The antimicrobial activity of pomegranate peel extract incorporated in edible chitosan and gelatin coatings against *Salmonella enterica* on Medjool dates

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Abstract

This study aims to investigate the *in vitro* antimicrobial activity of pomegranate peel extract (PPE) against five strains of *Salmonella enterica* at 37°C. The *in vivo* antimicrobial activities of 2.5% chitosan or 9% gelatin coatings containing 0.0–10.0% PPE against *S. enterica*, total mesophilic bacterial count, and yeasts and molds on Medjool dates at 4°C and 24°C for 56 days were also investigated. *Salmonella*-inoculated Majdool dates dipped in distilled water were deemed as the control. The pH, water activity (a_w), and color changes of Medjool date homogenates were also evaluated. The minimum inhibitory concentrations and minimum bactericidal concentrations of PPE ranged from 7.81 to 15.63 mg/mL and 15.63 to 31.25 mg/mL, respectively, against *S. enterica* at 37°C. In general, *S. enterica* survival on dates was more pronounced at 4°C than at 24°C, regardless of the treatment. Further, the initial numbers of *S. enterica* (3.9–5.5 log colony-forming unit [CFU]/g) in dates coated with either gelatin or chitosan containing 2.5–10.0% PPE at 24°C were reduced to non-detectable levels by enrichment (<1 CFU/15 g) after 56 days of storage. At 4°C, *S. enterica* was significantly reduced ($p \leq 0.05$) by 1.3 log CFU/g to >4.5 log CFU/g by day 56 on PPE-coated dates, compared to uncoated samples. Yeast and molds on the treated dates were reduced below detectable levels (2 log CFU/g) on day 42 at both 4°C and 24°C. Coating type or PPE did not affect the appearance of dates, but only slightly affected the overall acceptability of the product. The current study demonstrates that using PPE incorporated into chitosan or gelatin could be used as an effective natural strategy to enhance the safety of dates.

Keywords: antimicrobial coating; chitosan; gelatin; Medjool dates; pomegranate peel extract, *Salmonella enterica*

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Introduction

Foodborne infections caused by microbial contamination of foods are a problematic issue worldwide (Olaimat *et al*., 2020). In the last few decades, there have been numerous significant foodborne outbreaks worldwide, raising concerns about microbiological safety (Piližota, 2023). According to World Health Organization (WHO, 2018a) estimates, 420,000 people die from foodborne illness each year, and more than 33 million healthy life years are lost because more than 600 million individuals are being made ill by contaminated food. In addition, an estimated $110 billion is wasted annually due to medical costs and lost productivity because of eating unsafe food (Food Guard, 2024). Generally, the majority of foodborne illnesses are linked to foods with high water activity (a_w), where bacteria multiply and grow (Olaimat *et al*., 2020). However, recent foodborne illness outbreaks have been linked to low a_w ready-to-eat foods, such as dried fruits, nuts, and chocolate, and involved longer periods of foodborne pathogen (FBP) survival, which can increase risk of contracting food poisoning by individuals, especially children, pregnant women, elderly, and immunocompromised individuals (Brar and Danyluk, 2018; Olaimat *et al*., 2020).

Salmonella is a common pathogenic bacterium that frequently causes foodborne illness outbreaks, and it is still the leading cause of bacterial illnesses in the United States and other developed countries (Jayeola *et al*., 2021). It is estimated that more than a million Americans contract *Salmonella* yearly, of whom 26,500 require hospitalization and among these, roughly 420 deaths occur (Scallan *et al*., 2011). *S. enterica* can cause large numbers of outbreaks, especially in low a_w foods, due to its ability to survive under harsh environmental conditions (Wei *et al*., 2020). *Salmonella* was responsible for the vast majority (>83%) of food-borne outbreaks related to low a_w food in the United States between 2007 and 2018 (Jayeola *et al*., 2021).

Dried fruits have been connected to numerous foodborne outbreaks and recalls (Beuchat *et al*., 2013). In 2019, in Norway, exotic dried fruits served as a vehicle for the transmission of *S. Agbeni*, and it resulted in 56 confirmed cases of foodborne illness (Johansen *et al*., 2021). Also, in 2018 in the United States, dried coconut was implicated in *S.* Typhimurium contamination and infection, with 14 reported cases (Centers for Disease Control and Prevention [CDC], 2019). Imported Medjool dates in the United Kingdom were voluntary recalled in 2021 due to contamination with hepatitis A virus (Garcia Vilaplana *et al*., 2021). Date fruits may be contaminated at any stage from farm to table in several ways, and often by the use of uncontrolled harvesting and post-harvesting protocols, post-processing/packaging contamination, cross-contamination with ingredients or equipment, contaminated transport containers or vehicles, improper storage conditions, or human handling resulting from poor sanitation and hygiene (Olaimat *et al*., 2020; Wei *et al*., 2020).

Although using synthetic antioxidants (such as butylated hydroxyanisole [BHA], butylated hydroxytoluene [BHT], tertiary butylhydroquinone [TBHQ], and antioxidants propyl gallate and ethoxyquin) and antimicrobials (such as sodium benzoate, potassium sorbate, sorbic acid, and parabens) can be effective against FBP, there are serious risks associated with their excessive or long-term use in the food chain, including cytotoxic, genotoxic, and carcinogenic effects (Esazadeh *et al.*, 2024; Pisoschi *et al*., 2018). Consequently, the food industry has set out to pursue greater use of clean labels and replace synthetic preservatives with natural ones (Alizadeh Behbahani *et al*., 2020). Therefore, adoption of natural agents to enhance the safety of food is becoming more common in the food industry. Companies are eager to use ingredients that have good antimicrobial and antioxidant activities, have no or minimal negative effects on the organoleptic properties of food, and are in good supply, inexpensive, and easy to obtain and extract (Alexandre *et al*., 2019; Chen *et al*., 2020; Yousef *et al*., 2021).

The rind or peel of a pomegranate is considered as waste by consumers, but it is possible to recycle it as an ingredient in the formulation of an active antimicrobial agent useful for the safety of food products (Alexandre *et al*., 2019; Chen *et al*., 2020; Elsherbiny *et al*., 2016). Accordingly, several studies investigated the addition of pomegranate peel extract (PPE) as an antimicrobial agent in food packaging materials to improve the longevity, safety, quality, antioxidant, and organoleptic characteristics of food products (Kumar *et al*., 2022; Ramos *et al*., 2016; Saadat *et al*., 2021). However, only one study, conducted by Alqahtani *et al*. (2023), determined the antimicrobial activity of 0.1% aqueous PPE or 1% lactic acid, or their combination as disinfection solutions against yeasts and molds on fresh Barhi date fruits at the Khalal stage at 4°C for 6 weeks. This study focused on the physicochemical properties and antioxidant activity of PPE, color parameters, texture profile, sensory characteristics, and microbiological quality of date fruits treated with PPE. However, Alqahtani *et al*.'s (2023) study did not test the antibacterial activity of PPE against the total bacterial count or specific foodborne bacterium on the dates. In addition, a suitable carrier must be used to deliver naturally occurring bioactive substances by their controlled release at food surface (Surendhiran *et al*., 2020).

About one-third of food is wasted globally (Galanakis, 2020); therefore, developing new and creative ways to extend the shelf life of fresh foodstuffs, such as fruits

and vegetables, is highly desired in the food sector. Edible packaging materials are developed commercially in the food sector, primarily in the United States, with an expected annual expansion rate of 14.31% from 2022 to 2030. Also, global edible packaging use was estimated as US$0.84 billion in 2021 and is expected to increase to US$2.8 billion by 2030 (Nair *et al*., 2023). The use of edible coatings on fruits and vegetables has various advantages, including an increase in carbon dioxide concentration, decrease in moisture penetration, oxygen availability, slowing down the respiration rate and the ripening process, reduction of water loss, and production of ethylene gas (Lin and Zhao, 2007). In addition, coatings can be formulated to contain antimicrobial agents, thus leading reduced harmful effects on consumers, thereby enhancing food safety and quality (Dhall, 2013; Lin and Zhao, 2007). The concept of bioactive edible coatings is based on impregnating food-grade coating materials, such as chitosan and gelatin, with antimicrobial substances capable of imparting antioxidant or antibacterial properties to inhibit microbial growth, preserve freshness, and extend the shelf life of foods (Surendhiran *et al*., 2020). Chitosan is a polysaccharide-based compound with desirable characteristics like biocompatibility, biodegradability, antimicrobial activity, capacity to disrupt outer membrane of target cells, and gas and aroma barrier properties (Surendhiran *et al*., 2020). Gelatin is a protein-based material valuable in the creation of edible coatings because of its availability and biodegradability (Alparslan *et al*., 2019; Yousef *et al*., 2021). Furthermore, several methods of pomegranate peel extraction have been developed. Of these methods, the methanolic method outperformed petroleum ether, chloroform, and water extracts, especially in achieving better antimicrobial activity (Alexandre *et al*., 2019; Chen *et al*., 2020; Elsherbiny *et al*., 2016).

To the best of our knowledge, there have been no studies to date investigating the antimicrobial activity of PPE incorporated in edible coatings against FBP on date fruits. Therefore, this study was designed to investigate: (i) the *in vitro* antimicrobial activity of methanolic PPE against *S. enterica* strains at 37°C for 24 h; and (ii) the antimicrobial activity of different concentrations of methanolic PPE (0%, 2.5%, 5%, and 10%) incorporated into edible chitosan or gelatin coatings against *S. enterica*, total mesophilic bacterial count (TMC), and yeasts and molds on Medjool dates during storage at 4°C and 24°C for 56 days.

Materials and Methods

Bacterial strains and culture conditions

Five strains of *S. enterica*, such as *S. copenhagen* PT99, *S. enteritidis* CRIFS 1016, *S. heidelberg* 271, *S. kentucky* 64701, and *S.* Typhimurium 02:8423, were employed in the present study. Each strain was frozen at -40°C in brain–heart infusion broth (BHI broth; Oxoid Ltd., Basingstoke, UK) containing 30% glycerol. All strains were obtained from the culture collection of the Food Microbiology Laboratory of the Hashemite University. *S. enterica* strains were either human clinical or food (plant and animal) isolates.

