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Synergistic effect of gamma rays and citric acid to improve fresh sausage quality

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Abstract

Fresh sausage samples divided into 2 groups, one for the artificial inoculation by *Bacillus cereus* and *Staphylococcus aureus*, and the other group for quality evaluation, were treated by dipping in 5 and 10% citric acid or gamma irradiated at 1.5 and 3.0 kGy with dose rate 4.124 kGy/ h or their combination. Gamma rays (1.5 or 3.0 kGy) significantly reduced the average log count of *B. cereus* and *S. aureus* in treated samples. Dipping in 5% or 10% citric acid had no inhibitory effect on the *B. cereus* counts but had slight inhibitory effect on the *S. aureus* counts and this effect was proportional with the concentration. The firmness and cooking loss (%) of the study samples were nearly unaffected by citric acid or gamma rays treatments. TBARS were significantly ($p < 0.05$) reduced in all samples compared with the control. The application of both citric acid and gamma irradiation positively impacted color components. It could be conclude that dipping in citric acid (5 or 10%) enhanced the lethality of ionizing radiation (at 1.5 or 3.0 kGy) without negatively impacting sausage color, lipid oxidation and firmness and fatty acids profile.

Introduction

Control of microorganisms in meat products is the major concern in the preparation of high quality foods. During slaughtering process, meat is exposed to many sources of bacterial contamination (Jo *et al.*, 2004). The hygienic status of animals prior, during and after slaughter can be critical to the finished product quality (Satin, 2002). During the deboning process, meat undergoes extensive handling and may susceptible to bacterial contamination resulting in pigment decomposition, discoloration and development of off odors (Nel *et al.*, 2004).

Staphylococcus aureus is a facultative anaerobe, non motile, spherical, gram positive bacterium. Nausea, vomiting, retching, abdominal cramping and prostration are the most common symptoms of staphylococcal food poisoning (Seo and Bohach, 2007). It can be transferred to meat from various sources as animal skin, hide, equipment and infected personnel (Jay *et al.*, 2005).

Bacillus cereus is defined as gram positive, aerobic or facultative anaerobic, motile, peritrichous flagella and endospore-forming rod shaped microorganisms. It has been long known as ubiquitous organism found in air, soil and water (Claus and Berkeley, 1986). *Bacillus cereus* is the etiologic agent of two distinct types of food poisoning characterized either by diarrhoea and abdominal pain or by nausea and vomiting after ingestion of contaminated foods (Thayer and Boyd, 1994).

Because of consumer demand for fresh refrigerated meat with extended shelf-life, considerable research have been directed towards using various preservation technologies to preserve or prolong meat shelf-life, while ensuring safety. One of the newly emerging technologies to ensure the microbiological safety of meat products is radiation processing.

Irradiation is known to be the best method for the control of potentially pathogenic microorganisms in meat without affecting its physical state. Food irradiation is generally defined as the process in which foods are exposed to certain forms of ionizing radiation (γ-, x- rays or electron beams). It appears to be a simple feasible technique to reduce the load of microbes, avoid post-packaging recontamination and so extend the shelf-life of fresh foods. The radiation doses required to inactivate 90% of the colony forming units (CFU) of the common food-borne pathogens associated with meat and meat products are in the range of 1-4 kGy (Kanatt *et al.*, 2005). Food safety officials and scientists view irradiation as an effective point in Hazard Analysis and Critical Control Points (HACCP) established for meat and poultry processing because of its effectiveness in minimizing the possibility of cross-contamination prior to consumer use (Satin, 2002).

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Organic acids have a long history of being utilized as decontamination of meat from several bacteria including Salmonella (Mani-Lopez *et al*., 2012). They are generally recognized as safe (GRAS) antimicrobial agents. Various researchers have proven the antibacterial effect of organic acids on different types of pathogenic bacteria which is directly proportional to the concentration of organic acid used (Samelis *et al*., 2001; Raftari *et al*., 2009). The concentration of citric acid (CA), treatment time, temperature and the type of organism plays an important role in reducing the number of bacteria (Virto *et al*., 2005).

