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Antibacterial multilayer of chitosan and (2-carboxyethyl)- β-cyclodextrin onto polylactic acid (PLLA)

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ABSTRACT

There is a wide demand for new biodegradable materials with innovations to improve the basic functions of traditional food packaging and prolong the shelf life of food, enhancing its security and sensorial properties. Poly (L-lactic acid) (PLLA) is a biodegradable biopolymer used in traditional packaging of food because of its non-toxicity, biodegradability, biocompatibility and transparency. The aim of this work is to modify PLLA films in order to prepare an improved material for food active packaging. For this, PLLA was internally blended with zinc oxide (ZnO) nanoparticles and, it was also superficially modified by the deposition of chitosan (CHI) and β-cyclodextrins (β-CD) by layer-by-layer (LbL). These prepared multilayers were characterized by confocal fluorescence microscopy, contact angle measurements, transmission electron microscopy and UV-Vis spectroscopy. On one hand, the proposed multiple modification inhibits polymer-bacterial interactions and improves the biological properties of the PLLA due to the bactericide effect of ZnO nanoparticles and the contact killing capacity of chitosan. On the other hand, the release of the carvacrol incorporated in β-CDs improves the antibacterial and antioxidant properties of prepared films (more than 95 wt% of totally released carvacrol was released within 14 days). This investigation demonstrates that here specifically modified PLLA films could be promising materials in the new trends of biodegradable and active food packaging.

1. Introduction

Nowadays, food packaging, which plays an important role in the food supply chain and food processes, has to fulfill the increasingly stringent demands and requirements of the society (Yam, Takhistov, & Miltz, 2005).

Most of materials employed in the industry of food packaging are synthetic polymers derived from petrochemicals, which represent serious environmental issues since they are not biodegradable. This makes imperative the development of environmentally friendly polymers with an increased biodegradability. The *L*-isomer of polylactic acid, (PLLA), which displays good mechanical properties and high crystallinity (Hamad, Kaseem, Yang, Deri, & Ko, 2015) is used in the packaging industry of food because of its transparency, no toxicity and biodegradability. However, PLLA also presents some properties which are not beneficial for food packaging materials, such as its hydrophobicity. Because of this, bacteria are adsorbed on the surface of PLLA, favoring the creation of biofilm (Castro-Aguirre, Iñiguez-Franco, Samsudin, Fang, & Auras, 2016) that leads to the contamination and destruction of the food and even serious health problems (Srey, Jahid, & Ha, 2013).

Regarding antibacterial strategies on surfaces, it is well known that an effective *via* is the reduction of the adhesion of the microorganisms to the surface of the material, that can be endorsed by increasing the hydrophilicity of the surface (Guo, Xiang, & Dong, 2015; Nostro et al., 2010). Indeed bacteria adhere better in hydrophobic or non-polar surfaces than in hydrophilic or polar surfaces and therefore, decreasing the hydrophobicity of the surface is an interesting way to reduce bacteria adhesion (Zhu, Mao, & Gao, 2013). This increase of surface hydrophilicity can be achieved by functionalization with hydrophilic moieties (Sirelkhatim et al., 2015).

Another strategy to prolong the durability of the food is the destruction of bacteria adhered to the surface by the action of antibacterial agents. Among the agents that present antibacterial activity, inorganic metallic nanoparticles are widely employed in the last

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decades (Shaheen, El-Naggar, Abdelgawad, & Hebeish, 2016; Wan, Wu, Yu, & Wen, 2006). These nanoparticles can be added to the material by an internal modification and react with ultraviolet (UV) light to create OH^{-}, H_2O_2 eta $O_2{}^{2-}$, which interact with the cellular membrane of the bacteria and destroy them (Lizundia, Ruiz-Rubio, Vilas, & León, 2016; Pantani, Gorrasi, Vigliotta, Murariu, & Dubois, 2013). In addition to the antibacterial capacity, these nanoparticles give to the material additional beneficial properties for its application in the packaging industry. This is the case of zinc oxide (ZnO) nanoparticles (De Silva, Pasbakhsh, Lee, & Kit, 2015) that promote good transparency, high elastic modulus, photostability, non-toxicity and safety, as denoted by American Food and Drug Administration (FDA) approval (Xie, He, Irwin, Jin, & Shi, 2011).

