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Use of deodorized yellow mustard powder to control *Escherichia coli* O157:H7 in dry cured Westphalian ham

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ABSTRACT

Dry cured (uncooked) hams with low water activity and pH ≥5.6 seem a likely food vehicle for *Escherichia coli* O157:H7. In previous work, isothiocyanates produced from mustard glucosinolates by bacterial myrosinase-like activity converted deodorized mustard into a potent antimicrobial in dry sausage. In this study its value in controlling *E. coli* O157:H7 survival in Westphalian ham was investigated. Hams were inoculated with a 7.5 log cfu g^{-1} cocktail of *E. coli* O157:H7, 4% or 6% (w/w) deodorized yellow mustard powder was surface applied and monitored 80d for pathogen survival. In one trial to accelerate formation of isothiocyanate, a *Staphylococcus* (*S.*) *carnosus* meat starter culture was added to hams at 45d (after salt equilibration). At 21d, *E. coli* O157:H7 was reduced by 3 log cfu g^{-1} on hams treated with mustard powder compared to only a 1 log cfu g^{-1} reduction in the control. By 45d, mustard powder caused a reduction of >5 log cfu g^{-1} *E. coli* O157:H7, whereas it took 80d for numbers in control hams to be similarly reduced. Although the commercial process used caused a 5 log cfu g^{-1} reduction of *E. coli* O157:H7 in 80d, 4% or 6% deodorized mustard accelerated this reduction and the *S. carnosus* starter culture may have contributed to the maintenance of this effect.

1. Introduction

Dry cured Westphalian ham is an artisanal uncooked, ready-to-eat (RTE) meat product originally from the Westphalian region of Germany. It is made from fresh whole pork muscle preserved by aging with salt, cure agents and smoke followed by drying. The process can take up to 8 months and involves fermentation during which an indigenous microflora containing lactic acid bacteria (LAB) and staphylococci develops (Lücke, 1986). Though the preservation of meat by fermentation has been done for centuries, problems regarding fermented meat safety occur and are reported more frequently with dry sausages than dry cured ham.

Foodborne illness outbreaks caused by *Escherichia coli* O157:H7 in dry cured sausage were first reported 12 years after this organism was discovered to be foodborne in 1982 (Riley et al., 1983). Investigation has established that the preservation process used during dry sausage fermentation is inadequate to eliminate *E. coli* O157:H7 if present, since it tolerates the high salt, low pH, and low water activity (a_w) generated during the process (Hinkens et al., 1996; Paton et al., 1996; Tilden et al., 1996; Faith et al., 1998; Reynolds et al., 2001). If internalized in dry cured ham by injection, the organism can survive in the ripened product (Graumann and Holley, 2007). *E. coli* O157:H7 is especially dangerous since it has an infectious dose of ≤10 cells (Kiranmayi et al., 2010). Symptoms following infection include bloody diarrhoea, abdominal cramping, and in about 5% of cases, haemolytic-uraemic syndrome (HUS) (CDC, 2010).

Unfortunately, *E. coli* O157:H7 is only one of about 200 known serotypes of Enterohemorrhagic (EHEC) *E.coli* (Fratamico and Smith, 2006). Internationally, *E. coli* O157:H7 has caused the greatest number of illnesses from consumption of contaminated food and is a major problem in meat products, including fermented meats. However, other serotypes (including O26, O111, O103, O121, O45 and O145) share the same clinical, pathogenic and epidemiologic features with *E. coli* O157:H7 and are therefore of current interest to FSIS (USDA, 2008). Since 1990, there have been over 20 foodborne illness outbreaks caused by non-O157 VTEC. Although non-O157 VTEC are occasionally present in meat, only one outbreak has been reported due to their contamination of meat products and this involved serotype O111 in mettwurst which caused illness in Australia (Cameron et al., 1995). This outbreak demonstrates that other serotypes could become more problematic in meat products in the future.

