



Quality Characteristics of Cold Stored Fresh Beef Sausage Formulated with Incorporation of Wheat Germ and Treated by Gamma Rays

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ABSTRACT

The quality characteristics of fresh beef sausage formulated with incorporation of wheat germ and treated by gamma rays were studied during cold storage. Samples of fresh beef sausage were formulated by incorporation of wheat germ at level of 10%. The prepared sample were exposed to gamma rays at doses of 0, 3 and 4.5 kGy and the quality characteristics were examined every 3 days during refrigerated storage of samples at $4 \pm 1^{\circ}\text{C}$. Samples of irradiated and non-irradiated sausage were highly acceptable for their sensory properties. Irradiation of samples significantly decreased their total bacterial count and molds and yeasts counts, while total psychrophilic bacterial and coliform bacteria were not detected in all irradiated samples. All microbial counts showed gradual increases during cold storage of samples but the rate of increases was lower in irradiated samples except coliform bacteria which were not detected in all irradiated samples during their cold storage. On the other hand, cold storage induced gradual decreases in the values of PH for all non-irradiated and irradiated samples, meanwhile the contents of total volatile basic nitrogen (TVBN) and thiobarbituric acid (TBARS) gradually increased in all sample under investigation during their cold storage. A significantly drop in the counts of *Escherichia coli*, *Staphylococcus aureus*, and *Enterococcus faecalis* in irradiated samples, with no levels detected over the storage time. The bacteria count of control (non-irradiated) samples, on the other hand, gradually increased, demonstrating the limited efficacy of cold storage alone. Additionally, *Bacillus cereus* was completely suppressed by 4.5 kGy gamma irradiation, although 3 kGy treatment markedly inhibited its growth in comparison to the control. *Salmonella spp.* count was not detected in any of the samples. Irradiation of beef sausage at doses of 3 and 4.5 kGy extended the refrigerated shelf life of sample of 18 and 21 days respectively compared to 6 days for the non-irradiated samples. It is recommended to use 4.5 kGy gamma-irradiation to enhance cold-stored fresh beef sausage with wheat germ for the best overall outcomes. The product's safety is ensured by eliminating major microbial hazards, such as *Bacillus cereus*. Bacterial, yeast, and mold growth is significantly reduced during cold storage, and the cold storage shelf life is extended to 21 days at $4 \pm 1^{\circ}\text{C}$ while maintaining acceptable sensory qualities (color, odor, taste, and texture). Thus, for fresh beef sausage, this dosage achieves the best possible mix of shelf-life extension, microbiological safety, and sensory quality during cold storage.

Keywords: Meat products, Sausage, Gamma rays, wheat germ, Food Irradiation.

1. Introduction

Meat products are an important component of the human diet and sausage is one of the oldest processed meat products being a very desirable and enjoyable easy meal. However, high consumption of processed meats increased the risk of several diseases due to their high content of fats and saturated fatty acids. This led researchers to develop healthier meat products with improved composition using different strategies including incorporation of natural bioactive planed compounds (Gagaoua and Picard, 2020; Mohan, 2014 and Ursachi *et al.*, 2020).

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In processed meat products including sausage, different binders including cereal flour are used to improve their characteristics and reduce the cost of formulation (Heinz and Hautzinger, 2007 and Mohan, 2014). Wheat germ as a major by-product of the milling industry constitutes a natural source of concentrated compounds with high nutritional and biological value (Boukid *et al.*, 2018 and Khosroshahi and Razavi, 2023). It has many health benefits including lowering plasma triglycerides and cholesterol and could be a good binder in sausage production (Abd El- Rahman, 2015; Elbakheet *et al.*, 2017 and Zou *et al.*, 2015).

