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Improving Minced Meat Quality by Edible Antimicrobial Polymers and Gamma Radiation

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THE HYGIENIC quality of minced meat preserved by dipping in edible antimicrobial polymers was evaluated and compared with exposure to gamma-radiation. Solutions of Carboxymethylcellulose incorporated with zinc oxide (CMC/ ZnO), polycarbonate/ zinc oxide (PC/ ZnO) and chitosan (CS) were prepared and applied to 3 minced meat groups compared to an irradiated group at 3kGy γ-rays. The results indicated that dipping minced meat in polymers improved its microbiological, chemical and sensory hygienic quality. Control samples (dipped in distilled water) were rejected hygienically on the seventh day. Sensory property scores revealed improvement in color, odor and texture of polymer treated and irradiated meat samples and remained acceptable till the end of storage period. Sensitivity test conducted using the films of the prepared polymers revealed that *Staph. aureus* was only sensitive to CMC, but it was not sensitive to other polymers. *List. monocyt.* was more sensitive to CS, which showed no sensitivity to other polymers. *Candida albicans* was sensitive only to CMC. It could be concluded that antimicrobial polymers and irradiation at 3kGy γ-rays improved the quality parameters of minced meat and could be used in food preservation as it increased the chilling-life of minced meat.

Keywords: Minced meat, Antimicrobial polymers, Hygienic quality, Sensory evaluation, Food microorganisms.

Introduction

Recently, there has been an increase in consumer awareness regarding the use of chemical additives in food and food products; this has resulted in an increase in research on natural additives, such as using plant and animal derivatives (Faustino et al., 2019). Polymers with antimicrobial activity have the ability to inhibit the growth of food poisoning microorganisms such as bacteria and fungi; they are permitted to minimize the deterioration of food of animal origin (Jamróz et al., 2019).

Antimicrobial polymers are produced by attaching or inserting an active antimicrobial agent onto a polymer backbone via an alkyl or acetyl linker; they are generally nonvolatile and chemically stable which decreases associated environmental hazards (Pezzi et al., 2019). This makes these materials prime candidates for use in food and meat packaging industry to prevent bacterial contamination (Ahmed et al., 2018).

Zinc oxide is known as antibacterial agent; reducing the size of ZnO to nanoscale dimensions further enhances its antimicrobial properties (Bhattacharyya et al., 2018). The antibacterial effect of ZnO may be due to the direct interaction of ZnO nanoparticles and bacteria membrane wall, in addition to active oxygen (Kuang et al., 2019). Food Zinc oxide is a bio-safety chemical additive for consumer (Zabihi et al., 2019). Silver (Ag) nanoparticles modified by polymer showed high antimicrobial activity against Gram-positive and Gram-negative bacteria. The free-radical generation effect of Ag nanoparticles on microbial

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growth inhibition can be used as effective growth inhibitors in various food poisoning microorganisms (Pramanik et al., 2019).

CMC powder is widely used in the ice cream industry, in bakery products including breads and cake preparation. It is also used as an emulsifier in producing high quality biscuits. It can help to reduce consumption of egg yolk or fat used in making biscuits, also used in chewing gums, margarines and peanut butter as emulsifier, it is used in leather crafting to burnish the edges as well (Baldwin et al., 2011). Chitosan (CS) is a carbohydrate obtained via alkaline deacetylation of chitin which has antimicrobial activity without toxicity to humans as it is biodegradable, nontoxic, antigenic, antimicrobial and biocompatible (Sahariah et al., 2017). PC is a synthetic compound found in plastics used in food packaging (Bignardi et al., 2018).

Microbial contamination of foods during processing and storage are the major causes of foodborne illnesses and decreased shelf life, therefore it is desirable to use food irradiation as the destruction of food poisoning organisms leading to a significant increase in the acceptable shelf life (Gunther et al., 2019).

The current study was planned out to study the effect of dipping chilled minced meat in three polymers (CMC/ ZnO, PC/ ZnO and CS) compared to gamma radiation of meat at 3 kGy on its hygienic quality (bacteriologically, chemically and sensory) during seven days of chilling storage. Also, the study aims at determining the antimicrobial sensitivity of polymer films (that could be used in the packaging of foods) against 3 food poisoning microorganisms; *List. monocyt.*; *Staph. aureus* and *Candida albicans*.

