

The effect of irradiation and thermal process on beef heme iron concentration and color properties

Efeito da irradiação e processo térmico na concentração do ferro heme e nas propriedades de cor da carne

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Abstract

The aim of this study was to evaluate the influence of irradiation and thermal process on the heme iron (heme-Fe) concentration and color properties of Brazilian cattle beef. Beef samples (patties and steaks) were irradiated at 0-10 kGy and cooked in a combination oven at 250 °C for 9 minutes with 70% humidity. Total iron and heme iron (heme-Fe) concentrations were determined. The data were compared by multiple comparisons and fixed-effects ANOVA. Irradiation at doses higher than 5 kGy significantly altered the heme-Fe concentration. However, the sample preparation conditions interfered more in the heme-Fe content than did the irradiation. Depending on the animal species, meat heme iron levels between 35 and 52% of the total iron are used for dietetic calculations. In this study the percentage of heme-iron was, on average, 70% of the total iron showing that humidity is an important factor for its preservation. The samples were analyzed instrumentally for CIE L*, a*, and b* values.

Keywords: irradiation; beef; heme iron; color properties.

Resumo

O objetivo deste estudo foi avaliar a influência da irradiação e de processos térmicos na concentração do ferro heme (Fe heme) e nas propriedades de cor da carne do gado brasileiro. As amostras da carne (hambúrgueres e bifês) foram irradiadas com 0-10 kGy e foram cozidas em um forno combinado a 250 °C por 9 minutos com umidade de 70%. As concentrações de ferro total e de ferro heme foram determinadas. Os dados foram comparados por comparações múltiplas e por efeitos fixos, ANOVA. Irradiação em doses mais altas do que 5 kGy alteraram significativamente a concentração de Fe heme. Entretanto, as condições de preparo da amostra, interferiram muito mais na quantidade de Fe heme do que a irradiação. Dependendo da espécie animal, os níveis do ferro heme da carne estão entre 35 e 52% do ferro total e são usados para cálculos dietéticos. Em nosso estudo, a porcentagem de ferro heme foi em média, 70% do ferro total, mostrando que a umidade é um fator importante para sua preservação. As amostras foram analisadas instrumentalmente por CIE e valores de L* a* b*.

Palavras-chaves: irradiação; carne; ferro heme; propriedades de cor.

1 Introduction

In Brazil there are more than 200 million heads of cattle (IBGE, 2007), raised predominantly on grass. Beef consumption in the country has been estimated to be 21 kg/year (58 g/day) *per capita*. In the period from 1995 to 2005 (FAO, 2007), It increased 9%.

In Brazil, food irradiation at any dose is allowed once the food functional properties or sensory attributes are not compromised (ANVISA). With this recent regulation, it is believed that in the near future irradiation of beef as well as other food products of animal origin could be implemented, not only to ensure the reduction of pathogens - increasing their shelf-life- but also to ensure an off-season supply and to guarantee meat quality at a good price. Iron deficiency anemia is one of our country's major Public Health problems (BRITO et al., 2003; STOLTZFUS, 2001), and meat is the best source of bioavailable Fe (KALPALATHIKA et al., 1991) and an important Fe bioavailability enhancing factor (AMARO LÓPEZ; CÂMARA MARTOS, 2004).

Conrad et al. (1967) demonstrated a low bioavailability for pure heme. The explanation for such fact was that in neutral pH of duodenum and absence of binding compounds heme molecules are prone to form polymers of high molecular weight and low solubility with a consequent low absorption potential. However, when in the form of hemoglobin or its metabolites, bioavailability is high. Thus, the absorption of heme iron is related to its degree of polymerization but not to changes in its molecular structure.

