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Safety achievement and shelf-life prolongation of poultry breast meats by polylactic acid active packaging and gamma-irradiation

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Abstract

Active packaging incorporated with volatile oils is a promising technology to extend the shelf-life of perishable food. The present work aimed at producing composite pouches based on polylactic acid incorporated with a mixture of lemongrass and cumin essential oils (PLA/mix oil). The effect on the shelf-life of fresh poultry breasts was determined on samples packaged in the PLA/mix oil alone and in combination with gamma-irradiation, and stored under refrigeration through microbiological, physicochemical, and sensorial analyses. The effect of active packaging and gamma-irradiation on artificially inoculated foodborne bacteria (*Escherichia coli* O157:H7 ATCC 25922, *Salmonella enteritidis*, *Listeria monocytogenes* ATCC 35152) in poultry breasts was evaluated. When compared to control, poultry breast samples packaged in the PLA/mix oil and irradiated at 4 kGy alone decreased microbial count, maintained colour and pH values, and increased TBARS index at a lower rate, thus extended the shelf-life by 21 and 14 d, respectively. However, the combination of PLA/mix oil and gamma-irradiation at 2 kGy (PLA/mix oil + 2 kGy) was more effective in decreasing all microbial counts and extending the shelf-life by more than 28 d. Initial load of *S. enteritidis*, *E. coli*, and *L. monocytogenes* inoculated in poultry breasts decreased by 3.03, 2.98, and 3.19 log CFU/g, respectively, after 3 d of storage in PLA/mix oil packaging, while the combination between PLA/mix oil and gamma-irradiation at 2 kGy (PLA/mix oil + 2 kGy) caused a synergistic impact with an increase in radiosensitivity of *S. enteritidis*, *E. coli*, and *L. monocytogenes* by 3.53, 4.47, and 4.23 log CFU/g, respectively, after one day of storage as compared to the control. Active packaging (PLA/mix oil) alone and in combination with gamma-irradiation can be considered an innovative technology that could have a major effect on the prolongation of shelf-life and safety of poultry breast meats. Moreover, this new technology represents a promising alternative to commercial and unsustainable plastic films.

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Introduction

Poultry meat represents around 37% of the total production of meat. Consumers prefer poultry meat because of its nutritious value, having low fat and cholesterol, and favourable content of unsaturated fatty acids as compared to other meats. This is in addition to being highly digestible, tasty, and of low calories besides its low price and easy availability (Sharma *et al.*, 2017).

In most countries all over the world, poultry meats rank first or second in foods associated with diseases (Kozačinski *et al.*, 2006). Poultry products have a high number of spoilage microorganisms, and may contain pathogenic bacteria; the total bacterial count of poultry products ranged between 10^6 and 10^7 CFU/g (Balakrishnan *et al.*, 2018). The most dangerous poultry meats pathogenic bacteria are *Salmonella enteritidis*, *Escherichia coli*, and *Listeria monocytogenes* (Al-Jobori *et al.*, 2016).

Outbreaks and foodborne diseases are major economic burdens in both developed and developing countries (Adwan *et al.*, 2015). Therefore, it is necessary to find appropriate conservation techniques

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for perishable meat products to extend their shelf-life, which is of great concern to the meat industry (Quinlan, 2013).

Consumer concerns over synthetic preservatives have led researchers to develop healthier and more environmentally friendly meat storage technologies. Recently, much attention has been given to the manufacture of biodegradable compact packaging films with active naturally occurring functional substances to extend the raw meat shelf-life (Rahman *et al.*, 2017). Consumer acceptance of antimicrobial agents from natural sources (such as herbal extracts and essential oils) as healthy preservatives has increased, and these are increasingly used on packing as an alternative to synthetic chemical agents (Maherani *et al.*, 2019). By directly incorporating an active compound into a polymer matrix, coating the compound on the packaging surface, or immobilising it in sachets, antimicrobial active packaging can be produced (Chen *et al.*, 2020).

The growing demand for food safety, efficiency, suitability, and environmental challenges related to the handling of plastic waste has highlighted the value of producing biodegradable and healthy films from natural polymers such as polylactic acid. Polylactic acid (PLA), a biodegradable polymer that can be obtained from renewable natural resources such as sugarcane or corn starch, has attracted more attention due to its biocompatibility, low cost, processability, and manageability. PLA is an interesting candidate for food packaging production (Ambrosio-Martín *et al.*, 2014).

The development of active bio-mobilisation systems can be achieved by antimicrobial agent's incorporation into biopolymer-based coating for extending shelf-life and maintaining food safety. Along with the selective barrier properties of active packaging for moisture, gases, and dissolved salts, it can also reduce the migration of antimicrobial agents from coatings to the products in solid and semi-solid foods, consequently keeping up a high antimicrobial agent's concentration on the surface of food products for quite a long time (Medimagh *et al.*, 2016). Natural antimicrobial agents are not usually stable with time; usage of edible coatings, microencapsulation, and effective packaging can help in enhancing the stability of the antimicrobial preparations, and extend their vital activity (Lacroix and Follett, 2015).

Food irradiation can be considered among the most effective conservation techniques for food safety and quality, which fights pathogens, parasites, and insects that cause food damage and degradation (Mrityunjoy *et al.*, 2019). However, there are potentially undesirable changes that radiation can cause in food, for instance, browning, softening, and loss in nutritional constituents (Moreno *et al.*, 2006).

Microbiological quality targets have been achieved by incorporating the use of various preservation techniques. Therefore, food irradiation should be coupled with other conservation techniques. Hurdle technology is using two or more hurdles to enhance the microbial stability and sensory consistency of foods, thus enhancing their economic and nutritional properties (Perricone *et al.*, 2015).

Combining irradiation and other conservation techniques is an effective method for enhancing efficiency and reducing energy or dose requirements for the destruction of foodborne diseases and dangerous organisms while maintaining or improving the quality of the product. In an earlier study by Ibrahim *et al.* (2020), the PLA/mix oil film showed excellent activity against *S.* Enteritidis, *E. coli*, and *L. monocytogenes in vitro*; consequently, it was selected for the application studies on poultry breast meat in the present work.

The aim of the present work was the fabrication of an eco-friendly, natural antimicrobial and functional packaging of PLA/mix oil, and using it alone and in combination with a low dose of gamma-radiation in packing raw poultry breast meats to achieve quality and microbial safety, as well as to maintain the natural and organoleptic properties.

