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Evaluation of antilisterial action of cilantro oil on vacuum packed ham

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Abstract

Cilantro oil is an essential oil preparation extracted from the plant *Coriandrum sativium*. A series of experiments were conducted to evaluate the ability of cilantro oil to control the growth of *Listeria monocytogenes* on vacuum-packed ham. The *in vitro* minimal inhibitory concentration for five strains of *L. monocytogenes* was found to vary from 0.074% to 0.018% depending on strain. Cilantro oil treatments were then tested on ham disks inoculated with a cocktail of the five *L. monocytogenes* strains. The treatments studied were 0.1%, 0.5%, and 6% cilantro oil diluted in sterile canola oil or incorporated into a gelatin gel in which lecithin was used to enhance incorporation of the cilantro oil. Gelatin gel treatments were also conducted with 1.4% monolaurin with or without 6% cilantro oil to determine if an interaction between the antimicrobials could increase inhibition of *L. monocytogenes*. Treated ham was then vacuum-packed and stored at 10 °C for up to 4 weeks. The only cilantro oil treatment which inhibited growth of *L. monocytogenes* on the ham samples was 6% cilantro oil gel. Samples receiving this treatment had populations of *L. monocytogenes* 1.3 log CFU/ml lower than controls at week 1 of storage, though there was no difference between treatments from week 2 onward. It appears that immobilization of the antimicrobial in a gel enhanced the effect of treatments. Cilantro oil does not appear to be a suitable agent for the control of *L. monocytogenes* on ham. The possible reasons for reduced effectiveness of cilantro oil against *L. monocytogenes* on ham are discussed.

Keywords: *Listeria monocytogenes*; Ham; Cilantro; Antimicrobial; Shelf life

1. Introduction

Spices have been used as food preservatives throughout history and the antimicrobial activity of specific spices was documented as early as the late 19th century (Boyle, 1955). However, these compounds have received surprisingly little study as antimicrobials until comparatively recently (Shelef, 1983). In the late 1970s, there was a renewal of interest in the antimicrobial properties of spices as a consequence of changes in consumer attitudes to the use of agents such as nitrates and NaCl in foods (Shelef, 1983).

Cilantro oil is an essential oil preparation extracted from cilantro or coriander, *Coriandrum sativium.* Cilantro is used in food preparation in the Middle East

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and Southeast Asia and is ascribed medicinal properties in China (Brown, 1995). Cilantro oil contains a complex mixture of organic molecules, with C_{10} to C_{12} aldehydes predominating (Potter, 1996). Since it has been has reported that significant quantitative differences exist between samples of cilantro at different growth stages (Potter, 1996), the composition of the cilantro oil used in these experiments was determined by gas chromatography/mass spectroscopy.

The ability of spices to inhibit the growth of microorganisms is well established. The essential oils of spices have been reported to inhibit the growth of a wide range of pathogenic or food spoilage bacterial species (Aureli et al., 1992; Sivropoulou et al., 1995; Ouattara et al., 1997). Specific essential oil components have also been demonstrated to inhibit a wide range of bacterial species (Moleyar and Narasimham, 1992; Jay and Rivers, 1984).

The antimicrobial mechanism of spices remains unclear. Most reviews of the topic ascribe the effect to disruption of the cell membranes, leading to depletion of cellular energy by lipophilic molecules (Shelef, 1983; Conner, 1993). Recent studies suggest that the mechanism of action of spice components may be more complex. While searching for chemicals that could reduce the formation of biogenic amines in foods, Wendakoon and Sakaguchi (1995) found that spice oils can inhibit enzyme activity. Ethanol extracts of cloves, cinnamon, sage, nutmeg and allspice were observed to inhibit the enzymatic activities of histidine, lysine and ornithine decarboxylases in a crude extract of *Enterobacter aerogenes*. Eugenol and cinnamaldehyde, major components of the spice oils, were demonstrated to inhibit histidine decarboxylase (Wendakoon and Sakaguchi, 1995). Helander et al. (1998) studied the effects of spice essential oil components, carvacrol, carvone, thymol and cinnamaldehyde on *Escherichia coli* O157:H7, *Salmonella typhimurium* and *Photobacterium leiognathi*. Analysis of intracellular and extracellular ATP pools by luciferase analysis and release of cytoplasmic proteins indicated that carvacrol and thymol disrupt the cell's membranes. However, membrane disruption did not appear to be involved in growth inhibition by cinnamaldehyde and carvone.