Heme iron concentration may be reduced in meats submitted to thermal processing (IGENE et al., 1979; SCHRICKER et al., 1982; BUCHOWSKI et al., 1988; KALPALATHIKA et al., 1991; CARPENTER; CLARK, 1995; RAMOS, 1999; LOMBARDI-BOCCIA et al., 2002; KONGKACHUICHAJ et al., 2002; and TURHAN et al., 2004) due to the oxidation of the porphyrin ring and iron liberation (SCHRICKER; MILLER, 1983; GARCIA et al., 1996). The myoglobin presents a tighter structure, which is resistant to denaturation by irradiation. However, the secondary and tertiary

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protein structures may be altered with the breaking of hydrogen and other chemical bonds making it more sensitive to high temperatures and prone to iron liberation (URBAIN, 1986).

The aim of this study was to evaluate the influence of irradiation and thermal process on heme iron (heme-Fe) concentration and color properties of beef.

2 Materials and methods

2.1 Sample preparation

Muscles from round beef (*semimembranosus* muscle) available in S. Paulo, Brazil, were used. Patties were approximately 9 cm in diameter and 2 cm thick; steaks were about 8 cm wide, 12 cm long, and 0.7 cm thick.

Irradiation of samples

The samples were irradiated at 1.0; 2.0; 3.0; 4.0; 5.0; 7.5; and 10.0 kGy using a ^{60}Co source (Irradiator model JS-7500 Nordium Inc). Parts of the non-irradiated meat were used as control.

Sample cooking

Half of the sample was kept raw and the other half was broiled in a pre-heated combination oven (Rational ClimaPlus Combi CPC Oven) for 9 minutes at 250 °C, 70% internal humidity, and frozen at -18 °C until analyzed.

2.2 Total iron concentration

Total iron concentrations were determined by atomic absorption spectrophotometry (Polarized Zeeman AAS Hitachi® Z-5000) employing a hollow cathode lamp at 248,3 nm, and slit of 0,2 nm, after a wet digestion ($\text{HNO}_3:\text{H}_2\text{O}_2$, 5:1; v/v). The samples were digested with nitric acid and hydrogen peroxide (5:1, v/v) at 150 °C and diluted with deionized water. The working standard solution was prepared with, FeCl_3 (Titrisol, Merck, Darmstadt, Germany).

A fat free casein-based diet was used as the secondary reference standard (REEVES et al., 1993).

2.3 Heme iron determination

Heme iron concentrations were determined by the Hornsey method for total pigment analysis (Hornsey, 1956). Freeze-dried bovine hemoglobin serum (H-2500 Sigma) was used as the secondary reference standard. Total pigments were extracted with acetone/ L- cysteine (1% soln) / HCl. The extract was filtered and the absorption was measured at 640 nm against a blank reagent. The absorbance was multiplied by the factor 6800 and then divided by the sample weight to give the concentration of total pigments in the meat as μg hematin/g meat. The iron content was calculated by the factor of 8.82 μg iron/ μg hematin (MERCK, 1989).

2.4 Color analyses

Color measurements were conducted using a CN 508-D Minolta spectrophotometer, which expressed CIE color para-

eters L^* (lightness), a^* (redness), and b^* (yellowness) using non-irradiated meat as the control.

2.5 Statistical analysis

The following techniques were used: unidimensional descriptive analysis and analysis of variance (ANOVA) with fixed effects and multiple comparisons. Levels of 5% significance were adopted for all tests and 10% for % FeH/FeT.

3 Results and discussion

3.1 Total iron

The effect of irradiation on total Fe concentration showed a statistically significant difference in relation to meat presentation (patty or beef) to cooking conditions ($p < 0.001$) but not to irradiation dose level ($p > 0.089$) (Table 1).

The higher total iron concentration shown in the ground beef can be explained by the absorption of the meat exsudate in the groundmass during the homogenization process (Figures 1 and 2).

In steaks, this exsudate is lost probably because of its greater contact with the heating surface and reduced thickness along with the high processing temperature. Cooked patties presented a higher total iron concentration than raw ones.

Part of the exsudate in the raw beef was lost after defrosting, but this did not occur with the broiled beef.

3.2 Heme iron

The average heme-Fe concentration in irradiated meat at doses of 7.5 and 10 kGy was different from that of the non-irradiated (Table 2, Figures 3 and 4).

