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Effect of Gamma Irradiation on the Microbiological Analysis of the Green Banana Flour

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ABSTRACT: *Brazil accounts for about 9% of the world banana production, however, the production losses are high. Nevertheless, these losses can be reduced by processing the fruit "unsuitable" for consumption into products based on green banana (pulp, rind and flour). The green banana flour is a complex–carbohydrate source, mainly resistant starch with functional properties, for use in Brazilian irradiated ready-to-eat foods. Gamma irradiation is considered to be an alternative method for food preservation to prevent food spoilage, insect infestation and, also, to reduce the microbial load. The object of this work was to determine the effect of gamma irradiation on microbiological analyses of the Total Coliforms at 35°C, Coliforms at 45 ℃, Salmonella spp, Staphylococcus aureus and the number of mesophilic of the green banana flour, commercially found in the Brazilian market. The Microbiological analyses were carried out in conformity with the methodologies described at the Faculty of Veterinary Medicine, according to the current legislation. Irradiation was performed in a ^{60}Co source, at doses of 1kGy and 3kGy. In samples of green banana flour, irradiated at 1 and 3 kGy, the growth of the Staphylococcus coagulase positive could not be observed. Furthermore, the irradiation process showed to be effective to reduce the microbial load of mesophiles, with both doses. As a result, the application of the irradiation technique may be recommended to enhance the food safety.*

KEYWORDS: *microbiological analysis, food irradiation*

I. INTRODUCTION

Brazil accounts for about 9% of the world banana production, however, the production losses are high. However, these losses can be reduced by processing the fruit "unsuitable" for consumption into products based on green banana (pulp, rind and flour) (Sunthralingam & Ravindran, 1993; Cordenunsi *et al.*, 1998). The green banana flour is a complex carbohydrate source, mainly of resistant starch with functional properties, macronutrients and micronutrients, for the use in the Brazilian irradiated ready to eat foods, such as bread, macaroni and dietary products, among others (Tribes *et al.*, 2009; Borges *et al*, 2009; Taipina *et al*, 2008).

In general, the addition of banana starch promoted a dilution effect on protein, lipid, and ash content, while moisture content was not affected. On the other hand, the content of resistant starch increased significantly ($p<0, 05$) with an increase of banana starch (Hernandes-Nava, *et al* 2009).

Studies on the mature green banana fruit shows that it is rich in amylase-resistant starch, which simulates colony production of short- chain fatty acids and it is used for treating diarrheal diseases. Green banana diet improves clinical severity in childhood shigellosis and it could be a simple and useful adjunct for dietary management of this illness (Rabbani *et al*, 2009).

Other works described with the Prata green banana flour, obtained using the process exposed in this study, presented an ideal microbiological pattern for: *Coliforms*; filamentous *fungi* and *yeast*; *Bacillus cereus, Salmonella spp.; Staphylococcus coagulase positive* and the counting of *mesophiles* (Borges *et al*, 2009).

The possibility of using gamma irradiation to improve the microbiological and fungal quality of different foods has been studied and it is, currently, applied commercially in the USA and France, among other countries. In many cases, food irradiation is limited due to the fatty acid decomposition and subsequent off-flavor formation in the foodstuff. Gamma irradiation is considered to be an alternative method for food preservation, in order to prevent food spoilage, insect infestation and, also, to reduce the microbial load. Food irradiation does not replace proper food production or handling. Even with treatments that destroy 99,9% of a pathogen, some could still survive (Desmonts,1997; Agundez - Arvizu, *et al*, 2006;Wood & Bruhn, 2000).

