Agricultural Research Department
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The determination of sulphite in tropical shrimps. A WEFTA intercomparison exercise

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REPORT 94.003
April 1994

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RIVO Report 94.003

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Price: f 29,40 (incl. V.A.T.)

Cover photo: Flying Focus - Castricum

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Summary

An intercomparison exercise among the WEFTA laboratories on the sulphite determination in tropical shrimps has been carried out. Samples of tropical shrimps were spiked with sodiummetabisulphite ($Na_2S_2O_5$) and hydroxymethylsulfonate (HMS) at a level of 25-90 mg SO_2/kg. Most of the laboratories have determined the sulphite content with (modified) methods of Monier-Williams or De Vries et al. The overall mean recovery of sulphite was rather low (47-60%), which may be attributed to an irreversible reaction of sulphite with the tropical shrimps. The repeatability of the methods was good.
It has been shown that the sulphite content in tropical shrimps spiked with $Na_2S_2O_5$ decreased during storage at -20°C and at +4°C. Hydroxymethylsulfonate was stable during storage.

1. Introduction

Sulphites in various forms have been added to foods as preservative agents and for other purposes for centuries. Their use became an issue of concern when certain sensitive individuals exhibited adverse reactions to sulphite residues in foods. Analytical methods were developed to monitor these compounds at the regulatory limits.
In the meeting of the WEFTA working group "Analytical Methods" in 1990 it was decided to collaborate with regard to the measurement of sulphite in fishery products and in particular in tropical shrimps.
Fazio and Warner have reviewed analytical methods for determining sulphites in foods (1). In the WEFTA working group meeting in 1991 Vyncke (2) has presented a review of the methodology for the determination of sulphite in shrimps and the methods used by the different WEFTA laboratories.

In the WEFTA working group meetings in 1992 and 1993 the Netherlands Institute for Fisheries Research (RIVO-DLO), was asked to coordinate two intercomparison exercises (3,4) among the WEFTA laboratories with appropriate samples of tropical shrimps spiked with sodiummetabisulphite ($Na_2S_2O_5$) and with hydroxymethyl-sulphonate (HMS).

In addition RIVO-DLO has carried out experiments regarding the stability of $Na_2S_2O_5$ and HMS in relation to the storage temperature of tropical shrimps and the presence of TMAO.
In this report the final results are presented integrally.

2. Materials and methods

2.1. Intercomparison exercises

In some prelimenary experiments at RIVO-DLO it was shown that it was not possible to produce appropriate samples of canned tropical shrimps spiked with sulphite. Therefore it was decided to produce **frozen** samples of tropical shrimps spiked with $Na_2S_2O_5$ for the first exercise and frozen tropical shrimps spiked with HMS for the second exercise. HMS is a bisulphite addition product of formaldehyde which is stucturally similar to some combined forms of sulphite in foods.

Thirteen kg of peeled tropical shrimps were ground in a meat grinder. After thorough homogenisation 4 kg were transferred to a chilled cutter. After addition of 600 ml of an aqueous standard $Na_2S_2O_5$ solution (0.167 mg SO_2/ml or 0.590 mg SO_2/ml) or an aqueous standard of HMS (0.185 mg SO_2/ml or 0.447 mg SO_2/ml) the samples were homogenised and portioned into plastic containers of approximately 125 g. After deepfreezing the unspiked and spiked samples at two levels were packed in carbondioxide ice and distributed by courier to the participants.
The samples for the first exercise with $Na_2S_2O_5$ were prepared on November 12th, 1992 and distributed to the participants on November 16th, 1992. Preparation of the samples for the second exercise with HMS was carried on June 29th, 1993. These samples were sent to the participants on July 7th, 1993.

The participants were:

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SFI and IFL have participated only in the first exercise. Each participant was asked to analyse each sample of the tropical shrimps with $Na_2S_2O_5$ four times in two independent runs (set A and B). In the second exercise participants were asked to analyse each sample four times in only one run.

The details of the methods of Monier-Williams and De Vries et al (5) used by most of the participants, based on the provided standard operating procedures, are summarized in table 1.

The general principle of the Monier-Williams method involves reflux distillation of a sample with hydrochloric acid or phosphoric acid to convert sulphite into sulphur dioxide. A stream of nitrogen sweeps the sulphur dioxide through a water-cooled condensor, into a hydrogen peroxide solution. Sulphur dioxide is then oxidized to sulphuric acid which is titrated.

The method of de Vries et al involves a rapid distillation with steam of a sample with hydrochloric acid or phosphoric acid. The liberated sulphur dioxide is distilled into a solution of potassium iodide and iodine ((in)direct iodometric titration).
IFL obtained erroneous results with this method and used an enzymatic method of Boehringer instead.

In the second exercise FRCF has used their Monier-Williams-HPLC method in a somewhat different modification from the first exercise. In the second exercise the test portion was not further blended with ethanol while the volume of water was increased to 200 ml.

RIVO-DLO has applied a modified method of de Vries et al in the second exercise because the repeatability of some prelimenary results with the Monier-Williams method were unacceptable. Furthermore the short time of distillation of the method of de Vries et al is considered to be an advantage.
The analyses for the first exercise were carried out by the laboratories in the period from the end of November 1992 to January 1993. Therefore the period between sample preparation and analyses varied from two to eight weeks. In the second exercise samples were analysed in the period from the end of July 1993 to September 1993.

The Monier-Williams method, applied by the various participants, showed some variation in details. The main differences were:

1) Weight of the sample (10-100 g)
2) Dilution with water (1:1 - 1:10)
3) Use of alcohol (methanol or ethanol)
4) Type of acid (HCl versus H_3PO_4)
5) Quantitation (acidimetric or iodometric titration, HPLC (UV) of the derivative hydroxymethylsulphonate)
6) Concentration and volume H_2O_2 (3% - 7%, 15 - 25 ml)
7) Distillation time (5 - 105 min.)