Materials and methods

Antimicrobial film preparation: Essential oil extraction

Cumin (*Cuminum cyminum*) and lemongrass (*Cymbopogon flexuosus*) powders were purchased from a local market in Cairo, Egypt, and extracted by steam-distillation for 3 h using a Clevenger type apparatus and stored at 4°C until subsequent analysis.

Encapsulation of essential oils into the polylactic acid matrix by emulsion/solvent casting method

The film-forming polylactic acid was prepared by polymerisation of L-lactic acid (1.03 g) using *p*-toluenesulfonic acid and stannous octoate (0.3 wt %)

as a catalyst, and stirred for 12 h at 150°C. The collected insoluble white precipitate of polylactic acid was mixed with ethyl acetate (5%, w/v) over a magnetic stirrer until its complete solubilisation (10 h, 25 ± 2°C). A mixture of essential oils (lemongrass and cumin essential oils combination, 1:1) was incorporated in a proportion of 1% (v/w; mix essential oil/ polylactic acid), and Tween 80 (Sigma-Aldrich, Darmstadt, Germany) (0.125 g/g polylactic acid) was added as an emulsifying agent. The essential oil-added film-forming solution was homogenised using an ultrasonic homogeniser (Branson model, UK) at 80% amplitude for 20 min. Polylactic acid films without the incorporation of essential oils' mixture were produced in the same conditions. The films were obtained by distributing the mixture into glass Petri dishes, and drying first at 25°C in a forced-air oven for 24 h, and then at 40°C in a vacuum oven to yield a uniform thickness in all cases. Packaging pouches were prepared with a fixed dimension 10 × 10 cm from the prepared sheets, and welded with a Zepter plastic film (Vasile, 2018).

Characterisation of polylactic acid/mix essential oils

(a) Surface roughness parameters using AFM

PLA/mix oil were observed by atomic force microscopy (AFM). AFM images were obtained in the tapping mode with a multimode AFM Nanoscope IV (Digital Instruments). For tapping mode imaging, a spring frequency of 1.5 to 3.6 N/m with a resonance frequency of 45 to 65 kHz and a tip radius less than 10 nm was used. With this method, the phase and topographic measurement of areas can be scanned. Each sample was filmed in several places, but only the most representative one was illustrated (Wong *et al.*, 2009).

(b) Zeta potential measurement

Zeta potential was measured by dynamic light scattering (DLS) instrument (PSS, Santa Barbara, CA, USA), using the line at 632 nm of the HeNe laser, with the light drop at 90° angle, and zeta potential with external angle at 18.9°. During possible zeta measurements, temperature (23°C), liquid viscosity (0.933 copies), and the refraction index (1.333) were measured (Fernández-Calderón *et al.*, 2020).

(c) Measurement of the surface area and pore structure using nitrogen adsorption-desorption analysis

The Brunauer-Emmett-Teller (BET) surface area was estimated in nitrogen adsorption-desorption measurements at 77.35 K (Nova Touch LX4 Quantachrome, USA). The samples were stored in the desiccator prior to the estimation until the test was completed. Liquid nitrogen was used to cool the samples, and the samples were investigated by estimating the volume of N_2 gas adsorbed at specific pressures. From the adsorption branch of the isothermal curve, the pore volume was measured at P/Po = 0.995, assuming complete pore saturation (Clarkson *et al.*, 2012).

Fresh poultry breast meat samples

Poultry breast meat was purchased from a local poultry processing plant in Cairo, Egypt and transported to the laboratory in an icebox where it was stored at 4°C for a period not exceeding 2 h prior to its use.

Package design and gamma-irradiation

The effect of both active packaging (PLA/mix oil) and gamma-irradiation was studied individually and in combination to achieve safety and prolong the shelf-life of poultry breast meat.

Fresh poultry breast meat samples were packaged in pouches produced; approximately 20 g of breast meat were packaged in pouches under hygienic conditions and divided into five groups. The first group served as the control (samples packaged in polyethylene pouches as commercial pouches (PE)), while the other four groups were with or without irradiation namely PLA (samples packed in polylactic acid pouches); PLA/mix oils (samples packed in polylactic acid enriched with the mix between lemongrass and cumin essential oil pouches); PLA + gamma (samples packed in polylactic acid pouches and irradiated at 4 kGy); and PLA/mix oils + gamma (samples packed in polylactic acid enriched with mix oils pouches and irradiated at 2 kGy). Samples were irradiated at the National Center for Radiation Research and Technology (NCRRT), Atomic Energy Authority, Nasr City, Cairo, Egypt at 4 and 2 kGy using a ^{60}Co source "Indian Gamma Chamber 4000 A". The dose rate was 2.519 kGy/h during the experiment. All samples were transferred to a refrigerator, and stored at 4 ± 1°C for 28 d. Periodically, the breast meat was characterised in terms of microbiological quality and safety, physicochemical properties, and sensory evaluation at 0, 3, 7, 14, 21, and 28 d of storage.

Microbiological analysis

Plate Count agar, MRS agar, and Czapek-Dox Yeast Extract agar were used for enumerating total viable counts (TVC) (Downes and Ito, 2001), lactic acid bacterial counts (LAB) (Oxoid Limited, 1998) and yeast and mould counts (Oxoid Limited, 1998), respectively using the pour-plate technique.

Monitoring the effectiveness of the tested films by artificial inoculation

The antimicrobial effectiveness of PLA, PLA/mix oils, PLA/gamma, and PLA/mix oils + gamma films against the selected strains were tested in a model food (poultry breast) according to Ahmed *et al.* (2016).

Bacterial strains used in artificial inoculation (Gram-positive *Listeria monocytogenes*, and Gram-negative *Salmonella enteritidis* and *Escherichia coli* O157:H7) were obtained from the culture collections of the Microbiology Department, National Center for Radiation Research and Technology (NCRRT), Atomic Energy Authority, Nasr City, Cairo, Egypt. All the strains were aerobically grown at 37°C in Brain Heart Infusion (BHI) broth. Packaged poultry breast meat samples (20 g) used for artificial inoculation tests were first sterilised by gamma irradiation facility at 10 kGy in the NCRRT using a ^{60}Co source "Indian Gamma Chamber 4000 A" to ensure that the samples were free from any microbial contamination. Under aseptic conditions, the sterilised poultry breasts (20 g) were placed in Petri dishes, 1 mL of selected strain inoculum (prepared by diluting 10^6 CFU/mL microbial inoculum in peptone water) were spread independently over the poultry breast into each dish, then the dishes were left for 30 min. The samples were placed in the pouches under study following a previously described method. Survivors of the studied microorganisms were enumerated on plate count agar (PCA) medium (Downes and Ito, 2001) using the pour-plate technique after incubation at 35°C for 24 h, at different time intervals (0, 3, 7, 21, and 28 d).

