Research Article

# Incorporation of Gallic Acid Into Zein Wax Film to Improve the Quality and Safety of Chilled Veal Meat Chunks

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**Abstract** | As the food industry moves toward clean packaging, the replacement of synthetic preservatives with natural food additives has become more widely practiced. It was investigated the effects of the 2% and the 4% gallic acid/ zein wax film as an antioxidant and antimicrobial food packaging material, focusing on the quality, shelf life, and antioxidant properties of veal meat chunks during refrigerated storage. After coating meat samples, we analyzed their microbial, chemical, and sensory properties after 0, 3, 5, 7, 9, and 11 days of chilled storage. GA/ zein wax sheets significantly reduced ($p < 0.05$) the abundance levels of total bacteria, and psychrotrophic bacteria, as well as pH, thiobarbituric acid (TBA), and total volatile base nitrogen (TVB-N), especially for 4% GA/ zein wax film. The coatings resulted in the prolongation of the shelf life of meat chunks. The shelf lives of the control, the 2%, and the 4% GA-coated meats were 7, 9, and 11days, respectively. Coated meats had higher overall sensory acceptability scores compared to uncoated ones. The antioxidant capacity, the glutathione, and nitric oxide contents of the coated meats were higher than those of the control meat. Four percent of GA/ zein wax film significantly reduced the abundance of *E. coli O26* by 3 $\log_{10}$ CFU/g when compared by uncoated meat chunks. We conclude that incorporating GA into zein wax film can effectively improve the stability and shelf life of veal meat chunks during chilled storage.

**Keywords** | Gallic acid, Zein, *E. coli O26*, Glutathione, TAC, NO, TBA- TVB-N, Veal meat, Shelf life.

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## INTRODUCTION

The fresh meat chunks are easily spoiled by natural contaminants such as microorganisms that negatively affect the texture, flavor, color, and nutritional quality of the product, resulting in serious economic losses in both the retail market and meat industry (Lahmar et al., 2018; Alizadeh-Behbahani et al., 2020). Microorganisms that spoil chilled preserved food are called mesophilic bacteria, which are able to grow at 4 °C (Ballestra et al., 2010). Naturally occurring spoilage microorganisms reach meat through various sources during carcass processing and handling (Tango et al., 2014; Camargo et al., 2019). *E. coli* is one of the most widespread contaminant bacteria that affect beef products (Castro et al., 2017). While, the term Shiga toxin-producing (STEC) refers to *E. coli* that produces this type of toxin, which is similar to that produce by *Shigella dysenteriae* type 1 (Skinner et al., 2013).

The STEC are infective at low doses that may cause highly severe infection to humans. For example, serogroup $O_{26}$ causes STEC-associated disease and is frequent associated with diarrhea and hemolytic uremic syndrome (Bielaszewska and Karch, 2000). Bacterial contamination decreases the quality and safety of meat during chilled storage, and it increases health risks. Therefore, there is an increasing demand to develop effective methods to improve both the quality and safety of fresh meat during chilling.

