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intercomparison exercise on the determination of sulphite in tropical shrimps

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Thet first WEFTA intercomparison exercise on the determination of sulphite in tropical shrimps

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Summary

A first 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 at a level of 25-90 mg SO_2/kg.

Most of the laboratories have determined the sulphite content with the (modified) Monier-Williams method. The overall mean recovery of sulphite was rather low (50-60%), which may be attributed to an irreversible reaction of sulphite in the tropical shrimps. The repeatability of the methods was good.

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 recently 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 meeting in 1992 it was decided that the TNO Department of Fishery Products, integrated since 1993 in the Netherlands Institute for Fisheries Research (RIVO-DLO), should prepare appropriate samples of tropical shrimps spiked with sulphite for an intercomparison exercise among the WEFTA laboratories. The results of this first exercise are presented in this paper.

2. Materials and methods

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 sodiummeta-bisulphite.
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 sodium metabisulphite solution (0.167 mg SO_2/ml or 0.590 mg SO_2/ml) the samples were homogenised and portioned into plastic containers of approximately 100 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 were prepared on November the 12th, 1992 and distributed to the participants on November the 16th, 1992.

The participants were:

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Each participant was asked to analyse each sample of the tropical shrimps four times in two independent runs (set A and B).
The details of the methods normally used by the participants (except of IFL), based on the provided standard operating procedures, are summarized in table 1.

Almost all laboratories used the (modified) Monier-Williams method for the determination of sulphite. The general principle of the method involves reflux distillation of a sample with hydrochloric acid or phosphoric acid to convert sulphite into sulfur dioxide. A stream of nitrogen sweeps sulphur dioxide through a water-cooled condensor, into hydrogen peroxide solution. Sulphur dioxide is oxidized to sulfuric acid which is titrated.
SFI used a modified Monier-Williams method according to De Vries et al. (3). IFL obtained erroneous results with this method and used an enzymatic method of Boehringer instead.
The analyses 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.

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 hydroxymethylsulfonate)
6) Concentration and volume H_2O_2 (3% - 7%, 15 - 25 ml)
7) Distillation time (5 - 105 min.)

3. Results and discussion

3.1. Unspiked tropical shrimps

The sulphite content in the samples of unspiked tropical shrimps (table 2) measured by six of the participants was below the limit of detection (< 1-4 mg SO_2/kg).

INIP 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.

3.2. Spiked samples

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

3.2.1. Spiked level-1

The sulphite content in the tropical shrimps spiked at a level of 25 mg SO_2/kg, measured by INIP 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 INIP. 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 INIP 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 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 INIP). 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 INIP seemed to be less repeatable between runs.
The coefficient of variation within sample sets was less than 10% for FRCF, IFREMER, SFI, RSZ and IFL.

3.2.2. Spiked level-2

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

The overall mean recovery for the sulphite in tropical shrimps spiked 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.

The recoveries of sulphite from tropical shrimps spiked with sulphite, determined by FRCF, IFREMER, TRS, SFI, INIP and RSZ 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 recoveries for RIVO-DLO were in agreement with the recoveries obtained in earlier experiments.

The higher recoveries, mentioned in the standard operating procedures of the WEFTA laboratories, are based on the determination of sulphite immediately after spiking. The 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 induce a loss in sulphite due to an irreversible reaction of sulphite with aldehydes/carbonyls in tropical shrimps.
A reduction of 50% has been reported for sulphite added to seafood at a spiking level of 10-20 mg SO_2/kg (4). In this exercise the spiking levels were at the same (25 mg SO_2/kg) or considerably higher (88.5 mg SO_2/kg) level. It has been suggested to determine the recovery of sulphite by the addition of hydroxymethylsulfonate (HMS). HMS is a bisulfite addition product of formaldehyde which is stucturally similar to some combined forms of sulphite in foods.
It has also been suggested that TMAO may also react with sulphite (5).

4. Conclusion

From the results of this first WEFTA intercomparison exercise on the determination of sulphite spiked to tropical shrimps at an level of 25-90 mg SO_2/kg it is concluded that the overall mean recovery of the sulphite is rather low (50-60%).

At the higher levels of spiked sulphite (90 mg SO_2/kg) the recovery and repeatability of the methods used, mainly Monier-Williams, are better.

5. Recommendations

It seems to be necessary to evaluate the methods for the determination of sulphite of the WEFTA laboratories (mainly based on the principle of Monier-Williams) critically in order to improve the results.

It is recommended to use HMS in the next intercomparison WEFTA exercise on the determination of sulphite in tropical shrimps. A study about the stability of sulphite added to tropical shrimps during frozen storage is advised in order to evaluate the significance of a sulphite determination in commercial frozen samples with respect to future limits for sulphite in fishery products within the European Community.

