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# Inhibitory effects of microencapsulated allyl isothiocyanate (AIT) against *Escherichia coli* O157:H7 in refrigerated, nitrogen packed, finely chopped beef

Pedro A. Chacon, Roberto A. Buffo, Richard A. Holley *

*Department of Food Science, University of Manitoba, Winnipeg, Manitoba, Canada, R3T 2N2*

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**Abstract**

Allyl isothiocyanate (AIT) is an effective inhibitor of various pathogens, but its use in the food industry is limited by its volatility and pungency. The objective of this study was to overcome the volatility of AIT by microencapsulation and evaluate its antimicrobial effectiveness against *Escherichia coli* O157:H7 in chopped beef. Chopped beef was aseptically prepared and inoculated with a five-strain cocktail of *E. coli* O157:H7 to yield 4 or 8 $\log_{10}$ cfu/g. AIT was microencapsulated in gum acacia to yield 3.7–54.8 mg AIT/g at a ratio of 1:4 and freeze dried. Microcapsules at 5% or 10% (w/w) were then added to experimental samples that were packed under nitrogen, and stored at 4 °C for 18 days. Samples were analyzed for numbers of *E. coli* O157:H7 and the aerobic mesophilic bacteria (TAC) at 3-day intervals. AIT at 4980 ppm eliminated both low and high levels of inoculated *E. coli* O157:H7 after 15 and 18 days of storage, respectively. AIT at 2828 ppm reduced *E. coli* by 2.7 $\log_{10}$ cfu/g by 18 days of storage. AIT levels <1000 ppm were not more effective in reducing *E. coli* survival than the control treatment without AIT addition. AIT at 170–1480 ppm had negligible effects on the TAC, and while 4980 ppm kept TAC levels $\leq$3 $\log_{10}$ cfu/g during 18 days of storage, the TAC reached 7.25 $\log_{10}$ cfu/g in the control. It was found that AIT microencapsulated in gum acacia could be used in chopped refrigerated beef to reduce or eliminate large numbers of *E. coli* O157:H7.


*Keywords:* Allyl isothiocyanate; Microencapsulation; Chopped beef; *E. coli* O157:H7; Food safety

## 1. Introduction

*Escherichia coli* O157:H7 is an important pathogen responsible for causing serious disease in humans including hemolytic uremic syndrome and hemorrhagic colitis. Consumption of undercooked ground beef has been identified as one of the main causes of *E. coli* O157:H7 outbreaks in North America (Griffin and Tauxe, 1991; Waters et al., 1994). In 1994, the U.S. Food Safety and Inspection Service (FSIS) began a testing program for detection of *E. coli* O157:H7 in ground beef produced by meat plants and on retail sale in the US. Since then >260 samples of ground beef have tested positive for *E. coli* O157:H7 (Food Safety and Inspection Service, 2005). The World Health Organization has stated that more countries must implement better preventative measures to control infections associated with *E. coli* O157:H7 (World Health Organization, 1997). Alternative means for eliminating *E. coli* O157:H7 in food systems are being studied, and one of these is the addition to meat of the natural antimicrobial allyl isothiocyanate (AIT).

The aliphatic compound AIT is a major product (as well as nitriles, epinitriles and glucose) of the hydrolytic action of the endogenous enzyme myrosinase on the glucosinolate sinigrin in cruciferous plants such as mustard, horseradish and cabbage (Delaquis and Mazza, 1995; Whitmore and Naidu, 2000). AIT is known as both a strong, pungent flavoring agent and a potent inhibitor of a large number of pathogenic microorganisms (Kanemaru and Miyamoto, 1990; Delaquis and Sholberg, 1997; Kyung and Fleming, 1997; Lin et al., 2000a).

Several studies have shown the potential of AIT as a natural antimicrobial in different food matrices, including meat products such as ground or roast beef (Ward et al., 1998; Park et al., 2000; Mari et al., 2002; Muthukumarasamy et al., 2003). The mechanism of AIT antimicrobial action is not well

* Corresponding author. Tel.: +1 204 474 9601; fax: +1 204 474 7630.
*E-mail address:* rick_holley@umanitoba.ca (R.A. Holley).

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understood, but some suggestions have been made. Lin et al. (2000b) reported that AIT damages the bacterial cell membrane, producing leakage of essential cellular metabolites. Delaquis and Mazza (1995) thought that AIT might cause inactivation of essential intracellular enzymes through oxidative cleavage of disulfide bonds.

The application of AIT in the food industry has been limited due to its poor water solubility and volatility. Both these obstacles can be overcome through microencapsulation. This technique has been used for a long time by the food flavoring industry as a practical way to transform sensitive and volatile compounds into stable and free flowing powders which are easy to handle and incorporate as food ingredients. The process of microencapsulation consists of coating solid particles, droplets of liquid or even gas cells with a thin protective film termed the wall material (Young et al., 1993).