On the other hand, fresh sausage is extremely perishable meat products and an efficient technology is required for keeping its quality. Food irradiation technology, as a non-thermal process, has the ability to inactivate a wide spectrum of microorganisms covering all significant aspects of food processing and preservation being approved by the health authorities of different countries including Egypt (Asghar *et al.*, 2022; Rady *et al.*, 2020 and Singh and Singh, 2020). Previous studies showed that gamma irradiation at dose of 3kGy effectively inactivated many microorganisms of public healthy significance in different meat products (Arvanitoyannis, 2010), while an irradiation dose of 4.5 kGy can be the maximum dose for treatment of refrigerated uncooked meat products (Thayer, 2007).

Therefore, the aim of the present work was to study the quality characteristics of cold stored fresh beef sausage formulated by incorporation of wheat germ and treated by gamma irradiation. for extending fresh beef sausage formulated by incorporation of wheat germ shelf- life and improving its hygienic quality.

2. Materials and Methods

2.1. Materials

Wheat germ was obtained from El mothadone Milling Company Mini Elqamhia Al Sharqia Government. Freshly ground very lean beef meat, spices, salt (sodium chloride) and garlic were purchased from local markets in Zagazig city, Al sharqia Governorate, Egypt. Sheep tail fat and natural casings were purchased from a butcher's shop in Zagazig city.

2.2. Preparation of sausage

Samples of fresh beef sausage in this study were prepared according to Mohan (2014). Firstly, four formulations of beef sausage were prepared to choose the acceptable level of the added wheat germ. The main formula was prepared by inclusion of 20% of the sheep fat, while the other three formulations were prepared by inclusion of the wheat germ at levels of 5, 10 and 15% with reducing of the added fat as shown in Table (1). After mincing of the sheep fat, ingredients of each formula were mixed thoroughly and homogenized, then stuffed in the prepared natural sheep casings which were hand liked at about 15 cm intervals. Based on the results of sensory evaluation, the addition of wheat germ at level of 10% was chosen.

Table 1: Formulations of beef sausage were prepared to choose the acceptable level

Component	Ingredients (%)			
Lean beef	66	66	66	66
Beef fat	20	15	10	5
Wheat germ	-	5	10	15
Ice water	10	10	10	10
Salt	2	2	2	2
Garlic	1	1	1	1
Spices ⁽¹⁾	1	1	1	1

¹ White or black pepper, Red pepper (Paprika)

Then samples of fresh beef sausage were prepared with the chosen level of wheat germ as mentioned above packaged in polyethylene pouches. Packages were sealed by heat and divided into appropriate sample for irradiation treatment.

2.2. Irradiation of sausage

Packaged samples of the prepared fresh beef sausage were transferred in an ice chest and exposed to gamma irradiation at doses of 0, 3 and 4.5 kGy using a Cobalt 60 Russian Chamber (dose rate 272,2 Gy / h) belonging to Cyclotron Project, Nuclear Research Center, Egyptian Atomic Energy Authority,

Egypt. Then irradiated and non-irradiated samples were subjected to the periodical analysis during cold storage at $4\pm 1^{\circ}\text{C}$

2.3. Analytical methods

2.3.1. Microbiological analysis

Total bacterial count and total psychrophilic bacteria were enumerated on plate count agar media according to APHA (1992). The counts of molds and yeasts were determined using Oxytetracycline yeast extract agar media according to the Oxoid manual (2006) and the plates were incubated at 25°C for 5 days. The count of coliform group was enumerated using violet red bile agar according to the Oxoid manual (2006). *Stahylococcus aureus* was counted on Bird parker agar media after incubated at 35°C for 48 h. according to Oxoid manual (2006), MacConkey agar medium was used for *E coli* (Oxoid manual, 2006), *Enterococcus faecalis* was counted on kanamycin.

Aesculine azide agar medium using surface plating technique and incubation at 35°C for 16-24 h according to the Oxoid manual (2006). Colonies were considered as *Enterococcus faecalis* if they were porcelain white and surrounded by a black zone. *Bacillus cereus* was counted using Mannitol-egg Yolk-Polymyxin (MYP) agar and incubated at 37°C for 16-24 hours as described by Roberts *et al.*, (1995). The detection of *Salmonella spp* was carried out using the most probable number technique. After enrichment at 37°C for 24 h in selenite broth, the cultures were streaked on Brilliant green agar and incubated at 37°C for 24 h, then colonies were biochemically examined in triple sugar iron agar according to the Oxoid manual (2006). The results were calculated as \log_{10} cfu /g.

