fats, non-heme proteins, etc.) that can be oxidized to nitrate (NO_3) or converted to the oxide nitrite (NO) gas which is released from the product (Honikel, 2008). Hence, a good portion of the added nitrite is consumed by these reactions.

By the adjusted model, levels of residual nitrite were unaffected by irradiation. Lower residual nitrite values with the application of gamma radiation have been reported in many products, such as cooked ham (Houser et al., 2003, 2005) and sausages (Ahn et al., 2003, 2002, 2004a; Dutra et al., 2011), but the residual nitrite values depended on the applied dose and type of packaging system used during irradiation. In the majority of these studies, greater reductions in residual nitrite values between the control and the irradiated samples were observed in the samples with longer storage time, which corroborates the observation by Simie (1983) that the reduction of residual nitrite by irradiation most likely occurs through its reactions with hydroxyl radicals produced by the radiolysis of water.

Studies involving irradiation of products with different nitrite amounts are scarce. Dutra et al. (2011) evaluated the effects of gamma radiation applied at doses of 0, 7.5 and 15 kGy on mortadella elaborated with 0, 75 and 150 ppm of nitrite and did not observe significant differences in residual nitrite at time zero between irradiated and non-irradiated mortadella. According to Pegg and Shahidi (2000), a significant factor in N-nitrosamine formation was residual nitrite concentration, and consequently, nitrate was eliminated from most curing processes to achieve better control over residual nitrite concentrations. Although a reduction in residual nitrite levels may be not achieved by the use of gamma radiation, this would not be a problem, as radiolysis of N-nitrosamines by radiation has been observed in many studies (Ahn et al., 2003, 2002, 2004a, 2004b; Jo et al., 2003).

None of the complete model for the THP ($P = 0.0514$) or NHP ($P = 0.5327$) could be fitted (Table 2). However, it was possible to fit a model ($THP = 92.3100 + 11.0521X_1$; $P = 0.0001$; $R^2 = 0.77$) from the linear nitrite component (coded level X_1), being that high amounts of nitrite added induced an increase in the heme pigment content. The higher concentrations of nitrite and, consequently, of residual nitrite may have allowed a greater formation of nitrosomyoglobin pigment (NOMb) that, due to its higher stability under heat than the other myoglobin derivatives (Honikel, 2008; Ramos & Gomide, 2007), could have protected against the loss of heme pigments during the cooking process. Clark, Mahoney, and Carpenter (1997) reported that the content of heme iron in meat products decreases with cooking, and this decrease is attributed to the split of the heme group into iron ions and porfirin.

However, the greater protection of the heme pigments did not affect the percentage of nitroso heme pigments (nitrosyl hemochrome) formed after cooking, as none of the regression coefficients was significant ($P > 0.10$) for %NHP (Table 2). This observation corroborated the results reported by Dutra et al. (2011), who did not observe a decrease in the content of nitroso pigments in mortadella after irradiation at 7.5 and 15 kGy. Other studies (Ahn et al., 2003, 2004a, 2004b) reported a decrease in the nitroso pigment concentrations with the application of irradiation. However, those authors did not report these effects in relation to the content of total heme pigments, as in the present study.

3.2. Lipid oxidation

The effects of the treatments on the lipid oxidation of the samples were evaluated by measuring the content of hydroperoxides through the peroxide index (PI), and the malonaldehyde content was measured using the TBARS index. The coefficients of regression and the analysis of variance of the codified mathematical models for both indexes are described in Table 3. For both indexes, the complete mathematical model was significant ($P < 0.05$) and a response surface as a function of the nitrite levels and radiation doses could be constructed (Fig. 1).

Lipid peroxidation is a complex process which involves the formation and propagation of lipid radicals, the uptake of oxygen, a rearrangement of the double bonds in unsaturated lipids and the eventual destruction of membrane lipids, with the production of a variety of breakdown products, including alcohols, ketones, alkanes, aldehydes and ethers (Repetto, Semprine, & Boveris, 2012). During the initial phase, lipids-free radicals are formed and the unsaturated lipids are easily oxidized by the reactive-oxygen species (ROS) forming hydroperoxides (ROOH) that are considered the primary products of lipid oxidation (Estévez, Morcuende, & Ventanas, 2009). Therefore, once ionizing radiation generates hydroxyl radicals in aqueous systems and it was dose-dependent (Ahn, Olson, Jo, Love, & Jin, 1999; Byun et al., 2002), was expected that irradiation accelerate the oxidative changes in meat. This explain the higher IP values observed with higher irradiation levels (Fig. 1a).

