LWT - Food Science and Technology 79 (2017) 1–10

Contents lists available at ScienceDirect

LWT - Food Science and Technology

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

Optimisation of edible chitosan coatings formulations incorporating *Myrcia ovata* Cambessedes essential oil with antimicrobial potential against foodborne bacteria and natural microflora of mangaba fruits

Gladslene Góes Santos Frazão [a], Arie Fitzgerald Blank [b], Luciana Cristina Lins de Aquino Santana [a, *]

[a] Northest Biotechnology Network (RENORBIO)/Department of Food Technology, Federal University of Sergipe, Av. Marechal Rondon, S/N, São Cristóvão, Sergipe, CEP 49100-000, Brazil
[b] Department of Agronomy, Federal University of Sergipe, Av. Marechal Rondon, S/N, São Cristóvão, Sergipe, CEP 49100-000, Brazil

ARTICLE INFO

Article history:
Received 12 September 2016
Received in revised form
13 December 2016
Accepted 6 January 2017
Available online 6 January 2017

Keywords:
Active packaging
Antimicrobial agent
Mangaba

ABSTRACT

Various edible coating formulations were prepared from cassava starch, chitosan and *Myrcia ovata* Cambessedes essential oils (MYRO-174 or -175) according to a 2^3 experimental design including six factorial points and three central points. The antimicrobial activity of the coatings was evaluated against eight foodborne bacteria using the disc diffusion method. The coatings containing MYRO-175 essential oil were the most effective. Mathematical models describing *Bacillus cereus*, *B. subtilis* and *Serratia marcescens* inhibition were developed using response surface methodology. The edible coatings containing 0.5% cassava starch-0.5% chitosan-1.25% MYRO-174 or -175 essential oil were applied to mangabas and their microbiological quality was evaluated during storage at 10 °C for 12 days. The total aerobic mesophilic bacteria, mould and yeast counts were reduced by ~3 log from day 4–12. For coated mangabas inoculated with *B. cereus*, the counts were <1.0 log from day 2–12 of storage. These innovative coatings show potential for controlling foodborne bacteria and natural microorganism growth during the storage of fruit, particularly, mangabas.

1. Introduction

Quality food preservation is a serious concern in the food industry, particularly in the marketing phase, where the greatest losses are due to microbiological activities. In most fresh or processed foods, microbial contamination occurs to a greater degree on the food surface. Thus, packaging plays a fundamental role in controlling microbial growth. Active packaging materials, such as antimicrobial films and coatings that are in contact with the packaged product, have been developed to reduce, inhibit or delay the microbial growth on food surfaces (Appendini & Hotchkiss, 2002; Elsabee & Abdou, 2013). An edible coating is a thin layer of edible material applied in liquid form onto the food, usually by immersing the product in a solution. Conversely, an edible film is a preformed, thin layer of edible material, which, once formed, can be placed on or between food components (McHugh, 2000). These packages offer extra advantages such as edibility, biocompatibility, improved aesthetic appearance, barrier properties against gases, non-toxicity, lack of pollution and low cost (Han, 2000). Although edible antimicrobial films and coatings can be prepared from various polymers, chitosan has been most used due to its well-documented antimicrobial properties (Elsabee & Abdou, 2013). Some researchers have suggested that the antibacterial action of chitosan depends on the application technique used, with coating solutions being more effective than film matrices (Vásconez, Flores, Campos, Alvarado & Gerschenson, 2009).

