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Effect of Gamma Irradiation on the Bacteriological, Sensory and Physicochemical Qualities of Peanut Butter

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Authors' contributions

All authors contributed equally to this research and manuscript preparation. Authors WTT, AKS, DDB, and AAG designed the study, performed the statistical analysis, wrote the protocol and managed the literature searches. Authors WTT and AKS wrote the first draft of the manuscript. Authors WTT, AKS, DDB, SAA, JNOA and JA managed the analyses of the study. All authors read and approved the final manuscript.

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ABSTRACT

Aims: This study investigated the effect of gamma irradiation on the bacteriological and sensory qualities as well as some physicochemical properties of peanut butter irradiated at different doses.
Methodology: Peanut butter samples were pre-packaged and sealed in plastic jars with each jar containing 500 g of peanut butter. The samples were exposed to 0, 2, 3, 4 and 5 kGy absorbed doses of gamma radiation and bacteriological, sensory and physicochemical qualities were evaluated.
Results: The TVC of the irradiated peanut butter ranged between 3.33 and 2.0 cfu/g and *Staphylococcus aureus* count ranged between 2.63 cfu/g to below detection limit. A dose of 2 kGy

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resulted in a 0.93 log reduction whilst maximum dose of 5 kGy used resulted in a 1.33 log reduction in the total viable counts of the irradiated samples. *E. coli* and *Salmonella* sp were not detected in both the irradiated and unirradiated peanut butter. No significant effect was observed in the sensory properties of the irradiated peanut butter. Peroxide value (PV) and Malonialdehyde (MDA) content were observed to range between 42.00±2.83-44.00±0.00 (meq/kg) and $6.89 \times 10^{-7} \pm 2.26 \times 10^{-8}$ - $1.12 \times 10^{-6} \pm 5.44 \times 10^{-8}$(M) respectively.

Conclusion: The MDA concentration increased significantly with increasing dose though no significant differences were observed between the measured values for doses of 2 and 3 kGy as well as 4 and 5 kGy. Gamma irradiation proved effective in reducing microbial load in the peanut butter without significantly affecting the sensory attributes as well as the peroxide value.

Keywords: Peanut butter; irradiation; bacterial count; sensory; physicochemical properties.

1. INTRODUCTION

Peanut butter is a food paste made primarily from ground dry roasted peanut, *Arachis hypogaea* L. which is an important oilseed leguminous crop cultivated in over 100 tropical and subtropical countries. It is mainly used as a sandwich spread, sometimes in combination as peanut butter and jelly sandwich and therefore a popular addition to many snacks and confectionaries in the western world. In African countries such as Ghana and Nigeria, peanut butter is used in preparing soup. It is an excellent source of plant protein and also supplies essential fatty acids that are needed by the human body. However peanut butter is frequently contaminated with various bacterial and fungal pathogens. Contamination with various species and strains of *Salmonella* have been reported [1,2]. Toxigenic moulds such as *Aspergillus* occasionally have been detected in peanut kernels [3,4]. Peanut butter has been cited as a potential source of disease transmission in several countries. In the United States, peanut butter and peanut butter products have been implicated as the vehicle of multistate outbreaks of *Salmonella tennessee* and *typhimurium* [5,6]. In 2014 the European Union issued several food safety alerts on a number of non-traditional exports including peanut butter exports from some African countries. Production of aflatoxins and ochratoxin A (OTA), which are potent toxins known for the teratogenic, immunosuppressive and carcinogenic effects have been reported [7].

Generally, the use of good manufacturing practices are recommended for the control of peanut butter contaminations. Current processing methods include the use of thermal treatment in pasteurization of peanut butter. However, there are indications that thermal treatments are inadequate to consistently destroy *Salmonella* in contaminated peanut butter [8,9]. In most developing countries, peanut butter is produced traditionally on a small scale with limited quality control procedures to ensure microbiological and toxicological safety. There is therefore the need for new effective methods for the control of microbial contamination of peanut butter. Although the use of ionizing radiation to improve hygienic quality of foods is well-documented [10,11], there has been limited use of the technology to enhance the hygienic quality of peanut products. The aim of the present study was to evaluate the bacteriological, sensory and some physicochemical parameters of irradiated peanut butter.

