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Use of mustard flour to inactivate *Escherichia coli* O157:H7 in ground beef under nitrogen flushed packaging

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Abstract

This study was undertaken to determine whether the glucosinolates naturally present in non-deheated mustard flour could serve as a source of allyl and other isothiocyanates in sufficient quantity to kill *Escherichia coli* O157:H7 inoculated in ground beef at three different levels, during refrigerated storage of the meat under nitrogen. Mustard flour was mixed at 5%, 10% or 20% (w/w) with freshly ground beef, then the beef was inoculated with a cocktail of five strains of *E. coli* O157:H7 at either 3, 6 or ≤1.6 $\log_{10}$ cfu/g. The ground beef was formed into 100 g patties and each was placed in a bag of Nylon/EVOH/PE, which was back-flushed with 100% N_2, heat-sealed and stored at 4 °C for ≤21 days. During storage, the allyl isothiocyanate (AIT) levels in package headspaces were determined by gas liquid chromatography. By 21 days, the levels present in treatments were not significantly different. After 21 days storage, there were 0.5, 3 and 5.4 $\log_{10}$ decreases in numbers of *E. coli* O157:H7 from the initial levels of 6 $\log_{10}$ cfu/g in meat containing 5%, 10% and 20% mustard flour, respectively. When inoculated at 3 $\log_{10}$ cfu/g, *E. coli* O157:H7 was reduced to undetectable levels after 18, 12 and 3 days with 5%, 10% and 20% mustard flour, respectively. When immunomagnetic separation (IMS) was used for *E. coli* recovery following its inoculation at ≤1.6 $\log_{10}$ cfu/g, 5% mustard did not completely eliminate the pathogen from ground beef stored for 6 days. The natural microflora of the ground beef which developed in vacuum packages was unaffected by the addition of 5% mustard flour but some inhibition was found at higher concentrations. Sensory evaluation of the cooked ground beef showed that there were no significant differences in the acceptability of meat treated with 5 or 10% mustard flour. However, panelists could distinguish untreated controls from mustard treatments, but considered the mustard-treated meat to be acceptable. These results showed that it is possible to use mustard flour at levels of >5–10% to eliminate *E. coli* O157:H7 from fresh ground beef.

Keywords: *Escherichia coli* O157:H7; Allyl isothiocyanate; Mustard flour; Ground beef; Natural antimicrobial; Modified atmosphere packaging

1. Introduction

Escherichia coli O157:H7 causes life threatening hemorrhagic colitis, hemolytic uremic syndrome and thrombotic thrombocytopenic purpura in the young,

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old and immuno-compromised (Acheson et al., 1996; Rowe, 1995). *E. coli* O157:H7 has a low infectious dose in food (10 cfu/g) and 2000 people are estimated to become seriously ill each year in the US from this pathogen and require hospitalization; among these 50 deaths occur (Rasmussen and Casey, 2001). *E. coli* O157:H7 outbreaks in a number of countries have been linked to undercooked or raw hamburgers eaten during the summer (Waters et al., 1994). In the US, ground beef accounts for nearly 50% of all beef consumption, and *E. coli* O157:H7 has the potential to continue to cause significant morbidity, mortality and economic loss to the meat industry.

Different options are being investigated for elimination of *E. coli* O157:H7 from ground beef, which might be acceptable to regulatory authorities, the food industry and consumers. One promising approach is the addition of natural antimicrobials of plant origin. Natural extracts and essential oils from plants were historically used to extend shelf life by stabilizing or improving the sensory quality of food. Currently, a substantial amount of work is underway to determine whether natural antimicrobials from plants, animals or benign microorganisms can be used to kill pathogenic bacteria and improve the safety of packaged foods (Han, 2000, 2003). Spices and essential oils containing phenolic compounds such as cinnamic aldehyde (cinnamon), eugenol (cloves) and thymol (thyme) have been reported to have significant antimicrobial activities. However, these essential oil components are only effective against certain microorganism at high concentrations, which often detracts from the sensory quality of food (Davidson, 2001). Studies have demonstrated that allyl isothiocyanate (AIT), a naturally occurring non-phenolic volatile compound found in plants belonging to the *Crucifereae* family, effectively inhibits a variety of pathogenic microorganisms when used at low concentrations (Lin et al., 2000; Ohta et al., 1995; Isshiki et al., 1992; Delaquis et al., 1999). The *Crucifereae* include horseradish, mustard, Brussels sprouts, broccoli, kale and turnip (Clydesdale, 1999; Delaquis and Mazza, 1995). Rhee et al. (2002) reported that three strains of *E. coli* O157:H7 at 6–7 $\log_{10}$ cfu/g were reduced to <0.3 $\log_{10}$ cfu/g after 7 days when 10% mustard was used and treatments stored at 5 °C. Kanemaru and Miyamoto (1990) showed, during testing of AIT and mustard at the same concentrations, that mustard had significantly higher antimicrobial activity against non-O157:H7 strains of *E. coli*. Mayerhauser (2001) found that deli-style mustard alone was able to reduce 6 $\log_{10}$ cfu/g of *E. coli* O157:H7 to undetectable levels within 24 h at 5 and 25 °C. Mustard, therefore, appears to have potential to control *E. coli* O157:H7.

