Treating Prawns by Soaking in a 4-hexylresorcinol Solution

Steve Slattery, David Williams and Caterina Torrisi

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Treating Prawns by Soaking in a 4-hexylresorcinol Solution

Steve Slattery, David Williams and Catorina Torrisi

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Innovative Food Technology

Queensland Department of Primary Industries and Fisheries

19 Hercules Street

HAMILTON

Brisbane Qld 4007

Ph: 07 3406 8623

Email: Steve.Slattery@dpi.qld.gov.au

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GPO Box 46
Brisbane Queensland 4001

Technical inquiries should be addressed to:
Steve Slattery, Senior Seafood Technologist,
Innovative Food Technology19 Hercules Street
Hamilton Q 4007
+61 7 3406 8623

Steve.Slattery@dpi.qld.gov.au

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2003/417 Treating Prawns with an Extended Dip in Everfresh

PRINCIPAL INVESTIGATOR: Steve L Slattery
ADDRESS: Innovative Food Technology
Department of Primary Industries and Fisheries
19 Hercules Street
Hamilton QLD 4007
Telephone: 07 3406 8623 Fax: 07 3406 8698

OBJECTIVES:

1. Identify alternate methods for treatment of aquacultured prawns with Everfresh to the standard 2 minute dip.
2. Identify alternate methods for treatment of wild caught prawns with Everfresh to the standard 2 minute dip.
3. Identify treatments that will result in residues that will comply with overseas requirements.

NON TECHNICAL SUMMARY:

For years now the 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. The new alternative treatment 4-hexylresorcinol (available commercially as Everfresh®) can prevent blackspot for longer without the use of sulphites (Otwell et al.,1992, Slattery et al, 1995). There are a number of reasons why the Australian seafood industry has not adopted this treatment wholeheartedly. The main reason is that the supplier has only one type of application that it recommends, a once off dip in a solution of Everfresh (equivalent to 50mg/l 4-hexlyresorcinol). Another is the big difference in cost per treatment compared to using sodium metabisulphite. A survey conducted by the authors found that over 20% of fishers applied their blackspot treatment to refrigerated seawater tanks or ice slurries rather than a once off dip (Slattery & Williams, 1988 & 1989). Chemicals are often used in this way by both wild capture and aquaculture producers because it is convenient. The capacity of the standard Everfresh dip method was found to be limited when used on consecutive batches of prawns. Another concern with soaking is the increase in yield due to uptake of water by the prawns. Fortunately this did not exceed 10% even after three days. Several soaking methods were found that gave better protection than the conventional dip yet satisfied international residue standards and provided savings for processors. Many treatments were as cheap to use as a sodium metabisulphite dip.

The key findings were:

- 200g of Everfresh in 1000L water (5 mg 4-hexylresorcinol/L) and 500kg of ice will treat 1000kg prawns effectively, with regular mixing, after a long term exposure (24 or more hours) and is cheaper to use than a sodium metabisulphite dip
- 400g of Everfresh in 1000L water (10 mg 4-hexylresorcinol/L) and 500kg of ice will protect 1000kg prawns, with regular mixing, after just 6 hours, is much cheaper than a standard Everfresh dip and only slightly more expensive than a sodium metabisulphite dip
- 800g of Everfresh in 1000L water (20 mg 4-hexylresorcinol/L) and 500kg of ice will treat 1000kg prawns, with regular mixing, better than a Everfresh dip at half the cost
- Predipping is only necessary if processing delays occur (large catches) or it is to be used for deep water species
- Only 72hr soaks in 20mg/L led to residues higher than 1mg/kg so there is a soak method which can meet any overseas standard

KEYWORDS: Everfresh, 4-hexylresorcinol, prawn, soak

BACKGROUND

For years now the seafood industry has been searching for alternatives to sulphiting agents for the prevention of blackspot. Consumers and food authorities would like to see a reduction in the amount of sulphite present in food. The new alternative treatment Everfresh® can prevent blackspot for longer without the use of sulphites (Otwell *et al*.,1992; Slattery *et al*, 1995).

There a number reasons why the Australian seafood industry has not adopted this treatment wholeheartedly. Apart from being more expensive to purchase than sodium metabisulphite the supplier has only one type of application that it recommends, a once off dip in a solution of Everfresh (equivalent to 50mg/L 4-hexlyresorcinol). A survey conducted by this researcher in 1988 found that over 20% of fishers applied their blackspot treatment to refrigerated seawater tanks or ice slurries rather than a once off dip (Slattery, 1988 & 1989).

Even when using a 50mg/L 4-hexylresorcinol dip the protection provided is not uniform (Guandalini *et al*., 1998). The residue from a once off dip drops rapidly over a number of days storage. These authors found that towards the end of organoleptic acceptability only 20-30% of prawns were unaffected by blackspot. Similar uneven blackspot development was noted by this researcher during storage (Slattery *et al*., 1995). This behaviour is obviously due to uneven exposure and retention of the limited amount of 4-hexylresorcinol that a quick dip in a solution of the chemical can supply.

It is obvious that longer term treatment at lower concentrations can be more convenient for industry to use. There is only limited information on the effect of longer term treatment with 4-hexylresorcinol but only at the same concentration as the short dip (Iyengar *et al*., 1991 and Slattery *et al*., 1995). Providing industry with better and cheaper methods of application will lead to more adoption of safer and less chemical treatment of prawns.

NEED

This project meets the APFA Strategic Objective of “Consistent product quality” and were assist the FRDC program of Industry Development by increasing the quality and safety of Australian prawns. It also meets the key goal of the Queensland Seafood Marketers Association of “provision of high quality Queensland seafood which meets and exceeds the standards and expectations of our customers”.

The seafood industry is becoming more active about consumer safety and has been searching for alternatives to sulphiting agents for the prevention of blackspot on prawns. Consumers and food authorities world wide would like to see a reduction in the amount of sulphite present in food.

Long term treatment at lower concentration can be more convenient for industry to use. The effect of long term treatment with 4-hexylresorcinol but only at the same concentration as the short dip has been reported only by a few authors (Iyengar *et al*., 1991 and Slattery *et al*., 1995). Industry needs information on how to use this safer food chemical in ways that are as cost effective as sodium metabisulphite.

OBJECTIVES

1. Identify alternate methods of treatment of aquaculture prawns with Everfresh to the standard 2 minute dip.
2. Identify alternate methods of treatment of wild caught prawns with Everfresh to the standard 2 minute dip.
3. Identify treatments that will result in residues that will comply with overseas requirements.

METHODS

Terminology

To assist the reader with following the details of this report an explanation of the terms used is provided below.

Dip	Immersion in a solution containing potable water and chemicals for a brief period of time, usually less than half an hour
Standard Everfresh® dip	Immersion in a solution made by dissolving a 200g sachet of Everfresh® in 95L of potable water (this results in a 50mg/L solution of 4-hexylresorcinol) for two minutes
Sodium metabisulphite dip	There is an industry standard for treating prawns by immersion in a solution containing 1kg of sodium metabisulphite in 100L of potable water for 30 seconds for domestic product and 1 minute for export product
Soak	Immersion in a 4-hexylresorcinol solution for a long period of time, usually for several hours
Soak water	A solution containing food grade NaCl added to potable water to make a salt concentration of 3.5% and varying amounts of Everfresh powder to produce 4-hexylresorcinol concentrations of 5, 10 and 20mg/L.
Short soak	Immersion in a solution for less than 12 hours
Long soak	Immersion in a solution for more than 12 hour
Batch	A mass of prawns weighing either 2kg used for soaking or 16kg and 25kg used for dip treatment
Cephalothorax	The head section of the prawn. This contains the digestive organ of the prawn where there are many enzymes which impact on quality.
Abdomen	The tail of the prawn and the main edible portion.

Procedures used

The method described on the Everfresh® packaging recommends immersion in a solution made by dissolving the contents of a 200g sachet of Everfresh in 95L of potable water. The active component of Everfresh is 4-hexylresorcinol, an active inhibitor of the blackspot pigment-causing enzyme called polyphenol oxidase. The preparation of the Everfresh dip results in a solution concentration of 50mg/L 4-hexylresorcinol. Prawns should be immersed in this solution for 2 minutes soon after harvest. The standard Everfresh dip should be considered as one of several control treatments for these experiments, with the main control being no chemical treatment. Contol treatments are those which the reader may be already familiar with and should not change much from one experiment to another, unless something as for Trials 2, 3 and 4 occurs outside the parameters of the experiment. It is used as a reference point in which to compare the results of all the other treatments.

Sodium metabisulphite has long been recommended as a method of treatment for blackspot on prawns. It works by releasing sulphur dioxide which competes with oxygen, a key element in the enzyme reaction described above and by changing the pH. Unfortunately there are a number of individuals who are allergic to sulphur so this method does have public safety issues. It is used by immersing prawns in a solution containing 1kg of sodium metabisulphite in 100L of potable water; for 30 seconds for domestic product and 1 minute for export product. The latter has been used for Trial 4 of this investigation as an extra control.

The experimental design has been developed with the intention to provide guidance to both aquaculture and wild capture industries without the need for separate experiments. McEvily *et al.* (1992) found no difference whether the test solution was based on freshwater or salt, the pH was 2, 5 or 8, a post-treatment rinse was used, when dip times were extended for up to 15 minutes, or the prawns were wild or cultured. Most wild capture producers keep prawns in refrigerated seawater up to overnight if freezing or up to 3 days if selling fresh uncooked. Aquaculture prawns are often kept overnight before packing or freezing.

To keep the number of trips to the farm at a minimum, thus limiting the overall cost of this investigation and to minimise the effect of differences over the growing season and between ponds, all treatments were conducted on the day of each trial. The experiment was repeated with black tiger prawns when the initial storage trial with banana prawns was completed to enhance statistical validity. A total of 64kg of freshly harvested banana prawns were collected at the BIARC research farm at Bribie Island and black tiger prawns at a commercial farm, Gold Coast Marine Aquaculture. They were killed under ice directly after harvest (approximately 5 minutes). The prawns were weighed into batches of approximately 2kg and exposed to the following treatments:

- A control treatment 2kg batch was kept chemical free and stored under ice for up to 12 days. Other control treatments used were the Standard Everfresh Dip and for just Trial 4 a Standard sodium metabisulphite dip.

- Individual 2kg batches of prawns were treated by immersion in 2L of soak water of three different concentrations. One kilogram of ice was then added to the soak water, the contents of the bucket mixed and the prawns held for 3, 6, 24, 48 or 72 hours. After sampling the remaining prawns were poured into the mesh trays and stored under ice for up to 12 days.

- 2kg batch treated with a Standard Everfresh Dip. The solutions were sampled at the start, after one minute and at the end of the 2 minute dip. After sampling the prawns were stored under ice for up to 12 days.

- The final batches of prawns were dipped in 25kg batches as above using the same standard Everfresh dip and then treated by immersion in the range of soak solutions for the various times. After soaking and sampling the prawns were stored under ice for up to 12 days.

The soaking times used were based on normal operating times for a processing plant. It is unlikely that any processor would hold prawns for 12hrs before further processing whether from aquaculture or wild capture as this would result in a very long working day. If interested, the 12hr soaking curve for blackspot protection would obviously fall between the 6 and 24hr soaks for each concentration. Table 1 summarises the experimental design showing the treatments applied for most trials.

Table 1. Treatment regime for prawns.

Treatments before 12 days ice storage	4-hexylresorcinol concentration used to make the slurry (mg/kg)		
No dip or soak (main control)	No chemical		
3 hour soak in ice slurry containing 4-hexylresorcinol	5	10	20
6 hour soak in ice slurry containing 4-hexylresorcinol	5	10	20
24 hour soak in ice slurry containing 4-hexylresorcinol	5	10	20
48 hour soak in ice slurry containing 4-hexylresorcinol	5	10	20
72 hour soak in ice slurry containing 4-hexylresorcinol	5	10	20
2 min dip in 50 mg/kg 4-hexylresorcinol solution (2nd control)	50		
2 min dip + 3 hour soak in ice slurry containing 4-hexylresorcinol	5	10	20
2 min dip + 6 hour soak in ice slurry containing 4-hexylresorcinol	5	10	20
2 min dip + 24 hour soak in ice slurry containing 4-hexylresorcinol	5	10	20
2 min dip + 48 hour soak in ice slurry containing 4-hexylresorcinol	5	10	20
2 min dip + 72 hour soak in ice slurry containing 4-hexylresorcinol	5	10	20

Samples of chemical solutions were taken before, half way through treatment and after prawn treatment and tested for 4-hexylresorcinol concentration. Samples of prawn flesh were taken after treatment but before storage under ice and tested for 4-hexylresorcinol residues. The storage under ice involved placing prawns in a mesh based tray, being covered by a perforated nylon material and fresh ice placed on top each day. During storage all batches of prawns were kept in a cold room at 2°C.

Blackspot was evaluated every second day. The number of prawns with blackspot present anywhere was recorded. As the handling time needed to be kept to a minimum to avoid warming and limit this procedures influence on the rate of blackspot development, there were no separate counts recorded for pigment on the cephalothorax, abdomen or both sections of the prawn.

This aspect and the need to minimise the amount of prawns that were required for each experiment also prohibited a Quality Index Method approach for blackspot evaluation. After counting, the prawns were again covered in ice and returned to the cold room for another two days. Because of the various soaking times applied, trays of prawns were being evaluated every day over a 15 day period.

The graphs depicting blackspot development show the percentage of pigmented prawns from when they left the soak solution. In normal commercial environments prawns would leave the processing area or vessel under ice only after a treatment had been performed. While there is the opportunity for farms to ship prawns fully immersed while still under treatment, this method of transport is more expensive and be most unlikely.

RESULTS

The results from the first trial were presented as a scientific poster at the AustralAsian Aquaculture Conference in Sydney on 26-29th September 2004 and the results from all trials were presented as a paper at the WAS conference in Florence, Italy on 9th May 2006. The paper has been submitted as a scientific paper for publication and a technical article present in the Appendix was forwarded to the Australian Prawn Growers Association newsletter, FRDC R&D news and Nutrinova detailing the most appropriate treatments to use.

