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ABSTRACT

Beer is an extremely complex mixture of more than 3,000 different compounds in an aqueous environment. Thus, it is perhaps not surprising that the maintenance of beer quality throughout its lifetime has been a considerable challenge for brewers. Whilst it is inevitable that chemical changes will occur in beer with the passage of time, it is the formation of flavor-active components which is of immediate concern to an overview of beer shelf life stability. Sulfur dioxide has long been recognized by brewers as the most important factor in delaying flavor staling, and prolonging the shelf life of beer. However, nowadays, sulfur dioxide and sulfites are considered allergens and concerns about the safety of their use as food additives have been on the increase. The present review is structured into three main parts. Firstly, the chemical properties of sulfur dioxide are presented, along with the toxic effects and maximum legal levels permitted according to U.S. and EU legislation. As the accurate determination of the free, bound and total sulfur dioxide in beer is essential to ensuring regulatory compliance, several methods have been developed for analyzing sulfur dioxide in beer. Thus, secondly, various types of methods are reported and compared with the officially recommended ones. Finally, the crucial role of sulfite in the control of flavor instability of beer is discussed in light of the current data. Two courses of action have been proposed, which are elucidated in detail relating firstly to the fact that sulfite inhibits beer oxidation during storage by acting as an antioxidant and, secondly, sulfite reacts with the carbonyl staling compounds in beer, and thereby masks stale flavors.

Key words: beer flavour; sulfur dioxide; legislation toxic effects; antioxidants

Introduction

1. Chemical properties of sulfur dioxide, safety concerns and regulations

Sulfur dioxide (SO_2) is a colorless gas at room temperature, with a pungent odor which is readily soluble in water (3927 cm^3 SO_2 in 100 g H_2O at 20 ºC). Maximum permitted atmospheric concentration for humans is 5 ppm but many green plants suffer severe distress from concentrations as low as 1-2 ppm (Earnshaw, 1999). A number of molecular and physical properties of sulfur dioxide are listed in Table 1.

Table 1 – Selected molecular and physical properties of SO2 (adapted from Greenwood and Earnshow, 1999).

Property	Value	Property	Value
MP /ºC	-75.5	Electrical conductivity κ /ohm^{-1} cm^{-1}	< 10^{-8}
BP /ºC	-10.0	Dielectric constant ε (0º)	15.4
Critical temperature / ªC	157.5	Dipole moment m /D	1.62
Critical pressure / atm	77.7	Angle O-S-O	119º
Density (-10º) / g cm $^{-3}$	1.46	Distance r(S-O) / pm	143.1
Viscosity η (0 ºC) / centipoise	0.403	DH_f^o (g) / kJ mol^{-1}	-296.9

Sulfur dioxide and sulfur (IV) oxoanions in solution undergo pH-dependent equilibration reactions between sulfur dioxide, sulfurous acid (H_2SO_3), bisulfite ion (HSO_3^-), and sulfite ion (SO_3^{2-}):

$$S_2O_6^{2-} + 4\ H^+ + 2e^- \rightleftarrows 2H_2SO_3 \qquad E^\circ = 0.564V$$

$$SO_4^{2-} + 4\ H^+ + 2e^- \rightleftarrows H_2SO_3 + H_2O \qquad E^\circ = 0.119V$$

Sulfur dioxide dissolves fairly readily in water to give a rather complicated reaction mixture, strongly dependent on concentration, temperature and pH (). Therefore, the concentration of each of the different forms is a function of the pH medium and is determined by the equilibrium constants K_1 (1.6×10^{-2} mol dm^{-3}) and K_2 (1.0×10^{-7} mol dm^{-3}). At a pH lower than 2 most of the SO_2 is in a loosely hydrated undissociated form, due to its high water solubility. Spectroscopic studies revealed that undissociated sulfurous acid, H_2SO_3, may be assumed to be a short-lived intermediate in the acidification of sulfites, but does not exist in detectable amounts in aqueous solutions of sulfur dioxide (). At the usual pH of beers (3.8 – 4.4), most of the SO_2 is present as the bisulfite (or hydrogen sulfite) anion, as illustrated in Figure 1.