Materials and Methods

*Preparation of polymer films and solution***s**

Three polymer solutions were prepared for dipping meat groups, A: 1.5g CMC (El Nasr Pharmaceuticals Chemicals Company) was dissolved in 50ml distilled water then add 0.01g of ZnO and 0.05-0.07ml of MBA (p-methoxybenzoic acid). B: 0.25g CS of molecular weight ranged from 100,000 to 300,000 (Acros Organics, New Jersey, USA) was dissolved in 50ml distilled water; add 3ml of glacial acetic acid. C: 1.5g PC was dissolved in 50ml CH_2Cl_2, add 0.01g ZnO. The three mixtures and an irradiated sample were stirred on magnetic stirrer for two days, carefully poured and spread on a clean glass plate, left to dry at room temperature (the thickness of the films; used for sensitivity test ranged from 0.3-0.13mm). Discs of the prepared polymers films (6mm × 6mm) were cut and left in sterile poly ethylene bags till being used while polymer solutions were kept in sterile corked bottles.

ZnO nanoparticles were purchased from Sigma-Aldrich. The particle size was less than 100mm.

Irradiation process

Irradiation was carried out using the Indian Co-60 gamma cell (4000A) at the National Center for Radiation Research and Technology (NCRRT), Cairo, Egypt with dose rate of 2.6kGy/h. CMC/ ZnO polymer films were irradiated at 10kGy while other polymer films were irradiated at 5kGy for cross-linking simultaneously in a homogeneous medium. The 4^{th} minced meat group samples were exposed to γ-rays at 3kGy.

Sample preparation

Minced meat samples were divided into 5 groups (250g each). The 1^{st} group was dipped in distilled water (served as control) for a minute. Then 3 meat groups were dipped each in an examined polymer, the solutions covered the meat samples for a minute then poured the residue. The 5^{th} group was gamma irradiated at 3kGy. Each minced meat group was put in a polyethylene bag and stored in the refrigerator at 4±1°C until analysis. Five packages of each treatment were analyzed after 1, 3, 5 and 7 days.

Hygienic quality of meat samples

Microbial analysis

The total bacterial (TBC), total mold and yeast counts (M&Y); 10g of each sample were homogenized in 90ml sterile physiological saline solution using a Stomacher model-400 (Seward laboratory, London) for a minute, then decimal dilutions were prepared. The TBC and total mold and yeast counts were enumerated on plate count agar and oxytetracycline glucose yeast extract agar media, respectively using a pour plate technique then incubated at 35°C for 24hrs and 25°C for 5 days, respectively (FDA, 2001). All results are reported as mean log colony forming unit per gram (CFU/ g).

Thiobarbituric acid detection (TBA)

TBA detection of malonaldehyde (a common lipid peroxidation product) was performed in 5 times using trichloro acetic acid 7.5% freshly prepared 0.02M thiobarbituric acid solution and the absorbance of the developed red color was measured at wave length 538nm malonaldehyde formed during lipid oxidation in fresh meat samples were measured and reported in units of mg MDA/ kg sample (Tarladgis et al., 1960).

Sensory evaluation

The test was conducted by 9 panelists to detect whether meat changed in odor, color or texture during storage period at 4±1°C at 1, 3, 5 and 7 days. Control samples were not treated with polymers or irradiation. The test is 5 points hedonic scale: 1= Very poor, 2= Poor, 3= Common, 4= Good, 5= Very good (Szczesniak, 1988).

Statistical analysis

Whole experiment was conducted 5times and data were analyzed according to Steel & Torrie (1981). Statistical significance of the difference in values of the control and treated samples was calculated by *F*-test at 5% significance level. Data of the present study were statistically analyzed using Duncan's Multiple Range Test (SAS, 1986).

Sensitivity test

Pure strains of *List. monocyt., Staph. aureus* and *Candida albicans* were obtained from the Animal Research Institute, Giza, Egypt. The tested pathogenic microorganisms were selected based on their food borne activity. The strains were grown on their specific media at 37°C for 24hrs; *List. monocyt.* on *Listeria*-specific Palcam Agar (Difco, Becton Dickinson) with Palcam selective supplement were obtained from Oxoid, Basingstoke, UK. *Staph. aureus* on CMO275, Baird-Parker agar base (Oxoid) and *Candida albicans* on CMO549 Rose Bengal Chloramphenicol agar base (Oxoid). Each tested strain was streaked out over the surface of the specific media agar plates; 5 times each. Discs of the prepared polymers films (6mm × 6mm each) were put on the implanted Petri dishes with the microorganisms, incubated at 37°C for 24-48 hours as described by Coma et al. (2002). An inhibition zone was performed. Sensitivity was classified according to mean diameter of the halo as: Not sensitive (<8mm= -), sensitive (9-14mm= +), very sensitive (15-19mm= ++) and ultrasensitive (>20mm= +++). 3 controls without polymers was used.

