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Using of Combined Treatment between Edible Coatings Containing Ethanolic Extract of Papaya (*Carica Papaya* L.) Leaves and Gamma Irradiation for Extending Shelf-Life of Minced Chicken Meat

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Abstract The objective of this work was to study the combined effect of edible coating containing ethanolic extract of papaya (*Carica papaya* L.) leaves at ratio 2% and gamma irradiation at dose levels of 0, 2, 4 and 6 kGy on the chemical, microbiological and sensorial qualities of minced chicken thighs meat during cold storage (4 ± 1°C). Samples of minced chicken thighs meat were divided into three groups; uncoated (control), coated with edible coating (without any additives), and irradiated coated with edible coating samples at 0, 2, 4 and 6 kGy. The obtained results showed that gamma irradiation at 2, 4 and 6 kGy with edible coating reduced the initial total bacterial count, psychrophilic bacteria and lactic acid bacteria and prolonged shelf-life of the samples under investigation. Coated samples and irradiated at 2 kGy reduced the counts of Enterobacteriaceae, *Staphylococcus aureu*s and *Bacillus cereus* as well as eliminating *Salmonella* spp, while coated samples irradiated at 4 and 6 kGy completely eliminated these bacteria. On the other hand, combination treatments of minced chicken thighs meat samples showed slight increase in thiobarbituric acid reactive substances (TBARS) post irradiation and during cold storage, but had no effects on their total volatile nitrogen (TVN) contents, while a gradual increase in these chemical quality indexes was observed during cold storage. Finally, combination treatment had no adverse effects on the sensory properties of minced chicken thighs meat samples. Therefore the incorporation of ethanolic extract of papaya leaves in edible coating materials increases the bacterial inhibitory effect of gamma irradiation and is suitable for preservation of minced chicken thighs meat or meat and chicken products.

Keywords: *edible coating, ethanolic extract, papaya leaves, gamma irradiation, chicken minced*

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1. Introduction

In recent years there has been an increased consumer demand for convenience or “case ready” meat and meat products requiring minimal home preparation [1]. Accordingly, less poultry is marketed today as whole carcasses and more as a variety of further prepared or fully cooked products [2]. Processed chicken based products such as burgers have been distributed through wholesalers and restaurants, and also widely consumed by the people. Furthermore, local industries have grown up to accomplish the demands from these products [3]. The food poisoning microorganisms causing outbreaks were mainly Salmonellae and *S. aureus* with an incidence of 8.99 and 11.54, respectively, while incidence of other food poisoning microorganisms causing 4.07% of the cases [4].

Processed raw poultry meat naturally harbors bacteria, most of which are responsible for the spoilage of poultry meat. However, poultry products can harbor food-borne pathogens, like *Salmonella stereotypes*, *Campylobacter jejuni*, *Listeria monocytogens*, *C. perfringens* and *S. aureus* [5]. Poultry and poultry products rank first or second in foods associated with disease in most of the countries all over the world which in the USA ranked third of the reported foodborne disease outbreaks [6]. The total bacterial count of chicken products as sausage, burger, luncheon and frankfurter was 10^7, 10^7, 10^6 and 10^6 cfu/g, respectively, while *Staphylococcus aureus* was isolated from the same products at incidence of 40%, 70%, 20% and 40%, respectively [7].

Edible coatings or films (biodegradable) have been used as moisture barriers, oxygen barriers, mechanical property modifiers, and food additive carriers in various food products. Antimicrobial edible coatings or films acting as a protective barrier can be used to retard food spoilage, thus extending food shelf life [8,9]. Edible films that can be used as coatings for foods can be include proteins (gelatin, wheat protein, maize protein, casein, etc.), polysaccharides (cellulose and its derivatives, starch and its derivatives, etc.), and lipids [10]. A number of coating materials have been tested in attempt to maintain quality and prolong shelf life of meat products. The chitosan-coated Atlantic cod and herring had reduced moisture loss and lipid oxidation. Immobilizing antimicrobials into film forming solutions is a very advantageous technology for food preservation [11]. The resulting bio-active films or coatings provide more inhibitory effects against spoilage and pathogenic bacteria by lowering the diffusion processes and maintaining high concentrations of the active molecules on the food surfaces [12]. Applications of natural products with both antioxidants and antimicrobial additives in meat products may be useful to prolong the shelf life for pork. Therefore, minimizing lipid oxidation and delaying or inhibiting product contamination, growth of spoilage, and pathogenic organisms in the product are major keys for improving fresh meat shelf life and increasing consumer safety [13].

Plants have the major advantage of still being the most effective and cheaper alternative sources of. The local use of natural plants as primary health remedies, due to their pharmacological properties, is quite common in Asia, Latin America and Africa [14]. Papaya (*Carica papaya* L.) belongs to the family Caricaceae. It has the following common names; pawpaw tree, papaya, papayer, tinti, pepol, chich put, fan kua, wan shou kuo, kavunagaci, kepaya etc. The parts that are usually used include the leaves, fruit, seed, latex, and root. The plant is also described in a documented property forms and it act as analgesic, amebicide, antibacterial, cardiotonic, cholagogue, digestive, emenagogue, febrifuge, hypotensive, laxative, pectoral, stomachic and vermifuge [15]. Papaya leaves are known to contain proteolytic enzymes (papain, chymopapain), alkaloids (carpain, carpasemine), sulfurous compounds (benzyl isothiocyanate), flavonoids, triterpenes, organic acids and oils [16]. The papaya leaves had antimicrobial activity against both gram-negative and gram-positive bacteria is an indication that the plant is a potential source for production of drugs with a broad spectrum of activity and its extracts possess compounds with antibacterial properties that can be used as antibacterial agents in novel drugs for the treatment of gastroenteritis, uretritis, otitis media, and wound infections [17,18].