Trial 1

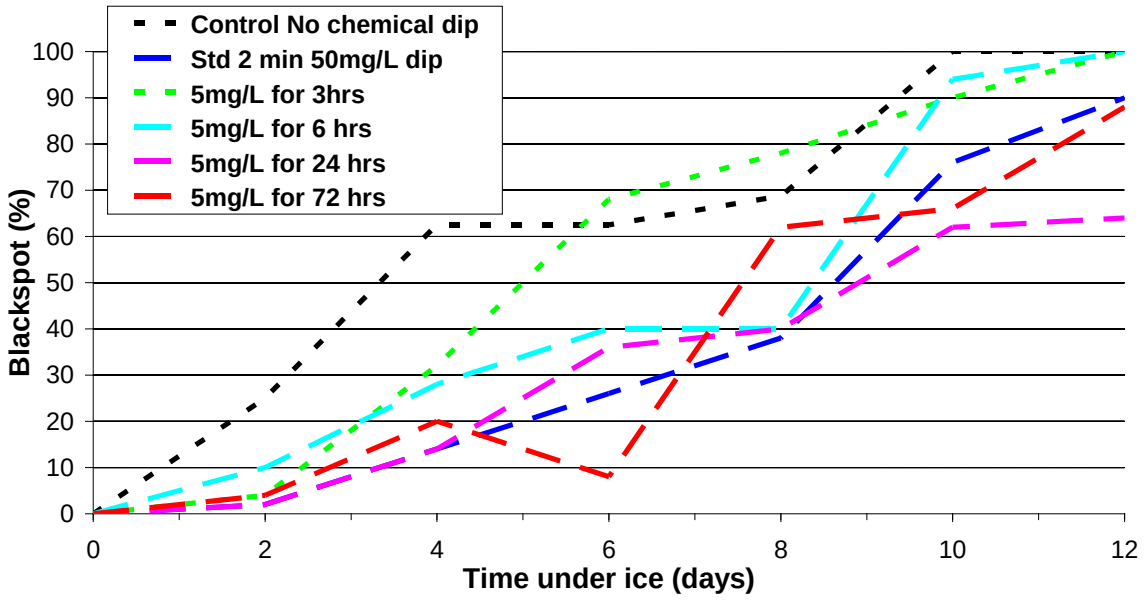
Handling conditions

Due to the timing of the completion of contracts it was too late to obtain any black tiger prawns from the industry partner farm for the first trial. Banana prawns (*Fenneropenaeus merguiensis*) were harvested from the DPI&F research BIARC facility for the first experiment. After treatment on the farm, the prawns were returned to the laboratory to monitor blackspot development and test for residues.

Blackspot protection

Figure 1 below shows the amount of blackspot that developed during storage under ice for the controls of prawns just left untreated or dipped in the standard Everfresh dip (50mg/kg 4-hexylresorcinol for 2 minutes) or prawns which were soaked for the various times in a 5mg/kg solution of 4-hexylresorcinol. The two controls will be present in all the graphs as reference treatments. For all of the blackspot development graphs in this report dotted lines were used to represent treatments that resulted in less blackspot protection than the standard Everfresh dip, dashed lines mean the treatment was equal to the standard Everfresh dip while solid lines mean the treatment provided better protection than the standard Everfresh dip.

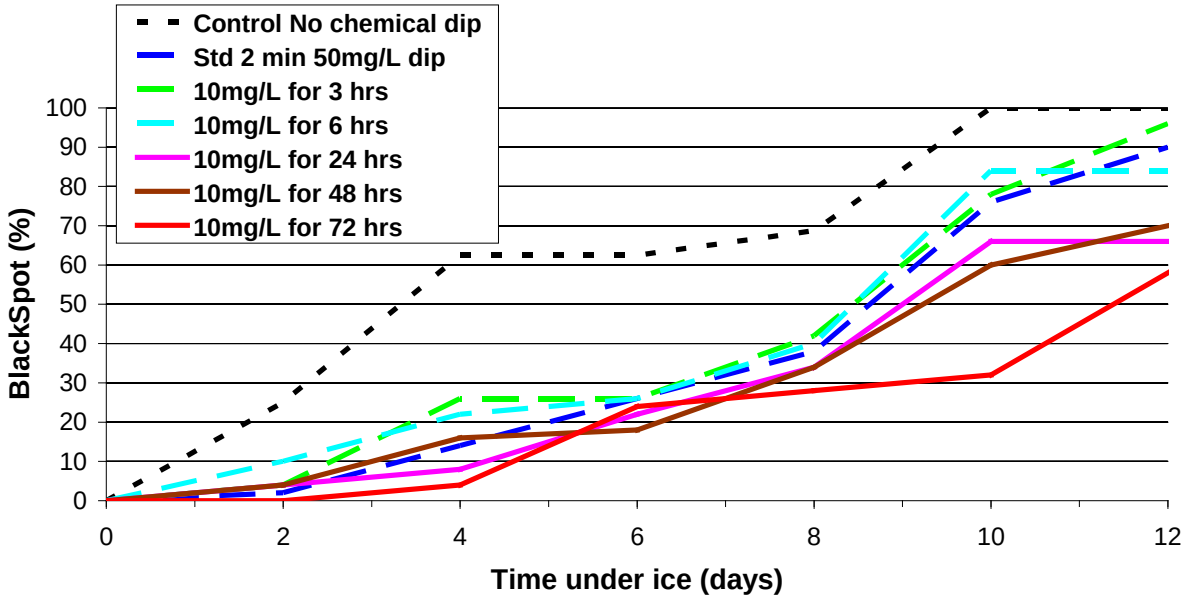
Figure 1. Banana prawns soaked in 5mg/kg 4-hexylresorcinol



Prawns soaked in solution of 4-hexylresorcinol of 5mg/L results in less blackspot, developing during 12 days of storage under ice than if there had been no chemical treatment, with the exception of a 5mg/L soak for 3 hours where this lasts for only four days. As exposure time increased the amount of blackspot inhibition became similar to the levels present on prawns exposed to the standard Everfresh dip. While the soak time of 48 hours was lost, it is likely that it would have performed as least as well as the 24 hour soak. A photograph of prawns soaked in a solution of this concentration is present in Appendix as Figure 22. This shows the prawns had only small amounts of blackspot on the cephaolthorax and it was present in very low amounts of the abdomen.

Soaking in a 5mg/L 4-hexylresorcinol solution for longer than 6 hours could successfully be substituted for the standard Everfresh dip. This directly results in savings for industry by reducing the amount of Everfresh required to effectively treat the prawns and is even cheaper than a standard sodium metabisulphite dip. Figure 2 below the protection provided by double this concentration.

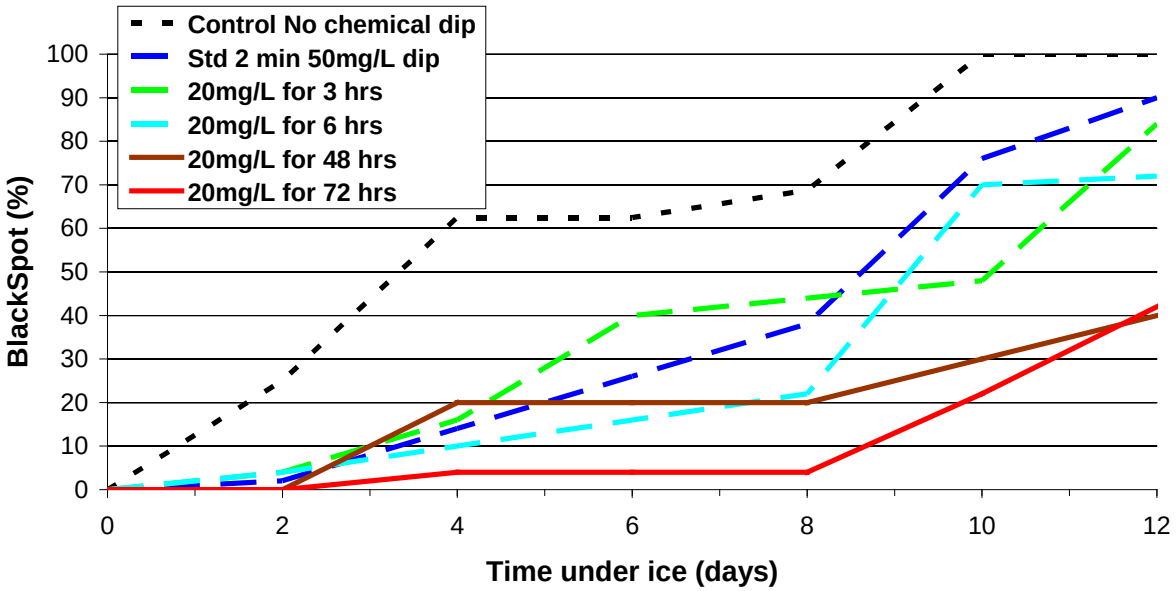
Figure 2. Banana prawns soaked in 10mg/L 4-hexylresorcinol



The higher concentration led to an improvement in the level of protection provided by a 3 hour soak and there was even not much difference to the 6 hour soak.. Soaking banana prawns in 10mg/L 4-hexylresorcinol for 3 hours or more provides a level of blackspot protection that is at least equal to that provided by the standard Everfresh dip.

Longer term treatments (>6hrs) resulted in better protection than the standard Everfresh dip. This outcome shows that not only are there savings to be made by changing from a standard Everfresh dip to a soaking system, there is much better blackspot protection when long term treatments are used. Figure 3 following shows the results for the 20mg/L soaks.

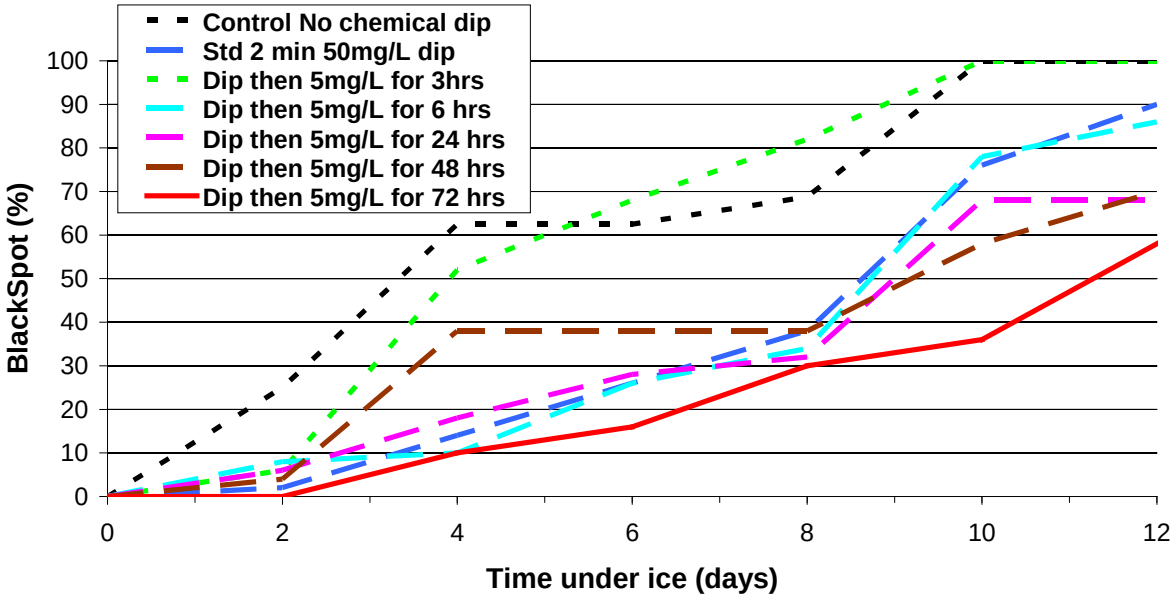
Figure 3. Banana prawns soaked in 20mg/L 4-hexylresorcinol



Again the 3 and 6hr soaks were equal to the standard Everfresh dip (although they were slightly better by day 10) while 48 and 72hr soaking led to much better protection. The 24hr treatment was lost but it could be expected to perform in a similar way to the other longer term treatments.

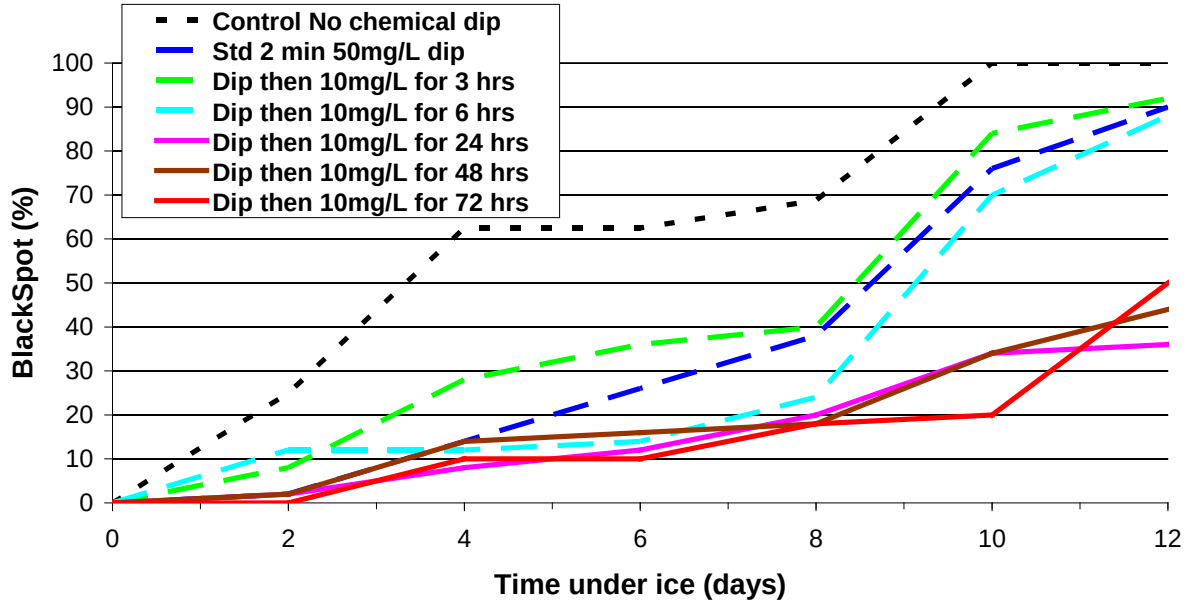
Under conditions that are highly conducive to blackspot development (deep water species or when there are very large catches that may take a long time to sort) or it is impossible to have large bins of the soak solutions present during harvesting, many fishers and prawn growers are known to apply two separate chemical applications (double dip) to their prawns. To see how a soaking method could replace this second dip the three different concentrations were evaluated. Figure 4 shows the performance of controls and soaking prawns in a 5mg/L solution of 4-hexylresorcinol after a standard Everfresh dip was applied.

