LWT - Food Science and Technology 182 (2023) 114839

Contents lists available at ScienceDirect

LWT

journal homepage: www.elsevier.com/locate/lwt

Development of novel antimicrobial coatings incorporating linalool and eugenol to improve the microbiological quality and safety of raw chicken

Sotirios I. Ekonomou [a,1], Damien J. Leech [b,c,1], Sonny Lightfoot [d], David Huson [d], Alexandros Ch Stratakos [a,*]

[a] College of Health, Science and Society, School of Applied Sciences, University of the West of England, Coldharbour Lane, Bristol, BS16 1QY, UK
[b] Centre for Additive Manufacturing, Faculty of Engineering, University of Nottingham, Nottingham, NG8 1BB, UK
[c] Imec, 3001, Leuven, Belgium
[d] Centre for Fine Print Research, University of the West of England, Coldharbour Lane, Bristol, BS16 1QY, UK

ARTICLE INFO

Keywords:
Antimicrobial coatings
Linalool
Eugenol
Chicken
Listeria monocytogenes

ABSTRACT

Incorporating natural antimicrobial agents in antimicrobial coatings has recently been gaining more attention. This study aimed to develop novel coatings with incorporated natural antimicrobial compounds (linalool, eugenol) to improve the quality and safety of raw chicken during storage at 4 °C. Coatings consisting of chitosan and gelatine (C/G) mixes (40:60 ratio) revealed lower viscosities above 30 °C, while the shear thinning behaviour of chitosan and gelatine was maintained. Furthermore, the antimicrobial coatings, when applied to raw chicken samples resulted in lower pH and TBARS levels compared to the control during storage. Also, raw chicken coated with the antimicrobial coatings containing 0.5 and 0.7 mg/mL of linalool and eugenol suppressed Lactic-acid-bacteria and Total-Plate-Counts under the spoilage level (7-logs CFU/g) and showed that they can increase the microbiological shelf-life of the product up to two days during storage at 4 °C. Results also showed that the coated samples exhibited a significantly lower *Listeria monocytogenes* population than the control samples during storage. Conclusively, this study revealed that adding linalool and eugenol in C/G coatings can enhance the shelf-life and safety of raw chicken.

1. Introduction

The foods we eat are never sterile, and different microorganisms are present with their composition depending on various factors, such as their growth, survival, and interaction with the food components over time (Adams & Moss, 2007). Chicken meat is a relatively cheap protein source, low in fat, and is consumed worldwide. However, it is perishable and has a relatively short shelf-life, even when stored under refrigeration temperatures. In this respect, and for the commercialisation of fresh chicken products, the rapid growth of spoilage bacteria and the contamination with pathogens are among the main limitations. *Listeria monocytogenes* is a ubiquitous bacterium that, due to its ability to survive under various conditions and even grow under temperatures as low as 0 °C, remains an important concern for the consumers' safety in refrigerated products (Ekonomou et al., 2020; Stratakos et al., 2015). Traditionally, to extend the shelf-life and maintain the quality of raw chicken products, brining, chilling, freezing, and irradiation treatments were carried out however these showed adverse effects on the sensory properties and loss of freshness of the food (Leygonie et al., 2012).

One common way to maintain this quality is through the production of safe and efficient food packaging or coatings. Food packaging remains an area of interest across many scientific fields, including chemistry, manufacturing, and microbiology, and is widely used to retain and extend the quality, shelf-life, and safety of many raw food products (Quintavalla & Vicini, 2002). Primarily, the packaging is intended to isolate and be the first line of defence against extrinsic factors such as impact forces, dust/particulate, gases and moisture, radiation, and contamination of food with pathogenic microorganisms and can often be one of the core parameters that determine the shelf-life of food. Antimicrobial packaging, or active packaging techniques, aims to combat the spread of foodborne pathogens that can cause severe human infections by incorporating antimicrobial materials into this design process (Al-Tayyar et al., 2020).

Many packaging approaches have been taken to preserve the quality

* Corresponding author.
E-mail address: alexandros.stratakos@uwe.ac.uk (A.C. Stratakos).
[1] The authors contributed equally to this work.

https://doi.org/10.1016/j.lwt.2023.114839
Received 14 March 2023; Received in revised form 1 May 2023; Accepted 3 May 2023
Available online 3 May 2023

and safety of raw food, including the incorporation of nanoparticles (L. Wang et al., 2021) and antimicrobial polymers directly into the packaging (Cagri et al., 2001; González-González et al., 2021; Ouattara et al., 2000) and post-processing techniques such as plasma treatment (Lei et al., 2014). However, many of these techniques require access to specialised equipment or complex material processing knowledge prior to packaging fabrication. Alternatively, others have proposed an approach that utilises biodegradable soft polymers as the core component of their coatings and films (Al-Tayyar et al., 2020; Cagri et al., 2001; Ouattara et al., 2000) that are typically manipulated by casting using pre-built moulds.

Chitosan, a polysaccharide found in the exoskeletons of many crustaceans, has previously shown promising results, specifically within antimicrobial food packaging applications and is one of the most widely used antimicrobial polymers to produce coatings for fresh meat products (Yuan et al., 2022). However, the extent of the antimicrobial activity has been shown to be dependent on various factors, including molecular weight, additives, and post-processing (Wang et al., 2021). In our study, a Chitosan/Gelatine (C/G) mix has been used as a basis mixture of our antimicrobial coatings. In the coatings prepared in the current study, the gelatine component seeks to increase the film's mechanical properties and provide a natural barrier against water, UV light, and oxygen, while the chitosan component increases the viscosity and setting time. Previously, C/G mixes of this type have also been shown to increase the shelf-life and sensory attributes of red bell peppers (Poverenov et al., 2014), fresh pork meat (Xiong et al., 2020), salmon (Gómez-Estaca et al., 2009), and chicken fillet (Hassan et al., 2022). The choice of chitosan and gelatine was intentional to provide a low-cost, approachable version of these antimicrobial polymers.

In order to develop a method that the food industry can readily adopt, it is required that we use natural compounds with high antimicrobial effects in low concentrations to avoid causing a negative effect on the sensory quality of the food products. Therefore, this study aimed to develop novel coatings with incorporated natural antimicrobials to improve the quality and safety of raw chicken during storage. Furthermore, due to these materials' generality and ease of use, we can extend this setup to audition various antimicrobial components to determine those with the most prominent antimicrobial properties. However, this study focused on two primary candidates with broad application in many sectors: linalool and eugenol. The rheological and chemical characterisation of the C/G coatings with 0.5 or 0.7 mg/mL of linalool or eugenol was performed with the materials identified. In addition, the pH, lipid oxidation, effect on spoilage microorganisms, and the antimicrobial effect of the coatings against *L. monocytogenes* were monitored. In the current study, we propose the application of a simple, low-cost method using antimicrobial and biodegradable coatings made from natural, food-grade materials that can be used as a preservation method for a wide variety of raw food products without the need for mould or casting equipment.

2. Materials and methods

2.1. Materials

Chitosan was purchased from Oxford Vitality (Bicester, UK) as a dietary supplement and the beef gelatine was purchased from MM Ingredients (Wimborne, UK), with a bloom value of 240 – both were used as received and chosen primarily for their low cost. All components within the C/G coating are considered safe for use in food. Linalool and eugenol were chosen as they are accessible and relatively cheap antimicrobial compounds that are considered safe for use in food and beverages by both the U.S. Food and Drug Administration (FDA) and the Joint FAO/WHO Expert Committee on Food Additives (JECFA) (World Health Organization, 2022). Linalool (3,7-Dimethylocta-1,6-dien-3-ol) is a monoterpenoid that has been isolated from a variety of plants and essential oils (EOs) and has displayed good antimicrobial activity against numerous foodborne pathogens, including *Escherichia coli* (Suppakul et al., 2008) and L. monocytogenes (Gao et al., 2019). Eugenol (4-Allyl-2-methoxyphenol) is another natural antimicrobial compound sourced most prominently from clove oil but also basil and cinnamon leaves and has shown strong antimicrobial activity against a large number of gram-positive and -negative pathogenic bacteria (Hu et al., 2018; Marchese et al., 2017). The linalool and eugenol were both purchased from Sigma-Aldrich, UK and used as received.