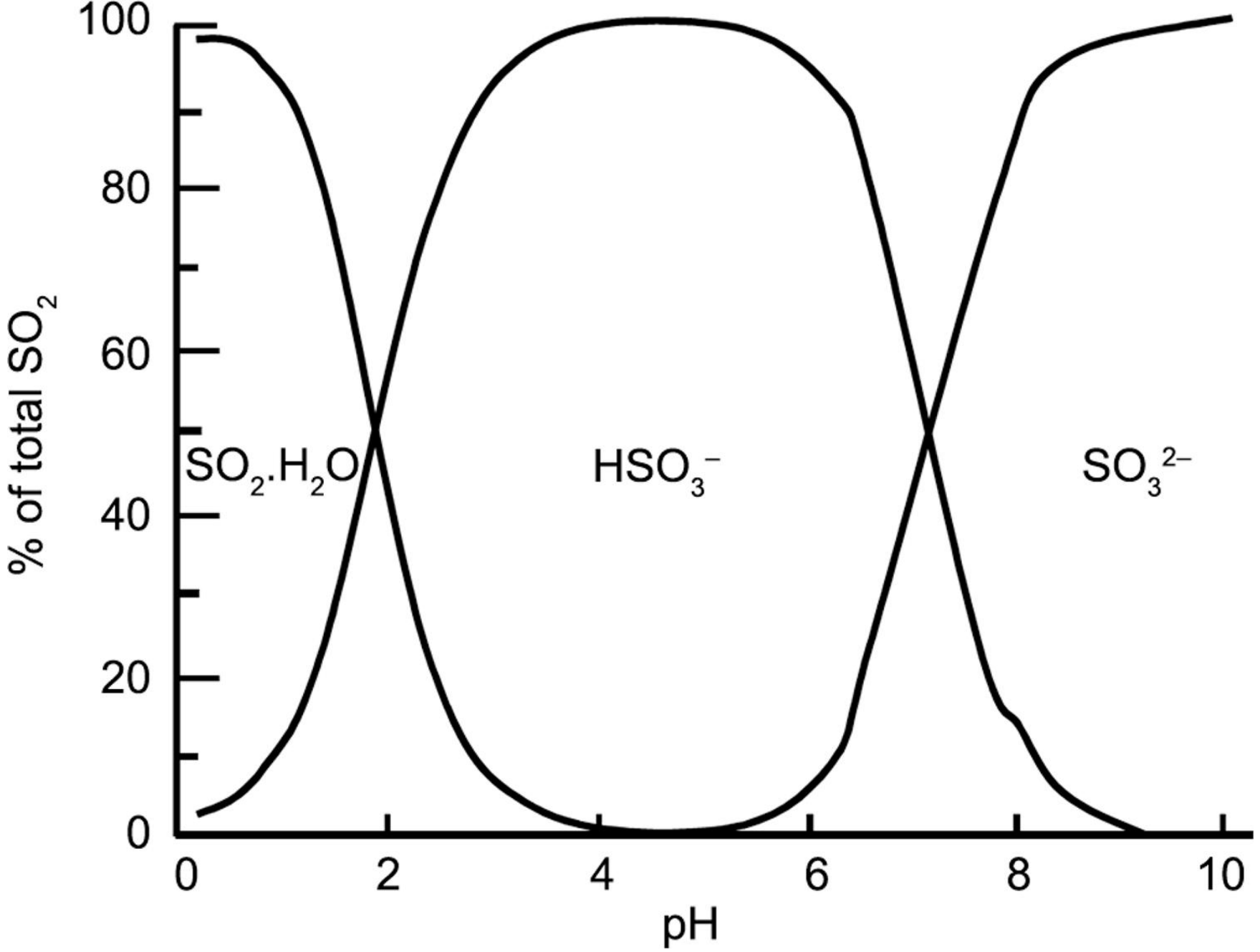

Figure 1 – The effect of pH on the equilibria of SO2 species in aqueous solution (adapted from Ilett, 1995).

Sulfur dioxide, sulfurous acid and sulfites can function as mild oxidizing agents; however, reactions in which these compounds react as reducing agents (and are oxidized to the sulfate ion, SO_4^{2-}) are more numerous and more important (Earnshaw, 1999), and a comprehensive monograph on the chemistry of sulfur dioxide in foods has been published (). In the present review, the terms sulfur dioxide and sulfite will be used interchangeably to represent all forms of SO_2.

Sulfites occur naturally in a number of foods and beverages as a result of fermentation, such as occurs in beer and wine. As a food additive, sulfites have been used since 1664 and have been approved for use in the United States since the 1800s (). With such a history of widespread application, sulfites have been generally regarded as safe (GRAS) by the FD; however, it is suspected that a low percentage of the population is sensitive to sulfites. The manifestations of sulfite sensitivity include a large array of dermatological, pulmonary, gastrointestinal, and cardiovascular symptoms. Asthmatics, for example, who

are steroid-dependent or have a great degree of airway hyperreactivity may be at an increased risk of having a reaction to a sulfite-containing food (). As a result, sulfiting agents are not considered GRAS for use in meats, i.e. foods recognized as a major source of vitamin B1 (thiamine), or “fruits or vegetables intended to be served raw to consumers or to be presented to consumers as fresh” ().

The European Commission’s Scientific Committee on Food (SCF) put forward in 1994 an acceptable daily intake (ADI) of 0.7 mg kg^{-1} body weight/day for sulfur dioxide and sulfur dioxide equivalents (). The ADI is the amount of food additive, expressed on a body weight basis, which can be ingested daily over a lifetime without appreciable health risk. Toxicity was in part attributed to the well-known destructive action of sulfite on thiamine. It has been suggested that the ingestion of SO_2 in a beverage may effectively reduce the level of thiamine in the rest of the diet (). Human studies over short periods showed that 400 mg d^{-1} produced no effect on thiamine excretion. Sulfites have also a certain irritant action on the skin, but the risks involved in handling these compounds are apparently rather small ().