On the other hand, irradiation as a method of meat preservation has excellent potential to improve meat safety and extend shelf-life [19]. The approval of meat irradiation by the FDA [20] has made consumers more confident and attracted the interest of industries concerned with food quality, and irradiation technology is rapidly entering into commercial reality throughout the world. The current trends consist to develop combination of mild treatments with low dose gamma irradiation to improve food safety and shelf-life extension [21]. The reduced the irradiation dose required for complete elimination of *Salmonella* on fresh poultry by combining gamma irradiation with marinating in natural plant extract [22].

Thus the present work was undertaken to study the effect of gamma irradiation at doses of 0, 2, 4 and 6 kGy in combination treatments with edible coatings containing ethanolic extract of papaya (*Carica papaya* L.) leaves as natural antimicrobial and antioxidant on quality of minced chicken thighs meat during cold storage (4 ± 1°C).

2. Materials & Methods

2.1. Preparation of Ethanolic Extract of Papaya (*Carica Papaya* L.) Leaves

Dried powdered of papaya (Carica papaya L.) leaves were macerated in ethanol (70%) by shaking for 7 days in the dark at room temperature. The ethanolic extract solution was then filtered and concentrated in a rotary evaporator under reduced pressure at 40°C.

2.2. Preparation of Coating Solution

The thirty gram pectin was dissolved in 990 ml distilled water. After the addition of 10 ml polyethylene glycol, the solution was homogenized with a homogenizer for 1 h and homogenized again for 30min. Finally, ethanolic extract of papaya (*Carica papaya* L.) leaves at ratio 2% (v / v) was added and the mixture was further stirred until the ethanolic extract of papaya leaves dissolved. Also, the final solution was sonicated about 1 h in order to remove air bubble or dissolved air.

2.3. Preparation of Minced Chicken Thigh Meat and Coating Application

Fresh chicken thighs samples were obtained from a local market and transported to the laboratory under chilled condition in ice boxes. Skin, bone and visible fat accompanying each type of meat were removed. The chicken thighs were minced in an electric mincer (either treated with 2% ethanolic extract of papaya or untreated) and homogenized to obtain minced chicken thigh meat which shaped handily to pieces of 50 ± 3 g. The fillet pieces were dipped in each freshly prepared coating solution for 1-2 min, drained for 2 min, and dried with mild flow of air in an air dryer at room temperature for 30 min. Then, the untreated and treated samples chicken minced chicken thigh meat were divided into three groups, the first used was control, second was minced chicken thighs meat treated with 2% ethanolic extract of papaya then irradiated at dose levels of 2, 4 and 6 kGy, then every group was packaged in low density polyethylene plastic bags.

2.4. Irradiation Treatments

For irradiation treatments, samples of coated minced chicken thighs meat containing ethanolic extract of papaya leaves at ratio 2% were exposed to gamma irradiation at doses of 0, 2, 4 and 6 kGy using an experimental ^{60}Co Russian gamma chamber, in Cyclotron Project, Nuclear Research Center, Atomic Energy Authority, Abou Zaabal, Egypt. After irradiation, all samples were stored at 4 ± 1°C.

2.5. Microbiological Analysis:

Colony forming units for total bacterial count were counted by plating on plate count agar medium and incubation at 30°C for 3-5 days [23]. Total psychrophilic bacteria were enumerated on plate count agar medium after incubation at 5°C for 7 days [24]. Lactic acid bacteria were counted by the pour plate over layer method as in [25]. Enterobacteriaceae were counted on violet red bile glucose agar medium after incubation for 20-24 h at 37°C [26]. *Staphylococcus aureus* was counted using Baird–Parker medium after incubated at 35°C for 24-48 h [27]. *Bacillus cereus* was counted using Mannitol-egg Yolk-Polymyxin (MYP) agar and incubated at 37°C for 16-24 hours as in [26]. The detection of *Salmonella* 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 [28].

2.6. Chemical Analysis

Total volatile nitrogen (TVN) was determined as in [29].
Measurement of lipid peroxidation:

2.6.1. Thiobarbaturic Acid Value (TBARs)

Lipid oxidation was determined as a 2-thiobarbituric acid reactive substances (TBARS) value by using the method described as in [30] with some modifications. Minced chicken thighs meat (5 g) and 15ml of deionized distilled water were homogenized with 50 μl butylated hydroxyanisol (7.2%) for 15 s. One milliliter of the meat homogenate was transferred to a disposable test tube and then 1ml of thiobarbituric acid/trichloroacetic acid (20mM TBA in 15% trichloroacetic acid) solution was added. The mixture was vortexed and incubated in a boiling water bath for 15 min, then cooled in cold water for 10min, and centrifuged for 15min at 2500 *g* at 4°C. Absorbance was measured at 532 nm and lipid oxidation was reported as mg malondialdehyde / kg meat.

2.7. Sensory Evaluation

Irradiated and non-irradiated coated minced chicken thighs meat containing ethanolic extract of papaya leaves at ratio 2% samples were periodically examined (every 3 days) for their appearance, texture and odor post treatments and during cold storage at 4 ± 1°C to determine the shelf-life of the samples. The panel consisted of ten members from our laboratory and scores were obtained as [31] by rating the above quality characteristics using the following rating 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.

2.8. Statistical Analysis

The obtained data were exposed to analysis of variance. Duncan's multiple range tests at 5% level was used to compare between means. The analysis was carried out using the PROC ANOVA procedure of Statistical Analysis System [32].

3. Resultes & Discussion

The composition of minced chicken meat makes it favorable for microbial growth, therefore minced chicken meat during storage spoils as a result mainly from microorganisms. The data in Table 1 exhibit that the effects of combination treatment between gamma irradiation and edible coating containing 2% ethanolic extract of papaya leaves during cold storage (4 ± 1°C) on the microbial load in minced chicken thighs meat. Samples of control had an initial counts of 7.8 x 10^5, 4.1.2 x 10^4, 3.6 x 10^3, 8.5 x 10^2 and 4.8 x 10^3 cfu/g for total bacterial count, Psychrophilic bacteria, lactic acid bacteria, Enterobacteriaceae and the total molds and yeasts, respectively. On the other hand, treated samples of minced chicken thighs meat with edible coating (without any additives) and edible coating containing 2% ethanolic extract of papaya leaves reduced the counts of total bacterial count, Psychrophilic bacteria, lactic acid bacteria, Enterobacteriaceae and the total molds and yeasts to 6.9 x 10^5, 3.6 x 10^4, 2.5 x 10^3, 7.4 x 10^2 and 4.3 x 10^3 & 3.4 x 10^5, 1.9 x 10^4, 8.7 x 10^2, 1.2 x 10^2 and 2.3 x 10^3 cfu/g, respectively.

