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Food additive–additive interactions involving sulphur dioxide and ascorbic and nitrous acids: a review

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This review evaluates the published work on sulphur dioxide and ascorbic and nitrous acid reactions with other food additives to form stable compounds. In some cases, such as between nitrite and sorbic acid, the compounds formed have a potentially higher toxicity than the original additives. No adverse effects have been demonstrated in real foods, however, probably due to the adoption of substantial safety margins between no-effect levels in animals and the maximum levels of additives to which humans could be exposed.

The reactions discussed in this review are those most likely to occur in current additive usage. However, due to the large numbers of permitted food additives, many more interactions occur in foods that could lead to chemical reactions under favourable conditions. © 1997 Published by Elsevier Science Ltd. All rights reserved

INTRODUCTION

In recent years there has been much publicity given to consumer concerns about the addition of chemicals to food. One area of concern is the interaction between additives and the possible health risks or joint effects of the cocktail of additives that is consumed each day.

Both weak and strong chemical interactions can occur between food additives when used in combination. Weak interactions involving changes in hydrogen-bonding, ionic or hydrophobic interactions, are readily annulled by relatively small changes in temperature, pH or salt concentrations. Many interactions in this category are known, particularly between polymeric food additives, as, for example the formation of gels between the thickeners xanthan gum and locust bean gum (Mannion *et al.*, 1992), and the loss of sweetness intensity when the protein sweetener thaumatin interacts with carrageenan (Ohashi *et al.*, 1991). Such weak interactions are unlikely to survive the aggressive conditions of the human digestive system and the structures that form during food preparation would not therefore be expected to cause any increase in additive toxicity.

This review is principally concerned with chemical interactions between additives that generate new covalent bonds. The stable compounds formed could potentially survive the digestive process and be carried into the bloodstream. Sulphur dioxide and ascorbic and nitrous acids are particularly reactive and are frequently used in combination with other additives. The interactions of these additives with other food additives are therefore considered, with special regard being paid to those foods in which interactions are likely to occur and to the potential for toxic products to be formed.

SULPHUR DIOXIDE INTERACTIONS

Sulphur dioxide, in its various forms, is added to food to inhibit and control the growth of microorganisms, to inhibit enzyme-catalysed reactions, to inhibit non-enzymic browning, and to act as an antioxidant and reducing agent. It is widely used to help preserve all manner of foods, including fruits and vegetables and drinks derived therefrom, jams, pickles, sauces, meat products, poultry, bakery products and dehydrated foods. Despite being chemically highly reactive, particularly the nucleophilic sulphite ion, potential interactions with other additives have been identified in relatively few cases. Interactions with azo and other dyes, with ascorbic acid and its degradation products, with sorbic acid and with nitrite ion are the major interactions reported in the literature.

With azo dyes

The interaction of sulphur dioxide with azo dyes is important because of their frequent use together,

particularly in fruit drinks, but also to a lesser extent in jams, sauces and other products.

The stability of the azo dyes to sulphur dioxide is variable (Wedzicha, 1984; Kroyer, 1986*a*). Recently, it has been suggested that the dyes fall into two classes: those that produce secondary dyes on reaction with bisulphite, such as Amaranth, Ponceau 4R and Sunset Yellow; and those that lose colour without change in the wavelength of maximum absorption, such as Carmoisine and Tartrazine (Adams & Langley, 1995). The former class have hydroxyl groups ortho to the azo bond as well as unsubstituted para positions in the naphthalene nucleus. Such dyes exist predominantly as hydrazone tautomers rather than strictly azo compounds. This facilitates the addition of bisulphite ion to the para position, as demonstrated by Damant *et al.* (1989) for the conversion of Sunset Yellow to a lemon yellow compound (Fig. 1).

Carmoisine and Tartrazine do not form secondary dyes on reaction with bisulphite. In the case of Carmoisine, a hydrazone tautomer is feasible, but addition of bisulphite ion at the para position appears not to take place, probably as a result of charge delocalisation via the fused aromatic ring of the naphthalene system. Wedzicha and Rumbelow (1981) have suggested that one molecule of the dye reacts with one molecule of bisulphite to form an unstable complex that hydrolyses to a colourless hydrazo product (Fig. 2). No structural evidence was presented for such a complex, however. In Tartrazine, the ortho-hydroxy group is a substituent of the pyrazoline ring that does not possess the pi-electron mobility of a truly aromatic system. A hydrazone tautomer is therefore unlikely to form and the dye is consequently very stable in the presence of bisulphite.

