Food Microbiology 54 (2016) 80–90

Contents lists available at ScienceDirect

Food Microbiology

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

# Control of *Listeria monocytogenes* by applying ethanol-based antimicrobial edible films on ham slices and microwave-reheated frankfurters

Anastasia E. Kapetanakou, Dimitrios Karyotis, Panagiotis N. Skandamis*

*Agricultural University of Athens, Department of Food Science and Human Nutrition, Laboratory of Food Quality Control & Hygiene, Iera Odos 75 GR-11855, Athens, Greece*

ARTICLE INFO

*Article history:*
Received 14 June 2015
Received in revised form
13 October 2015
Accepted 18 October 2015
Available online 21 October 2015

*Keywords:*
*Listeria monocytogenes*
Frankfurters
Ham slices
Distillery ethanol
Alcoholic beverages
Microwave reheating

ABSTRACT

The objectives of the study were to evaluate: (i) the efficacy of Na-alginate films immersed in 40% v/v distillery ethanol and traditional Greek alcoholic beverages (ABs), namely "tsipouro", "raki", and "ouzo" to inhibit *Listeria monocytogenes* growth on frankfurters and ham slices and (ii) the impact of edible films-ABs on heat sensitivity of the pathogen after microwave (MW) reheating of frankfurters in water during storage at 4 and 10 °C. Samples without edible films (controls) or with edible films but free of distillery ethanol or ABs were also tested. Two pieces of frankfurters were placed between two edible films, while every ham slice was covered by a film from both sides. On ham, all edible films immersed in ABs maintained levels of *L. monocytogenes* at least 5.0 log CFU/cm$^2$ lower ($p < 0.05$) than controls after 81 and 52 days at 4 and 10 °C, respectively. Moreover, on frankfurters packaged with edible films–ethanol or -ABs, *L. monocytogenes* population remained 4.0–4.5 log CFU/cm$^2$ lower ($p < 0.05$) than controls after 40 days of storage at 4 °C, while growth suppression of 0.8–2.0 log CFU/cm$^2$ was observed after 15 days at 10 °C, at all assays except for samples packaged with raki. However, the results from reheating experiments revealed that subsequent inadequate MW reheating of frankfurters packaged with edible films-ABs would not lead to remarkable inactivation of the pathogen, since cross-protection against thermal stress is been acquired as a result of products' exposure to sub-lethal stresses such as low pH formed by metabolic products, indigenous microbiota, and ethanol exposure (*ca.* 2.4–2.7% w/w) during cold storage. Our findings suggest that food safety regulations enabling antimicrobial interventions for the safety of ready to eat meat products should ensure both low levels of *L. monocytogenes* during storage and minimum tolerance to recommended consumer practices, such as MW reheating.

© 2015 Elsevier Ltd. All rights reserved.

## 1. Introduction

*Listeria monocytogenes* is a ubiquitous contaminant of low prevalence but of major safety concern on ready-to-eat (RTE) cooked meat products, since it has been repeatedly implicated in outbreaks of listeriosis (hospitalization and fatality rates up to 90% and 20% of infections, respectively) (CDC, 2000, 2002; USDHHS-FDA-CFSAN and USDA-FSIS, 2003). In this context, USDA and EU regulations have regulated a strict policy for *L. monocytogenes* in RTE products (Code of Federal Regulations, 2003; EU Reg. No 2073/2005). Considering the impact to health of consumers along with the consequent financial problems on RTE cooked meat industry caused *via* recalls, contamination by *L. monocytogenes* is a major issue for study.

Frankfurters and ham are among the most popular RTE cooked meat products. Even though both foods are submitted to a thermal treatment during production process enough to eliminate *L. monocytogenes*, they are prone to post-process contamination e.g., during peeling, slicing or packaging (Marcos et al., 2007). Moreover, previous studies have proved that the pathogen could potentially survive and/or grow during storage at retail and/or domestic refrigerators (Gombas et al., 2003; Wallace et al., 2003; Zhu et al., 2005). For these reasons, FDA and USDA have classified non-reheated frankfurters as a product of "high risk" for causing listeriosis (USDHHS-FDA-CFSAN and USDA-FSIS, 2003). Considering the latter, many manufacturing companies often suggest

* Corresponding author.
*E-mail address:* pskan@aua.gr (P.N. Skandamis).

http://dx.doi.org/10.1016/j.fm.2015.10.013
0740-0020/© 2015 Elsevier Ltd. All rights reserved.

reheating prior to consumption through labeling, for example MW reheating (i.e., in MWs with or without the presence of water). However, the intensity of thermal treatment is a matter of consumer choice, thus representing a potential risk of inadequate inactivation of *L. monocytogenes*. Moreover, the uneven distribution of heat is a major concern for foods reheated *via* MWs, resulting in the formation of hot and cold spots in the heated food, especially in non-uniform products, leading to survival of pathogens (Mudgett, 1989; Suga et al., 2007). Taking into account the latter considerations, FDA has regulated 74 °C as the minimum recommended temperature for foods reheated in MW oven (USDHHS-FDA, 2005). However, the probability of *L. monocytogenes* not to survive after MW treatment is much more complex than just to comply with the aforementioned regulation, since inactivation of the target organism is also dependent by several other factors such as the initial population of the pathogen, the levels of endogenous microbiota, the pH, the MW reheating time/power combination, and food ingredients such as fat and salt (Mudgett, 1989; Rodríguez-Marval et al., 2009).

