

MINISTERIE VAN LANDBOUW
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Rijkscentrum voor Landbouwkundig Onderzoek - Gent

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Directeur: Dr. P. HOVART

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W. VYNCKE



Paper presented at the 15th meeting of the Working Group on Analytical Methods of the West-European Fish Technologists' Association (WEFTA), Hamburg, May 1992.

Mededelingen van het Rijksstation voor Zeevisserij (C.L.O.-Gent)
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Summary

The efficiency of two types of steam distillation apparatus viz. a rapid distillation unit (Tecator) and a compact distillation apparatus according to Antonacopoulos was evaluated, using either iodimetric titration or colorimetric determination with Ellman's reagent.

There was no interference from the shrimp matrix.

Recoveries of sulfite added to shrimp averaged 99,6 % (s = 3,8 %) for concentrations of at least 3 $\mu\text{g/g}$ with Tecator and 10 $\mu\text{g/g}$ with Antonacopoulos.

Best results were obtained with Tecator distillation followed by colorimetry with Ellman's reagent. The detection limit was lower (1 vs 3 $\mu\text{g/g}$ for titrimetry) and the precision higher (0,2 vs 1 $\mu\text{g/g}$).

The other methods however were still satisfactory, which could be important for laboratories where sulfite is analyzed only sporadically and which do not possess automatic distillation equipment such as Tecator.

Samenvatting

De doeltreffendheid van twee types stoomdistillatie-apparaten nl. een snelle distillatie-eenheid (Tecator) en een compact distillatie-apparaat volgens Antonacopoulos werden geëvalueerd. Hierbij werden én jodimetrische titratie én colorimetrische bepaling met Ellman's reagens gebruikt.

Er bleek geen interferentie van de garnaalmatrix op te treden.

Recoveries van aan garnaal toegevoegd sulfiet bedroegen gemiddeld 99,6 % (s = 3,8 %) voor concentraties van ten minste 3 $\mu\text{g/g}$ met Tecator en 10 $\mu\text{g/g}$ met Antonacopoulos.

De beste resultaten werden met Tecator destillatie, gevolgd door colorimetrie met Ellman's reagens, bekomen.

De detectiegrens lag lager (1 t.o.v. 3 $\mu\text{g/g}$ voor titrimetrie) en de precisie hoger (0,2 t.o.v. 1 $\mu\text{g/g}$).

De andere methoden waren echter eveneens bevredigend. Dit kan belangrijk zijn voor laboratoria waar sulfiet alleen sporadisch wordt geanalyseerd en waar geen automatische distillatie-apparatuur zoals Tecator voorhanden is.

1. Introduction

Sulfite is often used to inhibit the formation of black-spot (melanosis) in crustaceans, especially shrimps (*). Many methods have been proposed for sulfite residue analysis. In the framework of the activities of the West-European Fish Technologists' Association (WEFTA), a review of methodology for the determination of total sulfite in shrimp was recently published (Vyncke, 1991). A more general review on analytical methodology is also available (Fazio and Warner, 1990).

In recent years, interest has increased in methods for determination of sulfite in foods which are fast, straightforward, low-cost, accurate and precise. One of these techniques is rapid Kjeldahl-type steam distillation (De Vries et al., 1986 ; Aberg and Persson, 1988, Williams et al., 1990).

The first aim of the present work was to evaluate the efficiency of two types of steam distillation apparatus : (a) a rapid distillation unit (Tecator) and (b) a compact distillation apparatus according to Antonacopoulos (1960), further called "Antona method". This latter equipment is widely used in fish laboratories e.g. for the determination of total volatile bases, protein nitrogen, etc. No data are available in literature on its suitability for sulfite distillation.

The second aim was to compare two determination methods : redox titration with iodine (De Vries et al., 1986) and colorimetry with 5,5'-dithiobis 2-nitrobenzoic acid (DTNB or Ellman's reagent). This method has been proposed for the determination of SO₂ in dehydrated vegetables, sausage and soft drinks (Wedzicha and Johnson, 1979 ; Wedzicha and Bindra, 1980 ; Banks and Board, 1982). No information on its use with shrimp or other seafood appears to have been published.

Special attention was paid to sensitivity, accuracy and precision of the methods.

