Development of Shelf-stable Intermediate-moisture Meat Products Using Active Edible Chitosan Coating and Irradiation

M. Shobita Rao, Ramesh Chander, and Arun Sharma

ABSTRACT: Shelf-stable intermediate-moisture (IM) meat products were developed using a combination of hurdles such as reduced \( a_0 \), active edible coating of chitosan, and irradiation. Chitosan prepared from chitin had a viscosity of 16 cP, molecular weight of 17.54 kDa, and a degree of deacetylation (DD) of 74%. The nitrogen content of the chitosan was estimated to be 7.56%. The antioxidant activity of chitosan increased upon irradiation without significantly affecting its antimicrobial property. The effect of irradiated chitosan coating in terms of its antimicrobial and antioxidant properties in IM meat products immediately after irradiation and during storage was assessed. The \( a_0 \) of meat products such as mutton sheek kababs and streaky bacon was first reduced to 0.85 ± 0.02. The products were then coated with chitosan and irradiated (4 kGy). No viable bacteria or fungi were detected in chitosan-coated, irradiated products. In contrast, IM meat products that were not subjected to gamma radiation showed visible fungal growth within 2 wk. The chitosan-coated products showed lower thiobarbituric acid-reactive substances (TBARS) than the noncoated samples for up to 4 wk of storage at ambient temperature. The studies thus clearly indicated the potential use of chitosan coating for the preservation of safe and stable meat products.

Keywords: intermediate moisture, irradiation, chitosan, antimicrobial, antioxidant

Introduction

With rapid urbanization and change in lifestyle, there has been a surge in demand for processed convenience food products. A variety of value-added, ready-to-cook/ready-to-serve meat products such as lollipops, fingers, patties, nuggets, and sausages have stormed urban Indian markets. These products have limited shelf life at ambient temperature and have to be stored and marketed in the frozen state (–18 °C). Freezing is energy-consuming and is therefore expensive. Furthermore, freezing does not eliminate pathogens (Geraldine 1992). Decreasing energy demand and improving safety of the preserved foods are therefore desirable. Shelf-stable foods can be prepared by using hurdle technology (Leistner 1994). Shelf-stable intermediate-moisture (IM) meat products have been developed in this laboratory using a combination of treatments. Water activity (\( a_w \)) in these products was reduced to 0.85. Although bacterial growth is not supported at this \( a_w \), it is conducive for fungal growth. Gamma irradiation at doses below 10 kGy was used to control fungal growth (Kanatt and others 2002). The shelf-stable IM meat products thus prepared, however, became vulnerable to fungal attack once the package was opened. Radiation processing accelerates lipid peroxidation (Klassen 1987; Kanatt and others 2005), and that also needs to be controlled. Synthetic antioxidants such as butylated hydroxytoluene (BHT), butylated hydroxyanisole (BHA), and others are being currently used to prevent oxidative changes in foods. But with increased dislike of consumers for synthetic antioxidants, efforts to find natural antioxidants as replacements are gaining momentum. Edible coatings of the various polysaccharides, proteins, and lipids have been reported to extend the shelf life of foods (Gennadios and others 1997) and could be used in conjunction with irradiation. The deacetylated form of chitin known as chitosan has been reported to possess antimicrobial and antioxidative properties that can be exploited to develop environmentally friendly active coating for radiation-processed shelf-stable foods. In this study, the use of chitosan coating as an edible packaging and a natural antioxidant for the preparation of safe and stable IM meat products has been investigated. Antioxidant and antimicrobial activity of chitosan and its efficacy as an active edible coating in combination with irradiation has been studied for development of safe shelf-stable IM meat products.

Materials and Methods

Chemicals

Chitin was purchased from SD Fine Chemicals (Mumbai, India). 1,1-Diphenyl-2-picrylhydrazyl (DPPH), BHT, nitro blue tetrazolium (NBT), and β-carotene were procured from Sigma Chemical Co. (St. Louis, Mo., U.S.A.). Thiobarbituric acid (TBA) was obtained from BDH Chemicals Ltd. (Poole, England). Phenazine methosulphate (PMS), deoxyribose, and nicotinamide adenine dinucleotide (NADH) disodium salt were from HiMedia (Mumbai, India). All other chemicals used were of analytical grade and procured from Qualigens Fine Chemicals (Mumbai, India) or SD Fine Chemicals. The microbiological media were obtained from Himedia Laboratories (Mumbai, India).

