

Aspects of the Analysis, Role, and Fate of Sulphur Dioxide in Beer - A Review

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ABSTRACT

Sulphur dioxide is present in all beers. It is produced by yeast and is sometimes added during the brewing process, or to beer. This review considers the role of SO_2 in masking stale flavors and in protecting beer from oxidation and microbial spoilage. The fate of SO_2 during beer storage and the analysis of SO_2 in beer are discussed. Alternatives to SO_2 are evaluated.

Key words: sulphur dioxide, sulphite, analysis, stale flavor

THE DIFFERENT FORMS OF SULPHUR DIOXIDE

In dilute aqueous solution, sulphur dioxide exists in several forms ($\text{SO}_2 \cdot \text{H}_2\text{O}$; HSO_3^- ; SO_3^{2-}) depending on the pH of the solution (Fig. 1). At low pH the undissociated form of SO_2 is weakly bound to water and is not in the free acid form, as might be expected.⁽⁶⁹⁾ At the usual pH of beers (3.8 - 4.4), most of the SO_2 is present as HSO_3^- (the bisulphite or hydrogensulphite anion). In this review the terms sulphur dioxide and sulphite will be used interchangeably to represent all forms of SO_2 .

"Free SO_2 " in beer includes gaseous SO_2 , $\text{SO}_2 \cdot \text{H}_2\text{O}$, HSO_3^- and SO_3^{2-} . "Bound SO_2 " includes that bound reversibly to compounds such as aldehydes, ketones and sugars. An equilibrium exists between free and bound SO_2 . Total concentrations of SO_2 in beer typically range from < 1 to 30 mg/l, but occasionally higher concentrations are found.

The flavor detection threshold of SO_2 in beer is approximately 20 mg/l.⁽⁴⁶⁾ At higher concentrations (e.g. >30 mg/l) it can adversely affect beer flavor, giving rise to undesirable flavors.⁽¹⁴⁾

Sulphur dioxide has three main actions in beer. Firstly, it reduces the rate of oxidation, causing a reduction in the rate of development of oxidation haze and stale flavors. Secondly, it forms adducts with carbonyl compounds to form α -hydroxy-sulphonates, thus limiting the flavor impact of any stale flavor due to these compounds.⁽⁵²⁾ Thirdly, at high concentrations, SO_2 has antimicrobial properties. The undissociated form ($\text{SO}_2 \cdot \text{H}_2\text{O}$) is the most potent bacteriostat, but HSO_3^- can still be effective when "free." In many modern beers the ability to protect against spoilage by yeast and bacteria is weak,⁽³¹⁾ because although the SO_2 is present as HSO_3^- at typical beer pH most of this is "bound."

SINTESIS

El dióxido de sulfuro esta presente en todas las cervezas. Es producido por la levadura y algunas veces anadido durante el proceso, o en la cerveza. Este resumen considera el rol del dióxido de sulfato en enmascarar sabores viejos y en proteger a la cerveza de la oxidacion y el estropeo mrocobial. Se discute el fuerte del dióxido de sulfato durante el almacenamiento de la cerveza y el analisis del dióxido de sulfato en la cerveza. Alternativas al dióxido de sulfato son evaluadas.

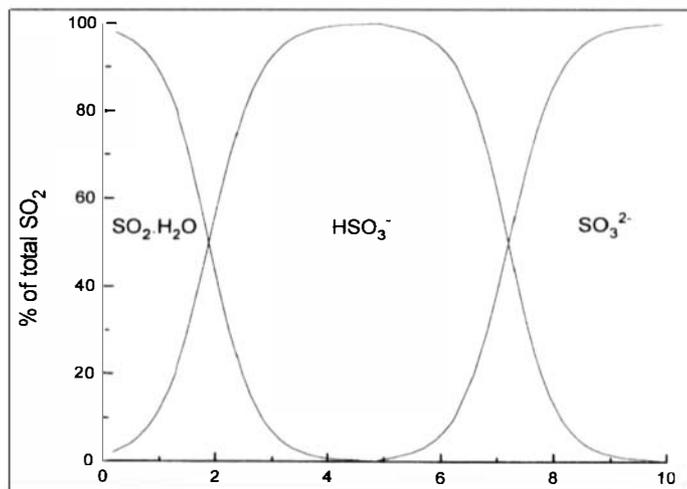


Fig. 1
The effect of pH on the equilibria of SO_2 species in aqueous solution

SOURCES OF SO_2 IN BEER

Table 1 shows the uses of SO_2 in the brewing industry. Sulphur dioxide can be derived from yeast metabolism, from addition of sulphiting agents (Table 2), or as a component of finings or primings.

