Effect of Antimicrobial Spice and Herb Extract Combinations on 
Listeria monocytogenes, Staphylococcus aureus, and Spoilage Microflora Growth on Cooked Ready-to-Eat Vacuum-Packaged Shrimp

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ABSTRACT

Two spice and herb extract combinations from galangal (Alpinia galanga), rosemary (Rosmarinus officinalis), and lemon iron bark (Eucalyptus staigerana) were evaluated for their ability to inhibit the growth of inoculated Listeria monocytogenes and Staphylococcus aureus and naturally present spoilage microflora on cooked ready-to-eat shrimp stored for 16 days at 4 or 8°C. A combination of galangal, rosemary, and lemon iron bark significantly reduced (P < 0.05) levels of aerobic bacteria and lactic acid bacteria at 4°C on day 12 by 1.6 and 1.59 log CFU/g, respectively. By day 16, levels of these bacteria were equivalent to those of controls. The shrimp treated with this spice and herb extract combination had significantly lower (P < 0.05) lipid oxidation from day 4 to day 16. Similarly, a combination of galangal and rosemary extract significantly reduced (P < 0.05) levels of aerobic bacteria and lactic acid bacteria at 8°C on day 8 by 2.82 and 2.61 log CFU/g, respectively. By days 12 and 16, levels of these bacteria were equivalent to those of controls. The shrimp treated with this spice and herb combination had significantly lower (P < 0.05) lipid oxidation on days 4 and 16. None of the spice and herb extract combinations had an effect on levels of L. monocytogenes or S. aureus or changed the color or pH of the shrimp during storage. The results of this study indicate that combinations of galangal, rosemary, and lemon iron bark extracts can be used to control the growth of spoilage microflora on ready-to-eat shrimp.

Spices and herbs have been used for centuries as food additives to enhance food flavor and as preservatives to extend shelf life and may act as natural preservatives to meet consumer demand for healthy and minimally processed ready-to-eat (RTE) foods (15). The antimicrobial properties of various spices and herbs have been studied extensively in vitro, and their major chemical compounds have been identified (24, 27, 30, 31). In addition to their antimicrobial properties, some spices and herbs have antioxidant activity and may therefore reduce oxidative rancidity in foods. Although spices and herbs have strong antimicrobial and antioxidant effects in vitro, some do not have these same properties when incorporated into a food matrix possibly because of insufficient concentrations of active components and/or reduced activity due to reactions with food ingredients (2). These inconsistent effects have limited the practical use of spices and herbs as natural preservatives. Thus, spice and herb extracts may be best considered as part of a microbial growth hurdle approach in combination with other preservation technologies such as modified atmosphere packaging (25), irradiation (16), high-pressure processing (20), and low temperature (25). A combination of physical hurdles rather than a single hurdle extends the shelf life of food products by controlling microbial population and retaining freshness and quality without altering the sensory and nutritive value of the product (17).

The consumption of raw and RTE shrimp has increased steadily over the years. In the United States, the per capita consumption of shrimp was 1.3 kg in 1998 and 1.9 kg in 2004 (11). Shrimp has a short shelf life because of its high protein content and high pH (~7.5) (12). Cooked shrimp is retailed as either a chilled or a frozen product. Frozen cooked shrimp are thawed prior to retail display or sale. During thawing, pre- and postprocessing microflora populations can increase, leading to spoilage, especially under abusive storage conditions. According to Food Standards Australia New Zealand (FSANZ) (10), the average temperatures recorded for chilled cooked prawn samples collected from retail outlets in Perth, Brisbane, Melbourne, and Sydney in a 4-week period during July and August 2002 ranged from −5.5 to 12.8°C. Temperatures higher than 5°C also were found in displayed cooked shrimp in retail outlets. Therefore, additional measures are needed to control the growth of naturally occurring microflora and pathogenic microorganisms under abusive storage conditions to ensure quality and safety.
Microbial contamination of shrimp can occur at various stages of production: on the ship, in the processing plant environment, during handling, during transportation, and during storage under fluctuating temperature conditions (10). In addition to spoilage microbes, shrimp may be contaminated with pathogenic microorganisms such as Listeria monocytogenes, Staphylococcus aureus, Salmonella Typhimurium, and Clostridium botulinum type E (20, 23). L. monocytogenes is a psychrotolerant bacterium that can grow close to 0°C and is an important pathogen in chilled RTE products such as cooked and peeled shrimp stored in modified atmosphere packaging (MAP) (3). RTE cooked shrimp and shrimp salad have been contaminated with L. monocytogenes (7, 14). Cooked, peeled shrimp can be vacuum packed to limit the passage of water vapor and oxygen and minimize dehydration and oxidation. Vacuum packaging also controls the growth of L. monocytogenes (Scott A) (13). S. aureus is a common foodborne pathogenic postprocessing contaminant of vacuum-packaged and reduced-moisture products (1). S. aureus can grow under temperature abuse conditions and produce enterotoxins without spoiling foods.

