Food Chemistry **39** (1991) 281–297

Reactivity of Sulphur Dioxide in Comminuted Meat

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(Received 2 February 1990; revised version received and accepted 15 March 1990)

ABSTRACT

The factors which determine the instantaneous irreversible binding of sulphur dioxide when sodium metabisulphite is added to comminuted meat are considered. The most important variable which affects the reactivity of sulphur dioxide in comminuted fillet steak, pork steak, pork chop, braising steak, shin beef and belly pork, is fat content, reactivity decreasing with increase in fat-content. This observation is explained in terms of liquid fat tending to smear, during comminution, over components of meat which are reactive towards the additive, thereby providing a protective barrier. In meat other than pork steak or chop, the reactive food components include disulphide bonds of proteins. The amount of S-sulphonate formed on addition of sulphur dioxide correlates with the cystine-content of the meat. The highest yield of S-sulphonate was obtained for shin beef where cleavage of disulphide bonds accounts for 48% of the additive which had undergone reaction. No S-sulphonate formation was observed for pork steak or pork chop samples.

INTRODUCTION

Sulphur dioxide is added to a limited number of meat products as an antimicrobial agent. In British fresh sausage it inhibits Enterobacteriaceae and especially salmonellae (Christian, 1963; Banks & Board, 1982). The spoilage of sulphited meat is limited to Gram-positive microflora consisting of Lactobacilli and *Brochothrix thermosphaeta* (Banks & Board, 1981) and leads to a sour odour unlike the putrid odour of spoiled untreated meat (Dyett & Shelley, 1966). The additive also has a beneficial effect on the colour

Food Chemistry 0308-8146/90/$03·50

of meat (Krol & Moerman, 1959). At neutral pH it can inhibit the oxidation of myoglobin to metmyoglobin. However, it cannot re-reduce metmyoglobin. Texture and flavour are also said to be affected by sulphur dioxide (Leads, 1979).

The manufacture of pork sausage with a target of 450 mg SO_2 per kg requires the addition of some 600 mg SO_2 per kg since approximately 25% is lost during the process. This amount is not recoverable by the Monier Williams distillation technique and is considered irreversibly bound. Some (*c.* 100 mg SO_2 per kg) of the remaining additive is bound reversibly, probably as hydroxysulphonate adducts formed between hydrogen sulphite ion and carbonyl constituents of the food. On subsequent storage, a further 100 mg SO_2 per kg may become irreversibly bound over a period of 8 days at 4°C (Banks & Board, 1982) but most of the remaining additive is progressively converted to a reversibly bound form. This is thought to be due to the formation of hydroxysulphonate adducts of carbonyl compounds of microbial origin. There is increasing evidence about the important role of acetaldehyde of yeast origin in reversible binding of S(IV) and the presence of the additive enriches such organisms as well as the spoilage organisms referred to above.

The purpose of this investigation is to identify the mechanisms contributing to the initial irreversible binding of sulphur dioxide when the additive is incorporated into comminuted meat. One possible pathway is reaction with disulphide bonds of meat proteins. In the case of sausage, cereal is added in the form of rusk and the cleavage of wheat flour protein could well also contribute to the reactivity of the additive. In order to separate any effects of rusk from those of meat proteins the present study involved meat samples with no added rusk.

MATERIALS AND METHODS

Wherever possible, reagents were of AnalaR grade and were obtained from BDH Chemicals Ltd (Poole, Dorset, UK). Meat was obtained locally from various retailers. The meat samples (500 g) were comminuted in a bowl chopper (Crypto Peerless R1) for varying lengths of time and, when required, the product was minced (Hobart AE200) through 2 mm holes. The comminuted product was divided into 5 g aliquots which, unless otherwise stated, were used immediately.

Defatted meat was prepared by exhaustive solvent extraction using a soxhlet apparatus. Comminuted meat samples were used without further treatment for extraction with acetone but were freeze-dried prior to

extraction with petroleum spirit. When required, the extract was recovered by evaporating the solvent (40°C, *in vacuo*).

Reactivity of meat samples

In order to assess the reactivity of the meat towards S(IV), samples (5 g) in 15 ml glass sample vials, were allowed to reach thermal equilibrium at the appropriate temperature, maintained to $\pm 0{\cdot}1$°C by a water bath, and solid $Na_2S_2O_5$ or a solution of the salt, added. The mixture was stirred for a given length of time with a nickel spatula and the S(IV)-content determined as described previously (Wedzicha & Bindra, 1980; Banks & Board, 1982) immediately or at timed intervals.

In experiments for which a controlled atmosphere was required, meat samples were placed in a desiccator which was evacuated and nitrogen or oxygen introduced. After 30 min the samples were mixed with solid $Na_2S_2O_5$ under the appropriate gas and immediately returned to the desiccator where the atmosphere in the headspace was re-established. Samples were analysed at timed intervals.

