APPLICATION OF LACTIC ACID IN COMBINATION WITH MELANOSIS INHIBITORS TO IMPROVE SHELF LIFE OF TEXAS BROWN SHRIMP (*PENAEUS AZTECUS*)

A Thesis

by

RONALD ALLEN BENNER, JR.

Submitted to the Office of Graduate Studies of
Texas A&M University
in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

December 1993

Major Subject: Food Science and Technology

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December 1993

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ABSTRACT

Application of Lactic Acid in Combination with Melanosis Inhibitors to Improve Shelf Life of Texas Brown Shrimp (*Penaeus aztecus*). (December 1993)

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The shelf life of fresh shrimp stored on ice is limited by two factors: melanosis (blackspot) and microbial spoilage. In this study L-lactic acid was tested, alone and in combination with sodium bisulfite and 4-hexylresorcinol, to determine their effectiveness as both melanosis and microbial spoilage inhibitors. Brown shrimp *(Penaeus aztecus)* were obtained from the Gulf of Mexico on board a commercial fishing vessel. Immediately after harvest, the shrimp were separated from the rest of the catch. The shrimp were then headed, washed, and sorted into six groups that were treated by dipping for 1 min in the following solutions: (1) Control (sea water), (2) 1.0% L-lactic acid, (3) 1.25% sodium bisulfite, (4) 0.0025% 4-hexylresorcinol, (5) 1.25% sodium bisulfite with 1.0% L-lactic acid, or (6) 0.0025% 4-hexylresorcinol with 1.0% L-lactic acid. Each of the six groups of shrimp was split into 2 subgroups. One subgroup of shrimp was treated immediately after sorting and the other subgroup was treated after a 2-hr delay on deck at ambient temperature to simulate normal practices during peak harvesting times. Each subgroup of shrimp was placed in a

nylon mesh bag, completely submerged in the appropriate dip solution for 1 min with moderate up and down agitation, and drained. Each treatment group was stored on ice for 16 days in separate ice chests, and examined over the storage period for aerobic plate count (APC), pH, and degree of melanosis.

Overall, this study demonstrated that treatment with L-lactic acid, alone or in combination with 4-hexylresorcinol, had no detectable effect on the microbiological shelf life of brown shrimp (*Penaeus aztecus*). In addition, 4-hexylresorcinol performed exceptionally well as a melanosis inhibitor, whether alone or in combination with L-lactic acid. However, extensive bacterial growth was demonstrated in the 4-hexylresorcinol dip solution during normal storage on deck, creating the potential for a heavy bacterial inoculation of shrimp during a typical dipping process. Addition of L-lactic acid to the 4-hexylresorcinol dip solution prevented bacterial growth in the dip solution and did not interfere with melanosis inhibition.

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TABLE OF CONTENTS

LIST OF TABLES

INTRODUCTION

Microbial spoilage and melanosis are the main causes of quality deterioration of shrimp. Melanosis in shrimp, commonly referred to as 'blackspot', is a surface color defect caused by activity of the enzyme polyphenoloxidase (PPO) which remains active during iced or refrigerated storage. Although melanosis on shrimp does not affect the eating quality of shrimp, it does result in devalued product with decreased consumer acceptance. In the shrimp industry, this enzymatic reaction has traditionally been controlled through the use of sulfiting agents, particularly sodium bisulfite. However, allergic reactions among sulfite-sensitive individuals and asthmatics have led to an increasing concern regarding the use of sulfite as a melanosis inhibitor in fresh shrimp.

4-Hexylresorcinol has been proposed as an alternative to sulfites for the inhibition of melanosis in shrimp. Advantages of 4-hexylresorcinol over traditional sulfiting agents include: chemical stability; lower concentration requirements; absence of acute and chronic toxicity; and an inability to reduce melanosis in shrimp that have previously blackened. Additionally, the use of 4-hexylresorcinol above the recommended level of 50 ppm will not enhance its melanosis-inhibiting effects; therefore, the likelihood of concentration abuse is decreased. Finally, the use of 4-hexylresorcinol as a processing aid for the prevention of melanosis in shrimp is considered generally recognized as safe (GRAS).

The citations on the following pages follow the style of the Journal of Food Science.

Application of lactic acid to other raw foods, such as red meats, has been shown to significantly reduce initial bacterial numbers and directly affect shelf life. It is expected that similar reductions can be obtained through the use of lactic acid applications on freshly harvested shrimp. Reduction of the initial bacterial population on freshly caught brown shrimp could substantially increase the expected shelf stability. It is also possible that a lactic acid application may control or enhance control of melanosis by lowering the pH at which the melanosis reactions occur.

The objective of this study was to provide information on the effect of lactic acid, alone and in combination with the melanosis inhibitors sodium bisulfite and 4-hexylresorcinol, on melanosis and microbiological shelf life of fresh brown shrimp (*Penaeus aztecus*).

REVIEW OF LITERATURE

Shelf life of shrimp

The shelf life of fresh shrimp is limited by two factors: melanosis and microbial spoilage (Fieger and Novak, 1961; Liston, 1980). Melanosis in shrimp, commonly referred to as 'blackspot', is a surface color defect caused by activity of the enzyme polyphenoloxidase (PPO) which remains active during iced or refrigerated storage (Otwell and Marshall, 1986). This enzymatic reaction and resulting color defects have historically been controlled through the use of sulfiting agents, particularly sodium bisulfite (Camber et al., 1957). Allergic reactions among sulfite-sensitive individuals and asthmatics have led to an increasing concern regarding the use of sulfite as a melanosis inhibitor in fresh shrimp (Taylor and Bush, 1986).

Previous investigators have studied many sulfite alternatives to inhibit melanosis in shrimp. Wagner and Finne (1986) dipped white shrimp (*Penaeus setiferus*) in solutions of 1%, 2%, and 5% citric acid for 5 min; 1% and 2.5% ethylenediamine tetraacetic acid (EDTA) and boric acid for 5 min; 1% and 2.5% ascorbate, isoascorbate, sodium ascorbate and glucose for 5 min; and control (no treatment) and 1.25% sodium bisulfite for 1 min. In this study, 2% citric acid and 2.5% boric acid showed the most promise. However, no treatment performed as well as the 1.25% sodium bisulfite solution after 7, 11, or 12 days of storage on ice, indicating that the use of sodium bisulfite was the best method tested to prevent melanosis of shrimp. In addition, boric acid is not included on the FDA's GRAS (generally recognized as safe) list (Otwell and Marshall, 1986). Otwell and Marshall (1986) evaluated various

dip solutions including controls (no treatment), sodium bisulfite in differing concentrations, and a variety of single compounds and/or mixtures of compounds in differing concentrations on their ability to inhibit melanosis on pink shrimp (*Penaeus duorarum*). They concluded that 2.5% sodium bisulfite dip for 1 min was more effective in preventing melanosis than was the legally recognized 1.25% sodium bisulfite dip for 1 min and that 1.25% sodium bisulfite dip for 1 min was more effective in inhibiting melanosis than any other treatment (single compounds or mixtures) tested.

Another possible alternative to sulfiting agents is 4-hexylresorcinol. The advantages of 4-hexylresorcinol over traditional sulfiting agents include: chemical stability; lower concentration requirements; absence of acute and chronic toxicity; and an inability to reduce melanosis in shrimp that have previously blackened. In addition, the use of 4-hexylresorcinol above the recommended level of 50 ppm will not enhance its melanosis-inhibiting effects; therefore, the likelihood of concentration abuse is decreased (McEvily et al., 1991b). Finally, the use of 4-hexylresorcinol as a processing aid for the prevention of melanosis in shrimp is GRAS (Frankos et al., 1991).

The second limiting factor in the shelf life of shrimp is microbial spoilage. Fresh Gulf of Mexico brown shrimp (*Penaeus aztecus*) commonly have bacterial counts of approximately 10^4 to 10^5 per g (Campbell and Williams, 1952; Vanderzant et al., 1970). Numerous bacterial types are present on Gulf shrimp, but the microflora is initially predominated by coryneform bacteria, *Pseudomonas*, *Moraxella*, and

Micrococcus (Vanderzant et al., 1970). The microflora shifts during iced storage to one dominated by Gram-negative, aerobic, psychrotrophic organisms including *Pseudomonas*, *Moraxella-Acinetobacter*, or a combination of both (Liston, J., 1980; Nickelson and Finne, 1992). These proteolytic bacteria (especially *Pseudomonas*) produce off-flavors and off-odors in shrimp during iced storage resulting in unacceptable product. Shelf life of shrimp stored on ice on a fishing vessel is expected to be 12 to 14 days (Cobb et al., 1976). Reduction of the initial bacterial population on freshly caught brown shrimp could substantially increase the expected shelf stability.

Application of lactic acid to other raw foods, such as red meats, has shown significant reduction of initial bacterial numbers and potential extension of shelf life (Woolthuis and Smulders, 1985). Snijders et al. (1985) concluded that the use of lactic acid as a terminal processing decontaminant provides both an immediate (bactericidal) and a delayed (bacteriostatic) effect which results in an extended shelf life of meat. In a study on immediate and delayed microbiological effects of carcass decontamination with 1.25% lactic acid, Smulders and Woolthuis (1985) achieved a bacterial reduction of 0.8/cm^2 from initial $\log_{10}$ counts of approximately 3.0/cm^2 on control carcasses. However, this $\log_{10}$ reduction increased to 1.3 at day 14, indicating some delayed effects of lactic acid. Lactic acid solutions of 1.0 and 2.0% have also been applied to broiler carcasses resulting in immediate post-treatment reductions of about 1 $\log_{10}$/g of skin (van der Marel et al., 1988).

Hamby et al. (1987) and Prasai et al. (1991) have demonstrated that the time of application of lactic acid is extremely important in reduction of bacterial numbers; acid treatments were most effective when applied as soon as possible after contamination and before bacterial attachment could occur. It is expected that similar reductions could be obtained through the use of lactic acid applications on freshly harvested shrimp. Conversely, lactic acid treatments applied after fabrication of meat have a minimal effect on bacterial numbers. Loins or steaks that were treated with various sprays (including lactic acid) did not differ significantly in APCs following storage and retail display (Acuff et al., 1987; Dixon et al., 1987). Based upon previous research on the use of lactic acid with red meats, treatment of the shrimp on board, immediately after harvest, should provide maximum reduction of bacterial numbers.

