Project Code:	A.MPT.0001
Prepared by:	Ian Eustace Food Science Australia
Date published:	April 2007

PUBLISHED BY
Meat and Livestock Australia Limited
Locked Bag 991
NORTH SYDNEY NSW 2059

Can sulphur-containing compounds inherent in meat affect test results for added sulphites in meat?

Executive summary

Sulphur dioxide (SO_2) is permitted in Australia as a food additive in certain meat products, including sausages. It can be added at a level up to 500 mg/kg. It is normally added as sodium metabisulphite. It is not permitted in mince and other fresh meat products and retailers have been prosecuted by regulatory authorities and/or exposed by media for illegal addition, particularly to mince.

Low levels of SO_2 have been found periodically by Australian regulatory authorities in samples of meat where contamination through accidental or deliberate addition during processing is strongly doubted. Some of the meat samples were taken directly from vacuum packs that were prepared in establishments where the presence and use of formulations containing sodium metabisulphite or other precursors of sulphur dioxide would have been very unlikely. There are published reports of meat samples that were known to contain no added sulphites giving ‘false positive’ or ‘blank’ values of 10 - 15 mg/kg, sometimes higher.

This report considers whether there are explanations other than addition of SO_2 for its detection. It provides information from a review of the scientific literature. It also provides details of a limited investigation undertaken with sulphur-containing compounds other than SO_2 or sodium metabisulphite.

Sulphur is present naturally in meat. The sulphur-containing amino acid cysteine is an important amino acid; cysteine-containing peptides in meat are believed to have a role in iron absorption in humans. Cysteine is present in meat at levels of 10,000 -16,000 mg/kg of protein. Sulphydryl and disulphide groups are integral components of a number proteins, particularly the myofibrillar ones. They are found in myosin, actin, tropomyosin, troponin and other myofibrillar proteins. The groups are also found in proteins of the sarcoplasmic reticulum and in cell organelles such as nuclei and mitochondria. Sulphydryl groups are present in bovine muscle tissue in amounts variously reported to range from 500 to 800 mg/kg.

Review of Monier-Williams method for quantifying SO_2

The modified Monier-Williams method is the recognised Association of Official Analytical Chemists (AOAC) method for SO_2 in meat and other food products. It is the normal method for compliance purposes. AOAC states that the Monier-Williams method is not applicable to dried onions, leeks and cabbage; they contain interfering sulphur-containing compounds.

There are published reports of bacteria from meat that are capable of metabolising sulphydryl, disulphide and other sulphur-containing groups that are relatively abundant in meat to produce hydrogen sulphide and other volatile sulphur-containing compounds. Compounds that have been detected recently in packaged beef of known history include methanethiol, dimethyl sulphide, diethyl sulphide, thioacetic acid methyl ester, and 2,3-dimethyl trisulphide.

Monier-Williams published his recommended procedure in 1927. Since then, changes adopted by AOAC have been limited to relatively minor changes in technique that permit determination of lower levels of sulphite. In 1986, the US Federal Drug Administration (FDA) introduced a requirement that sulphites be declared on labels of any food containing detectable (10 ppm) amounts of sulphite. As a result, more accurate measurement of sulphites at the 10 ppm level became necessary; FDA specified several aspects of the procedure more precisely than AOAC Method 962.16. The optimised procedure was adopted as AOAC Method 990.28.

From the review findings, several recommendations are made pertaining to the methodology. In circumstances where low levels of SO_2 are being tested for, analysts should use AOAC Method 990.28.

Investigation with sulphur-containing compounds

Of the compounds mentioned above as having been reported in packaged meat,

methanethiol is the only one with a boiling point lower than the normal condenser temperature where condenser water at ambient room temperature is used (AOAC 962.16). Unfortunately, due to its toxicity, methanethiol is difficult to handle in a general food laboratory such as that operated by Queensland Health Analytical Services where the investigation was undertaken. Therefore Queensland Health was not prepared to acquire and use this compound in its initial trials.

Queensland Health therefore undertook recovery trials with five compounds – ethanethiol, 1-propanethiol (n-propanethiol), carbon disulphide, ethylmethylsulphide, and diethyl disulphide. The distillation and titration procedure followed was essentially that prescribed by AOAC 962.16. There was no measurable SO_2 in the collection vessel.