Determination of S-sulphonates

The method is based on that of Nakamura & Tamura (1974). A sulphited meat sample (5 g) in a 15 ml sample vial was mixed thoroughly using a glass rod, with dithiothreitol (5 mM, 1 ml) in Tris-HCl buffer (0·05M, pH 9·2) containing EDTA (5 mM). The mixture was warmed (37°C, 5 min) and Hg_2Cl_2 (0·1M, 1·5 ml) added to remove any thiols liberated by DTT and which might interfere with the analysis for S(IV). Heating time and temperature had been varied in order to determine the optimum conditions on the basis of maximum recovery of S(IV). The suspension was shaken with water (20 ml) and the whole of the mixture analysed for S(IV) as above.

Analysis of cystine, water and fat-content

For analysis of cystine, samples were prepared as described by Paul and Southgate (1978) and the method was that of Moore (1963). Water-content was determined by the AOAC (1984) method and total fat-content according to Pearson (1970).

Experiments involving the use of malachite green

Pork steak which had been extracted with acetone and dried over P_2O_5 (2 g) was suspended in solutions of malachite green (1–30 mg/100 ml, 10 ml), the

mixtures allowed to stand for 5 min and filtered through Whatman No. 1 paper. The filtrate was made up to 10 ml and its absorbance measured at 616 nm. In order to correct for adsorption of the dye on to filter paper, the starting solutions were filtered through the paper and analysed as for the meat fractions. The data were used to plot adsorption isotherms.

The relationship between reflectance and the amount of malachite green adsorbed was investigated by packing the wet solid tightly into a 2·5 cm Petri dish and measuring the reflectance at 616 nm (Macbeth 'Color-Eye' spectrophotometer). The wet solid obtained above was also mixed intimately with varying weights of a lipid fraction extracted from pork steak with petroleum spirit. The reflectance of these samples was measured similarly, before and after the addition of water (5 ml) and solutions of $Na_2S_2O_5$ (5 ml, *c.* 1 and 2 mM).

RESULTS AND DISCUSSION

Preliminary experiments

In this work sulphur dioxide was added to meat in the form of sodium disulphite (metabisulphite, $Na_2S_2O_5$) because this salt is stable to autoxidation without the need for special precautions, e.g. inert atmosphere, and is used widely for this purpose in sausage manufacture. Approximately 4·5 mg of the salt (total sulphur(IV)-content = 47 μmol) is required for a 5 g sample of meat to give 600 mg SO_2 per kg. The ionic form of the additive depends on concentration and pH. In meat systems which are of relatively high water activity, disulphite ion is hydrolysed to hydrogen sulphite ion,

$$S_2O_5^{2-} + H_2O \rightleftharpoons 2HSO_3^-$$

and this species predominates in the pH range of many foods (pH 2–7). However, the pK_a of HSO_3^- is 7·2 and small but significant concentrations of sulphite ion may be present in some samples. Thus it is not realistic to express the total concentration of the additive in terms of individual species unless detail of their activity coefficient and pK_a, and ionic strength of the medium is known. To avoid these complications the amount of the additive will be expressed here as moles of S(IV), indicating the total number of moles of sulphur oxospecies in oxidation state +4.

The distillation/spectrophotometric analysis using 5,5-dithiobis(2-nitrobenzoic acid) was originally developed (Wedzicha & Johnson, 1979; Wedzicha & Bindra, 1980) for determination of sulphur dioxide in soft drinks and dehydrated vegetables, from a method reported by Humphrey *et al.* (1970). Banks and Board (1982) first applied it to the determination of the additive in sausage. When 5 g samples of pork steak, taken from a single

batch of the comminuted meat were each mixed (30 s) with 54·8 μmol S(IV) as solid $Na_2S_2O_5$, separate analysis of the samples gave a recovery of 39·4 ± 2·0 μmol (mean of eight determinations ± standard deviation). A distillation time of 10 min was found to give a maximum recovery of the additive. The loss of some 46% of the additive when comminuted pork is mixed with S(IV) is much greater than a loss of 26% reported by Banks and Board (1982) for sausage. One possible reason for the apparently lower recovery of S(IV) in the present investigation is incomplete liberation of reversibly bound S(IV). In order to determine whether *total* S(IV) was being measured, the pH of the meat–S(IV) mixture was raised to pH 11 with NaOH immediately prior to analysis. This releases reversibly bound S(IV). Analysis with and without addition of NaOH gave, respectively, 14·3 ± 0·3 and 14·4 ± 0·3 μmol S(IV) in 5 g samples (mean of four determinations ± standard deviation). It is concluded, therefore, that analysis was satisfactory and the difference between our result and that published previously was attributable to the composition of the samples or the way in which meat–S(IV) mixtures were prepared.

Other variables which need to be considered are (a) length of mixing time for incorporation of additive into the meat, (b) effect of oxygen, (c) temperature at which mixing is carried out, (d) presence of liquid water in the sample which may arise by disruption of cells during comminution, (e) age of the meat and (f) the effect of freezing.