Microencapsulation of liquid volatile compounds is generally accomplished in two steps. First, an emulsion of the volatile compound is made in an aqueous dispersion of a wall material which also functions as the emulsifier. Second, the microencapsulated emulsion must be dried under controlled conditions so as to diminish the loss of the encapsulated material by volatilization (Kim et al., 1996).

The objective of this study was to overcome the volatility of AIT by microencapsulation and to assess the antimicrobial potency of microencapsulated AIT against *E. coli* O157:H7 inoculated at two levels in refrigerated, chopped beef packed under nitrogen.

## 2. Materials and methods

### 2.1. AIT microcapsule preparation and analysis

#### 2.1.1. Microcapsule core material preparation

Ten and 60% (v/v) stock solutions of 94% pure AIT (Sigma, St. Louis, MO, USA) were prepared at 5 °C to minimize AIT volatilization during mixing. To prepare the stock solutions, 10 and 60 ml of AIT were mixed with 90 and 40 ml of commercial corn oil (Mazola, ACH, Memphis, TN, USA) respectively, using a high speed mixer (Omni-mixer, Ivan Sorvall Inc., Norwalk, CT, USA) set at 4800 rpm for 2 min.

#### 2.1.2. Gum acacia emulsion

The procedure of Buffo and Reineccius (2001) was modified and used to prepare four gum acacia emulsions. A 30% (w/w) solution of a commercial spray-dried gum acacia powder (SD prehydrated, Nealanders International Inc, Mississauga, ON, Canada) in deionized water was homogenized in the Omni-mixer for 25 min at 4800 rpm. The solution was divided into four parts, covered and held overnight at 5 °C to allow complete hydration of the gum acacia for use as the wall material. Four emulsions were prepared by adding different core materials to the gum acacia solutions at a core/wall ratio of 1:4. The first and second emulsions were prepared using, respectively, 10% and 60% solutions of AIT in corn oil as the core material. A third emulsion was made using a solution of 94% pure AIT as the core material. The fourth emulsion contained corn oil without AIT as the core material. The mixtures were emulsified using the Omni-mixer at 8000 rpm for 2 min.

#### 2.1.3. Freeze drying

Five hundred milliliters of each emulsion was spread over individual aluminium trays (19.4 cm × 19.4 cm × 4.4 cm) covered with wax paper to form a layer approximately 1.6 cm thick. Emulsions were frozen by holding them at −40 °C for about 8 h. Frozen emulsions were placed in a freeze dryer (Model 10-146 MP, Virtis Corp., Gardiner, NY, USA) and held initially at −70 °C and 0.5 mm Hg atmospheric pressure for about 70 h. Dried emulsions were chopped using a food processor (Model FP 1000-04, Black and Decker, Brockville, ON, Canada) for 30 s to provide four different microencapsulated powders.

#### 2.1.4. AIT calibration

Six solutions containing 20, 50, 100, 300, 500 and 1000 ppm of 94% of AIT in hexane (HPLC grade, Fisher Scientific, Ottawa, ON, Canada) were used as the AIT standard solutions for preparing the AIT calibration curve. A 10 μl aliquot of each solution was taken with a gas tight 10 μl syringe (Hamilton Co., Reno, NV, USA) and injected into a gas–liquid chromatograph (Varian Star 3400cx, Varian Chromatography Systems, Walnut Creek, CA, USA) operated using the following conditions: BD5MS column (30 m by 0.25 mm inside diameter; 25 μm wall thickness; J&W Scientific Inc., Folsom, CA); helium carrier gas; column temperature was increased from 60 to 95 °C at a rate of 12.5 °C/min and was held at 95 °C for 45 s. The inlet and flame ionization detector temperatures were set at 250 °C. The area under curve obtained from the chromatograph software (Varian Star chromatography software, Walnut Creek, CA, USA) was plotted against the AIT concentration to prepare the calibration curve.