The identity of the colourless breakdown products formed in the presence of bisulphite is unknown. Reduction is feasible, at least as far as the hydrazo product (Wedzicha & Rumbelow, 1981; Adams & Langley, 1995). The toxicity of such hydrazo compounds is unlikely to be any greater than that of the parent azo dyes from which they are formed *in vivo* as a result of the azoreductase activity of intestinal bacteria (Levine, 1991; R. Walker, personal communication, 1996).

Dis-azo and other more complex azo dyes have a fair to good stability to sulphur dioxide but the degradation products have yet to be identified.

With other dyes

The triarylmethane dyes also have variable stability to sulphur dioxide. Thus, Green S has good stability and is widely used in soft drinks in conjunction with sulphur dioxide, whereas Violet BNP is unstable. The products of decolorisation are probably sulphonates.

The indigoid dye, Indigo Carmine, has poor stability to sulphur dioxide whilst Quinoline Yellow, the only permitted quinoline food colour, has excellent stability.

The natural anthocyanin pigments form adducts with bisulphite ion. The ion forms a bond to position 2 or 4 of the flavylium nucleus which decolorises the pigment and simultaneously stabilises the glycosidic bond at position 3 (Adams & Woodman, 1973). Whilst the equilibrium constant for adduct formation is high at pH 3·0, acidification to pH≤1·0 leads to reversal of the reaction with quantitative recovery of the pigment. Such adducts are therefore likely to be unstable under gastric conditions and not contribute significantly to the toxicity of foods.

With ascorbic acid

Sulphur dioxide inhibits ascorbic acid oxidation and is therefore frequently used in combination with ascorbate, particularly in fruit drinks and sausages. Under anaerobic conditions, Davies and Wedzicha (1992) have suggested that hydrolysis of the ascorbic acid lactone ring could be catalysed by the highly nucleophilic sulphite ion. 3,4-Dideoxypentosulos-3-ene is then formed slowly as a result of decarboxylation and dehydration reactions. Bisulphite ion, the dominant sulphur dioxide species under acidic conditions, readily adds across the double bond to yield 3,4-dideoxy-4-sulphopentosulose (Fig. 3; Wedzicha, 1984).

Under aerobic conditions, the bisulphite ion forms a hydroxysulphonate of dehydroascorbic acid. Only the monoadduct is known due to the rigid ring structure of

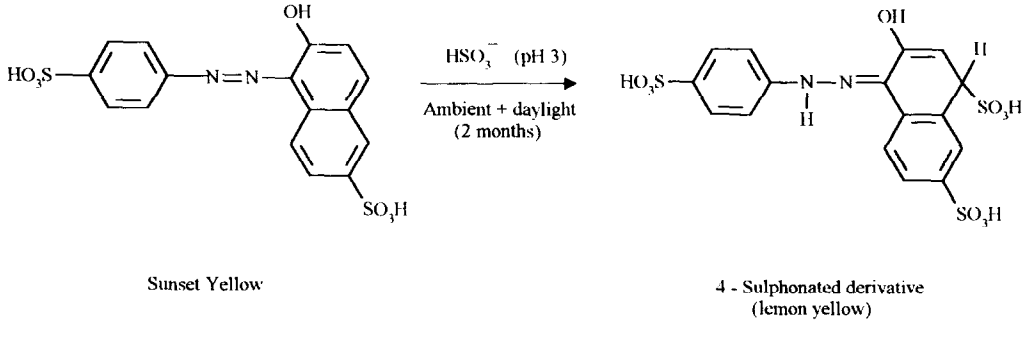
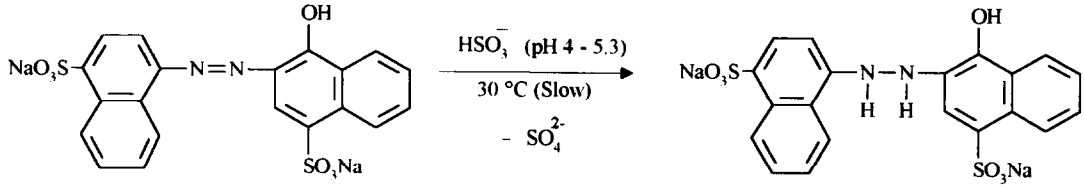


Fig. 1. The reaction of sulphur dioxide with Sunset Yellow.