The use of sulfites in food products became an issue of concern to both consumer and regulatory agencies when it was found that certain sensitive individuals exhibited adverse reactions to sulfite residues in foods. Further investigations into this potential health hazard led agencies to promulgate regulations concerning the usage and labelling of sulfiting agents. Brewers can produce beers with a sulfite content well below 10 mg L^{-1}, the level at which declaration of sulfites has been mandated for the labelling of alcoholic beverages by both US and EU legislation. The statement “Contains sulfites” or “Contains (a) sulfiting agent(s)” or a statement identifying the specific sulfiting agent where sulfur dioxide or a sulfiting agent is detected at a level of 10 or more parts per million (ppm), measured as total sulfur dioxide, is mandatory in the USA (). The 2000/13/EC Directive from the European Parliament and Council on the labelling of foodstuffs (EC, 2000) requires sulfur dioxide and/ or sulfites to be labeled when present in beer above 10 mg L^{-1} (calculated in terms of the total SO_2). For products where a list of ingredients is not required (e.g. alcoholic beverages with > 1.2 % by volume of alcohol) the presence of the allergen must still be shown on the label e.g. ‘contains sulfur dioxide’. In Canada, the new food allergen labelling regulations require that sulfites at a level of 10 ppm or more must be declared when present in alcoholic beverages. Beer, ale, stout, porter and malt liquor are exempt from the requirement to declare food allergen, gluten or added sulfites unless a list of ingredients is shown on the product's label in a statement entitled ‘Contains’. Any list of ingredients that is voluntarily provided must be complete, and declare all priority allergens and gluten sources, and added sulfites when present at 10 ppm or more

[B.01.010.2, B.01.010.3, Food and Drug Regulations] (). Brazil's regulatory agency (issued a resolution (Resolution RDC Nº 65 of 29 November 2011) establishing a maximum legal limit of 0.005 g / 100 g or 0.005 g / 100 mL to earn a free of total sulfur dioxide in beer classification (Brazil, 2011).

2. Sulfur dioxide analysis in beer

Potential problems with the usage of sulfiting agents in foods and the investigations revealing wide and increased utilization of these compounds in foodstuffs have raised questions as to the suitability of available methodologies for determining sulfites in food and beverages. Many methods are currently in use, varying in specificity and sensitivity, applicable to specific matrices but lacking a suitable data-base for comparison with each other.

Currently, many different procedures are being used by the industry to determine the sulfite content of their products including various versions of the classical Monier-Williams method, colorimetric procedures such as the *p* -rosaniline and to a limited extent, flow injection analysis, pulse polarography and ion chromatography. The Monier-Williams method has been the reference method for many years, but it has drawbacks at the 10 mg L^{-1} level. Minor procedural changes made to the Monier-Williams method by the Food and Drug Administration (FDA) improved accuracy at the 10 ppm level used in the labelling of sulfite content in commercial products (). The Monier-Williams technique, adopted by the Institute of Brewing, involves the removal of SO_2 from acidified beer in a stream of carbon dioxide and nitrogen at 100 ºC. The gas is absorbed in hydrogen peroxide and the sulfuric acid formed titrated against NaOH. Minor modifications made by the FDA include reduction in concentration of the titrant by a factor of 10, the replacement of the pyrogallol trap by a GLC-type oxygen scrubber and elimination of the hot condenser step (;). A coulometric adaptation of the Monier-Williams method yielded significant correlation with the *p* -rosaniline reference method, permitting a reduced sample size while improving precision and significantly reducing analysis time ().

The colorimetric method using rosaniline hydrochloride was adopted by the American Society of Brewing Chemists (ASBC). This method proved to be quite acceptable until the last few years when questions about the carcinogenicity of the *p* -rosaniline reagent were raised. This colorimetric analysis is based on the reaction between SO_2, *p* -rosaniline and formaldehyde. After reaction with *p* -rosaniline and development of the color for 30 minutes, the absorbance of the *p* -rosaniline-sulfite complex is measured at 550 nm. This method has been widely used as it is precise and allows for measurement of free and total SO_2. Another colorimetric method uses DTNB, which is 5.5-dithiobis (2-nitrobenzoic

acid), as the color reagent after a distillation step. It is a method for total SO_2 determination in beer recommended by the EBC. Sulfur dioxide is distilled from acidified 25 mL samples into a buffered DTNB solution, with a nitrogen carrier gas, and absorbance is measured at 415 nm. A simpler version of the DTNB method has been recently applied to beer samples with 97 % of sulfite recovery ().

During the past few years, flow injection analysis (FIA) methods for determining sulfites in beer and other beverages have been widely developed. Most flow injection analyses are adaptations of other existing methods, mainly spectrophotometric methods. Application of FIA systems for both the malachite green (;) and *p* -rosaniline () methods are known. The sample is injected into the carrier stream which contains NaOH for on-line release of bound sulfites. Sulfuric acid is introduced to lower the pH and convert all existing equilibrium products into the sulfur dioxide form which then diffuses across a gas permeable membrane. The sulfur dioxide is then reacted with malachite green or *p* -rosaniline to give a colored product which is measured using a spectrophotometer. Several applications of FIA towards the determination of sulfite in wine by spectrophotometric detection have been recently reported (; ; ;).

A chronopotentiometric method for the on-line determination of total sulfur dioxide in beer has been reported (). Free and bound sulfites were converted to sulfite anions on alkalising the sample solutions. On acidifying the solution, the sulfur dioxide released was separated on-line through a semipermeable membrane and transported by an electrolyte into the measuring cell and measured by stripping chronopotentiometry. The results corresponded well with those obtained with the alternative EBC methods, with the advantage of being much faster and simpler.

An enzymatic assay employing sulfite reductase is currently recommended by the EBC (Method 9.25.2, EBC, 1987). It is based on the oxidation of NADH by the hydrogen peroxide formed during the oxidation of sulfite by the action of sulfite oxidase. Changes in absorbance of NADH at 340 nm are used for determining the concentration of total sulfite.