Figure 4. Banana prawns dipped then soaked in 5mg/L 4-hexylresorcinol



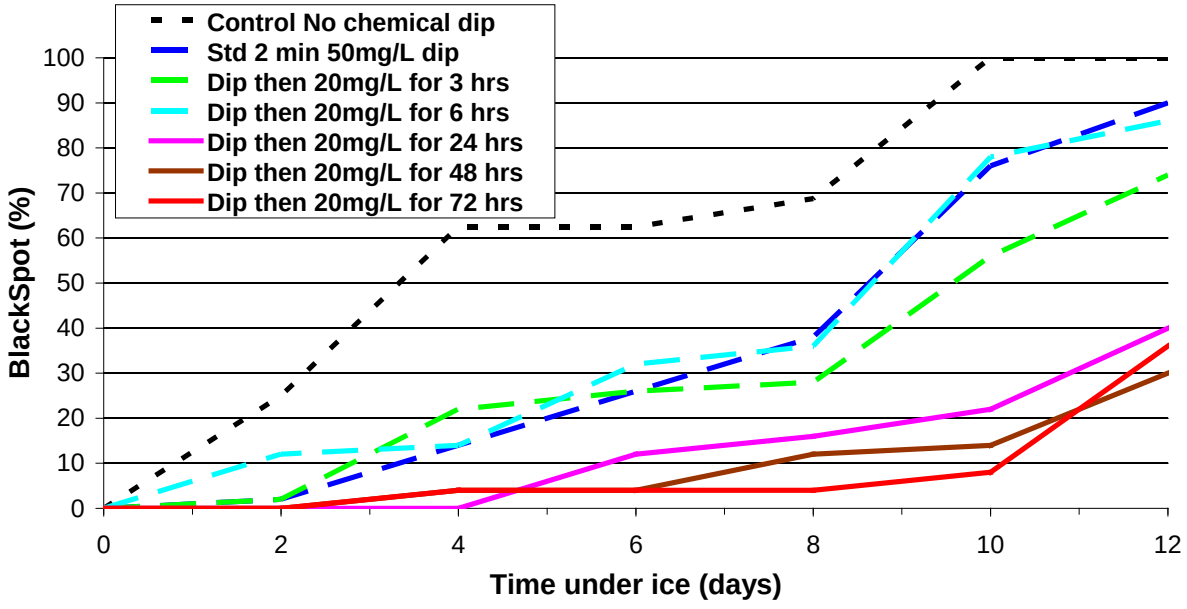
Again the 3 hour soak at 5mg/L was ineffective even though there was a dip before the soak. Only prawns soaked for 72hrs after the dip had much better protection from blackspot than those treated with the dip alone. There appears to be no advantage in soaking after a dip unless there is a long holding time before sale. Because of the large number of treatments it was not possible to evaluate a double dip treatment. One could safely assume however that a double dip would result in better protection than a single exposure but where this curve would lie in relation to those of the dipped and soaked prawns would only be a guess. Figure 5 shows the protection when a 10mg/kg soak is used after a dip.

Figure 5. Banana prawns dipped then soaked in 10mg/L 4-hexylresorcinol



Again the short term soaks were as good as the standard Everfresh dip while soaking for 24hrs or more for was much better at preventing blackspot for. Dipping prior to long term immersion resulted in better protection than the soak alone. Figure 6 shows the performance of the 20mg/L 4-hexylresorcinol soaks after a standard Everfresh dip.

Figure 6. Banana prawns dipped then soaked in 20mg/L 4-hexylresorcinol



The 20mg/L 4-hexylresorcinol soak after dipping performed even better than the 10mg/L treatments. Similar to the soak-only treatment, the long term immersed prawns did not exhibit blackspot on more than 40% of the batch even after 12 days. The dip before soaking did provide a little more protection but the combination method would not be needed unless it was impossible to place the prawns directly into the soaking container.

Residue analysis

The results of the chemical analysis are present in the following Table 2.

Table 2. Solution concentration and meat residues for 4-hexylresorcinol for dip and soak treatments for banana prawns.

Treatment	4-hexylresorcinol level (mg/kg)			
	Start of treatment	Half way through treatment	End of treatment	Meat residue
2 min dip in 50 mg/L 4-hexylresorcinol solution first 16kg batch	49.5	44.9	42.4	0.91
2 min dip in 50 mg/L 4-hexylresorcinol solution second 16kg batch	39.1	38.7	37.5	-
3 hours in ice slurry containing 5mg/L 4-hexylresorcinol	5.1	3.6	3.1	0
6 hours in ice slurry containing 5mg/L 4-hexylresorcinol	6.5	3.9	2.7	0
24 hours in ice slurry containing 5mg/L 4-hexylresorcinol	5.5	3.7	3.3	0.17
48 hours in ice slurry containing 5mg/L 4-hexylresorcinol	6.6	3.5	2.5	0.22
72 hours in ice slurry containing 5mg/L 4-hexylresorcinol	6.3	3.8	1.2	0.36
2 min dip + 3 hours in ice slurry containing 5mg/L 4-hexylresorcinol	6	2.6	1.6	0.18
2 min dip + 6 hours in ice slurry containing 5mg/L 4-hexylresorcinol	5.8	3	2.6	0.25
2 min dip + 24 hours in ice slurry containing 5mg/L 4-hexylresorcinol	4.6	3.5	1.7	0.31
2 min dip + 48 hours in ice slurry containing 5mg/L 4-hexylresorcinol	4.9	3.1	1.7	0.26
2 min dip + 72 hours in ice slurry containing 5mg/L 4-hexylresorcinol	6	4.2	1.8	0.44
3 hours in ice slurry containing 10mg/L 4-hexylresorcinol	7.5	6.5	6.2	0.2
6 hours in ice slurry containing 10mg/L 4-hexylresorcinol	10.6	8.7	8.1	0.4
24 hours in ice slurry containing 10mg/L 4-hexylresorcinol	11.3	7	4	0.44
48 hours in ice slurry containing 10mg/L 4-hexylresorcinol	10.1	7.1	4.3	0.33
72 hours in ice slurry containing 10mg/L 4-hexylresorcinol	8.2	6.2	3.2	0.4
2 min dip + 3 hours in ice slurry containing 10mg/L 4-hexylresorcinol	9.7	9	7.5	0.28
2 min dip + 6 hours in ice slurry containing 10mg/L 4-hexylresorcinol	9.8	7.6	6.2	0.33
2 min dip + 24 hours in ice slurry containing 10mg/L 4-hexylresorcinol	12.7	10.7	6.2	0.4
2 min dip + 48 hours in ice slurry containing 10mg/L 4-hexylresorcinol	10.3	m	m	0.67
2 min dip + 72 hours in ice slurry containing 10mg/L 4-hexylresorcinol	12.3	8.6	3.4	0.67
3 hours in ice slurry containing 20mg/L 4-hexylresorcinol	22.9	14.9	13.2	0.22
6 hours in ice slurry containing 20mg/L 4-hexylresorcinol	21.8	14	13	0.4
24 hours in ice slurry containing 20mg/L 4-hexylresorcinol	m	m	10.2	0.45
48 hours in ice slurry containing 20mg/L 4-hexylresorcinol	19.3	14	8.9	0.67
72 hours in ice slurry containing 20mg/L 4-hexylresorcinol	17	13	6.5	0.89
2 min dip + 3 hours in ice slurry containing 20mg/L 4-hexylresorcinol	19.5	16.8	15.1	0.26
2 min dip + 6 hours in ice slurry containing 20mg/L 4-hexylresorcinol	23.6	14.1	12.2	0.34
2 min dip + 24 hours in ice slurry containing 20mg/L 4-hexylresorcinol	24.6	21.6	6.2	0.89
2 min dip + 48 hours in ice slurry containing 20mg/L 4-hexylresorcinol	24.5	16.1	10.1	1
2 min dip + 72 hours in ice slurry containing 20mg/L 4-hexylresorcinol	24.2	19.2	13.9	1.09

There was a 25% drop in the standard Everfresh dip concentration for just 36kg of prawns. This outcome has ramifications for the predicted capacity of 250kg stated by the producer on the sachet. This aspect requires further investigation and some treatments may be applied for the next trial.

The data also shows, as could be expected, that as the exposure time and solution concentration increases, the amount of 4-hexylresorcinol removed from solution and entering the flesh of the prawn increases. Dipping prior to soaking reduces the amount of 4-hexylresorcinol removed from the solution but increases the amount of residue obtained. There were only two treatments, the two highest exposure treatments, that resulted in meat residues higher than the standard Everfresh dip and these were also above 1mg/kg. This outcome bodes well for Australian producers as well as overseas producers who use soaking as a method of application to meet the majority of importing country residue standards for 4-hexylresorcinol. The blackspot development curves and the residue data show that successful inhibition can be achieved using less chemical. The increased exposure time that soaking permits resulted in better penetration into the prawn tissue with more polyphenol oxidase being inhibited by the 4-hexylresorcinol.

Summary

Overall the majority of soaking treatments were as or more effective than the standard 2 minute dip in 50mg/L 4-hexylresorcinol and all of these provide savings for industry. When a second application of chemical is required, the dip followed by a soak method can still provide savings and possibly similar levels of protection. Most international residue standards can be met using a soak method. All that is needed is a repeated trial with similar outcomes to consolidate these findings.

Trial 2

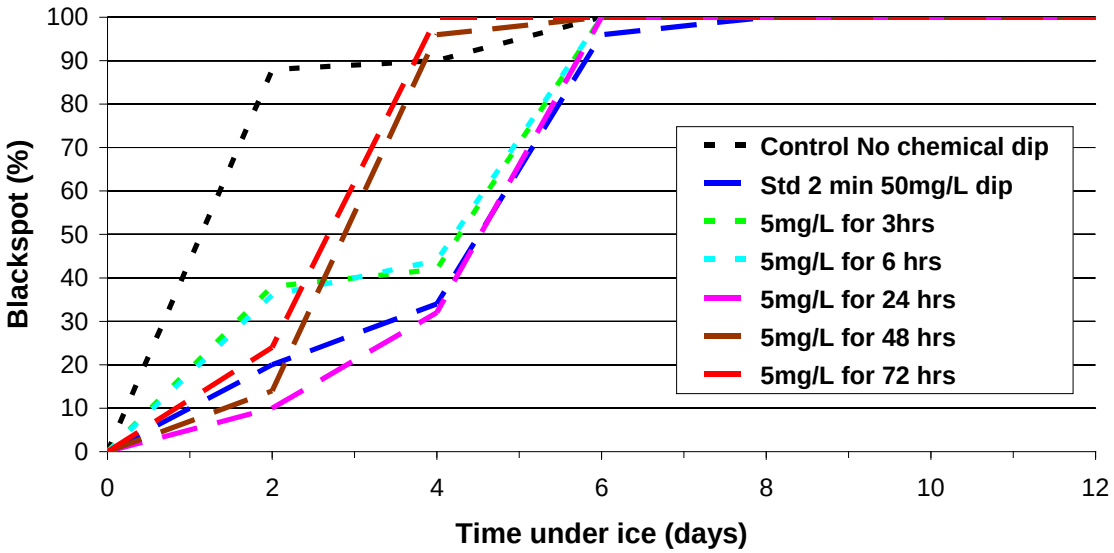
Handling procedures

This trial was conducted on the Gold Coast Marine Aquaculture farm by the Logan River. The treatments were conducted on prawns delivered directly from the ponds within 5 minutes. All of the treatments described in Trial 1 were applied plus some extras to replace those that were lost and to look at the impact of over-filling the treatment container. There were also some additional treatments included such as the tracking of 25kg batches through the recommended 250kg capacity of the standard Everfresh dip as well as a couple of soakings with increased loads of prawns. The treatments were applied and the prawns taken to the laboratory in good condition but four days into storage and over the weekend, the cold room compressor failed raising the temperature to 9°C after several hours.

Blackspot protection

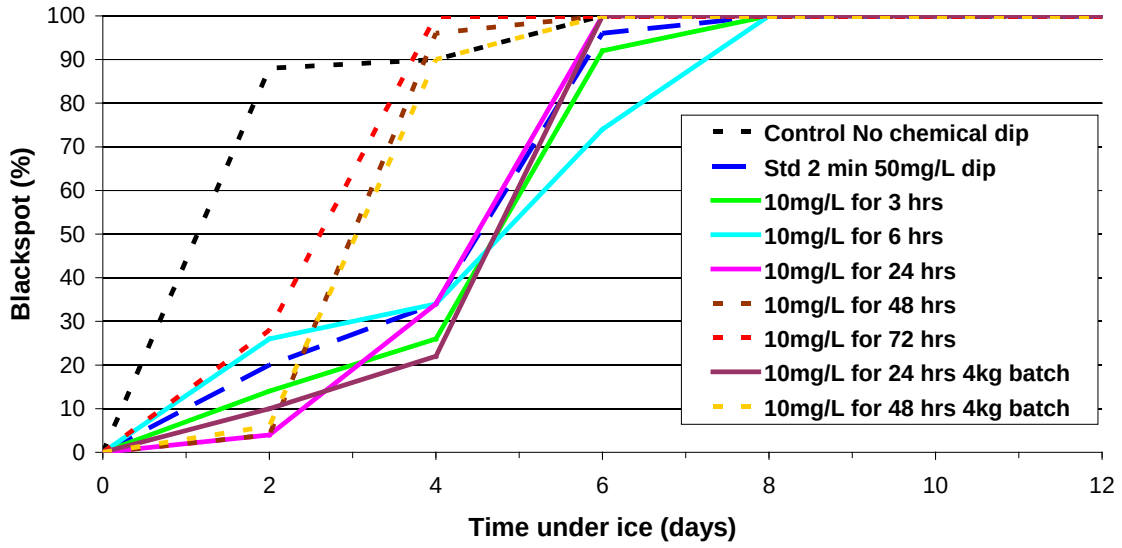
Even with ice covering all of the trays the heat caused a rapid acceleration in blackspot development. Figure 7 shows the profiles for the 5mg/kg soaks. When this batch of Everfresh was tested prior to the trial it was found to result in a higher concentration (60mg/L) than that used for the previous trial (50mg/L). Testing prior to the trial was necessary so that the appropriate concentrations could be successfully applied. This situation should not be a problem for industry as the calculations will be based on a sachet producing a solution of 50mg/L 4-hexylresorcinol. The higher concentration will only provide more protection than expected while the residues should still meet most import standards.