It is also possible that a lactic acid application may control, or enhance control of, melanosis. The activity of PPO may be reduced by lowering the pH at which the melanosis reactions occur (McCord and Kilara, 1983; Zemel et al., 1990). Madero (1982) demonstrated an absence of shrimp PPO activity at pH values of 5.0 and below. The PPO enzyme has an optimum activity in a pH range of 5 to 7 (Zawistowski et al., 1991) with shrimp PPO optimum activity range reported to be pH 6.5 to 7.5 (Simpson et al., 1987). Phenolase enzymes may be irreversibly inactivated at a pH of 3 (Richardson and Hyslop, 1985).

Shrimp harvest and processing

In the Gulf of Mexico, there are three major commercial species of shrimp: white shrimp (*Penaeus setiferus*), brown shrimp (*Penaeus aztecus*), and pink shrimp (*Penaeus duorarum*). On the Texas coast, white shrimp (*Penaeus setiferus*) and brown shrimp (*Penaeus aztecus*) are most commonly harvested for commercial processing. Shrimp are harvested in nets which are towed by fishing vessels for varying lengths of time. The time of tow or drag depends on the amount of catch calculated to be contained in the nets.

Upon completion of a drag, the content of the nets is released onto the back deck of the boat and the shrimp are separated from the remainder of the catch (fish, crabs, and other marine life) which is thrown back in the water. The shrimp are then "headed" (heads removed by hand), placed in baskets, and washed with sea water. In some cases, shrimp are stored on the boat with heads on. Washing of headed shrimp before storage on board the boat is very important because the tail meat has been exposed to contamination from deck surfaces, hands, baskets, etc. This washing decreases the bacterial load on the shrimp (Miget, 1991). Washing of headed shrimp may also remove some of the polyphenoloxidase enzyme responsible for melanosis on shrimp (Ward, 1990).

After the catch has been headed and washed, it can be preserved by either icing or brine freezing. Iced shrimp are usually dipped in a 1.25% sodium bisulfite solution for 1 min and placed in layers of ice in the boat hold for storage. Brine frozen shrimp may or may not be dipped in sodium bisulfite solution before freezing. These shrimp

(50 - 60 lbs.) are then placed in open mesh bags (onion sacks) and submerged in the brine tanks. The brine tanks typically contain a solution consisting of salt (approx 23%), corn syrup, and sodium bisulfite. Soaking time in the brine solution is approximately 15 - 20 min. The bags of shrimp are then removed from the brine tanks and placed in the on board freezer.

Once in port, the hold of vessels with iced shrimp are flooded with water to melt the ice. The shrimp are then vacuum pump into holding tanks in the processing plant. Bags of brine frozen shrimp are taken off the boat and emptied into holding tanks in the processing plant. The shrimp are soaked in these holding tanks for approximately 10 min to allow the shrimp to thaw and separate.

Further processing procedures for iced and brine frozen shrimp are very similar. The shrimp are separated by size according to the number of shrimp per pound, a process called grading. After grading, the shrimp may be block frozen in 5 lb. boxes in a plate or blast freezer, individually quick frozen (IQF), peeled and IQF, peeled and deveined and IQF, or be further processed into a breaded product.

Lactic acid

L-Lactic acid (2-hydroxypropionic acid) is a short-chain organic acid that in its edible form is a slightly yellow liquid available in 50%, 80%, or 88% aqueous solutions (Smulders, 1987). Lactic acid has recently been used in the meat industry as a microbial decontaminant on carcass surfaces. Dickson and Anderson (1992), in a review of past research, concluded that a decontamination step in processing can improve the microbial safety and shelf life of meat and should be regarded as an

integral part of the production process. This conclusion may also hold true for the shrimp production process.

Lactic acid along with other organic acids (acetic, propionic, citric, etc.) inhibits microbial growth by: (1) cell membrane gradient neutralization, (2) denaturation of intracellular proteins, and (3) lowering the internal pH of bacteria. When an undissociated organic acid molecule enters a bacterial cell, it will dissociate since the internal pH of the cell is much higher than the pK of the acid. This phenomenon lowers the internal pH of the cell. To compensate, a net transport of protons out of the cell must take place. The cell membrane, however, is proton impermeable resulting in an overall depletion of cellular energy which inhibits cell growth (Eklund, 1989).

In shrimp, lactic acid is present after rigor mortis as a result of anaerobic breakdown of carbohydrates. The quantity of lactic acid present is, however, very low due to the relatively low concentrations of glycogen and other sugars in shrimp tissue. Lactic acid and glycogen levels in 0-hr post-mortem brown shrimp are both reported to be 160 mg/100g (0.16%) which is low when compared to concentrations found in mammals (Flick and Lovell, 1972). Lactic acid and glycogen levels in marine animals are, however, generally lower than those of warm blooded animals (Flick and Lovell, 1972).

Sodium bisulfite

Sulfiting agents is a term that food scientists use to describe a group of chemical additives whose common characteristic is the release of sulfur dioxide

(Modderman, 1986). Sodium bisulfite is a salt that falls into this category. It has been used in the seafood industry to inhibit melanosis in shrimp since the early 1950's (Fieger, 1951). The recommended procedure is a 1 min dip in a 1.25% sodium bisulfite solution (Camber et al., 1957).

The mechanism by which sodium bisulfite inhibits enzymatic browning or melanosis in shrimp is not fully understood. Melanosis is caused by enzymatic oxidation of phenolic compounds to quinones which polymerize to form melanins. Ferrer et al. (1989) suggest that bisulfite inhibits melanosis by two mechanisms: (1) by reacting with intermediate quinones in the melanosis reactions, forming sulfoquinones, and (2) by irreversibly reacting with PPO causing complete inactivation. Another theory is that bisulfite is a competitive inhibitor of the PPO reaction (Madero, 1982). This research proposed that sulfite binds to a sulfhydryl group located at the active sight of the enzyme, preventing melanosis.

Ingestion of sulfites has been linked to many adverse reactions in sulfite-sensitive people; the most common of which is asthma. However, other reactions such as urticaria, pruritis, angioedema, swelling of the tongue, difficulty in swallowing, tightness in the chest, clammy skin, weakness, headache, dizziness, nausea, hives, and contact sensitivity have been documented (Taylor et al., 1986). Because sodium bisulfite can cause such adverse allergic reactions in certain individuals, the United States Food and Drug Administration (FDA) is under pressure to more strictly regulate the use of sulfites in shrimp (Wagner and Finne, 1986). This regulation has been restricted due to the absence of any viable alternatives to the use of sulfites.

Currently, with the introduction of 4-hexylresorcinol, an additive proven to inhibit melanosis, regulatory action may be eminent.

4-Hexylresorcinol

4-Hexylresorcinol is one of a number of 4-substituted resorcinols that are very good inhibitors of PPO activity in a variety of foods including shrimp (McEvily et al., 1991a). It is a dihydroxybenzene with a hexyl group in the 4 position and hydroxyl groups on positions 1 and 3 of the aromatic ring. This compound is considered GRAS for use as a processing aid to prevent melanosis in shrimp (Frankos et al., 1991). Shrimp tissues treated with 4-hexylresorcinol according to the recommended procedures are reported to have mean residuals of 1.18 ppm with a standard deviation of 0.13 ppm (King et al., 1991). This blackspot inhibitor can easily replace sulfiting agents in the on board dip tank and can be used under the same type of dipping protocol presently followed in the shrimp industry. Dipping of shrimp in 4-hexylresorcinol requires no changes in the post-treatment storage, shipping, or processing of the product. In light of the numerous safety and functional advantages, 4-hexylresorcinol could be considered an effective substitute for sulfites in the shrimp industry (McEvily, et al., 1991b).

The purpose of this study was to provide information on the effect of lactic acid, alone and in combination with the melanosis inhibitors sodium bisulfite and 4-hexylresorcinol, on melanosis and microbiological shelf life of fresh brown shrimp.

MATERIALS AND METHODS

Preliminary testing

Preliminary investigations were conducted to determine whether the treatment of shrimp with L-lactic acid, alone or in combination with 4-hexylresorcinol or sodium bisulfite, would result in product with objectionable sensory defects (discoloration, off-odor, etc.). Frozen Indian shrimp (*Penaeus indicus*, 1.8 kg) were obtained from a commercial source and thawed in a 1-gal Ziploc® freezer bag under running tap water at 29°C. Thawed shrimp were then separated into six groups (250 g per group) and treated as follows: (1) Control (1 min dip in Synthetic Sea Water; Acuff, 1992), (2) 1.0% L-lactic acid (Purac, Inc., Arlington Heights, IL) dip for 1 min, (3) 1.25% sodium bisulfite (William Blythe & Co. Ltd., Lancashire, England) dip for 1 min, (4) 0.0025% 4-hexylresorcinol (EverFresh™, Opta Food Ingredients, Inc., Cambridge, MA) dip for 1 min, (5) 1.25% sodium bisulfite with 1.0% L-lactic acid dip for 1 min, or (6) 0.0025% 4-hexylresorcinol with 1.0% L-lactic acid dip for 1 min.

Dip solutions were prepared using Synthetic Sea Water in 1,000 mL glass beakers filled to a volume of 500 mL. pH of each dip solution was determined with a Corning M140 pH meter (Corning, Scientific Instruments, Medfield, MA) equipped with a Corning combination electrode (#476483, Corning Scientific Instruments, Medfield, MA). The Corning combination electrode was placed into each dip solution while stirring and the pH meter was allowed to stabilize before the reading was recorded.

Each 250-g group of shrimp was submerged in the appropriate dip solution for 1 min with gentle agitation in a 1,000 mL glass beaker. After dipping, the treated shrimp and dip solution were poured through a strainer and allowed to drain for 10 to 15 seconds. Three shrimp were randomly selected from each group to determine the pH of the meat tissue after treatment. The pH measurement of the meat was obtained immediately after treatment by peeling back the first shell segment and placing the electrode of a Corning M140 pH meter (flat bulb design, Model #269-164, Curtin Matheson Scientific, Inc., Houston, TX) directly onto the surface of the meat. Shrimp used for pH measurements were discarded after examination. The shrimp were then stored in ice separated by treatment group within divided compartments of two ice chests at a ratio of approximately 2 parts ice to 1 part shrimp. Crushed ice was added and mixed in with the ice and shrimp throughout the 14-day storage period when necessary to compensate for melting ice.