Table 1. Descriptive levels of the ANOVA for total Fe concentration in irradiated beef (whole basis).

Effect	p-value
Dose	0.089
Presentation ¹	< 0.001
Cooking process ²	< 0.001
Presentation * Cooking process	0.168

1: steak or patties; 2: temperature; humidity.

Table 2. Confidence interval for differences on mean heme- Fe concentration in beef irradiated at different γ radiation doses (kGy) (whole basis).

Comparisons	Difference of means	Confidence Interval	p-values
1.0 kGy-0.0 kGy	-0.58	[-2.52; 1.37]	0.975
2.0 kGy-0.0 kGy	-0.91	[-2.85; 1.04]	0.790
3.0 kGy-0.0 kGy	-0.95	[-2.90; 1.00]	0.752
4.0 kGy-0.0 kGy	-1.55	[-3.49; 0.40]	0.215
5.0 kGy-0.0 kGy	-1.78	[-3.72; 0.17]	0.107
7.5 kGy-0.0 kGy	-2.78	[-4.72; -0.83]	0.003
10.0 kGy-0.0 kGy	-2.64	[-4.58; -0.69]	0.005

The heme iron concentration was irradiation dose-dependent ($p = 0.001$), with a considerable reduction from $62(2) \mu\text{g}\cdot\text{g}^{-1}$ to $46(2) \mu\text{g}\cdot\text{g}^{-1}$ in the broiled beef (Table 3).

Buchowski et al. (1988) assessed the effect of temperature on the release of total and heme iron to broth and observed that meat heated to 97°C retained more iron (85.3%) than meat heated to 60°C (81.6%) and 77°C (78.2%). To retain heme, the meat should be cooked at a temperature high enough to allow a rapid coagulation of heme-protein in order to prevent iron release. The authors also stated that slow heating and greater contact surface resulted in heme iron losses.

3.3 Heme iron/Total iron

The percentages of heme iron were not significantly different ($p < 0.1$) between the irradiated meat and the non-irradiated meat, but they differed depending on the conditions, whether raw or broiled (Table 4). This can be explained by the fact that the steaks were thinner than the patties and presented a larger heating contact surface. The result is in agreement with those of Buchowski et al. (1988) as mentioned above.

Schricker et al. (1982) observed differences in the concentration of total iron and heme iron in the meat of different animal

species as well as in different muscles of the same species. In this case, the differences may be due to the different amounts of blood that remain in the muscle as a result of the uncontrollable vasodilatation that occurs prior to slaughter. Differences in iron concentration in different animal species have been corroborated

Table 3. Heme iron concentrations in irradiated beef samples (patties and steaks): raw and broiled on dry and de-fatted basis (mean) (SD).

Dose (kGy)	Heme iron ($\mu\text{g}/\text{g}$)			
	Raw		Broiled	
	Patties	Steaks	Patties	Steaks
0	105 (1)	94 (5)	94 (3)	62 (2)
1	108 (4)	88 (7)	91 (4)	58 (2)
2	106 (1)	89 (7)	94 (2)	52 (2)
3	109 (3)	93 (6)	87 (7)	52 (4)
4	105 (1)	90 (5)	88 (5)	50 (4)
5	108 (1)	89 (3)	80 (11)	50 (4)
7,5	98 (3)*	92 (10)*	86 (5)*	47 (1)*
10	102 (1)*	84 (4)*	84 (5)*	46 (2)*

*Different from corresponding means at D_0 ($p < 0.05$).

Table 4. % Heme iron in irradiated beef samples (patties and steaks): raw and broiled on dry and de-fatted basis (mean) (SD).