Figure 7. Black tiger prawns soaked in 5mg/L 4-hexylresorcinol



The development of blackspot in the control treatment was much more rapid, even before the temperature increased, than observed during the previous trial with banana prawns suggesting that black tiger prawns may be more vulnerable to this condition. The graph appears to show differences between the various treatments for the onset times of rapid increases in blackspot but this is due to the different holding times while soaking. Remember the time scale is in days while the prawns were held under ice after treatment. As seen in Trial 1 the short term soaks at the low concentration were no better than the standard Everfresh dip. The graph below (Figure 8) shows blackspot development after soaking in 10mg/L 4-hexylresorcinol.

Figure 8. Black tiger prawns soaked in 10mg/L 4-hexylresorcinol



As seen with the banana prawns in Trial 1 the higher concentration provided the short and long soaks with similar or better protection to the standard Everfresh dip. Several batches of 4 kg were tested but the limited amount of solution kept some prawns exposed. An increase in the batch size to 4kg did not appear to have much impact. It would be best to keep the ratio to the 3 volumes of slurry to 2 kg of prawns so none of the prawns stick out above the solution during soaking. Figures 9 to 13 show the blackspot curves in the earlier style for all the other soaking treatments applied.

Figure 9. Black tiger prawns soaked in 20mg/L 4-hexylresorcinol

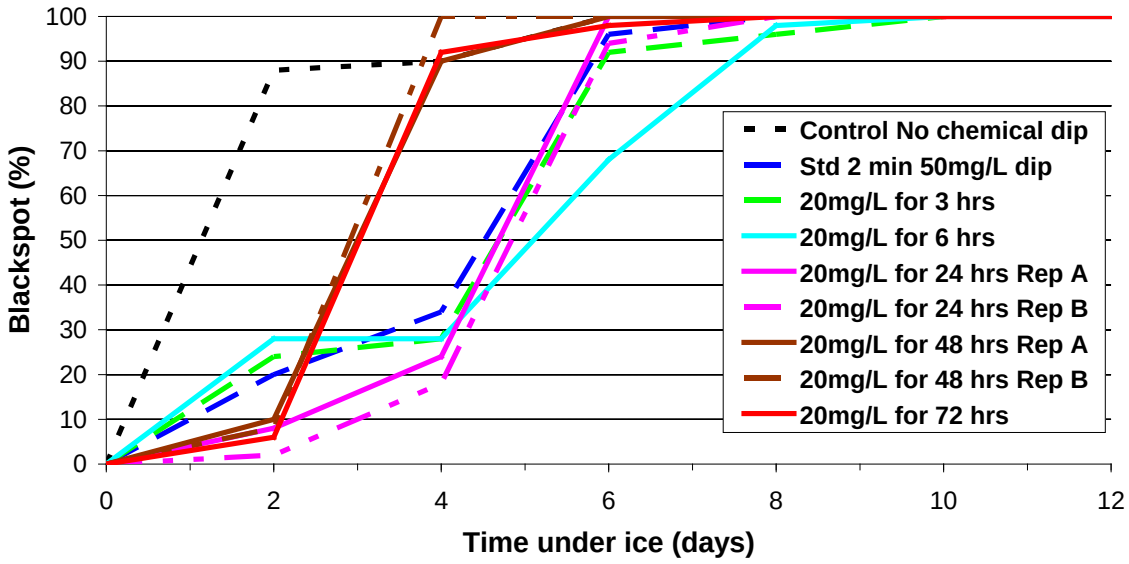


Figure 10. Black tiger prawns dipped then soaked in 5mg/L 4-hexylresorcinol

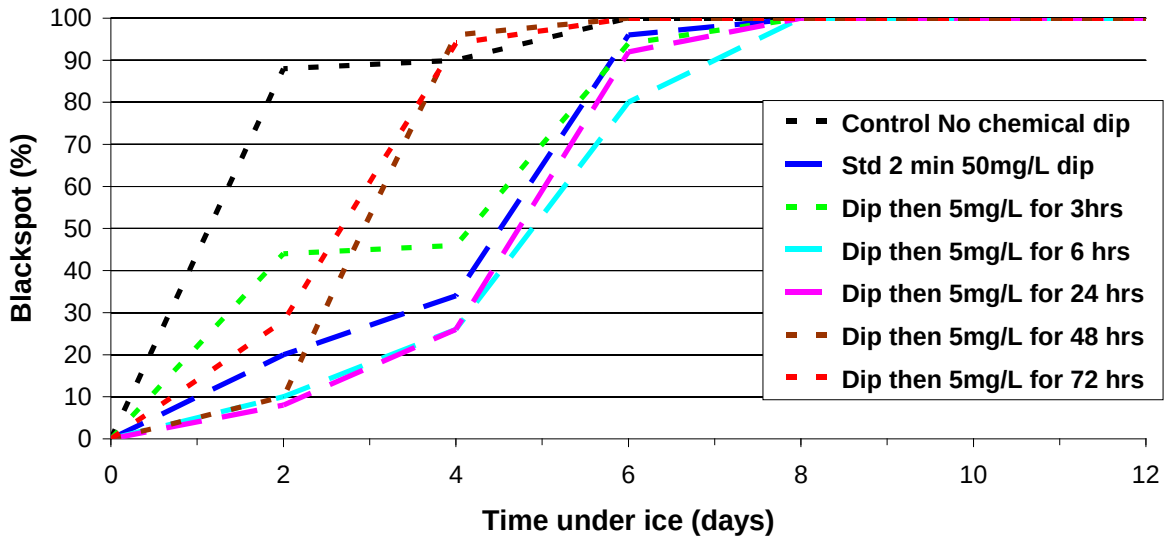


Figure 11. Black tiger prawns dipped then soaked in 10mg/L 4-hexylresorcinol

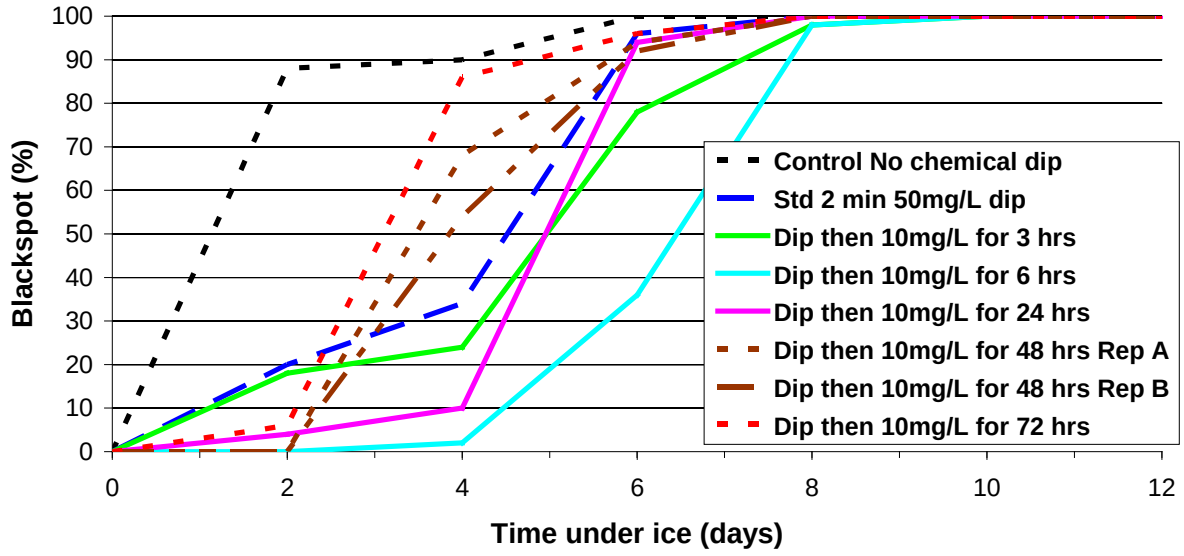
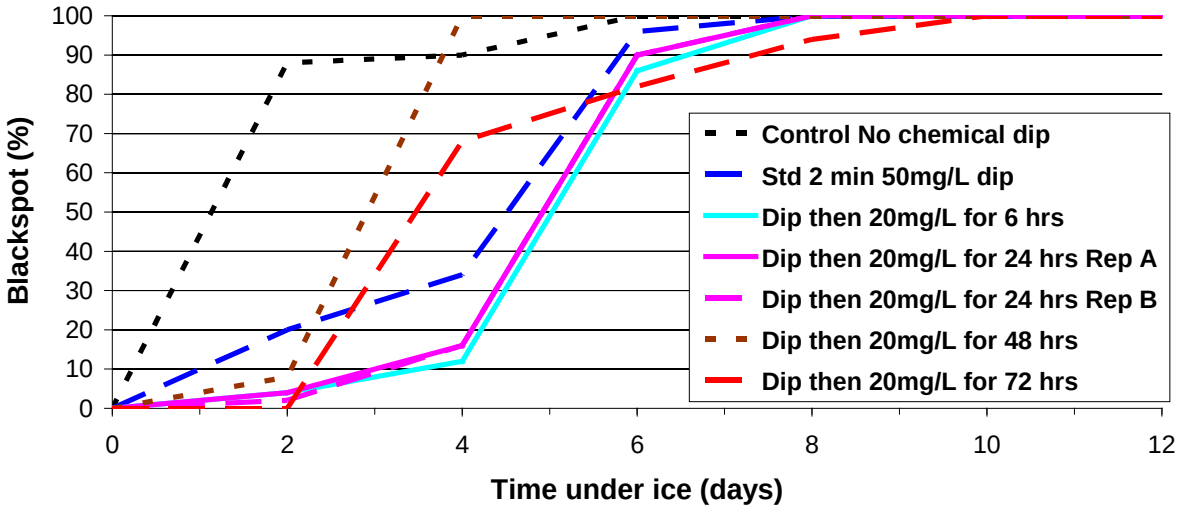


Figure 12. Black tiger prawns dipped then soaked in 20mg/L 4-hexylresorcinol



Because of the large number of trays present in the cold room those stacked closer to the door warmed up quicker. This led to differences between the treatments that would normally be unexpected. The position of the trays in the cold room was random and changed daily as the prawns were inspected for blackspot. Figure 12 is the best example of this condition where shorter soak times appeared to provide better protection.

Residue analysis

While the blackspot occurrence was affected by the heat the solution concentration and meat residue samples were not. Their analysis was continued to provide a more robust pool

of data to base decisions on. In this project because of the large number of treatments the amount of replication was minimal. Table 3 presents the chemical data for Trial 2.

Table 3. Solution concentration and meat residues for 4-hexylresorcinol for dip and soak treatments of black tiger prawns.

Treatment	4-hexylresorcinol level (mg/kg)			
	Start of treatment	Half way	End of treatment	Meat residue
2 min dip in 50 mg/L 4-hexylresorcinol solution first 25kg batch	60	54.6	50.8	0.48
3 hours in ice slurry containing 5mg/L 4-hexylresorcinol	5.1	4.7	4.3	<0.1
6 hours in ice slurry containing 5mg/L 4-hexylresorcinol	5.1	4.3	3.9	0.1
24 hours in ice slurry containing 5mg/L 4-hexylresorcinol	5.1	3.8	3.3	0.1
48 hours in ice slurry containing 5mg/L 4-hexylresorcinol	5.1	3.2	2.8	0.45
72 hours in ice slurry containing 5mg/L 4-hexylresorcinol	5.1	3.1	2.7	0.33
2 min dip + 3 hours in ice slurry containing 5mg/L 4-hexylresorcinol	5	4.3	4.1	<0.1
2 min dip + 6 hours in ice slurry containing 5mg/L 4-hexylresorcinol	5	4.2	3.8	<0.1
2 min dip + 24 hours in ice slurry containing 5mg/L 4-hexylresorcinol	5	3.7	3.1	0.1
2 min dip + 48 hours in ice slurry containing 5mg/L 4-hexylresorcinol	5	3.1	2.7	0.33
2 min dip + 72 hours in ice slurry containing 5mg/L 4-hexylresorcinol	5	3	2.6	0.4
3 hours in ice slurry containing 10mg/L 4-hexylresorcinol	9.9	9.5	8.3	0.1
6 hours in ice slurry containing 10mg/L 4-hexylresorcinol	9.9	8.9	7.5	0.2
24 hours in ice slurry containing 10mg/L 4-hexylresorcinol	9.9	6.8	5.7	0.32
24 hours in ice slurry containing 10mg/L 4-hexylresorcinol for 4kg	9.9	6.9	6	0.39
48 hours in ice slurry containing 10mg/L 4-hexylresorcinol	9.9	5.6	5	0.33
48 hours in ice slurry containing 10mg/L 4-hexylresorcinol for 4kg	9.9	m	5.5	0.38
72 hours in ice slurry containing 10mg/L 4-hexylresorcinol	9.9	4.6	m	0.29
2 min dip + 3 hours in ice slurry containing 10mg/L 4-hexylresorcinol	9.9	9.5	8.3	0.17
2 min dip + 6 hours in ice slurry containing 10mg/L 4-hexylresorcinol	9.9	8.9	7.5	0.31
2 min dip + 24 hours in ice slurry containing 10mg/L 4-hexylresorcinol	9.9	6.8	5.7	0.33
2 min dip + 48 hours in ice slurry containing 10mg/L 4-hexylresorcinol	9.9	5.6	5.5	0.45
2 min dip + 48 hours in ice slurry containing 10mg/L 4-hexylresorcinol	9.9	m	5	0.31
2 min dip + 72 hours in ice slurry containing 10mg/L 4-hexylresorcinol	9.9	4.6	m	0.62
3 hours in ice slurry containing 20mg/L 4-hexylresorcinol	20	19.4	18.1	0.38
6 hours in ice slurry containing 20mg/L 4-hexylresorcinol	20	17.6	15	0.34
24 hours in ice slurry containing 20mg/L 4-hexylresorcinol Rep A	20	16.6	14.7	0.45
24 hours in ice slurry containing 20mg/L 4-hexylresorcinol Rep B	20	15.6	10.3	0.67
48 hours in ice slurry containing 20mg/L 4-hexylresorcinol	20	13.4	9.2	0.89
72 hours in ice slurry containing 20mg/L 4-hexylresorcinol	20	11.4	10.8	1.39
2 min dip + 3 hours in ice slurry containing 20mg/L 4-hexylresorcinol	20	18.6	17	m
2 min dip + 6 hours in ice slurry containing 20mg/L 4-hexylresorcinol	20	17.3	16.4	0.34
2 min dip + 24 hours in ice slurry containing 20mg/L 4-hexylresorcinol Rep	20	16.6	14.7	1.3
2 min dip + 24 hours in ice slurry containing 20mg/L 4-hexylresorcinol Rep	20	15.6	m	1.19
2 min dip + 48 hours in ice slurry containing 20mg/L 4-hexylresorcinol	20	13.6	9.2	1.09
2 min dip + 72 hours in ice slurry containing 20mg/L 4-hexylresorcinol	20	11.4	10.8	1.9

The variability of sachet concentration is evident in the dip concentrations and residues obtained from the standard Everfresh dip used in both trials. As could be expected dipping a larger sized batch resulted in lower residues. The soak residue data was combined with the previous trial for statistical analysis. There were no differences between the residues from both species of prawn. The trends identified in the first trial remained present for second trial.

