

Elimination of *Escherichia coli* O157:H7 from Fermented Dry Sausages at an Organoleptically Acceptable Level of Microencapsulated Allyl Isothiocyanate

Pedro A. Chacon, Parthiban Muthukumarasamy, and Richard A. Holley*

Department of Food Science, Faculty of Agricultural and Food Science, University of Manitoba, Winnipeg, Manitoba R3T 2N2, Canada

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Four sausage batters (17.59% beef, 60.67% pork, and 17.59% pork fat) were inoculated with two commercial starter culture organisms ($>7 \log_{10}$ CFU/g *Pediococcus pentosaceus* and $6 \log_{10}$ CFU/g *Staphylococcus carnosus*) and a five-strain cocktail of nonpathogenic variants of *Escherichia coli* O157:H7 to yield 6 to $7 \log_{10}$ CFU/g. Microencapsulated allyl isothiocyanate (AIT) was added to three batters at 500, 750, or 1,000 ppm to determine its antimicrobial effects. For sensory analysis, separate batches with starter cultures and 0, 500, or 750 ppm microencapsulated AIT were produced. Sausages were fermented at $\leq 26^\circ\text{C}$ and 88% relative humidity (RH) for 72 h. Subsequently sausages were dried at 75% RH and 13°C for at least 25 days. The water activity (a_w), pH, and levels of starter cultures, *E. coli* O157:H7, and total bacteria were monitored during fermentation and drying. All sausages showed changes in the initial pH from 5.57 to 4.89 and in a_w from 0.96 to 0.89 by the end of fermentation and drying, respectively. Starter culture numbers were reduced during sausage maturation, but there was no effect of AIT on meat pH reduction. *E. coli* O157:H7 was reduced by $6.5 \log_{10}$ CFU/g in sausages containing 750 and 1,000 ppm AIT after 21 and 16 days of processing, respectively. *E. coli* O157:H7 numbers were reduced by $4.75 \log_{10}$ CFU/g after 28 days of processing in treatments with 500 ppm AIT, and the organism was not recovered from this treatment beyond 40 days. During sensory evaluation, sausages containing 500 ppm AIT were considered acceptable although slightly spicy by panelists.

In 1982 *Escherichia coli* O157:H7 was first recognized as a human pathogen which caused hemorrhagic colitis (29). Since then, human infections attributed to *E. coli* O157:H7 have been reported in over 30 countries, reaching alarming annual incidence rates of 8 per 100,000 inhabitants in some areas of Canada, the United States, and Scotland (23). Foods of animal origin are considered the main source of *E. coli* O157:H7 illnesses in humans (34). Most outbreaks of *E. coli* O157:H7 have been linked with the consumption of undercooked meats, especially ground beef or hamburger patties (4, 31). Fermented meat products had long been thought to be relatively safe due to their low pH, their low water activity (a_w), and the presence of curing salts (nitrite and nitrate). However, an important wake-up call occurred in 1994, when 23 individuals in the states of Washington and California were infected with *E. coli* O157:H7 as a consequence of consumption of dry-cured salami (5). After this outbreak, the U.S. Department of Agriculture Food Safety and Inspection Service required meat processors to validate that processing dry and semidry fermented sausages resulted in at least a 5- \log_{10} -unit reduction in numbers of *E. coli* O157:H7 cells (28). The ability of *E. coli* O157:H7 to adapt to acid environments (11) has caused this microorganism to be regarded as one of the most dangerous pathogens in fermented meat products. Several studies have shown that *E. coli* O157:H7 is able to survive the processes of fermentation, drying, and storage when this microorganism is

present at high numbers (14, 16, 27). The use of different starter cultures during fermentation has been unsuccessful in achieving the required 5- \log_{10} -unit reduction of *E. coli* O157:H7 (12, 22). Heat treatments seem to be the best way to reduce the numbers of this pathogen in semidry sausages; however, physical characteristics of dry sausage change during heating, and the products no longer resemble uncooked fermented sausages (16).

Alternative means for reducing the viability of *E. coli* O157:H7 in food systems are being studied, and one of these is the addition of the natural antimicrobial allyl isothiocyanate (AIT) to meat. AIT is one of several naturally occurring compounds formed when glucosinolates present in cruciferous vegetables are hydrolyzed by the enzyme myrosinase (8). AIT is a potent inhibitor of a large number of pathogenic microorganisms (9, 19), and it has been successfully used for elimination of *E. coli* O157:H7 from ground beef, roast beef, and hamburger patties (24, 26, 32). In general the application of AIT in the food industry has been limited due to its volatility; however, this obstacle was overcome by microencapsulation (6).

Microencapsulation of liquid volatile compounds can be successfully accomplished in two steps. First, an emulsion of the volatile compound is made in an aqueous dispersion of a suitable "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 (20).

The objectives of this study were to determine whether microencapsulated AIT was able to reduce the levels of *E. coli* O157:H7 in dry fermented sausages by at least 5 \log_{10} units and to evaluate the acceptability of uncontaminated AIT-treated products to consumers.

* Corresponding author. Mailing address: 228 Ellis Building, Department of Food Science, University of Manitoba, Winnipeg, Manitoba R3T 2N2, Canada. Phone: (204) 474-9601. Fax: (204) 474-7630. E-mail: rick_holley@umanitoba.ca.

