

# INHIBITORY EFFECT OF HEXYLRESORCINOL ON MELANOSIS AND DECOMPOSITION OF TRIMETHYLAMINE OXIDE (TMAO) IN SHRIMP ON ICE AND IN FROZEN STORAGE

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## ABSTRACT

The occurrence of melanosis, or blackspot, in shrimp during icing or frozen storage is a serious problem leading to considerable commercial losses for Brazilian producers. Sulfites have been used to prevent the occurrence of blackspot, but have also been known to compromise the quality of the product. The present study evaluates hexylresorcinol as an alternative to sulfites. After thirty days of storage Southern brown shrimp (*Farfantepenaeus subtilis*) treated with hexylresorcinol were shown to develop very little melanosis. Furthermore, hexylresorcinol did not favor the formation of DMA and FA in the muscle tissue of the shrimp.

## RESUMO

HEXYLRESORCINOL COMO INIBIDOR DE MELANOSE E DECOMPOSIÇÃO DO ÓXIDO DE TRIMETILAMINA EM CAMARÃO SOB

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ESTOCAGEM EM GELADO CONGELADO. A ocorrência de manchas pretas em camarão durante estocagem em gelado é um problema sério que tem resultado em grandes perdas comerciais para os produtores nacionais. O uso de sulfitos tem sido empregado para prevenir a melanose; no entanto, este composto pode comprometer a qualidade do produto. Neste estudo o uso de hexylresorcinol, uma alternativa aos sulfitos, foi avaliado. Foi demonstrado que o hexylresorcinol é eficiente na inibição da melanose em camarão-rosa, uma vez que baixos níveis na ocorrência de manchas pretas foram observados após 30 dias de estocagem. Além disso, o hexylresorcinol não favoreceu a formação de DMA e FA no músculo do camarão.

## INTRODUCTION

Penaeid shrimp fishing on the northern coast of Brazil is done along one of the world's most important shrimp fishing banks, namely the one which ranges from Tutóia (Maranhão state) to the Orinoco River delta (border between English Guiana and Venezuela), covering an area of some 233.000 km<sup>2</sup> (2). Four penaeid species occur in the region - *Xiphopenaeus kroyeri*, *Farfantepenaeus schmitti*, *F. brasiliensis* and *F. subtilis* - but the last one mentioned contributes with as much as 95% of the landings alone, and the second one mentioned is rarely processed by the fishing industry (4).

The price obtained for shrimp on the international market is closely tied up with the handling and maintenance given to the catch on board. The greatest problem in preserving the catch is the occurrence of black spot - a kind of melanosis spotting the head and segments of the tail. Sulfites are a low cost means of preventing blackspot in shrimp, but when used in excess have been proved to cause adverse reactions in sensitive people (such as asthmatics) and to contribute to the decomposition of trimethylamine oxide (TMAO) into dimethylamine (DMA) and formaldehyde (FA), which in turn are known to compromise the quality of the product (8). Alternatives have therefore been proposed, some of which include mixtures containing ice, salt, potassium sorbate and sodium erisorbate (5), 4-hexylresorcinol (6,7,9), and kojic acid, that is, 5-hydroxy-2-hydroxymethyl-1-pyrone (1,3). However, none of these alternatives has as yet become a common commercial procedure.

Since studies have indicated the efficiency of 4-hexylresorcinol as a means of inhibiting melanosis in crustaceans (10), this chemical agent was presently tested on the species *Farfantepenaeus subtilis* in order to determine whether its

use contributes to the inhibition of melanosis in shrimp and whether its use interferes with the decomposition of trimethylamine oxide (TMAO) into dimethylamine (DMA) and formaldehyde (FA) during icing and frozen storage.

## **MATERIALS AND METHODS**

### **Materials**

In the early morning, specimens of the Southern brown shrimp (*Farfantepenaeus subtilis*) were captured in trawls lasting 90 minutes or less, at an average depth of 37 meters. The fishing vessel operated at 00°43'73 N latitude and 47°47'39 W longitude. All specimens were handled on board, placed in plastic bags and stored on ice for five days, then deep frozen at a temperature lower than -18°C up to thirty days. Sodium metabisulfite (SM) and 4-hexylresorcinol (current trade name, Everfresh) (HR) were used for preparing the solutions which were tested for efficacy in the prevention of melanosis.