Also, irradiating edible coating containing 2% ethanolic extract of papaya leaves minced chicken thighs meat samples at dose levels of 2, 4 and 6 kGy markedly decrease the counts of total bacterial count, Psychrophilic bacteria and lactic acid bacteria by 93.21, 96.34 and 96.67; 99.22, 98.66 and 99.56 & 99.95, 99.94 and 99.17%, respectively. These results agreed with those of Ouattara et al. [33] found that combined effect of edible coating containing thyme oil and *trans*-cinnamaldehyde and gamma irradiation at dose of 3 kGy had synergistic effects in reducing the aerobic plate counts and *Pseudomonas putida* of pre-cooked shrimp. While, the irradiation at dose levels of 2, 4 and 6 kGy of samples under investigation completely eliminated Enterobacteriaceae bacteria from these samples as it remained undetectable upon cold storage periods of the treated samples [34,35]. Meanwhile, the irradiated samples under investigation at dose levels of 0, 2, 4 and 6 kGy inhibition the growth of lactic acid bacteria till 9, 18, 24 and 30 days of storage, respectively. Also, the Table 1 shows that the initial count of total molds and yeasts were 4.8 x 10^3 cfu/g in control samples of minced chicken thigh meat. The initial counts of molds and yeasts reached 4.5 x 10^3, 2.3 x 10^3 and 9.8 x 10^1 cfu/g in samples of coated, edible coating containing ethanolic extract of 2% papaya leaves & irradiated edible coating containing ethanolic extract of papaya leaves at dose level of 2 kGy, respectively. While, irradiated samples at dose levels 4 and 6 kGy induced inhibition growth till 21 and 30 days of storage, respectively. Cha´vez-Quintal et al. [36] found that the ethanolic extract of papaya leaves exhibited the broadest action spectrum against three phytopathogenic fungi, *Rhizopus stolonifer*, *Fusarium* spp. and *Colletotrichum gloeosporioides*. During cold storage, gradual increase in the total bacterial count and psychrophilic bacteria was observed in all samples, but the rate of increase was higher in control samples than irradiated samples. Furthermore, samples were rejected when the count total bacterial count reached 1 x 10^7 cfu/g. Thus, the shelf-life samples of uncoated control, coated with edible coating (without any additives), and irradiated coated with edible coating containing 2% ethanolic extract of papaya leaves at doses of 0, 2, 4 and 6 kGy by 6, 9, 15, 30, 39 and 54 days, respectively. These results are in agreement with the results of Yusha et al. [37], who reported that the ethanolic extract of powdered papaya leaves were active against *Escherichia coli*, *Klebsiella pneumoniae* and Proteus *mirabilis* all concentrations used in disc diffusion and microbroth dilution technique (In vitro). Romasi et al. [38] found that extracts of papaya leaves could inhibit the growth of *Rhizopus stolonifer*, *Bacillus stearothermophilus*, *Listeria monocytogenes*, *Pseudomonas* sp., and *Escherichia coli* by agar diffusion method. On the other hand, the growth of Enterobacteriaceae were not appeared in samples of combination treatment between gamma irradiation at doses 0 and 2 kGy and edible coating containing 2% ethanolic extract of papaya leaves till 9 and 15 day of cold storage, respectively. This is in agreement with the results of Alabi et al. [39] found that The aqueous, ethanol and acetone extract of both the dried and fresh papaya leaves had broad spectrum antimicrobial activity against Gram-negative and Gram-positive bacteria and fungi of medical importance.

Table 1. Effects of combination treatments between gamma irradiation and edible coating containing ethanolic extract of papaya leaves during cold storage (4 ± 1°C) on the microbial load in minced chicken thighs meat