Preparation of chitosan

Chitosan was prepared from chitin by refluxing in alkali as described by Shahidi and Synowiecki (1991). The 1% chitosan solution (w/v) prepared in 1% glacial acetic acid (v/v) was filtered (Whatman nr 4) to remove any undissolved particles. It was then sterilized either by autoclaving at 121 °C for 15 min at 15 psi or decontaminated by irradiation at a 4-kGy dose in a cobalt-60 Gamma Cell-220 (Nordion, Inti. Inc., Ottawa, Ont., Canada; dose rate of 9.0 Gy/min).
Characterization of chitosan

The viscosity of 1% chitosan solution (untreated, autoclaved, and irradiated) was measured with spindle nr 5 at 50 rpm using Brook field DV-II viscometer (Stoughton, Mass., U.S.A.) at 25 °C. Measurements were done in triplicate with values reported in centipoise (cP) units. Molecular weight was calculated using the Mark–Houwink equation (Kanatt and others 2004). The ninhydrin reaction was used to estimate the degree of deacetylation (Curotto and Aros 1993). The lyophilized powder was used for nitrogen content estimation by the microkjeldal method using KjelPlus (Pelican Instruments, Chennai, India).

Measurement of reducing power

The reducing power was quantified by the method described by Oyaizu (1986). Briefly, the reaction mixture containing chitosan (0.5 to 2.5 mg) in 2.5 mL phosphate buffer (200 mM, pH 6.6) was incubated with 2.5 mL of potassium ferricyanide (1% w/v) at 50 °C for 20 min. At the end of incubation, 2.5 mL of trichloroacetic acid solution (10%) was added and centrifuged at 8000 rpm for 10 min. The supernatant was mixed with 5 mL of deionized water and 1 mL of ferric chloride (0.1% w/v) solution. The absorbance was measured at 700 nm.

DPPH radical scavenging activity

The ability to scavenge DPPH radical by chitosan was estimated by the method of Yamaguchi and others (1998). Chitosan (0.5 to 2.0 mg) in 1 mL of 0.1 M Tris. HCl buffer (pH 7.4) was mixed with 1 mL of DPPH (250 μM) with vigorous shaking. The reaction mixture was stored in the dark at room temperature for 20 min and the absorbance measured at 517 nm. The scavenging activity was calculated by the following equation:

\[
\text{Scavenging activity} \% = \left( \frac{A_{\text{Blank}} - A_{\text{Sample}}}{A_{\text{Sample}} - A_{\text{Blank}}} \right) \times 100
\]

Superoxide radical scavenging activity

Measurement of superoxide radical scavenging activity was done based on the method described by Liu and others (1997). Briefly 1 mL of NBT solution (156 μM/L), 1 mL of NADH (468 μM/L), and chitosan (0.5 to 2.0 mg) in 0.1 M potassium phosphate buffer (pH 7.4) were mixed. The reaction was started by adding 100 μL of PMS solution (60 μM/L). The reaction mixture was incubated at 25 °C for 5 min, and the absorbance at 560 nm was measured against blank samples. The scavenging activity was calculated using the following formula:

\[
\text{Scavenging activity} \% = \left( \frac{A_{\text{Blank}} - A_{\text{Sample}}}{A_{\text{Sample}} - A_{\text{Blank}}} \right) \times 100
\]

Hydroxyl radical scavenging assay

The assay was carried out according to the method described by Halliwell and others (1987) with slight modifications. The reaction mixture containing chitosan, hydrogen peroxide (30 mM), H₂O₂, FeCl₃ (1 mM), EDTA (1 mM), and ascorbic acid (1 mM) was incubated at 37 °C. The reaction was terminated by adding 2 mL of TCA solution (2% w/v) and 2 mL of TCA solution (1% w/v) was then heated in a boiling water bath for 10 min. The contents were cooled, and the absorbance of the mixture was measured at 532 nm against reagent blank.

\[
\text{Scavenging activity} \% = \left( \frac{A_{\text{Blank}} - A_{\text{Sample}}}{A_{\text{Sample}} - A_{\text{Blank}}} \right) \times 100
\]