Physicochemical parameters of the treated poultry meat

(a) pH

The pH of poultry breast meat pouches was determined as reported by Troutt *et al.* (1992). For 1 min, an amount of 10 g of the sample was mixed with 50 mL of distilled water. The pH of the homogenate was recorded by dipping a combined digital pH meter electrode (type pioneer 10) with a Fisher pencil probe.

(b) Thiobarbituric acid reacting substances (TBARS) number

As per the method defined by Tarladgis *et al.* (1960), the TBARS value of poultry breast meat was calculated. The optical density was recorded at 538 nm by a spectrophotometer. To calculate TBARS, the O.D was multiplied by a factor of 7.8, and the resulting TBARS value was expressed in mg of malonaldehyde/kg from the poultry breast sample.

Sensory evaluation

Using an eight-point descriptive scale (Keeton, 1983), sensory properties for poultry breasts were evaluated, where eight scores were given for an excellent product, and one was for an extremely bad product. Panellists were asked to evaluate various characteristics including general appearance, flavour, binding, texture, juiciness, and overall product acceptability using sensory assessment proforma.

Statistical analysis

Statistical analyses were performed using SPSS statistical software (SPSS, version 20.0; SPSS Inc., Chicago, IL, USA). All assays were conducted in at least three replicates. The results were expressed as mean ± standard deviation (SD), and tests were carried out with a probability limit of $p > 0.005$.

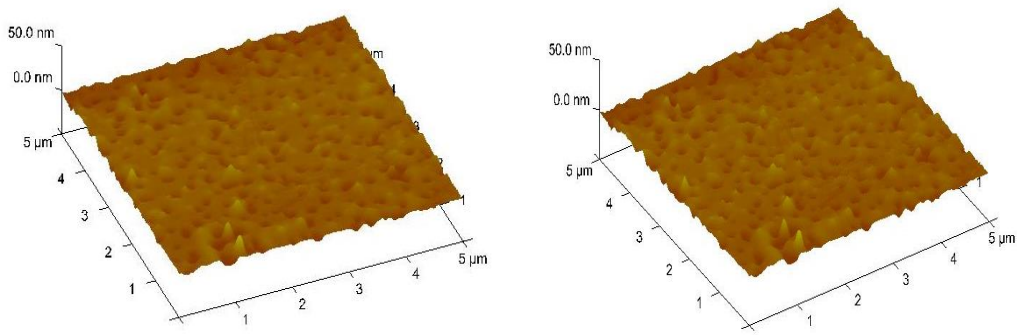
Results and discussion

Film characterisation

Atomic force microscopy (AFM)

AFM may be used to track and measure post-treatment surface evolution, interaction forces, or to explain antimicrobial or other active surface mechanisms. In the present work, the AFM 3-D images of PLA showed a surface that consisted of a ridge-and-valley structure (Figure 1A). The surface topography shifted from the structure of a ridged valley to a normal surface from a rough surface, with the encapsulation of the active mix of essential oil into the PLA matrix. AFM also showed that the incorporation of the mixed oil improved the roughness of PLA films, thus causing the amount of the mixed oil released from the active films to increase, which in turn improved the efficiency of the antimicrobial activity of these films (Figure 1B). Roughness modifications were therefore correlated

with changes in the adhesion, permeability, chemical functionality, and bioavailability of materials, and are important in the field of active packaging (Sadeghnejad *et al*., 2014).



(A) **(B)**

Figure 1. AFM surface topography images of PLA **(A)** before, and **(B)** after encapsulation with volatile oils.

Zeta potential and surface area

Zeta potential is the applied potential difference existing between the surface of a polymer particle dispersed in a conducting medium (*e.g.* aqueous), and the bulk of the surrounding medium. Moreover, zeta potential measurements could be considered as a key indicator of colloidal dispersion stability.

Average zeta potential measurements and its related factors of PLA in the presence and absence of the mix oils at different ratios were determined. The strength of zeta potential measurement can be attributed to the degree of repulsion force between PLA encapsulated essential oils. The prepared dispersion particles will resist the coagulation behaviour by increasing zeta potential values. Zeta potential of the dispersed PLA encapsulated essential oils mix in aqueous medium presented high value between -34.11 and -42.00 mV. These results confirmed the good stability of the prepared PLA/oils dispersion in water to be used as an antimicrobial coating in packaging materials.

Surface area

The ratio between volume/area is the key for desirable properties in nanomaterials. In addition, surface area measurement can indicate the efficiency of the antimicrobial behaviour of selected materials through the direct proportion of surface area and antimicrobial acting by surface contact area with microbial media.

Different isotherm adsorption aspects were measured. The correlation coefficient ratio was nearly ≈ 1, which showed a high homogeneity of the measured adsorption P/Po values. Additionally, the surface area of PLA (over 22 m^2/g) could increase the direct effect of PLA as antimicrobial behaviour and encapsulated bioactive oils with synergetic effect for both components in nanocapsules.

Effects of active packaging and gamma-irradiation on the shelf-life of poultry breast meats

Aerobic microflora were used as indicators for estimating the shelf-life of the stored samples in the composite pouches (Elzamzamy, 2014). The inhibitory effects of active packaging and gamma-irradiation (PLA/mix oils, PLA + gamma-irradiation at 4 kGy and its combination) on lactic acid bacteria (LAB), total viable counts (TVC), and yeast and mould counts (YMC) in poultry breast samples stored at refrigerated temperature (4 ± 1°C) for 28 d are shown in Figures 2A, 2B, and 2C, respectively. The poultry breast meats packed in PE (control) had initial counts of 4.36, 2.64, and 2.13 log CFU/g for TVC, LAB, and YMC, respectively. TVC were found to be in the acceptable range according to the Egyptian Organization for Standardization (EOS) for meats (1694/ 2005).

Microbial loads were directly related to the shelf-life of the poultry samples. The data of TVC, LAB, and YMC of the poultry meat samples packed in PE (control), PLA, and PLA/mix oils composite

pouches revealed that the composite pouches could improve the shelf-life of the poultry meat.