In order to determine whether any of the test compounds could be oxidised in the titration flask if they did distil over, solutions of the test compounds were added directly to the H_2O_2 solution in the flask. When the thiols were left to stand in the peroxide in the collection vessel for 90 mins, there was some evidence of oxidation, implying that if circumstances were such that they could distil over when the test meat samples were heated, they could test as trace quantities of SO_2.

Methanethiol and dimethyldisulphide are generated when florets of broccoli are crushed. In an attempt to obtain tentative information about methanethiol as a compound that can distil into the collection flask, broccoli was crushed and placed in the Monier-Williams flask. Distillation continued for approximately 4 hours. There was no measurable SO_2 in the collection vessel.

It is likely that methanethiol, with a boiling point of 6°C, would be the compound most likely to distil over into the H_2O_2 solution from meat and brassica samples. Its high toxicity precluded its being included in the group of compounds tested.

The compound can be sourced from the US but not in small, dilute quantities. At the time, it was decided not to proceed with the departmental approval process for the importation of methanethiol. However, if it can be obtained in small quantities, Departmental approval to use it in recovery trials would be possible.

Despite the initial lack of success by Queensland Health to use crushed broccoli as a substrate for generating adequate methanethiol, the scientific literature indicates this is a means of producing the compound as a model substrate for Monier–Williams distillation; it should be further investigated.

Before packaged meat investigations are carried out, it should be verified that known sulphur-containing compounds are sometimes ‘seen’ by the Monier-Williams method as SO_2 by one of the approaches indicated above.

Introduction

In Australia sulphur dioxide (SO_2) is permitted as a food additive in certain meat products, including sausages. It or sodium or potassium sulphites can be added at a level up to 500 mg/kg. It is normally added as sodium metabisulphite. It is not permitted in mince and other fresh meat products and retailers have been prosecuted by regulatory authorities and/or exposed by media for illegal addition, particularly to mince. As recently as January 2007, a Woollahra, NSW, butcher shop was fined $3,000 plus costs of $3,414 for an offence relating to use of SO_2.

Low levels of SO_2 have been found periodically by Australian regulatory authorities in samples of meat where contamination through accidental or deliberate addition during processing is strongly doubted. Some of the meat samples were taken direct from vacuum packs that were prepared in establishments where the presence and use of formulations containing sodium metabisulphite or other precursors of sulphur dioxide would have been very unlikely. There are published reports of meat samples that were known to contain no added sulphites giving ‘false positive’ or ‘blank’ values of 10-15 mg/kg, sometimes higher.

In 2005, Meat & Livestock Australia Ltd contracted Food Science Australia to investigate whether some sulphur-containing compounds that are known to occur in aged meat will lead to positive test results for sulphur dioxide with the intention of making the findings and any recommendations available to Food Standards Australia New Zealand.

Literature review

This review refers to almost 120 years of published research on quantification of sulphur dioxide and sulphites in foods. Two review papers are of particular note - that of Monier-Williams (1927) and that of Fazio and Warner (1990).

The normal method of analysis for SO_2 for compliance purposes is the modified Monier-Williams method. It is the recognised AOAC method for SO_2 in meat and other food products. AOAC states that the Monier-Williams method is not applicable to dried onions, leeks, and cabbage. This is because they contain sulphur-containing compounds. Monier-Williams (1927) cited a number of reports of detections of low levels (5-20 mg/kg) of SO_2 in "unsulphured" meat. Banks and Board (1982) commented about slight interference to the Monier-Williams procedure from volatile compounds in British fresh sausage.

Sulphur is present naturally in meat. The sulphur-containing amino acid cysteine is an important amino acid; cysteine-containing peptides in meat are believed to have a role in iron absorption in humans. Cysteine is present in meat at levels of 10,000 -16,000 mg/kg of protein. Sulphydryl and disulphide groups are integral components of a number of proteins, particularly the myofibrillar ones. They are found in myosin, actin, tropomyosin, troponin and other myofibrillar proteins. The groups are also found in proteins of the sarcoplasmic reticulum and in cell organelles such as nuclei and mitochondria. Sulphydryl groups are present in bovine muscle tissue in amounts variously reported to range from 500 to 800 mg/kg.