Summary

The trends for blackspot protection up until the refrigeration broke down during this trial, were similar to the first trial. The residue analysis supports this fact so that these methods can be applied successfully to a range of prawn species.

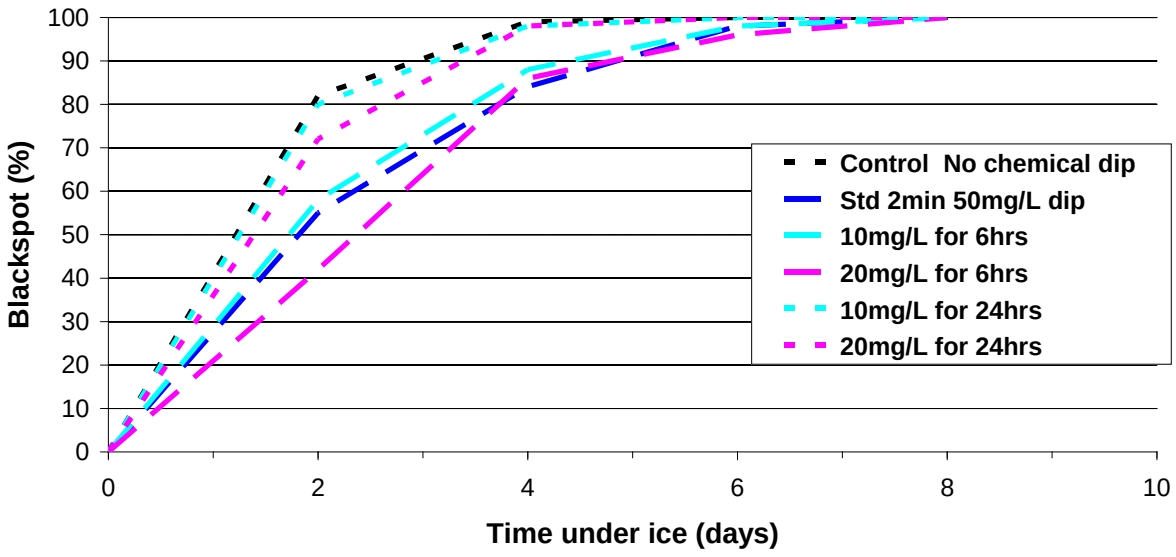
The duplicated treatments produced very similar results for both blackspot protection and residue showing that within the experiment there was consistent handling of the various batches. Differences can occur between trials due to a number of biological causes but this outcome shows that the batch size used for these experiments was sufficient to cater for whatever individual variation was present within the harvest.

Trial 3

Blackspot protection

To assist the farm in obtaining useful methods for their future operation and within the current year, several treatments were repeated as soon as it was known that the trial had been compromised. Unfortunately, being close to the end of the season, these prawns were harvested from the drained ponds and were kept under the sun for some time. Normally the farm cooks these prawns as they are highly prone to blackspot development. Figure 14 shows the performance of the treatments applied.

Figure 14. Black tiger prawns soaked in 4-hexylresorcinol



The more rapid development of blackspot in all of the above treatments from the start of storage, when compared with the performance of these treatments during Trial 2 up until the refrigeration breakdown, shows that the process of melanosis for many prawns had already begun even before any effective protective treatment could be applied. Because there is a series of enzymatic changes that operate during the development of blackspot and only the last of these result in a discernable pigment, there was no way of identifying this process had already started when the prawns were supplied. The researchers were not told of the source and condition of the prawns supplied until after the experiment had been conducted.

Residue analysis

Table 4. Solution concentration and meat residues for 4-hexylresorcinol from the standard Everfresh dip

Treatment	4-hexylresorcinol level (mg/kg)			
	Start of treatment	Half way	End of treatment	Meat residue
2 min dip in 60 mg/L 4-hexylresorcinol dip first 25kg batch	64.8		61.7	m
6 hours in ice slurry containing 10mg/L 4-hexylresorcinol	10.1	7.2	5.7	0.28
24 hours in ice slurry containing 10mg/L 4-hexylresorcinol	10.1	m	4.3	0.62
6 hours in ice slurry containing 20mg/L 4-hexylresorcinol	19.9	14.6	10.6	0.38
24 hours in ice slurry containing 20mg/L 4-hexylresorcinol	19.9	m	6.6	0.8

The residues for this trial were higher than those obtained for the same treatments from Trial 2 yet the level of blackspot was much worse than that observed up till day 4. Also the batch of prawns used for the 24hr treatments exhibited more blackspot than those soaked for only 6 hours. This supports the argument that blackspot had already been initiated before the 4-hexylresorcinol had been applied. While this analysis was carried out an insurance claim was initiated. Unfortunately no resolution was possible before the end of that growing season so an extension of the project was applied for and approved for the following year.

Summary

This trial shows that handling delays that occur before blackspot treatment have a huge impact on the development of pigment during storage regardless of the type of treatment. So now we had two unsuccessful trials, but at least there was the encouraging aspect that most of the soak treatments were still more effective than the standard Everfresh dip.

Trial 4

Handling conditions

While not part of the original design an extra reference treatment was included so that the ability of a soak in 4-hexylresorcinol to prevent black spot could be better understood by industry not familiar with this compound. A one minute dip in 1% sodium metabisulphite was used instead of the 30 second dip recommended for domestic production as this higher dosage is similar in sulphite concentration to that used by industry overseas. It is the intention of the authors to promote this research outside of Australia as well as domestically.

The trial was conducted on a day when there were high temperatures. The ponds were partially harvested using a net which means the prawns had not been starved overnight. When prawns are harvested having recently consumed food, a large amount of variously active enzymes are present in the stomach and the hepatopancreas. When the animal dies these enzymes start to attack the surrounding tissues causing major changes which lead to quality defects.

While the farm hands were told to deliver the baskets of prawns within 5 minutes of harvest, the first recorded blackspot levels back at the laboratory indicated that there may have been considerable delays that allowed enzymic action within the cephalothorax (head) of the prawn to occur.

On reflection it was noted that some of the batches obtained were quite warm (it had been a very hot day with the experiment being conducted at the entrance to a tin shed) and mostly containing dead prawns when received at the work area. At the time no suspicions were aroused because the prawns on the top of the basket were still jumping. Excess ice (over two tonne) was available throughout this trial so there was no concern about the soaking solutions getting too hot.

It may have taken some time to accumulate a whole basket of prawns when using a fixed net for harvesting. This was the experience of the author at another prawn farm just one week prior to this trial. The focus of the farm hands probably had been on delivering a full basket rather than the more important issue of delivering harvested prawns within 5 minutes.

Unfortunately the researchers were too occupied with the preparations and treatments to track individual baskets of prawns from the pond to the processing area to be aware of any handling delay and thus confirm whether this was the true cause of the high levels of blackspot. When baskets were delivered with some prawns still jumping on the top the scenario of extended delays in the sun would not have occurred to the experimenters. Only the officer weighing out the individual 2kg batches was in contact with individual prawns and it was he who recollected later that many were quite warm.

When the dead prawns were washed in the ice slurry, originally intended to kill and clean them, this action would have spread these enzymes about the cephalothorax. All of the blackspot observed in the early stages (day 2) of storage under ice was present only on the head. Some blackspot did develop on the tail section but only after several more days of storage, as would normally happen and as experienced in the first trial.

Blackspot on the tail section did remain at low levels throughout the trial. An example can be seen in Appendix 2. If the head had been scored separately to the body this aspect may have allowed better evaluation of the treatments but this would have doubled the handling time and in turn caused even more blackspot due to this extra warming.

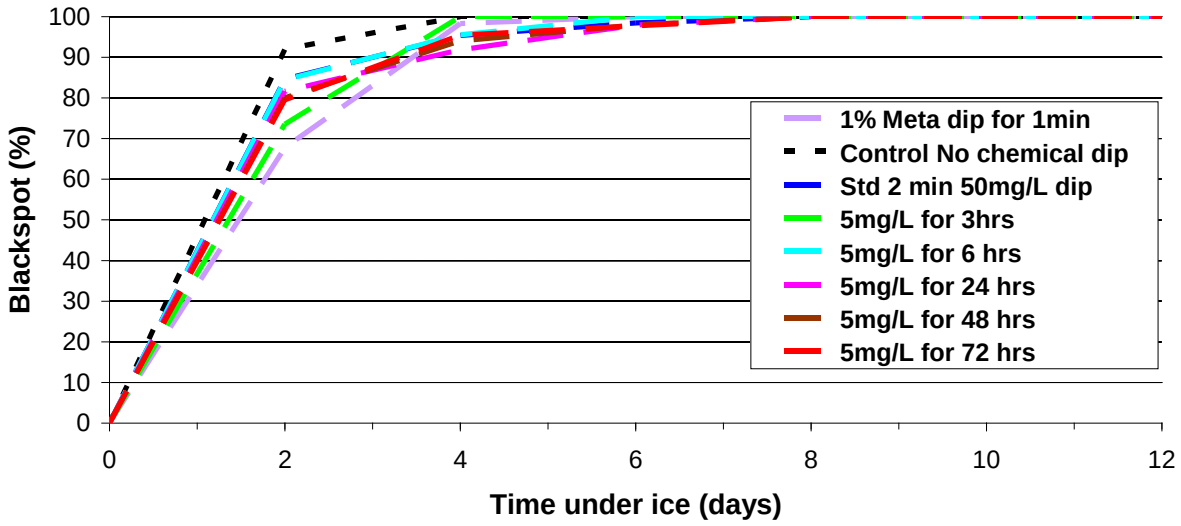
Within two days of treatment, blackspot was present on more than 50% of all the prawns used in this trial. The high levels of blackspot for all treatments, even the standard 4-hexylresorcinol and sodium metabisulphite dips, after two days storage under ice, support the theory that something had indeed gone wrong with the handling prior to treatment.

While this time the prawns were drowned in a freshwater (because the intended 3% salt had not been added) ice slurry, it is hard to believe that this alone was the major cause of the problem. The short immersion time should have limited much in the way of osmotic changes which could have drawn out the polyphenol oxidase from prawns that were still alive. Also prawns stored below water or with limited oxygen such as with modified packaging (MAP) do not develop blackspot until returned to the air. This researcher completed a trial of black tiger prawns which were grown organically, kept chemical-free and stored uncooked in MAP at 4°C for up to 20 days just prior to these experiments and no blackspot developed. These prawns were killed using an ice slurry before packaging.

Blackspot protection

Figures 15 to 20 below show the percentage of blackspot that developed during storage under ice.

Figure 15. Black tiger prawns soaked in 5mg/L 4-hexylresorcinol



Whatever the cause, the irreversible stage of pigment development was obviously underway in many prawns by the time of chemical treatment. For those prawns in the early phase where the enzyme action was still able to be inhibited, many of the previously successful treatments that were applied again give considerable protection. As there was no control over how long each basket of prawns was held at temperature, the amount of affected prawns could have varied between treatments. This could then override the tendency of longer soaking treatments to provide better protection than those that were of a slightly shorter duration.

Sufficient numbers of prawns in each batch were successfully treated so that the trends for soak concentration and duration can still be observed in the blackspot charts. In fact, if the blackspot counts were reduced by 50% the curves would look remarkably similar to the first trial. Unlike earlier comparisons of sodium metabisulphite with 4-hexylresorcinol (Slattery et al., 1995) the longer sodium metabisulphite dip used in this investigation led to more effective protection from blackspot than the standard Everfresh dip. This chemical was also more effective for up to four days than the 4-hexylresorcinol soaks.

As in Trial 1, a short soak in a 5 mg/L solution of 4-hexylresorcinol (see Figures 1 and 15) is not as effective in protecting prawns from blackspot as the long soak. Figure 23 in Appendix 2 shows how these prawns looked after two days storage under ice.

Figure 16 shows the blackspot development after the soaking solution was increased to 10mg/L. The longer term immersion resulted in better protection than either the standard Everfresh or sodium metabisulphite dip. This is certainly a good outcome for these treatments considering the gross trend towards blackspot appearing within two days storage under ice.

