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Effect of sulphite treatment on allyl isothiocyanate in mustard paste

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Allyl isothiocyanate (AITC), the major constituent of black and brown mustard oils, reacts with hydrogensulphite, used as an antioxidant in some types of mustard paste, to afford allylaminothiocarbonylsulphonate (AITC–SO_3^-). The reaction was studied in a model aqueous system of pH 4 and mustard paste of the Dijon type, respectively. The reaction rate was influenced considerably by temperature, but not by oxygen dissolved in the media. About 10% of total AITC (c_{tot}[AITC] = 1.81 g kg^{-1}) in the mustard paste treated with bisulphite ($c_0[K_2S_2O_5]$ = 1.5 g kg^{-1}) converted to AITC–SO_3^- within a period of four weeks regardless of storage temperature used. The primary reaction product, AITC–SO_3^-, showed good stability both in diluted acidic solutions and mustard paste. A rapid decomposition of this adduct was observed in diluted alkali (pH 9). A method for the simultaneous determination of AITC–SO_3^- and AITC in mustard paste was developed and its performance characteristics assessed. Sensory analysis revealed slight fading of characteristic mustard flavour and the appearance of garlic-like flavour in model solutions. In mustard pastes, the original pungent flavour became much milder, but the development of garlic-like notes was not detected. © 1998 Elsevier Science Ltd. All rights reserved.

INTRODUCTION

Mustards, in kind with other plants of the genus *Brassica* (Brassicaceae), contain glucosinolates and their breakdown products, which exhibit a variety of physiological effects on living organisms and affect the nutritional and organoleptic properties of many foods (Velíšek, 1995). Sinigrin is the principal glucosinolate of two of the most commercially employed species in mustard paste production — *Brassica juncea* (brown mustard) and *B. nigra* (black mustard). Allyl isothiocyanate (AITC), the main degradation product of sinigrin produced by the action of myrosinase (thioglucoside glucohydrolase, EC 3.2.3.1) in mild acidic media (Scheme 1), causes the typical pungent flavour of mustard paste.

AITC is a volatile compound that reacts easily with many natural food constituents such as proteins, alcohols and water, and food additives such as sulphur dioxide. The participation of AITC in nucleophilic addition reactions with amino, hydroxyl and thiol groups, β-dicarbonyls, carboxylic acids and many other substances has been described (Drobnica *et al.*, 1977).

Sulphiting agents are added to some types of mustard paste to protect them from oxidation and browning. Their ability to prevent undesirable reactions is the consequence of the nucleophilicity of the hydrogensulphite ion, the dominant form at pH values typical for mustard pastes. Therefore, the most important impact on the apparent reactivity of oxospecies of S(IV) is from the pH value affecting the equilibria among dissociation forms of sulphurous acid ($pK_1 \cong 1.8$ and $pK_2 \cong 7.2$ in dilute solutions at 25°C). The pK value of HSO_3^- in more complex media can be influenced significantly by the change of ionic strength (Wedzicha, 1985).

It has been known for a long time that organic isothiocyanates react irreversibly with HSO_3^- (Backer *et al.*, 1935; Dalton *et al.*, 1976). However, only a few papers have studied the reaction involving AITC (Scheme 2). The reaction in mustard paste prepared from brown mustard flour has been described by Frijters *et al.* (1981). The concentrations of AITC (10 and 20 mM) decreased in two sulphited pastes (both with 25 mM SO_2) by 85 and 79%, and in non-sulphited pastes by 55 and 48% to constant levels within 21 and 13 days, respectively.