Antimicrobial agents, such as plant essential oils (EO), also may be incorporated directly into food packaging characterising a form of active packaging. Traditionally, antimicrobial agents are added directly to foods, but their activity may be inhibited by substances in the food itself, diminishing their efficiency. In addition, other factors may diminish the efficiency of EOs such the low solubility of them due to their lipophilic nature. Accordingly, antimicrobial

* Corresponding author. Federal University of Sergipe, Department of Food Technology, Laboratory of Food Microbiology and Bioengineering, Avenida Marechal Rondon s/n, CEP: 49100-000, São Cristóvão, Sergipe, Brazil.
E-mail address: aquinoluciana@hotmail.com (L.C.L. de Aquino Santana).

http://dx.doi.org/10.1016/j.lwt.2017.01.011
0023-6438/

effects *in vivo* of EOs are lower than *in vitro* conditions. Thus, the use of antimicrobial films or coatings can be more efficient than adding antimicrobial agents directly to the food since these may selectively and gradually migrate from the packaging onto the surface of the food, thereby maintaining high concentrations where they are most necessary (Ouattara, Simard, Piette, Bégin, & Holley, 2000).

Many researchers have published studies concerning the antimicrobial activity of plant essential oils against foodborne pathogens. Since essential oils are rich in volatile terpenoids and phenolic compounds, they have the potential to inhibit a wide spectrum of microorganisms. Generally, the active components inhibit microorganisms through disturbance of their cytoplasmic membranes, disrupting the proton motive force, electron flow, active transport and inhibiting protein synthesis (Burt, 2004).

There are some reports related to the antimicrobial effectiveness of edible coatings incorporating essential oils. For instance, Sánchez-González et al. (2011) revealed that when edible chitosan coatings, with and without bergamot EO, were applied to Muscatel table grapes during postharvest cold storage, the coatings containing bergamot oil not only displayed the strongest antimicrobial activity but effectively inhibited the fruit respiration rates regarding both O_2 consumption and CO_2 generation. Perdones, Sanchez-Gonzalez, Chiralt, and Vargas (2012) verified that the addition of lemon EO enhanced the antifungal activity of chitosan coatings applied to cold stored strawberries. Vu, Hollingsworth, Leroux, Salmieri, and Lacroix (2011) also increased the shelf-life of strawberries by 21 days by coating with a modified chitosan-based formulation containing limonene. Ponce, Roura, del Valle, and Moreira (2008) inhibited *Listeria monocytogenes* on the surface of pumpkins with a chitosan-1% rosemary EO coating, while Ramos-García et al. (2012) used a 1.0% chitosan-0.1% beeswax-0.1% lime EO coating on tomatoes to reduce *Rhizopus stolonifer* and *Escherichia coli* DH5α contamination during storage at 12 and 25 °C. Santos et al. (2012) obtained growth inhibition of *Rhizopus stolonifer* URM 3728 and *Aspergillus niger* URM 5842 in artificially infected grapes coated with chitosan-*Origanum vulgare* L. EO coatings. Duan, Cherian, and Zhao (2010) obtained reductions in total plate and psychrotrophic counts in cold stored and frozen stored lingcod fillets coated with Chitosan–fish oil coatings. Ojagh, Rezaei, Razavi, and Hosseini (2010) studied the effect of chitosan coatings enriched with cinnamon oil on the quality of rainbow trout during refrigerated storage (4 °C) for 16 days. The authors observed that the total viable and psychrotrophic counts of coated fish samples were reduced during refrigerated storage compared with uncoated samples.

Myrcia ovata essential oils have shown antimicrobial activity against microorganisms related to gastric and intestinal disorders (Cândido et al., 2010) and against *Fusarium solani*, a phytopathogenic fungus of importance in agriculture (Sampaio et al., 2016). *Myrcia ovata* Cambessedes, popularly known as “laranjinha do-Mato”, is a shrub that grows to about eight meters in height and its leaves are commonly used in tea, folk medicine and treatment of gastritis and diarrhoea. *Myrcia*, comprising around 377 species, is one of the largest genera of the family Myrtaceae, recognised in the Brazilian cerrado and Atlantic forests (Lucas et al., 2011; Wubshet, Moresco, Tahtah, Brighente, & Staerk, 2015).