MATERIALS AND METHODS

AIT microcapsule preparation and analysis. (i) Gum acacia emulsion. The procedure of Chacon et al. (6) was used to prepare a gum acacia emulsion. A 30% (wt/wt) solution of a commercial spray-dried gum acacia powder (SD prehydrated; Nealanders International Inc., Mississauga, ON, Canada) in deionized water was homogenized using a high-speed mixer (Omni-mixer; Ivan Sorvall Inc., Norwalk, CT) set at 4,800 rpm for 25 min. The solution was covered and held overnight at 5°C to allow complete hydration of the gum acacia for use as the wall material. The emulsion was prepared by adding a solution of 94% pure AIT (Sigma, St. Louis, MO) as the core material to the gum acacia solution at a core/wall ratio of 1:4. The mixture was emulsified using the Omni-mixer at 8,000 rpm for 2 min.

(ii) Freeze drying. Five hundred milliliters of the gum acacia-AIT emulsion was spread over individual aluminum trays (19.4 cm by 19.4 cm by 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) 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 the microencapsulated powder.

(iii) AIT calibration. Six solutions containing 20, 50, 100, 300, 500, and 1,000 ppm of 94% AIT in hexane (high-pressure liquid chromatography grade; Fisher Scientific, Ottawa, ON, Canada) were used as standards for preparing the AIT calibration curve. A 10- μ l aliquot of each solution was taken with a gas-tight 10- μ l syringe (Hamilton Co., Reno, NV) and injected into a gas-liquid chromatograph (Varian Star 3400cx; Varian Chromatography Systems, Walnut Creek, CA) operated using the following: a BD5MS column (30 m by 0.25 mm [inside diameter]; 25- μ m wall thickness; J&W Scientific Inc., Folsom, CA), helium carrier gas, and a column temperature that 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 the curve obtained from the chromatograph software (Varian Star chromatography, Walnut Creek, CA) was plotted against the AIT concentration to prepare the calibration curve.

(iv) Determination of AIT in microcapsules. Lyophilized powder (0.5 g) from the chopped emulsion was placed in a screw-cap test tube (16 by 100 mm), which was closed and stored at -40°C for analysis of AIT by gas-liquid chromatography. Distilled water (2 ml) was added to each powder sample and mixed at 2,500 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 ensure the complete dissolution of the wall material, and then 6 ml of hexane (high-pressure liquid chromatography grade; Fisher Scientific) was added to the sample and mixed for 1 min. The precipitated wall material was allowed to stand for 30 min, mixed, and held again for 30 min. A 10- μ l aliquot of the hexane phase was taken with a gas-tight 10- μ l syringe (Hamilton Co., Reno, NV) and injected into the gas-liquid chromatograph, which was operated using the same conditions as for preparation of the AIT calibration curve. The amount of AIT in the powder was obtained by calculating the area under the AIT response curve by comparison with the calibration curve for AIT.

Bacterial strain preparation. Dry-cured sausages were fermented using the strains *Pediococcus pentosaceus* UM 116P and *Staphylococcus carnosus* UM 110M, which were isolated from lyophilized commercial meat starter cultures (Trumark LTII M and LTII, respectively; Rector Foods Ltd., Mississauga, ON, Canada). *P. pentosaceus* was maintained in deMan Rogosa Sharpe (MRS) broth (BBL, Becton Dickinson, Sparks, MD) containing 20% glycerol at -70°C. *S. carnosus* was maintained in Trypticase soy broth (TSB) (BBL, Becton Dickinson) containing 20% glycerol at -70°C. *P. pentosaceus* was subcultured twice in MRS broth (Trypticase soy agar was used for *S. carnosus*) incubated at 37°C before use in the experiments. Overnight cultures (16 h, at the late logarithmic phase of growth) were centrifuged at 8,000 \times g for 10 min, washed in 0.1% (wt/vol) peptone water, and suspended in 0.1% peptone water to achieve 9 log CFU/ml by standardization at an optical density of 600 nm using a spectrophotometer (Ultraspec 2000; Pharmacia Biotech, Baie d'Urfe, QC, Canada).

Five human isolates of *E. coli* O157:H7 which had mutated and become nonpathogenic (verotoxin negative) during storage (strains 3581, 0304, 0627, and 0628 and a nonmotile strain, 1840) were provided by Rafiq Ahmed, National Microbiology Laboratory, Public Health Agency, Canadian Science Centre for Human and Animal Health, Winnipeg, MB, Canada. These organisms were comparable in their resistance toward AIT to pathogenic human clinical and hamburger isolates of *E. coli* O157:H7 (6, 24, 26). These strains were maintained in TSB (BBL) 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 8,000 \times g for 10 min at 10°C (Sorvall RC-5; Du Pont, Newtown, CT). *E. coli*

TABLE 1. Formulation used for manufacture of dry fermented sausage

Ingredient(s)	Composition (% wt/wt)
Beef fronts (85% lean).....	17.59
Pork (90% lean).....	60.67
Pork fat.....	17.59
Spice mix.....	0.44
Salt.....	2.90
Dextrose + corn syrup solids (Rapidur) ^a	0.69
Pickle cure concentrate ^a	0.31

^a From Canada Compound Western Ltd., Winnipeg, MB, Canada.

