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RESEARCH ARTICLE

USE OF IRRADIATION TO CONTROL MICROORGANISMS AND EXTEND THE REFRIGERATED MARKET LIFE OF CHICKEN SAUSAGE

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Abstract

This study investigated the microbial, chemical and sensorial properties of chicken sausage exposed to gamma irradiation and stored at 0-4°C. Chicken sausage was treated with 0, 2, 4 or 6 kGy doses of gamma irradiation. Microbial, chemical, and sensory analyses of chicken sausage were evaluated at 0, 4, 8, 12, 16 and 20 weeks of storage. Irradiation at 2, 4 and 6 kGy significantly reduced the counts of total viable (mesophilic aerobic) plate counts (TPCs), fecal coliform and yeast load and prolonged the refrigerated shelf-life of chicken sausage. Irradiation significantly decreased their amount of total acidity, volatile basic nitrogen (VBN), and thiobarbituric acid reactive substances (TBARS), while storage increased the total acidity, VBN and TBARS for irradiated and non-irradiated samples. The percentage of protein slightly increased in irradiated samples with higher doses, while the percentage of fat significantly decreased. Gamma irradiation showed no significant effect on the sensory properties of chicken sausage.

Keywords: Gamma irradiation, microbial load, chicken sausage, sensory evaluation, shelf-life.

Introduction

On the market there is a growing interest in semi-prepared and prepared meals. Ready-to cook/eat products are manufactured, and frozen in the processing establishment, distributed, and sold in the frozen condition (Farkas *et al.*, 2005). Frozen foods are not always safe as freezing does not eliminate pathogens (Kanatt *et al.*, 2005; Yeboah-Manu *et al.*, 2010). It is known that neither traditional meat inspection nor supposedly good manufacturing practices can really assure the attainment and maintenance of high hygienic standards for meat and meat products with respect to contamination with pathogenic or spoilage microorganisms (Badr, 2007). Irradiation of foodstuffs is an effective, environmentally friendly solution which is authorized in more than 50 different countries all over the world and for various kinds of food products (Cutrubinis *et al.*, 2007). Food irradiation is being considered as an important tool, not only in ensuring safety but also in extending the shelf-life of chicken meat (Yoon, 2003), meat and meat products (Sommers and Fan, 2003; Savvaidis *et al.*, 2002), and ready-to-eat cooked meat products (Arizina *et al.*, 2012; Kim *et al.*, 2012; Inamura *et al.*, 2012; Al-Bachir *et al.*, 2010). Although, irradiation can provide pathogen–free raw materials, its application in meat production may be hampered by its known adverse effects on quality characteristics of fresh or processed meats (Brewer, 2009; 2004). The chemical changes of meat and poultry by

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irradiation, however, are concern to consumers, and the meat industry is having difficulties in using the technology to achieve its food safety benefits (Lee and Ahn, 2004; Nam *et al.*, 2007). The acceleration of lipid oxidation and off-odor production caused by irradiation processing in raw and cooked meat products has been reported and the rate of oxidation increased in a dose-dependant manner (Gomes *et al.*, 2003; Nam and Ahn, 2003).

Currently, limited information about the effects of irradiation on quality of chicken sausage is available. Therefore, the objective of this work is to determine an adequate value for radiation dose sighting to increase the shelf-life of the chicken sausage based on the evaluation of its microbiological, chemical, and sensorial characteristics after irradiation.

Materials and methods

Preparation and formulation of chicken sausage

The chicken sausage was prepared by a local caterer. No changes were made to the way in which the chicken sausage is usually prepared in this industry. Chicken sausage have two parts, in which the outer layer is sheep intestine with cemetery not more than 16 mm. Outer layer was stuffed with ground chicken meat. To each 3 kilogram of boneless ground chicken meat the following ingredients were added; 200 g ground Soya bean, (42 g flavorings in 400 g ice), 50 g allspice, 50 g garlic, 50 g special mixed spices, and 15 g NaCl. After preparation, 300 g of chicken sausage were placed on polystyrene trays and covered with lids made of polyethylene film. The film thickness is 0.087 mm and sealed properly. Each tray of chicken sausage is considered as a replicate

Treatments and analysis performed during storage

Samples from packed chicken sausage were exposed to gamma radiation at doses of 2, 4 and 6 kGy in a ^{60}CO package irradiator (dose rate 8.488 kGy h-1). The irradiation was performed at room temperature (15–20^{o}C). The absorbed dose was determined using alcoholic chlorobenzene dosimeter. Ethanol chlorobenzene is prepared in our lab by mixing 24 ml chlorobenzene, 4 ml distilled water, 0.04 ml acetone, 0.04 ml benzene to 100 ml ethanol. The absorbed dose is determined by the measurement of chloride ions or hydrogen ions by means Oscillotitrator (OK-302/2, Radelkisz, Budapest, Hungary) (Al-Bachir, 2005). For each treatment, 20 trays of chicken sausage were allocated and all were stored at 1–4 ^{o}C temperature. Microbiological and chemical analyses were performed on controls and treated samples immediately after irradiation, and monthly throughout the storage period, which lasted 5 months. Sensory evaluation and proximate analysis were done within two days of irradiation.

