Meat Science 95 (2013) 160–165

Contents lists available at SciVerse ScienceDirect

Meat Science

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

Effectiveness of a polyamide film releasing lactic acid on the growth of *E. coli* O157:H7, *Enterobacteriaceae* and Total Aerobic Count on vacuum-packed beef

F.J.M. Smulders, P. Paulsen *, S. Vali, S. Wanda [1]

Institute of Meat Hygiene, Meat Technology and Food Science, Department for Farm Animals and Veterinary Public Health, University of Veterinary Medicine Vienna, 1210 Vienna, Austria

ARTICLE INFO

Article history:
Received 12 January 2013
Received in revised form 19 April 2013
Accepted 23 April 2013

Keywords:
Lactic acid
Active packaging
Immediate release
Antimicrobial effect

ABSTRACT

The suitability of a polyamide 6 monolayer film containing lactic acid for use as an antimicrobial package for fresh beef cuts was studied. The release of lactic acid in an aqueous environment was immediate (within 1 h) and was from approx. 55 μg lactic acid/cm^2 film at 0–8 °C to approx. 67 μg lactic acid/cm^2 film at 12–20 °C. Beef was contaminated with an *Escherichia coli* O157:H7 isolate with known minimum inhibitory concentration against lactic acid (0.09% v/v), then wrapped with the lactic-acid polyamide film and vacuum packaged. During storage at 12 °C, the numbers of *E. coli* were 1 log unit lower than that of a control (untreated polyamide film) and decreased by an additional 1 log during storage for 14 days.

1. Introduction

In beef, Shiga toxin producing *Escherichia coli* are among the pathogens of high concern as most outbreaks of hemorrhagic colitis involving *E. coli* O157:H7 have been linked to the consumption of undercooked beef such as burgers (Bell et al., 1994; King et al., 2009), beef donair (Currie et al., 2007), beef tacos (Jay et al., 2004) or blade-tenderized steaks (Swanson-Laine et al., 2005). Due to its low infectious dose of only 10 to 100 organisms (Law, 2000; Reiss, Kunz, Koin, & Keeffe, 2006; Wachtel, McEvoy, Luo, Williams-Campbell, & Solomon, 2003) enterohemorrhagic *E. coli* (EHEC) represents one of the more serious agents of food borne illness, sometimes with severe post-diarrhea complications such as hemolytic uremic syndrome and thrombocytopenic purpura (Duffy, Cummins, Nally, O'Brien, & Butler, 2006; Rhoades, Duffy, & Koutsoumanis, 2009). Cattle are considered a major reservoir for *E. coli* O157:H7 and contamination of the beef carcass at slaughter might occur during such processes as hide removal and evisceration (Marshall, Niebuhr, Acuff, Lucia, & Dickson, 2005; Woody et al., 2000) and of meat products during processing, storage and handling (Sofos, Belk, & Smith, 1999). In addition, seasonal variation in fecal shedding of Shiga toxin producing *E. coli* has been reported, peaking in the warmer months (Barkocy-Gallagher et al., 2003; Rhoades et al., 2009), thus indicating a higher likelihood of carcass and product contamination. The latter raises concern as the warmer months in the year also pose a higher risk for temperature abuse in — and along the cold chain. Although refrigeration might be considered as one of the prime processes to control the growth of pathogenic microorganisms such as *E. coli* O157:H7 in meat, deviations from approved temperature ranges might occur, especially when the product moves along the cold chain (Koutsoumanis & Taoukis, 2005; Nychas, Skandamis, Tassou, & Koutsoumanis, 2008).

A number of intervention strategies targeted at *E. coli* O157:H7 have been studied on beef carcasses (e.g. rinses with organic acids or hot water, steam-vacuuming, application of trisodium phosphate and chlorine) and meat cuts (i.e. organic acids sprays/dips, irradiation, pasteurization) (Acuff et al., 1987; Castillo, Lucia, Mercando, & Acuff, 2001; Smulders & Greer, 1998; Sofos & Smith, 1998; Sofos et al., 1999). Not all of these techniques are compliant with the EU definition of "fresh meat" (EC, 2004).