Sulphur dioxide is used as a preservative for several raw materials and processing aids. During an investigation of the contribution of sulphited hops to the SO_2 content of beer, Klopfer⁽⁴¹⁾ found that the hop rate he used corresponded to an SO_2 dosage of 3 - 4 mg/l, but that this was almost entirely lost during wort boiling. Sulphur dioxide can be used as a preservative in syrups made from starch (2 - 40 mg/l). Sulphur dioxide

TABLE 1
Uses of SO₂ in the Brewing Industry

Stage of Beer Production	Use of SO ₂
malt kilning	control N-nitrosodimethylamine (NDMA) formation, bleach malt by burning sulphur
kilning of hops	bleaching agent, preservative
storage of syrups	preservative
storage of finings	preservative
fermentation vessels	antimicrobial agent
proteolytic enzymes	preservative
beer additive	antioxidant, preservative

can also be added indirectly to beer during processing since it is used as a preservative in isinglass finings.⁽²⁹⁾ Finings are stored at breweries with up to 500 mg/l sulphite. At typical finings dosing rates (1 part finings per 100 parts beer) this gives up to 5 mg/l in the final beer.

Some strains of *Saccharomyces cerevisiae* produce about 10 - 30 mg SO₂/l during the synthesis of the sulphur-containing amino acids, cysteine and methionine, from sulphate (Fig. 2).⁽¹⁸⁾ Lager strains generally produce more SO₂ than do ale strains.⁽³²⁾ Crumplen *et al.*⁽¹⁵⁾ used 12 different yeast strains and found that the ale yeasts produced less than 2 mg/l, while the lager strains produced more than 4 mg/l.

Sulphate is converted to APS (adenosine-5'-phosphosulphate) by the enzyme ATP-sulphurylase. APS is then converted to PAPS (3'-phosphoadenosine-5'-phosphosulphate) which gives sulphite and ADP. Sulphite reductase reduces the sulphite to sulphide which is used to produce amino acids. This pathway is controlled by feedback inhibition of the ATP-sulphurylase.⁽⁷⁾ If more sulphite is produced than is needed for amino acid synthesis, the excess is released into the beer.

Many factors influence sulphite production by yeast, e.g. pitching rate, fermentation temperature, wort pH, wort strength, pressure, trub content and wort aeration. Brewer and Fenton⁽⁷⁾ showed the optimum temperature for SO₂ formation by one strain of *Sacch. cerevisiae* to be about 16°C. Sulphur dioxide is first detected 15 - 20 hours after pitching. Sulphur dioxide formation is also increased by using high wort pH, low wort oxygenation or a low yeast pitching rate.⁽⁵¹⁾

TABLE 2
Chemicals Used as Sulphiting Agents

Chemical	E Number
sulphur dioxide (sulphurous acid)	E220
sodium sulphite	E221
sodium hydrogensulphite	E222
sodium metabisulphite	E223
potassium metabisulphite	E224
calcium sulphite	E226
calcium hydrogensulphite (bisulphite)	E227

Yeasts are able to reduce carbonyl compounds to alcohols during fermentation. However, if the carbonyls are bound to SO₂ this may protect them from being reduced.⁽¹⁷⁾ For instance, if large amounts of SO₂ are produced during fermentation this can result in a higher carbonyl content in the fresh beer in the form of carbonyl-bisulphite adducts. Carbonyls are then released as the SO₂ reacts with other beer components.⁽¹⁷⁾ It has been suggested that it may be better to use a sulphiting agent after fermentation to minimize the quantity of aldehydes present, rather than encourage production of SO₂ by yeast. Low alcohol beers generally have yeast-derived SO₂ levels that are too low to offer much protection against carbonyls.⁽⁵¹⁾

Some of the materials used as sulphiting agents are shown in Table 2. The rate of addition of SO₂ for beer stabilization, legislation permitting, is usually in the range of 10 - 25 mg/l⁽⁴⁴⁾, most beer contains < 10 mg/l.

There are problems associated with the addition of SO₂ to beer. Overuse can lead to sulphury off-flavors, and addition of excessive SO₂, prior to wort boiling, can have a detrimental effect on beer foam.^(6,14)