Since the advent of flame photometric detection (FPD), the use of various gas chromatographic methods has been reported () using GC to accurately quantify the amount of SO_2 in beer. A method for the routine determination of both free and total sulfur dioxide residues in malt and total SO_2 in beer using the technique of headspace GC was developed by Munar and co-authors (). They showed that both the colorimetric and the headspace methods are comparable. The use of a highly sensitive and selective chemiluminescence sulfur detector was reported (;). The sulfur chemiluminescence

detector was evaluated as a replacement for the FPD as it was found to have better selectivity, sensitivity, and linearity. It was not susceptible to hydrocarbon-quenching effects shown by the FPD detector. These methods have the additional advantage over the colorimetric methods for dark beers.

Ion exclusion chromatography with electrochemical detection was proven to be a lot simpler and quicker with the results compared more favourably with the Monier-Williams technique and to correlate well with the *p* -rosaniline technique (;). This method showed good reproducibility at levels close to 0.5 mg L^{-1} and an analysis time of approximately half an hour. Wagner and McGarrity used ion exclusion chromatography with pulsed amperometric detection to avoid the loss of detector sensitivity that occurs over time when direct amperometry is used (;).

A rapid and sensitive polarographic method was also presented for determining sulfiting agents in foods and beverages (;). This method is based on the modified Monier-Williams distillation followed by polarographic detection by differential pulse polarography or square wave voltammetry and is specific for total SO_2. A method involving voltammetric determination of free and total sulfur dioxide in beer was reported by . Voltammetric determinations are in agreement with those obtained by the *p* -rosaniline reference method, with the advantage of excluding the use of toxic reagents and providing more accurate and precise results.

More recently, a method was developed for the quantification of sulfite in beer based on derivatization with the maleimide-derived probe ThioGlo I followed by separation of fluorescent adducts by reversed-phase, high-performance liquid chromatography and fluorescence detection. ThioGlo I was found to have generated fluorescent adducts with both bound and free sulfites, providing a quantification of total sulfite content in beer. The limit of quantification of sulfite was 0.6 mg L^{-1} and the method can be used for quantification of sulfite in highly colored beers ().

Albeit numerous methods for determining sulfur dioxide in beer have been reported in the literature, the brewing industry is still searching for the ideal procedure. It should be fast, simple, inexpensive, allow for measurement of free or/and total SO_2 and exclude the use of toxic reagents. Most of these conditions, or at least a number of them, are met in the aforementioned methods.

3. Uses of sulfur dioxide in the brewing industry

Sulfites in various forms have been added to foods for centuries. The usage of sulfiting agents or S (IV) compounds to foods has become widespread because of their varied

functional applications such as bleaching agents, antimicrobials, oxygen scavengers, reducing agents and enzyme inhibitors. Sulfur dioxide and several forms of sulfites, which generate sulfur dioxide when used in food, can be used as sulfiting agents. These include sulfur dioxide (E220), potassium bisulfite (potassium hydrogen sulfite) (E228), potassium metabisulfite (E224), sodium bisulfite (sodium hydrogen sulfite) (E222), sodium metabisulfite (E223), sodium sulfite (E221), calcium sulfite (E226) and calcium hydrogen sulfite (E227) after the Nº 95/2/EC (EC, 1995) Directive. As all the above can convert to sulfur dioxide, sulfites are measured and expressed as sulfur dioxide. Sulfur dioxide found in beer is due not only to the addition of sulfiting agents (exogenous SO_2) but is also derived from the yeast metabolism or as a component of finings or primings (endogenous SO_2). Yeast has the ability to produce sulfur dioxide, from the reduction of sulfate in water and grist material. SO_2 levels will be increased if the sulfate supply to the yeast is increased, wort clarity is increased, wort oxygenation and pitching rate are lowered and fermentation temperature is reduced (). The sources of sulfur dioxide in beer were comprehensively reviewed by . Table 2 summarizes the uses of sulfur dioxide in the brewing industry. The different roles of sulfur dioxide on beer flavor stability will be emphasised and discussed below.

Table 2 – Uses of sulfur dioxide in the brewing industry (adapted from Ilett, 1995).

Stage of beer production	Use of sulfur dioxide
germinating grain malt kilning	reduce malting losses, enhanced yields of extracts control the formation of nitrosamines, bleach malt, increase soluble nitrogen
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

4. Antioxidant activity of sulfur dioxide in beer

Antioxidants can be broadly defined as compounds that inhibit an oxidative reaction. They can act by decreasing molecular oxygen levels, scavenging chain-initiating and chain-propagating free radicals, chelating metals, or decomposing peroxides (). They are, therefore, thought to have a significant effect in malting and brewing as inhibitors of oxidative damage. In their general reactions, sulfites and hydrogen sulfites are moderatetely strong reducing agents, and together with antioxidant properties, yield either dithionate ($S_2O_6^{2-}$) or sulfate (SO_4^{2-}) upon reaction. This reducing (antioxidant)

behavior under all pH conditions may be seen in the following redox potentials ():

$$S_2O_6^{2-} + 4\ H^+ + 2e^- \rightleftarrows 2H_2SO_3 \qquad E^\circ = 0.564V$$

$$SO_4^{2-} + 4\ H^+ + 2e^- \rightleftarrows H_2SO_3 + H_2O \qquad E^\circ = 0.119V$$

Beer flavor stability is influenced by a multiplicity of factors; some of them are certainly disputed, but there is no question of the crucial role of oxygen in packaged beer. It has been repeatedly demonstrated that high air levels in the final package greatly reduce shelf life (;). Therefore, modern fillers are designed to achieve very low O_2 levels and several measures have been adopted in order to keep ingress of oxygen into the packaged beer as low as possible. Various antioxidants may be used in beer (depending on the legislation), with the most prominent of these being sulfur dioxide and ascorbic acid (). These agents can only protect against new oxidation occurring in beer, and do not rectify any damage which has occurred upstream. Sulfur dioxide is the more effective, either in the role of carbonyl binder (discussed below) or radical scavenger.