In all experiments 47·4 μmol S(IV) as solid $Na_2S_2O_5$ were added to separate 5 g samples of comminuted pork steak. When samples were mixed for 30 s and 2 min, the recoveries of S(IV) were, respectively, 25·9 ± 0·8 and 24·5 ± 1·0 μmol (mean of six determinations ± standard deviation); though the difference between them is small these values are just significantly different at $p = 0{\cdot}05$ ($t_{calc} = 2{\cdot}45$; $t_{0{\cdot}05} = 2{\cdot}23$). The implication of this result is that it is necessary to control the mixing time and since it was desired to investigate reactions which take place on a short timescale, a mixing time of 30 s was adopted as a reasonable time during which incorporation of the additive into the meat sample could be carried out. Figure 1 illustrates the effect of time of storage, at 15°C, after mixing, on the loss of S(IV) from comminuted pork, in the presence and absence of an oxygen atmosphere. It is evident that the additive continues to react slowly after mixing but the effect of oxygen on the reaction is of particular interest. The longer term reaction is dependent on the presence of oxygen whilst the loss which took place during 30 s of mixing was independent of oxygen-content. This was confirmed by four replicate experiments with and without oxygen in the headspace which gave insignificantly different ($P < 0{\cdot}01$) means. Provided that samples are analysed immediately after mixing it is not necessary to take precautions to exclude air.

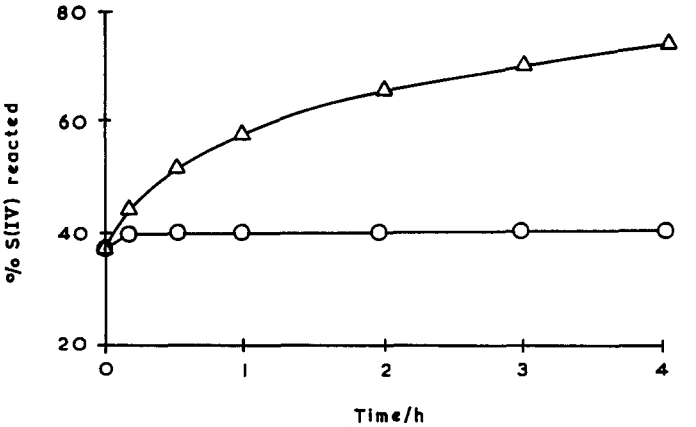


Fig. 1. Effect of oxygen on the amount of S(IV) reacted with time, when comminuted pork steak (5 g) is treated with $Na_2S_2O_5$ (4·5 mg) at 15°C. ○, Nitrogen headspace; △, Oxygen headspace.

An assessment of the effect of mixing temperature gave the results shown in Table 1.

Surprisingly, there is an increasing retention of S(IV) as mixing temperature is increased. This result is unexpected because the implication is that the rate of the reaction in question decreases with increasing temperature. It is, however, clear that temperature control during mixing is required to ensure reproducible results and a temperature of 15°C was adopted for the remainder of the work. The temperature in sausage production is not regulated but is kept below 16°C (Wilson, 1960).

A possible contributor to the effect of mixing time on the extent of loss of S(IV) is the fact that when solid $Na_2S_2O_5$ is first added, it is localised in the sample and mixing serves to disperse it. In order to assist dispersion, the additive was dissolved in varying volumes of water and solutions added to 5 g samples of meat. The residual levels of S(IV) found are shown in Table 2.

The addition of the S(IV) in up to 0·25 ml water does not cause a significant ($p < 0·05$) change in retention of S(IV) and it may be concluded that distribution of the additive in the liquid phase of the meat is probably not a rate determining factor.

When pork samples were stored at 4°C for varying lengths of time (1–4 days) before the addition of S(IV) as solid $Na_2S_2O_5$, the amounts of S(IV) recovered as a function of storage time are shown in Table 3.

There is no significant trend in these results ($p < 0·05$) and storage before addition of S(IV) does not alter its irreversible binding. On the other hand, it is likely that the tendency for *reversible* binding to occur does, in fact, increase with time (Banks & Board, 1982). If comminuted pork samples are frozen and stored at −30°C for 24 h and 2 weeks, before addition of S(IV),

TABLE 1
Effect of Mixing Temperature on Retention of S(IV) when Comminuted Pork Steak (5 g) is Mixed with S(IV) (47·4 μmol) as Solid $Na_2S_2O_5$

	Temperature (°C)			
	0	*15*	*20*	*25*
S(IV)-content (μmol)	24·1 ± 0·7	28·2 ± 0·8	29·0 ± 0·7	32·0 ± 0·7

S(IV)-contents are given as means ± standard deviations from four replicate experiments.

TABLE 2
Effect of Volume of Added Water to Comminuted Pork Steak (5 g) on Retention of S(IV) when Meat Sample is Mixed with S(IV) (47·4 μmol) in Solution

	Volume added (ml)				
	0	*0·05*	*0·10*	*0·25*	*0·50*
S(IV)-content (μmol)	28·5 ± 0·5	28·8 ± 0·7	28·4 ± 0·5	27·8 ± 0·7	26·9 ± 0·6

The results are means of four replicates ± standard deviation and do not depend on the fact that the mass of the sample had changed on addition of water.