6. References

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Food additives and contaminants 7(4), (1990), 433-454

(2) Vyncke, W.
Determination of total sulphite in shrimp: a review of methodology
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(3) De Vries, J.W., H. Ge, F.J. Ebert, J.M. Magnuson and M.K. Ogawa
Analysis for total sulphite in foods by using rapid distillation followed by redox titration
J. Assoc. Off. Anal. Chem. 69(5), (1986), 827-830.

(4) Hillery, B. R., E.R. Elkins, C.R. Warner, D. Daniels and T. Fazio.
Optimized Monier-Williams method for the determination of sulfites in foods: collaborative study
J. Assoc. Off. Anal. Chem. 72(3), (1989), 470-475

(5) Rehbein, H., personal communication at the meeting of the WEFTA working group "Analytical Methods", Madrid, 1993.

Table 1. Overview analytical procedures for determination sulphite

Parameter	RIVO-DLO	FRCF	IFREMER
Method	Monier-Williams	Monier-Williams (modified)	Monier-Williams
Applied to	Cockles, shrimps	Shrimps	Cod, crustaceans
Weight of sample	30 g	20 g	30 g (cod), 100 g (lobster, prawns)
Volume of water	280 ml	50 ml	100 ml
Volume of alcohol	-	100 ml 5% ethanol	-
Type of acid	HCl	HCl	HCl
Conc. of acid	25%	4 N	10%
Volume of acid	25 ml	45 ml	10 ml
Inert gas	Nitrogen	Nitrogen	Carbondioxide
Conc. H2O2	7%	-	3%
Volume of H2O2	25 ml	-	20 ml
Distillation time	90 min.	105 min.	90 min.
Quantification	Acidimetric titration	HPLC (UV) of the derivative HMS	Acidimetric titration
Indicator	Bromophenol blue	-	Bromophenol blue
Titrant	NaOH 0.05 M	-	NaOH 0.01 M or NaOH 0.1 M
Level added for recovery	20-100 mg SO2/kg	30-200 mg SO2/kg	30 mg SO2/kg
Recovery from water	70-90%	90-95%	96%
Recovery from samples	70-90%	60-85%	91%
Detection limit*	4 mg SO2/kg	2 mg SO2/kg	2 mg SO2/kg
Blank values	1-3 mg SO2/kg	-	3 mg SO2/kg

* = 3 x standard deviation of blank

Table 1. Overview analytical procedures for determination sulphite

Parameter	TRS	SFI	INIP
Method	Monier-Williams/ Tanner	Monier-Williams (modified)	Monier-Williams
Applied to	Crab, nephrops	Crustaceans	Crustaceans
Weight of sample	50 g	10 g	50 g
Volume of water	60 ml	50 ml	60 ml
Volume of alcohol	100 ml methanol	-	100 ml methanol
Type of acid	H3PO4	HCl	H3PO4
Conc. of acid	88%	33%	85%
Volume of acid	30 ml	30 ml	30 ml
Inert gas	Nitrogen	No gas	Nitrogen
Conc. H2O2	3%	-	3%
Volume of H2O2	20 ml	-	15 ml
Distillation time	30 min.	5 min.	30 min.
Quantification	Acidimetric titration	Iodometric titration	Acidimetric titration
Indicator	Methyl red	Starch	Methyl red
Titrant	NaOH 0.05 M	Thiosulfate 0.05 M	NaOH 0.05 M
Level added for recovery	90-900 mg SO2/kg	65-675 mgSO2/kg	150 mg SO2/kg
Recovery from water	97%	67-78%	88-90%
Recovery from samples	52-83%	71%	85%**
Detection limit*	1 mg SO2/kg	2 mg SO2/kg	3 mg SO2/kg
Blank values	< 1 mg SO2/kg	2 mg SO2/kg	-

*= 3 x standard deviation of blank
**= average with a large not specified variation

Table 1. Overview analytical procedures for determination sulphite

Parameter	RSZ
Method	Tecator-Kjeltec 1002
Applied to	Shrimps
Weight of sample	15 g
Volume of water	40 ml
Volume of alcohol	-
Type of acid	H3PO4
Conc. of acid	60%
Volume of acid	25 ml
Inert gas	-
Conc. H2O2	-
Volume of H2O2	-
Distillation time	5 min.
Quantification	Iodometric titration
Indicator	Starch
Titrant	I2 0.02 N
Level added for recovery	3-200 mg SO2/kg
Recovery from water	94%
Recovery from samples	95-102%
Detection limit*	3 mg SO2/kg
Blank values	< 3 mg SO2/kg

