Determination of Added Sulfites in Dried Allium (Modified Monier-Williams Method)

Purpose: This modification of the Monier-Williams method is suitable specifically for detection of sulfites in dried allium (i.e. garlic, onion, shallot, leek, and chive) (Reference 1). Organosulfur components are removed in a toluene trap before sulfur dioxide is collected and oxidized to sulfuric acid with hydrogen peroxide in a second trap. Sulfuric acid is titrated with standard sodium hydroxide solution. The limit of quantitation is 10 µg/g. Sulfites in non-allium foods should be analyzed by the optimized Monier-Williams method as described in AOAC 990.28 or EN 1988-1.

A. Apparatus

1. Distillation apparatus: as described in EN 1988-1 (1998) or AOAC 990.28 (2000) and with a dual bubble-through system (see Figure).

2. 1000 mL round-bottom flask with three 29/32 (or 24/40) tapered joints (vertical arms) and appropriate heating mantle.

3. 250 mL dropping funnel with stopcock and tapered joint to fit round bottom flask.

4. 25 mL buret.

5. Graduated cylinders of 25, 50, 100 and 500 mL.

6. 1 L volumetric flask.

7. 3 mL graduated pipet.

8. Cryostat set at - 5°C.

9. pH meter.

B. Reagents

1. Deionized water.

2. 0.01 N NaOH solution.

3. Indicator: Dissolve 250 mg of methyl red in 100 mL of ethanol (analytical grade). This indicator is red for pH < 4.4 and yellow for pH value > 6.2.

4. 30% w/w or 3% w/w hydrogen peroxide H_2O_2 solution, analytical grade.

5. 37% concentrated hydrochloric acid HCl for analysis.

6. 85% phosphoric acid H_3PO_4 for analysis.

7. 85% potassium hydroxide KOH in pellets for analysis.

8. Potassium dihydrogen phosphate KH_2PO_4 of purity > 99% for analysis.

9. Toluene, analytical grade.

10. Cryostat circulating fluid: mixture of monopropylene glycol/water (2/1).

11. Nitrogen flow with precision valve allowing a flow rate of about 200 mL/minute.

12. Buffer solution

Solution A (acid part): 0.59 M H_3PO_4 and 0.96 M HCl solution. Introduce approximately 200 mL of water into a 1 L volumetric flask, carefully add 40 mL of 85% H_3PO_4 followed by 80 mL of 37% HCl with, respectively, a 50 and 100 mL graduated cylinder and, finally, dilute to volume with water.

Solution B base part: 0.25 M KH_2PO_4 and 0.258 M KOH solution. Introduce 34 g KH_2PO_4 and 17 g KOH into a 1 L volumetric flask and dilute to volume with water.

Check that the buffer components are properly made by mixing solutions A and solution B in a 1:4 ratio (e.g. 15 mL of solution A and 60 mL of solution B). The pH must range from 2.3 to 2.5. Deoxygenate these 2 solutions by bubbling nitrogen through them for 15 min, less than 1 h before use.

13. Hydrogen peroxide (3%, H_2O_2) solution. Into a 50-mL graduated cylinder, add 3 mL of 30% H_2O_2 solution with a graduated pipet and water to give a volume of 30 mL. Alternatively, one may purchase and use 30 mL of 3% H_2O_2 solution directly for this purpose. Add 3 drops of methyl red indicator using a Pasteur pipet. With stirring, add 1 to 5 drops of 0.01 N NaOH and until the color of the indicator just turns yellow. This solution should be prepared fresh daily.

C. Preparation of Sample

Dried allium powders can be tested for evidence of added sulfite without preparation but quantitative precision will be improved if powders are finely ground to improve homogeneity (see Statistics section).

D. Procedure

Part A. Collection of sulfite from the sample.

1. Start circulating the coolant in the condenser, set the cryostat to -5°C and wait for approximately 30 minutes until this temperature is reached. (Note 1)

2. Weigh approximately 25 g of test sample, to the nearest 0.1 g, into a dry round-bottom flask. (Note 2)

3. Place the round-bottom flask in the heating mantle. Connect the dropping funnel (stopcock closed), the condenser and the nitrogen inlet to the round-bottom flask and the chain of 2 bubblers to the upper condenser outlet as shown in the Figure.