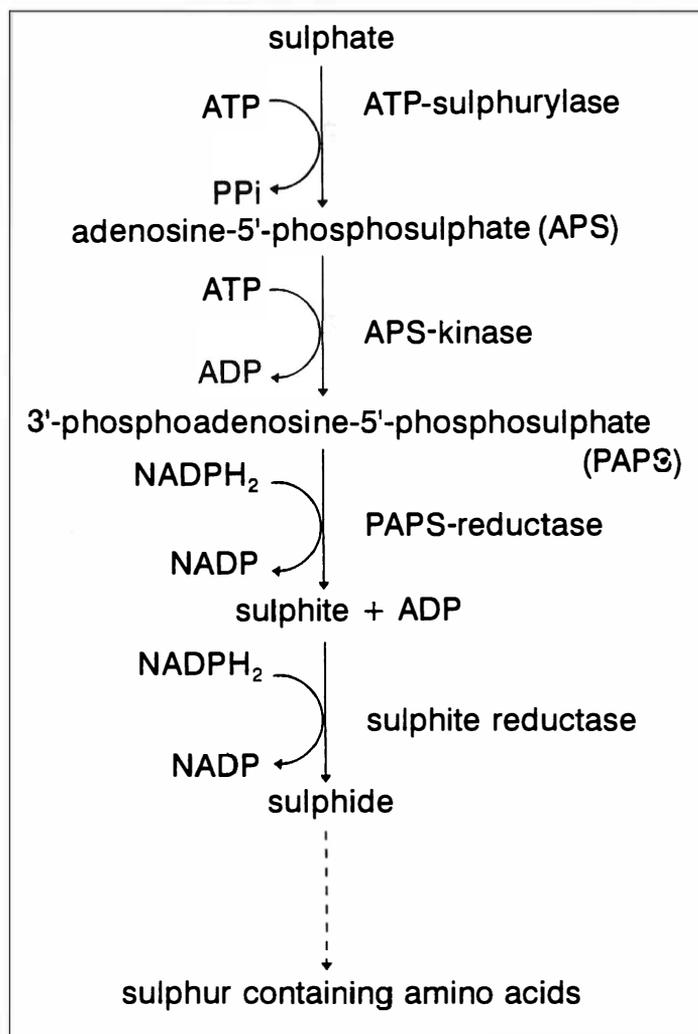


Fig. 2

The formation and use of SO₂ in yeast metabolism (ATP = adenosine triphosphate, ADP = adenosine diphosphate, NADP = nicotinamide adenine dinucleotide phosphate, PPi = inorganic pyrophosphate)

ANALYSIS OF SO₂ IN BEER

The ideal method for analysis of SO₂ in beer should (i) allow reliable determination of SO₂ at levels typically encountered in the product; (ii) be rapid and not labor-intensive; (iii) allow measurement of total and/or free SO₂; (iv) be inexpensive, and (v) not present any safety hazards.

Analysis methods can be split into direct and indirect methods. Indirect methods are those that require separation of SO₂ prior to analysis (e.g. distillation procedures).

The European Brewery Convention (EBC) currently recommends three methods: the Monier-Williams distillation method, a Spectrophotometric method using dithiobisnitrobenzoic acid (DTNB), and an enzymic procedure employing sulphite reductase.⁽²⁰⁾ The American Society of Brewing Chemists (ASBC) recommends a colorimetric method using *p*-rosaniline.⁽²⁾ The Institute of Brewing (IOB) recommends a distillation procedure based on the Monier-Williams method and also the *p*-rosaniline method.⁽³⁵⁾

Distillation methods

Distillation methods are among the most widely used procedures for analysis of SO₂ in food and beverages. Most are adaptations of the Monier-Williams⁽⁴⁸⁾ distillation to convert the bisulphite ion to the more volatile SO₂.H₂O. The solution is refluxed and released SO₂ is trapped and simultaneously converted to H₂SO₄ by reaction with H₂O₂. The H₂SO₄ produced is titrated against NaOH. This method has the advantage of simplicity and accuracy, but the distillation step can take an hour or more. Consequently it is not suited to situations in which a rapid turnover of samples is required.

Modifications include the use of *o*-phosphoric acid instead of HCl and addition of methanol to the sample prior to distillation to lower the reflux temperature.⁽⁷⁰⁾ Analysis time can be reduced using a downward condenser and titrating the H₂SO₄ with alkali. However, these modifications can also lead to co-distillation of aldehydes during the analysis. These aldehydes can bind to SO₂ in the distillate, leading to analysis errors. Interferences due to co-distillation of other reducing compounds have also been encountered. A rapid distillation method was recommended by the IOB for rapid quality control purposes (method 8.2.2), but it is no longer considered to be suitable because of its poor precision.⁽⁵⁾

Iodometric methods

Iodometric titrations have long been used for pale or uncolored foods/beverages. The earliest published method is that of Ripper.⁽⁵⁶⁾ Free and total SO₂ can be measured by careful pH adjustment, but interferences can occur due to other reducing materials in foods. Also at low concentrations (<32 mg/l SO₂) the speed of the reaction between iodine and SO₂ is very slow and causes the end point to be blurred.⁽¹³⁾ An alternative to visual determination of the end point is to use an electrometric procedure.⁽³⁴⁾ This also permits colored samples to be analyzed.

Spectrophotometric methods

Many of the direct spectrophotometric methods used to analyze SO₂ in foods and beverages are based on the reaction between SO₂, *p*-rosaniline and formaldehyde (Fig. 3). Originally fuchsin (a less pure form of *p*-rosaniline) was used in a test for

aldehydes.⁽⁶¹⁾ Steigman⁽⁵⁹⁾ modified the reaction conditions to measure sulphites, exploiting the reaction of the sulphite-fuchsin complex with formaldehyde. This dye produced in the reaction is acid resistant. Stone and Laschiver's method⁽⁶¹⁾ for SO₂ in beer has the advantage that it can measure both free and bound SO₂. The procedure relies on the ability of tetrachloromercurate (II) ions to bind to sulphite released by exposure of the samples to alkali, thus preventing the sulphite from recombining with carbonyls. The total SO₂ concentration can then be determined. Absorbance of the sample is measured at 500 nm and the SO₂ concentration is derived from calibration graphs. Drawbacks of this method include the fact that *p*-rosaniline is a potential carcinogen and that mercury is toxic.^(43,73) However, this has not prevented the method from being widely used as it is precise, allows measurement of free and total SO₂, and it can be automated.