2. Methods and material.

2.1 Apparatus.

- Distillation unit Tecator N° 1002 (Tecator, Höganäs, Sweden) modified with appropriate tubing to allow distillate to be received in beaker under buret outside of distillation unit (De Vries et al., 1986) (fig. 1).

- Antona still (Antonacopoulos, 1960), unmodified (fig. 2).

(*) The use of sulfite in crustaceaens is not allowed in Belgium at the present time (June 1993). Due to harmonization of the use of additives within the EEC, this ban could be lifted in the foreseeable future.

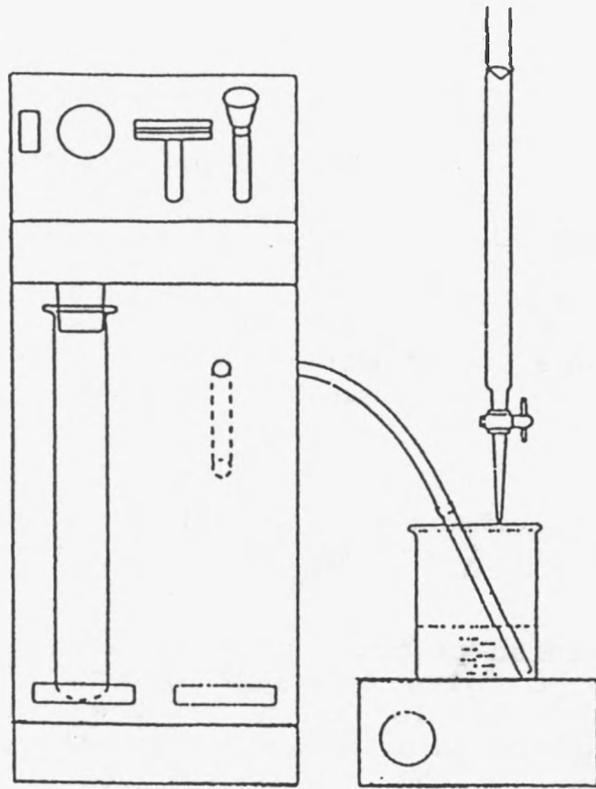


Fig. 1 Modified Tecator apparatus for sulfite analysis.

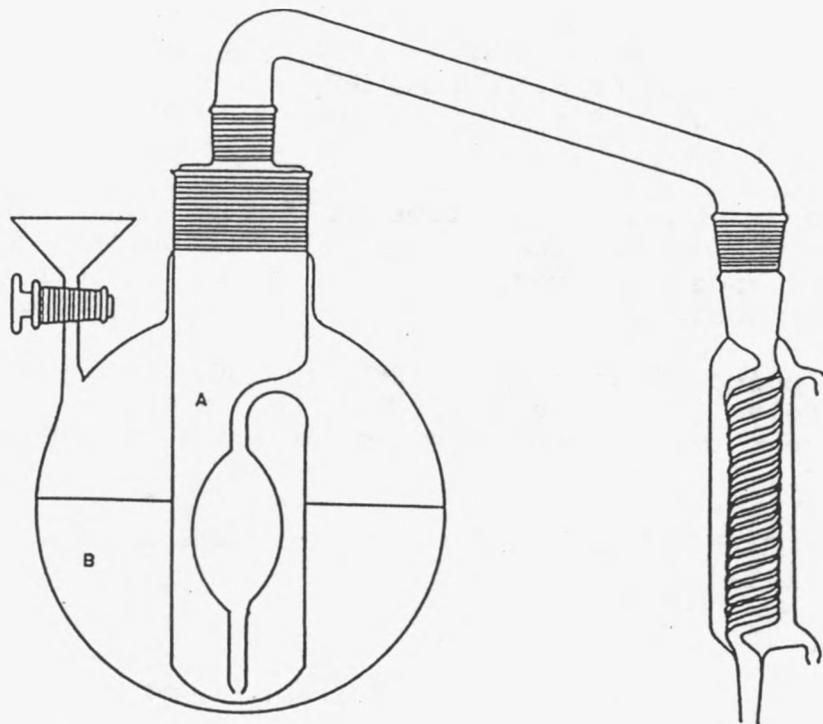


Fig. 2 Distillation apparatus according to Antonacopoulos
A : Distillation flask
B : Steam generator (2 l)

2.2 Shrimp.

Frozen tropical shrimp (*Penaeus* spp.) raw and cooked.