#### 2.1.5. Determination of AIT in microcapsules

Lyophilized powder (0.5 g) from each chopped emulsion was placed in a screw-cap test tube (16 × 100 mm), closed and stored at −40 °C for analysis of AIT by gas–liquid chromatography. Distilled water (2 ml) was added to 0.5 g of each powder sample and mixed at 2500 rpm (Vortex mixer, Fisher Scientific) for 1 min. Samples were left at 5 °C for 6 h with mixing at 2 h intervals in order to assure the complete dissolution of the wall material. Then 6 ml of HPLC grade hexane (Fisher Scientific) was added to the sample and mixed for one min. The precipitated wall material was allowed to stand for 30 min, mixed and held again for 30 min. A 10 μl aliquot of the hexane phase was taken with a gas tight 10 μl syringe (Hamilton Co.) and injected into the gas–liquid chromatograph operated using the same conditions as for preparation of the AIT calibration curve. The actual amount of AIT in the powder ($AIT_a$) was obtained by calculating the area under the AIT calibration curve. The percentage of AIT retention was calculated according to the expression:

$$Rt(\%) = 100 \times \frac{AIT_a}{AIT_t}$$

where Rt is the percent of AIT retained and $AIT_t$ is the theoretical quantity of AIT in the powder assuming 100% retention (4 g of core material in 16 g of dry carrier or wall material).

#### 2.1.6. Scanning electron microscopy (SEM)

Microcapsule morphology and surface structures were assessed using a cold stage scanning electron microscope (FEI, Quanta 200, Hillsboro, OR, USA) at an accelerating voltage of 5–15 kV. Microcapsules were mounted on stubs using double-sided sticky carbon tape. Stubs were then coated with a gold-palladium layer (15 nm thick) using a SEM sputter coater (Hummer VII Sputter Coater, Anated Ltd., Alexandria, VA, USA). Electron micrographs were taken at magnifications of 800× and 1600×.

### 2.2. Chopped beef preparation

Fresh beef chuck roasts were bought from a local supermarket. The outer surfaces of roasts were slightly frozen by exposure at −18 °C for 2 h. Frozen surfaces were trimmed away using a sterile knife to eliminate outer microbial contamination. Roasts were cut into 8×8 cm pieces and chopped in a sterile rotating bowl cutter (Titane 40 L, Dadaux SA, Le Bouchaud, France) to yield particles measuring ≤9 mm, typical of ground beef. Afterward, the chopped beef was held at 4 °C for 20 min and used for bacterial inoculation and treatment with AIT microcapsules.

### 2.3. Bacterial strain preparation

Five human isolates of *E. coli* O157:H7 which had mutated and become non-pathogenic during storage (3581, 0304, 0627, 0628 and a non-motile strain 1840) were provided by Rafig Ahmed, National Microbiology Laboratory, Public Health Agency, Canadian Science Centre for Human and Animal Health, Winnipeg, MB. These strains were maintained in trypticase soy broth (TSB) (BBL, Becton Dickinson, Sparks, MD, USA) and subcultured twice at 37 °C for 18 h before use in experiments. After the last incubation, 40 ml of each *E. coli* O157:H7 culture was centrifuged at 8000×*g* for 10 min at 10 °C (Sorvall RC-5, Du Pont, Newtown, CT, USA). *E. coli* O157:H7 cells were washed in 40 ml of 0.1% (w/v) peptone water and collected by centrifugation. Cell pellets were standardized at an optical density of 0.249 at 600 nm by dilution in peptone water and monitored with a spectrophotometer (Ultrospec 2000, Pharmacia Biotech, Baie d'Urfe, QC, Canada). *E. coli* O157:H7 dilutions were mixed to obtain an equal number of cells of each strain in the inoculation cocktail.

### 2.4. Preparation of inoculated chopped beef with microcapsules

Approximately 4.5 kg of chopped beef was inoculated with the five strain cocktail of *E. coli* O157:H7 to yield either 4 or 8 $\log_{10}$ cfu/g. Chopped beef was thoroughly mixed by hand while wearing sterile gloves, to facilitate even distribution of *E. coli* O157:H7 in the meat. Inoculated meat was divided in 8 groups of 550 g each. Gum acacia microcapsules were added to 7 of the 8 meat groups at each inoculation level. Table 1 presents the corresponding treatments. AIT was added to 6 of the 8 chopped beef treatments to obtain final concentrations from 174 to 4980 ppm. Of the other treatments, one contained encapsulated corn oil without AIT and the other had chopped beef without any microcapsule addition. Twenty-five grams of chopped beef from each treatment were placed in $O_2$ barrier plastic bags (Deli*1, Winpak, Winnipeg, MB, Canada) with $O_2$ transmission rates of 2.3 $cm^3/m^2$ per 24 h at 23 °C. Bags were flushed with 100% $N_2$ and sealed using a vacuum packaging machine (Model GM 2000, Bizerba, Mississauga, ON, Canada). Samples were stored at 4 °C for 18 days and were analyzed for *E. coli* O157:H7 and total numbers of bacteria at 3 day intervals.

