Wine & Health

Sulfur dioxide and the wine consumer

Creina Stockley

Health and Regulatory Information Manager

The Australian Wine Research Institute

Introduction

An adverse reaction to a food can either be a food allergy or a food intolerance. An immune or IgE-mediated allergy occurs on subsequent exposure of an individual to an allergen, such as a food protein, where the sensitised mast and blood basophil cells release histamine and other anaphylaxis-associated chemicals. In Australia, similar to the USA, approximately 2.5% of the population has a food allergy, and specifically, 4-8% of children under three years of age and 1-2% of the adult population have a food allergy.

While there are an estimated 2000-3000 food additives and processing aids, only a small number of these are associated with adverse reactions. For example, sulfur dioxide and the inorganic sulfites that liberate sulfur dioxide are additives that are commonly considered by consumers to elicit an adverse reaction. Sulfur dioxide is used worldwide in winemaking. Adverse reactions to sulfur dioxide include asthma and, anecdotally, wine has been associated with induced-asthma adverse reactions. In 2000, a community survey suggested that approximately 30% of asthmatic individuals believe that wine is associated with worsening asthma symptoms (Vally et al. 2000); approximately 2.2 million Australians have asthma (National Asthma Council of Australia 2005).

Few clinical studies have, however, assessed the degree to which sulfur dioxide additives contribute to wine-induced asthma, the results of these studies have been equivocal and hence direct evidence is limited (Halpern et al. 1985; Dahl et al. 1985). Furthermore, some individuals report reactions to red wines only, others to white wines only, some to both red and white wines and some to specific brands or types only. These are confounding factors, which are further confounded by the fact that wine consists of hundreds of components derived from the grape, yeast and bacteria as well as from additives and processes used in winemaking (Zuskin et al. 1997).

What is sulfur dioxide?

Sulfur dioxide is both an antimicrobial agent and an antioxidant, used in the food, pharmaceutical and wine industries.

Why is sulfur dioxide added to wine?

Antimicrobial activity

Sulfur dioxide has been added during winemaking since the 17th century when Dutch winemakers began burning elemental sulfur in empty wine barrels to enable their reuse without spoiling the stored wine (Blackburn 1988), and when Bordeaux winemakers found that the sulfur dioxide gas produced from burning elemental sulfur also protected the stored wine from spoilage. Thus sulfur dioxide has been primarily added to grapes, juice, must and wine to restrict the growth of indigenous microflora, such as yeast, mould and bacteria, the latter being the most sensitive to sulfur dioxide (Cruess 1912; Kunkee and Amerine 1970; Ough et al. 1983).

Sulfur dioxide is added to grape juice and wine to give a total concentration between 20 and 200mg/L, generally as potassium metabisulphite, a liquefied gas or as a solution made by dissolving sulfur dioxide in water (Beech et al. 1979; Ough et al. 1986). At the normal pH of juice or wine, 3.0-3.8, molecular sulfur dioxide undergoes ionisation to the bisulfite anion and sulfite anion. The primary antimicrobial form of sulfur dioxide is the unionised molecular form, followed by the bisulfite and sulfite forms, which have minimal antimicrobial activity (Ough et al. 1983). The unionised molecular form penetrates the yeast cell membrane by diffusion and subsequently inactivates the intracellular constituents (Stratford and Rose 1986). In the yeast cell, sulfur dioxide activates adenosine triphosphate (ATPase) and the ATPhydrolysing enzyme, which decreases the concentration of ATP. Furthermore, once sulfur dioxide has entered the yeast cell, it is ionised in response to the difference between the pH of the grape juice and that of the cell and becomes trapped. The bisulfite and sulfite ions then react with the cellular constituents. The combined effect of ATP depletion and cellular activity leads to inactivation and eventual death of the yeast cell (Beech and Thomas 1985; Stratford and Rose 1986).

