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Determination of free, bound and total sulphites in foods by indirect photometry-HPLC

Laura Pizzoferrato,* Giuseppe Di Lullo & Enrica Quattrucci

Istituto Nazionale della Nutrizione, via Ardeatina 546, 00178 Roma, Italy

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Sulphur dioxide and sodium, potassium, calcium salts of hydrogen-sulphite, disulphite and sulphite ions are extensively used as food additives for their technological efficacy and versatility. They are active as antimicrobial agents, enzyme inhibitors, antioxidants, structure modifiers, in the control of enzymatic and non-enzymatic browning reactions with stabilising and conditioning functions. In this study a modified Monier-Williams method has been utilised as a preparative procedure to obtain both the free and bound sulphite fractions. The two fractions have been analysed by HPLC with indirect photometric detection using a 250×4.6 mm LC-SAX column eluted with a solution of potassium hydrogen phthalate. Levels of 5–10 ppm of SO_2 in foods, corresponding to 30–60 ng injected are reliably detected by this method. The results confirm that the chromatographic method, unlike the Monier-Williams method, is able to avoid the potential interference of volatile substances derived from matrices or utilised chemicals. © 1998 Elsevier Science Ltd. All rights reserved.

INTRODUCTION

Sulphiting agents (Table 1) act as antimicrobial agents, enzyme inhibitors, antioxidants, in the control of enzymatic and non-enzymatic browning reactions with stabilising and conditioning functions (Walker, 1985) and for these characteristics they are widely used in foods such as dried fruits, dehydrated vegetables, biscuits, jellies, mustard and wine.

The useful properties of sulphiting agents are generally due to the nucleophilicity of the sulphite ion that may react by addition to carbonyl groups, carbon–carbon double bonds, quinones, heterocyclic nitrogen compounds or by cleaving disulphite bonds (Wedzicha, 1992). They are chemically equivalent compounds in foods since they are converted to the same ionic or non-ionic species at a given pH, ionic strength and non-electrolyte concentration.

The sulphite utilisation for technological purposes undergoes specific norms and in 1995 the European Community issued a directive reporting a detailed list of foods to which sulphited can be added with the relevant maximum permitted sulphite levels (EC Council Directive, 1995).

Since 1959 sulphiting agents have been listed in the US *Code of Federal Regulations* (CFR) as GRAS (generally recognised as safe) when used in accordance with good manufacturing practice, except that in meats or in foods recognised as a source of vitamin B_1.

In 1986–1988 the Food and Drug Administration made it illegal to add sulphiting agents to fresh fruits and vegetables intended to be sold in the raw state and required that sulphites be declared on the label of any food containing 10 ppm sulphites (Federal Register, 1986a,b). The accurate measurements of sulphites at very low level in foods then became a critical issue and various analytical approaches followed to quantify such a low level of sulphites in foods (Fazio and Warner, 1990).

In fact the GRAS status came under question when, in the early 1980s, ingestion of sulphiting agents was linked to severe reactions among asthmatics (Congressional Hearing on Sulphites, 1985).

Sulphites are also known to present some cytotoxic, mutagenic and antinutritional effects (Stammati *et al.*, 1992). In particular they interact with some vitamins, i.e. pyridoxal, nicotinamide, thiamine, folic acid, reducing the nutritional quality of treated foods (Pizzoferrato *et al.*, 1988).

On the other hand they can be oxidised to sulphate, an innocuous product, or volatilised and lost as SO_2 at pH <4 (Wedzicha, 1992).

Because of this, a distinction is often made between free and bound sulphite; the former refers to all the species that may rapidly and quantitatively be converted to SO_2, thereby acidifying a treated food; the latter

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*To whom correspondence should be addressed.