2.3 Distillation procedure.

The procedure was identical for both types of distillation equipment. Minced shrimp (15 g) was placed in the distillation tube and acidified with 25 ml 60 % orthophosphoric acid immediately before distillation.

2.4 Iodimetry.

The receiver beaker was primed with 20 ml water containing 1 ml of starch indicator 10 % and 0,1 ml 0,02 N iodine solution.

As the distillation proceeded any SO_2 was titrated in the receiver flask to give a slight excess of iodine (0,1 ml). When no discolouration of the iodine occurred (after 2-5 minutes) distillation was stopped by closing the steam valve.

It was found that using always the same amount of iodine reagent (0,1 ml) in the starting solution facilitated titration and increased accuracy.

2.5 Colorimetric determination

The outlet of the condenser was placed below the surface of a solution of DTNB reagent (50 ml $2,5 \cdot 10^{-3} \text{M}$) in phosphate buffer ($1,6 \cdot 10^{-2} \text{M Na}_2\text{HPO}_4 + 1,8 \cdot 10^{-3} \text{M KH}_2\text{PO}_4$, pH 8,0) containing 10 % v/v of ethanol to aid dissolution of the reagent.

After a distillation time of 5 min, the solution in the receiver was diluted to 100 ml with buffer pH 8,0 and the absorbance measured at 412 nm on a Shimadzu UV-190 spectrophotometer.

The coloured complex of DNTB and SO_2 can be measured immediately and is stable for at least 60 min. Ellman's reagent can be kept for up to 4 weeks (Banks and Board, 1982).

2.6 Determination of recovery, detection limits and reproducibility.

Comparison of distillation versus direct titration was carried out with amounts of SO_2 equivalent to 1 (3 for titrimetry), 5, 10, 25, 50, 100 and 200 $\mu\text{g/g}$ shrimp. Sodium metabisulfite (reagent grade) was used.

Recovery tests were performed by adding quantities of SO_2 ranging from 1 (3 for titrimetry) to 200 $\mu\text{g/g}$ immediately before distillation. Each concentration was determined

three times in duplicate.

The detection limits were determined as three times the standard deviation of the intercept.

The reproducibility of the methods (standard deviation) was calculated from the differences between the duplicates.

Significance level was set at minimum 95 %.

3. Results and discussion.

3.1 Iodimetry.

- Distillation of pure sulfite (without shrimp).

Fig. 3 shows the three regression lines ($n=28$ for each line). Table 1 reports the statistical data.

The loss of SO_2 during distillation appeared to be low : 4,6 % and 9,7 % on average for the Tecator and Antona methods respectively. The detection limits with distillation were about $3 \mu\text{g/g}$ (rounded off value) for both methods. In practice, $2 \mu\text{g/g}$ still gave a discolouration of iodine, $1 \mu\text{g/g}$ not. The Tecator company reports a value of $5 \mu\text{g/g}$ for foods in general (Aberg en Persson, 1988).

- Distillation of shrimp (without sulfite).

The results of twenty distillations of shrimp of different species, raw and cooked, were not significantly different from the blank (no discolouration of iodine). Only with clearly spoiled shrimp, a small amount of "false" SO_2 equivalent to maximum $5 \mu\text{g/g}$ was detected. This was shown to be caused by the formation of hydrogen sulphide.

- Distillation of sulfite added to shrimp.

Both raw and cooked shrimp were used. Differences were not significant and results were pooled.

Recoveries were calculated taking into account the titration value obtained without shrimp (fig. 3). Average results are reported in table 2. The shrimp matrix had little influence on the recoveries with Tecator. With Antona a marked decrease occurred when SO_2 concentrations were below $10 \mu\text{g/g}$. In the range $10-200 \mu\text{g/g}$ however, the same average recovery of almost 100 % was obtained. The standard deviations were not significantly different. De Vries et al. (1986) found a recovery of $97,9 \% \pm 6,4 \%$. Williams et al. (1990) reported a value of $95,1 \%$, with the same equipment.