Another spectrophotometric method uses DTNB [Dithiobis(2-nitrobenzoic acid)] as the color reagent after a distillation step. It was originally developed to analyze ginger ale.⁽⁷²⁾ It has been adapted as a recommended method for total SO₂ in beer (0 - 20 mg/l) by the EBC.⁽²⁰⁾ Sulphur dioxide is distilled from acidified 25 ml samples into a buffered DTNB solution, with a nitrogen carrier gas. The absorbance is measured at 415 nm.

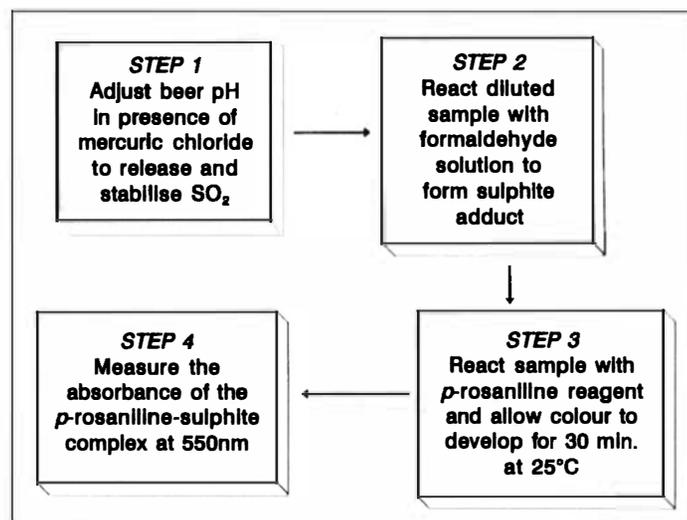


Fig. 3

Scheme for the colorimetric analysis of SO₂ using *p*-rosaniline

Flow Injection Analysis

In a flow injection analysis system the sample is injected into a carrier stream which merges and mixes with a reagent stream. The resultant reaction products are quantified using a flow-through detector (Fig. 4). Flow injection analyses offer several advantages over manual methods. Greater numbers of samples can be run for the same degree of operator effort. In addition, there is less operator contact with hazardous chemicals.

Most flow injection analyses are adaptations of spectrophotometric methods. In one system, the sample is injected into the carrier stream which contains NaOH to release bound sulphites. The pH is lowered with H₂SO₄ and the SO₂ released from the sample is carried to a gas diffusion membrane which it crosses, leaving the larger (usually colored) molecules behind. The SO₂ is then reacted with malachite green or *p*-rosaniline to give a

colored product which is measured using a spectrophotometer.⁽⁸⁾ A flow injection analysis system has also been described which allows analysis of sulphite in wine using an immobilized sulphite oxidase reactor.⁽⁴⁵⁾ Another system uses electrochemical detection with a specially-treated, glassy carbon electrode.⁽²³⁾

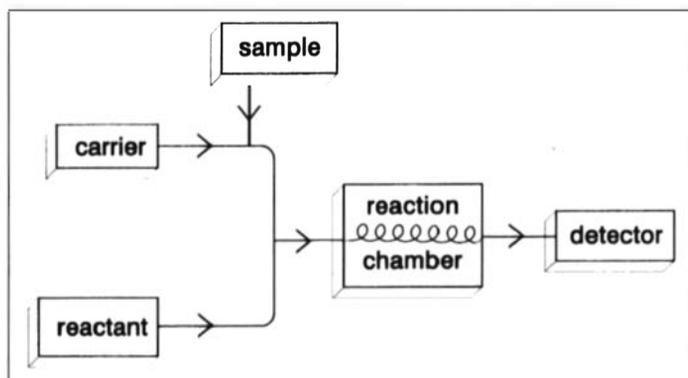


Fig. 4
A flow injection analysis system

Selective Electrodes

Selective Electrodes can be split into enzymic and non-enzymic systems, of which the enzymic types are most common. Enzymic electrodes are mostly based on the use of sulphite oxidase to catalyze oxidation of sulphite to sulphate.

Fassnidge and Van Engel⁽²¹⁾ evaluated an electrode for use in beer, which was based on the dissolved oxygen electrode and contained sulphite oxidase on the membrane. Sulphite was detected amperometrically as the oxygen in the sample was depleted during the oxidation process. The presence of yeast in the samples led to erratic results. Interference also resulted from the presence of either ascorbic acid or cysteine.

Other electrodes feature the use of gas-permeable membranes, through which SO_2 can pass into the filling solution (Fig. 5). Etherington⁽¹⁹⁾ compared the Tacussel ADS-1 with other methods of SO_2 analysis for wines and found it quicker and more accurate than the Ripper method (an iodometric titration).⁽⁵⁶⁾ In this electrode a 700 mV potential converts sulphite to sulphate. The current generated is proportional to SO_2 concentration. H_2S is the only other compound that is oxidized at this potential, but because it is present at low concentration in beer (typically $< 10\mu\text{g/l}$),⁽⁶⁴⁾ it causes little interference.

