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New Modified Dipping Method Using 4-hexylresorcinol for Preventing Blackspot Formation in Prawns

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*Industry acceptance of alternative sulphite treatments for preventing blackspot in prawns is limited. This study demonstrated that dipping prawns (*Penaeus monodon *and* Fenneropenaeus merguiensis*) in a less concentrated solution for longer times provides better control of blackspot formation than the recommended 2 minute dip in 50 mg/L 4-hexylresorcinol. Protection improved as modified dipping times and solution concentrations increased. Blackspot protection provided by most of the modified dip methods was more effective than a 1 minute dip in 1% sodium metabisulphite. The recommended 2 minute dip method should be limited to 125 kg when used on consecutive 25-kg batches of prawns. Yields increased by less than 10% even after 72-hour exposure in the modified dip. The new dipping method meets most international residue standards when applying the modified treatments evaluated.*

KEYWORDS 4-hexylresorcinol, dip, melanosis, blackspot, residues, prawns, yield

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INTRODUCTION

For years now, the international seafood industry has been searching for alternatives to sulphiting agents for the prevention of blackspot. Because sulphites cause allergic reactions in some sensitized individuals, consumers and food authorities would like to see a reduction in the amount of sulphite present in food. A number of researchers (Otwell et al., 1992; Slattery et al., 1995) have found that the compound 4-hexylresorcinol can prevent blackspot without the use of sulphites. The Australian seafood industry has not adopted this treatment wholeheartedly due to the fact that the cost per treatment is much higher than that for using sodium metabisulphite, and it has been reported as not always effective when applied.

Present application guidelines recommend a 200 g sample for treating 10 batches of 25 kg of prawns using a dip time of 2 minutes. This application was based on an extrapolation from a small volume experiment with a 2 minute dip in 50 mg/L 4-hexylresorcinol to 227–272 kg of shrimp (Otwell et al., 1992). Industry has expressed concern about the effectiveness and residue differences found in this application related to the amount of prawns treated.

Investigation of current industry practices identified the frequent misuse of the protocol given by the manufacturers and that handling practices had a major impact on effectiveness of the treatment, indicating that alternate treatment methods were needed. The data from these experiments will assist both wild capture and aquaculture industries for treatment in a more efficient manner, reduce the cost of using them, and encourage greater adoption of dipping methods that are safer for consumers while meeting export market requirements.

MATERIALS AND METHODS

Prawns

Two species of prawns were used in the study: banana prawns (*Fenneropenaeus merguiensis*) sourced from the Bribie Island Aquaculture Research Centre (BIARC) and black tiger prawns (*Penaeus monodon*) obtained from a commercial farm (Gold Coast Marine Aquaculture). Fresh prawns of mixed size grades were net harvested to simulate normal aquaculture or trawling operations where no possibility of sorting for size prior to treatment is available.

Dipping Solutions

Standard treatment solutions of 50 mg/L 4-hexylresorcinol were made by dissolving 200 g of 4-hexylresorcinol in 95 L of domestic tap water with salt to deliver a salinity of 3.5% according to the manufacturer's specifications (Nutrinova Australasia Pty Ltd.).

Modified Solutions

The stock solution was diluted in 10-L capacity plastic buckets to produce 2 L solutions of 5 mg/L 4-hexylresorcinol. Salt was added to each bucket to produce a salinity of 3.5%. When this had dissolved, 1 kg of ice was added. This procedure was repeated to produce treatment solutions containing 10 and 20 mg/L 4-hexylresorcinol. A ratio of 2 kg of prawns in 3 L of 4-hexylresorcinol solution (after the ice had melted) was used for all modified dip treatments. Care was taken to keep the buckets containing the solutions and prawns out of direct sunlight as the compound is affected by strong light.

Experimental Treatments

All treatments were performed on the same day with prawns from a single pond. The live prawns were killed by immersion in ice slurry for 5 min, drained for 5 min prior to weighing, and then treated as described below.