Carmoisine Hydrazo-compound

Fig. 2. The reaction of sulphur dioxide with Carmoisine.

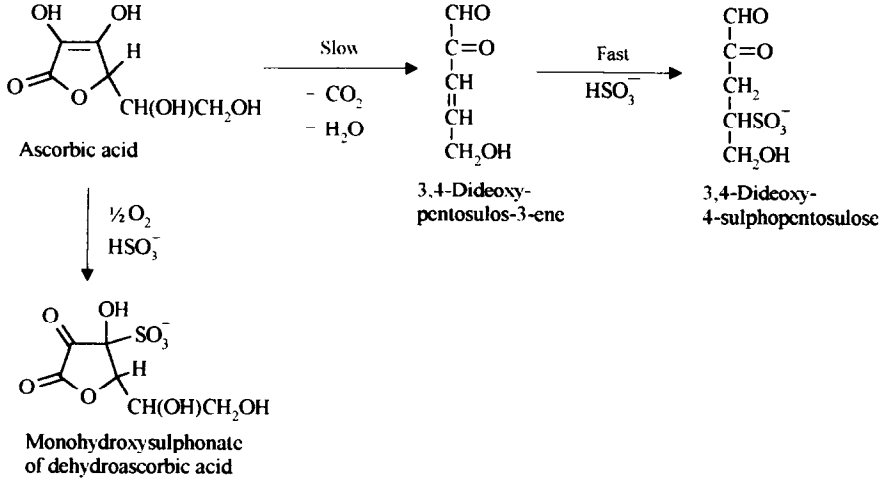


Fig. 3. The reaction of sulphur dioxide with ascorbic acid degradation products.

dehydroascorbic acid, which sterically hinders the approach of a second bisulphite to the free carbonyl group.

With sorbic acid

Sorbic acid is increasingly used as a replacement for sulphur dioxide and, occasionally, the two preservatives are used together. Under neutral conditions, sulphite ion reacts with sorbic acid, apparently by 1,2-addition across the diene (Fig. 4; Khandelwal & Wedzicha, 1990). Oxygen interferes with the reaction, hypothetically through sulphite-mediated oxidation in which free radicals such as $^{\cdot}OH$ and $^{\cdot}O_2^-$ oxidise sorbic acid (Goddard & Wedzicha, 1992). In aerobic food systems, such autoxidations are expected to prevail. Light has no effect on sulphite loss at neutrality.

Under acidic conditions, bisulphite ion causes the degradation of sorbic acid to many products, including minor amounts of α-angelica lactone and 2-methyl-5-acetylfuran (Saxby *et al.*, 1982). The major products were not identifiable by gas chromatography–mass spectrometry. However, the reaction was accelerated by light and could feasibly involve hydroxyl free radicals and superoxide anion that are capable of oxidising unsaturated organic compounds (Yang, 1984).

With nitrite ion

Sulphur dioxide is rarely used in combination with nitrite ion because the latter reacts with bisulphite ion to form products that are sulphonates of either hydroxylamine or ammonia (Wedzicha, 1984).

Such reactions would destroy the preservative activity of the individual additives. The reaction of alkali metal nitrites, in the cold, with an excess of bisulphite, leads to the formation of hydroxylamine *N,N*-disulphonate (Fig. 5). At elevated temperatures, complete substitution occurs at the nitrogen atom with the formation of the *N,N,N*-bonded trisulphonate. Under acidic conditions, the disulphonate can hydrolyse, yielding the monosulphonate and bisulphite ion, whilst the trisulphonate yields sulphamate. Further reaction of sulphamate with nitrous acid leads to production of bisulphate ion and nitrogen gas.