In the early days of the export of vacuum-packaged beef from Australia, sulphur compounds inherent in meat were periodically used by bacteria in the vacuum packs to produce hydrogen sulphide. On occasions, sufficient H_2S was produced to react with the myoglobin of the meat and give an undesirable green pigment, sulphmyoglobin. Based on the findings of later research on vacuum packs, other volatile and non-volatile sulphur-containing compounds were probably also produced.

There are now reports of bacteria isolated from vacuum packs of meat and similar systems that are capable of metabolising sulphydryl, disulphide and other sulphur-containing groups that are relatively abundant in meat to produce hydrogen sulphide and a range of other sulphur-containing compounds. Volatile sulphur-containing compounds that have been detected recently in packaged beef of known history include methanethiol, dimethyl sulphide, diethyl sulphide, thioacetic acid methyl ester, and 2,3-dimethyl trisulphide (Dainty et al, 1989; Mayr et al, 2003). The presence of all these compounds in onions and brassicas has been reported (Pino et al, 2001; Thrasher, 1966). Several species of foodborne bacteria are capable of producing these compounds (Dainty et al, 1989; Mayr et al, 2003). It appears possible, therefore, that proteins or other sulphur-containing compounds inherent in meat may give rise to compounds which assay as SO_2 when the modified Monier-Williams procedure is used to lead to what may be false detections of added SO_2.

The increased use of vacuum-packaged stored topsides and other meat for mince means that there is an increasing need to determine whether low levels of SO_2 in test samples can be attributed to metabolism by bacteria of sulphur-containing components inherent in the meat. If that is the case, the meat industry should be armed with independent evidence with which to defend possible prosecutions and adverse media coverage. Also, it is important that the limitations of the method and ways to optimize it are well known to meat industry and regulatory people alike.

Monier-Williams method

In the Monier-Williams method, SO_2 is released when a sample is mixed with an acid - normally hydrochloric acid but sometimes phosphoric acid - and heated. The SO_2 is distilled using a stream of nitrogen gas which carries the gaseous SO_2 into an absorbing solution of hydrogen peroxide where it is oxidised to sulphuric acid. The amount of SO_2 distilled into the H_2O_2 is determined by titration with 0.1M sodium hydroxide. 1 mL of 0.1M NaOH is equivalent to 3.203 mg SO_2.

AOAC states that the Monier-Williams method is applicable in the presence of other volatile sulphur compounds. However it is not applicable to dried onions, leeks, cabbage and certain other vegetables. They naturally contain dimethyl-disulphide, methanethiol and other interfering volatile sulphur containing compounds which cause the method to give high apparent SO_2 values. Attempts to distinguish between naturally occurring sulphite and added sulphite by modifying the method have been reported as unsuccessful. Reduction or omission of the 1.75 hours of boiling leads to lower assays from onions and Brassica vegetables but some SO_2 is still normally found.

Development of the Monier-Williams method

In an extensive 1927 review of published research and personal communication with UK and Continental European public analysts, Monier-Williams, then Head of the Chemical Laboratory of Britain's Ministry of Health, described the late nineteenth century and early twentieth century research into determination of sulphur dioxide in foods.

In his introduction, Monier-Williams commented that the method proposed by Haas in 1882 – distillation using CO_2 as the carrier gas - had been adopted widely and found to be satisfactory. Much of the investigation between 1882 and 1927 had been concerned with differentiating between 'free' and 'combined' sulphurous acid. He stated "It is chiefly in the case of foods containing small amounts of sulphur dioxide, of the order of 5 to 50 parts per million, that difficulties may arise, owing to uncertainty as to whether the analytical figures really represent sulphur dioxide or are due in some measure to volatile sulphur compounds of a different character, originating from the food itself".

According to Monier-Williams, the usual method employed early in the 20th century, which depended on distillation in a current of carbon dioxide into bromine or iodine solution followed by gravimetric determination as barium sulphate, was "somewhat tedious" and many analysts had tried to finder a quicker and equally accurate method. In his report, he reviewed the work already done prior to 1927 and suggested a method which, "while obviating to a great extent the errors due to volatile sulphur compounds, permits direct distillation of the distillate."