Mustard is the second most commonly consumed spice, after pepper. There are two types of mustard commercially available—white and black, but only black mustard has significant amounts of the essential oil AIT. Two species of black mustard commonly grown are *Brassica nigra* and *Brassica juncea*.

AIT occurs at levels of approximately 0.5–1% (w/w) of the mustard seed (Clark, 1992). It is extracted from the mustard press cake using steam distillation after oil has been removed by cold press methods. In this process, the enzyme myrosinase hydrolyses glucosinolates and releases AIT in the presence of moisture (Farrell, 1985; Verghese et al., 2000). Whole mustard seed has been used in pickles and salads as a spice and preservative (Raghavan et al., 1971). Mustard flour is prepared by removing the hull and then grinding the endosperm to finer particles without water. These products can be used in the meat industry as binders, especially in sausage, but they first must be prepared to eliminate AIT, which is the cause of bitter and pungent flavors. This can be done by adjusting seed moisture to ≥6.8% and heating to 91–105 °C (Holley and Timbers, 1983) to denature myrosinase or by grinding seeds at high moisture (RH), holding to allow dissipation of AIT and then by drying to form a powder. These processes yield deheated mustard.

Kanemaru and Miyamoto (1990) compared the antimicrobial effects of mustard and purified AIT at equal concentrations of AIT. They found that mustard was more effective against *E. coli* than purified AIT. They found that 0.1% mustard with 9.4 ppm of AIT was able to inhibit the growth of *E. coli* in culture medium within 24 h but 12.3 ppm of purified AIT was required to achieve the same level of inhibition. Mayerhauser (2001) found that retail style mustards eliminated 6 $\log_{10}$ of *E. coli* O157:H7 from trypticase soy broth within a few hours at refrigerator or room temperature. More recently, Rhee et al. (2002) showed that mustard flour alone or with acetic acid reduced 6 $\log_{10}$ of *E. coli* O157:H7 to <0.3 $\log_{10}$ in 24 h at room temperature. AIT from mustard provides a natural means of killing *E. coli* O157:H7 in food products

since mustard has been used as a spice for centuries. AIT is most effective in food systems when closed atmospheres are used to control its volatilization. Two approaches taken involve the use of impermeable plastic packaging films or glass containers to prevent AIT loss (Delaquis et al., 1999).

The objective of the present research was to inactivate *E. coli* O157:H7 in packaged ground beef through incorporation of mustard flour (non-deheated) as an ingredient and to evaluate the sensory acceptability of cooked ground beef patties containing the mustard flour.

2. Materials and methods

2.1. Dehulled non-deheated mustard flour

In order to maintain the highly potent antimicrobial properties of mustard, low moisture mustard seed is milled to flour under low humidity after being dehulled and dried. This hot or "non-deheated" flour still contains glucosinolates unreacted with myrosinase, which can form AIT upon contact with moisture. The mustard flour used was product #2232 kindly provided by Newly Weds Foods, UFL Division (Edmonton, AB, Canada).

For bacterial analysis, 10 g mustard flour was diluted in 90 ml 0.1% (w/v) peptone water (Sigma, St. Louis, MO, USA) mixed for 1 min with a stomacher (Model 400; A.J. Seward, London, UK) and surface plated immediately on prepoured BBL trypticase soy agar (TSA; Becton Dickinson, Cockeysville, MD, USA). TSA plates were incubated at 37 °C for 48 h.

2.2. Ground beef preparation

Fresh beef (chuck roast) was purchased from a local butcher shop the day before each experiment. The meat was stored at 4 °C overnight. On the day of the experiment, the meat was kept at −18 °C for 3 h until the outer surface was frozen. Ground beef was prepared using aseptic procedures, sterile utensils and sanitized equipment. The outer 2 mm of meat was trimmed using a stainless steel knife sanitized with 200 ppm aqueous chlorine solution (Canada Safeway, Calgary, AB, Canada). The meat was cut into 5×5-cm pieces and coarse ground through a die plate with 1.2-cm diameter perforations, using an electric meat grinder (Model 84142; Hobart Manufacturing, Tory, OH, USA). After the coarsely ground beef was held at 4 °C for 1 h, it was used for inoculation and treatment with mustard flour.