Results

Hygienic quality of meat samples

Bacteriological evaluation

Figures 1 and 2 show that mean TBC and M & Y counts of the control samples increased about 2 logs at the end of storage period; similarly, the counts increased slightly in all polymers treated groups and irradiated group with storage.

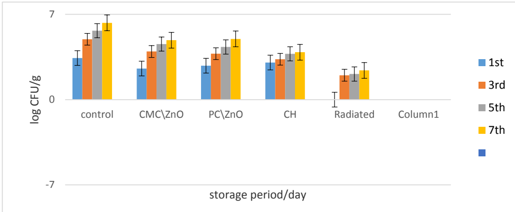

Fig. 1. Effect of dipping minced meat stored at 4±1°C in edible antimicrobial polymers and exposure to γ-rays at 3kGy on its total bacterial count [Control: Minced meat dipped in distilled water, CMC\ZnO: Meat dipped in Carboxymethylcellulose incorporated with zinc oxide, PC\ZnO: Meat dipped in polycarbonate incorporated with zinc oxide, CH: Meat dipped in chitosan, gamma radiated: Radiated minced meat exposed to γ-rays at 3kGy].

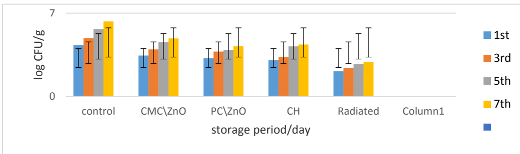

Fig. 2. Effect of dipping minced meat stored at 4±1°C in edible antimicrobial polymers and exposure to γ-rays at 3kGy on its on log mold and yeast count (CFU/g) [Legends as in Fig. 1].

Chemical evaluation

From the results given in Fig. 3, there was an increase in the TBA value in the control samples with storage time at refrigeration. TBA increased from an initial value of 0.18 to 0.66µg MDA/ g meat. Irradiated and polymers treated samples had relatively high TBA value.

Sensory evaluation

Sensory evaluation of the samples revealed that immediately after dipping meat samples in edible antimicrobial polymers and irradiation a day after at 4±1ºC, there were no significant differences between treated and non- treated meat samples (Figs. 4-6).

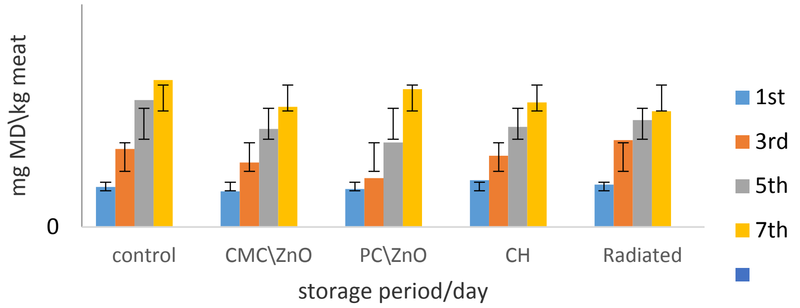

Fig. 3. Effect of dipping minced meat stored at 4± 1°C in edible antimicrobial polymers and exposure to γ-rays at 3kGy on thiobarbituric acid (TBA values) [Legends as in Fig. 1].

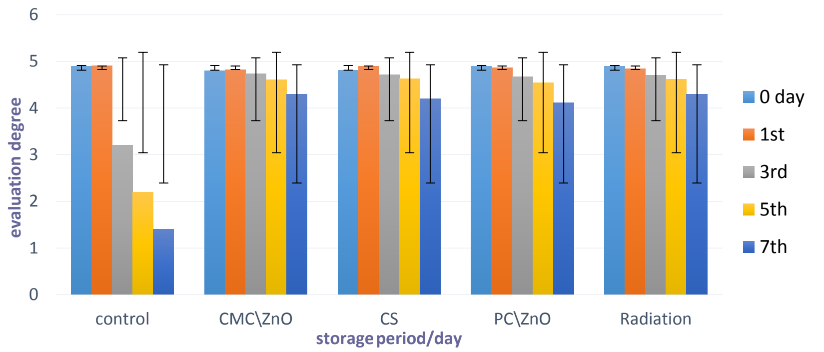

Fig. 4. Evaluation of minced meat color treated with edible antimicrobial polymers and γ-rays during chilling storage (4±1°C) 1= Very poor, 2= Poor, 3= Common, 4= Good, 5= Very good [Legends as in Fig. 1].