The TVC, LAB, and YMC of the poultry samples stored in PLA/mix oils composite pouches was significantly ($p < 0.05$) lower than PE and PLA pouches; the microbial growth rate of the stored poultry samples in composite pouches was relatively low during cold storage period, the films containing mix oils reduced the initial load of TVC, LAB, and YMC by 1.11, 0.66, and 0.49 log CFU/g, respectively (Figures 2A, 2B, and 2C). From the results, the active composite pouches were able to suppress the microbial growth in the poultry breast samples, and prolong their shelf-life.

The composite pouch's inhibition efficiency was due to the periodic release of volatile oils from the packaging film on the poultry surface. The content of the water in the poultry sample could induce the diffusion of volatile oil. Such findings are in a good agreement with a comparable observation made by Ahmad *et al*. (2012) who reported that in sea bass slices coated with a gelatine film containing lemongrass oil, TVC and psychrophilic bacterial counts were found to decrease as compared to uncoated ones. Due to post-processing handling, the microbial contamination of the processed meat occurs mainly on the surface; therefore, using packaging material carrying antimicrobial compounds might be more effective by slowing migration to the food surface, thus helping to retain high concentrations where necessary. Polylactide/polyethylene glycol/cinnamon oil (PLA/PEG/CIN) films reduced LAB, TVC, coliforms, and *Pseudomonas* spp. by 1 log CFU/g of a chicken sample (Ahmed *et al.*, 2016). Chitosan film incorporated with 5% *Herba lophatheri* extract was the best film, it extended the shelf-life of the fried bighead carp by 14 d (50%) as compared to control (Chen *et al.*, 2020).

Active films or coatings containing antimicrobial compounds can be combined at low doses of gamma-irradiation to achieve a synergistic inhibitory effect based on the microbiological hurdle concept (Lacroix and Ouattara, 2000).

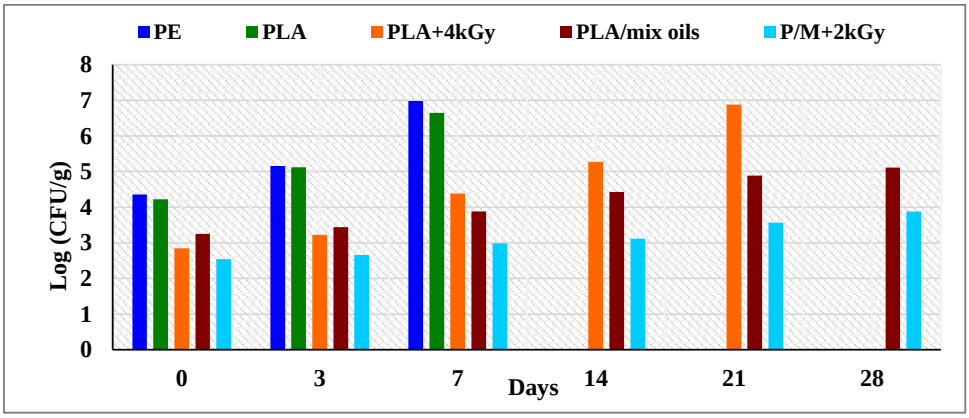
Figures 2A, 2B, and 2C show the effect of gamma-irradiation on poultry breast samples packaged in both PLA pouches (PLA + 4 kGy) and PLA/mix oils composite pouches (PLA/mix oils + 2 kGy). TVC, LAB, and YMC patterns in irradiated samples were different from unirradiated samples (PE pouches). The irradiation showed an increase in the lag phase ($p < 0.05$) before microbial growth began. For the poultry breasts packed in PLA bags and gamma-irradiated (4 kGy), the TVC (1.51 CFU/g), LAB (1.66 CFU/g), and YMC (undetected level) considerably decreased ($p < 0.05$) as compared to the control at 0 time. The combination between PLA/mix oils and gamma-irradiation at 2 kGy (PLA/mix oils + 2 kGy) was more effective in decreasing all microbial counts; this decrease was significant ($p < 0.05$), and in general, gamma-irradiation combination with the PLA/mix oils composite pouches increased microbial growth inhibition.

Irradiation is a simple practical mechanism to minimise microbial loads and avoid post-packaging recontamination, thus prolonging the shelf-life (Lacroix and Lafortune, 2004). The sharp decrease observed in the present work after gamma-irradiation might have been due to the immediate impact of gamma rays on the microbial cells by causing harm in the DNA of the cells, thus preventing cells from performing the vital processes essential for their continued survival. The effect of irradiation is more promising when applied in combination with other techniques (Ouattara *et al.*, 2002).

Combined treatment is recommended to control microbial contamination of foods. The main goal of combination treatments is to increase efficiency and decrease negative effect of the application by exposure to lower doses as compared to the individual application (Hussain *et al.*, 2016).

Aerobic microflora was used as a standard for predicting the shelf-life of foods (Elzamzamy, 2014), and 7 log CFU/g was the recommended maximum count limit set by the International Commission on Microbiological Specifications for Foods (ICMSF) for mesophilic aerobic bacteria (ICMSF, 1986). Figures 2A, 2B, and 2C indicate that the packaged samples pouches in PLA/mix oils and PLA/mix oils + 2 kGy stored at a refrigerator (4 ± 1°C) showed the longest shelf-life (28 d). The packaged samples pouches in PLA + 4 kGy (21 d) followed them, the packaged samples in PLA pouches lasted for 14 d, while the packaged samples in PE pouches (control samples) had the lowest shelf-life (7 d), respectively. This result could have been due to a time-late emission or distribution of the antimicrobial agents from the package surface to the inside air space or to the poultry breast samples.

A technique must be developed that combines many preservative methods to enhance their effectiveness. Therefore, there is a requirement for a system for combining a couple of methods together.



