Food Hydrocolloids 79 (2018) 544–559

Contents lists available at ScienceDirect

Food Hydrocolloids

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

Physico-chemical, antimicrobial and antioxidant properties of gelatin-chitosan based films loaded with nanoemulsions encapsulating active compounds

Luis J. Pérez-Córdoba [a, b, *], Ian T. Norton [b], Hannah K. Batchelor [c], Konstantinos Gkatzionis [b], Fotios Spyropoulos [b], Paulo J.A. Sobral [a]

[a] Department of Food Engineering, Faculty of Animal Science and Food Engineering, University of São Paulo, Pirassununga 13635-900, São Paulo, Brazil
[b] School of Chemical Engineering, University of Birmingham, Edgbaston, Birmingham B15 2TT, UK
[c] Pharmacy School, University of Birmingham, Edgbaston, Birmingham B15 2TT, UK

ARTICLE INFO

Article history:
Received 10 October 2017
Received in revised form
7 December 2017
Accepted 8 December 2017
Available online 9 December 2017

Keywords:
Biopolymer
Active films
Emulsion
α-tocopherol
Cinnamaldehyde
Garlic oil

Chemical compounds studied in this article:
Cinnamaldehyde (PubChem CID: 637511)
alpha-tocopherol (PubChem CID: 14985)
Garlic oil (PubChem CID: 6850738)
Tween 20 (PubChem CID: 443314)
Span 60 (PubChem CID: 14928)
Chitosan (PubChem CID: 21896651)
Acetic acid (PubChem CID: 176)
Glycerol (PubChem CID: 753)
2,2′-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) (PubChem CID: 16240279)
1,1-Diphenyl-2-picrylhydrazyl (PubChem CID: 2735032)

ABSTRACT

The aim of this research was to develop and characterize gelatin-chitosan (4:1) based films that incorporate nanoemulsions loaded with a range of active compounds; N_1: canola oil; N_2: α-tocopherol/cinnamaldehyde; N_3: α-tocopherol/garlic oil; or N_4: a-tocopherol/cinnamaldehyde and garlic oil. Nanoemulsions were prepared in a microfluidizer with pressures ranging from 69 to 100 MPa, and 3 processing cycles. Films were produced by the casting method incorporating 5 g $N_{1,2,3,4}$/100 g biopolymers and using glycerol as a plasticizer, and subsequently characterized in terms of their physico-chemical, antimicrobial and antioxidant properties. No differences ($p > 0.05$) were observed for all films in terms of moisture content (18% w/w), and thermal properties. The films' solubility in water and light transmission at 280 nm were considerably reduced as compared to the control, N_1 (15% and 60% respectively) because of the nanoemulsion incorporation. The film loaded with N_1 showed the greatest ($p < 0.05$) opacity, elongation at break and stiffness reduction, and was the roughest, whilst the lowest tensile strength and ability to swell were attained by films loaded with N_3 and N_4, respectively. DSC and X-ray analyses suggested compatibility among the biopolymeric-blend, and a good distribution of nanodroplets embedded into the matrix was confirmed by AFM and SEM analyses. Films loaded with nanoencapsulated active compounds (NAC) were very effective against *Pseudomonas aeruginosa*, and also showed high antioxidant activity. Overall, the present study offers clear evidence that these active-loaded films have the potential to be utilized as packaging material for enhancing food shelf life.

1. Introduction

The development of biodegradable packaging has been the focus of recent research, as an alternative to plastic material derived from petroleum, which due to their poor biodegradation generate a massive accumulation of plastic waste in the environment (Arancibia, Giménez, López-Caballero, Gómez-Guillén, & Montero, 2014; Rubilar et al., 2013). Films based on biopolymers do not have the same physical properties as synthetic plastics, but they present a promising application because they generally are from renewable sources, non-toxic, biodegradable, biocompatible, and sometimes could become edible material (Chen et al., 2016; Kurek, Galus, & Debeaufort, 2014; Pérez-Córdoba & Sobral, 2017).

