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Analytical Methods Section

Determination of ethylenethiourea and propylenethiourea in tomato products and in fruit purees

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An analytical method was developed for determining ethylenethiourea and propylenethiourea in tomato products (tomato pulp, tomato puree, tomato paste of 28° and 36° Brix, peeled tomatoes and ketchup) and in fruit purees (apricot, pear, peach, apple). The residues were extracted with dichloromethane on an Extrelut column and clean-up was carried out using a Sep-Pak C18 cartridge before injection into a liquid chromatograph with an electrochemical detector (coulometer).

With this method 100 samples of commercial tomato products were analysed. The positive samples were confirmed by capillary gas chromatography with flame photometric detector (sulphur mode filter).

The sensitivity of the method is 3 $\mu g\ kg^{-1}$ in the case of tomato products (peeled and strained tomatoes, tomato pulp) with a total solids content similar to that of fresh tomatoes and of fruit purees with a soluble solids content of 10° Brix.

INTRODUCTION

Ethylenebisdithiocarbamates are fungicides which are frequently used for the control of fungal diseases in a wide range of crops. These substances are not stable in the presence of moisture and/or oxygen, nor in biological systems; during their degradation several products are formed such as ethylenethiourea (ETU), ethylenebisdithiocyanate sulphide (EBIS), ethylenethiuram disulphide (ETD) and ethylenethiuram monosulphide (ETM) (Engst & Schnaak, 1974).

Many research workers in the food and ecological sectors are interested in ETU because this compound, which has been found to be carcinogenic and teratogenic for laboratory animals, is also considered dangerous to human health (Fishbein, 1976). In 1974 FAO/OMS began to set ETU limits for 11 types of vegetables: these values are quite low, ranging from 10 to 100 $\mu g\ kg^{-1}$ (FAO/WHO, 1978). There is an increasing need for simple and rapid methods to control ETU residues in foods.

Bottomley *et al.* (1985) reviewed the available methods for the determination of ETU residues by means of different chromatographic techniques: thin-layer chromatography (seven methods), gas chromatography (23 methods) and liquid chromatography (11 methods).

While thin-layer chromatography generally does not provide the required accuracy, gas chromatography gives unsatisfactory results since ETU, being a very polar compound, does not elute well from the gas chromatographic column unless derivatisation is applied. Derivatisation, however, requires further handling of the sample, which means longer preparation times, possibility of errors and low recoveries, especially when complex matrixes such as foods are used.

In this study, a method for the determination of ETU residues in tomato products and in fruit purees (apricot, pear, apple, peach) was developed using High Performance Liquid Chromatography (HPLC) with an electrochemical detector (coulometer) which provides the required sensitivity and selectivity. Using the same method, the propylenethiourea (PTU) residue was determined, too. The PTU is a degradation product of the widely used fungicide propylenebisdithiocarbamate (Propineb). The method was used to analyse 100 samples of commercial tomato products and 15 samples of fruit purees. Confirmation tests were carried out by capillary gas chromatography with flame-photometric detection (sulphur mode filter) according to method described in the literature (Nitz *et al.*, 1982).

MATERIALS AND METHODS

Reagents and standards

Solvents suitable for the analysis of residues and for HPLC analysis were obtained from Merck (Darmstadt,

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Table 1. Recoveries (%) of ETU and PTU from spiked tomato products

Added (μg kg^{-1})	ETU			PTU		
	Average recovery (n = 4)	Standard deviation	Coefficient of variation (%)	Average recovery (n = 4)	Standard deviation	Coefficient of variation (%)
5	76·2	7·3	9·6	89·4	7·3	8·1
20	90·4	6·4	7·1	93·2	8·4	9·1
50	96·2	7·5	7·8	100·5	8·1	8·1
500	92·5	6·5	7·0	91·2	10·5	11·5

Germany). Standards were obtained from S.I. Ehrenstorfer (Augsburg, Germany). ETU and PTU stock solutions for calibrating the coulometric detector and for sample fortification were prepared by dissolving the standards in bidistilled water. The solutions were then stored in a freezer.

The standard solutions for analytical determinations were obtained by dissolving the stock solutions with mobile phase while those for recovery were diluted with bidistilled water. ETU and PTU solutions for gas chromatographic determination were diluted with dichloromethane (CH_2Cl_2) from stock solutions in CH_2Cl_2.

