

EFFECT OF DIFFERENT IONIZING RADIATION DOSE RATES ON THE STAPHYLOCOCCAL ENTEROTOXIN IN MECHANICALLY DEBONED CHICKEN MEAT

Heliana de Azevedo¹, Poliana de Paula Brito¹, Henrique Takuji Fukuma¹, Cláudio Vítor Roque¹, Wilson Custódio¹, Yasko Kodama², Norma Terugo Nago Miya³ and José Luiz Pereira³

¹Brazilian Nuclear Energy Commission (CNEN - MG)
Rodovia Poços de Caldas - Andradas Km 13
37701-970 Poços de Caldas, MG - Brazil
hazevedo@cnen.gov.br
pbrito@cnen.gov.br
cvroque@cnen.gov.br
htfukuma@cnen.gov.br
wilsonc@cnen.gov.br

²Nuclear and Energy Research Institute (IPEN / CNEN - SP)
Av. Prof. Lineu Prestes, 2242 – Cidade Universitária
05508-000 São Paulo – SP – Brazil
ykodama@ipen.br

³Department of Food Sciences
Campinas State University (UNICAMP)
Cidade Universitária Zeferino Vaz
13081-970 Campinas - SP - Brazil
pereira@fea.unicamp.br
miya@fea.unicamp.br

ABSTRACT

Samples weighing 50g each were prepared from allotments of back with skin MDCM, to the EEB contamination or not (control). Each sample of MDCM contaminated or not with EEB was conditioned in low density polyethylene bag, frozen (-18 ± 1 °C) for one night in a tunnel and irradiated with gamma rays from ⁶⁰Co source in this state with doses of 0.0 kGy (control), 1.5 kGy (5.7 kGy.h⁻¹ – higher dose rate, 1.8 kGy.h⁻¹ – intermediary dose rate and 0.6 kGy.h⁻¹ – lower dose rate) and 3.0 kGy (8.4 kGy.h⁻¹ – higher dose rate, 2.4 kGy.h⁻¹ – intermediary dose rate and 0.6 kGy.h⁻¹ – lower dose rate). Irradiated or non irradiated MDCM samples were processed to the EEB extraction, according to the VIDAS Staph enterotoxin II kit (bioMérieux) manufacturer protocol. The calculation to determinate the MDCM EEB recovery after the sample (control or irradiated) processing were carried out applying the principle of mass balance, along the whole process. Described experiment was performed in triplicate. Results showed that the irradiation process was effective to remove the MDCM EEB, to both 1.5 kGy and 3.0 kGy. According to the expected, doses of 3.0 kGy showed the highest values of MDCM EEB removal. Regarding the effect of dose rate of radiation on the removal of EEB of the MDCM, it could be observed only for samples irradiated with 1.5 kGy radiation dose; in these processing conditions, the highest value of EEB removal was obtained for samples processed with low radiation dose rate.

1. INTRODUCTION

The mechanically deboned chicken meat (MDCM) is a product of the mechanical separation of meats in bone remnants, such as back, neck, bones of the thigh and chest, difficult to be

manually withdrawn in an inexpensive way, but still representing 24% of the edible portion of the carcass [1]. The MDCM can be used in traditional meat products such as sausages, bologna, or salami, in substitution of raw materials with more elevated costs. However, this material frequently contains some pathogens that are important in the development of foodborne diseases, as *Staphylococcus aureus*.

S. aureus is a colonizing bacterium from the mucosa of men and domestic animals; some strains are producers of enterotoxins [2]. Enterotoxins are proteins highly thermal stable [3], responsible for Staphylococcal food poisoning [4].

Several studies have been performed with irradiation of food trying to establish a dose capable of promoting a microbial decrease without significantly changing the sensory characteristics of food, but until now, little is known about the action of different dose rates of ionizing radiation in staphylococcal enterotoxins, which may be present in food.

2. MATERIALS AND METHODS

2.1. Materials

Samples of 50 g (M_i = initial mass) were prepared from MDCM batches (6 kg) produced from chicken backs with skin, in order to verify contamination or non-contamination with type B staphylococcal enterotoxin (BSE) - Sigma S4881. Each MDCM sample unit contaminated with 98.1084 ng of BSE (control) was packaged in bags of transparent low density polyethylene, frozen at -18 ± 1 °C for one night.

2.2. Irradiation

Irradiation was performed at Co-60 Multipurpose Irradiator of Ipen. Samples were irradiated at -18 ± 1 °C (dry ice) radiation doses of 0.0 kGy (control), 1.5 kGy and 3.0 kGy. Dose rate of 1.5 kGy: 5.7 kGy.h⁻¹ (higher), 1.8 kGy.h⁻¹ (intermediate) and 0.6 kGy.h⁻¹ (lower). Dose rate of 3.0 kGy: 8.4 kGy.h⁻¹ (higher), 2.4 kGy.h⁻¹ (intermediate) and 0.6 kGy.h⁻¹ (lower). Experiments were performed in triplicate. Dosimeters used Amber R 3042, batch GS. Readings were performed using Genesys Espectrophotometer.

Two dosimeters were used for each dose and each dose rate. One dosimeter was positioned before the sample and the other one after, referred to the radioactive source. It was considered the average of the two values of absorbed dose. Variation of dose rates occurred due to the distribution of radioactive pencils on the racks to the samples irradiated closer to the racks. The analyses procedures described above were performed for samples taken from each of the three collection campaigns.