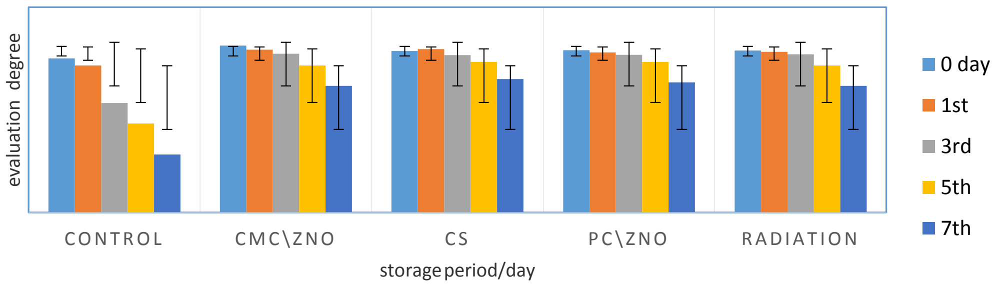

Fig. 5. Evaluation of minced meat odor treated with edible antimicrobial polymers and γ-rays during chilling storage (4±1°C), 1= Very poor, 2= Poor, 3= Common, 4= Good, 5= Very good [Legends as in Fig. 1].

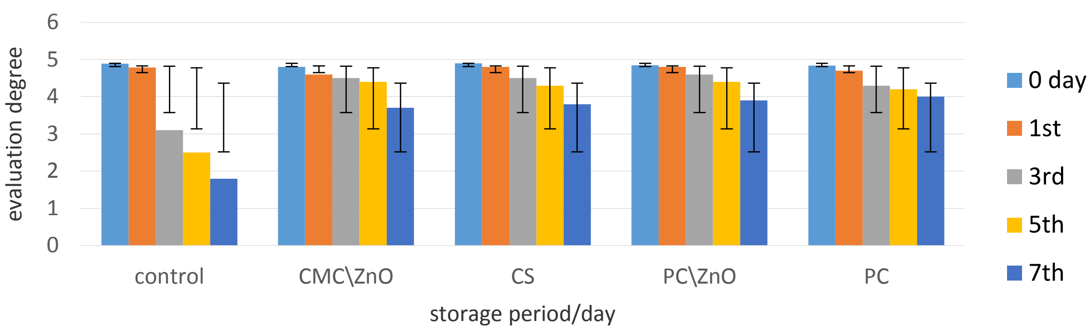

Fig. 6. Evaluation of minced meat texture treated with edible antimicrobial polymers and γ-rays during chilling storage (4± 1°C), 1= Very poor, 2= Poor, 3= Common, 4= Good, 5= Very good [Legends as in Fig. 1].

At the 3rd day; changes in color and texture were noticed in the control samples while its odor was rejected at the 5th day. At the 7th day, the control non treated meat samples were all rejected while the treated meat samples with edible antimicrobial polymers as well as irradiated meat samples were accepted for color, odor and texture. From the 3rd day of refrigerated storage, the majority of the panelists preferred the polymer treated and irradiated samples which maintained acceptable until the 7th day of refrigerated storage.

Sensitivity test

The results in Table 1 revealed that *Staph. aureus* was more sensitive to CMC/ZnO, while *List. monocyt*. was sensitive to CS but showed no sensitivity to the other polymers. *Candida albicans* was sensitive only to CMC/ZnO.

TABLE 1. Sensitivity of *Staph. aureus*, *List. monocytogenes* and *Candida albicans* against the examined polymers

Polymers	*Staph. aureus*	*List. monocyt.*	*Cand. albicans*
CMC/ZnO	+++	_	+
CS	+	+++	_
PC/ZnO	+	_	_

Legends as in Fig. 1.

As seen in Fig. 7 there is a clear zone of inhibition of growth of *List. monocyt.* under and around the disc film of CS; it was clear after 24hrs at 37°C. Figure 8 shows a clear zone of inhibition of growth of *Staph. aureus* under and slightly around CMC and CS films ,while it was not affected by the other polymers even after 48hrs of incubation. Figure 9 shows a clear zone of inhibition of growth *Candida albicans* against CMC/ ZnO (A).