Microbiological evaluation

Three replicates from each treatment, un-irradiated and irradiated, were aseptically opened, and 10 g of whole chicken sausage were transferred to a sterilized glass bottle containing 90 ml of sterile physiological water (9 g kg^{-1} NaCl). The bottle was shaken to homogenize the sample. Further dilutions were made as far as 10^{-6} (AOAC, 2010). The media used for the microbiological study were nutrient agar for the total viable (mesophilic aerobic) plate counts (TPCs), agar plate counts (APCs) (Oxoid, CM 325, UK) (48 h incubation at 30^{o}C). A cut-off value of 10^{7} CFU g^{-1} for TPCs was used for the unacceptable samples, and no further analyses were carried out when those indicator values were exceeded (Al-Bachir *et al.*, 2010). The fecal coliform colony count was determined on Violet Red Bile Agar (VRBA) (Oxoid, CM 485, UK) at 37 ^{o}C for 48 h. Yeast were enumerated on Dichloran Rose-Bengal Chloramphenicol Agar (DRBC) (Merck, 1.00466, Germany) after incubation at 25 ^{o}C for 5 days. Before irradiation and in order to determine the survival curves, the chicken sausage was artificially inoculated by thoroughly mixing it with a pure culture of *Salmonella* spp and *Escherichia coli*. The used *Salmonella* spp and *E. coli* have been isolated in our lab from contaminated local food. The above mentioned strains were identified by biochemical identification test. The initial numbers of artificial contamination were .2.0x10^{7} and 2.0x10^{6} CFU g^{-1} for *Salmonella* spp and *E. coli* respectively. The survival curve was estimated from irradiation doses of 0.2, 0.4, 0.6, 0.8 and 1.0

kGy. The survival level of *Salmonella* spp was determined by plate counting on Xylose Lysine Desoxycholate Agar (XLD) (Biolife, 402206, Italy) and the survival level of *E. coli* was determined by plate counting on Eosin Methylene Blue Agar (EMBA) (Oxoid, CM 69, UK), after 2 days of incubation at 37°C.

Chemical analysis

Approximately 150 g of chicken sausage were blended for 15 s in a laboratory blender, and was used in all the chemical analysis. Each sample was homogenized and analyzed in triplicates, to determine moisture and ash (drying for 6 h at 105°C, and ashing for 4 h at 550°C), crude fat (as extractable component in Soxhlet apparatus), crude protein (as Kjeldahl nitrogen) using standard methods (AOAC, 2010). pH values of the solutions of chicken sausage were determined using an HI 8521 pH meter (Hanna Instruments, Woonsocket, RI, USA). Water activity was determined using the reference solutions (Al-Bachir *et al*., 2010). The total acidity was obtained by a direct titration with (0.1 N) NaOH and phenolphthalein as an indicator. The total acidity was calculated as ml of (0.1 N) NaOH = 0.0090 g lactic acid. Thiobarbituric Acid (TBA) values were determined and expressed as mg malonaldehyde (MDA) /1 kg chicken sausage. Total volatile basic nitrogen in the sample in term of mg VBN per kg chicken sausage (ppm) was determined (Al-Bachir, 2005).

Sensory evaluation

The sensorial criteria, especially the taste, odor, color and texture of the irradiated and non-irradiated chicken sausage were evaluated within two days of irradiation. Each panelist received four coded pieces samples (one non-irradiated and three irradiated samples; one at each dose). All chicken sausage were tasted by 25 person. Before testing, chicken sausage were fried in sunflower oil for 5 min. Each member independently evaluated the chicken sausage samples for taste, odor, color and texture on a 5- point hedonic scale (1: extremely poor, 2: poor, 3: acceptable, 4: good, 5: excellent) (Al-Bachir *et al.*, 2010).

Statistical analysis

The four treatments were distributed in a completely randomized design with three replicates. Data were subjected to the analysis of variance test (ANOVA) using the SUPERANOVA computer package (Abacus Concepts Inc, Berkeley, CA, USA; 1998). A separation test on treatment means was conducted using Fisher's least significant differences (LSD) methods at 95% confidence level (Snedecor and Cochran, 1988). D_{10} value were calculated using Cricket graph computer package (40 Valley Stream Parkway Malvern,PA 19355, 1986/87/88 Cricket Software, Copyright, Version 1.3).