2.3.2. Chemical analysis

PH value was assessed as described by Carballo *et al.* (1995). Total volatile basis nitrogen contents were determined according to the Mwansyemela (1992). lipid oxidation was measurement by determine the Thiobarbituric acid-reactive substances (TBARS) using the method as described by Alasnier *et al.* (2000).

2.3.4. Sensory evaluation

Sensory evaluation was firstly carried out for beef sausage to choose the acceptable addition level of wheat germ. Samples of the different formulated sausage were pan fried and periodically evaluated (every 3 days) for their appearance, odor texture and juiciness, taste and general acceptability after irradiation of samples irradiated and non-irradiated beef sausage were subjected to sensory evaluation for their texture and taste at Zero time of storage, while samples were evaluated for their appearance during cold storage. In all experiments of sensory evaluation, the panelists consisted of 20 members of our laboratory from the Unit of Food Irradiation, Nuclear Research Center using the following scale: 9=Excellent, 8, very good, 7= good, 6 below good, above fair 5= fair, 4=below fair, above poor, 3, poor, 2, = very poor and 1= extremely poor as described by Wierbicki, (1985). The rejection of sample was based on the visual appearance of microbial growth on the surface of samples.

2.4. Statistical analysis

In all determination, three poaches from each of Samples of the different formulated sausage were used and analysis were carried out using triplicate samples ($n=3$). The results were statistically analyzed using SPSS (2009) and results were recorded as mean \pm standard error.

3. Results and Discussion

3.1. Choosing of the acceptable level wheat germ addition

The firstly prepared samples of fresh beef sausage which prepared by inclusion of wheat germ at levels of 0, 5, 10 and 15% were sensory evaluated to choose the acceptable level of addition. As shown in table (2), all formulated samples showed highly acceptable scores for their sensory characteristics. Based on these results, formulation of beef sausage with inclusion of wheat germ at level of 10% chosen.

Table 2: Choosing of the acceptable level wheat germ (WG) % addition.

Character WG %*	Appearance	Odor	Texture	Taste	General acceptability
0	9.00±.000 A	8.51±.018 A	8.50±.017 A	8.50±.015 A	9.00±.000 A
5	6.50±.015 D	7.50±.015 B	7.01±.012 C	5.10±.020 C	7.51±.015 D
10	8.51±.015 B	8.51±.023 A	7.10±.015 B	8.50±.006 A	8.34±.173 B
15	7.10±.015 C	7.51±.012 B	8.00±.023 B	7.51±.018 B	8.00±.020 C

* Germ Wheat Means with the same capital letter in the same rows are not significantly different, means with the same small letter in the same columns are not significantly different

3.2. Effects of gamma irradiation on microbiological properties of fresh beef sausage during cold storage at 4±1⁰c.

Irradiation of the prepared fresh beef sausage significantly decreases their initial total bacterial count (Fig. 1). However, cold storage induced gradual significant increases in the counts of total bacteria for both non- irradiated and irradiated samples but at lower rate of increase in the irradiated samples shown in Figure (1). The total microbial count were increased in control sample from (4.34 ±0.032 log₁₀ cfu / g) to (5.06±0.031 log cfu/g) during cold storage after 6 days and control sample was rejected according to NFSA (2021) which mentioned that the maximum acceptable count for meat and meat products is 10⁶ cfu /g. On other hand It is notable that the dried wheat germ extract is a potential source of antibacterial agents that act mainly on Gram-positive and Gram-negative bacteria. Thus, it can be an efficient protective agent for use as antioxidant and antibacterial additives in food systems (Awad *et. al.*, 2015). Meanwhile, the initial log₁₀ count for total psychrophilic bacteria was 2.94± 0.02 cfu/g in samples on non- irradiated sausage and psychrophilic bacteria were not detected in all irradiated sample at Zero time (Figure 2). The same Figure shows that the count of psychrophilic bacteria gradually increased during cold storage but also at lower rate of increase irradiated samples.

