

# Meat technology – information sheet

February 2006

## Sulphur dioxide, sulphites in meat products

**This information sheet discusses methods for quantifying low levels of SO<sub>2</sub> in meat products, and suggests reasons, other than addition of sulphites, for the apparent presence of SO<sub>2</sub> in meats to which sulphites have not been added.**

In Australia sulphur dioxide (SO<sub>2</sub>) is permitted as a food additive in certain meat products, including sausages. SO<sub>2</sub> as such is rarely, if ever, added to a meat product. It is normally added as sodium metabisulphite; however, it is quantified and reported as SO<sub>2</sub>. It is not permitted in minced meat products and retailers have been prosecuted by regulatory authorities for illegal addition.

Periodically, low levels of SO<sub>2</sub> have been found by Australian regulatory authorities in samples of meat where contamination through accidental or deliberate addition during processing is strongly doubted. In some cases, 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. The questions this raises are:

- 1) are there sulphur-containing compounds in meat that might test as SO<sub>2</sub>?
- 2) are the analytical methods appropriate and how do you avoid a false positive result?

This information sheet offers a possible explanation for the unexpected detections of small quantities of SO<sub>2</sub> in meat products, and specifies the conditions for analysis that should be followed when testing samples for the presence of low levels of SO<sub>2</sub>.

### Regulations

In the Food Standards Code, Food Standards Australia New Zealand (FSANZ) states that sodium or potassium sulphites can be added to specified meat products, at a permitted level up to 500 mg/kg. This includes deli meats, edible casings, and sausages and sausage meat (raw).

Where a food contains sulphites at a level of 10 mg/kg or higher, the label must state that they are present.

### Why the use of sulphites in foods is regulated

A small section of the population responds to sulphites with adverse reactions. Many of these people also suffer from allergies and asthma. In sulphite-sensitive people, sulphites can provoke asthma and other symptoms of an allergic response, such as skin rashes and irritations.

Sensitivity to sulphites in food is dependent on how much a person is exposed to them across his or her whole diet and from other non-food sources.

The ingredient lists of foods will alert people to the presence of added sulphites in foods—look for the additives numbered 220–225, and 228.

- 220 Sulphur dioxide
- 221 Sodium sulphite
- 222 Sodium bisulphite
- 223 Sodium metabisulphite
- 224 Potassium metabisulphite
- 225 Potassium sulphite
- 228 Potassium bisulphite

In August 2005, FSANZ published the results of the 21<sup>st</sup> Australian Total Diet Study (ATDS) sampling 59 types of food from all Australian States and Territories. The meat products included in the study were sausages, frankfurts, hamburger patties and strassburg. Minced meat (hamburger patties) was included in the study, even though there is no provision in the Food Standards Code for sulphite use in minced meat.

The survey indicated that illegal addition of sulphites to minced meat may occur, although this contributed little to the overall estimated intake of sulphites. FSANZ and State and Territory enforcement agencies will continue to target meat manufacturers to ensure compliance with the Food Standards Code.

### Why SO<sub>2</sub> is added to meat products

SO<sub>2</sub> is added to meat products as a preservative. It increases the lag phase of bacteria (the period of time before they begin to grow in a new environment), slows their growth rate, and selects against the species of bacteria that traditionally dominate in meat and eventually cause the putrid off-odours of spoilage. It is effective against *Salmonella* spp and many other Enterobacteriaceae, *Pseudomonas* spp, *Lactobacillus* spp and various species of yeasts.

In sausages and other finely comminuted fat-protein-water meat emulsions, it is an effective antioxidant. It also acts as a reducing agent for preventing the grey-brown discolouration of minced meat and fresh sausages.

### Why SO<sub>2</sub> addition must be included on labels

The label must state that sulphites are present if they are added to a food at a level of 10 mg/kg or higher so that sensitive individuals can avoid that food.

### Why SO<sub>2</sub> is not permitted in minced meat

The reason it is not permitted in minced meat dates back to the early 20<sup>th</sup> century when its addition was seen by regulators as masking the presence of high numbers of bacteria by delaying the onset of discolouration and putrid spoilage, thereby making it possible to get away with poor hygiene and storage temperatures.

### Permitted levels in meat products

Sausage and sausage meat containing raw unprocessed meat	500 mg/kg
Processed comminuted meat	500 mg/kg
Edible casings for sausages, processed comminuted meat	500 mg/kg

### Analysis for SO<sub>2</sub>

The normal method of analysis for SO<sub>2</sub> for compliance purposes is the Modified Monier-Williams method (Association of Official Analytical Chemists [AOAC] Official Method 962.16). It is the recognised AOAC method for SO<sub>2</sub> in meat and other food products. A more sensitive Optimized Monier-Williams method (AOAC Official Method 990.28) has been developed to test for SO<sub>2</sub> in samples at concentrations down to 10 ppm. These methods are described below.

