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Laboratory simulation of captan residues degradation during apple processing

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The degradation of captan residues during the processing of apple to sterilised purée was investigated using laboratory small-scale processing (125°C for 20 min and pH 4·0). [^{14}C]-cyclohexene ring-labelled captan was completely degraded, mainly to tetrahydrophthalimide (96·5%). Other minor products such as tetrahydrophthalic acid (0·3) and tetrahydrophthalamic acid (0·2) were identified by HPLC and mass spectrometry. [^{14}C]-trichloromethylthio-labelled captan was completely degraded essentially to [^{14}C]CO_2 (77%) accompanied by small amounts of [^{14}C]CS_2 (2%). Thiophosgene was not detectable. Approximately 11·5% of the radioactivity was non-extractable and was believed to result from the reaction of the trichloromethylthio moeity with endogenous compounds of the apple, e.g. protein. The results were compared with those obtained in buffer medium mimicking the same process.

INTRODUCTION

Following the use of a pesticide, raw commodities (RAC) frequently contain pesticide residues. International controls on these residues are based on maximum residue limits (MRL) which are established from the residual levels found in field trials carried out according to good agricultural practice. These values represent a 'worst case' and frequently result in over estimations of actual daily intakes. Many industrial or household processes which may quantitatively or qualitatively change the nature of the residue are generally applied to these commodities before being consumed (Holland *et al.*, 1994). The major factors influencing the stability of the residue in typical processes are temperature, pH, water content and chemical nature of the residues. In order to determine the extent and nature of the degradation in typical unit processes it is necessary to attempt to simulate these procedures on a laboratory scale. In cases where the basic mechanisms of the degradation may be assumed to be the hydrolysis and the thermal decomposition of the molecule, it could be anticipated that the degradation would be similar with and without the matrix. This would not, however, consider any interactions of the residue with some matrix components.

The objective of the present study was to compare the behaviour of a potentially reactive pesticide residue both *in vivo* and *in vitro* using laboratory conditions which simulate unit processes. Captan (*N*-trichloromethylthio-4-cyclohexene-1,2-dicarboximide), a fungicide widely used in apple treatment, contains an unstable trichloromethylthio moeity that has been shown to undergo rapid hydrolytic (Wolfe *et al.*, 1976; Buyanovsky *et al.*, 1988) and metabolic (Krieger & Thongsinthusak, 1993) degradation to tetrahydrophthalimide and tetrahydrophthalamic acid. Extensive degradation was observed when apples (Frank *et al.*, 1983), strawberries (Ritcey *et al.*, 1987) or tomatoes (El-Zemaity, 1988) containing residues were subjected to cooking. In this investigation the sterilisation of apple purée containing ^{14}C-captan labelled on either the stable cyclohexene cycle or the trimethylthio group was chosen as an experimental substance/matrix couple.

MATERIALS AND METHODS

Chemicals

Separate samples of captan labelled with ^{14}C in either the 1,2-position of the cyclohexene ring (1·265 GBq/mmol, radiopurity > 90%) or in the trimethylthiomoeity (1·535 GBq/mmol, radiopurity > 90%) were obtained from ICI Agrochemicals (Fernhurst, Haslemere, UK). Purification of both compounds was carried out in small batches, using thin-layer chromatography (TLC) on Silicagel 60 plates (Merck) and chloroform/methanol

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(100 : 1·5, v/v) as eluent. Silica corresponding to the main radioactive spot R_f 0·85, detected using a Berthold radioscanner, was scraped from the glass plate then extracted with chloroform. Once purified, both molecules had a measured radiopurity > 98%, and were stored in acetonitrile at 4°C.

Unlabelled captan was purchased from Riedel-de Haën (Seelze, Germany), *cis*-1,2,3,6-tetrahydrophthalic anhydride, *cis*-1,2,3,6-tetrahydrophthalimide (THPI) and 4-nitrothiophenol from Aldrich-Chemie (Steinheim, Germany). α-Amylase (EC. 3.2.1.1.) type 1A, pronase E, hemicellulase from *Aspergillus niger* and cellulase (EC 3.2.1.4.) from *Aspergillus niger* were supplied by Sigma-Chemie (L'isle d'Abeau, Chesnes, France). All other chemicals and solvents used were of reagent grade. The liquid scintillation cocktail Ultima Gold™ was obtained from Packard (Rungis, France). Viles reagent (Viles, 1940) was prepared by dissolving 50 mg copper acetate in 1 litre 95% ethanol containing 20 ml triethanolamine and 1 ml diethylamine. Standard bis(4-nitrophenyl)trithiocarbonate was synthesised by treatment of 4-nitrothiophenol with thiophosgene (Owens & Blaak, 1960).