TABLE 3
Effect of Storage Time of Pork Steak on Retention of S(IV) when Stored Samples (5 g) are Mixed with S(IV) (47·4 μmol) as Solid $Na_2S_2O_5$

	Time (days)			
	1	*2*	*3*	*4*
S(IV)-content (μmol)	28·4 ± 0·7	28·4 ± 0·5	28·1 ± 0·5	27·6 ± 0·7

Each value is the mean from four replicates ± standard deviation.

TABLE 4
Effect of Comminution Time on Retention of S(IV) when Comminuted Pork Steak (5 g) is Mixed with S(IV) (47·4 μmol) as Solid $Na_2S_2O_5$

	Time (s)			
	30	*60*	*90*	*90 + mincing*
S(IV)-content (μmol)	42·2 ± 1·4	38·3 ± 1·4	32·7 ± 1·1	29·7 ± 0·7

Each result is the mean of four replicates ± standard deviation.

the respective S(IV)-contents per sample were found to be 28·6 ± 0·5 and 27·5 ± 0·6 μmol (mean of five replicates ± standard deviation), compared with 28·3 ± 0·5 μmol for five samples of pork from the same batch before freezing. Freezing has no significant effect ($p < 0·05$) on reactivity despite its known effect on the texture of meat.

The most important experimental variable appeared to be the degree of comminution. When pork steak was comminuted in a bowl chopper for varying lengths of time (30–90 s) or a sample which had been chopped for 90 s was minced through a disc with 2 mm holes, before addition of S(IV), the S(IV)-contents found as a function of comminution time are shown in Table 4.

It is clear that the more finely chopped meat appears to be more reactive towards S(IV) and the results have a lower standard deviation. The latter may be due to improved homogeneity of the batch leading to more uniform samples and the normal practice adopted for preparing comminuted meat samples was the use of a bowl chopper for 90 s followed by mincing.

Effect of heat

A possible cause of the reactivity of S(IV) is the action of oxidising enzymes and a simple method of testing this possibility is to heat the meat sample

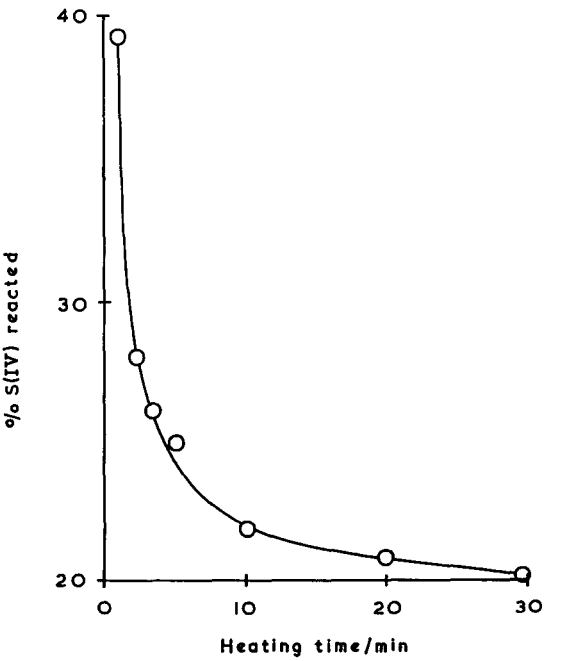


Fig. 2. Effect of time of heating at 80°C of comminuted pork steak (5 g) before the addition of $Na_2S_2O_5$ (4·5 mg), on the extent of reaction of S(IV).

prior to assay of its reactivity towards S(IV). Figure 2 shows the effect of heating at 80°C for varying lengths of time prior to addition of S(IV). It is clear that the effect of heat is to reduce the reactivity of the sample considerably, consistent with the denaturation of an enzyme.

Effect of fat-content

In exploratory work it was found that, if pork steak samples were extracted exhaustively with acetone before reactivity towards S(IV) was assessed, the result gave 75 ± 3% loss of S(IV) compared with 36% for the original meat sample. The extent of this loss did not change if a 'rehydrated' extracted pork sample was heated at 80°C for varying lengths of time. Furthermore, if lard (0·5 g) was added to such extracted samples (weight equal to the weight of fat extracted into acetone) and the mixture heated at 80°C for 10 min before assessment of reactivity towards S(IV), it was found that the loss of S(IV) was only 13·5%. It was suspected, therefore, that the effect of heat (Fig. 2) could be to cause melted fat to coat the substance which reacts with S(IV), thereby blocking access to S(IV). This suggestion is strengthened by the observation that when different meat samples (fillet steak, pork steak, pork chop, braising steak, shin beef, belly pork) were analysed for fat content and samples also assessed for their reactivity towards S(IV), the observed loss of S(IV) was