A different way to promote the destruction of bacteria, is the use of molecules with the so called antibacterial "contact killing" property (Aider, 2010). Although the specific mechanism of their action is not still well known, it is though that these molecules are able to kill bacteria and microorganisms when they interact with their cellular membrane (Je & Kim, 2006). Ammonium salts are typical examples of molecules that present this property (van de Lagemaat et al., 2017). This characteristic appears also in surfaces that contain ammonium quaternary groups or polycations, like the biopolymer chitosan (Murata, Koepsel, Matyjaszewski, & Russell, 2007).

Chitosan is a linear polysaccharide composed of randomly distributed β-(1–4)-D-glucosamine (deacetylated unit) and *N*-acetyl-*D*-glucosamine (acetylated unit), which is obtained by the deacetylation of chitin. Chitosan is a weak polyelectrolyte and their amine groups (-NH_2) can be ionized at acidic conditions (pkã6.5) (Manni, Ghorbel-Bellaaj, Jellouli, Younes, & Nasri, 2010). Due to this, chitosan can be dissolved only in acid solutions (pH < 6), when the protonation of amine groups (-$NH_3{}^{+}$) occurs. Moreover, chitosan possesses unique biological and physicochemical properties that have made it one of the most promising biomaterials in the past decades. Indeed, chitosan exhibits many exceptional properties for its use in the packaging industry of food, such as low toxicity, biodegradability, biocompatibility, immune stimulation, suppression of tumor growth, it is an enhancer of humoral and cell-mediated immune responses, presents cholesterol lowering effect, wound-healing, mucoadhesive properties, and as mentioned before, "contact killing" capacity against bacteria and microorganism (Cheba, 2011). For this reason, chitosan films have been investigated as materials for the packaging of food and promising results have been reported (Cazón, Velazquez, Ramírez, & Vázquez, 2017; Dutta, Tripathi, & Dutta, 2012; Tripathi, Mehrotra, & Dutta, 2009).

Additionally, the interest in packaging with the ability to encapsulate or release fragrances, odors, flavors and active molecules like antibacterials or antioxidants has increased in the industry of food in the last years (Rakmai et al., 2017). For this purpose, cyclodextrins (CD) are promising molecules for the encapsulation and release of hydrophobic compounds in aqueous mediums. Cyclodextrins are cyclic oligosaccharides produced from starch that contain six (α-CD), seven (β-CD) or eight (γ-CD) glucopyranose units linked by α-1,4-glucosidic bonds. The β-form is the most accessible and widely used cyclodextrin. In addition, β-CDs present different commercially available derivatives β-CDs for improving their stability and improving in some cases their water solubility, such as methylated cyclodextrins, hydroxyalkylated cyclodextrins and anionic (2-carboxyethyl)-β- cyclodextrin sodium salt (Rakmai et al., 2017). As a result of the restricted rotation of linked glucopyranose units CDs are toroidal or truncated cone shaped molecules with an internal cavity whose size is around 7.5–8.0 Å for β-CDs (Mura, 2014; Szente & Szejtli, 1999). CDs have hydrophilic hydroxyl groups on their outer surface, but a hydrophobic cavity in the center, which provides a non-polar matrix. As a result, on the one hand, CDs have a special capability to entrap hydrophobic guests, and therefore, to stabilize volatile or unstable compounds, this is, to reduce/promote specific tastes and odors. On the other hand, because of its hydrophilic outer nature CDs can make decrease the hydrophobicity of surfaces (Martin, Tabary, Leclercq, et al., 2013), which is interesting to decrease bacterial adhesion. Thus, CDs and their derivatives, have received increasing attention in the last years and have been extensively exploited to form host-guest inclusion complexes with a variety of drugs (Gharib, Greige-gerges, Fourmentin, Charcosset, & Auezova, 2015), food additives (Kayaci & Uyar, 2012), and antibacterial agents (Hill, Gomes, & Taylor, 2013), in different fields, such as pharmacy (Loftsson & Duchene, 2007), food industry (Astray, Gonzalez-Barreiro, Mejuto, Rial-Otero, & Simal-Gándara, 2009) and catalysis (Hapiot et al., 2017).