### 2.5. Microbial analysis of chopped beef

Eleven grams of chopped beef from each sealed bag were placed in a sterile stomacher bag (Filtra-bag, VWR International, Edmonton, AB, Canada) and homogenized in a stomacher (Model 400, A. J. Seward, London, UK) with 99 ml of 0.1% peptone water. The aerobic mesophilic bacteria were determined by serial dilution in 0.1% peptone water and plated on trypticase soy agar (TSA, BBL, Fisher Scientific) using a spiral plater (Autoplate 4000, Spiral Biotech, Norwood, MA, USA). *E. coli* O157:H7 numbers were obtained by spiral plating the diluted samples on Sorbitol MacConkey agar (BBL) modified with a cefixime tellurite supplement (Oxoid, Hampshire, England) to yield the medium CT-SMAC (Zadik et al., 1993). TSA and CT-SMAC plates were incubated for 48 and 24 h, respectively, at 37 °C. In order to determine if *E. coli* O157:H7 cells were killed or

Table 1
Treatments used to challenge *E. coli* O157:H7 at 4 or 8 $\log_{10}$ cfu/g in ground beef with allyl isothiocyanate (AIT)

| Treatment code | Microcapsule core material | % (w/w) Microcapsule addition to inoculated ground beef | AIT[a] concentration (ppm) |
|---|---|---|---|
| T1 (Control) | No microcapsules | No microcapsules | 0 |
| T2 | Corn oil only | 10 | 0 |
| T3 | AIT, 10% (v/v) in corn oil | 5 | 174 |
| T4 | AIT, 10% (v/v) in corn oil | 10 | 333 |
| T5 | AIT, 60% (v/v) in corn oil | 5 | 1481 |
| T6 | AIT, 60% (v/v) in corn oil | 10 | 2828 |
| T7 | 94% AIT | 5 | 2608 |
| T8 | 94% AIT | 10 | 4980 |

[a] AIT concentrations reached in the meat treatments after the addition of different percentages of microencapsulated AIT.

injured in the chopped beef as a result of AIT exposure, any cells in samples showing no growth were removed from AIT treatments using an immunomagnetic separation (IMS) technique (Dynabeads anti-*E. coli* O157:H7, Dynal, Oslo, Norway) performed according to the manufacturer's instructions. Fifty microlitres of the IMS-complex was spread onto CT-SMAC plates. Then plates were incubated at 37 °C for 24 h. Presumptive *E. coli* O157:H7 colonies from CT-SMAC plates were confirmed as *E. coli* using API 20E biochemical test strips (bioMerieux Vitek, Inc., Hazelwood, MO).

### 2.6. AIT odor intensity in the chopped beef

The odor of AIT in the chopped beef was evaluated informally by two of the persons performing microbial analysis immediately as packages were opened.

### 2.7. Statistical analysis

Microbiological data were analyzed by the general linear models (GLM) procedure using the Statistical Analysis System (SAS) software program, version 8.1 (SAS Institute Inc, Cary, NC, USA). Mean differences were compared using a Tukey test at the 95% significance level ($p < 0.05$).

## 3. Results and discussion

### 3.1. AIT microencapsulation in gum acacia

AIT was successfully microencapsulated in gum acacia at a core-wall material ratio of 1:4 and freeze dried. Microcapsule content of AIT was affected by the concentrations of AIT used in the core material solutions (Table 2). As expected, the use of a higher AIT core concentration yielded more AIT in the microcapsules. The highest concentration of AIT was achieved when a solution of 94% pure commercial AIT was encapsulated yielding 54.78 mg of AIT/g of microcapsule material. The percentage of AIT retained in the microcapsules after freeze drying was also highest (73%) in microcapsules made with the highest concentration of AIT (Table 2).

Dilutions in corn oil proved to be an adequate means for adjusting the final levels of AIT entrapped in the microcapsules. However, AIT retention was less in microcapsules containing AIT diluted in corn oil. Unsaturated fatty acids are believed by Nadarajah (2003) to complex with the lipophilic AIT molecule and slow its release. This may also explain the current observations regarding AIT retention by microcapsules. Additionally, a slight increase in the mixing temperature during the preparation of the AIT-corn oil core solutions could have accelerated AIT losses initially and produced microcapsules with lower AIT content.

AIT retention in the microcapsules during the freeze drying process may occur as a result of "microregion entrapment", which was explained by Flink (1975). Before freeze drying, AIT is present in the emulsion as a molecular dispersion in small droplets due to its poor solubility. Flink's theory states that at the beginning of the freezing process, water starts to freeze, forming ice crystals in the solution. Growing ice crystals gradually push solutes (including AIT) between them, creating small regions (microregions) of concentrated material. Low moisture content in these microregions, as a consequence of freezing and subsequent drying, create associations of carbohydrate molecules by hydrogen bonding. Carbohydrate–carbohydrate hydrogen bonds seal these microregions and enable entrapment of AIT.

