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The Effect of Sulphur Dioxide on Horseradish Paste

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ABSTRACT

The effect of adding sulphur dioxide (bisulphite) to horseradish paste has been studied using a combination of sensory and chemical techniques. Changes in the sensory quality of the sulphited pastes were greater than that in the non-sulphited control and were mainly related to the increased loss of the major flavour component of horseradish, 2-propenyl isothiocyanate. Treatment with bisulphite led to a decrease of total intensity and of the sensory qualities, horseradish and pungent, whilst the qualities root vegetable/earthy and watercress increased. It was found that the chemical reaction between bisulphite and 2-propenyl isothiocyanate (to form the substituted aminothiocarbonyl sulphonate salt) was more rapid than the reaction of the additive with 2-phenylethyl isothiocyanate. Differences were noted in the reaction between 2-propenyl isothiocyanate and bisulphite reported here and that previously described in mustard paste. In particular, the aminothiocarbonyl sulphonate salt did not appear to break down in the former system, and garlic-like off flavours, reported in mustard, were absent.

INTRODUCTION

Horseradish (*Armoracia lapathifolia* Gilib.), a member of the family Cruciferae, is primarily cultivated for its fleshy root which is used as a relish and is prized for its pungency and flavour. Approximately 6000 tonnes are cultivated annually. In the west, the root is generally shredded or ground and presented as a paste in, for example, water or vinegar

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(Sahasrabudhe & Mullin, 1980). In the orient the root of the Japanese horseradish, also called wasabi (*Wasabia japonica* Matsum.), is widely consumed after processing and the effect of this on the flavour and quality of wasabi has been well documented by Kojima and his coworkers (Kojima *et al.*, 1973, 1982; Kojima, 1977).

The pungency of the roots of these species is due to the production of volatile isothiocyanates which are formed enzymically when the tissue is damaged, cut or otherwise ruptured (Fenwick *et al.*, 1983). Isothiocyanates are formed from water-soluble, involatile glucosinolates, the presence of which is a chemotaxonomic feature of cruciferous plants (Fenwick *et al.*, 1983). Recent studies using capillary gas chromatography–mass spectrometry have indicated that the volatiles from horseradish are derived from many different glucosinolates (Grob & Matile, 1980), although the vast majority of these are present only in trace amounts. The two principal volatiles which have been described in horseradish essence (Sahasrabudhe & Mullin, 1980; Gilbert & Nursten, 1972) are 2-propenyl (or allyl) isothiocyanate (I), which possesses a pungency characteristic of brown mustard, and 2-phenylethyl isothiocyanate (II), which possesses an odour reminiscent of watercress. These volatiles are derived from the glucosinolates sinigrin and gluconasturtiin, respectively.

$R—NCS$	I	$R = CH_2{=}CH_2—CH_2—$
	II	$R = PhCH_2CH_2—$
$R—NHC(=S)SO_3^-$	III	$R = CH_2{=}CH—CH_2—$
	IV	$R = PhCH_2CH_2—$
	V	$R = PhCH_2—$
$CH_2{=}CH—CH_2SR'$	VI	$R' = H$
	VII	$R' = CH_2{=}CH—CH_2—$
	VIII	$R' = CH_2{=}CH—CH_2—S—$

Prepared horseradish may be subject to discoloration and loss of pungency (Weber *et al.*, 1969; Rhodes, 1977) and the use of certain additives has been suggested to alleviate these problems. One such additive is sulphur dioxide (bisulphite). There have also been reports (Sahasrabudhe & Mullin, 1980; Rhodes, 1977; Weber, 1964) of undesirable flavour changes, characterised by a loss of pungency, accompanying the use of this ubiquitous food additive.

Previous work in this Institute (Griffiths *et al.*, 1980; Frijters *et al.*, 1981) has shown that the addition of sulphur dioxide to brown mustard

(*Brassica juncea*) pastes leads to unexpected and undesirable flavour changes on storage—a reduction in pungency and a gradual increase in a garlic-like off flavour. These changes have been found to arise from a chemical reaction between the additive and the pungent principle, 2-propenyl isothiocyanate.

This latter compound is the major flavour volatile in horseradish essence, although the exact composition will depend upon such factors as the cultivar examined and the agronomic features associated with its growth; it was therefore felt that similar chemical reactions might occur in sulphited horseradish and might explain the sensory changes reported earlier. The present paper describes the results of an investigation of this possibility, using a combination of sensory and chemical techniques.