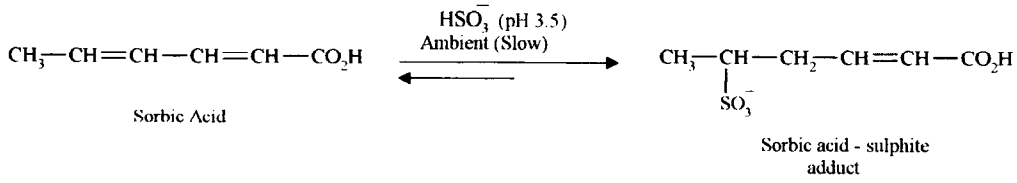


Fig. 4. The reaction of sulphur dioxide with sorbic acid.

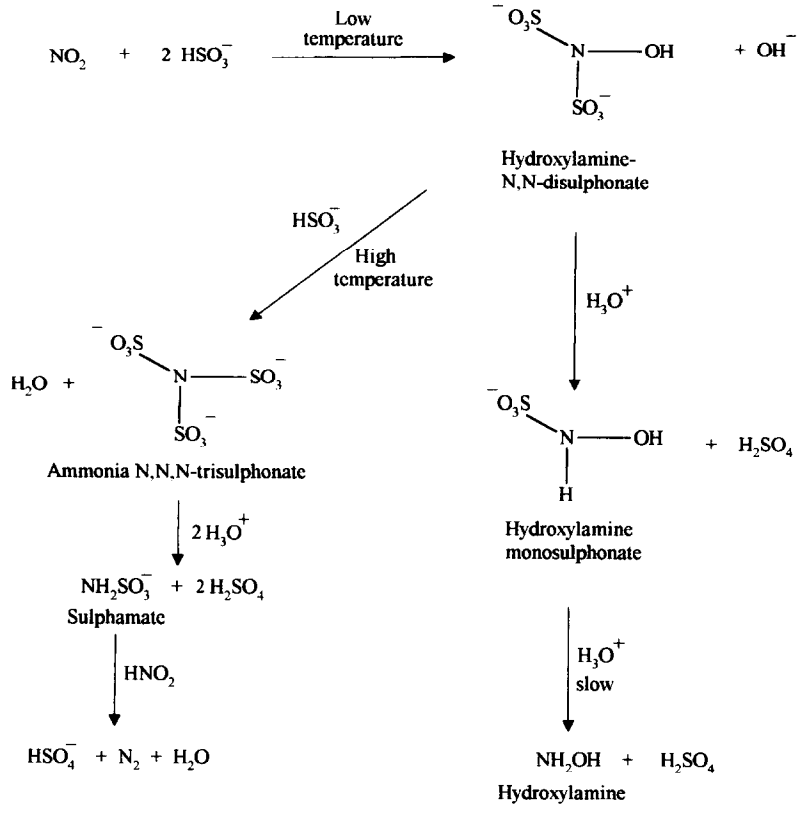


Fig. 5. The reaction of sulphur dioxide with nitrite ion.

Destruction of excess nitrite using bisulphite has been suggested in a patented process for producing cured meats (Coleman *et al.*, 1974). The reaction does not affect the nitrosyl pigments and so has no influence on the cured meat colour.

ASCORBIC ACID INTERACTIONS

Ascorbic acid is a widely used additive in the food industry, being added to fruit drinks, to curing solutions for meat products and to flour for bread-making. Potential interactions have been identified with azo dyes in fruit drinks and with nitrite used in curing meat products. Direct interactions with sorbic acid and, in the palmitate form, with vitamin E are also known, whilst reactions with artificial sweeteners and with benzoic acid require the presence of a heavy metal catalyst. The interaction of ascorbic acid degradation products with sulphur dioxide has been referred to above.

With azo dyes

As with sulphur dioxide, the interaction of ascorbic acid with azo dyes is important because of their frequent use in combination in fruit drinks. The stability of the most frequently used dyes in the UK has recently been studied (Adams & Langley, 1995). At pH 3·0 and 4·0, the following order of dye stability was observed in the presence of ascorbic acid: Tartrazine > Sunset Yellow≃ Amaranth≃Ponceau 4R > Carmoisine > Black PN. No stable secondary dyes were formed under these conditions. Model system studies using Sunset Yellow showed that exposure to fluorescent light greatly enhanced ascorbate-induced dye degradation.