Monier-Williams reviewed the various methods reported for trapping and oxidizing the SO_2 distilled into the collecting vessel, including neutralized hydrogen peroxide (H_2O_2) which had been used previously by Franz and Sonntag (1908) and others. He stated that H_2O_2 could not be used for wine or for foods containing volatile acids which may distil over and affect the titration. He cited Lange (1909) and Froboese (1920) who found that this method was not accurate for gelatine, owing to the distillation of volatile fatty acids.

Monier-Williams also cited Gutbier, Sauer and Brintziger (1921), who with a view to preventing the distillation of volatile acids from gelatine, used a reflux condenser so that only carbon dioxide and sulphur dioxide passed over into the receiver. He adopted the principle of a reflux condenser with distillation into pure H_2O_2 in the method that he described his 1927 report and commended to public analysts. He stated that the errors due to volatile organic compounds, with the exception of those containing sulphur, could be avoided if the SO_2 in the distillate was determined by oxidation to sulphuric acid and precipitation as barium sulphate. The AOAC official method retains this gravimetric step which may be performed after the titration.

Monier-Williams noted several reports of the presence of volatile sulphur compounds in the distillates from unsulphured foods:

i. Niemann and Rubner (1893) detected the presence of hydrogen sulphide and mercaptans in the distillates from vegetables;

ii. Schmidt (1904) found that fresh fruits and vegetables yielded traces of volatile sulphur compounds;

iii. König (1926) investigated the volatile substances from several foods and found that egg albumen on being heated to 100°C in a current of nitrogen yielded 28 mg/kg of hydrogen sulphide, while cauliflowers gave 5·6 mg/kg of hydrogen sulphide and 2·6 mg/kg of methyl mercaptan (now more commonly referred to as methanethiol);

iv. Mentzel (1906) found that unsulphured meat gave from 5 to 8 mg of barium sulphate per 100 g (equivalent to 14-22 mg/kg SO_2) when distilled into iodine solution, and that the addition of onions to the meat resulted in a large increase in the amount of "apparent" sulphur dioxide found. By distilling onions alone into iodine solution he obtained as much as 60 mg/kg of "apparent" sulphur dioxide. Mentzel considered that in the case of meat it was not safe to infer the presence of sulphur dioxide unless the amount indicated is greater than 40 mg/kg or 50 mg/kg from meat products containing onions.

Monier-Williams also reported the findings of Winton and Bailey (1907) who examined samples of beef, mutton, veal and pork both when fresh and after keeping for various periods up to 23 days. The amount of "apparent" sulphur dioxide in fresh meat was of the order of 4 mg/kg, while no hydrogen sulphide could be detected. On keeping veal for nine days the apparent sulphur dioxide went up to 48 mg/kg and the hydrogen sulphide to 72 mg/kg.

Mutton after nine days gave 84 mg/kg of apparent sulphur dioxide and 6 mg/kg of hydrogen sulphide. According to Monier-Williams, Winton and Bailey suggested that sulphites may have been formed in decomposing meat as intermediate products of the oxidation of sulphides to sulphates, but Monier-Williams considered that it was more likely that the apparent sulphur dioxide was due to volatile organic sulphur compounds.

Hydrogen peroxide, according to Lunge (1890), also oxidizes hydrogen sulphide to sulphur, but Monier-Williams found that when a weak solution of hydrogen sulphide (H_2S) is added to H_2O_2 containing barium chloride the liquid remains clear when the solution is at room temperature and no sulphur separates. On heating, barium sulphate is precipitated. In the presence of hydrochloric acid the solution still remains clear when cold, but barium sulphate is formed on heating.

Monier-Williams undertook a series of trials with pure H_2S and other sulphur-containing compounds and ones distilled from mixtures of foodstuffs. He concluded that hydrogen peroxide, when cold, does not oxidize appreciable amounts of hydrogen sulphide or volatile organic sulphur to sulphuric acid, but if the solution was heated (the usual manner at that time for the gravimetric procedure where precipitation with barium chloride produces barium sulphate), considerable oxidation to sulphuric acid takes place. He also concluded that if much protein or volatile products containing sulphur are present, the correction to be applied to the results to estimate added SO_2 may be rather large.