Citric acid solution may be applied to the surfaces of meat in concentrations up to 10% immediately prior to packaging as antibacterial agent (Code of Federal Regulations, 1998). Although the antibacterial mechanism(s) for organic acids (including citric acid) are not fully understood, they are capable of exhibiting both bacteriocidal and bacteriostatic depending on the physiological status of the organism and the physicochemical characteristics of the external environment (Ricke, 2003). Some studies suggest that organic acids may enhance the bacteriocidal effects of ionizing radiation (Bhide *et al*., 2001; Giroux *et al*., 2001). Relatively little data exist on the combined effects of ionizing radiation and organic acids on meat quality.

This work aimed to study the influence of both CA and/ or ionizing radiation, on the viability of *S. aureus* and *B. cereus* artificially inoculated into sausage, and whether it affects sausage quality parameters (shear force, lipid oxidation, color and fatty acids composition).

Materials and Methods

Sampling

Fresh beef Egyptian sausage samples were purchased from a local butcher in Giza governorate then transferred to the National Center for Radiation Research and Technology (NCRRT), Egypt, under refrigerated conditions in an ice-filled thermal container. In the lab, samples were divided into 2 groups, the first group for artificial inoculation test (each sample weighed 25 g), while the other group evaluated for quality parameters (each weighed 60-80 g).

Bacterial strains and inoculation

Bacterial strains used for artificial inoculation of the samples were standard *B. cereus* (strain ATCC 11778) and *S. aureus* (strain ATCC 6538P) that maintained on nutrient agar (Oxoid). The cultures were propagated for 16-18 h in 50 ml nutrient broth (Oxoid), which was agitated on rotary shaker at 150 rpm and incubated at 35°C. The cultures were serially diluted in sterile saline (0.85% NaCl) for standardization by pour plate assay in duplicate using nutrient agar plates incubated at 35°C for 18 h. Sausage samples used for artificial inoculation test were initially sterilized using 25 kGy by accelerated electrons in the NCRRT (energy: 1.5 Mev and current: 0.9 mA). One ml of the inoculum (10^7) of each individual strain was inoculated in a sample (3 sausage replicates for each treatment for each strain).

Citric acid dip and packaging

Sausage samples were dipped in sterile citric acid solution (CA) (0%, 5% and 10% w/v) for 60 seconds and then left 15 minutes for drying. Following dipping, the samples were organized, into four sausages per pack for evaluation of quality parameters (one sausage for moisture, the other for cooking loss and texture, the third for color and the forth for fatty acids profile) were randomly sealed in each polyethylene bag. Sausage samples used for artificial inoculation were individually packed. Packages were then stored at 4°C until subjected to gamma irradiation (approximately 30 min.).

Irradiation process

Packaged sausage samples were irradiated with 0.0, 1.5 and 3.0 kGy gamma irradiation at dose rate 4.124 kGy/ h using the "Indian Gamma Chamber 4000 A" with a 60°C source. The irradiation process was conducted at the NCRRT, Nasr city, Cairo, Egypt. After irradiation, all samples were transferred to a refrigerator and kept at 4ºC until examination. Three packages from each treatment were analyzed immediately after irradiation.

Microbiological analysis

Twenty five grams of each sample were homogenized in 225 ml sterile saline (0.85% NaCl) using a Stomacher model 400 (Seward laboratory, London) for 1-2 minutes, then decimal dilutions were prepared. Survivors of *B. cereus* and *S. aureus* were enumerated on plate count agar (PCA) medium (APHA, 2001) using pour plate technique after incubation at 35°C for 24 h.

Moisture content

Moisture content of sausage samples were determined according to the Association Official Analytical Chemists (AOAC, 1990), by drying about 10 g of the sample at 105°C until a constant weight

was recorded.

Texture analysis

Shear force and cooking loss of all sausage samples were measured. Shear force was measured after cooking, as an index of firmness, using Instron 1195 by shearing the sample with a (V-shape) knife blade. The scale load was 100 kg f, cross head speed was 10 mm/min. The shear force values of three samples per treatment were recorded and the mean and standard error were calculated. The results were expressed as kilogram force (kg f) to shear.