Blaszyk and Holley (1998) previously reported that monolaurin glycerol may interact with the spice component eugenol to increase inhibition of meat spoilage and pathogenic bacteria, including *Listeria monocytogenes* at 250 ppm, in broth media. The addition of monolaurin glycerol to agar plates has been reported to reduce the tolerance of *L. monocytogenes* to salt and low pH (Farid et al., 1996). Inhibition of *L. monocytogenes* growth by monolaurin glycerol has been reported in beef roasts (Stillmunkes et al., 1993) and beef and turkey frank slurries (Wang and Johnson, 1997).

In this paper, we describe studies evaluating the use of cilantro oil to control the growth of *L. monocytogenes* on ham. Since microbial growth occurs on the surface of processed meat products such as ham, we developed treatments that can be applied to the meat surface. The application of surface treatments to ham has been previously shown to enhance the effectiveness of lysozyme and nisin-based treatments (Gill and Holley, 2000). Due to the hydrophobic nature of the cilantro oil, the surface treatments were applied by using either a vegetable oil or a gelatin gel containing lecithin as an ampiphilic binding molecule. Lecithin was supplied in the form of powdered egg yolk or 40% soya bean lecithin. In one set of experiments, monolaurin glycerol was added to a gel containing cilantro oil to determine whether interactions with cilantro oil components could increase the antimicrobial effect.

2. Materials and methods

2.1. Cultures and incubation conditions

Experiments were conducted using five *L. monocytogenes* strains. These strains are meat plant isolates provided by Dr. M.W. Griffiths, University of Guelph, Guelph, ON. The Canadian Research Institute for Food Safety culture collection numbers for the strains are C716, C717, C718, C719 and C720. All the strains were identified as somatic serotype 1. C718, C719 and C720 have identical genetic fingerprints as determined by pulsed field electrophoresis using *Apa*1 and *Sma*1 restriction enzymes. C716 and C717 are unrelated to each other and the other three (Griffiths, 2001). Stock *L. monocytogenes* cultures were frozen at -85 °C in glycerol. For experimental use, each strain was streak-plated on BHI agar. Cultures for experiments were prepared by inocula-

tion of a single colony into BHI broth and incubated overnight at 30 °C. Selective enumeration of *L. monocytogenes* was conducted by spread plating on modified Oxford (MOX) agar plates incubated for 48 h at 24 °C.

2.2. Materials

Commercially prepared chopped ham mix (12.5% meat protein, pork, water, salt, sugar, dextrose, sodium phosphate, carrageenan, sodium erythorbate and sodium nitrite) was kindly provided by Maple Leaf Meats (Winnipeg, MB). Pasteurized dried egg yolk was provided by Canadian Inovatech (Winnipeg, MB). Cilantro oil was prepared by hydrodistillation of dried cilantro leaves and stems in a custom built, proprietary column. Lecithin (L-α-phosphatidyl-choline) and monolaurin glycerol (1-monolauroyl-rac-glycerol) were obtained from Sigma (St Louis, MO). Sausage casings (hog intestine, 30-mm diameter, Canada Compound Western, Winnipeg, MB) were stuffed with meat batter using a 9-l capacity mechanical sausage stuffer (F. Dick, Germany). Knox Gelatin (Tomas J. Lipton, Toronto, ON) and Canola Harvest, 100% canola oil (Canbra Foods Lethbridge), were used where required. Low oxygen permeable (2.3 cm^3/m^2/day atm 23 °C) polyethylene-nylon laminated plastic bags (Deli * 1) and (0.7 cm^3/m^2/day atm 23 °C) metal foil laminate bags (MESE 1250 R) were kindly provided by Winpak (Winnipeg, MB). Standard methods agar (SMA), BHI broth, granulated agar, proteose peptone No. 3, Oxford medium base and modified Oxford antimicrobic supplement (colistin sulfate 10 mg/l, moxalactam 20 mg/l) for MOX were all obtained from Difco, Becton-Dickinson (Franklin Lakes, NJ). An Autoplater 4000 equipped with a CASBA-4 automated counting system was used for surface plating on pre-poured agar media and enumeration (Spiral Biotech, Bethesda, MD). The anaerobic incubator (model 3640-6) was from the National Appliance (Portland, OR). Anaerobic conditions were created by flushing twice with 30% CO_2/70% N_2. A model GM 2002 vacuum packaging machine (Bizerba, Mississauga, ON) was used to vacuum package and seal pouches. A Tekmar LSC 2000 purge and trap unit supplied by Tekmar (Cincinnati, OH) was used to sample headspace gas composition for essential oil volatiles.