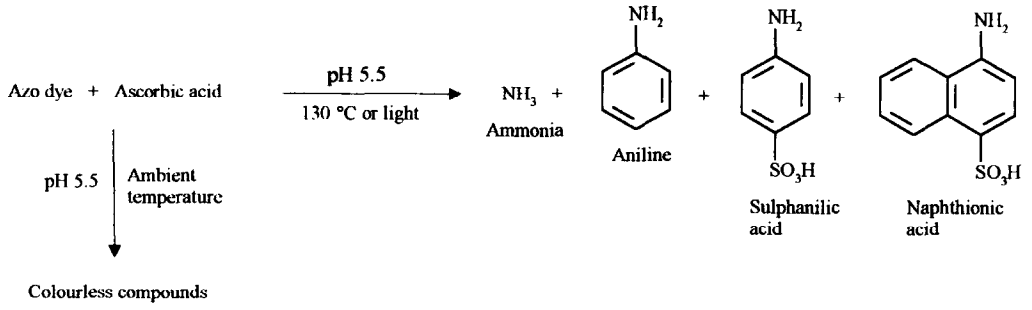


Fig. 6. The reaction of ascorbic acid with azo dyes.

At pH 5·5, in acetate buffer, Fogg and Summan (1983*a*,*b*, 1984) found a similar order of dye stability and sensitivity to light. This study also showed that inhibiting ascorbate oxidation with EDTA lowered the rate of azo dye degradation. In contrast, in studies on Amaranth, Marovatsanga and Macrae (1987) found that the presence of sucrose in a sample, or nitrogen in the headspace, factors expected to stabilise ascorbic acid caused an increase of dye loss in a soft drinks model system. Nitrogen purging was also shown to increase the rate of Sunset Yellow loss in a model system (Adams & Langley, 1995). It is therefore uncertain whether dye degradation is due to reduction by ascorbic acid or to reaction of the dyes with ascorbate oxidation products. Isolation and identification of the breakdown products is required to resolve this problem.

Nursten and Williams (1969) showed that Carmoisine and Ponceau 4R lost colour more rapidly on heating in the presence of ascorbic acid at pH 7 than at pH 3. The high stability of Ponceau 4R at pH 3 compared with Carmoisine was attributed to the sulphonic acid group peri to the azo bond. Black PN was particularly unstable at pH 3 and at pH 7, probably due to the reductive cleavage of both azo bonds to yield 1,4-diamino-naphthalene-6-sulphonic acid, a compound that had previously been shown to be capable of catalysing the breakdown of the original dye (Eisenbrandt & Lang, 1966). The dis-azo dye has also been shown to cause extensive destruction of ascorbic acid (Kroyer, 1986*b*).

Breakdown products of azo dyes have not been isolated from real foods or drinks containing ascorbic acid. This may be due mainly to difficulties in separating the azo dye breakdown products from ascorbic acid and its breakdown products (Adams & Langley, 1995). However, using diazotisation methods, Fogg and Summan (1983*a*) obtained evidence that amines such as aniline, sulphanilic acid and naphthionic acid are formed on reduction of azo dyes by ascorbic acid. Exposure to light caused degradation of the amines to ammonia (Fig. 6). Free aromatic amines are known to be relatively genotoxic and carcinogenic compared to their sulphonated derivatives (Jung *et al.*, 1992), and it is therefore important to know to what extent they are formed in real foods and drinks.

With nitrous acid

In meat curing at pH 4·5–6·5, the concentration of undissociated nitrous acid, HNO_2, would be expected to be low ($pK_a = 3{\cdot}4$ for HNO_2). Nevertheless, some very reactive nitrosating species can be generated (Sebranek & Fox, 1985). The reaction of excess nitrous acid with ascorbic acid (Fig. 7) has been considered to be a possible means of preventing nitrosation in cured meats (Basu *et al.*, 1984). The effect is apparently due to the formation of dinitrosyl ascorbate which readily breaks down to dehydroascorbic acid and nitric oxide (Fox *et al.*, 1981; Izumi *et al.*, 1989). The nitric oxide then reacts with aerial oxygen and water to produce a mixture of nitrous and nitric acids. As the nitrate is relatively unreactive, it acts as a sink to remove nitrous acid from the system.