There is a rapid test available, but it has limited use for minced meat.

### Modified Monier-Williams method

Monier-Williams was in charge of the chemical laboratory of the UK Ministry of Health during the 1920s. In 1927 he reviewed the various methods then available for quantifying SO<sub>2</sub> and, from them, he recommended a procedure to be followed by public analysts in the UK. His method was adopted in the US by AOAC as an official method some time prior to 1950. Modifications to the AOAC method since 1950 have been limited to relatively minor changes in the technique; the Official method 962.16 has remained unchanged since 1965.

In the modified Monier-Williams method, SO<sub>2</sub> is released from sulphites and some bound compounds when a sample is mixed with an acid—normally hydrochloric acid, but sometimes phosphoric acid—and heated. The SO<sub>2</sub> is distilled using a stream of nitrogen gas which carries the gaseous SO<sub>2</sub> into an absorbing solution of hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) where it is oxidised to sulphuric acid. The amount of SO<sub>2</sub> distilled into the H<sub>2</sub>O<sub>2</sub> is determined by titration with 0.1M sodium hydroxide. 1 mL of 0.1M NaOH is equivalent to 3.203 mg SO<sub>2</sub>.

AOAC states that the modified Monier-Williams method is applicable in the presence of some volatile sulphur compounds. It is not applicable to dried onions, leeks, cabbage and certain other vegetables however, because they naturally contain dimethyl-disulphide, methanethiol and other interfering volatile sulphur-containing compounds which cause the method to give high apparent SO<sub>2</sub> values. In these vegetables, attempts to distinguish between naturally occurring sulphite and added sulphite by modifying the method have been reported as unsuccessful.

Although it is time consuming, the modified 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 method measures free sulphite in food samples plus a reproducible portion of the bound sulphites, such as the carbonyl addition products.

AOAC method 962.16 is most suitable for testing samples that contain at least 45 mg of SO<sub>2</sub>. (A 50 g sample containing 900 mg/kg should yield 45 mg.) It may not be suitable for samples containing less than around 3 mg, i.e. for 50 g samples with less than 60 mg/kg.

For meat samples, the modified Monier-Williams method (AOAC Official Method 962.16) has been reported as giving false positive values of 10–15 mg/kg, sometimes higher.

### Optimized Monier-Williams method

In 1986, the US Food and Drug Administration (FDA) issued a final rule requiring that sulphites be declared on the label of any food containing detectable (10 mg/kg) amounts of sulphite. As a result of the FDA action, more accurate measurement of sulphites at the 10 mg/kg level in foods became necessary, than was possible with AOAC Method 962.16. The steps in the modified Monier-Williams procedure where FDA recommended tighter specification are summarised in Table 1. The method was adopted by AOAC as Official Method 990.28.

Table 1. Conditions for Monier-Williams method as used in FDA laboratories to permit quantitation of sulphites at 10 mg/kg in foods (Source: Fazio and Warner, 1990).

	Modified Monier-Williams (AOAC method 962.16)	FDA optimized (AOAC method 990.28)
Sample mixed with acid	✓	✓
Oxygen scrubber	Pyrogallol	GLC-type
Purge gas flow rate	Not specified	200 cm/min
Condenser length	Not specified	30 cm
Coolant temperature	Not specified	5°C
End of 1.75 hours condenser	Hot	5°C
Titration	0.1 M NaOH	0.01 M NaOH

The risk of interfering substances reaching the H<sub>2</sub>O<sub>2</sub> trap by aerosolisation, co-distillation or steam distillation is reduced if the optimized procedure is followed because values are specified for the condenser coolant temperature, reflux ratio (condenser length), and nitrogen flow.

Procedures that do not use the H<sub>2</sub>O<sub>2</sub> trap have been suggested, but they offer no significant advantages as long as the trap is titrated immediately—as specified in the original Monier-Williams method. This is because the oxidation of sulphur-containing substances to form sulphuric acid is slow relative to the rate of oxidation of sulphur dioxide.

### Rapid strip test

Sulphite test strips (Merckoquant strips, Merck Pty Ltd) are available commercially. Briefly, 10g of minced meat is mixed in 20 mL of water, filtered and the resulting filtrate is adjusted to pH 12 with 1 M NaOH. A strip is dipped into the filtrate and examined against a colour scale to give an approximate value. They can be used, and have been used, by concerned individuals; however, the manufacturer acknowledges that this method can have interference from the cysteine or glutathione in the meat which will give a false positive.

### Reasons for SO<sub>2</sub> detection

If the concentration of SO<sub>2</sub> detected in meat (as distinct from meat products containing garlic or certain other ingredients) is greater than, say, 30 mg/kg, it is almost certainly because of addition of sulphites. Detections at lower levels may also be due to addition of sulphites, but the modified Monier-Williams method has been reported as giving blank values of 10–15 mg/kg, sometimes higher. Are there explanations other than addition of sulphites for these detections of low concentrations?