However, higher hydroperoxide are formed by irradiation when larger quantities of nitrite were used. This might be due to the greater amount of residual nitrite formed with higher amounts of added nitrite. The FOX method is based on the ability of hydroperoxides to oxidize Fe^{+2} ions in acid medium, followed by the formation of blue-purple pigment from the complexation of the Fe^{+3} ions formed and the xylenol orange pigment (XO) (Gay & Ebicki, 2002). Wolff (1994) reports that compounds that bind to Fe^{+3} ions can interfere with the FOX analysis by competing with the XO indicator. Furthermore, the oxidizing potential of the nitrite present in the sample should be considered, which, in this case, could oxidize the Fe^{+2} ions added in the analytical assay, as the same way that it would oxidize the haematin of myoglobin (Pegg & Shahidi, 2000; Ramos & Gomide, 2007), overestimating the results. This would

Table 3
Regression coefficients (RC) and analysis of variance[1] of the regression models for peroxide and TBARS values of the experimental sausages.

	Peroxide values (mg CHP/kg)		TBARS values (mg MAD/kg)	
	RC	*p-value*	RC	*p-value*
Constant (β_0)	49.5438	***<0.0000***	0.5447	***0.0004***
NO_2	11.8005	***0.0032***	−0.1389	***0.0171***
$NO_2 \times NO_2$	2.9028	*0.3240*	0.1529	***0.0229***
Irrad	8.5521	***0.0121***	−0.0062	*0.8817*
Irrad × Irrad	6.4174	***0.0603***	−0.0988	***0.0904***
$NO_2 \times$ Irrad	13.0772	***0.0088***	0.1111	*0.1033*
R^2	0.94		0.75	
F-test (regression)		***0.0063***		***0.0330***

The abbreviations are as follows: NO_2 = level of sodium nitrite (ppm); Irrad = level of gamma irradiation (kGy); CHP = cumene hydroperoxide; and MAD = malonaldehyde.

[1] Significative values ($P < 0.10$ for regression coefficients; $P < 0.05$ for F-test) are shown in bold.