The pH of juice and wine influences the equilibrium of the ionisation and consequently the proportion of the three forms of sulfur dioxide present. Increasing acidity increases the concentration of unionised molecular sulfur dioxide. At pH 3.03.6, however, approximately 94-98% of the added sulfur dioxide exists as the bisulfite form and only approximately 1.8-5.5% exists as the primary antimicrobial form (King et al. 1981). Therefore, since wine has a pH value ranging from 2.8-4.4, with the majority in the range 3.0-3.8, the bulk of sulfur dioxide present in wine is generally in the less active bisulfite form (Ough and Crowell 1987). The constituents of juice and wine, including the fermentation products, also affect the antimicrobial activity of added sulfur dioxide. Ionised free sulfur dioxide, generally the bisulfite form, binds with a wide range of juice and wine constituents, in particular, acetaldehyde, pyruvic acid and 2-ketoglutarate, as well as with galacturonic acid, anthocyanins and sugars (Kielhofer and Wiirdig 1960; Rankine 1966; Burroughs and Sparks 1973; Beech and Thomas 1985; Lafon-Lafourcade 1985). Sulfur dioxide binds most strongly with acetaldehyde, which is a reversible reaction although dissociation is slow. This fraction of bound sulfur dioxide is, however, still active, although the free form is approximately five to six times more active than the bound form (Hood 1983, Stratford and Rose 1986). Sulfur dioxide, which is reversibly bound to sugars and certain phenolic compounds in the juice, provides a reservoir of free sulfur dioxide that is released when the juice free sulfur dioxide concentration depletes (Margalit 1990). Thus the total concentration of sulfur dioxide in juice or wine consists of free molecular sulfur dioxide, free ionic forms and ionic forms bound to juice or wine constituents. Consequently, it is necessary to add sufficient sulfur dioxide during winemaking to obtain an adequate concentration of the free molecular form to inhibit microbial activity, but not so much as to have an excess of the bisulphite form, which can adversely affect the sensory attributes or characteristics of the wine. Research undertaken by Beech et al (1979) in a model wine indicated that 0.825mg/L of molecular sulfur dioxide is necessary to control the growth of wine yeasts and bacteria. Subsequently, oenologists have generally accepted and adopted this concentration of molecular sufur dioxide to control growth in juice and wine, although some researchers advocate a higher concentration is necessary (Rankine et al. 1989; Margalit 1990; Boulton et al. 1996).

While bacteria are considered to be more sensitive to sulfur dioxide than yeast and moulds (Ough et al. 1983), the growth of moulds, such as Botrytis cinerea, on grapes, can be restricted by addition of sulfur dioxide to freshly harvested grapes prior to crushing. A higher concentration of sulfur dioxide should also be added to juice prepared from Botrytis-infected grapes, because such juice contains significant amounts of lactase (polyphenol oxidase) enzyme, which is a catalyst for oxidation and hence increases the need for sulfur dioxide, and aldehydes, which will bind a greater amount of the added sulfur dioxide (RibereauGayon et al. 1976).

Antioxidant activity

In addition to antimicrobial activity, sulfur dioxide also protects the phenolic compounds in the must from enzymic oxidation by lactase and tyrosinase (phenol- and polyphenol-oxidase) prior to fermentation. Tyrosinase catalyses the hydroxylation of monophenols into diphenols and the oxidation of orthodiphenols into quinones, and lactase catalyses the oxidation of not only mono-and ortho-diphenols but also meta- and para-phenols, diamines and ascorbic acid as well as degrading the major phenolic compounds which impart colour - the anthocyanins and procyanidins (Ribereau-Gayon et al. 1977; Somers et al. 1983). The minimum concentration of sulfur dioxide required to inhibit the activity of tyrosinase is influenced by factors such as grape cultivar, clarity and temperature of the must as well as the concentration of phenolic compounds in the must (Dubernet and Ribereau-Gayon 1973; Traverso-Rueda and Singleton 1973; White and Ough 1973; Amano et al. 1979; Hooper et al. 1985; Ough 1985). A higher concentration of sulfur dioxide (100mg/L) is necessary to modify, and not necessarily inhibit, lactase activity (Ribereau-Gayon et al. 1977; Somers et al. 1983), such that the antioxidants ascorbic and erythor6fc acid are generally added as an adjunct to a lower concentration of sulfur dioxide.