In our previous work, we identified combinations of three spice and herb extracts that had synergistic antimicrobial activity against foodborne pathogens (30). The combination of galangal (Alpinia galanga), rosemary (Rosmarinus officinalis), and lemon iron bark (Eucalyptus staigerana) extract had optimal synergistic activity against L. monocytogenes, and the combination of galangal and rosemary extract had optimal synergistic antimicrobial activity against S. aureus. The objectives of this study were to evaluate the effectiveness of combined spice and herb extracts to control inoculated L. monocytogenes and S. aureus and naturally occurring spoilage microflora on vacuum-packed cooked RTE shrimp stored at 4 and 8°C. The effects of the spice and herb extract combinations on lipid oxidation, color, and pH of the shrimp also were examined.

MATERIALS AND METHODS

Extraction of spices and herbs. Fresh galangal was purchased from a local shop (Brisbane, Australia). Fresh leaves of lemon iron bark were donated by Australian Rainforest Products (Lismore, New South Wales, Australia). A dried commercial preparation of rosemary (Masterfoods, Wyong, Australia) was purchased from a Brisbane supermarket. Fresh leaves (Lismore, New South Wales, Australia) and rhizome was sliced and dried at 40°C overnight and subjected to cryogrinding with a freezer mill (SPEX Sample Prep, Metuchen, NJ) for 5 min to make a fine powder. Fresh leaves of E. staigerana were dried at 40°C for 24 h and cryogrinding for 3 min. R. officinalis also was cryogrinding for 3 min to a fine powder.

Hexane was used to extract A. galanga, and ethanol was used to extract R. officinalis and E. staigerana as described in Weerakkody et al. (31). These extracts produced with these solvents possessed the highest levels of antimicrobial activity compared with extracts obtained with other solvents or water (30). Dried ethanol extracts were dissolved in 90% ethanol, and the dried galangal hexane extract was dissolved in glycerin (Melbourne Food Ingredient Depot, Melbourne, Australia) to make stock solutions (500 mg/ml), which were stored at 4°C until use.

Test microorganisms. L. monocytogenes Scott A (serotype 4b) and L. monocytogenes V7 (serotype 1/2a) were obtained from the U.S. Food and Drug Administration (Cincinnati, OH). S. aureus ATCC 25925, S. aureus ATCC 29213, and L. monocytogenes ATCC 7644 were obtained from the American Type Culture Collection (Manassas, VA). S. aureus SA113 was supplied by the Institute for Biomedical Innovation (Queensland University of Technology, Brisbane, Australia). All bacterial growth media used in this study were supplied by Oxoid (Basingstoke, UK). All bacteria were maintained as frozen stocks in 40% glycerin at −80°C. Working cultures were grown and maintained on tryptic soy agar.

Preparation and inoculation of shrimp. Frozen RTE cooked, peeled, and deveined shrimps (Peneaus vannamei), which had the last abdominal shell (plus telson and uropods) still present, were obtained from a Brisbane supermarket 1 day before the experiment. Shrimps were transported on ice to the laboratory and stored at 4°C to thaw overnight. The next day, the last abdominal shell with the telson and uropods was removed from each shrimp, and the shell-less shrimp abdomen was weighed. Shrimps that were 10 ± 1 g were selected for the experiments.