2.3. Essential oil analysis

Diluted oil aliquots (1 μl) were injected into a gas chromatograph (Model HP 5890, Model HP 3396A integrator) equipped with an autosampler (model 7673A, Hewlett Packard, Avondale, PA). Components were separated on a Supelcowax 10 fused silica capillary column (60-m length × 0.25-mm i.d. × 0.25-μm film thickness) with helium and nitrogen as carrier and make-up gases, respectively. Column headpressure was maintained at 207 kPa (30 psi) and the injector split ratio was adjusted to 20:1. The injector and detector (flame ionization) temperatures were both set at 250 °C; the oven was programmed to increase from 35 to 200 °C at a rate of 3 °C/min, followed by a final hold time of 10 min.

Mass spectra were recorded with an HP 5890-5970 GC–MSD system. The mass spectrometer was operated with an ion source at 250 °C, ionizing energy of 70 eV, scan range of 25–250 amu, threshold at 400, and a frequency of 2.6 scans/s. Column and temperature programming for separation on the gas chromatograph was similar to that described above. Eluted compounds were identified using HP G1034C MS ChemStation software containing a HP G1035A Wiley (138.1) PBM library. Confirmation of several compounds was accomplished by comparing retention times with reference standards (Aldrich Chem., Milwaukee, WI).

2.4. Determination of minimum inhibitory concentration

The minimum inhibitory concentration (MIC) of cilantro oil was determined for the individual *L. monocytogenes* strains by the resazurin indicator method of Mann and Markham (1998). The protocol of Mann and Markham was modified by conducting experiments in BHI broth with 0.15% agar at 24 °C, with an initial inoculum of 5.3 log CFU/ml^2. Eight replicates were prepared simultaneously in 96-well microtitre plate wells.

2.5. Preparation of ham sausage

A single batch of raw meat batter obtained from the manufacturer was stored at − 20 °C until needed. The batter was thawed and casings were soaked in distilled

water to remove salt for 24 h at 4 °C prior to processing. Ham sausages were prepared by filling batter into the casings with the stuffer. The sausages (approximately 30 cm) were clipped with a metal ring and cooked to an internal temperature of 69 °C in a jacketed steam kettle for three 20-min intervals at 53, 64, and 75 °C. The cooked sausages were then vacuum-packed in heat-sealed O_2 barrier plastic bags and frozen at −20 °C.

Ham sausage was thawed overnight at 4 °C and was cut into 14-mm-thick disks on a sterile board using a flame-sterilized knife. This produced discs of meat 29–31 mm in diameter and 14-mm thickness, with a surface area (maximum) of 28.73 cm^2, weighing 10 ± 1 g. Three disks were prepared for each treatment group and for each sampling time point.

2.6. Preparation of inoculum for ham experiments

A single colony of each of the five strains of *L. monocytogenes* was transferred to 9 ml of BHI broth, which was incubated aerobically overnight at 30 °C. A dipping bath for inoculation of the samples with *L. monocytogenes* was prepared by adding 0.3 ml of each of the five cultures to 1.5 l of 0.1% peptone. This produced a five-strain cocktail of *L. monocytogenes* that yielded an initial population of approximately 4 log CFU/cm^2 on the inoculated ham.

2.7. Sampling for ham experiments

Three coins were removed from the packages at each sampling time point, were placed separately in a stomacher bag with 90 ml of 0.1% peptone and blended for 2 min (Stomacher 400, A.J. Seward, Canlab, Toronto, ON). The mixed samples were then serially diluted to produce dilutions ranging from 10^{-3} to 10^{-5}. Each dilution was plated in duplicate on modified oxford media (MOX) and SMA using the spiral plater.

The MOX plates were incubated at 24 °C aerobically for 48 h to provide selective enumeration of *L. monocytogenes*. The SMA plates were incubated anaerobically for 48 h at 24 °C to provide an estimate of the total anaerobic number of bacteria (TAN). The number of CFU/ml per sample was converted to CFU/cm^2 of sample surface.

2.8. Treatment of ham sausage with cilantro oil in canola oil

The meat coins were inoculated by a 30-s immersion in the bacterial cocktail using a 21-cm diameter stainless steel mesh basket with 2-mm holes. The meat coins were then allowed to dry on sterile filter paper in a laminar flow hood for 15 min prior to treatment with cilantro oil.