Extremely rapid decrease (within a few hours) of AITC ($c_0 \cong 0.1$ g kg^{-1}) due to the reaction with 2 g kg^{-1}

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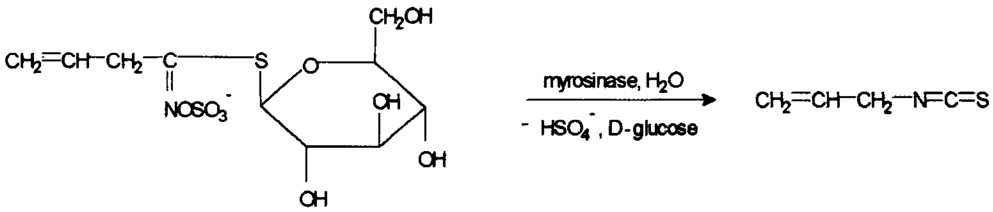
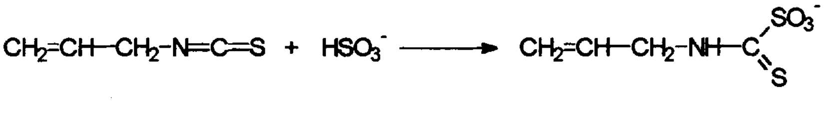


Scheme 1.



Allyl isothiocyanate

(AITC)

Allylaminothiocarbonylsulfonate

(the adduct, AITC-SO$_3^-$)

Scheme 2.

of sulphite was observed both in model experiments and in sulphited horseradish paste (Kroyer, 1986). About 40% of AITC was converted within 24 h of storage to the adduct when a treatment with 0.2% HSO$_3^-$ was performed.

The primary aim of this work was to quantify the reaction product of hydrogensulphite with AITC in both model solutions and mustard paste under varying storage temperatures and access of oxygen.

MATERIALS AND METHODS

Synthesis of allylaminothiocarbonylsulphonate (AITC–SO$_3$K)

The compound was synthesised according to the procedure of Backer *et al.* (1935) using allyl isothiocyanate (declared as >98%, m/m, Fluka Chemie, Buchs, D—actual AITC stock purity was 84% when applied because the concentrated AITC undergoes isomeration to give allyl thiocyanate) and potassium bisulphite (p.a., Lachema, Brno, CZ). The product was purified by a repeated crystallisation from hot ethanol. Structure and purity of the adduct were confirmed by IR, ^{1}H- and ^{13}C-NMR spectrometry using a FT-IR spectrometer Nicolet 740 (Nicolet, USA), resolution 2 cm^{-1}, KBr tablets, and FT-NMR spectrometer Gemini 300 HC (Varian, USA), deuterium oxide, respectively.

Model experiments

Solutions (100 ml) of pH 4 (in McIlvaine buffer–citric acid monohydrate/Na$_2$HPO$_4$.12H$_2$O—both p.a., Lachema, Brno, CZ) with AITC (4.7 mM) and K$_2$S$_2$O$_5$ (4.2 mM) were prepared and stored under different conditions (at 5 ± 0.2 and 23 ± 1°C, with and without nitrogen purging at a flowrate of 2.5 ml s^{-1} for 15 s after each sampling, respectively).

Mustard paste preparation

Mustard seed of *B. juncea* (300 g) was ground (Ultra-Turrax T25, Janke (Kunkel, Staufen, D) for 1 min and mixed well with 50 g NaCl (p.a., Lachema Brno, CZ), 200 ml vinegar (8% acetic acid—Labena Company, Ústí n. L., CZ), 1.50 g K$_2$S$_2$O$_5$ and 450 ml distilled water for 5 min. The maximum concentration of AITC found in the paste was 1.81 g kg^{-1}. The samples were stored at 5 ± 0.2 and 23 ± °C, respectively.

Commercial mustard paste

The decrease of AITC level was also investigated in sulphited Dijon paste produced by Boneco Ltd. (Benešov, CZ). The paste was divided into three parts which were stored in closed flasks at 5 ± 0.2, 20 ± 0.2 and 40 ± 0.2°C, respectively. K$_2$S$_2$O$_5$ was added to the level of 1.50 g kg^{-1}, the concentration of AITC in the paste was 1.22 g kg^{-1} after 48 h from paste formation.

Determination of sulphite

Sulphite concentration was determined spectrophotometrically at 405 nm (Merck, 1996) using Ellman's reagent (2,2′-dinitro-5,5′-dithiodibenzoic acid, Merck, Darmstadt, D) in Na$_2$HPO$_4$ solution and Pye Unicam 8700 spectrophotometer.