2.2. Stability of $Na_2S_2O_5$ and HMS in tropical shrimps during storage

Six kg of peeled tropical shrimps were ground in a meat grinder. After thorough homogenisation 3 kg were transferred to a chilled cutter. After addition of 150 ml of an aqueous standard $Na_2S_2O_5$ solution (3.04 mg $Na_2S_2O_5$/ml) or an aqueous standard of HMS (4.33 mg HMS/ml) the samples were homogenised and portioned (approximately 60-80 g).
The spiked content of $Na_2S_2O_5$ in shrimps was 97.5 mg SO_2/kg and of HMS 98.4 mg SO_2/kg. The samples were stored for a period up to 68 days at -20°C. At regular time intervals an appropriate number of samples were thawed in running tapwater. The sulphite content in the samples was determined at RIVO-DLO with the modified method of de Vries et al (table 1) directly after thawing and after of one and two days storage at +4°C.

2.3. Stability of HMS in relation to the presence of TMAO

An appropriate volume of a standard solution of TMAO (18 mg TMAO-N/ml) was added to 10 ml of an aqueous standard solution of HMS (0.328 mg SO_2/ml) or 30 g of tropical shrimps (97.5 mg SO_2/kg). The sulphite content in this mixture was determined at RIVO-DLO by the modified method of de Vries et al (table 1).

3. Results and discussion

3.1. First intercomparison exercise with $Na_2S_2O_5$

In the paragraphs 3.1.1. and 3.1.2 all the results of the first intercomparison exercise of the determination of sulphite added to tropical shrimps as $Na_2S_2O_5$ are discussed.

3.1.1. Unspiked tropical shrimps

The sulphite content in the samples of unspiked tropical shrimps from the first exercise (table 2) measured by six of the participants was below the detection limit (< 1-4 mg SO_2/kg).
IPIMAR reported that the poor reproducibility and relatively high sulphite content (approximately 10 mg SO_2/kg) in the unspiked samples may be regarded as a consequence of the inaccuracy of the method with samples containing low levels of sulphite.
IFL reported that the samples were refrozen two times before analysing which might have had an unknown effect on the sulphite content.

3.1.2. Spiked samples

The results of the sulphite content in the two samples of tropical shrimps spiked with $Na_2S_2O_5$ are shown in tables 3 and 4 and figures 1 and 2.

3.1.2.1. Spiked level-1 of $Na_2S_2O_5$

The sulphite content in the tropical shrimps spiked with $Na_2S_2O_5$ at a level of 25 mg SO_2/kg, measured by IPIMAR was 12 mg SO_2/kg which is almost equal to the sulphite content in the unspiked tropical shrimp sample (10 mg SO_2/kg) measured by IPIMAR. Correction for the sulphite content, by substraction these two values of the same magnitude, will result into an inaccurate very low recovery of the spiked sample. Therefore the results of IPIMAR are not taken into consideration for the overall recovery determination.

The results of the sulphite content in the unspiked tropical shrimp sample of IFL (8 mg SO_2/kg) was about 40% of the value for the spiked tropical shrimp sample. Correction for the sulphite content in the unspiked sample decreased the recovery of sulphite in the spiked tropical shrimp sample considerably from 79% to 48%.

The overall mean recovery of the sulphite content in the tropical shrimp sample, spiked with $Na_2S_2O_5$ at a level of 25 mg SO_2/kg was 51%. This overall mean recovery is based on all the results of all participants (except the results of IPIMAR). The results of IFL were corrected for the sulphite content of unspiked tropical shrimp sample.

A low recovery was measured by TRS (25%) while RIVO-DLO measured a high recovery (85%).
From the results of the two independent sets (A and B) of analyses it is concluded that the repeatability of the sulphite determination is good for most of the laboratories. The results of IPIMAR seemed to be less repeatable between runs. The coefficient of variation (CV) within sample sets was less than 10% for FRCF, IFREMER, SFI, RSZ and IFL.

3.1.2.2. Spiked level-2 of $Na_2S_2O_5$

The relative contribution of the sulphite content of the unspiked tropical shrimp sample to the result of the sulphite content in a sample spiked with $Na_2S_2O_5$ at a level of 88.5 mg SO_2/kg was 15% for IPIMAR and IFL.

The overall mean recovery for the sulphite in tropical shrimps spiked with $Na_2S_2O_5$ at a level of 88.5 mg SO_2/kg, based on all results and corrected for the sulphite content in the unspiked tropical shrimp sample, was 62% which is somewhat higher than at the lower level of spiking. The recovery ranged from approximately 50% (TRS and IFL) to 80% (RIVO-DLO).

The repeatability between the two independent sets of analyses and the coefficient of variation within each laboratory was much better than at the spiked level-1. The coefficient of variation within sample sets for all laboratories is less than 5% at this spiked level of sulphite.

3.2. Second intercomparison exercise with HMS

In the paragraphs 3.2.1 and 3.2.2. all the results of the second intercomparison exercise of the determination of sulphite added to tropical shrimps as HMS are discussed.

3.2.1. Unspiked tropical shrimps

The sulphite content in the samples of unspiked tropical shrimps from the second exercise (table 5) measured by all the participants was below the detection limit (< 1-3 mg SO_2/kg).

3.2.2. Spiked samples

The results of the sulphite content in the two samples of tropical shrimps spiked with HMS are shown in tables 6 and 7 and figures 3 and 4.

3.2.2.1. Spiked level-1 of HMS

The overall mean recovery of the sulphite content in the tropical shrimp sample, spiked with HMS at a level of 25 mg SO_2/kg was 47%. This recovery of sulphite spiked as HMS is somewhat lower than the recovery of sulphite (51%) spiked as $Na_2S_2O_5$ in the first exercise. These results do not confirm the observations of Hillery et al (6). They showed an increase in the recovery of HMS (80%) in comparison with $Na_2S_2O_5$ (69%) when added at a level of 10 mg SO_2/kg.

