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Sequencing and identification of different *Salmonella* species in cocoa beans treated with gamma irradiation

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Abstract

Gamma irradiation is an effective way to eliminate the bacterial load in cocoa beans. During harvesting these are exposed to various factors of contamination. The aim of this study was to analyze the effect of gamma irradiation on the presence of *Salmonella spp* considered a microbiological risk in chocolate. Cocoa samples (n = 31) were treated with three doses of gamma irradiation (2, 3 and 5 kGy) besides a control sample without irradiation. The PCR method revealed 22 bacteria (n = 124) with Salm3-Salm4 amplified. The DNA sequencing method confirmed the presence of two isolates who belong to *Salmonella spp.* and 20 belonging to *Klebsiella sp* and *Enterobacter sp*. These results were retrieved from the control samples and irradiation 2kGy, while radiation 3 to 5 kGy no growth of microorganisms.

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1. Introduction

Cocoa beans (*Theobroma cacao L.*) is the main raw material for the production of chocolate. During the primary processing, the fruits are harvested and opened with knives, to separate the seeds with pulp that are fermented in wooden boxes, for example. This is, one of the most important stages of cocoa processing[1,2]. During this stage, the cocoa seeds are constantly handling. The aeration of the mass, the conditions of room temperature, pH and acidity of the pulp contribute to the growth of specific microorganisms[3]. These microorganisms will play a role in the sensory aspect and quality of chocolate, but also a problem of food security, since the growth of pathogenic microorganisms and transmitter's foodborne diseases. Studies show that during the pre - processing of cocoa is no further contamination[4,5].

Salmonella is the leading known microbiological risk in chocolate. Its origin is not allocated to the cocoa or the environment where it grows, it is of fecal origin. It can be introduced from the hands of the workers during handling. The only stage at which is possible to eliminate the presence of *Salmonella* is the roasting of the beans or nibs. Subsequently, it is important to minimize the risk of contamination after this step because there is not another stage to be eliminated [1,6]. Da Silva demonstrated the presence of total *Enterobacteriaceae*, coliforms, *Escherichia coli* and *Salmonella* in chocolates produced in Brazil [4]. Although the aw and pH values are unsuitable for *Salmonella* survival, it can survive in those conditions. Moreover the high fat content in the chocolate (> 20%) increases the heat resistance of the *Salmonella* [6,7,8].

Food irradiation can control microbial development, contributes in reducing the incidence of some diseases, it will increase the shelf life of food [10]. They were used gamma irradiation sources cobalt 60, the main action of ionizing radiation. It occurs through changes in genetic material, this will depend on the intensity of treatment and the type of microorganism. Gram-positive bacteria are more tolerant to irradiation that Gram-negative bacteria [11]. Studies show that cocoa beans from Ghana, need a dose of 5 kGy to reduce the microbial burden [12]. In this context, this paper aims to contribute with necessary information regarding the use of this technique, controlling the growth of *Salmonella* in cocoa.

2. Materials and methods

2.1 Irradiation Test

Were analyzed 31 batches of cocoa beans the pre-processing stage. Each batch was subdivided into 4 subgroups. They were packed in polyethylene bags and were irradiated with Cobalt-60 source underwent to three doses of irradiation (2, 3 and 5kGy) and a control without irradiation in CBE (Companhia Brasileira de Esterilização) - São Paulo, Brazil. Furthermore, the samples were stored at 7 °C in the Microbiology Division of the Research Center for Chemistry, Biology and Agriculture - CPQBA/UNICAMP.

2.2 Isolation of *Salmonella*

It was performed by the method of the Food and Drug Administration using reference methods in the Manual for "Microbiological Analysis Methods of Food and Water" [13]. The pre-enrichment was performed with the milled cocoa beans and suspended in buffered peptone water (BPW), controlling pH 6.8 +/- 0.2 incubated at 35 +/- 2 °C for 18 to 24 h. The enrichment was made in Tetrationate (TT) broth and Rappaport - Vassiliadis Modified (RV) broth incubated at 35 °C and 42 °C / 24 h, respectively. For isolation of *Salmonella* were used the selective media of Hektoen Enteric agar (HE), Xilose Lysine Deoxycholate agar (XLD) incubated at 35 °C / 24 h and Bismuth Sulfite