2.3. Bacterial strain preparation

E. coli O157:H7 strains 7128, 7110, 7220 (human isolates), and 7282 and 7283 (hamburger isolates) were provided by the Laboratory Centre for Disease Control (Ottawa, ON, Canada). All strains were routinely preserved in glycerol and stored at −80 °C. Strains were activated by two consecutive transfers in 5-ml portions of BBL trypticase soy broth (TSB; Becton Dickinson) and incubated at 35 °C for 24 h. Following incubation, 30 ml of each *E. coli* culture was centrifuged at 12,000×*g* for 15 min at 10 °C (Sorvall RC-5 refrigerated centrifuge; Du Pont, Newtown, CT, USA). Cell pellets were washed once in 30-ml 0.1% peptone water and re-centrifuged at 12,000×*g* for 15 min at 10 °C. The cultures were then re-suspended in peptone water and diluted to an absorbance (A_{600}) of 0.3, determined by using a spectrophotometer (Ultrospec 2000; Pharmacia Biotech, Baie d'Urfe, QC, Canada). The bacterial numbers at $0.3A_{600}$ were 8 $\log_{10}$ cfu/ml. A five strain cocktail for meat inoculation was prepared by combining equal amounts (≤20 ml) of each standardized culture.

2.4. Preparation of inoculated patties with mustard flour

To coarsely ground beef weighing approximately 5 kg, 5%, 10% or 20% (w/w) non-deheated mustard flour were added by hand, using sterile gloves. The meat was then ground again through the 1.2-cm diameter die plate using the electric meat grinder. The meat weight was adjusted to 5 kg and the meat was inoculated with ≤100 ml of the five-strain *E. coli* O157:H7 cocktail to obtain final numbers of 3 or 6 $\log_{10}$ cfu/g. The meat was then re-ground using a manual meat grinder equipped with a die plate having 0.8-cm diameter perforations, to achieve as nearly as possible even distribution of *E. coli*. Ground beef weighing 100 g was formed into patties using a template 10-cm diameter and 1-cm deep. Each patty was placed in a bag made of nylon/ethylene vinyl alcohol/polyethylene

(Deli*1, Winpak, Winnipeg, MB, Canada), 75 μm thick with an oxygen transmission rate of 2.3 $cm^3 \cdot m^{-2} \cdot 24\ h^{-1}$ at 23 °C. The water vapor transmission rate was 7.8 $g \cdot m^{-2} \cdot 24\ h^{-1}$ at 37.8 °C and 98% relative humidity. Following vacuum application, bags were backflushed with 100% N_2, heat-sealed with a vacuum packaging machine (Model GM-2000; Bizerba Canada, Mississauga, ON, Canada) and stored at 4 °C for ≤21 days. During storage, the AIT levels in the package headspaces from mustard were determined by gas liquid chromatography.

An additional test was conducted using patties made with 5% mustard that were inoculated with 1.1–1.6 log_{10} cfu/g of *E. coli* O157:H7. Ground beef patties were prepared from 2.5 kg of freshly ground beef, 5% mustard flour was added and the meat was inoculated with the five-strain cocktail of *E. coli* O157:H7 to achieve the target number of organisms. The meat was re-ground, formed into patties and packaged as previously described. Samples were stored at 4 °C for 6 days. At days 0 and 6, three samples were analyzed in duplicate for *E. coli* O157:H7. For analysis, 25 g of ground beef was added to 225 ml of buffered peptone water (Becton Dickinson) in a stomacher bag (Fisher Scientific, Nepean, ON, Canada) and mixed for 1 min with a stomacher. The bags were then incubated at 35 °C for 6 h. After incubation the bags were mixed in the stomacher again for 1 min. One milliliter of liquid was transferred into an immunomagnetic separation (IMS) device (Dynal, Oslo, Norway), containing magnetic beads coated with antibody against *E. coli* O157:H7, and used according to the manufacturer's instructions. Concentrated samples were plated on Sorbitol MacConkey agar (Difco Division, Becton Dickinson, Sparks, MD, USA) supplemented with 50 μg/l cefixime and 2.5 mg/l potassium tellurite (Dynal, Lake Success, NY, USA) to obtain the medium cefixime tellurite sorbitol MacConkey (CT-SMAC) (Zadik et al., 1993). The plates were incubated at 35 °C for 24 h.

2.5. GLC analysis of AIT in package headspace

A gas chromatograph (Varian Star 3400 cx; Varian Chromatography Systems, Walnut Creek, CA, USA) equipped with a flame ionization detector and a BD5MS column measuring 30-m×0.25-mm internal diameter and 0.25-μm wall thickness (J&W Scientific, Folsom, CA, USA) was used to determine the amount of AIT released from mustard into the headspace. A sample of 500 μl was withdrawn from the headspace by piercing the bag with an airtight syringe (Precision Sampling, Baton Rouge, LA, USA) and was injected into the gas chromatograph. Each bag was sampled only once. The GLC was programmed to increase the initial column temperature of 60 °C after sample injection at a rate of 12.5 °C/min, to 90 °C, which was then maintained for 45 s. The injector and the flame ionization detector were programmed to operate at 250 °C. Hydrogen was used to fuel the flame at the detector. Helium was used as the carrier gas. Total running time was 4 min. Resulting peaks including that of AIT (from horseradish, 95% pure; Aldrich, Milwaukee, WI, USA) were identified using Varian star chromatography software (Varian Chromatography Systems).