S. enterica strains were revived by streaking a loopful of each frozen culture on tryptone soy agar (TSA; Oxoid Ltd.) plates, and incubated aerobically at 37°C for 18–24 h. After the growth of each strain, a single colony was plated on *S. shigella* (SS) agar (Oxoid Ltd.) and incubated aerobically at 37°C for 18–24 h. A single colony from the selective agars was placed in BHI broth and incubated for 18–24 h at 37°C. Then, 0.1 mL from the culture was inoculated into 10-mL sterile fresh BHI broth tubes, which were incubated at 37°C for 18–24 h and used in future steps of the experiments. For the cocktail preparation, 2 mL of *S. enterica* culture was pooled with each of four other strains in a new sterilized tube. The collected 10 mL cocktail containing each bacterium was centrifuged for three times for 18 min at 5,000 ×g. After each centrifugation, pellets were washed with 0.1% peptone water (Oxoid Ltd.). Finally, the pellets were diluted in 10-mL peptone water to yield ~8 log colony-forming unit (CFU)/mL.

Preparation of pomegranate peel extract by methanol

Pomegranate peels were extracted according to the method described by Elsherbiny *et al*. (2016) with some modifications. Dark red pomegranate fruits (Akkawi variety) were purchased from a local market in November 2022 (Althaqafah Stores, Irbid, Jordan). Initially, whole fruit were washed with distilled water to remove adhering dust and dirt. Peels were manually removed using a sharp knife, and the peeled fruit were washed again with distilled water. Next, the peels were allowed to air-dry for 3 h in a closed room using a fan to accelerate the drying process. Samples were then packed in sterilized bags and stored at -18°C till starting of the extraction process. For extraction, the peels were thawed at 4°C and placed in an oven (Microprocessor oven, Model 1350 GX-2CE, Sheldon; Cornelius, OR, USA) at 50°C for 24 h. The dried peels were treated in a grinder (National; Amman, Jordan) for 2 min to obtain a soft powder. To ensure consistency, 10 batches of powder were processed under identical conditions and all powder samples obtained from different batches was mixed together.

A peel powder sample of 100 g was soaked in an aluminum foil-wrapped flask containing 1 L of methanol for 2 days at room temperature with shaking. Then, the

mixture was filtered twice using double-layered Whatman No.1 filter paper (Double Rings Filter Papers qualitative No. 102, medium, Ø 125 mm; Marsfield, Australia) using a vacuum pump (KNF Neuberger N035.1.2.AN.18; Caerphilly, South Wales). Afterward, the extract was concentrated using a rotary vacuum evaporator (Buchi R-215 Rotavapor System w/ chiller; Flawil, Switzerland) at 60 rpm, with methanol as a solvent at 50°C. Finally, the concentrated viscous liquid extract, having a honey-like consistency, was placed in sterilized tubes and stored at −18°C before use.

Pomegranate peel yield

The final concentrated PPE yield based on dried peels was assessed according to the following equation:

$$\text{Yield (final concentrated PPE)} = \frac{W_2}{W_3} \times 100\%,$$

where W_2 is the weight of the final PPE sample after using a rotary evaporator, and W_3 is the weight of dried powdered peels.

PPE based on fresh peels was assessed according to the following equation:

$$\text{Yield (final concentrated PPE)} = \frac{W_2}{W_1} \times 100\%,$$

where W_2 is the weight of the final PPE sample after using a rotary evaporator, and W_1 is the weight of fresh peels.

Determination of minimum inhibitory (MIC) and minimum bactericidal (MBC) concentrations of PPE against *S. enterica* strains

The antimicrobial activity of PPE was tested against each *S. enterica* strain by determining MIC and MBC using the microdilution method in 96-well microtiter plates according to Jaradat *et al*. (2022) and Wiegand *et al*. (2008).

Each strain was grown on TSA plates and incubated aerobically at 37°C for 18–24 h, suspended in Mueller–Hinton broth (MHB; Oxoid Ltd.) and adjusted to 1×10^8 CFU/mL (0.5 McFarland scale, OD = 0.08-–0.13) at 625 nm using a spectrophotometer (JENWAY 6105 UV-visible spectrophotometer; Loughborough, UK), followed by serial dilutions of each strain to obtain the final inoculum of 5.0×10^5 CFU/mL in reaction well. Then, 1,000 mg/mL (50% w/v) of PPE as a stock solution was prepared by dissolving 1-g PPE in 1 mL MHB broth followed by vortex agitation to obtain a uniform mixture. The wells of microtiter plate were filled with 100-µL MHB broth, and 100 µL of PPE stock solution was added to the first well. Then, 100 µL was transferred into the adjacent well and this was repeated to complete dilution series. At the last well after mixing, 100 µL was discarded to maintain a uniform volume in tests. Then, 100 µL of each bacterial strain was added to each well in separate tests to obtain 10 different concentrations of PPE (250, 125, 62.5, 31.25, 15.6, 7.8, 3.9, 1.95, 0.98, and 0.49 mg/mL). Negative control wells were prepared by adding 100 µL of fresh sterile MHB into 100 µL of PPE, while positive control wells were prepared by adding 100 µL of bacterial culture into 100-µL MHB. The microtiter plates were incubated at 37°C for 16–20 h. Then, 100 µL of each well was diluted and 100 µL of an appropriate dilution was placed on TSA plates, which were incubated at 37°C for 24 h.

The lowest concentration of PPE that showed no visible growth of bacteria was defined as the MIC. While the MBC was determined as the lowest concentration of PPE causing a ≥3 log CFU/mL reduction of the initial number of each bacterial strain (Olaimat *et al*., 2014).

Preparation of edible coatings with PPE

Gelatin coating preparation

Gelatin powder from hydrolyzed fish collagen was used to prepare a 9% gelatin coating according to the method described by Yousef *et al*. (2021). This was done by dissolving 9-g gelatin (Foodchem; Shanghai, China) in 100 mL of distilled water at room temperature. The mixture was agitated by a magnetic stirrer until the gelatin was completely dissolved, heated to 45°C for 15 min using a hotplate (Kewlab; Melbourne, Australia) and 5-g glycerol was added. Finally, three different concentrations of PPE (2.5, 5, and 10 g) were dissolved separately in 5-mL sterile distilled water, and these PPE solutions were separately added to gelatin coating solutions to yield a final volume of 100 mL. The mixtures were agitated using a magnetic stirrer for 10 min and then treated in a blender until a uniform solution was obtained. Four coating treatments were prepared: control without PPE, and 2.5%, 5.0%, and 10.0% PPE.

Chitosan coating preparation

The chitosan coating solution was prepared by following the method described by Olaimat and Holley (2015). In brief, 2.5 g of chitosan powder (medium molecular weight, 75–85% deacetylated chitin, poly(d-glucosamine); Sigma-Aldrich, Germany) was added to 1% acetic acid (Carlo ERBA Reagents; GmbH, France) and stirred on a hotplate for 60 min at 60°C to achieve the final chitosan concentration of 2.5% (w/v). As a plasticizer, 1% (v/v) glycerol (Carlo ERBA Reagents) was added to the solution and agitated with a magnetic stirrer for 15 min at 60°C. Finally, three different amounts of PPE (2.5, 5, and 10 g)

were dissolved separately in 5-mL sterile distilled water, and these PPE solutions were separately added to chitosan coating solutions to yield a final volume of 100 mL. The mixtures were agitated using a magnetic stirrer for 10 min and treated in a blender until a smooth solution was achieved. This yielded four coating treatments: control without PPE, and 2.5%, 5.0%, and 10.0% PPE.

Medjool date sampling

Whole, riped, pre-sorted, and brushed Medjool date varieties were used in this study. The ultimate soft, completely ripe, dark brown Medjool dates at the Tamar stage were purchased from a local company (Palmera; Amman, Jordan). The dates were from palm date trees grown locally in Jordan Valley, harvested and collected in September 2023. Medjool dates were of medium size with a weight range of 15–17 g for each piece and graded as of premium quality that had no fruits with broken skins, scarring, sunburn, insect injury, or puffiness with skin separation of 0–10% and ~26% moisture content.

Inoculation of Medjool dates with *S. enterica*

For inoculation, each Medjool date sample was dipped into the inoculant solution of ~8 log CFU/mL *S. enterica* for 1 min to ensure bacterial attachment and uniform inoculum distribution. Samples were then picked using sterilized forceps and placed in sterilized (autoclaved at 121°C for 20 min) perforated trays under sterilized conditions and allowed to completely dry in a biohazard safety cabinet for 3 h.

Treatment of Medjool dates

The inoculated Medjool dates were dipped in chitosan or gelatin coating containing different concentrations of PPE (0.0, 2.5, 5.0, and 10.0%) for 90 s to allow the complete wetting of date surfaces. Control samples were dipped in distilled water. After that, the coating solution was allowed to drain from date surfaces and the fruit was subjected to air-drying under aseptic conditions in a safety cabinet for 3 h to form fully intact coatings on its surfaces. The inoculated date samples were stored in sterilized bags at 4°C±0.5°C or 24°C±0.5°C.

Antimicrobial activity of chitosan and gelatin coating containing PPE against *S. enterica* on Medjool dates during storage

A piece of treated Medjool dates was taken at 10 intervals (on day 0, 3, 7, 14, 21, 28, 35, 42, 49, and 56) using a sterile spoon and weighed in a sterile stomacher bag. A nine-fold dilution using 0.1% sterile buffered peptone water was added to solubilize samples using a stomacher for 1 min (Stomacher, Easy Mix; AES Laboratories, Combourg, France). Each sample was serially diluted and 0.1 mL was spread onto the surfaces of SS agar and TSA plates. Yeast and mold colonies were counted using Sabouraud agar plates (Oxoid Ltd.). The inoculated plates were aerobically incubated at 37°C for 24 h, and the typical colonies of *S. enterica*, TMC, and yeast and mold were enumerated.