Table 1. The sulphiting agents

Sulphur dioxide (E220)
Sodium sulphite (E221)
Sodium bisulphite (E222)
Sodium metabisulphite (E223)
Potassium metabisulphite (E224)
Calcium sulphite (E226)
Calcium bisulphite (E227)
Potassium bisulphite (E228)

represents hydroxysulphonate adducts formed by reaction of carbonyl groups with HSO_3^-. In fact, according to the chemical nature of food, the type and extent of the technological processes, and the conditions and time of storage, sulphites react with reducing sugars, aldehydes, ketons, proteins to form combined sulphites. These adducts are released *in vitro* only when acidified food solutions are boiled, but since they are reported to be also decomposed *in vivo* in the digestive tract (Wedzicha, 1992) it is necessary to quantify this bound fraction for purposes of legislation, nutrition and food safety.

In this research a modified (Quattrucci and Di Lullo, 1986) Monier-Williams method (Monier-Williams, 1927) has been utilised both as an analytical and a preparative tool to obtain free and bound sulphite fractions. Afterwards, the two fraction amounts have also been separately confirmed by a chromatographic method with indirect photometric detection (Pizzoferrato *et al.*, 1990).

The studied procedure, compared with the reference Monier-Williams method, has been tested in a number of different food matrices with particular reference to the sulphite distribution between the free and bound forms in grape must and potatoes.

MATERIALS AND METHODS

Chemicals

All reagents (Carlo Erba, Milano, Italy) were of analytical or HPLC grade as required. Hydrogen peroxide and sodium hydrogen sulphite 30% were obtained from Merck (Darmstadt, Germany).

Equipment

Chromatography was performed with an analytical HPLC system comprising a Waters model 510 solvent delivery system, a Gilson model 231-401 autosampling injector and a Waters Model 490 programmable multiwavelength specrophotometer. Results were elaborated by means of a Waters Millennium chromatography system.

A 250×4.6 mm, 5 μm Supelcosil LC-SAX column, maintained at room temperature was eluted with a solution of potassium hydrogen phthalate (0.15 g litre^{-1}, pH 5.7) at a flow rate of 3 ml min^{-1}. Analytes were detected at 280 nm by a Waters Model 490 programmable multi-wavelength spectrophotometer reversing the recorder polarity to obtain a positive peak.

Sample preparation and analysis

The traditionally adopted Monier-Williams method (Monier-Williams, 1927) is based on an acid distillation followed by vapour phase transfer of the SO_2, facilitated by a carrier gas stream, to an oxidising trapping solution. The sulphite content is then determined by tritation or gravimetrically as sulphate.

In this research a modified (Quattrucci and Di Lullo, 1986) Monier-Williams method was used as an analytical and preparative procedure to obtain both the free and bound sulphite fraction. A suitable amount of liquid or homogenised solid samples or suitable volumes of standard were put into a three necked round bottom flask and acidified with 5 ml of 37 %HCl. Two recovery flasks, respectively for free and bound sulphite, were prepared adding 5 ml of a 3% hydrogen peroxide solution with a few drops of methyl red and methylene blue indicator and adjusting the colour to light green with 0.01 N sodium hydroxide.

Nitrogen was flushed into the three-necked flask containing the sample through a capillary tube at a flow rate of 250 ml min^{-1} for 30 min to collect the free sulphite fraction and at 50 ml min^{-1} for 15 min to collect the bound sulphite. In the first part of the experiment (free fraction), the sample temperature was controlled by an ice bath, in the second part (bound fraction) the flask was heated and maintained at a gentle boiling.

The two collected fractions were titrated with 0.01 N sodium hydroxide to the initial green colour and the amount of sulphite was calculated and expressed as sulphur dioxide to complete the Monier-Williams procedure.

The distilled solutions, already neutralised for titration purposes were diluted, injected into the HPLC and detected by indirect photometry (Pizzoferrato *et al.*, 1990) as described above.

Total sulphur dioxide can be determined mathematically as a sum of the free and bound fractions or analytically by direct acid distillation at high temperature, without the step of distillation in ice.