To determine the retention of AIT within the Deli *1 bags, 1 ml of AIT was vortex-mixed with 5 ml corn oil, poured into a glass Petri dish, placed in a Deli *1 bag and heat sealed. Thirty bags were prepared and stored at 4 °C for ≤41 days. Three bags were removed at regular intervals (initially and every 2 days for the first week and weekly thereafter). At sampling, 500 μl of headspace was withdrawn from each bag with an airtight syringe and was injected into the GLC.

2.6. Microbial analysis of patties

Eleven grams of meat from each patty was weighed in a sterile stomacher bag, mixed with 99 ml of peptone water (0.1%, w/v) and treated in the stomacher for 30 s. Each sample was serially diluted with peptone water and total bacterial numbers were determined by spiral plating (Autoplate 4000, Spiral Biotech, Norwood, MA, USA) using BBL trypticase soy agar (TSA; Becton Dickinson). Viable *E. coli* O157:H7 were also determined by spiral plating using CT-SMAC agar, while lactic acid bacteria were determined using de Man Rogosa Sharpe (MRS) agar (Difco; Becton Dickinson). TSA plates were incubated at 22 °C for 48 h, while CT-SMAC agar plates were incubated at 35 °C for 48 h. The MRS agar was incubated anaerobically using Gas Pak jars with the Gas Pak Plus anaerobic system (Becton Dickinson) and palladium catalyst at 25 °C for 48 h. At desired times, three samples from three different experiments were each plated in duplicate.

2.7. Sensory evaluation

The fresh beef (chuck roast) used was previously described. The meat was trimmed to remove visible fat and coarse ground using an electric meat grinder. For the non-mustard control, 20 g salt was added to 1 kg of ground beef. For the 5% mustard treatment, 50 g non-deheated mustard flour plus 20 g salt, 5 g sugar and 60 ml vinegar were mixed with 1 kg of ground beef. For the 10% mustard treatment, 100 g mustard flour plus 20 g salt, 10 g sugar and 180 ml vinegar were mixed with 1 kg of ground beef. Ingredients were evenly mixed by hand. Hamburger patties made from these mixtures weighed 100 g. The samples were placed in a convection oven preheated to 230 °C and baked until the internal temperature reached 71 °C. After baking, the hamburger patties were held at 60 °C until they were served to the panelists.

Seventy-five students and staff at the University of Manitoba who had received no formal training in sensory evaluation participated. Panelists were 18–60 years old and 64% were female. Of panelists, 88% included hamburger in their diets weekly or monthly, with the remainder consuming hamburger less frequently. A 9-point hedonic scale was used to evaluate overall acceptability where 1=dislike extremely and 9=like extremely. Each panelist received three 10-g samples of cooked hamburger in a container coded with a randomly chosen three digit number, plus water and an unsalted cracker. The panelists tasted samples and recorded the overall acceptability of each product.

2.8. Statistical analysis

All chemical and microbial analysis were performed with duplicate measurements of three samples. Data were analyzed using the Statistical Analysis System software program, version 8.1 (SAS Institute, Cary, NC). Microbiological data were analyzed by the general linear models (GLM) procedure and Duncan's multiple range tests with examination for significant differences ($p<0.05$) at each storage interval for individual treatments. Sensory data were analyzed by ANOVA and *t*-tests for separation of mean differences.

3. Results

3.1. Headspace AIT analysis

Fig. 1 shows AIT levels from non-deheated mustard flour released into the headspaces of packaged ground beef patties. Data indicated that in all three treatments AIT headspace concentrations were similar after the first day, and after 9–12 days reached an equilibrium of 17–30 μg/ml, which was maintained up to 21 days. Headspace AIT concentrations from day 15 onward were not significantly different. The

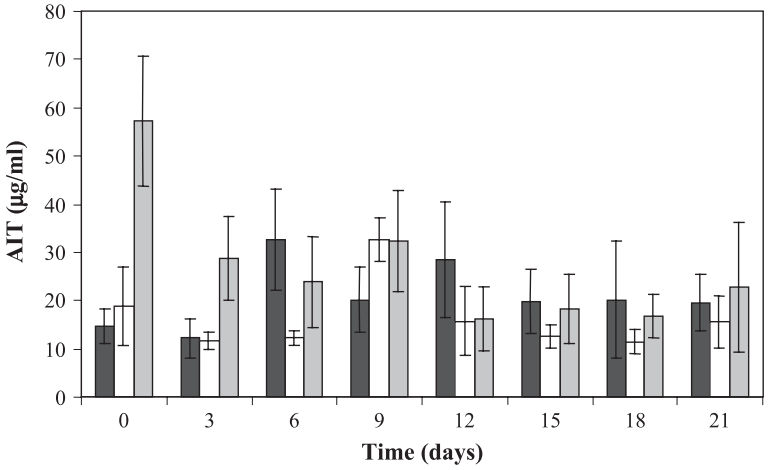

Fig. 1. AIT concentrations in the headspaces of packaged ground beef patties formulated to contain 5% (■), 10% (□), 20% (▨) non-deheated mustard flour and stored at 4 °C for ≤21 days. Vertical bars represent ±1 standard deviation.

packaging film used was only very slightly permeable to AIT. The initial AIT concentration in sealed bags containing only AIT and corn oil was 380 μg/ml and this concentration was maintained for 16 days at 4 °C. At day 23, this was reduced to 350 μg/ml and by day 41 the AIT level was 280 μg/ml.