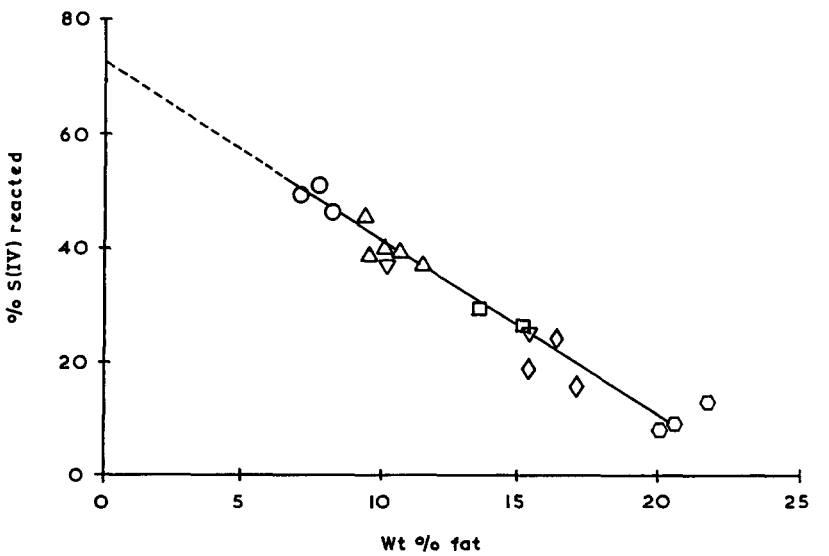


Fig. 3. Relationship between the fat-content of different cuts of meat from various sources and the amount of S(IV) reacted when comminuted meat samples (5 g) were mixed with $Na_2S_2O_5$ (4·5 mg) at 15°C. ○, Fillet steak; △, pork steak; □, braising steak; ▽, shin beef; ◇, pork chop; ⬡, belly pork.

strongly correlated to fat content as shown in Fig. 3. When freeze-dried meat samples were extracted with petroleum spirit and rehydrated with the original weight of water removed, the observed loss of S(IV) when samples were mixed with the additive as before was 33·9, 32·7, 33·0, 33·3, 33·9 and 33·9 μmol for the six different types of meat, respectively. This indicates that the potential reactivity of the non-fat solids of various cuts of meat from different animals is very similar and implies that the different reactivities of the fresh comminuted meats are due to their differing fat contents. If the values of the loss of S(IV) given above are assumed to come from the same population, their mean value of $33{\cdot}5 \pm 0{\cdot}5$ μmol is seen to be the intercept of the graph in Fig. 3. This observation adds credence to the idea that fat-content is the important variable which distinguishes the different meats.

Meat samples with different fat contents are also likely to contain different amounts of water and, hence non-fat solids. Figure 4 shows the relationship between the loss of S(IV) and the total non-fat solids-content of each sample (i.e. weight of meat—weight of fat—weight of water); this gives a significant ($p < 0{\cdot}05$) correlation ($r = 0{\cdot}92$) between the variables, though the result is less satisfactory than that shown in Fig. 3. It is noteworthy that the graph intersects the x-axis (when S(IV)-loss = 0) a long way from the origin suggesting that non-fat solids-content is perhaps the less likely variable.

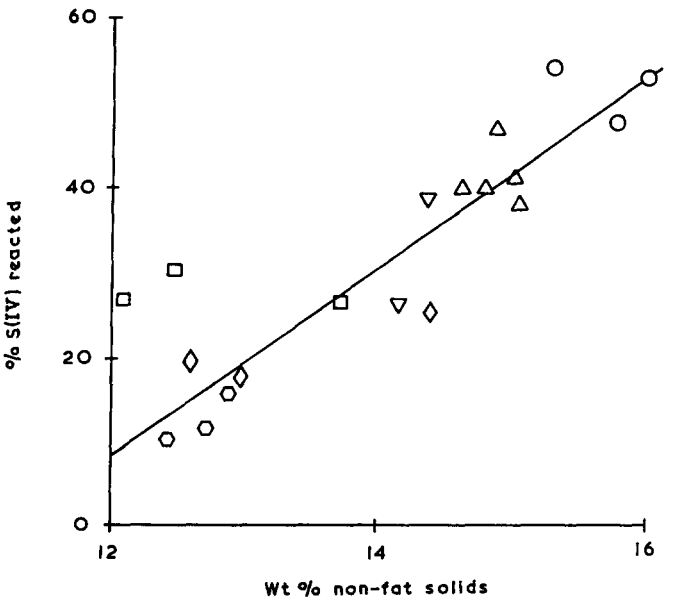


Fig. 4. Relationship between the non-fat solids-content of different cuts of meat from various sources and the amount of S(IV) reacted when comminuted meat samples (5 g) were mixed with $Na_2S_2O_5$ (4·5 mg) at 15°C. ○, Fillet steak; △, pork steak; □, braising steak; ▽, shin beef; ◇, pork chop; ⬭, belly pork.

Fat smearing, in which fat covers meat particles which are comminuted, is a common problem in sectioning meat samples for microscopy but this may be reduced by chilling samples prior to cutting (F. O. Flint, pers. comm.). The fat in, for example, pork melts over two ranges: 8–14°C and 18–40°C and, at 15°C, the lower melting fat will already be in the liquid phase. In order to assess whether smearing of fat over meat particles contributed to the reactivity of S(IV), samples of pork were comminuted at 0°C and their reactivities at 15°C compared with those of samples comminuted at 15°C. Respective losses of 48·7 and 42·0% (each result the mean of four determinations) were significantly different ($p < 0{\cdot}05$). The increased retention of S(IV) with temperature at which the samples were mixed with S(IV), reported above, is also consistent with this result and the possibility of fat being distributed during comminution leading to a reduction of reactivity.

