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Effect of irradiation in honey inoculated with *Bacillus sporothermodurans*

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ABSTRACT

Honey is a natural viscous product widely used for nutritional and medicinal purposes. Although it's a food that presents a high degree of resistance against the growth of microorganisms is not a sterile medium. Spore-forming bacteria, such as Clostridium botulinum, are present in honey and it's the only recorded source of food carrier of the agent that causes childhood botulism. The steps of honey processing do not include the heat treatment for its commercial sterility, due to the application of heat increase the presence of hydroxymethylfurfural. Food irradiation is a method of preservation that can be applied in the processing of honey by not physically altering the appearance, shape or temperature of the product. The objective of this work was to evaluate the effect oof gamma radiation on honey inoculated with Bacillus sporothermodurans spores. The inoculation was made of lyophilized culture this bacterium in the honey samples was carried out and the irradiation of the samples at 5, 10 and 15 kGy doses was carried out. After irradiation, the samples were incubated at 30 degrees Celcius for 72 hs in Brain Heart Infusion (BHI) agar.The count present reduction of one logarithmic cycle in the number of colonies in the 5 kGy irradiated honeys. In the samples irradiated with doses of 10 and 15 kGy there was no microbial growth and spore germination. It's concluded that irradiation constitutes an excellent conservation method, which can be used to guarantee the microbiological quality of the honey.

Keywords: **gamma radiation, honey, *Bacillus sporothermodurans***

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1. INTRODUCTION

Honey is a viscous natural product produced by bees, obtained from the nectar of flowers, secretions from living parts of plants or excretions from sucking insects [1, 2]. In honey processing, the beneficiation steps do not provide heat treatment with temperatures above 100 ° C to achieve commercial sterility. Because the application of heat increases the presence of hydroxymethylfurfural (HMF), which is a molecule that indicates the quality of honey as to its aging or alteration of its physicochemical properties, caused by overheating or incorrect storage [3, 2].

Honey marketed in six Brazilian states revealed a significant presence of *Clostridium botulinum*, therefore, it should not be included in the diet of children under one year of agedue to the immaturity of the intestinal flora, which allows spore germination and botulinum neurotoxin production that causes the childhood botulism [4]. Irradiation process in food is one of the alternative conservation methods that can be applied in honey processing because it does not physically alter the appearance, shape or temperature of the product and has no residual effects [5], besides being cold that can be used to destroy microorganisms [6]. The *Bacillus sporothermodurans* when compared to *Clostridium botulinium* presents similarities in its phenotypic and developmental characteristics, for these reasons it was chosen for the present study. The objective of this work was to evaluate the effect of gamma radiation on spores of *Bacillus sporothermodurans* inoculated on honey.

2. MATERIALS AND METHODS

2.1. Sample collection, inoculation of *Bacillus sporothermodurans* and irradiation of honey

The honey of the Apis mellifera multifloral bee used in the analyzes was donated by Agricultural Cooperative of Rural Producers of Arizona – COAPRO, located in the city 'Orizona' of State 'Goias' The samples were packed in 300 mL clear polypropylene pots and mellifer divided into four groups of 200 g. 106CFU/mL of lyophilized Bacillus sporothermodurans strain DSMZ (Deutsche Sammulung von Mickoorganismenund

Zellkulturen GmbH, Germani) was inoculated into each pot. The samples were then irradiated at the Center of Nuclear Energy in Agriculture (CENA/USP) in the 'Piracicaba' city, of State 'São Paulo', in irradiator type GammaCell-220, with of Cobalt-60 source, dose rate of 0.430 kGy/h at the doses of 0(control), 5 kGy, 10 kGy and 15 kGy.

2.2. Microbiological analysis

The samples were prepared according to the method of Adolf Lutz [7]. The honey pots were placed in a sealed vessel at 40 ± 1 ° C for 20 minutes, shaking each sample, then cooled to room temperature.

For the dilutions of the samples of each treatment, control (0 kGy), 5 kGy, 10 kGy and 15 kGy, 25 mL of the honey sample was added in 225 mL of peptone water with shaking until the total dilution of the honey. The dilution series was performed with 9 mL of peptone water and 1 mL of diluted sample after shaking in the stomacher.

Then, 0.1 mL of the 10^3, 10^4 and 10^5 dilutions were inoculated. The plating was performed on surface and in triplicate, incubated at 30° C for 72 h, in *Bacillus sporothermodurans* (BHI Agar) for the isolation of *Bacillus sporothermodurans* according to Brazil [8], with modifications in inoculation technique [9]. After incubation, colonies identified as typical (small, smooth and white to beige coloration and no soluble pigment) were enumerated. The final result was given in CFU / mL.