The first outbreak of *E. coli* O157:H7 from consumption of dry cured salami occurred in 1994 in the US (CDC, 1995). The USDA implemented strict guidelines to ensure a 5 log kill of *E. coli* O157:H7 in all dry and semi-dry uncooked fermented sausages

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containing beef ingredients (USDA, 2005). The CFIA adopted these regulations after Canada's first documented illness outbreak from fermented sausages (1998, British Columbia) which was followed by another outbreak in 1999 (the largest *E. coli* O157:H7 outbreak recorded in Canada), also due to dry cured salami (CFIA, 2010; Williams et al., 2000; MacDonald et al., 2004).

Cattle have been a major focus of *E. coli* O157:H7 investigations, as they are regarded as the primary reservoirs of the pathogen. Approximately 52% of *E. coli* O157:H7 outbreaks are associated with beef products, with undercooked ground beef most often the cause (Griffin and Tauxe, 1991; Brandt et al., 1994; Tuttle et al., 1999). However, hogs have been found to be potential *E. coli* O157:H7 reservoirs, and more alarmingly, in some cases the pathogen has been observed at higher levels in pigs (Borie et al., 1997; Doane et al., 2007). Though there is no documentation linking dry cured ham with an outbreak of *E. coli* O157:H7, pork in dry cured salami has been linked to an outbreak (Paton et al., 1996). However, facilities processing only pork are not required to follow the same guidelines for *E. coli* O157:H7 control as those manufacturing products that contain beef (CFIA, 2010).

To improve the safety of raw dry cured products, the addition of other agents during manufacture to control *E. coli* O157:H7 is of interest. One alternative with promise is mustard, which has natural antimicrobial properties. All plants in the Brassicaceae family contain glucosinolates as secondary metabolites, and yellow mustard (produced in largest amounts in Canada) contains the glucosinolate sinalbin. Upon physical damage of the plant tissue, hydrolysis of sinalbin is catalyzed by the endogenous enzyme myrosinase (EC 3.2.1.147) in the presence of moisture to produce the antimicrobial ρ-hydroxybenzyl isothiocyanate (PHBIT) (Kawakishi and Muramatsu 1966; Delaquis and Mazza, 1995; Ekanayake et al., 2006). The mechanism of PHBIT antimicrobial action is uncertain, but it may inhibit essential enzymes and cause membrane damage (Lin et al., 2000).

PHBIT is not only antimicrobial, it is also responsible for the hot, spicy characteristics of yellow mustard (Buskov et al., 2000). Since this compound can overpower other flavours, the use of yellow mustard as a food ingredient has been limited. However, the optional inactivation of myrosinase by thermal treatment to deodorize mustard has broadened the range of food products where it can be used as a neutral-flavoured, high protein ingredient. Deodorized (deheated) mustard is extensively used as a binder-extender in cooked meat products, contributing to texture without flavour impact. However, glucosinolates are still present. Interestingly, studies have shown that even with myrosinase inactivated, deodorized or deheated yellow mustard powder was lethal to *E. coli* O157:H7 (Graumann and Holley, 2008), which suggested that sinalbin was somehow hydrolyzed to produce PHBIT.

Recently it was shown that *E. coli* O157:H7, other pathogens and some meat starter cultures have myrosinase-like activity which converted glucosinolates sinalbin and sinigrin into antimicrobial isothiocyanates (Luciano et al., 2011, unpublished, this laboratory). By hydrolyzing the glucosinolate in mustard, myrosinase positive organisms assimilate energy from the resulting glucose, but this exposes the organisms to the lethal effects of the isothiocyanate PHBIT in the case of yellow mustard. Of further interest, it was found that of the microorganisms tested, *E. coli* O157:H7 had the greatest capacity for glucosinolate degradation, while the meat starter culture *Staphylococcus carnosus* UM123M showed the second greatest rate of glucosinolate hydrolysis (250.3 μM versus 507.9 μM for *E. coli* O157:H7, after 6d at 25 °C) (Luciano and Holley, 2011; Luciano et al., 2011).