Meats and their products are sources of natural enzymatic and non-enzymatic antioxidants (Jung et al., 2010; Moñino et al., 2008; Sacchetti et al., 2008). After slaughter, meat undergoes a number of chemical reactions, and during meat preparation for consumption, the changes in meat musculature also cause changes in the meat's antioxidative profile (Yu et al., 2017). Among the most important antioxidants, hydrophilic dipeptides such as carnosine (β-alanyl-l-histidine; the main antioxidant in meat) and anserine (*N*-β-alanyl-1-methyl-l-histidine) are known to be effective (Antonini et al., 2002). Other antioxidants found in meat are glutathione and nitric oxide. Glutathione is a tripeptide consisting of glycine, cysteine, and glutamate. It is the most important part of the antioxidant system in living organisms, as it maintains the balance between free radicals and antioxidants. (Forman et al., 2009). The primary function of NO is the relaxation of the inner muscles of blood vessels, which increases circulation. Therefore, NO facilitates the effective and efficient transport of blood, nutrients, and oxygen to every part of the body (Yang et al., 2018). Antioxidative agents delay the oxidative degradation of lipids; and they increase the quality, and maintain the nutritional value of meat (Bensid et al., 2020). Active packaging, combined with natural antioxidant and antimicrobial agents, has attracted much attention in recent years because of its ability to increase the shelf life and quality of food products. It prevents chemical, microbial, and physical damage to food; and it prevents health problems resulting from illness caused by food borne pathogens as well as the economic losses from spoilt food (Newell et al., 2010; Barzegar et al., 2020). Zein is a prolamin found in the corn-grain endosperm. It is a hydrophobic protein containing high levels of amino acids (Tapia-Hernández et al., 2019). Zein films are used as edible and biodegradable coatings on vegetables and nuts to delay rancidity, color change, weight loss, and to maintain firmness during storage (Cagri et al., 2004). Meanwhile, gallic acid (3, 4, 5 trihydroxybenzoic acid) is a water-soluble phenolic acid with potent antioxidant and antibacterial activities. It targets bacterial membranes, leading to cell rupture (Borges et al., 2013; Papuc et al., 2017). Several reports have focused on the antimicrobial activity of GA against spoilage bacteria such as Pseudomonas (Borges et al., 2013; Gutiérrez-Larraínzar et al., 2012; Jayaraman et al., 2010), however, most studies have been done in vitro. Incorporating GA into zein films can improve the porosity of films, thus increasing their flexibility and decreasing their brittleness (Alkan and Yemenicioglu, 2016).

We investigated the effects of the incorporation of gallic acid into zein film on the safety and quality of chilled meat. Specifically, we evaluated the shelf life of chilled meat chunks based on microbial (total bacterial and psychrotrophic abundance), chemical (TBA, pH, total volatile basic nitrogen (TVB-N)), and sensory qualities of meat during storage. The antioxidant capacity of GA/ zein wax sheets was also determined by measuring the total antioxidant capacity (TAC) and the levels of glutathione (GSH) and NO in stored meat chunks. Finally, we evaluated the ability of gallic acid zein wax films to control *E. coli O26*in chilled meat chunks.

## MATERIALS AND METHODS

### Meat chunks

Fresh, boneless veal meat was purchased from the Carrefour hypermarket in Cairo, Egypt and stored immediately at 4°C. The meat was cut into 5 × 5 $cm^2$ pieces weighing approximately 25 g.

### Zein wax film

Zein wax film was prepared according to Padgett et al. (1998). Briefly, 1.4 g of corn zein was stirred together with 8.1 ml of 97% ethanol using a magnetic stirrer for 30 min. Glycerol (0.5 ml) was added to the emulsion, which was then boiled for 5 min. After cooling, gallic acid was then added to the emulsion at final concentrations of 2% and 4%.The emulsions were then stirred for ten minutes and then poured into glass templates using a thin layer chromatography spreader apparatus. Films were dried at room temperature for 24 h, then the dried films were carefully peeled off the glass templates and utilized for packaging.

### Meat packaging

Meat chunks were packaged with 2 % GA and 4 % GA films, and control meat chunks were packaged with commercial sterile polyethylene bags. After packaging, all meat chunks were stored at 4°C until they were evaluated for microbial and chemical qualities. Each treatment, as well as the control, were replicated thrice.

### Microbial quality

Twenty-five grams of packed and unpacked meat chunks were placed in sterile stomacher bags. The contents of each bag were mixed with 225 ml of sterile peptone water, homogenized for 2 min by stomaching, and the homogenate was serially diluted. Aerobic and psychrotrophic bacteria were enumerated on plate count agar according to pub-

lished protocols (APHA, 2001). Aerobic bacteria were enumerated after incubation at 35˚C for 48 h, while psychrotrophic bacteria were enumerated after incubation at 7˚C for 10 days.

### Chemical quality

The pH of each sample was measured according to EOS: 63-11/2006), while the contents of thiobarbituric acid (TBA) and total volatile basic nitrogen were measured according to EOS: 63-10/2006and EOS: 63-9/2006, respectively.