4. Add 25 mL toluene into the first bubbler and place the 50 mL graduated cylinder with 3% H_2O_2 solution into position as the second bubbler (see Figure).

5. Add 120 mL of solution A via the dropping funnel with a 250 mL graduated cylinder. If the system is sealed properly, gas should begin to flow through the bubblers as liquid enters the round bottom flask.

6. Lower the height of the second bubbler cylinder to improve the flow and to ensure that no H_2O_2 solution goes up in the bubbler containing toluene. The tip of the bubbler tube should be submerged at least 1 cm below the surface of the H_2O_2 solution.

7. Close the dropping funnel tap when a few milliliters of solution A remain in the funnel and raise the second bubbler cylinder such that the tip of the second bubbler tube is approximately 1 cm from the bottom of the cylinder.

8. Open the nitrogen inlet valve slowly and check the various tapered joints for leaks.

9. Allow the nitrogen to continue bubbling for 30 minutes (without heating) and then stop the nitrogen flow. (Note 3)

10. Immediately add 480 mL of solution B with a 500 mL graduated cylinder via the dropping funnel.

11. Lower the height of the second bubbler cylinder as in step 6.

12. Close the dropping funnel tap when a few milliliters of solution B remain in the funnel and raise the second bubbler cylinder as in step 7.

13. Immediately re-open the nitrogen flow.

14. Turn on the heat to the round bottom flask and after the mixture starts to boil, let it reflux for 105 min.

15. Stop the heating but leave the nitrogen flow on. Lower the H_2O_2 cylinder and rinse the bubbler tip with water into the H_2O_2 solution.

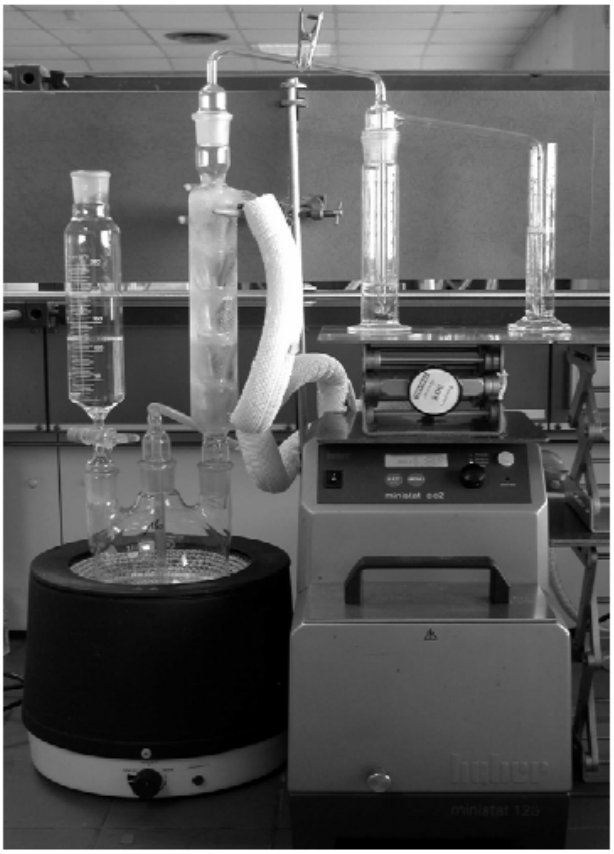
16. Turn off the nitrogen flow and immediately remove the dropping funnel from the round bottom flask to open the system and avoid suction of toluene from bubbler #1 back into the system as it cools.

