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Effects of Gamma Irradiation on Microbial of White Sandwich Bread

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Abstract: **Daily consumption of bread as staple food is enormous. However, the spoilage of the bread is a common problem and mainly caused by microbial. This study investigates the effects of gamma irradiation on microbial of white sandwich bread. Three different brands were irradiated using gamma radiation doses of 0.0, 0.2, 0.3, 0.4 and 0.5 kGy. Results show that 0.2 to 0.5 kGy gamma radiation exposure to the bread has the potential of eliminating or at least minimizing the ecology of spoilage-causing microorganisms in the bread. It is concluded that gamma irradiation could serve as an effective method in extending the shelf life of white sandwich bread.**

Keywords**: White sandwich bread, Gamma irradiation, microbial, shelf life, food spoilage.**

I. INTRODUCTION

Ever since the prehistoric times, bread have become the staple food for millions of people worldwide due to its well-balanced nutritional values which benefit folks from all walks of life. The rich carbohydrate content of bread for instance is capable of providing almost 50% of one's daily calorie requirement which is essential in maintaining a sufficient energy supply to the body [1]. Bread products also are normally baked with enriched flour which contains various micronutrients such as iron which serves as a major component of the haemoglobin and folate which is essential for cardiovascular well-being [2].

Despite the fact that it provides plethora of benefits to the consumers, one of the main issues related to bakery products is that they have a relatively shorter shelf life and are prone to spoilage as compared to other processed food products. This particular condition may be influenced by various factors and among them are moisture content, formulation of ingredients, and product handling [3]. Thus, continuous effort to improve the shelf life of bakery products is indeed essential in order to ensure that consumers can be supplied with food products of guaranteed quality.

Since decades before, the baking industry has been employing numerous conventional preservation methods such as addition of chemical preservatives and low temperature product storage with the aim of extending the shelf life of bakery products [4]. Bread preservation techniques such as chemical treatment, pasteurization, and low temperature storage are among the conventional practices in conserving the quality of bakery products [5]. However, some of these so called effective techniques such as addition of chemical preservatives in the products might actually jeopardize the health of consumers. This is because reports have shown that rats which were fed with 4% concentration of calcium propionate which are normally used in the baking process have developed cancer-like tumors [6]. In addition to that, prolonged preservation of bakery products with high concentration of calcium propionate also has the potential of stimulating the growth of resistant strains of *Penicillium roqueforti* on the bread [4].

However, with the rapid advancement in food processing technology, food irradiation approach also has been widely implemented due to its substantial capacity in minimizing food spoilage by combating microbial pathogens [7]. Food irradiation technology also significantly improves food safety apart from extending its shelf life by minimizing or eliminating the presence of microorganisms such as *Salmonella* and *Escherichia coli* (E. coli) on food [8]. Normally, the

irradiation process makes use of specific radioactive sources such as Cobalt-60 which emits ionizing radiation in an array of intense gamma photons. The ionizing radiation arrays which strike on food product is of high energy which is capable of disintegrating the chemical bonds in pathogenic microbial molecules that are essentially important for its cellular growth and genomic integrity [9]. Consequently, the spoilage and diseases-causing microorganisms die or can no longer proliferate due to extensively impaired cellular properties. Thus, the main purpose of conducting this research was to examine the efficacy of gamma irradiation as an alternative to other bread preservation techniques especially the controversial ones, in extending the shelf life of white sandwich bread. The study also aims at ascertaining the role of gamma irradiation in extending the shelf life of white sandwich bread by reducing the presence of bacteria and microorganism which causes its spoilage.

II. MATERIALS AND METHOD

2.1 White sandwich bread:

Three different brands of white sandwich bread were used in this study and were labelled as brand A, B and C, respectively. In ensuring that a bias-free study can be conducted, the uppermost and lowermost slices of bread crusts were excluded in this experiment. This is because based on an experiment conducted by [10], it was found that chemical reaction from the baking process actually produces a substance known as pronyl-lysine that is eight times more abundant in the crust than in the crumb. As, one of the factors influencing shelf-life of bakery products is the formulation of ingredients, thus content differential between the bread crusts and crumbs may affect the accuracy and significance of the results obtained from the experiment.

2.2 Cobalt-60 radioactive source:

For the purpose of this study, Cobalt 60 (Co-60) radioactive source was chosen due to the fact that besides its vast application in the medical field, it is also said to be effective in preserving food products by killing pathogenic microbes and preventing spoilage [11]. Co-60 is also capable of emitting two high energy gamma rays of 1.17 MeV and 1.33 MeV which have an excellent penetrating power on various types of materials [12].