### Biochemical analysis

We sampled 0.2 g of each meat treatment after three days of chilling. Each sample was minced into small pieces, homogenized with a glass homogenizer in 0.4 ml of 25% metaphosphoric acid (MPA) (ref. No.: 253-433-4, Sigma-Aldrich, Germany). Then 1.4 ml of distilled water was added, and the homogenates were mixed and incubated for 1 hour and then centrifuged for 10min at 3,000 r.p.m. The supernatant was collected and analyzed as follows: The total antioxidant capacity was measured according to Pisoschi and Negutescu (2011), glutathione content was measured according to Kand'ár et al. (2007) and NO content was measured according to Grisham et al. (1996).

### Sensory qualities

The sensory analyses of color, flavor, taste, and overall acceptability were based on sample examination by nine assessors, who were staff of the Food hygiene department, Animal Health Research Institute. The assessors were asked to score the meat on the 9-point scale of Rodríguez-Melcón et al. (2017), where a score of 9 is considered "excellent." Scores below five indicate sample deterioration, at which point, the assessment was terminated. Meat samples that were further analyzed were placed in a pot and covered with cold water. The meat was boiled with a lid on for 30 minutes, and after cooking, the odor of cooking liquid vapor was assessed immediately by opening the lid; and the odor and taste of the meat were also assessed. Meat chunks were evaluated after 0, 3, 7, 9, and 11 days of cold storage.

### Bacterial culture

*E.coli O26* was obtained from the strain bank of the Animal Health Research Institute, Agriculture research center. The strain was grown in tryptic soy broth supplemented with 0.5% yeast extract at 37 ˚C overnight (~$10^9$CFU/ml). One milliliter of the culture was centrifuged at 13,400 ×g for 5 min, and the cells were washed and serially diluted in 0.1% peptone water to yield cell suspensions containing 100 to$10^8$CFU/ml. Active cultures with bacterial densities of at least 1x$10^8$_1x$10^9$ CFU/ml were used to inoculate meat samples.

### Meat chunk inoculation

Fresh meat chunks were dipped in a previously prepared culture of *E.coli O26*. The inoculated meat chunks were divided into two groups: the first group was packed with zein wax film containing 4% GA, and the control group was packed with sterile commercial polyethylene bags. Both two groups were stored at 4˚C for 11 days.

*x. E. coli O26* enumeration

Twenty-five grams of each chilled beef treatment was placed in sterile stomacher bags, and the samples were mixed with 225 mL TSBY (treptic soya broth yeast) and homogenized for 2 min by stomaching. One milliliter of undiluted or serially diluted (1:10 in PBS) homogenized suspension was plated on duplicate cefixime rhamnose-sorbitol MacConkey agar (CR- SMAC) plates and incubated at 37˚C for 24 hrs. *E. coli O26* colonies are colorless (rhamnose non-fermenter) (Hiramatsu et al., 2002).

### Statistical analysis

All experiments were carried out in triplicate. The data are presented as means ± standard deviations. Treatment effects were evaluated by a one-way ANOVA t-tests, and a P-value of 0.05 was considered significant. All statistical analyses were performed using SPSS statistical software.

## RESULTS AND DISCUSSION

Gallic acid (GA) had antimicrobial activity against both gram-negative and gram-positive bacteria. Its effects include changing the cell surface hydrophobicity and charge, inducing propidium iodide uptake, and leakage of K+, resulting in cell rupture or pore formation in the cell membranes (Borges et al., 2013). (Figure 1) shows the effects of GA/ zein treatments on the abundance levels of aerobic and psychrotrophic bacteria on chilled meat. The use of both 2% and 4%GA resulted in significantly less aerobic and psychrotrophic bacteria from the 3rd day of storage, compared to levels on the uncoated meat chunks (control group). The abundance levels of aerobic bacteria were 5.7± 0.05 and 5.23± 0.25 $\log_{10}$ CFU/g after 9 days of chilled storage in the 2% and 4% GA groups, respectively. The control, 2%, and 4% GA were spoiled and putrefied after 7, 9, and 11 days of chilled storage, thus no further analyses were conducted.