2.3. Extraction of BSE

Non-irradiated or MDCM samples were subjected to treatment for extraction of BSE, according to the protocol recommended by the manufacturer of the VIDAS Staph enterotoxin II kit, from bioMérieux.

2.4. Determination of BSE Amount

Calculations to determine the amount of BSE (mini VIDAS equipment bioMérieux) recovered from the MDCM after processing with different doses of radiation, different rates of ionizing radiation or control samples were performed applying the principle of mass balance throughout the process, that is, since the contamination of MDCM with BSE, preparation of standard solutions and extraction of BSE from MDCM, to the determinations in mini VIDAS bioMérieux equipment. All the glassware used for the preparation of solutions and development of the experiment was calibrated to correct for volumes over the experiment.

3. RESULTS AND DISCUSSION

3.1. BSE Recovery

Results showed that there was an effect of the irradiation on the BSE removal from MDCM, both for the dose of 1.5 kGy, as well as 3.0 kGy (Table 1).

Baptista et al. [5], irradiated bothropstoxin-1 and concluded that irradiation of proteins leads to significant structural changes and also changes in the modulation of immunological response.

Table 1. Test Values and staphylococcal enterotoxin type B (BSE) in MDCM samples irradiated at doses of 1.5 and 3.0 kGy, versus rates of different doses of ionizing radiation.

Dose (kGy)	Dose Rate	VT \pm sd	BSE _{rec} (ng/500 uL) \pm sd
1.5	HIGHER	1.27 \pm 0.04	0.59 \pm 0.02
3.0		1.15 \pm 0.07	0.52 \pm 0.04
1.5	INTERMEDIARY	1.29 \pm 0.00	0.61 \pm 0.00
3.0		1.13 \pm 0.04	0.51 \pm 0.02
1.5	LOWER	1.17 \pm 0.07	0.53 \pm 0.04
3.0		1.06 \pm 0.06	0.46 \pm 0.04

VT = test value (result obtained in mini VIDAS equipment) sd = standard deviation, n = 3

The dose of 3.0 kGy showed the higher values of BSE removal from MDCM for all dose rates tested (lower, intermediary, and higher), respectively 34.8%, 35.12%, and 31.68% for higher, intermediary and lower dose rates. The values of removal for the dose of 1.5 kGy were respectively 11.73%, 9.23%, and 26.07% for higher, intermediary and lower dose rates (Table 2).

In relation the different radiation dose rates tested, results showed effect only for samples irradiated with dose of 1.5 kGy and lower dose rate radiation, indicating removal of 25, 5784 ng. Intermediary and higher dose rates for dose of 1.5 kGy had removal of 11.5084 ng and 9.0584 ng, respectively (Table 2).

Table 2. Values of staphylococcal enterotoxin type B (BSE) in MDCM samples irradiated at doses of 1.5 and 3.0 kGy, as opposed to different dose rates of ionizing radiation.

Dose (kGy)	Dose Rate	BSE		BSE	EEB
		total recovered (ng) ± sd	total recovered (%) ± sd	removed from MDCM (ng)	removed from MDCM (%)
1,5	HIGHER	86.60 ± 8.4	88.27 ± 8.6	11.5084	11.73
3,0		63.97 ± 10.4	65.20 ± 10.6	34.1384	34.80
1,5	INTERMEDIARY	89.05 ± 5.6	90.76 ± 5.8	9.0584	9.23
3,0		63.65 ± 9.3	64.88 ± 9.5	34.4584	35.12
1,5	LOWER	72.53 ± 4.5	73.93 ± 4.6	25.5784	26.07
3,0		67.03 ± 10.2	68.32 ± 10.4	31.0784	31.68

sd = standard deviation, n = 3

4. CONCLUSIONS

Results showed that the dose of 3.0 kGy was the most effective in reducing enterotoxin B from MDCM (lower readings in the test VIDAS). As regards to dose rates, there was an effect only for the 1.5 kGy dose and lower dose rate of ionizing radiation.

ACKNOWLEDGMENTS

The authors are indebted to Armando Luiz Brusqui for technical assistance. This research has been financial supported by CNPQ (151592/2005-5).

REFERENCES

1. C.V.A. Xavier, N. J. Beraquet, "Vida-de-Prateleira de Carne Mecanicamente Separada de Frango Estocada sob Refrigeração", *Coletânea ITAL*, **24**, PP.91-104 (1994)
2. B. D. G. M. Franco, M. Landgraf, *Microbiologia de Alimentos*. São Paulo & Brasil (1996).
3. P. M. Germano, M. I. S. Germano, *Higiene e Vigilância Sanitária de Alimentos*. Barueri & Brasil (2008).
4. M. S. Bergdoll, I. Y. Huang, E. J. Schantz, "Chemistry of the staphylococcal enterotins", *Journal of Agricultural Food Chemistry*, **22**, PP 9-13 (1974).
5. J. A. Batptista, D. P. Vieira, A. J. G. Junior, C. M. Yonamine, P. Caproni, M. Casare, H. C. A. Júnior, P. J. Spencer, N. Nascimento, "Structure Alteration and Immunologiacl Properties of ⁶⁰CO Gamma Rays Irradiated Bothropstoxin-1", *International Nuclear Atlantic Conference (INAC)*, Santos, outubro (2007).