The increased interest of consumers in minimally processed foods with natural flavor has prompted researchers to focus on the application of alternative and innovative technologies for developing products of fresh-like quality without compromising food safety. The last decade, active packaging has been defined as a mode of packaging in which the package, the product, and the environment interact during storage in order to prolong shelf-life and/or improve quality and increase safety of the product (Coma, 2008). Different active packaging systems have been developed, including oxygen scavengers, moisture absorbers, $CO_2$ controllers, ethanol emitters, etc. Antimicrobial active packaging has received a lot of attention by researchers and thus, it is of high importance for development of commercial applications (Coma, 2008; Suppakul et al., 2003). Antimicrobial compounds may either be released through evaporation in the headspace (i.e., essential oils, food-grade ethanol) of the packaging or migrated into the food through diffusion and partitioning (i.e., plant extracts, nisin, organic acids, and/or salts) by direct addition onto the surface of the food product or indirect incorporation into edible carrier materials such as coatings and/or films (i.e., cellulose; alginate; zein; whey protein; pectin-based apple, carrot, and hibiscus) (Gadang et al., 2008; Marcos et al., 2007; Oussalah et al., 2007; Ravishankar et al., 2012; Santiago-Silva et al., 2009). Edible coatings and/or films may assist in the controlled release of the antimicrobials during storage, enhancing in parallel the physical properties and sensory attributes of the food product e.g., alginate coatings and films are oxygen and moisture barriers as well as lipid oxidation inhibitors. As such, antimicrobial active packaging by using edible coatings and/or films may also be a promising method for controlling spoilage and pathogenic microorganisms.

Ethanol is a common natural compound with strong antimicrobial activity (Larsen and Morton, 1991). Different direct applications of ethanol such as dipping and spraying have been reported as significantly effective against spoilage and pathogenic microorganisms on several food products i.e., fruits and bakery products (Bai et al., 2011; Lurie et al., 2006). However, the aforementioned applications in industrial scale could have some drawbacks. For instance, the applied concentration and volume should be optimized, since high ethanol concentrations (*ca.* 30–50%) may lead to increased costs, safety hazards, ethical issues, and sensory problems with respect to RTE products, thus reducing its feasibility (Karabulut et al., 2005). In order to overcome the latter considerations, the indirect application of ethanol *via* its incorporation to carriers such as edible coatings and/or films is a promising alternative, since by this way, ethanol could be released gradually and in controlled rate to the applied food product. In this context, in Japan and USA, ethanol generating films (i.e., adhesive-backed film that can be taped on the inside of a package) or sachets have been patented, marketed, and applied in foods, such as bakery products for shelf-life extension (Floros et al., 1997; Smith et al., 1987, 1995; Suppakul et al., 2003). In previous studies, our research group reported the significant effect of distillery ethanol or alcoholic beverages (as an alternative to pure ethanol) on the shelf-life extension of animal (pork meat) and plant origin (pomegranate arils) foods (Kapetanakou et al., 2014, 2015). In the present work we aimed: i) to evaluate the efficacy of edible films immersed in distillery ethanol and traditional, Greek alcoholic beverages such as "tsipouro", "ouzo", and "raki" to inhibit growth of *L. monocytogenes* on frankfurters and ham slices under vacuum at different storage temperatures and ii) to assess the impact of those edible films with alcoholic beverages followed by MW reheating on the reduction of pathogen on frankfurters at different times during storage at 4 and 10 °C.

## 2. Materials and methods

### 2.1. Antimicrobial compounds

Three commercial, traditionally Greek alcoholic beverages (ABs), namely "tsipouro" (41% v/v ethanol), "raki" or "tsikoudia" (39.6% v/v ethanol), and "ouzo" (40% v/v ethanol) were the antimicrobials used in the current study. As previously reported by Kapetanakou et al. (2014), "tsipouro" and "raki" are produced by single and double distillation of grape pomace, respectively, while "ouzo" is being produced by infusing pure white ethanol, after double or triple distillation process, with various herbs (i.e., aniseed). Moreover, aqueous equal-strength solution of distillery ethanol (40% v/v) was also applied among the treatments in order to evaluate the contribution of pure ethanol content of ABs in the antimicrobial activity.

### 2.2. L. monocytogenes strains and inoculum preparation

Four *L monocytogenes* strains were used in the present study, two belonging to serotype 1/2a and two to 4b. All strains were maintained on slants of Tryptic Soy Agar supplemented with 0.6% w/v yeast extract (TSAYE) (Lab M, Lancashire, UK) and sub-cultured once a month. Each strain was grown separately in Tryptic Soy Broth supplemented with 0.6% w/v yeast extract (TSBYE) (Lab M, Lancashire, UK) for 24 h at 30 °C and subsequently, 100 μL of each overnight culture was transferred to fresh TSBYE for 18 h incubation and reached cell density of *ca.* $10^9$ CFU/mL. Following activation, strains were separately harvested by centrifugation (3600 rpm; 4 °C; 15 min) (Megafuge 1.0 R, Heraeus Instruments), washed and re-suspended in 10 mL of 1/4 strength Ringers' solution (Lab M, Lancashire, UK) twice, and finally mixed in equal volumes.

### 2.3. Preparation of Na-alginate films

Quantity of 1.5 g of Na-alginate (Applichem GmbH, Darmstadt, Germany) was added gradually in 100 mL of pre-warmed (65 °C) distilled sterile water and under stable agitation for complete dissolution. One mL of glycerol (plasticizer) was added in order to improve film flexibility and reduce brittleness. Plasticizer may also affect moisture and $O_2$ permeability of edible films (Han and Gennadios, 2005). Films, round (9 cm diameter) and square (10 cm × 10 cm) were produced in different Petri-dishes using 10 and 17 g of Na-alginate solution, respectively. All Petri-dishes were placed in a laminar flow cabinet to dry at ambient temperature for 12 h. Following drying process, aliquots of 10 and 15 mL of 2% w/v

Download English Version:

https://daneshyari.com/en/article/4362631

Download Persian Version:

https://daneshyari.com/article/4362631

Daneshyari.com