Microbial determinations (cfu/g)	Storage (days)	Control	Edible coating	Edible coating with ethanolic extract of papaya leaves			
				0 kGy	2 kGy	4 kGy	6 kGy
Total aerobic count	0	7.8x10^{5}	6.9x10^{5}	3.4x10^{5}	5.3x10^{4}	6.1x10^{3}	3.7x10^{2}
	3	5.2x10^{6}	9.2x10^{5}	6.7x10^{5}	6.4x10^{4}	8.3x10^{3}	5.2x10^{2}
	6	9.3x10^{6}	3.2x10^{6}	8.3x10^{5}	7.6x10^{4}	9.7x10^{3}	7.2x10^{2}
	9	8.4x10^{7}R	9.6x10^{6}	9.5x10^{5}	8.8x10^{4}	2.7x10^{4}	8.3x10^{2}
	12		7.1x10^{7}R	4.2x10^{6}	9.5x10^{4}	5.2x10^{4}	9.8x10^{2}
	15			9.4x10^{6}	3.6x10^{5}	6.7x10^{4}	2.9x10^{3}
	18			6.5x10^{7}R	6.9x10^{5}	8.2x10^{4}	5.7x10^{3}
	21				8.5x10^{5}	1.3x10^{5}	6.6x10^{3}
	24				1.6x10^{6}	3.8x10^{5}	8.9x10^{3}
	27				4.1 x10^{6}	6.7x10^{5}	3.8x10^{4}
	30				7.9 x10^{6}	8.3x10^{5}	7.5x10^{4}
	33				3.9x10^{7}R	4.2x10^{6}	1.9x10^{5}
	36					7.5 x10^{6}	4.7x10^{5}
	39					9.1x10^{6}	8.5x10^{5}
	42					4.3x10^{7} R	9.7x10^{5}
	45						2.9 x10^{6}
	48						6.1x10^{6}
	51						7.6 x10^{6}
	54						8.9x10^{6}
	57						5.8 x10^{7}R
Psychrophilic bacteria	0	4.1x10^{4}	3.5x10^{4}	1.9x10^{4}	1.5x10^{3}	5.5x10^{2}	2.3x10^{1}
	3	5.9x10^{4}	6.5x10^{4}	4.4x10^{4}	6.6x10^{3}	9.2x10^{2}	4.6x10^{1}
	6	9.6x10^{4}	9.2x10^{4}	7.9x10^{4}	7.9x10^{3}	2.6x10^{3}	6.1x10^{1}
	9	5.2x10^{5}R	3.8x10^{5}	1.3x10^{5}	2.8x10^{4}	5.5x10^{3}	8.5x10^{1}
	12		6.9x10^{5}R	5.6x10^{5}	4.9x10^{4}	7.2x10^{3}	3.1x10^{2}
	15			9.6x10^{5}	7.8x10^{4}	9.8x10^{3}	5.8x10^{2}
	18			3.5x10^{6}R	9.6x10^{4}	3.1x10^{4}	7.5x10^{2}
	21				2.4x10^{5}	6.6x10^{4}	8.9x10^{2}
	24				5.1x10^{5}	8.8x10^{4}	9.7x10^{2}
	27				7.2x10^{5}	1.8x10^{5}	3.9x10^{3}
	30				9.5 x10^{5}	4.6x10^{5}	6.4x10^{3}
	33				4.6 x10^{6}R	6.3x10^{5}	8.4x10^{3}
	36					8.9 x10^{5}	1.7x10^{5}
	39					1.2 x10^{6}	4.3x10^{5}
	42					3.3 x10^{6} R	6.4x10^{5}
	45						7.8 x10^{5}
	48						9.8x10^{5}
	51						3.6 x10^{6}
	54						5.2x10^{6}
	57						8.7x10^{6}R
Lactic acid bacteria	0	3.6x10^{3}	2.5x10^{3}	8.7x10^{2}	1.2x10^{2}	1.6x10^{1}	3.0x10^{1}
	3	4.6x10^{3}	2.7x10^{3}	<10	<10	<10	<10
	6	8.7x10^{3}	5.4x10^{3}	<10	<10	<10	<10
	9	5.2x10^{4} R	7.8x10^{3}	<10	<10	<10	<10
	12		2.6x10^{4} R	6.2x10^{2}	<10	<10	<10
	15			8.7x10^{2}	<10	<10	<10
	18			3.9x10^{3} R	<10	<10	<10
	21				1.6x10^{1}	<10	<10
	24				3.3x10^{1}	<10	<10
	27				6.5 x10^{1}	1.2x10^{1}	<10
	30				9.8 x10^{1}	3.8x10^{1}	<10
	33				7.9 x10^{2}R	6.7x10^{1}	4.0x10^{1}
	36					8.2 x10^{1}	2.1x10^{1}
	39					2.7 x10^{2}	5.7x10^{1}
	42					6.8 x10^{2}R	8.3x10^{1}
	45						9.7 x10^{1}
	48						2.7 x10^{2}
	51						6.9x10^{2}
	54						8.5x10^{2}
	57						4.6 x10^{3}R
Enterobacteriaceae	0	8.5x10^{2}	6.4x10^{2}	1.2x10^{2}	Nil	Nil	Nil
	3	4.2x10^{3}	9.2x10^{2}	<10	Nil	Nil	Nil
	6	8.8x10^{3}	4.9x10^{3}	<10	Nil	Nil	Nil
	9	5.9x10^{4} R	7.9x10^{3}	<10	Nil	Nil	Nil
	12		1.6x10^{4} R	1.2x10^{1}	Nil	Nil	Nil
	15			4.6x10^{1}	Nil	Nil	Nil

	18			7.1x10^{1}R	Nil	Nil	Nil
	21				Nil	Nil	Nil
	24				Nil	Nil	Nil
	27				Nil	Nil	Nil
	30				Nil	Nil	Nil
	33				Nil R	Nil	Nil
	36					Nil	Nil
	39					Nil	Nil
	42					Nil R	Nil
	45						Nil
	48						Nil
	51						Nil
	54						Nil
	57						Nil R
Total molds and yeasts	0	4.8 x10^{3}	4.3 x10^{3}	2.3 x10^{3}	2.3 x10^{1}	<10	<10
	3	8.5 x10^{3}	7.2 x10^{3}	5.7x10^{3}	3.9 x10^{1}	<10	<10
	6	2.7 x10^{4}	9.8 x10^{3}	8.9x10^{3}	6.7x10^{1}	<10	<10
	9	7.6 x10^{4}R	3.7 x10^{4}	2.1x10^{4}	9.4x10^{1}	<10	<10
	12		8.7 x10^{4}R	6.8x10^{4}	3.4x10^{2}	<10	<10
	15			1.9x10^{5}	5.6x10^{2}	<10	<10
	18			5.8x10^{5}R	7.2x10^{2}	<10	<10
	21				9.5x10^{2}	<10	<10
	24				4.7x10^{3}	7.0 x10^{1}	<10
	27				7.9x10^{3}	1.2 x10^{2}	<10
	30				2.6x10^{4}	2.3 x10^{2}	<10
	33				6.9x10^{4}R	4.1 x10^{2}	<10
	36					6.2 x10^{2}	4.0 x10^{1}
	39					8.7 x10^{2}	9.0x10^{1}
	42					3.6 x10^{3}R	1.8x10^{2}
	45						2.8x10^{2}
	48						5.3x10^{2}
	51						6.9x10^{2}
	54						8.4x10^{2}
	57						1.8x10^{3}R

R= Rejected

3.1. Food Borne Pathogens

Table 2 illustrates the effects of combination treatment gamma irradiation and edible coating during cold storage (4 ± 1°C) on food borne pathogens in minced chicken thighs meat. The results show that the initial counts of control samples were 7.8 x 10^2 and 8.9 x 10^2 cfu/g for *Staphylococcus aureus* and *B. cereus*, respectively. Moreover, bacteria of *Salmonella* spp were detected in these samples. AL-Dughaym and Altabari [40] revealed that revealed that the mean total bacterial count was ranged from 2.7 × 10^4 cfu/g for nuggets A to 3.3 × 10^7 cfu/g for burger B and the other products in the range of 10^5-10^6 cfu/g. While *Staphylococcus aureus* mean count ranged from less than 10^2 cfu/g for all samples, accept 10^4 and 10^6 cfu/g for mince and frankfurter samples, respectively. *Escherichia coli* isolated from 70% of the samples and *Salmonella arizona* was isolated at once from thigh samples from poultry meat products were collected from local markets.