Results and discussion

Chicken sausage characteristics

Table 1. shows the chemical characteristics of chicken sausage as function of irradiation doses. It can be seen that the proximate chemical contents were: crude fat (5.82 ± 0.04 %), crude protein (18.68 ± 0.67 %), ash (1.94±0.13 %) and moisture (72.67 ±0.05 %). The water activity value for chicken sausage was 0.92 at 24°C and the pH value was (5.57±0.05). Considering the nature of ingredients, chicken sausage is rich with nutritional constituents. In general, increase trend was observed in protein content with the higher irradiation doses. There was no significant ($p>0.05$) difference between the protein contents of the non-irradiated and irradiated samples. Lipid is reported to be the most sensitive food components to the irradiation process (Venugopal *et al*., 1999). In current study, also, the lipid of the samples was affected by irradiation treatment. There was significant ($p>0.05$) differences in lipid contents between the non-irradiated and samples irradiated with 4 and 6 kGy. The pH values of samples were ranged between 5.57- 5.68. However, well aspect that there was no significant ($p>0.05$) changes, after the irradiation treatment. Irradiation had no effects on the pH of the fermented sausages (Kim *et al*., 2012).

Table 1. *Effect of gamma irradiation on moisture, protein, fat and ash contents of chicken sausage (%)*

Treatment	0kGy	2kGy	4kGy	6kGy	LSD 5%
Moisture	72.67±0.05	72.25±0.05	72.88±0.63	73.46±0.36	**0.69**
Protein	18.68±0.67	18.31±0.49	19.79±0.45	19.98±1.21	**1.45**
Fat	5.82±0.04	5.96±0.06	4.97±0.28	4.96±0.23	**0.35**
Ash	1.94±0.13	2.08±0.18	1.96±0.09	1.63±0.11	**2.25**
PH	5.57±0.05	5.62±0.02	5.68±0.02	5.66±0.02	**0.06**

LSD, Least significant difference

Table 2. *Effect of gamma irradiation on the microbial load of chicken sausage stored at 1-4 ^{0}C (log_{10} CFU/g)*

Treatment	0kGy	2kGy	4kGy	6kGy	LSD 5%
Storage period(Weeks)					
		Total Count (log_{10} CFU/g)			
0	7.23±0.99	4.79±0.07	3.99±0.20	3.20±0.12	0.96
4	R	4.44±0.10	3.71±0.09	3.19±0.09	0.19
8	R	4.46±0.10	3.57±0.08	3.11±0.06	0.16
12	R	5.70±0.06	3.85±0.02	3.03±0.01	0.08
16	R	R	5.82±0.12	3.43±0.06	0.22
20	R	R	R	5.93±0.38	
		Total Coliforme (log_{10} CFU/g)			
0	6.46±0.20	2.77±0.12	<1	<1	0.38
4	6.55±0.04	2.54±0.08	<1	<1	0.14
8	R	2.50±0.08	<1	<1	
12	R	R	<1	<1	
16	R	R	<1	<1	
20	R	R	<1	<1	
		Total Yeasts (log_{10} CFU/g)			
0	2.26±0.05	<1	<1	<1	
4	2.32±0.09	<1	<1	<1	
8	R	2.33±0.03	<1	<1	
12	R	2.72±0.06	2.39±0.06	<1	0.14
16	R	3.47±0.04	2.46±0.06	<1	0.11
20	R	R	2.66±0.11	<1	

R= Rejected

Microbial quality of irradiated chicken sausage

Microbial assessment, i.e. total viable (mesophilic aerobic) plate counts (TPCs), fecal coliform and yeast load of chicken sausage stored at 1-4^{o}C before and after irradiation and throughout storage periods are summarized in table 2. The results from table 2 shows that chicken sausage contaminated a TPCs, fecal coliform and yeast level of 7.23, 6.46 and 2.26 log CFU/g before irradiation, respectively. This suggested that preparation of chicken sausage in the trimming in the plant's chicken sausage area might have been contaminated with high population of microorganisms, and from the raw materials used for made chicken sausage in particular chicken meat and ingredients, that may contained high amounts of microorganisms. The particular range of total TPCs and yeast counts defined the spoilage

condition. The spoilage occurs, when TPCs exceed 10^7 (Al-Bachir *et al*., 2010), and yeast and mold counts exceed 10^4 (CUMARIA, 2003).

