Food Control 85 (2018) 29–41

Contents lists available at ScienceDirect

Food Control

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

Evaluation of antibacterial activity of two natural bio-preservatives formulations on freshness and sensory quality of ready to eat (RTE) foods

Mehdi Harich, Behnoush Maherani, Stephane Salmieri, Monique Lacroix*

INRS-Institut Armand-Frappier, Research Laboratories in Sciences Applied to Food, Nutraceutical Institute and Functional Foods (INAF), Canadian Irradiation Centre, 531, Boulevard des Prairies, Laval, Québec, H7V 1B7, Canada

ARTICLE INFO

Article history:
Received 12 May 2017
Received in revised form
15 August 2017
Accepted 8 September 2017
Available online 19 September 2017

Keywords:
Ready-to-eat vegetables
Bio-preservative
Citrus extract
Essential oils
Foodborne pathogens
Sensorial analysis

ABSTRACT

The antibacterial activity of two natural antibacterial formulations based on lemongrass essential oil/citrus extract/lactic acid for F2 (with ratio of 0.01:0.1:1) and oregano/citrus extract/lactic acid for F6 (with ratio of 0.01:0.1:1) was studied against several pathogens (*Listeria monocytogenes, Escherichia coli* and *Salmonella* Typhimurium) and non-pathogenic bacteria (*Listeria Innocua*, *Escherichia coli* ATCC 25922, and *Salmonella enterica*).

The *in vitro* inhibition capacity (IC %) of F2 and F6 formulations demonstrated their high antimicrobial potential against pathogenic and non-pathogenic bacteria. In addition, *in vitro* minimum inhibitory concentration (MIC) of F2 and F6 formulations exhibited a lower MIC (higher antibacterial properties) against pathogenic and non-pathogenic bacteria compared to sodium benzoate.

Furthermore, *in situ* antimicrobial capacity of two natural formulations (F2 and F6) was assessed on ready to eat vegetables and fruits (pre-cut red peppers, cranberries and pre-cut/pre-fried potatoes) against targeted bacteria; it's important to mention that peppers and cranberries were frozen and then thawed before treatment and storage, however pre-fried potatoes were fresh.

The *in situ* results showed that both coatings allowed a significant reduction ($P \leq 0.05$) of pathogenic bacteria in cranberries (e.g. in presence of F2 formulation: 1.5, 2.3, 1.7 log reduction of *L. monocytogenes, E. coli* and *S.* Typhimurium populations, respectively) and in red peppers (1.3, 0.85 and 2 log reduction of *L. monocytogenes, E. coli* and *S.* Typhimurium in presence of F6). Results obtained on pre-fried sliced potatoes packed under modified atmosphere (MA), showed that F2 allowed a 0.5 and 1.5 log reduction of *E. coli* and *S.* Typhimurium respectively at day 10. On the other hand, F6 allowed a 0.5 and 1.1 log reduction of *E. coli* and *S.* Typhimurium respectively at day 10. The sensory analysis of red peppers and potatoes treated with F2 and F6 formulations suggested that both formulations were acceptable in terms of organoleptic attributes. Therefore, their sensorial attributes could be accepted for further commercialization.

1. Introduction

Changes in consumer trends, search for healthier and convenient products, and also new life styles have brought a noticeable increasing demand (over 30% during the last 10 years) for fresh and ready-to-eat (RTE) food production (Millan-Sango, McElhatton, & Valdramidis, 2015; Oliveira et al., 2015). This has led to the development of a new range of ready to eat (RTE) products. However, it has changed the status of foodborne diseases and had an important economic and social impact in the world (Millan-Sango et al., 2015; Oliveira et al., 2015). As fruits and vegetables can be contaminated with pathogens during all steps from the harvest to the process, and until the consumption. The RTE foods are no longer considered as low-risk foods in terms of safety (De Medeiros Barbosa et al., 2016).