In addition, sulfur dioxide prevents the chemical oxidation of juice and wine during processing and maturation, which is initiated by the reaction of phenolic compounds with dissolved oxygen. For example, oxygen dissolved in wine can be converted to hydrogen peroxide, which can then convert ethanol to acetaldehyde, alcohols to their corresponding aldehydes and catalyse the oxidative polymerisation of phenolic compounds (Allen 1983). Molecular sulfur dioxide rapidly reacts with hydrogen peroxide, which is the by-product of phenol oxidation, thus protecting the must from secondary reactions with hydrogen peroxide (Allen 1983). Sulfur dioxide also reacts with other constituents of juice and wine, including oxidised phenolic compounds and aldehydes, to form compounds less deleterious to the sensory characteristics of the wine (Peynaud 1984; Ough and Crowell 1987).

How much sulfur dioxide can be added to Australian wine?

The Australia New Zealand Food Standards Code in Standards 2.7.4 (Wine and wine product) and 4.5.1 [Wine production standards (Australia only)] specifies that "Wine, sparkling wine and fortified wine must contain no more than 250mg/L in total of sulfur dioxide in the case of products containing less than 35g/L of sugars, or 300mg/L in total of sulfur dioxide in the case of other products". This is consistent with the FAO/WHO Joint Expert Committee on Food Additives (JECFA) acceptable daily intake (ADI) for all sulfur dioxide of 0-0.7mg/kg body weight, which was re-evaluated in 1998.

How much sulfur dioxide is in Australian wine?

Approximately 20-200mg/L of sulfur dioxide may be added during winemaking (Ough 1986) and approximately 10-50mg/L is formed by the yeast during fermentation, which is usually bound to acetaldehyde on formation. Therefore, when wine is analysed for the concentration of total sulfur dioxide, a small amount will always be measured regardless of whether sulfur dioxide was added during the course of winemaking. In addition to seeking sufficient antimicrobial and antioxidant activity, the style of wine produced and whether the wine is intended for short, medium or long-term storage will also influence the amount that will be added during winemaking (Rankine 1989, Robinson and Godden 2003).

The mean concentration of total sulfur dioxide observed in 9477 white wines and 18,421 red wines analysed at The Australian Wine Research Institute from 1990 to 2001 was 121 mg/L and 33.5 mg/L, respectively (AWRI, unpublished data).

In 2004, the mean concentration of total sulfur dioxide in red wine was approximately 55 mg/L; it increased steadily from 40mg/L in 1987 to 58 mg/L in 2001 and has subsequently stabilised. The mean concentration of free sulfur dioxide in red wine has also increased steadily from 1986 and in 2004 was 25 mg/L; it is the free form of sulfur dioxide that has the major antimicrobial and antioxidant activity. As total sulfur dioxide is the sum of free and bound sulfur dioxide, a consequent decrease in the concentration of bound sulfur dioxide in red wine has correspondingly occurred, and in 2004 was 35mg/L. The ratio of free to total sulfur dioxide in red wine has thus increased, relatively steadily, from 1987 to 2004 (AWRI, unpublished data; see Figure 1).

In 2004, the mean concentration of total sulfur dioxide in white wine was approximately 125mg/L; it has been relatively constant since 1987. As with red wine, the mean concentration of free sulfur dioxide in white wine increased steadily from 1987 to 2001, and in 2004 was approximately 29mg/L. The mean concentration of bound sulfur dioxide in white wine has thus decreased since 1987, and in 2004 was 100mg/L. There has been, however, no significant increase in the ratio of free-to-total sulfur dioxide in white wine (AWRI, unpublished data,- see Figure 2).