Canola oil sterilized by autoclaving was used for dilution of the cilantro oil. The meat coins were apportioned to receive the following treatments: no further treatment (control), dipping in sterile canola oil, and dipping in 0.1%, 0.5% or 6.0% (v/v) cilantro oil. To apply the oil coating, the inoculated meat pieces were dipped with flame-sterilized tweezers into the oil for 5 s. Excess oil was allowed to drain for 15 s and the sample was immediately placed into packaging bags.

Three coins from each sample group were retained as time zero samples. These samples were stored at 4 °C and analyses were started within 4 h. The remaining samples were divided into groups of three for each time point and vacuum-packed in heat-sealed, polyethylene-nylon plastic pouches. The samples were incubated in their sealed pouches at 10 °C for up to 4 weeks after inoculation. Packaging of the 6% (v/v) cilantro oil-treated samples differed. Vacuum packages were placed in gasket-sealed 1-l glass Mason jars to limit changes in headspace volatile composition that could result from gas penetration in or diffusion through the packaging film.

2.9. Treatment of ham sausage with cilantro oil in gelatin gel

The ham disks received the following treatments prior to inoculation: no further treatment (control), coating with 7% (w/v) gelatin gel containing egg yolk, and coating with 7% (w/v) gelatin gel containing egg yolk with 0.1% or 0.5% (v/v) cilantro oil.

The gelatin gel was prepared as follows: an emulsion containing 1 g pasteurized dried egg yolk with 3 ml sterile distilled water was prepared by mixing for 5 min in a sterile beaker with a sterile magnetic mixing bar. Cilantro oil (0.1 or 0.5 ml) was added to the egg yolk emulsion as appropriate and mixed for a further 10 min. The egg yolk emulsion was then added to a 7% gelatin solution in boiling sterile water. The ham disks

where then treated by dipping into the gelatin solution. The gel was set by placing the coated disks on a sterile grill at 4 °C for 5 min before a second dipping in the gel followed by setting for a further 10 min at 4 °C.

The treated ham disks were inoculated as described above. Time zero samples were put aside for immediate sampling and the remaining samples were vacuum-packed in heat-sealed, polyethylene-nylon barrier bags. The samples were incubated in their pouches at 10 °C for up to 4 weeks after inoculation.

2.10. Treatment of ham sausage with cilantro oil and monolaurin in gelatin gel

In a refinement of the preparation of the gelatin gel, pure lecithin was substituted for powdered egg yolk in these experiments. The gelatin gel was prepared with 7% (w/v) gelatin and 2 g lecithin. Four treatment gels were prepared, gel without antimicrobials (control), gel with 6% (v/v) cilantro oil, or 1.40% (w/v) monolaurin glycerol (dissolved in ethanol, 0.3 g/ml) or gel with both 6% cilantro oil and 1.40% monolaurin glycerol. The 6% (v/v) cilantro oil or 1.40% (w/v) monolaurin glycerol were equivalent to approximately 1000 and 250 ppm, respectively, per ham disk with an average 0.2 g coating of gelatin per 10 g ham sample.

The treated ham disks were inoculated as described above. Time zero samples were put aside for immediate analysis and the remaining samples were vacuum-packed in heat-sealed, foil laminate (MESE 1250 R) plastic pouches. The samples were incubated in their bags at 10 °C for up to 4 weeks after inoculation.

Table 1
Major components of cilantro oil separated by gas chromatography and identified by mass spectroscopy

Compound	Percentage (v/v)
Nonane	2.54
α-Pinene	2.74
p-Cymene	3.51
Linalool oxide	1.52
Decanol	8.42
Camphor	1.85
Linalool	25.86
Terpinene	1.09
(*E*)-2-Decenal	20.22
Carvone	1.20
1-Decanol	3.88
(*E*)-2-Decen-1-ol	7.90
(*E*)-2-Dodecenal	3.72
Octanoic	1.69
Minor components	13.86

Table 2
Minimal inhibitory concentrations of cilantro oil as determined for *L. monocytogenes* strains in BHI broth at 24 °C

Strain	MIC (v/v)[a]	Proportion[b]
C716	0.018	3
C717	0.074	4
C718	0.074	6
C719	0.037	7
C720	0.037	3

[a] Minimal inhibitory concentration, no growth observed in any replicates.

[b] Proportion of replicates, out of eight, with growth at next serial dilution after MIC.