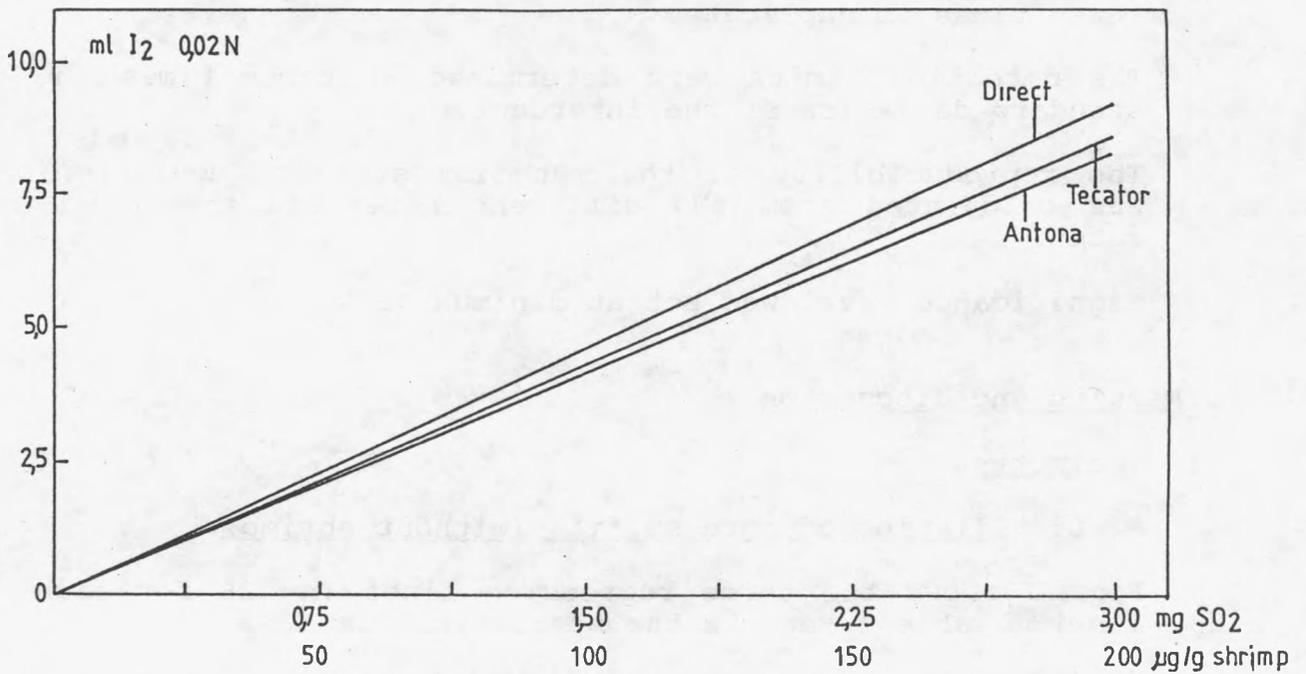


Fig. 3 Regression lines for iodimetric determination of sulfite.

