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Standardization of sodium metabisulfite solution concentrations and immersion time for farmed shrimp *Litopenaeus vannamei*

Padronização da concentração da solução de metabissulfito de sódio e do tempo de imersão para camarão cultivado *Litopenaeus vannamei*

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ABSTRACT

*Sodium metabisulfite is the main additive used in the prevention of melanosis in shrimp; however, it has currently been employed with great variation in concentration by producers. Thus, the aim of the present study was to determine the correlation between the concentration of the sodium metabisulfite solution and immersion time of the whole shrimp to obtain the concentration of sulfur dioxide (SO_2) in the edible muscle of farmed shrimp (**Litopenaeus vannamei**) in accordance with the limit established by law. For this, solutions of sodium metabisulfite at different concentrations (1%, 2 %, 3 %, 4% and 5%) were prepared and samples of **L. vannamei** shrimp (100g) were immersed during 10, 20 or 30 minutes at temperature of 7°C. For all treatment assayed the concentration of SO_2 was determined in the edible muscle of farmed shrimp (**L. vannamei**). The results show that for the conditions used in this study, the correlations were linear, with significant increase ($P<0.05$) in the SO_2 concentration in the edible muscle of shrimps both increasing sodium metabisulfite concentration as increasing immersion times, suggesting the immersion of shrimps in a 3% solution for a time of 13 minutes in order to obtain SO_2 concentration of 100ppm in its edible muscle in accordance with Brazilian legislation.*

Key words: *sulfur dioxide, edible muscle, farmed shrimp.*

RESUMO

*O metabissulfito de sódio é o principal aditivo usado na prevenção da melanose em camarão, porém, atualmente, é empregado com grande variação de suas concentrações pelos produtores. Assim, o objetivo deste estudo foi determinar a correlação entre a concentração da solução de metabissulfito de sódio e do tempo de imersão do camarão inteiro para obter a concentração final de dióxido de enxofre (SO_2) no músculo comestível de camarão cultivado (**Litopenaeus vannamei**), de acordo com os limites estabelecidos pela legislação. Para isso, foram preparadas soluções de metabissulfito de sódio em diferentes concentrações (1%, 2%, 3%, 4% e 5%); e amostras de camarão **L. vannamei** (100g) foram imersas durante 10, 20 e 30 minutos à temperatura de 7ºC. Para todos os tratamentos, foram realizadas análises da concentração de SO_2 no músculo comestível do camarão cultivado (**L. vannamei**). Os resultados demonstraram que, para as condições empregadas nesta pesquisa, as correlações encontradas foram lineares, ocorrendo um aumento significativo ($P<0,05$) nos teores de SO_2 no músculo comestível do camarão, tanto com o aumento da concentração das soluções de metabissulfito de sódio, quanto com o aumento no tempo de imersão, sendo possível sugerir a imersão dos camarões em solução a 3% por um tempo de 13 minutos, de forma a se obter, em seu músculo comestível, a concentração de 100ppm de SO_2, de acordo com o recomendado pela legislação brasileira.*

Palavras-chave: *dióxido de enxofre, músculo comestível, camarão cultivado.*

INTRODUCTION

Marine ***Litopenaeus vannamei*** shrimp is currently the main shrimp species cultivated in Brazil due to its excellent growing conditions and adaptability, easy nutrition, management and high productivity and profitability levels (PEREZ - VELAZQUEZ et al., 2012).

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One of the limiting factors to increase shrimp marketing both domestically and externally are the losses of freshness that shrimp is subjected after collection, and melanosis is one of the main problems, being responsible for the darkening of its carapace. It occurs due to the action of an endogenous enzyme complex present in shrimp, polyphenol oxidase (PPO), in which tyrosinase is the main active enzyme (HUANG et al., 2010).

The method most commonly used for inhibiting enzymatic browning in shrimp is the use the sulfite preservatives, since they act by removing oxygen and reducing pH, which are essential conditions for the enzymatic reaction (ROCHA, 2000). Their use at concentrations below ideal may contribute to melanosis or result in rejection by the buyer / importer and in cases where the concentration exceeds the limit established by law, sulfite preservatives cause nausea, abdominal pain, vomiting, skin reactions, as well choking and chemical pneumonitis in consumers or handlers. In addition, the overuse of these compounds can be harmful for the environment, since their residues when discarded, alkalinize the water causing death of several aquatic species (ARAÚJO & ARAÚJO, 2011; LIMA, 2008; PEDALE et al., 2012).