Monier-Williams concluded that:

- In the determination of SO_2 in foods containing volatile sulphur compounds, H_2O_2 will give reliable results, provided that precipitation and filtration of barium sulphate are carried out at room temperature.
- Titration of the sulphur dioxide in the distillate, after oxidation to sulphuric acid, gives accurate results if precautions are taken to eliminate volatile organic compounds.

He considered the most important points to be: (i) to ensure that the whole of the sulphur dioxide has been driven over into the distillate, (ii) to prevent oxidation of sulphur dioxide during distillation, and (iii) to correct the results for volatile sulphur compounds oxidized to sulphuric acid in the distillate.

Modifications to Monier-Williams method

The Monier-Williams method was adopted by AOAC as an official method prior to 1950. While various modifications have been proposed to the Monier-Williams method that either vary the conditions of the distillation or substitute a different measurement system for sulphur dioxide, adopted modifications of the AOAC procedure have been limited to relatively minor changes in technique that permit determination of lower levels of sulphite in food and better control of the distillation and titration steps.

In 1954, Shipton recommended changes to the distillation apparatus to reduce the risk of leaks. To that time, the AOAC method used carbon dioxide as the carrier gas as specified by Monier-Williams. CO_2 prevents a sharp end-point to the titration so Shipton recommended the use of nitrogen at a flow rate of 6-12 bubbles per minute. He also stated that the time for refluxing could be reduced to 30 minutes. AOAC adopted the recommendation to use nitrogen. In the official method the reflux time remains at 1.75 h, however.

Thrasher (1966) recommended an alternative system to the U-tubes for trapping the sulphur dioxide distilled over from the refluxing apparatus. In 1965 AOAC adopted Thrasher's recommendation that the alternative trap be specified in the official method. The design of the reflux and trapping apparatus for the official method (AOAC Official Method 962.16) has remained unchanged since 1965.