All six bacterial strains were grown separately in 2 ml of tryptic soy broth at 37°C for 18 h to a population of ~9.0 log CFU/ml. Cells were harvested by centrifugation at 9,000 × g for 10 min at room temperature. The supernatant was decanted, and the pellet was resuspended in sterile 0.85% saline by vortexing. A 100-µl aliquot of each S. aureus strain or 50 µl of each L. monocytogenes strain was added to 500 ml of 0.85% saline solution to provide a three-strain mixture containing ~5.0 to 6.0 log CFU/ml, which resulted in an attachment level of ~4.0 to 5.0 log CFU/g of shrimp.

Shrimps were inoculated with a three-strain mixture of L. monocytogenes or a three-strain mixture of S. aureus or were not inoculated. The noninoculated shrimps were included to determine the growth of natural microflora and pH, color, and oxidation changes. Shrimps were inoculated by immersion for 5 min at 25°C in the bacterial suspensions and agitation with a glass rod to ensure an even distribution of the organisms. After inoculation, shrimps were removed and air dried for 15 min in a biosafety cabinet to allow bacterial attachment.

Treatment of shrimps. In a previous study, we investigated various combinations of three herb and spice extracts (galangal, rosemary, and lemon iron bark) for synergistic activity against foodborne pathogens in laboratory growth media (30). Galangal and rosemary extracts had synergistic antimicrobial activity against S. aureus, and galangal, rosemary, and lemon iron bark extracts had synergistic antimicrobial activity against L. monocytogenes (30).

The stock solutions (500 mg/ml) of galangal, rosemary, and lemon iron bark extracts were diluted in 80% glycerin to prepare lower concentrations. Food grade glycerin was used as a solvent instead of ethanol because glycerin is a generally recognized as safe (GRAS) substance. The S. aureus–inoculated and some noninoculated shrimps were treated with a mixture of 5 mg/ml galangal and 10 mg/ml rosemary extracts, and the L. monocytogenes–inoculated shrimps and some noninoculated shrimps were treated with a mixture of 5 mg/ml galangal, 10 mg/ml rosemary, and 2.5 mg/ml lemon iron bark extracts.

Aliquots of 500 µl of the mixtures were spread on each side of single shrimp surfaces and left for 10 min. Controls for this experiment included sets of shrimps that were inoculated with equivalent volumes of 80% glycerin only or with water containing 0.25 µl/ml ethanol (ethanol was added at 0.025%, similar to the
concentration present in the spice combination mixtures) and left for 10 min. These controls allowed determination of the antimicrobial activity due to glycerol and allowed comparison of bacterial levels found in shrimps that were not treated with extracts or glycerol.

Each shrimp was placed in a separate vacuum bag (Micris Pty. Ltd., Cleveland, Queensland, Australia) to which an additional 1 ml of the corresponding spice and herb extract mixture or control liquid (80% glycerol or water plus 0.025% ethanol) was added. The vacuum bags have an oxygen transmission rate of 80 cc/m²/24 h/atm at 23°C and a water vapor transmission rate of 7 g/m²/24 h at 38°C. The bags were vacuum sealed (Vacumatic 282, Vacumatic Australia, Narre Warren, Victoria, Australia) at 80 mm Hg. After vacuum packaging, L. monocytogenes–inoculated shrimps were stored at 4°C for 16 days, and S. aureus–inoculated shrimps were stored at 8°C for 16 days. We selected two temperatures, 4 and 8°C, for the two pathogenic bacteria, which would allow growth during storage. Glycerol (80%)-treated and water (plus 0.025% ethanol)-treated control samples were stored separately at both temperatures. Shrimps were analyzed for bacterial populations, pH, color, and lipid oxidation after 0, 4, 8, 12, and 16 days of storage. This entire shrimp trial was repeated independently two more times.

