S-Sulfonate Determination in Shrimp

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The treatment to which shrimp must be subjected to release protein-bound sulfite, followed by HPLC determination involving ion exclusion and electrochemical detection, is studied. The two agents assayed, dithiothreitol (DTT) and cyanide, are efficient at releasing sulfite from solutions of *S*-sulfocysteine, although DTT is not a suitable agent for the determination of *S*-sulfonates in shrimp. This aspect was confirmed both by HPLC and by the optimized Monier−Williams method. For the analysis of protein-bound sulfite in shrimp, sample treatment with cyanide is proposed. In assays of the reproducibility of the method, standard deviations were 14.8 and 9.1 for mean *S*-sulfonate values of 105 and 47 μg of SO_2/g, respectively. Mean recovery was 96.3% for different amounts (51, 102, and 205 μg of SO_2/g) of *S*-sulfocysteine added. The contents of *S*-sulfonates in 11 batches of commercial shrimp were found to vary between 30 and 175 μg of SO_2/g. It was not possible to establish a direct relationship between total SO_2 (free plus reversibly bound fraction) and protein-bound sulfite.

Keywords: *S-Sulfonates; sulfites; shrimp; cyanide; dithiothreitol (DTT)*

INTRODUCTION

The fate of sulfites added to foods depends on the nature of the food, the level of addition, the type and length of the processing, the conditions and time of storage, the characteristics of the packages used, and the method of preparation (Armentia-Alvarez *et al.*, 1993a, 1994; Taylor *et al.*, 1986; Wedzicha, 1984).

Following the addition of sulfites to foods, these may be found as sulfurous acid and free inorganic sulfites and as a whole range of forms of bound sulfites. Complex balances dependent upon several factors determine the amount of sulfite in each of these forms. Additionally, sulfites may be partially oxidized to sulfates. With respect to free forms, the most important factor is pH. In this sense, in the usual pH range of foods the predominant species will be the bisulfite ion; if the pH of the food is lower than 4, some of the sulfite added may become volatilized as sulfur dioxide. In general, an equilibrium exists between free and bound forms of sulfite, although some bound forms are irreversible.

Sulfites can cleave the disulfide bonds of cystine, peptides, and proteins, giving rise to the formation of a thiol (R−SH) and an *S*-sulfonate ($R-S-SO^{3-}$). Disulfide bonds strongly affect the properties of proteins, it being possible to alter these by reducing disulfide bonds. Such alterations may have technological and nutritional repercussions (Friedman, 1994; González and Damoradan, 1990a; Wedzicha, 1984).

The stability of the compounds arising from the interaction of sulfite with proteins and amino acids in foods is poorly known, although such compounds are considered to be irreversibly bound forms (Daniels *et al.*, 1986; Fazio and Warner, 1990; Taylor *et al.*, 1986). This means that with the analytical methods usually employed, the fraction of sulfite bound to proteins is not determined. In sulfited foods, the fraction of sulfite bound to proteins is unknown and only the results obtained in model experiments carried out on meat products and biscuits sulfited at the laboratory are available (Thewlis and Wade, 1974; Wedzicha and Mountfort, 1991). In biological media, methods for the identification and quantification of *S*-sulfocysteine have been described (Bethizy and Street, 1980; Kagedal *et al.*, 1983), and also the protein-bound sulfite fraction has been determined after bond cleavage (Bechtold *et al.*, 1993; Gunnison and Benton, 1971; Gunnison and Palmes, 1973; Gunnison *et al.*, 1987; Togawa *et al.*, 1992).

Both in food and in biological media, the procedure involves sample treatment with an agent, dithiothreitol (DTT) or cyanide, able to release the sulfite bound to proteins. This sulfite is later determined by any of the methods used in the analysis of total sulfite (free plus reversibly bound).

Currently, sulfites are the agents of choice for the control and prevention of blackspot in crustaceans. Since such foods have high protein contents, the sulfite bound to proteins may become an important fraction, and its determination should help to clarify the fate of the additive in crustaceans. Additionally, the existence of *S*-sulfonates may represent an additional exposure to sulfite arising in the organism through metabolic processes (Gibson and Strong, 1974, 1976; Gunnison and Palmes, 1973).

Today, the safety of sulfites is debatable owing to the appearance of adverse reactions associated with the consumption of some foods containing them (Bush *et al.*, 1990; FAO/WHO, 1987; *Federal Register*, 1986a,b; Taylor *et al.*, 1986). It should be stressed that this type of reaction only appears in susceptible individuals and that there is no evidence of risk for the general population when sulfiting agents are used in permitted amounts (Gunnison and Jacobsen, 1987).