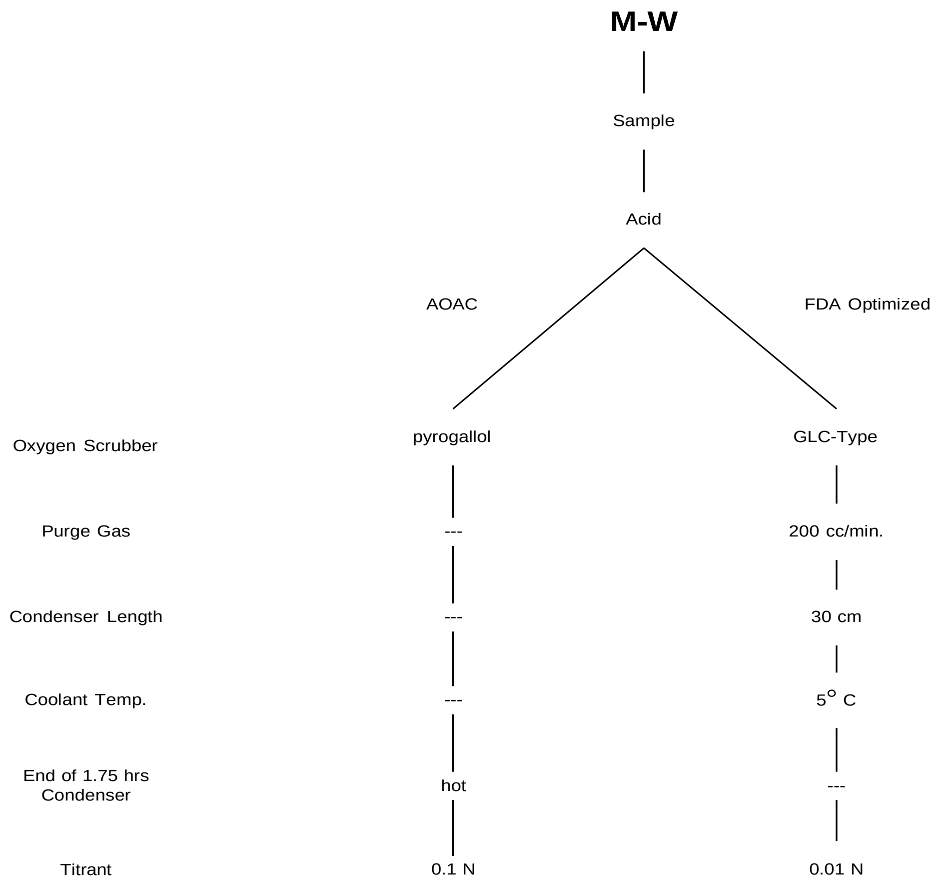
FDA optimized procedure

In their comprehensive review, Fazio and Warner (1990) state that the distillation methods reviewed by and recommended by Monier-Williams in 1927 have been remarkably successful over the years because relatively few substances are commonly found in food that are sufficiently volatile to pass through a cold condenser and yield sulphuric acid upon reaction with hydrogen peroxide in the trap.

When the use and determination of sulphites in foods were studied during the early part of the 20th century, most of the uses of sulphite were regarded as innocuous and application limits were 100 ppm and (well) above. Studies of recoveries of sulphur dioxide often involved spiking samples with the equivalent of 100 mg of SO_2 at concentrations of 100-1000 ppm.

About 40 years ago the GRAS status of sulphites came under question when scientific findings began linking ingestion of sulphiting agents to severe reactions among sensitive individuals, asthmatics particularly. In 1986 FDA issued a final rule requiring that sulphites be declared on the label of any food containing detectable (10 ppm) amounts of sulphite (FDA 1986). As a result of the FDA action, more accurate measurement of sulphites at the 10 ppm level in foods became necessary. When the various methods available were evaluated, the most generally useful was the modified Monier-Williams procedure. FDA specified several aspects of the procedure more precisely than Method 962.16, permitting reliable determination of sulphites at 10 ppm. The optimized procedure is AOAC Official Method 990.28. The procedural changes are summarised in the figure below.

The risk of interfering substances reaching the hydrogen peroxide trap by aerosolisation, co-distillation or steam distillation is reduced if the optimized procedure is followed because values are specified for condensor coolant temperature, reflux ratio, and nitrogen flow.



Schematic of procedural changes made by FDA in the official AOAC Monier-Williams method to permit quantitation of sulphites at 10ppm in foods. (After Fazio & Warner, 1990)

Procedures that do not use the hydrogen peroxide trap offer no significant advantages as long as the trap is titrated immediately as specified in the original Monier-Williams method because the oxidation of sulphur-containing substances to form sulphuric acid is slow relative to the rate of oxidation of sulphur dioxide.

Although it is time consuming, the Monier-Williams method has been proven to be the most reliable and has been the reference method against which the accuracy and precision of new methods have been compared. The Monier-Williams method measures free sulphite plus a reproducible portion of the bound sulphites, such as the carbonyl addition products.

Quantification techniques such as colourimetry, ion chromatography and polarography are more sensitive than titration but as Fazio and Warner state, this is seldom an advantage in food analysis because ample test sample is usually available. They comment that the fact that the Monier-Williams procedure requires a 50 to 100 g test portion is actually an advantage because a representative test portion can be selected without extensive comminution. Excessive comminution leads to some loss of sulphite.

Interferences

As stated earlier, very few foods not treated with sulphite give positive test results, calculated as sulphur dioxide, even at levels below 10 ppm. There are known exceptions however. Fresh untreated onions and cabbage typically give responses in the range of 3-20 ppm sulphur dioxide while isolated soy protein and garlic assay at around 30 and 200 ppm respectively. In 1966, Thrasher as an associate referee of AOAC, concurred with Zonneveld and Meyer (1960) who reported that certain dried vegetables, specifically onions, leeks and cabbage, contain volatile compounds that co-distil along with the true SO_2. Thrasher recommended that AOAC include an introductory statement "…not applicable to dried onions, leeks and cabbage". This recommendation was adopted and remains within the official methods 962.16 and 990.28 as introductory statements.