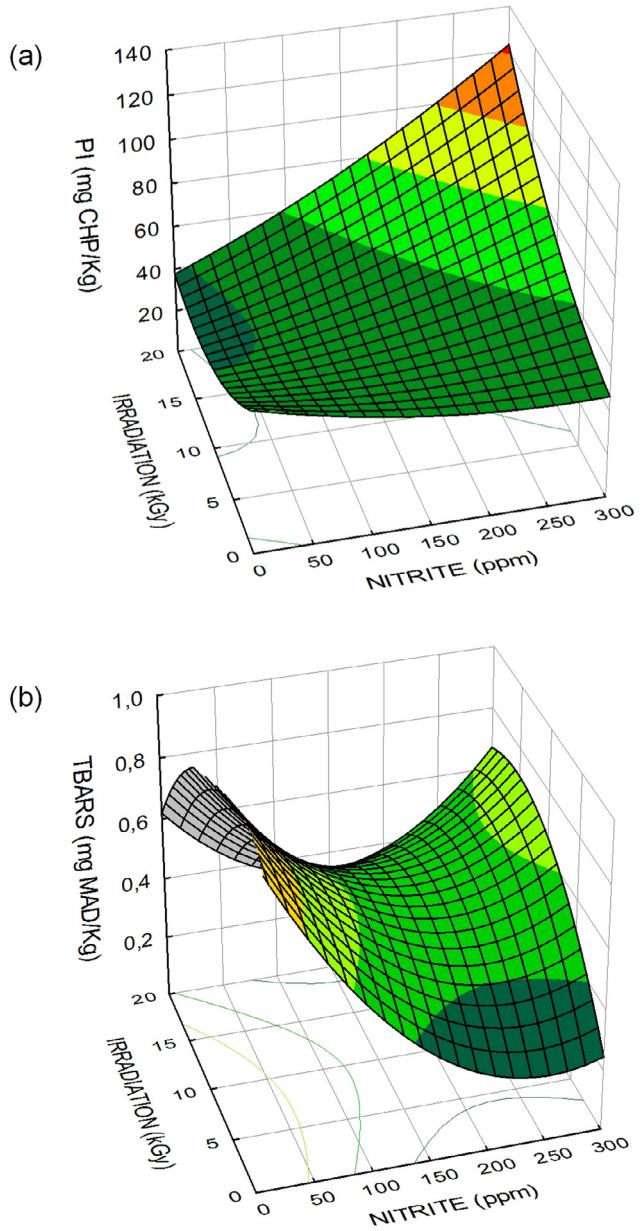

Fig. 1. Effects of added sodium nitrite and gamma irradiation on the (a) peroxide index ($IP = 49.5438 + 11.8005X_1 + 2.9028X_1^2 + 8.5521X_2 + 6.4174X_2^2 + 13.0772X_1X_2$; $R^2 = 0.94$) and (b) TBARS values ($TBARS = 0.5447 - 0.1389X_1 + 0.1529X_1^2 - 0.0062X_2 - 0.0988X_2^2 + 0.1111X_1X_2$; $R^2 = 0.75$) of mortadella samples. X_1 = coded variable for nitrite added; and X_2 = coded variable for gamma irradiation.

explain the effect observed in the PI values, as higher residual nitrite contents were observed in samples with higher amounts of nitrite. Additionally, higher amounts of residual nitrite would imply the presence of considerable amounts of nitrate (NO_3), as described by Honikel (2008), which also acts as a powerful oxidant (Pegg & Shahidi, 2000).

Otherwise, it should be taken into account that hydroperoxides are labile species, of very transitory nature, which undergo changes and deterioration with the radicals (Estévez et al., 2009). So, the PI is a very sensitive indicator of lipid oxidation only in the initial oxidation stage because when it approaches a given concentration, complex changes take place, degrading hydroperoxides and forming compounds of low molecular weight, such as aldehyde (Kolodziejska, Skonieczny, & Rubin, 1990). Hence, depending on the oxidation degree of the samples, the TBARS index values, which measure the presence of malonaldehydes in the product, may be more efficient in the elucidation of the effects.

Although, even at high irradiation doses, the amount of hydroperoxide was low in the samples with low amount of nitrite added, it is likely that the initial hydroperoxides formed have already been broken into secondary products such as pentanal, hexanal, and malonaldehyde (MAD). This explains the high MAD values observed in samples with low nitrite content (Fig. 1b). Moreover, lower TBARS values are observed as greater amounts of nitrite are added. This observation is in accordance with the antioxidant effect attributed to nitrite, which is most likely caused by the same reaction responsible for the color development (Ramos & Gomide, 2007; Shahidi, Pegg, & Shamsuzzaman, 1991). The free ionic iron released from heme pigments by heating is the most probable catalyst for lipid peroxidation (Apte & Morrissey, 1987). Free iron, especially in its reduced form (ferrous state), catalyzes the generation of hydroxyl radical (·OH), which is a highly oxidizing free radical and will initiate lipid peroxidation by hydrogen abstraction (Hogg & Kalyanaraman, 1999). In cured meat products, however, part of the nitric oxide (NO) formed from added nitrite is bound to myoglobin, forming the heat stable NOMb (Honikel, 2008). Therefore, by binding to myoglobin, nitrite prevents the release of heme iron and its subsequent catalysis of lipid oxidation. Furthermore, the malonaldehyde formed may react with the residual nitrite or its reactive form, nitrous acid (Kolodziejska et al., 1990), resulting in lower TBARS values. However, as the radiation dose was increased, the protective effect of nitrite was reduced.