Bhat & Sridhar (2008) described nutritional qualities and functional properties of both raw and electron beam - irradiated (doses: 0; 2,5; 5; 7,5; 10; 15 e 30 kGy) lotus seed flour. The seeds showed to be rich in protein, amino acids, unsaturated fatty acids and minerals. Irradiation of seeds revealed a decrease in crude protein and fiber, which was not significant at any of the doses. However, significant increases of ash (10 kGy onwards) and carbohydrates (at 30 kGy) were recorded, after irradiation. As a physical method of preservation, electron beam irradiation was effective in the retention of the nutritional qualities of lotus seeds. The green banana flour also possesses great potential for development of new food products and formulations. Bakery and intermediate moisture food products, such as enriched bread, cereals, cookies and crackers may benefit from irradiation processing. However, irradiated lipid rich products should be assayed, carefully, in terms of safety, nutrition and acceptability (Skrbic & Filipcev, 2008).

The object of this work is to determinate the effect of gamma irradiation on the microbiological analyses of: the number of aerobic *mesophilic, Total Coliforms at* 35°C, *Coliform at* 45°C, *Staphylococcus coagulase positive* and the *Salmonella* spp of the green banana flour, commercially found in the Brazilian market.

II. MATERIAL AND METHODS

2.1 Material

Eight packets of green banana flour were used found in the market, in 200g pouches. Lots of the green banana flour were employed and kept at a refrigerator (4-7ºC), before and after irradiation. A portion of 25g of flour, homogenized with 225 ml of sterile peptone water (1%) in a sterile plastic bag, was analyzed for *Salmonella spp*; another plastic bag containing 225ml of sterile 0.1% peptone water with other homogenized 25g flour was kept for further analyses. The samples were homogenized in a stomacher for about a minute. This was considered the dilution of 10^{-1}.The enumeration of *Total Coliform at* 35°C, *Colif*orm *at* 45°C and *Staphylococcus coagulase positive* expression of the results were performed by CFU/g (Brazil, 2003; Brazil, 2001; Bennett,& Lancette, 2001).

2.2. Irradiation

Irradiation was performed in a ^{60}Co Gammacell 220 (AECL) source, at a dose rate of about 1.96 kGy/h with doses of 1kGy and 3kGy, dose uniformity factor of 1.13. Dosimetric mappping was previously performed by Fricke dosimetry.

2.3. Microbiogical *A*nalysis

Microbiological analysis of green banana flour samples were analyzed at the Faculty of Veterinary Medicine and Animal Science, before and after irradiation at IPEN-CNEN/SP. The microbiological analyses were carried out in conformity with the methodologies described at the Faculty of Veterinary Medicine, according to the current legislation, with the following determinations: *Total Coliforms* at 35°C,, *Colif*orm at 45°C, *Salmonella spp*, *Staphylococcus coagulase positive* and the *mesophiles* (Brazil, 2003; Bennett,& Lancette, 2001; Brazil, 2001). The analysis of variance was applied and the mean comparisons were performed by Dunnett test, considering an error of 5%.

Standard Count of microorganisms, facultative viable aerobic *mesophiles* and *Staphylococcus* coagulase showed to be positive. After the initial dilution (10^{-1}), the other dilutions (to 10^{-5}), in saline Peptone 0.1%, were done.

One ml of each dilution was inoculated into sterile Petri dishes and then covered with about 15 ml of Plate Count Agar (PCA), merged and maintained between 46 and 48 °C by the method "pour plate" (plating in depth). The samples were homogenized, left to solidify at room temperature and incubated inverted at 36° C, for 48 hours. To read the mesophiles, the plates of the dilutions that had 25 to 250 colonies were counted and the results expressed in CFU /g.

For *Staphylococcus coagulase positive* reading, the plates containing between 20 and 200 colonies were selected for counting. The number of typical and atypical colonies was counted (Bennett & Lancette, 2001).

The culture medium used for detection and enumeration of Coliforms was the chromogenic medium Rapid E. coli 2 Biorad®. The Principle of the medium was based on simultaneous detection Coliforms and *Escherichia coli*. The hydrolysis of chromogenic substrates included, in the product, results in blue Coliforms colonies (β-D-glucoronidase negative / β-D-galactosidase positive) and in purple *Escherichia coli* colonies (β-D-glucoronidase positive / β-D-galactosidase positive) (Brazil, 2003).