**Microbial enumeration.** Each shrimp was transferred to an individual sterile stomacher bag. 0.85% saline was added to produce a 1:10 (wt/vol) dilution, and the mixture was homogenized in a stomacher (240 rpm per paddle; BA6021, Colworth Stomacher 400, Seward, London, UK) for 60 s. Serial dilutions of the bacterial suspensions were prepared with 0.85% saline, and 0.1 ml of the dilutions was spread onto appropriate media. PALCAM agar was used to enumerate L. monocytogenes from inoculated samples, and plates were incubated at 37°C for 48 h. Baird-Parker agar was used to enumerate Staphylococcus aureus from inoculated samples, and plates were incubated 37°C for 24 h. From the noninoculated shrimp samples, native microflora including aerobic bacteria and lactic acid bacteria were enumerated. Aeroebic bacteria were enumerated by plating 100 μl on plate count agar, which was incubated at 30°C for 2 days. Lactic acid bacteria were enumerated by plating 100 μl on de Man Rogosa Sharpe agar, which was incubated at 25°C for 3 days anaerobically in a GasPak Anaerobic System (Oxoid). For each sample, at least two appropriate dilutions were enumerated. Bacteria were enumerated on plates with 30 or more colonies.

**Determination of TBARS.** Thiobarbituric acid reactive substances (TBARS) in the shrimp samples were determined as described by Nirmal and Benjakul (21). A 1-g sample of ground shrimp was mixed with 9 ml of a solution containing 0.375% thiobarbituric acid (Sigma-Aldrich, Steinheim, Germany), 15% trichloroacetic acid (Ajax Finchem, Cheltenham, Victoria, Australia), and 0.25 N HCl. The mixture was heated in a boiling water bath for 10 min, cooled with running water, and then centrifuged at 4,000 × g for 20 min. The supernatant was collected, and the absorbance was read at 532 nm with a spectrophotometer (Lovibond, Dortmund, Germany). The TBARS value was calculated from a standard curve of malonaldehyde (1,1,3,3-tetramethoxypropane; Sigma-Aldrich) (0 to 2 ppm) and expressed as milligrams of malonaldehyde per kilogram of shrimp meat.

**pH measurement.** Ten grams of shrimp sample was transferred to a stomacher bag with 90 ml of 0.85% saline solution and homogenized for 60 s with a stomacher. The pH of this homogenized sample was measured with a pH meter (TPS Pty. Ltd., Brisbane, Australia).

**Color measurement.** Color changes occurring on the surface of shrimp samples were quantified with a chromameter (Konica Minolta, Sensing, Japan) based on CIE L, a, and b values (L, lightness; a, redness and greenness; b, yellowness and blueness). Color measurements were conducted at predetermined storage intervals. Measurements were taken perpendicular to the sample surface at three places on each shrimp specimen.

**Statistical analysis.** Shrimp samples were analyzed in triplicate on different days. Microbial counts were log transformed before the analysis of variance was conducted in MINITAB (Minitab, Inc., State College, PA). Tukey’s test for comparison of means was performed with the same program. The significance level was defined at P < 0.05.

**RESULTS**

Effect of spice and herb extract mixture on L. monocytogenes and spoilage microflora on shrimps stored at 4°C. Counts of naturally occurring spoilage bacteria and inoculated L. monocytogenes on shrimps stored at 4°C are given in Table 1. The galangal, rosemary, and lemon iron bark extract mixture did not significantly inhibit (P > 0.05) the growth of inoculated L. monocytogenes over the 16 days of storage as compared with glycerin-treated or

| TABLE 1. Antimicrobial activity of spice and herb extracts against L. monocytogenes and native microflora on shrimp stored at 4°C |
|---|---|---|---|---|
| Bacteria | Treatment | 0 | 4 | 8 | 12 | 16 |
| L. monocytogenes | No spice control | 5.34 ± 0.30 | 6.55 ± 0.24 | 7.40 ± 0.74 | 8.19 ± 0.36 | 8.77 ± 0.18 |
| | Glycerin control | 5.28 ± 0.33 | 5.82 ± 0.37 | 6.57 ± 0.58 | 7.90 ± 0.73 | 8.45 ± 0.84 |
| | X3 spice mix | 4.99 ± 0.24 | 5.61 ± 0.54 | 5.89 ± 0.56 | 7.43 ± 1.08 | 8.21 ± 0.89 |
| Aerobic bacteria | No spice control | <3.48 | <3.48 | <3.48 | <3.48 | <3.48 |
| | Glycerin control | <3.48 | <3.48 | <3.48 | <3.48 | <3.48 |
| | X3 spice mix | <3.48 | <3.48 | <3.48 | <3.48 | <3.48 |
| Lactic acid bacteria | No spice control | <3.48 | <3.48 | <3.48 | <3.48 | <3.48 |
| | Glycerin control | <3.48 | <3.48 | <3.48 | <3.48 | <3.48 |
| | X3 spice mix | <3.48 | <3.48 | <3.48 | <3.48 | <3.48 |

* a No spice control shrimp were treated with water (plus ethanol) only. Glycerin control shrimp were treated with glycerol only. X3 spice mix shrimp were treated with extracts of galangal, rosemary, and lemon iron bark.