Figure 16. Black tiger prawns soaked in 10mg/L 4-hexylresorcinol

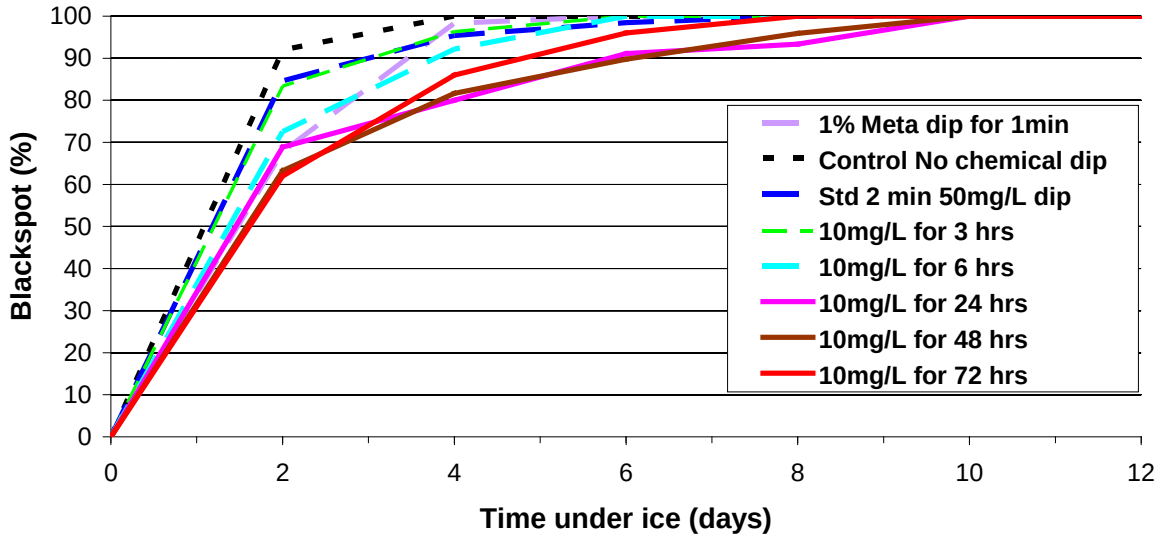
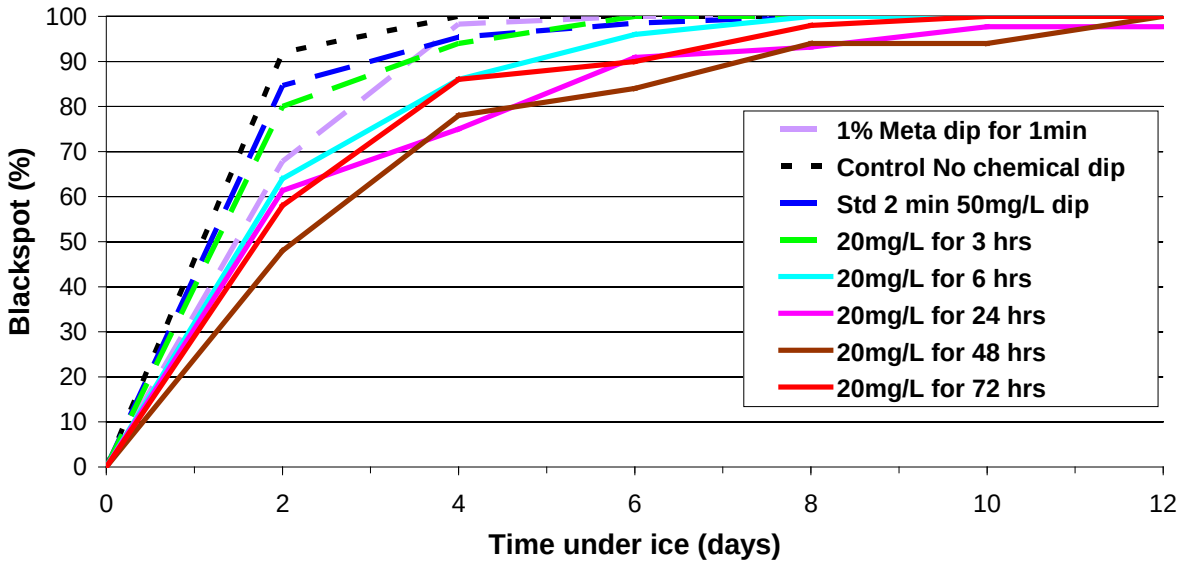


Figure 17 shows the blackspot development with a soaking solution concentration of 20mg/L. Long term soaking was more effective than short immersion periods. As with the previous concentration the level of protection did not increase directly with the increase in soak time for the long term exposures.

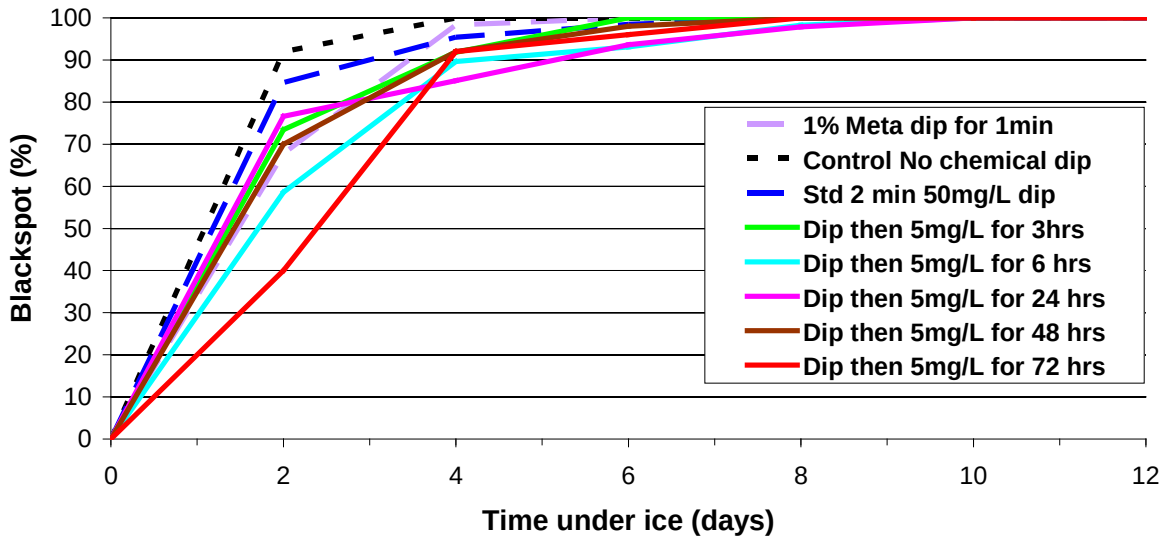
Figure 17. Black tiger prawns soaked in 20mg/L 4-hexylresorcinol



As seen in Trial 1, immersion in 20mg/L 4-hexylresorcinol (Figure 17) provides even better protection than a soak in 10mg/L for even shorter treatment times. After just 6 hours of soaking the blackspot levels were much lower than the standard or a sodium metabisulphite dip. Again there was no better reduction in blackspot incidence wrt to increases in the long term soaking time. Figure 24 in Appendix 2 shows how these prawns looked after 6 days storage under ice. Again there is little black spot apparent on the abdomen of the prawn.

The added treatment of dipping before soaking would still have been effected by similar high levels of blackspot described earlier. Figures 18 to 20 present the blackspot levels for these treatments.

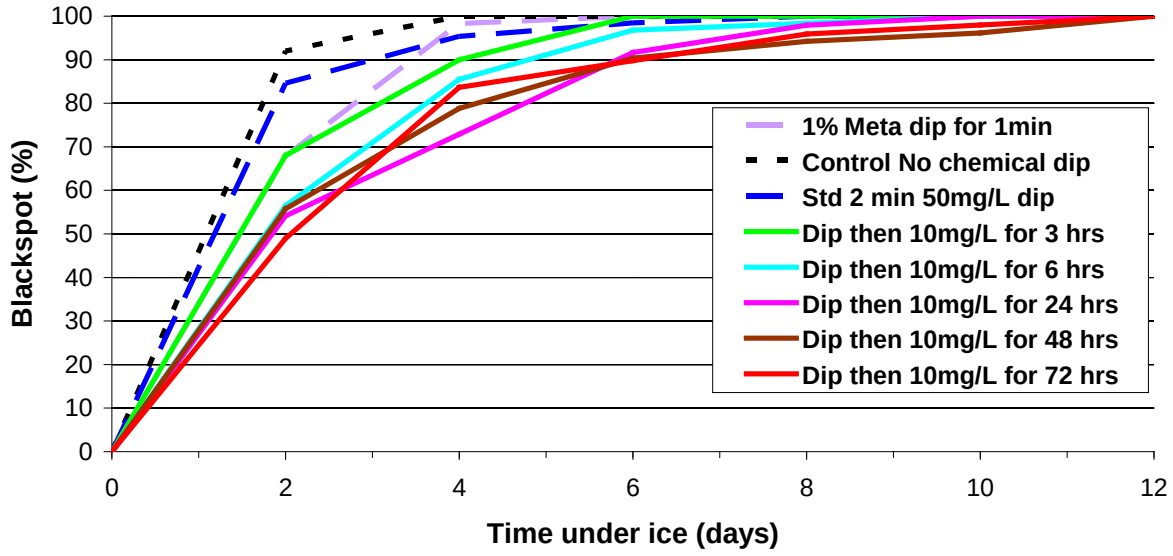
Figure 18. Black tiger prawns dipped then soaked in 5mg/L 4-hexylresorcinol



By the end of storage all of the soaking treatments after the dip in 50mg/L 4-hexylresorcinol led to better blackspot protection that the standard dip, the sodium metabisulphite or soaking alone for a similar time. Previously the shortest term soak after dipping was not very affective at this concentration. It is prossible that the 3hr soak-only batch contained more live prawns when processed than the other treatments.

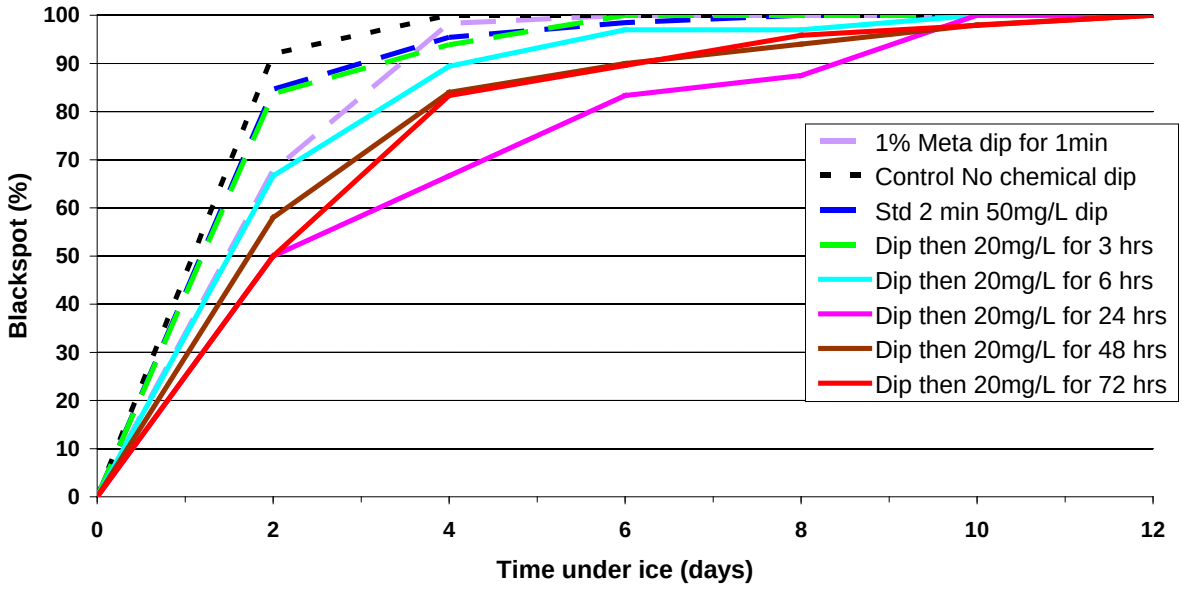
The treatments were somewhat randomly applied so there would be no sequential effects. While there may have been some mixing during washing and dipping the top layer of each 25kg batch would have been the most recently caught so there could have been some bias unwittingly introduced when weighing out 2kg for a particular treatment. All of these treatments performed better than the standard Everfresh dip alone or after two days for sodium metabisulphite.

Figure 19. Black tiger prawns dipped then soaked in 10mg/L 4-hexylresorcinol



Dipping in 50mg/L 4-hexylresorcinol before a soak in 10mg/L 4-hexylresorcinol did not lead to better blackspot protection to that provided by the soak alone, for all of the soaking times. All of these treatments, however, were more effective than just a dip. Soaking for 3 was not as affective as a longer term immersion. Figure 20 shows the performance of the highest concentration used as a soak after dipping.

Figure 12. Black tiger prawns dipped then soaked in 20mg/L 4-hexylresorcinol



The only improvement in blackspot protection provided by dipping prior to soaking in 20mg/L 4-hexylresorcinol occurred after the 24hr soak. Again the long term exposures were much better at preventing blackspot under these extreme conditions than 3 or 6 hr soaks.

Resiue analysis

There were difficulties with the HPLC equipment which led to incorrect results for this trial. Most flesh samples were retested but many solution samples had become rancid by the time a final accurate measurement could be made. The data is presented here but it would be better to rely on the residue analysis from the previous trial as an indicator of possible residue attainable. The residue data from Trials 1 and 2 will be used for industry recommendations.

Table 5. Solution concentration and meat residues for 4-hexylresorcinol for dip and soak treatments of black tiger prawns.

Treatment	4-hexylresorcinol level (mg/kg)			
	Start of treatment	Half way	End of treatment	Meat residue
2 min dip in 50 mg/L 4-hexylresorcinol solution first 25kg batch	35.6	32.3	31.5	0.22
3 hours in ice slurry containing 5mg/L 4-hexylresorcinol	4.2	0.7	0.6	0
6 hours in ice slurry containing 5mg/L 4-hexylresorcinol	4.2	2.9	0.9	0
24 hours in ice slurry containing 5mg/L 4-hexylresorcinol	4.4	0	0	0.27
48 hours in ice slurry containing 5mg/L 4-hexylresorcinol	4.2	0.4	0.4	0
72 hours in ice slurry containing 5mg/L 4-hexylresorcinol	4.2	0.5	1.2	0
2 min dip + 3 hours in ice slurry containing 5mg/L 4-hexylresorcinol	4.4	0.3	0.5	0.71
2 min dip + 6 hours in ice slurry containing 5mg/L 4-hexylresorcinol	4.2	0.9	2.4	0
2 min dip + 24 hours in ice slurry containing 5mg/L 4-hexylresorcinol	4.2	0.3	0.1	0.3
2 min dip + 48 hours in ice slurry containing 5mg/L 4-hexylresorcinol	4.3	0	0	0.68
2 min dip + 72 hours in ice slurry containing 5mg/L 4-hexylresorcinol	4.5	0	0	0.43
3 hours in ice slurry containing 10mg/L 4-hexylresorcinol	10.6	1.2	1.2	0
6 hours in ice slurry containing 10mg/L 4-hexylresorcinol	10.7	3.5	1.9	0.3
24 hours in ice slurry containing 10mg/L 4-hexylresorcinol	10.4	1	1.4	0.11
48 hours in ice slurry containing 10mg/L 4-hexylresorcinol	10.7	1.8	1.3	0.06
72 hours in ice slurry containing 10mg/L 4-hexylresorcinol	10.9	1.2	1.6	0.33
2 min dip + 3 hours in ice slurry containing 10mg/L 4-hexylresorcinol	8.9	1.2	1.6	0.3
2 min dip + 6 hours in ice slurry containing 10mg/L 4-hexylresorcinol	10.6	2.1	3.9	0.21
2 min dip + 24 hours in ice slurry containing 10mg/L 4-hexylresorcinol	8.9	0.7	0.5	0.43
2 min dip + 48 hours in ice slurry containing 10mg/L 4-hexylresorcinol	8.9	0.7	0	0.54
2 min dip + 72 hours in ice slurry containing 10mg/L 4-hexylresorcinol	8.9	0	0.9	0.5
3 hours in ice slurry containing 20mg/L 4-hexylresorcinol	20.1	5.6	5.6	0.3
6 hours in ice slurry containing 20mg/L 4-hexylresorcinol	20.1	4.1	4.1	0.1
24 hours in ice slurry containing 20mg/L 4-hexylresorcinol	20.9	3.2	2.5	0.06
48 hours in ice slurry containing 20mg/L 4-hexylresorcinol	20.1	3.3	2.5	0.28
72 hours in ice slurry containing 20mg/L 4-hexylresorcinol	19.3	2.4	3.6	0.57
2 min dip + 3 hours in ice slurry containing 20mg/L 4-hexylresorcinol	18.1	3.1	3.5	0.36
2 min dip + 6 hours in ice slurry containing 20mg/L 4-hexylresorcinol	17.9	6.6	5.7	0.77
2 min dip + 24 hours in ice slurry containing 20mg/L 4-hexylresorcinol	18.3	2.8	2.2	0.42
2 min dip + 48 hours in ice slurry containing 20mg/L 4-hexylresorcinol	18.2	5.6	5	0.3
2 min dip + 72 hours in ice slurry containing 20mg/L 4-hexylresorcinol	17.9	2.4	3.6	0.3

There appears to be an underestimation of the concentration of the soak solution and final residue for many of the treatments. It is not possible to repeat the testing of these samples so for industry recommendations the residue data from Trial 2 was used.