Antioxidant activity

The antioxidant activity of chitosan was determined by the coupled oxidation of β-carotene and linoleic acid, according to the methodology described by Velioglu and others (1998). β-Carotene emulsion was prepared by taking 200 μL of β-carotene solution in chloroform (1 mg/mL) in a round-bottom flask containing 20 mg linoleic acid and 200 μL Tween-80. Chloroform was evaporated under a stream of nitrogen gas and 100 mL of distilled water was added with vigorous shaking until a stable emulsion was formed. The reagent (4.9 mL) was mixed with 0.1 mL of 1% chitosan solution, and absorbance was measured at 470 nm, which corresponded to zero time absorbance. BHT was taken as standard. The samples were then incubated in a water bath at 50 °C for 60 min to induce oxidation. Absorbance was again measured at the end of incubation. Antioxidant activity coefficient (AAC) was calculated as follows:

\[
\text{AAC} = \left[ \frac{A_{\text{Sample}} - A_{\text{Blank}}}{A_{\text{Sample}} - A_{\text{Blank}}} \right] \times 1000
\]

Antimicrobial activity

The efficacy of chitosan as an antimicrobial agent was analyzed by testing against 4 organisms in pH 6.2 according to the method of Knowles and Roller (2001). The organisms used were *Staphylococcus aureus*, *Bacillus cereus*, *Pseudomonas spp.*, and *Escherichia coli*. The cultures were maintained on nutrient agar slants at 4 °C. The organisms were grown in nutrient broth for 18 h, and the cells were collected by centrifugation and washed with normal saline. The pellets were suspended in 50 mL of normal saline (pH 6.2) containing various concentrations (0.25% and 0.5%) of chitosan. Samples were withdrawn at intervals of 15 min up to 1 h, and viable counts were estimated using plate count agar.

Chitosan coating of IM meat products

Fresh mutton kababs were obtained in chill condition from local retailer. The aw of kababs was reduced to 0.85 ± 0.02 by grilling at 250 °C for 2 min. The streaky bacon was purchased from local meat processing industry and dried in an oven at 60 °C for 35 min with air circulation to bring the aw to 0.85 ± 0.02. Both products were divided into 3 lots individually. One lot had no chitosan coating and no irradiation treatment. The 2nd lot had no chitosan coating but was given irradiation treatment at 4 kGy. The 3rd lot was coated with chitosan by dipping in 1% chitosan solution and irradiated at 4 kGy in Gamma Cell 220. All the samples stored at ambient temperature were analyzed at 1, 7, 14, 21, and 28 d. (Day 1 corresponds to day of irradiation.)

Microbiological analysis of products

The sample (10 g) was aseptically transferred in duplicate to stomacher bags containing 90 mL sterile saline. The samples were homogenized for 2 min using a Stomacher 400 laboratory blender (Seward Medical, London, U.K.). Serial dilutions were poured plated. The various media used were plate count agar (PCA), Baird-Parker agar, and potato dextrose agar (PDA) for total viable counts (TVC), *Staphylococcus spp.*, and mold, respectively. The Baird-Parker agar plates were incubated at 37 °C, and the PCA and PDA plates were kept at room temperature. The *Staphylococcus* counts and TVC were enumerated, after 24 and 48 h of incubation, respectively, and molds were counted after 5 d. Results were expressed as the log of the number of colony-forming units (CFU) per gram of sample.

Determination of lipid peroxidation

The lipid oxidation was studied in terms of thiobarbituric acid-reactive substances (TBARS) by the method of Alasnier and others (2000). Briefly, sample (4 g) was homogenized in 16 mL of 5% trichloroacetic acid. BHT (10 μL of 0.1%) was added to prevent the oxidation due to homogenization. The homogenate was filtered through
a Whatman filter (nr 4), and 2 mL of the filtrate was mixed with 2 mL of 0.02 M TBA. All samples were tested in triplicates. The samples were incubated in a boiling water bath for 30 min and after cooling were centrifuged at 8000 rpm for 10 min to obtain a clear supernatant. The absorbance of the pink-colored complex was measured at 532 nm, and values were expressed as milligrams of malonialdehyde per kilogram of sample.

**Statistical analyses**

All the experiments were done in triplicate at different time points, and the values reported are the mean of these independent experiments. Differences between variables were tested for significance by 1-way analysis of variance (ANOVA) with Tukey’s post-test using GraphPad InStat version 3.05 for Windows 95, GraphPad Software (San Diego, Calif., U.S.A., www.graphpad.com). Differences at $P < 0.05$ were considered to be statistically significant.