Table 1. Sulphur-containing compounds detected in meat and alliums and/or brassicas

Compound	Reference	
	Meat	Alliums / brassicas
Hydrogen sulphide	Dainty *et al* (1989) Egan *et al* (1989) Stutz *et al* (1991)	Chin *et al* (1994)
Benzothiazole	King *et al* (1995)	
Methanethiol	Dainty *et al* (1989) Gardner, Patterson (1975) Mayr *et al* (2003)	Dan *et al* (1999) Chin *et al* (1994)
Diethylsulphide	Mayr *et al* (2003) Stutz *et al* (1991)	
Dimethylsulphide	Dainty *et al* (1989) Mayr *et al* (2003) Stutz *et al* (1991)	Chin *et al* (1994)
Dimethyldisulphide	Dainty *et al* (1989) Mayr *et al* (2003) Stutz *et al* (1991)	Chin *et al* (1994) Cha *et al* (1998)
2,3-dimethyltrisulphide	Dainty *et al* (1989) Mayr *et al* (2003)	Chin *et al* (1994) Cha *et al* (1998)+
Dimethyl sulphone	King *et al* (1995)	
Thioacetic acid methyl ester	Mayr *et al* (2003)	
Methylthioacetate	Dainty *et al* (1989)	
Methylthiopropanoate	Dainty *et al* (1989)	Cha *et al* (1998)
bis(methylthio)methane	Dainty *et al* (1989)	
Methyl-n-propylene disulphide	Stutz *et al* (1991)	

The ability to distinguish between naturally occurring sulphite and sulphite that is added during processing is a problem. Brassica and allium vegetables are examples of foods with natural substances that hydrolyse to yield sulphur dioxide. It has been determined that the sulphur dioxide-yielding substances are partly sulphur-containing compounds that decompose in refluxing hydrochloric acid. With onions and brassicas though quantities are released even when hydrolysis in not involved.

Direct reports of detection of SO_2 in 'unsulphured' meat are limited to the early 20 th century ones cited by Monier-Williams (1927) and that of Banks and Board (1982) Banks and Board reported a blank value of about 15 mg/kg for unsulphited samples of fresh sausage. However there are several recent reports of volatile sulphur compounds that may distil in the H_2O_2

collection vessel and be assayed as added SO_2 if the test conditions vary at all from those specified by Monier-Williams himself and by AOAC in the FDA-optimised official AOAC method 990.28.

Some of these reports are mentioned in the table.

Conclusions to be drawn from literature review

1. The AOAC Official Method 962.16, the modified Monier-Williams method, is recognised as being unsuitable for dried onions, leeks and cabbage. They contain volatile sulphur-containing compounds.
2. For meat samples, the Monier-Williams method has been reported as giving blank values of 10-15 mg/kg, sometimes higher.
3. Monier-Williams and others stress that during the tests, effective condensers are necessary to limit, as far as possible, the carryover of compounds other than SO_2 during refluxing.
4. In situations where there is a possibility that detected SO_2 is attributable to sulphur-containing volatile and non-volatile compounds, Monier-Williams and others suggest the gravimetric the gravimetric estimation of SO_2 as barium sulphate after titration of the sulphuric acid in the H_2O_2 For both the titration and the precipitation of barium sulphate the H_2O_2 should be cold.
5. The titration should be done as soon as the refluxing step is completed.
6. Where the level in the product is expected to be low (0-50 mg/kg) the AOAC Official Method 990.28, optimized Monier-Williams method, should be used. For this method, a number of conditions are specified more closely than in Method 962.16, including flow rate of nitrogen carrier gas, length of condenser, condenser coolant temperature and concentration of titrant.
7. Monier-Williams cited a study that indicated that while samples of veal and mutton tested fresh had very low levels of SO_2, samples stored for nine days or longer contained appreciable quantities of ‘apparent’ SO_2.
8. Meat stored aerobically or in vacuum packs contains volatile and non-volatile sulphur-containing compounds. At least some of these are also present in onions and/or cabbage and may contribute to false detections of SO_2.
9.