O157:H7 cells were washed in 40 ml of 0.1% peptone water and collected by centrifugation. Cell pellets were standardized at an optical density of 0.250 at 600 nm by dilution in peptone water and were monitored with the spectrophotometer. *E. coli* O157:H7 suspensions were mixed to obtain an equal number of cells of each strain in the inoculation cocktail.

Dry fermented sausage manufacture for *E. coli* O157:H7 challenge. Four salami batches of approximately 10 kg each were processed in the Department of Food Science pilot plant. The batch composition is shown in Table 1. Sausage batters were prepared by chopping 1°C pork fat, pork, and beef (purchased from a local wholesaler) in a precooled (1°C) Titane 40 rotating bowl meat cutter (Dadaux, Bersaillin, France). *P. pentosaceus* and *S. carnosus* were added to reach a final inoculation level of 7 log₁₀ CFU/g. All batters were also inoculated with the five-strain cocktail of nonvirulent *E. coli* O157:H7 to yield approximately 7 log₁₀ CFU/g. To these batters, cervelat spice mix (Hermann Laue Spice Co. Inc., Uxbridge, ON, Canada), Rapidur (a proprietary mixture of corn syrup solids and dextrose), pickle cure concentrate containing 6.25% NaNO₂ (which gave a final concentration of 193 ppm of nitrite) plus 1% NaHCO₃, and 2.9% (wt/wt) salt (Canada Compound Western Ltd., Winnipeg, MB) were added and chopped (\leq 5 min) to achieve a granular (3-mm) consistency of meat and fat particles. AIT microcapsules (containing 44.14 mg of AIT/g of microcapsules) were added to the first three sausage batters at 1.13%, 1.69%, and 2.26% (wt/wt) to yield 500, 750, and 1,000 ppm of AIT, respectively. These concentrations were used because preliminary testing indicated that <500 ppm AIT would not cause the extent of *E. coli* O157:H7 lethality desired. No AIT was added to the control salami batter. Fibrous casings (55-mm diameter; Kalle GmbH & Co., Wiesbaden, Germany) were presoaked in lukewarm water (40°C for 30 min) before use. Sausage batters at 4°C were stuffed with a piston stuffer (Mainca model EM30; Equipamientos Carnicos, S. L. Barcelona, Spain) into the casings to achieve a final weight of about 500 g. Sausages were transferred to a single-cage smokehouse (Allroundsystem Rondette with a Titan programmable controller; Maurer AG, Reichenau, Germany) and processed by fermentation at 26°C and 88% relative humidity (RH) for 24 h to reach a pH of <5.3. Thereafter the fermentation temperature and RH were decreased stepwise (six times at 12-h intervals, where temperature and RH were reduced by 2°C and 2.2%, respectively) from 26 to 14°C and 88% to 75% RH over 72 h, with intermittent smoking (from electrically fired hardwood chips) for 30 min at 48, 60, and 72 h. The degree-hour guidelines of Agriculture and Agri-Food Canada (1) were followed when calculating the permitted time and temperature combinations during fermentation. Fermentation was followed by drying at 13°C and 75% RH for 25 days.

Microbial analysis of dry fermented sausages. Each dry fermented sausage treatment was analyzed microbiologically after stuffing (0 days) and after 3, 6, 9, 16, 21, and 28 days of fermentation and ripening. For *E. coli* O157:H7, additional analyses were made after 35, 40, and 45 days of processing. For microbial analysis, 11 g sausage meat was taken in triplicate from each of two cross-sectioned sausages, placed in a sterile stomacher bag (Filtira-bag; VWR International, Edmonton, AB, Canada), and homogenized in a stomacher (model 400; A. J. Seward, London, United Kingdom) with 99 ml of 0.1% peptone water. The total number of bacteria and numbers of *P. pentosaceus*, and *S. carnosus* cells were determined by serial dilution in 0.1% peptone water and plating in duplicate on Trypticase soy agar, MRS agar, and mannitol salt agar (BBL), respectively, using a spiral plater (Autoplate 4000; Spiral Biotech, Norwood, MA). *E. coli* O157:H7 numbers were obtained by spiral plating of the diluted samples on sorbitol MacConkey agar (BBL) modified with a cefixime tellurite supplement (Oxoid, Hampshire, England) to yield the medium CT-SMAC (35). All plates were incubated at 37°C for 24 to 48 h aerobically, except for *P. pentosaceus* plates, which were incubated at 45°C anaerobically using the BBL GasPak system. In order to determine if *E. coli* O157:H7 cells were killed or injured in the dry