The aim of the present work was to study the treatment to which shrimp should be subjected to release sulfite from *S*-sulfonates, later determining this by ion exclusion HPLC with electrochemical detection (Armentia-Alvarez *et al.*, 1993b). Before the study in shrimp was begun, it was essential to conduct studies

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with *S*-sulfocysteine to shed light on the stability of sulfonates and the effectiveness of the treatments designed to release sulfite from them since little information concerning this is available (Daniels *et al.*, 1986; Fazio and Warner, 1990). Once the analytical procedure to be used had been chosen, commercial samples were determined with a view to estimating the real amounts of *S*-sulfonates present in them.

MATERIALS AND METHODS

Reagents. *S*-Sulfocysteine (cysteine-*S*-sulfonic acid) was purchased from Fluka Chemie AG (Buchs, Switzerland); dithiothreitol (DTT) was obtained from Sigma Chemical Co. (St. Louis, MO); potassium cyanide, sodium hydroxide, sodium sulfite, tris(hydroxymethyl)aminomethane (Tris), and aluminum oxide 90 were supplied by Merck (Darmstadt, Germany).

Apparatus. A Milton Roy Model CM-4000 HPLC system equipped with an electrochemical detector (Metrohm, Model 6565) with a glassy carbon electrode at 1150 mV was used with an Ag/AgCl reference electrode. Output from the detector was fed to a Milton Roy Model CI-4000 integrator. Separation was accomplished on a 150 × 7.8 mm anion exclusion column (Waters Chromatography, Milford, MA).

Samples. All samples used were commercial shrimps (*Parapenaeus longirostris*) frozen at sea and previously treated with a mixture of four sulfiting agents except batches C, D, and H, to which only sodium metabisulfite had been added. All batches also contained a preservative (benzoic acid or sodium benzoate) and a synergic antioxidant agent (disodium calcium EDTA), except batch C, to which the latter agent had not been added.

Assays were carried out on the edible part, which was obtained by removing the head and peeling the tails.

For the determination of *S*-sulfonates, 11 batches of commercial shrimp were purchased from different suppliers. Each batch (2 kg) was divided into portions of 250 g. For the analyses, three of these portions were taken and each was considered a sample. Samples were stored at −18 °C (the temperature recommended for the storage of frozen crustaceans) until determination.

To compare the results found in samples treated and or not with DTT with the optimized Monier−Williams method, 1 kg of shrimp from the same batch was acquired. The peeled tails were ground and carefully mixed, and the homogenate thus obtained was divided into portions of 25 g, which were stored at −30 °C until analyzed. Each of these portions was considered one sample. Also in this case, the shrimps had been treated with a mixture containing four sulfiting agents, sodium benzoate, and EDTA.

Nonsulfited shrimps, used in the study on recovery, were supplied by a fishing company in Huelva, Spain, and had been frozen (−30 °C) immediately after capture.

Procedure for the Determination of *S*-Sulfonates in Shrimp. Approximately 50 g of the edible part of the shrimp was ground and homogenized in a domestic blender. Two grams of aluminum oxide was added to 1 g of previously homogenized sample, and this was mixed until the paste acquired a loose dry consistency.

(a) Treatment with Cyanide. Ten milliliters of alkaline cyanide solution (0.125 M KCN and 0.03 M NaOH) was added, and the resulting suspension was incubated in a water bath with stirring at 37 ± 1 °C over 1 h. After the mixture had cooled, keeping it at 4 °C for 10 min, 20 mL of the extractant solution used for the determination of total sulfite was added and the sulfite was then analyzed by the HPLC method developed at the authors' laboratory (Armentia-Alvarez *et al.*, 1993b). In this method, total sulfite is extracted with a 0.020 M Na_2HPO_4 aqueous solution containing 0.1% (v/v) glycerol, adjusted to pH 12 with NaOH, and then determined by ion exclusion chromatography with electrochemical detection.

Treatment with cyanide quantifies free sulfite plus the reversibly bound form and also the protein-bound sulfite. It is therefore necessary to quantify total sulfite (free and reversibly bound) in parallel in another aliquot of sample subjected to an identical treatment but in which cyanide is replaced by phosphate adjusted to pH 12. Protein-bound sulfite is calculated by difference between sulfite quantified after cyanide treatment and sulfite quantified after treatment at pH 12.