2.11. Data treatment

The data generated from this experiment was analyzed to determine if the presence of cilantro oil in the oil or gel-coated samples resulted in significantly different bacterial populations of the test microorganism and TAN compared to control and treated samples.

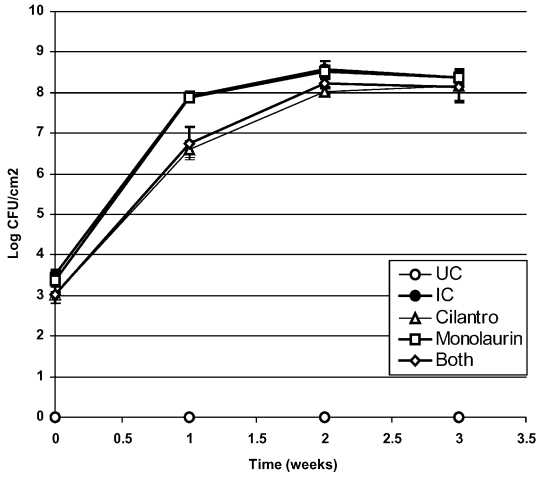

Fig. 1. Growth of *L. monocytogenes* on vacuum-packaged ham at 10 °C, recovered on aerobic modified oxford media. UC—uninoculated control; IC—inoculated control. Cilantro—samples coated with gelatin gel containing 6% cilantro oil. Monolaurin—samples coated with gelatin gel containing 1.4% monolaurin glycerol. Both—samples coated with gelatin gel containing 6% cilantro oil and 1.4% monlaurin. Error bars indicate 1 standard deviation interval.

The data were first screened to determine if there were practical differences between bacterial populations in control and treated samples at each time point during storage. A practical difference in populations was defined as a difference between the means for the control and treatment ≥ 1 log CFU/cm^2. A value of 1 log was chosen for practical significance as differences of one order of magnitude are generally regarded as being of microbial significance (Gill and Baker, 1998; Jarvis, 1989). If the difference of the two means was of practical significance, a two way *t*-test with an $\alpha = 0.05$ was conducted to determine whether the difference between the means was statistically significant. For results to be considered significant, the difference between means of control and treatment were required to meet the conditions of both practical and statistical significance.

2.12. Analysis of the permeability of plastic bags to cilantro oil

Filter paper disks (Whatman #1) were placed in glass petri dishes (50-mm internal diameter). Except for two control dishes, 0.5 ml of cilantro oil was added to the petri dishes. The petri dishes were vacuum packed in 15×15-cm bags composed of either the polyethylene-nylon or foil laminates. Care was taken to avoid contact between the film and oil, and the films were tightly stretched across the open face of the dish. The packaged petri dishes were then placed in 1-l glass Mason jars and sealed with canning jar lids modified to accommodate a septum. The jars were stored at 12 °C.

The headspace volatiles were recovered from each jar after 24 h by a needle introduced through the septum