2.2. Preparation of the mixes

The C/G mixes were formed by mixing powdered 10% chitosan with 90% acetic acid and storing it for 48 h at room temperature or until fully dissolved. Then, a solution of 25% (w/v) 250 Bloom gelatine was prepared in warm water (40 °C) and refrigerated for 48 h. The C/G mix was produced from a 40:60 ratio of these to produce a water-loaded hydrogel with the desired setting properties. This ratio attempts to balance the setting time (generally driven by the gelatine) with the viscosity of the mix (controlled by the chitosan) to produce an easy-to-use and malleable mix that can be used in coating, film-forming and printing techniques with minimal variation.

The addition of 0.055 mg/mL Polysorbate 20 incorporated into the mix can mean that up to 0.7 mg/mL of essential oils can easily be added and retained during the film formation process. Our primary testing formulations were comprised of the core C/G mix, with the addition of 0.5 and 0.7 mg/mL of linalool or eugenol.

2.3. Rheological analysis

Rheological measurements were taken using a rheometer (Kinexus Pro+, Netzsch, UK) that allows the rheological response to be probed with varying shear rates and temperature. Each of the four C/G mixes was tested separately in a temperature range of 30–55 °C by placing 2 g of material onto the heated plate and heating it at 30 °C. This ensures that all the tested materials were in a liquid form to better simulate the conditions in which they can be applied as films or coatings.

2.4. Fourier-transform infrared (FTIR) spectroscopy

FTIR spectra were obtained using a PerkinElmer FTIR Frontier and used to analyse the chemical nature of the mixtures. Solid, room temperature samples of each of the four mixtures were placed onto the crystal cell of the FTIR spectrophotometer, with the platform cleaned thoroughly in-between to remove any presence of residual liquid. The measurements were performed in the range of 750–4000 cm^{-1} at room temperature, and the responses were collected from 10 scans at a resolution of 4 cm^{-1}.

2.5. Determination of pH

The pH values were measured using a pH meter (FiveEasy Plus, Mettler Toledo, UK). Briefly, five (5) grams of coated and uncoated chicken samples were added in 45 mL of distilled water into a stomacher bag (Seward, UK) separately. Then, the samples were homogenised at 200 rpm for 2 min using a stomacher (Stomacher® 400 Circulator, Seward, UK).

2.6. Thiobarbituric acid reactive substances (TBARS) analysis

Lipid oxidation was measured by TBARS value as described by González-González et al. (2021). Coated and uncoated chicken samples of 5 g were added separately in 35 mL of 7.5% trichloroacetic acid (TCA). All samples were standardised after homogenisation for 30 s, centrifugation at 3000×*g* for 2 min (Allegra X-30 Series, Beckman Coulter, US), and filtration through a Whatman filter paper with a diameter of 125 mm (Grade 1, Thermo Fischer Scientific, UK). Then (5)

millilitres of the standardised samples were transferred into plastic universal polystyrene containers (Sterilin, UK), with 5 mL of 0.02 mol/L thiobarbituric acid (TBA) to a ratio of 1:1, TBA: standardised sample. The samples were vortexed and kept at room temperature (20 °C) for 20 h. After this step, the samples were vortexed again, and an aliquot of 200 μL of each sample was transferred into a 96-well plate to measure their absorbance at 532 nm, using a microplate reader FLUOstar Omega (BMG Labtec, UK). A blank sample was prepared by adding 5 mL of both TCA and TBA. For negative control, the uncoated chicken sample was washed with distilled water. The calibration curve was prepared using 1, 1,3,3-tetra methoxy propane. The TBARS value was expressed as mg of malondialdehyde (MDA)/kg of chicken. The values were expressed as the mean of 4 different samples tested twice (two technical replicates).

2.7. Bacterial cultivation and growth conditions

2.7.1. Total plate counts (TPC) and lactic acid bacteria (LAB)

Ten (10) grams from each chicken sample were transferred aseptically into stomacher bags with 90 mL of Maximum Recovery Diluent (0.1% w/v peptone, 0.85% w/v NaCl; MRD) and homogenised at 200 rpm for 2 min using a Stomacher. A volume of 0.1 mL from the appropriate 10-fold serial dilutions, using the spread plate technique, was used to enumerate the total plate counts (TPC) on plate count agar (PCA) incubated at 25 °C for 48–72 h. A volume of 1 mL from the appropriate 10-fold serial dilutions, using the pour plate technique, was used to enumerate the lactic acid bacteria (LAB) on De Man, Rogosa, Sharpe agar (MRS), incubated at 25 °C for 72 h. The microbiological analysis was carried out every 2 days. All results were expressed as Log CFU/g. All microbiological media were supplied by Oxoid, UK.

2.7.2. L. monocytogenes

Listeria monocytogenes NCTC 7973 from a frozen stock stored at −80 °C in Cryoinstant vials with porous beads (Microbank, Pro-Lab Diagnostics, UK) was used. A single bead was transferred aseptically in sterile Mueller Hinton broth (MHB) to activate the culture and incubated overnight at 37 °C. From the overnight culture, a volume of 0.1 mL was transferred in 10 mL MHB and incubated at 37 °C for 24 h. To prepare the working culture, the cells were harvested by centrifugation at 6500×*g* for 10 min, washed twice with phosphate-buffered saline (pH 7.4; PBS), and finally resuspended in 10 mL of MRD to an appropriate bacterial population before use. An inoculum of 0.1 mL was added on the surface of the chicken meat samples to reach a bacterial population of 10^3 Log CFU/g, and the samples were stored at 4 °C for 8 days. Control samples were inoculated with 0.1 mL of sterile distilled water. As mentioned in section 2.7.1, 10 g from each chicken sample were added aseptically in a Stomacher bag with 90 mL of MRD and homogenised at 200 rpm for 2 min using a Stomacher. Finally, the 10-fold dilutions were prepared in MRD, and an aliquot of 0.1 mL from the appropriate 10-fold serial dilution was plated onto Brilliance Listeria agar [formerly Oxoid Chromogenic Listeria Agar (OCLA)] and incubated at 37 °C for 24 h. The microbiological analysis was carried out every 2 days. All results were expressed as Log CFU/g.

2.8. Statistical analysis

All experiments were performed at least in triplicate and were analysed twice. Statistical analysis was done using Excel Microsoft® Office 365 (ver. 16.48). When required, the data were subjected to a one-way ANOVA followed by Tukey post hoc test using IBM® SPSS® statistics 26 software for macOS (SPSS Inc.). All data showed a normal distribution. Student's t-test was used to determine significant differences between two groups at a 5% level of significance. Thus, results were considered statistically significant when p was less than 0.05 ($p < 0.05$).

3. Results and discussion

3.1. Rheological properties of the mixes

Fig. 1a shows the results for the mixture containing 0.5 mg/mL eugenol and how they change with varying temperatures. The solid eugenol samples were heated at 30 °C to ensure that were fully liquid before being introduced to the rheometer and utilised the in-built heating plate to move to the desired measurement temperature. All temperatures were examined in our "liquid window" of functionality - above 30 °C (below the mixture's setting temperature) and below 55 °C (above which the antimicrobial activity can be negatively affected). As expected, larger temperatures result in lower viscosities and, therefore, an easier method of depositing and moving material, making temperature control the most direct and simplest method of controlling the viscosity of the mixture. As the primary components of the mixture were gelatine and chitosan, the shear thinning behaviour of those components (Payet et al., 2010) was maintained in this mixture. The other antimicrobial mixtures used in this study revealed the same trend (results are not shown). In addition, comparisons between the four mixtures at a similar temperature (35 °C), in Fig. 1b, show a similar shear viscosity response with increasing shear rate and compare well with other literature (Bertolo et al., 2021; Wang et al., 2009) with a higher average value due to the higher loading of gelatine and chitosan. The variations seen between the differing mixtures may be due to inhomogeneity within the mixture, possibly arising from incomplete mixing and the lack of purity of the core biopolymers in the mixture. As such, due to the large amount of variation inherent in the chosen biopolymers arising from differing sources and production methods (Schnepp, 2013), direct comparisons are not easy and would not produce many valuable insights.