On 0, 1, and 6 days of storage, duplicate shrimp samples (50 g each) for each treatment group were examined for total aerobic plate count (APC). A 50-g sample (2-4 shrimp) was placed in 450 mL of sterile 0.1% peptone (Difco, Detroit, MI) diluent and blended for 2 min in a sterile Waring Blendor 700 (Model 1120, Waring Products Division, New Hartford, CT). One mL (divided over 4 plates), 0.1 mL, and 0.1 mL volumes of appropriate decimal dilutions (sterile 0.1% peptone diluent) of each sample homogenate were surface plated on pre-poured and dried Tryptic Soy Agar (TSA, Difco, Detroit, MI) plates. The plates were incubated at 25°C for 72 hr, after which the APC was determined from countable (25 to 250 colonies) plates (Swanson

et al., 1992). Counting was assisted by the use of a darkfield Quebec Colony Counter (American Optical, Scientific Instrument Division, Keene, NH). Before each 50-g shrimp sample was examined for APC, a pH measurement of a representative shrimp from each treatment group was conducted as described above. Each treatment group was also inspected before microbiological sampling during the 14 day storage period for any visual or odor defects.

Procurement of shrimp

Brown shrimp (*Penaeus aztecus)* were obtained during two separate fishing trips (March 12, 1992 and April 29, 1992) from the Gulf of Mexico on board a 22.5 m, 13,600 kg (shrimp capacity) fishing vessel (Gulf King 36) supplied by Gulf King, Inc., Aransas Pass, TX. The two trips served as replications of the experimental treatments. For both trips, as is customary when fishing for Texas brown shrimp, the trawl or drag took place at night. Nets were dropped in the water 35 miles off the coast of Port Aransas, TX at approximately 16:30 and were harvested at approximately 06:30. Immediately after the harvest was placed on deck, the shrimp were separated from the rest of the catch which was returned to the Gulf. The shrimp were headed and washed by boat personnel and then sorted by Texas A&M University personnel into six groups (approx. 4.5 kg per group) that were treated as follows: (1) Control (sea water dip for 1 min), (2) 1.0% L-lactic acid dip for 1 min, (3) 1.25% sodium bisulfite dip for 1 min, (4) 0.0025% 4-hexylresorcinol dip for 1 min, (5) 1.25% sodium bisulfite with 1.0% L-lactic acid dip for 1 min, or (6) 0.0025% 4-hexylresorcinol with 1.0% L-lactic acid dip for 1 min. Each of the six groups of

shrimp was split into 2 subgroups (approx. 2.3 kg each). One subgroup of shrimp was treated with the dip solutions immediately after sorting and the other subgroup was treated after a 2-hr delay on deck at ambient temperature to simulate normal practices during peak harvesting times.

On board preparation of dip solutions

Each dip solution was prepared (1 hr before use) on board the boat in six 18.9-L plastic buckets using sea water filled to a volume of 11.4 L of the appropriate dip per bucket. Pre-measured portions of L-lactic acid (129.05 mL to be added to 11.4 L), 4-hexylresorcinol (24 g to be added to 11.4 L), and sodium bisulfite (147.4 g to be added to 11.4 L) were brought aboard the boat in sealed containers to facilitate rapid dip mixing on board the vessel. L-Lactic acid was stored in 200-mL plastic dilution bottles. The 4-hexylresorcinol and sodium bisulfite were each stored in separate 1-pint Ziploc® freezer bags. Each 18.9-L bucket was filled to the 11.4 L level and appropriate pre-measured quantities of treatment chemical(s) were added while stirring. Temperature of the dip solutions was measured once, after mixing, using a digital thermometer (Digi-Thermo, Model TX-201F, Soar Corporation, Cherry Hill, NJ). The external sensor of the digital thermometer was placed into the dip solutions for approximately 30 sec before reading and recording. The pH of each dip solution was determined using a Markson Model 612 portable pH meter equipped with a Combination pH\Reference Electrode (Model #34607, Markson Science Inc., Phoenix, AZ). The Combination pH\Reference Electrode was placed into each dip solution while stirring and the pH meter was allowed to stabilize before the pH reading was

recorded. A pH measurement of each dip solution was also conducted after each shrimp treatment during Trip 2.

Treatment of shrimp

Each 2.3-kg subgroup of shrimp was placed in a nylon mesh bag, immediately after heading and separating, and completely submerged in the appropriate dip solution for 1 min with moderate up and down agitation. This process of dipping a batch of shrimp for 1 min in a particular solution was designed to minimize on board activities by simulating the current dip procedure, calling for a 1 min dip in 1.25% sodium bisulfite solution (Camber et al., 1957). After dipping, the mesh bag containing the shrimp was allowed to drain for 10 to 15 sec. The bag was opened and 1 shrimp was removed for a pH measurement of the meat directly below the shell on the first segment of the shrimp. This measurement was obtained by peeling back the shell and placing the portable pH meter equipped with a Combination pH\Reference surface electrode directly onto the surface of the meat. Each treatment group was immediately placed in ice for transport and storage in separate 68.1-L ice chests (Igloo® Corporation, Houston, TX) at an approximate ratio of 2 parts ice to 1 part shrimp. Crushed ice was added daily throughout the 16-day storage period to compensate for melting ice. Treatment groups that were dipped immediately after washing and heading were stored in the left side of the ice chest. Shrimp treated after the 2-hr delay were stored in the right side of the ice chest. The two treatment subgroups were separated in the ice chests by a folded Labcraft® biohazard bag (Curtin Matheson Scientific, Inc., Houston, TX) placed as a center divider. All ice chests containing

shrimp were then transported to the Food Microbiology Laboratory at Texas A&M University for sampling and storage.

During storage, the 6 ice chests were placed on 1-m high stainless steel tables. Each ice chest was arranged so that the drain spout extended approximately 10 cm over the edge of the table, and was elevated opposite the drain spout to allow water from melting ice to drain continuously.

Microbiological examination

Microbiological examination of shrimp. On 1, 3, 7, 10, 13, and 16 days of storage for Trip 1, and 0, 1, 3, 7, 10, 13, and 16 days of storage for Trip 2, duplicate samples (50 g each) were randomly selected from each treatment group and examined for APC. Day 0 samples were collected on board the boat, placed in 1-pint Ziploc® freezer bags, and stored in ice for transport. A 50-g sample (1-4 shrimp) was placed in 450 mL of sterile 0.1% peptone diluent and blended for 2 min in a Waring Blendor as described earlier. One mL (divided over 4 plates), 0.1 mL, and 0.1 mL volumes of appropriate decimal dilutions (sterile 0.1% peptone diluent) of each sample homogenate were surface plated on pre-poured and dried TSA plates. The plates were incubated at 25°C for 72 hr, after which the APC was determined from countable plates as described previously.

From plate counts obtained from shrimp harvested during Trip 1, 1 to 3 representatives of each colony type appearing on countable plates were picked and transferred to separate TSA slants (Vanderzant et al., 1985). Slants were incubated for 48 hr at 25°C. The identity of each isolate was determined by diagnostic tests and

schemes as described by Vanderzant and Nickelson (1969) and Holt (1984,1986). Each of the colony types was identified to generic level and then expressed as a percentage of the total number of colonies appearing on the countable plates. This procedure was followed to produce a bacterial type distribution for samples from each sampling day throughout the storage period of Trip 1. Bacterial type distributions for samples collected on Trip 2 were not conducted.

Microbiological examination of ice. Duplicate samples of ice from the fishing vessel (Gulf King 36) and the laboratory ice maker were examined for APC. Two samples of ice from the vessel were collected directly from the ice hold using sterile 16 x 150-mm screw cap culture tubes. Ice from 3 different locations in the hold was aseptically collected in each tube to obtain a pooled sample. The tubes were then capped, stored on ice, and transported to Texas A&M University for examination. Two ice samples from the laboratory ice maker were collected during shrimp storage periods in sterile 16 x 150-mm screw cap culture tubes. The ice for each sample was taken from 3 separate locations in the ice maker and pooled as described above. Ice samples from the boat and the ice maker were examined for APC in exactly the same manner. The ice was thawed at ambient temperature. Then one mL (divided over 4 plates), 0.1 mL, and 0.1 mL volumes of appropriate decimal dilutions (sterile 0.1% peptone diluent) of each ice sample were surface plated on pre-poured and dried TSA plates. Duplicate sets of plates were incubated at 25°C for 72 hr and 7°C for 16 days (laboratory ice maker only), after which the APC and psychrotrophic plate counts (PsPC) were determined as described earlier.

At a later date (April 5, 1993), duplicate samples of ice from another fishing vessel (Gulf King 18) were examined for APC and PsPC. Four samples of ice from the vessel were collected directly from the ice hold using sterile 100-mL glass dilution bottles. Aerobic plate counts and PsPC were determined as described above. In addition, a bacterial type distribution for these ice samples was determined as previously described.

Microbiological examination of dip solutions. On Trip 2, a 15-20 mL sample from each on board dip solution was obtained and examined for APC. Each dip solution was collected in a sterile 16 x 150-mm screw cap culture tube: (1) after the initial preparation, (2) after the first treatment (0 hr), (3) after the second treatment (2-hr delay), and (4) at the end of the day (3-hr delay). The sample tubes were sealed, stored on ice, and transported to Texas A&M University for microbiological examination. One mL (divided over 4 plates), 0.1 mL, and 0.1 mL volumes of appropriate decimal dilutions (sterile 0.1% peptone diluent) of each sample were surface plated on pre-poured and dried TSA plates. The plates were incubated at 25°C for 72 hr, after which the APC was determined from countable plates as described earlier.

pH measurement of shrimp

Before each 50-g shrimp sample was examined for APC, a representative shrimp (1) was selected to determine the pH of the meat tissue. Each pH measurement of the meat was obtained by peeling back the first shell segment and placing a surface pH electrode directly onto the surface of the meat. For pH

measurements of shrimp in the laboratory, a Corning M140 pH meter equipped with a flat bulb design electrode was used as described earlier.

Sensory evaluation

All shrimp treatments were evaluated before each sampling by a trained 3-member panel for degree of melanosis (blackspot) according to a melanosis scale (Otwell and Marshall, 1986) used to describe and rate the occurrence of melanosis on shrimp (Table 1). This melanosis scale can be related to recommendations developed by the National Marine Fisheries Service for grading of raw shrimp (Code of Federal Regulations, 1991). A score of 4 or less corresponded to Grade 1 whereas a score between 4 and 8 (Grade 2) was considered indicative of shrimp with a measurable quality defect resulting in a devalued product. Any score of 8 or greater represented a severe defect, approaching an unacceptable product (Otwell and Marshall, 1986; McEvily et al., 1991b). Development of off-odors during storage was also noted and recorded.

Statistical analysis of data

Data was analyzed statistically, where applicable, by analysis of variance. Means were separated using Duncan's multiple range test (SAS Institute, 1982).