### **Methods**

#### **Immersion in solutions of 4-hexylresorcinol and sodium metabisulfite**

The experiments were carried out on board the research vessel Almirante Paulo Moreira. The shrimp were immediately separated from the by-catch and other collected debris upon landing, after which all were washed and some were beheaded. A second wash was performed in order to remove eventual dirt and organic material. Then 500-gram batches were placed in baskets and immersed in different treatment solutions. The solutions used in each treatment were prepared with seawater. The treatments were as follows:

T1- Control treatment, immersion in seawater;

T2- immersion in 1.25% sodium metabisulfite solution;

T3- immersion in 0.005% 4-hexylresorcinol solution.

Seawater and solution temperature were around 25°C and volume of liquid

used was twice the volume of shrimp. Three distinct 500-gram batches were immersed in each of the solutions above for 5 min. Then the shrimp were stored on ice for five days, and then frozen at a temperature lower than -18°C. The occurrence of melanosis was registered visually according to the method developed by OTWELL *et al.* (10). After thirty days the shrimp batches were thawed and analyzed (in triplicate) as to the content of DMA and FA present in the muscle (Figure 1).

### **Chemical analyses**

#### *Preparation of extracts for analysis:*

The shrimp tails were thawed at room temperature and peeled. Fifty-gram muscle samples were used after being cut in pieces, macerated and mixed with 7.5% trichloroacetic acid in the proportion of 100 ml per 50 g of sample. In the case of FA determination, after this initial procedure, extracts were mixed with 6.0% perchloric acid solution in the proportion of 100 ml per 50 g of sample.

#### *DMA and FA determination*

The DMA contents were determined according to the method described by WOYEWODA *et al.* (11), whereby the colored copper-dimethyl-dithiocarbamate complex is formed. The FA analysis was performed according to the Nash method also described by WOYEWODA *et al.* (11).

Analytical determinations were run in triplicate with five determinations for each analysis.

## **RESULTS AND DISCUSSION**

Figure 2 shows that in whole shrimp melanosis began developing after only one day on ice. In the control samples 12% of the specimens were affected by melanosis, displaying black spots on these segments and mainly on the head. The specimens treated with sodium metabisulfite (SM) and 4-hexylresorcinol (HR) were also affected, however, in a lesser proportion, namely 4% and 3%, respectively. The concentration of spots was much less accentuated on the

specimens treated with these compounds indicating that both have an inhibitory effect on melanosis in trawled shrimp.

On the fifth day of storage on ice, over 50% of the control samples presented black spots. During this same period, only 9% of the sample treated with HR and 12% of the sample treated with SM were affected by melanosis. McEVILY *et al.* (7) likewise observed a faster development of melanosis in their control samples of *Farfantepenaeus aztecus* and *F. duorarum*. Melanosis development depends on the concentration of reaction components (substrate and enzyme) on the affected region. The higher the concentration, the earlier melanosis develops (8).

After 30 days of storage 70% of the control samples displayed black spots, while only 32% of the sample treated with SM were affected. The most efficient treatment, however, was immersion in HR with as little as 18% of samples affected. OTWELL *et al.* (10) have also demonstrated HR to be more efficient than sulfites in preventing melanosis in *Farfantepenaeus aztecus*.

The tests performed with beheaded shrimp (Figure 2) showed a quite similar profile for each type of treatment, but with an overall lower occurrence of melanosis. For example, after 30 days of storage 62% of the control samples, 24% of the sample treated with SM and mere 12% of the sample treated with HR were affected by black spots.

