LETHAL EFFECTS OF NON-DEHEATED (HOT) MUSTARD FLOUR ON *E. COLI* O157:H7 IN REFRIGERATED NITROGEN PACKAGED GROUND BEEF

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Background

The incidence of *E. coli* O157:H7 which was first reported to cause hemorrhagic colitis and hemolytic uremic syndrome two decades ago (Riley et al. 1983) has been increasing year after year. Consumption of undercooked ground beef has been a common cause of *E. coli* O157:H7 outbreaks (hamburger disease) in North America. United States and Canada have the highest frequencies of foodborne illness caused by *E. coli* O157:H7 in the world. *E. coli* O157:H7 is a "zero-tolerated" pathogen in beef in the U.S., and as a result thousands of tons of ground beef are recalled every year for possible *E. coli* O157:H7 contamination. This has led to considerable financial loss for the meat industry. Canada is the single largest mustard exporter in the world and is one of the world's top five mustard producers. Mustard has been used as a spice or a condiment for centuries throughout the world. Mustard flours when deheated are used as emulsifiers, binders and bulking agents, but non-deheated mustard is used as a spice in seasonings and for flavouring in meat formulations, particularly in sausage and salami manufacture (Cui, 1997). Brown or oriental mustard (*Brassica juncea*) and yellow or white mustard (*Sinapis alba*) contain glucosinolates, which on enzymatic breakdown by myrosinase yield isothiocyanates, cyanides and thiocyanates (Tsao et al. 2002). The heat or flavour of the mustard is due to the presence of isothiocyanates and when used as binders the flour is treated to inactivate the myrosinase. Allyl isothiocyanate, one of the predominant breakdown products of glucosinolates, has been reported to have antimicrobial effects against bacteria including *E. coli* O157:H7 in ground beef, pre-cooked roast beef, alfalfa seeds, lettuce, and 'Asazuke' (a low salt vegetable) (Muthukumarasamy et al. 2003; Ward et al. 1998; Park et al. 2000; Lin et al. 2000; Ogawa et al. 2000). Although pure AIT from natural sources can be used as a food preservative in Japan, its use in food is restricted as a flavouring agent in North America. Moreover, use of pure AIT in food has limitations because of the strong pungent smell associated with volatile AIT which causes eye and nasal irritation and a burning sensation of the skin and tongue. Mustard flour, being a natural source of AIT, may be used as an alternative approach in food systems to eliminate *E. coli* O157:H7.

Objectives

The objective of the research was to study the lethality of mustard flour toward *E. coli* O157:H7 in refrigerated nitrogen packed ground beef. The effect of deheated mustard, which is incapable of producing isothiocyanates due to enzyme inactivation, was compared with non-deheated mustard to understand if isothiocyanates from the flour were responsible for lethal effects on *E. coli* O157:H7. The effect of pure allyl isothiocyanate, on *E. coli* O157:H7 was also studied.

Materials and methods

Five strains of *E. coli* O157:H7, 7128, 7110, 7236 (human isolates) and 7282 and 7283 (hamburger isolates) were kindly donated by Dr. R. Khakria, Laboratory Center for Disease Control, Ottawa, Canada. *E. coli* O157:H7 strains were maintained in tryptic soy broth at 37º C. Inside round beef roasts were obtained from a local supermarket and aseptically ground manually in a meat grinder (9.5 mm plate). The five strain cocktail of *E. coli* O157:H7 prepared in peptone water was inoculated in the meat to reach a level of 3 log CFU/g. Deheated and non-deheated mustard flour at 10 and 20% were added to the ground beef and re-ground to evenly mix the mustard flour. Twenty five grams of meat were placed in Deli*1 bags (Winpak, Winnipeg, Canada), a vacuum was generated, back flushed with nitrogen and the bags were sealed. Samples to study the effect of AIT were prepared by adding 1 ml of pure allyl isothiocyanate (Acros Organics, Geel, Belgium) and a commercial corn oil mixture (AIT: corn oil at a ratio of 7:3) to sterile filter paper inserts and one insert was placed in each bag containing 25g of *E. coli* O157:H7 - inoculated beef before packaging. The packaged ground beef was stored at 4º C. Triplicate samples for each treatment were analysed for *E. coli* O157:H7 and