2.3 Gamma irradiator:

The gamma irradiator unit used in this experiment was located at the Gammacell Irradiation Lab of National University of Malaysia (UKM), Bangi Campus. The specific type of gamma irradiator used is known as Gammacell 220 Excel manufactured by Nordion Incorporated. Basically, the Gammacell is loaded with eight Co-60 sealed sources in the form of plated pellets. The unit comprises of an annular source permanently enfolded with a lead shield, a cylindrical drawer, and a dedicated drive mechanism to shift the drawer up or down along the source center-line. The drawer is integrated with a chamber which carries the sample of interest towards the Co-60 source for irradiation. The chamber is designed to accommodate a variety of sample sizes up to six inches in diameter and eight inches in height.

2.4 Packaging of white sandwich bread samples:

Individual slices of the white sandwich bread were carefully packed into transparent polyethylene bags measuring 15x22cm each with sealable opening. The transparent polyethylene bags help to ensure that minimal direct contact can be established between samples and the operator to maintain high level of hygiene. Once the individual slices were inserted into their respective bags, the opening of the bags was properly sealed to minimize samples contamination from the surrounding atmosphere by keeping them in a well preserved enclosure. Each bag was labelled according to its respective brands namely A, B and C followed by their gamma exposure magnitudes namely 0.0, 0.2, 0.3, 0.4 and 0.5kGy as shown in Table 1.

Table.1: Control & irradiated groups of white sandwich bread samples

	Brand/ Gamma dose		
Control (non-irradiated) group	A/ 0.0 kGy	B/ 0.0 kGy	C/ 0.0 kGy
Irradiated group	A/ 0.2 kGy	B/ 0.2 kGy	C/ 0.2 kGy
	A/ 0.3 kGy	B/ 0.3 kGy	C/ 0.3 kGy
	A/ 0.4 kGy	B/ 0.4 kGy	C/ 0.4 kGy
	A/ 0.5 kGy	B/ 0.5 kGy	C/ 0.5 kGy

2.5 Irradiation of white sandwich bread samples:

Once all the samples are packed and labelled accordingly, they were brought into the Gammacell Irradiation Lab to be exposed at 0.2, 0.3, 0.4 and 0.5 kGy doses of gamma which is an effective exposure range in sterilizing grain and cereal products [9]. However, the 0.0 kGy group of samples were not irradiated as it serves as a control group to ensure necessary comparison can be made between irradiated and non-irradiated groups.

Firstly, 3 slices (one from each brand) of the bread samples were placed on stack into the gamma irradiator chamber and the collar doors of the chamber were locked once all the samples were in place. Then, the necessary exposure time was set on the digital timer on the control panel. Upon pressing the start button, the motor-driven drawer drives the irradiator chamber downward to the center of the source. The chamber remains in such position until the preset time interval has elapsed and eventually it rise automatically allowing the operator to collect the samples back.

2.6 Microbial analysis of white sandwich bread samples:

Following the irradiation process at Gammacell Irradiation Lab of UKM Bangi Campus, samples were brought to the Food Sciences Laboratory of Kuliyyah of Allied Health Sciences, International Islamic University Malaysia (IIUM) Kuantan Campus for microbiological analysis. At the Food Sciences Laboratory, samples weighing 25g were taken from each slice of white sandwich bread and were diluted into individual beakers containing 225ml of peptone water. Then, the dilution was carefully poured into a sample bag before being homogenized by a stomacher blender. Following the homogenization process, the samples were further diluted into 25ml of peptone water producing two additional concentrations, 10^{-2} and 10^{-3} by pipetting 1ml of homogenate from the initial dilution into the subsequent ones. Then, about 0.1ml of homogenate is pipetted from each dilution ($10^{-1,}$ $10^{-2,}$ and 10^{-3}) into their respective nutrient agar plates, and for each dilution 2 sets of agar plates were prepared. By using a sterilized hockey stick, the dilutions were spread uniformly across the agar medium so that the number of microbial colonies present on the plate can be easily quantified. Once all the sample plates were prepared, they were stored in an oven under controlled temperature of 37°C.

2.7 Quantification of colony forming unit (CFU) on the samples:

The presence of microorganism on the samples was observed on daily basis for 5 days and the number of colonies present on each agar plate was recorded accordingly. The calculation method used in quantifying the colony forming unit (CFU) on each sample plate is based upon the following equation:

cfu/g = number of colony x dilution factor / volume of cultured sample

The microbial load (cfu/g) present in each sample of varying dilution factors namely 10^{-1}, 10^{-2} and 10^{-3} were recorded and tabulated accordingly. However, one of the main purposes of preparing the 10^{-2} and 10^{-3} sample dilutions was to aid in the quantification of individual microbial colony present in the samples as the number of microbes in the most concentrated dilution (10^{-1}) could be too dense and can't be quantified effectively. However, for the purpose of results analysis, only the CFU from 10^{-1} sample plates were employed as they were able to provide quantifiable number of microbes from each individual plate of varying brands and doses. The number of microbial colony forming unit present on the sample plates were counted by marking each visible colony with a red marker pen on the surface of the agar plate.