References

Ahn, D U Nam, K C, Du, M, and Jo C (2001). Volatile production in irradiated normal, pale soft exudative (PSE) and dark firm dry (DFD) pork under different packaging and storage conditions. *Meat Science 57*, 419-426

AOAC International (2000) Official Methods of Analysis 17th Ed. Official method 962.16. Sulfites in foods; modified Monier-Williams method

AOAC International (2000) Official Methods of Analysis 17th Ed. Official method 990.28. Sulfites in foods; optimized Monier-Williams method

Banks, J G, and Board, R G (1982). Comparison of methods for the determination of free and bound sulphur dioxide in stored British fresh sausage. *J Sci Food Agric. 33*, 197-203.
Dainty, R H, Edwards, R A, Hibbard, C M, and Marnewick, J J (1989).
Volatile compounds associated with microbial growth on normal and high pH beef stored at chill temperatures. *J Appl Bact. 66*, 281-289.

Food and Drug Administration Center for Food Safety and Applied Nutrition (1985). Appendix A to Part 101 – Monier-Williams procedure (with modifications) for sulfites in food. www.accessdata.fda.gov

Fazio, T and Warner, C R. (1990). A review of sulphites in foods; Analytical methodology and reported findings. *Food additives and Contaminants 7*, 433-454.

+Franz, F, and Sonntag, G, (1908) Distillation into hydrogen peroxide and titration of sulphuric acid formed. *J Chem Soc 94* A. ii 714.

+Froboese, V (1920) Distillation into sodium bicarbonate, oxidation with hydrogen peroxide and titration. *Arbeiten aus dem Kaiserlichen Gesundheitsamte 52*, 657

+Gutbier, A, Sauer, E and Brintzinger H (1921). Determination of sulphur dioxide in gelatine by direct iodine titration, also by distillation into hydrogen peroxide through reflux condenser. *Kolloid-Z 29*,130

+Lange, W (1909) Sulpur dioxide in commercial gelatine. *J Soc. Chem. Ind. 28*, 995.

+Lunge G J (1890) Determination of sulphur dioxide in presence of hydrogen sulphide by hydrogen peroxide. *J Soc. Chem. Ind. 9*, 1013.

+Konig, J (1926) Hydrogen sulphide and methyl mercaptan from various foods. *Chem-Ztg, 50*, 992.

+Konig, J and Schreiber, W Z (1927). Volatile substances, including sulphur compounds, obtained on heating various foods. *Unters. der Lebensmittel 53*, 1

Mayr, D, Margesin, R, Klingsbichel, E, Hartungen, E, Jenwein, D, Schinner and Mark, T D (2003). Rapid detection of meat spoilage by measuring volatile organic compounds by using proton transfer reaction mass spectrometry. *Appl. Environ. Microbiol. 69*, 46974705.

Monier-Williams, G W (1927) The determination of sulphur dioxide in foods. Public Health and Medical Subjects Report No. 43 Ministry of Health London 56 pp.

+Mentzel, C Z (1906) Volatile sulphur compounds in meat and their effect on sulphur dioxide determination. *Genussm. 11*, 320.

+Niemann, F, and Rubner, M, (1893). Volatile sulphur compounds given off on heating foods. *Arch. Hygiene 19*, 126, 136.

Pino, J A, Fuentes, V, and Correa M T (2001). Volatile constituents of Chinese chive. *J Agric and Food Chem. 49*, 1328-1330. (2001).

Schmidt, H, (1904). Sulphur dioxide in dried fruit and other foods. *Gesundheitsante 21*, 226

Stutz, H K, Silverman, G J, Angelini, P, and Levin, R E (1991). Bacteria and volatile compounds associated with ground beef spoilage. *J Food Science 56*, 1147-1153.

Shipton, J, (1954). Sulphur dioxide in dried foods. *Food Preserv. Quart. 14*, 54-56.

Thrasher J L, (1966). Sulfur dioxide determination by modified Monier-Williams method. *J of the AOAC 49*, 834-836.

+Winton A L, and Bailey, E M J (1907). Volatile sulphur compounds in meat. J *Amer. Chem. Soc. 29,* 1499.

Zonneveld H and Meyer, A (1960). Determination of sulphur dioxide , particularly in dried food. *Zeit. Fur Lebensmittel Untersuchung und Forschung 111*, 198-207

+ As cited by Monier-Williams, (1927).