Subjecting samples of minced chicken thighs meat to edible coating (without any additives) and edible coating containing 2% ethanolic extract of papaya leaves reduced the counts of *Staphylococcus aureus* to 4.1 x 10^2 and 2.7 x 10^2 cfu/g and counts of *B. cereus* to 5.3 x 10^2 and 1.6 x 10^2 cfu/g, respectively. While, subjecting coated samples under investigation to gamma irradiation at dose levels of 2, 4 and 6 kGy completely eliminated *Staphylococcus aureus* bacteria from these samples as it remained undetectable upon cold storage periods [34,35]. Meanwhile, bacteria of *Salmonella* spp was detected in samples edible coating (without any additives) while, these bacteria were not detected in samples of minced chicken thighs meat treated by edible coating containing 2% ethanolic extract of papaya leaves & irradiated edible coating containing 2% ethanolic extract of papaya leaves at dose of 2 kGy. These results are in agreement with previous reports Abdeldaiem [41], found that edible coatings containing 1% oregano essential oil samples of bolti fish fillets and irradiated at 1 kGy reduced the counts of Enterobacteriaceae, *Staphylococcus aureus* and *Bacillus cereus* as well as eliminating *Vibrio* spp and *Salmonella* spp, while coated samples irradiated at 3 and 5 kGy completely eliminated these bacteria. Also, Anibijuwon and Udeze [17] and Okunola et al. [18] concluded that papaya leaves had antimicrobial activity against both gram-negative and gram-positive bacteria is an indication that the plant is a potential source for production of drugs with a broad spectrum of activity and its extracts possess compounds with antibacterial properties that can be used as antibacterial agents in novel drugs for the treatment of gastroenteritis, uretritis, otitis media, and wound infections. Also, Romasi et al. [38] reported that the extracts of papaya leaves showed could inhibit *B. stearothermophilus, L. monocytogenes, Pseudomonas* sp., and *E. coli* by using agar diffusion method. Moreover, Rahman et al. [42] revealed that the ethanolic extract of papaya leaves inhibit the growth of selected microorganisms. Various Gram negative (*Escherichia coli, Proteus mirabilis, Pseudomonas aeruginosa, Salmonella typhi, Salmonella paratyphi A, Shigella flexenert)* and Gram positive bacteria (*Staphylococcus aureus, Staphylococcus epidermidis, Bacillus subtilis, Micrococcus luteus*) in vitro. During cold storage, gradual increases of *Staphylococcus aureus* and *B. cereus* were observed during cold storage in samples of control, edible coating containing 2% ethanolic extract of papaya leaves & irradiated edible coating containing 2% ethanolic extract of papaya leaves at dose of 2 kGy. While, irradiation at doses of 4 and 6 kGy combined with edible coating containing 2% ethanolic extract of papaya leaves completely eliminated these pathogen from samples minced chicken thighs meat. Moreover, Alabi et al. [39] mentioned that the plant kingdom synthesizes diverse active compounds, some of the active compounds do occur singly or in combination with other inactive substances which inhibit greatly the life processes of microbes, especially the pathogenic microbes. Medicinal plants are cheap and renewable source of pharmacologically active substances. Also, Azmi et al. [43] mentioned that the mainly component in papaya leaves were flavonoids, alkaloids, saponins, xanthine alkaloids, terpenoids and anthranol glycosides which could partially explain the pharmacological properties of this plant and demonstrates its importance in alimentation and daily intake especially for gout patient.

Table 2. Effects of combination treatments between gamma irradiation and edible coating containing ethanolic extract of papaya leaves during cold storage (4 ± 1°C) on food borne pathogens in minced chicken thighs meat

Microbial determinations (cfu/g)	Storage (days)	Control	Edible coating	Edible coating with ethanolic extract of papaya leaves			
				0 kGy	2 kGy	4 kGy	6 kGy
Staphylococcus aureus	0	$7.8x10^2$	$4.1x10^2$	$2.7x10^2$	Nil	Nil	Nil
	3	$1.9x10^3$	$6.2x10^2$	$3.6x10^2$	Nil	Nil	Nil
	6	$4.7x10^3$	$7.7x10^2$	$6.2x10^2$	Nil	Nil	Nil
	9	$8.5x10^3$R	$9.3x10^2$	$7.4x10^2$	Nil	Nil	Nil
	12		$4.2x10^3$R	$8.7x10^2$	Nil	Nil	Nil
	15			$3.3x10^3$	Nil	Nil	Nil
	18			$6.8x10^3$R	Nil	Nil	Nil
	21				Nil	Nil	Nil
	24				Nil	Nil	Nil
	27				Nil	Nil	Nil
	30				Nil	Nil	Nil
	33				Nil R	Nil	Nil
	36					Nil	Nil
	39					Nil	Nil
	42					Nil R	Nil
	45						Nil
	48						Nil
	51						Nil
	54						Nil
	57						Nil R
Bacillus cereus	0	$8.9x10^2$	$5.3x10^2$	$1.6x10^2$	$4.5x10^1$	N.D	N.D
	3	$9.6x10^2$	$7.5x10^2$	$2.9x10^2$	$6.9x10^1$	N.D	N.D
	6	$3.5x10^3$	$8.9x10^2$	$4.1x10^2$	$7.7x10^1$	N.D	N.D
	9	$8.1x10^3$R	$2.6x10^3$	$5.8x10^2$	$8.1x10^1$	N.D	N.D
	12		$6.7x10^3$R	$7.8x10^2$	$9.3x10^1$	N.D	N.D
	15			$9.8x10^3$	$2.8x10^2$	N.D	N.D
	18			$4.2x10^3$R	$4.3x10^2$	N.D	N.D
	21				$6.5x10^2$	N.D	N.D
	24				$7.2x10^3$	N.D	N.D
	27				$8.9x10^2$	N.D	N.D
	30				$2.1x10^3$	N.D	N.D
	33				$5.5x10^3$R	N.D	N.D
	36					N.D	N.D
	39					N.D	N.D
	42					N.D R	N.D
	45						N.D
	48						N.D
	51						N.D
	54						N.D
	57						N.D R
Salmonella spp	0	+	+	N.D	N.D	N.D	N.D
	3	+	+	N.D	N.D	N.D	N.D
	6	+	+	N.D	N.D	N.D	N.D
	9	+ R	+	N.D	N.D	N.D	N.D
	12		+ R	N.D	N.D	N.D	N.D
	15			N.D	N.D	N.D	N.D
	18			N.D R	N.D	N.D	N.D
	21				N.D	N.D	N.D
	24				N.D	N.D	N.D
	27				N.D	N.D	N.D
	30				N.D	N.D	N.D
	33				N.D R	N.D	N.D
	36					N.D	N.D
	39					N.D	N.D
	42					N.D R	N.D
	45						N.D
	48						N.D
	51						N.D
	54						N.D
	57						N.D R