Summary

While the actual percentage of blackspot was higher from the start for this trial, the trends were similar to those observed in Trial 1 with the higher concentration being more effective than the standard Everfresh dip alone. This time it was better for even shorter treatment times. This combination of treatments shows that soaking can be effective even under the most extreme conditions. The difficulties with the residue analysis suggest that the data from the first two trials be used as the guide for selecting the most appropriate soak method to adopt.

Bulk dip capacity

Handling procedures

For most chemical treatments the theoretical total capacity of a solution is usually defined by the ability of the product being treated to be taken up by the same amount as the previous batch while still using the same exposure time even though some chemical has been removed. There is a flaw in recommending capacities based on this concept. The capacity of the standard Everfresh dip was something that was of interest to industry because of the drop in solution concentration identified during Trial 1.

If industry followed the recommendation present on the packaging, which may be incorrect, they could have problems with blackspot developing on their product. Trial 1 found that a massive 25% of the dip concentration was removed after treating just 32kg of prawns. If this amount of chemical was removed for each batch of prawns treated sequentially and the same dosage time was applied then there would not have been sufficient chemical to provide adequate protection even for half of the recommended 250kg capacity stated on the Everfresh sachet.

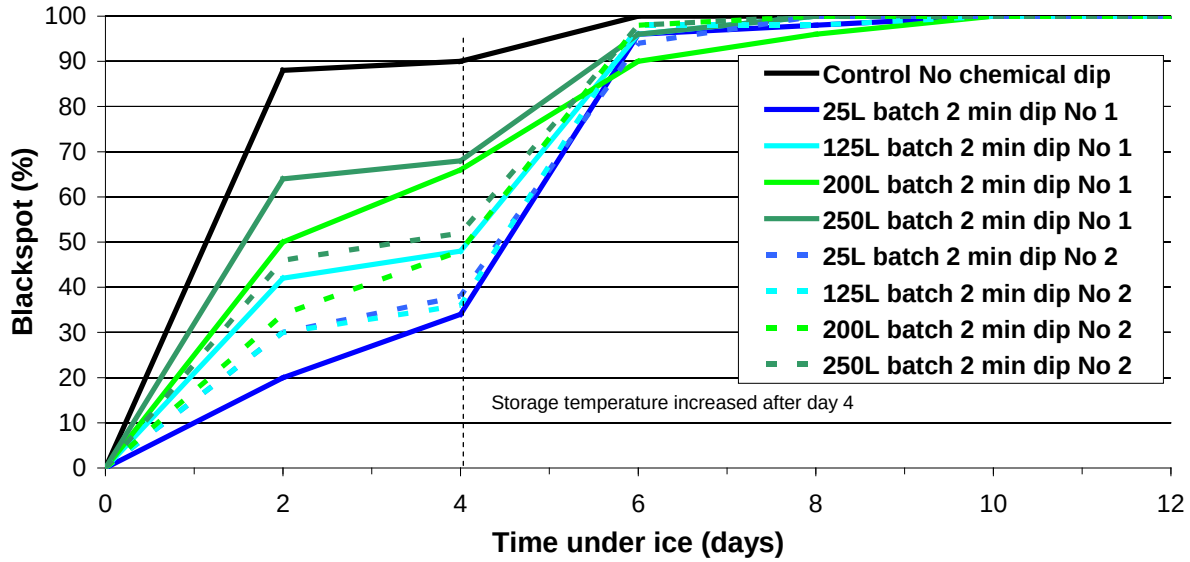
As a large volume of prawns was available on harvest day at Gold Coast Marine Aquaculture during Trial 2 it was decided to take on the extra burden of more samples to investigate the performance of the standard Everfresh dip. Prawns were treated in 25kg batches (this load is based on OWH&S principles) for a total of 250kg.

The solutions and meat residues were sampled for the 25kg batch at the 25kg, 125kg, 200kg and 250kg loads. A new dip was then prepared and the experiment repeated. From each of these 25kg batches, a 2kg subsample was taken and stored under ice for blackspot studies. Because of the already large number of samples to be analysed in the main experiment not all of the possible samples could be taken for this adjunct experiment.

Blackspot protection

The blackspot protection provided by treating 25kg batches of black tiger prawns in sequence for a total of 250kg for two minutes for two freshly made dips is shown in Figure 21.

Figure 21. Black tiger prawns treated with 2 minute dip in 50mg/kg 4-hexylresorcinol



While the heat that developed during Trial 2 did impact on the amount of blackspot present after day 4, blackspot levels of prawns from three of the chemical treatments had already risen to above 40% within the first two days. There was sufficient separation of the treatments to show that using a consistent 2 minutes per dip results in a progressive loss of protection as the load is applied to the solution. The last batch of prawns at the 250kg mark have significantly more blackspot ($P<0.01$) than the first batch.

There also appears a difference between the two dips in their ability to prevent blackspot. This was due to the first dip having a lower starting concentration than the second dip (see Table 6). As noted earlier there is a reasonable amount of variation between sachets. From the number of packs tested it has been noted that the content of the sachets can vary. The last 25kg batch put through the first dip behaved more like the control with no chemical present, suggesting that after treating 200kg of prawns the residue obtained from dipping for two minutes must have been quite low.

Residue analysis

The chemical data obtained from the dip solution and meat samples is presented in Table 6 following.

Table 6. Solution concentration and meat residues for 4-hexylresorcinol from the standard Everfresh dip

Treatment	Dip	4-hexylresorcinol level (mg/kg)			
		Start of treatment	Half way	End of treatment	Meat residue
25kg batch 2 min dip	1	60	54.6	50.8	0.48
125kg batch 2 min dip	1	m	m	45.8	0.27
200kg batch 2 min dip	1	m	m	37	0.1
250kg batch 2 min dip	1	m	m	33	<0.1
25kg batch 2 min dip	2	69.8	64.4	58.4	0.62
125kg batch 2 min dip	2	m	m	53	0.29
200kg batch 2 min dip	2	m	m	42.2	0.1
250kg batch 2 min dip	2	m	m	38.8	<0.1

When the results for the standard Everfresh dip from Trail 1 are compared with the above dips, it becomes apparent that the larger the load of prawns treated, the lower the residue attained. In this trial there was also a difference between the two dips for starting concentration. As the water used to make the dips was measured fairly accurately there is obviously a reasonable amount of variation between sachets for 4-hexylresorcinol content.

The first standard Everfresh dip contained 15% less 4-hexylresorcinol. This proportion remained throughout the process until the last batch. While there was no statistical difference between the two dips for residue and end of treatment concentration, this lower initial dip concentration explains the difference in blackspot protection observed between the two dips where, after the first batch was treated, the lower concentration dip resulted in higher levels of blackspot in the following batches. This outcome indicates that more research is needed so that industry can get the maximum use out their treatments without losing efficacy.

Cost Comparison

Table 7 below shows the costs for application of the various soaking and dipping treatments.

Table 7. Cost of application.

Compound	Package size	Cost	Dosage	Treatment	Treatment capacity	Cost per kg
sodium metabisulphite	1kg	A$2.60	1kg/100L	60s dip	100kg	A$0.026
Everfresh	200g	A$16.00	1 sachet per 95L (50mg/L)	2min dip	125kg	A$0.128
Everfresh	200g	A$16.00	1 sachet per 1000L (5mg/L)	24hr or more soak	960kg	A$0.017
Everfresh	200g	A$16.00	1 sachet per 500L (10mg/L)	6hr or more soak	475kg	A$0.034
Everfresh	200g	A$16.00	1 sachet per 250L (20mg/L)	3hr or more soak	237kg	A$0.067

A 1kg bag of sodium metabisulphite, when bought in bulk, costs $2.60. The sodium metabisulphite is added to 100L of water to make a dip solution. This will treat 100kg of prawns effectively, although some individuals have extrapolated from limited experiments that it could treat more. The blackspot treatment cost is 2.6 cents per kilogram of prawns.

A 200g sachet of Everfresh costs $16. It should be dissolved in 95L of water. Although Otwell et al. (1992) extrapolated that 500lbs of frozen prawns could be treated, the earlier results (see Figure 14) show that a 2 minute dip, if used sequentially, in the 50mg/kg 4-hexylresorcinol solution will only effectively treat 125kg of prawns. This results in a blackspot treatment cost of 12.8 cents per kilogram, a lot more than the cost of a sodium metabisulphite dip and the main reason used by industry for not adopting 4-hexylresorcinol.

If a 10mg/kg 4-hexylresorcinol solution is used for soaking for 6 hours or longer then a sachet will treat 475kg of prawns and the cost per kilogram will be 3.4 cents per kilogram. This is 25% of the cost of a standard Everfresh dip and only 0.8 cents/kg more than the cost of using sodium metabisulphite. While a concentration of 5mg/kg of this compound is only effective when used for 24 or more hours, this treatment works out to be cheaper per kilogram of prawns than sodium metabisulphite.

RECOMMENDATIONS FOR INDUSTRY

The key recommendations are:

- 5mg 4-hexylresorcinol/L soaking (made by dissolving a 200g Everfresh sachet in 1000L of water before adding 500kg of ice) will treat 1000kg of prawns as effectively as a standard Everfresh dip after 24 or more hours. Regular mixing of the prawns in the solution is advised. This treatment is cheaper to use than sodium metabisulphite or a standard Everfresh dip.
- 10mg 4-hexylresorcinol/L soaking (made using two 200g Everfresh sachets, 1000L of water, 500kg of ice, 1000kg of prawns and mixed regularly) for 3 hours or more can replace a standard Everfresh dip and provide savings
- 20mg 4-hexylresorcinol/L soaking (made using four 200g Everfresh sachets, 1000L of water, 500kg of ice, 1000kg of prawns and mixed regularly) can replace a standard Everfresh dip and provide less savings than a 10mg/L solution.
- 10mg/kg or stronger soaking for 24 or more hours inhibits blackspot much better than a standard Everfresh dip or a 1% sodium metabisulphite 1 minute dip.
- A dip prior to soaking is only necessary if processing delays occur or it is to be used for deep water species or large catches.
- Only 20mg/L for 48 and 72 hours following a dip led to higher residues than the standard Everfresh dip
- All overseas standards (2mg/kg 4-hexylresorcinol) with the exception of Canada and China (which have a 1mg/kg 4-hexylresorcinol residue limit) can be met using any of the soak methods. Do not use a soak in 20mg/L 4-hexylresorcinol for 72 hours if product is to be exported to these two countries.

BENEFITS

This research identified the 15 following treatments that are as effective in inhibiting blackspot as the standard Everfresh dip:

- 24 hours in ice slurry containing 5mg/L 4-hexylresorcinol
- 48 hours in ice slurry containing 5mg/L 4-hexylresorcinol
- 72 hours in ice slurry containing 5mg/L 4-hexylresorcinol
- 2 min dip + 6 hours in ice slurry containing 5mg/L 4-hexylresorcinol
- 2 min dip + 24 hours in ice slurry containing 5mg/L 4-hexylresorcinol
- 2 min dip + 48 hours in ice slurry containing 5mg/L 4-hexylresorcinol
- 2 min dip + 72 hours in ice slurry containing 5mg/L 4-hexylresorcinol
- 3 hours in ice slurry containing 10mg/L 4-hexylresorcinol
- 6 hours in ice slurry containing 10mg/L 4-hexylresorcinol
- 2 min dip + 3 hours in ice slurry containing 10mg/L 4-hexylresorcinol
- 2 min dip + 6 hours in ice slurry containing 10mg/L 4-hexylresorcinol
- 3 hours in ice slurry containing 20mg/L 4-hexylresorcinol
- 6 hours in ice slurry containing 20mg/L 4-hexylresorcinol
- 2 min dip + 3 hours in ice slurry containing 20mg/L 4-hexylresorcinol
- 2 min dip + 6 hours in ice slurry containing 20mg/L 4-hexylresorcinol

There were another 12 treatments that are better than the standard Everfresh dip:

- 24 hours in ice slurry containing 10mg/L 4-hexylresorcinol
- 48 hours in ice slurry containing 10mg/L 4-hexylresorcinol
- 72 hours in ice slurry containing 10mg/L 4-hexylresorcinol
- 2 min dip + 24 hours in ice slurry containing 10mg/L 4-hexylresorcinol
- 2 min dip + 48 hours in ice slurry containing 10mg/L 4-hexylresorcinol
- 2 min dip + 72 hours in ice slurry containing 10mg/L 4-hexylresorcinol
- 24 hours in ice slurry containing 20mg/L 4-hexylresorcinol
- 48 hours in ice slurry containing 20mg/L 4-hexylresorcinol
- 72 hours in ice slurry containing 20mg/L 4-hexylresorcinol
- 2 min dip + 24 hours in ice slurry containing 20mg/L 4-hexylresorcinol
- 2 min dip + 48 hours in ice slurry containing 20mg/L 4-hexylresorcinol
- 2 min dip + 72 hours in ice slurry containing 20mg/L 4-hexylresorcinol

Of these treatments 6 (3 if direct soaking and 3 if there was a second dip) were cheaper to use than sodium metabisulphite:

- 24 hours in ice slurry containing 5mg/L 4-hexylresorcinol
- 48 hours in ice slurry containing 5mg/L 4-hexylresorcinol
- 72 hours in ice slurry containing 5mg/L 4-hexylresorcinol
- 2 min dip + 24 hours in ice slurry containing 5mg/L 4-hexylresorcinol
- 2 min dip + 48 hours in ice slurry containing 5mg/L 4-hexylresorcinol
- 2 min dip + 72 hours in ice slurry containing 5mg/L 4-hexylresorcinol

The rest of the treatments were cheaper to use than the standard Everfresh dip. Industry will be more likely to adopt these new methods because of the savings.