As in the first exercise TRS has measured a low recovery for sulphite (25% for $Na_2S_2O_5$ respectively 20% for HMS).

3.2.2.2. Spiked level-2 of HMS

The overall mean recovery for sulphite in this exercise with HMS was 54% which is higher than at the lower level of spiking with HMS of 25 mg SO_2/kg. Also this recovery of sulphite as HMS is somewhat lower than the recovery in the first exercise with $Na_2S_2O_5$ (62%).

3.3. General remarks

The recoveries of sulphite from tropical shrimps spiked with different forms of sulphite, determined by FRCF, IFREMER, TRS, SFI, IPIMAR, RSZ and RIVO-DLO (second exercise) were significantly lower than the recoveries mentioned in their standard operating procedures (table 1). For IFL no recoveries were given in their standard operating procedure.
The higher recoveries, mentioned in the standard operating procedures of the WEFTA laboratories, are based on the determination of sulphite immediately after spiking. This difference in recovery can probably be explained by the fact that the samples in this exercise were analysed after a few weeks of the preparation and storage at -25°C. This may probably induce an increased loss in sulphite due to an irreversible reaction of sulphite with aldehydes/carbonyls in tropical shrimps during storage.

A reduction of 50% has been reported for sulphite added to seafood as $Na_2S_2O_5$ at a spiking level of 10-20 mg SO_2/kg (5). In this exercise the spiking levels were at the same (25 mg SO_2/kg) or considerably higher (58.3 and 88.5 mg SO_2/kg) level.
It seems that spiking with HMS does not improve the recovery of sulphite as suggested by Hillery et al (5).

3.4. Stability of $Na_2S_2O_5$ and HMS in tropical shrimps during storage

In table 8 and figure 5 the sulphite in the spiked tropical shrimps is given in relation to the storage conditions. Analysis of variance on the the sulphite content in tropical shrimps with the storage time at -20°C as well with the storage time at +4°C after thawing has been carried out.
The results of the analysis of variance showed that there is a significant decrease of the sulphite content in tropical shrimps, if $Na_2S_2O_5$ is applied, from approximately 70 mg SO_2/kg to 60 mg SO_2/kg during storage at -20°C ($P<0.001$) or after thawing and storage at +4°C ($P<0.01$). The results showed also that sulphite in tropical shrimps, spiked as HMS, was stable during storage at -20°C and after thawing and storage at +4°C.

3.5. Stability of HMS in relation to the presence of TMAO

The sulphite content measured in the samples with increasing amounts of TMAO is presented in figure 6.
The results show that the sulphite content in aqueous solutions of HMS was independend of the presence of TMAO. However, as suggested (7), in tropical shrimps the measured sulphite content decreased with an increasing content of TMAO. However, this reduction occurred at rather high concentrations of TMAO. Therefore it is expected that at natural TMAO content in the tropical shrimps (approximately 50 mg TMAO-N/kg) this reduction will be rather low.

4. Conclusions

From the results of both WEFTA intercomparison exercises on the determination of sulphite spiked to tropical shrimps at a level of 25-90 mg SO_2/kg it is concluded that the overall mean recovery of the sulphite is rather low (47-62%).
At the higher levels of spiked sulphite (60-90 mg SO_2/kg) the recovery and repeatability of the methods used, mainly Monier-Williams and de Vries et al, are better.
The content of sulphite in tropical sgrimps spiked as $Na_2S_2O_5$ decreases during storage at temperature from -20°C to +4°C while HMS is stable under these conditions. A high content TMAO may reduce the content of sulphite in tropical shrimps.

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Table 1. Overview analytical procedures for determination sulphite (continued)

	RIVO-DLO		FRCF
	First exercise	Second exercise	First exercise
Method	Monier-Williams	De Vries et al	Monier-Williams (modified)
Applied to	Cockles, shrimps	Shrimps	Shrimps
Weight of sample	30 g	30 g	20 g
Volume of water	280 ml	30 ml	50 ml
Volume of alcohol	-	-	100 ml 5% ethanol
Type of acid	HCl	HCl	HCl
Conc. of acid	25%	25%	4 N
Volume of acid	25 ml	25 ml	45 ml
Inert gas	Nitrogen	No gas (steam)	Nitrogen
Conc. H_2O_2	7%	-	-
Volume of H_2O_2	25 ml	-	-
Distillation time	90 min.	10-15 min	105 min.
Quantification	Acidimetric titration	Iodometric titration	HPLC (UV) of the derivative HMS
Indicator	Bromophenol blue	Starch	-
Titrant	NaOH 0.05 M	Thiosulfate 0.02 M	-
Level added for recovery	20-100 mg SO_2/kg	100 mg SO_2/kg	30-200 mg SO_2/kg
Recovery from water	70-90%	65-70%	90-95%
Recovery from samples	70-90%	75%	60-85%
Detection limit*	4 mg SO_2/kg	2 mg SO_2/kg	2 mg SO_2/kg
Blank values	1-3 mg SO_2/kg	1 mg SO_2/kg	-
* = 3 x standard deviation of blank			

Table 1. Overview analytical procedures for determination sulphite (continued)