cis-1,2,3,6-Tetrahydrophthalamic acid (THPMA) was prepared by fractionated addition of *cis*-1,2,3,6-tetrahydrophthalic anhydride into an excess of ammonium hydroxide at room temperature. Ammonia was evaporated by heating under vacuum, then hydrochloric acid was added in excess. A precipitate was obtained when the mixture was left at room temperature which was washed with distilled water then characterised by mass spectrometry using a Nermag R-10-10 (Asnières, France) quadrupolar apparatus. Following direct introduction, positive chemical ionisation using ammonia as reagent gave $[M + H]^+$ at *m/z* 170 and $[M + NH_4]^+$ at *m/z* 187. Confirmation was obtained by GC-MS analysis using on-column methylation with trimethylanilinium hydroxide (Methelut reagent, Pierce), which showed the $M^{+\cdot}$ ion at *m/z* 197(1) that corresponds to the dimethylated form and six main fragments ions at *m/z* 165(90), 150(5), 136(10), 107(5), 80(100) and 79(90).

cis-1,2,3,6-Tetrahydrophthalic acid (THPA) was prepared by heating the corresponding anhydride with sodium hydroxide at 100°C for 30 min. After cooling and addition of hydrochloric acid a precipitate of THPA was obtained, which was washed with distilled water then analysed by mass spectrometry. Following direct introduction and positive ionisation using ammonia as reacting gas the $[M + H]^+$ at *m/z* 171, $[M + NH_4]^+$ at *m/z* 188 and $[M + N_2H_7]^+$ at *m/z* 205 ions were obtained that correspond to the THPA structure. The identity of the compound was confirmed by GC-MS analysis in similar conditions as those described for THPMA, that showed a $M^{+\cdot}$ ion at *m/z* 198(1) corresponding to the dimethyl ester form and three main fragment ions at *m/z* 167(15), 138(40) and 79(100).

Experimental protocol

Captan-^{14}C-cyclohexene(CHR)-ring labelling

After peeling and removing the core, apples (Golden delicious) were pureed under nitrogen using a domestic food mixer. A 5 g subsample of the puréed apple was introduced into a 10 ml Pyrex PTFE capped vial. One millilitre of distilled water and 50 μl of an acetonitrile solution of ^{14}C-CHR captan containing 1–5 × 10^6 dpm were added. Ten microlitres of an acetonitrile solution of unlabelled captan was added to achieve a final concentration of 5 ppm in the apple. The hermetically closed vial was immersed for 20 min in an oil bath set at 125°C. After cooling, the sample was transferred to a centrifuge tube and the reaction vial was rinsed with 10 ml distilled water. The sample was centrifuged at 105 000 × *g* and 4°C for 1 h. The supernatant was removed and the pellet was washed three times with 15 ml ethylacetate then once with 15 ml methanol. The organic solvent fraction was evaporated to dryness and the residue was either taken up with methanol for separate HPLC analysis or taken up with the aqueous supernatant. The residual pellet was combusted (Oxidizer Packard 306) and the radioactivity measured by liquid scintillation counting (LSC) in a Packard Tri-carb 2200 CA apparatus.

Additional experiments using 5 ml citric acid–Na_2PO_4 buffer (pH 4), in place of the apple purée, were also carried out at 125°C for 20 min and will be further referred to as control conditions. Experiments were also carried out to collect and trap volatile compounds. The apparatus used is described below.