AITC and AITC-SO$_3$K analysis

Both the analytes in model solutions were analysed simultaneously by RP-HPLC/UV with Nova-Pak® C18, 250×4.6 mm, 4 μm, with Guard-Pak® Nova-Pak® C18 (Waters, Milford, MA, USA). Gradient elution was performed by mixing of Sörensen buffer (Table 1; sodium citrate–HCl) of pH 2 and methanol (Lichrosolv® gradient grade, Merck, Darmstadt, D) at a flowrate of 1 ml min^{-1}, ambient temperature and $\lambda = 254$ nm using Thermo Separation Products HPLC system consisting of binary system of high pressure pumps constaMetric®3500 and constaMetric®3200 equipped with high pressure gradient dynamic mixer (Watrex Praha, CZ), autosampler AS 100 and spectrophotometric detector spectroMonitor®3200 (Thermo Separation Products, Inc., Riviera Beach, FL, USA). AITC was extracted by acetonitrile (3×15 ml) and the adduct extracted by water (3×15 ml) from the mustard paste (10 g). The reliability of the chromatographic separation was confirmed by peak purity and UV spectra comparisons of the analytes in an HP 1090 HPLC/DAD (diode array) system with the same column and eluent.

Stability of the adduct

Stability was tested in water media of pH 4 (McIlvaine buffer–citric acid/hydrogenphosphate) and pH 9 (Britton-Robinson buffer–$H_3PO_4/CH_3COOH/H_3BO_3/NaOH$) with initial AITC–$SO_3^-$ concentration of 251 mg dm^{-3}.

RESULTS AND DISCUSSION

The reaction of AITC with hydrogensulphite was studied in model systems as well as in mustard pastes prepared in the laboratory and/or produced by regular technological procedures. The concentrations of both reactants in model solutions were chosen with respect to the limited solubility of AITC in aqueous solutions and AITC/SO_2 ratio commonly occurring in mustard pastes, namely 1:0.28–1:2.25 (mol/mol) (Vangheesdaele and Fournier, 1980).

In model experiments, the adduct formation was approximately 10 times faster at room temperature (23°C) than at 5°C (Fig. 1, Table 2). No substantial impact of nitrogen treatment on the rate of the reaction was observed because no significantly slower oxidative decrease of sulphite was found. Molecular sulphur dioxide is nearly absent at pH 4, and therefore the effect of stripping process on removing SO_2 was negligible. However, the concentration of AITC, and consequently of the adduct, were lower owing to the partial evaporation of AITC by nitrogen stream. Almost 9% of AITC was removed during 10 samplings with nitrogen purging within 5 weeks of storage.

The formation of AITC–SO_3^- in mustard paste at two different temperatures is shown in Fig. 2. In comparison to model experiments, the adduct in the mustard paste was formed in lower amounts and at a slower rate due to the competitive reactions of SO_2 and AITC in biotic matrix, different oxygen concentration and different initial AITC/SO_2 ratio (18.3 mM AITC/13.5 mM HSO_3^-). More than 60% (at 23°C) and 36% (at 5°C) of AITC was incorporated into the adduct within 7 weeks of storage in model systems (and this trend would have probably continued). About 10% of total AITC

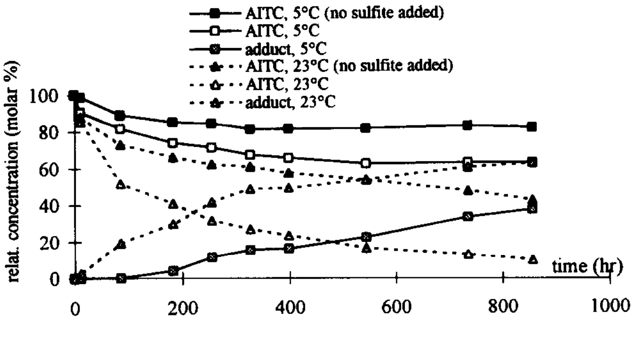


Fig. 1. Effect of storage on concentrations of the compounds in model solutions.

