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Analysis of Sulfites in Shrimp Using Rapid Distillation Followed by Redox Titration[1]

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ABSTRACT

The use of rapid steam distillation followed by redox iodine titration provides a rapid and accurate determination of total sufite residual in shrimp. Values obtained for sulfite-treated shrimp using the rapid distillation method gave comparable results to those of the officially recognized Monier-Williams method. Values for the rapid distillation method ranged from 6 to 212 ppm while those of the Monier-Williams procedure ranged from 6 to 241 ppm for untreated and treated shrimps, respectively. Statistical analysis using two-sample Student's t-test indicated that there were no significant differences ($p>0.05$) for residual levels below 100 ppm but the values obtained by the rapid distillation method and the Monier-Williams procedure were significantly different ($p<0.05$) at concentrations near and above 100 ppm.

Sulfiting agents have been proven as effective and necessary controls to prevent adverse melanosis ("blackspot") on penaeid shrimp (*2,3,8*). More recent work has concluded sulfites represent the most effective and practical control as compared to a large variety of chemical alternatives (*12*). Prior sanctioned for use on shrimp in 1956 (*3*) and eventually granted GRAS status in 1959; sulfites to control shrimp melanosis is currently restricted to treatments which impart residuals less than 100 ppm as SO_2 on the raw, edible portion. Residuals in excess of 10 ppm constitute labeling requirements (*7*). Thus residual screening is deemed necessary to provide a routine quality control during processing and to protect the interest of concerned consumers.

Sulfites are typically applied on the shrimp vessel immediately post-harvest. Attempts to monitor residuals at this primary level of production are restricted by limited laboratory space and minimal technical training not amenable to dockside settings or primary shrimp processing locations. Since subsequent storage and cooking do not significantly diminish residual levels (*12*), sulfite screening is usually applied at any distribution point after immediate processing or packaging. Although a variety of simple sulfite test strips can be used to detect the presence of sulfites on shrimp, these strips are limited to qualitative assessments and have been noted to produce "false positive" readings (*11*). Likewise, a variety of simplified analytical methods have been introduced; p-rosanile (*1*), ion specific electrode (*14*), malachite green as modified by General Mills, Inc. (*5*), potassium permanganate colorimetric screening (*9*), drop and digital titration kits (Lamotte SO_2 test kit, Thomas Scientific), iodate-iodide titret kits (Sulfite test kit, Chemetrics, Inc.), and an enzymatic (UV) method (Mannheim Boehringer cat. No. 725854). These methods are limited to qualitative assessments, subjective quantitation, or tedious application. These methods have not been compared with the official Monier-Williams method (*1*). Also, comparable ion chromatographic methods have been introduced to replace or compliment the Monier-Williams method, but these methods require expensive instrumentation and an experienced analyst (*4,10*).

Thus a reliable and simplified method has yet to evolve for use at the primary level of sulfite treatment for shrimp. The rapid distillation method introduced by DeVries et al. (*6*) may offer a practical solution, but this method requires further verification across a variety of SO_2 residual concentrations typically encountered on sulfited shrimp. This study was devised to evaluate the DeVries et al. (*6*) method utilizing an available pre-assembled apparatus to measure SO_2 residuals on sulfite treated penaeid shrimp.

MATERIALS AND METHODS

Preparation and storage of samples

Raw iced shrimp (*Penaeus setiferus*) purchased from a seafood retailer in Gainesville, Florida were divided randomly into six batches. One batch was left untreated while the remaining five batches were dipped in various concentrations of sodium metabisulfite solutions ranging from 0.156 to 2.5% for 1 min, and then drained for 3 min at room temperature (about 25°C). For the control batch, this procedure was repeated with a water dip. All treated batches were frozen at -20°C until analyzed. For analysis, the shrimp samples were thawed, peeled (shell removed by hand), and finely minced. Eight subsamples were taken from each batch, four for the rapid distillation analysis and four for the Monier-Wiliams determination of sulfites. This entire sampling scheme was duplicated.

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Sulfite determination by the rapid distillation method:

The method used was by DeVries et al. (*6*). Residual sulfite on shrimp was analyzed using a Kjeltec rapid distillation unit (Tekator No. 1002; Fisher Scientific) equipped with several 500-ml sample tubes modified for side discharge. Approximately 10 g of minced (finely chopped with knife) shrimp were placed in a sample tube with 50 ml of de-ionized water. The tubes with wet samples were placed under the distillation unit and 30 ml of 33% HCl were dispensed into each tube. Each sample was then steam-distilled while the condensate was being titrated simultaneously with a 0.02 *N* iodine solution containing starch as indictor. The iodine solution was standardized against 0.1 *N* sodium thiosulfate. Concentration of sulfite was calculated using the following equation (equation 1):

$$\text{ppm } SO_2 = VxNx32x1000/W \quad [1]$$

where V is the volume (ml) of I_2 consumed, N is the normality of the I_2 working solution, 32 is the equivalent weight of SO_2, and W is the weight (g) of the sample.