RESULTS AND DISCUSSION

The chromatographic system performance has already been reported in a previous paper (Pizzoferrato *et al.*, 1990) which refers to total sulphur dioxide determination in foods. To summarise, the peak area values are linearly correlated ($r^2 = 0.9997$) to the additive concentration in the considered range from 0 to 300 ng SO_2 injected. The detection limit, represented by the analytical noise plus three times the standard deviation, corresponds to a value of 0.42 ng of SO_2 on the calibration curve. Therefore level of 5–10 ppm of SO_2 in foods, corresponding to 1.5–3.0 ppm in the final solution or to 30–60 ng in a 20 μl injection, are reliably detected.

As an example, Fig. 1 shows the recovery from different food matrices of a known amount of added bisulphite, distilled at high temperature by the Monier-Williams procedure, evaluated titrimetrically or by the HPLC method, and expressed as ppm of total sulphur dioxide on an 'as is' basis.

Comparable results are obtained with beer, brussel sprouts, grapefruit juice and prunes. Differences in total sulphite levels of anchovy paste, potato and sugar samples are not statistically significant (t-test, $p > 0.05$). The sulphur dioxide content is more reliably evaluated by the HPLC method in shrimps, dried onions, wine and other foods containing volatile compounds of an acidic nature that may positively interfere in the titrimetric Monier-Williams method. In fact, volatile organic sulphur compounds and inorganic or organic acids are liable to be co-distilled interfering with the final Monier-Williams titration.

With the aim of extending the method applicability to the determination of the free and bound fractions of sulphite present in foods, and to understand better the hypothesized overestimation in the Monier-Williams methodology, grape must and mashed potatoes have been added with known amounts of bisulphite and analysed repeatedly in order to follow over time the fate of this additive and its distribution into the free and bound forms in foods.

In Figs 2 and 3 the sulphite distribution between the free and the bound forms in potato and grape must samples, added with an amount of bisulphite corresponding to 100 ppm of sulphur dioxide, is reported as a function of time following the addition.

The total level of sulphur dioxide analysed even immediately after the addition is lower than the actual added level (100 ppm). In fact, particularly in a semi-solid sample such as mashed potatoes, volatile sulphur dioxide is lost during mechanical homogenisation and the residual level is nearly 60% in potatoes and 80% in grape must.

The free form levels, evaluated at a fixed time by the two methods, do not show significant differences due to the analytical temperature (ice bath) which is unfavourable to the co-distillation of interfering compounds. On the whole, the free form decreases with time in consequence of the volatilisation of sulphur dioxide and the