Captan-^{14}C-trichloromethylthio (TCM) labelling

Incubates of apple purée and buffer solution fortified with ^{14}C-TCM were prepared as described above. Control experiments with citric acid–Na_2PO_4 buffer instead of the puréed apple were also performed. The reaction vessels for these experiments were 100 ml two-necked stoppered glass reaction flasks connected to three glass traps with fritted disc in series. The first two traps contained 250 ml 5M sodium hydroxide for carbon dioxide and dihydrogen sulphide trapping. The third one contained 200 ml of Viles reagent in order to trap carbon disulphide (CS_2) and carbonyl sulphide (COS). The series of flasks used for gas collection had been validated by previous workers. The data showed that concentrated aqueous NaOH traps absorbed neither COS (Stock & Kuß, 1917; DeBaun *et al.*, 1974) nor CS_2 (Lopatecki & Newton, 1952). In contrast, Viles reagent was shown to be effective in trapping both COS (DeBaun *et al.*, 1974) and CS_2 (Viles, 1940). The reaction flask was incubated for 20 min at 125°C, then the traps were connected and the gaseous compounds carried away by a nitrogen flow (1 litre/min) for 5 min using a pump placed after the last trap. For thiophosgene trapping, the reaction flask was connected to an ice water-cooled condenser the outlet of which was plunged in an ice-cooled glass trap containing a mixture of 400 mg *p*-nitrothiophenol in 30 ml acetone and 5 ml of 6M sodium acetate. The cooled trap system was connected to the reaction flask either during the course of the reaction carried out under N_2 flow or at the end of the reaction. The condenser was rinsed with trapping solution at the end of the experiment.

After removal of the radioactive volatile compounds

the remaining suspension was extracted by stirring with 50 ml 70% ethanol for 15 h at 0°C. The suspension was filtered on a glass fibre filter (GF/F Whatmann) and the insoluble residue was washed twice with 50 ml of 70% ethanol followed by 50 ml of a mixture of methanol/acetone (1 : 1). The filtrates were pooled, neutralised with dilute sodium hydroxide, concentrated at 40°C under vacuum then stored at 4°C until analysis. The insoluble residue was dried and either combusted or sequentially extracted using the method of Langebartels and Harms (1985) to determine the amount of radioactivity associated with each fraction. In brief, the residue was treated successively with α-amylase (starch), pronase E (protein), EGTA (pectin), 1M potassium hydroxide at 25°C (hemicellulose), dioxane/hydrochloric acid (9 : 1) at 70°C (lignin) and cellulase (cellulose). After each treatment the soluble fraction was separated (105 000 × *g* centrifugation or 0·45 μm filtration) from the insoluble residue which was washed before applying the next treatment. The radioactivity in the final non-extractable residual matrix was measured following combustion.

Analytical methods

Analysis of volatile traps

[^{14}C]-CO_2 was determined by direct LSC using a 0·5 ml aliquot of the 5M sodium hydroxide trapping solution and 20 ml of Ultima Gold. [^{14}C]-CS_2 collected in Viles reagent was determined specifically as copper diethyldithiocarbamate. The trapping solution was adjusted to pH 9 with dilute hydrochloric acid then evaporated to dryness under vacuum, and the residue was taken up with 50 ml of citric acid/ammonium hydroxide buffer pH 8·5. The copper complex was extracted three times with 20 ml chloroform. The solvent was filtered on a phase separation filter (Whatman IPS) then evaporated to dryness, and the radioactivity of the residue was measured by LSC.

A qualitative determination of hydrogen sulphide production was performed on samples fortified with unlabelled captan (100 μg), using a filter paper impregnated with saturated lead acetate solution placed in the head space of the reaction flask.

Thiophosgene production was investigated by analysis of the bis(4-nitrophenyl)trithiocarbonate formed with 4-nitrothiophenol. The trapping solution was evaporated under vacuum to remove acetone and the aqueous phase was extracted five times with 10 ml diethylether. The ethereal phase was washed with 50 ml of a 6M sodium acetate solution in order to remove excess of 4-nitrothiophenol, then filtered on a phase separation filter (IPS) and concentrated under nitrogen. TLC on Silicagel 60 plates with toluene as eluent indicates R_f values of 0·67 and 0·60 for standard bis(4-nitrophenyl)trithiocarbonate and 4-nitrothiophenol, respectively.

Analysis for captan

An aliquot (2 ml) of the 105 000 × *g* supernatant was transferred to a Sep-Pak Plus C18 (Waters) cartridge pre-treated with methanol and distilled water. The cartridge was washed with 5 ml distilled water and captan was eluted with 5 ml acetonitrile. The solvent was evaporated to dryness. The residue was dissolved in 250 μl acetonitrile and analysed by HPLC. The operating parameters were as follows: column, Spherisorb ODS2 5 μm (250 × 4·6 mm i.d.), protected by a pre-column with a 10 × 4 mm cartridge packed with Nucleosil C18 5 μm; solvent system, acetonitrile/water (40 : 60); flow rate, 1 ml/min. In these chromatographic conditions captan has a retention time of 20 min. Fractions (250 μl) were collected (Gilson 202 collector) and radioactivity measured by LSC.