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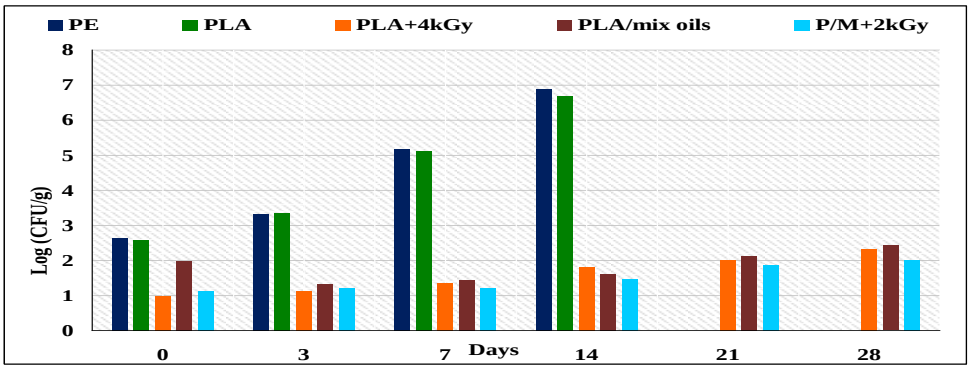

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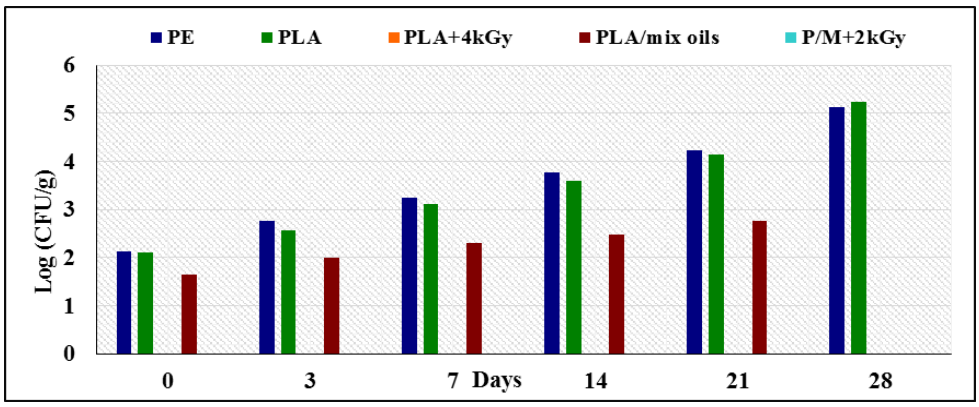


(C)

Figure 2. Effect of active packaging and gamma--irradiation on the poultry breasts' microbial load, **(A)**: total viable counts (TVC), **(B)**: total lactic acid bacterial counts, and **(C)**: yeast and mould counts. PE: polyethylene; PLA: polylactic acid; PLA + 4 kGy: polylactic acid + 4 kGy; PLA/mix oils: polylactic acid with mix oils; P/M + 2 kGy: polylactic acid with mix oils + 2 kGy.

Combinations can be irradiation with cooling, heating, and antimicrobial packaging. This result indicated that the combination of PLA/mix oils film and gamma-irradiation (2 kGy) (PLA/mix oils +2 kGy) was effective in the reduction of all the microbial counts with the extension of the poultry breast meats shelf-life for more than 28 d. The enhanced antimicrobial packaging was also reported by several researchers (Hussain *et al.*, 2016). Using natural antimicrobial agents in concentrations that have no effect on the sensory characteristics can increase the bacterial sensitivity by more than four-folds, and can reduce the radiation dose needed for pathogen elimination. The natural antimicrobials are usually unstable over time; microencapsulation and edible coatings can improve the stability of the antimicrobial formulations, and extend their bioactivity (Lacroix and Follett, 2015).

Effect of active packaging and gamma-irradiation on artificially inoculated pathogenic bacteria in poultry breast meats

Figures 3A, 3B, and 3C show the *S. enteritidis*, *E. coli* O157:H7, and *L. monocytogenes* growth, respectively, on the artificially-contaminated poultry breast meats packaged in PLA/mix oils films as compared to PE and PLA control samples during storage at 4°C for 28 d. The bacterial population decreased from day 3 ($p < 0.05$), and the decrease continued until day 28.

PLA/mix oils film decreased the initial load of *E. coli* O157:H7, *S. enteritidis*, and *L. monocytogenes* in poultry breast samples by 2.98, 3.03, and 3.66 log CFU/g from the third day of the storage as compared to PLA.

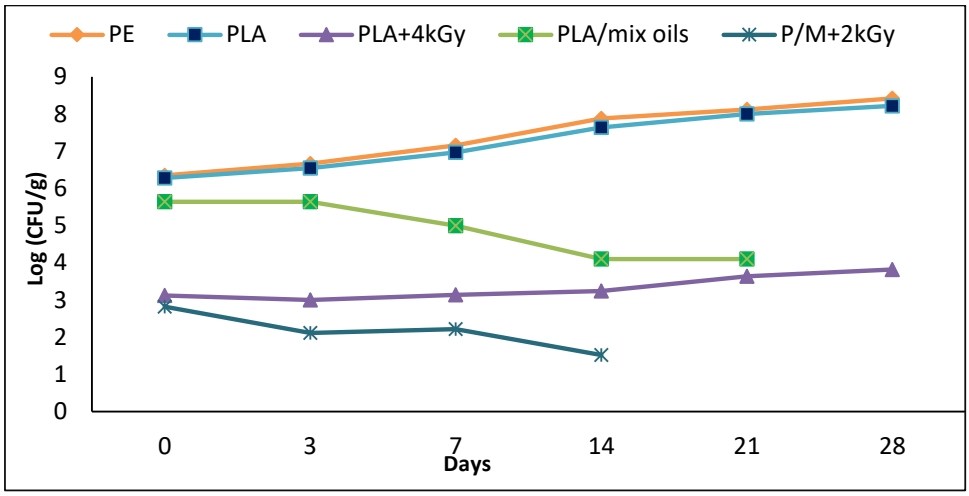
The bacterial populations of the samples packaged in PE and PLA pouches (control) increased steadily. In contrast to this, the samples packaged in PLA pouches containing mix oils showed decreased population, which did not undergo significant growth for 28 d.

L. monocytogenes and *S. enteritidis* were completely eliminated in poultry breast samples packed in active films after 28 d. These results revealed that the incorporation of mixture oils into the film prevented bacterial growth. This might have been due to the gradual release over time of the essential oils' mixture, thus permitting their continuous availability into the bacteria cell membranes. Phenols, terpenes, and aldehydes are the main active components of essential oils; they act mainly against the cytoplasmic membrane of the cells due to their hydrophobic nature, thus affecting the unsaturated fatty acid on the bacterial cell wall, which in turn changing its structure (Severino *et al.*, 2015).

