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Whey protein isolate/cellulose nanofibre/TiO_2 nanoparticle/rosemary essential oil nanocomposite film: Its effect on microbial and sensory quality of lamb meat and growth of common foodborne pathogenic bacteria during refrigeration

Mahmood Alizadeh Sani [a], Ali Ehsani [a,*], Mohammad Hashemi [b]

[a] Department of Food Sciences and Technology, Faculty of Nutrition and Food Sciences, Tabriz University of Medical Sciences, Tabriz, Iran
[b] Department of Nutrition, Faculty of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran

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ABSTRACT

The use of biodegradable nanocomposite films in active packaging is of great importance since they can have a controlled release of antimicrobial compounds. This study was conducted to evaluate the efficacy of whey protein isolate (WPI)/cellulose nanofibre (CNF) nanocomposite films containing 1.0% (*w*/*w*) titanium dioxide (TiO_2) and 2.0% (*w*/*v*) rosemary essential oil (REO) in preserving the microbial and sensory quality of lamb meat during the storage at 4 ± 1 °C. Initially, the best concentration of each compound to be added to the film was determined by micro-dilution and disc diffusion methods. The microbial and sensory properties of lamb meat were controlled in two groups (control and treatment) over 15 days of storage. Then, the samples were analysed for total viable count (TVC), *Pseudomonas* spp. count, *Enterobacteriaceae* count, Lactic acid bacteria (LAB) count, inoculated *Staphylococcus aureus* count, *Listeria monocytogenes* count, and *Escherichia coli* O_{157}:H_7 count. Microbial analysis and nine-point hedonic scale was applied for the sensory analysis. Results indicated that the use of nanocomposite films significantly reduced the bacterial counts of treatment group. Higher inhibition effect was observed on Gram-positive bacteria than on Gram-negative bacteria ($P < 0.05$). The microbial and sensory evaluations also showed that the use of nanocomposite films significantly increased the shelf life of treated meat (15 days) compared to the control meat (6 days). Based on the results of this study, the edible nanocomposite films were effective in preserving the microbial and sensory qualities of lamb meat; therefore, this application is recommended in meat especially red meat.

1. Introduction

In recent years, the use of chemical preservatives in food products has increased consumer concerns (Huang et al., 2011). One reason regarding this issue might be the long-term application of chemical preservatives in large quantities causing multiple disorders such as cancers (Kim et al., 2013). Therefore, natural origin preservatives have been suggested as alternatives here, as they could extend foods' shelf life with no side effects. This has led to an increasing tendency towards the application of natural preservatives by producers and researchers (Zhou et al., 2010).

Although meat is an efficient deliverer of protein, essential amino acids, minerals, vitamins and also energy, it is one of the most perishable foods and an excellent substrate for the growth of pathogenic bacteria such as *Staphylococcus aureus*, *Listeria monocytogenes*, *Salmonella* spp., and *Escherichia coli* O_{157}:H_7 as well as spoilage; *Pseudomonas* spp., *Enterobacteriaceae*, and Lactic acid bacteria. Due to the limitations of storing fresh meat and the importance of preserving its quality before consumption, researchers are looking for different methods to extend the shelf life and to preserve the optimal quality (Jay et al., 2005).

Needless to say that food packaging has been significantly developed in preserving the quality of meat for instance the use of edible and biodegradable films. The main advantage of edible films is their function as a carrier for food additives such as flavours, antimicrobials, antioxidants, enzymes, and colors. Biopolymer nanocomposite films with the controlled release of preservative compounds could reduce the speed of biological and chemical corruption in foodstuff (Cutter, 2006).

A recent study has been conducted on the antimicrobial effects of essential oils (EOs), extracts and spices (Burt, 2004; Ehsani et al., 2016; Raeisi et al., 2016), their ingredients are of natural source and pose little risk to human health and environment compared to chemical

* Corresponding author at: Golgasht Ave, Attar-Neishaboori St., Department of Food Sciences and Technology, Faculty of Nutrition and Food Sciences, Tabriz University of Medical Sciences, Tabriz, Iran.
E-mail address: ehsani@tbzmed.ac.ir (A. Ehsani).

