Food Control 63 (2016) 21–27

Contents lists available at ScienceDirect

Food Control

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

Antibacterial effects of 16 formulations and irradiation against *Clostridium sporogenes* in a sausage model

Mina Ghabraie, Khanh Dang Vu, Sarra Tnani, Monique Lacroix*

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

ARTICLE INFO

Article history:
Received 12 June 2015
Received in revised form
16 October 2015
Accepted 12 November 2015
Available online 17 November 2015

Keywords:
Antibacterial formulations
Clostridium sporogenes
Irradiation
Meat sausage

ABSTRACT

The anti-Clostridial effects of 16 formulations consisting of nisin (12.5–25 ppm), nitrite (100–200 ppm), mixed essential oils (EOs) of Chinese cinnamon plus Cinnamon bark (0.025–0.05%) and mixed of potassium lactate plus sodium acetate (1.55–3.1%) against *Clostridium sporogenes* in a sausage model were evaluated. Further, the anti-Clostridial effects of combined treatments using these formulations with irradiation at 1.5 kGy were also conducted to see if there is any synergistic effect. The antimicrobial effects were evaluated at day 1, 4 and 7 during storage of sausage at 4 °C. Eight formulations that contained low nitrite concentration (100 ppm) and contained either low or high concentrations of other agents could reduce *C. sporogenes* by 0.69–1.39 log CFU/g meat during 7 days of storage. Eight formulations that contained high nitrite concentration (200 ppm) and contained either low or high concentration of other agents could reduce *C. sporogenes* by 0.93–1.93 log CFU/g meat during storage. The combined irradiation (1.5 kGy) and formulations are less anti-Clostridial effects than formulations alone, except for the formulations 10, 13 and 16 at day 1 as compared to their equivalents alone. The anti-Clostridial effects of the combined treatments were significantly decreased at day 4 and day 7 while formulations alone maintained well their activity during storage. It is possible that irradiation treatment caused a stress on vegetative cells of *C. sporogenes* and therefore, there might have the endospores formation which are difficult to eliminate them.

1. Introduction

Clostridium botulinum is a Gram positive bacterium strictly anaerobic, rod-shape bacteria with the ability of spore forming under non-optimal growth condition. It is widely distributed in nature especially in soil (Cui, Gabriel, & Nakano, 2010). *C. botulinum* is among concerning bacteria because it can produce botulinum neurotoxin (BoNT) which is the most potent known toxin that can affect humans, animals and cause fetal at 30–100 ng (Peck, 2009). Foodborne botulism resulted by ingestion of BoNT in food is the most common botulism (Lindström, Kiviniemi, & Korkeala, 2006). *Clostridium sporogenes* is a non-toxic analog of *C. botulinum* (Cammack et al., 1999). *C. sporogenes* can be used as a model organism representing for *C. botulinum* in studies due to having similar metabolism without toxins formation (Cammack et al., 1999).

Meat and meat products, due to their high level of nutrients, pH (5.5–7.0) and high water activity, offer a congenial environment for the growth of bacteria (Dave & Ghaly, 2011). Hence these products should be handled and preserved properly. Recent years, food companies are interested to use natural food additives to replace completely or partially synthetic additives (Jayasena & Jo, 2013). Essential Oils (EOs) are among of natural compounds that can be used in food products due to their GRAS (Generally Recognized As Safe) status and their antimicrobial efficacy against several foodborne pathogens (Burt, 2004; Fratianni et al., 2010). The European Commission and the United State Food and Drug Administration (FDA) accepted some EOs constituents like cinnamaldehyde, thymol, eugenol, carvacrol as well as crude EOs such as cinnamon, mustard, oregano, thyme, clove and so forth to be used in food products (Hyldgaard, Mygind, & Meyer, 2012). It has been known that EOs at high concentration may cause sensorial side effects on food products (Abdollahzadeh, Rezaei, & Hosseini, 2014; Burt, 2004). Thus, a synergetic or additive antibacterial effects of combined EOs with other antimicrobial components can be useful for food

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

http://dx.doi.org/10.1016/j.foodcont.2015.11.019
0956-7135/

application because lower concentration of EOs can be used and can be organoleptically acceptable (Jayasena & Jo, 2013; Solomakos, Govaris, Koidis, & Botsoglou, 2008).

Nisin is a "broad-spectrum" bacteriocin which is effective against many Gram-positive organisms and also effective against bacterial spores (Zacharof & Lovitt, 2012). It is approved and widely used in more than 50 countries (Abdollahzadeh et al., 2014). Combined nisin (500 and 1000 IU) and thyme EO (0.6%) have been found to be more effective in controlling Escherichia coli O157: H7 than each individual (Solomakos et al. (2008)).

Potassium lactate (PL) and sodium acetate (SA) or sodium diacetate are organic acid salts having antimicrobial properties and are recognized as safe (GRAS). These organic acid salts are widely used as food preservatives (Nerbrink, Borch, Blom, & Nesbakken, 1999; Perumalla et al., 2012). Nitrite is also a food antimicrobial agent which extends the shelf life of meat products. It contributes to color stability along with improving sensory quality of meat products by giving unique color, texture and flavor (Cui et al., 2010; Sindelar & Milkowski, 2011). It has been reported that germination of *C. sporogenes* spores in meat was reduced by sodium nitrite (Dave & Ghaly, 2011; Sindelar & Milkowski, 2011). The highest acceptable concentration of nitrite in meat products is 200 ppm (Cui et al., 2010; Nyachuba, Donnelly, & Howard, 2007). However, it is suggested to use nitrite in food products as lower concentration than 200 ppm when it is possible (Sindelar & Milkowski, 2011).