	FRCF	IFREMER	TRS
	Second exercise	First and second exercise	First and second exercise
Method	Monier-Williams (modified)	Monier-Williams	Monier-Williams Tanner
Applied to	Shrimps	Cod, crustaceans	Crab, nephrops
Weight of sample	20 g	30 g (cod), 100 g (lobster, prawns)	50 g
Volume of water	200 ml	100 ml	60 ml
Volume of alcohol	-	-	100 ml methanol
Type of acid	HCl	HCl	H_3PO_4
Conc. of acid	4N	10%	88%
Volume of acid	45 ml	10 ml	30 ml
Inert gas	Nitrogen	Carbondioxide	Nitrogen
Conc. H_2O_2	-	3%	3%
Volume of H_2O_2	-	20 ml	20 ml
Distillation time	105 min.	90 min.	30 min.
Quantification	HPLC (UV) of the derivative HMS	Acidimetric titration	Acidimetric titration
Indicator	-	Bromophenol blue	Methyl red
Titrant	-	NaOH 0.01 M or NaOH 0.1 M	NaOH 0.05 M
Level added for recovery	30-200 mg SO_2/kg	30 mg SO_2/kg	90-900 mg SO_2/kg
Recovery from water	90-95%	96%	97%
Recovery from samples	60-85%	91%	52-83%
Detection limit *	2 mg SO_2/kg	1 mg SO_2/kg	1 mg SO_2/kg
Blank values	-	3 mg SO_2/kg	> 1 mg SO_2/kg
*= 3 x standard deviation of blank			

Table 1. Overview analytical procedures for determination sulphite

	SFI	IPIMAR	RSZ
	First exercise	fFrst and second exercise	First and second exercise
Method	De Vries et al	Monier-Williams	De Vries et al. Tecator-Kjeltec
Applied to	Crustaceans	Crustaceans	Shrimps
Weight of sample	10 g	50 g	15 g
Volume of water	50 ml	60 ml	40 ml
Volume of alcohol	-	100 ml methanol	-
Type of acid	HCl	H_3PO_4	H_3PO_4
Conc. of acid	33%	85%	60%
Volume of acid	30 ml	30 ml	25 ml
Inert gas	No gas (steam)	Nitrogen	No gas (steam)
Conc. H_2O_2	-	3%	-
Volume of H_2O_2	-	15 ml	-
Distillation time	5 min.	30 min.	5 min.-
Quantification	Iodometric titration	Acidimetric titration	Iodometric titration
Indicator	Starch	Methyl red	Starch
Titrant	Thiosulfate 0.05M	NaOH 0.05 M	I_2 0.02 N
Level added for recovery	65-675 mg SO_2/kg	150 mg SO_2/kg	3-200 mg SO_2/kg
Recovery from water	67-78%	88-90%	94%
Recovery from samples	71%	85%**	95-102%
Detection limit *	2 mg SO_2/kg	3 mg SO_2/kg	3 mg SO_2/kg
Blank values	2 mg SO_2/kg	-	<3 mg SO_2/kg
*= 3 x standard deviation of blank			
**= average with a large not specified variation			

Table 2. Sulphite content in unspiked tropical shrimps (first exercise).

		Sulphite (mg SO_2/kg)					
Laboratory	Sample set	anal 1	anal 2	anal 3	anal 4	mean	Standard deviation
RIVO-DLO	A	<4	<4	<4	<4	<4	-
	B	<4	<4	<4	4,3	<4	-
FRCF	A	<2	<2	<2	<2	<2	-
	B	<2	<2	<2	<2	<2	-
IFREMER	A	2,0	<2	<2	<2	-	-
	B	3,0	<2	<2	2,6	-	-
TRS	A	<1	<1	<1	<1	<1	-
	B	<1	<1	<1	<1	<1	-
SFI	A	<2	<2	<2	<2	<2	-
	B	<2	<2	<2	<2	<2	-
IPIMAR	A	7,5	7,5	5,9	7,5	7,1	0,8
	B	13,3	12,3	13,3	14,4	13,3	0,9
RSZ	A	<3	<3	<3	<3	<3	-
	B	<3	<3	<3	<3	<3	-
IFL	A	7,5	7,5	7,5	7,9	7,6	0,2
	B	8,3	8,3	8,0	8,3	8,2	0,2

Table 3. Sulphite content in spiked ($Na_2S_2O_5$) tropical shrimps (first exercise).*

		Spiked level (25.0 mg SO_2/kg)							
Laboratory	Sample set	anal 1	anal 2	anal 3	anal 4	mean	CV (%)	Rec. (%)	Recovery mean (%)
		mg SO_2/kg							
RIVO-DLO	A	22,6	20,3	16,9	19,8	19,9	11,9	80	85
	B	22,8	24,1	23,3	19,4	22,4	9,2	90	
FRCF	A	11,7	10,8	11,0	11,6	11,3	3,9	45	42
	B	9,3	9,3	10,4	9,1	9,5	6,2	38	
IFREMER	A	13,9	13,6	13,3	13,6	13,6	1,8	54	56
	B	14,5	14,5	12,7	15,4	14,3	7,8	57	
TRS	A	8,3	9,5	5,3	6,8	7,5	24,3	30	25
	B	5,8	4,6	4,7	4,7	4,9	11,2	20	
SFI	A	13,4	14,1	14,9	15,4	14,5	5,9	58	53
	B	11,8	12,2	11,9	12,2	12,0	1,3	48	
IPIMAR**	A	7,5	10,7	10,7	10,7	9,9	16,2	-	
	B	13,9	13,9	16,0	15,5	14,8	7,3	-	
RSZ	A	11,7	12,6	11,7	12,1	12,0	3,6	48	49
	B	12,6	11,9	13,0	12,6	12,5	3,7	50	
IFL	A	18,3	18,3	17,7	17,7	18,0	1,9	72	79 (48***)
	B	21,7	21,1	22,3	21,1	21,6	2,7	86	
								Overall mean recovery*	55
								Overall mean recovery ***	51

* not corrected for sulphite content in unspiked tropical shrimps
** excluded due to high sulphite content in unspiked tropical shrimps
*** corrected for sulphite content in unspiked tropical shrimps

Table 4. Sulphite content in spiked ($Na_2S_2O_5$) tropical shrimps (first exercise).*