The four mixtures used are solid at room temperature and become liquid in the region of 30–40 °C, meaning that their properties can be probed via characterisation methods that require both liquid and solid samples. The core formulation is cheap, easy to make, and accessible in producing an antimicrobial coating. When using these materials as malleable coatings with a low phase transition temperature, their liquid and general fluid flow response can be useful in probing how these materials could be used in forming antimicrobial films, surfaces and/or coatings. This manipulation could even be extended to the production of more complex textures and surfaces through three-dimensional patterning techniques, like direct ink writing (Gutierrez et al., 2019; Pattinson & Hart, 2017). In the current study, however, all raw chicken samples were coated by dipping the samples into the antimicrobial mix while in a liquid form, as explained earlier.

3.2. FTIR analysis

FTIR analysis was used to probe the chemical composition of the mixtures. Fig. 2 shows the FTIR spectra for two representative samples of C/G mixtures loaded with either linalool or eugenol at 0.7 mg/mL. As the majority of the mixture is comprised mostly of the C/G component, much of the FTIR response is the same and overlaps, however the two formulations can be distinguished by the fingerprint region of 750–2000 cm^{-1}, alongside the region of 2500–3000 cm^{-1}. Distinctive peaks, such as the dominant 3300 cm^{-1} peak (representing $-NH_2$ stretching) and the peak at 1670 cm^{-1} (representing the C═O stretching), can be found in gelatine-chitosan mixes within the literature (Pezeshki-Modaress et al., 2018). Fig. 2a shows the FTIR results for a linalool loaded ink - the 2971 cm^{-1} peak found in the spectra is the aliphatic C–H stretching (Jabir et al., 2018), C═C stretching of the allyl groups at 1634 cm^{-1}, N–H bending at 1550 cm^{-1} and a C–O stretching band at 1094 cm^{-1} (Menezes et al., 2014). Fig. 2b shows the FTIR results for a eugenol loaded mix with peaks at 1640 cm^{-1} and 1510 cm^{-1} that are also found in eugenol spectra and can correspond to the C═C stretching of the aromatic moiety (Nuchuchua et al., 2009; Yang & Song,

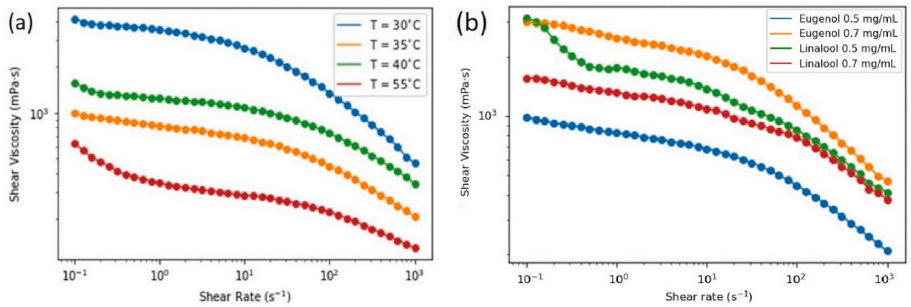

Fig. 1. (a) Rheological response of a C/G antimicrobial mix formulation, loaded with 0.5 mg/mL eugenol, displaying the large differences in material response at 30, 25, 40, and 55 °C. (b) Rheological response of a C/G antimicrobial mix, loaded with 0.5 and 7 mg/mL eugenol or linalool. The temperature was kept at a constant 35 °C for each measurement.

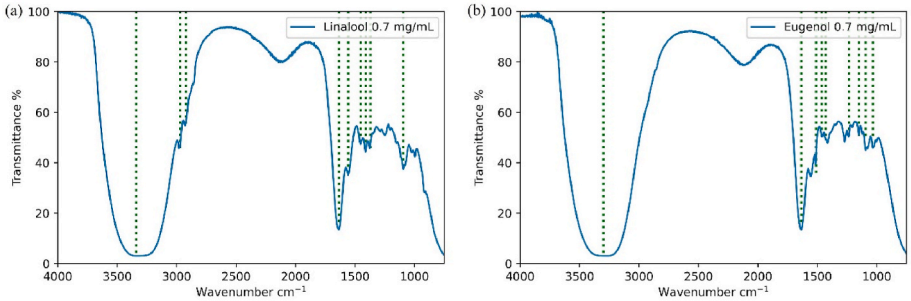

Fig. 2. FTIR spectra of a standard C/G mix, prepared as documented in the main text and loaded with a) 0.7 mg/mL linalool and b) 0.7 mg/mL eugenol.

2005). The stronger peak at 1157 cm^{-1} also corresponds to C–O stretching (Pezeshki-Modaress et al., 2018). Similar results were seen for mixtures containing 0.5 mg/mL of linalool and eugenol.

3.3. pH profile of chicken coated with the antimicrobial mixtures

In order to show the efficacy of the antimicrobial coatings, each variation of the mixture was applied to raw chicken breast, kept at 4 °C and monitored across 8 days of storage. Fig. 3 shows the raw data for this pH response for uncoated (control) and coated with 0.5 and 0.7 mg/mL of eugenol and linalool samples across the storage period at 4 °C. It can be observed that raw chicken breast when coated with the antimicrobial mixtures maintained a lower pH for longer periods of storage and the rate of pH reduction increases as the concentration of the antimicrobial compounds increase. The higher pH value is an indicator that microbial growth is faster, and that spoilage is occurring (Adams & Moss, 2007). This is in accordance with our microbiological results presented in the next section where the uncoated samples reached the spoilage level (7-logs CFU/g) after 6 days of storage and the coated samples with 0.5 mg/mL linalool on the last day of storage (Day 8). Similar to our results,

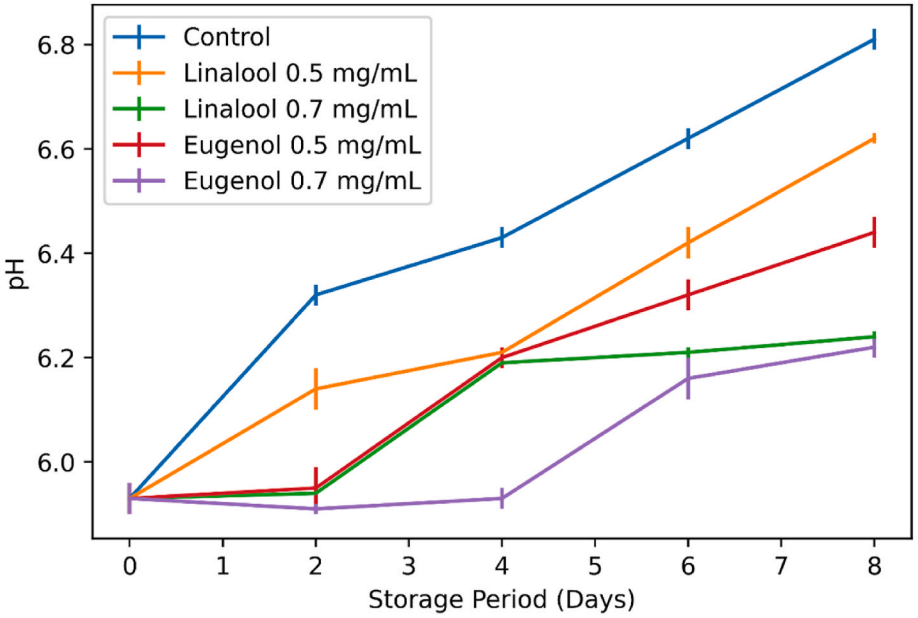

Fig. 3. pH response of raw chicken breast, during storage at 4 °C across 8 days, both with and without an applied antimicrobial coating.