Monier-Williams method for the determination of sulfites:

The modified SO_2 method was directly from AOAC (*1*) with a slight modification in using Grade 5 nitrogen to replace the pyrogallol trap. Approximately 50 g of the minced shrimp were distilled for 125 min in 350 ml water containing 90 ml of 33.33% HCl. The SO_2 evolved was collected in 10 ml 3% H_2O_2 and titrated with 0.1 *N* NaOH using methyl red as indicator. The concentration of sulfite was calculated using the following equation (equation 2):

$$\text{ppm } SO_2 = (3.203)x(\text{ml } 0.1\ N\ \text{NaOH})/\text{weight of sample (kg)} \quad [2]$$

Results for the distillation method and Monier-Williams procedure were statistically analyzed using the two sample Student's t-test.

RESULTS AND DISCUSSION

The results presented in Table 1 indicate that the two procedures achieved similar values at the various levels of sulfites tested and for residual levels below 100 ppm the values obtained by the two methods are not statistically different ($p>0.05$). With both methods, the variation among the samples at concentrations greater than 200 ppm is significantly greater than the variation at the lowest three concentrations ($p<0.05$). Moreover, the coefficient of variation for the rapid distillation (33, 11, 8, 5, 6, and 4%) and the Monier-Williams method (17, 25, 14, 12, 5, and 5%) indicates the two methods to be similar in precision with increasing levels of concentration and both possessing greater variation relative to the mean at lower concentration levels. Similar precision was achieved with various levels of sulfite on dehydrated vegetables using a distillation method followed by spectrophotometric measurement (*13*). Apparent underestimation at higher concentrations with the rapid distillation procedure may result due to a more subjective end-point determination while overestimation at higher concentrations with the Monier-Williams methods may be due to the extensive digestion (120 min) compared to the rapid distillation (10 min). With the rapid distillation method, it was necessary to compare the color of the solution at the end-point to that of standards prepared at the beginning of the titration. This comparison is recommended to better familiarize the analyst with the proper end-point determination. This comparison was not necessary for the distillation/spectrophotometric procedure (*13*) but this procedure requires more involved instrumentation and has not been verified for shrimp or other major protein sources. After mincing the shrimp, the Monier-Williams method requires approximately 120 min for a complete analysis as compared to 10 min for the rapid distillation and distillation/spectrophotometric (*13*) methods.

TABLE 1. *Summary of average sulfite residuals (ppm) determined on shrimp by two methods.*

Sample	Rapid distillation[a]	Monier-Williams[a]
Untreated	6±2	6±1
0.156% sulfite	19±2	16±4
0.312% sulfite	29±4	29±4
0.652% sulfite	64±6	58±6
1.25% sulfite	102±5	114±4
2.5% sulfite	212±9	241±12

[a]Data are means ± standard deviation for two complete experiments with n=4 for each dip treatment in each experiment. Values are corrected for blanks.

SUMMARY

The rapid distillation method appears to be a very straightforward for the routine assay of sulfites in shrimp. Slight variation can be expected, particularly at higher concentrations (SO_2), due to subjective end point determinations; yet this procedure is rapid and convenient. The method required substantially shorter times than the official Monier-Williams procedure and employs standard, inexpensive laboratory glassware, careful assembly, and constant monitoring during the actual analysis. The distillation/spectrophotometric method (*13*) would require an additional purchase of a spectrophotometer or colorimeter. Thus the rapid distillation method should be considered for use in routine quality control programs while acknowledging that the current officially recognized method is still Monier-Williams.

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curacy by weight. This procedure is recommended for mechanical pipets.

Test Frequency

Perform a single sample measurement following a change of any removable parts.

Perform a four-sample test monthly – or more frequently, depending on use.

Perform a ten-sample test quarterly.

Test Method

The test is based upon the determination of weight of water picked up and delivered by the instrument.

Equipment and test water should be placed in the test environment of 19-24°C (66-75°F) for at least two hours before testing.

Test Procedure

1. Place a 50 cc or smaller beaker or vessel containing 20-25 g of water onto an analytical balance and record its weight.
2. Using another container of water, double-rinse the mechanical pipet tip by aspirating the quantity of sample to be transferred.
3. If sample is to be transferred without diluting it within the pipet tip, aspirate water from beaker on analytical balance equal to the quantity of sample to be pipeted and record weight of the beaker. Expel the quantity back into the same beaker and note the weight.
4. If 1.0 mL of 1:10 dilution is to be made within a pipet tip, aspirate 0.9 mL then 0.1 mL into the tip noting the weight of each volume separately. Expel the 1.0 mL volume back into the same beaker and note the weight.
5. Repeat steps 1-3 or 1, 2, and 4 as appropriate for the number of samples to be examined and divide by the number of measurements to obtain the average value.
6. Calculate weights of sample removed from and returned to the vessel on the balance. Both weights should fall within tolerance limits listed below:

   Tolerances

   a. 0.1 mL should weigh 0.0975-0.1025 g
   b. 0.9 mL should weigh 0.890-0.910 g
   c. 1.0 mL should weigh 0.990-1.101 g

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