EXPERIMENTAL

Materials

Dried and powdered horseradish root was supplied by Dr J. H. Merz, Colman's Food, Norwich. Commercially dehydrated samples were obtained from local supermarkets. 2-Propenyl and 2-phenylethyl isothiocyanates were obtained from Kodak Chemicals Ltd, Kirkby, Great Britain. They were redistilled before use and were >99% odour pure by odour evaluation of the effluent from a gas chromatograph. Sinigrin and gluconasturtiin were available from previous studies (Hanley *et al.*, 1983). Sulphur dioxide was added as potassium metabisulphite (BDH, laboratory reagent grade). 2-Propenyl-, 2-phenylethyl- and benzyl aminothiocarbonyl sulphonates (III)–(V) were prepared by refluxing the appropriate isothiocyanate and potassium metabisulphite in ethanol and subsequently recrystallising to constant melting point. Compounds thus prepared were chromatographically pure and possessed analytical and physical data (ir, nmr and FAB-ms) consistent with their expected structure (Sankaran & Narashima Rao, 1977). All samples were stored in the refrigerator prior to use.

Preparations of pastes

Horseradish pastes were prepared from the ground root (50 g) and glass distilled water (500 ml). After thorough mixing, samples were stored in

stoppered glass flasks (1 litre). Where required, potassium metabisulphite was mixed with the paste to obtain a final concentration of 2000 mg sulphur dioxide per kilogram.

Methods of analysis

Sensory analysis

The prepared horseradish paste was diluted ten times with distilled water and the resultant slurry presented for odour assessment. Such a sample was of at least moderate, and generally strong, intensity to all assessors. Twenty-five millilitre aliquots of these samples were dispensed into brown screw capped bottles, capacity 125 ml, and the samples allowed to equilibrate for at least 1 h at room temperature (approximately 18 °).

Assessments were conducted by fourteen assessors who had previous experience of odour profiling a similar system based on mustard paste (Frijters *et al.*, 1981). The terms for the profile were derived by the panel who used their own terms to describe the odour characteristics of sulphited and non-sulphited horseradish pastes which had been stored for 0 to 4 days. In addition, they were familiarised with solutions having a mustard-like odour (2·4 mM 2-propenyl isothiocyanate), a watercress-like odour (2·9 mM 2-phenylethyl isothiocyanate) and a mixture of the two (2·0 mM and 0·25 mM, respectively) to represent a horseradish-like odour. The final terms used for the odour profile were agreed by discussion. These were: total intensity, horseradish, pungent, mustard, root vegetable/earthy, green vegetable/crushed leaves, watercress and garlic. Each term was rated on the following scale: absent, very weak, weak, moderate, strong and very strong (these were assigned values of 0, 1, 2, 3, 4 and 5 respectively for calculation of results). The panel members were familiarised with the procedure by assessing, on two occasions, sulphited and non-sulphited pastes which had been stored for 0, 4, 6 and 11 days. During the storage experiment proper, panel sessions were held at 1400 h on days 0 (2 h), 1, 2, 4, 7 and 14. At each session four coded samples were assessed, two from each treatment, the time interval between assessments being 1 min.

Chemical analysis

Sulphur dioxide was measured using the colorimetric method of Wedzicha & Johnson (1979). Isothiocyanates were analysed by gas chromatography in the following manner. Horseradish paste (filtered,

2 ml) was extracted with dichloromethane (1 ml) by vigorous shaking. The resultant mixture was separated into two phases by centrifugation (3000 rpm for 5 min) and the lower phase analysed directly on a Pye GCV gas chromatograph fitted with a flame ionisation detector. Compounds were separated on a 1·52 m × 3 mm inside diameter glass column packed with 5% OV-17 on Chromosorb WHP (80–100 mesh). The carrier gas was argon with a flow rate of 50 ml min^{-1}. The column temperature was maintained at 100 °C for 5 min after injection, then programmed 10 ° min^{-1} to 200 °C and maintained there for 5 min. Solutions of 2-propenyl and 2-phenylethyl isothiocyanates were used to determine response factors; both showed a linear response over the concentration ranges employed in the present study.