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preservatives (Burt, 2004). EOs are rich in phenolic compounds, such as monoterpenes, flavonoids, and phenolic acids (Baser and Kirimer, 2006). Rosemary (*Rosmarinus officinalis*) essential oil (REO) which is broadly known as a spice, is successfully used for food preservation due to its antioxidant and antimicrobial properties (Pintore et al., 2002; Raeisi et al., 2016; Seydim and Sarikus, 2006; Uçak et al., 2011). Moreover, among inorganic antimicrobial compounds, titanium dioxide (TiO_2) is a metal oxide, non-toxic, abundant and cheap component which represents photocatalytic sterilization properties (Gelover et al., 2006). Today, it is widely used in the production of antimicrobial nanocomposite films. TiO_2 has antimicrobial activity against a broad spectrum of organisms, including bacteria, fungi, and cancer cells (Blake et al., 1999; Luo et al., 2015).

There has not been enough information about the antibacterial activity of TiO_2 and REO on lamb. Only a few antimicrobial agents have been incorporated to edible coatings on lamb meat in order to restrict quality changes during the storage (Babuskin et al., 2015; Morsy et al., 2014; Quintavalla and Vicini, 2002; Zhang et al., 2009). Therefore, the present study was conducted to determine the shelf life and quality changes of lamb meat packed in nanocomposite films containing REO and TiO_2 nanoparticles during the storage at 4 °C.

2. Materials and methods

2.1. Materials

Whey protein isolate (WPI, 92 wt% protein) was obtained from Davisco Foods Intl., Inc. (Eden Prairie, Minn., U.S.A.). TiO_2 nanoparticles (anatase, purity > 99%) were purchased from US Research Nano materials, Inc., Houston, U.S.A. Cellulose nanofibre (CNF) was supplied from Nano Novin Polymer, Mazandaran, Iran. REO was supplied by Barijessence Company, Kashan, Iran. Medium cultures including Plate Count agar (PCA), Violet Red Bile Glucose agar (VRBA), De Man, Ragusa, Sharpe agar (MRS), Palcam agar, Eosin Methylene Blue agar (EMB), Baird-Parker agar, *Cetrimide Fusidin Cephaloridine* agar, Nutrient broth, Nutrient agar, and Mueller-Hinton agar were all purchased from Micromedia, Canada. All the applied reagents were of analytical grade. Bacterial strains were obtained from Biological and Genetic Resources Center, Tehran, Iran.

2.2. GC-MS analysis of REO

Gas Chromatography-Mass spectrophotometry (GC-MS) analysis of REO was performed by an Agilent 6890N gas chromatography equipped with a mass spectrophotometry with an HP-5MS capillary column (30 × 0.25 mm × 0.25 μm). Helium was used as a carrier gas with a flow rate of 1 mL/min and the injection volume was 1 μL with a split ratio of 1:1. The initial temperature was 50 °C and was held at this temperature for 5 min; then programmed to 240 °C at a rate of 3 °C per min, kept for 3 min and finally increased to 300 °C (Raeisi et al., 2016). The compounds were determined by comparing them with both authentic samples and mass spectral information of computerized library of Wiley-VCH 2001, Weinheim, Germany (Ehsani et al., 2016).

