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A novel antifungal surface-coating application to limit postharvest decay on coated apples: Molecular, thermal and morphological properties of electrospun zein–nanofiber mats loaded with curcumin

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ABSTRACT

Coating surfaces of fruit with electrospun zein mats with functionalized antimicrobial properties can be a novel strategy to prevent fungal colonization on fruit surfaces. In this study, we tested curcumin-loaded electrospun zein nanofibers (CLZN) in terms of limitation of postharvest decay on CLZN–coated apples infected with *Botrytis cinerea* and *Penicillium expansum*. Mixtures of zein and curcumin (the curcumin amounts of 2.5 and 5 wt% based on the weight of zein powder) were electrospun to yield cylindrical and ultrafine (<350 nm in diameter) polymeric nanofibers. In addition, molecular, thermal, zeta potential and morphological properties of the CLZN as well as their encapsulation efficiency and releasing kinetics were determined, revealing that the developed zein-based scaffolds showed high encapsulation efficiency, molecular interactions with curcumin within nanofibers, alterations in physical states of these components, smooth beadless structure and good thermal (an endothermic peak at 152 °C) and dispersion stability (−24 mV of ζ potential) properties. *In vitro* antifungal activity tests conducted at 27 °C for six days showed that CLZN were effective against growth of the tested fungal pathogens, exhibiting almost 40–50% inhibition of mycelial growth of the fungal pathogens; but the antifungal effect against *P. expansum* was but two-fold higher than that against *B. cinerea*. *In vivo* tests conducted at 23 °C with 75% humidity for six days confirmed *in vitro* test results in terms of both visual inspections on uncoated and coated apples, revealing almost 50% reduction in lesion diameter measured on coated apples infected with *Penicillium expansum*. Our results suggest that CLZN mats open up new opportunities for a novel application of edible and biodegradable antifungal coating material with the ability to hinder fungal proliferation on coated apples during storage period.
Industrial relevance: We coated the surfaces of fruits with electrospun mats with functionalized antimicrobial properties to prevent fungal colonization on fruit surface. The coating of apples with curcumin-loaded zein nanoparticles (CLZNs) limited the postharvest decay caused by the fungal pathogens, *Penicillium expansum* and *Botrytis cinerea*. This study showed that by encapsulation of curcumin into zein-based nanofibers considerably increased the antifungal effectiveness of curcumin. Our results highlighted the potential use of the CLZN as an effective fungicidal coating material against *P. expansum* and *B. cinerea* and suggested that CLZNs can be promising tools to compete with synthetic fungicide counterparts of curcumin. The results of this study should be of great importance to industrial applications in terms of development of natural, but effective preservatives as alternative to synthetic ones.

1. Introduction

Zein as a mixture of proteins with different molecular weights in corn gluten exhibits important properties such as low hydrophilicity, high elasticity, compressibility, glossiness and film-forming capabilities that widen its potential in biomedical applications as being a scaffold and matrix for novel drug delivery systems (Huang et al., 2013) in terms of serving as carriers of proteins, cells, drugs and growth factors (Jain & Banerjee, 2008; Maham, Tang, Wu, Wang, & Lin, 2009). Several studies have shown the successful encapsulation of different bioactive molecules within protein-based polymer fibers such as zein nanofibers (Corradini et al., 2014).

Curcumin is a natural polyphenolic bioactive compound isolated from the dry rhizomes of *Curcuma longa* (Vitaglione et al., 2012, Yen, Wu, Tzeng, Lin, & Lin, 2010). It is widely used in the food industry especially

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as flavor and food-coloring agents (Khalil et al., 2012). Curcumin is also a promising bioactive component and is highly biocompatible and biodegradable, which allows it to be metabolized very easily (Mishra, Narain, Mishra, & Misra, 2005). Furthermore, it is demonstrated to have important therapeutic functions such as antibacterial (Negi, Jayaprakasha, Jagan Mohan Rao, & Sakariah, 1999), antifungal (Khalil et al., 2012), anticancer (Du et al., 2015), anti-inflammatory and antioxidant effects (Kocaadam & Sanlier, 2015) and anti-HIV activity (Mazumder et al., 1996; Sui, Salto, Li, Craik, & Ortiz de Montellano, 1993). It was also reported to be effective against cystic fibrosis (Egan et al., 2004) and Alzheimer's disease (Ono, Hasegawa, Naiki, & Yamada, 2004). Despite the wide range of biological activities that this polyphenolic substance possesses, curcumin has poor solubility and dissolution properties that lower its bioavailability and it is markedly sensitive to metabolic processes, which limits its potential in these applications. Several different methodologies were shown to be applied for the enhancement of the dissolution and bioavailability as well as that of resistance properties of curcumin such as triggering its potential with different molecules (Khalil et al., 2012), dissolving curcumin in the oil body of emulsions (Wang et al., 2008a) and preparation of curcumin in nano sizes (Bhawana, Basniwal, Buttar, Jain, & Jain, 2011). Among these methodologies, encapsulation of curcumin within different nanofibers appears to be one of the most highly preferred and promising tools to overcome the poor solubility and low bioavailability problems (Prasad, Tyagi, & Aggarwal, 2014). These nanofibers can only be prepared by application of an electrospinning technique, which can be applied on a wide variety of dissolved or melted polymer solutions (Greiner & Wendorff, 2007) containing curcumin, which results in the redesigning of this polyphenol within the nanofiber in order to improve its delivery and bioavailability by providing a controlled releasing profile and enhancing its dispersibility and surface area (Prasad, Tyagi, & Aggarwal, 2014). In this respect, curcumin has been encapsulated in different nanoparticles and nanofibers including liposomes (Wang et al., 2008b), bovine serum albumin (Gupta, Aseh, Rios, Aggarwal, & Mathur, 2009), chitosan (Das, Kasoju, & Bora, 2010), cyclodextrin (Yallapu, Jaggi, & Chauhan, 2010) and zein (Hu et al., 2015) for different purposes.

