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DECONTAMINATION OF BIOLOGICAL FERMENT BY GAMMA RADIATION

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ABSTRACT

Biological ferment is a product obtained from pure yeast (*Saccharomyces cerevisiae*) culture by a suitable technological process and employed to increase the size and porosity of the baker's products. Foods containing high microorganisms count indicate that Good Manufacturing Practices were not applied. The aim of this study was to observe the viability of Dry Biological Ferment after the radiation process using different doses of ^{60}Co gamma rays and different storage times. Dry baker's yeast *Saccharomyces cerevisiae* samples were purchased from a local supermarket in São Paulo (Brazil) and irradiated at IPEN in a Gammacell source at 0.5, 1.0, 2.0 and 3.0kGy doses (dose-rate of 3.51kGy/h) at room temperature (25°C). The fluorescent method was performed to observe the viability of yeast cells. The viability decrease with the increase of the radiation dose, as shown: the amount of the viable cell found in the non-irradiated samples (control) at 0 day was 87.2%; 30 days 67.7%; 60 days 77.4% and 90 days 60.1%. With 1.0kGy at 0 day was 61.4%; 30 days 22.7%; 60 days 56.9% and 90 days 24.2%. With 3.0kGy at 0 day was 57.00%; at the next periods the most of the cells become not viable.

1. INTRODUCTION

Yeast is an important component in microbe based industrial technologies [1]. The biological ferment is a product obtained from pure yeast (*Saccharomyces cerevisiae*) culture by a suitable technological process and employed to increase the size and porosity of the baker's products.

The biological ferments, in agreement with moisture content, were classified in fresh ferment and dry biological ferment [2]. The *Saccharomyces cerevisiae* used as fermentation agent (bread, beer, production of ethylic alcohol) is produced under a controlled process with high degree of purity [3].

Measurements to clarify the factors responsible for a higher rate of CO_2 production of microbial populations exposed to ionizing radiation stem-med from the desire of investigators to relate cell death resulting from radiation to some molecular change or process initiated within the cells by the radiation [4].

Among food preservation methods, food irradiation is considered the most versatile and effective treatment, nowadays [5,6]. Food irradiation has been shown to be an effective tool

to eliminate certain foodborne-pathogens from food. Safety and efficiency of food irradiation has been approved by several authorities (FDA, USDA, WHO, FAO, etc.) and scientific societies based on extensive research [7,8].

Food irradiation is the processing of food products by ionizing radiation in order to reduce microbial load and insect infestation, inhibit the germination of root crops, and extend the durable life of perishable produce [9]. Radiation treatment causes practically no temperature raise in the product. Irradiation can be applied through packaging materials including those, which cannot withstand heat [10].

The mechanism of microbial inactivation by ionizing radiation is mainly due to the damage of nucleic acids, direct damage or indirect damage, affected by oxidative radicals originating from the radiolysis of water. Differences in radiation sensitivities among the microorganisms are related to differences in their chemical and physical structure, and in their ability to recover from radiation injury [10].

The methods for evaluation of the viability of yeast cells have been based until this date, in the use of vital dyes or, more frequently, growth in appropriate medium. There are few methods used to determine the viability of cells. Living fungi cells have the capacity of accumulating fluorescein diacetate (FDA). Fluorescein can be visualized through fluorescence microscopy by its brilliant green fluorescence. Ethidium bromide (EB) is fluorescent compound that penetrate whole cells, rapidly enters damage cells giving a bright red fluorescence [11,12,13]. The combination of these two fluorescent compounds results in an intense contrast between living cells and dead cells [13,14]

The aim of this study was to observe the viability evaluated by FDA-EB fluorescent of Dry Biological Ferment after the radiation process using different radiation doses of ^{60}Co and different storage times.

2. MATERIAL AND METHODS

2.1. Material

Three different samples of dry biological ferment were purchased from a local supermarket in São Paulo, Brazil. Each original package containing 10g of sample was stored at room temperature until irradiation process.