These methods will be easy to adopt as they have been tailored to fit in with many different types of operation.

Consumers will benefit because more prawns will be produced without allergenic sulphite present.

Consumers will also benefit from safer to eat prawns that have a better shelf life than before.

FURTHER DEVELOPMENT

As identified in the need section of the grant application a lot of the prawn production is treated using a bulk dip. While many may adopt a soaking method not all would be able to adopt this type of practice and would continue to use a short dip in a chemical solution.

This project found that bulk dips were ineffective at protecting about one third of the amount of prawns that the supplier recommended the standard Everfresh dip was capable of treating. This aspect needs further research to ensure that industry does not use methods that are not appropriate. Any increased treatment time will also have to have upper limits to accommodate overseas residue standards if our industry wants to export their product. By incrementally increasing the dip time the amount of effectively treated prawns will be less than the current recommended capacity. Fishers wishing to treat the same amount of prawns as before will have to increase their consumption of this chemical to ensure effective treatment.

It is likely that an increasing dip time is required but the literature is lacking in any data that may be successfully used to extrapolate reliable times. The amount recommended by the manufacturer has been extrapolated from a small scale experiment (Otwell et al., 1992) indicating that there are some unanticipated dynamics that may be operating when the method is scaled up. Only by conducting experiments using normal commercial volumes can effective dip times be recommended.

The same situation has occurred for the use of sodium metabisulphite with recent recommendations doubling the levels originally developed from small scale experiments. The other chemical used by industry for prawns, ascorbic acid, also needs to comply with residue standards.

To ensure that industry is supplied with reliable methods that will result in better shelf life than currently achievable a project proposal to investigate this aspect has been develop for submission to the FRDC and also the presently being developed Australian Seafood CRC.

REFERENCES

Guandalini, E., Ioppolo, A., Mantovani, A., Stacchini, P. and Giovannini, C. 1998. 4-hexylresorcinol as inhibitor of shrimp melanosis: efficacy and residues studies; evaluation of possible toxic effect in a human intestinal *in vitro* model (Caco-2); preliminary safety assessment. Food Additives and Contaminants 15 (2):171-180.

Iyengar, R., Bohmont, C.W. and McEvily, A.J. 1991. 4-Hexylresorcinol and prevention of shrimp blackspot: Residue analyses. Journal of Food Composition and Analysis 4:148-157.

McEvily, A.J., Iyengar, R. and Otwell, W.S. 1992. A new processing aid for the inhibition of shrimp melanosis. Proceedings of the Fifteenth Tropical and Subtropical Fisheries Technology Conference:147-153.

Otwell, S.W. and McEvily, A.J. 1992. Inhibiting melanosis in trawled and pond-reared shrimp species. Proceedings of the Fifteenth Tropical and Subtropical Fisheries Technology Conference:369-372.

Otwell, S.W., Iyengar, R. and McEvily, A.J. 1992. Inhibition of shrimp melanosis by 4-hexylresorcinol. Journal of Aquatic Food Product Technology 1(1):53-65.

Slattery, S.L. 1988. Questionaire on meta use. The Queensland Fisherman March:42.

Slattery, S.L. 1989. Meta studies. The Queensland Fisherman March:27.

Slattery, S.L. 1995. A sulphite-free treatment inhibits blackspot formation in prawns. Food Australia 47 (11):509-514.

APPENDIX 1 TECHNICAL ARTICLE FOR INDUSTRY

Soaking in Everfresh is cheaper and better at preventing blackspot in prawns than the standard 2 minute dip.

By Steve Slattery

A recently FRDC/SSA funded project identified numerous alternate ways of using the prawn blackspot inhibiting compound sold as Everfresh. While the active compound, 4-hexylresorcinol, is much safer for consumers the cost of using an Everfresh dip (A$0.128/kg) to treat prawns is much higher than the alternate sodium metabisulphite dip (A$0.026/kg). By using a longer term treatment of soaking at a lower concentration, better protection can be achieved at lower cost.

This research investigated soaking at three different concentrations and for five different soak times. All prepared solutions then had ice added to ensure rapid chilling and were stored in a cold room at 2°C. To cater for those difficult handling conditions the application of a dip prior to soaking was also investigated but the main focus of this article is a once off treatment. Table 1 below shows how effective a soak is when compared with a normal 2 minute dip in Everfresh as recommended on the sachet.

Table 1. Blackspot protection provided by a soak in Everfresh as compared with that provided by a standard 2 minute Everfresh dip.

4-hexylresorcinol solution concentration and soak time	Level of protection provided by soak verses dip for	
	Banana prawns	Black tiger prawns
5mg/L for 3hrs	worse	similar
5mg/L for 6 hrs	worse	similar
5mg/L for 24 hrs	similar	similar
5mg/L for 48 hrs	similar	similar
5mg/L for 72 hrs	similar	similar
10mg/L for 3 hrs	similar	similar
10mg/L for 6 hrs	similar	similar
10mg/L for 24 hrs	better	better
10mg/L for 48 hrs	better	better
10mg/L for 72 hrs	better	better
20mg/L for 3 hrs	similar	similar
20mg/L for 6 hrs	similar	better
20mg/L for 24 hrs	better	better
20mg/L for 48 hrs	better	better
20mg/L for 72 hrs	better	better

The table shows that when compared to a normal 2 minute dip in Everfresh, most of the soak treatments provide similar or better protection from blackspot.

Residue issues

While no residue limit applies to Australia and New Zealand, the need to export to obtain better prices means that the treatments applied by industry when processing export product

should comply with overseas residue standards for 4-hexylresorcinol. Figure 1 following shows what residues will be attained by the individual treatments.

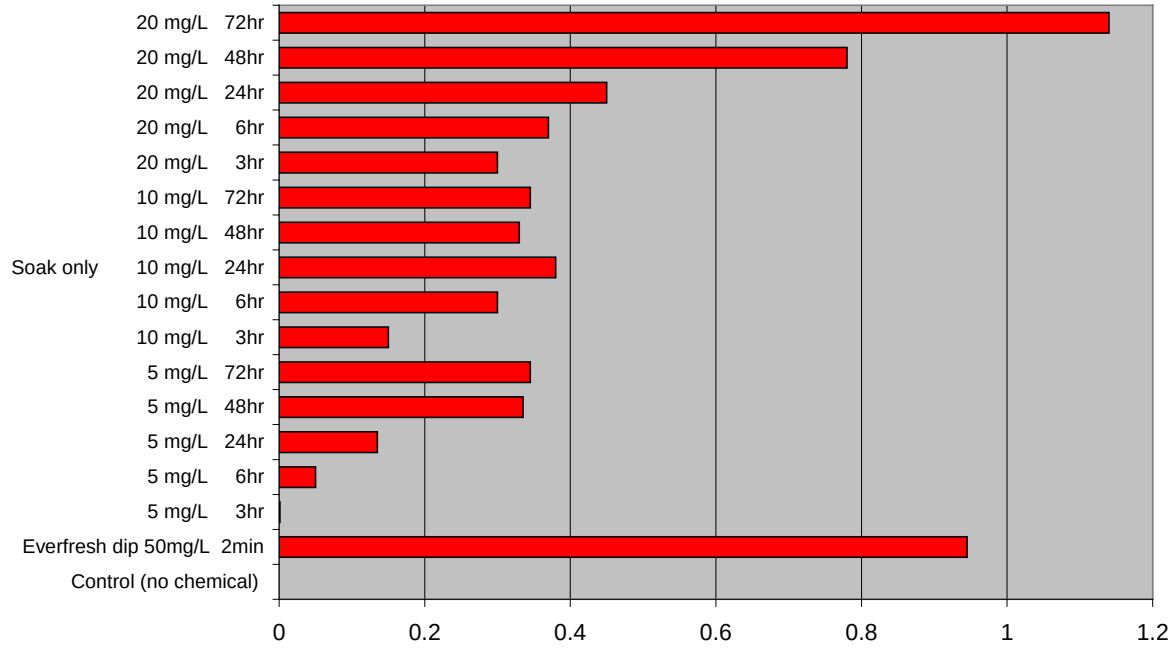


Figure 1. Residue measured as mg/kg 4-hexylresorcinol

All of the soak treatments will meet the US and EU 2 mg/kg residue standard while only one treatment is unsuitable for countries such as Canada and China where there is a 1 mg/kg residue standard.

The adoption of any new treatment must also be cost effective for industry. Table 2 below shows the amount each treatment is capable of effectively protecting and the cost per kilogram of prawns treated.

Table 2. Cost of application.

Compound	Package size	Cost	Dosage	Treatment	Treatment capacity	Cost per kg prawns
sodium metabisulphite	1kg	A$2.60	1kg/100L	60s dip	100kg	A$0.026
Everfresh	200g	A$16.00	1 sachet per 95L (50mg/L)	2min dip	125kg	A$0.128
Everfresh	200g	A$16.00	1 sachet per 1000L (5mg/L)	24hr or more soak	960kg	A$0.017
Everfresh	200g	A$16.00	1 sachet per 500L (10mg/L)	6hr or more soak	475kg	A$0.034
Everfresh	200g	A$16.00	1 sachet per 250L (20mg/L)	3hr or more soak	237kg	A$0.067

A 5 mg/L 4-hexylresorcinol soak solution can be made by dissolving a 200g Everfresh sachet in 1000L of clean water and 500kg of ice. This will, with regular mixing, treat nearly 1000kg prawns effectively, after a long term exposure (24 or more hours) and is cheaper to use than a sodium metabisulphite dip. A 10 mg/L 4-hexylresorcinol soak is similar to that described above but is made using two sachets of Everfresh while the 20 mg/L 4-hexylresorcinol soak solution requires four 200g sachets of Everfresh. Predipping is only necessary if processing delays occur (large catches) or it is to be used for deep water species. A paper will be published which describes this aspect and more detail.

APPENDIX 2 PHOTOGRAPHS OF PRAWNS RECORDED DURING STORAGE

Trial 1 Photographs

Figure 22. Banana prawns soaked in Everfresh and then stored under ice.

Trial 4 Photographs

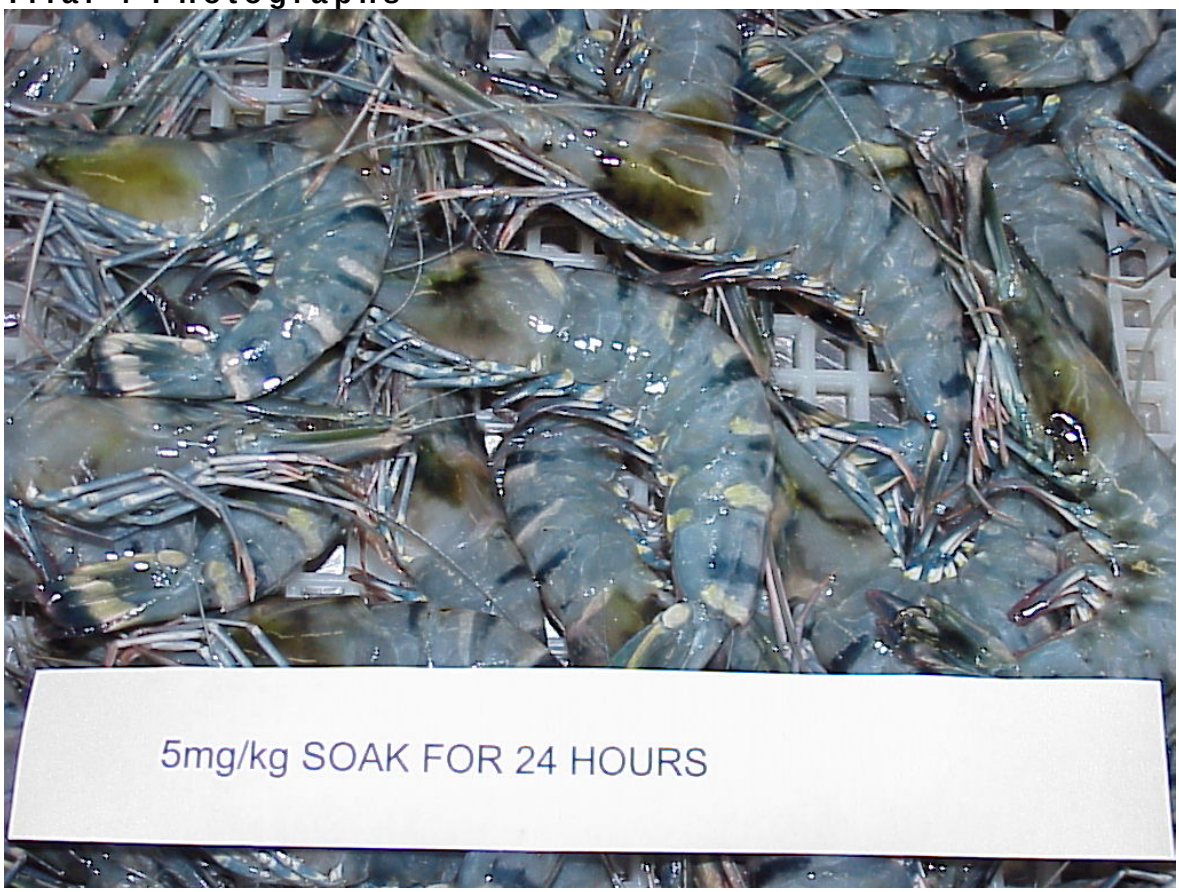


Figure 23. Blackspot present on head only after two days storage under ice.

Figure 24. Blackspot present mainly on head after six days storage under ice.