Several studies have reported the use of metabisulfite solution concentrations between 1.25% and 12% aiming to control melanosis in shrimp during storage, with immersion times ranging from 1 to 20 minutes under cooling temperatures (CINTRA et al., 1999; GÓES et al., 2006; BARBIERI JR. & OSTRENSKY, 2001; OGAWA et al., 2003; ARAÚJO & ARAÚJO, 2011). Thus, it is clear that there is a large variation among procedures and, consequently, a great difference of residual SO_2 concentrations found in the final product.

Given the importance of controlling the SO_2 levels in the edible muscle of shrimp, the aim of the present study was to determine the correlation between different concentration of the sodium metabisulfite solution and immersion times of the whole shrimp to establish the value of these variables which permit to obtain the concentration of sulfur dioxide (SO_2) in the edible muscle of cultured shrimp (***L. vannamei***) in accordance with the maximum limit established by law (100ppm).

MATERIAL AND METHODS

Litopenaeus vannamei shrimp with average weight of 10g, equivalent to 81/100 classification (individuals per kilogram) was obtained from shrimp farm located at the municipality of Pilar - PB, in which collection was randomly performed.

Immediately after collection, shrimps were immersed in drinking water at temperature close to 0°C for 10 minutes, resulting in killing by thermal shock, being then transported to the Laboratory of Meat and Fish Technology and Processing - UFPB in thermal box containing ice, using an ice / shrimp ratio of 2:1 (approximately 4°C), which were placed in 500g LDPE plastic bags, being submitted to slow freezing in domestic freezer for later analysis.

To characterize the sample, water activity analyses were performed on electronic meter AQUALAB model CX2 (Decagon Devices, Washington, USA); pH, according to parameters described by method No. 947.05 of the AOAC (2000) and proximate composition with moisture, ash and protein analyses performed as described in AOAC (2000) items 950.46.41, 920 153 and 928.08, respectively, while the lipid content was determined by FOLCH, LEES & SLAON - STANLEY (1957).

The residual sulfite in edible muscle of shrimp was analyzed for different immersion times (10, 20 and 30 minutes) and concentrations of sodium metabisulfite solutions (1%, 2%, 3%, 4% and 5%). Samples of 100g of whole shrimp were dipped into solutions of sodium metabisulfite adjusted to the tested concentration and pre-chilled at 7°C for the different immersion times assayed. To avoid loss by evaporation of sulfates, the sodium metabisulfite solutions were prepared just before the assays. Then the excess water was drained for 3 minutes, the carapace and exoskeleton of whole shrimp were removed and the residual sulfite in edible muscle was directly determined according the optimized procedure described by Monier-Williams in accordance with Brazilian Legislation (BRAZIL, 2011). The temperature of 7°C was used considering that this is the usual temperature employed in the shrimp farms during the immersion in the metabisulfite solution. All analyzes were performed in triplicate in two different experiments.

The experimental design was completely randomized, and the results of triplicates were evaluated by analysis of variance and differences between means were treated using the Tukey test (COCKRAN & COX, 1957) with the aid of the SAS System software (2001).

RESULTS AND DISCUSSION

The physical and chemical characteristics of the edible muscle of shrimp

Table 1 - Physical and chemical characteristics of edible muscle of farmed shrimp (***Litopenaeus vannamei***).

Sample	Moisture	Protein	Lipids	ASH	pH	Aw
Edible muscle of shrimp	76.02 (± 0.24)	19.22 (± 0.67)	0.46 (± 0.11)	2.33 (±0.26)	6,75 (±.11)	0.979 (± 0.002)

studied (Table 1) are in accordance with the previously related in studies that described characterization of farmed shrimp of the same specie (SRIKET, 2007; GONÇALVES GOMES, 2008; ARAUJO et al., 2012).