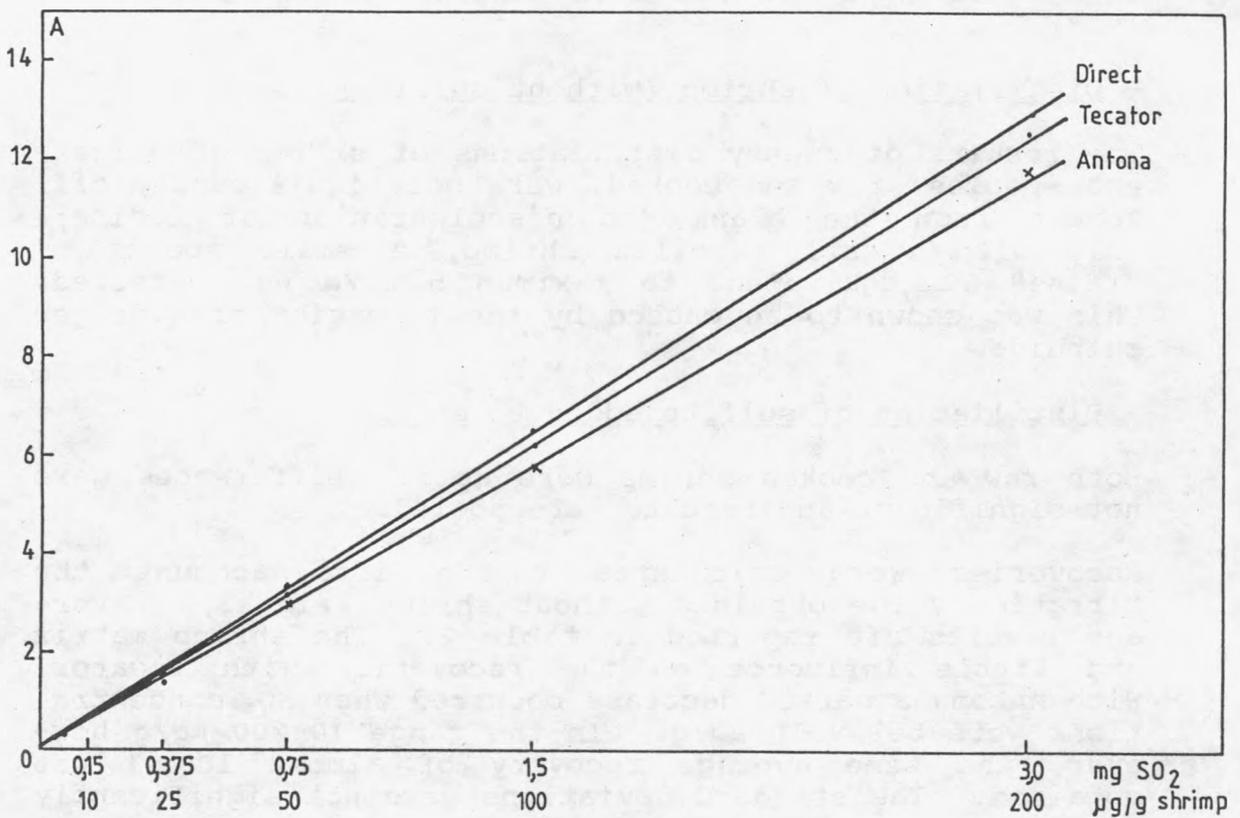


Fig. 4 Calibration lines for colorimetric determination of sulfite (samples were appropriately diluted from 0,75 mg SO₂ on).

Table 1. Iodimetric titration of SO₂ (pure solutions) (a)

	Correlation	Equation	Detection limit
Direct titration	0,9999	$y = 3,07 x + 0,022$ (b)	0,05 ml
Tecator	0,9997	$y = 2,93 x + 0,032$ (b)	0,11 ml (2,5 µg/g shrimp)
Antona	0,9997	$y = 2,80 x + 0,006$ (b)	0,11 ml (2,6 µg/g shrimp)

(a) y = ml 0,02 N I₂; x = mg SO₂ distilled over

(b) not significantly different from 0.

Table 2. Recovery (%) of sulfite added to shrimp (redox titration) (a)

SO ₂ (µg/g)	Tecator	Antona
200	101,1	101,9
100	97,9	98,7
50	102,4	100,7
25	102,2	97,3
10	98,5	100,4
5	97,8	(85,6) (b)
3	95,2	(77,5) (b)
Mean	99,3	99,8
n	21	15
Standard deviation	3,1	4,2

(a) Calculated on distillation values of pure sulfite.

(b) Not used for calculation of the mean and standard deviation.

The reproducibility of the methods was calculated from the difference between the duplicates. The standard deviations were 0,045 ml, corresponding to 1 $\mu\text{g/g}$ SO_2 for Tecator and 0,087 ml, corresponding to 2 $\mu\text{g/g}$ SO_2 for Antona. The two values were significantly different. The reproducibility of Tecator is about twice as high as Antona, which however is still very acceptable.

As these reproducibilities could be different with shrimp where sulfite is partially bound to the matrix, 16 determinations with concentrations between 10 and 60 $\mu\text{g/g}$ were carried out (only Tecator was used). The sulfite had been added 3-4 weeks before freezing and further analysis. The standard deviation was 1,1 $\mu\text{g/g}$, which was similar to that of the tests where SO_2 was determined without delay after addition to the shrimp.