The abundance levels of psychrotrophic bacteria (Figure 2) were 5.15± 0.14 and 4.67± 0.15 $\log_{10}$ CFU/g after 9 days of chilled storage in the 2%, and 4% GA groups, respectively. However, the control meat chunks spoiled after 7 days of chilled storage. This indicates that during refrigerated storage, fresh meat undergoes physical, chemical, and microbial changes because it acts as a perfect environment for the growth of microbes. Under cold, aerobic conditions, psychrotrophic bacteria are predominantly responsible for

**Table 1:** Effect of coating sheet on pH values (mean ± SD) of chilled meat chunks.

| Days | Zero | 3rd | 5th | 7th | 9th | 11th |
|---|---|---|---|---|---|---|
| Control | 5.6$^{a}$± 0.1 | 6.05$^{a}$ ± 0.1 | 6.4$^{a}$±0.1 | Ss* | Ss* | Ss* |
| 2% GA | 5.6$^{a}$±0.1 | 5.68$^{b}$±0.1 | 5.85$^{b}$±0.1 | 5.64 $^{a}$±0.49 | 6.19 $^{a}$±0.1 | Ss* |
| 4% GA | 5.6$^{a}$±0.1 | 5.62$^{c}$±0.1 | 5.75$^{c}$±0.1 | 5.84 $^{a}$±0.1 | 6.12 $^{a}$±0.1 | 6.4±0.05 |

Means followed by different letters are significantly different (P<0.05) on the same day.
Ss*=Spoiled samples.

**Table 2:** Effect of coating sheet on TBA values (mean ± SD) of chilled meat chunks.

| Days | Zero | 3rd | 5th | 7th | 9th | 11th |
|---|---|---|---|---|---|---|
| Control | 0.47$^{a}$±0.05 | 0.81$^{a}$±0.14 | 0.9$^{a}$±0.05 | Ss* | Ss* | Ss* |
| 2% GA | 0.47$^{a}$±0.05 | 0.6$^{a}$±0.1 | 0.75$^{ab}$±0.1 | 0.85 $^{a}$±0.1 | 0.9 $^{a}$±0.05 | Ss* |
| 4% GA | 0.47$^{a}$±0.05 | 0.54$^{a}$±0.1 | 0.65$^{b}$±0.1 | 0.78 $^{a}$±0.1 | 0.81$^{a}$±0.1 | 0.9±0.05 |

Means followed by different letters are significantly different (P<0.05) on the same day.
Ss*=Spoiled samples.

**Table 3:** Effect of coating sheet on TVB-N values (mean ± SD) of chilled meat chunks.

| Days | Zero | 3rd | 5th | 7th | 9th | 11th |
|---|---|---|---|---|---|---|
| Control | 7.5$^{a}$±0.1 | 14$^{a}$±1 | 20$^{a}$±1 | Ss* | Ss* | Ss* |
| 2% GA | ±7.5$^{a}$±0.1 | 9.38$^{b}$±0.1 | 15$^{b}$±1 | 18.9$^{a}$±1 | 20$^{a}$±1 | Ss* |
| 4% GA | 7.5$^{a}$±0.1 | 8.4$^{b}$±0.1 | 11.9$^{c}$±1 | 14.6$^{b}$±0.5 | 19.5$^{a}$±1 | 20±0.5 |

Means followed by different letters are significantly different (P<0.05) on the same day.
Ss*=Spoiled samples.

**Table 4:** Effect of coating sheet on nitric oxide, total glutathione, and total antioxidant capacity values (mean ± SD) of chilled meat chunks.

| | NO(U/g) | GSH(U/g) | TAC (n.mol/g) |
|---|---|---|---|
| Control | 42.7 $^{a}$±0.2 | 4.2 $^{a}$±0.2 | 1.35 $^{a}$±0.02 |
| 2% GA | 56.6$^{b}$±0.2 | 4.46$^{b}$±0.02 | 1.46 $^{b}$±0.02 |
| 4% GA | 72.6$^{c}$±0.2 | 4.58 $^{b}$±0.02 | 1.52 $^{c}$±0.02 |

Means followed by different letters are significantly different (P<0.05) in the same column.

food spoilage (Djenane and Roncales 2018).