Treatment with cyanide was based on the method described by Gunnison *et al.* (1987) for biological samples.

(b) Treatment with DTT. DTT (5 or 20 mM) in 0.05 M Tris-HCl buffer (pH 9.2), containing 5 mM EDTA was added to 1 g of sample, homogenized as above. The mixture was incubated at 37 ± 1 °C for 5 min. After the sulfite had been released, HPLC determination was implemented. As in the previous case (treatment with cyanide), it was necessary to carry out the determination of total sulfite in aliquots of the same sample and calculate the protein-bound sulfite by the difference. Treatment with DTT was based on the procedure reported by Nakamura and Tamura (1974) and its application to sulfited meats according to Wedzicha and Mountfort (1991).

Optimized Monier−Williams Method. This was used to confirm some of the results because it is considered to be the reference method for the determination of total SO_2 (*Federal Register*, 1986a).

Statistics. An *F*-test for comparing standard deviations and a *t*-test for comparison of means were used (Miller and Miller, 1988).

Table 1. Recovery of Sulfite from *S*-Sulfocysteine

sulfite present in *S*-sulfocysteine (SO_2, μg)	treatment	recovery of sulfite (%)	SD	*n*
13	DTT[a]	103.8	1.9	10
102	DTT[b]	102.4	2.8	5
13	KCN[c]	96.4	5.3	10
102	KCN[d]	49.8	8.8	5

[a] One milliliter of 5 mM DTT. [b] Two milliliters of 5 mM DTT. [c] One milliliter of alkaline cyanide solution (0.125 M KCN and 0.03 M NaOH). [d] Two milliliters of alkaline cyanide solution (0.125 M KCN and 0.03 M NaOH).

RESULTS AND DISCUSSION

Effectiveness of Different Treatments for the Release of Sulfite from *S*-Sulfocysteine. It was initially observed that sulfite was not displaced from *S*-sulfocysteine with alkaline extraction and that no free sulfite was present in the standard used. This assay was carried out with an aqueous solution of *S*-sulfocysteine to which the HPLC method was applied (Armentia-Alvarez *et al.*, 1993b).

After treatment of solutions of *S*-sulfocysteine under the conditions described by Nakamura and Tamura (1974) with regard to the amount of SO_2 coming from *S*-sulfonates and the amount of DTT necessary to cleave these adducts, a mean recovery of 103.8% was obtained (Table 1). According to prospective studies carried out at this laboratory, the amount of sulfite bound to proteins in shrimp would be expected to be greater than that used in the above-mentioned assay. Accordingly, another experiment in which the amounts of *S*-sulfocysteine and DTT were increased was designed. The results obtained (Table 1) show that also in this case a good recovery of sulfite is obtained, mean recovery being 102.4%.

When the treatment with cyanide was applied to different amounts of *S*-sulfocysteine using different volumes of reagent, it was observed that for sulfite to be released a suitable amount of cyanide was necessary; this depended on the sulfonates present (Table 1). A new assay was therefore implemented using 10 mL of the cyanide reagent and different amounts of SO_2 in the form of *S*-sulfocysteine. The results, shown in Table 2, show that the addition of larger amounts of cyanide permits the recovery of sulfite (mean value 97.2%).

Table 2. Recovery of Sulfite from *S*-Sulfocysteine by Treatment with Alkaline Cyanide (10 mL)

sulfite present in *S*-sulfocysteine (SO_2, μg)	sulfite found (SO_2, μg)					mean recovery (%)
51	49	50	51	50	51	98.4
102	101	99	100	101	102	98.6
204	196	192	192	190	190	94.0
						97.2

Table 3. Sulfite Determined in Shrimp, Treated or Not with DTT, by the Optimized Monier−Williams Method[a]

	total SO_2			
	$\bar{x}$ (μg/g)	SD (μg/g)	CV (%)	*n*
shrimp	323	14.1	4.4	10
shrimp treated with DTT	334	11.8	3.5	10

[a] $F = 1.428$; $t = 1.795$.

It is generally accepted that the Monier−Williams method does not determine the sulfite bound to proteins; however, we thought it suitable to test this with standard sulfonate. For this, a solution of *S*-sulfocysteine (1273 μg of SO_2) was prepared in water, and then the sulfite was analyzed. It was apparent that it was impossible to determine this with the reference method. Additionally, as expected, previous treatment with DTT of the solution of *S*-sulfocysteine permitted release and later determination of sulfite according to the Monier−Williams method. The mean recovery found in four assays was 89.5%, with a standard deviation of 2.6, from solutions of *S*-sulfocysteine (1273 μg of SO_2) to which 5 mL of 20 mM DTT had been added.