Dose (kGy)	Heme iron/total iron (%)			
	Raw		Broiled	
	Patties	Steaks	Patties	Steaks
0	91(2)	102 (6)	80 (3)	72 (2)
1	94 (4)	97 (8)	75 (3)	73 (2)
2	93 (2)	102 (9)	80 (3)	68 (2)
3	92 (4)	100(7)	66 (8)	70 (4)
4	94 (2)	96 (6)	75 (4)	72 (4)
5	98 (5)	95(5)	63 (7)	64 (4)
7,5	87 (3)	102 (11)	73 (5)	65 (2)
10	91 (2)	95 (4)	71 (4)	60 (3)
Mean*	92 (3) ^a	99 (3) ^b	73 (6) ^c	68 (4) ^d

*Means with different superscripts are statistically different ($p < 0.1$).

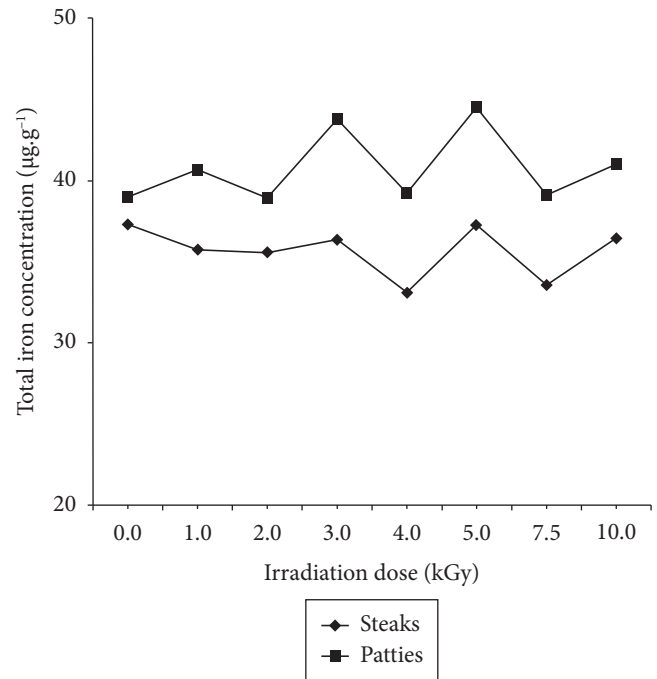


Figure 1. Total Fe in cooked beef (whole basis).

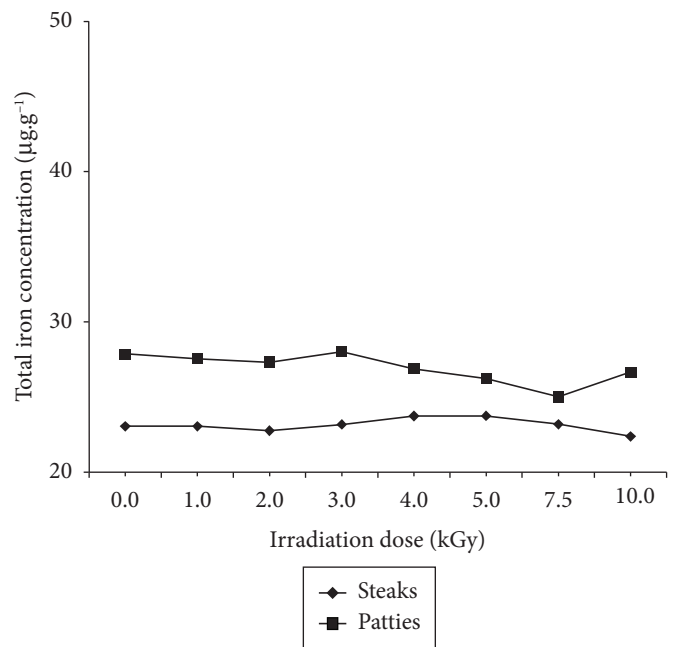


Figure 2. Total Fe in raw meat (whole basis).