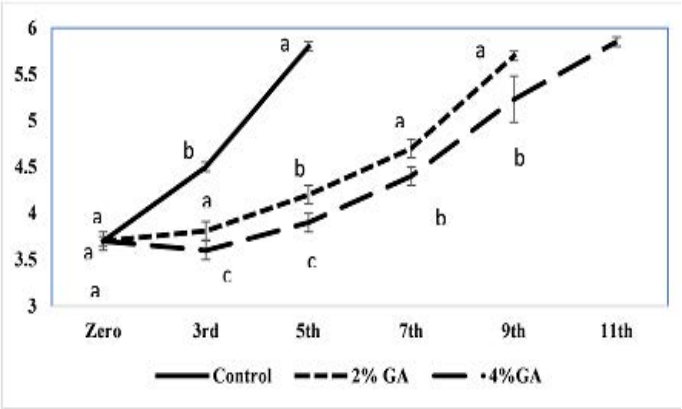


**Figure 1:** Effect of coating sheets on APC (mean ± SD log CFU/g) of chilled meat chunks. Means followed by different letters are significantly different (P<0.05) on the same day.

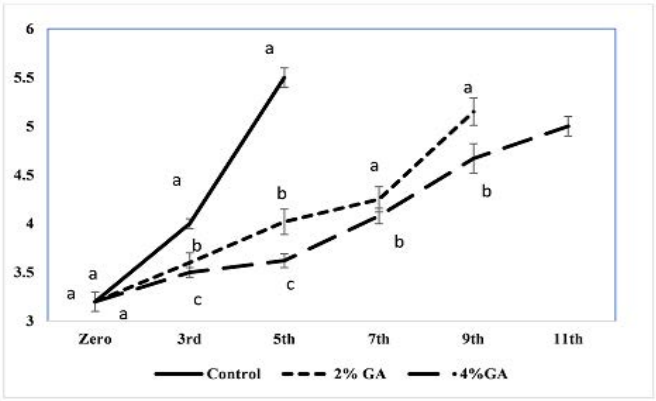


**Figure 2:** Effect of coating sheets on psychrotrophic count (mean ± SD log10 CFU/g) of chilled meat chunks. Means followed by different letters are significantly different (P<0.05) on the same day.

The pH of meat is a good indicator of its quality. Volatile gases such as trimethylamine and ammonia are produced either microbially or endogenously (by meat enzymes) during storage, and these gases may cause the PH to rise. The PH values of chilled meat in (Table 1) show gradually increasing values during storage. Chilled meat had a starting PH of 5.6, which increased through storage time. The PH values of control and coated samples were significantly different from the 3rd day of storage. According to the Egyptian Standard (EOS.36-11. 2006), chilled meat starts spoiling at PH 6.4.

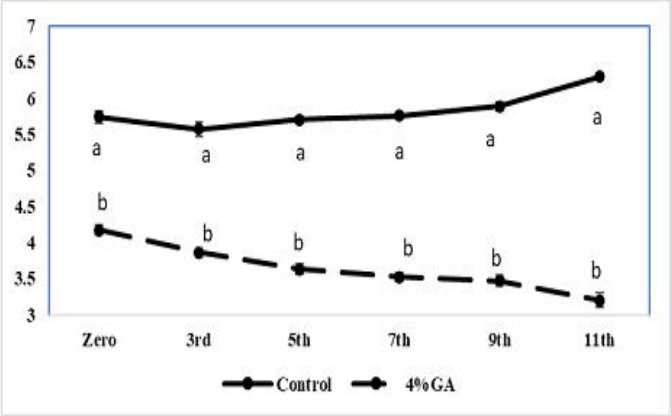


**Figure 3:** Effect of coating sheets on *E. coli* count (mean ± SD $\log_{10}$ CFU/g) of chilled meat chunks. Means followed by different letters are significantly different (P<0.05) in the same day