HPLC analysis

The HPLC system consisted of a Model 510 pump (Waters Associates, Milford, MA) with a Model LP-21 LO-Pulse damper (Scientific Systems, Inc., State College, PA), Rheodyne injector, Model 5100A coulometric detector (ESA, Inc., Bedford, MA) with a Model 5011 analytical cell. Detection conditions were: detector 1 + 0·60 V, detector 2 + 0·70 V gain × 20. The column used was a Supelcosil LC-8 (25 cm × 0·4 cm i.d.) with a particle size of 5 μm. The mobile phase was a sodium acetate trihydrate 0·025 M solution, adjusted to pH 6·9 with acetic acid; the flow was 0·9 ml min^{-1}.

Fifty grams of product taken from a homogeneous sample (tomato pulp, tomato puree, peeled tomatoes), after pH adjustment to 7·5 with 5 M NaOH were centrifuged at 12 000 rpm for 15 min. After filtration through two Minisart NML filters (Sartorius AG, Göttingen, Germany) in series (5 and 1·2 μm), serum (1 ml) was loaded onto an Extrelut 1 column (Merck) and, after adsorption for 10 min, elution was performed with 10 ml dichloromethane/methanol (98:2, v/v). The eluate was evaporated to dryness with a rotary evaporator at a bath temperature of 30°C; the residue was taken up with methanol (1 ml) and loaded onto a Sep-pak C18 cartridge (Millipore Corporation, MA) preconditioned with methanol (10 ml). The sample was eluted with 3 ml methanol in a 20 ml volumetric flask, rotary-evaporated at a bath temperature of 30°C to a volume of 0·5 ml; the last solvent traces were then removed with a gentle stream of nitrogen at room temperature and the sample was finally taken up with mobile phase (200 μl) with an automatic pipette. An aliquot (20 μl) was injected into the chromatograph. As regards samples of tomato paste of 28 and 36°Brix as well as ketchup, the preparation was the same after dilution by four, five and three times, respectively.

GC analysis

An HP 5890 gas chromatograph equipped with a flame photometric detector with sulphur filter was used. Injector and detector temperatures were 250°C; helium carrier flow rate was 4 ml min^{-1}. A Stabilwax (Restek Corporation, Bellefonte, PA) bonded-phase polyethylene glycol column (8 m × 0·25 mm i.d.; 0·25 μm film) was used. The initial temperature of 60°C was increased to 220°C at a speed of 30°C min^{-1}. The sample was treated as in the HPLC analysis until filtration. Filtrate (20 ml) was collected and KF dihydrate (10 g) was added. After stirring with a magnetic stirrer, the solution was poured onto an Extrelut 20 column; after 15–20 min the column was eluted with dichloromethane/methanol (98:2, v/v). The solvent was evaporated to dryness and the residue was taken up with dichloromethane (200 μl): a sample (1 μl) was injected into the gas chromatograph using the splitless injection technique. The purge valve was switched off after 0·8 min.

RESULTS AND DISCUSSION

ETU and PTU retention times under the HPLC conditions applied were 6·9 and 13·6 min, respectively.

Figure 1 shows the HPLC chromatograms of a

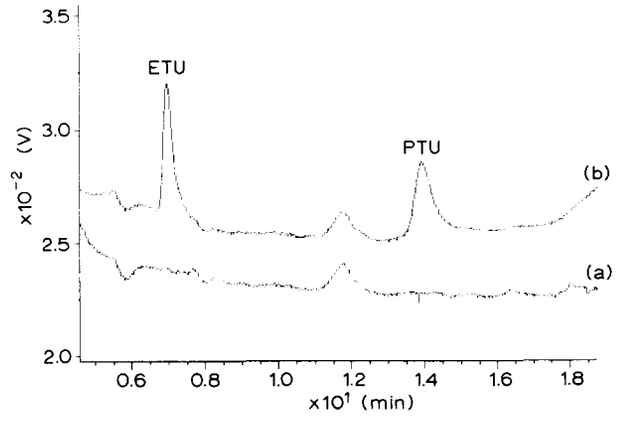

Fig. 1. HPLC chromatograms of (A) tomato pulp free from ETU and PTU, (B) tomato pulp fortified with 0·005 mg kg^{-1} of ETU and PTU.