		Spiked level 2 (88.5 mg SO_2/kg)							
Laboratory	Sample set	anal 1	anal 2	anal 3	anal 4	mean	CV (%)	Rec. (%)	Recovery mean (%)
		mg SO_2/kg							
RIVO-DLO	A	70,2	64,5	71,5	69,8	69,0	4,5	78	80
	B	72,5	71,7	72,3	75,1	72,9	2,1	82	
FRCF	A	55,6	55,9	58,1	56,4	56,5	2,0	64	63
	B	56,0	56,1	53,8	55,6	55,4	1,9	63	
IFREMER	A	55,6	53,2	60,2	60,5	57,4	6,2	65	65
	B	55,6	60,3	56,7	59,1	57,9	3,7	65	
TRS	A	43,6	44,7	42,5	44,6	43,9	2,3	50	51
	B	44,1	48,4	47,8	43,8	46,0	5,2	52	
SFI	A	52,2	54,1	55,0	54,4	53,9	2,3	61	64
	B	59,5	58,9	57,6	61,4	59,4	2,7	67	
IPIMAR	A	60,8	64,0	64,0	64,0	63,2	2,5	71	74 (62**)
	B	64,5	70,9	69,8	66,1	67,8	4,5	77	
RSZ	A	56,3	55,4	53,7	51,9	54,3	3,6	61	60
	B	51,9	53,7	51,9	50,6	52,0	2,4	59	
IFL	A	49,8	49,2	49,8	50,6	49,9	1,2	56	61 (52**)
	B	58,2	57,5	58,2	57,5	57,9	0,7	65	
							Overall mean recovery*		65
							Overall mean recovery **		62

* not corrected for sulphite content in unspiked tropical shrimps

** corrected for the sulphite content in unspiked samples

Table 5. Sulphite content in unspiked tropical shrimps (second exercise).

Laboratory	Sulphite (mg SO_2/kg)				
	anal 1	anal 2	anal 3	anal 4	mean
RIVO-DLO	<2	<2	<2	<2	<2
FRCF	<2	<2	<2	<2	<2
IFREMER	<2	<2	<2	<2	<2
TRS	<1	<1	<1	<1	<1
IPIMAR	<3	<3	<3	<3	<3
RSZ	<3	<3	<3	<3	<3

Table 6. Sulphite content in spiked (HMS) tropical shrimps (second exercise).

Laboratory	Spiked level 1 (24,1 mg SO_2/kg)						
	anal 1	anal 2	anal 3	anal 4	mean	CV (%)	Recovery mean (%)
	mg SO_2/kg						
RIVO-DLO	17,2	17,8	15,9	16,2	16,8	5,3	70
FRCF	14,6	14,6	15,3	15,2	14,9	2,5	62
IFREMER	12,4	13,3	11,6	12,0	12,3	6,2	51
TRS	5,0	4,1	5,7	4,5	4,8	14,0	20
IPIMAR	6,4	6,6	6,0	6,4	6,3	4,2	26
RSZ	13,1	13,5	13,1	12,7	13,1	2,5	54
Overall mean recovery							47

Table 7. Sulphite content in spiked (HMS) tropical shrimps (second exercise).

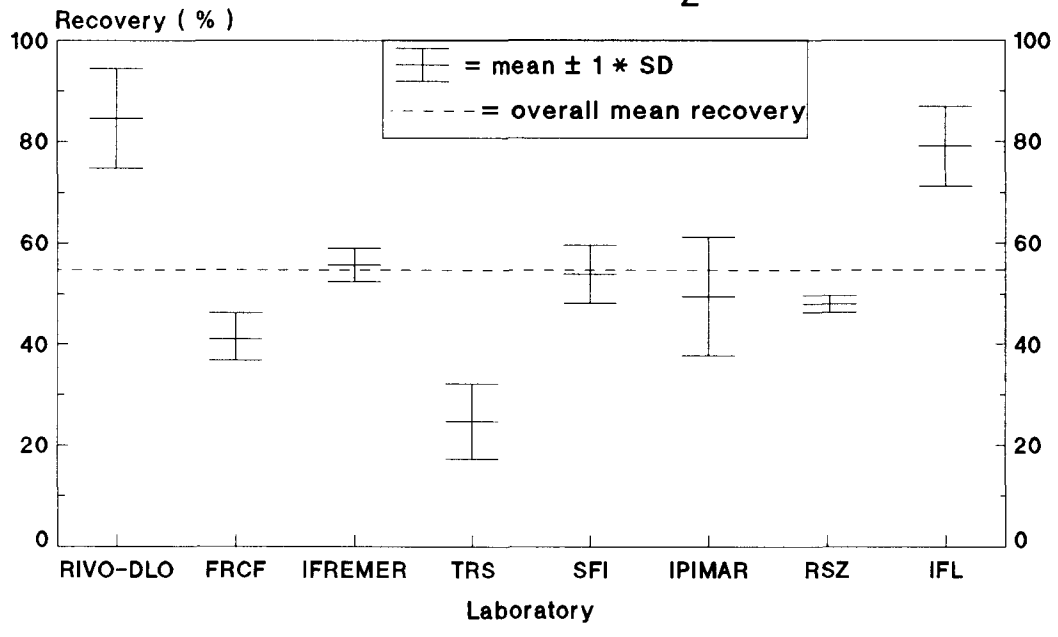
Laboratory	Spiked level 2 (58,3 mg SO_2/kg)						
	anal 1	anal 2	anal 3	anal 4	mean	CV (%)	Recovery mean (%)
	mg SO_2/kg						
RIVO-DLO	36,4	33,4	36,4	35,9	35,5	4,0	61
FRCF	43,3	44,1	42,4	43,2	43,3	1,6	74
IFREMER	35,6	35,6	35,6	37,3	36,0	2,5	62
TRS	18,6	18,4	18,6	19,3	18,7	2,2	32
IPIMAR	25,0	24,8	24,1	24,5	24,6	1,5	42
RSZ	31,3	31,7	30,4	31,7	31,3	2,0	54
Overall mean recovery							54

Table 8. Sulphite content in spiked tropical shrimps during storage at -20°C and +4°C.

