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EFFECT OF DIFFERENT IONIZING RADIATION DOSES AND DOSE RATES, USING COBALT-60 AND ELECTRONS BEAM SOURCES, ON THE STAPHYLOCOCCAL ENTEROTOXIN INOCULATED IN MECHANICALLY DEBONED CHICKEN MEAT

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ABSTRACT

The purpose of food irradiation is the destruction of present pathogenic microorganisms and the increase of shelf life of foods. To achieve this process, the source of cobalt-60 and the electron accelerator can be used. The mechanically deboned chicken meat (MDCM) is used for the production of traditional meat products, and it may come to present pathogenic microorganisms such as staphylococcus aureus, a bacterium that produces enterotoxin, which causes food poisoning. The objective of this study is to analyze the effect of ionizing irradiation with different doses and dose rates, deriving from different radiation sources, on staphylococcal enterotoxin type B (SEB) in the MDCM. 50 g samples of MDCM were prepared in a batch of 6 kg of MDCM. The samples were contaminated, with the exception of the control, with SEB in amounts of about 100 ng. Then they were conditioned in a transparent bag made of low density polyethylene, frozen at -18±1 ºC overnight and irradiated in these conditions with doses of 0.0 kGy (control), 1.5 kGy and 3.0 kGy, and with three different dose rates, both in the Cobalt-60 and the electron accelerator. The experiments were conducted in quintuplicate. The SEB extraction from the MDCM was performed according to the protocol recommended by the manufacturer of the kit VIDAS Staph Enterotoxin II (bioMérrieux). The principle of mass balance was used to determine the actual amount of SEB removed by irradiation. The treatment that presented the best results was the one with a dose of 1.5 kGy, high dose rate of the electron accelerator.

1. INTRODUCTION

According to the IAEA (International Atomic Energy Agency), food irradiation is a treatment that consists of exposing foods, already packaged or in bulk, to a carefully controlled amount of ionizing radiation, for a predetermined time and well-defined goals. The process does not increase the level of radioactivity on foods and it can prevent the division of microorganisms that cause the deterioration of foods, such as bacteria and fungi, altering their molecular structure.

According to the technical regulation of identity and quality, the MDCM is obtained by a mechanical grinding process and separation of bones and carcasses intended for the preparation of specific meat products. *Staphylococcus aureus* is a Gram-positive bacterium [1], which may be found colonizing skin and mucous membranes of humans and animals, including birds, as it may produce heat-stable enterotoxins, causing human food poisoning [2]. Enterotoxins are proteins with high thermal stability [3].

Cobalt-60 is one of the radioisotopes used for food irradiation and it is obtained from metallic Cobalt-59. Its decay is accomplished by two possible beta (β) emissions to stable nickel ($^{60}_{28}$Ni), with energy of 1.486 and 0.313 MeV, and two photons with energies of 1.17 and 1.33 MeV, which are responsible for the processing of materials by radiation in gamma irradiators. The half-life of Cobalt-60 is 5.261 years [4]. Another source of ionizing radiation used is the electron accelerator, where a constant voltage is used to accelerate electrons emitted by a filament heated in the region of highest potential, resulting in a constant electron beam [5].

Both gamma radiation and electron beams produced by accelerators are sources of ionizing energy, as they transfer energy through interaction with electrons from the orbits of atoms that constitute the product. These interactions provoke excitation of electrons to higher energy levels or pull them out of their orbits, with enough energy to interact with orbital electrons of other atoms. The products of ionization are responsible for the chemical, physical and biological effects on the irradiated materials [4].

The penetrating power of gamma rays is much higher than the penetrating power of the electron beam. The electromagnetic radiations present an exponential characteristic of matter absorption, and have no defined scope as in the case of charged particles. The latter lose their energy during the trajectory because of a large number of collisions with atomic electrons [5].

The aim of the current work is to analyze the effect of ionizing radiation on staphylococcal enterotoxin type B (SEB) inoculated in mechanically deboned chicken meat (MDCM), being that this radiation is originated from two sources, Cobalt-60 and the electron accelerator, with different doses and dose rates.