Degradation products from ring-labelled captan

The investigation was carried out on the combined organic extract and 105 000 × *g* supernatant fraction of the cooked puréed apple. A 500 μl aliquot was filtered (0·2 μm) and 250 μl were injected directly into HPLC. Chromatographic separations were performed on a Spherisorb ODS2 C18 column as already described. The mobile phase (Eluent A) consisted of a mixture of acetonitrile/water (10 : 90) containing 5 mM tetrabutylammonium hydrogen sulphate (TBA) and 10 mM Na_2HPO_4 (final pH: 7·2). All experiments were performed isocratically at ambient temperature with a flow rate of 1 ml/min. This ion-pair system allowed separation of THPMA (R_t 7·0 min), THPI (11·0 min) and THPA (18·0 min). As ion-pairing is not appropriate for further MS analysis of ionised compounds, the acidic products formed during the degradation process were separated from neutral compounds using a 500 mg Anion Exchange cartridge Chromabond SB (Macherey Nagel, Strasbourg, France). The cartridge pre-conditioned with methanol and water was loaded with 1 ml aliquots of the supernatant (pH 8). The cartridge was washed with distilled water (5 ml) then eluted with 5 ml 0·1M H_3PO_4. The eluate was concentrated to 1 ml under vacuum (Speed Vac) and extracted with ethyl acetate. The organic extract was analysed by TLC using Silicagel 60 plate and chloroform/methanol/acetic acid (95 : 5 : 5) as eluent. The radioactive spots were located by scanning and the standard compounds were detected after reaction with iodine vapours. In these conditions the following R_f values were observed: THPI (0·70), THPA (0·40) and THPMA (0·25). The neutral compounds were separated by HPLC using the same column and a mobile phase (Eluent B) consisting of acetonitrile/water (10 : 90) with 10 mM Na_2HPO_4 brought to pH 7·2 with dilute sulphuric acid.

RESULTS AND DISCUSSION

Compounds resulting from the degradation of ring-labelled captan

The distribution of the radioactivity in different fractions from the sterilised puréed apple was determined in five separate experiments. Total recovery of the

added radioactivity was 97·9 ± 1·2%. This suggests that no radioactive volatile compound was evolved at a significant level during the process. Confirmation was derived from two separate experiments carried out with gas collection which showed that no measurable radioactivity was recovered from the gas traps. The majority of the radioactivity (94 ± 0·9%) was present in the aqueous supernatant following centrifugation whereas organic washings accounted for 3·8 ± 0·5% only. The remaining radioactivity in the pellet measured after combustion represented 0·12 ± 0·08%. These results indicate that non-extractable products resulting from the reaction of ring-labelled captan with apple endogenous compounds are not significantly formed. Moreover, four separate determinations showed that captan had disappeared completely (limit of detection 0·1% of the dose).

Direct HPLC analysis of the residue from the organic solvent extract of the pellet showed a single radioactive compound which co-eluted with standard THPI. Figure 1 shows a typical ion-pair HPLC profile of the supernatant fraction. About 10 degradation products appeared, among them three exhibiting retention times similar to synthesised standards. The major peak (A) that accounted for 96·5% corresponds to THPI. This compound was isolated by successive HPLC runs (Eluent B) and fraction collection. Its identity was confirmed by mass spectrometry following direct introduction and using electron impact ionisation that gave the molecular ion at *m/z* 151 (Fig. 2a). The other compounds (B) and (C) exhibited retention times corresponding to THPMA and THPA, respectively. Confirmation was obtained following GC-MS analysis which gave spectra (Figs 2b and c) identical to those obtained with the corresponding standard compounds.

Unresolved polar compounds contributed to 0·7% of the total radioactivity in the supernatant. When isolated (HPLC, Eluent B) and hydrolysed in the presence of 0·1M hydrochloric acid (25°C, 30 min), this polar fraction was partially decomposed, yielding small amounts (0·1%) of THPI. The formation of THPI following the acidic treatment of a substance more polar than this compound could be due to the cleavage of THPI thiocarbonate, an intermediate of captan hydrolysis postulated by other authors (Wolfe *et al.*, 1976). This is consistent with the generally observed degradation of thiocarbonates in acidic conditions.