2. MATERIALS AND METHODS

Samples of peanut butter were obtained from the Food Research Institute of the Council for Scientific and Industrial Research, Accra-Ghana. The samples were pre-packaged and sealed in plastic jars with each jar containing 500 g of peanut butter. The samples were exposed to 0, 2, 3, 4 and 5 kGy absorbed doses of gamma radiation from a cobalt-60 source at the Gamma Irradiation Facility of the Radiation Technology Centre, Ghana Atomic Energy Commission. The absorbed dose was confirmed by ethanol-chlorobenzene (ECB) dosimetry.

2.1 Microbiological Analysis

The irradiated and unirradiated samples were microbiologically analyzed to determine the populations of indicator and pathogenic microorganisms. From each sample 10 g was weighed and blended with 90 ml diluents (0.1% peptone + 0.5 NaCl) for 90 min in a Waring Blender and stirred on a mechanical shaker (Junior Orbit Shaker, Lab-Line In-struments, United States of America) for 30 min. Total viable count was determined on Plate Count Agar (Oxoid, England); total coliform count was

determined on Violet Red Bile Agar (Oxoid, England); *Staphylococcus aureus* was estimated on Baird-Parker Agar (Oxoid, England) and *Escherichia coli* was estimated on Eosine Methylene Blue Agar (Oxoid, England). The detection of *Salmonella spp*. was done using 25 g of sample on Xylose Lysine Deoxycholate Agar (Oxoid, England). All samples were incubated at 37℃ for 48 hours.

Counts are expressed as $\log_{10}$ cfu/g.

2.2 Sensory Analysis

Sensory analysis was conducted for attributes of colour, aroma, taste, flavour, texture and overall acceptability. A total of 35 panelists analysed the samples on two different days in a preference test. Panelists scored for 5 samples of irradiated peanut butter according to a 9-point hedonic scale. Data obtained was then pooled together and analysed with Statgraphics Centurion XVI.

2.3 Peroxide Value

Peroxide value was analysed according to AOAC 965.33 (2000). 5 g of oil was weighed into an Erlenmeyer flask and 30 ml of acetic acid - chloroform solution was added and swirled to dissolve oil. 0.5 ml of saturated Potassium Iodide solution was added and allowed to stand for 1 min with occasional shaking after which 30 ml of distilled water was added. The mixture was then titrated slowly against Sodium Thiosulfate standard solution until the yellow colour was almost gone. 0.5 ml of 1% starch solution was then added as indicator and titration was continued until blue colour just disappeared.

Peroxide value was calculated as follows:

Peroxide value = S x M x 1000/g sample,
where S = ml $Na_2S_2O_3$ (blank corrected) and
M = molarity $Na_2S_2O_3$ solution

2.4 Lipid Peroxidation MDA Assay

Malonialdehyde content of the peanut butter was quantified as a measure of rancidity. A lipid peroxidation assay kit (Biomedical Research Services and Clinical Application, Bufffalo, New-York) was used for the analysis. 1 g of sample was homogenized in 10 ml of ice cold Trichloroacetic acid (TCA) for 5 mins and clarified by centrifugation at maximal speed for 5 mins. 2 ml of the TCA treated sample was then mixed with 2 ml of the working solution (6.5 mg of Thiobarbituric acid (TBA) per ml of MDA Assay Solution) in a centrifuge tube and heated at 95℃ for 30 mins. The tube was allowed to cool down to room temperature and spinned briefly in a centrifuge to deposit contents. 4 ml of n-butanol was then added to the sample and agitated for 1 min. The tube was then centrifuged for 3min and an equal volume of the upper layer was recovered for measurement. Absorbance was read at 523 nm using n-butanol as blank. MDA concentration was calculated using a molar extinction coefficient of 1.56 $x10^5 cm^{-1}M^{-1}$

2.5 Statistical Design and Analysis

Statistical significance was established using one-way analysis (ANOVA) and data were reported as mean ± standard deviation. Statistical analyses were carried out using SPSS for Windows, version 17.0 (SPSS Inc. Chicago, IL.USA). Significance was established at $P<0.05$.