Substituted aminothiocarbonyl sulphonates were analysed by high performance liquid chromatography. Horseradish pastes were filtered and applied to a reverse phase column (Spherisorb ODS 10, 25 cm × 4·6 mm inside diameter). The column was operated isocratically using 0·5M sodium chloride solution/methanol (6:4 v/v) at a flow rate of 1 ml min^{-1}. The eluant was monitored at 276 nm. Both 2-propenyl and 2-phenylethyl aminothiocarbonyl sulphonates gave linear responses across the concentrations found in the present study.

RESULTS

Sensory analysis

The averages (two sessions) of the mean panel ratings (all assessors) for the five characteristics—total intensity, horseradish, pungent, root vegetable/earthy and watercress—are shown in Figs 1 and 2. These show that in the non-sulphited paste during the first 7 days total intensity decreased by 0·8 units, the pungent and horseradish notes decreased by 1·5 and 1·2 units, respectively; the latter characteristics were closely related (Kendall's $\tau = 1{\cdot}000$). There was no further change after this time. The other qualities were hardly perceived.

The sulphited paste was lower in total intensity and pungency than the non-sulphited paste after 2 h. The total intensity thenceforth only decreased by approximately 1 unit during the remainder of the storage period. In contrast, pungency declined markedly (by 2·5 units) over the first 2 days and then levelled off. There was a gradual loss of horseradish

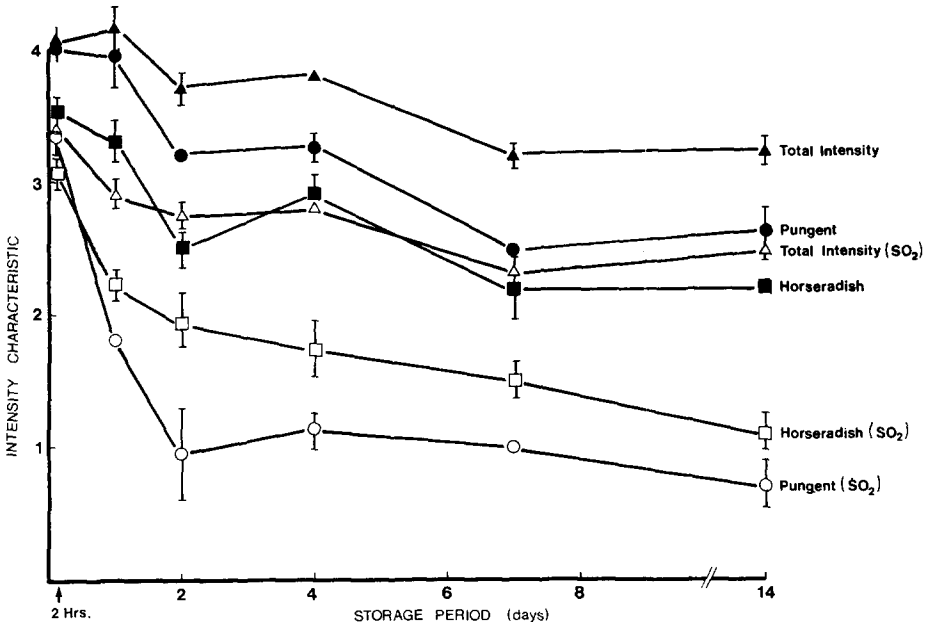

Fig. 1. Odour changes in total intensity and the qualities pungent and horseradish during storage of sulphited and non-sulphited horseradish paste. ▲—▲—▲, total intensity (non-sulphited); △—△—△, total intensity (sulphited); ●—●—●, pungent (non-sulphited); ○—○—○, pungent (sulphited); ■—■—■, horseradish (non-sulphited); □—□—□, horseradish (sulphited).

intensity throughout the experiment (total loss 2·1 units); the correlation between the two characteristics was $\tau = 0{\cdot}8281$. The qualities watercress and root vegetable/earthy were marginally stronger in the sulphited system after 2 h and continued to increase during the first days of storage. Both qualities, however, reached a plateau after approximately 1 week; thereafter the watercress decreased.

Only slight changes were noted in the strength of the mustard, green vegetable/crushed leaves and garlic qualities during the storage period. Throughout the period of the experiment the values were higher for mustard in the non-sulphited paste (average 1·8 units as compared with 0·92 units for the sulphited sample). The green vegetable/crushed leaves note was, however, always higher for the sulphited paste (average 1·2 units) than in the non-sulphited paste (average 0·47 units). It was noticeable that, in marked contrast to the earlier study on mustard paste (Frijters *et al.*, 1981), the term garlic was only rarely used by the assessors.