The DMA and FA analyses (Figure 3) showed that treatment with SM favored the formation of these components, whether the shrimp had been beheaded or not. DMA content for whole shrimp treated with SM was six times higher (1,52 0,15 mg/100g) than that treated with HR (0,25 0,01 mg/100g). For beheaded shrimp, SM-treated samples showed DMA values 3,5 times higher (0,56 0,04 mg/100g) than HR-treated samples (0,16 0,01 mg/100g). A higher FA content in both whole and beheaded shrimp was also observed for SM-treated samples when compared to sample treated with HR. For whole shrimp, the FA content reached values of 2,77 0,11 mg/100g (HR-treated samples) and 7,02 0,39 mg/100g (SM-treated samples). For beheaded shrimp, FA values were 1,23 0,09 mg/100g in sample treated with HR and 8,54 0,30 mg/100g in sample treated with SM. However, the control samples and the sample treated with HR contained about the same content of DMA and FA. These facts evidenced the interference of the use of sulfite on the formation of these compounds (DMA and FA) and showed that HR had just a smaller influence on the decomposition of trimethylamine oxide (TMAO) during icing and frozen storage. OGAWA *et al.* (8) have observed that the use of sulfites favors the formation of DMA and FA in lobster tails as well.

## CONCLUSIONS

The results indicate that the use of 4-hexylresorcinol as an inhibitor of melanosis in the Southern brown shrimp (*Farfantepenaeus subtilis*) is efficient whether the shrimp have been beheaded or not. The easy handling on board the fishing vessel and the low concentrations required of this compound make it a strong candidate for the replacement of sulfites in the prevention of melanosis in crustaceans, considering that the cost compensates.

## ACKNOWLEDGEMENTS

The authors would like to thank CEPNOR - Centro de Pesquisa e Extensão Pesqueira do Norte do Brasil [Fisheries Research and Extension Center of Northern Brazil]/IBAMA-Instituto Brasileiro do Meio Ambiente e dos Recursos Naturais Renováveis [Brazilian Institute for the Environment and Renewable Natural Resources] for their technical assistance and for placing their research vessel Almirante Paulo Moreira at our disposal. Appreciation is extended to Mr. Almiro Bonifácio e Silva (Cultor Food Science Brasil) for providing Everfresh. This research was funded in part by the CNPq (Conselho Nacional de Desenvolvimento Científico e Tecnológico) program.

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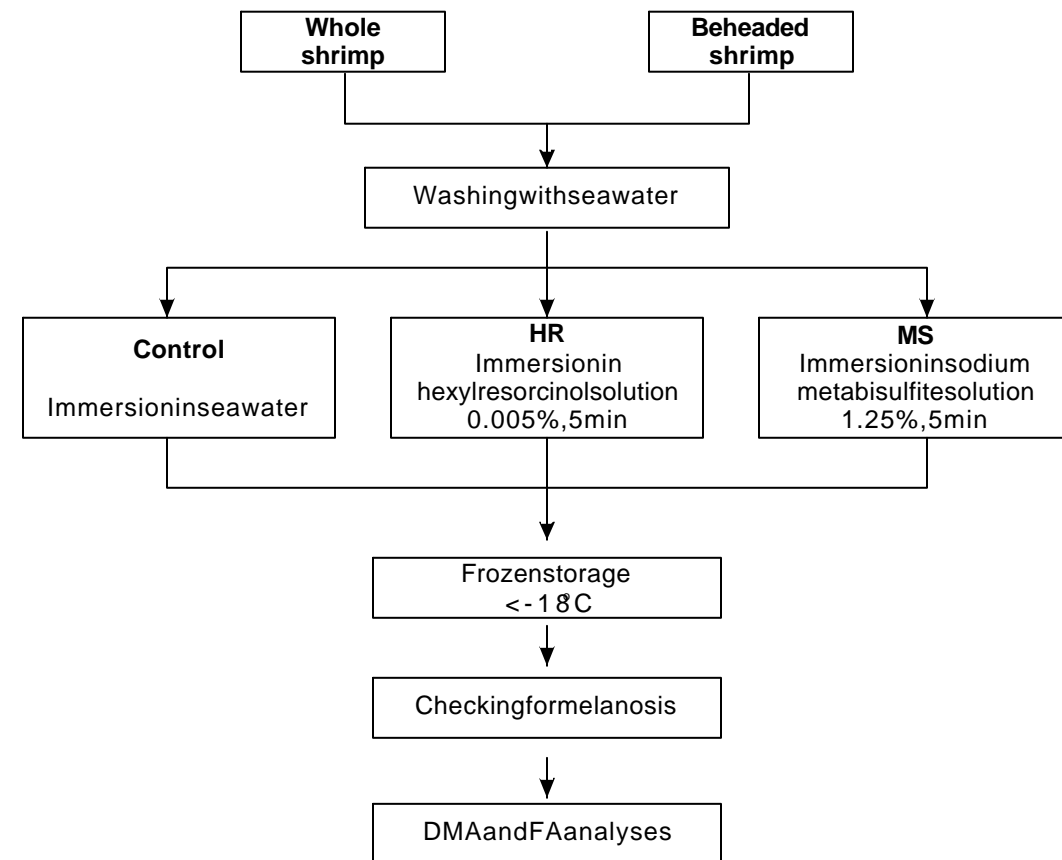