3.2. Antimicrobial effects of mustard flour in uninoculated meat

Mustard flour contained 2.3±0.3 log cfu bacteria/g when received from the manufacturer. Fig. 2 shows the antimicrobial effects of mustard flour on the natural microflora in ground beef patties stored at 4 °C. The numbers of bacteria making up the natural microflora in ground beef patties formulated with 5% and 20% mustard flour were significantly lower ($p<0.05$) than in the 0% mustard treatment on days 0–3, while in the 10% mustard treatment numbers present on days 3–21 were significantly lower. Fig. 3 shows the antimicrobial effects of mustard flour at 10% (w/w) on the natural anaerobic microflora recovered as presumptive lactic acid bacteria on MRS agar from ground beef patties stored at 4 °C.

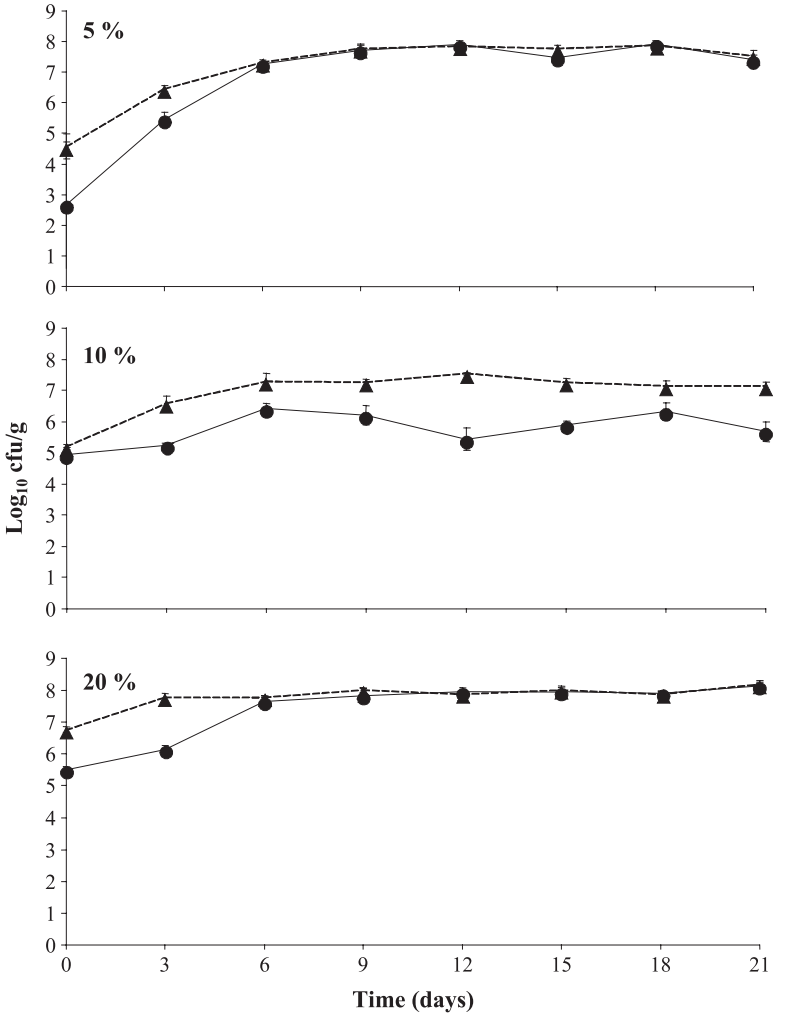

Fig. 2. Changes in numbers of aerobic bacteria recovered on TSA from uninoculated ground beef containing 5%, 10% or 20% (w/w) non-deheated mustard flour, packaged in nitrogen and stored at 4 °C for ≤21 days. Total bacterial numbers without mustard (▲ - - -), with mustard (● ——). Six replicates were used to generate the standard deviation bars.