*= 3 x standard deviation of blank

Table 2. Sulphite content in unspiked tropical shrimps

Laboratory	Sample set	Sulphite (mg SO2/kg) anal 1	anal 2	anal 3	anal 4	Mean	SD
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	-
INIP	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 tropical shrimps*

SPIKED LEVEL 1 (25.0 mg SO2/kg)

Laboratory	Sample set	anal 1	anal 2	anal 3	anal 4	Mean	CV	Rec.	Rec. mean
		<-------- mg SO2/kg -------->					(%)	(%)	(%)
RIVO-DLO	A	22.6	20.3	16.9	19.8	19.9	11.9	80	
	B	22.8	24.1	23.3	19.4	22.4	9.2	90	85
FRCF	A	11.7	10.8	11.0	11.6	11.3	3.9	45	
	B	9.3	9.3	10.4	9.1	9.5	6.2	38	42
IFREMER	A	13.9	13.6	13.3	13.6	13.6	1.8	54	
	B	14.5	14.5	12.7	15.4	14.3	7.8	57	56
TRS	A	8.3	9.5	5.3	6.8	7.5	24.3	30	
	B	5.8	4.6	4.7	4.7	4.9	11.2	20	25
SFI	A	13.4	14.1	14.9	15.4	14.5	5.9	58	
	B	11.8	12.2	11.9	12.2	12.0	1.3	48	53
INIP**	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	
	B	12.6	11.9	13.0	12.6	12.5	3.7	50	49
IFL	A	18.3	18.3	17.7	17.7	18.0	1.9	72	
	B	21.7	21.1	22.3	21.1	21.6	2.7	86	79
									48***
						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 tropical shrimps*

SPIKED LEVEL 2 (88.5 mg SO2/kg)

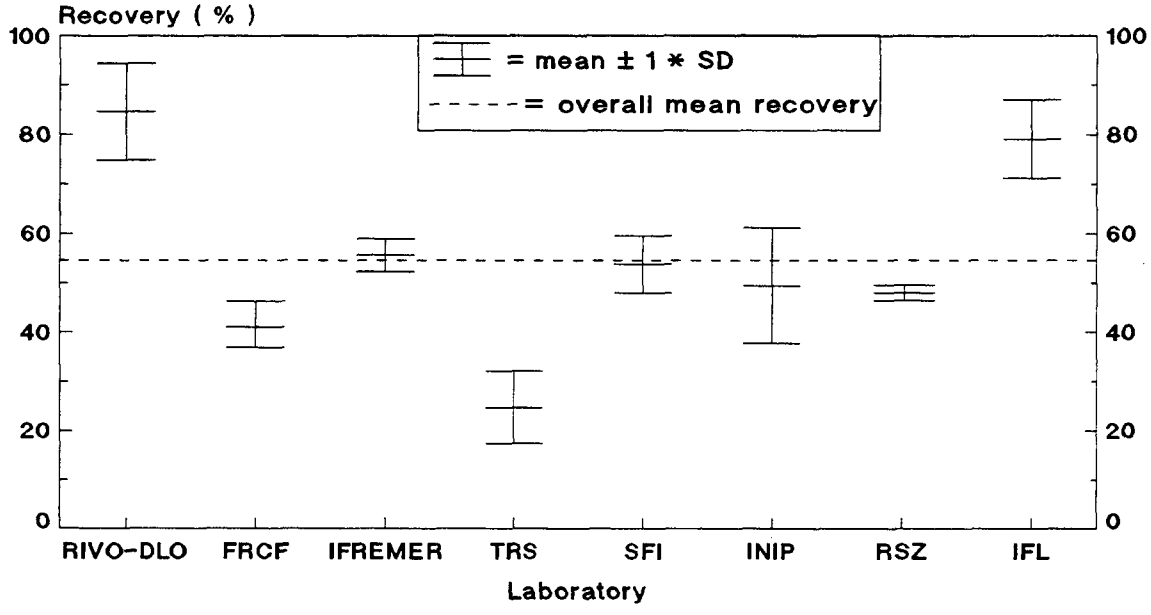
Laboratory	Sample set	anal 1	anal 2	anal 3	anal 4	Mean	CV	Rec.	Rec. mean
		<-------- mg SO2/kg -------->					(%)	(%)	(%)
RIVO-DLO	A	70.2	64.5	71.5	69.8	69.0	4.5	78	
	B	72.5	71.7	72.3	75.1	72.9	2.1	82	80
FRCF	A	55.6	55.9	58.1	56.4	56.5	2.0	64	
	B	56.0	56.1	53.8	55.6	55.4	1.9	63	63
IFREMER	A	55.6	53.2	60.2	60.5	57.4	6.2	65	
	B	55.6	60.3	56.7	59.1	57.9	3.7	65	65
TRS	A	43.6	44.7	42.5	44.6	43.9	2.3	50	
	B	44.1	48.4	47.8	43.8	46.0	5.2	52	51
SFI	A	52.2	54.1	55.0	54.4	53.9	2.3	61	
	B	59.5	58.9	57.6	61.4	59.4	2.7	67	64
INIP	A	60.8	64.0	64.0	64.0	63.2	2.5	71	
	B	64.5	70.9	69.8	66.1	67.8	4.5	77	74 62**
RSZ	A	56.3	55.4	53.7	51.9	54.3	3.6	61	
	B	51.9	53.7	51.9	50.6	52.0	2.4	59	60
IFL	A	49.8	49.2	49.8	50.6	49.9	1.2	56	
	B	58.2	57.5	58.2	57.5	57.9	0.7	65	61 52**
						Overall mean recovery*			65
						Overall mean recovery**			62