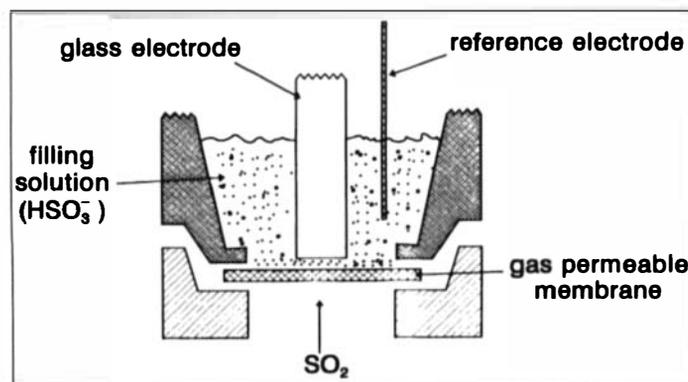
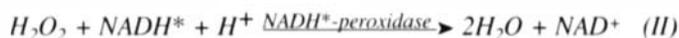


Fig. 5
A sulphite selective electrode

Enzymic determination

Boehringer-Mannheim produce a kit for total sulphurous acid (free and bound sulphite) determination which exploits the following reactions:



(*NADH = Nicotinamide Adenine Dinucleotide, reduced form)

The concentration of sulphite can be calculated from the changes in absorbance of NADH at 340 nm.

Jacobsen *et al.*⁽³⁶⁾ compared results obtained with the EBC distillation method with those obtained from this enzymic method. Beer samples were degassed and either analyzed directly or pretreated with bentonite to remove interfering substances. Absorbance values were measured after 30 minutes and thereafter every 5 minutes until a change in reaction rate occurred. Both treated and untreated samples were measured, and the results were calculated with and without correction (by graphical extrapolation) for interference reactions caused by other NADH-oxidizing compounds present in beer.

Gas chromatographic methods

Headspace analysis

Free and bound SO_2 can be measured by headspace gas chromatography using a flame photometric detector (FPD). In one procedure, bound SO_2 is released from solution by addition of alkali, then trapped using tetrachloromercurate (II) ions. The sample is then acidified to restore it to its original pH value. Such GC methods can be calibrated using a separately prepared standard curve,⁽⁴⁹⁾ or by using a sulphur-containing compound as an internal standard.

Chemiluminescence detection provides an alternative to FPD. Osborne⁽⁵³⁾ found that it was more sensitive than the available FPD and gave a linear rather than logarithmic response to the analyte.

Ion chromatography

Anderson *et al.*⁽³⁾ developed a method to analyze sulphite in foods which requires a 10-minute flash distillation. The SO_2 is reduced by phosphoric acid and is collected in an ice-cold trapping solution, consisting of 0.1M NaOH and 1 g/l formaldehyde. The samples are analyzed by ion chromatography, using electrochemical detection, to give a value for total SO_2 . Free SO_2 is measured by mixing the homogenized food directly with the trapping solution.

Problems can occur using ion chromatography in beer due to the high sulphate and low sulphite levels present.⁽⁵⁵⁾ Careful selection of chromatography conditions is needed to avoid interference problems.

Ion exclusion chromatography

These methods use a liquid chromatography system equipped with a strong anion exclusion column and electrochemical detection. The interferences associated with ion chromatography can be overcome using a mobile phase containing sulphuric

acid, so that the sulphite is present as $\text{SO}_2 \cdot \text{H}_2\text{O}$.⁽⁴⁰⁾ Frost⁽²⁵⁾ used a 25 mM sulphuric acid eluent with a total analysis time of 6 min per sample. D-Mannitol can be used to minimize oxidation of sulphite. Wagner and McGarrity⁽⁶³⁾ used pulsed amperometric detection to avoid the loss of detector sensitivity that occurs with time when direct amperometry is used. This occurs as the working electrode becomes contaminated and can lead to a loss of up to 40% sensitivity over an 8-hour period.⁽⁴⁰⁾

High performance liquid chromatography (HPLC)

Akasaka *et al.*⁽¹⁾ described a fluorometric method for determination of sulphite in wine using *N*-(9-acridinyl)-maleimide (NAM). The sulphite reacts with the NAM to give a fluorescent product. The fluorescence intensity at 436 nm is proportional to the sulphite content of the sample. The method can be adapted to provide an HPLC method. This method requires sample volumes of less than 100 μl . The time-limiting factor for both assays is the reaction between sulphite and NAM (2 h at 35°C for batch method or 30 min. at 50°C using HPLC). The HPLC determination takes 10 minutes.