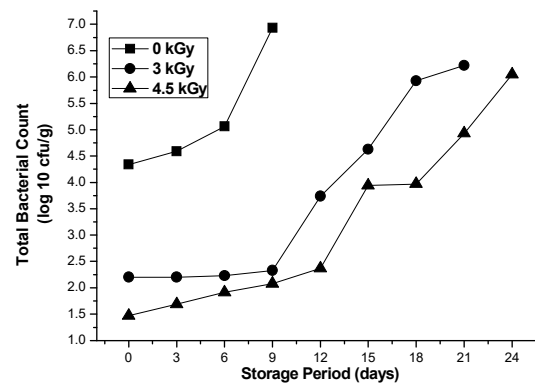


Fig. 1: Effects of gamma irradiation on total bacterial count of fresh beef sausage during cold storage at 4±1⁰c.

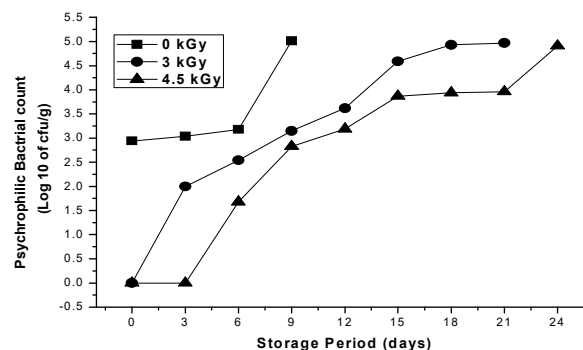


Fig. 2: Effects of gamma irradiation on psychrophilic bacteria count of fresh beef sausage during cold storage at 4±1⁰c.

The initial \log_{10} count of total molds and yeasts was 2.48 ± 0.017 cfu/g and molds and yeasts were not detected in all irradiated samples on day zero of cold storage as shown in Figure (3). However, gradual significant increases in the counts of molds and yeasts were observed upon cold storage of samples (Figure 3).

On the other hand, non- irradiated samples of beef sausage had an initial \log_{10} count of 1.47 ± 0.015 cfu/g for coliform bacteria and the counts of coliforms significantly increased during cold storage. However, coliform bacteria were not detected in all irradiated samples post treatment and during storage (Figure 4).

The efficiency of irradiation treatment in microbial destruction has been clearly illustrated (Indiarto *et al.*, 2023 and Islam *et al.*, 2023).

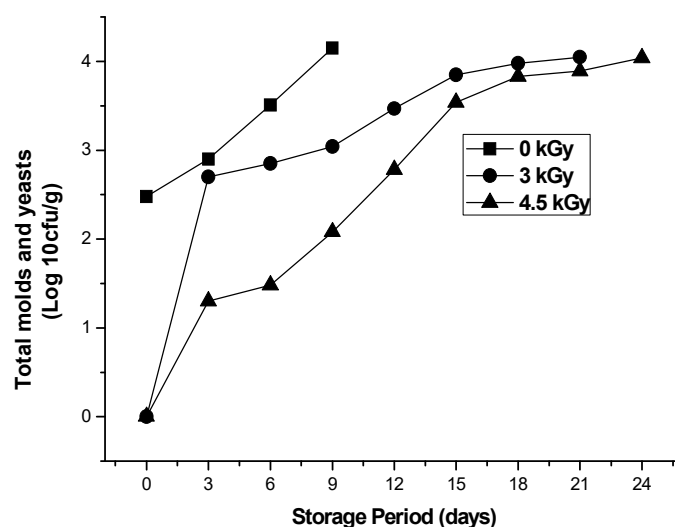


Fig. 3: Effects of gamma irradiation on total mold and yeast count of fresh beef sausage during cold storage at $4 \pm 1^\circ\text{C}$.