* not corrected for sulphite content in unspiked tropical shrimps
** corrected for the sulphite content in uspiked samples

Figure 1

Recovery of sulphite from tropical shrimps*

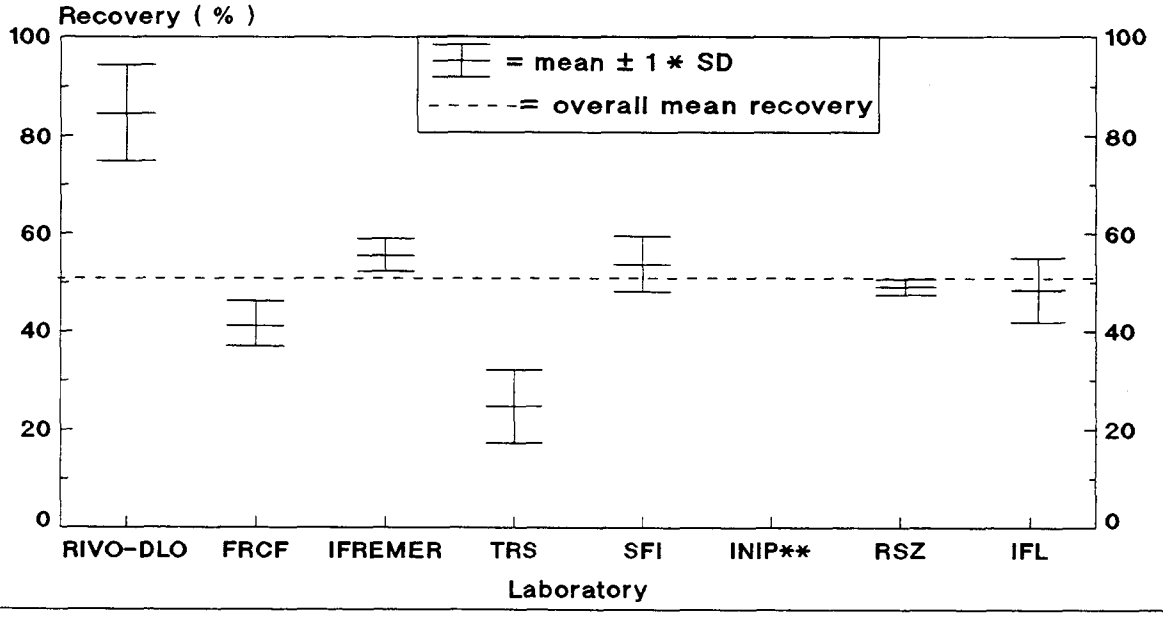
(level 1 : 25.0 mg SO2/kg)



* not corrected for sulphite content in unspiked tropical shrimps

Recovery of sulphite from tropical shrimps*

(level 1 : 25.0 mg SO2/kg)