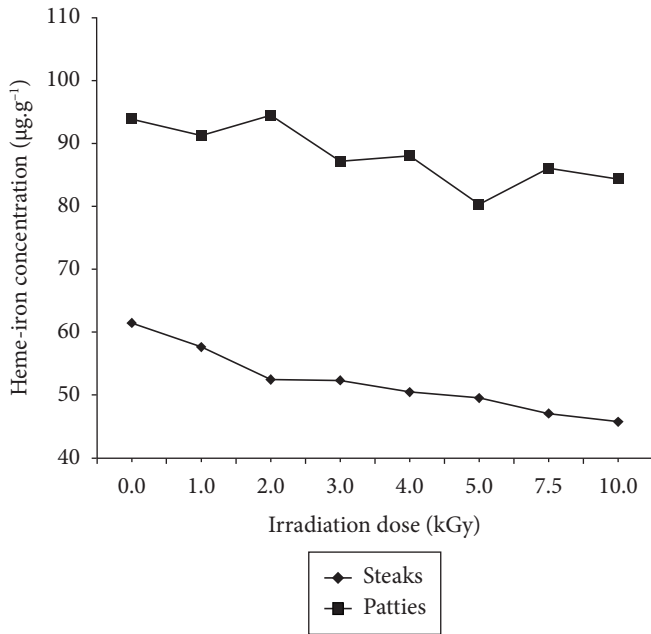


Figure 3. Total Fe-heme in cooked beef (whole basis).

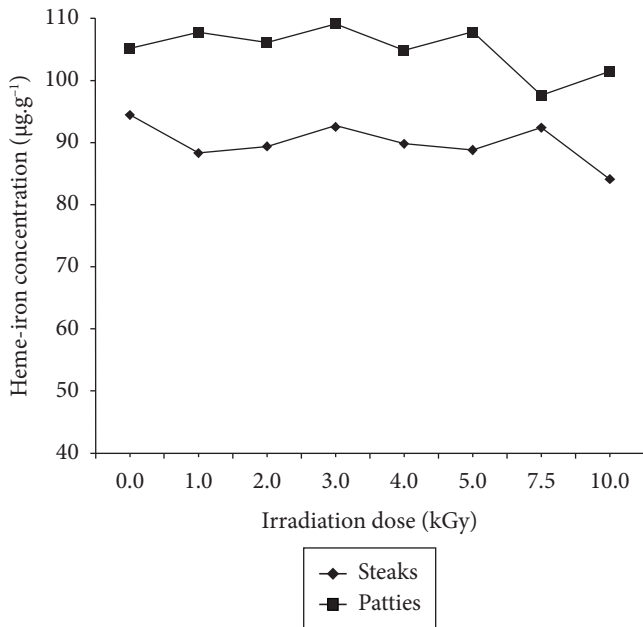


Figure 4. Total Fe-heme in raw beef (whole basis).

ted by recent studies of Ramos (1999), Lombardi-Boccia et al. (2002), Kongkachuichai et al. (2002), Zapata et al. (2003), and Turhan et al. (2004).

In this study, heme iron losses reached 30% although other authors obtained values between 50 to 79%, in different processing conditions (KALPALATHICA et al., 1991; CARPENTER; CLARK, 1995; RAMOS, 1999). This equipment was designed to promote broiling by either humid heat or dry heat or even both preserving nutritional and sensorial characteristics such as color, tenderness, and flavor of foods. In this process, internal moisture control allows the adjustment of internal moisture of the meat avoiding heme-Fe losses. (MURPHY et al. 2001)

In most dietetic calculations, it is assumed that heme iron corresponds to 40% of total iron in meat (MONSEN, 1978). However, Hallberg and Hulten (2000) obtained percentages between 35 to 52% depending on the species and the processing. The data shows that the percentage of heme iron in meat may be frequently underestimated since it depends on the conditions of processing, which must be specified.

3.4 Color analyses

The values of L^* , a^* , and b^* remained unaltered with no significant differences, (0,05) either in the patties or in the beefs, within the evaluated irradiation dose intervals (Table 5). Still, there was discoloration of the meat due to an increase in the metamyoglobin resulting from long periods of storage under these conditions (reduction of L^* and of a^* and increase of b^*). The irradiation and vacuum storage insured the stability of the oxymyoglobin (LUCHSINGH et al., 1997a; LUCHSINGH et al., 1997b; and MURANO et al., 1998). These variations do not affect the availability of heme iron, but the red bright color of the meat is directly related to consumer acceptance.