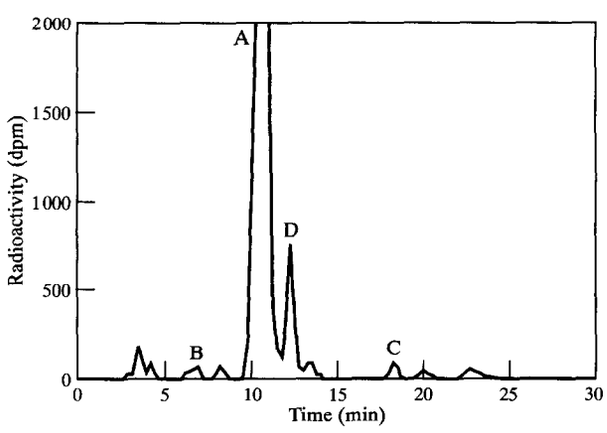

Fig. 1. Typical ion pair radio-HPLC chromatogram of [^{14}C] ring-labelled captan degradation products. ^{14}C ring-labelled captan was degraded in the presence of apple purée at 125°C for 20 min. The supernatant fraction was filtered and directly injected A, THPI; B, THPMA; C, THPA; D, THPI *N*-Thiol (?).

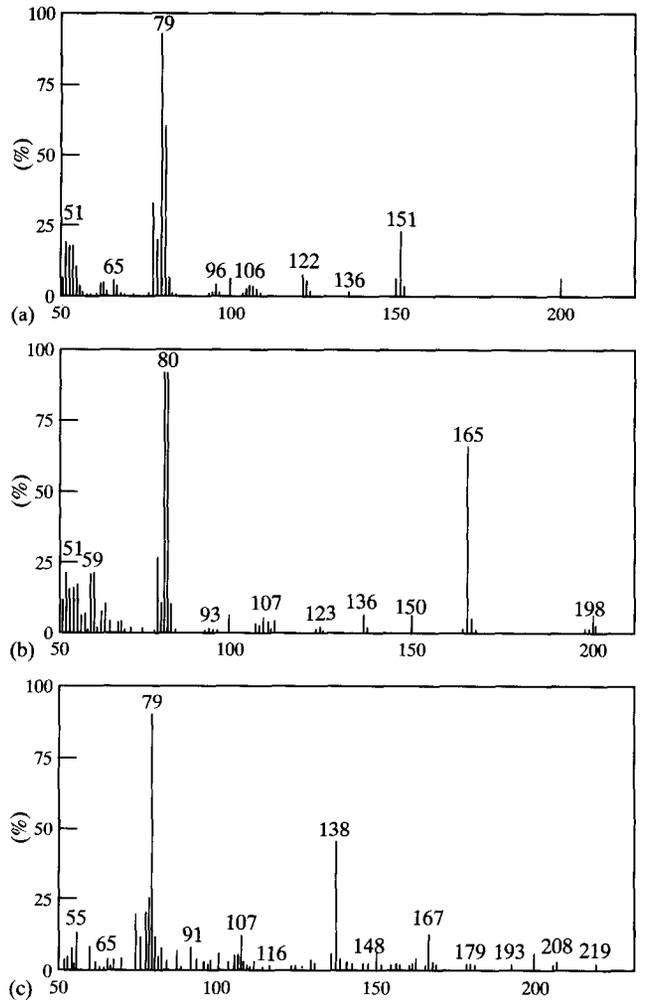

Fig. 2. Mass spectra of captan degradation products: (a), THPI after direct introduction and electron impact ionisation; (b), THPMA after methylation and GC-MS retention time 6·0 min; (c), THPA after methylation and GC-MS retention time 5·5 min.

A further unknown component, compound D, accounted for 1·6% of the total radioactivity in the supernatant. Although this product has not been identified the following structural information has been derived. (i) The retention time of this compound was not affected by absence of TBA (Eluent B), a result which is consistent with the lack of an acidic group in the molecule. The above hypothesis was confirmed by the fact that the unknown product was not retained by the anion-exchange cartridge. (ii) When labelled THPI was submitted to degradative conditions similar to those applied to captan, the unknown compound was not present, suggesting that it should contain at least part of the trichloromethylthio group. (iii) Compound D did not occur during the degradation of [^{14}C-trichloromethylthio]captan suggesting that compound