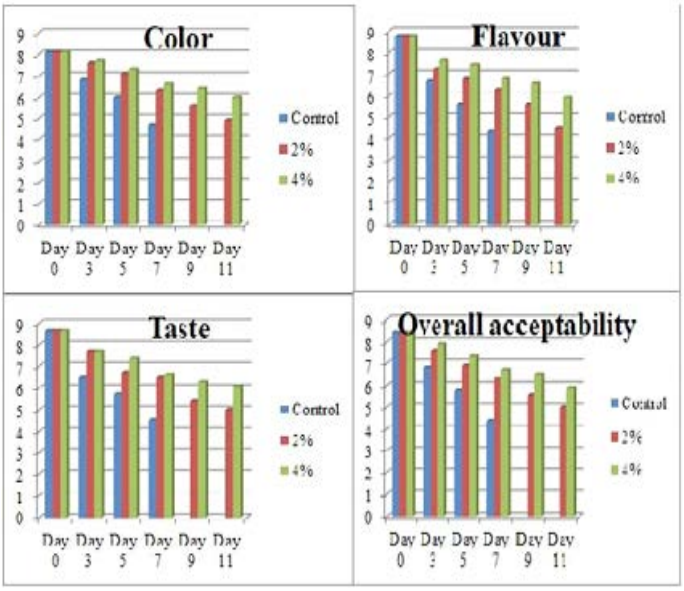


**Figure 4:** Sensory evaluation of control, 2%, and 4% gallic acid zein wax sheets cooked meat chunks (values represent the mean scores of nine panelists).

Lipid oxidation is a major problem of stored, chilled meat (Hansen et al., 2004) because it leads to an unpleasant rancid flavor. The chilled conditions of storage promote oxygen attack on the double bonds of fatty acids, producing free radicals (Kamaleldin et al., 2003). Lipid oxidation products, such as hydroperoxides, may also form, in addition to aldehydes, ketones, and alcohol compounds produced from oxidative polymerization. TBA is a universal test for secondary lipid oxidation (Kamaleldin et al., 2003). (Table 2) shows the TBA values resulting from GA/ zein wax coating of meat samples during chilled storage. The TBA value before storage was 0.47 mg MDA/kg. This value increased throughout the storage period in all treatments. The TBA value at 4% GA differed significantly from that of the control on day five of chilled storage. According to the Egyptian Standard (EOS 3602/2013), the TBA of fresh meat should be lower than 0.9 mg MAD/kg. The TBA value of the control meat reached 0.9 mg MDA/ kg, five days after chilled storage. However, with 2% and 4% GA/ zein wax coatings, TBA values reached this upper limit at the 9th and 11th day of chilled storage, respectively. Therefore, incorporation of GA into the zein wax sheets can enhance the barrier features of zein wax to oxygen, thus lowering the exposure of meat to oxygen and the resulting oxidation (Sun et al., 2014).

TVB-N is the main indicator of the freshness of chilled meat. The TVB-N values in (Table 3) indicate increasing values with storage time. According to the Egyptian Standard (EOS. 3602/2013), meat products start spoiling when the TVB-N value reaches20 mg/100 g sample. Based on this standard, it took 5 days for the control meat to spoil, whereas the samples coated with 2% and 4% GA/ zein wax sheets began spoiling on the 9th and 11thdays of chilled storage, respectively. This result may be due to the superior antioxidant and antiseptic capacities of GA. Previous research has noted that lower bacterial abundance and higher antioxidant levels are associated with lower activity of the enzyme decarboxylase, which then results in higher TVB-N values (Wook et al., 2016).

The results of the evaluations of color, flavor, taste, and overall acceptability of cooked meat chunks during the cold storage period show high scores of 8.1, 8.78, 8.67, and 8.4, respectively, at the start of the storage time (Figure 4). The sensory evaluation scores decreased gradually through storage time. The GA/ zein wax sheets did not modify the sensory characteristics of meat chunks, and they also tended to improve the sensory scores when compared with the control. Higher scores were obtained by meat treated with the higher GA concentration, resulting in prolonged shelf life.

Based on chemical, bacteriological, and sensory analyses, the shelf lives of the control meat, and meats coated with2% and 4% GA were 7, 9, and 11 days, respectively. Therefore, GA/ zein wax coating sheets increased the shelf

life of chilled meat chunks. Without treatment, meats were considered unacceptable by sensory evaluation after 7 days of storage, whereas meats coated by 2% and 4% GA were acceptable until 9 and 11 days of storage, respectively. This result may be attributed to that the higher levels of oxidative and microbial stability of coated meat chunks, which are, in turn, mainly due to the antimicrobial and antioxidant activities of GA and the ability of zein wax sheets to act as a barrier to oxygen and water. Thus, GA and zein wax sheets inhibit oxidation and microbial growth, therebypreserving the quality of meat (Alizadeh-Behbahani, et al., 2021; Barzegar et al., 2020; Kiarsi et al., 2020).