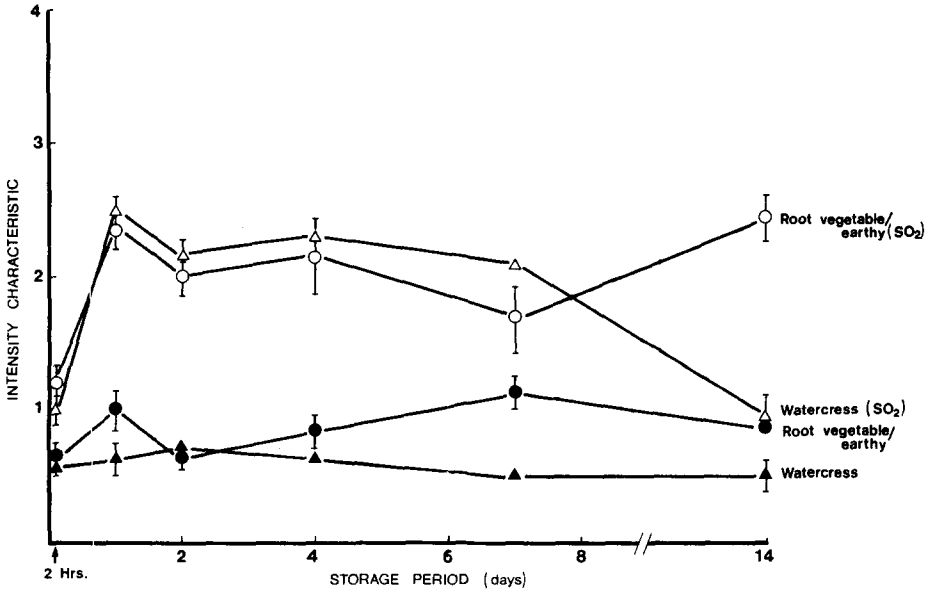

Fig. 2. Odour changes in the qualities watercress and root vegetable/earthy during storage of sulphited and non-sulphited horseradish paste. ▲—▲—▲, watercress (non-sulphited); △—△—△, watercress (sulphited); ●—●—●, root vegetable/earthy (non-sulphited); ○—○—○, root vegetable/earthy (sulphited).

Chemical analysis

Chemical analysis of the volatile isothiocyanates gave results generally in agreement with earlier workers (Sahasrabudhe & Mullin, 1980; Gilbert & Nursten, 1972), 2-propenyl isothiocyanate being the major volatile. Loss of 2-propenyl isothiocyanate was extremely rapid, less than 50% of the original amount remaining after 6 h and 10% after 2 days (Fig. 3). In contrast, the content of this compound in the non-sulphited paste was 90% after 6 h and 60–80% after 2 days. 2-Phenylethyl isothiocyanate concentrations were very little affected by the addition of bisulphite or the length of the storage period. The stabilities of aqueous solutions of sinigrin and gluconasturtiin (the glucosinolate precursors of 2-propenyl and 2-phenylethyl isothiocyanates) were unaffected by the addition of bisulphite.

The reduction of 'free' sulphur dioxide in the sulphited horseradish paste is shown in Table 1. Initially this loss is extremely rapid, less than 30% remaining after 2 days. There is, however, a small amount of free