Table 1 - Scale to describe and rate the occurrence of melanosis (blackspot) on shrimp (Otwell and Marshall, 1986).

Melanosis Score	Description
0	Absent
2	Slight, noticeable on some shrimp
4	Slight, noticeable on most shrimp
6	Moderate, noticeable on most shrimp
8	Heavy, noticeable on most shrimp
10	Heavy, totally unacceptable

RESULTS

Preliminary testing

Preliminary investigations were conducted to determine whether the treatment of shrimp with L-lactic acid, alone or in combination with 4-hexylresorcinol or sodium bisulfite, would result in product with objectionable sensory defects (discoloration, off-odor, etc.). Each dip solution was prepared using Synthetic Sea Water (Acuff, 1992). pH measurements of preliminary test shrimp tissue samples stored on ice for 0-6 days and dip solutions are presented in Table 2. pH measurements of dip solutions ranged from 2.2 for the 1.0% L-lactic acid and the 0.0025% 4-hexylresorcinol with 1.0% L-lactic acid solutions to 6.5 for the control and 0.0025% 4-hexylresorcinol solutions. Shrimp tissue pH values exhibited a much smaller pH range initially (day 0) and throughout the storage period for all treatments. In general, pH values for shrimp tissue increased from storage day 0 to day 6 with the exception of decreases of 7.1 to 6.5 from day 0 to day 1 for 1.0% L-lactic acid shrimp and 7.9 to 7.6 from day 0 to day 1 for 0.0025% 4-hexylresorcinol treated shrimp.

Aerobic plate counts (log_{10}/g) for preliminary test shrimp stored on ice for 0-6 days varied and displayed no consistent pattern throughout the storage period (Table 3). Counts (log_{10}/g) ranged from 6.0 for day 1 0.0025% 4-hexylresorcinol with 1.0% L-lactic acid treated shrimp to 7.4 for day 1 1.25% sodium bisulfite with 1.0% L-lactic acid treated shrimp.

Table 2 - pH measurements of preliminary test shrimp tissue samples[a] stored on ice for 0-6 days and dip solutions.

Days of Storage	Treatment[b]					
	C	L	S	E	LS	LE
0	7.5	7.1	6.7	7.9	5.9	7.0
1	7.4	6.5	7.4	7.6	7.1	7.7
6	7.9	7.5	7.9	7.8	7.9	7.8
Dip Solutions	6.5	2.2	3.9	6.5	2.6	2.2

[a] pH measurements of shrimp tissue were obtained by peeling back the first shell segment and placing the surface electrode of a Corning M140 pH meter (Corning, Scientific Instruments, Medfield, MA) directly onto the surface of the meat.

[b] C = Control (1 min dip in Synthetic Sea Water; Acuff, 1992), L = 1.0% L-lactic acid dip for 1 min, S = 1.25% sodium bisulfite dip for 1 min, E = 0.0025% 4-hexylresorcinol dip for 1 min, LS = 1.25% sodium bisulfite with 1.0% L-lactic acid dip for 1 min, LE = 0.0025% 4-hexylresorcinol with 1.0% L-lactic acid dip for 1 min.

Table 3 - Aerobic plate counts (APC, log_{10}/g) of preliminary test shrimp samples stored on ice for 0-6 days.

Days of Storage	Treatment[a]					
	C	L	S	E	LS	LE
0	6.6	6.6	6.9	6.6	6.4	6.4
1	6.8	6.3	6.3	6.7	7.4	6.0
6	7.1	7.0	7.0	7.2	6.4	6.9

[a] C = Control (1 min dip in Synthetic Sea Water; Acuff, 1992), L = 1.0% L-lactic acid dip for 1 min, S = 1.25% sodium bisulfite dip for 1 min, E = 0.0025% 4-hexylresorcinol dip for 1 min, LS = 1.25% sodium bisulfite with 1.0% L-lactic acid dip for 1 min, LE = 0.0025% 4-hexylresorcinol with 1.0% L-lactic acid dip for 1 min.

Sensory analyses of preliminary test shrimp were conducted on days 4 and 14 of storage. All treatment groups emitted a stale to putrid smell on day 14 but had acceptable odor evaluations on day 4. There were no other indications of any unusual visual or odor defects for any of the treatment groups of preliminary test shrimp.

pH and temperature of on board dip solutions

pH measurements of on board dip solutions for Trip 1, obtained immediately before shrimp were dipped ranged from 2.2 for 1.0% L-lactic acid and 0.0025% 4-hexylresorcinol with 1.0% L-lactic acid to 8.2 for the sea water control (Table 4). The temperature of all dip solutions was 19.4°C. Trip 2 on board dip solution pH measurements (Table 5) taken immediately before dipping (Initial) were similar to those for Trip 1 with only slightly higher pH readings recorded for 1.25% sodium bisulfite, 0.0025% 4-hexylresorcinol, and 1.25% sodium bisulfite with 1.0% L-lactic acid solutions. Temperature of all dip solutions for Trip 2 was 23.3°C. Trip 2 dip solutions demonstrated measurable pH changes with respect to time of measurement (Table 5). The pH of control and 0.0025% 4-hexylresorcinol solutions decreased 0.8 and 0.6 respectively, whereas 1.0% L-lactic acid, 1.25% sodium bisulfite, 1.25% sodium bisulfite with 1.0% L-lactic acid, and 0.0025% 4-hexylresorcinol with 1.0% L-lactic acid solutions showed increases of 0.2, 0.2, 0.3, and 0.1 respectively.

pH measurements of shrimp tissue

pH measurements of shrimp tissue for Trips 1 and 2 obtained after the 0-hr and 2-hr dips are presented in Table 6. Trip 1 pH readings were similar to Trip 2 with the exceptions of post 2-hr 1.0% L-lactic acid and 1.25% sodium bisulfite with 1.0%

Table 4 - pH measurements of on board dip solutions (Trip 1).

	Treatment[a]					
	C	L	S	E	LS	LE
Dip Solutions	8.2	2.2	4.4	7.8	2.5	2.2

[a] C = Control (1 min dip in sea water), L = 1.0% L-lactic acid dip for 1 min, S = 1.25% sodium bisulfite dip for 1 min, E = 0.0025% 4-hexylresorcinol dip for 1 min, LS = 1.25% sodium bisulfite with 1.0% L-lactic acid dip for 1 min, LE = 0.0025% 4-hexylresorcinol with 1.0% L-lactic acid dip for 1 min.

Table 5 - pH measurements of on board dip solutions (Trip 2).

Time of Measurement[b]	Treatment[a]					
	C	L	S	E	LS	LE
Initial	8.2	2.2	4.5	8.1	2.6	2.2
Post 0-hr	8.0	2.3	4.6	7.8	2.7	2.3
Post 2-hr	7.5	2.4	4.7	7.5	2.8	2.3
Post 3-hr[c]	7.4	2.4	4.7	7.5	2.9	2.3

[a] C = Control (1 min dip in sea water), L = 1.0% L-lactic acid dip for 1 min, S = 1.25% sodium bisulfite dip for 1 min, E = 0.0025% 4-hexylresorcinol dip for 1 min, LS = 1.25% sodium bisulfite with 1.0% L-lactic acid dip for 1 min, LE = 0.0025% 4-hexylresorcinol with 1.0% L-lactic acid dip for 1 min.

[b] Indicates the time at which the pH measurements were obtained.

[c] End of day.

Table 6 - pH measurements of shrimp tissue (Trip 1 and Trip 2).

Time of Measurement[b]	Treatment[a]					
	C	L	S	E	LS	LE
	Trip 1					
Post 0-hr	6.7	5.1	5.8	6.6	5.1	6.0
Post 2-hr	6.6	5.8	6.1	6.7	6.1	5.5
	Trip 2					
Post 0-hr	6.8	5.1	5.8	6.8	4.9	5.2
Post 2-hr	6.8	5.0	6.2	6.6	4.9	5.5

[a] C = Control (1 min dip in sea water), L = 1.0% L-lactic acid dip for 1 min, S = 1.25% sodium bisulfite dip for 1 min, E = 0.0025% 4-hexylresorcinol dip for 1 min, LS = 1.25% sodium bisulfite with 1.0% L-lactic acid dip for 1 min, LE = 0.0025% 4-hexylresorcinol with 1.0% L-lactic acid dip for 1 min.

[b] Indicates the time at which the pH measurements were obtained.

L-lactic acid and post 0-hr 0.0025% 4-hexylresorcinol with 1.0% L-lactic acid treatments. The differences in pH measurements ranged from a 0.8 decrease for Trip 2 on the post 2-hr 1.0% L-lactic acid treatment and post 0-hr 0.0025% 4-hexylresorcinol with 1.0% L-lactic acid treatment to a 1.2 decrease for Trip 2 on the post 2-hr 1.25% sodium bisulfite with 1.0% L-lactic acid treatment.

Mean pH measurements of shrimp obtained on Trip 1 (0-hr and 2-hr treatments) and Trip 2 (0-hr and 2-hr treatments) increased overall throughout the 16-day storage period for all treatment groups (Table 7 and Table 8). pH values ranged from 6.55 to 8.04 for Trip 1 (0-hr) shrimp, 6.40 to 8.04 for Trip 1 (2-hr) shrimp (Table 7), 5.89 to 7.89 for Trip 2 (0-hr) shrimp, and 6.09 to 7.73 for Trip 2 (2-hr) shrimp (Table 8). Significant statistical differences between treatments within the same day of storage occurred on days 1, 13, and 16 for Trip 1 (0-hr) and on days 1, 4, 10, and 16 for Trip 1 (2-hr) (Table 7). Other significant differences between treatments within the same day of storage were demonstrated on days 0, 1, 7, and 13 for Trip 2 (0-hr) and on days 0, 1, 7, and 16 for Trip 2 (2-hr) (Table 8).

