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Use of growth inhibitors for control of *Listeria monocytogenes* in heat-processed ready-to-eat meat products simulating post-processing contamination

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ABSTRACT

The main factor responsible for occurrence of listeriosis associated to ready-to-eat meat products is assumed to be post-processing contamination during operations at retail and consumption. This study evaluated the antilisterial activity of growth inhibitors in bolognas and frankfurters, during storage at 8 °C for 30 days, simulating post-processing contamination with *Listeria monocytogenes*. Bolognas were manufactured with nisin-based NovaGARD®LM100, NovaGARD®NR100 and the individual components of these growth inhibitors. Frankfurters were manufactured with sodium lactate (Purasal S®) and immersed in liquid smoke (AM5®). On the 10th day at 8 °C, counts of *L. monocytogenes* in experimentally contaminated bolognas containing NovaGARD®LM100 and in frankfurters containing sodium lactate and treated with liquid smoke were 3 log lower than in controls, and in the products manufactured with the individual components of NovaGARD®LM100 and NovaGARD®NR100. These growth inhibitors, when used correctly, can increase the robustness of existing safety and quality assurance programs in the meat industry.

1. Introduction

Proper heat treatment during processing is enough for elimination of most contaminating microorganisms in processed meat products, especially non-sporogenic pathogens (Jiang & Xiong, 2015). Nevertheless, several outbreaks of foodborne diseases have been associated to heat treated ready-to-eat (RTE) meat products (Cartwright et al., 2013; Gottlieb et al., 2006; Thomas et al., 2015), and occurrence of pathogens such as *Listeria monocytogenes* and *Salmonella* spp is common (Garrido, Vitas, & García-Jalón, 2009; Gomez et al., 2015; Meyer et al., 2012; Modzelewska-Kapitula & Maj-Sobotka, 2014; Osaili et al., 2014). The main factor responsible for their occurrence is cross-contamination during post-processing operations (slicing, chopping, comminuting) at retail establishments and meals preparation sites, including homes (Simmons et al., 2014).

Listeria monocytogenes is of particular concern in RTE foods due to its psychrotrophic characteristics, resistance to high salt concentration and capability to survive and grow in refrigerated foods, aerobically and anaerobically, in a wide range of pH, from 4.6 to 9.4. *L. monocytogenes* is the causative agent of listeriosis, a severe disease that affects immunocompromised individuals, pregnant women, the elderly and young children (Ferreira, Wiedmann, Teixeira, & Stasiewicz, 2014). Different from most pathogens, *L. monocytogenes* is ubiquitous and a common contaminant in the food processing environment, mainly due to formation of resistant biofilms on surfaces (Manios & Skandamis, 2014; Silva & Martinis, 2012). Absence of competing microbiota in heat processed foods favors its growth (Gombas, Chen, Clavero, & Scott, 2003). Consequently, processed meat products that become contaminated with *L. monocytogenes* after heat treatment may contain high numbers of the pathogen due to the extended shelf-life under refrigeration, posing risk to consumers. Risk assessments have shown that delimeats belong to “very high risk” category of RTE foods (FDA-FSIS, 2003).

A number of studies have evaluated and modeled the transfer of

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L. monocytogenes from equipment (slicers), utensils (knifes, choppers) and food handlers to RTE products (Chaitiemwong, Hazeleger, Beumer, & Zwietering, 2014; Keskinen, Todd, & Ryser, 2008; Mataragas, Zwietering, Skandamis, & Drosinos, 2010; Pérez-Rodríguez, Valero, Carrasco, García, & Zurera, 2008; Sheen, 2008; Vorst, Todd, & Ryser, 2006), evidencing the importance of post-processing cross-contamination. Some quantitative risk assessments considered the effect of growth inhibitors (lactate, diacetate) added to the RTE meat products (ham and turkey), showing numerically how they were able to decrease the relative risk of listeriosis-associated deaths (Pradhan et al., 2009, 2010, 2011).

Because of the high prevalence of *L. monocytogenes* in RTE meat products, the high mortality rate of listeriosis (30%) and absence of a killing step before consumption, extensive research has been conducted on the application of growth inhibitors for control of *L. monocytogenes* in these products (Ahmed et al., 2015; Lavieri et al., 2014; Terjung, Loeffler, Gibis, Hinrichs, & Weiss, 2014). While sodium and potassium lactates are well known antimicrobials used in processed meat products, others, like those based on bacteriocins produced by GRAS (Generally Recognized As Safe) lactic acid bacteria and liquid smoke have not been sufficiently evaluated as tools for the control of *L. monocytogenes* and spoilage microorganisms (Balciunas et al., 2013; Guilbaud et al., 2008; Zacharof & Lovitt, 2012).

Nisin, a bacteriocin produced by *Lactococcus lactis* subsp *lactis*, is a biopreservative widely used in many countries (Jozala, Novaes, & Pessoa, 2015), but its effectivity in meat products remains controversial (Deegan, Cotter, Hill, & Ross, 2006; Devlieghere, Vermerein, & Debevere, 2004). Recent studies have shown that nisin combined with other antimicrobials can act synergistically as they have different modes of action in the target cells (Field et al., 2015; Govaris, Solomakos, Pexara, & Chatzopoulou, 2010; Turgis, Vu, Dupont, & Lacroix, 2012). The effectivity of these combinations needs to be better evaluated as growth of microorganisms depends on the complexity of the meat matrix, especially content of fat, protein, carbohydrates and salt, and pH (Zhang, Kong, Xiong, & Sun, 2009).