Table 2
Influence of input allyl isothiocyanate (AIT) concentrations on the retention of AIT in gum acacia microcapsules[a] after freeze drying

| Core material solution | mg AIT/g microcapsules | AIT retention (%) |
|---|---|---|
| 94% pure AIT | 54.8 | 73 |
| 56% pure AIT in corn oil | 31.1 | 69 |
| 9.4% pure AIT in corn oil | 3.7 | 50 |

[a] Core (AIT), wall (gum acacia) ratio of 1:4.

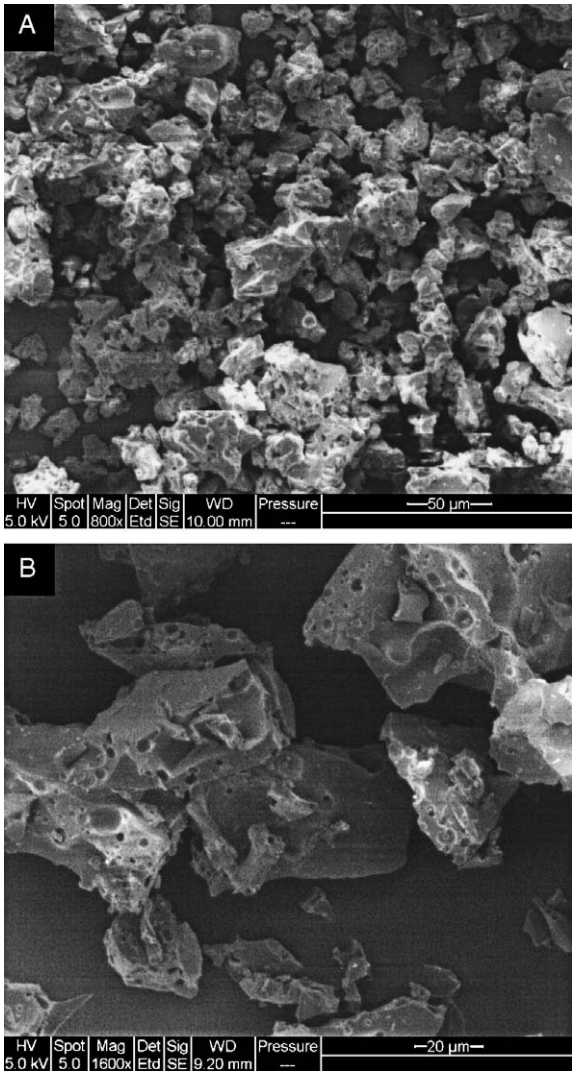


Fig. 1. Scanning electron micrographs showing the appearance of AIT-gum acacia microcapsules. Samples were examined on a cold stage at 800× (A) and 1600× (B) magnification.

### 3.1.1. Microcapsule characteristics

Scanning electron micrographs did not show structural differences among the powder particles obtained after chopping the freeze-dried emulsions. The shapes of the microcapsules obtained were in agreement with the report by Buffo and Reineccius (2001), where microcapsules were described as irregular, rock-like particles. Fig. 1 shows microcapsule structures viewed at different magnifications. All microcapsules obtained exhibited good flow properties and had a creamy-white appearance.

### 3.2. Effect of AIT microcapsules on E. coli O157:H7 inoculated in chopped beef and stored at 4°C

The bactericidal effect of microencapsulated AIT against *E. coli* O157:H7 was apparent at the highest concentration used. Microencapsulated AIT completely eliminated 4 $\log_{10}$ cfu/g *E. coli* O157:H7 at 4980 ppm after 15 days of storage (Table 3). Microcapsules without AIT or those containing 174 and 333 ppm did not have any significant effect ($p>0.05$) on the viability of *E. coli* O157:H7 in comparison with the control at 18 days storage. At 1481 ppm, AIT showed a slight (0.6 $\log_{10}$ cfu/g) but significant ($p<0.05$) reduction, while AIT at 2608 and 2828 ppm reduced viable *E. coli* 0157:H7 cells by >1 $\log_{10}$ cfu/g at the end of storage, which was also significant.

A similar antimicrobial effect occurred when these concentrations of AIT were used in chopped beef inoculated with 8 $\log_{10}$ cfu *E. coli* 0157:H7/g (Table 4). AIT concentrations lower than 333 ppm did not show significant ($p>0.05$) reductions of *E. coli* O157:H7 numbers after 18 days of storage. Initial levels of *E. coli* O157:H7 were reduced >2.5 $\log_{10}$ cfu/g by 18 days of storage with AIT concentrations of 2608 and 2828 ppm. *E. coli* O157:H7 was undetectable at day 18 when 4980 ppm AIT was used.