Table 1. Degradation products of [cyclohexene ring-1,2-^{14}C] captan[a]

Compound	% of the dose
Unchanged captan	< 0·1
Tetrahydrophthalic acid	0·30 ± 0·1
Tetrahydrophthalamic acid	0·25 ± 0·1
Tetrahydrophthalimide	96·5 ± 1·1
Compound D (THPI *N*-thiol ?)	1·6 ± 0·3
Unknown compounds	1·1 ± 0·3
Non-extractable	0·10 ± 0·5

[a]5 g of apple purée was added with 1–5 × 10^6 dpm of [^{14}C]cyclohexene ring-labelled captan and the mixture was incubated at 125°C for 20 min. (n = 5).

D does not contain the ^{14}C-labelled atom. (iv) This product was stable in acidic medium but was cleaved to the extent of 90% in alkaline solution (0·1M NaOH, 100°C, 30 min) yielding THPI (20%) and THPMA (70%) just as THPI does when submitted to similar experimental conditions. (v) Degradation of the compound was observed also during GC-MS analysis, giving only the THPI spectrum. The absence of the parent ion may reflect the instability of this intermediate compound. Considered together, these chemical and structural data suggests that compound D could correspond to the THPI *N*-thiol.

In control conditions, similar chromatograms were obtained (not shown), which confirms the absence of chemical interaction between ring-labelled captan and the components of the apple. Moreover, this similar behaviour enables the isolation and preparation of captan degradation products with very limited amounts of interfering substances that would facilitate further structural analysis. A full description of the degradation products is shown in Table 1.

The demonstration that captan is very sensitive to the process conditions used to prepare sterilised puréed apple confirms earlier observations (Frank *et al.*, 1983); however the nature of the degradation products is now clearly established. The implications of the data are that captan residues will only be found in the RAC. In processed commodities captan residues will be significantly reduced. The major degradation product will be THPI, a hydrolysis product from captan.

Degradation compounds derived from [trichloromethylthio-^{14}C] captan

The results are given in Table 2. Following processing, the mean recovery of the radioactivity added to the puréed apple was 95 ± 2% of the dose (five trials). In contrast with the results obtained with ring-labelled captan, most of the radioactivity was recovered as gaseous compounds (80 ± 5%) while the remaining was associated with the puréed apple suspension and distributed between the 70% ethanol extractable fraction (4·4 ± 2%) and the residual non-extractable matter (11·5 ± 3%). In control conditions only 1·5% of the radioactivity remained in the solution after treatment.

Table 2. Degradation products of ^{14}C[trichloromethylthio]captan

Compound	% of the dose
Carbon dioxide	77 ± 3
Carbon disulphide	2 ± 0·5
Carbonyl sulphide	ND[b]
Thiophosgene	ND
Bicarbonate ion	2 ± 1
Unknown	1·5 ± 0·2
Bound residues	11·5 ± 2·4
Starch	0·5
Protein	3·1
Pectin	0·3
Hemicellulose	3·8
Lignin	0·8
Cellulose	0·3
Residual matrix	1·8

[a]5 g of apple purée was added with 1–5 × 10^6 dpm of [^{14}C trichloromethylthio]captan and the mixture was incubated at 125°C for 20 min and then gassed off for 5 min at 125°C (n = 5). [b]ND, not detectable.

This strongly suggests that products originating from the degradation of the trichloromethylthio moeity of captan interact with the apple matrix.

Analysis of volatile components

The first sodium hydroxide trap contained 96% and the second only 1% of the evolved radioactivity. These data confirm the high efficacy of the trapping system for [^{14}C]-CO_2 and the fact that the radioactivity recovered further in the Viles reagent trap did not result from an incomplete absorption in the former traps. The major product is carbon dioxide which accounts for 77% of the applied radioactivity. A further 2–3% of the radioactivity was recovered in the Viles reagent which was attributable to CS_2 or COS. Extraction of copper diethylthiocarbamate from the Viles reagent showed that CS_2 accounted for 80% of the radioactivity trapped, in the presence of apple purée. It can therefore be concluded that significant amounts of carbonyl sulphide (COS) were not detected in spite of the fact that this compound was postulated to be an intermediate in thiophosgene hydrolysis (Wolfe *et al.*, 1976). However, it must be noted that in our experimental conditions COS is probably hydro-lysed into CO_2 and H_2S, as the formation of the latter was evidenced qualitatively using lead acetate paper.