The production of reactive oxygen species (ROS) is promoted by light and certain enzyme systems. Of particular importance are transition metal ions, such as iron and copper, which are effective in stimulating the formation and multiple-interconversions of radicals from oxygen (). The deleterious effect of ROS has led most brewers to make efforts to eliminate these substances from their processes and products. The reactivity of oxygen and its activation by acquisition of electrons was described by and . As oxygen passes successively through superoxide ($O_2^{\cdot -}$), peroxide (O_2^{2-}) and hydroxyl ($HO^\cdot$) it becomes increasingly reactive. The hydroxyl radical is an immensely reactive species generated in wort and beer, which reacts instantly with a great many types of molecule. Superoxide, on the other hand, is less reactive. At beer pHs, the majority of the superoxide will be in a perhydroxyl form ($HO_2^\cdot$). Hydroxyl and perhydroxyl are capable of reacting with unsaturated fatty acids, such as linoleic acid, and thereby set in motion a chain reaction that leads to stale flavor development ().

The 1-hydroxyethyl radical, which can be formed by reaction between hydroxyl radical and ethanol, was recently found to be, quantitatively, the most important radical in beer, detectable by electron spin resonance (ESR). Oxidation processes were characterised in beer involving reaction of the 1-hydroxyethyl radical in an important reaction with oxygen, converting it into hydroperoxyl radicals (). A number of potential antioxidants have been evaluated for their effect on the formation of radicals in beer using the ESR lag phase method. Sulfite was found to be the only compound that was able to delay the formation

of radicals, whereas phenolic compounds such as phenolic acids, catechin, epicatechin, and proanthocyanidin dimers had no effect on the formation of radicals. It was suggested that antioxidants must be able to either scavenge peroxides or trap metal ions in order to be effective in beer. The effectiveness of sulfite was suggested to be a consequence of its two-electron nonradical producing reaction with peroxides (). Additionally, the concept of Endogenous Antioxidant (EA) value, which is the time taken before an ESR signal is developed in an aging test, showed a clear relationship between the EA value and sulfite level in beer (). More recently, Karabín and co-authors have shown that one of the reasons for the decline in the endogenous antioxidative potential of beer during storage is a significant decrease in SO_2 content, probably caused by chain oxidation reactions catalysed by transition metal ions, particularly ferric ions ().

It is postulated that the deterioration rates of beers might be assessed from the chemiluminescence (CL) producing patterns in fresh beer before storage. The presence of sulfite in beer depressed CL production during its storage indicating that there is some contribution of sulfite to flavor stability due to its inhibitory effect on radical reactions (). Formaldehyde- and acetaldehyde-bisulfite adducts inhibited the chemiluminescence production of beer and Cypridina lucifern analog dependent luminescence in beer, indicating that they inhibit free radical reactions. Therefore, it could be concluded that aldehyde-bisulfite adducts have radical scavenging activity and protect free radical chain reactions during beer storage, leading to stability of beer quality (). Additionally, it was shown by Kaneda and co-authors that the lower the dissolved oxygen in pitching wort, the higher the pitching rate of yeast. Clearer pitching wort leads to higher sulfite content level, inhibition of CL production, and better flavor stability of the resulting beers (). Lermusieau and co-authors confirmed in laboratory-scale experiments that SO_2 can reduce both lipid autooxidation and the nonenal potential rise while the wort is boiling ().

Much of the SO_2 added to beers is rapidly bound up with components other than carbonyls, with an addition of 20 ppm revealing only approximately 12 ppm of measurable total SO_2. The majority of this is lost through aging, especially in the presence of high levels of air (). Recent sensory and chemical evidence from Bushnell and co-authors support the argument that sulfites exert their protective effect as antioxidants rather than as agents that bind carbonyls as adducts.

5. Formation of adducts with carbonyl compounds

Sulfur dioxide reacts with a wide range of food components. It forms adducts by reversible action with aldehydes and ketones (including reducing sugars, acetaldehyde, quinones, and ketoacids), with anthocyanins, and with cysteine residues in proteins. In

most foods and beverages, adducts with carbonyl compounds, the hydroxysulfonates (Figure 2), comprise most of the bound sulfite, and this equilibrium reaction has been studied in detail. In the range pH 1 to 8 the hydroxysulfonates predominate, while at higher pH values dissociation occurs (). It is also believed that sulfite stabilizes intermediates of the Maillard reaction by forming adducts. For example, glyceraldehyde forms stable hydroxysulfonate adduct, which could contribute to the mechanism of the inhibition of Maillard browning by sulfite species (). Dissociation constants of carbonyl bisulfite compounds at pH 3.5 have been reported by . They revealed that aliphatic aldehydes form adducts with S (IV), whereas ketones, cyclic aldehydes, and trans-alkenes interact weakly and are found predominantly in the free form. Range of dissociation constants of bisulfite compounds are 1.09×10^{-7} for formaldehyde, 2.06×10^{-6} for acetaldehyde, 3.45×10^{-6} for hexanal, 2.52×10^{-6} for octanal and 1.33×10^{-4} for hexan-2-one. Determination of the predicted sulfite binding power was carried out by Lea and co-authors. They found equilibrium constants of 1.6×10^{-4} for pyruvate, 1.8×10^{-2} for galacturonic acid and 6.4×10^{-1} for glucose (). The equilibrium constants remain fairly constant between pH 2 to 6, a pH range that encompasses all beers. At pH > 7 dissociation of adducts is favored to give the free carbonyl.