An attempt to illustrate the possibility of fat preventing access of S(IV) to meat fibres was made by labelling, with malachite green, pork samples which had been exhaustively extracted with acetone. This dye reacts quickly with S(IV) to yield a colourless product. The purpose of labelling was, therefore, to measure the effect of added fat on the bleaching of adsorbed malachite green by S(IV). Malachite green is a stain for proteins and is, therefore, a good choice for the experiment attempted here when the principal structural

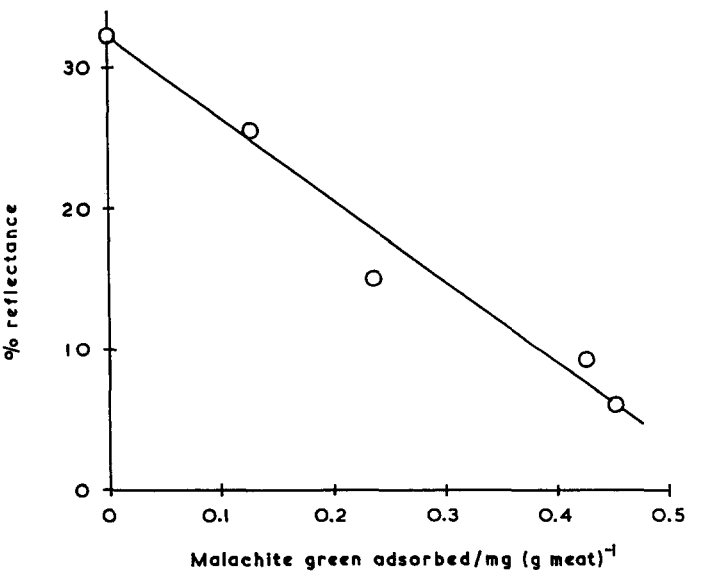


Fig. 5. Relationship between reflectance at 620 nm and the weight of malachite green adsorbed on to pork steak which had been exhaustively extracted with acetone. The amount of malachite green adsorbed is expressed in terms of the weight of the meat sample before extraction with acetone.

component is proteinaceous. When samples of acetone-extracted pork steak, each from 5 g meat, were allowed to stand in solutions of the dye, maximum adsorption of the dye occurred after 5 min and this time was adopted for adsorption studies. The reflectance of the sample at 620 nm was found to be linearly related to the weight of malachite green adsorbed as illustrated in Fig. 5.

A pork fat fraction was obtained by extraction of a similar pork sample with petroleum spirit. Figure 6 shows the percent change in reflectance as fat is incorporated into the extracted meat sample (amounts expressed on a wet weight basis) with and without the addition of S(IV) (47·7 μmol per 5 g of *original* meat sample). The graph shows that the more fat is present, the less able is the S(IV) to bleach malachite green, consistent with the idea of a barrier effect. Furthermore, the linear relationship between reflectance and adsorbed malachite green concentration (Fig. 5) and the linearity of the relationship between % change in reflectance and fat content on addition of S(IV) (Fig. 6), suggest that the extent of the chemical reaction of malachite green with S(IV) is proportional to fat content. This is, of course, reminiscent of the behaviour of S(IV) in comminuted meat samples.

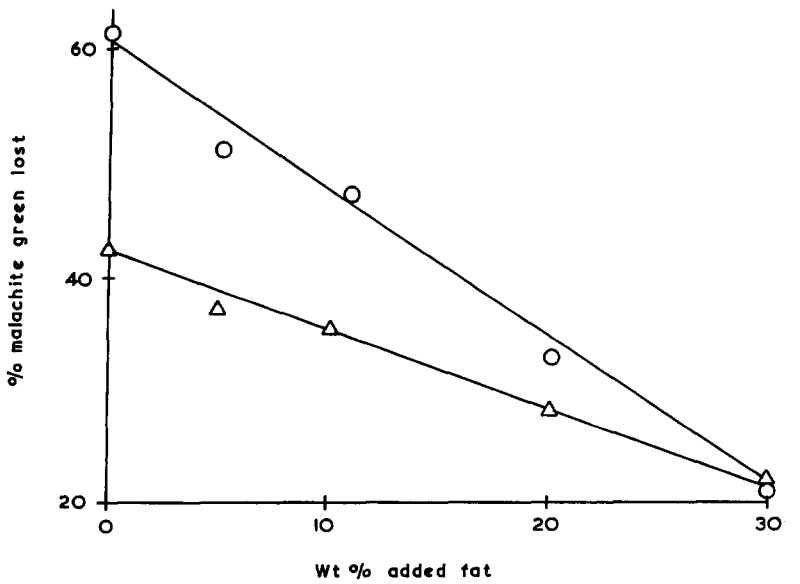
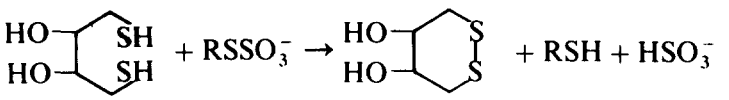


Fig. 6. Effect of addition of fat to pork steak, exhaustively extracted with acetone and labelled with malachite green, on the extent of bleaching when $Na_2S_2O_5$ (4·5 mg per 5 g of original meat sample) is added. Experiments carried out at two levels of adsorbed malachite green: △, 64 μg malachite green (g meat)$^{-1}$; ○, 121 μg malachite green (g meat)$^{-1}$. Amounts of malachite green expressed in terms of the weight of meat sample before extraction with acetone.