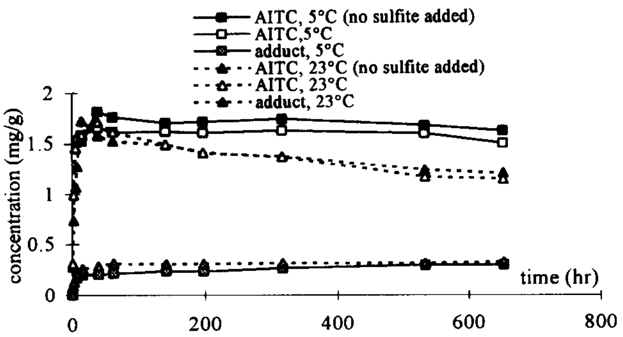


Fig. 2. Effect of storage on concentrations of the analytes in mustard paste.

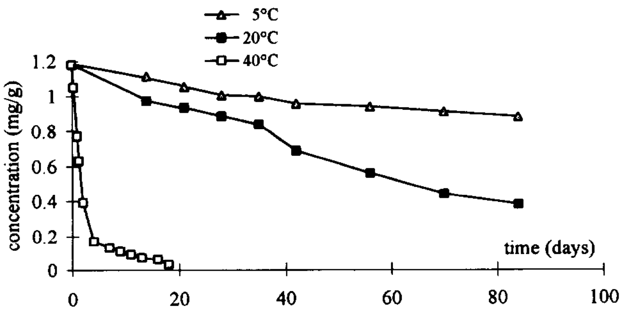


Fig. 3. Decrease of AITC in commercial paste stored at various temperatures.

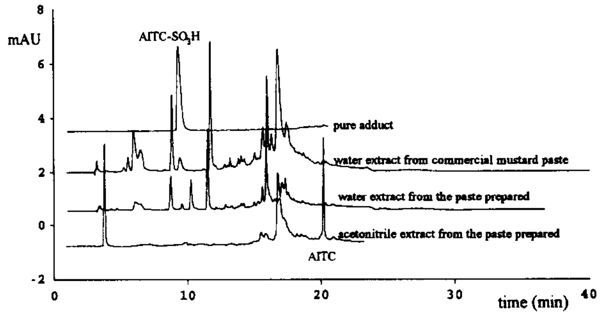


Fig. 4. HPLC analyses of extracts of mustard paste.

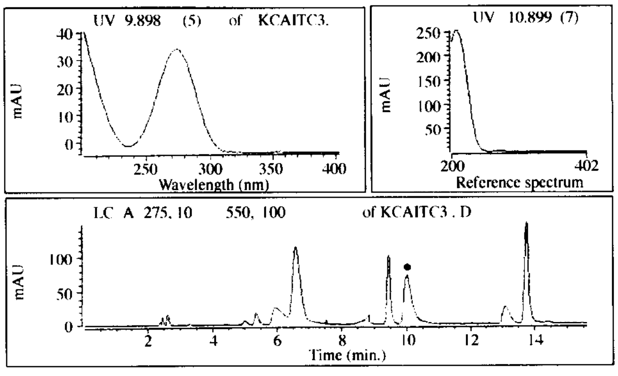


Fig. 5. UV spectrum analysis of AITC–SO_3^- separated by HPLC.

converted to the adduct at both storage temperatures during 8 weeks of storage. The constant concentration of the adduct was reached around 3 days and 4 weeks of storage at 23°C and 5°C, respectively.

The decrease of AITC level in commercially produced sulphited mustard paste at different temperatures is shown in Fig. 3. The rate of AITC decrease in the sulphited paste produced by the regular technological procedures was in a good agreement with that found in the paste prepared in the laboratory.

AITC degradation in non-sulphited aqueous solutions is controlled by pseudo-first-order reaction kinetics (Ohta *et al.*, 1995). Though the reactions in sulphited solutions and mustard pastes have involved more than one species whose concentrations change with time, the data found indicate that the reaction rates correspond approximately to the first-order reaction with respect to AITC. Therefore, the apparent first-order constants were used for evaluation of the decrease in rate of AITC concentration in sulphited solutions and mustard pastes. Second-order reaction kinetics can be employed for the reaction of AITC with HSO_3^-, when molar concentrations of both reactants are similar. The second-order rate constants were calculated from the equation for parallel first- and second-order reactions (Table 2).