The effect of gamma-irradiation (4 kGy) on *E. coli* O157:H7, *L. monocytogenes*, and *S. enteritidis* artificially inoculated on poultry breast samples packed in PLA film and refrigerated at 4 ± 1°C are shown in Figures 3A, 3B, and 3C. Gamma irradiation (4 kGy) induced the highest reduction of *S. enteritidis* (3.23 log CFU/g), *E. coli* O157:H7 (3.35 log CFU/g), and *L. monocytogenes* (4.7 log CFU/g). The reduction in the survivors of all tested bacteria was significant ($p < 0.05$) as compared to the control throughout the storage period (28 d). Initial loads of *S. enteritidis*, *E. coli* O157:H7, and *L. monocytogenes* inoculated in poultry breast samples and packaged in PLA/mix oils pouches with 2 kGy (PLA/mix oils + 2 kGy) were reduced by 3.53, 4.47, and 4.23 log CFU/g, respectively after 1 d of storage as compared to PLA pouches. No survival was found for *S. enteritidis* and *L. monocytogenes* after 14 and 21 d of storage in poultry breast samples packaged in PLA/mix oils + 2 kGy films. Using active packaging with gamma-irradiation resulted in a synergistic impact, which strengthened the effect of gamma-irradiation against the bacteria tested, or in other words, the presence of active packaging increased the radiosensitivity of tested bacteria.

In a previous study (Caillet *et al.*, 2006), the combination treatment between gamma-irradiation and other non-thermal treatments indicated that microorganisms that were able to survive after irradiation treatment were more sensitive than untreated microbial cells that could represent harmful environmental factors such as the presence of antimicrobial agnate or changed atmosphere.

The radio sensitisation of *L. monocytogenes* inoculated on broccoli florets covered with palmitoylated chitosan containing a nanoemulsion of mandarin EO has been confirmed. Gamma-irradiation damages bacterial cells in several ways such as the breakdown of chemical DNA bonds, the change in membrane permeability, and the change in cell function which can facilitate the contact between the antimicrobial compounds and cell membranes, thus leading to an increase in bacterial sensitivity to antimicrobial agnate (Lopez-Gonzalez *et al.*, 1999).



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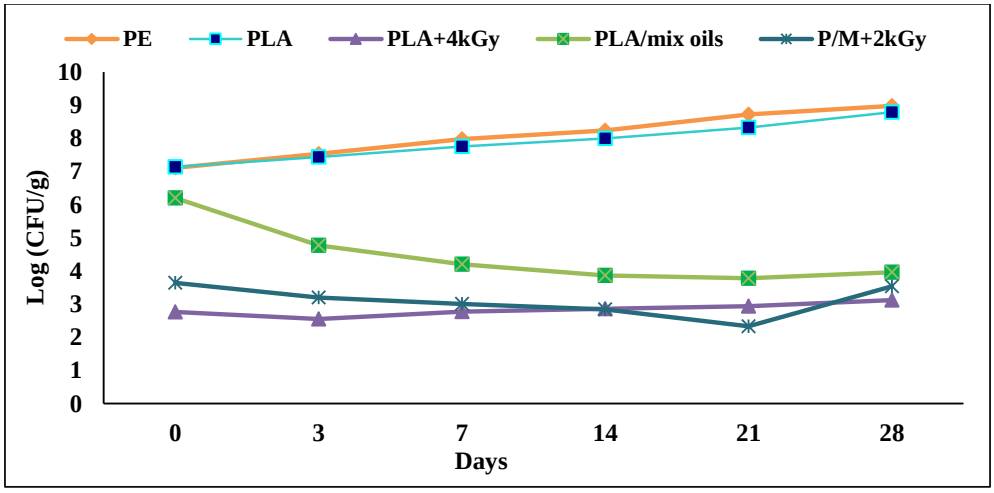

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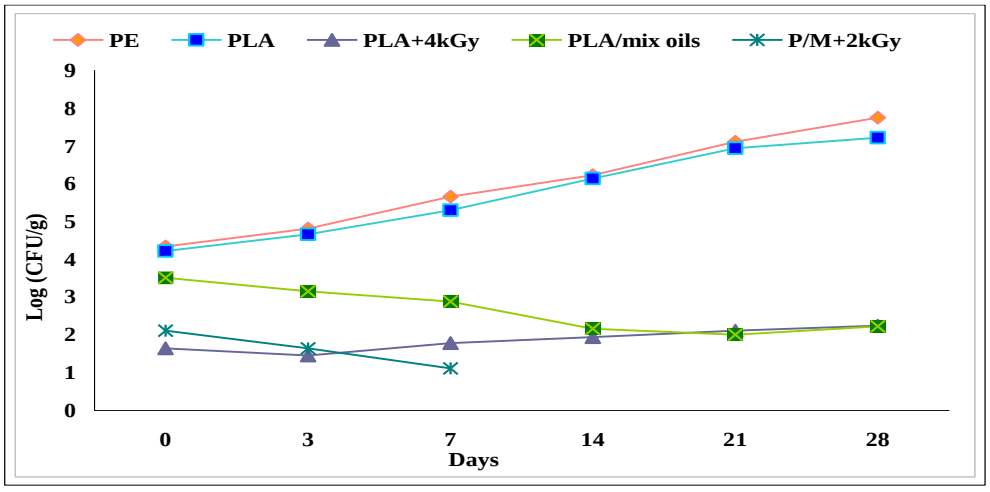

(C)

Figure 3. Effect of active packaging and gamma--irradiation on the poultry breasts inoculated microbial load. **(A)**: Inoculated *S. enteritidis* in poultry breasts, **(B)**: Inoculated *E. coli* O157:H7 ATCC 25922 in poultry breasts, and **(C)**: Inoculated *L. monocytogenes* ATCC 35152 in poultry breasts. PE: polyethylene; PLA: polylactic acid; PLA + 4 kGy: polylactic acid + 4 kGy; PLA/mix oils: polylactic acid with mix oils; P/M + 2 kGy: polylactic acid with mix oils + 2 kGy.

pH

Physicochemical evaluation of the poultry breast meats packaged in active packaging alone and in combination with gamma-irradiation is shown in Table 1. An increasing trend in pH for all samples was observed as the storage progressed, but the increase rate was lower for active packaging (PLA/mix oils) alone and in combination with gamma-irradiation.

At each storage interval, the increase in the pH values of PE samples (control) and PLA samples were significant ($p \leq 0.05$), where the pH values of PLA/mix oils increased ($p \leq 0.05$) from day 14 onwards.