Volpe et al. (2015), also found a lower pH increase for the Carrageenan-based coated fresh trout fillets with or without essential lemon oil compared to uncoated samples.

3.4. Determination of lipid oxidation

The TBARS value was selected as a useful chemical indicator for the quality assurance of chicken during storage. TBARS is one of the most widely used methods for measuring malondialdehyde (MDA), the most predominant end product of lipid peroxidation (Singh et al., 2016). The TBARS value of uncoated (control) and coated chicken meat with linalool and eugenol are presented in Fig. 4.

The TBARS value for all samples increased during storage. On Day 4, the TBARS values for the coated samples with linalool and eugenol 0.5 and 0.7 mg/mL reached 0.49, 0.45, 0.50, and 0.43 mgMDA/kg, respectively and remained lower than the control samples (0.61 mgMDA/kg). According to (Reitznerová et al., 2017) there is no legislative limit on MDA concentrations in meat products indicating lipid oxidation. However, numerous studies indicated that TBARS values lower than 0.50 mgMDA/kg indicate oxidation at a tolerable level, while values above 1.00 mgMDA/kg are an index of oxidation (Frigg, 1992; Warriss, 2000). At the end of the storage period (Day 8), the TBARS values for the control samples (1.39 mgMDA/kg) were higher than the coated samples with linalool and eugenol 0.5 and 0.7 mg/mL, ranging from 0.79 to 0.85 mgMDA/kg (Fig. 4). Our results gave evidence that the coating formulations with linalool and eugenol can extend the shelf-life of the raw chicken compared to the uncoated samples. In contrast, the TBARS values for the uncoated raw chicken on Day 8 (end of storage) were above the acceptable level of 1.00 mgMDA/kg, showing its oxidation during storage at 4 °C. This effect can be attributed to the reduced oxygen atmosphere of the coated samples and the high antioxidant effect of the natural antimicrobials in the coatings and has been observed by other authors investigating the antimicrobial effect of edible films enriched with pomegranate peel powder on chicken nuggets (Bashir et al., 2022), ginger (*Zingiber officinale*) EO on chicken breast (Noori et al., 2018), tea polyphenol and rosemary extract on raw fish (Li et al., 2012), and apple peel extract (rich on polyphenols) on meat products (Maroufi et al., 2022). As demonstrated in the study of Liu et al. (2021), the edible coating on hairtail fish meat with eugenol-chitosan nano-emulsions showed a high preservative effect with the TBARS values remaining above the acceptable level after 16 days of storage at 4 °C. Furthermore, the antioxidant properties of the various substances, such as gums and gelling agents, that are traditionally used to form coatings have been shown by numerous researchers (Criado et al., 2020; Panahi & Mohsenzadeh, 2022). In particular, edible alginate films loaded with cellulose nanocrystals revealed high antioxidant properties in chicken breast samples during storage at 4 °C (Criado et al., 2020). This effect was correlated to both the presence of the cellulose nanocrystals and the reduced oxygen permeability of the alginate based edible films.

3.5. Microbiological analyses

3.5.1. Microbial population changes during storage

It is known that during aerobic storage of chicken meat at refrigeration temperatures, the most frequently isolated bacteria contributing to spoilage include *Pseudomonas* spp., Enterobacteriaceae, *Brochothrix thermosphacta,* and LAB (Katiyo et al., 2020). However, the C/G coating applied on the samples was expected to act as a very good barrier to oxygen during storage (Elsabee & Abdou, 2013). Indeed, as seen from the TBARS values, at the end of the storage period (Day 8), the coating provided an efficient oxygen barrier to the coated samples with linalool and eugenol 0.5 and 0.7 mg/mL, since TBARS remained under 1.00 mgMDA/kg. Under these conditions, the growth of aerobic spoilage microbiota can be significantly reduced (Narasimha Rao & Sachindra, 2002), and for this reason, in the current study, the growth of LAB during storage, was investigated. The microbiological changes in the TPC and LAB counts for uncoated (control) and coated raw chicken breast during storage at 4 °C are presented in Figs. 5 and 6.

The initial (Day 0) population of TPC and LAB was 4.40 and 1.66 log CFU/g, respectively. These results were within the range of other studies that reported that the initial levels of mesophiles and LAB on refrigerated chicken were 3–5 and 1 to 3-log CFU/g, respectively (Park et al., 2013). The TPC counts increased and were significantly higher from Day 2 to Day 8 for the control samples compared to all coated raw chicken samples ($p < 0.05$, Fig. 5). In particular, the TPC counts on Day 6 reached 7.08 log CFU/g which is above the level of 7-logs that is considered as the first indication of spoilage in fresh meat (Adams & Moss, 2007) and fish products (Ekonomou et al., 2022). On the same day, the TPC numbers of raw chicken meat coated with antimicrobial mixtures containing linalool and eugenol 0.5 and 0.7 mg/mL remained under this limit showing their protective effect against the spoilage of the raw chicken. At the end of storage (Day 8), the TPC numbers of the control were 7.63 log CFU/g, while the bacterial counts of TPC only for

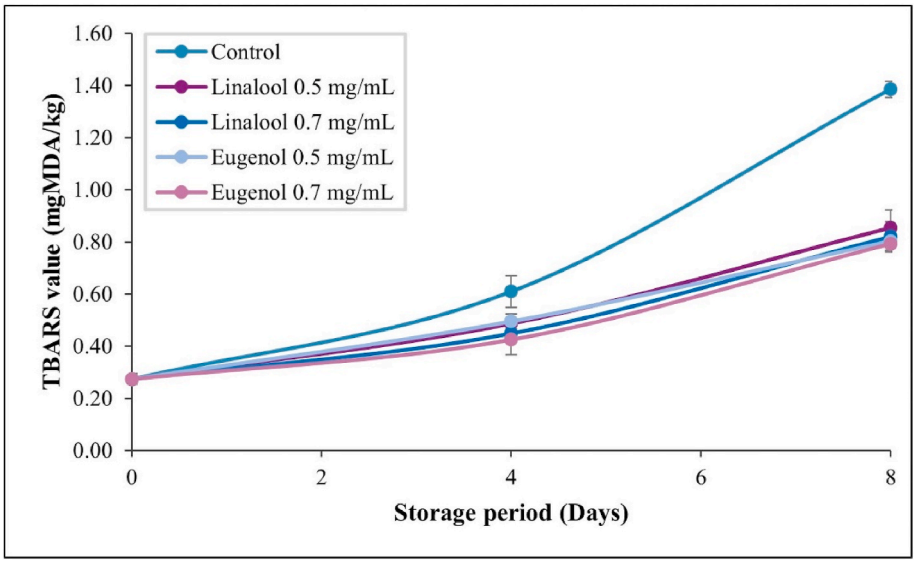

Fig. 4. Effect of the antimicrobial coatings containing 0.5 and 0.7 mg/mL linalool and eugenol on lipid oxidation in chicken meat during storage at 4 °C for 8 days. The data represent the mean mg MDA of eight replicates and their standard deviations (S.D.), some of which however lies within the data points.