The reduction of *E. coli* O157:H7 inoculated at both levels in chopped beef was notable in treatments with ≥1481 ppm AIT. The progressive bacterial reduction observed in these treatments was likely due to the continuous release of AIT from the gum acacia microcapsules during storage at high relative humidity (RH). This assertion is based on unpublished work in our laboratory where it was found that AIT release occurred over a period of days and at 85% RH was significantly faster than at 11% RH. At higher RH, 12% was released at day 5 and >85% at 20 days. Since fresh chopped beef contains 75% moisture (96% RH), the gum acacia microcapsules would have been less stable, facilitating AIT release. Rosenberg et al. (1990) found that the retention of volatiles by gum acacia microcapsules was significantly reduced by increasing the RH from 75% to 97%. RH values close to 97% completely destroyed the microcapsule structure, causing total release of the volatiles within 35 days at 25 °C. It is likely in the present tests that AIT was completely released from the microcapsules into the meat during storage.

Table 3
Effect of microencapsulated allyl isothiocyanate (AIT) on the viability of *E. coli* O157:H7 inoculated at 4 $\log_{10}$ cfu/g in chopped beef stored at 4 °C

| Treatment | Storage time (days) | | | | | | |
|---|---|---|---|---|---|---|---|
| | 0 | 3 | 6 | 9 | 12 | 15 | 18 |
| Control[a] | 4.38a[b] | 3.97a | 3.91b | 3.82bcd | 4.30a | 3.99bc | 3.99c |
| No AIT[c] | 4.38a | 3.93a | 4.35a | 4.45a | 4.44a | 4.41a | 4.44a |
| 174 ppm AIT | 4.38a | 3.95a | 4.37a | 4.19ab | 4.12ab | 4.04bc | 4.00bc |
| 333 ppm AIT | 4.38a | 3.95a | 4.30a | 4.25a | 4.21ab | 4.20ab | 4.21ab |
| 1481 ppm AIT | 4.38a | 3.94a | 4.11ab | 4.07abc | 3.91bc | 3.90c | 3.44d |
| 2608 ppm AIT | 4.38a | 3.92a | 3.80bc | 3.67d | 2.97d | 3.19d | 2.91e |
| 2828 ppm AIT | 4.38a | 3.94a | 3.94b | 3.75cd | 3.59c | 3.32d | 2.90e |
| 4980 ppm AIT | 4.38a | 3.04b | 3.50c | 2.18e | 1.80e | Nv[d]e | Nv[d]f |

[a] Inoculated chopped beef with *E. coli* O157:H7.
[b] Means in the same column with the same letters are not significantly different ($p>0.05$). Tabulated values are the means of three experiments.
[c] Microcapsules with corn oil only.
[d] Nv—No viable cells were detected. Immunomagnetic separation (IMS) was performed for each presumptive negative sample after 24 h of enrichment in TSB at 37 °C.

Table 4
Effect of microencapsulated allyl isothiocyanate (AIT) on the viability of *E. coli* O157:H7 inoculated at 8 $\log_{10}$ cfu/g in chopped beef stored at 4 °C

| Treatment | Storage time (days) | | | | | | |
|---|---|---|---|---|---|---|---|
| | 0 | 3 | 6 | 9 | 12 | 15 | 18 |
| Control[a] | 8.12a[b] | 7.92a | 7.46a | 7.31a | 7.44ab | 7.46ab | 7.43ab |
| No AIT[c] | 8.12a | 7.92a | 7.52a | 7.51a | 7.58a | 7.56a | 7.53a |
| 174 ppm AIT | 8.12a | 8.04a | 7.31ab | 7.38a | 7.54a | 7.50a | 7.33ab |
| 333 ppm AIT | 8.12a | 8.06a | 7.72a | 7.36a | 7.42ab | 7.50a | 7.13b |
| 1481 ppm AIT | 8.12a | 8.09a | 7.30ab | 7.21ab | 7.17b | 6.18c | 5.81c |
| 2608 ppm AIT | 8.12a | 7.91a | 6.97b | 6.78c | 6.83c | 6.21bc | 5.39d |
| 2828 ppm AIT | 8.12a | 7.86a | 6.87b | 6.81bc | 6.81c | 6.13c | 5.53cd |
| 4980 ppm AIT | 8.12a | 7.16b | 6.34c | 5.35d | 4.44d | 3.68d | Nv[d]e |

[a] Inoculated chopped beef with *E. coli* O157:H7.
[b] Means in the same column with the same letters are not significantly different ($p>0.05$). Tabulated values are the means of three experiments.
[c] Microcapsules with corn oil only.
[d] Nv—No viable cells were detected. Immunomagnetic separation (IMS) was performed for each presumptive negative sample after 24 h of enrichment in TSB at 37 °C.