In the United States, the major foodborne pathogens include *Escherichia coli* O157:H7, *Listeria monocytogenes*, *Salmonella* Typhimurium, and *Staphylococcus aureus*. More than 61 deaths per year resulting from 73,000 cases of *E. coli* O157:H7 infections, have been observed according to the US CDC (Rangel, Sparling, Crowe,

* Corresponding author.
E-mail address: monique.lacroix@iaf.inrs.ca (M. Lacroix).

https://doi.org/10.1016/j.foodcont.2017.09.018
0956-7135/

Griffin, & Swerdlow, 2005). In the United States it was estimated that the cost of disease caused by pathogenic bacteria was 77 billion $/year (Scharff, 2012). Therefore, a new and effective approach to overcome the bacterial survival and growth in RTE foods is inevitable.

A variety of disinfectants, including chlorine and hydrogen peroxide have been used to reduce the initial bacterial populations on vegetables but the possibility of formation of carcinogenic derivatives of chlorine (chloramines and trichloromethane) during treatment in presence of chlorine in water has raised concerns about its use in food processing (Harich et al., 2017).

Most of the countries try to proffer new methods to make the food safer for consumption. In this regard, bio-preservation or biocontrol is an interesting alternative to be considered. Bio-preservation or biocontrol refers to the use of natural antibacterial products to extend the shelf life of products and enhance the safety of foods (Daglia, 2012).

Herbs and spices have been recognized to possess a broad spectrum of active constituents that exhibit naturally antibacterial, antifungal, antiparasitic, and/or antiviral activities (Daglia, 2012). Essential oils (EOs) extracted from plant and fruits have been used as bio-functional components for centuries as part of natural traditional medicine. They are aromatic oily liquids with natural antibacterial activity, obtained from plant material (flowers, seeds, leaves, herbs, wood, fruits and roots). The major components that make essential oils effective antimicrobials include polyphenols (flavonoids, flavonoids and acid phenols), terpenes, and their precursors (Daglia, 2012; Manach, Scalbert, Morand, Rémésy, & Jiménez, 2004). Polyphenols are secondary metabolites produced by plants, which have potential healthy properties on human organism, mainly as antioxidants, anti-allergic, anti-inflammatory, anticancer, antihypertensive, and majority antimicrobial activity (Bakkali, Averbeck & Idaomar, 2008; Rivera et al., 2015).

Essential oils can cause structural damages in microorganisms by diffusion through the cellular membrane and this ability is due to the hydrophobic nature of essential oil (Lambert, Skandamis, Coote, & Nychas, 2001). Teissedre and Waterhouse (2000) defined essential oils as a complex mixture of cyclic and acyclic monoterpenes responsible for biological properties such as antifungal, antibacterial and antioxidant. The activity of major components, the structure, size and hydrophobicity of functional groups and the possible synergy interactions of essential oils is responsible for the biological function of essential oil (Burt et al., 2007; Dorman & Deans, 2000; Koroch, Juliani, & Zygadlo, 2007).

Furthermore, Govaris, Solomakos, Pexara, and Chatzopoulou (2010) expressed carvacrol and thymol (78–85%) as the major phenolic compounds in oregano, are able to lead membrane expansion and disturbs embedded proteins because of their lipophilic character (Cristani et al., 2007).

Regarding to EOs benefits, the objective of this study was to evaluate *in vitro* antimicrobial properties of two natural formulations F2 and F6 based respectively on lemongrass and oregano by measuring inhibition capacity (IC %) using agar diffusion assay, and determining minimum inhibitory concentration (MIC) against foodborne pathogenic bacteria. Furthermore, the antimicrobial capacity of the two natural formulations (F2 and F6) was assessed on ready to eat vegetables (pre-cut red peppers, pre-cut/pre-fried potatoes) and on cranberries against pathogenic bacteria (*Listeria monocytogenes, Escherichia coli* and *Salmonella* Typhimurium) and non-pathogenic bacteria (*Listeria innocua, Escherichia coli* ATCC 25922, and *Salmonella enterica*).