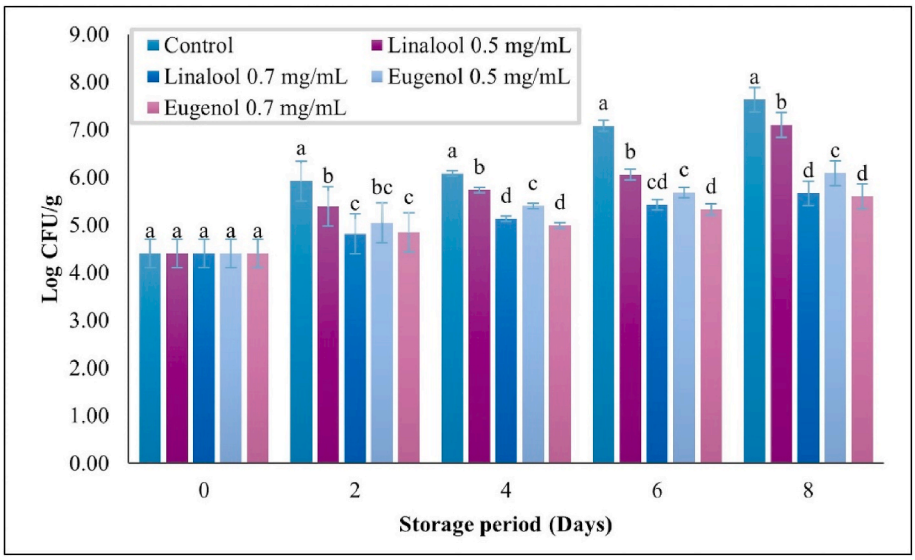

Fig. 5. Microbiological changes of TPC of chicken meat coated with antimicrobial mixtures containing 0.5 and 0.7 mg/mL linalool and eugenol during storage at 4 °C for 8 days. Each column represents the mean of 6 replicates (3 batches from each and 2 technical). Error bars represent the S.D. The values followed by different lowercase letters differed significantly within each day of storage ($p < 0.05$).

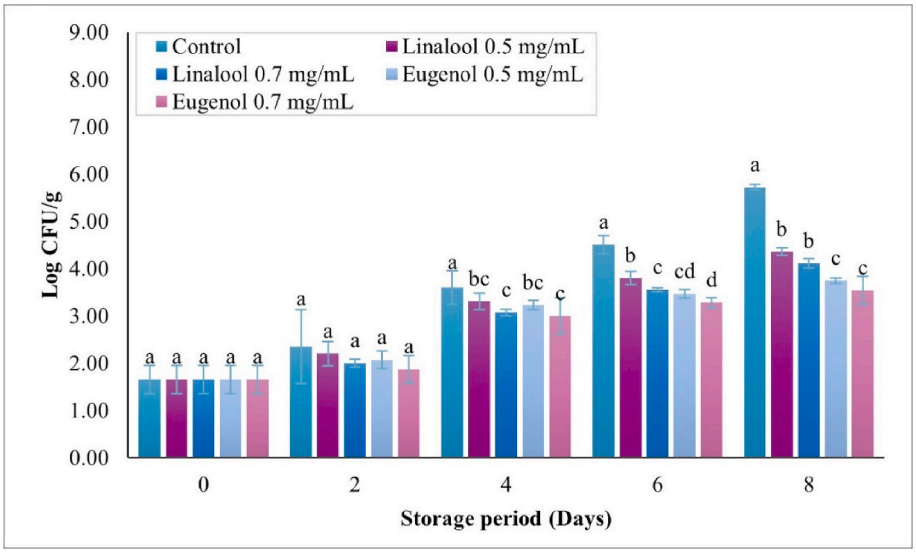

Fig. 6. Microbiological changes of LAB of chicken meat coated with antimicrobial mixtures containing 0.5 and 0.7 mg/mL linalool and eugenol during storage at 4 °C for 8 days. Each column represents the mean of 6 replicates (3 batches from each and 2 technical replicates). Error bars represent the S.D. The values followed by different lowercase letters differed significantly within each day of storage ($p < 0.05$).

the coated sample with linalool 0.5 mg/mL reached 7.10 log CFU/g. Our results clearly demonstrate that raw chicken breast coated with the antimicrobial mixtures with linalool 0.7 mg/mL and eugenol 0.5 and 0.7 mg/mL delayed spoilage and can potentially increase the shelf-life of the product. This result can be due to the decrease in oxygen levels caused by the C/G coating that is known that act as an oxygen barrier and can inhibit the growth of aerobic microorganisms (Devlieghere et al., 2004). In addition, chitosan has been shown to exhibit antimicrobial properties (L. Wang et al., 2021). However, the antimicrobial activity observed was also due to the incorporation of the antimicrobial compounds of linalool and eugenol. The mode of action of both linalool and eugenol is related to the disruption of the cell membranes, causing increased permeability and uptake of compounds (Mohammadi Nejad et al., 2017), as has been seen for other natural antimicrobial compounds (Stratakos et al., 2015). This fact explains the lower levels of TPC and LAB found in the coated raw chicken breast samples. Moreover, Oyedemi et al. (2009) observed that the time of exposure of bacterial cells to eugenol is an important factor as the cell wall and membrane damage was increased after a longer exposure time.

A similar pattern was observed for the LAB counts after Day 4, where the population on the control samples was significantly higher compared to the coated ones and equal to 3.60 log CFU/g ($p < 0.05$, Fig. 6). On Day 4, the LAB population of raw chicken samples coated with antimicrobial mixtures containing 0.7 mg/mL linalool (3.07 log CFU/g) and eugenol (3.00 log CFU/g) was significantly lower compared to the samples coated with the mixtures incorporated with 0.5 mg/mL of the same antimicrobials ($p < 0.05$, Fig. 6). At the end of the storage period (Day 8), the antimicrobial coatings with 0.5 and 0.7 mg/mL eugenol were the most effective against the growth of LAB, showing the highest inhibitory effect. As a result, it can be concluded that the effect of eugenol was more pronounced on the counts of LAB than on TPC counts. Our results are in agreement with other recent studies using

antimicrobial compounds and EOs incorporated in food coatings to reduce the microbial population of TPC, LAB, and H_2S-producing bacteria in raw chicken coated with guar gum and oregano EO formulations (Garavito et al., 2020), and other food products such as fish (Volpe et al., 2015; Yuan et al., 2022) and meat with edible kappa-carrageenan coatings incorporated with cinnamon EO (He & Wang, 2022). In addition, the findings of Liu et al. (2021) suggested that eugenol-chitosan nano-emulsions can inhibit the growth of TPC on fish meat and prevent the entry of other microorganisms on the food product during storage.

3.5.2. *Effect of linalool and eugenol-based antimicrobial coatings on L. monocytogenes*

In addition to the microbiological quality, it was essential to assess the safety of the coated raw chicken samples by monitoring the effect of the antimicrobial coatings in inhibiting *L. monocytogenes*. The effect of the antimicrobial coatings against *L. monocytogenes* during storage at 4 °C for 8 days is presented in Fig. 7. The initial (Day 0) population of *L. monocytogenes* on raw coated and uncoated chicken samples was 3.13 log CFU/g (Fig. 7). After two days of storage at 4 °C *L. monocytogenes* population significantly increased for all samples except the coated samples with the highest concentration (0.7 mg/mL) of eugenol showing an inhibitory effect with a population of 3.12 log CFU/g ($p < 0.05$, Fig. 7). During storage, from Day 4 to Day 8 all the coated samples revealed a significantly lower population for *L. monocytogenes*, compared to the control samples that the final population of the pathogen on the last day of storage (Day 8) reached at 4.94 log CFU/g ($p < 0.05$, Fig. 7). Finally, the antimicrobial coatings incorporated with 0.7 mg/mL eugenol were the most effective against *L. monocytogenes* with a final population on Day 8 of only 3.80 log CFU/g (Fig. 7). The high antimicrobial effect of eugenol-loaded coatings is mainly related to the nature of the compound, which is a volatile bioactive naturally occurring phenolic monoterpenoid. Noori et al. (2018) observed a similar effect in inhibiting *L. monocytogenes* and *Salmonella* Typhimurium on the raw chicken breast by incorporating ginger EO in edible coatings. The authors explained that this effect was mainly attributed to the phenolic compounds that are presented in ginger EO. It is well known that phenols can damage the bacterial membrane leading to microbial death, and inhibit the growth of various foodborne pathogens, including L. monocytogenes (Ekonomou et al., 2020; Noori et al., 2018).