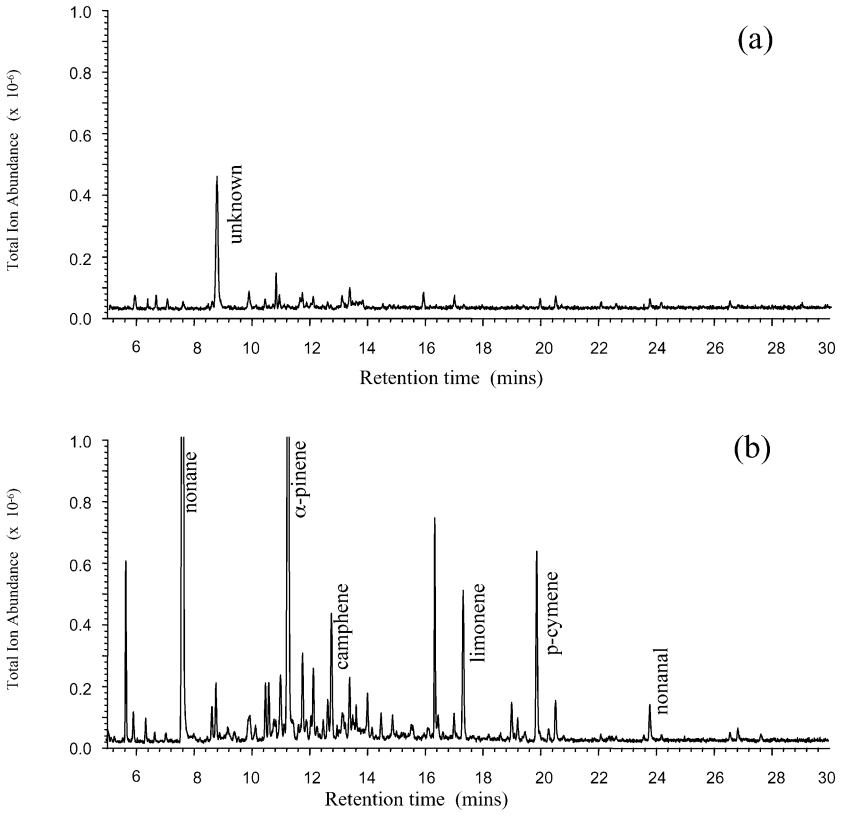

Fig. 2. Gas chromatograms of samples withdrawn from the glass jar headspace above cilantro oil packed in a polyethylene–nylon pouch. a—without cilantro oil. b—with cilantro oil. Analysis of headspace after 1 day in a glass container at 12 °C.

and connected to a Tekmar LSC 2000 purge and trap unit. Using purified nitrogen at a purging rate of 100 ml min^{-1}, volatiles were collected for 5 min on the glass trap. Separation and identification of volatile compounds by gas chromatography and mass spectrometry was done as previously described (Section 2.3).

3. Results

The results of the cilantro oil analysis are presented in Table 1. The two single largest components were linalool at 25.86% and (*E*)-2-decenal at 20.22%.

Minimal inhibitory concentrations of cilantro oil determined by *in vitro* tests were found to depend on the isolate of *L. monocytogenes* tested, with values ranging between 0.074% and 0.018% obtained in BHI broth at 24 °C (Table 2). Since the highest value for MIC was 0.074%, 0.1% cilantro oil was used as the lowest value for ham surface treatment experiments.

No differences in *L. monocytogenes* and total anaerobic populations were observed between untreated controls and samples receiving coating with oil or gel without antimicrobial (results not reported). Cilantro oil applied to ham at concentrations of 0.1%, 0.5% and 6.0% in canola oil had no effect on the growth of *L. monocytogenes* or total anaerobic populations. Treatment of ham with cilantro oil incorporated into a gelatin gel at 0.1% or 0.5% also had no

