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Light-activated antimicrobial activity of turmeric residue edible coatings against cross-contamination of *Listeria innocua* on sausages

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ABSTRACT

The high number of foodborne outbreaks in ready-to-eat (RTE) food such as cooked sausages illustrate the importance of controlling microbial safety during post-processing stages of food products. Edible antimicrobial coatings can be applied to food after processing and sanitation with the goal of preventing microbial cross-contamination, decreasing the risk of foodborne illness and increasing the product shelf-life. In this study, edible hydrogel coatings that can present strong antimicrobial activity when combined with UV-A light were prepared. The hydrogels coatings were prepared using either turmeric residue and gelatin hydrogels (TGH) or cassava starch and gelatin hydrogels with added purified curcumin (CGH). The coatings were characterized regarding their thickness, encapsulated curcumin concentration and water swelling, in addition to their light-activated antimicrobial activity against different initial loads of *Listeria innocua* at different incubation temperature. Additionally, the coatings were applied to the surface of cooked sausages and evaluated for their ability to prevent bacterial cross-contamination. It was observed that UV-A light-exposed hydrogels coatings could inactivate more than 5 log CFU/mL of *L. innocua* after light treatments as short as 5 min. In addition, the light-activated antimicrobial activity of the hydrogel coatings were not affected by the incubation temperature. Hydrogel-coated sausages exposed to UV-A light experienced a reduction from 4 log CFU/mL of incubated bacteria to levels below the detection limit of 1 log CFU/mL after 5 and 15 min of light exposure, for CGH and TGH, respectively. Further mechanistic studies suggested that *L. innocua* inactivation was due to the photo-irradiation of low levels of curcumin released from the coatings to solution. Lastly, it was shown that the combination of curcumin-loaded hydrogels coatings and UV-A light have great potential as antimicrobial coatings to prevent cross-contamination of *L. innocua* in refrigerated sausages.

1. Introduction

Food contamination continues to be a big concern for public health, consumer, regulatory agencies and food industries around the world (Giaouris et al., 2014). Read-to-eat (RTE) meat products are subjected a proper heat treatment for elimination of non-sporogenic pathogens (Brasileiro et al., 2016). Nevertheless, in America and in Europe several outbreaks of foodborne diseases in RTE have been reported by *Listeria monocytogenes* contamination (Buchanan, Gorris, Hayman, Jackson, & Whiting, 2017). The main factor responsible for their occurrence is cross-contamination during post processing operations (slicing, chopping, comminuting) and the potential of *L. monocytogenes* to grow under various conditions, such as the presence or absence of oxygen, over a large pH range (4.7–9.2) and at low temperature (Magalhães et al., 2016).

Post-processing protection using antimicrobial edible films or coatings has been proposed as a potential approach to prevent or minimize cross-contamination of sausages (Cagri, Ustunol, & Ryser, 2002; Cagri, Ustunol, Osburn, & Ryser, 2003; Gadang, Hettiarachchy, Johnson, & Owens, 2008; Marchiore et al., 2017; Nguyen, Gidley, & Dykes, 2008; Siripatrawan & Noipha, 2012). Natural biopolymers such as proteins, polysaccharides and lipids or

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mixture of them, have been used to develop biodegradable coatings and films (Alemán et al., 2016). Furthermore, the use of industrial residues to form edible coatings has become popular in the recent years (Maniglia, Domingos, de Paula, & Tapia-Blácido, 2014). Turmeric (*Curcuma longa* L.) residue is a by-product obtained after supercritical fluid extraction (SFE) and pressurized liquid extraction (PLE) of curcumin and curcuminoids from turmeric rhizomes. The turmeric residue obtained is mostly composed of starch, fibers and traces of oleoresin and curcuminoids, which confers the turmeric residue great potential to be used for the preparation of edible coatings (Maniglia et al., 2014; Osorio-Tobón, Carvalho, Rostagno, Petenate, & Meireles, 2014). In Brazil, sausage are commonly wrapped using natural or artificial coatings and immersed in yellow colorant (MAPA, 2000). In that scenario, turmeric residue could be applied on sausage from an antimicrobial coating with the goal of preventing microbial cross-contamination during food handling (Maniglia et al., 2014).

During the last decade, food industries have explored the use of non-thermal processes such as UV-C light irradiation, ionizing radiation or hydrostatic high pressure to inactivate microorganisms or to reduce microbial cross-contamination on food, with minimal impacts on the sensorial properties of food (Bayındırlı, Alpas, Bozoğlu, & Hızal, 2006; Janisiewicz, Takeda, Glenn, Camp, & Jurick, 2015; Mukhopadhyay, Ukuku, & Juneja, 2015; Shahbaz et al., 2014; Sommers, Sheen, Scullen, & Mackay, 2017; Tawema, Han, Vu, Salmieri, & Lacroix, 2016). However, many of these non-thermal processes have disadvantages such as significant investment in specialized equipment that may limit their use on food. Thus, there is an unmet need for technologies which are safe, efficient, practical and preferably inexpensive for inactivating pathogenic microorganism at a local food processing, handling and service facilities (Liu & Avena-Bustillos et al., 2016).