Storage period at -20°C (days)	Recovery HMS (%)			
	Storage period at +4°C after thawing (days)			mean
	0	1	2	
0	68,6	71,4	74,3	71,4
1	76,7	73,5	67,3	72,5
2	70,7	66,3	72,6	69,9
3	68,2	61,4	65,7	65,1
7	71,7	68,2	65,1	68,3
14	73,6	69,3	66,7	69,9
21	70,3	66,8	65,9	67,7
68	72,3	69,3	67,5	69,7
mean	71,5	68,3	68,1	69,3
Storage period at -20°C (days)	Recovery $Na_2S_2O_5$ (%)			
	Storage period at + 4°C after thawing (days)			mean
	0	1	2	
0	78,1	69,2	70,3	72,5
1	73,3	70,1	56,2	66,5
2	73,9	61,7	68,9	68,2
3	59,4	64,6	55,4	59,8
7	70,1	60,0	61,4	63,8
14	69,4	58,0	61,9	63,1
21	58,7	55,9	52,5	55,7
68	52,8	50,1	50,7	51,2
mean	67,0	61,2	59,7	62,6

Figure 1
Recovery of sulphite ($Na_2S_2O_5$) from tropical shrimps*

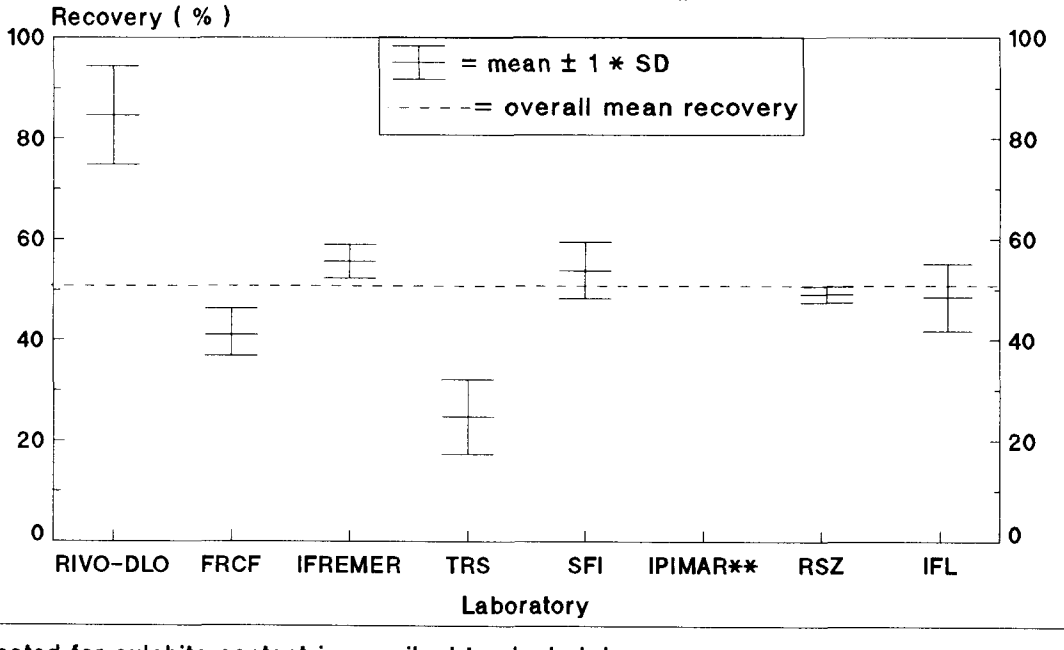
(level 1 : 25.0 mg SO_2/kg)



* not corrected for sulphite content in unspiked tropical shrimps

Recovery of sulphite ($Na_2S_2O_5$) from tropical shrimps*

(level 1 : 25.0 mg SO_2/kg)



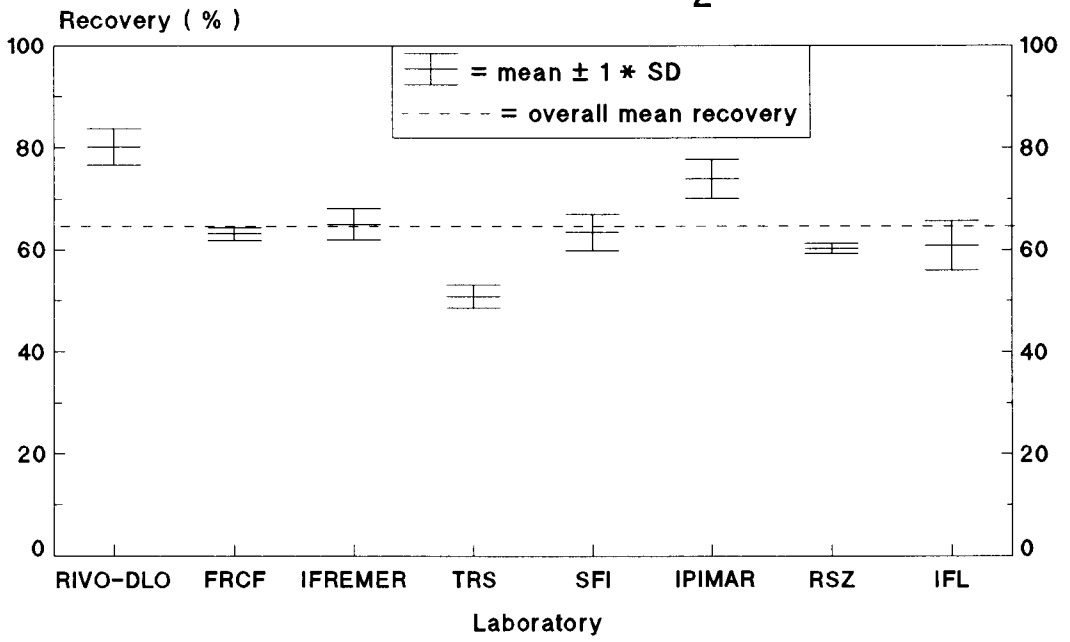
* corrected for sulphite content in unspiked tropical shrimps