Figure 1 - Steps in the preparation of the treatments and analyses of the study

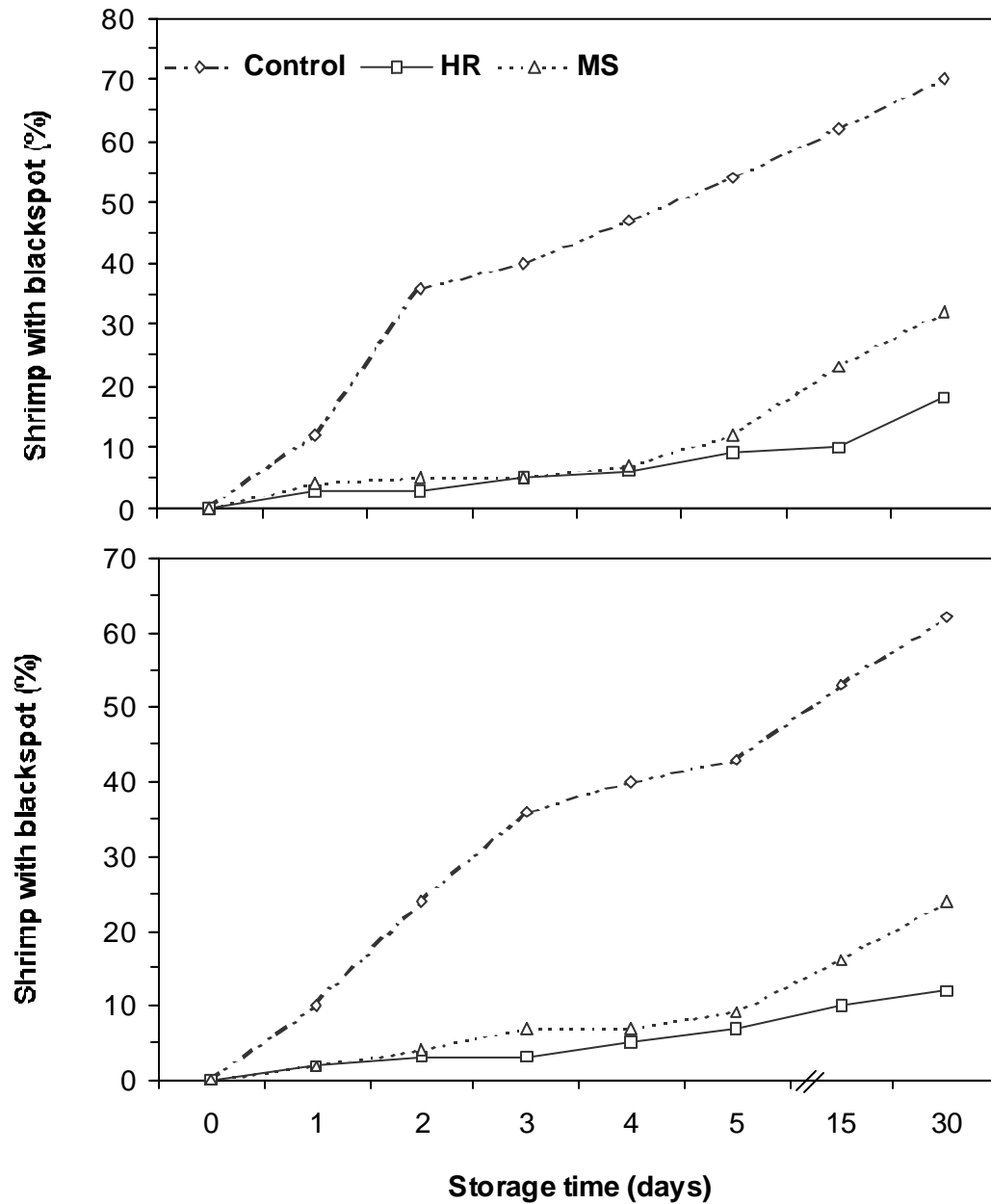


Figure 2 - Inhibition of melanosis in whole (A) and beheaded (B) shrimp (*Farfantepenaeus subtilis*) using hexylresorcinol (HR) and sodium metabisulfite (SM), compared to control group, during five days of ice storage and then 25 days of frozen storage.