The potential effect of GA against *E. coli* O26 is shown in (Figure 3). Throughout the storage period, *E. coli* O26 was significantly less abundant on meat coated with zein wax sheets containing 4% GA compared to levels in the control meat chunks. After 11 days of chilled storage, the abundance levels of *E. coli* O26 were 6.3± 0.05 and 3.2± 0.1 $\log_{10}$ CFU/g in the control and coated meat chunks, respectively. These results are similar to those obtained by Rodríguez Vaquero et al. (2010). The inhibitory effect of GA/ zein wax coating sheets on *E. coli* is likely due to the ability of GA to disrupt peptidogly can and/or disintegrate the outer membrane of bacteria through the chelation of divalent cations (Nohynek et al., 2006; Neo et al., 2013).

The antioxidant capacity of the coated meat chunks with 4% GA/ zein wax sheet was significantly higher than those of the control and 2% GA/ zein wax sheet-treated meat chunks (Table 4). The antioxidant capacities of the control and the 2% and 4% GA/ zein wax treatments were 1.35± 0.02, 1.46± 0.02, and 1.52± 0.02 nmol/g, respectively. This result can be explained by the effectiveness of GA as an antioxidant that can sustain free radicals and terminate the oxidation chain reaction (Badhani et al., 2015). Gallic acid can be adsorbed on the surface of coated meat chunks and antioxidant components can also diffuse from coating materials into the meat. The glutathione content of coated meat chunks was significantly higher than that of the control; however, the glutathione contents of the two GA treatments did not differ significantly. This result may be because GA improves both the shelf life and the quality of meat. The concentration of GSH concentration in freshly prepared meats is relatively high (Cobiac and Syrette 2000). The concentration of NO was much higher in both GA treatments (42.7± 0.2, 56.6± 0.2, and 72.6± 0.2U/g in the control, 2% and 4% GA/ zein wax sheet treatments, respectively). NO is an unstable molecule and its levels in the bloodstream diminish rapidly, requiring it to be continuously restored. The consumption of antioxidants can improve its stability and limit its disintegration. Antioxidants can prevent NO from breaking down, thus increasing its lifespan (Yang et al., 2018).The primary cause of meat deterioration is the oxidation of lipids high in polyunsaturated fatty acids (Mukumbo et al., 2020). Lipid oxidation produces many by-products, such as cholesterol oxides, malonaldehyde, and 4–hydroxynonenal, which are all potential carcinogens (Csala et al., 2015).The free radicals that are generated during oxidative processes can increase oxidative stress in the consumer's body (Chen et al., 2018). The increase in levels of antioxidant compounds plays a special role in improving the shelf life of meat (Ribeiro et al., 2019).

## CONCLUSION

This study has demonstrated the antimicrobial and antioxidant properties of GA. Its incorporation into zein films increased the shelf life and antioxidant properties of meat chunks. GA/ zein wax film reduced microbial growth and inhibited the occurrence of undesirable chemical reactions in meat during chilled storage. Therefore, GA can extend the shelf life of veal meat chunks. Moreover, it preserves the antioxidant capacity and maintains the glutathione and NO contents of stored meat chunks. It also reduced the abundance of *E. coli* O26 by 3 $\log_{10}$ when compared to control samples. Therefore, the incorporation of GA into zein wax film can effectively improve the stability and shelf life of veal meat chunks during chilled storage.

### The study time limitation

The study was designed to evaluate the effect of treatment on extending the shelf life of the refrigerated veal meat chunks and ended while the meat cuts of the treated group be spoiled.

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## CONFLICT OF INTEREST

The authors have declared no conflict of interest.

## NOVELTY STATEMENT

GA is known as a potent antioxidant and antibacterial. Zein films are used as edible and biodegradable coatings on vegetables and nuts. This study aimed to evaluate the effect of GA/ zein wax sheets on shelf life and control of E. coli O26 contamination of chilled meat chunks.

## AUTHORS CONTRIBUTION

Asmaa Sh. Fayed: Preparation of gallic acid/zein, Microbial quality wax film, Chemical quality, and writing. Safaa, M. Abo El-Soud: Biochemical analysis and reviewing.

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