The significance of sulfur dioxide in wine for the consumer

A relatively small amount of a food is needed to trigger an IgE-mediated food allergy. Symptoms attributed to the ingestion of sulfite compounds or sulfite-containing foods include asthma (bronchospasms and wheezing), urticaria and angioedema,

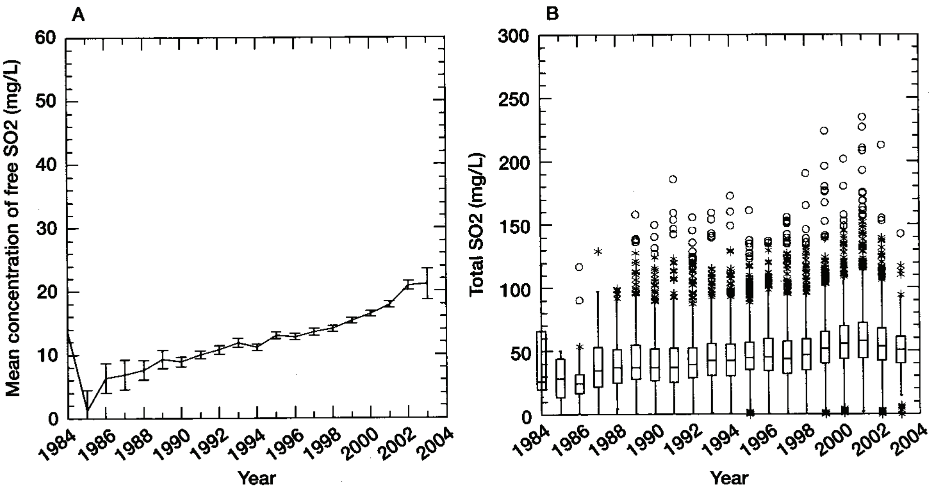

Fig. 1. Mean concentration of free (A) and total sulfur dioxide (B) in Australian red wine from 1984 to 2004 (AWRI unpublished data)

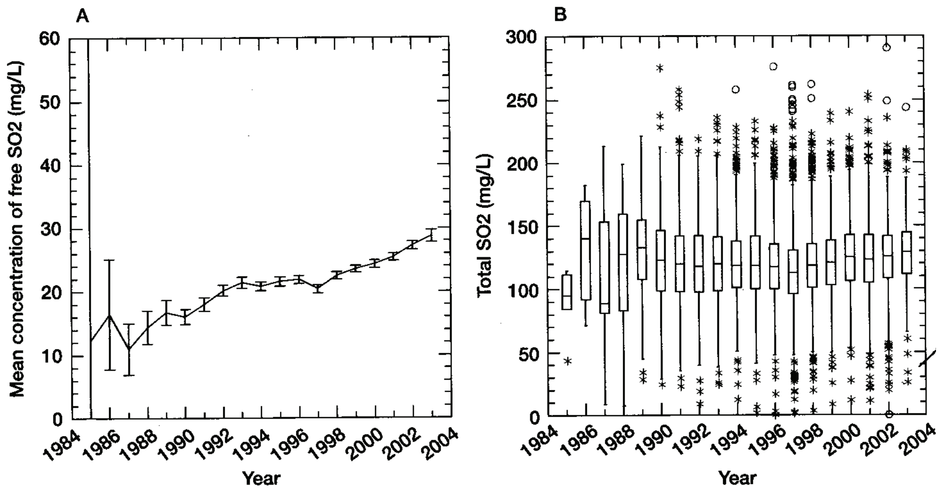

Fig. 2. Mean concentration of free (A) and total (B) sulfur dioxide in Australian white wine from 1984 to 2004 (AWRI unpublished data)

nausea, abdominal pain and diarrhoea, hypotension and anaphylactic shock, seizures and death, where symptoms may occur immediately or be delayed. The most common adverse reaction to sulfite compounds is asthma. Foods and food additives, however, are not common triggers for asthma (National Asthma Council of Australia 2005). Furthermore, adverse reactions to sulfite compounds in non - asthmatic and non-sensitive individuals are rare (Bush et al. 1986).

Approximately 30 years ago it was purported that 1-3mg sulfur dioxide released from wine and inhaled by a sulfite sensitive individual may trigger an adverse reaction (Freedman 1977; Baker et al. 1981; Ough et al 1983). It has since been clinically demonstrated, however, that sulfur dioxide will generally only precipitate an adverse reaction in sulfitesensitive asthmatics, which comprise approximately 1.7% of all asthmatics. Steroid-dependent asthmatics are most at risk of an adverse reaction. The threshold for an adverse reaction varies between 5200mg sulfur dioxide (Taylor et al. 2002) where foods containing greater than

100mg/L sulfur dioxide may elicit no reaction in some sulfite-sensitive individuals. Usually the minimum threshold is considered to be l0mg/L (Hefle and Taylor 2002; Taylor et al. 2002), which reflects existing Australian and international legislation stipulating that "added sulphites in concentrations of 10mg/kg or more" must be stated on the label of a food product such as wine. Indeed, adverse reactions elicited by additives and processing aids are relatively rare.