Irradiation significantly ($p<0.05$) improved the microbiological quality of the chicken sausage by reducing the TPCs and fecal coliform. The numbers increased with storage time and there was a significant ($p<0.05$) difference between the irradiation doses. At the beginning of the storage period non-irradiated chicken sausage had TPCs greater than 7 log CFU/g, while in irradiated (2, 4 and 6 kGy) it did not reach these numbers even after 12, 16 and 20 weeks of storage at 0- 4 ^{O}C, respectively. The decrease in TPCs as a result of irradiation was in agreement with other studies on chilled meat (Sweetier *et al.,* 2005; Chouliara *et al*., 2006), luncheon meat (Al-Bachir, 2005), borak (Al-Bachir, 2007), chicken kabab (Al-Bachir *et al*., 2010), and fermented sausages (Kim *et al*., 2012).

Radiation processing had a significant effect on reduction/elimination of fecal coliform (table 2). In chicken sausage non-irradiated control samples had initial fecal coliform count of 6.46 log cfu/g. and irradiation at 2 kGy reduced the fecal coliform counts by about 4 log cycles. In samples irradiated at 4 and 6 kGy this organisms were not detected throughout the storage period. No coliform bacteria were found in fermented sausage samples irradiated at 1 kGy during storage (Kim *et al*., 2012). Chouliara *et al.* (2006) reported that, irradiation at 4 kGy had a significant decontamination effect, and improved hygienic quality of fermented sausages. It can be seen from table 2 that irradiation doses of 2, 4 and 6 kGy could eliminate the yeast load may be present in the chicken sausage. Irradiated samples with 2 and 4 kGy showed absence of yeast count till 4 and 12 weeks, respectively. However, in samples irradiated with 6 kGy the yeast were not detected until the end of the storage periods (20 weeks).

The dose needed to decrease by 1 log CFU/g (D_{10} value) of *Salmonella* spp and *E coli* numbers in chicken sausage was 345 and 250 Gy, respectively. D_{10} value in the range of 0.40 – 0.46 kGy for *Salmonella* spp in different meat systems has been reported (Sweetier *et al.,* 2005; Chouliara *et al.,* 2006; Al-Bachir, 2005; 2007). D_{10} value was 0.24 kGy for *E coli* in meat has been reported (Olson, 1998). Our results are in agreement with these studies as a dose D_{10} value of *E coli* in chicken sausage was 250 Gy.

Chemical quality of irradiated chicken sausage

Total acidity

There was an interaction between treatment and storage time on the total acidity (Table 3). Immediately after treatment, the percentage of total acidity of irradiated chicken sausage with 2, 4 and 6 kGy doses of gamma irradiation were significantly ($p<0.05$) lower than those of the control. The results in general, are in good agreement with those of Kanatt *et al.* (1997) who indicated that free fatty acid content (FFA) in meat decreased after irradiation. Lescano *et al.* (1991), found that free fatty acids were reduced in chicken breast irradiated with 2.5 kGy dose. Throughout storage periods, the total acidity of both irradiated and non-irradiated chicken sausage increased. The increase was higher in the control than those of irradiated samples.

Lipid oxidation

As the storage time increased overall lipid oxidation increased, and the rate of lipid oxidation was faster in irradiated than non-irradiated chicken sausage (table 3). The rapidly increase of TBA contents through out storage periods may be attributes to using ground chicken meat and salt as additive when preparing the chicken sausage. It has been pointed out that grinding of meat also speeds up the oxidation of myoglobin (Toores *et al.,* 1988). On other hand, Addition of salt and cooking significantly ($p<0.05$) increased the TBARS contents after preparation and during storage of raw and cooked meat products (Badr, 2007). Present oxidation results of chicken sausage are in general agreement with those of Javanmard, *et al.* (2006) who showed that peroxide value at low irradiation dose (up to 5 kGy) there was no significant change in chicken meat. On other hand, this findings are directly contradictory to those reported in previous studies which found significant changes in the TBA counts of fermented sausage (Kim *et al.,* 2012; Cutrubinis *et al.,* 2007) and pork salami (Kanatt *et al.,* 2005)

after irradiation. Since there are some differences in experimental design among the previous studies, such as the source of irradiation, the properties of the raw materials, and the preparation methods of end products, etc. it is not easy to compare the results of all previous studies with those of this study. However, these contradictory findings may reveal the complexity in understanding of chemical characteristics of irradiated poultry meat (Yoon, 2003).