Over the years, mathematical models has been widely applied in several fields to estimate parameters (Valipour & Eslamian, 2014; Valipour, 2015a, 2015b, 2016). Response surface methodology (RSM) is a statistical tool that enables the evaluation of the effects of many factors and their interactions on response variables. The RSM has as advantages the reduction in the number of experimental runs to evaluate multiple variables and the ability of the statistical tool to identify interactions (Chen, Chen, & Lin, 2004). Consequently, it is less laborious and time consuming compared with one variable at a time. Yet, there are few reports about the use of RSM to optimise edible coating formulations (Arismendi et al., 2013; Peretto et al., 2014; Dave, Rao, & Nandane, 2016; Malmiri, Osman, Tan, & Rahman, 2012). Hence, this work aimed optimise the antimicrobial properties of edible chitosan-cassava starch coatings containing *M. ovata* Cambessedes EO against foodborne bacteria (*in vitro* tests) using RSM. The most effective formulations were then applied to mangabas and the inhibition of natural microflora on the fruit during refrigerated storage was evaluated.

2. Material and methods

2.1. Material

Two essential oils obtained from *Myrcia ovata* Cambessedes plants (named MYRO-174 and -175) were provided by the Department of Agronomy of the Federal University of Sergipe, Brazil. The plants were collected in the municipality of Japaratuba, State of Sergipe, Brazil, in November 2013 and the oils were extracted by hydrodistillation using a modified Clevenger apparatus. The chemical composition of the EOs was assessed according to Sampaio et al. (2016) using gas chromatography coupled with mass spectrometry and flame ionisation detection (GC–MS/FID; QP2010 Ultra, Shimadzu Corporation, Kyoto, Japan). A Rtx-5MS (Restek) fused silica capillary column (5%-diphenyl–95%-dimethylpolysiloxane; 30 m × 0.25 mm i.d., 0.25 μm film thickness) was used. The helium gas flow rate was maintained at 1.2 mL/min. The oven temperature was programmed to 50 °C (isothermal for 1.5 min) and increased at 4 °C/min to 200 °C, then at 10 °C/min to 250 °C, and finally maintained at 250 °C for 5 min. MYRO-174 contained 27.50% isopulegol, 19.61% linalool and 10.29% iso-isopulegol, while MYRO-175 contained 14.97% linalool and 52.61% nerolic acid as the major compounds.

Chitosan, in powder form, (80 mesh particle size, 85.9% deacetylation degree, pH 8.2), was obtained from Polymar (Fortaleza-CE, Brazil). Cassava starch (“Dinha Bá" brand) was purchased at the municipal market of Aracaju, SE. Mangabas, with green-yellow peel, were acquired from the city of Caueira, Sergipe directly from the trees.

2.2. Bacteria

Pseudomonas aeruginosa (INCQS 00025), *Staphylococcus aureus* (INCQS 00014), *Bacillus cereus* (INCQS 00003), *Bacillus subtilis* (INCQS 00002), *Enterococcus faecalis* (INCQS 00531), *Serratia marcescens* (INCQS 00131), *Escherichia coli* (INCQS 00032) and *Salmonella enteritidis* (INCQS 00258) were provided by the National Institute of Health and Quality Control/Oswaldo Cruz Foundation (Manguinhos, Rio de Janeiro, Brazil). The strains were stored in brain heart infusion broth with 20% glycerol at −80 °C. All culture media were purchased from Oxoid (Brazil).

2.3. Preparation of edible chitosan coatings incorporating MYRO essential oils

The edible coating formulations were prepared according to Azevedo et al. (2014). Chitosan (w/v) was dissolved in a 1.5% acetic acid solution with 1.28% glycerol (w/v) added to obtain a final volume of 100 mL, by agitation at room temperature (25 °C) for 10 min. Then, 100 mL of cassava starch solution (w/v) containing 0.64% glycerol (w/v) was heated (not exceeding 70 °C) in a water bath with stirring. The cassava starch and chitosan solutions were then mixed until completely homogenised. The mixture was autoclaved in glass bottles at 121 °C for 15 min. Finally, the EO (MYRO-174 or -175) was added to the mixture. The cassava starch,