2.3. Determination of minimum inhibitory concentration

The antimicrobial activity of TiO_2 nanoparticles and REO was determined by micro dilution method on 96-well micro-plates according to the previously reported method (Ehsani et al., 2016; Tajik et al., 2015). Five bacterial suspensions including: *Listeria monocytogenes* (IBRC-M 10671), *Escherichia coli* O_{157}:H_7 (IBRC-M 10698), *Staphylococcus aureus* (IBRC-M 10690), *Salmonella. enteritidis* (IBRC-M 10954) and *Pseudomonas fluorescens* (IBRC-M 10752) were prepared after 18 h cultures on Nutrient broth, adjusted to 0.5 McFarland standard turbidity 1.5×10^8 CFU/mL and diluted to the desired bacterial density (1.5×10^6 CFU/mL). Then, target bacterial suspensions (20 μL) with different concentrations of TiO_2 in distilled water (0.25, 0.5, 1, 2, 3, 4, 5, 6, 7, and 8 mg/mL; homogenized by ultrasonic homogenizer for better dispersion) and REO in Dimethyl Sulfoxide (DMSO) (0.25, 0.5, 1, 2, 3, 4, 5, 6, 7, and 8 mg/mL) were separately added to the tested wells containing 160 μL of Nutrient broth (20 μL each) and in combination (10 μL each). As for the combined mode, TiO_2 at a constant concentration of 1 mg/mL along with different concentrations of REO (0.25–8 mg/mL) were added to the tested wells containing 20 μL bacterial suspension and 160 μL culture medium. The last wells of micro-plates were considered as negative controls containing un-inoculated broth with antimicrobials and as positive controls containing inoculated broth without antimicrobials. The final volume of each well was 200 μL, the final concentration of bacterial suspensions was approximately 1.5×10^5 CFU/mL, and the final concentration of antimicrobials was in the range of 0.25–8 mg/mL. The micro-plates were incubated at 37 °C for 18–24 h under constant shaking (50-100 rpm) by a micro-plate shaker (Boeco, Hamburg, Germany). MIC values were determined as the lowest concentration with no visible bacterial growth. The best concentration of each antimicrobial agent which was to be added to the edible films was determined by this method.

2.4. Preparation of WPI/CNF/TiO_2/REO nanocomposite films

The WPI film was prepared according to a previously described method (Seydim and Sarikus, 2006; Zhou et al., 2009) with some modifications. Whey protein isolate (10% *w*/*v*) was dissolved in distilled water for 2 h, and pH was adjusted to 8.0 with 2N NaOH. The solution was heated to 90 °C for 30 min while being stirred continuously and later was filtered through a layer of cheesecloth. Glycerol (6% *w*/*w*) was added to the solution as a plasticiser along with CNF (7.5% *w*/*w*), TiO_2 (1% *w*/*w*) and REO (2% *w*/*v*) were incorporated into the solution (separately and in combination) as antibacterial agents, then, the solution was stirred continuously using ultrasonic homogenizer (Model 3000MP, Biologic's, Inc. Manassas, U.S.A) in order to obtain a good dispersion. Then the solution was vacuumed for 40 min to eliminate dissolved air (The weights were based on WPI). The film's solution (14 ± 0.2 g) was cast into 8 cm circular sterile plates, dried by oven at 30 °C for 24 h, peeled from the plates and stored in a desiccator for further use. The concentrations for each component were calculated and adjusted based on whey protein isolate (WPI) weight. Final concentrations of dried matter were: TiO_2 (0.1 g), REO (0.2 g), CNF (0.75 g) and glycerol 6 g in 100 mL final solution.

2.5. Determination of antibacterial activity of WPI/CNF/TiO_2/REO nanocomposite films

Antibacterial activity of the films was evaluated by disc diffusion method (Ehsani et al., 2016; Seydim and Sarikus, 2006). *L. monocytogenes*, *S. enteritidis*, *E. coli* O_{157}:H_7, *S. aureus*, and *P. fluorescens* suspensions were prepared from 18 h Nutrient broth cultures, adjusted to 0.5 McFarland standard turbidity and diluted (1:10) to the desired bacterial density (1.5×10^6 CFU/mL). The films were aseptically cut into pieces with 10 mm inner diameter and were placed on the surface of Mueller-Hinton agar plates, and finally inoculated with 0.1 mL of the bacterial suspensions (final concentration of 1.5×10^5 CFU/mL). The plates were then incubated at 37 °C (*L. monocytogenes*, *S. enteritidis*, *E. coli* O_{157}:H_7, and *S. aureus*) and 23 °C (*P. fluorescens*) for 24 h and inhibition zones around the discs were measured by a calliper. In order to select the best film to be applied in meat samples, the films were prepared in three separate groups, including films containing REO and TiO_2 separately and in combination,

2.6. Preparation of meat samples and treatments

Fresh lamb meat samples were obtained from an abattoir, immediately transferred to the laboratory and cut under aseptic conditions.