3. RESULTS AND DISCUSSION

The inoculation of *Bacillus sporothermodurans* in the sample of honeys after the irradiation had results in all doses of radiation studied (Figure 1), with the highest doses, 10 and 15 kGy, being the most effective. Gamma radiation has a high penetrating power that causes lesions in the DNA, and the damages caused can compromise its reproduction and most of the functions of the microbial cell, resulting in cell death [10].

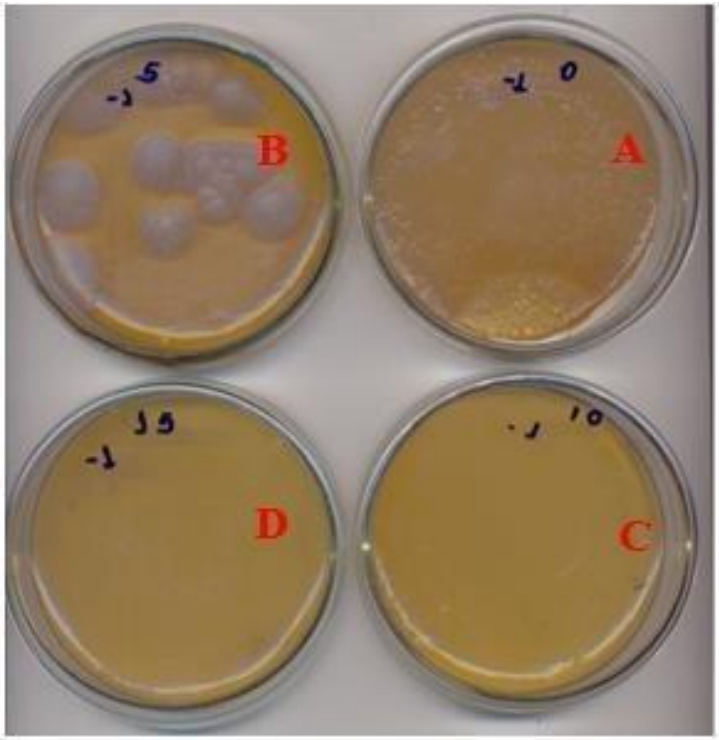

Figure 1 - Counting of *B. Sporothermodurans* in irradiated honey (A - Control, B - 5 kGy, C - 10 kGy, D - 15 kGy) in a dilution of 10^{-1} .

In addition to the effect on the genetic material, the radiation has a variety of effects on other cellular components, resulting in the formation of ions and free radicals that can react with proteins and with the DNA of the microorganisms, as a consequence of this reaction, mutations and cell death can occur [11].

The results of *B. sporothermodurans* counts of irradiated honey samples (Table 1) showed a reduction of one logarithmic cycle in the number of colonies in honey samples inoculated with *B. sporothermodurans* irradiated with a dose of 5 kGy corresponding to 6.25% in relation to the control sample count. The sensitivity of the microorganism to radiation is expressed by the dose of irradiation capable of reducing the microbial population to 10% of the initial amount, called Dose D_{10} [11]. It was reported that in some studies spores of various species of *Bacillus ssp typically* exhibited log reduction values at doses between 1.8 and 5.5 kGy [12].

Table 1. Mean CFU / mL count in BHI culture media for *Bacillus sporothermodurans* in honey for the 0, 5, 10 and 15 kGy treatments.

Doses (kGy)	UFC/mL
0	$4.0x10^6$
5	$2.5x10^5$
10	0.0
15	0.0

The growth conditions for *B. sporothermodurans* were favorable for the four treatments, however at the 10 and 15 kGy doses, there was no microbial growth and germination of the spores, presenting a 100% reduction in counting when compared to the control sample. The same result was observed in studies with honey samples inoculated with *Escherichia coli, Aspergilus niger, Clostridium esporogeneses*, submitted to gamma radiation (5 and 10 kGy), which obtained a reduction in the microbial load from the application of the dose of 5 kGy reaching the absence with the application of 10 kGy dose [13]. The massive destruction of bacterial spores to obtain sterilized food requires doses greater than 10 kGy [14].

In studies carried out to eliminate vegetative cells and spores of *Bacillus cereus* in raw rice, treated with doses of 15 and 25 kGy, showed to be effective in the elimination of microorganisms [15]. It was also found in studies with honey inoculated with 10^6 spores of *C. botulinum* and *B. subtilis* in 50 g of sample, in which honey was sterile after irradiation process with a dose of 25 kGy [16].

Radiation doses necessary for the destruction of *C. botulinum* spores in various foods have been established. As a conservation method the use of gamma radiation at doses of 10 kGy had an effect on the destruction of the microorganism in studies for the control of spores and vegetative cells of *Clostridium botulinum* in mortadella [17].

4. CONCLUSION

The irradiation was able to inactivate the spores of *B. sporothermodurans* present in the samples of honey, from the dose of 10 kGy. It's verified that the irradiation is an excellent method of conservation, that can be used to guarantee the microbiological quality of the honey.

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