The pH value found (6.75) indicates that the shrimp has met the maximum limit established by RIISPOA, which is from 6.5 to 6.8 (BRASIL, 1997). The water activity found (0.979) was similar to that observed by SANTOS et al. (2011), when analyzing ***Macrobrachium olfessi***.

After analysis of the SO_2 levels in the edible muscle of shrimps that were previously submitted to peeling and immersion in concentration of sodium metabisulfite solutions of 1%, 2% , 3%, 4% and 5% on times of 10, 20 and 30 minutes at temperature of 7°C, it was possible to construct the curves shown in figure 1.

Figure 1 shows that the correlation between median value of residual concentration of SO_2 in the edible muscle of farmed shrimps and the concentration of sodium metabisulfite solutions is linear over the range of concentrations used in this study for all immersion times studied. GÓMEZ-GUILLÉN et al. (2005) studied the effect of SO_2 on the melanosis inhibition in ***Parapenaeus longirostris*** shrimp after different treatments with metabisulfite solutions and found an exponential increase in SO_2 residues when related to treatments of 0-9%. However, it was observed that up to 5% concentration, there is a trend of linearity in its points.

A significant increase ($P<0.05$) was also found in the SO_2 contents in the edible muscle of shrimps with increasing concentration of sodium metabisulfite solutions used in this study, and this increase was also observed by VIEIRA et al. (2008) for most concentrations and analytical methods used by the authors. Similarly, when analyzing the increase in SO_2 concentration in the edible muscle of shrimps with increased immersion times, a significant variation was also observed ($P<0.05$). GOES et al. (2006) reported that for the concentration of sodium metabisulfite solutions tested in their experiment (1% and 10 %), there was a significant influence of the exposure time on the SO_2 levels. These results confirm the results found by WEDZICHA (1992), who reported the influence of some factors such as concentration and immersion time on the use of sulfur dioxide.

It is noteworthy that all concentration of sodium metabisulfite solutions used in this study exceeded the SO_2 concentration of 100 ppm established by law (BRASIL, 1988), at least in two immersion times (20 and 30 minutes), except for concentration of 1%, where, even after immersion for 30 minutes, maximum SO_2 concentration of 67.62ppm was reached. According to VIEIRA et al. (2008), in practice, the use of much higher sodium metabisulfite concentrations is observed (about 10%) for preventing melanosis, which may suggest that the shrimp marketed presents residual SO_2 contents above limits established by Brazilian (100ppm) and European legislation (300ppm). Studies by OGAWA et al. (2003) and HARDISSON et al. (2002) with ***L. vannamei*** shrimps proved this finding, since excessive levels of this compound were observed in more than 50% and 40% of the analyzed samples, respectively. This excess SO_2 found in shrimp may cause harm to consumer's health, handlers and refusal by the consumer market.

When analyzing the correlation between median value of residual concentration of sulfur dioxide (SO_2) in the edible muscle of farmed shrimps and the immersion time of whole shrimp in sodium metabisulfite solution, it was possible to construct curves, which are shown in figure 2 for each sodium metabisulfite concentration used at temperature of 7°C.

By means of equations shown in figure 2, it was possible to obtain the immersion time required to achieve SO_2 concentration of 100ppm, established by Brazilian law for each sodium metabisulfite concentration used, except for the 1% solution, which did not reach this value. Therefore, in order to optimize the shrimp collection stages, it is recommended to immerse ***L. vannamei*** on a 3% sodium metabisulfite solution for a time of 13 minutes, and this time was calculated so as not to extrapolate the minimum and maximum values