Many authors (Ahn et al., 1999; Dutra et al., 2011) have also reported that TBARS values in cured irradiated products are dose-dependent. Houser et al. (2003) and Ahn et al. (1999) reported that irradiated cooked ham and sausages, respectively, with 4.5 kGy displayed significant increases in the TBARS values, although they were considered to be low. In turn, Houser et al. (2005) did not observe changes in the TBARS values for hams and sausages irradiated with up to 2 kGy. Other authors, irradiating sausage (Jo et al., 2003) and mortadella (Byun et al., 2000) samples with 5 kGy, or restructured cooked ham (Shahidi et al., 1991) even at doses as high as 10 KGy, did not observe a significant effect on TBARS values at time zero. The absence of effect or the small variations in TBARS values with irradiation are explained in these studies as the result of the antioxidant action of the nitrite present in the products. Moreover, according to Shahidi et al. (1991), proteins, and possibly products from the interaction of proteins with carbohydrates, have been reported as capable of exerting antioxidant effects that increase with the increase in irradiation dose, therefore protecting lipids from the oxidizing changes induced by irradiation. Østdal, Skibsted, and Andersen (1997) suggested that the protein (globin) of the myoglobin molecule acts as a "radical dissipator", transferring hydroxyl radicals ($OH^{\cdot}$) to antioxidants of low molecular weight, such as glutathione and ascorbates present in meat products.

Therefore, even at high levels of gamma radiation doses, the antioxidant activity of nitrite derivatives in products added with at least 50–75 ppm of nitrite most likely outweighed the deleterious effects promoted by free radicals generated from water hydrolysis during irradiation.

3.3. Color

The regression coefficients and analysis of variance of the mathematical models coded for lightness (L^*), redness (a^*), yellowness (b^*), chroma (C^*) and hue angle ($h°$) of cooked and irradiated mortadella are described in Table 4.

For lightness (L^*), the complete mathematical model could not be fitted ($P = 0.1266$) and only the linear coefficient for the variable

Table 4
Regression coefficients (RC) and analysis of variance[1] of the regression models for color indexes of the experimental sausages.

	L^*		a^*		b^*		C^*		$h°$ (graus)	
	RC	*p-value*	RC	*p-value*	RC	*p-value*	RC	*p-value*	RC	*p-value*
Constant (β_0)	58.6531	***0.0001***	10.0009	***0.0001***	13.2657	***<0.0000***	16.6199	***0.0001***	53.0151	***<0.0000***
NO_2	−1.6292	***0.0285***	2.7024	***0.0033***	−1.2791	***0.0026***	0.1074	*0.7452*	−10.1698	***0.0008***
$NO_2 \times NO_2$	1.1551	*0.1299*	−1.6264	***0.0453***	0.8793	***0.0238***	0.2352	*0.5560*	6.5467	***0.0119***
Irrad	0.6947	*0.2503*	−0.6191	*0.2821*	−0.1013	*0.6776*	−0.4999	*0.1707*	1.4154	*0.3657*
Irrad × Irrad	−0.5425	*0.4337*	1.0697	*0.1415*	0.0745	*0.7966*	0.6200	*0.1574*	−2.7737	*0.1634*
$NO_2 \times$ Irrad	0.0675	*0.9322*	0.0900	*0.9061*	0.3825	*0.2913*	0.3725	*0.4373*	0.3300	*0.8760*
R^2	0.70		0.82		0.89		0.59		0.88	
F-test (regression)		*0.1266*		***0.0206***		***0.0170***		*0.3873*		***0.0059***

The abbreviations are as follows: NO_2 = level of sodium nitrite (ppm); Irrad = level of gamma irradiation (kGy); L^* = lightness; a^* = redness; b^* = yellowness; C^* = chroma; and $h°$ = hue angle.
[1] Significative values ($P < 0.10$ for regression coefficients; $P < 0.05$ for F-test) are shown in bold.

nitrite (coded level X_1) was significant. However, it was possible to fit a mathematical model ($L^* = 59.0972 - 1.6292X_1$; $P = 0.0033$; $R^2 = 0.64$) describing this pattern: higher amounts of nitrite meant implies in lower L^* values and, therefore, darker mortadella. Dutra et al. (2011) also observed lower L^* values for samples with added nitrite in relation to the control (uncured samples). However, these authors did not observe differences in the L^* values between samples with 75 and 150 ppm of nitrite.