A recent methodology proposed to act as an additional hurdle for the growth of pathogenic and/or spoilage microorganisms in the meat chain, is Antimicrobial Packaging (AMP), i.e. packages that release compounds into the packaged food. The antimicrobial agent is either embedded into — or coated onto the polymer matrix of the packaging system or the polymeric material itself has antibacterial properties (i.e. polylactic acid; Han, 2000). Antimicrobial substances considered for food packaging application need to be approved as food additives, food contact material (FCM) or be generally considered as safe (GRAS) for the consumer. In this respect, organic acids, their salts and anhydrides are frequently employed as antimicrobials combined with film materials (Joerger, 2007). In particular lactic acid, a natural component of meat, fulfills the legal requirements (EC,

* Corresponding author. Tel.: +43 1 25077 3318; fax: +43 1 25077 3390.
E-mail address: peter.paulsen@vetmeduni.ac.at (P. Paulsen).
[1] Current address: Institute of Veterinary Public Health, Department for Farm Animals and Veterinary Public Health, University of Veterinary Medicine Vienna, 1210 Vienna, Austria.

0309-1740/$ – see front matter © 2013 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.meatsci.2013.04.058

2002; Van Netten, Huis In't Veld, & Mossel, 1994). Currently, consumers mostly lack knowledge on active packaging techniques, which makes them reluctant to accept such strategies to improve meat safety (Van Wezemael, Ueland, & Verbeke, 2011). It could be argued that the incorporation of a natural compound of meat into a meat packaging film would be acceptable for most consumers.

Among the polymer films, polyamides provide the characteristics necessary in meat applications, namely gas (e.g. oxygen) and aroma barrier function combined with good mechanical, thermal, chemical and fatty resistance (Araùjo, Fèlix, Manzoli, Padula, & Monteiro, 2008; Camacho, Hedenqvist, & Karlsson, 2002). Due to their hygroscopic properties, water-soluble antibacterial compounds can be embedded within the film matrix, and a prototype film has been described ("BOPA-L80F"; Wanda, Paulsen, Budai, Vali, & Smulders, 2013).

The aim of the study was to assess how far a polyamide based antimicrobial packaging film incorporating lactic acid can contribute to beef safety and shelf life by affecting Total Plate Count and numbers of *E. coli* O157:H7 and *Enterobacteriaceae*.

2. Material and methods

2.1. Antimicrobial film

Bi-axially oriented polyamide 6 (BOPA6), 15 μm thickness (CFP Flexible Packaging S.p.A., Cesano Maderno; Italy) produced by flat die extrusion technology was chosen as a packaging matrix. Food-grade L-lactic acid, 80% w/w (PURAC FCC 80; Purac, Tambon Banchang, Rayong, Thailand) was used as antibacterial agent.

Antimicrobial film preparation was conducted as described previously (Wanda et al., 2013). Briefly, BOPA6 films were cut to size, pre-dried over silica-gel at 24 °C, 3% relative humidity (RH) for 72 h, then immersed for 2 h in 80% (w/w) lactic acid and dried for 24 h at 40 °C and 10% RH. In the following, such films are termed "BOPA-L80F", whereas untreated films are termed "uBOPA".

2.2. Effect of temperature on the release of lactic acid

The time–temperature effect on lactic acid release from BOPA-L80F films was assessed for five temperatures (0, 4, 8, 12, 20 °C) over 14 days. The 0–4 °C temperature range reflects the appropriate chilling regime in the meat chain, and 8 °C, 12 °C and 20 °C simulate slight, moderate and high temperature abuse, respectively. As meat exudate is supposed to enter the polymer matrix and dissolve lactic acid, an aqueous environment (*aqua bidest.*) was chosen to simulate the environment normally encountered at the surface of vacuum packaged beef. Films were inserted in glass tubes with screw caps and covered with 10 mL of *aqua bidest.* Tubes were fitted in floating devices and immersed in water at the above temperatures in a cooling bath (CC1-K25-NR, huber, Offenburg, Germany). To prevent ice formation, a defrosting agent (Adamol, Vienna, Austria) was added in the water baths maintained at 0 and 4 °C. After 1 h, 2 days, 5 days, 9 days and 13 days, 100 μL were sampled and lactic acid concentration was measured by HPLC, as described by Grosheny, Isengard, and Phillipp (1995) and Wanda et al. (2013).