Determination of *S*-Sulfonates in Shrimp by DTT-Induced Sulfite Release. After the treatment conditions had been established, the protein-bound sulfite in shrimp was determined. Determination was carried out in three batches divided into portions, each of these being considered a sample for analysis. It is mandatory to proceed in this way because of the variability found in the contents of free and total sulfite among samples from the same batch (Armentia-Alvarez *et al.*, 1994). The results show that under the conditions of treatment with DTT described here, it is not possible to determine protein-bound sulfite in shrimp.

Since DTT had proved to be effective in the experiments conducted with *S*-sulfocysteine, in sulfite determination by both HPLC and the Monier−Williams method, determination was performed in shrimp following the reference method. It should be noted that in a previous work (Armentia-Alvarez *et al.*, 1993b) no statistically significant differences between the amounts of total sulfite (free plus reversibly combined), as determined by either method, had been found.

Aliquots of 3 g each were taken from the ground samples destined for this assay, and half of them were subjected to treatment with 5 mL of 20 mM DTT. Table 3 shows the sulfite contents determined and includes the statistics calculated. In the light of these results, the concentrations of sulfite determined by previous treatment with DTT can be said to show no significant differences ($p = 0.05$) from those determined when the reducing agent was not employed. Accordingly, under these conditions DTT can be said to be inefficient at releasing protein-bound sulfite from shrimp. As far as we are aware, this can be attributed to certain intrinsic factors that, in this particular case, would affect the accessibility of the reducing agent to sulfonates. A similar effect has been observed by other authors (Carbonaro *et al.*, 1992; Wolf, 1993) in beans and soybean proteins when attempting to reduce disulfide bonds with DTT. This hypothesis is corroborated by the experimental observation that the sulfite added to shrimp in the form of *S*-sulfocysteine is recovered following treatment with DTT. In the two experiments in which *S*-sulfocysteine was added to 3 g of shrimp homogenate (1273 μg of SO_2), the recoveries obtained with the Monier−Williams method were 76.3 and 77.8%.

Table 4. Reproducibility of the Method for *S*-Sulfonate Analysis in Shrimp

shrimp	parameter	total SO_2 treatment with CN^-	SO_2 from *S*-sulfonates
sample 1	$\bar{x}$, μg/g	302	105
	SD, μg/g	14.8	14.8
	CV, %	4.9	14.1
	n	8	8
sample 2	$\bar{x}$, μg/g	296	47
	SD, μg/g	9.0	9.1
	CV, %	3.0	19.4
	n	6	6

It should be pointed out that treatment with DTT has allowed some authors to determine *S*-sulfonates in sulfited meat products in the laboratory. In this case, the sulfite released was separated by distillation and collected on 5,5-dithiobis(2-nitrobenzoic acid) (DTNB), after which spectrophotometric determination was carried out. This demands the addition of $HgCl_2$ after treatment with DTT and before distillation since the thiols react with the DTNB. In initial assays we observed that the addition of $HgCl_2$ shortens the life of the chromatographic columns, and since spectrophotometry was not used, this step was not included.

The effectiveness of DTT for the determination of *S*-sulfonates in meat products may be due to better accessibility to these compounds than in shrimp or perhaps to the effect exerted by $HgCl_2$ on proteins. Alternatively, but less likely, is the possibility of a blockade of the reversibility of the reaction that would be exerted by this latter agent when the thiols formed are precipitated.

Determination of *S*-Sulfonates in Shrimp by Cyanide-Induced Sulfite Release. The treatment employing cyanide was applied to different samples of shrimp. The results obtained show that in this type of food it is possible to release protein-bound sulfite with this treatment. Cyanide was therefore used for the determination of *S*-sulfonates. Below we describe the assays carried out to establish the reproducibility and recovery of the method.

The assays on the reproducibility of the method were performed on two different batches of shrimp. Two hundred fifty grams of the edible parts from each of the batches was homogenized, taking the portions necessary for each determination from these homogenates. The mean values, standard deviations, and coefficients of variation (CVs) are shown in Table 4. Reproducibility includes the variability itself of the analysis of total sulfite (free plus reversibly bound) in shrimp together with the variability arising from having to calculate the *S*-sulfonate content by the difference between two aliquots from the same sample, even though great care was taken to set up working conditions that would avoid this effect as far as possible. Logically, this aspect is more pronounced when the concentration of sulfite coming from *S*-sulfonates is lower.