Linalool is another efficient antimicrobial compound that has been found to be effective against *Pseudomonas aeruginosa* (X. Liu et al., 2020), *Staphylococcus aureus* (Silva et al., 2015), and *L. monocytogenes* (Gao et al., 2019). The mechanism of action of linalool remains unclear. However, Gao et al. (2019) tried to describe the primary mechanism of the anti-listeria effects of linalool. The authors observed that the cells treated with linalool initially lost their cellular wall, followed by the rupture of their cytoplasmic membrane, causing the final lysis of the cells (cytoplasm separation from the cells) and finally leading to the up-regulation of genes related to peptidoglycan biosynthesis as a response to the cellular damage. Moreover, Garavito et al. (2020) demonstrated the synergistic antimicrobial effect of nisin (1 mg/mL) as a naturally occurring food preservative in combination with oregano EO (0.05 mg/mL) incorporated in Guar Gum edible coatings on raw chicken breast fillets and observed an inhibitory effect against *Pseudomonas* spp.

4. Conclusions

In the framework of this study, a novel antimicrobial coating was formulated from inexpensive and malleable core ingredients and, as such, can readily be utilised in coatings. Rheological measurements showed a broad change in the overall viscosity of the mixture in a relatively low temperature range (30–55 °C), and the mixture allowed for the retention of antimicrobial components (eugenol and linalool) that are liquid at room temperature and therefore challenging to utilise in coatings. Collectively, the microbiological results indicated that the C/G antimicrobial coatings coupled with the presence of 0.5 and 0.7 mg/mL linalool and eugenol can inhibit bacterial growth for at least 2 days and can be considered as a promising preservation method. Furthermore, it was shown that the coatings incorporated with 0.5 and 0.7 mg/mL eugenol were the most effective among the others in inhibiting the growth of *L. monocytogenes* on raw chicken during storage at 4 °C. It was also observed that the presence of linalool and eugenol prevents lipid oxidation in raw chicken during the first 8 days of storage as shown by the TBARS value. Taking into account the preferences of modern consumers for natural additives, the proposed antimicrobial coatings can potentially be adopted by the food industry to help reduce the risk of foodborne disease, maintain quality and extend the shelf-life of raw chicken meat to help ensure food safety and decrease food waste.

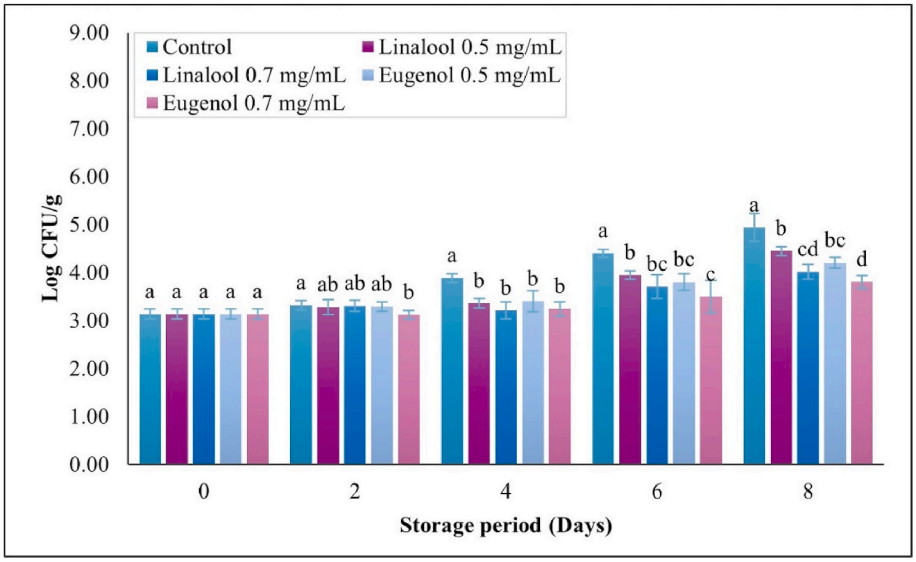

Fig. 7. Microbiological changes of artificially inoculated *L. monocytogenes* on chicken meat coated with antimicrobial mixtures containing 0.5 and 0.7 mg/mL linalool and eugenol during storage at 4 °C for 8 days. Each column represents the mean of 6 replicates (3 batches from each and 2 technical replicates). Error bars represent the S.D. The values followed by different lowercase letters differed significantly within each day of storage ($p < 0.05$).

Funding

This research was funded by an internal competitive grant awarded from the University of the West of England, Bristol.

CRediT authorship contribution statement

Sotirios I. Ekonomou: Writing – original draft, Software, Methodology, Validation, Investigation, Data curation, Formal analysis. **Damien J. Leech:** Writing – original draft, Software, Methodology, Validation, Investigation, Data curation, Formal analysis, Funding acquisition. **Sonny Lightfoot:** Validation, Investigation. **David Huson:** Validation, Investigation. **Alexandros Ch Stratakos:** Writing – review & editing, Supervision, Resources, Conceptualization, Funding acquisition.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

References

Adams, M. R., & Moss, M. O. (2007). The microbiology of food preservation. In *Food microbiology* (pp. 65–120). Royal Society of Chemistry. https://doi.org/10.1039/9781847550880-00065.

Al-Tayyar, N. A., Youssef, A. M., & Al-hindi, R. (2020). Antimicrobial food packaging based on sustainable bio-based materials for reducing foodborne pathogens: A review. In *Food chemistry* (Vol. 310)Elsevier, Article 125915. https://doi.org/10.1016/j.foodchem.2019.125915.

Bashir, S., Arshad, M. S., Khalid, W., Nayik, G. A., Al Obaid, S., Ansari, M. J., Moreno, A., & Karabagias, I. K. (2022). Effect of antimicrobial and antioxidant rich pomegranate peel based edible coatings on quality and functional properties of chicken nuggets. *Molecules, 27*(14). https://doi.org/10.3390/molecules27144500

Bertolo, M. R. V., Leme, R., Martins, V. da C. A., Plepis, A. M. de G., & Junior, S. B. (2021). Rheological characterization of the influence of pomegranate peel extract addition and concentration in chitosan and gelatin coatings. *Polysaccharides, 2*(3), 648–660. https://doi.org/10.3390/POLYSACCHARIDES2030039, 2021, Vol. 2, Pages 648-660.

Cagri, A., Ustunol, Z., & Ryser, E. T. (2001). Antimicrobial, mechanical, and moisture barrier properties of low pH whey protein-based edible films containing p-aminobenzoic or sorbic acids. *Journal of Food Science, 66*(6), 865–870. https://doi.org/10.1111/J.1365-2621.2001.TB15188.X

Criado, P., Fraschini, C., Salmieri, S., & Lacroix, M. (2020). Cellulose nanocrystals (CNCs) loaded alginate films against lipid oxidation of chicken breast. *Food Research International, 132*, Article 109110. https://doi.org/10.1016/j.foodres.2020.109110

Devlieghere, F., Vermeulen, A., & Debevere, J. (2004). Chitosan: Antimicrobial activity, interactions with food components and applicability as a coating on fruit and vegetables. *Food Microbiology, 21*(6), 703–714. https://doi.org/10.1016/J.FM.2004.02.008

Ekonomou, S. I., Bulut, S., Karatzas, K. A. G., & Boziaris, I. S. (2020). Inactivation of Listeria monocytogenes in raw and hot smoked trout fillets by high hydrostatic pressure processing combined with liquid smoke and freezing. *Innovative Food Science and Emerging Technologies, 64*. https://doi.org/10.1016/j.ifset.2020.102427

Ekonomou, S. I., Parlapani, F. F., Kyritsi, M., Hadjichristodoulou, C., & Boziaris, I. S. (2022). Preservation status and microbial communities of vacuum-packed hot smoked rainbow trout fillets. *Food Microbiology, 103*, Article 103959. https://doi.org/10.1016/J.FM.2021.103959

Elsabee, M. Z., & Abdou, E. S. (2013). Chitosan based edible films and coatings: A review. *Materials Science and Engineering: C, 33*(4), 1819–1841. https://doi.org/10.1016/j.msec.2013.01.010

Frigg, M. (1992). *Research experiences with vitamin E for poultry meat quality* (p. 2). Basel, Switzerland: Hoffmann-La Roche Ltd.