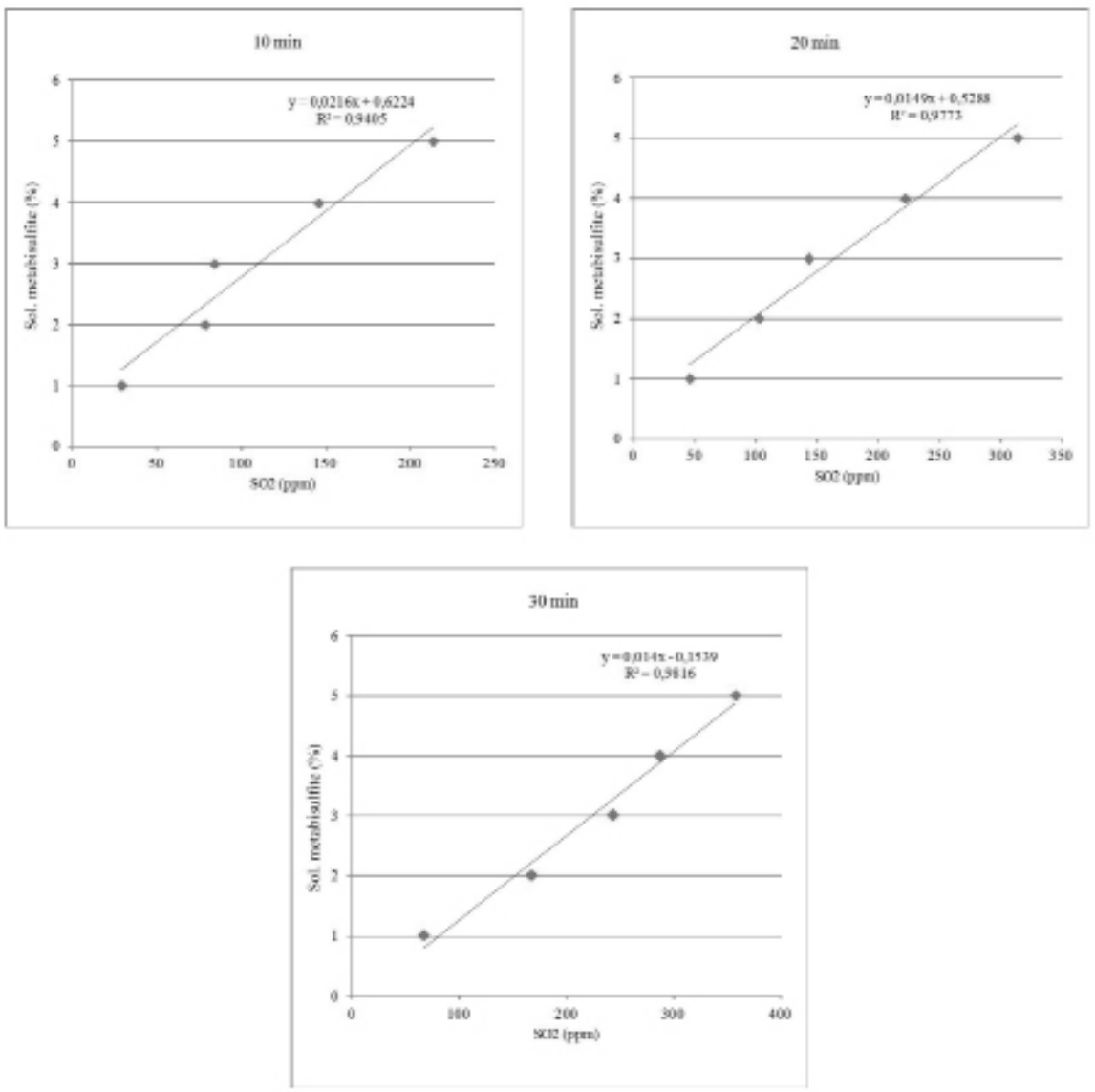

Figure 1 - Correlation between median value of residual concentration of sulfur dioxide (SO_2) in the edible muscle of farmed shrimps (***Litopenaeus vannamei***) and the concentration of sodium metabisulfite solutions at different immersion times.

found in this study, thus obtaining the shorter immersion time with the lowest concentration as possible in order to reduce costs with reagents and save time for companies working in this area. However, further studies should be carried out in order to verify the possibility of obtaining a threshold SO_2 concentration in shorter immersion times, thus enabling the application and standardization of these parameters by shrimp-producing companies.