Apples are among the most common fruits that are prone to such postharvest losses mostly caused by *Penicillium expansum* and *Botrytis cinerea* (Calvo, Calvente, de Orellano, Benuzzi, & Tosetti, 2007). Public concerns over the fungicides due to their harmful effect on health (Singh, Al-samarai, & Syarhabil, 2012) and resistances that pathogens could acquire, have prompted investigators to seek for novel alternatives to the fungicides (Combrink, Regnier, & Kamatou, 2011). Therefore, nowadays, a great effort is being exerted to develop novel antimicrobial agents in order to control microbial spoilage.

Electrospun mats can be used for encapsulation of a number of antimicrobial compounds in order to prevent microbial contamination and infection (de Faria, Perreault, Shaulsky, Chavez, & Elimelech, 2015), revealing that it would be also possible to fabricate antimicrobial electrospun scaffolds using electrospun polymer solutions including antimicrobial compounds (Botes & Cloete, 2010). Functionalization of nanofibers capable of inhibiting fungal growth can be an effective strategy to fabricate polymer-based coating materials in terms of prevention of fungal growth on fruit surfaces (de Faria, Perreault, Shaulsky, Chavez, & Elimelech, 2015). Given that, coating surfaces of fruits with electrospun mats with functionalized antimicrobial properties would be a novel strategy to prevent fungal colonization on fruit surface. Therefore, we aimed at using curcumin as a bioactive component and zein as a nanocarrier polymer in order to prepare curcumin-loaded zein nanofibers (CLZN) and to use CLZN as a coating material to coat apple surface to understand to what extent postharvest decay on apples would be limited. We also aimed at characterizing of CLZN in terms of molecular, thermal, zeta potential and morphological properties as well as their encapsulation efficiency and releasing kinetics. Our results highlight the potential use of the curcumin-loaded zein nanofibers as an edible and effective fungicidal coating material against *P. expansum* and *B. cinerea* for a novel postharvest application, along with their molecular, thermal, zeta potential, encapsulation efficiency and morphological properties.

2. Materials and methods

2.1. Materials

Purified zein (Acros Organics, New Jersey, USA), curcumin ($C_{21}H_{20}O_6$, M.W.: 368.38; Sigma-Aldrich Company, China), dialysis membrane (Molecular cut off: 12 kDa; Sigma-Aldrich, China), ethanol (Düzey LAB Ltd., Turkey) and phosphate-buffered saline (pH: 7.4; Gibco, Invitrogen, USA) were used in this study. PDA (potato dextrose agar), streptomycin and Ringer's solution were purchased from Merck (Darmstadt, Germany). The fruit material used in this study was the apple cultivar, 'Golden Delicious' harvested from the Eğirdir region in Isparta, Turkey and were sourced from "İstanbul" Wholesale Market Hole. The apples were harvested at optimal maturity, selected for uniformity in size, appearance, ripeness, absence of physical defects and manually sorted to remove those with blemishes were stored at −0.5 °C for a period of 2 months after harvest. Before analysis, the fruits were removed from the cold storage and left overnight at room temperature. The selected fruits were randomized before being used for treatments. In this study, all the process are represented in the Fig. 1 where the antifungal surface-coating application process was summarized to produce electrospun zein–nanofiber mats loaded with curcumin along with their molecular, thermal and morphological characterization.

2.2. Production of electrospun nanofibrous scaffolds

2.2.1. Preparation of spinning dope solutions

A single solvent system was used to prepare the spinning dope solutions. The zein solution was prepared by dissolving the zein in ethanol/water solution (85% v/v) under constant stirring for 24 h in order to form a homogeneous solution. The curcumin-loaded zein solutions were prepared by dissolving curcumin and zein powders in the amounts of 2.5 and 5 wt% of curcumin based on the weight of 30% zein powder in 85% (v/v) aqueous ethanol solution. The weight percentage of the curcumin and zein in total spinning dope solution was calculated according to the following Eqs. (1), (2):

$$\%\mathrm{Wt}=^{\mathrm{powder}}\frac{\text{Powder materials (curcumin + zein)}}{\text{Powder materials + EtOH weight}}\times 100 \quad (1)$$

$$\%30\mathrm{Wt}_{\text{Curcumin and zein}} = \text{Curcumin } (2.5 \text{ or } 5\%) + \%\text{Zein weight } (27.5 \text{ or } 25\%) \quad (2)$$

The solution was withdrawn into a 10-mL plastic syringe equipped with 18-gauge needles, which was inserted into a syringe pump to initiate electrospinning process.

2.2.2. Electrospinning process

Electrospinning process was performed using a benchtop electrospinning equipment (Nanodev Scientific, Ankara, Turkey) (Fig. 2A). The electrospinning apparatus was consisted of followings: a syringe pump (New Era Pump Systems Inc., NE-300, Hauppauge, NY), a high voltage supply (Spellman, CZE 1000R, High Voltage Electronics Corporation, Hauppauge, NY) and a spinneret made of stainless steel with an outer diameter of 1.2 mm and an inner diameter of 0.94 mm (Terumo Co., Japan) and fitted with a single 18-gauge needle to which a single needle was attached (Fig. 2B).

Zein nanofibers (ZN) and curcumin-loaded zein nanofibers (CLZN) were electrospun using a 30-mL syringe (TOP, Japan) containing 10 mL of the spinning dope solutions, collected on grounded aluminum foil, and dried in a vacuum oven. The distance between the collection plate and the needle tip were adjusted to 10 cm and the electric field