Sample enrichment was done when bacterial cells were below the detection level (<2 log CFU/g). This was done by combining diluted samples in stomacher bags with ~150 mL of double-strength BHI broth and incubating them for 24 h at 37°C. A loopful from each bag was then streaked on proper selective and general agar and incubated at 37°C for 24 h. The detection level of positive samples by enrichment in BHI broth was ≥1 CFU/15 g.

Color evaluation

The date skin color was assessed objectively using a Hunter colorimeter (Color TEC-PCMTM, Cole-Parmer; Accuracy Microsensors, Pittsford, NY, USA) at the end of storage (56 days). Observations were expressed using a three-value scale of L*, a*, and b*. L* value in this coordinate system represented lightness and ranged from 0 (black) to 100 (white). Green intensity (-100) to red intensity (+100) was measured by a* value. While blue intensity (-100) to yellow intensity (+100) was measured by b* value. A single measurement was expressed by the average of the three readings taken from different places of the outer shell of the whole fruit of Medjool dates at 4°C and 24°C (Mehyar *et al.*, 2014).

pH and a_w evaluations

The pH and a_w values of all treated or non-treated date samples were measured at room temperature at initial and final storage periods (0 and 56 days of storage) at 4°C and 24°C. The pH values were measured using an Adwa pH meter (AD1000; Adwa, Romania). The a_w values of date samples were measured using an electronic a_w meter (Aqualab Series, Hygrolab; Rotronic Instr. Corp, Huntington, NY, USA).

Statistical analysis

All samples were examined in four independent trials (n = 4). The data were analyzed using IBM SPSS, version 26 (IBM, New York, USA), and $p \leq 0.05$ was considered

statistically significant. The results were expressed as mean ± standard deviation (SD). A one-way ANOVA test was used to compare mean microbial numbers within variable treatments (the same treatment with different concentrations and/or times), while the Tukey's Honest Significant Difference (HSD) test was used to compare microbial numbers between the treated samples.

Results and Discussion

PPE yield

The final average yield of methanolic PPE was 28.0±2.4% on a dry matter basis after oven-drying and 9.3±1.0% based on fresh peels. The PPE yield in this study at 28.0% was greater than that reported by Ghasemi *et al*. (2023), who reported that PPE yield was 18.9% using methanol/water (50:50 v/v) as a solvent and this difference could have resulted from differences in polarity between the two solvents, raw materials, and pomegranate variety. Indeed, other studies showed greater PPE yields than that of the current study. Nair *et al*. (2018) indicated that using 80% ethanol as a solvent for the extraction of pomegranate peels gave a yield of 43.6%. Ali and Kumar (2014) also reported that the final yields of a methanolic extract using soxhlet and ultrasound-assisted extractions were 36.8% and 44.0%, respectively. In addition, Shiban *et al*. (2012) reported that their highest extract yield of 45.4% was obtained using 80% methanol, rather than water and diethyl ether. However, the extract yield of PPE in the current study was roughly consistent with 23.6–29.2% reported by Iqbal *et al*. (2008), Kennas and Amellal-Chibane (2019), and Padmaja and Prasad (2011), who used methanol as a solvent for extraction.

MIC and MBC of methanolic PPE against different *S. enterica* strains

The MIC and MBC values of PPE against the five strains of *S. enterica* using the broth microdilution method were 15.63 mg/mL and 31.25 mg/mL at 37°C, respectively, for all strains except for *S*. Typhimurium 02:8423, which had significantly lower values of MIC (7.81 mg/mL) and MBC (15.63 mg/mL) (Table 1). In recent studies, the antimicrobial activity of PPE was tested against different microorganisms. Alexandre *et al*. (2019) investigated the antimicrobial activity of PPE prepared by two combined extraction methods involving enzymes (pectinase and cellulase) and high pressure (300 MPa), and it was reported that the MIC and MBC values of PPE were 15.63 mg/mL and >125 mg/mL, respectively, against *L. monocytogenes*, and 62.5 and 125 mg/mL, respectively, against *S. enteritidis*. The current study found lower MIC values against *S. enterica* strains (Table 1). Tinrat and Singhapol (2017) also investigated PPE with 95% ethanol against different FBP and found that MIC and MBC were 0.781 mg/mL and 12.50 mg/mL, respectively, against *B. cereus*; 0.39 mg/mL and 6.25 mg/mL, respectively, against *S. aureus*; 3.125 mg/mL and >50.00 mg/mL, respectively, against *S.* Typhimurium; and 0.781 mg/mL and 25.00 mg/mL, respectively, against *E. coli*.

In addition, Wafa *et al*. (2017) reported that MIC and MBC of PPE extracted by a mixture of solvents (water, methanol, and ethanol) were 10.75 mg/mL and 12.75 mg/mL, respectively, against *S. enteritidis*; and 12.50 mg/mL and 12.75 mg/mL, respectively, against *S. kentucky*. These results were consistent with the results of the present study, where MIC and MBC were 7.81–15.63 mg/mL and 15.63–31.25 mg/mL, respectively, against *S. enterica* strains.

On the other hand, Yassin *et al*. (2021) reported that the MIC and MBC of methanolic PPE against *E. coli*, *S.* Typhimurium, *S. aureus*, and methicillin-resistant *S. aureus* (MRSA) ranged from 0.125 to 0.50 mg/mL and 0.25 to 2.00 mg/mL, respectively. However, several factors could be responsible for variation observed in MIC and MBC values. These include the content and type of antimicrobial substances, bacterial strains tested, target bacterial resistances, timing of harvesting, climate, geographical location, plant age, processing used, drying and extraction techniques, and growth stage; all these factors play a crucial role in the antimicrobial activity of PPE (Alexandre *et al*., 2019; Duman *et al*., 2009; Rosas-Burgos *et al*., 2016; Yassin *et al*., 2021). The major antimicrobial components of PPE in the current study were 5-hydroxymethylfurfural (5-HMF), 3-aminopyrazine 1-oxide, cirsiumaldehyde, and furfural (results not shown).

Table 1. Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of methanolic pomegranate peel extract against different strains of *S. enterica* incubated at 37°C for 24 h.

S. enterica strains*	MIC (mg/mL)	MBC (mg/mL)
S. copenhagen PT99	15.63±0.08^{a,A}	31.25±0.01^{a,B}
S. enteritidis CRIFS 1016	15.63±0.07^{a,A}	31.25±0.05^{a,B}
S. heidelberg 271	15.63±0.05^{a,A}	31.25±0.00^{a,B}
S. kentucky 64701	15.63±0.09^{a,A}	31.25±0.07^{a,B}
S. Typhimurium 02:8423	7.81±0.09^{b,A}	15.63±0.08^{b,B}

*The initial number of *S. enterica* strains was 5.8–6.0 log CFU/mL.
Mean values in the same row with different uppercase superscript letters are significantly different ($p \leq 0.05$) by *t*-test.
Mean values in the same column with different lowercase superscript letters are significantly different ($p \leq 0.05$).

Survival of *S. enterica* on Medjool dates stored at 4°C and 24°C

The initial number of *S. enterica* on day 0 in the control samples was 5.8 log CFU/g. When stored at 4°C for 56 days, *S. enterica* numbers were significantly ($p \leq 0.05$) reduced by 1.3 log CFU/g (Table 2). Additionally, the number of *S. enterica* was further reduced to 3.8 log CFU/g between day 21 and day 42 at 24°C (Table 3). It again declined significantly ($p \leq 0.05$) to become undetectable on day 49 by direct plating (<2 log CFU/g) or on day 56 after enrichment (1 CFU/15 g) (Tables 2 and 3); this could be due to low a_w (0.64–0.69) of dates that lacked sufficient available water to sustain bacterial growth and/or the intrinsic antimicrobial activity of dates (Beuchat *et al*., 2013; Hussain *et al*., 2019).

Antimicrobial activity of chitosan and gelatin coatings containing PPE against *S. enterica*, total mesophilic bacteria, and yeasts and molds in S. enterica-inoculated Medjool dates during storage at 4°C and 24°C

The dates are susceptible to microbial contamination as they are produced and harvested in hot climates (Sarraf *et al*., 2021). In addition, the natural microbiota could be introduced to dates during harvesting, post-harvest handling, or from microorganisms present in the soil (Jdaini *et al.,* 2022). Because of their high sugar content and low water activity, the dates are usually considered a shelf-stable product and can be stored in room temperature (20–25°C). However, dates can be stored at lower temperatures (-18–5°C) to prevent disease incidence, sugar spots, color changes, syrupiness processes, and insect infestation (Sarraf *et al*., 2021). The antimicrobial activity of chitosan and gelatin coatings containing PPE increased with increase in concentration and temperature. Significant reduction was noted in *S. enterica* numbers on inoculated Medjool date samples treated with either gelatin or chitosan coatings containing 2.5%, 5.0%, and 10.0% PPE from day 0 compared to the control. Gelatin coating containing 5.0% and 10.0% PPE significantly ($p \leq 0.05$) reduced the number of *S. enterica* on Medjool dates by 0.8 log CFU/g and 1.2 log CFU/g, respectively, on day 0, compared to the control at 24°C. On the other hand, the chitosan coating containing 5.0% and 10.0% PPE significantly ($p \leq 0.05$) reduced the number of *S. enterica* on day 0 by 1.7 log CFU/g and 1.9 log CFU/g, respectively, compared to the control (untreated dates) at 24°C. *S. enterica* numbers on Medjool dates were sharply reduced to reach undetectable levels by direct plating in gelatin-coated treatments without PPE or by enrichment in chitosan-coated treatments without PPE after 35 days at 24°C. However, the addition of 2.5%, 5.0%, and 10.0% PPE to gelatin or chitosan coatings reduced *S. enterica* numbers to undetectable levels by enrichment at 14, 14, and 7 days, respectively (Table 3). On the other hand, *S. enterica* numbers on Medjool dates treated with gelatin coatings containing 2.5%, 5.0%, and 10.0% PPE were 3.2 log CFU/g, 2.8 log CFU/g, and 2.1 log CFU/g, respectively, compared to untreated dates (control) and gelatin-coated dates, in which numbers were 4.5 log CFU/g and 4.4 log CFU/g, respectively, at the end of storage at 4°C. While chitosan coating containing 2.5%, 5.0%, and 10.0% PPE significantly reduced *S. enterica* numbers to 2.9 log CFU/g, 2.4 log CFU/g, and <2.0 log CFU/g, respectively, after 56 days, compared to numbers 4.5 log CFU/g of untreated dates or numbers 3.8 log CFU/g of chitosan-treated dates. It should be noted that *S. enterica* numbers were not detected by enrichment (<1 CFU/15 g) of Medjool dates treated with chitosan coating containing 10.0% PPE.