3.3. Effects of gamma irradiation on pathogenic bacteria counts of fresh beef sausage during cold storage at $4 \pm 1^\circ\text{C}$.

As shown Table (3) The findings show a significant lowering in *Escherichia coli*; *Staph. aureus* and *Enterococcus fecalis* (\log_{10} cfu/g) count in fresh beef sausage and the effect of gamma radiation at doses of 3 and 4.5 kGy throughout 24 days of cold storage at $4 \pm 1^\circ\text{C}$. Control samples (0 kGy) presented with an initial *E. coli*; *Staph. aureus* and *Enterococcus fecalis* have \log_{10} cfu/g counts of 2.2 ± 0.06 ; 1.51 ± 0.01 and 2.6 ± 0.04 respectively, however irradiated samples with 3 and 4.5 kGy showed no detectable levels of *E. coli*; *Staph. aureus*, and *Enterococcus fecalis*, while during cold storage control (non-irradiated) samples showed a gradual significant ($p < 0.05$) increase in *E. coli*, *Staph. aureus*, and *Enterococcus fecalis* counts, reaching 2.7 ± 0.028 ; 2.34 ± 0.059 , and 2.99 ± 0.045 \log_{10} cfu/g, respectively, by day 9, whereas, *E. coli*, *Staph. aureus*, and *Enterococcus fecalis* counts for samples treated with gamma irradiation (3 and 4.5 kGy) still undetectable during cold storage period.

The inhibition of *Staph. aureus*, *Enterococcus fecalis*, and *E. coli* counts in irradiated treatments is consistent with other research showing how effective gamma radiation is in microbial decontamination. Lacroix and Ouattara (2000) state that common foodborne bacteria such as *E. coli* can be rendered inactive by low-dose gamma irradiation (2–4.5 kGy),

The antibacterial properties of gamma irradiation with doses (3 and 4.5 kGy) were also evaluated against *Bacillus cereus*, a foodborne pathogen that forms spores and produces toxins. *B. cereus* was first identified in untreated samples (0 kGy) at a level of 2.3 ± 0.05 \log_{10} cfu/g at the initial time of cold storage. During cold storage, these counts gradually increase reaching 3.1 ± 0.019 \log_{10}

Table 3: Effects of gamma irradiation on pathogenic bacteria counts of fresh beef sausage during cold storage at 4±10c

Storage period (days)	γ - radiation dose (kGy)														
	<i>E. coli</i> (Log 10 cfu/g)			<i>Staph. aureus</i> (Log 10 cfu/g)			<i>Salmonella spp.</i> (Log 10 cfu/g)			<i>Enterococcus fecalis</i> (Log 10cfu/g)			<i>B. cereus</i> (Log 10 cfu/g)		
	0	3	4.5	0	3	4.5	0	3	4.5	0	3	4.5	0	3	4.5
0	2.2 ±0.0609 A	ND	ND	1.51 ±0.0114A	ND	ND	ND	ND	ND	2.6 ±0.0488 A	ND	ND	2.3 ±0.0550Aa	1.21 ±0.0505Ab	ND
3	2.43 ±0.0304B	ND	ND	1.73 ±0.0499B	ND	ND	ND	ND	ND	2.73 ±0.0606AB	ND	ND	2.89 ±0.016Ba	1.34 ±0.0251ABb	ND
6	2.59 ±0.0050C	ND	ND	1.9 ±0.0514C	ND	ND	ND	ND	ND	2.88 ±0.0452AB	ND	ND	2.94 ±0.0457Ba	1.47 ±0.0629BCb	ND
9	2.7 ±0.0286C	ND	ND	2.34 ±0.0595D	ND	ND	ND	ND	ND	2.99 ±0.1654B	ND	ND	3.1 ±0.1925Ba	1.61 ±0.0431Cb	ND
12		ND	ND		ND	ND		ND	ND		ND	ND		1.82 ±0.0489D	ND
15		ND	ND		ND	ND		ND	ND		ND	ND		2.5 ±0.059E8	ND
18		ND	ND		ND	ND		ND	ND		ND	ND		2.96 ±0.0569F	ND
21		ND	ND		ND	ND		ND	ND		ND	ND			ND
24		ND	ND		ND	ND		ND	ND		ND	ND			ND