Reaction with disulphide bonds

A possible reaction of S(IV) within the solid matrix of meat is with disulphide bonds of proteins. A simple method of measuring the amount of the additive present in the form of thiosulphonates, the products of disulphide cleavage, is to allow the product to react with dithiothreitol, DTT. This displaces sulphite ion from thiosulphonates as follows:



The amount of S-sulphonate present may be determined from the amount of S(IV) released on treatment of the sample with DTT. In order to validate the procedure used in this work, two identical samples of bovine serum albumin, BSA, were mixed with sufficient S(IV) to cleave approximately half of the disulphide bonds (17 total) in the protein molecule. To one sample was added an excess of DTT and both assayed for S(IV). The absorbances at 412 nm of analytes in DTNB reagent were 0·254, 0·138 and 0·247 for the total amount of S(IV) added to the protein, the reaction mixture of protein and S(IV) and the reaction mixture after treatment with DTT, respectively. It is seen, therefore, that on mixing of S(IV) with BSA, 54% of the S(IV) becomes bound to the protein. Addition of DTT leads to a 97% recovery of S(IV) added.

The effect of DTT on the recovery of S(IV) when the additive is mixed with different types of meat is summarised in Table 5. It is seen that S(IV)-treated pork steak and chop appear to contain no S-sulphonate product whilst the formation of these products accounts for nearly 50% of the loss of the S(IV) in some of the beef samples. The role of disulphide cleavage is consolidated by considering analytical data for cystine-content of the meat samples in

TABLE 5

Effect of Adding Dithiothreitol, DTT, on the Recovery of S(IV) from Sulphited Meat (Initial S(IV)-content = 9·48 mmol/kg meat)

Meat sample	*S(IV) bound (mmol/kg)*	*Increase in S(IV) on adding DTT (mmol/kg)*	*% S(IV) as S-sulphonate*
Pork chop	1·90	0·00	0
Pork steak	3·74	0·00	0
Belly pork	0·96	0·26	27
Fillet steak	4·66	0·51	11
Braising steak	2·50	1·06	42
Shin beef	2·28	1·09	48

question. These are shown in Fig. 7 as the amount of S(IV) released on addition of DTT versus cystine-content and it is seen that the two are linearly related. It is evident that the proportion of disulphide bonds cleaved by the reagent (increase in S(IV)-content on addition of DTT divided by the cystine content) is less than 2%. No cystine was detected in the case of pork fillet or chop.

The data reported here relate to the instantaneous irreversible binding of S(IV) when the preservative is added to comminuted meat. In this respect the work covers one of the stages during which S(IV) is 'lost' in meat products.

Preliminary experiments (Fig. 1) suggest that the extent of this initial reaction does not depend on the presence of oxygen in the head space. The fact that, in the absence of oxygen, there is no subsequent time-dependent change in S(IV)-content indicates that in the initial stages one is observing the total extent of reaction and not interrupting a partially complete reaction. Thus, this evidence suggests that the extent of the observed reaction is not kinetically controlled. A subsequent reaction takes place in the presence of oxygen at a much slower rate where kinetic effects would be significant.

The idea that an oxygen-independent reaction is the cleavage of disulphide bonds of proteins is attractive but it should be noted that the data illustrated in Fig. 1 were for pork steak which, despite its considerable reactivity towards S(IV), did not originally contain a measurable amount of

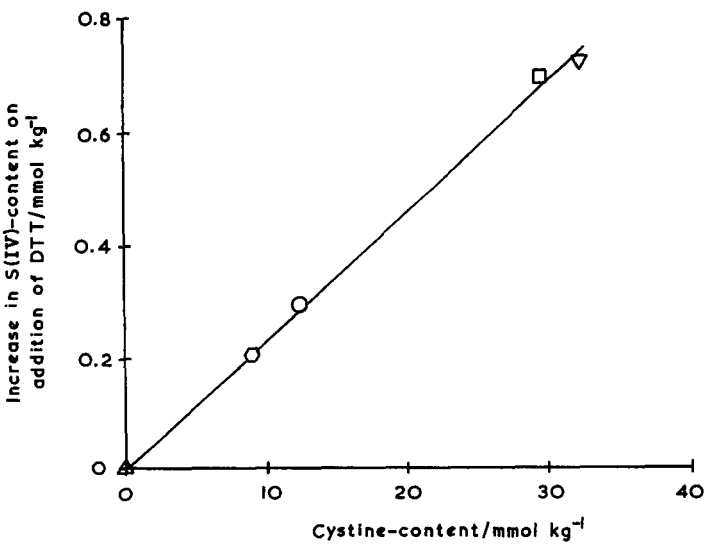


Fig. 7. Relationship between the cystine-content of different cuts of various meats and the extent of formation of S-sulphonates on addition of $Na_2S_2O_5$ (4·5 mg) to comminuted meat samples (5 g) at 15°C. The amount of S-sulphonate is equal to the increase in S(IV)-content on addition of DTT to the sulphited meat sample. ○, Fillet steak: □, braising steak; ▽, shin beef; ⬭, belly pork; △, pork steak and pork chop.