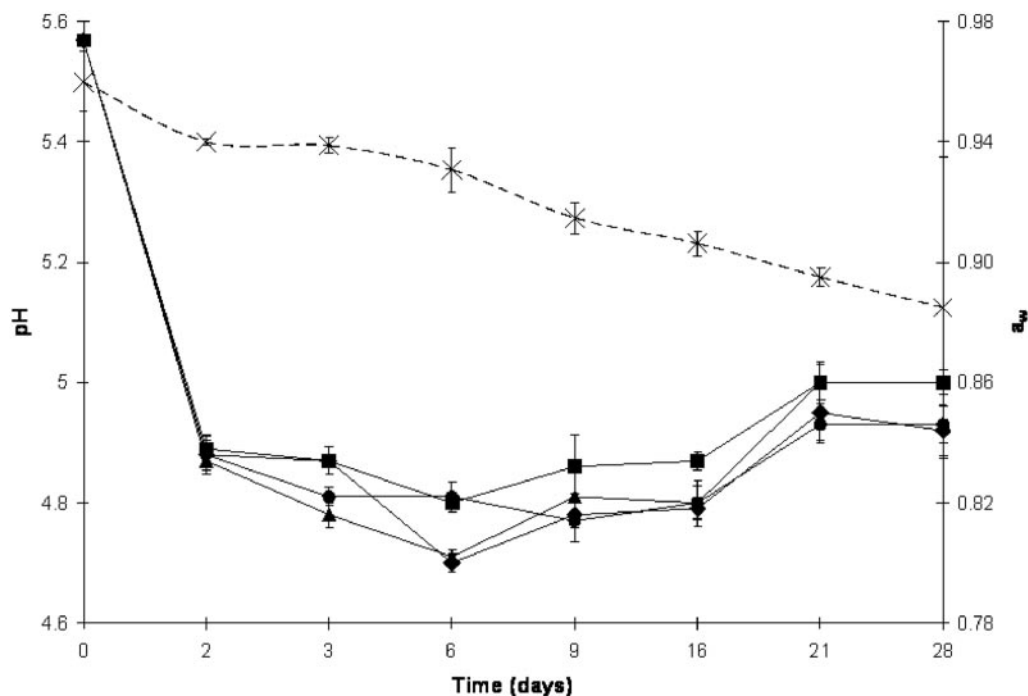


FIG. 1. Changes in pH and a_w of salami during fermentation (first 3 days) and drying (next 25 days) after treatment with different concentrations of microencapsulated AIT. ◆, control salami pH; ●, salami pH with 500 ppm of AIT; ▲, salami pH with 750 ppm of AIT; ■, salami pH with 1,000 ppm of AIT; ×, a_w averages for all treatments. Error bars represent standard deviations.

fermented sausage samples as a result of AIT exposure, an additional 25 g of meat from samples showing no growth after AIT treatment was examined for viable *E. coli* O157:H7 cells after 24 h of resuscitation in 225 ml TSB, using an immunomagnetic separation (IMS) technique (Dynabeads anti-*E. coli* O157:H7; Dynal, Oslo, Norway) performed according to the manufacturer's instructions. Fifty microliters of the IMS complex was spread onto CT-SMAC plates and incubated at 37°C for 24 h. Presumptive *E. coli* O157:H7 colonies from CT-SMAC plates were confirmed to be *E. coli* by using API 20E biochemical test strips (bioMérieux Vitek, Inc., Hazelwood, MO).

Chemical analysis. (i) **Water activity and pH.** The pHs of triplicate samples were recorded using a pH meter (Sentron Titan pH meter) equipped with a Lancefet probe inserted directly into cross-sectioned samples of sausages. Triplicate measurements were taken and averaged. Water activity was analyzed in triplicate (Novasina AW Sprint TH 500 a_w -measuring unit; Axair AG, Pfäffikon, Switzerland).

(ii) **Determination of percent fat in sausages.** The Soxhlet method (3) was used for crude fat determination. Three replicates of each treatment of salami were placed in the freeze-dryer for 72 h. Five grams of freeze-dried salami was placed into thimbles and covered with glass wool. Thimbles were placed inside the extracting tubes. Flat-bottom flasks (250 ml) containing boiling chips were treated at 125°C for 30 min in a drying oven. Subsequently, the flasks were cooled inside a desiccator and then weighed on an analytical balance. One hundred milliliters of hexane was added to each flask. The extracting tubes were assembled with the flasks, and the Soxhlet system was refluxed for approximately 16 h. Subsequently, flasks were evaporated to dryness in a heating mantle and then placed inside the oven for 1 h at 100°C. Following heating, flasks were placed in a desiccator for 1 h and then weighed. The fat content in the salami was calculated using the following equation: percent crude fat = (grams crude fat/grams sample) × 100.

Dry fermented sausage manufacture for sensory evaluation. Three separate 10-kg batches of salami were prepared using the same formulation and manufacturing conditions as used for the salami pathogen inoculation experiment, but no *E. coli* O157:H7 was used. To two batches of salami either 1.11% or 1.66% (wt/wt) microcapsules (containing 45.16 mg of AIT/g of microcapsules) were added to yield 500 or 750 ppm AIT in the sausages. A third batch, identified as control salami, did not have any microencapsulated AIT added. Sausages containing 1,000 ppm AIT were not included in the sensory study because prelim-

inary tests indicated that these samples had a bitter, strong flavor which could generate bias against the other AIT-treated samples among panelists.