** results excluded due to high sulphite content in unspiked tropical shrimps

Figure 2

Recovery of sulphite ($Na_2S_2O_5$) from tropical shrimps*

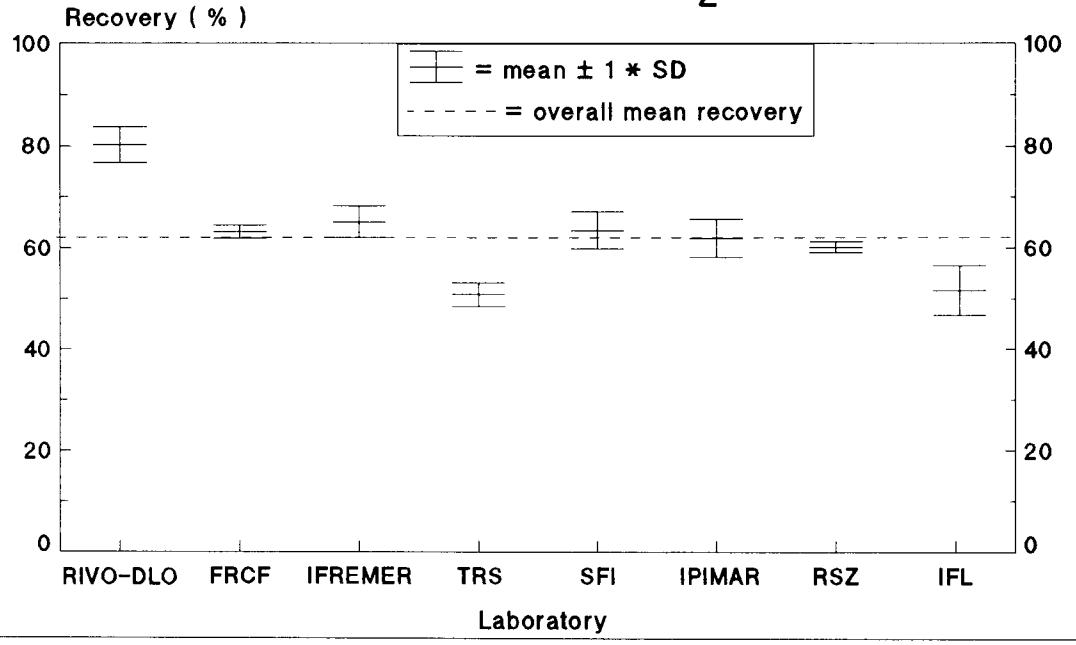
(level 2 : 88.5 mg SO_2/kg)



* not corrected for sulphite content in unspiked tropical shrimps

Recovery of sulphite ($Na_2S_2O_5$) from tropical shrimps*

(level 2 : 88.5 mg SO_2/kg)



* corrected for sulphite content in unspiked tropical shrimps

Figure 3

Recovery of sulphite (HMS) from tropical shrimps (level 1: 24.1 mg SO_2/kg)

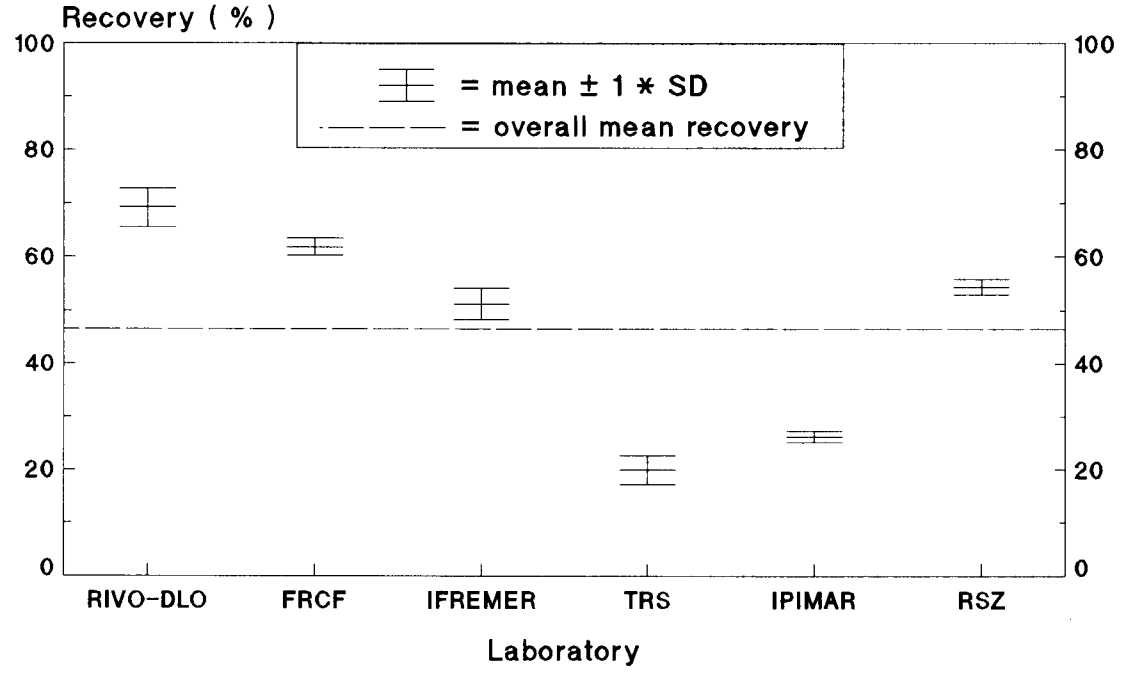


Figure 4

Recovery of sulphite (HMS) from tropical shrimps (level 2: 58.3 mg SO_2/kg)