* Corresponding author. Department of Food Engineering, Faculty of Animal Science and Food Engineering, University of São Paulo, Pirassununga 13635-900, São Paulo, Brazil.
E-mail address: luchop283@usp.br (L.J. Pérez-Córdoba).

https://doi.org/10.1016/j.foodhyd.2017.12.012

Furthermore, these films are excellent vehicles for incorporating a wide variety of active agents, such as antioxidant and antimicrobial compounds, and thus, these biodegradable materials can be used for active packaging (Abdollahi, Rezaei, & Farzi, 2012; Rhim & Ng, 2007).

According to Gennadios, McHugh, Weller, and Krochta (1994), gelatin (G) was one of the first materials used as a carrier of bioactive components. Gelatin is a protein obtained by hydrolyses of the collagen from bones and skin via exposure to acidic (type-A) or alkaline (type-B) pre-treatment conditions (Gómez-Guillén et al., 2009). Gelatin has excellent film-forming properties and can generally form films with good mechanical characteristics that also act as barriers to oxygen, carbon dioxide, and volatile compounds (Tongnuanchan, Benjakul, & Prodpran, 2012); they form however a relatively poor barrier to moisture mainly due to the hydrophilic nature of the gelatin molecules (Ahmad, Benjakul, Prodpran, & Agustini, 2012). Moreover, gelatin has the ability to blend well with others biopolymers, such as chitosan (Bonilla & Sobral, 2016; Pérez-Córdoba & Sobral, 2017).

Chitosan (Ch) is a linear polysaccharide consisting of β-(1–4)-2-acetamido-D-glucose and β-(1–4)-2-amino-D-glucose units, derived from chitin through deacetylation in alkaline media, and it is the second most abundant polysaccharide found in nature, after cellulose (Baron, Pérez, Salcedo, Pérez-Córdoba, & Sobral, 2017; Elsabee & Abdou, 2013). Similar to gelatin, chitosan has excellent film-forming properties and offers great potential as the basis for active packaging material due to its intrinsic antimicrobial activity (Kanatt, Rao, Chawla, & Sharma, 2012). Blending chitosan with gelatin can produce films with improved properties, showing antimicrobial or antioxidant activity due to the presence of chitosan, or following the incorporation of hydrophilic bioactive agents (Benbettaïeb, Kurek, Bornaz, & Debeaufort, 2014; Bonilla & Sobral, 2016; Hosseini, Rezaei, Zandi, & Ghavi, 2013; Jridi et al., 2014; Pereda, Ponce, Marcovich, Ruseckaite, & Martucci, 2011; Rivero, García, & Pinotti, 2009).

More recently, a number of studies have reported biopolymer films loaded with lipophilic compounds that are dispersed within the hydrophilic film structure as nanodroplets (nanoemulsions) (Acevedo-Fani, Salvia-Trujillo, Rojas-Graü, & Martín-Belloso, 2015; Alexandre, Lourenço, Quinta Barbosa Bittante, Moraes, & Sobral, 2016; Chen et al., 2016; Otoni, Avena-Bustillos, Olsen, Bilbao-Sáinz, & McHugh, 2016; Sasaki, Mattoso, & de Moura, 2016). In parallel to these studies, other works have focused on the encapsulation of essential oils within a nanoemulsion microstructure (Sasaki et al., 2016), flavonoids, such as rutin (Dammak & Sobral, 2017), curcumin (Sari et al., 2014) and other compounds like α-tocopherol (Cheong, Tan, Man, & Misran, 2008; Yang & McClements, 2013), cinnamaldehyde (Donsì, Annunziata, Vincensi, & Ferrari, 2012) or garlic oil (Wang, Cao, Sun, & Wang, 2011). Potential applications of nanoemulsions for the encapsulation of bioactive components, either as a viable and efficient approach to increase their physical stability or in order to minimize their potentially detrimental sensorial effects, have been well documented within the food sciences research arena (Donsì, Annunziata, Sessa, & Ferrari, 2011; Fathi, Mozafari, & Mohebbi, 2012).