Sensory evaluation. A consumer taste panel was used to compare the sensory quality of sausages containing different levels of microencapsulated AIT with that of control salami. A group of 51 untrained volunteers who consumed fermented sausages at least four times a year comprised the sensory panel. The sensory analysis facility in the Department of Food Science was used, and panelists made their evaluations in private sensory booths. Panelists were given three slices of numerically identified, blindly coded sausage samples from the different treatments (control, AIT at 500 ppm, and AIT at 750 ppm) and asked to evaluate them for overall acceptability, appearance, flavor, and texture on a nine-point Hedonic scale, with 9 being extremely good and 1 being extremely poor (33). On the questionnaire, panelists were asked whether they regularly consumed spicy food and whether they would buy any of the tested sausage samples.

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

RESULTS AND DISCUSSION

Effect of microencapsulated AIT on starter culture performance and on pH and a_w changes of dry fermented sausages. AIT levels ranging from 500 ppm to 1,000 ppm did not significantly affect ($P > 0.05$) the acidification performance of *Pedococcus pentosaceus* compared with the control during salami fermentation. All salami treatments achieved the guidelines for fermentation safety (degree-hour factor) established by Agriculture and Agri-Food Canada (1); that is, the time above 15°C required to reach pH 5.3 was <65 h. The pHs of the different sausage treatments dropped initially from 5.57 to less than 4.89 within 48 h of fermentation at 26 to 24°C. pH values remained steady until the later phases of drying at 16 days,

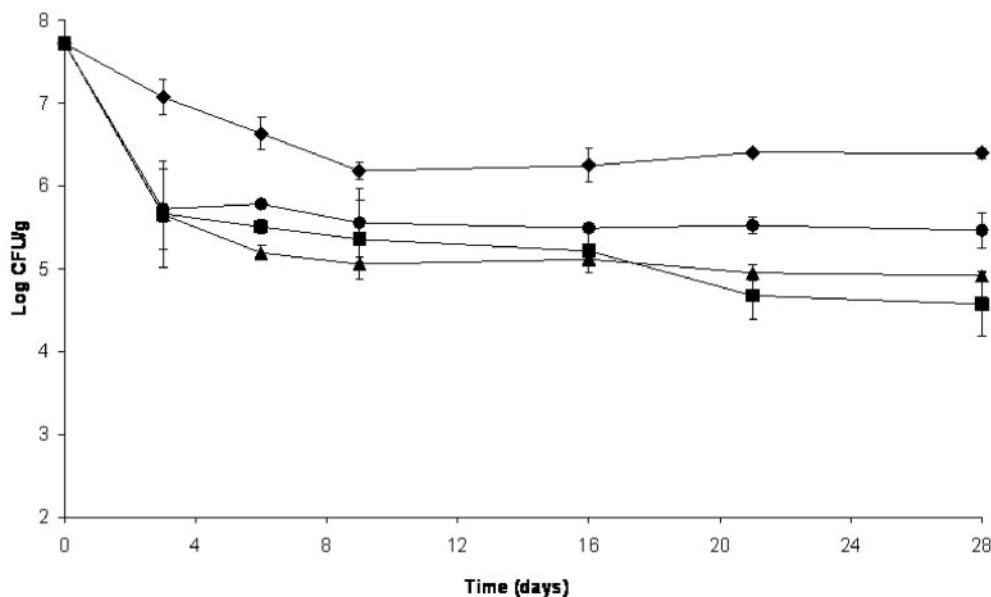


FIG. 2. Effect of different concentrations of microencapsulated AIT on the number of *Pediococcus pentosaceus* cells during fermentation (first 3 days) and drying (next 25 days) of salami. ◆, control salami; ●, salami with 500 ppm of AIT; ▲, salami with 750 ppm of AIT; ■, salami with 1,000 ppm of AIT. Organisms were recovered on MRS agar incubated anaerobically at 45°C. Error bars represent standard deviations.

where a slight increase in the pH was observed (Fig. 1). This is regarded as a normal increase in pH as a consequence of production of ammonia and buffering peptides by bacterial metabolism (10).

The level of *P. pentosaceus* decreased from 7.72 to 6.39 log₁₀ CFU/g in the control salami by the end of drying. Initial numbers of *P. pentosaceus* were significantly reduced to levels close to 5.7 log₁₀ CFU/g in all treatments containing AIT after 3 days of fermentation and thereafter decreased slowly until levels reached from 5.7 to 4.6 log₁₀ CFU/g at the end of drying (Fig. 2). This reduction in the number of these gram-positive bacteria by AIT was expected on the basis of previous evaluations (2, 21), which were confirmed by our preliminary results (data unpublished). *S. carnosus* numbers decreased by ≤2 log CFU/g at the end of drying in all treatments (Fig. 3). A reduction in the number of *Staphylococcus* cells has been reported by several researchers (12, 18), and it is considered normal in the processing of dry fermented sausage.

The *a_w* of control sausage dropped from 0.960 ± 0.010 at the beginning of fermentation to 0.885 ± 0.050 by the end of 28 days of processing. No significant differences in *a_w* were found among the control salami and the three treatments containing 500, 750, or 1,000 ppm of AIT, and therefore the mean *a_w* value is presented for all treatments (Fig. 1).