### Sulphur compounds in meat

Brassica (cabbage, broccoli, and turnip) and allium vegetables (onions, leeks, chives, garlic, shallots) and isolated soy protein are examples of foods with natural substances that hydrolyse during the Monier-Williams method 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 is not involved.

Sulphur is present naturally in meat. The sulphur-containing amino acid cysteine is an important amino acid and believed to have a role in iron absorption in humans. 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 beef muscle tissue in amounts variously reported to range from 500 to 800 mg/kg.

Can these compounds be converted to compounds that are the same as, or similar to, those in cabbages and onions? There are various studies that indicate this can happen. One early British study indicated that while samples of veal and mutton tested fresh had very low levels of SO<sub>2</sub>, samples stored for nine days or longer contained appreciable quantities of 'apparent' SO<sub>2</sub>. After that period the number of spoilage bacteria would have been high.

It appears possible that proteins or other sulphur-containing compounds inherent in meat may give rise to compounds which assay as SO<sub>2</sub> and may lead to false detections of added SO<sub>2</sub>.

Several species of food-borne bacteria are capable of producing sulphur-containing compounds. There are several reports of bacteria isolated from vacuum packs of meat and similar systems that are capable of metabolising the sulphydryl, disulphide and other sulphur-containing groups 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. The presence of all these compounds in onions and brassicas (cabbage, broccoli, and

turnip) has also been reported and probably contribute to the 'SO<sub>2</sub>' detected in these crops.

Vacuum-packaged (VP) chilled meat has a considerably longer shelf life than fresh meat stored in the air. The ultimate shelf-life of VP meat is determined by spoilage—often caused by the microbial population that develops within the pack. Thus, the increased use of VP-stored topsides and other meat for mince means that there is an increasing need to determine whether low levels of SO<sub>2</sub> in test samples can be attributed to the metabolism by bacteria of sulphur-containing components inherent in the meat. An investigation has begun.

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<sub>2</sub>.

### Trouble shooting

As stated earlier, very few foods not treated with sulphite give positive test results, calculated as sulphur dioxide, even at levels below 10 mg/kg. There are known exceptions however. Fresh untreated onions and cabbage typically give responses in the range of 3–20 mg/kg sulphur dioxide, while isolated soy protein and garlic assay at around 30 and 200 mg/kg respectively.

Where the level in the product is expected to be low (0–50 mg/kg) the AOAC Official Method 990.28, i.e. the 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. Monier-Williams and others stress that during the tests, effective condensers are necessary to limit the carryover of compounds other than SO<sub>2</sub> during refluxing. A coolant temperature of 5°C is desirable and is specified by FDA; AOAC Method 990.28 specifies 15°C or colder.

In situations where there is a possibility that detected SO<sub>2</sub> is attributable to sulphur-containing volatile and non-volatile compounds, Monier-Williams and others suggest the gravimetric estimation of SO<sub>2</sub> as barium sulphate after titration of the sulphuric acid in the H<sub>2</sub>O<sub>2</sub>. For both the titration and the precipitation of barium sulphate, the H<sub>2</sub>O<sub>2</sub> should be cold. The titration should be done as soon as the refluxing step is completed. AOAC methods 962.16 and 990.28 include gravimetric estimation as an option.

If a processor gets a positive sulphur dioxide reading and they believe that sulphites have not been added to the meat product, then they should make sure that the analytical laboratory test was conducted using the optimized Monier-Williams method, and that flavour ingredients such as onions and garlic are not the source of the positive reading.

### Further reading

Literature Review: Can sulphur-containing compounds inherent in meat affect test results for added sulphites in meat? Meat & Livestock Australia Project Number RMISCC.001.

Fazio, T. and Warner, C. R. (1990) A review of sulphites in foods: analytical methodology and reported findings. Food Additives and Contaminants 7(4): 433–454.

*The information contained herein is an outline only and should not be relied upon in place of professional advice on any specific matter.*

## Contact us for additional information

Meat Industry Services is supported by the Australian Meat Processor Corporation (AMPC) and Meat & Livestock Australia (MLA).

**Brisbane:**

Food Science Australia  
PO Box 3312  
Tingalpa DC QLD 4173

Ian Eustace

**T** +61 7 3214 2117

**F** +61 7 3214 2103

**M** 0414 336 724

Neil McPhail

**T** +61 7 3214 2119

**F** +61 7 3214 2103

**M** 0414 336 907

**Sydney:**

Bill Spooner  
PO Box 181  
KURMOND NSW 2757

**T** +61 2 4567 7952

**F** +61 2 4567 8952

**M** 0414 648 387

**Adelaide:**

Chris Sentance  
PO Box 178  
FLAGSTAFF HILL SA 5159

**T** +61 8 8370 7466

**F** +61 8 8370 7566

**M** 0419 944 022