Comparison of methods

There are many examples in the literature of method comparisons and it is usual when a new method is published to compare the results with those obtained using established methods, e.g. Monier-Williams distillation.^(8,37) In reviewing methodology for measuring SO_2 , Fazio and Warner⁽²²⁾ found little comparative data at, or around the legal limit, for SO_2 in beer in the USA. However, they also found that the development of methods was continuing.

The IOB Analysis Committee carried out a collaborative study involving eight laboratories in which four methods of SO_2 analysis (*p*-rosaniline, Monier-Williams, DTNB and IOB rapid distillation) were compared.⁽⁵⁾ Beers containing 1 - 40 mg/l SO_2 were included in the trial. The best precision was obtained using the *p*-rosaniline method. This was closely followed by the Monier-Williams distillation.

LEGAL STATUS OF SO_2 IN BEER

Permissible limits and purity criteria

Restrictions apply in many countries to the levels of SO_2 permitted in beer. The limits are set for total SO_2 (free and reversibly bound SO_2). Current limits are summarized in Table 3. A European Community directive on food additives sets limits of 20 mg SO_2 /l in beer and 30 mg/l in cask-conditioned beers. Generally, limits for ciders and wine are higher, e.g. 200 mg/l for cider and 450 mg/l for wine in the UK.⁽⁴⁷⁾ Sulphiting agents for use in beer must meet certain criteria of purity. Table 4 shows the purity requirements which apply in the USA.⁽²⁴⁾

Another additive that can increase the SO_2 levels in beer is sodium dithionite. This is added to beers in some countries as an antioxidant. It oxidizes in aqueous solution to give SO_2 , using a molecule of oxygen in the process.⁽⁶⁰⁾

TABLE 3
Legal Limits for Total SO_2 in Beers
(as of September 1994)

Country	Limit (mg/l)	Notes
UK ⁽¹⁾	70	-
USA	25	>10 mg/l must be labeled
Canada	15	-
Australia	25	-
New Zealand	25	-
Denmark ⁽¹⁾	20	-
France ⁽¹⁾	100	residual SO_2
	50	sulphurous acid and alkali sulphites
Ireland ⁽¹⁾	70	-
Italy ⁽¹⁾	20	not allowed as an additive
Luxembourg ⁽¹⁾	10	20mg/l for beer or original gravity not less than 15.5 degrees Plato
Netherlands ⁽¹⁾	10	20mg/l for beer of original gravity not less than 15.5 degrees Plato
Belgium ⁽¹⁾	10	20 mg/l for beer of original gravity not less than 15.5 degrees Plato

(1) will implement EC limits 20 mg/l.

TABLE 4
Purity Requirements for Sulphiting Agents in the USA

	Sodium bisulphite	Sodium metabisulphite	Sodium sulphite	Sulphur dioxide*
Purity	>58.5% and <67.4% SO_2	>90.0% $\text{Na}_2\text{S}_2\text{O}_5$	>95.0% Na_2SO_3	99.9% SO_2 by weight
Lead	ns	<10 mg/kg	ns	<10 mg/kg
Heavy Metals	<10 mg/kg	<20 mg/kg	<10 mg/kg	<30 mg/kg
(as Pb)				
Arsenic	<3 mg/kg	<3 mg/kg	<3 mg/kg	<3 mg/kg
Iron	<50 mg/kg	<20 mg/kg	ns	ns
Selenium	<30 mg/kg	<30 mg/kg	<30 mg/kg	<20 mg/kg

* Also non-volatile residue <0.05% by weight and H_2O <0.05% by weight.⁽²⁴⁾ ns = not specified.

Toxicological aspects

Free sulphites are not especially toxic to man ($\text{LD}_{50} = 65 - 2000$ mg sulphiting agent/kg body weight for various mammals).⁽⁶²⁾ α -Hydroxysulphonates usually decompose in the gastrointestinal tract, due to the low pH, to release SO_2 .⁽²⁷⁾

Sulphites can induce asthma in certain individuals (5 - 11% of asthmatics are affected). Physiological effects include anaphylactic shock, headaches, abdominal pains, nausea, dizziness and hives.⁽²²⁾ Ingestion of high levels of SO₂ has been implicated in the deaths of a small number of sulphite-sensitive asthmatics.⁽²²⁾

The acceptable daily intake (ADI) of SO₂ is up to 245 mg/person/day. The estimated intake of sulphite from beer is 0.38 mg/person/day. Estimated intakes for other foods include: wine 0.81 - 3.68, dried fruit 0.59, pickles 0.008 and canned vegetables 0.325 mg/person/day.⁽⁶²⁾

In the human body, the enzyme sulphite oxidase converts sulphite to sulphate, which is then excreted in urine. This mechanism is more than adequate to cope with the amounts of SO₂ ingested in a normal diet.⁽²⁶⁾

ROLES OF SO₂ IN BEER

Antioxidant

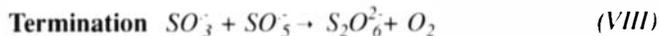
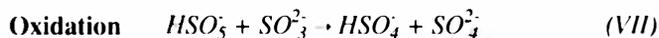
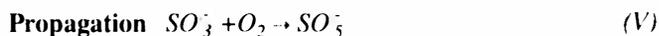
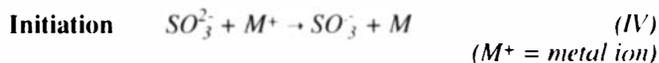
The presence of antioxidants in beers is important to help maintain fresh beer flavor. When oxidation occurs in beer, cardboard-like off-flavors develop due to the formation of carbonyl compounds, some of which have low flavor thresholds.