Irradiation is an efficient method for eliminating pathogenic bacteria in food and food products (Farkas, 2006). The appropriate dose of irradiation to control microorganisms in foods depends on several factors such as target microorganism, their quantity, composition of food matrix, moisture, presence of oxygen, fresh or frozen food and the temperature during the irradiation (Farkas, 2006).

Encapsulation of antibacterial agents in edible polymers is an effective technique to prolong activity of the antimicrobial compounds during food storage (Huq, Vu, Riedl, Bouchard, & Lacroix, 2015). Proteins, alginates, starches, and other polysaccharides are usually used as materials for encapsulation of antimicrobial agents (Huq et al., 2015; Neetoo, Ye, & Chen, 2010). It has been demonstrated that combined irradiation with microencapsulated beads containing EOs could improve the antibacterial stability and efficiency against *Listeria monocytogenes* in ham during storage (Huq et al., 2015).

Hurdle technology is combined technologies or combined treatments consisting of several antimicrobial factors at their sub-inhibitory concentrations for food preservation against foodborne pathogenic bacteria (Manju, Jose, Srinivasa Gopal, Ravishankar, & Lalitha, 2007). Hurdle technology helps to reduce dose or concentration of individual component in a combined treatment since the combined treatment may cause synergistic effects in reduction of pathogenic bacteria in food products (Zhou, Xu, & Liu, 2010).

There were studies on antibacterial effects against *Clostridium* sp. using EOs and nitrite (Dias, Rogrigues, Palhares, Ramos, & Piccoli, 2015; de Oliveira et al., 2011), or plant extracts and nitrite (Cui et al., 2010), etc; however, there is no study on applying a hurdle technology using four antimicrobial agents to control *C. sporogenes* in a meat model.

The objective of this study was to evaluate the antibacterial activity of microencapsulated antimicrobial formulations containing essential oils, nisin, nitrite and organic acid salts against *C. sporogenes* in fresh pork sausage during 7 days of storage at 4 °C. The possible synergy between antimicrobial formulations and gamma irradiation at 1.5 kGy against *C. sporogenes* in fresh pork sausage during 7 days of storage at 4 °C was also evaluated.

2. Materials and methods

2.1. Materials

Reinforced Clostridial medium and Peptone were purchased from Alpha Biosciences Inc. (Baltimore, MD, USA). AnaeroGen sachet was bought from Fisher Canada. Alginate and $CaCl_2$ were purchased from Sigma–Aldrich Canada Ltd. (Oakville, ON, Canada). Cellulose nanocrystals (CNC) was supplied by FPInnovations pilot plant (Pointe-Claire, QC, Canada). Nisin power (Niprosin™, containing 2.5% pure nisin, 77.5% salt and 20% vegetable protein) was purchased from Pro-food International Inc (Profood, Naperville, IL, USA). Ground lean pork meat was purchased from a local grocery store (IGA, Laval, Quebec, Canada). Binding agent and sodium erythorbate were delivered from BSA Food Ingredients (St-Leonard, Quebec, Canada). Chinese cinnamon and Cinnamon bark EOs were provided by Aliksir Inc. (Grondines, Québec, Canada). EOs were mixed together at a ratio of 1: 4. The chemical composition of these EOs is presented in Table 1.

2.2. Bacterial strain

C. sporogenes was stored at −80 °C in Reinforced Clostridial Medium (RCM) containing glycerol (10% v/v). Before each experiment the bacteria were propagated through two consecutive 24 h in 9 ml of RCM at 37 °C under anaerobic condition. The incubation of this bacterium was conducted in sealed jar with AnaeroGen sachet. The final concentration of bacteria after two times of propagation was approximately 10^6 CFU/ml.

2.3. Experimental design for antimicrobial formulations

In this study four antimicrobial factors at two levels (maximum acceptable concentration and half of that concentration) were chosen to be combined together. Based our previous study, a mixed organic acid salts consisting of 0.40% (w/w) sodium acetate and 2.70% (w/w) potassium lactate (3.1%, w/w) was selected and used in this study due to its antimicrobial activity and organoleptically acceptance. Nitrite was another antimicrobial agent was used in this study. The maximum acceptable concentration of nitrite in food is 200 ppm. In our previous study, it was found that the mixed Chinese cinnamon and Cinnamon bark EOs (0.05%, v/w) were efficient in controlling the growth of pathogenic bacteria such as *L. monocytogenes*, *Escherichia coli*, *Salmonella* Typhimurium and *Staphylococcus aureus*. Based on the results of sensorial analysis, this combination of EOs are organoleptically acceptable at maximum of 0.05%. Nisin was the fourth antimicrobial agents which used in this study, it was reported that nisin at the concentration of 1000 IU/g mined beef (25 ppm) can reduce around 1 log CFU/g minced fish during storage of 12 days (Abdollahzadeh et al., 2014). Thus, in this current study, we selected to use 25 ppm nisin as the highest concentration.

A standard experiment with 4 independent factors at 2 levels (4^2) was conducted using STATISCA 8 (STATSOFT Inc., Thulsa, US). The dependent factor was the count (log CFU/g) of *Clostridium monocytogenes* during storage of 7 days. All the independents factors with their values (concentrations) are presented in Table 2.

2.4. Nisin preparation

Nisin powder was mixed in 0.01 M $CaCl_2$ solution to have final concentration of 0.25 w/v. The pH of this solution was adjusted to 3 by diluted lactic acid. The solution was centrifuged for 15 min at 3500 × g at 4 °C, undissolved particles were removed and nisin supernatant was collected (Huq et al., 2015).