2.2. Irradiation Treatment

The samples of biological ferment were irradiated at room temperature, in original packaging (three for each dose assay), using a ^{60}Co gamma ray facility (Gammacell 220) installed in Instituto de Pesquisas Energéticas e Nucleares - IPEN/CNEN (São Paulo, Brazil). The applied doses were 0 (control), 0.5, 1.0, 2.0 and 3.0kGy. The dose rate was of 3.51kGy/h with uncertainly of ± 1. Harwell Amber 3042 dosimeters were used for the measurement of radiation dose. After irradiation, fluorescent viability test was performed.

2.3. Fluorescent Viability Test

After irradiation samples were submitted to a viability test. Around 1g of each sample was suspended in 1.5mL of sterile distilled water. Next, 0.1mL of a 1:1 mixture of a fluorescein diacetate solution and ethidium bromide was added to 0.1mL of the suspension. The material was covered with a glass slide and examined under a fluorescent microscope. One hundred (100) fluorescent cells were counted on each prepared slide. From this count, viable and dead cells percentage was calculated. This experiment was repeated during different storage times (0, 30, 60 and 90 days).

3. RESULTS AND DISCUSSION

The results can be visualized in table 1 and figure 1. Analysing and comparing data in table 1, it was realized that yeast viability during time decreased even in the control sample. It also happened with the irradiated processed samples even if results obtained after 30 days showed a lower percentage of viable cells than percentage viable cells after 60 days.

In the control samples and those irradiated with 0.5kGy, it was found the lowest percentage of dead cells, which indicates a directly proportional relation between irradiation dose and cell viability. It was confirmed with higher irradiation doses, such as 2.0 and 3.0kGy.

Once increasing irradiation dose and storage time, the number of viable cells in the dry biological ferment decreased (Figure 1).

Table 1. **Frequency of viable and dead cells of yeast using fluorescent method of control and irradiated samples during different storage time.**

Storage (Days)	0kGy		0.5kGy		1.0kGy		2.0kGy		3.0kGy	
	VC (%)	DC (%)	VC (%)	DC (%)	*VC (%)	*DC (%)	*VC (%)	*DC (%)	*VC (%)	*DC (%)
0	*87.2	*12.8	*65.3	*34.7	*61.4	*38.6	*63.5	*36.5	*57.0	*43.0
30	*67.7	*32.3	*50.3	*49.7	*22.7	*77.3	*10.7	*89.3	*4.5	*95.5
60	*77.4	*22.6	*79.9	*20.1	*56.9	*43.1	*3.2	*96.8	*0	*100
90	*60.1	*39.9	*32.0	*68.0	*24.2	*75.8	*0.8	*99.2	*0	*100

VC – Viable cells; DC – Dead cells; * Values obtained for average of three different samples.