Additionally, the current study examined the antimicrobial activity of chitosan and gelatin coatings containing PPE against TMC and yeasts and molds in *S. enterica*-inoculated Medjool dates during storage at 4°C and 24°C. The results of TMC indicated approximately similar patterns to *S. enterica* (Tables S1 and S2). The initial number of yeasts and molds in *S. enterica*-inoculated Medjool dates on day 0 in uncoated (control) samples was 3.0 log CFU/g at room temperature, and the microorganisms showed fluctuations in their behavior during storage at both 4°C and 24°C. However, at the end of storage, the number was not significantly different ($p > 0.05$), compared to the initial number (2.9–3.1 log CFU/g) (Tables 3, 4). Gelatin coating without PPE did not inhibit yeasts and molds at 4°C during storage for 56 days, while chitosan coating caused a reduction of 0.7 log CFU/g, compared to the control. However, both coatings significantly reduced ($p \leq 0.05$) the number of yeasts and molds by 0.7–1.1 log CFU/g at 24°C after 56 days. Further, the addition of 2.5%, 5.0%, and 10.0% PPE to gelatin coating significantly ($p \leq 0.05$) reduced yeast and mold numbers to undetectable levels after 42, 21, and 14 days of storage, respectively, at 4°C, or after 42, 28, and 21 days of storage, respectively, at 24°C (Tables 3, 4). Further, the incorporation of chitosan with 2.5%, 5.0%, and 10.0% PPE reduced yeasts and molds to undetectable levels (<2.0 log CFU/g) after 49, 14, and 14 days of storage at 4°C, respectively, or after 42, 14, and 7 days of storage at 24°C, respectively (Tables 3, 4).

Of the two edible coatings used in the present work, chitosan normally exhibits antibacterial and antifungal effects (Elsabee and Abdou 2013; Ing *et al*., 2012; Muñoz-Tebar *et al*., 2023). As expected, the chitosan coating used here exhibited an antimicrobial effect against *S. enterica* and yeast and molds on dates during storage at 4°C and 24°C. These results agreed with those of Al-Nabulsi *et al*. (2020), who found that *E. coli* O157:H7 in chitosan-coated white brined cheese was reduced by

Table 2. Antimicrobial activity of chitosan and gelatin coatings containing pomegranate peel extract against *Salmonella enterica* on Medjool dates during storage at 4°C.

Day	Cell density of *S. enterica* (log CFU/g) on Medjool dates treated with gelatin (G) or chitosan (CH) coatings containing different concentrations of PPE during storage at 4 °C								
	Control	G 0%	G 2.5%	G 5%	G 10%	CH 0%	CH 2.5%	CH 5%	CH 10%
0	$5.82\pm0.04^{A,a}$	$5.72\pm0.08^{A,b,a}$	$5.52\pm0.08^{B,a}$	$5.01\pm0.15^{C,a}$	$4.67\pm0.18^{D,a}$	$5.68\pm0.06^{A,B,a}$	$5.21\pm0.16^{C,a}$	$4.13\pm0.13^{E,a}$	$3.94\pm0.12^{E,a}$
3	$5.69\pm0.13^{A,a,b}$	$5.57\pm0.07^{A,b,a}$	$5.05\pm0.16^{C,D,b}$	$4.61\pm0.18^{E,b}$	$4.24\pm0.17^{F,b}$	$5.31\pm0.11^{B,C,b}$	$4.97\pm0.15^{D,a}$	$3.82\pm0.10^{G,b}$	$3.55\pm0.18^{G,b}$
7	$5.42\pm0.28^{A,b,c}$	$5.31\pm0.14^{A,b}$	$4.75\pm0.16^{B,b}$	$4.28\pm0.03^{C,c}$	$3.91\pm0.10^{D,c}$	$5.18\pm0.13^{A,b,c}$	$4.68\pm0.16^{B,b}$	$3.48\pm0.04^{E,c}$	$3.15\pm0.15^{F,c}$
14	$5.40\pm0.15^{A,b,c}$	$5.31\pm0.11^{A,B,b}$	$4.41\pm0.18^{C,c}$	$4.05\pm0.16^{D,c}$	$3.52\pm0.17^{E,d}$	$5.11\pm0.07^{B,b,c}$	$4.44\pm0.05^{C,b}$	$3.26\pm0.15^{E,c,d}$	$2.85\pm0.13^{F,d}$
21	$5.05\pm0.19^{A,d,e}$	$5.20\pm0.07^{A,b,c}$	$4.29\pm0.15^{B,c}$	$3.73\pm0.12^{C,d}$	$3.35\pm0.09^{D,d}$	$5.00\pm0.14^{A,c}$	$4.11\pm0.12^{B,c}$	$3.04\pm0.16^{E,d,e}$	$2.68\pm0.15^{F,d,e}$
28	$5.22\pm0.19^{A,c,d}$	$5.00\pm0.14^{A,B,c,d}$	$3.93\pm0.15^{C,d}$	$3.68\pm0.14^{C,d,e}$	$3.26\pm0.05^{D,d,e}$	$4.73\pm0.16^{B,d}$	$3.80\pm0.17^{C,d}$	$2.90\pm0.12^{E,e}$	$2.54\pm0.07^{F,e}$
35	$4.99\pm0.11^{A,d,e,f}$	$4.87\pm0.12^{A,d,e}$	$3.67\pm0.16^{C,d,e}$	$3.43\pm0.15^{C,e,f}$	$3.01\pm0.15^{D,e,f}$	$4.46\pm0.18^{B,e}$	$3.48\pm0.16^{C,e}$	$2.87\pm0.16^{D,e}$	$2.23\pm0.15^{E,f}$
42	$4.82\pm0.17^{A,e,f,g}$	$4.67\pm0.13^{A,e,f}$	$3.69\pm0.12^{C,d,e}$	$3.34\pm0.13^{D,f}$	$2.88\pm0.09^{E,f}$	$4.31\pm0.10^{B,e}$	$3.25\pm0.18^{D,e,f}$	$2.56\pm0.11^{F,f}$	$2.15\pm0.17^{G,f}$
49	$4.67\pm0.13^{A,f,g}$	$4.53\pm0.08^{A,f,g}$	$3.41\pm0.17^{C,e,f}$	$3.05\pm0.04^{D,g}$	$2.43\pm0.09^{E,g}$	$3.95\pm0.08^{B,f}$	$3.00\pm0.09^{D,f,g}$	$2.46\pm0.12^{E,f}$	ND (0/4)^{*F,g}
56	$4.52\pm0.09^{A,g}$	$4.39\pm0.11^{A,g}$	$3.22\pm0.17^{C,f}$	$2.77\pm0.11^{D,h}$	$2.07\pm0.15^{F,h}$	$3.77\pm0.13^{B,f}$	$2.94\pm0.08^{D,g}$	$2.39\pm0.10^{E,f}$	ND (0/4)^{G,g}

Mean values in the same row with different uppercase superscript letters are significantly different ($p \leq 0.05$)
Mean values in the same column with different lowercase superscript letters are significantly different ($p \leq 0.05$)
The values are the average of four separate trials (n = 4) ± standard deviation (SD)
ND: *S. enterica* cells were not detected (the detection limit was 2.0 log CFU/g)
*The number of positive samples by enrichment in brain heart infusion (BHI) broth (≥1 CFU/15 g)

Table 3. Antimicrobial activity of chitosan and gelatin coatings containing pomegranate peel extract against *Salmonella enterica* on Medjool dates during storage at 24°C.

Day	Cell density of *S. enterica* (log CFU/g) on Medjool dates treated with gelatin (G) or chitosan (CH) coatings containing different concentrations of PPE during storage at 24°C								
	Control	G 0%	G 2.5%	G 5%	G 10%	CH 0%	CH 2.5%	CH 5%	CH 10%
0	$5.82\pm0.04^{A,a}$	$5.72\pm0.08^{A,B,a}$	$5.52\pm0.08^{B,a}$	$5.01\pm0.15^{C,a}$	$4.67\pm0.18^{D,a}$	$5.68\pm0.06^{A,B,a}$	$5.21\pm0.16^{C,a}$	$4.13\pm0.13^{E,a}$	$3.94\pm0.12^{E,a}$
3	$5.86\pm0.11^{A,b}$	$3.84\pm0.10^{B,b}$	$3.28\pm0.15^{C,D,b}$	$3.23\pm0.17^{D,b}$	$2.08\pm0.15^{E,b}$	$3.50\pm0.11^{C,b}$	$3.16\pm0.14^{D,b}$	$3.09\pm0.13^{D,b}$	$2.00\pm0.00^{E,b}$
7	$4.07\pm0.19^{A,c}$	$2.71\pm0.08^{B,c}$	$2.23\pm0.15^{C,c}$	$2.08\pm0.15^{C,c}$	ND (0/4)^{*D,c}	$2.75\pm0.18^{B,c}$	$2.15\pm0.17^{C,c}$	$2.15\pm0.17^{C,c}$	ND (0/4)^{D,c}
14	$3.27\pm0.32^{A,d}$	$2.00\pm0.00^{B,d}$	ND (0/4)^{C,d}	ND (0/4)^{C,d}	ND (0/4)^{C,c}	$2.00\pm0.00^{B,d}$	ND (0/4)^{C,d}	ND (0/4)^{C,d}	ND (0/4)^{C,c}
21	$2.00\pm0.00^{A,e}$	$2.00\pm0.00^{A,d}$	ND (0/4)^{B,d}	ND (0/4)^{B,d}	ND (0/4)^{B,c}	$2.00\pm0.00^{A,d}$	ND (0/4)^{B,d}	ND (0/4)^{B,d}	ND (0/4)^{B,c}
28	$2.00\pm0.00^{A,e}$	$2.00\pm0.00^{A,d}$	ND (0/4)^{B,d}	ND (0/4)^{B,d}	ND (0/4)^{B,c}	$2.00\pm0.00^{A,d}$	ND (0/4)^{B,d}	ND (0/4)^{B,d}	ND (0/4)^{B,c}
35	$2.00\pm0.00^{A,e}$	ND (2/4)^{B,e}	ND (0/4)^{B,d}	ND (0/4)^{B,d}	ND (0/4)^{B,c}	ND (0/4)^{B,e}	ND (0/4)^{B,d}	ND (0/4)^{B,d}	ND (0/4)^{B,c}
42	$2.00\pm0.00^{A,e}$	ND (2/4)^{B,e}	ND (0/4)^{B,d}	ND (0/4)^{B,d}	ND (0/4)^{B,c}	ND (0/4)^{B,e}	ND (0/4)^{B,d}	ND (0/4)^{B,d}	ND (0/4)^{B,c}
49	ND (1/4)^{B,f}	ND (2/4)^{B,e}	ND (0/4)^{B,d}	ND (0/4)^{B,d}	ND (0/4)^{B,c}	ND (0/4)^{B,e}	ND (0/4)^{B,d}	ND (0/4)^{B,d}	ND (0/4)^{B,c}
56	ND (0/4)^{B,f}	ND (1/4)^{B,e}	ND (0/4)^{B,d}	ND (0/4)^{B,d}	ND (0/4)^{B,c}	ND (0/4)^{B,e}	ND (0/4)^{B,d}	ND (0/4)^{B,d}	ND (0/4)^{B,c}