Experiment 1

This experiment was performed to test the effectiveness of the current dipping method relative to the quantity of prawns used. Effectiveness was evaluated by processing 250 kg of prawns in just one 95 L dip solution. A freshly made 50 mg/L 4-hexylresorcinol dip solution was prepared, and 25 kg batches of black tiger prawns were dipped consecutively for 2 min. The basket of prawns was moved up and down within the solution to ensure good mixing during the 2 min. A batch of 2 kg was kept for evaluation after 1, 5, 8, and 10 consecutive treatments which corresponds to 25, 125, 200, and 250 kg of prawns having passed through the solution. Prawns were stored in ice for 4 days and evaluated for blackspot formation. The experiment was repeated a second time.

Experiment 2

Effectiveness of modified dipping methods was evaluated using 2 kg of prawns (an average of 95 for banana prawns and 150 for black tiger prawns). For each species, a total of 15 batches were treated with 5, 10, or 20 mg/L 4-hexylresorcinol for 3, 6, 24, 48, or 72 h. The containers were covered with a lid during treatment. The modified dip solutions containing prawns were kept under ice in a cold room. The prawns in the solutions were stirred vigorously at the start of the modified dip and when later sampled.

A 2 kg batch of prawns was dipped in a fresh 50 mg/L 4-hexylresorcinol solution for 2 min (standard dip) while another 2 kg batch was kept untreated as a control.

After all chemical treatments, the prawns were drained for 5 min and weighed. Changes were expressed as a percentage of original weight. The prawns were then placed in grated plastic bins and covered with a plastic mesh, which kept the prawns separate from a covering of ice, and stored for 12 days at 2°C. The melted ice was replaced daily.

Sampling and Evaluations

Prawns were drained for 5 min and weighed pretreatment and posttreatment. Treatment solutions were stirred using a plastic ladle and then sampled (100 mL) for 4-hexylresorcinol concentration prior to the introduction of prawns, halfway through, and at the end of treatment. After treatment, a 100 g sample of whole prawns was removed from each 2 kg batch and frozen for later analysis of 4-hexylresorcinol residue.

Residue levels in the peeled flesh (50% meat recovery) and standard dip and modified dip solution concentrations of 4-hexlyresorcinol were evaluated by high performance liquid chromatography (HPLC) according to the method of King et al. (1991). Analytical sensitivity of the method was determined at 0.1 mg/kg 4-hexylresorcinol.

Formation of blackspot was evaluated every second day of ice storage for a total of 12 days. The number of prawns exhibiting any blackspot was counted, and the amount was expressed as a percentage of the total number present.

Statistical Analysis

Treatments were compared for residues and solution concentration. This was performed with one-way analysis of variance (ANOVA) using the Analytical Software Statistix Version 1.0 (1996).

RESULTS AND DISCUSSION

Experiment 1

Table 1 contains the results of the current dipping method using 25 kg batches of prawns which were consecutively dipped for 2 min for a total load of 250 kg.

No significant differences were found between the two separate dipping sessions for residue, solution concentration, or blackspot incidence after 4 days storage. There were, as could be expected after the large amount of prawns being dipped, significant differences between the

TABLE 1 Residues, Dip Concentration, and Blackspot Levels of Black Tiger Prawn Treated Consecutively in a Standard Dip in 25-kg Batches for a Total of 250 kg for Two Replicate Sessions

Load treated by standard dip(kg)	4-hexylresorcinol residue(mg/kg)[1]	4-hexylresorcinol concentration after load(mg/L)[2]	Blackspot incidence after 4 days under ice(%)
25	0.55[a]	54.6[a]	36
125	0.28[b]	49.4[ab]	42
200	0.1[bc]	39.6[bc]	57
250	0[c]	35.9[c]	60
Control (no chemical)	0	0	90

[1]Significant differences between loads for residue ($p < .01$).
[2]Significant differences between loads for solution concentration after load ($p < .05$).
Means in the same column followed by a different subscript are significantly different at the above level.

sampling times for residue ($p < .01$) and solution concentration ($p < .05$). One other aspect identified was that there was a difference between sachet concentrations. This sachet variability has been observed before, fortunately with no pack resulting in a solution under 50 mg/L 4-hexylresorcinol with a maximum concentration of 59. The next important outcome was when 25 kg of prawns were dipped, the residues were much lower than that obtained when a 2 kg batch was treated which shows that even with good mixing, bulk dipping will result in lower residues.