Liquid smoke is an antimicobial produced by condensing wood smoke created by the pyrolysis of sawdust or wood chips. The carcinogenic polyaromatic hydrocarbons are removed and the resulting phenols, carbonyls and organic acids are responsible for the flavor, color and antimicrobial activity (Lingbeck et al., 2014b). The response of *L. monocytogenes* to liquid smoke was evaluated *in vitro* (Guilbaud et al., 2008; Lingbeck et al., 2014a) and as ingredient in chicken and pork frankfurters (Morey, Bratcher, Singh, & McKee, 2012) or sprayed on the products after peeling (Gedela, Escoubas, & Muriana, 2007; Martin et al., 2010).

This study evaluated the activity of antimicrobials added to two types of heat processed RTE meat products (bolognas and frankfurters), for the control of *L. monocytogenes* during refrigerated storage, simulating the occurrence of post-processing contamination. The antimicrobials were two nisin-based growth inhibitors (NovaGARD®LM100 and NovaGARD®NR100), the individual components of these two products, sodium lactate (Purasal S®) and liquid smoke (AM5®). Lactic acid bacteria and yeasts and molds were also monitored in frankfurters, as indicators of shelf-life.

2. Materials and methods

2.1. Strains

The study was conducted with a pool of six *Listeria monocytogenes* strains (five meat isolates and one ATCC 7644 strain). Four meat isolates belong to the culture collection of the Food Research Center, University of Sao Paulo, Sao Paulo, Brazil, and the fifth isolate was donated by Fundação Oswaldo Cruz, Rio de Janeiro, Brazil. Stock cultures were maintained at −80 °C in Brain Heart Infusion (BHI - Oxoid, Basingstoke, UK) with 10% glycerol (v/v).

Before use, the six strains were tested for antagonism among them, using the spot on-the-lawn bioassay (Lewus & Montville, 1991), adapted to *Listeria* spp (Bruhn, Vogel, & Gram, 2005). Briefly, the strains were grown separately overnight in Tryptic Soya Broth (TSB - Oxoid, Basingstoke, UK) at 37 °C, diluted to 10^7 CFU mL^{-1} in 0.1% sterile peptone water (PW), and an aliquot of 0.1 mL was transferred to a petri dish containing liquid TSB plus 1% microbiological agar. After mixing and solidification of the agar, drops of 10 μL of each culture were spotted on the surface of the agar plates containing each *L. monocytogenes* culture separately. Plates were incubated at 37 °C for 24 h and examined for growth inhibition zones around the spots, indicating antagonism and a reason for exclusion of the strain from the study.

For experimental contamination of the RTE meat products, the strains were grown overnight separately in BHI at 37 °C, transferred to fresh BHI and incubated at 37 °C for 10 h, correspondent to early stationary phase, as indicated by a standard growth curve. Equal volumes of each culture were mixed and centrifuged at 6000 × g for 10 min under refrigeration. The supernatants were discarded and the pellets re-suspended in sterile 0.1% PW. The procedure was repeated three times. The last pellets were re-suspended in sterile 0.1% PW until a suspension with OD_{595} = 0.5 (Ultrospec 2000; Pharmacia, USA) (10^8 CFU mL^{-1}) was obtained.

2.2. Growth inhibitors

The study was conducted with one batch of each of the following growth inhibitors:

a) NovaGARD®LM100 (Danisco do Brasil, Ltda, Brazil): patented product that contains encapsulated nisin, free nisin, rosemary extract and sodium diacetate, for use in cooked or cured RTE meat products at 0.25%;
b) NovaGARD®NR100 (Danisco do Brasil, Ltda, Brazil): patented product that contains free nisin and rosemary extract, for use in cooked or cured RTE meat products at 0.05%;
c) Purasal S® (Purac Sínteses Indústria e Comércio Ltda, Brazil): sodium lactate, a liquid product obtained by sugar fermentation, for use in meat products at 2%;
d) AM5® (Kraki Kienast & Kratschmer Ltda, Brazil): liquid smoke (acidity 0.12%, pH 6.0, specific gravity 1.057 and phenolics 0.03 mg L^{-1}), for immersion of meat products.

2.3. Production of bolognas

The bolognas were manufactured at Solae do Brasil Indústria e Comércio de Alimentos Ltda, Rio Grande do Sul, Brazil, using mechanically deboned chicken meat (60%), pork fat (10%), pork skin emulsion (7%), pork meat (4.1%), ice or water (5%), cassava starch (5%), salt (3%), sodium tripolyphosphate (0.3%), isolated soy protein (2.5%), sodium nitrite (0.016%), sodium erythorbate (0.05%) and cochineal carmine dye (0.02%). The ingredients were mixed in a cutter and stuffed in caliber 60/90 mm polyamide casings, prehydrated in water. The bolognas were cooked in boiling water to a core temperature of 82 °C (app. 10 min), cooled in cold water and stored in a chamber at 4 °C.

Two 1 kg lots of bolognas were manufactured. The first lot comprised bolognas prepared with the commercial growth inhibitors in the concentrations recommended by the manufacturers. Negative controls, with no growth inhibitors, were also prepared. The second lot comprised bolognas prepared with each component