The antioxidant effect of SO₂ is due in part to its reaction with oxygen to produce sulphate.



However, this reaction is not as simple as it first appears. It can take place alone, or catalytically in the presence of transition metal ions. There have been many mechanisms proposed to explain the antioxidant properties of SO₂ in foods.⁽⁶⁹⁾ All of them are possible schemes rather than proven pathways.

Bäckström⁽⁴⁾ proposed the following mechanism:



This is the most widely quoted mechanism, but others have been proposed. Hayon *et al.*⁽³⁰⁾ included SO₄^{·-} in the scheme. Larson *et al.*⁽⁴²⁾ suggested the involvement of the hydroxyl (OH[·]) radical in the propagating stages. Yang⁽⁷⁴⁾ proposed a mechanism in which superoxide and sulphite radicals were implicated.

Both free and less strongly bound sulphite can act as antioxidants, although whether strongly bound sulphites have antioxidant activity is questionable⁽¹³⁾ Kaneda *et al.*⁽³⁰⁾ used chemiluminescence detection as an indicator of beer staling. Their experiments showed 98-100% of the SO₂ to be bound, and so the effect of free SO₂ would be very small. However, production of chemiluminescence was inhibited by bound sulphite as well as by free sulphite, suggesting the bound forms could scavenge

active oxygen and inhibit free radical reactions in beer.

Masking stale flavors

SO₂ can react reversibly with the carbonyl staling compounds in beer to form hydroxysulphonates (Fig. 6). The adducts formed are non-volatile and therefore have much higher flavor thresholds than the free carbonyls. Gjertson and Schouboe⁽²⁸⁾ conducted taste tests using beer, beer with 13 mg/l acetaldehyde and beer with 13 mg/l acetaldehyde-sulphite adduct. They found that there was no statistical difference between the beer and beer with added adduct. However, both of these were preferred to the beer to which acetaldehyde had been added, thus showing the masking effect of the SO₂.

Acetaldehyde has a high affinity for forming sulphite adducts. It has an apparent equilibrium constant of 1.4 x 10⁻⁶ at pH 4, compared with 2.2 x 10⁻⁴ for pyruvic acid and 6.9 x 10⁻² for xylose.⁽⁹⁾ The equilibrium constant (K) for the reaction between sulphite and carbonyls is given by the equation:

$$K = \frac{[SO_2] \times [\text{free carbonyl}]}{[\text{carbonyl-sulphite adduct}]} \quad (IX)$$

The equilibrium constants of the adducts remain fairly constant between pH 2 - 6: a pH range encompassing that for all beers. At pH > 7 dissociation of the adducts is favored to give the free carbonyl. At pH > 2 the adducts are less stable due to the formation of SO₂.H₂O which does not act as an efficient nucleophile. The only competing reaction is that with water or hydroxide ions.⁽⁶⁹⁾

The structure of the carbonyl itself also influences formation of adducts. Steric hindrance, due to the shape of the carbonyl molecule can interfere with the approach of sulphite to the electron

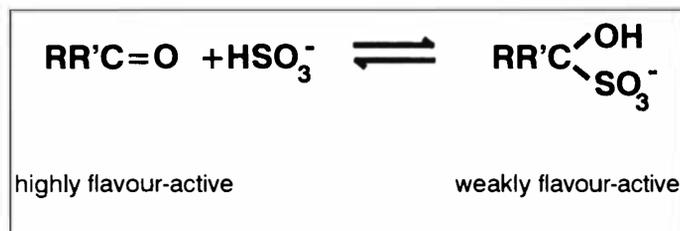


Fig. 6
Formation of α-hydroxysulphonates

orbitals of the carbon atoms, thus making them less reactive.

Antimicrobial activity

The use of SO₂ to control microbial growth dates at least from the Romans. The biocidal/biostatic activity of sulphiting agents is usually in the order: Gram-negative bacteria > Gram-positive bacteria > molds > yeasts.⁽⁶⁶⁾

The undissociated form of SO₂ is the most effective and the antimicrobial action is therefore pH dependent.⁽²⁶⁾ Bound forms of SO₂ (e.g. carbonyl-sulphite adducts) are ineffective as antimicrobial agents.^(10,38)

There are various theories as to the mechanism(s) of the antimicrobial action. Formation of ATP in yeast under aerobic conditions is prevented by sulphites. It has also been suggested that sulphites interact with nucleic acids,⁽⁵⁸⁾ interrupt glycer-aldehyde-3-phosphate conversion to 1,3-diphosphoglycerate in yeasts and also interrupt the NAD dependent formation of

oxalate from malate in *Escherichia coli*.^(26,27)

Mutagenic effects on *E. coli* have been reported but require SO₂ concentrations several thousands times higher than those found in beer.^(50,58,68)

FATE OF SO₂ IN BEER

SO₂ levels in beers decrease on storage.⁽⁵²⁾ Ilett and Simpson⁽³³⁾ showed that the rate of SO₂ loss is pseudo first-order and that the rate of loss increases with storage temperature. The kinetics of the reaction are such that the rate of loss is not greatly affected by initial SO₂ content. Half-lives for total SO₂ loss from small packed beers lie in the range 3 - 6 months.