Mean values in the same row with different uppercase superscript letters are significantly different ($p \leq 0.05$).
Mean values in the same column with different lowercase superscript letters are significantly different ($p \leq 0.05$).
The values are the average of four separate trials (n = 4) ± standard deviation (SD).
ND: *S. enterica* cells were not detected (the detection limit was 2.0 log CFU/g).
*The number of positive samples by enrichment in brain heart infusion (BHI) broth (≥1 CFU/15 g).

Table 4. Antimicrobial activity of chitosan and gelatin coatings containing pomegranate peel extract against yeasts and molds in *Salmonella enterica*-inoculated Medjool dates during storage at 4°C.

Day	Cell densityof yeasts and molds (log CFU/g) on Medjool dates treated with gelatin (G) and chitosan (CH) coatings containing different concentrations of PPE during storage at 4°C							
	Control	G 0%	G 2.5%	G 5%	G 10%	CH 0%	CH 2.5%	CH 5%	CH 10%
0	2.95±0. 26^{A,c}	2.84±0. 28^{A,a}	2.80±0. 40^{A,a}	2.78±0. 32^{A,a}	2.51±0. 39^{A,a}	2.67±0.54$^{A,a-c}$	2.50±0. 43^{A,a}	2.46±0.45^{A,a}	2.45±0. 17^{A,a}
3	3.91±0.07^{A,a,b}	3.01±0.19^{B,a}	2.23±0.15^{C,b}	2.96±0.08^{B,a}	2.39±0.10^{C,a}	2.88±0.25^{B,a,b}	2.46±0.33^{C,a}	2.27±0.37^{C,a}	2.08±0.15^{C,b}
7	4.13±0.08^{A,a}	3.18±0.33^{B,a}	2.29±0.40^{C,a,b}	2.39±0.45^{C,b}	2.25±0.33^{C,a}	2.79±0.22$^{B,C,a-c}$	2.39±0.40^{C,a}	2.21±0.42^{C,a}	2.15±0.17^{C,b}
14	3.06±0.19^{A,c}	3.04±0.16^{A,a}	2.15±0.30^{B,b}	2.31±0.23^{B,b}	ND^{C,b}	2.85±0.22$^{A,a-c}$	2.31±0.44^{B,a}	ND^{C,b}	ND^{C,c}
21	3.22±0.25^{A,c}	3.16±0.21^{A,a}	2.15±0.30^{B,b}	ND^{C,c}	ND^{C,b}	2.91±0.29^{A,a,b}	2.23±0.15^{B,a}	ND^{C,b}	ND^{C,c}
28	3.11±0.15^{A,c}	2.95±0.35^{A,a}	2.23±0.29^{B,b}	ND^{C,c}	ND^{C,b}	3.12±0.15^{A,a}	2.23±0.15^{B,a}	ND^{C,b}	ND^{C,c}
35	3.61±0.15^{A,b}	2.86±0.39^{B,a}	2.79±0.22^{B,a}	ND^{D,c}	ND^{D,b}	2.96±0.13^{B,a,b}	2.23±0.15^{C,a}	ND^{D,b}	ND^{D,c}
42	3.75±0.18^{A,b}	2.77±0.31^{B,a}	ND^{D,c}	ND^{D,c}	ND^{D,b}	2.56±0.10$^{B,a-c}$	2.19±0.24^{C,a}	ND^{D,b}	ND^{D,c}
49	3.17±0.24^{A,c}	2.56±0.35^{B,a}	ND^{C,c}	ND^{C,c}	ND^{C,b}	2.42±0.38^{B,b,c}	ND^{C,b}	ND^{C,b}	ND^{C,c}
56	2.91±0.05^{A,c}	2.83±0.12^{A,a}	ND^{C,c}	ND^{C,c}	ND^{C,b}	2.23±0.15^{B,c}	ND^{C,b}	ND^{C,b}	ND^{C,c}

Mean values in the same row with different uppercase superscript letters are significantly different ($p \leq 0.05$).
Mean values in the same column with different lowercase superscript letters are significantly different ($p \leq 0.05$).
The values are the average of two separate trials with two replicates for each trial (n = 4) ± standard deviation (SD).
ND: yeasts and molds on *S. enterica*-inoculated Medjool dates were not detected (the detection limit was 2.0 log CFU/g).

2.3 log CFU/g and 1.0 log CFU/g after 28-day storage at 4°C and 10°C, respectively, compared to the control.

Chitosan antimicrobial activity is attributed to its capacity to disrupt the outer membrane of target cells following the interaction of the negatively charged surface of bacteria with positively charged chitosan residues. Similarly, chitosan primarily targets fungal plasma membrane. Positively charged chitosan interacts with negatively charged phospholipid components of fungal membranes. This increases membrane permeability and allows cellular contents to seep out, which consequently leads to cell death (Ing *et al*., 2012). Further, low-molecular weight chitosan and oligo-chitosan may target the DNA of fungi by penetrating their cell walls. This prevents mRNA from being synthesized and halts the synthesis of vital proteins and enzymes (Ing *et al*., 2012). In addition, chitosan binds to trace elements and functions as a chelating agent, rendering vital nutrients inaccessible for the normal growth of bacteria or fungi (Elsabee and Abdou, 2013; Ing *et al*., 2012). Abu-Shama *et al*. (2020) reported that the antibacterial effect of a 0.75% chitosan coating was higher against total bacteria on Barhi dates after 7 weeks of storage at 6°C, compared to 2% gelatin coating. It is likely that the preparation of liquid chitosan with 1% (v/v) acetic acid in the current study increased its positive charge that augmented its antimicrobial activity due to lower pH. The antimicrobial activity of chitosan coating is affected by a_w of food matrix, where it is more inhibitory in high-a_w foods, as it can more effectively interact with microbial cells (Muñoz-Tebar *et al*., 2023). A number of variables in addition to pH and a_w are known to influence the antibacterial activity of chitosan coatings. These include the bacterial strain and its growth phase, temperature, food product, chitosan concentration, and its molecular weight and source (Muñoz-Tebar *et al*., 2023).

It was apparent in the current study that the antimicrobial activity of the coatings was chiefly the result of PPE action, compared to the control (uncoated) and PPE free-coated samples. The antimicrobial activity of PPE increased proportionally to its concentration in the formulated coatings applied to Medjool dates. PPE is rich in its content of bioactive substances, including phenolic compounds, flavonoids, and tannins, which facilitate its action as both preservative and antimicrobial agent in food systems (Chen *et al*., 2020; Ghasemi *et al*., 2023; Lisyanti *et al*., 2022; Saadat *et al*., 2021).

Several studies demonstrated the antimicrobial effect of PPE either *in vitro* or *in vivo* (Al-Zoreky, 2009; Devatkal *et al*., 2011). Nair *et al*. (2018) reported that the use of chitosan with or without 1% PPE extracted by 80% methanol reduced fungal growth on bell pepper capsicum by 0.9 log CFU/g and 1.1 log CFU/g, respectively, at 25 days

Table 5. Antimicrobial activity of chitosan and gelatin coatings containing pomegranate peel extract against yeasts and molds in *Salmonella enterica*-inoculated Medjool dates during storage at 24°C.