CONCLUSION

Based on equations relating residual concentration of SO_2 in the edible muscle of shrimps with concentration of sodium metabisulfite solutions and residual concentration of SO_2 in the edible muscle of shrimps with immersion time of whole shrimp in sodium metabisulfite solution at different concentrations, it is possible to suggest immersing

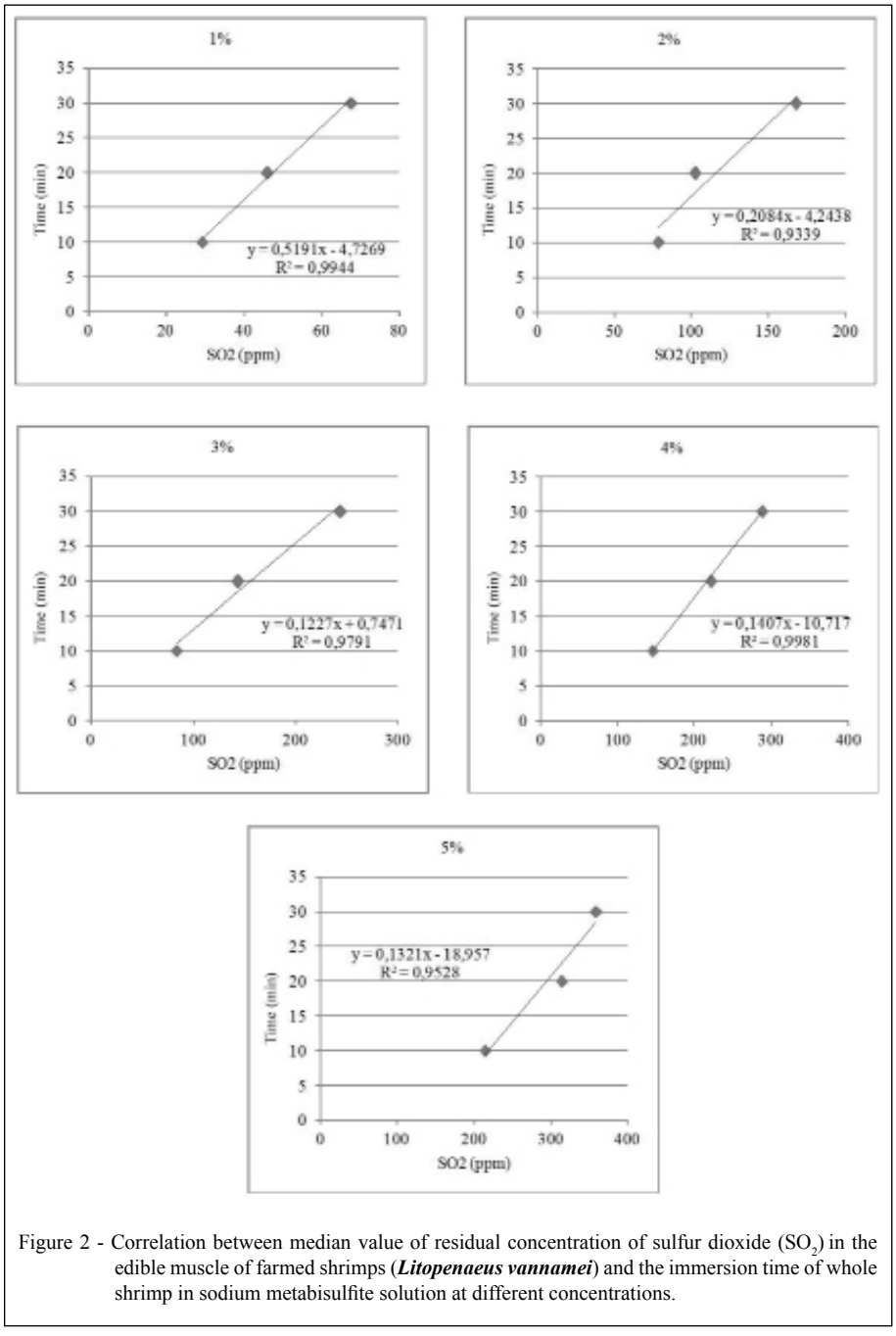

Figure 2 - Correlation between median value of residual concentration of sulfur dioxide (SO_2) in the edible muscle of farmed shrimps (***Litopenaeus vannamei***) and the immersion time of whole shrimp in sodium metabisulfite solution at different concentrations.

shrimp in a 3% solution for a time of 13 minutes so as to achieve SO_2 concentration of 100ppm, as recommended by Brazilian law (BRASIL, 1988).

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