Table 2. Recoveries (%) of ETU and PTU from spiked fruit purees

Added ($\mu g\ kg^{-1}$)	ETU			PTU		
	Average recovery ($n = 4$)	Standard deviation	Coefficient of variation (%)	Average recovery ($n = 4$)	Standard deviation	Coefficient of variation (%)
5	68·4	2·8	4·1	99·0	2·0	2·0
20	73·0	4·5	6·1	85·2	10·9	12·8
50	76·0	4·8	6·4	87·1	9·9	11·4
500	87·2	4·2	4·8	90·4	3·6	4·0

tomato pulp sample not treated with dithiocarbamates (ethylene and propylene) and of the same sample fortified with 5 $\mu g\ kg^{-1}$ of ETU and PTU. As can be seen, chromatograms are satisfactory even when the ETU and PTU amounts are very low; there are no peaks interfering with the active compounds analysed. However, when a sample was injected, interferences were noticed due to matrix substances retained by the column for a long time. These interferents elute giving very large peaks, thus making it necessary to clean the system for an hour. In these cases, the use of a double-column system is recommended so that column-switching could allow work to be continued with one column while the other is being cleaned after use (Hogendoorn *et al.*, 1991).

The techniques described by Banfi (Plasmon, Italy; personal communication) and Krause (1989), on which this work is based, were actually used only as a starting point; in fact, even though these techniques are applicable to a wide range of foods they proved unsatisfactory in the specific case of tomato products.

Correction of pH suggested by Krause (1989) for apple sauce markedly increased ETU and PTU recoveries.

Compared with the method used by Banfi, further clean-up of the sample with Sep-pak C18 cartridge significantly reduced the problem of interference. A longer chromatographic column (LC-8, 250 mm long) was used, which allowed the removal of negative peaks in the immediate vicinity of the PTU peak.

The minimum detectable level of ETU and PTU is 0·3 ng, equivalent to a 3 $\mu g\ kg^{-1}$ residue in the sample. This value represents the sensitivity of the proposed analytical method in the case of tomato products (peeled and strained tomatoes, tomato pulp) which have a total solids content similar to that of fresh tomatoes and of fruit purees with a soluble solids content of 10° Brix. As regards 28 and 36°C Brix tomato pastes as well as ketchup, the method was applied in the same way but starting, of course, with a smaller sample. The sensitivity of the method was therefore lower depending on dilution: 12 $\mu g\ kg^{-1}$ for 28° Brix tomato paste, 15 $\mu g\ kg^{-1}$ for 36° Brix paste and 9 $\mu g\ kg^{-1}$ for ketchup. The ETU and PTU response is linear in the 0·3–50 ng range.

Table 1 reports the percent recoveries of ETU and PTU added to samples of tomato products (peeled and strained tomatoes, tomato pulp) prepared with tomatoes not treated with ethylenebisdithiocarbamates or which, in any case, were found negative when analysed for the same active substances. Recoveries are higher than 80% for all levels except for the lowest ETU addition.

Table 2 reports the percent recoveries of ETU and PTU added to fruit purees. The fruit purees used were semiprocessed products directly supplied by the manufacturers, which proved free from ETU and PTU. PTU recoveries were good; ETU recovery levels were lower except for one case (500 $\mu g\ kg^{-1}$).

Table 3 reports the ETU residue values in tomato products purchased from the market or supplied by different manufacturers. The 100 samples were divided on the basis of the production year (1990 or 1991) and of the type of product. Analyses were carried out after the 1991 season. As Table 3 shows, it was difficult to obtain tomato paste samples from the 1990 production season. HPLC results show that eight samples were positive with rather low residue levels. These values were confirmed using capillary gas chromatography.

Table 3. Results of ETU residues analysis in 100 samples of commercial tomato products

Production year	Product	Samples analysed	Samples with ETU	Highest residue found ($\mu g\ kg^{-1}$)
1990	Peeled tomatoes	12	2	13
	Tomato pulp	8	2	7
	Tomato puree	12	0	—
	Tomato paste (28° Brix)	3	0	—
	Tomato paste (36° Brix)	1	0	—
	Ketchup	5	0	—
1991	Peeled tomatoes	16	1	3
	Tomato pulp	18	0	—
	Tomato puree	11	1	4
	Tomato paste (28° Brix)	11	1	26
	Tomato paste (36° Brix)	3	0	—

Confirmation by gas chromatography proved to be very useful; in fact, in spite of the specificity of the coulometric detector, there were some cases of interference. Only in one case was ETU residue, determined by liquid chromatography, not confirmed by gas chromatography, but the samples analysed (peeled tomatoes) belonged to a particular cultivar seldom used for processing.

As regards PTU residues, gas chromatography did not confirm the results for the two samples which had turned out positive when analysed by means of HPLC.

With this method, 15 fruit puree samples were also analysed. ETU was present in 10 samples, with residue values ranging between the detection limit and 50 $\mu g\ kg^{-1}$.

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