Gao, Z., Van Nostrand, J. D., Zhou, J., Zhong, W., Chen, K., & Guo, J. (2019). Anti-listeria activities of linalool and its mechanism revealed by comparative transcriptome analysis. *Frontiers in Microbiology, 10*, 2947. https://doi.org/10.3389/FMICB.2019.02947/BIBTEX

Garavito, J., Moncayo-Martinez, D., & Castellanos, D. (2020). *Evaluation of antimicrobial coatings on preservation cold storage*.

Gómez-Estaca, J., López De Lacey, A., Gómez-Guillén, M. C., López-Caballero, M. E., & Montero, P. (2009). Antimicrobial activity of composite edible films based on fish gelatin and chitosan incorporated with clove essential oil. *Journal of Aquatic Food Product Technology, 18*(1–2), 46–52. https://doi.org/10.1080/10498850802581252

González-González, C. R., Labo-Popoola, O., Delgado-Pando, G., Theodoridou, K., Doran, O., & Stratakos, A. C. (2021). The effect of cold atmospheric plasma and linalool nanoemulsions against Escherichia coli O157:H7 and Salmonella on ready-to-eat chicken meat. *Lebensmittel-Wissenschaft und -Technologie, 149*, Article 111898. https://doi.org/10.1016/J.LWT.2021.111898

Gutierrez, E., Burdiles, P. A., Quero, F., Palma, P., Olate-Moya, F., & Palza, H. (2019). 3D printing of antimicrobial alginate/bacterial-cellulose composite hydrogels by incorporating copper nanostructures. *ACS Biomaterials Science & Engineering, 5*(11), 6290–6299. https://doi.org/10.1021/acsbiomaterials.9b01048

Hassan, A. H. A., Korany, A. M., Zeinhom, M. M. A., Mohamed, D. S., & Abdel-Atty, N. S. (2022). Effect of chitosan-gelatin coating fortified with papaya leaves and thyme extract on quality and shelf life of chicken breast fillet and Kareish cheese during chilled storage. *International Journal of Food Microbiology, 371*, Article 109667. https://doi.org/10.1016/j.ijfoodmicro.2022.109667

He, S., & Wang, Y. (2022). Antimicrobial and antioxidant effects of kappa-carrageenan coatings enriched with cinnamon essential oil in pork meat. *Foods, 11*(18), 2885. https://doi.org/10.3390/FOODS11182885, 2022, Vol. 11, Page 2885.

Hu, Q., Zhou, M., & Wei, S. (2018). Progress on the antimicrobial activity research of clove oil and eugenol in the food antisepsis field. *Journal of Food Science, 83*(6), 1476–1483. https://doi.org/10.1111/1750-3841.14180

Jabir, M. S., Taha, A. A., & Sahib, U. I. (2018). Linalool loaded on glutathione-modified gold nanoparticles: A drug delivery system for a successful antimicrobial therapy. *Artificial Cells, Nanomedicine, and Biotechnology, 46*(sup2), 345–355. https://doi.org/10.1080/21691401.2018.1457535

Katiyo, W., de Kock, H. L., Coorey, R., & Buys, E. M. (2020). Sensory implications of chicken meat spoilage in relation to microbial and physicochemical characteristics during refrigerated storage. *Lebensmittel-Wissenschaft und -Technologie, 128*, Article 109468. https://doi.org/10.1016/j.lwt.2020.109468

Lei, J., Yang, L., Zhan, Y., Wang, Y., Ye, T., Li, Y., Deng, H., & Li, B. (2014). Plasma treated polyethylene terephthalate/polypropylene films assembled with chitosan and various preservatives for antimicrobial food packaging. *Colloids and Surfaces B: Biointerfaces, 114*, 60–66. https://doi.org/10.1016/J.COLSURFB.2013.09.052

Leygonie, C., Britz, T. J., & Hoffman, L. C. (2012). Impact of freezing and thawing on the quality of meat: Review. *Meat Science, 91*(2), 93–98. https://doi.org/10.1016/j.meatsci.2012.01.013. Elsevier.

Li, T., Hu, W., Li, J., Zhang, X., Zhu, J., & Li, X. (2012). Coating effects of tea polyphenol and rosemary extract combined with chitosan on the storage quality of large yellow croaker (Pseudosciaena crocea). *Food Control, 25*(1), 101–106. https://doi.org/10.1016/j.foodcont.2011.10.029

Liu, X., Cai, J., Chen, H., Zhong, Q., Hou, Y., Chen, W., & Chen, W. (2020). Antibacterial activity and mechanism of linalool against Pseudomonas aeruginosa. *Microbial Pathogenesis, 141*, Article 103980. https://doi.org/10.1016/J.MICPATH.2020.103980

Liu, J., Shao, Y., Yuan, C., Takaki, K., Li, Y., Ying, Y., & Hu, Y. (2021). Eugenol-chitosan nanoemulsion as an edible coating: Its impact on physicochemical, microbiological and sensorial properties of hairtail (Trichiurus haumela) during storage at 4 °C. *International Journal of Biological Macromolecules, 183*, 2199–2204. https://doi.org/10.1016/J.IJBIOMAC.2021.05.183

Marchese, A., Barbieri, R., Coppo, E., Orhan, I. E., Daglia, M., Nabavi, S. F., Izadi, M., Abdollahi, M., Nabavi, S. M., & Ajami, M. (2017). Antimicrobial activity of eugenol and essential oils containing eugenol: A mechanistic viewpoint. *Critical Reviews in Microbiology, 43*(6), 668–689. https://doi.org/10.1080/1040841X.2017.1295225

Maroufi, L. Y., Shahabi, N., Ghanbarzadeh, M. dokht, & Ghorbani, M. (2022). Development of antimicrobial active food packaging film based on gelatin/dialdehyde quince seed gum incorporated with apple peel polyphenols. *Food and Bioprocess Technology, 15*(3), 693–705. https://doi.org/10.1007/S11947-022-02774-8/METRICS

Menezes, P. P., Serafini, M. R., Quintans-Júnior, L. J., Silva, G. F., Oliveira, J. F., Carvalho, F. M. S., Souza, J. C. C., Matos, J. R., Alves, P. B., Matos, I. L., Hădărugă, D. I., & Araújo, A. A. S. (2014). Inclusion complex of (-)-linalool and β-cyclodextrin. *Journal of Thermal Analysis and Calorimetry, 115*(3), 2429–2437. https://doi.org/10.1007/S10973-013-3367-X/METRICS

Mohammadi Nejad, S., Özgüneş, H., & Başaran, N. (2017). Öjenolün farmakolojik ve toksikolojik özellikleri. In *Turkish journal of pharmaceutical sciences* (Vol. 14, pp. 201–206). Turkish Pharmacists' Association. https://doi.org/10.4274/tjps.62207, 2.