Cooking loss

The samples were cooked to an internal temperature of 74°C in a conventional electric oven set at 177°C for 20 min and cooled to room temperature (22°C) for 1 h before measuring the weight of the cooked beef sample. The difference in weight of the sample before and after cooking was calculated as cooking loss (%) and the sample mean (n = 3) with standard error were calculated.

Instrumental color measurements

Instrumental color determinations were made on the sausage samples using a micro color unit attached to a data station (Brano Lange – Germany) using the standard CIE LAB color system as follows: a-value (redness/green), b-value (yellowness/blue) and L-value (lightness/darkness). Color measurements were determined in triplicate on each treatment group. All samples were measured in polyethylene bags. Six readings were taken at various points on each sample (CIE, 1978).

TBARS

A common method used for quantitating malondialdehyde (MDA), a major lipid peroxidation product, was performed in triplicates by the extraction methods according to (Vyncke, 1970). The results were expressed as mg MDA/kg sample.

Fatty acids profile

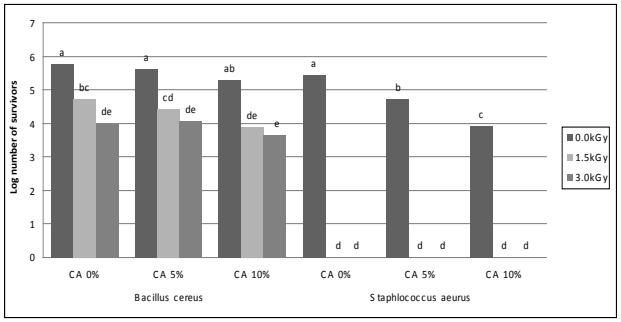
For preparing the fatty acids, total lipids were saponified by boiling under reflux with an excess of dilute aqueous ethanolic alkali. Ether containing the water soluble hydrolysis products (mainly soap solution and glycerol) was acidified by sulphuric acid to liberate the free fatty acids. The free fatty acids were extracted with diethyl ether, recovered, dried over anhydrous sodium sulphate and transferred to their methyl ester for GC-MS analysis (Varso, 1972). Fatty acids profile was determined quantitatively using Gas Chromatograph-Mass selective detector instrument "GC-MS" type HP type 6890 series.

Statistical analysis

Sausage samples from 9 different groups were analyzed. The values given in each treatment category are the mean values of three individual samples. Mean ± standard errors (SE) were calculated. Analysis of variance (F-test) was done for sausage samples. Least significant difference (LSD) ($P < 0.05$) was performed on the tested parameters (SAS, 2002).

Results and Discussion

The average log number of artificially inoculated *Bacillus cereus* and *Staphylococcus aureus* in sausage samples as influenced by gamma rays (1.5 or 3.0 kGy) and citric acid (5 or 10%) are represented in figure 1. There was significant ($p < 0.05$) reduction in the average log number of *B. cereus* and *S. aureus* by gamma irradiation. The immediate reduction after gamma irradiation may be mainly due to the direct effect of gamma rays on the microbial cell by causing lesions in the genetic material of the cell, effectively preventing it from carrying out the biological processes necessary for its continued existence (Murano, 1995).



Bars with different letters are significantly different ($p < 0.05$).
LSD*: 0.64 and LSD**: 0.24.

Figure 1. Log number of *B. cereus** and *S. aeurus*** in treated sausage by citric acid (CA) and gamma rays

Presence of *S. aureus* gives rise to a significant risk of contamination by food handlers (Hatakka *et al*., 2000). It is worthy to mention that the least irradiation dose (1.5 kGy) used in the present study with or without dipping in CA was enough for complete elimination of *S. aureus* indicating the sensitivity of this organism to irradiation as documented by many investigators (Hammad *et al.*, 2000; Zahran *et al*., 2009).