4 Conclusions

Up to 5 kGy, the irradiation process does not affect heme iron concentration in raw or broiled steaks or patties. The heme-iron accounts for, on average, 70% of the total iron concentration. Above this irradiation dose (7.5 and 10 kGy), the loss of heme iron varies between 3% and 25% according to the presentation conditions (as patties or steaks) and the processing conditions (different temperatures, cooking times and moisture). The values of L^* , a^* , and b^* remained unaltered with no significant differences, either in the patties or in the beefs, within the dose intervals evaluated.

Table 5. CIE color values related to intervals of irradiation dose of patties and steaks – Mean (SD).

Dose (kGy)	L^*		a^*		b^*	
	patties	steaks	patties	steaks	patties	steaks
0	37.9 (5.2)	39.2 (0.8)	15.7 (7.9)	13.9 (0.7)	12.5 (3.3)	13.3 (2.2)
0–2.5	35.8 (4.3)	40.4 (1.0)	15.1 (2.2)	13.5 (0.8)	12.6 (2.3)	14.1 (1.7)
2.5–5	37.8 (5.4)	39.5 (1.2)	13.3 (2.1)	13.5 (1.2)	11.7 (0.7)	12.6 (1.4)
5–10	37.5 (5.8)	38.3 (2.6)	12.1 (0.4)	13.4 (0.5)	11.2 (0.3)	12.2 (2.7)

No significant differences ($p > 0.05$).