total bacterial numbers from 0 to 18d at 3d intervals. Eleven grams of beef were mixed with 99ml of peptone water and stomached for 1 min. Serial dilutions were prepared in peptone water and plated with a spiral plater. Sorbitol MacConkey agar supplemented with cefixime-tellurite (CT-SMAC) and tryptic soy agar (TSA) were used for enumerating *E. coli* O157:H7 and total bacteria, respectively. The plates were incubated at 37º C aerobically for 24 and 48h for CT-SMAC and TSA, respectively. In order to determine if the *E. coli* O157:H7 cells were killed or injured as a result of mustard flour or AIT treatment, a resuscitation step on TSA (incubated at 37º C for 3h) was used followed by over-laying with CT-SMAC agar. Survivors on the TSA over-laid CT-SMAC plates indicated recovery of injured *E. coli* O157:H7 cells. All data were analysed by Statistical Analysis System (version 8.1) software. Analysis of variance by the General Linear models procedure and Duncan's multiple range tests were used to find significant differences ($P< 0.05$) among and between treatments.

Results and discussion

Bactericidal effects on *E. coli* O157:H7

The effect of AIT, deheated and non-deheated mustard flour on the viability of *E. coli* O157:H7 in ground beef stored under nitrogen at 4º C are shown in Fig 1. Non-deheated mustard at 20% was most effective in killing *E. coli* O157:H7 compared to deheated mustard at both 10 and 20% or AIT at ca. 1300 ppm. Non-deheated mustard at 20% completely eliminated *E. coli* O157:H7 at an initial level of 3 log CFU/g from ground beef by day 3, whereas AIT at ca. 1300 ppm and non-deheated mustard at 10% required 15 and 18 d respectively to reduce *E. coli* O157:H7 below detectable levels (40 CFU/g). Deheated mustard at both levels did not completely kill *E. coli* O157:H7 in ground beef stored under nitrogen at 4º C even after 18 days. On TSA over-laid with CT-SMAC plates, no recovery of injured cells occurred, indicating that *E. coli* O157:H7 were killed in treatments where no *E. coli* O157:H7 grew on CT-SMAC plates.

The results presented clearly show that non-deheated mustard flour at 20% had strong bactericidal effects against *E. coli* O157:H7 in ground beef stored under nitrogen at 4º C. Non-deheated mustard flour at 10% and AIT were able to completely eliminate *E. coli* O157:H7 but required significantly longer than 20% non-deheated mustard flour. The only difference between the two types of mustard flour used was the presence of active myrosinase in the non-deheated product. Lethal effects of the latter flour were attributed to myrosinase action, causing formation of isothiocyanates in the presence of moisture from the meat. Deheated mustard, with inactive myrosinase, was not significantly lethal to *E. coli* O157:H7. Allyl isothiocyanate is the main component of mustard that might be responsible for bactericidal effects (Isshiki et al. 1992). The average allyl isothiocyanate levels in mustard ranges from 7 to 13 mg/g. The results showed that non-deheated mustard flour at 20% was more effective than AIT (ca. 1300 ppm) in killing *E. coli* O157:H7. The increased lethality of mustard flour may be due to synergistic effects of AIT with other isothiocyanates present in mustard. The bactericidal activity of mustard flour may provide an opportunity for its use in meat and more widely in the food industry as a secondary preservative to control pathogenic microorganisms. This should provide added assurance of safer food for consumers.

Effect on total bacterial numbers

The effect of AIT, deheated and non-deheated mustard flour on the total bacterial numbers in ground beef stored under nitrogen at 4º C is shown in Fig 2. All treatments significantly delayed the growth of total aerobic mesophiles in ground beef stored at 4º C. In the untreated control the total bacterial count was 6.88 log CFU/g by 18d, indicating that the beef could be close to spoilage if untreated with any antimicrobials. Non-deheated mustard flour at 20% and AIT were able to maintain the total bacterial numbers at < 4 log CFU/g by the end of 18d.