Furthermore, the sensory analysis (odor, color, taste, global appreciation, and texture) of the red peppers and potatoes treated with F2 and F6 formulations were evaluated.

2. Materials and methods

2.1. Raw materials

Pre-cut red peppers (10 mm × 10 mm) and frozen cranberries (*Vaccinium macrocarpon*) were kindly provided by Bonduelle Americas Inc. (Saint-Denis-sur-Richelieu, QC, Canada) and Atoka Cranberries Inc. (Manseau, QC, Canada), respectively. Both red peppers and cranberries were stored at −20 °C until used as recommended by the food company. Pre-cut/pre-fried potatoes (50 mm × 10 mm) were also provided by Michel St-Arneault Co. (St-Hubert, QC, Canada) and were stored at 4 °C under modified atmosphere packaging (MAP) before testing.

2.2. Preparation of pathogens cultures

Stock cultures of *E. coli* O157:H7 EDL 933, *S.* Typhimurium SL 1344, *L. monocytogenes Six strains HPB 2812*, 2558, 2569,1043,2371,2739, were stored at −80 °C in Tryptic Soy Broth (TSB) medium (Alpha Biosciences Inc., Baltimore, MD, USA) containing glycerol (10% v/v). Prior to each experiment, stock cultures were grown through two consecutive 24–48 h growth cycles in TSB at 37 °C. Working cultures were diluted in peptone water to obtain the bacterial concentration of 10^6 CFU/mL for MIC determination or 10^3–10^4 CFU/mL for *in situ* tests.

2.3. Preparation of non-pathogens cultures

Stock cultures of *E. coli* ATCC 25922, *S. enterica* Typhimurium ATCC 53648 chi 4064., *L. innocua* LSPQ 3285 were stored at −80 °C in Tryptic Soy Broth (TSB) medium (Alpha Biosciences Inc., Baltimore, MD, USA) containing glycerol (10% v/v). Prior to each experiment, stock cultures were grown through two consecutive 24–48 h growth cycles in TSB at 37 °C. Working cultures were diluted in peptone water to obtain the bacterial concentration of 10^6 CFU/mL for MIC determination or 10^3–10^4 CFU/mL for *in situ* tests.

2.4. Preparation of antimicrobial formulations

Formulations F2 and F6 were prepared under sterile conditions, according to the method developed in our laboratories (Tawema, Han, Vu, Salmieri, & Lacroix, 2016). Formulations were prepared in sterile distilled water in presence of previously filtered (0.2 microns) Tween 80 (0.06% (w/v)) (sigma-Aldrich Ltd, Oakville, ON, Canada) used as an emulsifying agent. F2 formulation containing lemongrass essential oil, citrus extract and lactic acid at a ratio of 0.01: 0.1:1 and F6 formulation containing oregano essential oil, citrus extract and lactic acid at a ratio of 0.01: 0.1:1. Lemongrass, oregano essential oils and lactic acid were provided by BSA Food Ingredients Inc. (St-Leonard, QC, Canada) and citrus extract (Biosecur F440D®) was provided by Biosecur lab (Mont St-Hilaire, Quebec, Canada). Sodium benzoate (E211), as synthetic preservative, kindly provided by Skjodt-Barrett Foods Inc. (QC, Canada).

2.5. Inhibition capacity (IC %) determination by agar diffusion assay

Agar diffusion assay was carried out according to a modified procedure from Cardiet, Fuzeau, Barreau, and Fleurat-Lessard (2012) to assess bactericide activity of F2 and F6 formulations by measuring microbial growth inhibition zones. Tryptic soy agar (TSA; Alpha Biosciences Inc., Canada) were surface-layered by 100 μL of diluted bacteria at 10^6 CFU/mL. Growth inhibition diameters (mm) were determined by agar diffusion from the deposition of 10 μL of the antibacterial formulation on a 12-mm