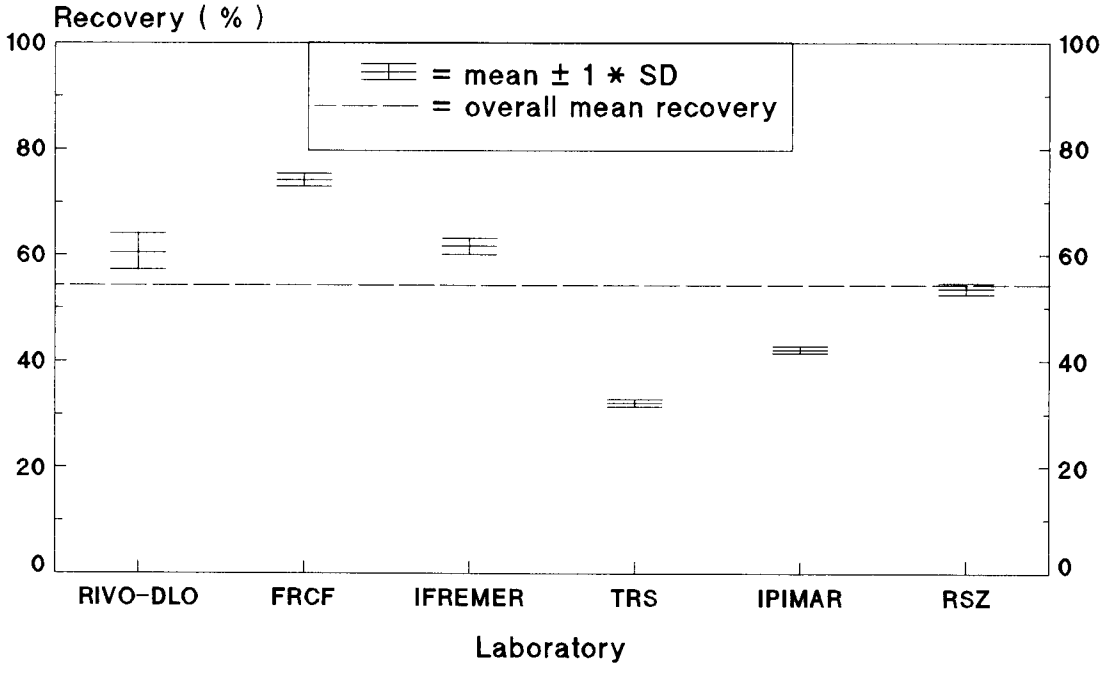


Figure 5. Recovery of sulphite from HMS and $Na_2S_2O_5$ in tropical shrimps during storage at -20°C.

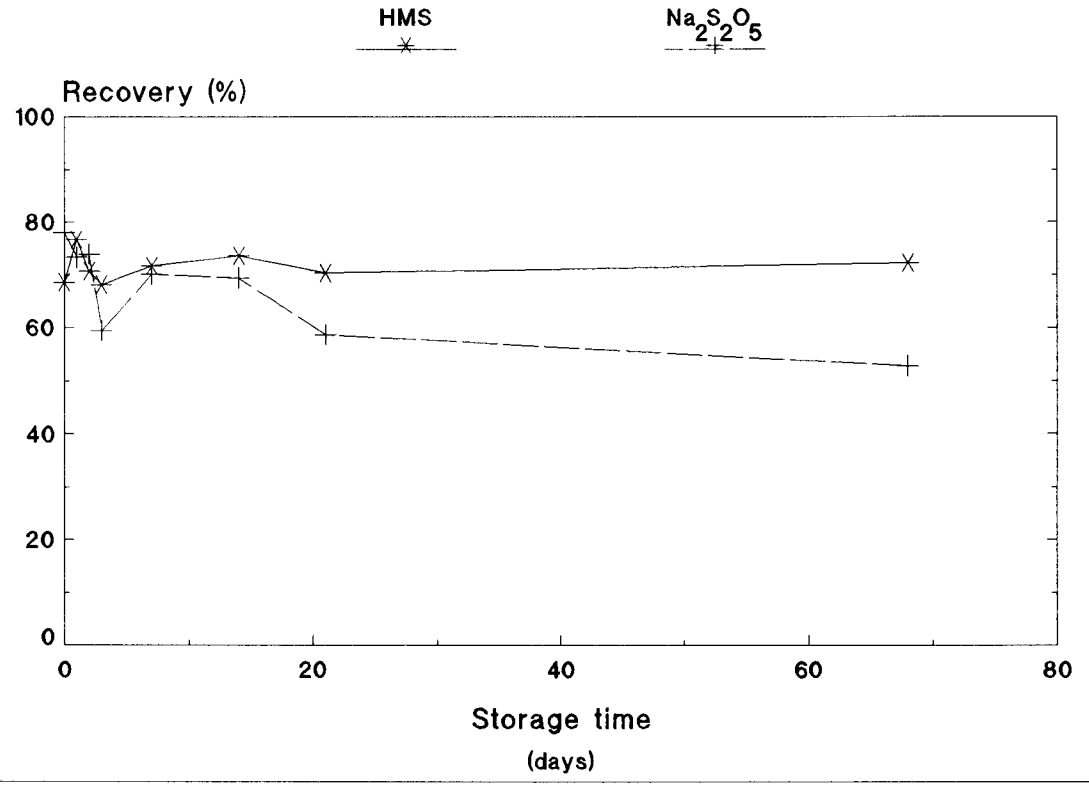


Figure 6. Recovery of sulphite from HMS in relation to the presence of TMAO