Microbiological examination

Microbiological examination of shrimp. Mean APCs (log_{10}/g) of shrimp obtained on Trip 1 (0-hr and 2-hr treatments) and Trip 2 (0-hr and 2-hr treatments) increased over the 16-day storage period for all treatment groups (Table 9 and Table 10). For most treatments, a decrease in bacterial numbers occurred on day 4, followed by increases throughout the remainder of the storage period. APCs (log_{10}/g) ranged from 3.90 to 8.80 for Trip 1 (0-hr) shrimp, 4.10 to 8.65 for Trip 1 (2-hr)

Table 7 - Mean pH measurements of Gulf of Mexico brown shrimp (Penaeus aztecus) *obtained on Trip 1.*

Days of Storage	Treatment[a] C	L	S	E	LS	LE	Order of Means[b]
			0-hr[c]				
1	7.14	6.59	6.85	6.87	6.55	6.65	C E S LE L LS
4	7.00	7.25	7.26	7.19	7.00	6.90	S L E C LS LE
7	7.52	7.31	7.23	7.54	7.42	7.29	E C LS L LE S
10	7.37	7.50	7.54	7.64	7.64	7.49	LS E S L LE C
13	7.65	7.76	7.59	7.83	7.51	7.60	E L C LE S LS
16	7.95	8.04	7.99	8.02	7.89	7.81	L E S C LS LE
			2-hr[c]				
1	7.19	6.57	6.83	6.99	6.40	6.77	C E S LE L LS
4	7.47	7.07	7.06	7.14	7.03	7.08	C E LE L S LS
7	7.26	7.35	7.47	7.16	7.23	7.20	S L C LS LE E
10	7.33	7.64	7.65	7.66	7.77	7.46	LS E S L LE C
13	7.64	7.60	7.74	7.73	7.56	7.57	S E C L LE LS
16	7.84	7.87	7.97	7.93	7.94	8.04	LE S LS E L C

[a] C = Control (1 min dip in sea water), L = 1.0% L-lactic acid dip for 1 min, S = 1.25% sodium bisulfite dip for 1 min, E = 0.0025% 4-hexylresorcinol dip for 1 min, LS = 1.25% sodium bisulfite with 1.0% L-lactic acid dip for 1 min, LE = 0.0025% 4-hexylresorcinol with 1.0% L-lactic acid dip for 1 min.

[b] Mean values within the same day of storage and underscored by a common line do not differ significantly ($p > 0.05$).

[c] Shrimp were treated on board the boat immediately after harvest (0-hr) or following a 2-hr post-harvest delay (2-hr).

Table 8 - Mean pH measurements of Gulf of Mexico brown shrimp (Penaeus aztecus) *obtained on Trip 2.*

Days of Storage	Treatment[a] C	L	S	E	LS	LE	Order of Means[b]
			0-hr[c]				
0	6.95	6.49	6.58	7.26	6.50	5.89	E C S LS L LE
1	7.01	6.50	6.80	6.85	6.57	6.53	C E S LS LE L
4	7.00	6.93	7.09	7.01	7.02	7.11	LE S LS E C L
7	7.17	7.12	7.23	7.43	7.52	7.20	LS E S LE C L
10	7.36	7.43	7.42	7.46	7.47	7.33	LS E L S C LE
13	7.38	7.48	7.42	7.89	7.54	7.70	E LE LS L S C
16	7.62	7.55	7.54	7.68	7.54	7.43	E C L LS S LE
			2-hr[c]				
0	6.97	6.38	6.84	6.95	6.09	6.45	C E S LE L LS
1	7.15	6.61	6.75	7.04	6.55	6.58	C E S L LE LS
4	7.13	7.20	7.03	7.19	6.99	6.87	L E C S LE LS
7	7.25	7.37	7.33	7.58	7.36	7.29	E L LS S LE C
10	7.47	7.36	7.31	7.32	7.36	7.25	C LS L E S LE
13	7.49	7.51	7.61	7.60	7.59	7.50	S E LS L LE C
16	7.45	7.55	7.54	7.60	7.57	7.73	LE E LS L S C

[a] C = Control (1 min dip in sea water), L = 1.0% L-lactic acid dip for 1 min, S = 1.25% sodium bisulfite dip for 1 min, E = 0.0025% 4-hexylresorcinol dip for 1 min, LS = 1.25% sodium bisulfite with 1.0% L-lactic acid dip for 1 min, LE = 0.0025% 4-hexylresorcinol with 1.0% L-lactic acid dip for 1 min.

[b] Mean values within the same day of storage and underscored by a common line do not differ significantly ($p > 0.05$).

[c] Shrimp were treated on board the boat immediately after harvest (0-hr) or following a 2-hr post-harvest delay (2-hr).

Table 9 - Mean aerobic plate counts (APC, log_{10}/g) of Gulf of Mexico brown shrimp (Penaeus aztecus) *obtained on Trip 1.*

Days of Storage	Treatment[a]						Order of Means[b]
	C	L	S	E	LS	LE	
	0-hr[c]						
1	5.15	4.75	4.75	5.10	4.70	3.90	C E L S LS LE
4	4.80	4.95	4.60	4.75	4.35	4.35	L C E S LS LE
7	5.40	5.45	4.95	5.55	5.00	4.90	E L C LS S LE
10	6.55	6.35	6.70	6.95	5.80	5.75	E S C L LS LE
13	7.65	7.65	7.50	8.10	7.15	7.20	E C L S LE LS
16	8.80	8.45	8.35	8.65	8.50	8.45	C E LS LE L S
	2-hr[c]						
1	4.95	4.75	4.70	4.50	4.65	4.10	C L S LS E LE
4	4.15	4.40	5.05	5.10	4.65	4.60	E S LS LE L C
7	5.55	4.85	4.85	5.20	4.80	4.30	C E L S LS LE
10	6.10	6.15	6.00	5.95	5.95	5.85	L C S E LS LE
13	7.45	7.85	7.45	7.40	7.40	7.30	L C S E LS LE
16	8.65	8.50	8.20	8.25	8.55	8.55	C LE LS L E S

[a] C = Control (1 min dip in sea water), L = 1.0% L-lactic acid dip for 1 min, S = 1.25% sodium bisulfite dip for 1 min, E = 0.0025% 4-hexylresorcinol dip for 1 min, LS = 1.25% sodium bisulfite with 1.0% L-lactic acid dip for 1 min, LE = 0.0025% 4-hexylresorcinol with 1.0% L-lactic acid dip for 1 min.

[b] Mean values within the same day of storage and underscored by a common line do not differ significantly ($p > 0.05$).

[c] Shrimp were treated on board the boat immediately after harvest (0-hr) or following a 2-hr post-harvest delay (2-hr).

Table 10 - Mean aerobic plate counts (APC, log_{10}/g) of Gulf of Mexico brown shrimp (Penaeus aztecus) *obtained on Trip 2.*

Days of Storage	Treatment[a] C	L	S	E	LS	LE	Order of Means[b]
			0-hr[c]				
0	4.55	4.45	4.45	4.50	4.35	5.00	LE C E L S LS
1	4.55	4.65	4.40	4.55	4.30	3.85	L E C S LS LE
4	4.70	4.45	4.00	4.20	4.70	3.90	C LS L E S LE
7	5.30	4.95	5.00	5.00	5.15	4.60	C LS E S L LE
10	6.45	5.90	6.10	6.50	5.90	5.80	E C S L LS LE
13	7.50	7.45	7.20	7.60	6.90	7.20	E C L LE S LS
16	8.15	8.00	7.75	8.15	7.90	8.05	C E LE L LS S
			2-hr[c]				
0	4.50	4.65	4.25	4.10	4.75	4.15	LS L C S LE E
1	4.25	5.05	4.45	4.90	4.00	4.75	L E LE S C LS
4	4.30	4.55	4.30	4.75	4.05	4.45	E L LE C S LS
7	4.75	5.00	4.90	5.60	4.40	4.45	E L S C LE LS
10	6.35	6.20	5.55	6.30	5.60	6.20	C E L LE LS S
13	7.55	7.40	7.15	7.25	6.70	7.35	C L LE E S LS
16	7.85	8.15	7.75	7.85	7.70	8.00	L LE C E S LS

[a] C = Control (1 min dip in sea water), L = 1.0% L-lactic acid dip for 1 min, S = 1.25% sodium bisulfite dip for 1 min, E = 0.0025% 4-hexylresorcinol dip for 1 min, LS = 1.25% sodium bisulfite with 1.0% L-lactic acid dip for 1 min, LE = 0.0025% 4-hexylresorcinol with 1.0% L-lactic acid dip for 1 min.

[b] Mean values within the same day of storage and underscored by a common line do not differ significantly ($p > 0.05$).

[c] Shrimp were treated on board the boat immediately after harvest (0-hr) or following a 2-hr post-harvest delay (2-hr).

shrimp (Table 9), 3.85 to 8.15 for Trip 2 (0-hr) shrimp, and 4.00 to 8.15 for Trip 2 (2-hr) shrimp (Table 10). Significant statistical differences in APC between treatments within the same day of storage for Trip 1 (0-hr) shrimp occurred on days 1, 7, 10, and 13, while, Trip 1 (2-hr) shrimp demonstrated significant differences on days 1, 7, 13, and 16 (Table 9). Trip 2 (0-hr) shrimp showed significant differences in APC between treatments within the same day of storage on days 4, 7, 10, 13, and 16, differing slightly from Trip 2 (2-hr) shrimp, demonstrating significant differences on days 1, 4, 7, 13, and 16 (Table 10).

Mean APCs and distributions of microbial types on the shrimp from all treatments of Trip 1 are presented by treatment and time of treatment in Tables 11 through 22. With the exception of the 0.0025% 4-hexylresorcinol with 1.0% L-lactic acid treatment groups, the microflora of all treatment groups (0-hr and 2-hr) was dominated initially (day 1) by *Pseudomonas* spp. (Tables 11 through 20). The 0.0025% 4-hexylresorcinol with 1.0% L-lactic acid treatment groups (0-hr and 2-hr) were dominated initially by coryneform bacteria (Tables 21 and 22). During storage on ice, type distributions for all treatment groups showed increases in the percentage of coryneform bacteria and then a gradual shift toward gram-negative psychrotrophic bacteria (*Pseudomonas, Moraxella-Acinetobacter* spp.). However, by the end of the storage period on day 16, *Pseudomonas* spp. was dominant or a major part of the bacterial population in all treatments. *Lactobacillus* spp., *Micrococcus* spp., *Staphylococcus* spp., *Streptococcus* spp., *Aeromonas spp., Alcaligenes* spp.,

Table 11 - Mean aerobic plate counts (APC, log_{10}/g) and distribution of microbial types on Gulf of Mexico brown shrimp (Penaeus aztecus) *obtained on Trip 1 (control[a], 0-hr[b]).*

Days of Storage	APC	Percent Distribution of Microflora[c]					
		Cor	Lact	Alc	Flavo	M/A	Ps
1	5.2	21					79
4	4.8	27				5	68
7	5.4	60		5		24	11
10	6.6	49	5			40	6
13	7.7	30			2	19	49
16	8.8	13	7				80

[a] Control = 1 min dip in sea water.

[b] Shrimp treated on board the boat immediately after harvest (0-hr).