* Within a column, mean values followed by the same uppercase letter are not significantly different (P > 0.05). Within a row, mean values with the same lowercase letter are not significantly different (P > 0.05).
untreated shrimps. On day 12 of storage, aerobic bacteria and lactic acid bacteria counts on extract-treated shrimps were significantly lower ($P < 0.05$) than those on glycerin-treated shrimps. The difference in these counts were 1.60 and 1.59 CFU/g, respectively. However, by day 16 levels of aerobic and lactic acid bacteria in extract-treated shrimps were equivalent to those in glycerin-treated shrimps. The glycerin-treated shrimp did have significantly lower aerobic and lactic acid bacteria counts ($P < 0.05$) on days 8 and 12 compared with these counts on untreated shrimps. However, glycerin did not inhibit growth of $L$. monocyctogenes.

Lipid peroxidation in shrimp stored at 4°C. The TBARS values of treated and untreated shrimps stored at 4°C for 16 days are listed in Table 2. The TBARS values for shrimp treated with the galangal, rosemary, and lemon iron bark extract mixture were significantly lower ($P < 0.05$) than those for glycerin-treated and untreated shrimps on all days except day 0. The glycerin treatment did not have an effect on TBARS values (compared with the untreated control) throughout the storage period, except on day 8 when significant higher values were obtained for glycerin-treated shrimp.

Effect of spice and herb extract mixture on $S$. aureus and spoilage microflora on shrimps stored at 8°C. Counts of spoilage bacteria and inoculated $S$. aureus on shrimps stored at 8°C are shown in Table 3. Galangal and rosemary extract mixtures did not significantly inhibit ($P > 0.05$) the growth of inoculated $S$. aureus during 16 days of storage as compared with glycerin-treated or untreated shrimps. The levels of aerobic bacteria and lactic acid bacteria were significantly lower (2.82 and 2.61 log CFU/g, respectively) in shrimps treated with the spice and herb extract mixture only on day 8 of storage compared with the glycerin-treated control. However, aerobic and lactic acid bacterial growth increased by days 12 and 16, and no difference in counts between extract-treated shrimp and glycerin-treated shrimp was found on those days. Glycerin did not have an effect on spoilage microflora except on day 4, when significant inhibition ($P < 0.05$) was noted for aerobic and lactic acid bacteria.

Lipid peroxidation in shrimp stored at 8°C. The TBARS values of shrimps stored at 8°C for 16 days are listed in Table 4. Significantly lower TBARS values ($P < 0.05$) were obtained on days 4 and 16 of storage for extract-treated shrimp compared with glycerin-treated and untreated shrimp controls. The glycerin treatment did not have an effect on TBARS values (compared with the untreated control) throughout the storage period. The TBARS values did not significantly change over time except when day 16 values were compared with day 0 values for glycerin-treated and untreated shrimp.

pH of shrimp stored at 4 and 8°C. Neither of the two spice and herb extract mixtures caused a significant change.
**TABLE 4. TBARS values for shrimp treated with spice and herb extracts, vacuum packaged, and stored at 8°C**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>0</th>
<th>4</th>
<th>8</th>
<th>12</th>
<th>16</th>
</tr>
</thead>
<tbody>
<tr>
<td>No spice control</td>
<td>5.06 ± 0.66 a A</td>
<td>4.87 ± 0.32 A a</td>
<td>5.27 ± 0.48 A a</td>
<td>5.79 ± 0.15 A a</td>
<td>6.98 ± 0.75 B a</td>
</tr>
<tr>
<td>Glycerin control</td>
<td>4.72 ± 0.30 A a</td>
<td>5.68 ± 0.27 A a</td>
<td>5.90 ± 0.69 A a</td>
<td>5.48 ± 0.27 A a</td>
<td>6.82 ± 1.12 A b</td>
</tr>
<tr>
<td>X2 spice mix</td>
<td>5.16 ± 0.14 A a</td>
<td>3.79 ± 0.53 B a</td>
<td>4.82 ± 0.87 A a</td>
<td>4.18 ± 0.07 A a</td>
<td>3.90 ± 0.04 B b</td>
</tr>
</tbody>
</table>

a No spice control shrimp were treated with water (plus ethanol) only. Glycerin control shrimp were treated with glycerin only. X2 spice mix shrimp were treated with extracts of galangal and rosemary.