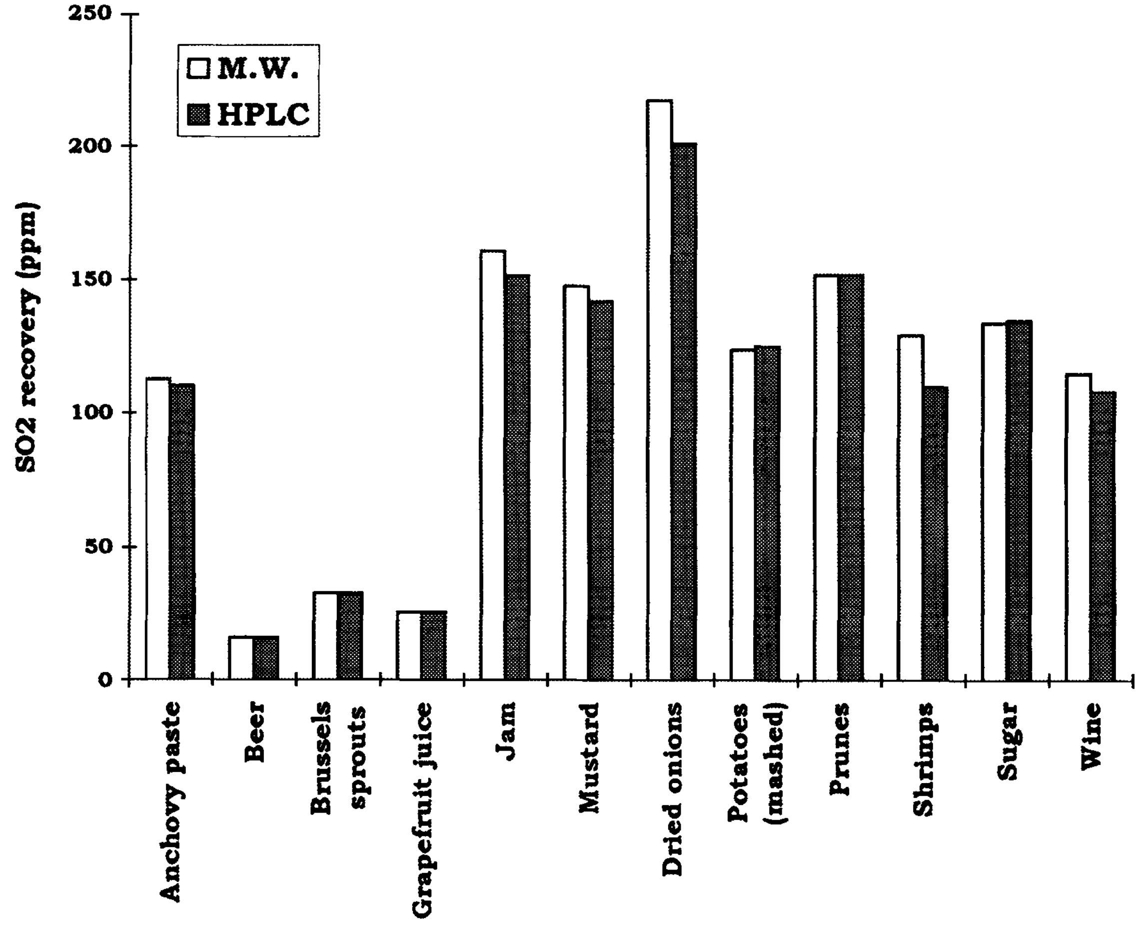

Fig. 1. Recovery of added sulphite from food samples: comparison between Monier-Williams (M.W.) and chromatographic (HPLC) methods. Values are expressed as ppm of sulphur dioxide.