disulphide, or of S-sulphonate after reaction. In all experiments the yield of sulphonate was far from quantitative (0–48%). The technique for analysing S-sulphonates relies on the action of DTT in liberating a quantitative amount of sulphite ion for analysis. The procedure used was such as to maximise the yield of sulphite ion, but there is no guarantee that the DTT had penetrated the protein structure to the same extent as had S(IV) in the cleavage reaction. However, the results that pork chop and pork steak apparently contain no measurable disulphide suggests that an alternative mechanism for the binding of S(IV) must be considered.

One possibility is oxidation of S(IV) to sulphate by the direct or indirect action of enzymes or a naturally present oxidising agent. Whilst the presence of oxygen in the headspace does not affect the initial reaction, small amounts of residual oxygen in the meat tissues could contribute to this initial change; further oxidation would be limited in rate by transport of oxygen from the headspace. The autoxidation of S(IV) is catalysed by transition metal ions (Bäckström, 1934) and our unpublished observations suggest that haematin is capable of causing a loss of S(IV); it could be argued that processed meat offers a good source of such catalysts. However, the autoxidation reaction is homolytic and involves reactive radicals such as $OH^{\cdot}$ which are readily scavenged by organic constituents of the mixtures, particularly alcohols (Hayon *et al.*, 1972). It is known that these compounds are, in general, good inhibitors of autoxidation (Schroeter, 1966). An alternative pathway for oxidation involves the enzyme sulphite oxidase which acts through a two-electron transfer mechanism thereby avoiding the formation of radicals. Although this enzyme exists mainly in the liver (Cohen *et al.*, 1973), small amounts may occur in other tissues. On the other hand oxidation could take place through an, as yet, unknown coupled oxidation with, say, an oxidase enzyme system.

The effect of heat treatment of the meat prior to testing for reactivity towards S(IV) (Fig. 2) does not unequivocally point towards enzyme action because it is likely that the elevated temperature causes the fat to melt and thus provides a barrier to attack by S(IV) on other food components. However, the participation of an enzymic oxidation cannot be ruled out.

A further possibility is that pre-formed oxidising agents may react with S(IV). Thus, for example, Fe(III) would oxidise sulphite ion to sulphate or dithionate according to whether a two- or one-electron transfer takes place (Carlyle & Zeck, 1973), i.e.,

$$2Fe^{3+} + SO_3^{2-} + H_2O \rightarrow 2Fe^{2+} + SO_4^{2-} + 2H^+$$

or,

$$Fe^{3+} + SO_3^{2-} \rightarrow Fe^{2+} + SO_3^{\cdot-}$$

$$2SO_3^{\cdot-} \rightarrow S_2O_6^{2-}$$

The behaviour of denatured haemoproteins or haematin in this respect is not known for certain, but it is possible that such a redox reaction is responsible for the loss of S(IV) when the oxospecies are mixed with haematin as referred to above. Evidence for this type of oxidation, which might be effected by organic oxidising agents, could be obtained from analysis of sulphate and dithionate contents of S(IV)-treated meat and work on these lines is in progress.

Whilst the reaction does not appear to be kinetically controlled, the evidence for a 'barrier-effect' of fat indicates a transport-limited process. A practical consequence of this concerns the adjustment of the final level of preservative in a comminuted meat product; any estimate of the amount of S(IV) to be added should take account of the fact that locally high fat contents could lead to an unexpectedly high local retention of S(IV).

CONCLUSION

The data presented here allow a simple model for the reactivity of S(IV) in comminuted meat to be proposed. In this, the additive dissolved in the aqueous phase is involved in a transport controlled reaction with components of the solid matrix of the meat. It is not known whether the components in question are soluble or not, but the fact that fat impedes the transport is beyond doubt. The substances which react with S(IV) in meat from different species of animal, and the cut of meat from a given animal, are different. Quantitative analysis of S-sulphonates shows different contributions from disulphide cleavage to the overall binding of the additive; in some pork samples no S-sulphonate is formed and alternative pathways for the irreversible binding of S(IV) have to be found. On the other hand, in belly pork, disulphide cleavage is an important contributor to S(IV)-reactivity. The surprising observation is that despite differences in composition, the non-fat solids of different meat samples appear to react to a similar extent with S(IV).

ACKNOWLEDGEMENT

The authors are grateful to the Agricultural and Food Research Council for a Research Studentship to one of us (K.A.M.) The authors are also grateful to Dr. J. Nobbs, Department of Colour Chemistry, University of Leeds, for the use of the Macbeth spectrophotometer and for helpful advice.

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