No detectable amounts of thiophosgene were found in the presence of the apple purée, as shown by the absence of bis(4-nitrophenyl)trithiocarbonate in the trap. However, if the nitrogen flow was maintained through the reaction flask during the process, trace amounts of a radioactive compound (0·1%) co-eluting with standard bis(4-nitrophenyl)trithiocarbonate were recovered from the trap. This result suggests that this compound might be produced to a greater extent under the experimental conditions, but rapidly hydrolysed or reacted with some endogenous components of the apple. This latter hypothesis is consistent with the results of earlier studies which showed that thiophosgene was produced

during the reaction of captan with thiols due to the cleavage of the N–S link (Lukens & Sisler, 1958). The presence of CS_2 in the evolved gas emphasises this proposal as CS_2 cannot arise directly from captan hydrolysis but more likely from unstable trithiocarbonate or/and dithiocarbamates derived from thiophosgene.

Extractable compounds

After purging the reaction flask the remaining suspensions retained 15 ± 3% of the initial radioactivity. About 25% of the residue was extractable with 70% ethanol which was used to precipitate both the cell proteins and cell wall polysaccharides. This extractable fraction gave two major peaks when analysed by HPLC (Eluent A). One of them exhibited the same retention time (R_t 4 min) as standard bicarbonate ion and disappeared after bringing the sample to pH 1. The other peak (R_t 4·5 min) was stable in acidic medium and was also present in control samples. No further investigation was conducted to characterise this compound.

Non-extractable fraction

The radioactivity which remained associated to the apple solid components accounted for 11·5 ± 2·4% of the dose. This result suggests that adducts were formed by reaction between endogenous substances and degradation compound(s) derived from the trichloromethylthio moiety of the captan molecule. Table 2 indicates that small amounts of radioactivity were recovered at every step of the fractionation, but that protein and hemicellulose fractions contained the majority of the radioactivity. Further separate experiments designed to evaluate the interaction of captan with purified pectin, starch, cellulose, lignin and proteins (bovine serum albumin and ovalbumin) were carried out in similar conditions (pH 4, 125°C 20 min). The results showed that starch pectin, lignin and cellulose each retained about 1·5% of the initial radioactivity, a value not significantly different from that obtained with the buffer alone. In contrast, considerable retention (7%) occurred with the two proteins. The association of the radioactivity with proteins is in accordance with data already published which showed that the degradation products originating from the [^{35}S]-trichloromethylthio moeity of captan bind preferentially to the proteins of *Neurospora crassa* spores (Richmond & Somers, 1968) as well as the nucleoproteins of rat liver (Couch *et al.*, 1977). The labelling of the hemicellulose fraction determined after solubilisation of the polymer into potash was surprisingly high (3·8%). However, this result was not confirmed by hemicellulase treatment. It may be that the harsh and non-specific chemical treatment used might have released some radioactivity adducted to other macromolecules and not completely freed by the former and successive enzymatic treatments. It must be noted that the two highly reactive chlorine atoms of thiophosgene may react towards thiol, hydroxy and amino groups to give single adducts or bridges between different biopolymers that may limit the enzymatic attacks. However, the exact nature of the adducts remains to be determined.

CONCLUSION

When the most drastic thermal treatments prevailing in the apple products technology were applied to captan residues, an extended degradation of this fungicide occurred leading to its complete disappearance and genesis of derived compounds including THPI, gases and adducts to the apple matrix. The same pattern of degradation was observed either under small-scale laboratory conditions modelling the apple treatment or *in vitro* in the absence of the food matrix but under physicochemical conditions mimicking the process. These data support the postulation that the degradation conditions of a molecule in a complex biological medium may be modelled and reduced to a set of physicochemical parameters such as the reaction medium (water contents), concentration of the molecule to be tested, pH, temperature or reaction time, mimicking the technological process. Reactions *in vitro*, however, do not take into account the formation of adducts which may be formed with highly reactive molecules. However, by studying the reactivity of the molecule towards the main constituents of the food matrix taken separately and using the physicochemical conditions used in the unit process, a model can be developed. It is therefore proposed that this two step model provides a simplified approach to the complex problem of the fate and eventual interaction of xenobiotic residues with biological components during food processing. It is further proposed that this approach should be validated using different compounds belonging to different chemical classes and using sets of conditions corresponding to different processes.

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