Food Control 84 (2018) 255–262

Contents lists available at ScienceDirect

Food Control

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

Efficacy of *N*-halamine compound on reduction of microorganisms in absorbent food pads of raw beef

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ARTICLE INFO

Article history:
Received 12 April 2017
Received in revised form
8 August 2017
Accepted 10 August 2017
Available online 16 August 2017

Keywords:
Antimicrobial
Absorbent pad
N-halamine
MC
Beef

ABSTRACT

The antimicrobial activity of *N*-halamine, 1-chloro-2,2,5,5-tetramethyl-4-imidazolidinone (MC) loaded absorbent pads in meat packaging was investigated. Absorbent pads treated with MC reduced the levels of total aerobic bacteria, *Pseudomonas* spp., and lactic acid bacteria to under the detection limit (2 log CFU/g) for day 0 and day 1. On days 4, 7 and 11, the microbial loads were significantly reduced ($p < 0.05$) in all MC treated absorbent pads. The levels of Enterobacteriaceae on the treated samples remained under the detection limit during the entire storage period. The lightness of packed beef with MC treated absorbent pads was not significantly increased until day 11, while in the control group there were significant differences compared to the initial meat from day 1. Microbial loads in meat samples with MC treated pads were also significantly lowered ($p < 0.05$) compared to the control on day 11. MC treated absorbent pads were able to reduce microbial loads in beef samples by 1 log CFU/g on average. MC was able to extend the shelf life of refrigerated raw beef.

1. Introduction

Beef is considered one of the most perishable foods due to its high moisture content and abundance of nutrients for microbial growth. Various microorganisms, including spoilage and foodborne pathogens, can utilize the nutrients and colonize on the meat surface. Raw beef naturally contains liquids, which can accumulate in the packaging container and leak during transportation and storage. The exudate is a sign of an unsanitary product from most consumers' perception. Absorbent pads are widely used in the meat industry packing systems to immobilize the exudates, isolate the meat from unsanitary juices, and create an appealing package. However, immobilized unsanitary exudates may generate undesirable odors and promote the growth of microorganisms. Therefore, reducing microbial loads in absorbent pads is an important avenue for improving food quality and safety during food packaging and storage (Quintavalla & Vicini, 2002).

Packaging materials can promote food products to consumers in an attractive and hygienic way. To prevent the spread of spoilage and pathogenic microorganisms through meat and meat products, antimicrobial packaging materials have been studied intensively and can be a potential alternative technique (Coma, 2008). Instead of mixing antimicrobials directly with food, incorporating antimicrobials such as essential oils (Skandamis & Nychas, 2002) and nisin (Cutter, Willett, & Siragusa, 2001; Ercolini et al., 2010b; Natrajan & Sheldon, 2000) into food packaging films or absorbent pads have shown benefits. Traditional applications of antimicrobial agents result in neutralization on the contact surface or diffusion into the food, reducing their efficacy (Juven, Kanner, Schved, & Weisslowicz, 1994). Antimicrobial packaging materials may slowly release the active antimicrobial compound to the food contact surface, and thus extend their antimicrobial activity during transportation and storage (Quintavalla & Vicini, 2002). In addition, some antimicrobials have negative impacts on physical properties, such as color, and are not allowed to be used in certain food products (Lone et al., 2016). Active antimicrobials in packaging materials that are not directly in contact with food have more applications to improve the safety of products.

N-halamine is a group of compounds containing one or more nitrogen-halogen covalent bond. They have been intensively investigated as potential antimicrobial agents in the past decade. 1-chloro-2,2,5,5-tetramethyl-4-imidazolidinone (MC, Fig. 1), is a

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http://dx.doi.org/10.1016/j.foodcont.2017.08.006
0956-7135/

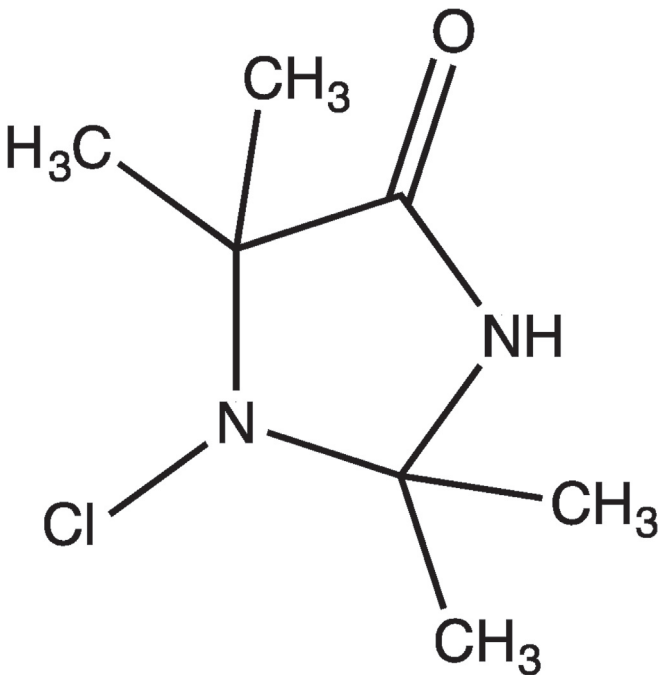

Fig. 1. Structure of MC (1-chloro-2,2,5,5-tetramethyl-4-imidazolidinone).