Day	Cell densityof yeasts and molds (log CFU/g) on Medjool dates treated with gelatin (G) or chitosan (CH) coatings containing different concentrations of PPE during storage at 24°C							
	Control	G 0%	G 2.5%	G 5%	G 10%	CH 0%	CH 2.5%	CH 5%	CH 10%
0	$2.95\pm0.26^{A,b,c}$	$2.84\pm0.28^{A,a}$	$2.80\pm0.40^{A,a}$	$2.78\pm0.32^{A,a}$	$2.51\pm0.39^{A,a}$	$2.67\pm0.54^{A,a}$	$2.50\pm0.43^{A,a}$	$2.46\pm0.45^{A,a}$	$2.45\pm0.17^{A,a}$
3	$3.00\pm0.17^{A,b}$	$2.54\pm0.47^{A,B,C,a}$	$2.34\pm0.49^{B,C,a,b}$	$2.00\pm0.00^{C,b}$	$2.00\pm0.00^{C,b}$	$2.70\pm0.38^{A,B,a}$	$2.30\pm0.35^{B,C,a}$	$2.08\pm0.15^{B,C,b}$	$2.00\pm0.00^{C,b}$
7	$3.80\pm0.16^{A,a}$	$2.36\pm0.46^{B,C,a}$	$2.39\pm0.33^{B,C,a,b}$	$2.00\pm0.00^{C,b}$	$2.00\pm0.00^{C,b}$	$2.68\pm0.25^{B,a}$	$2.12\pm0.24^{C,a}$	$2.00\pm0.00^{C,b}$	$ND^{D,c}$
14	$4.00\pm0.08^{A,a}$	$2.38\pm0.38^{B,C,a}$	$2.21\pm0.42^{B,C,a,b}$	$2.15\pm0.17^{B,C,b}$	$2.00\pm0.00^{C,b}$	$2.56\pm0.11^{B,a,b}$	$2.08\pm0.15^{C,a}$	$ND^{D,c}$	$ND^{D,c}$
21	$2.48\pm0.21^{A,d}$	$2.39\pm0.33^{A,a}$	$2.15\pm0.17^{A,b}$	$2.15\pm0.17^{A,b}$	$ND^{B,c}$	$2.35\pm0.26^{A,a,b}$	$2.15\pm0.30^{A,a}$	$ND^{B,c}$	$ND^{B,c}$
28	$3.02\pm0.20^{A,b}$	$2.21\pm0.42^{B,a}$	$2.15\pm0.17^{B,b}$	$ND^{C,c}$	$ND^{C,c}$	$2.84\pm0.15^{A,a}$	$2.17\pm0.35^{B,a}$	$ND^{C,c}$	$ND^{C,c}$
35	$3.13\pm0.16^{A,b}$	$2.24\pm0.28^{C,a}$	$2.15\pm0.17^{C,b}$	$ND^{D,c}$	$ND^{D,c}$	$2.64\pm0.20^{B,a}$	$2.19\pm0.24^{C,a}$	$ND^{D,c}$	$ND^{D,c}$
42	$2.95\pm0.19^{A,b,c}$	$2.39\pm0.10^{B,a}$	$ND^{C,c}$	$ND^{C,c}$	$ND^{C,c}$	$2.50\pm0.14^{B,a,b}$	$ND^{C,b}$	$ND^{C,c}$	$ND^{C,c}$
49	$2.57\pm0.06^{A,c,d}$	$2.30\pm0.35^{B,a}$	$ND^{C,c}$	$ND^{C,c}$	$ND^{C,c}$	$2.43\pm0.09^{A,B,a,b}$	$ND^{C,b}$	$ND^{C,c}$	$ND^{C,c}$
56	$3.12\pm0.27^{A,b}$	$2.45\pm0.17^{B,a}$	$ND^{D,c}$	$ND^{D,c}$	$ND^{D,c}$	$2.00\pm0.00^{C,b}$	$ND^{D,b}$	$ND^{D,c}$	$ND^{D,c}$

Mean values in the same row with different uppercase superscript letters are significantly different ($p \leq 0.05$).
Mean values in the same column with different lowercase superscript letters are significantly different ($p \leq 0.05$).
The values are the average of two separate trials with two replicates for each trial (n = 4) ± standard deviation (SD).
ND: yeasts and molds on *S. enterica*-inoculated Medjool dates were not detected (the detection limit was 2.0 log CFU/g).

of storage at 10°C, compared to the control. Mehdizadeh *et al*. (2020) reported that chitosan-starch films with 0.5–1% PPE (extracted by 70% ethyl alcohol and 30% distilled water) reduced the number of *L. monocytogenes* on fresh beef stored at 4°C by 1.0 log CFU/g after 21 days, compared to the control. Alqahtani *et al*. (2023) reported that disinfection solutions of 0.1% aqueous PPE, 1% lactic acid, or their combination significantly inhibited the growth of yeasts and molds on fresh Barhi date fruits at 4°C for 6 weeks. In the current study, incorporation of 5.0–10.0% PPE to gelatin or chitosan coatings reduced the number of yeast and mold to undetectable levels at 7–28 days at both 4°C and 24°C.

Additionally, the storage temperature of treated Medjool dates (4°C and 24°C) had a strong effect on microbial reduction. Higher reduction of *S. enterica* and yeast and molds was observed at 24°C, compared to 4°C. Generally, when the temperature increases, the metabolic activity of bacterial cells typically increases, which results in depletion of the ATP required to adapt to the presence of antimicrobial agents in PPE (Olaimat *et al*., 2022; Zaidi and Imam, 2008). However, the induction of cold shock proteins at 4°C may enhance the bacterial tolerance to the antimicrobial coatings (Dawan and Ahn, 2022).

Changes in the color values of uncoated and coated Medjool dates

The lightness (L*), redness (a*), and yellowness (b*) of the samples were determined to evaluate color variations after storage of both uncoated and coated Medjool dates at 4°C and 24°C (Table 6). The color of Medjool dates was not affected by either the type of coating (gelatin or chitosan) or the concentration of PPE (2.5%, 5.0%, or 10.0%), and there were no significant differences in L*, a*, and b*color values between treated and untreated Medjool dates stored at 4°C or 24°C, except for b* values. For example, the highest yellowness values of 20.4 and 19.6 occurred in dates treated with gelatin coatings containing 5.0% and 10.0% PPE, respectively, while the lowest value was in dates treated with chitosan containing 10.0% PPE (13.3) but stored at 4°C. Furthermore, there were no significant differences ($p > 0.05$) in the color values of dates stored at 4°C and 24°C, except for b* values of dates treated with chitosan coating containing 10.0% PPE.

Color is considered as one of the most crucial factors influencing fruit attractiveness to consumers (Nair *et al*., 2018). The current study showed a significant difference ($p \leq 0.05$) in b* values, which occurred due to high yellowness (20.4 and 19.6) in dates treated with gelatin coatings containing 5.0% and 10.0% PPE, respectively. The lowest b* value (13.3) occurred in dates treated with chitosan containing 10.0% PPE at 4°C. This difference

was attributed to the nature of gelatin coating color, which was characterized as white or slightly yellow. In contrast to the current study, Nair *et al*. (2018) reported significant differences in L*, a*, and b* values between bell pepper (capsicum) samples coated with chitosan or alginate containing 1% PPE, compared to uncoated samples during storage at 10°C. Mehdizadeh *et al*. (2020) also reported significant differences in L*, a*, and b* values between samples coated with chitosan containing 0.5% and 1% PPE, compared to chitosan coating without PPE. In the current study, the addition of PPE at 2.5%, 5.0%, and 10.0% to either chitosan or gelatin coatings on Medjool dates did not affect color, compared to uncoated samples. This result could be due to a number of factors, including the naturally intense brownish color of Medjool dates, the thickness and type of edible coating, storage temperature, and PPE concentration.

In an evaluation of sensory characteristics of treated and untreated Medjool dates conducted using an ethics-approved protocol with 40 trained panelists at the Department of Clinical Nutrition and Dietetics, Hashemite University using a 9-point Hedonic scale, it was found that gelatin and chitosan coatings without or with 2.5%, 5.0%, and 10.0% PPE did not significantly affect both appearance and color. Also, the texture and overall acceptability scores of Medjool dates treated with gelatin coatings alone or with 2.5–5.0% PPE decreased slightly. However, the texture and overall acceptability scores of Medjool dates treated with chitosan alone or chitosan containing 2.5–5.0% PPE were not significantly different, compared to untreated samples (Figure S1). Hari and Carvalho (2023) reported that the majority of natural plant extracts that possesses good antimicrobial activities might tend to inflict unpleasant taste and affect the organoleptic properties of treated food items. In the current study, the PPE incorporated into the coating materials imparted a considerable antimicrobial activity against *S. enterica*; however, it did not affect the appearance of the product, yet it only slightly decreased the overall acceptability of the dates.

Changes in pH values of uncoated and coated Medjool dates

The initial pH of uncoated *S. enterica*-inoculated Medjool dates (whole piece) was 6.4 and decreased significantly ($p \leq 0.05$) to 6.0 at both 4°C and 24°C, while the initial pH values of Medjool dates coated with gelatin containing 5.0–10.0% PPE decreased slightly to 6.2, compared to uncoated samples. Similarly, the pH values of Medjool dates coated with chitosan containing 10.0% PPE decreased significantly to 6.0. However, other coatings did not affect the initial pH values of Medjool dates (Table 7).

The final pH values of *S. enterica*-inoculated Medjool dates coated with gelatin or chitosan without or with PPE (5.8–6.0%) were not significantly different ($p > 0.05$), compared to the pH of uncoated samples (6.0) at 4°C, except for gelatin coatings containing 0.0–2.5% PPE, which was significantly higher (≈6.2). However, all coating types significantly reduced ($p \leq 0.05$) the pH values of dates at 24°C, and ranged from 5.6 to 5.9. It is worth mentioning that pH values decreased with increase in PPE concentrations of the coating. Further, it was evident that Medjool dates treated with different coatings and

Table 6. Effect of gelatin and chitosan coatings containing different concentrations of pomegranate peel extract on the color of Medjool dates stored for 56 days at 4°C and 24°C.