Among such bioactive compounds recently studied, α-tocopherol (α-t), cinnamaldehyde (Cin), and garlic oil (GO) have been shown to exhibit a wide range of biological effects including antimicrobial and/or antioxidant properties (Donsì et al., 2012; Wang et al., 2011; Yang & McClements, 2013). α-tocopherol is an isomer and the most naturally abundant and biologically active form of vitamin E in humans (Yang & McClements, 2013) and it has been shown to have high antioxidant activity in both biological and food systems (Saberi, Fang, & McClements, 2013). Cinnamaldehyde is a hydrophobic aromatic compound with a benzene ring and an aldehyde group. It is the main active component of cinnamon oil (Chen et al., 2016) and it has been shown to be active against a broad range of foodborne pathogens bacteria, fungi and viruses (Wei, Xiong, Jiang, Zhang, & Ye, 2011). Garlic oil is an essential oil extracted from garlic bulbs, which contains a range of compounds; mainly diallyl disulfide (60%), diallyl trisulfide (20%), allyl propyl disulfide (16%), a small quantity of disulfide and possibly diallyl polysulfide (Pranoto, Rakshit, & Salokhe, 2005). It is also used as a food preservative and it has been shown to inhibit the growth of a wide range of pathogens and spoilage microorganisms, including bacteria, mold, fungi, parasites and viruses (Sung, Sin, Tee, Bee, & Rahmat, 2014). All three of these active compounds have been categorized as safe (GRAS) for use in food by the US Food and Drug Administration (FDA) (Chen et al., 2016; Wei et al., 2011) and have been independently used as active additives within a range of packaging formulations (Noronha, De Carvalho, Lino, & Barreto, 2014; Otoni et al., 2016; Pranoto et al., 2005). However, they are poorly soluble in water and as such extremely difficult to incorporate within film formulations, which are usually hydrophilic/aqueous systems (Alexandre et al., 2016).

The present study reports on a microstructural approach that involves the encapsulation of active compounds within oil-in-water (O/W) nanoemulsions, before incorporating these into a biopolymer film formulation, in order to facilitate dispersion of the bioactive species into the biopolymer matrix (Chen et al., 2016). To the best of the authors' knowledge, the joint incorporation of nanoencapsulated active compounds (NAC), such as α-t, plus Cin and/or GO, within gelatin-chitosan (G-Ch) based films, in order to improve the films' physicochemical, antimicrobial and antioxidant properties, has not been previously reported. The objective of this work was to successfully produce G-Ch based films loaded with O/W nanoemulsions containing the encapsulated α-t, and Cin and/or GO active compounds and then characterize these formulations in terms of moisture content, solubility in water, swelling, light transmission, opacity, crystallinity, mechanical and thermal properties, microstructure, as well as their antioxidant and antimicrobial activities, thus enabling future development and application of such composite systems as food packaging material.

2. Material and methods

2.1. Material

Garlic oil (purity >99%), cinnamaldehyde (>95%), and α-tocopherol (>96%), Span 60, medium molecular weight chitosan (degree of deacetylation: 75–85% and viscosity: 200–800 cps), Trolox, TPTZ (2,4,6-tripyridyl-*s*-triazine), chloride acid, Iron trichloride, and ethanol were purchased from Sigma-Aldrich and Labsynth (São Paulo, Brazil). Pigskin gelatin (type A, bloom 260° and molecular weight ~5.2×10^4 Da) was supplied by GELNEX (Itá, SC, Brazil). Acetic acid, glycerol, Tween 20, DPPH (2,2-diphenyl-1-picrylhydrazyl), potassium persulfate, $ABTS^{\bullet +}$ [2,2′-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid)], sodium bromide, sodium hydroxide, nutrient broth, and Mueller Hinton agar were obtained from Sigma-Aldrich (Dorset, England, UK). Canola oil was purchased from a local supermarket. Deionized Millipore water (Elix® 5UV, essential), tetracycline, and strains of bacteria *P. aeruginosa* (ATCC 15692) and *L. monocytogenes* (ATCC 35152) were provided by the microbiology laboratory at the School of Biochemical Engineering of the University of Birmingham.

2.2. Nanoemulsion preparation

The α-tocopherol and cinnamaldehyde and/or garlic oil were encapsulated in nanoemulsions using the microfluidization