The ascorbic acid reaction with nitrous acid has been shown to eliminate *N*-nitrosopyrrolidine formation in bacon frying at levels below 300 ppm ascorbate (Walters *et al.*, 1976). Nitrosamine formation in frying bacon takes place mainly in the adipose tissue, and model system studies have confirmed the importance of the phase in which nitrosamine formation occurs and the solubility of the secondary amines in that phase (Massey *et al.*, 1982). As a consequence of the preferred formation in the lipid phase, it has been suggested that only fat-soluble nitrite scavengers will effectively eliminate nitrosamine formation in bacon (Bharucha *et al.*, 1979). This may be a major reason why ascorbylpalmitate is more effective than sodium ascorbate (Sen *et al.*, 1976). The water-soluble derivatives of ascorbic acid, ascorbic acid 2-phosphate and ascorbic acid 2-sulphate, form more stable adducts with nitrous acid than does ascorbic acid itself (Izumi, 1992). Although the effect on nitrosamine formation is unknown, this enhanced adduct stability could be the cause of low nitrosomyoglobin colour development in some cured meat products.

Although not a direct reaction between additives, degradation products of ascorbic acid can react with nitrite ion (Fig. 7). Thus, 3-deoxypentosulose, arising from the spontaneous anaerobic decomposition of ascorbic acid during non-enzymic browning, shows significant reactivity at the pH of food products (Wedzicha *et al.*, 1982).

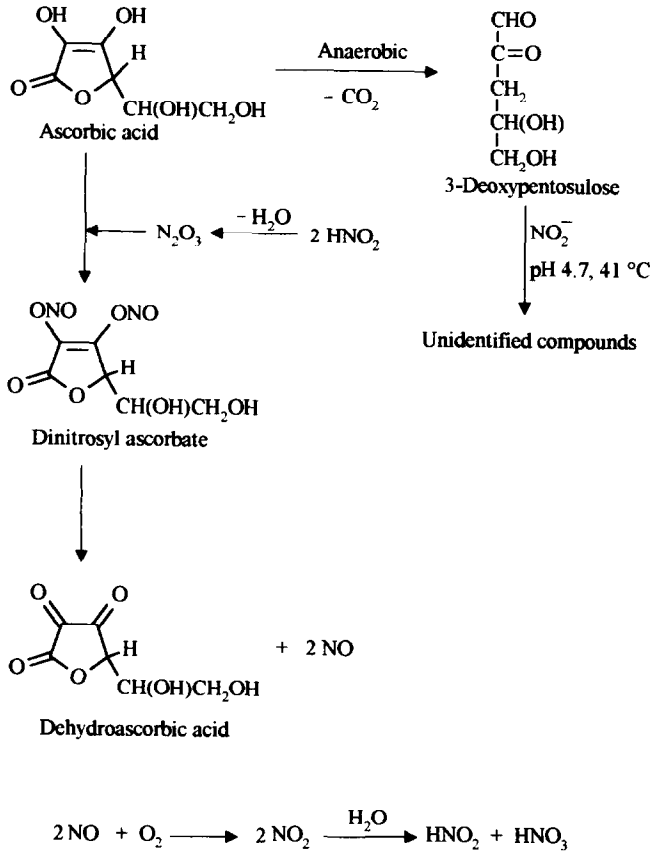


Fig. 7. The reaction of ascorbic acid with nitrite ion.