R = Rejected + = Positive N.D = not detected

3.2. Chemical Evaluation

Moreover, TVN can be considered as a reliable indicative measure for the quality of various food articles especially chicken meat and chicken cut-up meat. In general, TVN in chicken cut-up meat may be increased as the days of storage increased [44]. As the results in Table 3 show the changes in the total volatile basis nitrogen (TVBN) and thiobarbituric acid reactive substances (TBARs) as a chemical indexes due to combination treatment and cold storage, respectively. The TVBN maybe considered as a quality index for minced chicken meat because its increase is related to the activity of spoilage bacteria and endogenous enzymes [45]. Moreover, Mexis et al. [46] mentioned that As autolysis of muscle proteins proceeds, compounds of alkaline reaction such as amines and ammonia build up producing objectionable odors. Thus, TVB-N has been used as a spoilage indicator in several animal food substrates. Furthermore, samples treated by edible coating (without any additive) or by combination treatment between gamma irradiation at doses of 0, 2, 4 and 6 kGy and edible coating containing 2% ethanolic extract of papaya leaves induced no changes in the contents of TVBN. During cold storage, an increase in these compounds was observed, but the rate of increase was lower in samples of combination treatment especially at higher doses. Mexis et al. [46] reported that the samples of ground chicken were rejected when the TVB-N ranged between 42.5 and 57.5 mg/kg. Balamatsia et al. [47] who reported TVB-N values of 45.8, 40.0 and 36.0 mg N/100 g for chicken breast fillets packaging in air, under vacuum and modified atmosphere packaging (MAP) respectively, at the limit of sensory acceptability (6 and 9-10 day of storage respectively at 4°C). Patsias et al. [48] reported initial TVB-N values of 12 mg N/100 g which increased to 13.0 and 49.0 mg N/100 g for raw chicken fillets stored in air and under MAP after 9 day of storage.

Table 3. Effects of combination treatments between gamma irradiation and edible coating containing ethanolic extract of papaya leaves during cold storage (4 ± 1°C) on chemical characteristics in minced chicken thighs meat

Chemical quality index	Storage period (days)	Control	Edible coating	Edible coating with ethanolic extract of papaya leaves			
				0	2	4	6
The total volatile basic nitrogen (TVBN mg N/100g wet matter)	0	11.28	11.79	11.35	11.74	11.92	12.05
	3	18.07	18.42	17.14	13.43	12.58	12.44
	6	26.15	22.57	19.62	15.34	13.93	12.98
	9	35.73 R	27.29	24.08	18.17	14.77	13.02
	12		31.14	27.13	21.48	16.61	13.66
	15		35.06R	29.26	22.93	18.44	13.12
	18			32.41	24.60	19.96	14.35
	21			35.18R	27.83	22.38	14.94
	24				30.07	24.84	15.62
	27				31.68	25.99	16.77
	30				33.54	27.56	18.48
	33				36.12R	29.16	20.63
	36					30.09	21.87
	39					32.74	22.42
	42					35.66R	22.91
	45						24.46
	48						26.55
	51						28.78
	54						32.12
	57						36.09R
Thiobarbituric acid-reactive substances (mg malonaldehyde/kg)	0	0.41	0.43	0.42	0.48	0.55	0.59
	3	0.54	0.58	0.47	0.51	0.56	0.60
	6	0.62	0.62	0.52	0.53	0.58	0.61
	9	0.79 R	0.69	0.59	0.56	0.59	0.62
	12		0.73	0.68	0.58	0.61	0.63
	15		0.89R	0.74	0.61	0.62	0.65
	18			0.79	0.67	0.64	0.66
	21			0.89R	0.71	0.65	0.67
	24				0.75	0.67	0.69
	27				0.77	0.69	0.72
	30				0.79	0.71	0.73
	33				0.91R	0.73	0.74
	36					0.78	0.76
	39					0.85	0.77
	42					0.96 R	0.79
	45						0.81
	48						0.83
	51						0.86
	54						0.91
	57						0.98 R

R = Rejected

Table 4. Changes in sensory attributes of minced chicken thighs meat as affected by combination treatment between gamma irradiation and edible coating containing ethanolic extract of papaya leaves during cold storage (4 ± 1°C)

