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Antimicrobial evaluation of novel poly-lactic acid based nanocomposites incorporated with bioactive compounds in-vitro and in refrigerated vacuum-packed cooked sausages

Mohammadreza Rezaeigolestani[a], Ali Misaghi[a,*], Ali Khanjari[a], Afshin Akhondzadeh Basti[a], Ali Abdulkhani[b], Samira Fayazfar[a]

[a] Department of Food Hygiene, Faculty of Veterinary Medicine, University of Tehran, Tehran 14155-6453, Iran
[b] Department of Wood and Paper Science and Technology, Faculty of Natural Resources, University of Tehran, Karaj, Iran

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ABSTRACT

Biodegradability and antimicrobial activity of food packaging materials are among the most attractive parameters in modern food industries. In order to develop biodegradable poly-lactic acid (PLA) film to antibacterial nanocomposites, different concentration of *Zataria multiflora* Bioss. essential oil (ZME), propolis ethanolic extract (PEE) and cellulose nanofiber (CNF) were incorporated to the polymer by solvent casting method. The resulting films were characterized by mechanical and physical tests and their antimicrobial application was evaluated in-vitro against four common foodborne pathogens and in vacuum-packed cooked sausages during refrigerated storage. Mechanical examination revealed that addition of ZME and PEE made films more flexible and incorporation of CNF improved almost all mechanical parameters tested. Moreover, according to physical analysis, incorporation of 0.5% *v*/v ZME to the composite primary solutions improved water vapor permeability of the resulting films. Almost all of the active films were effective against the tested bacteria except for PLA/PEE films, and maximum antibacterial effects recorded for the films containing both ZME and PEE. Based on the microbiological and sensory evaluation of the sausages, all of the PLA/1%ZME/PEE composites increased the shelf life to > 40 days. The results indicate that incorporation of natural antimicrobial substances such as ZME and PEE to packaging material could be an interesting approach in development of active packaging material without significant negative effect on polymer technical properties.

1. Introduction

Vacuum-packed cooked sausages are one of the most popular ready-to-eat food products that generally recognized as long-life meat products. However, during production of these types of sausages, particularly after heat treatment, many of the food spoilage microorganisms and also foodborne pathogens may enter the products, which along with poor post-process storage can threaten products' shelf life (Korkeala and Björkrot, 1997; Reij and Den Aantrekker, 2004).

Nowadays, antimicrobial food packaging as a type of active packaging, can be viewed as one of the best strategies to control microbial growth in food products while keeping them fresh, safe and in good quality. A variety of antimicrobial food packaging films have been developed by addition of different antimicrobial agents to various packaging materials (Malhotra et al., 2015).

In this context, poly-lactic acid (PLA) films have shown great promise in food packaging applications. Biodegradable PLA is one of the most interesting and significant food packaging biomaterials due to its biodegradability, comparable cost, biocompatibility, superior transparency, and excellent thermal and mechanical properties comparing to other popular and important biodegradable polymers (Shameli et al., 2010). Recently antibacterial PLA films were successfully developed with the help of some chemical or natural antibacterial compounds (Javidi et al., 2016; Salmieri et al., 2014; Woraprayote et al., 2013).

Natural antimicrobial agents have respectable potential to be applied as food preservatives and different kinds of them have been used in development of active food packaging materials (Irkin and Esmer, 2015). Plant essential oils (EOs) are important groups of natural antibacterial compounds which act against both Gram-positive and Gram-negative bacteria (Burt, 2004; Golestani et al., 2015).

Among the main herbal EOs, *Zataria Multiflora* Bioss. essential oil

* Corresponding author.
E-mail addresses: rezaei.reza@ut.ac.ir (M. Rezaeigolestani), amisaghi@ut.ac.ir (A. Misaghi), khanjari@ut.ac.ir (A. Khanjari), aakhond@ut.ac.ir (A.A. Basti), abdolkhani@ut.ac.ir (A. Abdulkhani), samirafayaz@gmail.com (S. Fayazfar).

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(ZME) is well known, especially for its strong antibacterial effects (Parsaeimehr et al., 2015; Sajed et al., 2013). In some regions, *Zataria multiflora* Boiss. widely added to foods as a flavouring agent and also used in traditional folk medicine, particularly for its antiseptic, analgesic and carminative properties (Akrami et al., 2015). ZME as an herbal EO has been generally recognized as safe (GRAS) for using as a food additive (by the US Food and Drug Administration), and this advantage along with its hydrophobic and antibacterial nature, make it an applicable candidate for using in antibacterial food packaging films in combination with hydrophobic plastic materials.

Propolis is another natural bioactive substance collected by *Apis mellifera* and used for building strength and protection of honeycombs (Maurício and Bankova, 2011). Flavonoids and phenolic compounds are major antibacterial constituents of propolis that originally derived from plant materials (Probst et al., 2011). In most of the published studies on propolis properties, ethanolic extract of propolis was employed and evaluated in different conditions (Alencar et al., 2007; Bryan et al., 2015; Probst et al., 2011).