The pH value of the control samples (PE) was significantly higher ($p \leq 0.05$) than that of the PLA/mix oil samples from day 3 onwards; however, on day 21 of storage, the pH value of the PLA/mix oil + 2 kGy samples was significantly lower ($p \leq 0.05$) than that of the PLA/mix oil samples. The increase in the pH value during the storage might have been due to the microbial metabolites and endoprotease activity. After using glucose, bacteria operate on amino acids produced during protein breakdown, thus producing ammonia, which results from the amino acid breakdown and therefore increases the pH value (Zahid *et al*., 2018). As compared to the control, lower pH values of the active packaging samples indicated that active components of essential oils such as phenols, terpenes, *p*-cymene, and terpenoids might have been effective in inhibiting or decreasing the growth of spoilage microorganisms (Mandic *et al*., 2018).

Thiobarbituric acid reacting substances (TBARS)

A substantial increase in TBARS values of all samples was observed with increasing storage period; but, the rate of the increase in the active packaging (PLA/mix oil) was comparatively slower, thus suggesting greater oxidative stability of the treated samples (Table 1). A significant increase ($p \leq 0.05$) in TBARS was observed during the storage in the control and PLA samples at each storage interval of time. Moreover, the PE and PLA samples showed a significant increase ($p \leq 0.05$) from day 3 forward.

Among treatments, a significant difference ($p \leq 0.05$) in TBARS was observed from day 7 onwards, the TBARS value of the PLA/mix oil was significantly lower ($p \leq 0.05$). In addition, during the storage period, the PLA/mix oil + 2 kGy samples showed the lowest increase in TBARS, followed by PLA/mix oil samples.

Increased TBARS of products may be due to lipid oxidation and volatile production of metabolites in the presence of air during storage (Sharma *et al*.,

Table 1. Effect of active packaging and gamma--irradiation on pH and TBARS values of poultry breasts.

Sample	Storage (day)					
	1	**3**	**7**	**14**	**21**	**28**
			pH			
PE	5.82 ± 0.2^{aA}	5.94 ± 0.1^{bB}	6.18 ± 0.3^{cB}	6.42 ± 0.1^{dC}	6.56 ± 0.4^{dD}	6.83 ± 0.2^{eD}
PLA	5.78 ± 0.4^{aA}	5.99 ± 0.1^{bB}	6.12 ± 0.4^{cB}	6.38 ± 0.2^{dC}	6.59 ± 0.2^{dD}	6.88 ± 0.3^{eD}
PLA + γ	5.80 ± 0.2^{aA}	5.82 ± 0.4^{aA}	5.94 ± 0.1^{aA}	6.12 ± 0.3^{bB}	6.33 ± 0.1^{bC}	6.62 ± 0.1^{cC}
PLA/oil	5.78 ± 0.1^{aA}	5.80 ± 0.1^{aA}	5.86 ± 0.2^{aA}	5.94 ± 0.4^{bA}	6.14 ± 0.2^{bB}	6.24 ± 0.4^{cB}
PLA/oil + γ	5.75 ± 0.3^{aA}	5.79 ± 0.2^{aA}	5.81 ± 0.5^{aA}	5.89 ± 0.1^{aA}	5.96 ± 0.3^{aA}	6.11 ± 0.3^{bA}
			TBARS (mg/MDA/kg)			
PE	0.16 ± 0.1^{aA}	0.30 ± 0.1^{bB}	0.49 ± 0.02^{bC}	0.84 ± 0.2^{cC}	1.18 ± 0.1^{dC}	1.65 ± 0.1^{eD}
PLA	0.18 ± 0.2^{aA}	0.28 ± 0.5^{bB}	0.44 ± 0.3^{bC}	0.80 ± 0.3^{cC}	1.14 ± 0.3^{dC}	1.58 ± 0.3^{eD}
PLA + γ	0.18 ± 0.4^{aA}	0.20 ± 0.2^{aA}	0.32 ± 0.1^{bB}	0.44 ± 0.1^{bB}	0.64 ± 0.2^{cB}	0.89 ± 0.4^{dC}
PLA/oil	0.14 ± 0.1^{aA}	0.17 ± 0.1^{aA}	0.26 ± 0.3^{aA}	0.34 ± 0.4^{bA}	0.48 ± 0.1^{bA}	0.61 ± 0.1^{cB}
PLA/oil + γ	0.16 ± 0.4^{aA}	0.16 ± 0.1^{aA}	0.20 ± 0.1^{aA}	0.26 ± 0.2^{aA}	0.39 ± 0.3^{bA}	0.49 ± 0.2^{bA}

PE: polyethylene; PLA: polylactic acid; PLA + γ: polylactic acid + 4 kGy; PLA/oil: polylactic acid with mix oils; and PLA/oil + γ: polylactic acid with mix oils + 2 kGy. Means followed by different lowercase superscripts (within rows) and different uppercase superscripts (within columns) are significantly different ($p \leq 0.05$).

2014). TBARS reduction in treated products could have been due to polyphenols in essential oils with antioxidant effects. Phenolic compounds are able to prevent oxidation while they react with oxygen and get oxidised in the process (Falowo *et al.*, 2014). Polyphenol delayed or inhibited oxidation by two processes, either by serving as an electron donor to end the oxidation cycle (Allen and Cornforth, 2010), or by eliminating free radical initiators (Antolovich *et al*., 2002).

Effect of active packaging and gamma-irradiation on the sensory evaluation of poultry breast meats

Sensory evaluation of the poultry breast meat samples packaged in active packaging alone and in combination with gamma-irradiation is shown in Table 2. Varied results were observed. A significant decrease in the PE and PLA (control) samples overall appearance scores was observed from day 3 onwards.

The general appearance scores of the active packaging (PLA/mix oils) samples were significantly higher ($p \leq 0.05$) than those of the samples irradiated at 4 kGy. However, the difference was insignificant ($p > 0.05$) in the general appearance scores of the active packaging (PLA/mix oils) and the active packaging with gamma-irradiation (PLA/mix oils + 2 kGy) samples until 21 and 28 d, respectively. Reduced appearance scores of the sample packaged might have been attributed to surface dehydration in aerobic packaging.