[c] Cor = coryneform bacteria, Lact = *Lactobacillus*, Alc = *Alcaligenes*, Flavo = *Flavobacterium*, M/A = *Moraxella/Acinetobacter*, Ps = *Pseudomonas*.

Table 12 - Mean aerobic plate counts (APC, log_{10}/g) and distribution of microbial types on Gulf of Mexico brown shrimp (Penaeus aztecus) *obtained on Trip 1 (control[a], 2-hr[b]).*

Days of Storage	APC	Percent Distribution of Microflora[c]				
		Cor	Lact	M/A	Ps	Uni
1	5.0	9			91	
4	4.2	74		7	19	
7	5.6	47		40	13	
10	6.1	65	3	20	11	
13	7.5	42		39	19	
16	8.7	32			59	9

[a] Control = 1 min dip in sea water.
[b] Shrimp treated on board the boat after a 2-hr post-harvest delay.
[c] Cor = coryneform bacteria, Lact = *Lactobacillus*, M/A = *Moraxella/Acinetobacter*, Ps = *Pseudomonas*, Uni = Unidentified.

Table 13 - Mean aerobic plate counts (APC, log_{10}/g) and distribution of microbial types on Gulf of Mexico brown shrimp (Penaeus aztecus) *obtained on Trip 1 (lactic acid[a], 0-hr[b]).*

		Percent Distribution of Microflora[c]				
Days of Storage	APC	Cor	Flavo	M/A	Ps	Uni
1	4.8	4		2	94	
4	5.0	4			96	
7	5.5	41		44	15	
10	6.4	50	1	16	34	
13	7.7	13			87	
16	8.5	8			75	17

[a] Lactic acid = 1.0% L-lactic acid dip for 1 min.
[b] Shrimp treated on board the boat immediately after harvest (0-hr).
[c] Cor = coryneform bacteria, Flavo = *Flavobacterium*, M/A = *Moraxella/Acinetobacter*, Ps = *Pseudomonas*, Uni = Unidentified.

Table 14 - Mean aerobic plate counts (APC, log_{10}/g) and distribution of microbial types on Gulf of Mexico brown shrimp (Penaeus aztecus) *obtained on Trip 1 (lactic acid[a], 2-hr[b]).*

Days of Storage	APC	Percent Distribution of Microflora[c]					
		Cor	Staph	Strep	Flavo	M/A	Ps
1	4.8	5	1				94
4	4.4	80		5			15
7	4.9	27				37	36
10	6.2	16			6	32	46
13	7.9	44			5	13	38
16	8.5	3					97

[a] Lactic acid = 1.0% L-lactic acid dip for 1 min.

[b] Shrimp treated on board the boat after a 2-hr post-harvest delay.

[c] Cor = coryneform bacteria, Staph = *Staphylococcus*, Strep = *Streptococcus*, Flavo = *Flavobacterium*, M/A = *Moraxella/Acinetobacter*, Ps = *Pseudomonas*.

Table 15 - Mean aerobic plate counts (APC, log_{10}/g) and distribution of microbial types on Gulf of Mexico brown shrimp (Penaeus aztecus) *obtained on Trip 1 (sodium bisulfite[a], 0-hr[b]).*

		Percent Distribution of Microflora[c]				
Days of Storage	APC	Cor	M/A	Ps	Y/M	Uni
1	4.8	21	1	78		
4	4.6	61		9		30
7	5.0	82		16	1	1
10	6.7	55	11	34		
13	7.5	34	28	38		
16	8.4	11		89		

[a] Sodium bisulfite = 1.25% sodium bisulfite dip for 1 min.
[b] Shrimp treated on board the boat immediately after harvest (0-hr).
[c] Cor = coryneform bacteria, M/A = *Moraxella/Acinetobacter*, Ps = *Pseudomonas*, Y/M = Yeast/Mold, Uni = Unidentified.

Table 16 - Mean aerobic plate counts (APC, log_{10}/g) and distribution of microbial types on Gulf of Mexico brown shrimp (Penaeus aztecus) *obtained on Trip 1 (sodium bisulfite[a], 2-hr[b]).*

Days of Storage	APC	Percent Distribution of Microflora[c]			
		Cor	M/A	Ps	Uni
1	4.7	23	7	67	3
4	5.1	1		99	
7	4.9	35	21	44	
10	6.0	42	20	37	
13	7.5	32	24	44	
16	8.2	23	12	65	

[a] Sodium bisulfite = 1.25% sodium bisulfite dip for 1 min.
[b] Shrimp treated on board the boat after a 2-hr post-harvest delay.
[c] Cor = coryneform bacteria, M/A = *Moraxella/Acinetobacter*, Ps = *Pseudomonas*, Uni = Unidentified.

Table 17 - Mean aerobic plate counts (APC, log_{10}/g) and distribution of microbial types on Gulf of Mexico brown shrimp (Penaeus aztecus) *obtained on Trip 1 (4-hexylresorcinol[a], 0-hr[b]).*

		Percent Distribution of Microflora[c]			
Days of Storage	APC	Cor	Flavo	M/A	Ps
1	5.1	2			98
4	4.8	46	8		46
7	5.6	64		34	2
10	7.0	50		17	33
13	8.1	42		42	16
16	8.7	25			75

[a] 4-hexylresorcinol = 0.0025% 4-hexylresorcinol dip for 1 min.
[b] Shrimp treated on board the boat immediately after harvest (0-hr).
[c] Cor = coryneform bacteria, Flavo = *Flavobacterium*, M/A = *Moraxella/Acinetobacter*, Ps = *Pseudomonas*.

Table 18 - Mean aerobic plate counts (APC, log_{10}/g) and distribution of microbial types on Gulf of Mexico brown shrimp (Penaeus aztecus) *obtained on Trip 1 (4-hexylresorcinol[a], 2-hr[b]).*

		Percent Distribution of Microflora[c]				
Days of Storage	APC	Cor	Alc	Flavo	M/A	Ps
1	4.5	2	2		2	94
4	5.1	9			3	88
7	5.2	40		7	44	8
10	6.0	57			26	17
13	7.4	74			26	
16	8.3	12			16	72

[a] 4-hexylresorcinol = 0.0025% 4-hexylresorcinol dip for 1 min.

[b] Shrimp treated on board the boat after a 2-hr post-harvest delay.

[c] Cor = coryneform bacteria, Alc = *Alcaligenes*, Flavo = *Flavobacterium*, M/A = *Moraxella/Acinetobacter*, Ps = *Pseudomonas*.

Table 19 - Mean aerobic plate counts (APC, log_{10}/g) and distribution of microbial types on Gulf of Mexico brown shrimp (Penaeus aztecus) *obtained on Trip 1 (lactic acid + sodium bisulfite[a], 0-hr[b]).*

		Percent Distribution of Microflora[c]						
Days of Storage	APC	Cor	Mic	Aero	Flavo	M/A	Ps	Uni
1	4.7	25		7			64	4
4	4.4	13	6				81	
7	5.0	48				17	35	
10	5.8	35			8	46	11	
13	7.2	11			5	33	51	
16	8.5	41				11	48	

[a] Lactic acid + sodium bisulfite = 1.25% sodium bisulfite with 1.0% L-lactic acid dip for 1 min.
[b] Shrimp treated on board the boat immediately after harvest (0-hr).
[c] Cor = coryneform bacteria, Mic = *Micrococcus*, Aero = *Aeromonas*, Flavo = *Flavobacterium*, M/A = *Moraxella/Acinetobacter*, Ps = *Pseudomonas*, Uni = Unidentified.

Table 20 - Mean aerobic plate counts (APC, log_{10}/g) and distribution of microbial types on Gulf of Mexico brown shrimp (Penaeus aztecus) *obtained on Trip 1 (lactic acid + sodium bisulfite[a], 2-hr[b]).*

		Percent Distribution of Microflora[c]							
Days of Storage	APC	Cor	Lact	Staph	Flavo	M/A	Ps	Vib	Y/M
1	4.7	11					89		
4	4.7	23				9	58		10
7	4.8	64	2		2	23	5	4	
10	6.0	57				20	23		
13	7.4	8			4	32	56		
16	8.6	6		3		19	72		

[a] Lactic acid + sodium bisulfite = 1.25% sodium bisulfite with 1.0% L-lactic acid dip for 1 min.
[b] Shrimp treated on board the boat after a 2-hr post-harvest delay.
[c] Cor = coryneform bacteria, Lact = *Lactobacillus*, Staph = *Staphylococcus*, Flavo = *Flavobacterium*, M/A = *Moraxella/Acinetobacter*, Ps = *Pseudomonas*, Vib = *Vibrio*, Y/M = Yeast/Mold.

Table 21 - Mean aerobic plate counts (APC, log_{10}/g) and distribution of microbial types on Gulf of Mexico brown shrimp (Penaeus aztecus) *obtained on Trip 1 (lactic acid + 4-hexylresorcinol[a], 0-hr[b]).*

		Percent Distribution of Microflora[c]					
Days of Storage	APC	Cor	Lact	Aero	M/A	Ps	Y/M
1	3.9	60		3	2	35	
4	4.4	71			13	15	1
7	4.9	33	1		5	61	
10	5.8	47			26	27	
13	7.2	27			6	67	
16	8.5	4			17	79	

[a] Lactic acid + 4-hexylresorcinol = 0.0025% 4-hexylresorcinol with 1.0% L-lactic acid dip for 1 min.
[b] Shrimp treated on board the boat immediately after harvest (0-hr).
[c] Cor = coryneform bacteria, Lact = *Lactobacillus*, Aero = *Aeromonas*, M/A = *Moraxella/Acinetobacter*, Ps = *Pseudomonas*, Y/M = Yeast/Mold.

Table 22 - Mean aerobic plate counts (APC, log_{10}/g) and distribution of microbial types on Gulf of Mexico brown shrimp (Penaeus aztecus) *obtained on Trip 1 (lactic acid + 4-hexylresorcinol[a], 2-hr[b]).*

Days of Storage	APC	Percent Distribution of Microflora[c]			
		Cor	Staph	M/A	Ps
1	4.1	61	4	1	35
4	4.6	10		15	75
7	4.3	52			48
10	5.9	30		28	42
13	7.3	30		22	48
16	8.6	20		11	69

[a] Lactic acid + 4-hexylresorcinol = 0.0025% 4-hexylresorcinol with 1.0% L-lactic acid dip for 1 min.
[b] Shrimp treated on board the boat after a 2-hr post-harvest delay.
[c] Cor = coryneform bacteria, Staph = *Staphylococcus*, M/A = *Moraxella/Acinetobacter*, Ps = *Pseudomonas*.