**Results and Discussion**

**Characterization of chitosan**

Chitosan was characterized in terms of its intrinsic properties such as viscosity, molecular weight, degree of deacetylation, and nitrogen content. The results are shown in Table 1. The viscosity of 1% chitosan solution was 16 cP and the molecular weight was calculated to be 17.54 kDa. In the literature, the reported viscosity of chitosan solutions varies widely (Rhoades and Roller 2000). The viscosity of chitosan varies with the raw material used and the period of the deacetylation process (Kamil and others 2002). It has been reported that gamma irradiation of chitosan at sterilizing doses (up to 25 kGy) caused chain scissions and that the viscosity and the average molecular weight of the polymer decreased with the increasing irradiation dose (Lim and others 1998). The viscosity of chitosan solution in this study was not affected as a low dose of irradiation (4 kGy) was used, and the initial viscosity of the solution was low.

The degree of deacetylation measured by the ninhydrin reaction was 74%. The degree of deacetylation (DD) has been found to influence the physical and chemical properties and the biological activities of the chitosan. The process of deacetylation leads to removal of acetyl groups from the molecular chain of chitin, leaving behind a free amino group. The versatility of chitosan mainly depends on the presence of highly chemically reactive free amino groups (Khan and others 2002). The ninhydrin reaction estimates the degree of deacetylation by direct detection of the $\text{–NH}_2$ group on the glycoside repeat unit of chitosan, but the values are lower than the actual DD of the sample because of the fading of color intensity with time after the boiling process (Tan and others 1998). Many other methods such as circular dichroism (Domard 1987), nuclear magnetic resonance (Hiral 1991), and gel permeation chromatography (Alba 1986) have been reported for estimation of DD but are not suitable for routine purposes because of the cost, expertise, and sophistication involved, whereas the amount of chitosan required for the ninhydrin reaction is much less, and the assay can be performed in normal laboratory settings.

The $N_2$ content in the chitosan preparation was found to be 7.56%. The term chitosan can be used only when the degree of deacetylation is more than 70% and the nitrogen content is >7 % (Muzzarelli 1985). No effect of gamma radiation was observed on the degree of deacetylation and nitrogen content.

**Reducing power of chitosan**

Figure 1 depicts the reducing power of chitosan. The reducing power of chitosan increased significantly ($P > 0.05$) upon irradiation and was twice that of an autoclaved sample at the same concentration. Autoclaving did not affect the reducing power, since there was no significant increase in absorbance compared with untreated chitosan. A direct correlation between antioxidant activity and reducing power has been reported (Tanaka and others 1988; Meir and others 1995). Breaking the free radical chain by donating a hydrogen atom induces antioxidant activity (Gordon 1990). The increase in reducing power upon irradiation indicated enhanced antioxidant activity that can be exploited for radiation-processed food in reducing radiation-induced lipid peroxidation.

**DPPH radical scavenging activity**

The effect of chitosan on DPPH free radical scavenging is depicted in Figure 2. A dose dependent increase in the ability to scavenge the radical was observed. Irradiated chitosan showed approximately 4 times more radical scavenging than the autoclaved chitosan. The activity of chitosan was lower than that of BHT at the same concentrations (results not shown). The scavenging activity of chitosan may be due to the reaction between the free radicals and the residual free amino group to form stable macromolecule radicals or amino groups can form ammonium groups by absorbing hydrogen ions from the solution and then reacting with free radicals through an addition reaction (Xie and others 2001). Park and others (2004) reported that the scavenging activity depended on the degree of deacetylation of chitosan. The chitosan with a higher degree of deacetylation has better scavenging activity, thereby, suggesting the action of nitrogen at the C-2 position in elimination of free radicals. Irradiation treatment of chitosan has been reported to increase DPPH free radical scavenging activity (Kanatt and others 2004).

**Table 1—Characteristics of chitosan**

<table>
<thead>
<tr>
<th>Property</th>
<th>Value $^a$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Viscosity (cP)</td>
<td>16 ± 0.27</td>
</tr>
<tr>
<td>Molecular weight (Da)</td>
<td>$1.754 \times 10^4$</td>
</tr>
<tr>
<td>Degree of deacetylation (%)</td>
<td>74 ± 1.21</td>
</tr>
<tr>
<td>Nitrogen content (%)</td>
<td>7.56 ± 0.18</td>
</tr>
</tbody>
</table>

$^a$Denotes the mean values of 3 independent experiments.