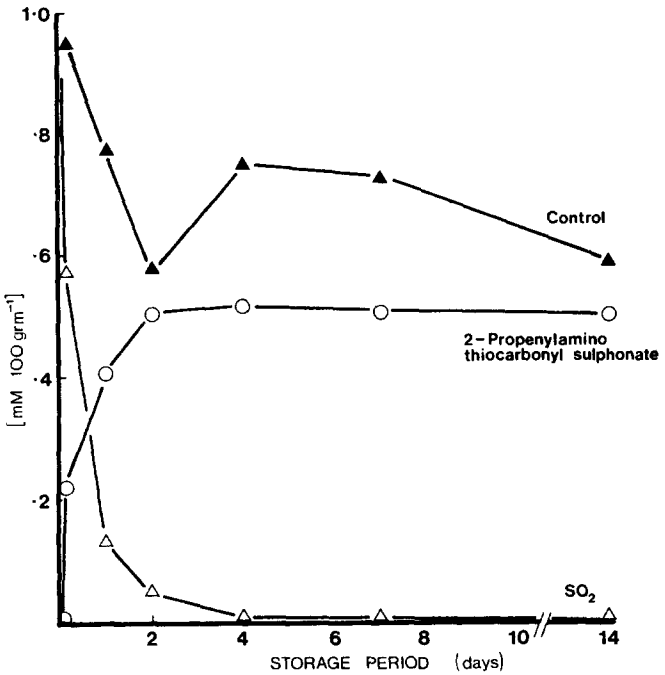

Fig. 3. Changes in chemical content of stored horseradish pastes. 2-Propenyl isothiocyanate in sulphited (△—△—△) and non-sulphited (▲—▲—▲) horseradish and 2-propenylaminothiocarbonyl sulphonate (○—○—○) in sulphited horseradish.

TABLE 1
Levels of Free SO_2 Horseradish After Storage

SO_2 level ($mg\,kg^{-1}$)	*Time*
2 000 (added)	0
1 460	2 h
1 040	6 h
768	1 day
515	2 days
302	4 days
194	7 days
116	14 days

bisulphite still recoverable after 2 weeks. Figure 3 shows the increase in the amount of 2-propenylaminothiocarbonyl sulphonate. Maximum concentrations were obtained after 4 days' storage and correspond to over 50% of the original 2-propenyl isothiocyanate measured. In marked contrast, very little of the 2-phenylethyl adduct was formed during the reaction period (less than 5% of the 2-propenyl isothiocyanate adduct) and the amount was still increasing after 14 days.

The much greater reactivity of 2-propenyl isothiocyanate, rather than 2-phenylethyl isothiocyanate, towards bisulphite was unexpected and prompted further investigation. The result was confirmed when solutions of these isothiocyanates (either individually or in combination) were stirred with bisulphite, a thirty- to forty-fold greater rate being observed in the reaction between 2-propenyl isothiocyanate and bisulphite.

DISCUSSION

Previous studies (Griffiths *et al.*, 1980; Frijters *et al.*, 1981), conducted with brown mustard (*Brassica juncea*) pastes, had shown that 2-propenyl isothiocyanate and bisulphite reacted readily to yield the involatile 2-propenyl aminothiocarbonyl sulphonate salt. In the horseradish pastes examined in the present study the rate of this reaction was much faster than that observed in the mustard pastes; for example, a loss of half of the content of this volatile occurred within 2–3 days and a plateau was reached after approximately 7 days' storage of the mustard paste compared with less than 1 day's storage and 3 days' storage, respectively for the horseradish paste. The rate of reaction between 2-propenyl isothiocyanate and bisulphite in aqueous solution was comparable with that observed in the horseradish paste, suggesting that the reaction in mustard may have been inhibited. The chemical nature of sulphur dioxide in water is very dependent upon the pH of the solution (Schroeter, 1966) and this might be expected to have a significant effect on the rate of reaction with 2-propenyl isothiocyanate. However, the pHs of the horseradish and mustard pastes were found to be very similar (5·6). The presence of residual oil in the mustard flour might have reduced the ease of contact between the glucosinolate sinigrin and the enzyme, myrosinase, necessary for its breakdown (Fenwick *et al.*, 1983). It should be noted here that the mustard pastes were prepared by 1:4 dilution with water compared with the greater dilution (1:10) of the horseradish; this

was necessary as a 1:4 mixture did not allow proper mixing. There is another possibility which cannot be ruled out. As mentioned above, myrosinase (thioglucoside glucohydrolase EC3:2:3:1) is necessary for flavour production. Some studies have been conducted into the nature and properties of seed myrosinase (usually that in rapeseed (*B. napus*) and white mustard (*S. alba*)) but very little is known about the nature of myrosinase in root tissue. It may, however, be significant that recently Ohtsuru & Kawetani (1979) have reported a molecular weight of 580 000 daltons for the myrosinase isolated from *Wasabia japonica*, considerably higher than that found in mustard seed (120 000–150 000 daltons) (Björkmann, 1976). This difference may also be reflected in the properties of the myrosinase; for example, with respect to ascorbate or bisulphite concentrations. Studies are in progress to examine these aspects of the properties of leaf and root myrosinase.