Flavobacterium spp., *Vibrio* spp., yeasts/molds, and unidentified bacteria were also detected, but constituted a much smaller part of the microflora throughout storage.

Microbiological examination of ice. Ice from the fishing vessel was used initially to ice down all treatment groups in ice chests on board the boat. Laboratory ice maker ice was then added to the ice chests throughout the 16-day storage period to replenish melting ice. Mean APC (log_{10}/mL) of ice collected from the fishing vessel was 4.00. APC and PsPC (log_{10}/mL) of ice from the laboratory ice maker were 1.04 and <0.00, respectively (data not shown in tabular form). Mean APC and PsPC (log_{10}/mL) of ice collected on the follow-up trip from the second fishing vessel were 3.4 and 3.7, respectively. The bacterial type distribution from this ice showed a microflora consisting of 75% *Pseudomonas* spp. and 25% coryneform bacteria (data not shown in tabular form).

Microbiological examination of dip solutions. APCs (log_{10}/mL) of on board dip solutions for all treatments ranged from 0.0 ESPC on the initial 1.25% sodium bisulfite with 1.0% L-lactic acid dip solution to 4.1 on the post 2-hr and post 3-hr samples for control dip solutions (Table 23). All dip solutions showed an overall increase in bacterial numbers from the initial to the post 3-hr time of measurement. However, increases in APC recorded throughout the day for dip solutions containing L-lactic acid were low (1.0% L-lactic acid = 0.1 ESPC, 1.25% sodium bisulfite with 1.0% L-lactic acid = 0.8 ESPC, and 0.0025% 4-hexylresorcinol with 1.0% L-lactic acid = 0.2 ESPC) compared to other dip solutions (control = 1.8, 1.25% sodium bisulfite = 1.1, and 0.0025% 4-hexylresorcinol = 1.0).

Table 23 - Aerobic plate counts (APC, log_{10}/mL) of on board dip solutions obtained on Trip 2.

Time of Measurement[b]	Treatment[a]					
	C	L	S	E	LS	LE
Initial	2.3	0.6[c]	1.0[c]	1.9	0.0[c]	0.3[c]
Post 0-hr	3.1[c]	0.3[c]	1.0[c]	1.9	0.0[c]	0.8[c]
Post 2-hr	4.1	0.5[c]	2.2	3.0	0.0[c]	0.0[c]
Post 3-hr[d]	4.1	0.7[c]	2.1	2.9	0.8[c]	0.5[c]

[a] C = Control (1 min dip in sea water), L = 1.0% L-lactic acid dip for 1 min, S = 1.25% sodium bisulfite dip for 1 min, E = 0.0025% 4-hexylresorcinol dip for 1 min, LS = 1.25% sodium bisulfite with 1.0% L-lactic acid dip for 1 min, LE = 0.0025% 4-hexylresorcinol with 1.0% L-lactic acid dip for 1 min.

[b] Indicates the time at which the samples were obtained.

[c] Indicates estimated standard plate count (ESPC, Messer et al., 1985).

[d] End of day.

Sensory evaluation

Mean melanosis scores of shrimp obtained on Trip 1 (0-hr and 2-hr treatments) and Trip 2 (0-hr and 2-hr treatments) increased throughout the 16-day storage period for all treatment groups with the exception of Trip 1 (0-hr and 2-hr) 0.0025% 4-hexylresorcinol with 1.0% L-lactic acid treated shrimp which showed decreases on days 13 and 16 (Table 24 and Table 25). Mean melanosis scores ranged from 0.00 to 10.00 for Trip 1 (0-hr and 2-hr) shrimp (Table 24), 0.00 to 7.67 for Trip 2 (0-hr) shrimp, and 0.00 to 9.00 for Trip 2 (2-hr) shrimp (Table 25). Statistically significant differences in melanosis scores occurred on all but day 1 for Trip 1 (0-hr and 2-hr) and Trip 2 (0-hr and 2-hr) shrimp.

All treatment groups of Trip 1 shrimp displayed acceptable odor characteristics until day 11 when the control and 0.0025% 4-hexylresorcinol treatment groups produced unacceptable off-odors. By day 13, all treatment groups of Trip 1 shrimp were evaluated as having noticeable off-odors. For trip 2, all treatment groups of shrimp began to produce noticeable off-odors on day 11 (data not presented in tabular form).

Table 24 - Mean melanosis scores[a,b] of Gulf of Mexico brown shrimp (Penaeus aztecus) *obtained on Trip 1.*

Days of Storage	Treatment[c] C	L	S	E	LS	LE	Order of Means[d]
			0-hr[e]				
1	0.00	0.00	0.00	0.00	0.00	0.00	C E L LE LS S
4	2.00	2.00	0.00	0.00	0.00	0.00	C L E LE LS S
7	4.00[f]	2.00	2.67	0.67	2.00	1.33	C S L LS LE E
10	6.00[f]	6.00[f]	2.67	2.00	2.67	2.00	C L S LS LE E
13	6.00[f]	9.33[g]	3.33	2.00	4.00[f]	0.67	L C LS S E LE
16	8.67[g]	10.00[g]	6.67[f]	2.00	4.67[f]	0.00	L C S LS E LE
			2-hr[e]				
1	0.00	0.00	0.00	0.00	0.00	0.00	C E L LE LS S
4	2.00	2.00	0.67	0.00	0.00	0.00	C L S LE LS E
7	4.67[f]	3.33	1.33	0.67	0.67	1.33	C L S LE LS E
10	6.00[f]	6.00[f]	3.33	2.00	2.00	2.00	C L S LE LS E
13	6.00[f]	8.67[g]	6.00[f]	2.00	4.00[f]	1.33	L S C LS E LE
16	8.67[g]	10.00[g]	6.67[f]	2.00	4.67[f]	0.67	L C S LS E LE

[a] 0 = Absent; 10 = Heavy, totally unacceptable (detailed melanosis scale evaluation description in Table 1).

[b] Individual means are based on data from 3 evaluators scoring a group of 10 randomly selected shrimp.

[c] C = Control (1 min dip in sea water), L = 1.0% L-lactic acid dip for 1 min, S = 1.25% sodium bisulfite dip for 1 min, E = 0.0025% 4-hexylresorcinol dip for 1 min, LS = 1.25% sodium bisulfite with 1.0% L-lactic acid dip for 1 min, LE = 0.0025% 4-hexylresorcinol with 1.0% L-lactic acid dip for 1 min.

[d] Mean values within the same day of storage and underscored by a common line do not differ significantly ($p > 0.05$).

[e] Shrimp were treated on board the boat immediately after harvest (0-hr) or following a 2-hr post-harvest delay (2-hr).

[f] Indicates shrimp with a measurable quality defect resulting in a devalued product.

[g] Represents a severe defect, approaching unacceptable product.

Table 25 - Mean melanosis scores[a,b] of Gulf of Mexico brown shrimp (Penaeus aztecus) *obtained on Trip 2.*

Days of Storage	Treatment[c]						Order of Means[d]
	C	L	S	E	LS	LE	
	0-hr[e]						
1	0.00	0.00	0.00	0.00	0.00	0.00	C E L LE LS S
4	4.00[f]	2.00	0.00	0.00	2.00	0.00	C L LS E LE S
7	4.67[f]	4.00[f]	2.00	0.33	1.00	0.00	C L S LS E LE
10	6.00[f]	6.33[f]	4.00[f]	0.00	1.33	0.00	L C S LS LE E
13	5.67[f]	7.33[f]	5.00[f]	1.67	5.67[f]	0.00	L C LS S E LE
16	7.00[f]	7.67[f]	6.33[f]	0.67	5.67[f]	0.67	L C S LS LE E
	2-hr[e]						
1	0.00	0.00	0.00	0.00	0.00	0.00	C E L LE LS S
4	4.00[f]	3.00	2.00	0.00	2.00	0.00	C L S LS LE E
7	6.00[f]	6.00[f]	3.00	1.00	2.00	0.00	C L S LS E LE
10	6.00[f]	7.00[f]	5.33[f]	0.67	3.00	0.67	L C S LS LE E
13	7.00[f]	8.33[g]	6.33[f]	2.00	7.00[f]	1.00	L C LS S E LE
16	8.00[g]	9.00[g]	7.33[f]	1.67	7.33[f]	1.33	L C S LS E LE

[a] 0 = Absent; 10 = Heavy, totally unacceptable (detailed melanosis scale evaluation description in Table 1).

[b] Individual means are based on data from 3 evaluators scoring a group of 10 randomly selected shrimp.

[c] C = Control (1 min dip in sea water), L = 1.0% L-lactic acid dip for 1 min, S = 1.25% sodium bisulfite dip for 1 min, E = 0.0025% 4-hexylresorcinol dip for 1 min, LS = 1.25% sodium bisulfite with 1.0% L-lactic acid dip for 1 min, LE = 0.0025% 4-hexylresorcinol with 1.0% L-lactic acid dip for 1 min.

[d] Mean values within the same day of storage and underscored by a common line do not differ significantly ($p>0.05$).

[e] Shrimp were treated on board the boat immediately after harvest (0-hr) or following a 2-hr post-harvest delay (2-hr).

[f] Indicates shrimp with a measurable quality defect resulting in a devalued product.

[g] Represents a severe defect, approaching unacceptable product.

DISCUSSION

Preliminary investigations were conducted to determine whether the treatment of shrimp with L-lactic acid, alone or in combination with 4-hexylresorcinol or sodium bisulfite, would result in product with objectionable sensory defects (discoloration, off-odor, etc.). No objectionable sensory defects resulting from preliminary treatments were observed. Since pH measurements of shrimp tissue were obtained by peeling back the first shell segment and placing the surface electrode of a Corning M140 pH meter directly onto the surface of the meat, high initial (day 0) pH measurements of preliminary test shrimp treated with L-lactic acid (1.0% L-lactic acid, 1.25% sodium bisulfite with 1.0% L-lactic acid, and 0.0025% 4-hexylresorcinol with 1.0% L-lactic acid) may indicate a lack of consistent penetration of the dip solutions below the shell to the tissue of the shrimp (Table 2). High initial (day 0) APCs (all treatments $\geq$ 6.4) also indicate that the shrimp used for preliminary testing were approaching spoilage levels (APC $> 10^7$, Cobb and Vanderzant, 1975) when testing began (Table 3). All treatment groups also contained shrimp that had reddening of the legs and lower shell extremities, which is characteristic of temperature abused shrimp (Cobb et al., 1977).