III. RESULTS AND DISCUSSION

3.1 Gamma dose and colony forming unit (cfu) relationship:

Hypothetically, the number of colony forming unit on each individual sample is inversely proportional to the radiation dose exposed on the samples. The higher radiation dose given, the lower will be the microbial load in the form of cfu/g on the samples.

3.2 Microbial load of control group samples in 5 days:

The histogram in Fig. 1 represents the average microbial load of Brand A, B and C control group samples in five days as a whole.

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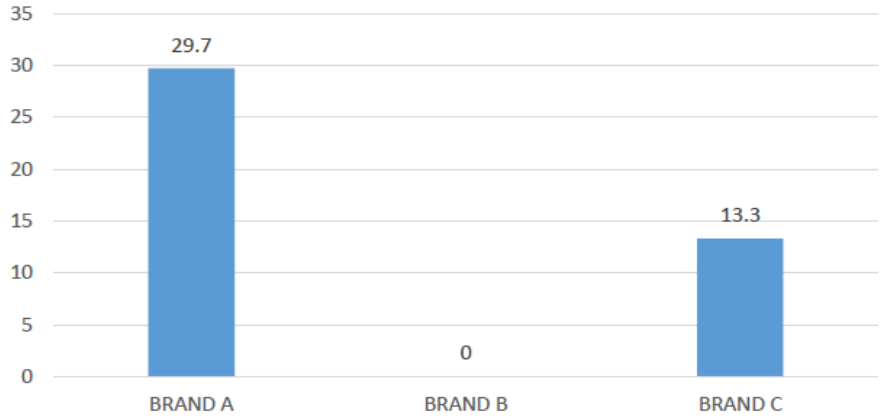

Fig.1: Mean microbial load of Brand A, B and C control group samples in 5 days.

By referring to the histogram, it is evident that among the three brands used, samples of Brand A control group recorded the highest amount of microbial load with an average of 29.7 followed by Brand C samples with an average of 13.3. However, throughout the total of five days observation period, zero CFU was recorded for the control group of Brand B samples. This is suggestive that there is no quantifiable microbial ecology present in the control group of Brand B samples throughout the period of study.

3.3 Microbial load of irradiated group samples in 5 days:

The histogram in Fig. 2 represents the averaged mean microbial load of Brand A, B and C irradiated group samples in five days period as a whole. Based on the histogram, the mean number of microbial loads of irradiated Brand A samples increases consistently from gamma exposure of 0.2 kGy to 0.4 kGy. However, Brand A samples recorded a relatively lower number of colonies at gamma dose of 0.5 kGy. In other words, the microbial ecology in irradiated Brand A samples were effectively reduced beyond the 0.4 kGy of gamma exposure. Similarly, the mean microbial load of irradiated Brand C samples increases consistently from 0.2 kGy to 0.3 kGy followed by a decreasing pattern beyond 0.3 kGy towards 0.5 kGy. Meanwhile, the mean microbial load of irradiated Brand B samples increases directly proportional with gamma dose increment up to the maximum dose limit which is 0.5 kGy.

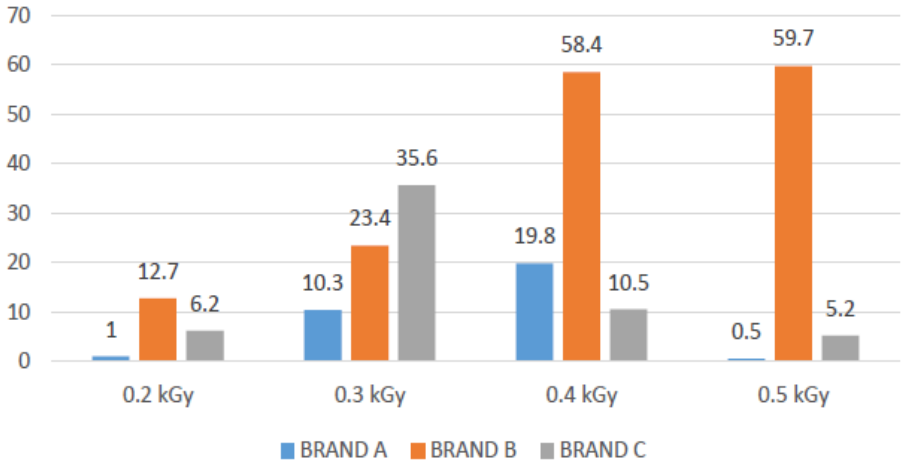

Fig.2: Mean microbial load of Brand A, B and C irradiated group samples in 5 days.