Sulfite recoveries were evaluated by adding different amounts of *S*-sulfocysteine to homogenates of the edible

Table 5. Recovery of Sulfite Added as *S*-Sulfocysteine from the Edible Part of Shrimp

sulfite added[a] (μg/g)	sulfite found after addition[a] (μg/g)					mean recovery (%)
51	48	47	48	57	57	100.8
102	103	100	101	105	100	99.8
204	176	184	181	181	178	88.2
						96.3

[a] Expressed in μg of SO_2/g of edible part of shrimp.

parts of nonsulfited shrimp. A suitable volume (250−500 μL) of an aqueous solution of *S*-sulfocysteine, at a concentration necessary for the levels of addition shown in Table 5 to be reached for the samples, was added to 1 g of the homogenate and the material was then carefully mixed. After 5 min, the mixture was treated with cyanide and the sulfite determined as explained under Materials and Methods. The mean recovery calculated for 15 determinations was 96.3%. In several works on the presence of *S*-sulfonates in biological media, the methods used have been evaluated using sulfited seroalbumin or by adding sodium sulfite. As far as we know, only Kagedal *et al.* (1983) used *S*-sulfocysteine for recovery assays, finding a mean recovery in urine of 91 ± 11% for 320 μg of SO_2/L.

Analysis of *S*-sulfonates in foods has only been performed in model experiments essentially aimed at establishing the distribution of sulfite in meat products and biscuits (Thewlis and Wade, 1974; Wedzicha and Mountfort, 1991). In biscuits, the method used determines nearly all of the sulfite bound to organic compounds, which in this case would be combined with proteins. In meat products, treatment with DTT, at least in some samples, does release sulfite. However, it should be stressed that the effectiveness of this dissociation has been checked with bovine serum albumin, and it has therefore not been possible to establish the effect of the food on the accessibility of the reducing agent to the sulfonates. In view of the results obtained in the present work, this aspect deserves further attention.

Contents of *S*-Sulfonates in Frozen Shrimp. Table 6 shows the contents of *S*-sulfonates and total sulfite (free plus reversibly bound) determined in 11 batches of frozen shrimp acquired from commercial sources. Three samples from each batch were analyzed. Regarding total sulfite, the standard deviations include the variability among samples in a single batch; this has been described and discussed in an earlier work (Armentia-Alvarez *et al.*, 1994). Again, the dispersion in the values of SO_2 found in shrimp from the same batch may be seen, together with the presence of residual levels above the permitted maximum.

The mean content of *S*-sulfonates varied between 30 and 175 μg of SO_2/g; in some of the batches fairly similar amounts were found (A−D), while in others the content was in fact higher (I−K). Within the same batch, the variation coefficient with respect to the mean value in most cases is higher than that established in the assay on the reproducibility of the analytical method such that it can be attributed to the variability of the samples.

It is not possible to establish a direct correlation between the contents of sulfonates and total sulfite, as reported by other authors in sulfited meat products in laboratory trials under controlled conditions. In the assays of those authors, the values of sulfonates for four samples, different species and cuts, corresponded to 17, 33, 68, and 70 μg/g, respectively, in all cases with the addition of 607 μg of SO_2/g of sample (Wedzicha and Mountfort, 1991).

Sulfitolysis reactions are governed by several factors including the concentration of sulfite in the form of SO_3^{2-} and the net charge in the vicinity of the disulfide bond (Cecil and McPhee, 1955a,b; Cecil and Wake, 1962; McPhee, 1956). Both variables are affected by the pH of the medium, which in turn increases with the addition of sulfite and, during the storage of frozen shrimp, may undergo small increases. According to previous results (Armentia-Alvarez *et al.*, 1994), in shrimp free sulfite corresponds to 43% of total sulfite; of this, an important fraction could in principle continue to react with disulfide bonds.

Furthermore, there are reversibly combined forms whose formation and stability again involve the pH of the food such that sulfite may be released from them. It is also possible that they could form new combined forms through reaction between free sulfite and certain food components. Among such components, the formaldehyde generated in shrimp may be very important owing to its ability to interact with proteins and sulfites (Flores and Crawford, 1973; Yoshida and Imaida, 1980).