- Means with the same capital letter in the same columns for each pathogenic microorganism are not significantly different ($p > 0.05$).
- Means with the same small letter in the same rows for each pathogenic microorganism are not significantly different ($p > 0.05$).
- ND = Not Detected

cfu/g on 9th day. This pattern suggests that, without any antimicrobial intervention, cold storage was not enough to inhibit the growth of *B. cereus*. However, samples exposed to 3 kGy gamma radiation exhibited noticeably lower *B. cereus* levels. The numbers steadily increase from $1.21 \pm 0.05 \log_{10}$ cfu/g at the beginning of cold storage to $2.96 \pm 0.05 \log_{10}$ cfu/g on the day 18. In comparison to the control, this treatment

Slowed and decreased microbial growth, but it fell short of complete suppression. Interestingly, *B. cereus* was not found (ND) at any time of cold storage in samples treated with 4.5 kGy gamma radiation, indicating total inactivation and/or suppression of viable cells and spores. Regarding to *Salmonella spp.* (\log_{10} cfu/g) count it was undetected either in control untreated samples or samples treated with gamma irradiation and still not detected during cold storage period. The effectiveness of irradiation treatment in eliminating microorganisms has been demonstrated thoroughly (Indiarto *et al.*, 2023 and Islam *et al.*, 2023).

3.4. Physicochemical properties of fresh beef sausage as effected by gamma irradiation and cold storage at $4 \pm 1^{\circ}\text{C}$.

The prepared samples of fresh beef sausage were examined for their PH value and their contents form total volatile basic nitrogen (TVBN) and thiobratbituric acid (TBARS) post irradiation treatment and during their cold storage at $4 \pm 1^{\circ}\text{C}$. As shown in Figure (5), cold storage of beef sausage samples induced significant gradual decreases in their pH value. In addition Wheat germ extract could serve as a safe and inexpensive natural antimicrobial against foodborne pathogens for use in food and cosmetic applications. Wheat germ, a by-product generated in large quantities by the flour milling industry, could thus be utilized as a source of this antimicrobial agent (Kim *et al.*, 2010)

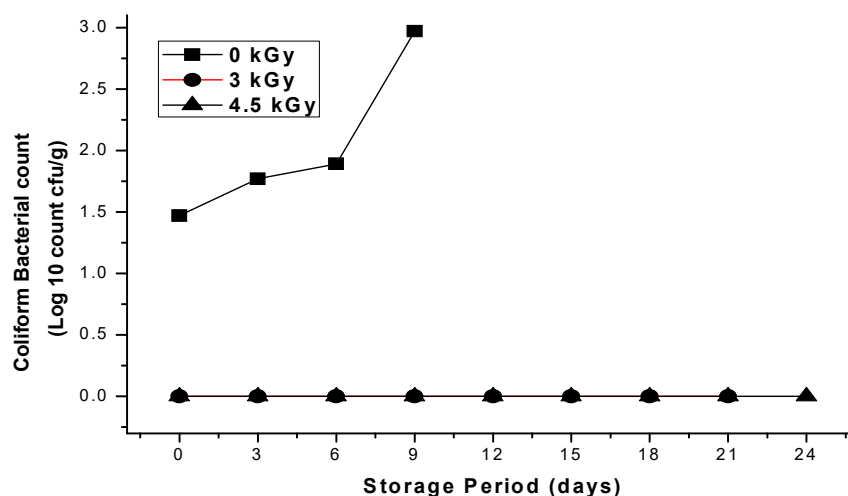


Fig. 4: Effects of gamma irradiation on coliform count of fresh beef sausage during cold storage at $4 \pm 1^{\circ}\text{C}$.