Table 1. Eluent composition during gradient elution

Time (min)	0	3	8	18	20	25	30
A (%, v/v)	100	100	90	0	0	100	100
B (%, v/v)	0	0	10	100	100	0	0

A, sodium citrate–HCl buffer; B, methanol:sodium citrate–HCl buffer (80:20, v/v).

The binding of HSO_3^- to AITC can be considered to be a reversible reaction, as the adduct disappears almost immediately at pH 9. The concentration of the adduct dropped to about one-tenth of the initial level within 30 min. Good stability of the adduct was found in solutions of pH similar to that of mustard paste (pH 4). In this case, AITC–SO_3^- level decreased by 20% after 40 days storage at ambient temperature. In mustard paste, the adduct serves probably as a reservoir of SO_2 for reactions with other components of the matrix, mainly saccharides and proteins.

An HPLC method on a reversed phase column with UV detection was developed to achieve reliable determination of the adduct and confirm its identity and purity in the analysed samples. The optimisation criteria for eluent composition were resolution $R > 1.5$, peak shape and minimum time of analysis. Confirmation of identity and purity of the separated adduct was carried out using diode array detection (Fig. 4). The chromatograms of extracts of the analysed mustard paste are presented in Fig. 5.

The simultaneous analysis of AITC and AITC–SO_3^- in one run was limited to model solutions due to insufficient extractability of both analytes in one extraction step from mustard paste. The extractability of AITC and AITC–SO_3^- to water and acetonitrile, respectively, was poor for the levels of the analytes in mustard. Nor did extraction by any mixture of water and acetonitrile bring satisfactory yields. Significant parameters of the analytical procedure are summarised in Table 3.

Table 2. Rate constants for AITC decrease and AITC–SO_3^- formation

Medium	Temperature (°C)	Apparent rate constants (AITC decrease)		Rate constants (AITC–SO_3^- formation)
		$k'\,10^7$ (s^{-1})		$k_2\,10^4$ ($M^{-1}\,s^{-1}$)
		Non-sulphited	Sulphited	Sulphited
Commercial mustard paste	5	—	0.5 ± 0.1	—
	20	—	1.5 ± 0.2	—
	40	—	54 ± 5	—
Paste prepared in laboratory	5	0.32 ± 0.08	0.5 ± 0.1	0.0069 ± (0.0002
	23	1.8 ± 0.3	2.1 ± 0.2	0.024 ± 0.008
Model solution	5	0.83 ± 0.08	2.3 ± 0.1	0.15 ± 0.02
	(1.6 ± 0.1)[a]	(2.7 ± 0.3)[a]	(0.19 ± 0.04)[a]	
	23	3.7 ± 0.2	12.4 ± 0.8	1.9 ± 0.2
	(5.3 ± 0.2)[a]	(14.6 ± 1.2)[a]	(2.0 ± 0.2)[a]	

[a] Under nitrogen atmosphere.

Table 3. Performance characteristics of analytical procedure

	MW (Da)	LOQ (g litre^{-1}) model systems	LOQ (g kg^{-1}) mustard paste	Extraction yield[a] (%)	
AITC	99.16	0.002	0.01	Acetonitrile extraction:	92 ± 9
AITC–SO_3K	219.33	0.01	0.05	Water extraction:	97 ± 5

[a] $n = 4$; 95% confidence intervals of the mean. Acquired with standard addition method: spiked by 1 g of AITC and 0.5 g of the adduct per 1 kg of mustard paste.

In model mixtures, the characteristic mustard flavour of AITC became much slighter and garlic-like notes were perceptible. After dissolving of the adduct in water, the formation of garlic off-flavour arose as well. The flavour resembling garlic was caused by transformation of AITC to diallyl disulphide and by changes in amounts of the individual flavour-active compounds. In treated mustard paste, the garlic-like flavour was not evident, but the original pungent flavour became much milder. These observations were in good agreement with previous observations of Frijters *et al.* (1981).

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