3.4 Radiation induced cellular damage:

As high energy spectrum of ionizing radiation is capable of damaging the nucleic acids of living cells which eventually may lead to cell killing. Their interaction at the cellular level of the microorganisms could severely affect the integrity of critical targets of the cells especially on its deoxyribonucleic acid (DNA) [13]. In the context of this study, reduction in microbial load as shown in Fig. 2, the mean microbial load of some of the samples might be caused by three main types of radiation induced DNA damages namely base damage, single strand break (SSB), and double strand break (DSB) [14].

According to [15], following radiolysis, molecules containing an unpaired electron in the valence shell are highly reactive and may cause a severe chemical and biological damage by breaking the chemical bonds of other molecules. Besides that, about two third of all radiolysis-induced damages are caused by the hydroxyl free radicals as they have excess energy enabling them to interact destructively with other molecules distant from radical's origin. More importantly, chemical bonding of two hydroxyl radicals may yield a hydrogen peroxide molecule which is evidently poisonous to the cells.

This situation indirectly explains the possibility that the reduction in the mean microbial load of the Brand A and Brand C irradiated samples as shown in the results from day 1 to day 5 might be caused by the interaction of free radicals or hydrogen peroxides with the microflora in the samples. This is because, a study conducted by [16] revealed that hydrogen peroxide is capable of damaging cellular components such as proteins and DNA of *Escherichia coli* which can be found in certain infected foods, leading to mutagenesis and cell death.

3.5 Radioresistance of microbial ecology in bread samples at varying gamma doses:

The interesting properties of radioresistant cells actually suggest that the reason why irradiated Brand A samples demonstrates increment in the mean microbial load despite being exposed at 0.2 to 0.4 kGy might be due to their radioresistance towards gamma exposure at that particular dose range. However, as the radiation dose increases to 0.5kGy, the mean number of colonies of irradiated Brand A samples demonstrate a significant reduction, suggesting that the microbial protection or repair mechanisms might no longer able to resist the damaging effects of exposure increment. Similarly, irradiated Brand C samples also demonstrate an increment in their mean microbial load despite being exposed at 0.2 to 0.3 kGy of gamma which is most probably due to its radioresistance at that particular dose range. However, the histogram in Fig. 2 shows that from 0.3 kGy onwards the mean CFU of irradiated Brand C samples demonstrate a reduction of about half of its initial value, suggesting that radioresistance of the microorganism is no longer effective beyond the 0.3 kGy threshold level. Thus, we may assume that the microbial ecology in the Brand A samples are relatively more radioresistance than the Brand C samples as the cellular lethality threshold level of Brand A sample is rather higher than the Brand C samples.

Meanwhile, even after being observed on daily basis for 5 continuous days, the whole plates of irradiated Brand B samples still failed to demonstrate any significant reduction in their mean number of colonies. Instead, the data collected shows that they continuously demonstrate a consistent increasing pattern of the mean microbial load from day to day even after being exposed to gamma radiation as high as 0.5 kGy. The occurrence of this particular condition is most probably due to the presence of a relatively high radioresistance microbial ecology in the Brand B samples as compared to the Brand A and C samples. This is because, certain lactic acid bacteria which are widely used in the fermentation of bakery products such as *Lactobacillus acidophilus* are highly radioresistant towards gamma radiation as high as 0.9 kGy, thus making them difficult to be reduced or eliminated at gamma exposure between 0.2 kGy to 0.5 kGy [17].

In this study, investigations into the effects of gamma radiation on the shelf life of white sandwich bread has strongly suggested that gamma irradiation could serve as an effective method in extending the shelf life of the product. This is mainly due to the substantial ability of gamma radiation in reducing the microbial load in the bread which as discussed before is the main factor influencing the shelf life of food products. Thus, by effectively reducing the microbial ecology in the bread samples, it is highly suggestive that the bread's shelf life can be extended at a relatively longer period of time as the main factor causing its spoilage has been efficiently tackled.

The results obtained from this study also has proven that exposure of white sandwich bread at certain range of gamma doses has the potential of eliminating or at least minimizing the ecology of spoilage-causing microorganisms in the bread. It can be assumed that the threshold level of gamma exposure in reducing the microbial load present in the bread is at 0.3 kGy and 0.4 kGy for breads of Brand C and Brand A respectively. Meanwhile the threshold level of gamma exposure for breads of Brand B could not be determined based on this work due to certain limiting factors such as high radioresistance of microbial ecology in that particular brand.

IV. CONCLUSION

There is a significant difference in terms of microbial ecology between different brands of bread at varying gamma doses. The overall results show that, the microbial ecology in bread of Brand B has the highest level of radioresistance followed by the bread of Brand A, while microbial ecology in bread of Brand C has the lowest level of radioresistance. In conclusion, effects of gamma radiation on the microbial of white sandwich bread have strongly suggested that gamma irradiation could serve as an effective method in extending the shelf life of the product.

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