Storage period (days)	Appearance						Flavor						Color					
	Control	Edible coating	Edible coating with ethanolic extract of papaya leaves				Control	Edible coating	Edible coating with ethanolic extract of papaya leaves				Control	Edible coating	Edible coating with ethanolic extract of papaya leaves			
			0 kGy	2 kGy	4 kGy	6 kGy			0 kGy	2 kGy	4 kGy	6 kGy			0 kGy	2 kGy	4 kGy	6 kGy
0	8.9^{Ba}±0.07	9.5^{Aa}±0.28	9.5^{Aa}±0.15	9.5^{Aa}±0.36	9.4^{Aa}±0.13	9.4^{Aa}±0.37	9.5^{Aa}±0.17	9.4^{Aa}±0.36	9.4^{Aa}±0.21	9.3^{Aa}±0.47	9.4^{Aa}±0.026	9.3^{Aa}±0.18	9.7^{Aa}±0.63	9.6^{Aa}±0.19	9.7^{Aa}±0.08	9.7^{Aa}±0.54	9.7^{Aa}±0.72	9.7^{Aa}±0.35
3	8.3^{Bb}±0.12	9.4^{Aa}±0.12	9.4^{Aa}±0.24	9.5^{Aa}±0.42	9.4^{Aa}±0.07	9.4^{Aa}±0.26	9.4^{Aa}±0.25	9.4^{Aa}±0.42	9.4^{Aa}±0.25	9.3^{Aa}±0.25	9.4^{Aa}±0.07	9.3^{Aa}±0.18	8.7^{Bb}±0.42	9.6^{Aa}±0.11	9.7^{Aa}±0.08	9.7^{Aa}±0.51	9.7^{Aa}±0.64	9.7^{Aa}±0.58
6	7.8^{Cc}±0.08	9.1^{Aa}±0.15	9.2^{Aa}±0.33	9.5^{Aa}±0.51	9.4^{Aa}±0.25	9.4^{Aa}±0.15	9.3^{Aa}±0.29	9.4^{Aa}±0.33	9.4^{Aa}±0.36	9.3^{Aa}±0.36	9.4^{Ba}±0.23	9.3^{Aa}±0.25	8.6^{Bb}±0.13	9.5^{Aa}±0.28	9.6^{Aa}±0.15	9.6^{Aa}±0.42	9.7^{Aa}±0.68	9.7^{Aa}±0.76
9	3.4^{Cd}±0.24R	8.9^{Bb}±0.07	9.2^{Aa}±0.12	9.5^{Aa}±0.38	9.3^{Aa}±0.29	9.4^{Aa}±0.18	3.1^{Cb}±0.14R	8.9^{Bb}±0.27	9.4^{Aa}±0.57	9.3^{Aa}±0.34	9.4^{Aa}±0.43	9.3^{Aa}±0.29	3.1^{Bc}±0.07R	9.5^{Aa}±0.26	9.5^{Aa}±0.16	9.6^{Aa}±0.12	9.7^{Aa}±0.73	9.6^{Aa}±0.64
12		3.5^{Cc}±0.35R	8.7^{Bb}±0.17	9.3^{Aa}±0.23	9.3^{Aa}±0.53	9.4^{Aa}±0.06		4.3^{Bc}±0.34R	9.3^{Aa}±0.56	9.2^{Aa}±0.16	9.4^{Aa}±0.38	9.2^{Aa}±0.22		4.1^{Bb} 0.28 ±R	9.5^{Aa}±0.28	9.5^{Aa}±0.19	9.6^{Aa}±0.18	9.6^{Aa}±0.12
15			8.7^{Bb}±0.18	9.5^{Aa}±0.12	9.2^{Aa}±0.58	9.3^{Aa}±0.51			9.3^{Aa}±0.12	9.2^{Aa}±0.58	9.4^{Aa}±0.29	9.2^{Aa}±0.41			8.9^{Bb}±0.27	9.4^{Aa}±0.25	9.6^{Ab}±0.47	9.5^{Aa}±0.11
18			4.3^{Bc}±0.09R	9.2^{Aa}±0.28	9.2^{Aa}±0.34	9.3^{Aa}±0.42			3.2^{Ba}±0.09R	9.1^{Aa}±0.12	9.3^{Aa}±0.16	9.2^{Aa}±0.46			4.3^{Bc}±0.64R	9.4^{Aa}±0.29	9.6^{Aa}±0.38	9.4^{Aa}±0.09
21				9.2^{Aa}±0.19	9.2^{Aa}±0.12	9.3^{Aa}±0.46				9.1^{Aa}±0.08	9.3^{Aa}±0.44	9.2^{Aa}±0.52				9.3^{Aa}±0.35	9.6^{Aa}±0.69	9.4^{Aa}±0.25
24				8.9^{Bb}±0.06	8.9^{Bb}±0.18	9.3^{Aa}±0.55				8.9^{Bb}±0.06	9.3^{Aa}±0.31	9.2^{Aa}±0.16				9.1^{Aa}±0.48	9.4^{Aa}±0.07	9.3^{Aa}±0.48
27				8.9^{Bb}±0.11	8.9^{Bb}±0.05	9.2^{Aa}±0.32				8.9^{Bb}±0.29	9.3^{Aa}±0.22	9.2^{Aa}±0.19				8.9^{Bb}±0.27	9.4^{Aa}±0.15	9.3^{Aa}±0.39
30				8.7^{Bb}±0.32	8.9^{Bb}±0.26	9.2^{Aa}±0.24				8.8^{Bb}±0.45	9.3^{Aa}±0.57	9.2^{Aa}±0.25				8.7^{Bb}±0.71	9.1^{Aa}±0.19	9.2^{Aa}±0.59
33				4.3^{Cc}±0.42R	8.8^{Bb}±0.46	9.1^{Aa}±0.19				6.4^{Bb}±0.52R	9.1^{Aa}±0.42	9.2^{Aa}±0.36				5.8^{Cc}±0.52R	9.1^{Ba}±0.27	9.2^{Ac}±0.67
36					8.8^{Bb}±0.42	9.1^{Aa}±0.13					8.9^{Bb}±0.08	9.2^{Aa}±0.28					8.9^{Bb}±0.54	9.1^{Aa}±0.74
39					8.7^{Bb}±0.19	9.1^{Aa}±0.15					8.9^{Bb}±0.16	9.1^{a}±0.41					8.8^{Bb}±0.65	9.1^{Aa}±0.53
42					4.2^{Cc}±0.27R	8.9^{Bb}±0.12					7.5^{Cc}±0.15R	9.1^{a}±0.49					6.2^{Bc}±0.39R	9.1^{Aa}±0.41
45						8.9^{b}±0.09						9.1^{a}±0.65						8.9^{b}±0.26
48						8.9^{b}±0.24						9.1^{a}±0.43						8.9^{b}±0.32
51						8.8^{b}±0.29						8.9^{b}±0.09						8.8^{b}±0.18
54						8.7^{b}±0.45						8.9^{b}±0.17						8.8^{b}±0.14
57						4.6^{c}±0.53R						8.6^{b}±0.47R						7.5^{c}±0.27R