Initial pH measurements of on board dip solutions were slighty elevated from Trip 1 to Trip 2 for 1.25% sodium bisulfite (4.4 to 4.5), 0.0025% 4-hexylresorcinol (7.8 to 8.1), and 1.25% sodium bisulfite with 1.0% L-lactic acid (2.5 to 2.6) (Tables 4 and 5). These differences might be attributed to the increase in dip solution temperatures from 19.4°C for Trip 1 to 23.3°C for Trip 2. pH changes were

observed in Trip 2 on board dip solutions with respect to time of measurement. The basic/neutral solutions (control and 0.0025% 4-hexylresorcinol) showed an overall decrease in pH from the initial time of measurement to the post 3-hr measurement whereas, the acidic solutions (1.0% L-lactic acid, 1.25% sodium bisulfite, 1.25% sodium bisulfite with 1.0% L-lactic acid, and 0.0025% 4-hexylresorcinol with 1.0% L-lactic acid) showed an overall increase in pH from the initial time of measurement to the post 3-hr measurement (Table 5). These changes in pH values of the dip solutions may have been due to the addition of proteins, washed off of the shrimp into the dip solutions, which buffered the solution pH levels (Lindsay, 1985; Cheftel et al., 1985).

The pH values of shrimp tissue obtained on board the fishing vessel were consistently lower on Trip 2 compared with Trip 1 in three of the treatments. The differences in pH measurements ranged from a 0.8 decrease for Trip 2 on the post 2-hr 1.0% L-lactic acid treatment and post 0-hr 0.0025% 4-hexylresorcinol with 1.0% L-lactic acid treatment to a 1.2 decrease for Trip 2 on the post 2-hr 1.25% sodium bisulfite with 1.0% L-lactic acid treatment. These lower pH values could be due to decreases in time from removal of shrimp from the dip solution to taking of the pH measurements on Trip 2 (Table 6). Because of the excellent buffering capacity of the muscle tissue of shrimp (Lindsay, 1985), shrimp that were allowed time to sit before sampling may have had adequate time to buffer and alter pH measurements. These inconsistencies may also be due to a lack of consistent penetration of the dip solutions below the shell to the tissue of the shrimp, as stated previously.

Early in this study, it was theorized that a lowering of the pH in shrimp might provide an inhibition of the PPO enzymes and prevent melanosis. pH decreases on L-lactic acid treated shrimp (1.0% L-lactic acid, 1.25% sodium bisulfite with 1.0% L-lactic acid, and 0.0025% 4-hexylresorcinol with 1.0% L-lactic acid) apparently were insufficient to deactivate PPO (pH $\leq$ 3) (Richardson and Hyslop, 1985). Melanosis scores on shrimp treated only with L-lactic acid were not better than control shrimp melanosis scores.

Although some significant statistical differences in shrimp tissue pH measurements were shown between treatment groups on the same day of storage (Tables 7 and 8), the differences were varied and showed no consistent pattern. Therefore, conclusions drawn from these statistically significant differences probably have no realistic value. Additionally, Trip 2 shrimp had lower pH readings at the end of the 16-day storage period as compared to Trip 1, ranging from 7.43 for Trip 2 (0-hr) 0.0025% 4-hexylresorcinol with 1.0% L-lactic acid treated shrimp to 8.04 for Trip 1 (0-hr) 1.0% L-lactic acid and Trip 1 (2-hr) 0.0025% 4-hexylresorcinol with 1.0% L-lactic acid treated shrimp (Tables 7 and 8). This probably resulted from the fact that less shrimp was stored in each ice chest on Trip 2 which could have kept the temperature in the ice chests lower and slowed spoilage.

In this study, APCs are considered separately from melanosis scores since melanosis on shrimp is not necessarily indicative of microbial spoilage. As was the case with pH measurements of shrimp tissue, statistical differences in APCs between treatment groups on the same day of storage were varied and showed no consistent

pattern (Tables 9 and 10). All treatment groups of shrimp were considered unacceptable by odor evaluation and microbial numbers (Cobb and Vanderzant, 1975) by day 13. Therefore, statistically significant differences in APCs between treatments have very little value since all treatment groups spoiled, based on odor and APCs, at essentially the same rate. Shelf life of Gulf of Mexico shrimp stored on ice is expected to be 12-14 days (Cobb et al., 1976).

Microbial type distributions of shrimp by treatment group and time of treatment showed no consistent differences in microflora throughout the 16-day storage period (Tables 11 through 22). The predominance of *Pseudomonas* spp. initially (day 0) in most of the type distributions (Tables 11 through 20) is inconsistent with previous reports (Campbell and Williams, 1952; Vanderzant et al., 1970). This predominance of *Pseudomonas* spp. initially (day 0) may be due to an unavoidably low plate count incubation temperature (18.8°C) in the laboratory during incubation of day 1 and 4 APCs resulting in limited bacterial colony growth, difficulty in colony differentiation, and subsequent altered type distributions. This problem was solved by increasing laboratory incubation temperature to 25°C and increasing incubation time from 48 hr to 72 hr. Another possible explanation could be that the ice from the fishing vessel used to store the shrimp initially may have contained a large number of *Pseudomonas* spp. Although this ice was not typed, follow-up research on ice taken from another fishing vessel showed mean APC and PsPC ($\log_{10}$/mL) of 3.4 and 3.7, respectively containing 75% *Pseudomonas* spp. and 25% coryneform bacteria. Starting at storage day 7, type distributions appeared to progress more normally when plate incubation

temperatures were corrected and the original ice in the ice chests from the fishing vessel was progressively replaced due to melting and drainage by much cleaner ice (log_{10}/mL APC and PsPC of 1.04 and <0.00, respectively) from a laboratory ice maker.

APCs of on board dip solutions indicate that bacterial growth occurred during the time on deck. Higher bacterial numbers in on board dip solutions not containing L-lactic acid (control, 1.25% sodium bisulfite, and 0.0025% 4-hexylresorcinol treatments) after 3 hours (Table 23) may suggest that these solutions, if allowed to sit on deck all day and incubate, could inoculate shrimp with high numbers of microorganisms which could result in decreased shelf life. However, APCs of solutions containing L-lactic acid (1.0% L-lactic acid, 1.25% sodium bisulfite with 1.0% L-lactic acid, and 0.0025% 4-hexylresorcinol with 1.0% L-lactic acid) all showed much lower counts (Table 23) demonstrating an ability of L-lactic acid to control microbial growth in these solutions. Microbial growth in on board dip solutions could become a problem if the dip solutions are mixed in the afternoon (4:00 p.m.) and allowed to sit on deck, at elevated temperatures, until the next morning (14 to 18 hr incubation) when final shrimp from the night-long drag are dipped.

Mean melanosis scores for 0.0025% 4-hexylresorcinol and 0.0025% 4-hexylresorcinol with 1.0% L-lactic acid over the 16-day storage period (melanosis scores all $\leq$ 2.00) (Tables 24 and 25) showed that these treatments prevent melanosis better than any of the other treatments tested. These treatments produced shrimp with melanosis scores of less than 4.00. A melanosis score between 4.00 and 8.00 is

indicative of shrimp with a measurable quality defect resulting in a devalued product. 0.0025% 4-hexylresorcinol with 1.0% L-lactic acid performed equally as well as 0.0025% 4-hexylresorcinol. The 1.25% sodium bisulfite and 1.25% sodium bisulfite with 1.0% L-lactic acid treatments inhibited melanosis somewhat (Tables 24 and 25) but not as well as the 0.0025% 4-hexylresorcinol and 0.0025% 4-hexylresorcinol with 1.0% L-lactic acid treatments. These treatments produced shrimp with melanosis scores between 0.00 and 7.33 over the 16-day storage period. A melanosis score of 8.00 or greater represents a severe quality defect, approaching unacceptable product. The L-lactic acid treatment did not prevent melanosis at all (Tables 24 and 25). In fact, melanosis progressed at faster rate with this treatment than on the control shrimp.

This study showed that treatment with L-lactic acid, alone or in combination with 4-hexylresorcinol or sodium bisulfite, had little effect on the shelf life of Texas brown shrimp (*Penaeus aztecus*). However, 4-hexylresorcinol did perform exceptionally well as a melanosis inhibitor, alone and with L-lactic acid. On the other hand, high bacterial counts in the 0.0025% 4-hexylresorcinol dip solution combined with similar melanosis scores over the 16-day storage period for both the 0.0025% 4-hexylresorcinol and the 0.0025% 4-hexylresorcinol with 1.0% L-lactic acid treated shrimp suggest that a 4-hexylresorcinol/L-lactic acid dip solution may be better than 4-hexylresorcinol alone. Future research should be conducted in a large scale commercial setting to determine whether L-lactic acid in combination with 4-hexylresorcinol is a more feasible sulfite alternative for the inhibition of melanosis than 4-hexylresorcinol alone.

CONCLUSIONS

Within the limits of the experimental procedures used and the number of samples, the following conclusions can be drawn from the data presented in this study:

1. Treatment of shrimp with 1.0% L-lactic acid resulted in no unusual or objectionable sensory defects.
2. The treatment of Texas brown shrimp (*Penaeus aztecus*) with L-lactic acid, alone or in combination with 4-hexylresorcinol or sodium bisulfite, had no effect on the shelf life of the shrimp.
3. L-Lactic acid demonstrated an ability to control microbial growth in on board dip solutions.
4. 4-Hexylresorcinol performed exceptionally well as a sulfite alternative for the inhibition of melanosis in shrimp, alone and in combination with L-lactic acid.
5. Treatments tested in this study inhibited melanosis and overall devaluation of shrimp in the following order: 1.0% L-lactic acid $<$ control $<$ 1.25% sodium bisulfite $\approx$ 1.25% sodium bisulfite with 1.0% L-lactic acid $<$ 0.0025% 4-hexylresorcinol $\leq$ 0.0025% 4-hexylresorcinol with 1.0% L-lactic acid.
6. L-Lactic acid did not interfere with the ability of 4-hexylresorcinol to inhibit melanosis on shrimp at the concentrations tested.

Overall, this study demonstrated that treatment with L-lactic acid, alone or in combination with 4-hexylresorcinol, had no detectable effect on the microbiological shelf life of brown shrimp (*Penaeus aztecus*). In addition, 4-hexylresorcinol

performed exceptionally well as a melanosis inhibitor, whether alone or in combination with L-lactic acid. Since microbial growth was demonstrated in the 4-hexylresorcinol on board dip solution while on deck before treatment of shrimp, this study indicates that a potential abnormally high bacterial inoculation of shrimp during the dip process can be avoided through the use of a dip solution containing 4-hexylresorcinol in combination with L-lactic acid.

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