Apart from the factors mentioned above, the effects of temperature and time on the reaction of sulfite with disulfide bonds are also important. At room temperature, the process is slow and may be favored by heating and using Cu^{2+} as a catalyst and protein denaturants (González and Damodaran, 1990b; Gunnison and Benton, 1971; Kella and Kinsella, 1985; Thanhauser *et al.*, 1984). Regarding the effect of temperature, new sulfonates would not be expected to appear under storage conditions of −18 °C. Despite this, in stored frozen fish cleavages and rearrangements of disulfide bonds and also noncovalent bonds are produced; these affect the stability of *S*-sulfonates. According to Bhobe and Pai (1986), in nonsulfited frozen shrimp kept at −18 °C the content in reactive sulfhydryl groups increases with storage time (Table 7).

The content of sulfonates that might be found if the sulfite were to interact with the disulfide susceptible to reduction would be 37 μg/g at the start, values of 550 μg/g being reached at 6 months of storage. Accepting this approach and the fact that free sulfite did exist in our samples, it is possible that new sulfonates could be formed during storage. In all of the shrimp, the determination of *S*-sulfonates was performed between 2 and 6 months after the freezing date stated by the manufacturer, except in batches A and G, the storage times for which were 15 and 10 months, respectively, and it was precisely these batches that did not have the highest amounts of sulfonates. If storage temperatures are not suitable, above −18 °C, protein denaturation would be accelerated and this could be related to the relatively high sulfonate concentrations found in samples I−K.

Thus, the greater or smaller content of sulfonates in the samples studied here should not be attributed to a single factor but are rather the result of the interaction among several factors: sulfiting conditions, time elapsed since capture until freezing, period and temperature of storage. Since the samples studied here were from commercial sources, of all these factors only the storage time was known.

The percentages of SO_2 in the form of *S*-sulfonates with respect to total sulfite, including that irreversibly bound, are 9 and 28%, excluding batches I−K in which this proportion was considerably increased (Table 6).

Table 6. Contents in *S*-Sulfonates in the Edible Parts of Different Samples ($n = 3$) of the Same Batch of Frozen Shrimps

		batch										
		A	B	C	D	E	F	G	H	I	J	K
total sulfite[a] (SO_2, μg/g)	$\bar{x}$	315	384	142	139	333	399	301	457	152	232	217
	SD	28.7	45.6	23.7	32.0	22.9	83.9	65.5	80.7	30.5	9.7	44.9
	CV, %	9.1	11.9	16.7	23.0	6.9	21.0	21.8	17.7	20.0	4.2	20.7
S-sulfonates (SO_2, μg/g)	$\bar{x}$	30	41	45	54	62	64	74	92	120	155	175
	SD	12.9	13.6	5.3	14.8	4.4	18.5	6.9	7.2	23.9	18.6	18.5
	CV, %	43.3	33.2	11.8	27.4	7.1	28.9	9.3	7.8	19.9	12.0	10.6
S-sulfonates (%)	$\bar{x}$	8.6	9.6	24.3	27.9	17.6	13.7	20.0	17.1	44.1	40.0	45.0
	SD	3.6	2.1	3.4	4.8	3.4	2.3	2.3	2.6	2.0	3.6	3.5

[a] Free + reversibly bound.

Table 7. Theoretical Formation of *S*-Sulfonates during Storage of Frozen Shrimp

days of storage[a] (−18 °C)	reactive SH[a] (mg/100 g)	*S*-sulfonates (μg of SO_2/g)
0	3.8	37
30	11.0	107
60	20.9	203
120	34.0	330
150	47.6	462
180	56.7	550

[a] Results taken from Bhobe and Pai (1986).

These concentrations are higher than those reported in the literature for meat products, and this could be related to stronger alterations in protein fractions in the case of shrimp. The formation of sulfonates in these samples cannot be attributed to the catalyzing effect of the Cu^{2+} present in hemocyanin since all of them, with the exception of batch C, had been treated with EDTA.

From the results obtained in the present work, it may be concluded that there is no correlation between the protein-bound fraction of sulfite and total sulfite and the importance of some factors linked to quality maintenance of the product. Knowledge of the influence of such factors could allow control of sulfonate formation with a view to determining the active fraction of the additive and fixing the amount to be added to achieve suitable residual levels. This kind of information would also facilitate better evaluation of the health risks involved in exposure to sulfiting agents.

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