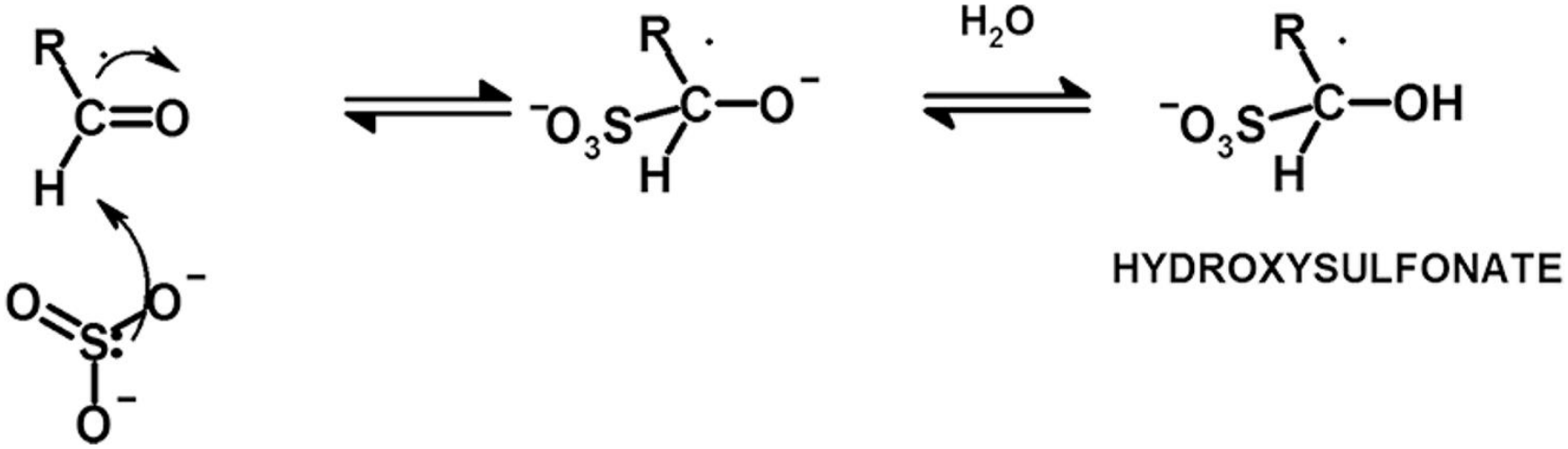

Figure 2 – Formation of α-hydroxysulfonates by the addition reaction of sulfite to the carbonyl group.

Beer flavor instability is caused by the formation of volatile, long chain, unsaturated carbonyls with low flavor thresholds and unpleasant flavors. Long chain unsaturated aldehydes, such as *E* -2-nonenal which contributes a cardboard-like flavor, are prime contributors (). Sulfite, produced by yeast during fermentation or added before bottling, readily form adducts with carbonyl compounds, rendering them nonvolatile and flavour-inactive (). As a result, SO_2 plays a role in masking the stale flavors that develop in beer during storage. Appearance of the cardboard flavor in aged beer is strongly retarded when such beers are supplemented with sulfite. The papery, cardboard stage of beer staling was ascribed to a decrease in bisulfite concentration and subsequent bisulfite

transfer from unsaturated aldehyde bisulfite addition complexes (). Nyborg and co-authors demonstrated that flavor-active *E* -2-nonenal has disappeared from the beer upon addition of sulfite, suggesting the formation of flavor-inactive adducts (). They confirmed the adduct formation between *E* -2-nonenal and sulfite in aqueous solution at beer pH indirectly by ^{1}H NMR spectroscopy and directly by liquid chromatography coupled to mass spectrometry (LC-MS). Dufour and co-authors have showed, using the unsaturated aldehyde *E* -2-butenal to model the behaviour of *E* -2-nonenal, that the mechanism of the addition reaction proceeds by a two-step process, to initially give a carbonyl adduct and ultimately yield a disulfonate as the thermodynamic product, as illustrated in the Figure 3 (). A reversible binding occurs between the carbonyl functional group and bisulfite, whereas the addition of bisulfite to the double bound of unsaturated aldehydes was shown to be irreversible. The stability of such adducts does not support the generally accepted mechanism for the release of unsaturated aldehydes from non-volatile species.