2. MATERIALS AND METHODS

Samples of 50 g of mechanically deboned chicken meat (MDCM) were prepared in a batch of 6kg of MDCM, contaminated or not with staphylococcal enterotoxin type B (SEB) (Sigma S4881) and submitted or not to the process of irradiation, using the electron accelerator or the source of Cobalt-60.

2.1. Irradiation

The samples were conditioned in transparent bags of low density polyethylene, frozen at -18±1 ºC overnight and irradiated in this condition. In order to irradiate the MDCM samples, two different sources of ionizing radiation were used. A radiation source of Cobalt-60 of multipurpose type (Nuclear and Energy Research Institute - IPEN), with doses of: 0.0 kGy (control), 1.5 kGy (dose rates: 5.7 $kGy.h^{-1}$ - high, 1.8 $kGy.h^{-1}$ – intermediate and 0.6 $kGy.h^{-1}$ - low) and 3.0 kGy (dose rates: 8.4 $kGy.h^{-1}$ - high, 2.4 $kGy.h^{-1}$ - intermediate and 0.6 $kGy.h^{-1}$ - low). And an electron accelerator source (Nuclear and Energy Research Institute - IPEN), with energy 1.500 MeV, beam width of 100 cm and tray speed of 6.72 m/min for both used doses of 1.5 KGy and 3 KGy (dose rates: 3.37 $kGy.s^{1}$). For the irradiation, two dosimeters were used (Amber R 3042, GS batch), positioned at frontal and foreside ends of the samples.

2.2. SEB Extraction

The SEB extraction in the MDCM samples after the process of irradiation was conducted according to the protocol recommended by the manufacturer of the kit VIDAS Staph enterotoxin II (bioMérrieux).

2.3. Quantity Determination of SEB

To determine the amount of SEB found in the MDCM samples after the ionizing radiation treatment, mass balance calculations were applied, where all masses that entered and exited the system were taken into account, both in the contamination of MDCM samples and in the SEB extraction from them. By making a standard curve, it was possible to obtain the amount of SEB ($ng.500\mu L^{-1}$), with the result in value and test (VT), obtained by the kit VIDAS Staph Enterotoxin II (bioMérrieux). All glassware used was calibrated, allowing the application of mass corrections in the system.

For each treatment performed, the arithmetic mean of all five samples was found. To obtain the amount of SEB removed by irradiation, the irradiated samples suffered the decrease in mass of the sample that was not irradiated (control).

3. RESULTS AND DISCUSSION

The results obtained in the current study are described on Table 1. During the entire experiment, from the contamination of MDCM samples to the SEB extraction, all incoming and outgoing masses were registered. After these considerations, the corrected mass was obtained, from where the aliquot of 500 µL was retreated for reading in the miniVIDAS equipment. From the test value (TV), the SEB recovery was calculated on the corrected mass, thus allowing the calculation of the amount of SEB removed from MDCM through balance and mass correction calculations.

Between the two irradiation sources used, as shown on Fig. 1, the electron accelerator obtained better results than Cobalt-60. The treatment that presented the best result was

that of the sample irradiated in the electron accelerator with dose of 1.5 KGy, presenting SEB removal of 13.63%.

On the samples irradiated on Cobalt-60, the sample with dose of 1.5 KGy and high dose rate presented the best result – 2.54% of SEB removal from MDCM. The dose rate presented great influence on the percentage of SEB removal; the intermediate dose rate of the treatment with doses of 1.5 KGy and 3.0 KGy of Cobalt-60 presented very similar removal results – approximately 1% of SEB removal.

Even presenting a higher penetration power than the electron accelerator, Cobalt-60 obtained less efficient results. The electron accelerator presented a dose rate value of 3.37 $kGy.s^{-1}$ – much higher than the most elevated dose rate of Cobalt-60, which was 5.7 $kGy.h^{-1}$.