The absence of a significant effect for irradiation coefficients is consistent with other studies that also reported no effect of irradiation on the L^* values of different cured products. Dutra et al. (2011) reported no effect of the application of irradiation (7.5 and 15 kGy) on the L^* values in mortadella. Ahn et al. (2004b) also did not observe any significant effect of irradiation on the L^* values in irradiated (5–20 kGy) sausages vacuum packed under modified atmosphere or an aerobic environment. Houser et al. (2003) did not find any difference in the L^* values with the irradiation (4.5 kGy) of cooked ham, whereas Byun et al. (2002) reported a slight decrease in samples irradiated at 5 kGy.

The complete mathematical model was significant for the redness (a^*; *P = 0.0206*) and yellowness (b^*; *P = 0.0170*), from its coefficients, it was possible to create a response surface as a function of nitrite levels and radiation doses (Fig. 2a and b). Among the variables studied, the strongest effect was clearly caused by the addition of nitrite, for which greater additions meant higher a^* values and lower b^* values. This observation is in accordance with the higher THP values observed in the mortadella exposed to this treatment (fitted model for THP).

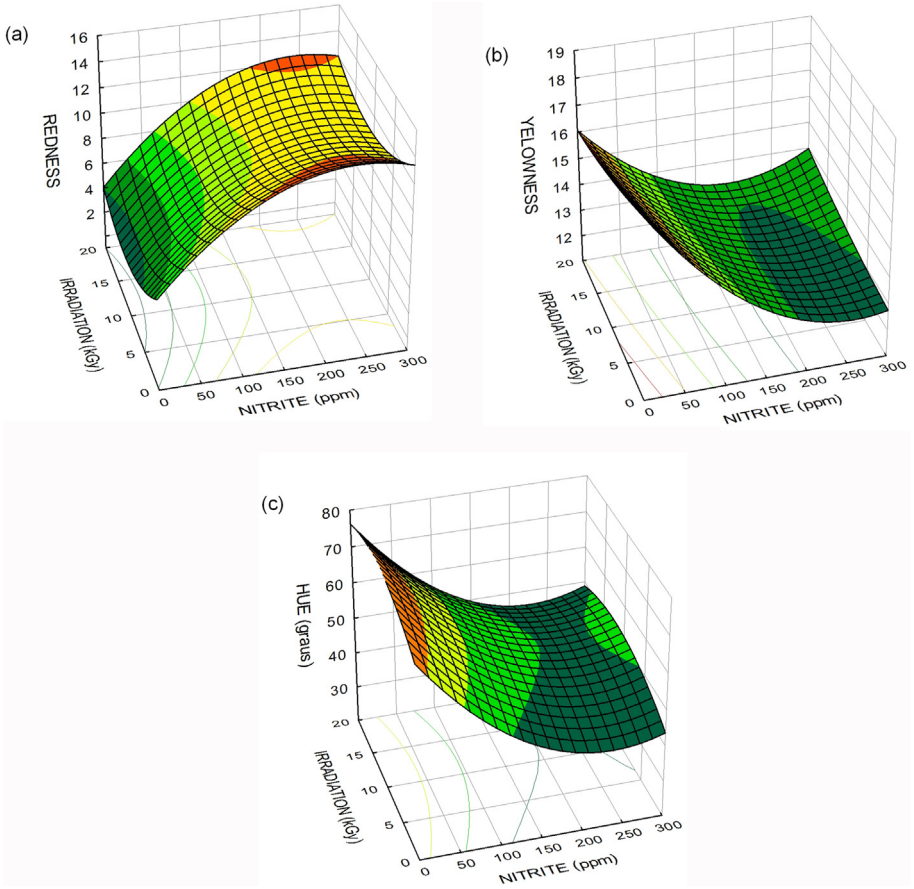

Fig. 2. Effects of added sodium nitrite and gamma irradiation on the (a) redness ($a^* = 10.0009 + 2.7024X_1 - 1.6264X_1^2 - 0.6191X_2 + 1.0697X_2^2 + 0.0900X_1X_2$; $R^2 = 0.82$), (b) yellowness ($b^* = 13.2657 - 1.2791X_1 + 0.8793X_1^2 - 0.1013X_2 + 0.0745X_2^2 + 0.3825X_1X_2$; $R^2 = 0.89$) and (c) hue angle ($h° = 53.0151 - 10.1698X_1 + 6.5467X_1^2 + 1.4154X_2 - 2.7737X_2^2 + 0.3300X_1X_2$; $R^2 = 0.88$) of mortadella samples. X_1 = coded variable for nitrite added; and X_2 = coded variable for gamma irradiation. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

None of the irradiation coefficients were significant and considering that in the complete model, the application of gamma radiation induced only slight changes in the a^* and b^* values. This result corroborates the observations described by Shahidi et al. (1991) and Dutra et al. (2011), who reported the absence of effects by irradiation on the a^* values in cured products. In contrast, many studies (Ahn et al., 2003, 2004b; Houser et al., 2005; Jo et al., 2003) have reported a decrease in the a^* values in cured irradiated meat products, and this decrease was attributed to the dissociation of NO from the nitroso pigment and the consequent formation of brown metmyoglobin. However, in the present study, irradiation caused no effects on the percentage values of nitroso heme pigments (Table 2); therefore, changes in the a^* values with irradiation cannot be attributed to the oxidation of nitroso pigments. Furthermore, it should be considered that changes in the red color of irradiated meat products have been reported (Ahn et al., 2003; Dutra et al., 2011; Jo et al., 2003; Shahidi et al., 1991) as being dependent on many factors, such as the irradiation dose, animal species, muscle type (associated with the amount of heme pigments), processing of product (emulsified, fermented, etc.), ingredients, and additives in the formulation and packaging system used during irradiation. Regarding yellowness, the b^* values are usually neglected in studies concerning the color of fresh and processed meats.