Table 5 shows differences in the antimicrobial activity of AIT when used as a gas, or liquid or in microcapsules against *E. coli* O157:H7 in broth, agar and food systems. The present tests with AIT showed that higher initial levels of AIT were needed to achieve the same bactericidal effects against *E. coli* O157:H7 when delivered from microcapsules than when volatilized into the package headspace. Muthukumarasamy et al. (2003) found that 1300 ppm AIT volatilized from a paper insert eliminated 3 $\log_{10}$ cfu/g *E. coli* O157:H7 from vacuum packed ground beef within 15 days at 4 °C, while Nadarajah et al. (2005) found 1800 ppm AIT delivered in the same way eliminated 3 $\log_{10}$ cfu/g of the same organisms in 18 days under similar conditions. These results are in agreement with several studies which showed that AIT vapor is more antimicrobially effective than the liquid form (Inouye et al., 1983; Isshiki et al., 1992; Delaquis and Sholberg, 1997; Ward et al., 1998). Unfortunately, it is difficult to generalize and accurately predict AIT concentrations sufficient to eliminate *E. coli* O157:H7 given differences in substrates, temperatures and modes of application used in other work.

The interaction of AIT with meat constituents may decrease AIT antimicrobial activity. For example, Kawakishi and Nakimi (1969) and Pechacek et al. (1997) reported that AIT

Table 5
Antimicrobial activity of allyl isothiocyanate (AIT) applied as liquid, microencapsulated liquid and gaseous forms against *E. coli* O157:H7 in different substrates

| Model System | AIT State | AIT concentration | Reduction of *E.coli* O157:H7 ($log_{10}$ cfu) | Temperature (°C) | Time (days) | Reference |
|---|---|---|---|---|---|---|
| Broth | Liquid | 12.3 ppm | 6/ml | 30 | 1 | Kanemaru and Miyamoto (1990) |
| Broth | Liquid | 2500 ppm | 8/ml | 37 | 0.04=1 h | Lin et al. (2000b) |
| Agar disk | Gaseous | 1500 μg/l | 6/$cm^2$ | 35 | 2 | Delaquis and Sholberg (1997) |
| Lettuce | Gaseous | 400 μl/l | 4/g | 4 | 2 | Lin et al. (2000a) |
| Roast beef | Gaseous | 20 μl/l | ~0.8/$cm^2$ | 12 | 7 | Ward et al. (1998) |
| Ground beef | Gaseous | 1300 ppm | 3/g | 4 | <15 | Muthukumarasamy et al. (2003) |
| Ground beef | Gaseous | 1300 ppm | 5/g | 4 | 25 | Muthukumarasamy et al. (2003) |
| Ground beef | Gaseous | 1800 ppm | 3/g | 4 | 18 | Nadarajah et al. (2005) |
| Ground beef | Liquid microencapsulated | 2828 ppm | <3/g[a] | 4 | 18 | Present study |
| Ground beef | Liquid microencapsulated | 4980 ppm | 8/g | 4 | 18 | Present study |

[a] Reductions were 1.5–2.6 $log_{10}$ cfu/g.

is decomposed through nucleophilic attack by water and hydroxide ions in aqueous media. This interaction may be an important reason for the lower bactericidal effect of AIT microcapsules in comparison with AIT vapor. In addition, AIT is known to react with thiols, sulphydryls, and free amino groups of proteins, causing faster depletion of AIT in meat systems (Kawakishi and Kaneko, 1987; Ward et al., 1998; Nadarajah et al., 2005). Another important factor that may affect the antimicrobial activity of liquid AIT is its solubility in meat fatty acids. So far, there is no clear explanation of the fate of AIT in meat lipids. Fat may provide a protective layer around contaminating bacteria, or possibly absorb the lipophilic AIT and reduce its effectiveness in the aqueous phase of the product (Holley and Patel, 2005). It should be noted that the fat content of the chopped beef was expected to be <5% (w/w) which is similar to the fat content of the beef used by Muthukumarasamy et al. (2003). While this is lower than the 7–30% (w/w) fat content of ground beef commonly used for hamburger preparation, less interference by fat with the antimicrobial action of AIT might be expected.