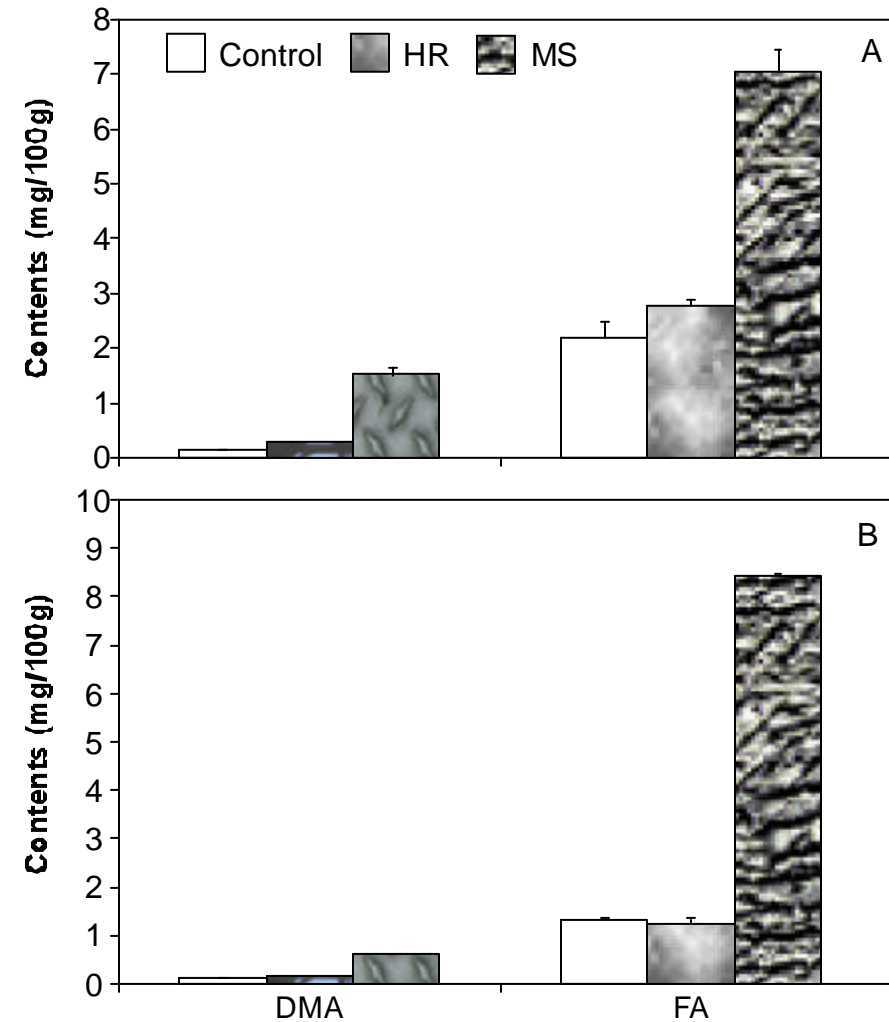


Figure 3 - Formation of DMA and FA in the muscle of whole (A) and beheaded (B) shrimp (*Farfantepenaeus subtilis*) treated with hexylresorcinol (HR) and sodium metabisulfite (SM), compared to control group, during five days of ice storage and then 25 days of frozen storage.