For *Salmonella*, the pré-enrichment took place through the incubation of sample aliquots prepared in Peptone Water, at a concentration of 1% and incubated at 36º C, for at least 16 hours and no more than 20 hours. From the pré-enrichment procedure established, 0.1 ml sample tube containing 10 ml of Rappaport Vassiliadis broth was inoculated and incubated at 41 ± 0.5 º C, in a 24-hour water bath. The selective media chosen were the XLT4 Agar Agar and Hektoen. If the incubations in the culture media reveal suspected colonies of *Salmonella*, this will be confirmed by biochemical and serological tests.

III. RESULTS AND DISCUSSION

The American FDA (1997) considered a dose between 0.2 and 0.5 kGy effective to eliminate spoilage fungus from wheat flour and wheat grains, for instance. Maintaining the irradiation doses below 1 kGy could be the best procedure to treat this type of food, without detrimental radiation effects.

In this investigation, commercial Mexican bread, made with wheat flour, was irradiated at 1,0 kGy using a Co-60 Gammabeam 651 PT irradiator facility, the obtained results confirm that gamma irradiation is effective to reduce the microbial load in bread. The total aerobic microorganisms, yeast and mold counts were reduced by 96%, 25% and 75%, respectively, with the irradiation process (Agundez - Arvizu, et al 2006).

Another work, initial microbial load of instant cup noodle(ICN),was investigated and gamma

irradiation applied to develop immune-compromised patients food for their safe consumption. Gamma irradiation could improve the microbial quality of instant cup noodle (Lee et al, 2012)

In the table 1 shows the results of the microbiological analyses of the number of mesophilic, total Coliforms at 35°C, Coliforms at 45°C, Staphylococcus coagulase positive and Salmonella spp of industrialized green banana flour, non-irradiated and irradiated with 1 and 3kGy. Only for mesophiles, a significant difference (error 5%) was observed between irradiated and non-irradiated samples. As it can be seen, gamma irradiation is effective to reduce the number of aerobic mesophilic of industrialized green banana flour. Some microorganisms: Salmonella spp, Total Coliforms at 35°C and Coliforms at 45°C, were not found. Moreover, the growth of the Staphylococcus coagulase positive could not be observed.

Table 1- The microbiological analyses of the number of *mesophilic* , total *Coliforms at 35℃, Coliforms at 45℃, Staphylococcus coagulase positive, Salmonella* spp, of industrialized green banana flour, non-irradiated and irradiated with 1 and 3kGy.

Microbiological Analysis	Dose (kGy)		
	0	1	3
Number of aerobic *mesophilic* in CFU/g	$7.4 \times 10^{4\,a} \pm 707.11$	$1.0 \times 10^{4\,b} \pm 707.11$	$6.0 \times 10^{2\,b} \pm 7.07$
Most probable number of *Total Coliforms at 35℃* in MPN/ g	$3^{a} \pm 0$	$3^{a} \pm 0$	$3^{a} \pm 0$
Most probable number of *Coliforms at 45℃*, in MPN/ g	$3^{a} \pm 0$	$3^{a} \pm 0$	$3^{a} \pm 0$
Number of *Staphylococcus* coagulase positive in CFU/g	$100^{a} \pm 0$	$99.5^{a} \pm 0.70$	$99^{a} \pm 1.4$
Presence of *Salmonella* spp (in 25g)	Absence	Absence	Absence

n = duplicate; Means ± standard deviation

a [b]Medium values followed by the different letters, on the same line, differ significantly from the control-sample, at 5% significance (error 5%)

IV. CONCLUSION

The present study does not allow conclusions about the effect of radiation of green banana flour, in relation to *Total Coliforms at 35℃ , Coliforms at 45℃* and *Salmonella* spp, since we did not find such microorganisms.

In samples of green banana flour, irradiated at 1 and 3 kGy, the growth of the *Staphylococcus coagulase positive* could not be observed. Furthermore, the irradiation process showed to be effective to reduce the microbial load of *mesophiles*, with both doses. As a result, the application of the irradiation technique may be recommended to enhance the food safety.

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