Fig. 7. Microbial sensitivity of *List. monocyt.* under and around the disc film of CS [Legends as in Fig. 1].

Fig. 8. Microbial sensitivity of *Staph. aureus* against CMC/ZnO and CS [Legend as in Fig. 1].

Fig. 9. Microbial sensitivity of *Candida albicans* against CMC/ ZnO (A) [Legend as in Fig. 1].

From Fig. 7 clear zones around discs of CMC/ ZnO indicated +ve result

Discussion

Hygienic quality of meat samples

Bacteriological evaluation

Total bacterial count (TBC) and mold & yeast counts (M & Y) are indicative as means of measuring the hygienic quality of minced meat. Figures 1 and 2 show that mean TBC and M & Y counts of control samples increased 2 logs at the end of storage period so rejected after 7 days, while the counts increased slightly in all the polymers treated groups and irradiated group and were still within the permissible limits (10^6CFU/g) approved by the Egyptian Organization for Standardization, EOS (2005) till the end of storage period. These results indicated that treatment with edible antimicrobial polymers and irradiation at 3 kGy increased the chilling life of minced meat as the samples maintained acceptable microbiologically until the 7th day of refrigeration storage. The use of edible polymers in food coating was mentioned by many authors; Pattanayaiying etal. (2019) found that a thermoplastic starch/ polybutylene adipate terephthalate (TPS/ PBAT; 40/60) film displayed excellent inhibition against foodborne pathogens on chilled and frozen seafood products. Yang et al. (2019) concluded that the antimicrobial film; xylan and hydroxyethyl cellulose with citric acid and polyethylene glycol has great potential in the field of sustainable food packing materials. Moreover, Guo et al. (2017) established innovative effective antimicrobial materials to reduce food borne pathogenic contaminants on ready-to-eat meat, strawberries, or other food.

Chemical evaluation

Lipid oxidation in minced meat is one of the major degradation processes responsible for loss of meat quality; it results in the formation of warmed off flavor, destruction of essential fatty acids, and loss of vitamins so TBA-values have been commonly considered as an index of lipid rancidity. Gamma irradiation and antimicrobial polymers induce the oxidation of lipids especially the unsaturated lipid and produce lipid oxides; subsequent decomposition of the unstable lipid oxides produces malonaldehydes (El-Dein et al., 2018).

From the results given in Fig. 3 and 4 there was an increase in the TBA value in control samples with storage time at refrigeration, but within the permissible limits (0.90 mg MD/ kg meat) approved by the EOS (2005). A significant increase in TBA contents was observed in treated meat samples. Similar results were reported by Medić et al. (2018) in pork meat. These increases are due to production of free radicals, which attach the unsaturated fatty acids leading to formation of peroxides and hydroperoxides; its breakdown resulted in unacceptable odors and flavors (Raeisi et al., 2018). In agreement with Naveen et al. (2016), irradiation and subsequent refrigeration storage of the control significantly increased the formation of peroxides and TPA.

Sensory evaluation

Sensory evaluation of the samples revealed that immediately after treating meat samples with the polymers and irradiation, a day after at 4±1ºC there were no significant differences between treated and non-treated meat samples (Fig. 4, 5 and 6) at the 3rd day; changes in color and texture were noticed in control samples while its odor was rejected at the 5th day (Zhang et al., 2017).

At the 7th day, the control non treated meat samples were all rejected while the polymers treated meat samples as well as irradiated meat samples were accepted for color, odor and texture. From the 3rd day of refrigerated storage the majority of the panelists preferred the polymer sprayed and irradiated samples which were maintained acceptable until the 7th day of refrigerated storage. Ramezani (2019) reported improvement of the sensory properties of quail meat under chilled conditions packaged in chitosan. Dutra et al. (2017) found that irradiation at medium doses preserved the sensory attributes of meat products and they distinguished that meat color is perceived by consumers as indicative of freshness against meat that turned to brown in color. Sensory panelists confirmed that the odor intensity of the irradiated meat were irradiation dose-dependent (Nam et al., 2011). This tendency was obvious as the irradiation dose increased to 6-8 kGy. 4 kGy was suitable for pasteurization beef meat (Zhao et al., 2017).