On the other hand, the initial contents of TVBN were 10.92, 9.88 and 9.69 mg/100g in control samples and those irradiated at doses of 3 and 4.5 kGy respectively, while the initial contents of TBARS were 0.44, 0.46 and 0.59 mg malonaldehyde /kg in these samples respectively (Figures 6 & 7). As shown in these Figures, the contents of TVBN and TBARS showed significant gradual increases during cold storage of both non irradiated and irradiated samples of fresh beef sausage. Abdeldaiem *et al.* (2017) and Tawfik *et al.* (2007) they reported that TVBN value of beef burger steaks irradiated at dose level 3 and 4 kGy were less than the accepted limits. Also, Dried wheat germ extract exhibits antioxidant activity in DPPH radical scavenging assay, ABTS radical scavenging assay, and oxidative stability in Rancimat method (Awad *et al.*, 2015).

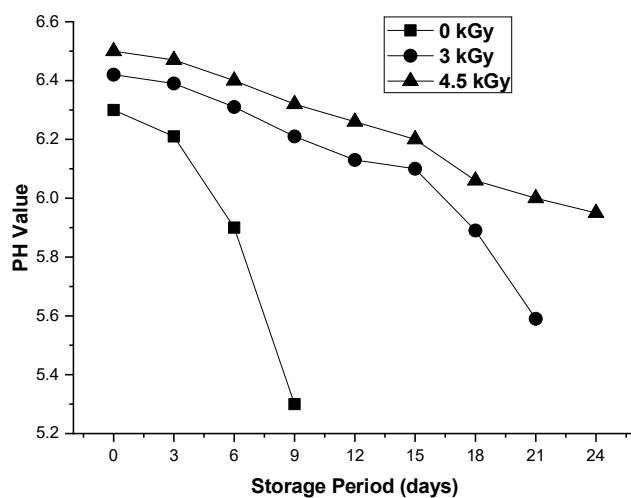


Fig. 5: Effects of gamma irradiation on PH value of fresh beef sausage during cold storage at $4\pm1^{\circ}\text{C}$.

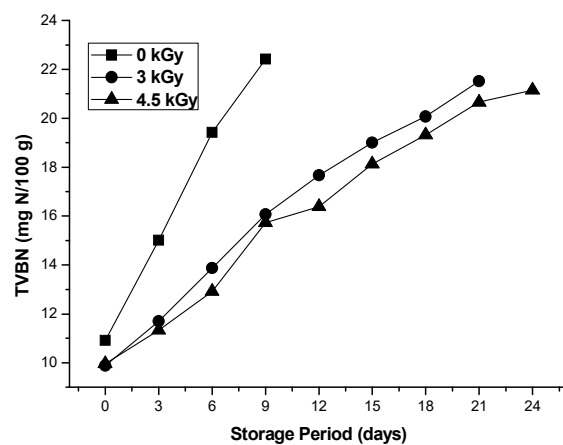


Fig. 6: Effects of gamma irradiation on Total Volatile Bases Nitrogen TVBN of fresh beef sausage during cold storage at $4\pm1^{\circ}\text{C}$.

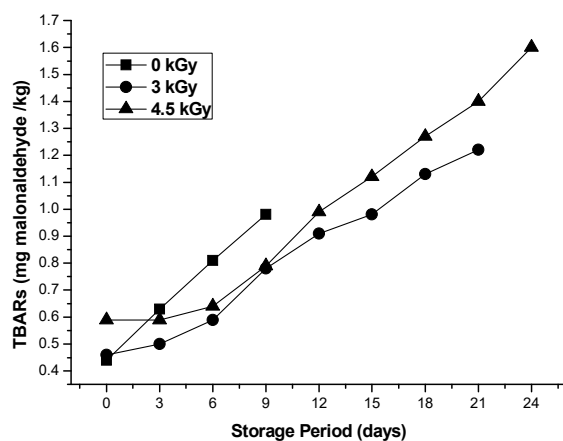


Fig. 7: Effects of gamma irradiation on TBARS of fresh beef sausage during cold storage at $4\pm1^{\circ}\text{C}$.