Capital and small letters were used for comparing between means in the rows and columns, respectively.
Means with the same letters are not significantly different (p>0.05).
R = Rejected

The TBARs value is an index of lipid oxidation measuring malondialdehyde (MDA) content. MDA formed through hydroperoxides, which are the initial reaction product of polyunsaturated fatty acids with oxygen [49]. The oxidative rancidity in fresh, frozen and cooked chicken breast and leg meat was evaluated by measuring malonaldehyde in fat with an improved thiobarbituric acid (TBARs) assay with antioxidant protection [50]. Exposing samples of coated minced chicken thighs meat containing 2% ethanolic extract of papaya leaves to gamma irradiation at doses levels of 2, 4 and 6 kGy induced slight increase in their TBARs values as compared to values of control, edible coating (without any additive) and edible coating containing 2% ethanolic extract of papaya leaves samples of minced chicken thighs meat. The slight increase in values of TBARs in irradiated samples may be mainly attributed to the strong antioxidant effect of ethanolic extract of papaya leaves which acts as a radical scavenger [51]. Imaga et al. [52] found that the extracts of papaya leaves contained flavonoids and the antioxidant vitamins A and C these compounds may be due to their inherent antioxidant nutrient composition. Ayoola et al. [53] reported that the ethanolic extract of papaya leaves showed high antioxidant (free radical scavenging) activities when compared to vitamin C and these extract showed the presence of flavonoids, terpenoids, saponins, tannins and reducing sugars. Abdeldaiem [41] showed slight increase in thiobarbituric acid reactive substances (TBARS) post irradiation and during cold storage of edible coatings containing 1% oregano essential oil of bolti fish fillets samples, but had no effects on their total volatile nitrogen (TVN) contents, while a gradual increase in these chemical quality indexes was observed during cold storage. The acceptability limit of TBARS value in this study was 1.0. Earlier workers reported that meat sample containing TBARS value less than 1 possesses no off odor [54]. Meanwhile, Jayasingh et al. [55] reported that for secondary oxidation products, such as, TBA, no legal threshold exists, but a limit of 1 mg malonaldehyde/kg meat has been suggested for sensory perceived rancidity. Shon et al. [56] illustrated that the soy protein isolate coating reduced thiobarbituric acid-reactive substances (TBARS) and peroxide value (PV) of cut pork stored at 4°C for 5 days compared with controls.

3.3. Sensory Evaluation

Table 4 showed that from these data all samples under investigation had an excellent scores for flavor and color, but appearance of coated samples with edible coating (without any additive) and coated with edible coating containing 2% ethanolic extract of papaya leaves treated by gamma irradiation at doses of 2, 4 and 6 kGy had higher scores than control samples. These may be due to brightness of coatings that coat minced chicken thighs meat samples. Similar results were observed as in [57] reported that irradiated pork patty coated with pectin-based material containing green tea leaf extract powder had high scores for appearance compared with control samples. Upon cold storage, samples of control and coated showed similar scores till the detection off odor and their total bacterial count more than 1 x 10^7 cfu/g and their rejection on day 9 and 12 of storage, respectively. While coated samples of minced chicken thighs meat with edible coating containing ethanolic extract of papaya leaves and treated by gamma irradiation at doses of 0, 2, 4 and 6 kGy were scored as good samples their rejection due to increasing their total bacterial count to more than 1 x 10^7 cfu/g on day 21, 33, 42 and 57 of storage, respectively. These results are in agreement with those of Ouattara et al. [58] they found that the shelf-life extension periods ranged from 3 to 10 days for uncoated shrimps and pizzas and from 7 to 20 days for irradiated edible coated shrimps and edible coated pizzas at dose of 4 kGy, respectively, compared to uncoated / unirradiated products. Furthermore, Lacroix et al. [59] mentioned that shelf-life extension periods for irradiated edible coated ground beef samples at doses 1, 2 and 3 kGy were 4, 7 and 10 days, respectively. Javanmard et al. [60] reported that the irradiation dose of 5 kGy can be effective to control bacterial pathogens in chicken meat, through its effectiveness in extending their frozen shelf-life without any significant effects on the sensory quality. Meanwhile, Abdeldaiem [41] concluded that the coated samples of bolti fish fillets with edible coating containing oregano EO and treated by gamma irradiation at doses of 0, 1, 3 and 5 kGy were scored as good samples their rejection due to increasing their total bacterial count to more than 1 x 10^7 cfu/g on day 18, 24, 33 and 39 of storage, respectively

4. Conclusion

In conclusion, The results obtained from this study showed that combination treatments between gamma irradiation at doses of 2, 4 and 6 kGy and edible coating films containing 2% of ethanolic extract of papaya leaves for improving the quality and safety of minced chicken thighs meat through its effectiveness in eliminating bacteria of public health and extending the refrigerated shelf-life to 30, 39 and 54 days for irradiated coated samples under investigation, respectively, compared to 6 days for uncoated control samples without any adverse changes in their chemical and sensory properties. Therefore the incorporation of ethanolic extract of papaya leaves in edible coating materials increases the bacterial inhibitory effect of gamma irradiation and is suitable for preservation of minced chicken meat.

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