**Figure 1—Reducing power of chitosan. Results shown are mean values. n (number of observations) = 3.**
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Superoxide radical scavenging activity of chitosan

The ability of chitosan to scavenge the superoxide radicals generated in a test system is shown in Figure 3. Untreated and autoclaved chitosan had similar activity at lower concentrations. Irradiation led to a several-fold increase in superoxide radical scavenging activity of chitosan. The scavenging of the superoxide radical was significantly higher at all tested concentrations of irradiated chitosan. Superoxide is a relatively weak oxidant, but it decomposes to form stronger reactive oxidative species, such as singlet oxygen and hydroxyl radicals, which initiate peroxidation of lipids. Superoxide radical is a zwitterionic radical (Xing and others 2005). It can react with free hydroxyl and amino groups of chitosan, thereby leading to the scavenging effect of chitosan. Chitosan has a compact structure due to intramolecular hydrogen bonding, so the scavenging effect is relatively less in untreated and autoclaved chitosan, since the reactive groups are not exposed. The pronounced superoxide scavenging activity of irradiated chitosan could be due to an increase in the availability of reactive hydroxyl and amino groups on irradiation. Low-molecular-weight chitosan has been reported to possess higher scavenging activity than high-molecular-weight chitosan because of having more free hydroxyl and amino groups (Xing and others 2005).

Hydroxyl radical scavenging activity of chitosan

Hydroxyl radicals react with deoxyribose to form products that react with TBA under acid conditions to yield a pink-colored complex. Hydroxyl radical scavengers compete with deoxyribose for hydroxyl radicals, which are generated by a reaction of iron–EDTA complex with H₂O₂ in the presence of ascorbic acid. The result of hydroxyl radical scavenging activity of chitosan is represented in Figure 4. The irradiated chitosan showed up to 50% hydroxyl radical scavenging activity, whereas untreated and autoclaved chitosan showed very low activity. The free radical scavenging activities are closely related to bond dissociation energy of O-H or N-H and the stability of the formed radicals. Chitosan has strong intramolecular and intermolecular hydrogen bonds. The OH and NH₂ groups are difficult to dissociate and react with hydroxyl radical. Thus, chitosan has very low intrinsic scavenging activity (Xie and others 2001). Irradiation is known to cause the breakage of different kinds of bonds.

The hydrogen bonds are weak, with bond energy of only 4.5 kcal/mol, compared with covalent bonds that have high bond energy. It is possible that the hydrogen bonds in chitosan are broken by the irradiation treatment, thereby increasing the availability of OH and NH₂ groups in the solution, which can react with the hydroxyl radicals. The hydroxyl groups in the polysaccharide unit can react with hydroxyl radical by typical H-abstraction reaction. The amino groups can form ammonium groups by absorbing hydrogen ions from the solution and then reacting with hydroxyl radical through an addition reaction. The exact mechanism of scavenging of hydroxyl radicals by chitosan needs to be further studied.

Antioxidant activity of chitosan

Figure 5 shows the antioxidant activity coefficient (AAC) of BHT and chitosan. The AAC values for BHT and irradiated chitosan were 1246 and 501, respectively. Irradiation led to 65% and 41.3% increas-

Figure 3—Superoxide radical scavenging activity of chitosan. Results shown are mean values. n (number of observations) = 3.

Figure 4—Hydroxyl radical scavenging activity of chitosan. Results shown are mean values. n (number of observations) = 3.
es in AAC value of chitosan over untreated and autoclaved chitosan, respectively. Although the AAC of irradiated chitosan was found to be less than that of BHT, the use of natural nontoxic compound in food can be advantageous. It is well known that the maximum lawful levels of synthetic antioxidants are established from different toxicological parameters that may not apply to naturally occurring compounds such as chitosan (Bonilla and others 1999).

Effect of chitosan on the test organisms

The inhibition of test organisms by chitosan is depicted in Figure 6 and 7. The viable counts of *E. coli* decreased from 8.53 to 4.51 Log CFU/mL within 15 min with 0.5% of autoclaved chitosan. In case of *Pseudomonas* spp., 3-log cycle reduction was observed, whereas, only about 1-log cycle kill was observed with *S. aureus*. Similarly, the effect of chitosan was also low against *Bacillus cereus*. Maximum antimicrobial activity of chitosan was observed within 15 min of addition, after which it was more or less constant. The induction of antibacterial activity of chitosan by irradiation has been reported previously (Le and others 2001). The minimum inhibitory concentrations of chitosan vary widely from 0.01% to 1.0% (Sagoo and others 2001). The antimicrobial activity of chitosan depends on various factors such as deacetylation degree, temperature, and molecular weight. The chitosan with a high degree of deacetylation is more effective against microorganisms. The chitosan with low molecular weight enters the microbial cells and disturbs the metabolism of the cell. Similarly, the high-molecular-weight chitosan forms a film around the cell, thereby preventing the entry of nutrients into the cell (Zheng and Zhu 2003). Inhibition of mRNA and protein synthesis has also been suggested for the antimicrobial action of chitosan (Sudharshan and others 1992).