Sensitivity test

The results in Table 1 revealed that *Staph. aureus* was less sensitive to all examined polymers, but it was more sensitive to CMC/ ZnO, while *List. monocyt*. was more sensitive to CS, but showed no sensitivity to the other polymers. Candida albicans was sensitive only to CMC/ZnO. Analyzing the antimicrobial activity of many polymers has been reported by many authors (Alzagameem et al., 2019; Vaidya et al., 2019; Yang et al., 2019). Suchomel et al. (2015) reported that polymers containing Ag nanoparticles (Ag NPs) showed a high antimicrobial activity against Gram-positive and Gram-negative bacteria. As seen in Fig. 7 there is a clear zone of inhibition of growth of *List. monocyt.* under and around the disc film of CS; it was clear after 24hours at 37°C. Similar results were recorded by Jin et al. (2009).

Figure 9 shows a clear zone of inhibition of growth of *Staph. aureus* under and slightly around CMC and CS films, while it was not affected by the other polymers even after 48hrs of incubation. Kim et al. (2007) studied the antimicrobial activity of Ag nanoparticles and ZnO containing film against *Staph. aureus* and found that the growth-inhibitory effects were mild. The obtained results by Haciu et al. (2013) indicated that agar films incorporated with ZnO were promising candidates in the packaging sector as antibacterial food packaging materials, to increase the safety of food.

From Fig. 7 clear zones around discs of CMC/ ZnO indicated +ve result. Hence, CMC/ ZnO blends made by γ-rays were selected to be used as materials having antifungal activity. CMC/ ZnO was used in food packaging (Ibrahim & El Salmawi, 2013).

Conclusion

Data from this study revealed that packaging of meat with edible antimicrobial polymers keeps effectively its hygienic quality and normal odor, color and texture, so they can be used to extend the shelf-life of fresh minced meat stored under refrigerated conditions. No significant differences were noticed among the tested polymers affecting the hygienic quality of meat as that of γ-rays of meat at 3kGy. This may lead to interesting applications in the non-thermal preservation of foods. It was found that *Staph. aureus* was less sensitive to the used edible antimicrobial polymers in this experiment, but it was more sensitive to CMC/ZnO; while *List. monocyt*. was more sensitive to CS, but showed no sensitivity to the other polymers. Candida albicans was sensitive only to CMC/ZnO. Therefore, the use of antimicrobial polymers and γ-rays at 3kGy for food preservation is highly recommended.

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تحسين جودة اللحم المفروم بواسطة البوليمرات الآمنة والمضادة للميكروبات وأشعة جاما

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نم دراسة وتقييم الجودة الصحية للحوم المفرومة والمحفوظة عن طريق الغمس في البوليمرات الآمنة والمضادة للميكروبات، ومقارنتها بطريقة الحفظ بأشعة جاما. تم تحضير محلول كربوكسي مثيل السيليلوز وأكسيد الزنك (Carboxymethylcellulose/ zinc oxide)، وبولي كربونات/ وأكسيد الزنك و الشيتوزان (chitosan) وتجربته علي 3 مجموعات من اللحم المفروم ومقارنته بمجموعة تم تعريضها لجرعة 3 كيلوجراي من أشعة جاما.

أظهرت النتائج أن غمس اللحم المفروم في محلول البوليمرات اللآمن حسن من جودته الصحية، والمعايير الكيميائية والحسية والفحص الميكروبي. وقد تم الرفض الصحي للعينات الضابطة (المغمورة في الماء المقطر) في اليوم السابع.

أظهر التقييم الحسي لعينات اللحم المغموسة في محلول البوليميرات والمعرضة لأشعة جاما، حدوث تحسن في اللون والرائحة والملمس، والتي ظلت مقبولة للإستهلاك حتى نهاية فترة التخزين.

نم اجراء اختبار حساسية للميكروبات المعزولة، وتبين أن ميكروب استاف اوريس (*Staph. aureus*) كان حساسا لمحلول كربوكسي مثيل السيليلوز (CMC) بينما لم يظهر حساسية للبوليميرات الأخرى. وكان ميكروب ليستريا مونوسيتوجينز (*List. monocyt.*) أكثر حساسية للشيتوزان ولم يظهر حساسية لبقية البوليميرات الأخرى. أظهرميكروب كانديدا البيكانز (*Candida albicans*) حساسية لمحلول كربوكسي مثيل السيليلوز فقط.

الخلاصة: أن البوليمرات الآمنة والمضادة للميكروبات وجرعة 3 كيلوجراي من أشعة الجاما تحسن جودة اللحم المفروم، ومن الممكن استخدامها في حفظ الطعام وأنه يزيد من فترة تخزين اللحم المفروم بالمبردات.