Among various technologies, light-mediated inactivation of microbes has emerged as a potential approach that could be implemented in various settings with low investment costs. One of the challenges with UV-C light-mediated approaches to inactivate microbes is the potential loss in organoleptic processes induced by oxidation processes triggered by UV-C radiation (Guerrero-Beltran & Barbosa-Canovas, 2004). To address this challenge few studies have evaluated the use of food grade compounds with longer wavelength light frequencies to inactivate a diversity of microbes (Cossu et al., 2016; Dovigo et al., 2011; Shirai, Kajiura, & Omasa, 2015; Yin et al., 2013). In this process, which is similar to the conventional photo-dynamic therapy (PDT) approach used in biomedical applications, food grade compounds such as organic acids, food grade dyes and polyphenolic compounds can generate oxidative stress species such as free radicals and singlet oxygen species that can lead to microbial inactivation (Haukvik, Bruzell, Kristensen, & Tønnesen, 2009; Qian et al., 2016; Sarkar & Hussain, 2016). The use of longer wavelengths such as blue light or UV-A can reduce the photonic energy required for the light-mediated process and thus reduce any significant impact on the organoleptic properties of food (Liu & Avena-Bustillos et al., 2016). Many of the current studies using light-activated compounds have been conducted without the presence of food materials and are often conducted only in aqueous solutions. To the best of our knowledge, only purified food grade compounds have been used as photo-activated compounds in food-related applications (Buchovec, Lukseviciute, Marsalka, Reklaitis, & Luksiene, 2016; López-Carballo, Hernández-Muñoz, Gavara, & Ocio, 2008; Oliveira, Cossu, Tikekar, & Nitin, 2017; Penha et al., 2016; Temba, Fletcher, Fox, Harvey, & Sultanbawa, 2016; Tiwari et al., 2009; Wu et al., 2016). This could represent a potential limitation as purified compounds can add a significant cost to the process.

In this study, turmeric residue obtained after the combination of supercritical fluid extraction and pressurized liquid extraction processes was used to prepare antimicrobial hydrogels to inhibit bacterial cross-contamination in meat products (Maniglia, de Paula, Domingos, & Tapia-Blácido, 2015; Osorio-Tobón et al., 2014). The selection of curcumin as a photo-activated antimicrobial agent was inspired by photodynamic therapy (PDT) treatments used in oral or dental applications (Araújo, Fontana, Bagnato, & Gerbi, 2012; Dovigo et al., 2011; Paschoal et al., 2013). Furthermore, applications of the PDT approach based on the combination of light and curcumin in biomedical applications suggest that the generation of toxic products was not of significant concern (Araújo, Fontana, Bagnato, & Gerbi, 2012; Dovigo et al., 2011; Paschoal et al., 2013). In contrast to biomedical applications, this study is focused on using a food-grade by-product of the curcumin extraction process for the inactivation of microorganisms on a meat surface (Sandikci Altunatmaz et al., 2016). Unlike previous studies with food-grade compounds in aqueous solution, this study is focused on application of a photo-activated antimicrobial agent on a solid food product (López-Carballo et al., 2008). To deliver curcumin from turmeric residue to a meat surface, the hydrogel was selected as a model delivery system. One of the potential advantages of the hydrogel approach are that this proposed antimicrobial treatment could be combined with edible coatings on food surfaces. These edible coatings can provide controlled release of encapsulated residual curcumin from the turmeric residue maintaining a significant concentration of the photo-activated antimicrobial compound on the surface of a meat product (Liu & Li et al., 2016).

In this way, edible hydrogels of turmeric residue and gelatin that could be activated in combination with UV-A light showing enhanced antimicrobial activity were prepared and characterized. In addition, hydrogels of cassava starch and gelatin supplemented with purified curcumin were prepared and characterized in order to compare with turmeric residue hydrogels which have residual curcumin naturally present. The hydrogels were used to form coatings and the UV-A light-activated antimicrobial activity of these coatings was assessed against *Listeria innocua*, a non-pathogenic surrogate for *L. monocytogenes*, at both 23 °C and 4 °C. The hydrogels were also applied on sausages and the ability of sausage-coated hydrogels to prevent *L. innocua* cross-contamination were evaluated. Furthermore, the possible light-mediated antimicrobial mechanisms of the hydrogel coatings were investigated by monitoring curcumin release from the hydrogels and UV-A light-mediated curcumin degradation within the hydrogels.

2. Materials and methods

2.1. Materials

Glycerol, Curcumin and Gelatin were purchased from Sigma-Aldrich (St. Louis, MO, USA). Turmeric was supplied by Office Herbal Pharmacy Handling Ltda (Ribeirão Preto, SP, Brazil). Phosphate-buffered saline (PBS), Tryptic Soy Broth (TSB) and Tryptic Soy Agar (TSA) were obtained from Fisher Scientific (Pittsburgh, PA, USA). Commercial cassava starch (Bob's Red Mill®, Milwaukie, OR, USA) and commercial sausages (Chicken Franks Foster Farms®, Livingston, CA, USA) were purchased from local markets. The commercial sausages used in this study were 57% water, 28% total fat, 12.5% protein and 1.78% carbohydrate. Ultrapure water was obtained using a Milli-Q filtration system (EDM Millipore; Billerica, MA, USA).