Table 1. Values of Staphylococcal Enterotoxin type B found in the MDCM samples irradiated with different doses and dose rates of ionizing radiation

Radiation Source	Dose (KGy)	Dose Rate	SEB (ng) ± sd recovered from the corrected mass	SEB (%) ± sd recovered from the corrected mass	SEB (ng) removed from MDCM	SEB (%) removed from MDCM
Cobalt-60	1.5	5.7 KGy/h	73.50 ± 1.30	73.75 ± 1.38	1.86	2.54
	1.5	1.8 KGy/h	74.60 ± 1.97	74.84 ± 1.92	0.78	1.05
	3	2.4 KGy/h	74.77 ± 0.65	74.84 ± 0.78	0.85	1.13
	3	0.6 KGy/h	74.67 ± 1.70	75.01 ± 1.58	0.58	0.78
Electron Accelerator	1.5	3.37KGy/s	66.26 ± 2.10	66.53 ± 2.10	9.03	13.63
	3		69.27 ± 1.90	69.43 ± 1.93	6.21	8.96

sd = standard deviation; n = 5

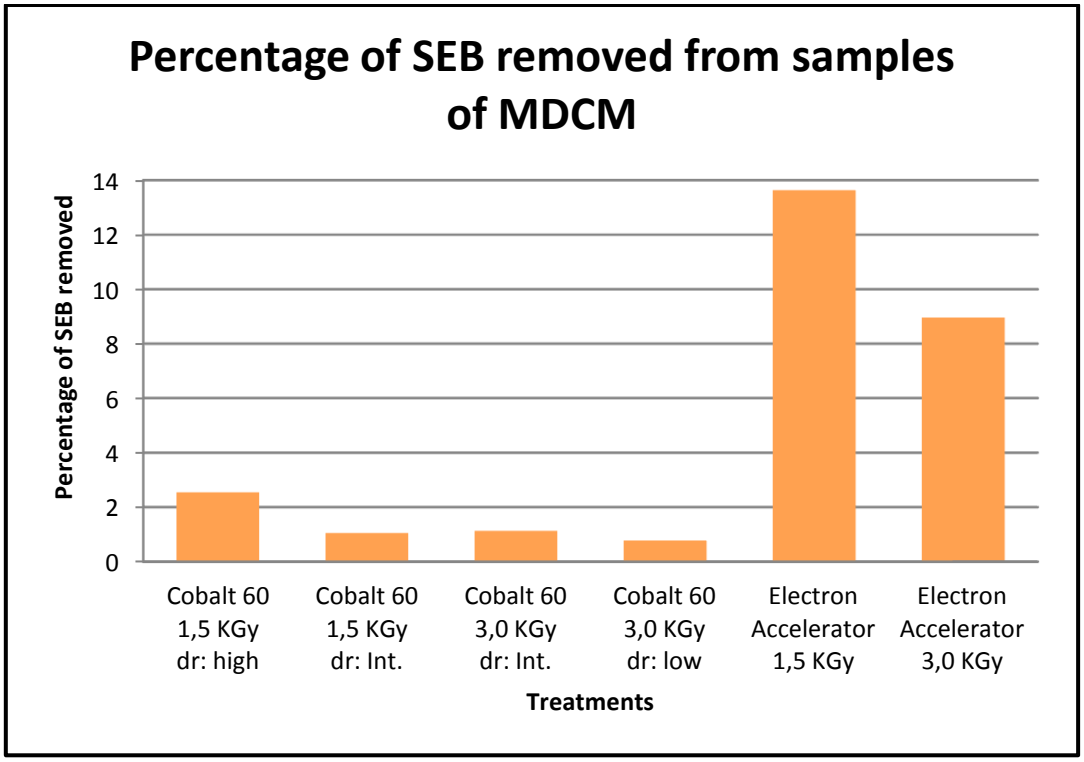

Figure 1. Graphic of the percentage of SEB removed from MDCM on the different treatments with ionizing radiation. (dr: dose rate; int.: intermediate)

4. CONCLUSION

Upon facing the results obtained in the current study, it was observed that the samples subjected to treatments with the electron accelerator presented the best results. The best treatment was that of the sample irradiated in the electron accelerator with a dose of 1.5 KGy. The treatments with higher dose rates presented greater efficiency on SEB removal.

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