3. RESULTS AND DISCUSSION

3.1 Effect of Gamma Irradiation on Bacteriological Quality of Peanut Butter

The assessment of the quality and safety of food is important in human health. High bacterial counts alone does not make food unsafe but it does suggest non-hygienic handing, poor storage, inadequate general hygiene during processing and/or poor quality raw materials [12]. The microbiological quality of the peanut butter irradiated at different doses is presented in Table 1. The total viable count (TVC) and count of S. aureus (CSA) of the unirradiated peanut butter were 3.33 - 2.00 $\log_{10}$ cfu/g and 2.63 $\log_{10}$ cfu/g to below detection limit respectively. No *E. coli* and *Salmonella sp* were detected in the unirradiated peanut butter. These values compare well with those of a similar study [2] where TVC and CSA of peanut butter were within the ranges of 2.54 – 3.36 $\log_{10}$ cfu/g and 2.08 – 3.32 $\log_{10}$ cfu/g respectively. A dose of 2 kGy resulted in a 0.93 log reduction whilst maximum dose of 5 kGy used resulted in a 1.33 log reduction in the TVC of the irradiated samples.

In the case of CSA, a 2 kGy reduced the population by 0.68 log cycle whilst a dose of 5 kGy eliminated all cells in the peanut butter. This confirms the efficacy of irradiation in eliminating the pathogen from peanut butter. *Staphylococcus aureus* continues to be major cause of community-acquired and health-care

related infection throughout the world. Animals, humans, food and inanimate environment can provide a favourable environment for the transmission of *S. aureus* [13,14]. *Staphylococcus aureus* as an indicator of contamination of processed foods could come from the skin, mouth, or nose of handlers [15].

Since *E. coli* and *Salmonella sp* were not detected in both irradiated and unirradiated samples of peanut butter, it is not possible to assess the effect of irradiation on them. However, this finding shows that local processing procedures are capable of eliminating some pathogens from peanut butter.

3.2 Effect of Irradiation on Sensory Qualities of Peanut Butter

Results for the sensory evaluation of peanut butter irradiated at different doses are presented in Table 2. The sensory scores for colour of samples ranged between a minimum of 6.94 and a maximum of 7.20. There were no significant differences between the samples (P=0.8339). For aroma of samples, values ranged between a minimum of 5.89 and a maximum of 6.63. No significant differences were observed in the values of aroma of samples (P=0.2313). The values for taste ranged between a minimum of 6.46 and maximum of 7.03 with no significant differences between the samples (P=0.2601). Also a minimum of 6.09 and a maximum of 6.69 were observed for flavour values, showing no significant differences (P=0.3346). Values for texture of sample gave a minimum of 6.51 and a maximum of 7.17 with no significant differences (P=0.2299) between some samples. For overall acceptability values ranged between 6.40 and 7.11 with no significant differences (P=0.1035) observed between the samples.

3.3 Effect of Irradiation on Rancidity of Peanut Butter

Peroxide value and MDA concentration were determined as a measure of oxidative rancidity. The results are shown in Table 3.

3.3.1 Peroxide value

No significant differences (P=0.5736) were observed for peroxide value of peanut butter upon irradiation. This could be as a result of the time elapsed before the analysis was conducted. Peroxide value is indicative of the degree of primary oxidation that has taken place in a substance. Fats and oils have double bonds which are susceptible to autoxidation, which is a free radical reaction involving oxygen and resulting in the formation of peroxides and hydroperoxides [16]. These peroxides, however, also break down after a while (secondary oxidation) resulting in a lower measured peroxide value than expected for the extent of rancidity that may have occurred [17]. This deterioration also leads to the formation of off - flavours and odours. Hence, the need for complimentary analysis such as the TBA analysis to determine the amount of secondary oxidative products in the sample [18]. Since the samples were analysed three days after the irradiation, it is possible that most of the peroxides initially formed were broken down into secondary products before analysis and thus, no significant differences were observed between doses.

Table 1. Bacteriological counts of peanut butter irradiated at different doses

Bacterial count	Dose (kGy)				
	0	2	3	4	5
Total viable count	3.33±0.10	2.4 ±0.11	2.18±0.11	2.05±0.07	2.0±0.12
Count of *E. coli*	ND	ND	ND	ND	ND
Count of *S. aureus*	2.63±0.18	1.95±0.07	1.87±0.08	1.60±0.10	ND
Count of salmonella	ND	ND	ND	ND	ND