The reduction in juiciness scores of the PE and PLA samples from day 3 onwards was significant ($p \leq 0.05$), while the active packaging samples showed a significant decrease ($p \leq 0.05$) from day 21 onwards; however, juiciness scores of the irradiated samples (4 kGy) decreased significantly ($p \leq 0.05$) from day 7 onwards. There was an insignificant difference ($p > 0.05$) in the juiciness scores of the active packaging with gamma-irradiation (PLA/mix oils + 2 kGy) samples until 28 d. The decrease in the juiciness of the packaged samples could have been

Table 2. Effect of polylactic acid active packaging and gamma--irradiation on sensory evaluation of packaged poultry breast meat.

Sample	Storage (day)					
	1	**3**	**7**	**14**	**21**	**28**
Appearance						
PE	7.73 ± 0.4^{aA}	6.11 ± 0.2^{bB}	R	R	R	R
PLA	7.78 ± 0.5^{aA}	6.28 ± 0.4^{bB}	R	R	R	R
PLA + γ	7.71 ± 0.1^{aA}	7.66 ± 0.2^{aA}	6.73 ± 0.2^{bA}	6.11 ± 0.2^{bB}	5.80 ± 0.2^{cB}	5.11 ± 0.2^{dB}
PLA/oil	7.79 ± 0.3^{aA}	7.75 ± 0.1^{aA}	7.73 ± 0.1^{aA}	7.63 ± 0.1^{aA}	7.54 ± 0.1^{aA}	7.13 ± 0.1^{bA}
PLA/oil + γ	7.75 ± 0.2^{aA}	7.77 ± 0.3^{aA}	7.74 ± 0.3^{aA}	7.72 ± 0.3^{aA}	7.68 ± 0.3^{aA}	7.44 ± 0.3^{aA}
Juiciness						
PE	7.34 ± 0.2^{aA}	6.10 ± 0.2^{bC}	R	R	R	R
PLA	7.35 ± 0.4^{aA}	6.14 ± 0.4^{bC}	R	R	R	R
PLA + γ	7.12 ± 0.2^{aA}	6.96 ± 0.2^{aB}	6.14 ± 0.2^{cB}	5.92 ± 0.2^{cB}	5.64 ± 0.2^{dC}	5.33 ± 0.2^{dC}
PLA/oil	7.36 ± 0.1^{aA}	7.35 ± 0.1^{aA}	7.31 ± 0.1^{aA}	7.24 ± 0.1^{aA}	6.84 ± 0.1^{bB}	6.47 ± 0.1^{cB}
PLA/oil + γ	7.35 ± 0.3^{aA}	7.36 ± 0.3^{aA}	7.31 ± 0.3^{aA}	7.32 ± 0.3^{aA}	7.26 ± 0.3^{aA}	7.16 ± 0.3^{aA}
Overall acceptability						
PE	7.23 ± 0.2^{aA}	6.33 ± 0.2^{bB}	R	R	R	R
PLA	7.21 ± 0.4^{aA}	6.38 ± 0.4^{bB}	R	R	R	R
PLA + γ	7.22 ± 0.2^{aA}	7.14 ± 0.2^{aA}	6.55 ± 0.2^{bB}	6.12 ± 0.2^{cB}	5.65 ± 0.2^{dB}	5.12 ± 0.2^{eB}
PLA/oil	7.23 ± 0.1^{aA}	7.22 ± 0.1^{aA}	7.14 ± 0.1^{aA}	7.12 ± 0.1^{aA}	7.00 ± 0.1^{aA}	6.97 ± 0.1^{aA}
PLA/oil + γ	7.22 ± 0.3^{aA}	7.22 ± 0.3^{aA}	7.20 ± 0.3^{aA}	7.17 ± 0.3^{aA}	7.11 ± 0.3^{aA}	7.00 ± 0.3^{aA}

PE: polyethylene; PLA: polylactic acid; PLA + γ: polylactic acid + 4 kGy; PLA/oil: polylactic acid with mix oils; and PLA/oil + γ: polylactic acid with mix oils + 2 kGy. Means followed by different lowercase superscripts (within rows) and different uppercase superscripts (within columns) are significantly different ($p \leq 0.05$). R: reduced.

attributed to losing water during storage.

Overall acceptability is based on colour and texture of the poultry breast samples. Results indicated that for both the PE and PLA samples, there was an insignificant difference ($p > 0.05$) in the overall acceptability of poultry breast samples up to 3 d of refrigerated storage. When comparing with active packaging samples alone and in combination with gamma-radiation, the overall acceptance of 4 kGy irradiated samples was lower ($p \leq 0.05$); this pattern in the overall acceptability was noted even after 14 d of chilled storage.

The overall acceptability of the active packaging (PLA/mix oil) and in combination with gamma-irradiation (PLA/mix oil + 2 kGy) was above the acceptable level as compared to individual treatments after 28 d of chilled storage.

In active packaging samples alone and in combination with gamma-irradiation, a significant influence ($p \leq 0.05$) on the maintenance of the overall acceptance of the poultry breasts meat during the storage conditions was observed. The rapid reduction in the overall acceptability of EP and PLA samples could have been linked to the reduction scores in texture, colour, and the loss of volatile aroma due to microbial spoilage.

The synergistic impact of combination treatment of active packaging and irradiation is related to microbial inhibition, retention of texture, and colour, thus maintaining a higher overall acceptability of the poultry breasts during storage.

Conclusion

The incorporation of a mixture of lemongrass and cumin essential oils (1:1) into polylactic acids film (PLA/mix oil) could be a promising new source of biodegradable polymer and antimicrobial packaging which offers a new commercial opportunity for foods processing. To date, there are scarce information and studies on the incorporation of volatile oils into PLA polymer, and their applications with gamma--irradiation on poultry meat packaging, which is an important area of research. The present work demonstrated the importance of PLA/mix oil packaging and in combination with a low dose of gamma--irradiation (2 kGy) to inhibit microbial spoilage of poultry breast meats. Results showed complete inhibition of Gram-negative *E. coli* O157:H7 ATCC 25922 and *S. enteritidis*, and Gram-positive *L. monocytogenes* ATCC 35152 inoculated in the poultry breast meats. The combined treatment besides maintaining storage quality resulted in an extension of 28 d in the shelf-life of the poultry breast meats during refrigerated storage. PLA/mix oil alone and in combination with gamma--irradiation could be a new alternative to synthetic or conventional polymers and food preservatives for safety and prolongation of poultry breast meats' shelf-life. This will induce a significant impact to the economy in the developing countries, especially in Egypt, in the field of food processing.

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