***Table* 3.** *Effect of gamma irradiation on total acidity (Lactic acid %), thiobarbituric acid-reactive substances TBARS (mg MDA/kg meat) and volatile basic nitrogen (VBN) (ppm) of chicken Sausage stored at 1-4 ^0C*

Treatment	0kGy	2kGy	4kGy	6kGy	LSD 5%
Storage period (Weeks)					
		Total acidity (Lactic acid %)			
0	1.47±0.07	1.20±0.13	1.16±0.10	1.20±0.06	0.17
4	1.63±0.14	1.28±0.07	1.33±0.08	1.14±0.06	0.17
8	R	2.06±0.08	1.47±0.11	1.30±0.09	0.19
12	R	1.72±0.69	1.51±0.20	1.45±0.23	0.87
16	R	1.43±0.21	1.29±0.05	1.38±0.19	0.33
20	R	R	2.18±0.79	1.85±0.32	1.36
		TBARS (mg MDA/kg meat)			
0	0.67±0.03	0.75±0.20	0.57±0.01	0.61±0.04	0.20
4	0.94±0.02	1.48±0.17	2.23±0.50	2.07±0.19	0.53
8	R	2.55±0.19	4.60±0.36	4.95±0.26	0.45
12	R	2.53±0.10	4.52±0.17	4.86±0.24	0.30
16	R	2.86±0.06	4.87±0.35	5.70±0.47	0.55
20	R	R	5.04±0.14	6.31±0.16	0.20
		Volatile basic nitrogen (VBN) (ppm)			
0	990.1±14.2	843.6±36.7	830.6±24.6	912.0±77.8	85.2
4	1350.8±19.1	1183.1±62.0	1212.9±74.0	1007.4±41.8	100.6
8	R	853.7±36.3	849.6±32.1	830.7±47.5	78.3
12	R	2720.6±149.9	203±515.4	1837.4±267.3	691.8
16	R	4359.6±519.4	2878.2±962.2	2064.2±111.7	1267.8
20	R	R	4932.9±228. 5	2703.8±335.3	650.3

R: Rejected
LSD, Least significant difference

***Table* 4.** *Effect of gamma irradiation on the taste, texture, color and flavor of chicken sausage*

Treatment	0kGy	2kGy	4kGy	6kGy	LSD 5%
Taste	3.92±0.83	3.5±0.72	3.42±0.78	3.46±0.78	0.45
flavor	4.08±0.78	3.58±0.65	3.79±0.88	3.83±0.82	0.45
color	4.04±0.69	3.71±0.69	3.54±0.83	3.17±0.7	0.42
Texture	3.96±0.75	3.79±0.78	3.58±0.78	3.92±0.83	0.45

[1] Data represent a 5 point scale ranging from 1 (very bad) to 5 (very good).

Total volatile basic nitrogen (TVBN)

The effect of various levels of gamma irradiation on total volatile basic nitrogen (VBN) contents of chicken sausage is shown in table 3. There was no significant ($p>0.05$) deference between irradiated and control groups. In the irradiated groups, no significant ($p>0.05$) deference was found as irradiation dose level increased. While, storage

significantly increased the TVBN for irradiated and non-irradiated samples. Ahn and Nam (2004) shows similar results with ground beef. In contrast Nam *et al.* (2007) reported that; almost 3 times higher amounts of volatiles were found in irradiated meats compared with the non-irradiated samples. VBN of fermented sausages was increased with increasing the irradiation doses of gamma irradiation (Kim *et al*., 2012). When irradiated chicken sausage were stored for 4 weeks, the VBN amount different from those at day 1. Irradiation of "long-time-aged" chicken sausage produced greater amount of VBN than 'pre-aged' and aged chicken sausage. This could be related to more severe structural disintegration in 'long-term-aged' than 'pre-aged' and 'aged' chicken meat, and the structural damage should have made the meat susceptible to the attacks of free radicals produced by irradiation (Ahn and Nam, 2004).

Sensory quality of irradiated chicken sausage

Table 4 illustrates the results of the initial sensory evaluations carried out for the chicken sausage products. For safety reasons the samples were not submitted to the panelists throughout the storage periods, when their microbiological examination of some samples revealed a count of 10^6 CFU/g or more. In case of chicken sausage it was found that immediately after irradiation the overall sensory scores of irradiated and non-irradiated samples were not significantly ($p<0.05$) different. Taste, odor, color and texture of irradiated samples were not different from its non-irradiated control and all the samples were acceptable. Our observation is in agreement with Sweetie *et al.* (2005) who found that irradiation did not affect the sensory attributes of some ethnic Indian meat products.

Conclusion

In conclusion, this study demonstrates that the used doses (2, 4, and 6 kGy) of gamma irradiation causing significant reduction of microbial load leading to shelf-life extension of chicken sausage, without adversity effect of nutrition value, chemical and sensorial characteristics of its products.

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