In this study, the population of *B. cereus* and *S. aureus* artificially inoculated in sausage samples decreased after being exposed to all treatments. The reduction rate was proportional to the concentration of CA and irradiation dose. Log reduction analysis showed that the increase in the concentration of CA resulted in increasing the antibacterial effect, also the effect of CA was more pronounced with *S.*

aureus. These findings are similar to those found by Raftari *et al.* (2009) who found that the population reduction of *E. coli* O157:H7 and S. aureus rose by increasing concentration of organic acids used. Carpenter and Broadbent (2009) mentioned that although the mechanisms by which organic acids inhibit growth of bacteria in foods are not full understood, it is clear that intracellular accumulation of anions is a primary contributor to inhibition of bacterial growth. In addition to the environmental factors that can influence the efficacy of organic acids as antimicrobial chemicals, inherent resistance of the target microorganism to these compounds is also a factor (Davidson, 2001). Microbial sensitivity to antimicrobial agents determines relative tolerance and is dependent on the type of organism (fungi, virus or bacteria), strain-to-strain variation and form (endospore versus vegetative cell) with vegetative cells being more susceptible and bacterial spores being the most resistant (Heinzel, 1998). Improving effectiveness of organic acids will require more understanding of general and specific stress response capabilities of food-borne pathogens. This would include development and application of molecular tools for studying pathogen behavior in microbial ecosystems in a variety of pre-harvest and post-harvest food production environments (Theron and Lues, 2007).

The moisture content, shear force, cooking loss and TBARS in sausage samples as influenced by gamma rays (1.5 or 3.0 kGy) and citric acid (CA) (5 or 10%) were represented in table (1). Moisture content of sausage samples were unaffected ($p > 0.05$) by all treatments compared with the control. This finding agreed with many investigators who reported that moisture contents were neither affected ($p > 0.05$) by gamma irradiation (Zahran, 2008a) or CA (Ke *et al*., 2009).

Table 1. Moisture, shear force, cooking loss and TBARS in treated sausage by citric acid (CA) and gamma rays

CA %	Radiation dose (kGy)	Moisture (%)	Shear force (kgf)	Cooking loss (%)	TBARS (mg / kg)
CA 0%	0.0	70.65 ± 2.66^{a}	6.25 ± 0.04^{a}	36.85 ± 0.81^{ab}	0.83 ± 0.01^{a}
	1.5	72.14 ± 1.91^{a}	5.56 ± 0.12^{a}	37.38 ± 0.2^{ab}	0.67 ± 0.04^{b}
	3.0	70.60 ± 0.24^{a}	5.28 ± 0.69^{a}	37.44 ± 1.09^{ab}	0.58 ± 0.01^{cd}
CA 5%	0.0	72.39 ± 0.01^{a}	5.77 ± 1.41^{a}	38.70 ± 1.00^{ab}	0.40 ± 0.01^{e}
	1.5	70.95 ± 1.48^{a}	5.75 ± 0.91^{a}	38.90 ± 0.86^{a}	0.64 ± 0.003^{bc}
	3.0	70.31 ± 0.43^{a}	5.71 ± 0.84^{a}	36.76 ± 0.42^{b}	0.55 ± 0.03^{d}
CA 10%	0.0	71.11 ± 0.64^{a}	5.97 ± 0.74^{a}	38.07 ± 0.45^{ab}	0.46 ± 0.03^{e}
	1.5	69.93 ± 2.21^{a}	5.43 ± 0.04^{a}	38.80 ± 0.48^{ab}	0.54 ± 0.03^{d}
	3.0	71.79 ± 0.53^{a}	5.68 ± 1.08^{a}	37.69 ± 0.43^{ab}	0.57 ± 0.02^{cd}
LSD		4.282	2.372	2.082	0.072

Means with different letters within column are significantly different ($p < 0.05$)

Table 2. Color components (L^*, a^* and b^*) of sausage treated by citric acid (CA) and gamma rays

CA %	Radiation dose (kGy)	L*	A*	B*
CA 0%	0.0	38.26 ± 0.77^{b}	3.50 ± 0.41^{c}	1.41 ± 0.18^{c}
	1.5	40.28 ± 1.07^{b}	3.96 ± 0.34^{bc}	1.50 ± 0.37^{c}
	3.0	39.68 ± 2.07^{b}	3.72 ± 0.31^{bc}	1.89 ± 0.45^{c}
CA 5%	0.0	45.52 ± 0.20^{a}	5.54 ± 0.59^{a}	2.79 ± 0.16^{b}
	1.5	45.52 ± 1.53^{a}	4.71 ± 0.54^{ab}	2.84 ± 0.33^{b}
	3.0	46.18 ± 0.85^{a}	4.92 ± 0.19^{ab}	3.52 ± 0.33^{ab}
CA 10%	0.0	47.63 ± 0.31^{a}	5.47 ± 0.34^{a}	2.82 ± 0.18^{b}
	1.5	47.97 ± 0.29^{a}	5.68 ± 0.54^{a}	3.27 ± 0.21^{ab}
	3.0	48.17 ± 0.35^{a}	5.44 ± 0.34^{a}	3.91 ± 0.10^{a}
LSD		3.04	1.21	0.83

Means with different letters within column are significantly different ($p < 0.05$).