Work has shown that yellow mustard was dependably effective in eliminating *E. coli* O157:H7 from dry cured meats (Graumann and Holley, 2008, 2009; Luciano et al., 2011). The purpose of the present study was to investigate whether the surface application of deodorized yellow mustard powder to dry cured Westphalian ham at levels of 4% or 6% would consistently prevent *E. coli* O157:H7 survival. The study was also designed to determine whether the starter culture *S. carnosus* UM123M could contribute toward the elimination of *E. coli* O157:H7 from ham in the presence of deodorized mustard by increasing the extent of glucosinolate hydrolysis and PHBIT production.

2. Material and methods

2.1. Preparation of E. coli O157:H7

A five strain mixture of *E. coli* O157:H7 was prepared for inoculation of dry cured Westphalian hams. Strains of *E. coli* O157:H7 (non-pathogenic, human isolates) included 00:3581, 02:0304, 02:0628, 02:0627, and 02:1840, and were supplied by Rafiq Ahmed, National Microbiology Laboratory, Public Health Agency, Canadian Center for Human and Animal Health, Winnipeg, MB, Canada. Non-pathogenic strains were used in this study since previous work demonstrated that they can survive the dry fermented sausage manufacturing process (Graumann and Holley, 2008; Chacon et al., 2006) just as well as the toxigenic strains that have caused food-borne illness (CDC, 1995; Williams et al., 2000; MacDonald et al., 2004). Therefore, they would be expected to behave in dry ham manufacture the same as the toxigenic strains, though toxigenic *E. coli* have not yet caused problems in ham (Graumann and Holley, 2009).

Strains were revived from frozen stock and streaked on tryptic soy agar (TSA; Oxoid Ltd, Basingstoke, UK) twice and incubated at 37 °C for 24 h. All strains were grown separately by transferring one colony of each strain to a tube of 10 ml tryptic soy broth (TSB; Oxoid Ltd.) and grown overnight at 37 °C. From each of the 5 tubes, 100 μl was transferred into 1 L TSB separately (5 L total) and grown overnight at 37 °C. The optical density (OD) (600_{nm}) of each strain was measured in a spectrophotometer (Ultrospec, 2000, Pharmacia Biotech, Cambridge, UK) to check bacterial growth. When culture OD_{600nm} reached an absorbance (A) of 1.3–1.5, it was centrifuged at 1643×*g* at 4 °C for 10 min (Sorvall Instruments RC-5C, DuPont, Newton, CT, USA), washed with 5 L (1 L per strain) 0.1% (w/v) peptone water (Fisher Scientific, Fair Lawn, NJ, USA), and recentrifuged as before. Finally, cultures were resuspended in peptone water and combined in a large pail to generate 5 L of a 5 strain *E. coli* O157:H7 mixture sufficient to yield a level of 7–7.5 log cfu g^{-1} on the ham surfaces. This inoculation level was chosen to accommodate a >5 log cfu g^{-1} decrease of the pathogen during the experiments.

2.2. Preparation of S. carnosus UM123M

S. carnosus UM123M (isolated from a commercial meat starter culture mixture Lactacel 115, Microlife Technics, Sarasota, FL, USA) was revived from a frozen stock culture and transferred to TSA (Oxoid Ltd.) twice and incubated at 37 °C for 24 h. One representative colony was transferred to 10 ml TSB (Oxoid Ltd.) and grown overnight at 37 °C, from which 300 μl was then transferred into 3.5 L TSB (Oxoid Ltd.). On the day of inoculation, OD (600_{nm}) was measured and when an OD_{600nm} of $A = 1.3–1.5$ was reached it was centrifuged at 1643×*g* and 4 °C for 10 min, washed with 0.1% peptone water and recentrifuged as before. The culture was then resuspended with 0.1% peptone water to a volume of 3.5 L, which was inoculated onto the hams at day 45 of trial 2. The delayed inoculation avoided exposure of *S. carnosus* to high initial NaCl concentrations resulting from surface rubbing of salt and cure. Culture addition took place after salt had equilibrated in the tissue