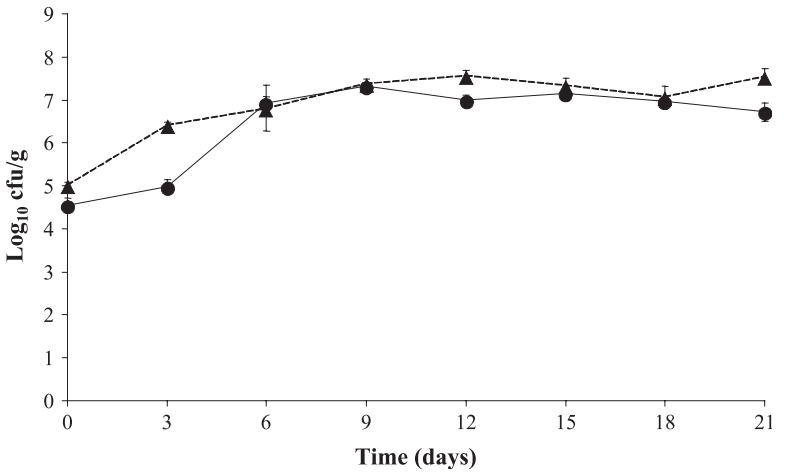

Fig. 3. Changes in numbers of presumptive lactic acid bacteria recovered on MRS agar from nitrogen packed ground beef patties formulated with 10% (w/w) non-deheated mustard flour stored at 4 °C for ≤ 21 days. Lactic acid bacteria from meat without mustard (▲ - - -), with 10% mustard (● ——). Six replicates were used to generate the standard deviation bars.

The natural anaerobic microflora in 10% mustard-treated meat was significantly lower on days 3, 12 and 21.

3.3. Antimicrobial effects of mustard flour in E. coli O157:H7-inoculated meat

The antimicrobial effects of mustard flour on the natural microflora and on *E. coli* O157: H7 when the latter was inoculated at 3 log cfu/g in ground beef patties are shown in Fig. 4. At these *E. coli* numbers, mustard flour was able to reduce *E. coli* O157:H7 to undetectable levels within 3–18 days, and showed some inhibitory effect at 10% and 20% on the total numbers of bacteria naturally present in the meat.

Fig. 5 shows the antimicrobial effects of mustard flour on the natural microflora and *E. coli* O157:H7 when the latter was inoculated at 6 $\log_{10}$ cfu/g in ground beef patties. At this inoculation level, the effect of 5% mustard was smaller on both the total bacterial population and *E. coli* O157:H7 than when *E. coli* was inoculated at 3 $\log_{10}$ cfu/g. However, ground beef patties with 10% mustard showed >2 $\log_{10}$ reduction in *E. coli* O157:H7 after 21 days. Increasing the mustard concentration to 20% led to a decrease of 5.6 $\log_{10}$ cfu/g of *E. coli* O157:H7 after 21 days. Total bacterial numbers were lower from days 0 to 15 in mustard-treated samples than in treatments without mustard.

When ground beef containing 5% mustard was inoculated with *E. coli* 0157:H7 at 1.1–1.6 $\log_{10}$ cfu/g, viable organisms were found on CT-SMAC when meat stored for 6 days at 4 °C was analyzed by IMS following resuscitation.

3.4. Sensory analysis of cooked ground beef patties containing 5% and 10% mustard

Results from the sensory analysis of cooked ground beef containing mustard flour indicated that panelists could not tell whether patties contained 5% or 10% mustard ($p>0.05$), but were able to distinguish the untreated control from both 5% and 10% mustard-containing meat ($p<0.05$). Overall, the panelists did not identify the 5% and 10% mustard-containing meat as being objectionable since the mean acceptability score for each was 5.9 and 5.8, respectively, (neither like nor dislike), while the untreated control was scored at 6.9.

4. Discussion

AIT has been reported in other work to have antimicrobial activity against pathogenic bacteria including *E. coli* O157:H7 (Chinen et al., 2001; Delaquis et al., 1999; Lin et al., 2000; Mayerhauser, 2001; Muthukumarasamy et al., 2003; Rhee et al.,