2.3. Assessment of antimicrobial potential

2.3.1. Bacterial strain

Experiments were performed using an isogenic mutant of EHEC strain EDL 933 (constructed and described by Gobert et al., 2007), belonging to the serotype O157:H7, from C. Martin, Unité Microbiologie, INRA Clermont-Ferrand-Theix; France. In the mutant *stx*1 and *stx*2 are not expressed and no Shiga-toxin is synthesized. The wild type strain EDL 933 was isolated from a hamburger incriminated in the first documented EHEC outbreak in the United States in 1982 (Marouani-Gadri, Augier, & Carpentier, 2009). Attachment genes, e.g. *eae* (Law, 2000), are still present, thus meat attachment properties should be similar to that of the wild type.

2.3.2. Inoculum

The mutant *E. coli* O157:H7 strain was kept in 20% glycerol solution at −80 °C. For preparation of the inoculum, one loop of frozen stock was added to 10 mL of LB broth acc. to Miller (Merck KgA, Darmstadt, Germany) and incubated overnight at 37 °C to reach the stationary phase (~10^7 CFU/mL).

For minimum inhibitory concentration (MIC) determination, a 1 mL portion of the bacterial suspension was transferred to flasks containing 50 mL of fresh LB broth and incubated under shaking at 37 °C to reach late exponential phase. The liquid culture was then diluted in 0.1% Maximum Recovery Diluent (Oxoid, Basingstoke, UK) to render an inoculum of 10^4 CFU/mL.

The bacterial suspension used for meat inoculation was grown until the stationary phase was reached. A suspension of stationary phase cells was then diluted in 0.9% sterile sodium chloride to a cell density of 10^5 CFU/mL to be used as inoculum for beef portions.

Cell concentrations in inocula were determined by plating out decimal dilutions onto enriched Plate Count Agar (Merck, Germany; plus 0.5% Lab Lemco Powder, and 0.3% yeast extract; Oxoid) and incubation at 30 °C for 48 h.

2.3.3. Enumeration of bacteria

Enterobacteriaceae were enumerated on VRBD (Violet Red Bile Dextrose) Agar (Merck, Darmstadt, Germany) after an incubation period of 24 h at 37 °C. *E. coli* O157:H7 colonies were counted on Chrom ID O157:H7 Agar (bioMerieux, Marcy L'Etoile, France), after incubation at 37 °C for 48 h. For each sample plated on Chrom ID O157:H7 Agar three single representative colonies were picked, sub-cultured on a non selective medium (Plate Count Agar) and incubated at 30 °C for 48 h. Colonies were confirmed with an agglutination test (Wellcolex *E. coli* O157:H7; Remel Inc., Lenexa, USA).

2.3.4. Determination of minimum inhibitory concentration (MIC)

Tests were carried out on an INFINITE F200 (Tecan Group Ltd., Männedorf, Switzerland) multimode microplate reader, and the experimental set up was based on the use of Costar 96 Well Cell Culture Clusters (Corning Costar, Corning, New York, USA). Method of susceptibility testing and determination of MIC was as described in Lambert and Pearson (2000), with minor modifications. Lactic acid (PURAC FCC 80) was diluted in MH Broth (Merck) to give final concentrations of 2.9%, 2.2%, 1.5%, 0.7%, 0.35%, 0.07% and 0.02% (v/v) of the antimicrobial under test, respectively. Addition of dilutions, growth medium and inoculum followed as described by the authors, only volume ratios were converted to fit the smaller wells given for the above mentioned plates. Plates were incubated at 37 °C under continuous shaking for 20 h, and optical density (OD) of each well was recorded at 610 nm. Each concentration was tested in triplicate.

2.4. Preparation and examination of meat samples

2.4.1. Beef samples

Beef primals (*M. longissimus*) of 12 refrigerated halves of bull carcasses (18 months old) were obtained at 4 days post mortem from an EU approved slaughterhouse and transported (approx. 45 min. transport in a refrigerated truck) to the laboratory, where they were stored in air and processed in a chamber at 0–2 °C within 2 days. Primals were flamed with a torch and burnt surfaces were removed with sterile knives to eliminate microbial surface load. Lean muscle was then cut in 9 × 3 × 2 cm portions with the help of sterile stainless steel templates, resulting in 124 portions. Before inoculation two samples from each primal (each with approx. 50 cm^2 surface) were analyzed to exclude the presence of naturally occurring *E. coli* O157:H7 (as described under Section 2.3.3.). The remaining 100 meat portions were randomly