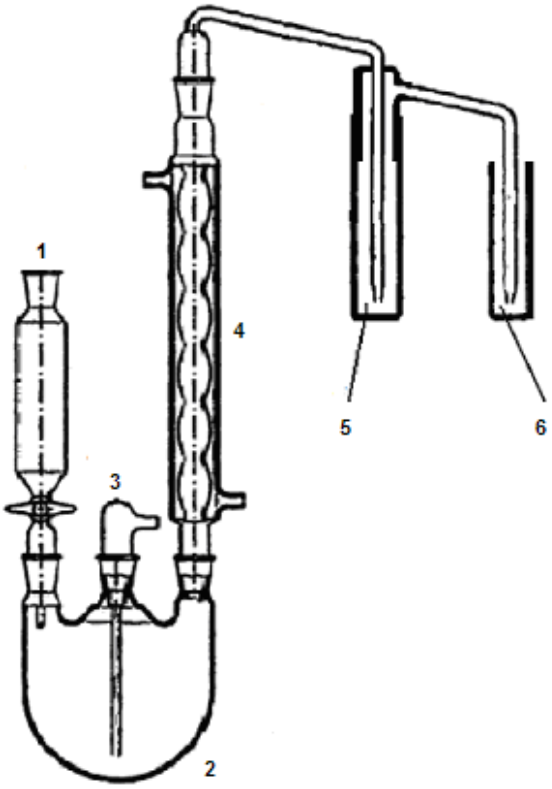
Part B. Titration of sulfite.

1. If the H_2O_2 solution remains yellow, the added sulfite content is below the quantification limit of the method (< 10 µg/g) and there is no need to titrate with NaOH. Stop here.

2. If the H_2O_2 solution has turned red, decant and rinse the solution quantitatively from the bubbler cylinder into a beaker.

3. Titrate the bubbler solution with 0.01 N NaOH solution to the yellow end point that persists for > 20 s. Record the volume of NaOH solution.





1. Dropping funnel
2. Round bottom flask
3. Nitrogen inlet tube
4. Allihn condenser
5. Bubbler #1 (toluene)
6. Bubbler #2 (H_2O_2 solution)

Figure. Assembled apparatus for the modified Monier-Williams method.

E. Calculation

The added sulfite content, expressed in µg/g (ppm) equivalent SO_2, is equal to:

$$C_{SO2} = \left(\frac{V}{W}\right)\left(\frac{0.01\,meq\,NaOH}{mL}\right)\left(\frac{32\,mg\,SO_2}{1\,meq\,SO_2}\right)\left(\frac{1000\,\mu g}{mg}\right) = 320\left(\frac{V}{W}\right)$$

Where V = the end point volume of 0.01 N NaOH expressed in mL
W = the mass of dried garlic expressed in grams

Example :
If V = 10 mL and W = 25 g, then C_{SO2} = 320 x 10 / 25 = 128 µg/g of added sulfites.

F. Statistics

An international collaborative trial between eight laboratories was completed in June 2010 (Reference 2). The results of four samples (duplicate analyses per lab) are shown in the table below.

	Sample 1	Sample 2	Sample 3	Sample 4
Average (µg/g)	37	561	44	<10
Interlaboratory reproducibility (µg/g)	±14	±54	±17	-
Interlaboratory reproducibility (%)	±38%	±10%	±39%	-

Outlying results (not shown) were disqualified in two cases due to high (> 0ºC) condenser temperature. Distinction of samples with detectable and nondetectable (< 10 µg/g) levels of sulfite was achieved in 7 of 8 labs (the 8th was an outlier). Samples 1 & 3 were coarsely granulated solids. The summary suggests that precision for these low level samples would be improved by grinding them into a finer, more homogeneous powder prior to analysis.

G. Notes

1. The condenser temperature must not exceed -3.5°C during distillation or slight false positive interference might occur. The condenser should be covered with insulating material (not shown in Figure) to maintain low temperatures. Avoid insulating with vulcanized rubber because this could introduce residual levels of sulfite to the test environment.

2. All the glassware must be well dried before use.

3. If a sample contains a high content of added sulfites, the H_2O_2 solution may turn red rapidly before heating.

H. References

1. Lafeuille, J.-L.; Lefevre, S.; Achouri, D. "Determination of Added Sulfites in Dried Garlic with a Modified Version of the Optimized Monier-Williams Method," *J. AOAC Intl.* **2007**, *90,* 1090-1097.

2. Lafeuille, J.-L. (McCormick France, EMEA Central Analysis Laboratory, Carpentras, France) "Final Statistical Results of the Collaborative Study: Added Sulfite in Dried Garlic," Special report to the European Spice Association and American Spice Trade Association, 23 June 2010.

I. Revision History

10/01/10 First version.