CA and/or irradiation of sausage had no significant ($p < 0.05$) effect on shear force. Cooking loss was unaffected ($p > 0.05$) by both gamma rays and CA compared with the control. This was corresponding to that reported by Sommers *et al.* (2003) that frankfurter firmness as measured by maximum shear force was not affected ($p > 0.05$) by ionizing radiation or citric acid. On the contrary, Ke *et al.* (2009) found that citric acid both improved texture and cook yield of beef muscle compared with the control. On the other hand, Yoon (2003) reported that irradiation significantly ($p < 0.05$) affected the texture quality of cooked chicken breast meat which was directly contradicting to those reported by Abu-tarboush *et al.* (1997). However, these contradictory findings may reveal the complexity in understanding of textural characteristics of irradiated meat. O'Bryan *et al.* (2008) mentioned that even though instruments detect a tougher texture in irradiated foods, consumers do not find a significant difference in texture between irradiated and non irradiated foods.

Concerning lipid oxidation, the concentration of MDA decreased significantly ($p < 0.05$) in all treated sausage samples compared with the control. The greatest decrease was found in samples dipped in CA. Similar results were reported by Ke *et al.* (2009) who reported that citric acid was responsible for inhibiting lipid oxidation in beef and the reason was that citric acid is a strong metal chelator (Decker, 2002).

The decrease in MDA concentration disagree with that observed by Sommers *et al.* (2003) who reported that TBARS was not affected by citric acid or ionizing radiation. Although Al-Bachir and Zeinou (2009) reported no significant difference in TBA value of camel meat due to irradiation, in the recent literature, there are clear contradictions concerning this indicator. Some of them indicate that TBA increased in irradiated lamb meat (Sweetie *et al.*, 2006) and in rabbit meat (Badr, 2004), whereas

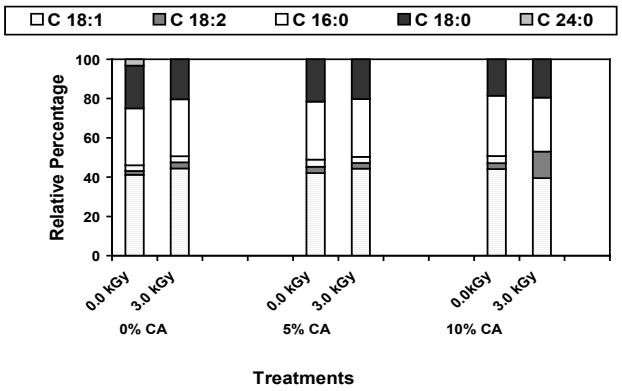


Figure 2. Relative percentage of fatty acids in treated sausage by citric acid (CA) and gamma rays

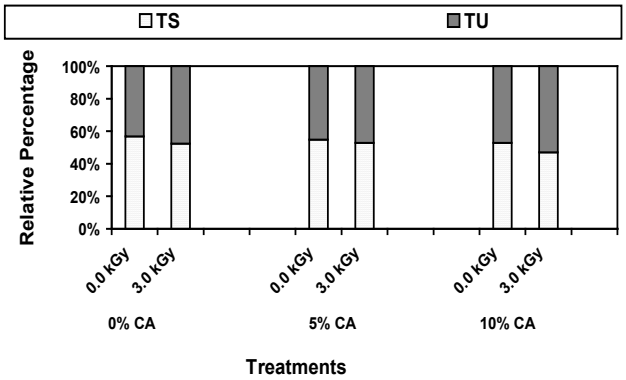


Figure 3. Relative percentage of total saturated fatty acids (TS) and total unsaturated fatty acids (TU) in treated sausage by citric acid (CA) and gamma rays

Sorman *et al*. (1987) found that with increasing radiation doses (2-6 kGy) TBA in beef decreased.