b Within a column, mean values followed by the same uppercase letter are not significantly different (P > 0.05). Within a row, mean values with the same lowercase letter are not significantly different (P > 0.05).

(P > 0.05) in the pH of the shrimp immediately after application or during storage (data not shown).

**Color changes of shrimp stored at 4 and 8°C.** No significant differences were found in L, a, and b values of treated and untreated shrimps stored at 4 and 8°C (data not shown).

**DISCUSSION**

Spice and herb extracts with antimicrobial properties can be used to control the growth of foodborne pathogenic bacteria and spoilage bacteria, which can in turn lead to an extension of the shelf life of processed food products. According to FSANZ guidelines (9), RTE food products are considered satisfactory when total aerobic bacterial counts are ≤6 log CFU/g and pathogenic bacteria are undetectable. According to this guideline, when stored at 4°C untreated shrimp would be unfit for consumption between days 5 and 8, glycerin-treated shrimp would be unfit between days 9 and 12, and shrimp treated with the galangal, rosemary, and lemon iron bark extract mixture would be unfit between days 13 and 16. A similar pattern was seen for shrimp stored at 8°C; untreated shrimp were unfit for consumption between days 1 and 4, glycerin-treated shrimp were unfit between days 5 and 8, and shrimp treated with galangal and rosemary extract mixture were unfit between days 9 and 12. Generally, chilled cooked crustaceans have a 10-day shelf life at −1°C (6, 10). This shelf life was achieved at higher temperatures (4 and 8°C) when treatment with spice and herb extracts was combined with vacuum packaging.

We found an inhibitory effect of spice and herb extract combinations on tested spoilage microorganisms, including aerobic bacteria and lactic acid bacteria. Galangal and rosemary extracts have produced antibacterial activity against the lactic acid bacteria Lactococcus lactis (8, 24, 26), which agrees with the results of our study. However, inoculated S. aureus and L. monocytogenes were not inhibited by treatment with spice and herb extracts during the 16 days of storage, even though the extracts previously had strong synergistic antimicrobial activity (30). The reason for this difference in findings is not clear, but this situation highlights the fact that results obtained with laboratory media can be different from those obtained in food matrices. One possible explanation for the lack of bacterial inhibition in shrimp is that the spice and herb extracts more actively inhibited competing spoilage microflora (initial levels of <3.48 log CFU/g) and therefore allowed the higher initial levels (~4 to 5 log CFU/ml) of inoculated S. aureus and L. monocytogenes to grow on the shrimp. The low growth of S. aureus in untreated shrimp also may be due to its well known poor ability to compete with other bacteria (3). The total aerobic bacterial counts in shrimp were ~100 times greater than the S. aureus counts after 4 days of storage. Application of the spice and herb extract mixtures may have been patchy due to poor contact on some places of the shrimp surface, thereby allowing enhanced bacterial growth. The naturally occurring microflora on shrimp also may be have been sublethally injured or stressed during frozen storage before the assays and therefore may have been more sensitive than the inoculated pathogens to the herb and spice extract mixtures.

L. monocytogenes populations increased over the 16-day storage period in shrimp stored at 4°C. These results are in agreement with those of Farber (7), who found that Listeria increased by 2 to 3 log CFU/g in cooked shrimp stored at 4°C for 7 days. MAP with CO2 was more effective against L. monocytogenes than vacuum packaging and air packaging (25). In addition to the spice and herb extract treatment, the combination of different physical hurdles such as vacuum packaging and refrigerated storage used in our study extended the shelf life of RTE shrimp by controlling the populations of spoilage microorganisms and the physiological quality of the shrimp during the storage period. Other researchers also have used various hurdle technologies in food preservation. Mastromatteo et al. (19) found that a combination of MAP and thymol coating (1,000 ppm) on shrimp improved the shelf life by more than 9 days at 4°C compared with samples packaged in air and stored at 4°C. In this same study, MAP alone did not improve the shelf life of uncoated shrimp. In another study, L. monocytogenes growth in RTE shrimp under MAP was inhibited by higher numbers of inoculated lactic acid bacteria at 2°C but only for low initial inoculum levels (2 log CFU/g) of L. monocytogenes (20).