Narasimha Rao, D., & Sachindra, N. M. (2002). Modified atmosphere and vacuum packaging of meat and poultry products. *Food Reviews International, 18*(4), 263–293. https://doi.org/10.1081/FRI-120016206

Noori, S., Zeynali, F., & Almasi, H. (2018). Antimicrobial and antioxidant efficiency of nanoemulsion-based edible coating containing ginger (Zingiber officinale) essential oil and its effect on safety and quality attributes of chicken breast fillets. *Food Control, 84*, 312–320. https://doi.org/10.1016/J.FOODCONT.2017.08.015

Nuchuchua, O., Saesoo, S., Sramala, I., Puttipipatkhachorn, S., Soottitantawat, A., & Ruktanonchai, U. (2009). Physicochemical investigation and molecular modeling of cyclodextrin complexation mechanism with eugenol. *Food Research International, 42*(8), 1178–1185. https://doi.org/10.1016/J.FOODRES.2009.06.006

Ouattara, B., Simard, R. E., Piette, G., Bégin, A., & Holley, R. A. (2000). Diffusion of acetic and propionic acids from chitosan-based antimicrobial packaging films. *Journal of Food Science, 65*(5), 768–773. https://doi.org/10.1111/j.1365-2621.2000.tb13584.x

Oyedemi, S. O., Okoh, A. I., Mabinya, L. V., Pirochenva, G., & Afolayan, A. J. (2009). The proposed mechanism of bactericidal action of eugenol, A-terpineol and Y-terpinene against Listeria monocytogenes, Streptococcus pyogenes, Proteus vulgaris and

escherichia coli. *African Journal of Biotechnology, 8*(7), 1280–1286. https://doi.org/10.4314/ajb.v8i7.60106

Panahi, Z., & Mohsenzadeh, M. (2022). Sodium alginate edible coating containing Ferulago angulata (Schlecht.) Boiss essential oil, nisin, and NaCl: Its impact on microbial, chemical, and sensorial properties of refrigerated chicken breast. *International Journal of Food Microbiology, 380*, Article 109883. https://doi.org/10.1016/j.ijfoodmicro.2022.109883

Park, H. R., Kim, Y. A., Jung, S. W., Kim, H. C., & Lee, S. J. (2013). Response of microbial time temperature indicator to quality indices of chicken breast meat during storage. *Food Science and Biotechnology, 22*(4), 1145–1152. https://doi.org/10.1007/S10068-013-0196-4/METRICS

Pattinson, S. W., & Hart, A. J. (2017). Additive manufacturing of cellulosic materials with robust mechanics and antimicrobial functionality. *Advanced Materials Technologies, 2* (4). https://doi.org/10.1002/admt.201600084

Payet, L., Ponton, A., Grossiord, J. L., & Agnely, F. (2010). Structural and rheological properties of chitosan semi-interpenetrated networks. *The European Physical Journal E, 32*(2), 109–118. https://doi.org/10.1140/EPJE/I2010-10602-7, 2010 32:2.

Pezeshki-Modaress, M., Zandi, M., & Rajabi, S. (2018). Tailoring the gelatin/chitosan electrospun scaffold for application in skin tissue engineering: An in vitro study. *Progress in Biomaterials, 7*(3), 207–218. https://doi.org/10.1007/S40204-018-0094-1/FIGURES/10

Poverenov, E., Zaitsev, Y., Arnon, H., Granit, R., Alkalai-Tuvia, S., Perzelan, Y., Weinberg, T., & Fallik, E. (2014). Effects of a composite chitosan–gelatin edible coating on postharvest quality and storability of red bell peppers. *Postharvest Biology and Technology, 96*, 106–109. https://doi.org/10.1016/J.POSTHARVBIO.2014.05.015

Quintavalla, S., & Vicini, L. (2002). Antimicrobial food packaging in meat industry. *Meat Science, 62*(3), 373–380. https://doi.org/10.1016/S0309-1740(02)00121-3

Reitznerová, A., Uleková, M., Nagy, J., Marcinčák, S., Semjon, B., Čertík, M., & Klempová, T. (2017). Lipid peroxidation process in meat and meat products: A comparison study of malondialdehyde determination between modified 2-thiobarbituric acid spectrophotometric method and reverse-phase high-performance liquid chromatography. *Molecules, 22*(11). https://doi.org/10.3390/molecules22111988

Schnepp, Z. (2013). Biopolymers as a flexible resource for nanochemistry. *Angewandte Chemie International Edition, 52*(4), 1096–1108. https://doi.org/10.1002/ANIE.201206943

Silva, V. A., Sousa, J. P., Guerra, F. Q. S., Pessôa, H. L. F., Freitas, A. F. R., Coutinho, H. D. M., Alves, L. B. N., & Lima, E. O. (2015). Antibacterial activity of the monoterpene linalool: Alone and in association with antibiotics against bacteria of clinical importance. *International Journal of Pharmacognosy and Phytochemical Research, 7*(5), 1022–1026. www.ijppr.com.

Singh, S., ho Lee, M., Park, lnsik, Shin, Y., & Lee, Y. S. (2016). Antimicrobial seafood packaging: A review. *Journal of Food Science and Technology, 53*(6), 2505–2518. https://doi.org/10.1007/s13197-016-2216-x

Stratakos, A. C., Delgado-Pando, G., Linton, M., Patterson, M. F., & Koidis, A. (2015). Synergism between high-pressure processing and active packaging against Listeria monocytogenes in ready-to-eat chicken breast. *Innovative Food Science and Emerging Technologies, 27*, 41–47. https://doi.org/10.1016/j.ifset.2014.11.005

Suppakul, P., Sonneveld, K., Bigger, S. W., & Miltz, J. (2008). Efficacy of polyethylene-based antimicrobial films containing principal constituents of basil. *LWT - Food Science and Technology, 41*(5), 779–788. https://doi.org/10.1016/J.LWT.2007.06.006

Volpe, M. G., Siano, F., Paolucci, M., Sacco, A., Sorrentino, A., Malinconico, M., & Varricchio, E. (2015). Active edible coating effectiveness in shelf-life enhancement of trout (Oncorhynchusmykiss) fillets. *Lwt, 60*(1), 615–622. https://doi.org/10.1016/j.lwt.2014.08.048

Wang, L., Periyasami, G., Aldalbahi, A., & Fogliano, V. (2021). The antimicrobial activity of silver nanoparticles biocomposite films depends on the silver ions release behaviour. *Food Chemistry, 359*, Article 129859. https://doi.org/10.1016/j.foodchem.2021.129859

Wang, Y., Qiu, D., Cosgrove, T., & Denbow, M. L. (2009). A small-angle neutron scattering and rheology study of the composite of chitosan and gelatin. *Colloids and Surfaces B: Biointerfaces, 70*(2), 254–258. https://doi.org/10.1016/J.COLSURFB.2008.12.034

Warriss, P. D. (2000). Meat science: An introductory text. Meat Science: An Introductory Text https://doi.org/10.1079/9780851994246.0000.

World Health Organization, F.and A.O.of the U.N. (2022). Safety evaluation of certain food additives: Prepared by the ninety-second meeting of the Joint FAO/WHO Expert committee on food additives (JECFA). In *WHO food additives Series*. https://apps.who.int/iris/handle/10665/354558.

Xiong, Y., Chen, M., Warner, R. D., & Fang, Z. (2020). Incorporating nisin and grape seed extract in chitosan-gelatine edible coating and its effect on cold storage of fresh pork. *Food Control, 110*, Article 107018. https://doi.org/10.1016/j.foodcont.2019.107018

Yang, Y., & Song, L. X. (2005). Study on the inclusion compounds of eugenol with α-, β-, γ- and heptakis (2,6-di-O-methyl)-β-cyclodextrins. *Journal of Inclusion Phenomena, 53* (1–2), 27–33. https://doi.org/10.1007/s10847-005-0247-4

Yuan, D., Hao, X., Liu, G., Yue, Y., & Duan, J. (2022). A novel composite edible film fabricated by incorporating W/O/W emulsion into a chitosan film to improve the protection of fresh fish meat. *Food Chemistry, 385*(8). https://doi.org/10.1016/j.foodchem.2022.132647