After 4 days storage under ice, there was a progressive increase in blackspot for each consecutive load threshold. While there were no significant statistical differences between batches, the outcome was certainly significant in a commercial sense. The first 25 kg batch of prawns contained 36% with blackspot while the last of the 250 kg load contained 60%. This outcome does bring into question how acceptable dip methods are when treating up to 250 kg of prawns. If the recommended procedure is followed, it is really only capable of effectively protecting 125 kg of prawns before there is a major increase in blackspot; so, this amount will be used for the cost effectiveness evaluations. As the dip solution lost only half the chemical after 250 kg, there is potential to increase residues and thus improve blackspot protection by modifying the standard dip method.

Experiment 2

The analysis of the prawn 4-hexylresorcinol residue data from the modified dip methods found that there was no statistical difference ($p > .05$) between the two trials for all treatments applied to the two different species of prawn. With no major residue difference between the trials, the combined data for the various treatments was analyzed. The result of using an increasing dip solution concentration and time can be seen in Table 2.

TABLE 2 Comparison of no Chemical, 1% Sodium Metabisulphite, and 50mg/L 4-hexylresorcinol Short Dips with Modified Dipping 2 Kg of Prawns in Various 4-hexylresorcinol Solutions

Dip type, concentration, and time	Weight gain (%)	4-hexylresorcinol residue (mg/kg)	Blackspot protection of modified dip vs. standard dip	Cost of treatment per kilogram of prawns
Sodium metabisulphite Dip	0	-	less	A$0.026
No Chemical	0	0[e]	less	-
Standard Dip	0	0.95[ab]	-	A$0.132
5 mg/L for 3 h	4	0[e]	less	A$0.017
5 mg/L for 6 h	3	0.05[de]	less	A$0.017
5 mg/L for 24 h	4	0.14[cde]	same	A$0.017
5 mg/L for 48 h	5	0.34[cde]	same	A$0.017
5 mg/L for 72 h	6	0.35[cd]	same	A$0.017
10 mg/L for 3 h	4	0.15[cde]	same	A$0.035
10 mg/L for 6 h	4.5	0.3[cde]	same	A$0.035
10 mg/L for 24 h	4.7	0.38[cd]	more	A$0.035
10 mg/L for 48 h	5.8	0.33[cde]	more	A$0.035
10 mg/L for 72 h	7.4	0.35[cd]	more	A$0.035
20 mg/L for 3 h	5	0.3[cde]	same	A$0.069
20 mg/L for 6 h	3.5	0.37[cd]	same	A$0.069
20 mg/L for 24 h	6.7	0.45[cd]	more	A$0.069
20 mg/L for 48 h	7.3	0.78[bc]	more	A$0.069
20 mg/L for 72 h	7.2	1.14[a]	more	A$0.069

Means in the residue column for both types of modified dip treatments followed by a different letter are significantly different ($p < .01$).

The most important aspect of the residue data is whether they are acceptable for the importing country. As the residue standards for 4-hexylresorcinol vary from 0.5 through to 2 mg/kg for different overseas countries (there is no residue standard for Australia), industry can still select a number of modified dipping methods which will satisfy them. The residue in the edible portion from dipping 2 kg of prawns in a 50 mg/L 4-hexylresorcinol solution for 2 min, from either trial, was less than 1 mg/kg. This is much lower than what Guandalini et al. (1998) found, but the prawns used here were freshly harvested. The results are similar to those obtained previously by the authors (Slattery et al., 1995) and others (Lyengar et al., 1991; Otwell et al., 1992). None of the modified dip treatments led to residues greater than 1.5 mg/L. Levels were lower when a larger amount of prawns were processed in the standard dip (see Table 1).

Only prawns dipped for 72 h in a 20 mg/L 4-hexylresorcinol solution had residues in excess of 1 mg/L. A total of 13 treatments resulted in residues below 0.5 mg/L. While low residues will allow the prawns to meet regulatory requirements, there needs to be a sufficient amount present to provide effective protection from blackspot. As indicated by the blackspot development graphs for the first trial (a representative graph for banana