Antimicrobial effects of microencapsulated AIT on *E. coli* O157:H7 inoculated in dry fermented sausages. The antimicrobial effects of microencapsulated AIT on *E. coli* O157:H7 inoculated at 6.45 log₁₀ CFU/g in dry fermented sausage are shown in Fig. 4. At these *E. coli* O157:H7 numbers, 1,000 and 750 ppm of AIT in the salami batter reduced *E. coli* O157:H7 to undetectable levels (<1 CFU/25 g meat) after 16 and 21 days of processing, respectively. In dry fermented sausages treated with 500 ppm of AIT, *E. coli* O157:H7 numbers were reduced (*P* < 0.05) by 4.75 log₁₀ CFU/g at the end of 28 days

of processing. An extension of the drying time showed that after 40 days, *E. coli* O157:H7 numbers were reduced by 6.5 log₁₀ CFU/g in sausage samples containing 500 ppm AIT. *E. coli* O157:H7 was able to survive 45 days of processing in the untreated salami, with a reduction in its numbers of only 2.4 log₁₀ CFU/g. *E. coli* O157:H7 not only showed its high acid tolerance but also survived exposure to an *a_w* level of 0.87.

The antimicrobial effect of gaseous AIT against *E. coli* O157:H7 has been broadly studied in meat systems; however, there is not much information about the antimicrobial potency of this compound used in its liquid or microencapsulated liquid state. Chacon et al. (6) successfully used microencapsulated AIT powder for decontamination of *E. coli* O157:H7 from ground beef. They found that 2,828 ppm of encapsulated AIT reduced *E. coli* O157:H7 by 2.7 log₁₀ CFU/g in inoculated ground beef stored at 4°C for 18 days. High concentrations of AIT (close to 5,000 ppm) were needed to eliminate initial *E. coli* O157:H7 levels of 8 log₁₀ CFU/g from the ground meat. In the present experiments it was found that 1,000 ppm of microencapsulated AIT was able to eliminate 6.45 log₁₀ CFU/g *E. coli* O157:H7 from sausages in 16 days. It is probable that factors such as the presence of ≥3% NaCl, a pH of <5.3, lower *a_w* (0.88, compared with 0.96 in ground beef), ≤200 ppm nitrite, and a higher storage temperature (13°C) may have contributed to the greater effectiveness of AIT against *E. coli* O157:H7 in the sausages than in ground beef. In addition, the antimicrobial activity of microencapsulated AIT may be enhanced by the higher fat concentrations in the fermented sausages, which were 33 to 35%, in comparison with the <5% fat in the ground beef. Hasegawa et al. (15) reported similar augmentation of AIT antimicrobial activity when they challenged *Vibrio parahaemolyticus* in tuna containing 20.8 or 0.04% fat. They found that *V. parahaemolyticus* was inhibited significantly better in fatty than in lean tissue suspensions,

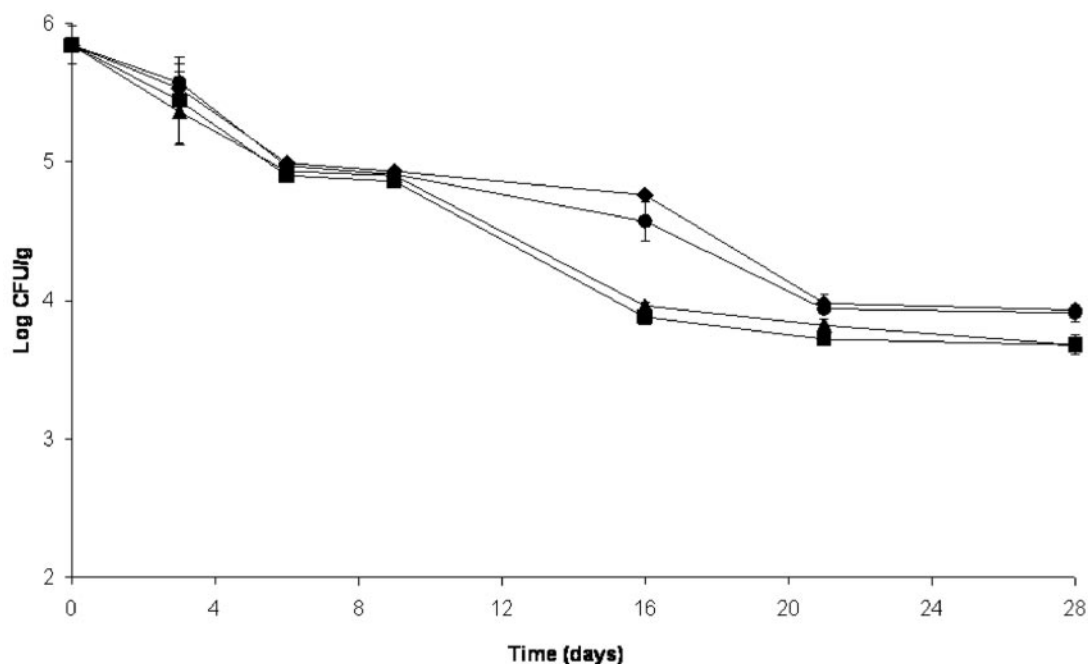


FIG. 3. Effect of different concentrations of microencapsulated AIT on the number of *Staphylococcus carnosus* cells during fermentation (first 3 days) and drying (next 25 days) of salami. ◆, control salami; ●, salami with 500 ppm of AIT; ▲, salami with 750 ppm of AIT; ■, salami with 1,000 ppm of AIT. Organisms were recovered on mannitol salt agar incubated at 37°C. Error bars represent standard deviations.

perhaps as a consequence of partial AIT dissolution in the fatty acids of the tuna meat. Nadarajah (25) reported that the high levels of *cis*-oleic acid in beef fat may complex with the AIT molecules, producing a reduction in volatilization of AIT. Additionally, the high fat (low moisture) content of sausages has

been reported to significantly reduce survival of *E. coli* O157:H7 in such products (13).