The results of instrumental color of sausage samples as influenced by gamma rays (1.5 or 3.0 kGy) and citric acid (CA) (5 or 10%) are presented in Table 2. It was obvious that L^* (lightness), a^* (redness) and b^* (yellowness) values increased significantly ($p < 0.05$) in all sausage samples dipped in CA 5% or 10% with or without irradiation compared with those undipped in CA (distilled water). These values did not differ significantly ($p > 0.05$) in samples dipped in 0% CA and exposed to 1.5 and 3.0 kGy compared with those exposed to 0.0 kGy (unirradiated). This finding was contradicting with that found by Sommers *et al.* (2003) who reported that ionizing radiation induced a small, but visually imperceptible, loss of redness (a^*), yellowness (b^*) and lightness (L^*). At the same time the application of CA did not affect a- value or b-value, but significantly ($p < 0.05$) reduced L- value. However, the application of both CA and gamma rays positively impacted both b-value and L-value. Also Giroux *et al.* (2001) reported significant ($p < 0.05$) reduction in L^*, a^* and b^* by irradiation.

In the present study, irradiation (1.5 and 3.0 kGy) did not influence ($p > 0.05$) values of L^*. This finding was similar to that reported by Nam and Ahn (2003). Sammel and claus (2006) also reported that citric acid increased b^* (yellowness) and L^* (lightness) values but had no effect on a^* (redness) values. Nam and Ahn (2002) mentioned that lighter color is a desirable trait and attributed the increase in L values to loosened muscle structure and therefore increased light scattering imparted by the low pH.

Color changes in irradiated raw meat differ significantly by animal species (O'Bryan *et al*., 2008), irradiation dose, packaging and among muscles within animal species (Ahn *et al*., 1998). Those authors reported that a^* values of patties made from *L. dorsi* increased after irradiation while those made from the psoas and *R. femoris* decreased. The color change apparently involves an interaction with the heme pigments (O'Bryan *et al*., 2008).

The relative percentage of fatty acids of sausage samples as influenced by the higher dose used of gamma rays (3.0 kGy) and CA are illustrated in figure 2. It was clear that the major fatty acids found were myristic (14:0), palmitic (16:0), stearic (18:0), oleic (18:1) and linoleic (18:2). Palmitic acid and stearic acid was the predominant saturated fatty acids, while oleic acid was the predominant unsaturated fatty acid. This finding was in agreement with other investigators (Valsta *et al.*, 2005, Zahran, 2008b). The ratio of total unsaturated fatty acids (TU) to total saturated fatty acids (TS) was calculated to precisely indicate the changes in fatty acids and shown in figure 3. Results indicated that TS in control was higher than that of all other treated samples indicating that the treatment of samples with irradiation or irradiation in combination with CA decreased TS. Similarly, total unsaturated fatty acids (TU) increased in all treated samples compared with the control, with the highest increase in TU in samples dipped in 10% CA followed by irradiation at 3.0 kGy. Approximately 60% of the saturated fatty acids in US and European diet are obtained from meat (Dupont *et al.*, 1991). Also, unsaturated fatty acids are thought to have beneficial effects on health (Belury, 2002).

Auto oxidation of unsaturated fatty acids and lipids is a well known phenomenon. The severity of oxidation depends on the degree and amount of unsaturated fatty acids present (Melton, 1983). It was also clear from figure 3 that different treatments caused an increase in the relative percentage of linoleic acid (C 18:2) with the greatest increase in sausage samples dipped in CA 10% and exposed to 3.0 kGy. Abd El-Fattah *et al.* (1979) found that 5.5 kGy of irradiation significantly reduced the levels of linoleic acid in meat by 18.4%.

Conclusion

Overall, CA and gamma radiation had little effect on the tested quality factors at the irradiation doses used. It may be possible to use combination treatments for sausages constitute citric acid (5 or 10%) and ionizing radiation (1.5 or 3.0 kGy) to minimize negative impacts on quality factors and improve microbiological safety that would be observed with individual treatment alone.

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