The disparity between the rates of reaction of bisulphite and 2-propenyl and 2-phenylethyl isothiocyanates is presumably a reflection of the ability of the former to more effectively delocalise electrons from the —NCS grouping, hence making it more susceptible to attack by the bisulphite anion. Support for this argument is obtained when the reaction between benzyl isothiocyanate and bisulphite is examined. This is more rapid than that of 2-propenyl isothiocyanate (Fig. 4), in agreement with the greater electron withdrawing effect of the aromatic, rather than the isolated π bond, system.

A significant feature of the addition of sulphur dioxide to mustard paste was the development on storage of garlic-like odour (Griffiths *et al.*, 1980; Frijters *et al.*, 1981). In the purified state 2-propenylaminothiocarbonyl sulphonate is odourless but it readily develops a garlic odour on storage, in both the solid phase and in solution. This has been found to be due to the formation of trace amounts of highly odoriferous 2-propenyl thiol (VI), 2-propenyl sulphide (VII) and 2-propenyl disulphide (VIII) (P. G. Jones *et al.*, unpublished work). In the present study the same sulphonate is clearly formed in the horseradish paste but, as far as can be ascertained from sensory and chemical (gc/ms) analyses, breakdown (at least to garlic-like volatiles) does not occur. Reasons for this difference are obscure.

Sahasrabudhe & Mullin (1980) found that sulphitation of horseradish root resulted in almost total loss of odour, which could not be regenerated upon addition of myrosinase. These observations would be consistent with the isothiocyanates reacting with the additive during drying. It has

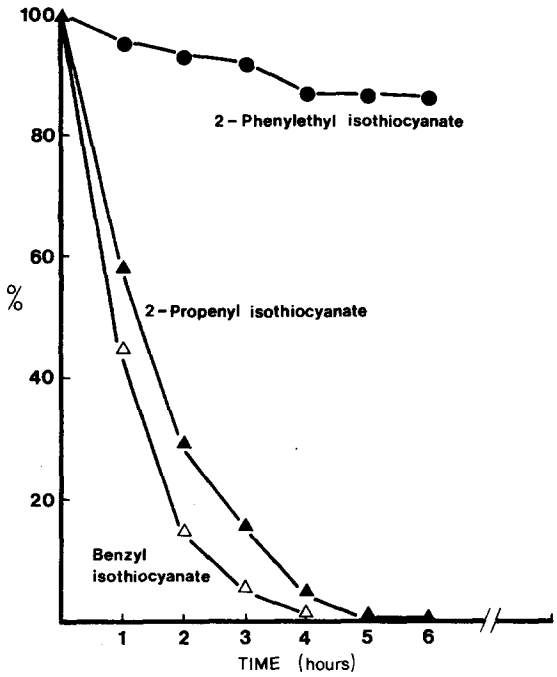

Fig. 4. Reaction of 2-propenyl (▲—▲—▲), benzyl (△—△—△) and 2-phenylethyl (●—●—●) isothiocyanates with bisulphite. Aqueous solutions of the isothiocyanate (10 mM) and bisulphite (30 mM) were stirred at room temperature, residual isothiocyanate contents being determined by gas chromatography.

been possible to reproduce the above experiment, and it was found that while 86% of the 2-propenyl isothiocyanate was lost after sulphitation and during drying, the major part (58%) was bound as the involatile sulphonate salt.

The odour qualities perceived in the odour changes during storage of horseradish paste—horseradish, pungent, watercress, green vegetables/crushed leaves and root vegetable/earth—have also been described in an earlier investigation (Gilbert & Nursten, 1972) of the gas chromatographic effluent from the analysis of the headspace above fresh (coarse grated) horseradish.

The chemical composition and sensory quality of the non-sulphited paste changed little during storage. The sulphited horseradish paste, however, became less pungent and horseradish-like at a similar rate to the loss of 2-propenyl isothiocyanate (Kendall's τ 1·0000). The greatest

changes in both chemical composition and sensory quality occurred in the first day; by this time the watercress and root vegetable/earthy characteristics were present. Loss of pungent 2-propenyl isothiocyanate from the system (Fig. 3) would allow the 2-phenylethyl isothiocyanate (watercress-like) to be detected.

The close agreement between the duplicate samples (Figs 1 and 2) suggests that the differences observed between sulphited and non-sulphited pastes after 2 h are real effects and that the sensory changes commenced soon after the addition of sulphur dioxide. Thus, once again it has been shown that the addition of a recognised food additive to a food system can have a significant effect on its resultant flavour and acceptability.

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