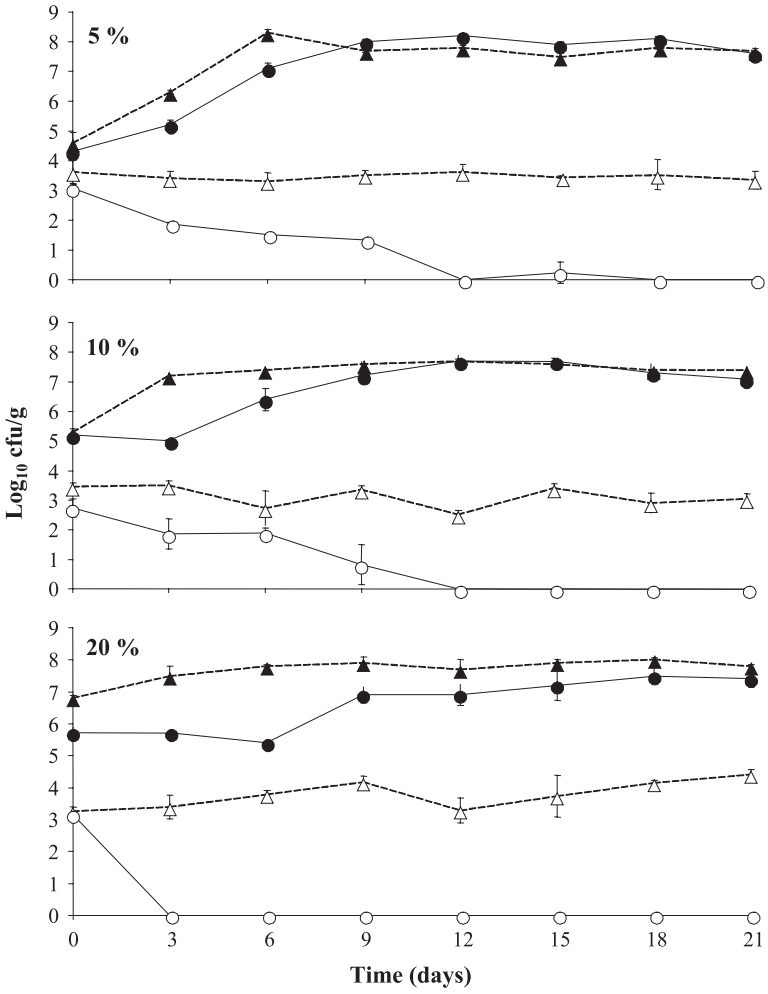

Fig. 4. Changes in numbers of aerobic bacteria recovered on TSA and *E. coli* O157:H7 on CT-SMAC agar from nitrogen packed ground beef patties formulated with 5%, 10% or 20% (w/w) non-deheated mustard flour, inoculated with 3 $\log_{10}$ cfu/g of a five strain cocktail of *E. coli* O157:H7 and stored at 4 °C for ≤ 21 days. Total bacterial numbers without mustard (▲ - - -), with mustard (● ——). *E. coli* O157:H7 numbers from meat without mustard (△ - - -), with mustard (○ ——). Six replicates were used to generate the standard deviation bars.

2002; Ward et al., 1998), other bacteria (Isshiki et al., 1992; Kanemaru and Miyamoto, 1990; Kojima and Ogawa, 1971; Ono et al., 1998) and fungi (Nielsen and Rios, 2000). Our results here with mustard against *E. coli* O157:H7 in ground beef are consistent with reports of the effectiveness of mustard against *E. coli* (Kanemaru and Miyamoto, 1990; Mayerhauser, 2001).

In the 5% and 20% mustard flour treatments, the natural microflora had decreased on day 0 but gradually increased to the control levels by day 6. Gram-negative bacteria were probably reduced in numbers initially as AIT was released from the mustard flour, but this group was not specifically monitored. During further storage, the Gram-positive lactic acid bacteria, which are resistant to AIT, would probably dominate the natural microflora at 4 °C (Ward et al., 1998). Differences in the initial total numbers of bacteria in mustard-treated meats was not due to the mustard flour since it contained about 100 bacteria/g.

To understand the effects of hydrated mustard on bacteria other than *E. coli* O157:H7 in the meat, bacterial growth during storage was considered with

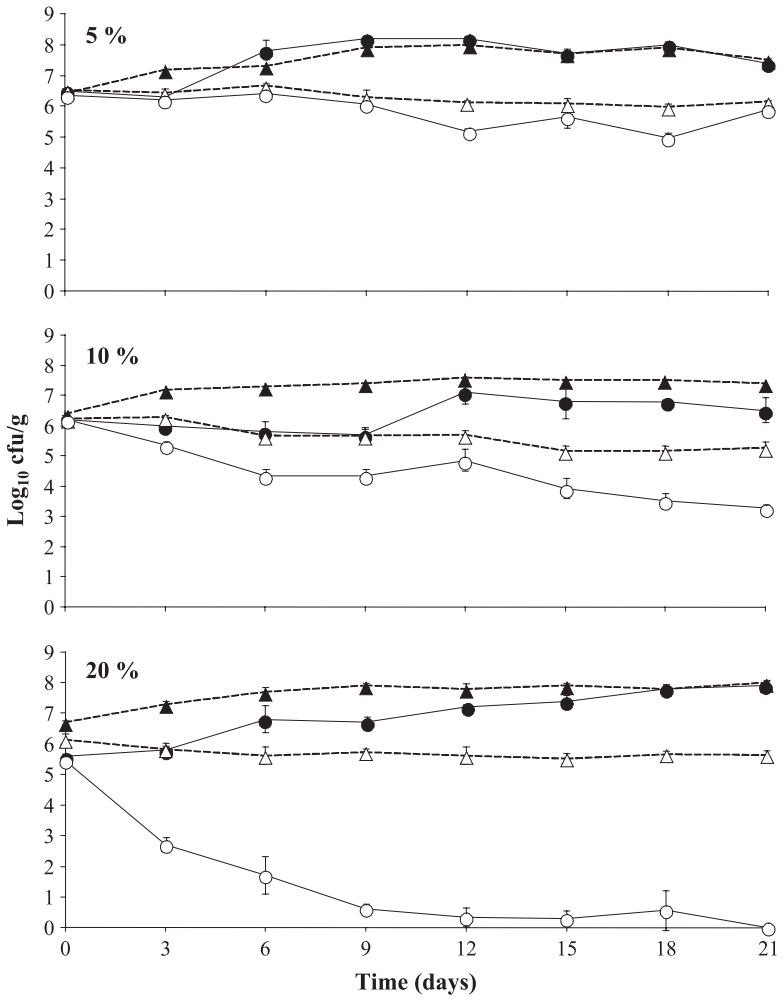

Fig. 5. Changes in numbers of aerobic bacteria recovered on TSA and *E. coli* O157:H7 on CT-SMAC agar from nitrogen packed ground beef patties formulated with 5%, 10% or 20% (w/w) non-deheated mustard flour, inoculated with 6 $\log_{10}$ cfu/g of a five strain cocktail of *E. coli* O157:H7 and stored at 4 °C for ≤ 21 days. Total bacterial numbers without mustard (▲ - - -), with mustard (● ——). *E. coli* O157:H7 numbers from meat without mustard (△ - - -), with mustard (○ ——). Six replicates were used to generate the standard deviation bars.