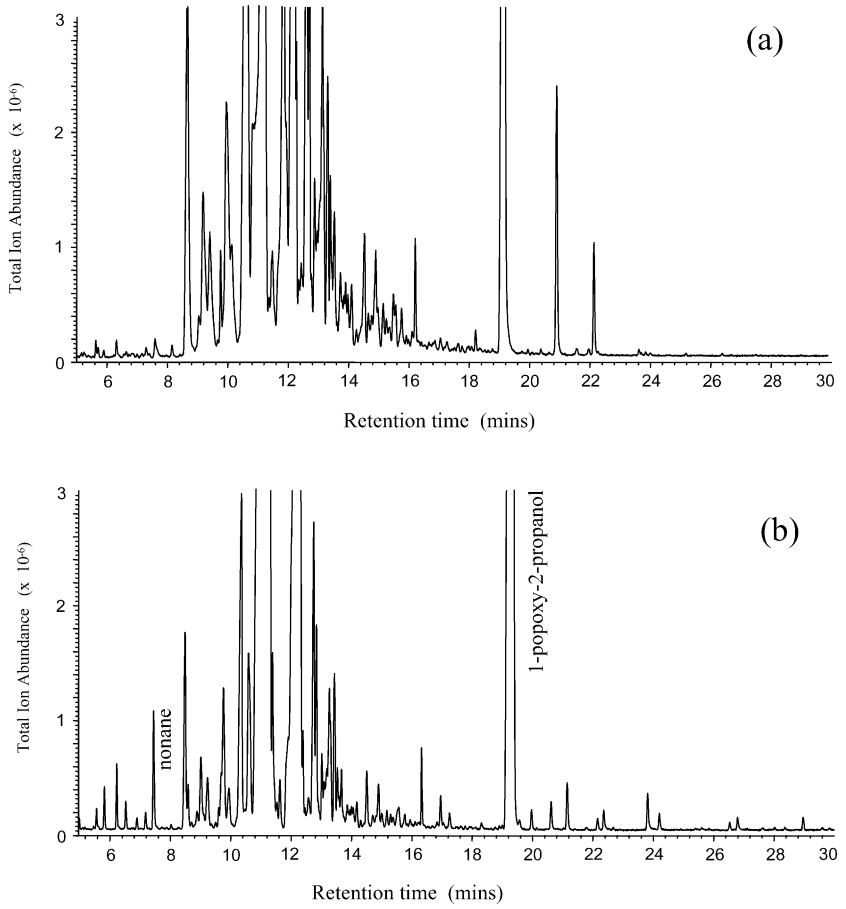

Fig. 3. Gas chromatograms of samples withdrawn from the glass jar headspace above cilantro oil packed in a foil laminate pouch. a—without cilantro oil. b—with cilantro oil. Analysis of head space after 1 day in a glass container at 12 °C.

significant effect on *L. monocytogenes* growth or total anaerobic populations (results not reported).

Coating with a gelatin gel containing 6% cilantro oil emulsified with lecithin was the only treatment found to have any effect on the fate of *L. monocytogenes* on ham (Fig. 1). There was no significant difference between the number of colonies recovered on aerobic MOX or anaerobic SMA media from the samples. Samples treated with 6% cilantro alone or with 1.4% monolaurin glycerol had numbers of *L. monocytogenes* 1.3 log CFU/cm^2 lower than the other treatments at week 1. No significant differences were observed in *L. monocytogenes* populations between control samples and samples treated with 1.4% monolaurin glycerol. There was also no significant difference between samples treated with cilantro oil or both cilantro oil and monolaurin glycerol together. Thus, it appears that treatment with monolaurin glycerol at 1.4% had no effect on *L. monocytogenes*. The experiments were discontinued after week 3 as the numbers of *L. monocytogenes* were greater than 8 log CFU/cm^2 on both control and treated samples.

To determine if the low antimicrobial activity of cilantro oil in the packaged ham experiments was due to diffusion of cilantro oil, the permeability of the polyethylene-nylon and foil laminate films was examined. The polyethylene-nylon film used in these experiments was observed to be permeable to several cilantro oil components (Fig. 2A,B) that were detected by gas chromatography. The release of volatiles from the foil laminate film makes it difficult to determine whether the permeability to cilantro oil components influenced the outcome (Fig. 3A,B). The detection of peaks for nonane and 1-propoxy-2-propanal suggests that the foil laminate was permeable to some cilantro oil components, although this was limited compared to the polyethylene-nylon film.

4. Discussion

The composition of the cilantro oil used in these experiments was considerably different from the composition of two samples as described by Potter (1996). However, the values for the two largest components, (*E*)-2-decenal (20.22%) and linalool (25.86%), fall within the range of reported values (Potter, 1996; Potter and Fagerson, 1990). The composition of cilantro oil is highly dependent upon the portion of the plant used and the stage of growth at harvest (Potter, 1996).

(*E*)-2-decenal has been previously reported to have antimicrobial activity against *E. coli*, *Haemophilus influenzae*, *Moraxella catarrhalis*, *Staphylococcus aureus*, *S. epidermidis*, *Streptococcus pneumoniae* and *S. pyogenes* at concentrations of 500–15.6 μg/ml as measured by disc diffusion assay (Bisignano et al., 2001). Linalool is a major component (greater than 10%) of several essential oils with known antimicrobial action, including cinnamon, pimento, black pepper, oregano and thyme (Ouattara et al., 1997). Moleyar and Narasimham (1992) reported that 1000 μg/ml of linalool slowed the growth of unspecified *Staphylococcus*, *Micrococcus* and *Bacillus* species on solid agar medium. Carvone, a minor component of cilantro oil (1.2%) has also been demonstrated to have an antilisterial effect at a concentration of 5 mM at 45 °C but not at 8 °C (Karatazas et al., 1999). Interestingly, Bisignano et al. (2001) reported that a combination of four olive aldehydes was significantly more effective at inhibiting bacterial growth than any of the aldehydes singly. This suggests that spice oil extracts may be more effective antimicrobials than their purified components. The variation in cilantro oil composition may explain why Aureli et al. (1992) previously reported that cilantro oil had a limited inhibitory effect on the growth of five strains of *L. monocytogenes* as measured by disc diffusion assay.

The MIC determinations conducted in these experiments indicated that cilantro oil inhibited the growth of *L. monocytogenes* in broth media. The effectiveness of the cilantro oil against *L. monocytogenes* was found to vary among strains, with values ranging from 0.074% and 0.018%. Since the difference in MIC between the closely related strains C719, C720 and C718 was a two-fold dilution, it is likely that this difference reflects the sensitivity of the technique used. Nevertheless, this plant extract was found to exert a potent in vitro antimicrobial effect against these species at relatively low concentrations.