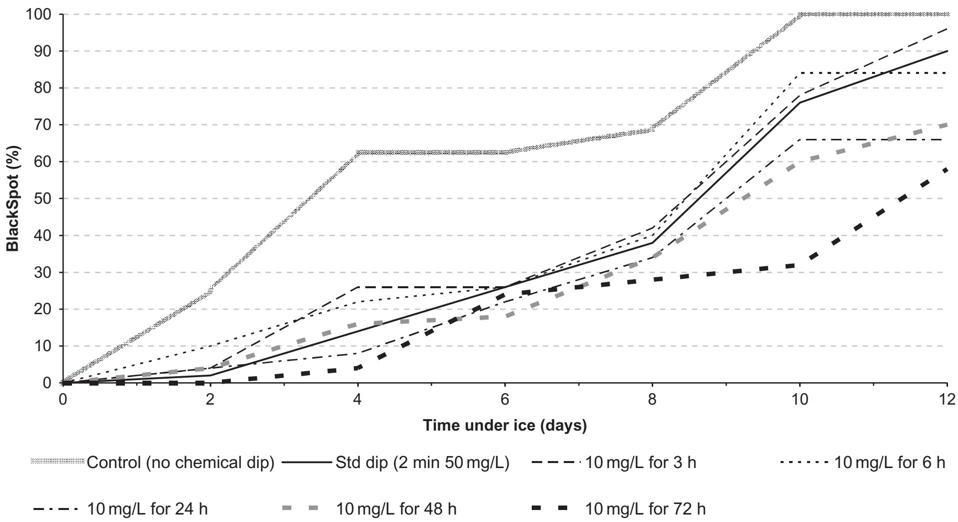

FIGURE 1 Blackspot on banana prawns in 10 mg/L 4-hexylresorcinol.

prawns can be seen in Figure 1), there was good correlation between increasing concentration and modified dip time resulting in better protection from black spot.

Only two treatments applied to banana prawns resulted in less protection than the standard dip (Table 2). This was due to the low residues obtained from dipping the prawns in 5 mg/L solutions for short periods of time.

While there was no difference between the trials for residue, there was for blackspot development. The second trial encountered major blackspot problems almost immediately after the return to the laboratory, as seen in Figure 2. Unknown delays at harvest on a hot day led to high initial blackspot counts during storage under ice. Details from the farm staff afterward confirmed that it took some time to collect full baskets of prawns and instead of delivering what they had, the baskets were left on the side of the pond until full. While the incident of blackspot was higher, the trends of increased protection with increased concentration and exposure were present in the same pattern as found in the first trial. What this outcome does show is that the modified dip treatments still achieved better protection than short dips even under extreme circumstances. An earlier trial with black tiger prawns exhibited similar blackspot levels to the banana prawns until a refrigeration breakdown interfered with the trial.

All of the black tiger prawn modified dip treatments were at least as effective as the standard dip. Once residues greater than 0.1 mg/kg 4-hexylresorcinol were present, protection from blackspot was as effective as the standard dip even though this latter treatment resulted in much higher residues. This

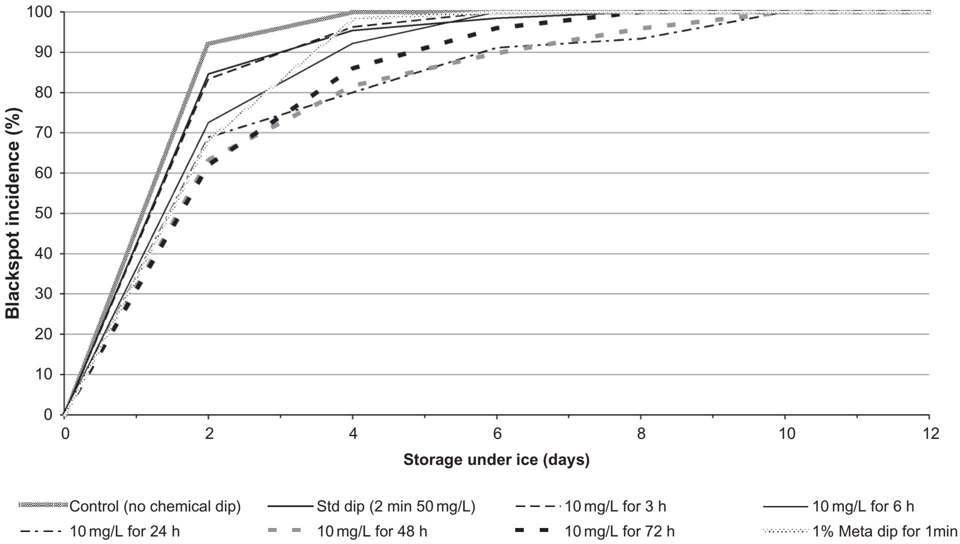

FIGURE 2 Blackspot on black tiger prawns dipped in 10 mg/L 4-hexylresorcinol.

indicates that while the residue is much lower, extended dipping allows the chemical more time to penetrate the site where it is really needed. At the other end of the spectrum, prawns dipped in 20 mg/L solutions for the longest periods of time had residues that were excessive for some importing countries. The treatments in between these should be suitable for most processors' operating environments.