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References

- AMARO LÓPEZ, M. A.; CÁMARA MARTOS, F. Iron availability: an updated review. **International Journal of Food Sciences and Nutrition**, v. 55, n. 8, p. 597-606, 2004.
- Agência Nacional de Vigilância Sanitária – ANVISA. Disponível em: <<http://www.anvisa.com.br>>. Acesso em: 15 Ago. 2007.
- BRITO, L. L. et al. *Fatores de risco para anemia por deficiência de ferro em crianças e adolescentes parasitados por helmintos intestinais*. **Revista Panamericana de Salud Pública**, v. 14, n. 6, p. 422-431, 2003.
- BUCHOWSKI, M. S. et al. Heating and the distribution of total and heme iron between meat and broth. **Journal of Food Science**, v. 53, n. 1, p. 43-45, 1988.
- CARPENTER, C. E.; CLARK, E. Evaluation of methods used in meat iron analysis and iron content of raw and cooked meats. **Journal of Agricultural and Food Chemistry**, v. 43, n. 7, p. 1824-1827, 1995.
- CONRAD, M. E. et al. Human absorption of hemoglobin-iron. **Gastroenterology**, v. 53, n. 1, p. 5-10, 1967.
- Food and Agriculture Organization - FAO. **Food Balance Sheet**. Disponível em: <http://faostat.fao.org/faostat/collections?version=ext&hasbulk=0&subset=agriculture>. Acesso em: 15 Aug. 2007.
- GARCIA, M. N. et al. Heat treatment on heme iron and iron-containing proteins in meat: iron absorption in humans from diets containing cooked meat fractions. **Journal of Nutritional Biochemistry**, v. 7, n. 1, p. 49-54, 1996.
- HALLBERG, L.; HULTHÉN, L. Prediction of dietary iron absorption: an algorithm for calculating absorption and bioavailability of dietary iron. **American Journal of Clinical Nutrition**, v. 71, n. 5, p. 1147-1160, 2000.
- HORNSEY, H. C. The colour of cooked cured pork. **Journal of the Science of Food and Agriculture**, v. 7, n. 8, p. 534-540, 1956.
- Instituto Brasileiro de Geografia e Estatística - IBGE. **SIDRA**. Disponível em: <<http://www.sidra.ibge.gov.br/bda/pecua/default.asp?t=2&z=t&o=21&u1=1&u2=1&u3=1&u4=1&u5=1&u6=1&u7=1>>. Acesso em: 15 Ago. 2007.
- IGENE, J. O. et al. Influence of heme pigments, nitrite, and non-heme on development of warmed-over flavor (WOF) in cooked meat. **Journal of Agricultural and Food Chemistry**, v. 27, n. 4, p. 838-842, 1979.
- KALPALATHICA, P. V. M.; CLARK, E. M.; MAHONEY, A. W. Heme iron content in selected ready-to-serve beef products. **Journal of Agricultural and Food Chemistry**, v. 39, n. 6, p. 1091-1093, 1991.
- KONGKACHUICHAI, R.; NAPATTHALUNG, P.; CHAROENSIRI, R. Heme and nonheme iron content of animal products commonly consumed in Thailand. **Journal of Food Composition and Analysis**, v. 15, n. 4, p. 389-398, 2002.
- LOMBARDI-BOCCIA, G.; MARTINEZ-DOMINGUEZ, B.; AGUZZI, A. Total heme and non-heme iron in raw and cooked meats. **Journal of Food Science**, v. 67, n. 5, p. 738-1741, 2002.
- LUCHSINGER, S. E. et al. Color and oxidative properties of irradiated ground beef patties. **Journal of Muscle Foods**, v. 8, n. 4, p. 445-464, 1997a.
- LUCHSINGER, S. E. Color and oxidative properties of irradiated whole muscle beef. **Journal of Muscle Foods**, v. 8, n. 4, p. 427-443, 1997b.
- MERCK index. **An encyclopedia of chemicals, drugs, and biologicals**. 11 ed. Rahway: Merck, 1989. 1605 p.
- MONSEN, E. R. Estimation of available dietary iron. **American Journal of Clinical Nutrition**, v. 31, p. 134-141, 1978.
- MURANO, P. S.; MURANO, E. A.; OLSON, D. G. Irradiated ground beef: sensory and quality changes during storage under various packaging conditions. **Journal of Food Science**, v. 63, n. 3, p. 548-551, 1998.
- MURPHY, R. Y. Heat transfer properties, moisture loss, product yield, and soluble proteins in chicken breast patties during air convection cooking. **Poultry Science**, v. 80, n. 4, p. 508-514, 2001.
- RAMOS, K. S. **Efeito do processamento sobre a concentração de ferro heme em carnes e em pulmão bovino**. São Paulo, 1999. Dissertação (Mestrado) - Faculdade de Ciências Farmacêuticas – USP.
- REEVES, P. G.; NIELSEN, F. H.; FAHEY JR, G. C. AIN-93 purified diets for laboratory rodents: final report of the American Institute of Nutrition ad hoc writing committee on the reformulation of the AIN-76A rodent diet. **Journal of Nutrition**, v. 123, n. 11, p. 1939-1951, 1993.
- SCHRICKER, B. R.; MILLER, D. D.; STOUFFER, J. R. Measurement and content of nonheme and total iron in muscle. **Journal of Food Science**, v. 47, p. 740-743, 1982.
- SCHRICKER, B. R.; MILLER, D. D. Effects of cooking and chemical treatment on heme and nonheme iron in meat. **Journal of Food Science**, v. 48, n. 4, p. 1340-1343, 1349, 1983.
- STOLTZFUS, R. J. Defining iron-deficiency anemia in public health terms: a time for reflection. **Journal of Nutrition**, v. 131, p. 565S-567S, 2001.
- TURHAN, S.; ALTUNKAYNAK, T. B.; YAZICI, F. A note on total and heme iron contents of ready-to-eat doner kebabs. **Meat Science**, v. 67, n. 2, p. 191-194, 2004.
- URBAIN, W. M. **Food Irradiation**. London: Academic Press Inc., 1986. 351 p.
- ZAPATA, J. F. F. et al. Características da carne de pequenos ruminantes do nordeste do Brasil. **Boletim da Sociedade Brasileira de Ciências e Tecnologia de Alimentos**, v. 37, n. 2, p. 146-153, 2003.