A large reduction of *E. coli* O157:H7 numbers occurred during the first 3 days of fermentation in all treatments as a consequence of the rapid drop in pH. However, the reduction

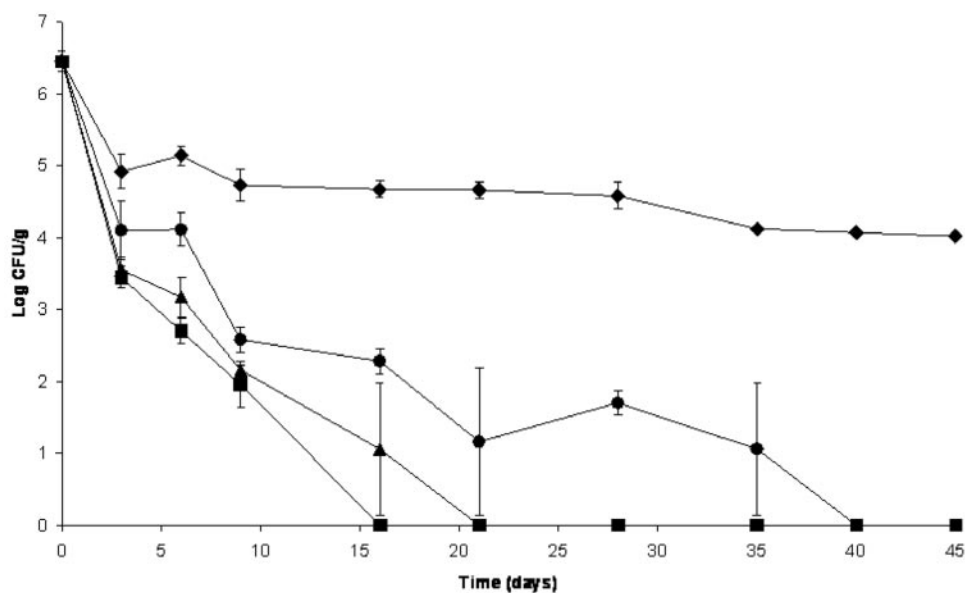


FIG. 4. Survival of *Escherichia coli* O157:H7 treated with different concentrations of microencapsulated AIT during fermentation (first 3 days) and extended drying (next 42 days) of salami. ◆, control salami; ●, salami with 500 ppm of AIT; ▲, salami with 750 ppm of AIT; ■, salami with 1,000 ppm of AIT. Organisms were recovered on CT-SMAC agar incubated at 37°C. IMS was performed for each presumptively negative sample after 24 h of enrichment in TSB at 37°C. Error bars represent standard deviations.

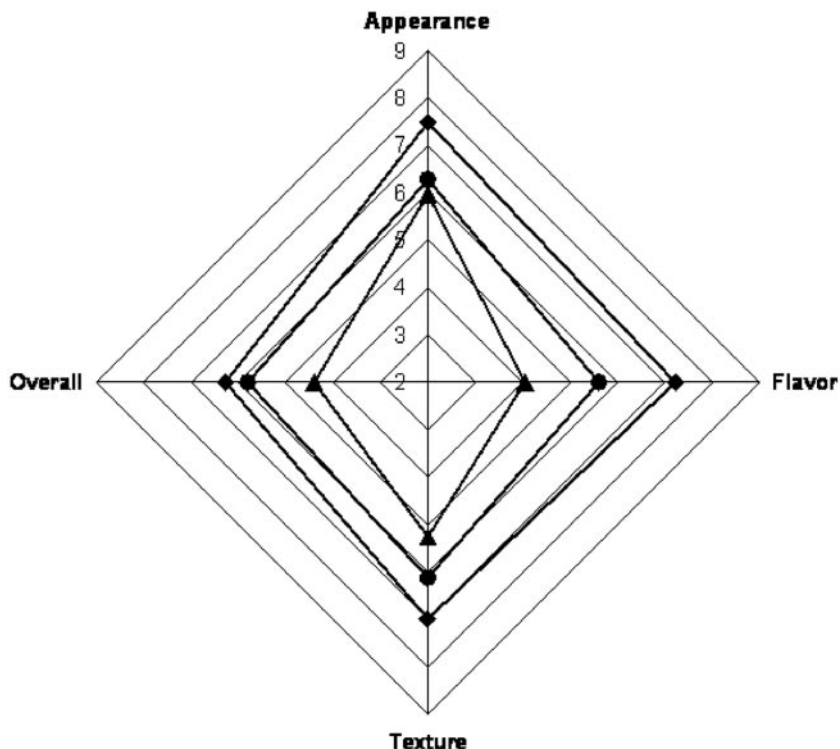


FIG. 5. Sensory analysis of dry fermented sausages at the end of ripening (nine-point Hedonic scale, where 9 and 1 represent “extremely good” and “extremely poor,” respectively, and a score of 6 represents “like slightly”). ◆, control salami; ●, salami with 500 ppm of AIT; ▲, salami with 750 ppm of AIT.