A recent study assessed the potential sensitivity of 16 individuals with asthma to red and white wines containing a low (3.7 and 18.9mg/L), a high (290.7mgL) concentration of total sulfur dioxide and model wine solutions containing no sulfur dioxide (Vally et al. 1999); the concentration of free sulfur dioxide in the wines was 1.9, 2.1 and 238.9mg/L, respectively. These individuals had selfreported wine-induced asthma, and were initially subject to a double-blind study of the low sulfite-containing wines, conducted between two and eight days apart, at the same time on each occasion; 175mL was consumed over a period of three minutes, and measurements of lung function and asthmatic symptoms were subsequently made.

Only three of the 16 asthmatic individuals experienced a rapid and significant change in lung function and worsened asthmatic symptoms: one subject to a low sulfite-containing wine, one to both low sulfite-containing wines and one placebo wine, and one to one low sulfite containing wine and one placebo wine. The fact that two of the three individuals experienced an adverse reaction to both the low sulfite-containing wines and the placebo suggests that compounds other than sulfur dioxide may have also elicited an adverse reaction in these individuals. In addition, four other individuals, however, perceived a significant reaction to one or more of the wines without a change in lung function. Ten of these asthmatic individuals were then subject to a double-blind study of the high sulfite-containing wines, and only two experienced an adverse reaction. This suggests that unless the asthmatic individuals were also sulfite-sensitive then even the maximum permissible concentration of sulfur dioxide wine, 300mg/L, may not elicit an adverse reaction, although some individuals may only react

~ adversely when their asthma is unstable or exacerbated by environmental condition, such as cigarette smoke (Dahl 1986).

An additional study then formally assessed sulfite reactivity in wine-sensitive asthmatics using medium and high sulfite containing wines (Vally and Thompson 2001). Wine containing 300mg/L sulfur dioxide elicited a positive asthma challenge •

in four of 24 patients reporting wine-sensitive asthma but with no response to wines containing 20, 75 or 150mg/L sulfur dioxide. The researchers concluded that only a small number of historically wine-sensitive asthmatic patients responded to a single dose challenge with sulfited wines under laboratory conditions.

These data suggest that the role of sulfur dioxide and/or wine in triggering asthmatic responses has been over estimated, or alternatively they suggested that cofactors or other components of wine may be important influences, although none of the asthma individuals assessed in either study by Vally et al. (1999, 2001)

were also allergic to egg, fish, milk and nuts, which are the common food allergens used in winemaking.

Conclusions

The concentration of sulfur dioxide in Australian wines has been continuously monitored since 1991 when Food Standards Australia New Zealand (formerly the National Food Authority) commenced its review of the then [Australian] Food Standards Code. Advances in techniques and technologies, and research into

practices and problems over the past 30 to 40 years (Rankine et al. 1976; Sneyd et al. 1993) have enabled the Australian wine industry

to respond to general community health concerns to reduce the dietary intake of sulfur dioxide by optimising the use of sulfur dioxide in winemaking. For example, understanding the properties of sulfur dioxide and its relationship with other winemaking variables such as pH, clarity and temperature as well as [dissolved] oxygen, has led to a general increase in the ratio of free-to-total sulfur dioxide, which maximises its effectiveness as an antioxidant and antimicrobial agent (Robinson and Godden 2003).

Furthermore, data from the relatively recent research undertaken by the Department of Medicine of The University of Western Australian now indicates that the low concentration of sulfur dioxide observed generally in Australian wine is not problematic for a significant proportion of sensitive individuals

(Vally et al. 1999, 2001). These data thus imply that the influence or role of sulfur dioxide and/or wine in triggering asthmatic adverse reactions may have been overestimated in the community.

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