According to the literatures, the integration of polymers with active agents such as antimicrobials, allows them to be gradually released from the polymeric matrix during an extended period, which would prolong their effects and consequently extend the product shelf-life (Iturriaga et al., 2012). Discontinuities in polymer structure due to the incorporation of active agents may lead to production of physico-mechanically weak composites (Javidi et al., 2016). In such cases, addition of a reinforcement compound to polymers can be considered as a solution to improve overall structural properties of the resulting composites. Between common reinforcement agents, nanocellulose is important non-toxic bio-based material used successfully in combination with various polymers (Aitomäki and Oksman, 2014; Jonoobi et al., 2010).

During the past couple of years, number of publications focused on development of antibacterial PLA films and their application in different foods such as salad and raw meats (Javidi et al., 2016; Llana-Ruiz-Cabello et al., 2015; Woraprayote et al., 2013). Only few efforts have been made to investigate addition of EOs to food packaging materials, and many aspects of their interactions and potential application in food products are still unknown.

Our objectives were to develop and characterize antibacterial PLA-based nanocomposites imbued with *Zataria multiflora* EO, propolis ethanolic extract (PEE) and cellulose nanofibers (CNF) by solvent casting method, and antibacterial evaluation of resultant composite films in two phases: first, their antibacterial activities against four common foodborne bacteria by means of agar diffusion agar; second, their ability to control microbial growth in sliced vacuum-packed cooked sausages during refrigerated storage. Finally, in order to evaluate the effect of treatment with the active films on sensorial quality of the products, sensory examination was carried out during the study period.

2. Materials and methods

2.1. Preparation of active PLA films

2.1.1. Exraction of propolis and ZME

Zataria Multiflora Bioss plants and crude propolis samples were supplied by Faculty of agriculture, University of Tehran (Iran). The dried aerial parts of plant were grounded and submitted to steam distillation for 2.5 h using a Clevenger-type system. The obtained EO was dried over anhydrous Na_2SO_4 and then stored in dark at 4 °C until tested.

PEE was prepared according to Bodini et al. (2013) with some modification; briefly 30 g of crude porpolis samples were grounded and extraction made under stirring in 100 ml of 80% ethanol (Merck, Germany). Then mixture stirred at 50 °C for 30 min and filtered with Whatman No. 1 filter paper to produce PEE. Finally, the resultant PEE stored in dark at 4 °C until used.

2.1.2. Analysis of chemical composition of ZME and PEE

Analysis of ZME and PEE were performed by Agilent 7890A/5975C GC-MS system, equipped with a capillary column (30 m length × 0.25 mm diameter, film thickness 0.25 μm). The oven temperature programmed from 50 °C (held for 3 min), to 240 °C (held for 1 min) at a rate of 5 °C/min. The Injector port temperature was 250 °C and carrier gas (helium) flow rate was set at 1 ml/min. The resulting GC-MS peaks were identified by the comparison with data from the literature and the Wiley (2001 data software) and National Institute of Standards and Technology (NIST 08) commercial libraries.

2.1.3. Preparation of active films

The PLA composite films were prepared according to Abdulkhani et al. (2014) using solvent casting method. In brief, a mixture of PLA granules (FkuR kunststoffm GmbH, Germany, Density: 1.3 g/cm^3, molecular weight: 197,000 g/mol) in chloroform (1%, *w/v*) was prepared and vigorously stirred by magnetic bar for 8 h until PLA granules completely dissolved. For preparation of antibacterial composite films, different concentrations of ZME (0, 0.5 and 1% *v*/v), PEE (0 and 2% *v*/v) and CNF gel (provided by Nano Novin polymer Co., Sari, Iran) (0 and 1% *v*/v) were added to the solution (Table 1), and the mixtures were homogenized at 8000 rpm for 2 min by means of a homogenizer (IKA T25-digital ultra turrax, Germany) with a S25 N-25F probe. The resultant solutions were poured into glass Petri dishes (diameter: 100 mm, deep: 15 mm), and then chloroform was allowed to evaporate under a chemical hood at room temperature for 24 h. Afterward, the 12 types of films were aseptically peeled and cut into 9 mm-diameter discs, and finally placed in a sealed container containing silica gel until used. The thicknesses of composites were measured using a digital micrometer and the averages of ten measurements were reported (Table 1).

Table 1
Composition of PLA master solutions and thickness of the resulting films.

Sample codes	Thickness (mm)[a]	PLA content (% *w/v*)	ZME content (% *v*/v)	CNF content (% *v*/v)	PEE content (% *v*/v)
PLA	0.06 ± 0.02	1	0	0	0
PLA/CNF	0.06 ± 0.03	1	0	1	0
PLA/PEE	0.08 ± 0.06	1	0	0	2
PLA/CNF/PEE	0.08 ± 0.05	1	0	1	2
PLA/0.5%ZME	0.07 ± 0.04	1	0.5	0	0
PLA/0.5%ZME/CNF	0.07 ± 0.03	1	0.5	1	0
PLA/0.5%ZME/PEE	0.08 ± 0.06	1	0.5	0	2
PLA/0.5%ZME/CNF/PEE	0.09 ± 0.04	1	0.5	1	2
PLA/1%ZME	0.08 ± 0.03	1	1	0	0
PLA/1%ZME/CNF	0.08 ± 0.04	1	1	1	0
PLA/1%ZME/PEE	0.9 ± 0.01	1	1	0	2
PLA/1%ZME/CNF/PEE	0.9 ± 0.02	1	1	1	2

[a] Data are expressed as mean ± standard deviation (n = 10).