log_{10} cfu/g ±SD, Mean of two replicates, ND- Below detection limit

Table 2. Sensory scores of peanut butter irradiated at different doses

Dose	Colour	Aroma	Taste	Flavour	Texture	Overall acceptability
0 kGy	7.08 ±1.15^a	6.57±1.56^a	6.97±1.25^a	6.51±1.27^a	6.92±1.24^a	6.86±1.06^a
2 kGy	6.97 ±1.07^a	6.29±1.47^a	6.91±1.09^a	6.34±1.16^a	6.86±1.03^a	6.71±0.86^a
3 kGy	7.20 ±0.90^a	6.63±1.42^a	7.03±1.07^a	6.69±1.30^a	7.17±1.20^a	7.11±1.07^a
4 kGy	6.94 ±1.14^a	6.14±1.35^a	6.46±1.42^a	6.09±1.20^a	6.51±1.38^a	6.40±1.1^a
5 kGy	7.11± 0.90^a	5.89±1.81^a	6.69±1.21^a	6.23±1.54^a	6.97±1.04^a	6.69±1.34^a

Means ± standard deviations in the same column with same superscripts are not significantly different (P>0.05)

Table 3. Peroxide value and MDA concentration of peanut butter irradiated at different doses

Dose	Peroxide value (meq of active oxygen / kg of oil)	MDA concentration (M)
0 kGy	44.00±0.00^{a}	$6.89x10^{-7}$±$2.26x10^{-8a}$
2 kGy	44.00±0.00^{a}	$9.36x10^{-7}$±0.00^{b}
3 kGy	43.00±1.41^{a}	$9.97x10^{-7}$±$4.53x10^{-9b}$
4 kGy	44.00±0.00^{a}	$1.14x10^{-6}$±$2.67x10^{-8c}$
5 kGy	42.00±2.83^{a}	$1.12x10^{-6}$±$5.44x10^{-8c}$

a-c: Means ± standard deviations in the same column with different superscripts are significantly different (P< 0.05)

A peroxide value of 42-44 meq/kg is however excessively high and indicative of rancidity. The [19] specifies a maximum peroxide value of 1.5 meq/ kg for peanut products to be used for "domestic programs" within the United States. The peroxide value obtained for unirradiated peanut butter in this study was approximately 28 times more than the stated limit. This is likely to be as a result of lapses in processing operations. The Codex Alimentarius Commission committee [20] also suggested that the peroxide value of peanuts should not exceed 5 meq/kg while that of peanut oil should be less than 10 meq/kg. Even though the presence off-flavours and odours were not detected in sensory evaluation it would be necessary for the producers to review their process operations to ensure that rancidity levels are kept at a minimum.

3.3.2 MDA concentration

Significant differences (P=0.0001) were observed for MDA concentration between some of the doses of gamma irradiation. The MDA concentration increased significantly with increasing dose though no differences were observed between the measured values for doses of 2 and 3 kGy as well as 4 and 5 kGy. This is in agreement with the findings of [21] who irradiated peanut butter with e-beam at a range of doses from 0 to 27.7 kGy. Increase in MDA concentration is likely to be as a result of the oxidative effects of gamma radiation. Exposure to gamma radiation has been found to induce oxidative rancidity in oils and oil-rich foods [22]. The increased levels of MDA concentration indicate an increase in rancidity of the peanut butter. Rancidity often results in the formation of off-flavours and odours which could make the product unpalatable to the consumer. Even though the off-flavours and odours were not detected by the sensory evaluation, it is possible that the levels were low and therefore undetected by the untrained senses. There is however a possibility that off-flavours and odours would increase in storage due to free radicals action that can continue after irradiation.

Malondialdehyde as a chemical, is known to be reactive and potentially mutagenic [23]. The consumption of rancid oils is not known to cause illnesses in the short term but the presence of free radicals that can cause cellular damage may increase one's potential of developing diseases such as cancer, diabetes and Alzheimer's [24]. Though rancidity in itself cannot be quantified, the presence of malondialdehyde is indicative of it. The concentration of malondialdehyde should therefore be kept as low as possible and processes which are likely to increase the MDA concentrations significantly should be avoided.

4. CONCLUSION

This study shows that gamma irradiation can be effective in reducing microbial load in the peanut butter without significantly affecting the sensory attributes as well as the peroxide value. The microbial quality of the irradiated peanut butter falls within the acceptable standards ensuring the safety of the consumer. The concentration of malondialdehyde increased significantly with increasing irradiation dose though no significant differences were observed between the measured values for doses of 2 and 3 kGy as well as 4 and 5 kGy. However, storage and the fact that the samples were not analysed immediately after irradiation may have affected the differences observed in the malondialdehyde values.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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