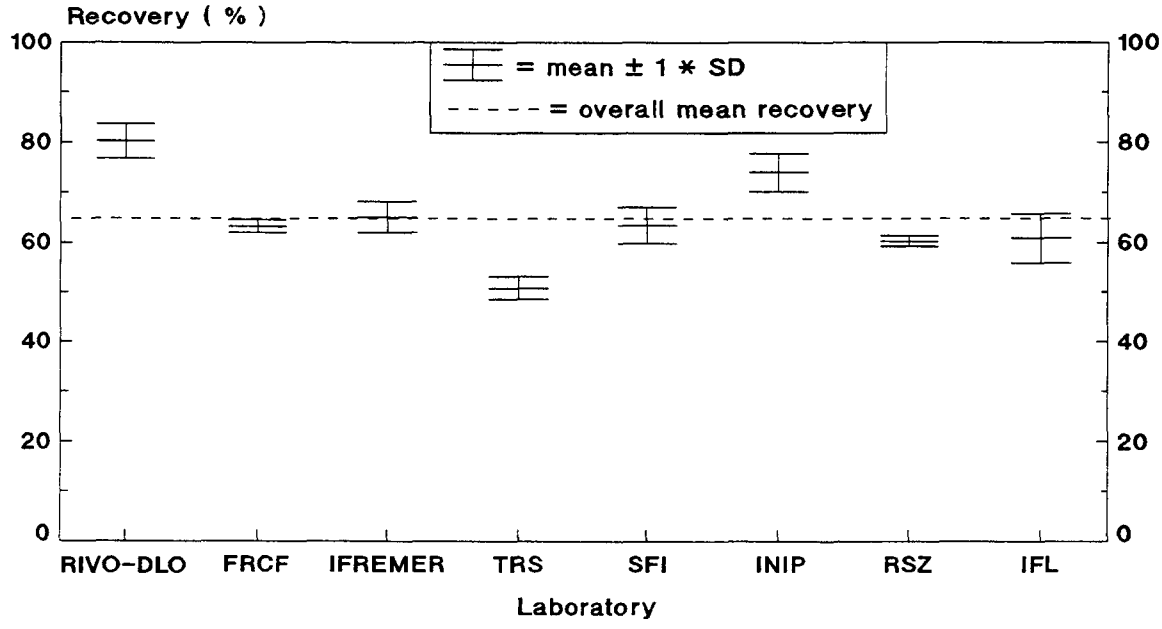
* corrected for sulphite content in unspiked tropical shrimps

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

Figure 2

Recovery of sulphite from tropical shrimps*

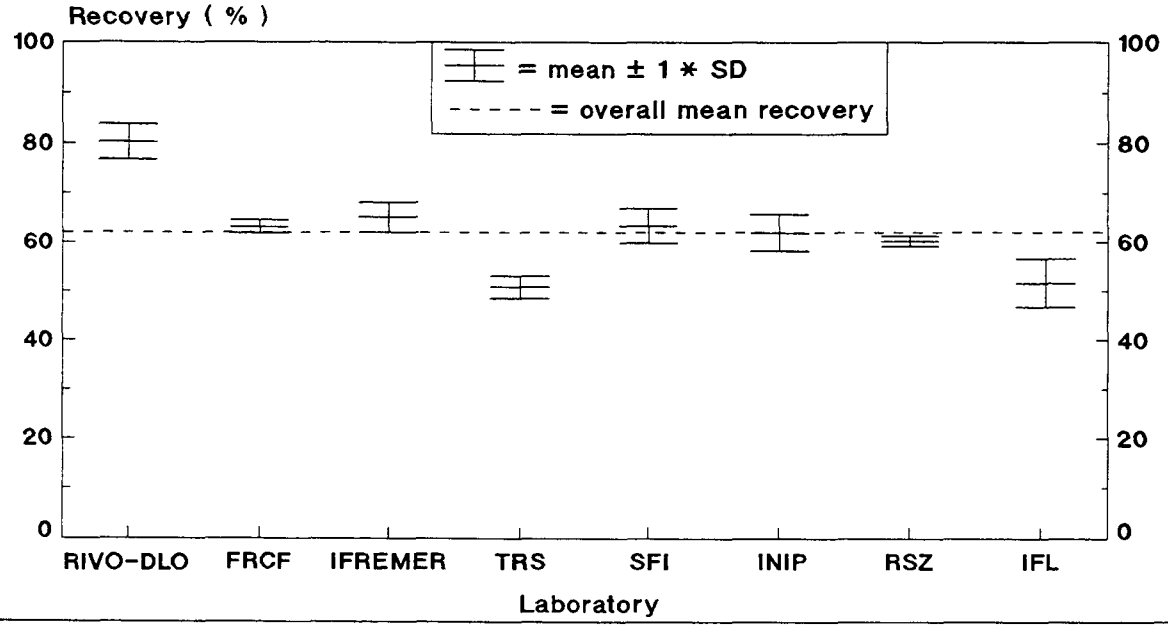
(level 2 : 88.5 mg SO2/kg)



* not corrected for sulphite content in unspiked tropical shrimps

Recovery of sulphite from tropical shrimps*

(level 2 : 88.5 mg SO2/kg)



* corrected for sulphite content in unspiked tropical shrimps