With artificial sweeteners

Artificial sweeteners are commonly used as sugar substitutes in beverages containing ascorbic acid. Interactions between the sweeteners and the vitamin are therefore of particular interest. Ascorbic acid was found to enhance the rate at which Aspartame and Acesulfame K degraded in acidic, aqueous solutions in the temperature range 120–200°C (Kroyer *et al.*, 1993). The ascorbic acid degraded more rapidly under these conditions as well. The high temperatures required in this work could indicate that some ascorbic acid degradation was necessary before any reaction with the artificial sweeteners could occur. At 30°C, Hsieh and Harris (1991) found that the oxidation of ascorbic acid occurred more rapidly in the presence of Aspartame only when cupric ion was present. This was unexpected as Aspartame, due to its chelating properties, was predicted to lower the activity of copper, so that the ascorbate oxidation should have been less. Presumably the copper complex with Aspartame was a more effective autoxidation catalyst than cupric ion alone.

With vitamin E

Evidence has been presented for the interactions between radicals of ascorbylpalmitate and vitamin E generated by aerial oxidation of subcutaneous chicken fat (Lambelet *et al.*, 1985). Electron spin resonance spectroscopy was used to show that hydrogen atom exchange took place between the ascorbylpalmitate and vitamin E radicals. Sequential consumption of the two vitamins, first vitamin C and then vitamin E, was demonstrated by differential pulse polarography.

The nitrite scavenging effect of ascorbic acid is enhanced by α-tocopherol and a complementary effect of the two vitamins was observed on the formation of nitrosopyrrolidine in fried bacon (Lathia & Blum, 1989). In fish oils, which contain abundant quantities of unsaturated fatty acids that have been associated with the prevention of heart disease, δ-tocopherol and ascorbic acid act synergistically in their antioxidant properties (Yi *et al.*, 1991).

With benzoic acid

Evidence has been presented that ascorbic acid can interact with benzoic acid in the presence of a transition-metal catalyst to yield benzene, a known carcinogen (Gardner & Lawrence, 1993). This apparently occurs as a result of the metal-catalysed reduction of oxygen and hydrogen peroxide by ascorbic acid forming hydroxyl radical which can then decarboxylate benzoic acid. The conditions for this reaction in a model system suggest that benzene could be produced in low yield in food products, especially acid beverages, containing the combination of ascorbic acid and sodium benzoate. The findings of a recent survey have confirmed this suggestion and although levels of benzene were only slightly raised, many soft drink manufacturers in the USA have taken action to avoid its formation (McNeal *et al.*, 1993).

NITRITE ION INTERACTIONS

The main use of nitrite is in the curing of meat where, in conjunction with sodium chloride, it inhibits the growth and toxin production of *Clostridium botulinum*. As the sodium salt, it is generally used at parts per million levels that are chosen to represent a compromise between the potential for formation of nitroso compounds at high nitrite levels and the potential for *Clostridium botulinum* toxin formation at low nitrite levels (Sebranek & Fox, 1985).

In addition to the interactions with sulphur dioxide and ascorbic acid discussed above, interactions with chloride ion, sorbic acid and lecithin have been reported.

With chloride ion

Sodium chloride added to cured meat at about 2–2·5% does not appear to influence nitrosamine levels. However, Sebranek and Fox (1985) have suggested that

sodium chloride can affect nitrosation reactions through the formation of nitrosyl chloride. Fox *et al.* (1994) have recently offered kinetic evidence for nitrosyl chloride production in meat model systems. This suggested that nitrous acid and nitrosyl chloride could be independently reduced by ascorbic acid to yield nitrous oxide which then reacted with myoglobin to form the nitrosylmyoglobin derivative. The proposed mechanisms involved formation of a semi-stable mononitrosoascorbyl dimer, in the absence of chloride, while the chloride-catalysed reaction proceeded by the facile reduction of nitrosyl chloride by ascorbic acid.

Whilst nitrosyl chloride is known to exist under strongly acidic conditions, it has not been isolated and identified under the mildly acidic conditions that prevail on meat curing. The evidence for its existence therefore remains tenuous.