The data obtained for weight increase due to modified dipping shows that water uptake due to extended dipping is not excessive. As could be expected, there was a corresponding increase in prawn weight with extended dip time but this did not exceed 10% of the original weight for even the longest treatment.

Chemical Cost Analysis

Farm operators and nonfreezer boats hold freshly harvested prawns between 1 and 3 days before dispatch to buyers; therefore, using modified dip treatments is not considered a sufficient difference in current practice to warrant including the cost of storage in this analysis. The chemical cost analysis was based on a purchase price in Australia of AUS$16.47 for a 200-g sachet containing 4-hexylresorcinol and AUS$2.60 for a 1-kg bag of sodium metabisulphite (prices were for delivery and includes tax, September 2007). The dip cost per kilogram of prawns treated was based on the identified effective treatment capacity (125 kg) from the bulk dipping experiment and 100 kg for 1 min dip in 1 kg/100 L of sodium metabisulphite (AUS$0.026/kg).

The modified dip capacity (using the above ratio of prawns to modified dip solution of 1:1 before the ice melted) was calculated from the volume of modified dip solution that can be made from a 200 g sachet containing 2.2% 4-hexylresorcinol and the amount of prawns this would treat. A ratio of 1:1.5 resulted in some prawns remaining uncovered by the solution so it could not be used. The theoretical capacity of a modified dip treatment from a 200-g sachet was 950 kg for a 5mg/L solution, 475 kg for 10mg/L, and 237 kg for 20mg/L.

Based on the effective treatment capacity, as identified in the bulk dip experiment one, the cost benefit analysis for the recommended method was AUS$0.132 per kg. For further comparison, a 1% sodium metabisulphite dip of 1 min had a cost efficiency of AUS$0.026 per kg. While cheaper to use than 4-hexylresorcinol, the level of protection from blackspot provided by sodium metabisulphite was less when a dip time of 30 s was applied (Slattery et al., 1995), or similar when the dip time was increased to the 1 min used in these experiments. The slightly stronger sulphite treatment used by Otwell et al. (1992), a 1 min dip in 1.25% sodium bisulphite, led to lower blackspot protection than a 1 min dip in 50 mg/L 4-hexylresorcinol (standard dip).

Table 2 shows that there are six modified dipping treatments which give better protection from blackspot than a quick 2 min 4-hexylresorcinol dip and are less costly to use. Another seven modified dipping treatments provided similar protection to the standard dip but cost less to use while only two treatments resulted in less protection for prawns from blackspot. Overall, there are 13 treatments which are more cost effective to use than a standard dip with similar or better ability to prevent blackspot. Three of the modified dip treatments were found to be more cost effective to use than sodium metabisulphite at AUS$0.017 per kg.

CONCLUSIONS

These experiments show that there is a range of treatments which can provide industry with an inexpensive and more effective means of protection than the recommended dip in a 50 mg/L solution of 4-hexylresorcinol while still meeting importing country residue standards. It is a matter of choosing the one that best fits the processing conditions and intended market.

Wild capture can successfully use a 3 h dip in a 10 or 20 mg/L 4-hexylresorcinol solution before freezing as this is approximately the time between individual trawls, and the prechilling will speed the freezing process. As aquaculture operations routinely hold prawns overnight, the 24 h modified dip methods will suit their operations. For the retail trade, where prawns are held for several days, even the 72 h modified dips would be convenient as a predip does not lead to excessive residues (data obtained from these experiments but not presented in this article).

For those individuals who are only concerned with the cost of chemicals, they will find that extended dipping of prawns in a solution of 4-hexylresorcinol will be cheaper than a short dip. Finally, a modified dip in 5mg/L 4-hexylresorcinol for 24 to 72 h can be cheaper even than using sodium metabisulphite.

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