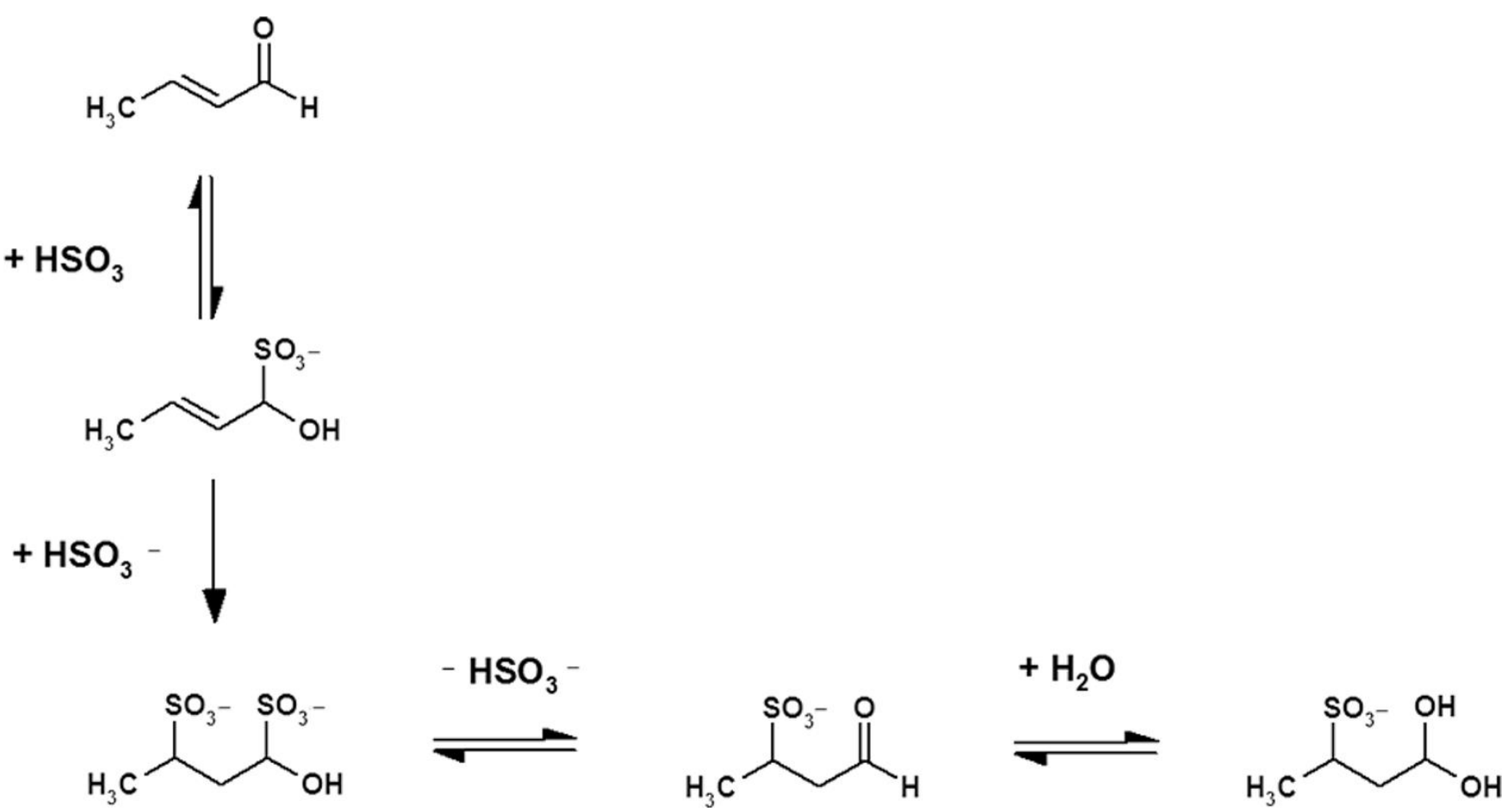

Figure 3 – Equilibria of E-2-butenal based on the 1H-NMR kinetic data proposed by Dufour et al., (1999).

The protective effect of sulfite against beer staling is not a passive event. During fermentation, the bisulfite excreted by yeast can potentially bind to the carbonyl compounds present in the medium, and prevent them from being reduced by yeast into the corresponding alcohols. These adducts might pass into beer, where they progressively break down to free the carbonyls which render the beer stale. Thus,

brewers have contradictory opinions on the efficiency of naturally produced bisulfite by yeast as compared to that added to the beer before bottling (). Partial or full elimination of MET10 gene activity in brewer's yeast resulted in increased sulfite accumulation. Beer produced with such yeasts was quite satisfactory and showed increased flavor stability ().

Acetaldehyde has a far greater capacity for binding bisulfite than have the staling aldehydes and its presence interferes with the ability of agents to bind staling substances (). Studies on the behavior of sulfites during fermentation and storage of beer revealed that acetaldehyde-bisulfite adduct and free sulfite produced during fermentation are oxidized by free radical reactions during beer storage and inhibit the oxidation of the other beer components (;). These observations most likely explain the increase obtained for the ratio of acetaldehyde and SO_2 during extended storage of beer. Using a voltammetric-based approach, the formation of an acetaldehyde-sulfite adduct was followed, and showed that the reaction is rapid and equilibrium is reached after 30 minutes ().

The complexity of carbonyl-bisulfite adducts formation is enhanced if the release mechanism of the carbonyl compounds from their adducts with amino acids and proteins of the wort is considered. Within the pH range of wort, a Schiff base formation is proposed between carbonyl compounds and either free amino acids or proteins (;). ^{1}H-NMR evidence of model systems suggest that N-adducts of alkenals, Michael adducts and Schiff bases, are relatively unstable particularly in the presence of bisulfite (). Formation of carbonyl-adducts and their subsequent release appears to interact in a complicated and dynamic way. Various linked equilibria, including those involving sulfite, are responsible for the release of carbonyl compounds and, thus, for the stale character typical of aged beers.