3.2 Colorimetric determination

- Distillation of pure sulfite (without shrimp).

Fig. 4 shows the three regression lines ($n = 28$ for each line). Table 3 reports the statistical data. The rather low loss of SO_2 during distillation, assessed with redox titration was confirmed by the colorimetric tests : 3,5 % and 9,9 % on average for Tecator and Antona respectively. The detection limits however were lower : 1 $\mu\text{g/g}$ against 3 $\mu\text{g/g}$.

- Distillation of shrimp (without sulfite).

Twenty distillations of shrimps of different species, raw and cooked, showed that Ellman's reagent gave slight blank values ranging from 0,010 to 0,025 absorbance units. As the detection limit was set at 0,060 however (table 3) these blanks can be ignored.

As with iodimetry, clearly spoiled shrimp gave equivalent amounts of SO_2 up to 5 $\mu\text{g/g}$, due to the formation of hydrogen sulphide. DTNB is known to react with organic thiols and inorganic sulphide (Wedzicha and Johnson, 1979).

- Distillation of sulfite added to shrimp.

Average recoveries are reported in table 4. They were similar to those obtained with redox titration.

Neither the recoveries nor the standard deviations obtained with the four methods were significantly different. The pooled standard deviation ($n = 72$) was 3,8 % giving a confidence interval of $\pm 7,5$ % ($p = 0,05$) (individual determinations). With the Antona still, a marked decrease in recovery was also noted for SO_2 concentrations below 10 $\mu\text{g/g}$, indicating the loss of SO_2 being due to the type of distilling equipment used and not to the determi

Table 3. Colorimetric determination of SO₂ (pure solutions) (a)

	Correlation Coefficient (r)	Equation	Detection limit
Direct determination	0,9999	$y = 4,33 x - 0,006$ (b)	0,066 A
Tecator	0,9999	$y = 4,20 x - 0,036$ (b)	0,063 A (0,9 µg/g shrimp)
Antona	0,9997	$y = 3,87 x - 0,009$ (b)	0,070 A (1,2 µg/g shrimp)

(a) : y = Absorbance units ; x = mg SO₂ distilled over

(b) : not significantly different from 0.

Table 4. Recovery (%) of sulfite added to shrimps (colorimetric determination) (a).

SO ₂ (µg/g)	Tecator	Antona
200	103,3	96,6
100	94,2	106,9
50	96,3	96,3
25	94,7	102,6
10	105,5	96,7
5	101,5	(77,6) (b)
1	101,0	(68,4) (b)
Mean	99,5	99,8
n	21	15
Standard deviation	3,8	4,4

(a) Calculated on distillation values of pure sulfite.

(b) not used for calculation of the mean and standard deviation

nation method (titrimetry or colorimetry).

The standard deviations, calculated from the differences between the duplicates were 0,014 absorbance units or 0,21 $\mu\text{g/g}$ SO_2 for Tecator and 0,025 absorbance units or 0,38 $\mu\text{g/g}$ SO_2 for Antona. As with titrimetry, the reproducibility of Tecator was significantly higher than that of Antona. On the other hand, data obtained with Ellman's reagent were about five times more precise than with iodimetry.

4. Conclusions.

The four methods tested showed no interference from the shrimp matrix.

SO_2 liberated by acid treatment was distilled over with an accuracy of $\pm 7,5\%$ with both stills. With analyses performed in duplicate, the accuracy was 5,3 %.

Best results were obtained with Tecator distillation followed by DTNB-colorimetry. The detection limit was lower (1 vs 3 $\mu\text{g/g}$ for titrimetry) and the precision higher (0,2 vs 1 $\mu\text{g/g}$).

Iodimetry however is also satisfactory, especially in laboratories where sulfite is analyzed only sporadically.

The Antona method was somewhat less precise, the standard deviations being twice as high as with Tecator. With sulfite concentrations of at least 10 $\mu\text{g/g}$ however, this method is applicable. This could be important for laboratories which do not possess automatic distillation equipment such as Tecator.

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