This decrease is due to the reaction of SO₂ with components in beer. We have already seen that SO₂ will react with oxygen to give sulphate and can react reversibly with carbonyls. The reactions of SO₂ with other components in beer have not been studied in depth. However, the fate of SO₂ has been studied in other foodstuffs. The results of these studies showed that losses of SO₂ were due to reactions with quinones, thiamine, disulphide bonds, polyphenols, carbon-carbon double bonds or hydroperoxides (Fig. 7).⁽⁶⁹⁾ Another reaction which may account for considerable SO₂ losses in beer during storage is the inhibition of non-enzymic browning. The SO₂ reacts with the carbon-carbon double bonds in 3-deoxysuloses, intermediates formed during Maillard browning, which occurs when reducing sugars react with amino acids, peptides or proteins. The sulphonates which are formed remove SO₂ irreversibly.⁽⁷¹⁾

Sulphur dioxide is also lost from cask-conditioned ales, which contain live yeast, during storage. This can result from conversion of SO₂ to H₂S by the yeast enzyme sulphite reductase, a reaction which can result in formation of large amounts of H₂S and an unacceptable change in beer flavor. Walker and Simpson⁽⁶⁵⁾ showed that minimization of the SO₂ content of such beers is a viable strategy for control of H₂S.

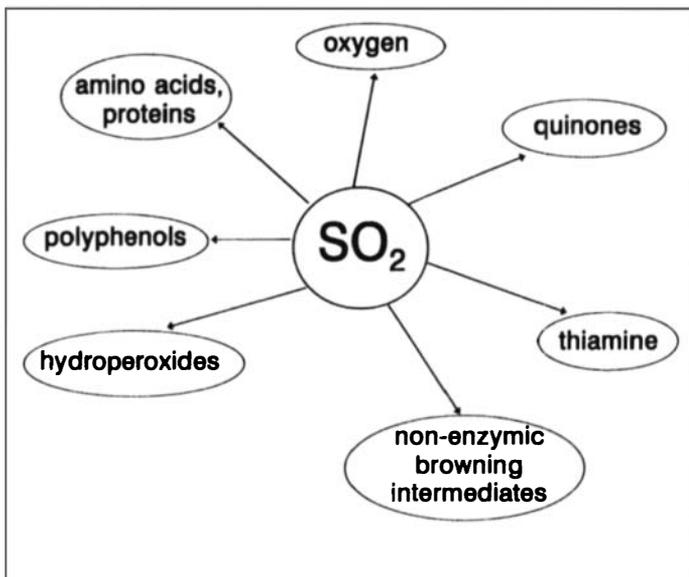


Fig. 7

Routes of SO₂ loss in food and beverages

ALTERNATIVES TO SO₂

Any attempt to replace the use of SO₂ in beer has to take into account the fact that SO₂ acts as an antioxidant, an antimicrobial agent and has the ability to reduce the flavor activity of carbonyls. Ascorbic acid (E300) is a commonly used antioxidant in the brewing industry, either alone or in combination with SO₂. It is an α -ketolactone, a weak acid and has a high reducing power. It protects beer against haze formation, stabilizes beer color and improves flavor stability.⁽⁵⁴⁾ The main disadvantage of its use is the possibility of coupled oxidation between ascorbic acid and other compounds in beer.⁽¹²⁾ Ascorbic acid fixes one atom of the oxygen molecule so that activation of the remaining oxygen atom may lead to oxidation of non-antioxidizable material. This is a particular problem in the presence of heavy metal cations and in beer with a high oxygen content.⁽¹⁶⁾

Beers also contain several naturally-occurring antioxidant materials and improved understanding and utilization of these materials is a current target.

Endogenous substances, which provide protection from microbial attack, are also found in beers. These include ethanol, CO₂ and hop bitter acids. To some extent the antimicrobial role of SO₂ is the least important of its properties for beer. However, SO₂ is used as a preservative in certain processing aids such as isinglass finings. The search for alternatives in this area continues, driven in part by the conversion of SO₂ to H₂S in cask-conditioned beer.⁽⁶⁵⁾

There are no acceptable alternatives to SO₂ as a binder for carbonyl compounds. Other compounds which react with carbonyls (e.g. 2,4-dinitrophenylhydrazine) are toxic. However, they can be used for analysis purposes.^(11,57)

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