Variation in the effectiveness of spices against different serotypes of *L. monocytogenes* has been reported by Hefnaway et al. (1992). The spices sage, allspice, cumin, garlic powder, paprika, and red pepper were all effective against *L. monocytogenes* Scott A (serotype 4b), but only sage was observed to have

any activity against *L. monocytogenes* V7 (serotype 1) (Hefnaway et al., 1992). Since all five of the strains used in our experiments had the same somatic serotype, our results show that nonserotype strain variation can result in a variable response to spice oil extract.

The surface treatments examined in these experiments were found to be ineffective in controlling the growth of the five *L. monocytogenes* strains on ham. Significantly fewer viable cells of *L. monocytogenes* were recovered after 1 week on samples treated with 6% cilantro (with or without monolaurin) immobilized in a surface gelatin gel. It appears that the immobilization of cilantro oil in a surface gel increases the effectiveness of the treatment, an observation in agreement with previous work conducted with other antimicrobials (Gill and Holley, 2000). It is possible that the slight increase in effect could be due to the use of the foil laminate film, which has a lower permeability to cilantro oil components or the presence of bioactive volatile compounds released from the film. The absence of any immediate antibacterial effect (time zero) by cilantro oil or significant effect by 6% cilantro oil in canola oil when packaged in impermeable glass jars suggests that diffusion through the packaging film is not the primary reason for the ineffectiveness of the cilantro treatments.

The ineffectiveness of the cilantro oil treatments when applied to ham may be due to a number of factors. It is possible that the antibacterial cilantro oil components remained partitioned in hydrophobic environments created by lecithin, canola oil or fat in the ham. Thus, it is possible that there was little opportunity for cilantro oil to come into contact with bacterial cells growing on more hydrophilic ham surfaces.

A correlation between fat content and the effectiveness of spices is supported by the observation of Ceylan et al. (1998) that the effectiveness of spices against *E. coil* O157:H7 was considerably greater in agar than ground beef or salami with 20% fat content. Partitioning of spice components into meat lipids was also suggested by Cutter (2000) as an explanation for the ineffectiveness of spice treatments in ground beef against *E. coli*, *L. monocytogenes* and *S. typhimurium*.

Another possibility is that cells attached to the meat surface were in a different physiological state than those in 0.15% agar, which could have rendered them less susceptible to the antimicrobial properties of cilantro oil. The physiological state of bacterial cells has been shown to affect the activity of spice components. In a study of the response of *L. monocytogenes* to combined carvone and mild thermal treatment (45 °C), Karatazas et al. (1999) found that the treatment had a bactericidal effect resulting in a reduction of 2 log CFU/ml on exponential phase cells grown at 8 °C. The same treatment had no effect on stationary phase cells grown at 8 °C or on cells from either phase grown at 35 °C (Karatazas et al., 1999).

A number of different physiological changes may be proposed to explain the increased resistance of bacterial cells localized at the surface of meats. One possibility is that membrane composition is altered by changes in temperature and growth rates, resulting in altered interaction with the antimicrobial. Ming and Daeschel (1995) observed that nisin resistant strains of *L. monocytogenes* Scott A had significantly lower levels of the phospholipids phosphatidylglycerol, diphosphatidylglycerol and bis-phosphatidylglyceryl phosphate, compared to sensitive strains. Another possibility is that the cells growing in a complex, nutrient-rich environment such as meat will reach their maximum replication rate and still have excess nutrients available for the repair or increased turnover of cellular components. Such cells can be expected to have increased resistance to many different stresses. To our knowledge, this possibility has yet to be investigated. Alternatively, cells growing on the meat surface may form a microbial biofilm community. Cells in such communities have been observed to possess higher resistance than planktonic cells to a wide range of antimicrobials (Kumar and Anand, 1998; Bower and Daeschel, 1999).

In conclusion, it appears that cilantro oil is not a suitable agent for applications to control *L. monocytogenes* on meat products. The phenomenon of a significant reduction in the effectiveness of antimicrobial essential oil treatments when they are moved from model media to food applications is well established. Determining whether this effect is due to interactions of the agent with environmental components or physiological differences exhibited by target organisms in these different environments may allow for better assessment of novel antimicrobial agents for food use.

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