Temperature/ treatments	L*		a*		b*	
	4°C	24°C	4°C	24°C	4°C	24°C
Control	19.76±1.04^{A}	19.90±0.97^{A}	1.79±0.32^{A}	1.58±0.65^{A}	14.81±2.26^{A,B}	13.94±2.35^{A}
Gelatin 0%	20.21±1.95^{A}	20.94±0.86^{A}	2.55±0.57^{A}	3.00±2.77^{A}	14.74±1.45^{A,B}	14.86±1.50^{A}
Gelatin 2.5%	21.58±2.48^{A}	20.26±1.79^{A}	1.34±0.14^{A}	2.13±0.87^{A}	15.37±0.94^{A,B}	18.56±5.19^{A}
Gelatin 5%	21.58±2.16^{A}	20.36±3.82^{A}	3.46±2.20^{A}	3.42±1.86^{A}	20.37±0.25^{A}	20.07±1.68^{A}
Gelatin 10%	21.59±0.36^{A}	21.34±1.77^{A}	2.97±1.06^{A}	2.85±0.91^{A}	19.55±2.57^{A}	17.23±1.51^{A}
Chitosan 0%	21.81±1.66^{A}	20.90±1.36^{A}	1.47±0.44^{A}	1.48±0.29^{A}	17.19±2.33^{A,B}	17.67±4.98^{A}
Chitosan 2.5%	19.78±0.99^{A}	17.76±4.81^{A}	4.14±2.44^{A}	1.83±0.37^{A}	15.93±0.96^{A,B}	14.12±2.16^{A}
Chitosan 5%	15.97±5.77^{A}	21.58±1.96^{A}	3.73±2.20^{A}	2.83±0.97^{A}	16.34±3.45^{A,B}	12.57±1.25^{A}
Chitosan 10%	21.03±2.83^{A}	21.55±2.22^{A}	5.11±3.81^{A}	2.24±0.67^{A}	13.34±3.05^{B*}	19.96±0.90^{A*}

Mean values in the same column of each color value of the same temperature with different uppercase superscript letters are significantly different ($p \leq 0.05$).
*Significant difference between storage temperatures using t-test.
The values are the average of three replicates ± standard deviation (SD).

Table 7. Initial and final pH values of *S. enterica*-inoculated Medjool dates treated with gelatin and chitosan coatings containing different concentrations of pomegranate peel extract at different storage temperatures.

Treatment	Initial pH	Final pH of *S. enterica*-inoculated dates after 56 days	
		4°C	24°C
Control	$6.41\pm0.08^{A,a}$	$5.96\pm0.13^{B,b}$	$6.00\pm0.10^{B,a}$
Gelatin 0%	$6.26\pm0.05^{A,b,c}$	$6.19\pm0.08^{A,a}$	$5.85\pm0.04^{B,b}$
Gelatin 2.5%	$6.34\pm0.09^{A,a,b}$	$6.25\pm0.08^{A,a}$	$5.85\pm0.02^{B,b}$
Gelatin 5%	$6.19\pm0.04^{A,c}$	$5.92\pm0.12^{B,b}$	$5.73\pm0.09^{C,b–d}$
Gelatin 10%	$6.23\pm0.09^{A,b,c}$	$5.96\pm0.13^{B,b}$	$5.67\pm0.08^{C,d}$
Chitosan 0%	$6.38\pm0.04^{A,a}$	$5.92\pm0.07^{B,b}$	$5.81\pm0.05^{C,b,c}$
Chitosan 2.5%	$6.32\pm0.04^{A,a,b}$	$5.97\pm0.10^{B,b}$	$5.81\pm0.08^{C,b,c}$
Chitosan 5%	$6.42\pm0.08^{A,a}$	$5.79\pm0.04^{B,b}$	$5.69\pm0.09^{C,c,d}$
Chitosan 10%	$5.99\pm0.06^{A,d}$	$5.93\pm0.15^{A,b}$	$5.56\pm0.08^{B,e}$

Mean values in the same row with different uppercase superscript letters are significantly different ($p \leq 0.05$).
Mean values in the same column with different lowercase superscript letters are significantly different ($p \leq 0.05$).
The values are the average of two independent trials ± standard deviation (SD).

Table 8. Initial and final water activity (a*w*) values of *S. enterica*-inoculated Medjool dates treated with gelatin and chitosan coatings containing different concentrations of PPE at different storage temperatures.

Treatment	Initial a_w	Final a_w of *S. enterica*-inoculated dates on day 56	
		4°C	24°C
Control	$0.71\pm0.01^{A,e}$	$0.68\pm0.02^{B,b,c}$	$0.65\pm0.01^{C,b}$
Gelatin 0%	$0.74\pm0.01^{A,b–d}$	$0.68\pm0.01^{B,b,c}$	$0.66\pm0.00^{C,b}$
Gelatin 2.5%	$0.75\pm0.01^{A,b,c}$	$0.65\pm0.00^{B,d}$	$0.65\pm0.00^{B,a,b}$
Gelatin 5%	$0.74\pm0.00^{A,d}$	$0.69\pm0.00^{B,b}$	$0.66\pm0.01^{C,b}$
Gelatin 10%	$0.76\pm0.00^{A,b}$	$0.70\pm0.01^{B,a,b}$	$0.64\pm0.01^{C,b}$
Chitosan 0%	$0.74\pm0.00^{A,c,d}$	$0.71\pm0.03^{A,B,a}$	$0.69\pm0.03^{B,a}$
Chitosan 2.5%	$0.76\pm0.00^{A,b,c}$	$0.66\pm0.02^{B,c,d}$	$0.66\pm0.00^{B,a,b}$
Chitosan 5%	$0.76\pm0.01^{A,b,c}$	$0.70\pm0.01^{B,a,b}$	$0.66\pm0.01^{C,b}$
Chitosan 10%	$0.78\pm0.02^{A,a}$	$0.70\pm0.01^{B,a,b}$	$0.64\pm0.05^{C,b}$

Mean values in the same row with different uppercase superscript letters are significantly different ($p < 0.05$).
Mean values in the same column with different lowercase superscript letters are significantly different ($p < 0.05$).
The values are the average of two independent trials ± standard deviation (SD).

stored at 24°C had significantly lower ($p \leq 0.05$) pH values than those stored at 4°C.

Changes in the water activity of coated and uncoated Medjool dates

The initial a_w of whole uncoated *S. enterica*-inoculated Medjool dates was 0.71 at room temperature, and this decreased significantly ($p \leq 0.05$) to 0.68 at 4°C and to 0.65 at 24°C by day 56; this may contribute to reductions in the number of *S. enterica* and TMC during storage. However, coatings significantly increased the initial a_w of *S. enterica*-inoculated Medjool dates to 0.74–0.78, but decreased significantly to 0.64–0.71 during storage at both 4°C and 24°C (Table 8). It should be noted that gelatin or chitosan coatings containing 5.0–10.0% PPE had significantly lower a_w when stored at 24°C than at 4°C, and this may rapidly decrease *S. enterica* numbers to undetectable levels.

With the a_w values of <0.85, Medjool dates are considered a low a_w food (Beuchat *et al.*, 2013). The results of this study were in agreement with those of Ghafoor *et al.* (2022), who reported reduction in a_w values of cucumber samples after 21 days at 4°C following chitosan coating with or without 1–2% orange peel extract and 1–2% olive cake extract. Also, Alqahtani *et al.* (2023) reported a decreased a_w values from 0.94 to 0.91 during 6-week storage of dipped dates with 0.1% PPE.

Conclusions

Numerous factors contribute to the microbial contamination of dates, and this necessitates applying preventive methods to reduce microbial impact for product's safety. Without due care, FBP might be able to thrive on dates or the products containing dates. Consumer interest in natural; hence, use of environmentally venerating and less toxic agents for food safety is increasing. Incorporation of PPE as an antimicrobial agent in edible coatings, such as chitosan and gelatin, on Medjool dates is a promising and eco-friendly technique that has the potential to minimize agricultural and packaging waste and enhance the fruit's safety by eliminating FBP. The current study demonstrated that gelatin and chitosan coatings containing 2.5–10.0% PPE were effective against *S. enterica*, TMC, and yeasts and molds on Medjool dates during storage at both refrigerator and room temperatures. The antimicrobial activity of coatings with PPE was concentration- and temperature-dependent, where higher concentrations of PPE and higher temperatures showed greater microbial reduction. The type of coatings and the concentration of PPE had a slight influence on both pH and a_w values of Medjool dates, but had no effect on L*, a*, and b* color values. The current study demonstrated that applying PPE incorporated into coating materials, such as chitosan and gelatin, could be used as an effective natural mitigation to enhance the safety of dates by reducing the risk of *S. enterica*. Natural antimicrobial agents, such as PPE, as a prevention against pathogenic microbes could be used globally by date farms and the industry.

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Data Availability

Data are provided upon request.

Author Contributions

AN Olaimat, S Hamaideh, MA Al-Holy, and AA Al-Nabulsi designed and performed the experiments, and acquired the data. AN Olaimat, S Hamaideh, MA Al-Holy, AA Al-Nabulsi, MH Abughoush, TM Osaili, S Hamed, B Khataybeh, AM Ababneh, T Osaili, and N Elsahoryi drafted the manuscript. RA Holley critically revised the manuscript. All authors read and approved the final version of the manuscript.

Conflict of Interest

The authors declared that they had no competing interests.

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Supplementary

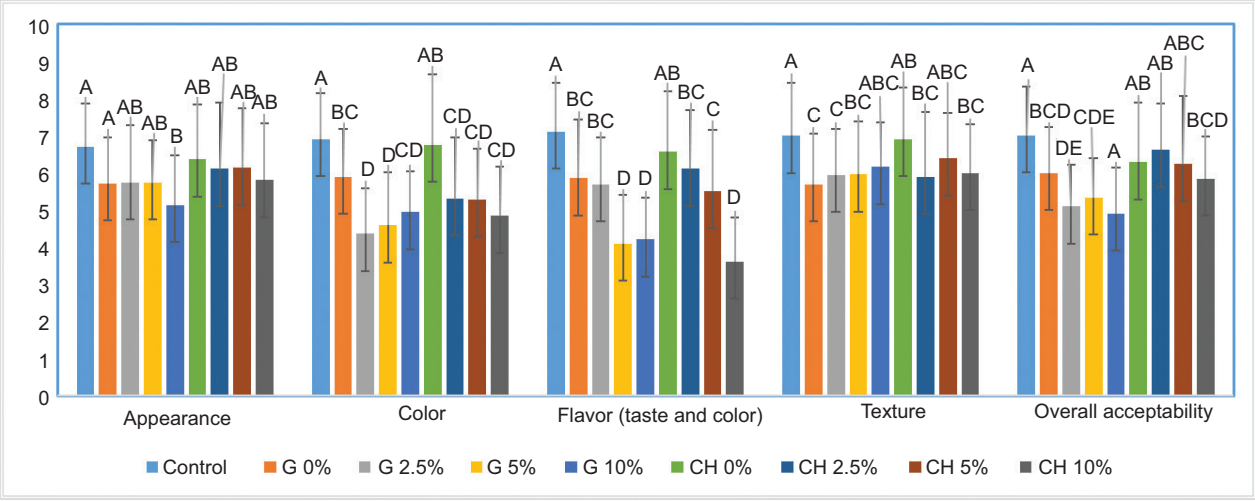

Figure S1. Sensory evaluation of gelatin (G) and chitosan (CH) coatings on Medjool dates without or with different concentrations of PPE (2.5, 5.0, and 10.0%). The values are the average of parameter scores ± standard error. Means with the same uppercase letters are not significantly different ($p > 0.05$). 1 = dislike extremely; 2 = dislike very much; 3 = dislike moderately; 4 = dislike slightly; 5 = neither like nor dislike; 6 = like slightly; 7 = like moderately; 8 = like very much; 9 = like extremely.