However, the a^* and b^* values are the sample chromaticity indices that together describe the color attributes chroma and hue, represented by the angular coordinates C^* and h°, respectively. Use of the angular coordinates of the CIELCH system can be, in many cases, more useful and sensitive for detecting changes in the red color of meats and meat products (Ramos & Gomide, 2007).

For the C^* values, neither the complete model ($P = 0.3873$) nor any of the regression coefficients ($P > 0.10$) were significant, thus indicating the absence of effects by the use of nitrite and irradiation. This is due to the inverse behavior observed on the chromaticity coordinates (a^* and b^*) and demonstrates the importance of evaluating these effects together. Thus, the color intensity of the mortadellas was note affected by the treatments, showing average C^* values of 18.64 ± 0.93.

Both irradiation and the addition of nitrite affected the hue angle (h° values) of the product, and the complete mathematical model ($P = 0.059$) is represented by the response surface curve in Fig. 2c. Small effects were observed in h° values with gamma radiation doses. This result contradicts the observation by Byun et al. (2002) that the desirable color in cured products (cooked ham in this case) could be obtained by gamma irradiation with no addition of nitrite in their formulation. Moreover, Jo et al. (2003) and Dutra et al. (2011) reported deleterious changes in the color of emulsified uncured sausages when irradiated.

Greater additions of nitrite mean lower h° values, corresponding to a sample with a redder hue (Ramos & Gomide, 2007). This decrease in h° values takes place up to 150 ppm of nitrite addition, beyond which the changes are very slight. Despite the observed increase in the h° values of samples with less than 100 ppm of nitrite, the results from the present study appear to corroborate the observation by Dutra et al. (2011) that the addition of 75 ppm of nitrite is sufficient to maintain the cured color of mortadella, even with the application of high doses of irradiation. Sensory evaluations must be employed to determine whether the hue differences observed in the samples are perceptible by consumers.

4. Conclusions

The amount of added nitrite had a stronger effect on the color and lipid oxidation of mortadella than the gamma radiation doses applied, reducing, even at levels of approximately 75 ppm, the deleterious effects of irradiation at doses as high as 20 kGy.

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