of *E. coli* O157:H7 viability continued progressively in treatments containing microencapsulated AIT until the organism was eliminated. This extended reduction was explained by Chacon et al. (6) as a result of the continued AIT release from microcapsules in the presence of elevated relative humidity.

Effects of microencapsulated AIT on the total bacterial numbers in dry fermented sausages. The effect of microencapsulated AIT on the total numbers of viable bacteria in dry fermented sausages was minimal. Numbers of organisms recovered from treatments showed the same profiles as recorded for *P. pentosaceus* in Fig. 2. In the untreated control, the total bacterial numbers decreased from an initial level of 7.9 log₁₀ CFU/g to 6.9 log₁₀ CFU/g at 28 days of processing. This reduction in the total number could be related in part to the reduction of *E. coli* O157:H7 in the control treatment but is more likely related to a gradual reduction of *P. pentosaceus* viability during sausage maturation, since the latter organism was the predominant component of the microflora. In treatments containing microencapsulated AIT, the total bacterial numbers decreased to levels ranging from 5.2 to 6.0 log₁₀ CFU/g at 28 days of processing, and these levels were lower than those in the control without AIT. It is notable that gram-positive lactic acid bacteria are the predominant component of the microflora recovered from fermented sausages on nonselective media (17) and that gram-positive bacteria, including the lactic acid bacteria, are more AIT tolerant than gram-negative bacteria (30, 32). In the present work, while AIT reduced the viability of the *Pediococcus* starter culture to some extent, there was no significant effect on the rate of meat

acidulation (which is primarily due to lactic acid production by this component of the starter culture mixture).

Sensory evaluation. Fermented sausages containing 1,000 ppm AIT were prescreened and found to have a strong and somewhat bitter mustard flavor. To avoid introducing bias in tests, sausages with 1,000 ppm AIT were not presented to panelists. The addition of microencapsulated AIT at 500 or 750 ppm significantly affected the sensory attributes (flavor, appearance, texture, and overall impression) of dry fermented sausages measured (Fig. 5). The appearance of the control salami received a significantly (*P* < 0.05) better score than that of those treated with 500 or 750 ppm of AIT, probably because of slight yellow color development and more brittle texture in some AIT-treated samples. Texture differences, which were apparent in samples treated with 750 ppm AIT but absent from sausages treated with 500 ppm AIT, were probably due to the inclusion of 1.69% (wt/wt) gum acacia in the former samples. The appearance of the control salami was scored by the panelist as “like moderately,” while the appearance of the salami from the AIT treatments was scored as “like slightly.” Significant differences were found in the flavor, texture, and overall impression of all sausages tested, where the scores for the control and the 500 ppm AIT and 750 ppm AIT treatments were “like moderately,” “like slightly,” and “dislike slightly,” respectively. Ninety percent of the panelists ate spicy food at least four times per year. This preference for spicy food had a positive impact on the acceptability of samples containing microencapsulated AIT at the lowest concentration (which yielded a slightly spicy sensation). Clark (7) noted that allyl

isothiocyanate has been used as a flavor fortifier when a sharp spice effect is needed to simulate the mustard or horseradish taste. The spicy effect in treatments containing 500 ppm was acceptable to the panelists, and 41% indicated that they would buy this product. However, only 13% of the panelists indicated that they would buy dry salami containing 750 ppm AIT.

Microencapsulated AIT did not affect meat acidulation by the starter cultures used in the manufacture of dry fermented sausages. AIT at 1,000 and 750 ppm eliminated $>6 \log_{10}$ CFU/g of *E. coli* O157:H7 in sausages after 16 and 21 days of processing, respectively. The 5- \log_{10} -unit reduction of *E. coli* O157:H7 necessary to validate the process of dry fermented sausage production (28) was nearly achieved (reduction of 4.75 \log_{10} CFU/g) at 28 days of processing using 500 ppm AIT, and this treatment was successful in eliminating $>6 \log_{10}$ CFU/g *E. coli* O157:H7 at day 40. It is possible that microencapsulated AIT at <500 ppm could eliminate 5 \log_{10} CFU/g *E. coli* O157:H7 if sausages were dried for ≥ 40 days at 13°C. The total bacteria enumerated were mainly lactic acid bacteria, which were slightly inhibited at the end of drying by the AIT-containing microcapsules.

Sausages containing 500 ppm AIT were the most acceptable AIT-treated sausages as assessed by the sensory panel, and these sausages could be marketed as a safe specialty product such as a hot salami. Sausages treated with 750 ppm of AIT had a strong (pungent) flavor and odor which were unacceptable to many of the panelists.

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