monochlorinated *N*-halamine and has shown broad-spectrum antimicrobial activities with long-term biocidal properties (Demir, Cerkez, Worley, Broughton, & Huang, 2015; Lauten et al., 1992; Worley et al., 1992). Demir et al. (2015) showed that MC coated polypropylene nonwoven fabric material was able to cause a 6-log reduction of *Staphylococcus aureus* (complete inactivation) and a 4-log reduction of *Escherichia. coli* O157: H7 within 5 min of contact time. MC in a mineral oil suspension has been used to disinfect *Salmonella* Enteritidis on eggshells within 72 h, and is recommended as a possible disinfectant for the egg-processing industry (Worley et al., 1992). MC coated fabrics in a dark environment are able to retain the initial active chlorine content for up to 6 months (Demir et al., 2015). Moreover, MC is considered a low toxic *N*-halamine compound. The acute oral LD50 of MC was reported as 338 mg/kg (Kern, Hurley, & Williams, 2000) and no indication of mutagenicity was observed (manuscript submitted for publication), indicating MC has a potential application in food related areas. In addition, MC has been patented in treating fish diseases (Bridges, Palczewski, Scott, Suess, & Nichols, 2007) using in fish tank and in preventing human infections when contained in fibrous compositions (Worley, Broughton, Cerkez, & Demir, 2016). Cellulose materials in absorbent pads can be easily incorporated with MC by immersion in 1% MC ethanol solution for industrial applications.

The objectives of this study were to evaluate the effectiveness of MC coated cellulose pads in reducing major spoilage-related microorganisms in air-permeable packaging materials during 11 days of storage at 4 °C. Additionally, meat quality was evaluated by measuring the changes of surface color and microbial loads on meat samples during storage. An *in vitro* study of the antimicrobial activities of MC against two foodborne pathogens *S. aureus* and *E. coli* O157:H7 were tested due to their common association with foodborne diseases from meat and meat products (Hanson et al., 2011; Millette, Le Tien, Smoragiewicz, & Lacroix, 2007; Rangel, Sparling, Crowe, Griffin, & Swerdlow, 2005; Scallan et al., 2011; Scanga et al., 2000; Wells et al., 1991).

2. Materials and methods

2.1. Materials

Absorbent pads Dri-loc® AC-40 were purchased from Novipax (Reading, PA). The absorbent part is a layer of highly absorbent fluff pulp, separated from food products by a non-permeable polyethylene film top layer and polyethylene film perforated with one-way valves. The absorbency is around 20 g per absorbent pad (2 g). White foam trays (1004D, length: 9 ¼″, width: 7 ¼″, depth 1 ¼″) were purchased from Genpak® LLC (Glens Falls, NY).

MC was supplied by Cangzhou Jincang Chemicals, LTD (China). Tryptic soy broth (TSB), Sorbital MacConkey agar (SMAC), buffered peptone water (BPW), Baird-Parker agar (BPA) and plate count agar (PCA) were purchased from BD (Sparks, MD); *Pseudomonas* agar base with cetrimide-fucidin-cephaloridine (CFC), de Man Rogosa Sharpe agar (MRS), and egg yolk tellurite emulsion were purchased from HiMedia (Nashik, MH). Crystal violet neutral red bile glucose agar (VRBG) was purchased from Hardy Diagnostics (Santa Maria, CA).

2.2. Preparation of cellulose-MC hybrid materials

MC was dissolved in 95% ethanol solution to prepare a 1% (10 g/L) stock solution. The stock solution was diluted with 95% ethanol to produce 0.01%, 0.05%, and 0.1% of MC working solutions. Cellulose (2 g/pad) from absorbent pads was soaked in 10 mL of working solutions for 1 min. The soaked pads were air dried at room temperature for 48 h. The controls were the cellulose treated with 95% ethanol.

2.3. Characterization of cellulose-MC hybrid materials

2.3.1. Verification of MC on absorbent pad

MC on cellulose materials was verified by Fourier Transform Infrared Spectroscopy (FT-IR) spectrum (Nicolet 6700 FT-IR Spectrometer, Thermo Scientific, Madison, WI). MC contains prominent C=O bands at 1715 and 1673 cm^{-1} on FTIR spectrum. If MC is successfully incorporated onto the absorbent pad, the MC coated materials will show these unique peaks, which are absent from cellulose alone.

2.3.2. Quantification of remaining active chlorine

Active chlorine loads in the cellulose over the 11 days of storage were determined by the modified iodometric/thiosulfate titration method (Worley et al., 2005). Active chlorine content was calculated according to the following equation:

$$Cl^{+}(\text{atoms/g}) = \frac{6.02 \times 10^{23} \times N \times V}{2 \times W}$$

where, N and *V* are the normality (equiv/L) and volume (L) of the sodium thiosulfate (titrant), respectively, and W is the weight of pads in gram.

2.4. Antimicrobial activity in vitro

A cocktail of *E. coli* O157: H7 ATCC 11229 and *S. aureus* ATCC 6538 was prepared. Bacterial suspension was dispersed evenly on 0.05 g cellulose materials, achieving 10^8 CFU/g for each sample. After 2 h, samples were placed into sodium thiosulfate solution to quench the chlorine residuals and wash off the attached bacteria by vortexing. Then, all the solutions were diluted by BPW (1:10) to make serial dilutions. Diluted samples were spread-plated on SMAC and BPA with egg yolk tellurite emulsion to determine