In the last years there has been a growing interest in the use of natural antimicrobials and antioxidants for application in food products (Rakmai, Cheirsilp, Mejuto, Simal-Gándara, & Torrado-Agrasar, 2018). Carvacrol (5-isopropyl-2-methylphenol) is a phenolic monoterpene constituent of essential oils produced by numerous aromatic plants and species such as oregano and black cumin (Miladi et al., 2016). Carvacrol has many interesting biological effects for food industry: a higher antioxidant activity *in vitro* than other compounds like thymol and antibacterial and antifungal activity against bacteria and microorganisms. Besides, it is proved that carvacrol inhibits oxidation for 30 days and it also exhibits antitumor and anti-inflammatory activities (Beena Kumar, & Rawat, 2013). Furthermore, carvacrol is Generally Recognized as Safe (GRAS) for consumption and it is approved by the US Food and Drug Administration for food use (Calo, Crandall, O'Bryan, & Ricke, 2015). However, carvacrol presents some disadvantages that limit its use as antimicrobial and antioxidant in food products, such as, its high insolubility in water and, that it is oxidized, decomposed, or evaporated when is exposed to the air, light or heat, respectively. To overcome these concerns Golparvar et al. (Golparvar, Gheisari, Hadipanah, & Khorrami, 2018) investigated the inclusion of complexes formed between carvacrol and β-CDs and reported that the volatility, oxidation and heat decomposition of carvacrol was effectively reduced, and its solubility in water was greatly increased.

A versatile way to modify polymeric surfaces is the development of coatings by the technique of layer-by-layer (LbL) deposition (LbL) which has been developed over the past several years as a tool for surface modification on different materials (Li & Peng, 2015). Among the possible LbL deposition techniques the polyelectrolytes multilayer build-up by dip-coating method is one of the most widely used. It consists on dipping samples alternatively in anionic and cationic polyelectrolyte solutions, allowing adsorption by electrostatic interactions and enabling the formation of multilayer assemblies over the substrate (Oliveira, Hatami, & Mano, 2016). This technique is fast, easy to use and seems to be adjustable to any kind of substrates.

A novel and interesting contribution to surface modification would be the assembling of positively charged polyelectrolytes like chitosan and negatively charged anionic β-CDs (2-carboxyethyl)-β-CDs). Chitosan as positive polyelectrolyte can be incorporated in the LbL coating offering its intrinsic antimicrobial properties (Fu, Ji, Yuan, & Shen, 2005), while (2-carboxyethyl)-β-CDs contribute with their hosting properties acting as reservoir of drugs and additives (Auzély-Velty & Rinaudo, 2001).

It is worth to mention that chitosan presents a high ability to be incorporated in multilayer assemblies on solid surfaces that allows the insertion of anionic CDs in the multilayered system, and enhances multilayer stability, as a result of its low degradation (Lin, Ren, & Ji, 2009). Few works have been reported about ionic CDs multilayered systems. In those cases, different multilayer systems with cationic and anionic β-CDs were constructed on glass slide or silicon substrates (Yang, Hoonor, Jin, & Kim, 2013), onto poly (ethylene terephthalate) (Pérez-Álvarez, Ruiz-Rubio et al., 2017) and polyacrylonitrile (Pérez-Álvarez, Matas, et al., 2017) polymers, and the effective loading and release of antibacterial/antifungal agents such as triclosan were reported. Martin et al. (Martin, Tabary, Chai, et al., 2013; Martin, Tabary, Leclercq, et al., 2013) also exploited chitosan and β-CDs properties for the development of a coating prepared by LbL built up of chitosan and polymeric anionic β-CDs. This CD derivative was synthesized by