Table S1. Antimicrobial activity of chitosan and gelatin coatings containing PPE against total TMC in *S. enterica*-inoculated Medjool date during storage at 4 °C.

Day	Numbers of TMC (log CFU/g) on Medjool date treated with gelatin (G) or chitosan (CH) coatings containing different concentrations of PPE during storage at 4°C								
	Control	G 0%	G 2.5%	G 5%	G 10%	CH 0%	CH 2.5%	CH 5%	CH 10%
0	5.92 ± 0.03^{Aa}	5.81 ± 0.06^{ABa}	5.62 ± 0.09^{Ba}	5.18 ± 0.16^{CDa}	4.94 ± 0.12^{Da}	5.68 ± 0.14^{ABa}	5.27 ± 0.17^{Ca}	4.50 ± 0.13^{Ea}	4.15 ± 0.15^{Fa}
3	5.86 ± 0.10^{Aa}	5.70 ± 0.13^{Aa}	5.40 ± 0.02^{Bb}	4.92 ± 0.03^{Cb}	4.62 ± 0.05^{Db}	5.81 ± 0.14^{Aa}	5.10 ± 0.15^{Cab}	4.31 ± 0.15^{Eab}	3.92 ± 0.14^{Fb}
7	5.67 ± 0.17^{Aab}	5.63 ± 0.16^{Aab}	5.33 ± 0.16^{Bbc}	4.48 ± 0.07^{Dc}	4.35 ± 0.14^{DEc}	5.66 ± 0.07^{Aa}	4.92 ± 0.07^{Cbc}	4.10 ± 0.18^{Ebc}	3.70 ± 0.14^{Fc}
14	5.43 ± 0.09^{Abc}	5.42 ± 0.16^{Abc}	5.13 ± 0.14^{Bc}	4.21 ± 0.13^{Dd}	4.10 ± 0.16^{Dd}	5.36 ± 0.11^{ABb}	4.68 ± 0.14^{Ccd}	3.97 ± 0.06^{Dc}	3.54 ± 0.08^{Ec}
21	5.14 ± 0.20^{Acd}	5.35 ± 0.14^{Ac}	5.16 ± 0.11^{Ac}	3.92 ± 0.11^{Ce}	3.83 ± 0.16^{Ce}	5.23 ± 0.20^{Ab}	4.42 ± 0.09^{Bd}	3.68 ± 0.17^{Cd}	3.20 ± 0.13^{Dd}
28	5.37 ± 0.26^{Ac}	5.27 ± 0.14^{Acd}	4.05 ± 0.15^{Bd}	3.75 ± 0.13^{BCDef}	3.60 ± 0.16^{CDef}	5.13 ± 0.14^{Abc}	3.90 ± 0.18^{BCe}	3.54 ± 0.07^{Dd}	2.94 ± 0.05^{Ee}
35	5.21 ± 0.09^{Acd}	5.04 ± 0.07^{Ade}	3.93 ± 0.03^{Bde}	3.64 ± 0.13^{CDfg}	3.42 ± 0.14^{Df}	5.11 ± 0.04^{Abc}	3.66 ± 0.15^{Cef}	3.16 ± 0.14^{Ee}	2.78 ± 0.15^{Fef}
42	5.18 ± 0.10^{Acd}	4.91 ± 0.14^{Bef}	3.86 ± 0.06^{Cdef}	3.42 ± 0.15^{Dgh}	3.09 ± 0.13^{Eg}	4.96 ± 0.13^{Bcd}	3.47 ± 0.14^{Dfg}	2.97 ± 0.09^{Eef}	2.63 ± 0.05^{Ff}
49	4.94 ± 0.05^{Ade}	4.85 ± 0.11^{Aef}	3.74 ± 0.05^{Bef}	3.20 ± 0.18^{Ch}	2.810 ± 0.04^{Eh}	4.84 ± 0.07^{Ad}	3.26 ± 0.11^{Cg}	3.00 ± 0.09^{Def}	2.00 ± 0.00^{Eg}
56	4.86 ± 0.04^{Ae}	4.76 ± 0.09^{Af}	3.68 ± 0.11^{Bf}	3.14 ± 0.17^{Ch}	2.00 ± 0.00^{Ei}	4.71 ± 0.08^{Ad}	3.21 ± 0.14^{Cg}	2.75 ± 0.10^{Df}	2.00 ± 0.00^{Eg}

Means in the same row with different uppercase letters are significantly different ($p \leq 0.05$).
Means in the same column with different lowercase letters are significantly different ($p \leq 0.05$).
The values are the average of two separate trials with two replicates for each trial (n=4) ± standard deviation.
ND: TMC in *S. enterica*-inoculated Medjool dates were not detected (the detection limit was 2.0 log CFU/g).

Table S2. Antimicrobial activity of chitosan and gelatin coatings containing PPE against total TMC in *S. enterica*-inoculated Medjool date during storage at 24 °C.

Day	Numbers of TMC (log CFU/g) on Medjool date treated with gelatin (G) or chitosan (CH) coatings containing different concentrations of PPE during storage at 24°C								
	Control	G 0%	G 2.5%	G 5%	G 10%	CH 0%	CH 2.5%	CH 5%	CH 10%
0	5.92 ± 0.03^{Aa}	5.81 ± 0.06^{ABa}	5.62 ± 0.09^{Ba}	5.18 ± 0.16^{CDa}	4.94 ± 0.12^{Da}	5.68 ± 0.14^{ABa}	5.27 ± 0.17^{Ca}	4.50 ± 0.13^{Ea}	4.15 ± 0.15^{Fa}
3	5.12 ± 0.03^{Ab}	4.30 ± 0.05^{Bb}	3.94 ± 0.16^{Cb}	3.72 ± 0.15^{CDb}	2.08 ± 0.15^{Fb}	3.62 ± 0.17^{Db}	3.22 ± 0.18^{Eb}	3.15 ± 0.12^{Eb}	2.00 ± 0.00^{Fb}
7	4.32 ± 0.04^{Ac}	4.02 ± 0.09^{Bc}	2.27 ± 0.20^{Dc}	2.08 ± 0.15^{Dc}	ND (0/4)*^{Ec}	3.44 ± 0.09^{Cc}	2.23 ± 0.15^{Dc}	2.15 ± 0.17^{Dc}	ND (0/4)^{Ec}
14	3.51 ± 0.13^{Ad}	2.00 ± 0.00^{Bd}	ND (0/4)^{Cd}	ND (0/4)^{Cd}	ND (0/4)^{Cc}	2.00 ± 0.00^{Bd}	ND (0/4)^{Cd}	ND (0/4)^{Cd}	ND (0/4)^{Cc}
21	2.35 ± 0.09^{Ae}	2.00 ± 0.00^{Bd}	ND (0/4)^{Cd}	ND (0/4)^{Cd}	ND (0/4)^{Cc}	2.00 ± 0.00^{Bd}	ND (0/4)^{Cd}	ND (0/4)^{Cd}	ND (0/4)^{Cc}
28	2.00 ± 0.00^{Af}	2.00 ± 0.00^{Ad}	ND (0/4)^{Bd}	ND (0/4)^{Bd}	ND (0/4)^{Bc}	2.00 ± 0.00^{Ad}	ND (0/4)^{Bd}	ND (0/4)^{Bd}	ND (0/4)^{Bc}
35	2.00 ± 0.00^{Af}	ND (2/4)^{Be}	ND (0/4)^{Bd}	ND (0/4)^{Bd}	ND (0/4)^{Bc}	ND (0/4)^{Be}	ND (0/4)^{Bd}	ND (0/4)^{Bd}	ND (0/4)^{Bc}
42	2.00 ± 0.00^{Af}	ND (2/4)^{Be}	ND (0/4)^{Bd}	ND (0/4)^{Bd}	ND (0/4)^{Bc}	ND (0/4)^{Be}	ND (0/4)^{Bd}	ND (0/4)^{Bd}	ND (0/4)^{Bc}
49	ND (1/4)^{Ag}	ND (1/4)^{Ae}	ND (0/4)^{Ad}	ND (0/4)^{Ad}	ND (0/4)^{Ac}	ND (0/4)^{Ae}	ND (0/4)^{Ad}	ND (0/4)^{Ad}	ND (0/4)^{Ac}
56	ND (0/4)^{Ag}	ND (0/4)^{Ae}	ND (0/4)^{Ad}	ND (0/4)^{Ad}	ND (0/4)^{Ac}	ND (0/4)^{Ae}	ND (0/4)^{Ad}	ND (0/4)^{Ad}	ND (0/4)^{Ac}

Means in the same column with different lowercase letters are significantly different ($p < 0.05$).
Means in the same row with different uppercase letters are significantly different ($p \leq 0.05$).
Means in the same column with different lowercase letters are significantly different ($p \leq 0.05$).
The values are the average of two separate trials with two replicates for each trial (n=4) ± standard deviation.
ND: TMC in *S. enterica*-inoculated Medjool dates were not detected (the detection limit was 2.0 log CFU/g).
*Numbers of positive samples by enrichment in BHI broth (≥ 1 CFU/15 g).