3.5. Effects of gamma irradiation and cold storage on the sensory properties of fresh beef sausage.

Samples of fresh beef sausage formulated by inclusion of wheat germ were evaluated for their odor and taste post irradiation treatment at zero time of storage while all sample were evaluated for their appearance treatment and during their cold storage at $4\pm 1^{\circ}\text{C}$.

As shown in table 4 non irradiated and irradiated fresh beef sausage were highly acceptable for their odor and taste. Also, all irradiated and non-irradiated samples of beef sausage were highly acceptable for their appearance at Zero time of storage, while all samples showed significant gradual decreases in their scores for appearance during cold storage at $4\pm 1^{\circ}\text{C}$ but sample were still acceptable until their rejection due to appearance of microbial growth on the surface of samples (table 5). As shown, samples of non-irradiated beef sausage were rejected at the 9 days of their cold storage while samples of sausage irradiated at doses of 3 and 4.5 kGy were rejected after 21 and 24 days of their storage due to appearance of microbial growth on sample (table 5).

Table 4: Effects of gamma irradiation on Odor and taste of fresh beef sausage at zero time.

Properties	Gamma Rays (kGy)		
	0	3	4.5
Odor	8.10 \pm .003	8.51 \pm .021	8.50 \pm .017
Taste	8.99 \pm .007	8.89 \pm .006	8.51 \pm .020

Means with the same capital letter in the same rows are not significantly different, means with the same small letter in the same columns are not significantly different ® Rejected Samples

Table 5: Effects of gamma irradiation on appearance of fresh beef sausage during cold storage at $4\pm 1^{\circ}\text{C}$.

Storage/days	Gamma Rays (kGy)		
	0	3	4.5
0	9.00 \pm .000 A	8.83 \pm .088 A	8.51 \pm .021 A
3	8.80 \pm .015 B	8.49 \pm .012 B	8.31 \pm .024 B
6	7.50 \pm .012 C	7.90 \pm .012 C	8.00 \pm .012 C
9	3.30 \pm .015 DR	7.50 \pm .009 D	7.81 \pm .023 D
12		7.10 \pm .012 E	7.50 \pm .009 E
15		7.00 \pm .006 F	7.30 \pm .012 F
18		5.10 \pm .009 G	6.09 \pm .012 G
21		3.80 \pm .012 HR	4.90 \pm .015 H
24			3.70 \pm .012 IR

R Rejected Means with the same capital letter in the same rows are not significantly different, means with the same small letter in the same columns are not significantly different

4. Conclusion

The aim of this study was to investigate the possibility of formulation of fresh beef sausage with inclusion of wheat germ and studying the effect of gamma irradiation and cold storage on sample. Gamma irradiation and the addition of wheat germ greatly increased the fresh beef sausage's shelf life and microbiological safety while it was being stored in cold conditions. Key pathogens like *Escherichia coli*, *Staphylococcus aureus*, *Enterococcus faecalis*, and *Bacillus cereus* were successfully reduced by gamma irradiation, especially at 4.5 kGy. *Salmonella* spp. was not detected in any of the samples. In contrast, the bacteria count in non-irradiated control samples gradually increased, highlighting the limited effect of cold storage alone. According to these results, gamma irradiation has promise as a preservation technique for preserving the quality and safety of meat products enhanced with useful components like wheat germ.

It is recommended to use 4.5 kGy gamma-irradiation to enhance cold-stored fresh beef sausage with wheat germ for the best overall outcomes. The product's safety is ensured by eliminating major microbial hazards, and the cold storage shelf life is extended to 21 days at $4 \pm 1^{\circ}\text{C}$ while maintaining

acceptable sensory qualities (color, odor, taste, and texture). Thus, for fresh beef sausage, this dosage achieves the best possible mix of shelf-life extension, microbiological safety, and sensory quality during cold storage.

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