Conclusions

Non-deheated mustard flour at 20% was more lethal than deheated flour to *E. coli* O157:H7 in ground beef. Non-deheated mustard flour may be used as a natural antimicrobial in ground beef to eliminate *E. coli* O157:H7 and has potential for similar use in other susceptible foods where mustard flavour is compatible. The use of mustard flour may not only increase the safety of ground beef but also extend its shelf life.

References

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Fig 1: Viability of *E. coli* O157:H7 in ground beef treated with AIT, deheated and non-deheated mustard flour, stored at 4º C under nitrogen packaging.

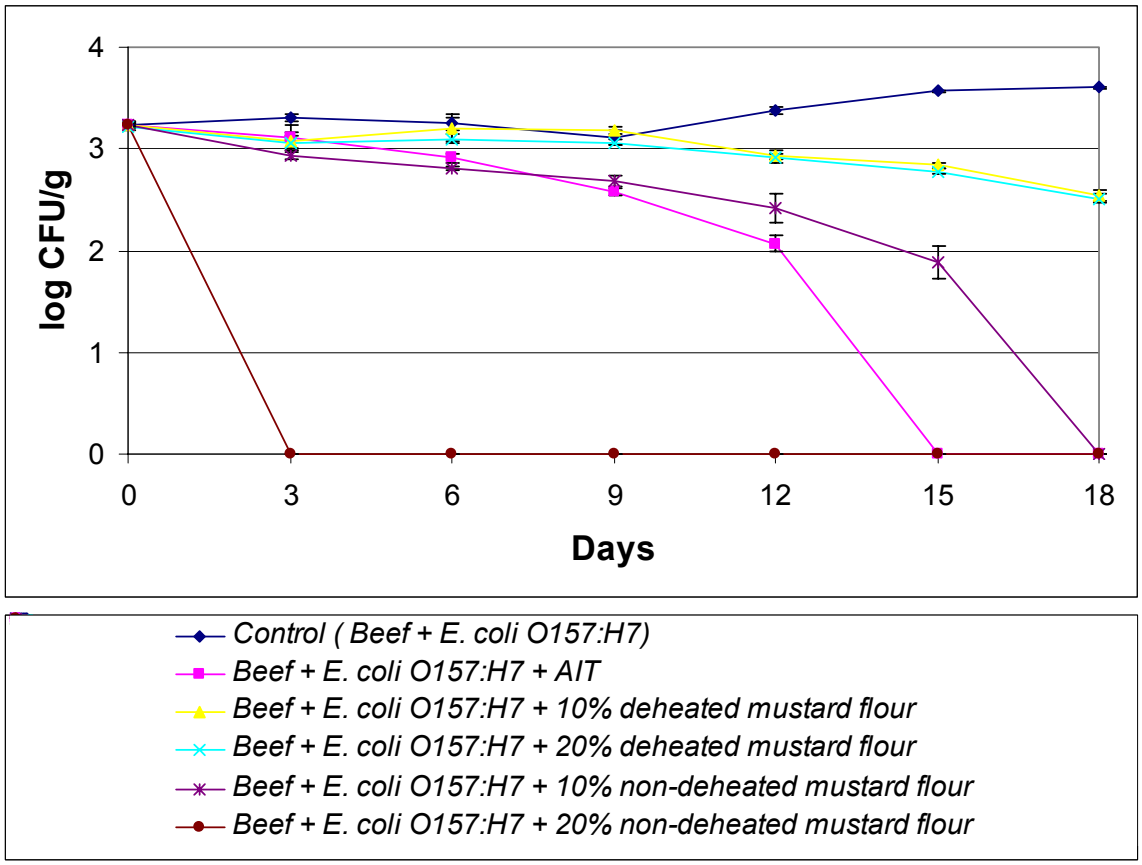

Fig 2: Changes in total number of viable bacteria in ground beef stored at 4º C under nitogen packaging after treatment with AIT, deheated and non-deheated mustard flour.