In the present study we used glycerin, which is a GRAS food additive (28), as a solvent for the extracts. Glycerin absorbs moisture from the environment, causing a reduction in the water activity of the product (28). The 80% glycerin used in our study did not have a significant inhibitory effect on S. aureus and L. monocytogenes. According to Litsky et al. (18), a higher concentration of glycerin (88.8%) had a bacteriostatic effect on S. aureus, and Escherichia coli.
Salmonella Typhimurium, and Pseudomonas aeruginosa were unable to grow in solutions of 58.6, 48.1, and 40.5% glycerin, respectively.

None of the spice and herb extract combinations used in this study changed the color or pH of shrimp during storage. Shrimp tissue has a natural buffering capacity, and the lactic acid generated by the growth of natural lactic acid bacteria on shrimp during storage can be neutralized by ammonia produced from the concurrent proteolysis of shrimp protein (19, 22). This buffering effect may explain the lack of significant changes in pH of shrimps throughout the storage period despite significant increases in the levels of lactic acid bacteria.

Lipid oxidation was significantly reduced ($P < 0.05$) in shrimp treated with the galangal, rosemary, and lemon iron bark extract mixture compared with the glycerin control shrimp during storage at $4^\circ$C. Spices and herbs contain phenols, which can act as antioxidants combating lipid oxidation. In our previous study, we found that lemon iron bark ethanol extract (386.43 mg gallic acid equivalent per g of dry sample) and rosemary ethanol extract (56.76 mg gallic acid equivalent per g of dry sample) had higher total phenolic contents than galangal hexane extract (1.33 mg gallic acid equivalent per g of dry sample) (31). Even though galangal is low in total phenols, Cheah and Gan (5) reported that a 10% galangal extract was as effective as 0.02% butylated hydroxytoluene and 0.1% $\alpha$-tocopherol for minimizing lipid oxidation in raw meat. The activity of our spice and herb extract against lipid oxidation during shrimp storage was less efficient at $8^\circ$C than at $4^\circ$C, possibly because higher levels of enzymatic activity the higher temperature lead to more rapid oxidation. Similar observations were reported for fresh frozen shrimp after 3.5 and 9 months of storage under air (29). Significantly increased levels of lipid oxidation were observed for shrimp stored at $-5$ and $-8^\circ$C compared with shrimp stored at $-12$ and $-15^\circ$C.

TBARS values of 7 to 8 mg malonaldehyde per kg are considered the limit for food safety (4). However, TBARS values in untreated shrimp were below the safe consumption limits even after 16 days of storage at $8^\circ$C. TBARS values in the shrimp that were not treated with spices and herbs or glycerin and were stored at $8^\circ$C were not significantly different at day 16 from those values at day 0. Therefore, vacuum packaging may slow lipid oxidation in shrimp.

In conclusion, the spice and herb extract mixtures used in this study significantly reduced the populations of aerobic bacteria and lactic acid bacteria naturally present on shrimps throughout the storage period. No significant effect on inoculated S. aureus and L. monocytogenes on shrimps was found despite previous findings of significant antimicrobial activity of these extracts in laboratory media. The combination of galangal, rosemary, and lemon iron bark extracts extended the shelf life of cooked RTE vacuum-packaged shrimp to $>12$ days when stored at $4^\circ$C. The combination of galangal and rosemary extracts extended the shelf life of cooked RTE vacuum-packaged shrimp to $>8$ days when stored at $8^\circ$C. Because of these antimicrobial effects and the concomitant reduction of lipid oxidation, these herb and spice extracts might be useful as a shrimp preservative. Further studies are required to determine the effect of these spice and herb extracts at different concentrations and to identify other suitable solvents that diffuse the antimicrobial compounds efficiently.

REFERENCES