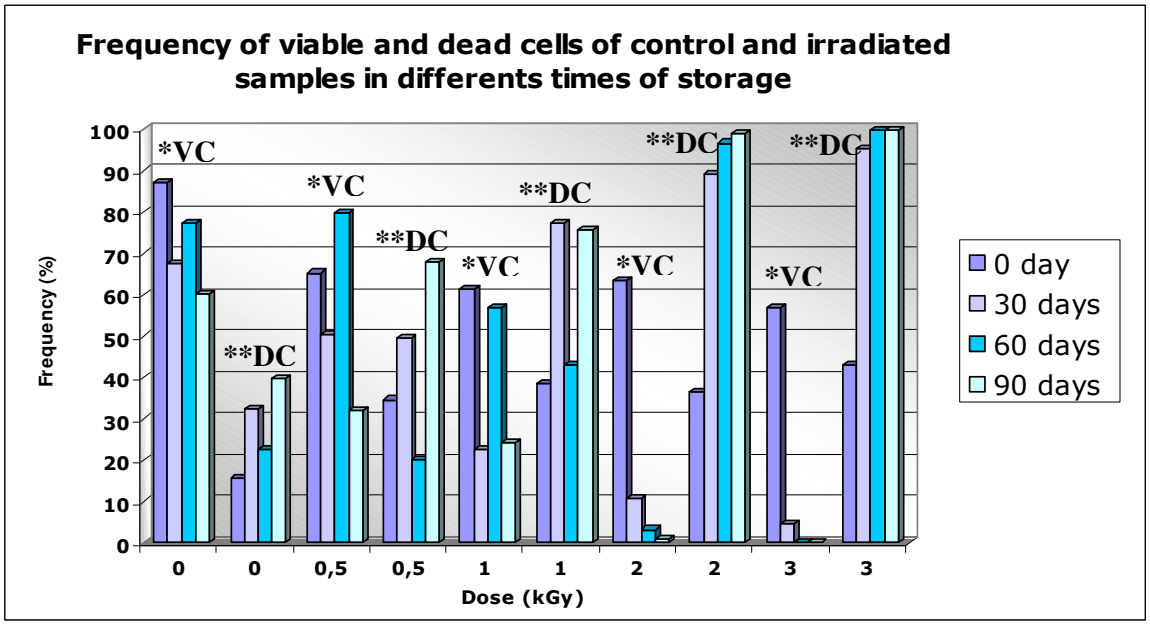

Figure 1. Frequency of viable and dead cells of control and irradiated samples in different storage times (*VC – Viable cells; **DC – Dead cells).

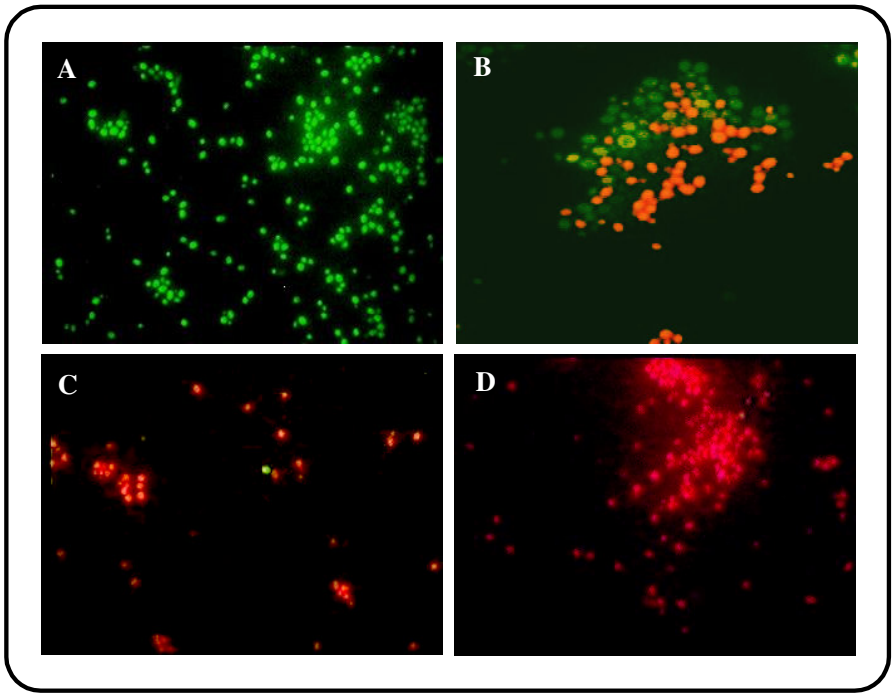

Figure 2. Viable (green) and dead (red) cells of yeast using the method of viability by fluorescence (FDA-EB). A: 0kGy, 0 day; B: 0.5kGy after 30 days; C: 1.0kGy after 30 days; D: 3.0kGy after 30 days.

Alcarde *et al.*[15] and Domarco *et al.* [16] realized that in general yeasts are more resistant than moulds. They also asserted that irradiation doses responsible for almost total reduction of these microorganisms are between 4.65 and 20.0kGy for yeasts and 2.5 and 6.0kGy for moulds. They proposed a range of 5.40 and 5.80kGy irradiation dose as a lethal dose for *Saccharomyces sp.* specifically.

Iemma *et al.* [17] noticed irradiation doses between 1 and 5.0kGy were effective in the reduction of *Saccharomyces cerevisiae* even after 90 days.

According to Jay [18] a dose of 3.0kGy was not sufficiently to cause yeast death, however in this study we observed that using the same dose and a 60 day storage time it was found 100% of dead cells.

4. CONCLUSION

From data obtained, we conclude that the radiation gamma initially inhibits viability of yeast cells and this effect was intensified over storage time both in control and irradiated samples. Doses of 0.5, 1.0 and 2.0kGy caused a reduction in the number of viable cells as well as increasing storage time. A dose of 3 kGy caused total death of yeast cells after 60 days of storage.

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