With sorbic acid

Sorbic acid has been proposed as a partial replacement for nitrite in meat curing, since it inhibits *Clostridium botulinum* growth and also reduces the formation of nitrosamines. However, this practice may lead to other toxicological problems since sorbic acid reacts with nitrite to yield mutagenic reaction products (Namiki *et al.*, 1981). At pH 3·5–4·2, the main mutagens have been proposed to be 1,4-dinitro-2-methylpyrrole (DNMP) and ethylnitrolic acid (Fig. 8; Osawa & Namiki, 1982). The reaction requires several days to occur at 4°C but takes place in hours at 60°C. The highest yield was obtained when the nitrite was in eight-fold excess over the sorbic acid. Optimum conditions do not exist in curing brines, and negative results have been obtained in mutagenicity assays in brines containing sorbic acid (Walker, 1990). However, the presence of both sorbic acid and nitrite in the diet of mice apparently leads to genotoxic compounds being formed *in vivo* (Banerjee & Giri, 1986).

Ascorbic acid completely abolishes the mutagenicity of DNMP by reduction of the C-4 nitro group to a C-amino group (Osawa & Namiki, 1982; Osawa *et al.*, 1986). The 1-nitro-2-methyl-4-amino pyrrole formed is non-mutagenic. Osawa *et al.* (1986) suggested that similar reducing substances were responsible for the desmutagenic activity of vegetable juices.

With lecithin

Lecithin preparations are used commercially as emulsifying agents, as dietary supplements and in sprays used to prevent food sticking to pots and pans during cooking. Lecithins have also been proposed to prevent bacon slices from sticking together (Wrobel & Rendek, 1973). They are a source of the quaternary ammonium compound choline, which can decompose on heating to yield trimethylamine. Demethylation can then produce dimethylamine which can react with nitrite to form dimethylnitrosamine, a known carcinogen. The formation of dimethylnitrosamine has been found to occur on heating sodium nitrite and lecithin at pH 5·6 in a model system (Pensabene *et al.*, 1975). Thus, nitrosamine formation is possible in foods containing both lecithin and nitrite.

CONCLUSIONS

Consideration has been given in this review to interactions of sulphur dioxide, ascorbic acid and nitrite with other additives. Interactions that lead to the formation of new covalent bonds have been discussed, with particular attention being paid to those foods in which the interactions are likely to occur, and with regard to the potential formation of toxic products. It should be noted that no adverse effects have, in fact, been demonstrated in real foods that could be attributed to additive interactions, and any possibility of such interactions taking place is taken into account by the adoption of substantial safety margins between the no-effect levels observed in animals and the maximum levels of additives to which humans could be exposed.

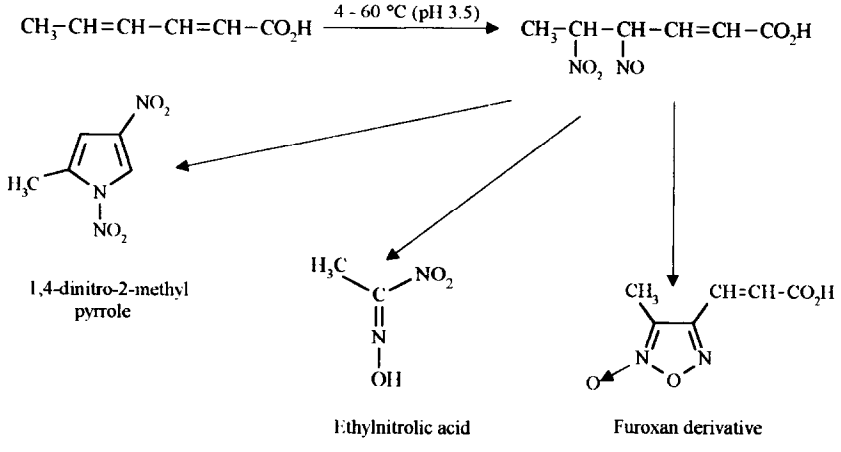


Fig. 8. The reaction of nitrite ion with sorbic acid.

Considering the large numbers of food additives permitted for use, many more interactions could occur than have been considered in this review. A strategy is therefore required that will assist in identifying those interactions that could occur in foods leading to new compounds with potentially higher toxicity than the original additives. This could involve (1) determining all food additive combinations in current use and (2) evaluating the potential for additive–additive reactions occurring by using chemical reaction databases in conjunction with appropriate expert systems. Such an exercise should enable interactions to be identified that could potentially lead to toxic products. Real food studies would then be required to confirm these 'theoretical' findings.

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