Chopped beef treated with concentrations lower than 1480 ppm had a faint AIT odor upon opening the packages after 18 days of storage. However, treatments containing between 2600 and 2800 ppm had a stronger smell and gave a spicy sensation just after opening the packages. The odor dissipated a short time later. This odor was not intolerable and might be pleasing for some individuals (Delaquis and Mazza, 1995). Treatments containing ~5000 ppm AIT had a very strong odor upon package opening after 18 days of storage, and caused lachrymation (eye watering) by some persons exposed. Although highly effective for *E. coli* O157:H7 elimination, AIT application at this level would be limited by its lachrymatory effects. Lin et al. (2000a) suggested that the strong odor of AIT could be reduced if permeable packages were used during storage; however, reduced antimicrobial potency would be anticipated.

### 3.3. Effect of AIT microcapsules on the natural flora of ground beef

The number of mesophilic bacteria in the control group increased progressively from 4.53 to 7.25 $log_{10}$ cfu/g at 18 days of storage (Table 6). A slight reduction in the number of mesophilic bacteria was observed in all treatments with AIT during the first 6 days of storage when compared with the control. No significant ($p>0.05$) differences in bacterial viability were found among the control, the treatment with microcapsules containing only corn oil and the group containing 174 ppm of AIT after 12 days of storage. Samples containing 333 ppm of AIT showed suppressed growth of mesophilic bacteria during the first 15 days; however, at the end of storage no significant differences were found between this treatment and the control. Inhibition of growth was more evident during the first 12 days when AIT levels ranging from ~1500 to 2828 ppm were used. Again, mesophilic bacterial populations were almost the same in these treatments and the control by 18 days of storage. The growth of mesophilic bacteria was significantly inhibited by the AIT level close to 5000 ppm. This treatment reduced the number of bacteria initially present by >1 $log_{10}$ cfu/g at 18 days.

The delay in growth observed in all AIT treatments after the first 6 days of storage may have been due to a change in the flora, from Gram-negative organisms to Gram-positive lactic acid bacteria (LAB). The latter form the dominant microflora during anaerobic storage of fresh meat (Nadarajah et al., 2005). Gram-positive bacteria have been reported to be more AIT tolerant than Gram-negative bacteria (Lin et al., 2000a; Shofran et al., 1998;

Table 6
Effect of microencapsulated allyl isothiocyanate (AIT) on the viability of mesophilic bacteria in chopped beef stored at 4 °C

| Treatment | Storage time (days) | | | | | | |
|---|---|---|---|---|---|---|---|
| | 0 | 3 | 6 | 9 | 12 | 15 | 18 |
| Control[a] | 4.53a[b] | 4.91a | 5.05a | 6.39a | 7.06a | 7.16ab | 7.26ab |
| No AIT[c] | 4.53a | 4.31ab | 4.72ab | 6.91a | 6.97a | 7.36a | 7.33a |
| 174 ppm AIT | 4.53a | 4.18bc | 4.19bc | 5.77b | 6.90a | 7.03ab | 7.26ab |
| 333 ppm AIT | 4.53a | 3.54c | 3.89c | 4.75c | 5.92b | 6.27c | 7.29ab |
| 1481 ppm AIT | 4.53a | 4.15bc | 4.14bc | 4.84c | 5.49c | 6.86b | 7.20ab |
| 2608 ppm AIT | 4.53a | 3.53c | 3.83c | 4.55c | 5.45c | 6.85b | 7.05ab |
| 2828 ppm AIT | 4.53a | 4.86a | 4.37abc | 4.55c | 5.31c | 6.29c | 6.99b |
| 4980 ppm AIT | 4.53a | 4.28ab | 3.97bc | 3.71d | 3.49d | 3.11d | 3.16c |

[a] Ground beef inoculated with 4 $log_{10}$ cfu *E. coli* O157:H7/g.
[b] Means in the same column with the same letters are not significantly different ($p>0.05$). Tabulated values are the means of three experiments.
[c] Microcapsules with corn oil only.

Ward et al., 1998). It is likely that LAB were still able to grow and reach normal levels at AIT concentrations <2828 ppm.

## 4. Conclusions

Microencapsulation of AIT in gum acacia generated, after lyophilization and chopping, a powder with mild odor which could be conveniently added as an ingredient to ground meat or other foods. Dilutions of AIT in corn oil permitted adjustment of the final concentration of AIT in the microcapsules.

*E. coli* O157:H7 inoculated at 4 or 8 $\log_{10}$ cfu/g chopped beef was inhibited by ≥1481 ppm AIT in microcapsules and was killed by 4980 ppm encapsulated AIT. Growth of the natural Gram-positive microflora of LAB was unaltered by the application of AIT microcapsules containing ≤2828 ppm AIT. While the use of microencapsulation facilitated handling of AIT during product formulation, its overall effectiveness against *E. coli* O157:H7 was reduced in comparison with AIT vaporized in the package headspace.

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