zero time taken as a baseline. With 5% mustard flour, the total numbers of bacteria increased by 5.3 $\log_{10}$ cfu/g during storage. At 10% and 20% mustard, the increases were only 1.1 and 1.2 $\log_{10}$ cfu/g. Thus, there was little inhibition of growth by 5% mustard flour, but both 10% and 20% mustard gave substantial inhibition when the results are considered in this manner. It must also be noted that, because of high initial numbers in the untreated control samples for the 10% and 20% mustard tests, growth was only about 2 $\log_{10}$ cfu/g during storage. Thus, the reduced growth in mustard treatments may be an artifact caused by high initial bacterial numbers. Nonetheless, when 10% mustard was used, the number of bacteria recovered on TSA agar was lower than in either the 5% or 20% mustard treatments. This result may simply reflect differences in the types of organisms present, which may have had different sensitivities to AIT, in meat purchased on different days. It is important when secondary antimicrobials like mustard flour are used for pathogen reduction that the profile of adventitious microorganisms present not become unbalanced, as this may provide opportunity for the growth of

previously unimportant pathogens. The initial inhibitory effect of 10% mustard flour on presumptive lactic acid bacteria was transient and by 6 days this group of organisms had probably adapted to its presence.

A greater reduction in viable *E. coli* O157:H7 when mustard flour was used instead of pure AIT has been reported (Nadarajah et al., 2003). It was also reported that the package headspace concentration of AIT was lower and was less well correlated with antimicrobial effectiveness when mustard flour was used as a meat ingredient than when pure AIT was added to filter paper and packaged with meat patties (Nadarajah et al., 2003; Muthukumarasamy et al., 2003). More direct contact between AIT produced by glucosinolate hydrolysis in the hydrated flour when mixed with meat is presumed to have improved antimicrobial effectiveness.

Another possible reason for mustard flour having greater antimicrobial activity than the concentrated liquid AIT is that, in mustard flour, there are other components present in small amounts, such as 3-methylthiopropyl isothiocyanate, 3-butenyl isothiocyanate, butyl isothiocyanate, phenyl isothiocyanate, 2-phenylethyl isothiocyanate, hexyl isothiocyanate, 4-phenethyl isothiocyanate and benzyl isothiocyanate, which may play a synergistic role against *E. coli* O157:H7 (Delaquis and Mazza, 1995).

When *E. coli* O157:H7 was inoculated at the low level of ≤40 cfu/g (1.6 $\log_{10}$ cfu/g), which is roughly representative of the commercial situation (Uhitil et al., 2001) 5% mustard did not completely eliminate the organism within 6 days at 4 °C. This was not surprising. At 5% mustard flour, *E. coli* O157:H7 was reduced by 1.5 $\log_{10}$ cfu/g within 6 days from an initial inoculum of 3 $\log_{10}$ cfu/g. This means that the probability of its survival under this condition is 1/anti-log of 1.5 or 1/34. This also means that in order to have survivors up to day 6, there should be >34 cells/g present initially. Thus, the results for meat inoculated with 3 $\log_{10}$ cfu/g agree with the result from IMS recovery of *E. coli* O157:H7 following its inoculation at ≤40 cells/g. Therefore, a higher level of mustard may be necessary to provide assurance that viable *E. coli* O157:H7 are absent from ground beef within 6 days.

Although AIT from plant sources has been demonstrated to have strong antimicrobial activity against other pathogenic bacteria, it has rarely been used in food systems as an antimicrobial agent since the odor is not acceptable (Delaquis et al., 1999; Muthukumarasamy et al., 2003). AIT at higher concentrations (>500 μg/ml) has been shown to stimulate eye watering, and cause a burning sensation on the tongue as well as nasal cavity irritation. The sensory evaluation of cooked ground beef showed that there were no significant differences between the overall sensory acceptability of ground beef formulated with 5% or 10% mustard. In the tests reported here, sugar, salt and vinegar were used to modulate the sensory effects of mustard addition according to normal culinary practice. The 20% mustard treatment was not included in sensory tests. It is likely that formulation of hamburgers with between 5% and 10% mustard flour would eliminate *E. coli* O157:H7 from ground beef contaminated at levels of about 10 cfu/g likely to be found in commerce (Uhitil et al., 2001). The lowest concentration of mustard necessary to dependably achieve this goal needs to be determined.

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