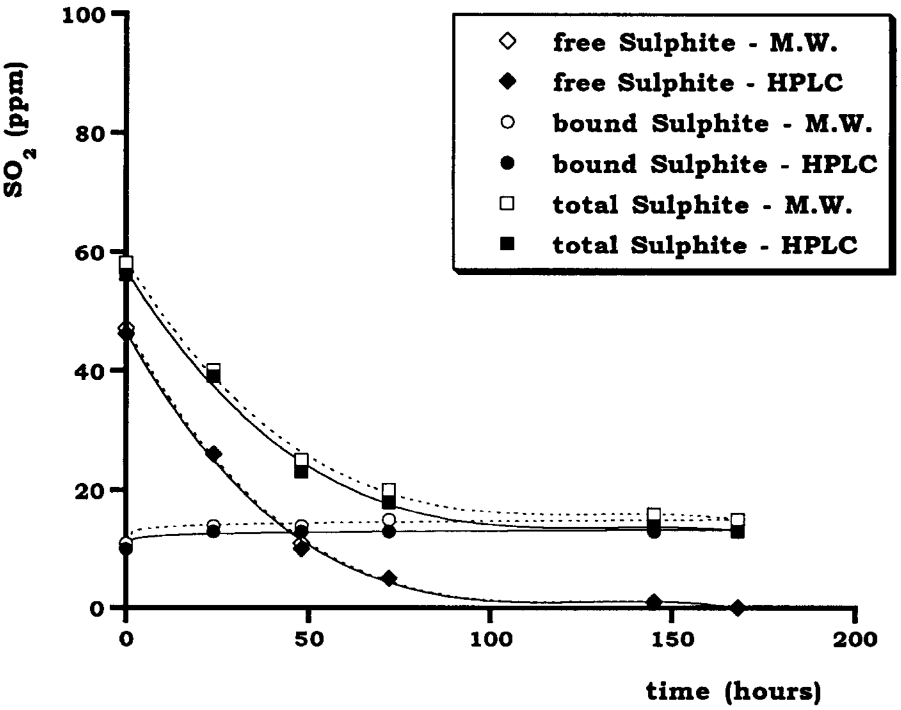

Fig. 2. Sulphur dioxide distribution between the free and bound forms in mashed potatoes added of known amount of bisulphite corresponding to 100 ppm of sulphur dioxide.

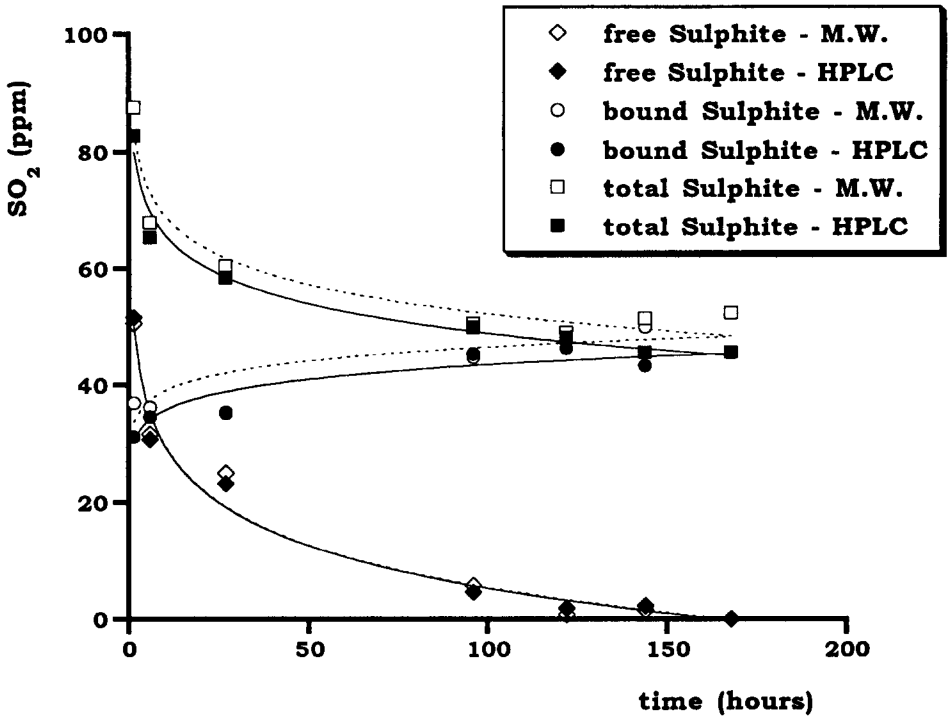

Fig. 3. Sulphur dioxide distribution between the free and bound forms in grape must added of known amount of bisulphite corresponding to 100 ppm of sulphur dioxide.

increase of the bound fraction. Particularly evident is the increase of the bound form in the grape must sample, a rich source of aldehyde and keton groups.

Comparing the two methodologies, bound form levels evaluated by the Monier-Williams procedure after heating, are confirmed to be affected, particularly in grape must, by interferences due to co-distilled volatile anions. As a consequence, total sulphite, calculated as the sum of free and bound fractions, are overestimated by the Monier-Williams method.

This over-estimation of the sulphite levels in foods can be avoided by using a separative method of analysis such as the proposed HPLC method that, unlike the Monier-Williams analysis, avoids the potential interference of volatile substances other than sulphur dioxide, derived from matrices or from utilised chemicals.

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