Effect of chitosan coating on the microbial load of IM meat products

The effect of chitosan coating on the microflora present in IM meat products was examined, and the data are compiled in Table 2. The total viable counts (TVC) were 1.21 Log CFU/g in mutton kababs, which increased to 2.89 in uncoated, nonirradiated samples on the 7th day. No *Staphylococcal* counts were detected on the 1st day, however, 1.28 Log CFU/g counts were observed after 1 wk of storage. Visible fungal growth was seen on these samples within 2 wk and thus discarded. However, although no bacterial counts were detected in irradiated uncoated mutton kababs, the samples spoiled due to fungal growth. Chitosan coating followed by irradiation of mutton kababs resulted in undetectable levels of viable counts, and no fungal growth in samples was observed up to 28 d of storage at ambient temperature.

In IM bacon samples, the TVC increased from 3.01 to 4.93 Log CFU/g in uncoated, nonirradiated samples in 28 d. The *Staphylococcal* counts increased from 1.17 to 3.46 Log CFU/g during the storage period. These samples showed no fungal growth. No bacterial counts were observed in uncoated irradiated samples on the 1st d of analysis but TVC reached 2.12 Log CFU/g during the storage period; in the chitosan-coated irradiated samples, no bacterial count was detected. No fungal growth was observed in these samples during the entire storage period.

Under normal circumstances, $a_0$ of 0.85 or less is sufficient to prevent bacterial spoilage (Leistner 1994), but the fungal spore germi-
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Table 2—Microbiological analyses of intermediate-moisture (IM) mutton kababs and IM bacon during storage period at ambient temperaturea,b,c,d

<table>
<thead>
<tr>
<th>Sample</th>
<th>Storage time (d)</th>
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<tr>
<td></td>
<td>1</td>
<td>7</td>
<td>14</td>
<td>21</td>
<td>28</td>
</tr>
<tr>
<td>Total viable counts Log CFU/g</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>M. K-1</td>
<td>1.21</td>
<td>2.07</td>
<td>#</td>
<td>#</td>
<td>#</td>
</tr>
<tr>
<td>M. K-2</td>
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<tr>
<td>M. K-3</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
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<tr>
<td>Bacon-1</td>
<td>3.86</td>
<td>4.33</td>
<td>4.18</td>
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<tr>
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<td>ND</td>
<td>1.32</td>
<td>1.97</td>
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<td>Bacon-3</td>
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Staphylococcal counts Log CFU/g

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<tr>
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<td>*</td>
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</tr>
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<td>ND</td>
<td>ND</td>
<td>*</td>
<td>*</td>
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<tr>
<td>Bacon-3</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
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</table>

aThe results are mean values of 3 independent experiments.
b CFU = colony-forming units; M. K = mutton kabab; ND = not detected; 1 = uncoated, nonirradiated; 2 = uncoated, irradiated; 3 = chitosan coated, irradiated.
c, * = analysis not done because the sample had spoiled.
d ND = no organisms were detected by the method used.

Figure 8—Thiobarbituric acid-reactive substances (TBARS) values of irradiated intermediate-moisture (IM) mutton kababs treated with 1% chitosan during storage period at ambient temperature. The results are mean values of 3 experiments. Mean values with different letters (a, b, c) are significantly different (P ≤ 0.05).

Figure 9—Thiobarbituric acid-reactive substances (TBARS) values of irradiated intermediate-moisture (IM) bacon treated with 1% chitosan during storage period at ambient temperature. The results are mean values of 3 independent experiments. Mean values with same letters (a, b, c) are not significantly different (P ≤ 0.05).
oxidation during irradiation and also during post-irradiation storage at ambient temperature.

**Conclusions**

Our study showed the potential of irradiated chitosan as an antioxidant that substantially retarded lipid peroxidation and could prevent the spoilage due to microbial growth in the irradiated IM meat products. The use of chitosan coating in place of polyethylene packaging for the preparation of safe and stable IM meat products was thus demonstrated.

**References**