6. The dualistic mode of action of sulfites

In terms of the contribution of sulfite to control flavor instability of beer, a dualistic mode of action has been proposed (Figure 4). Firstly, sulfite inhibits beer oxidation during storage by acting as an antioxidant. Sulfite has radical scavenging activity and avoids the free radical chain reactions during beer storage, and contributes to the stability of beer quality. Secondly, sulfite can react with the carbonyl staling compounds in beer, which masks stale flavors. Adducts formed are non-volatile and, therefore, have much higher flavor thresholds than free carbonyls.

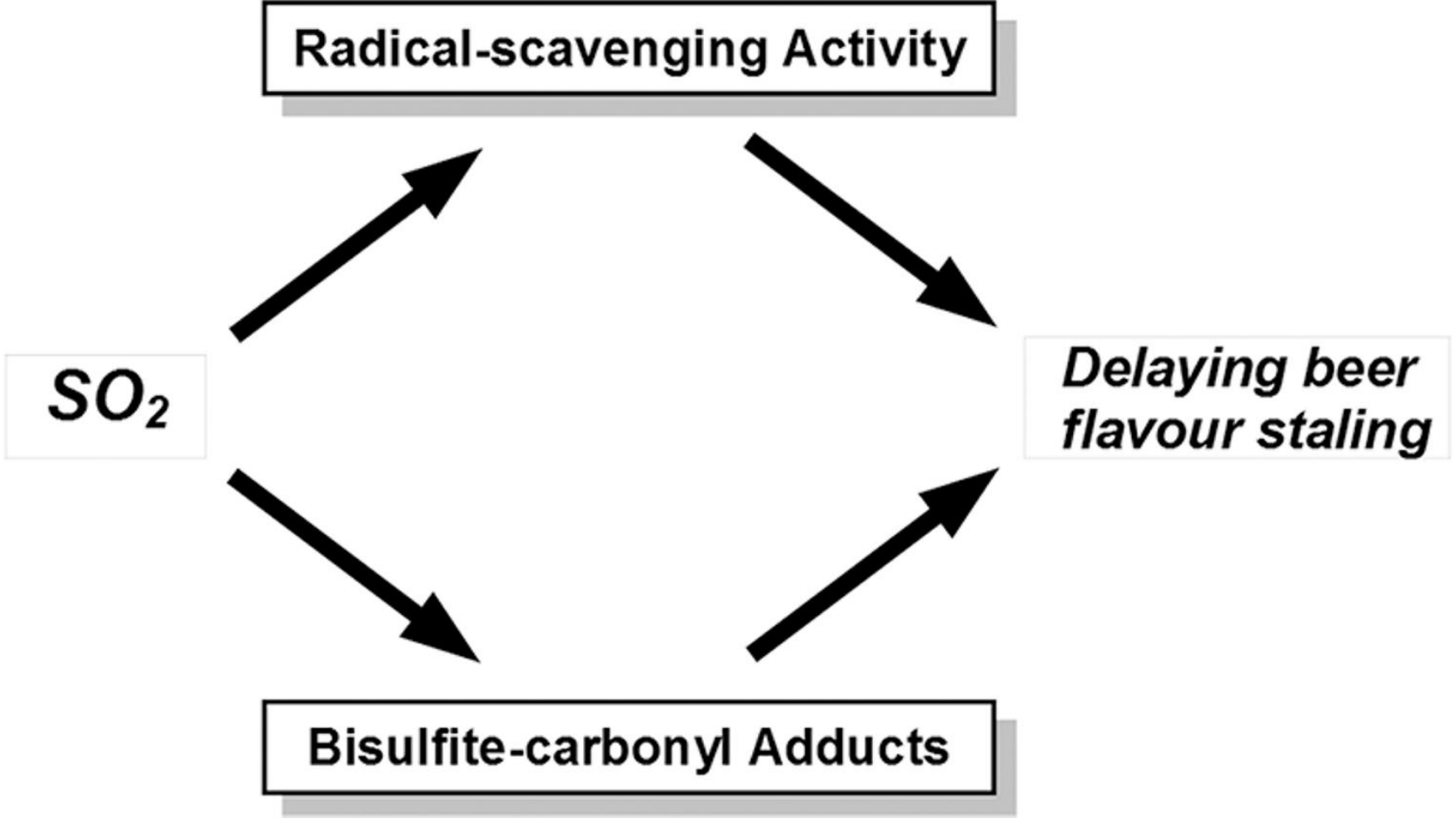

Figure 4 – The dualistic mode of action of sulfites to control the flavor instability of beer.

The most effective role of SO_2 in delaying flavor staling of beer, beit acting as an antioxidant or masking stale flavors, is still an open question. Sulfur dioxide is progressively lost from beer in a first order reaction. The constant rate for the reaction increased as the temperature increased in the range of 0 to 40 ºC and was dependent to some extent, on the initial SO_2 concentration. Storage of beer at 0 ºC considerably reduced the rate of SO_2 loss, but did not prevent it (). Lowering the temperature is, thus, the main measure that must be adopted by the brewer in an attempt to minimize the loss of SO_2 and to successfully preserve the overall flavor quality of the final product.