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Food Chemistry 87 (2004) 383–386
Food Chemistry
www.elsevier.com/locate/foodchem

Determination of carbendazim, thiabendazole and thiophanate-methyl in banana (*Musa acuminata*) samples imported to Italy

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Received 15 August 2003; received in revised form 1 December 2003; accepted 1 December 2003

Abstract

The occurrence of benzimidazole fungicides (benomyl and its metabolites carbendazim, thiabendazole and thiophanate-methyl) in 50 banana samples imported to Italy from Ecuador, Panama and Costa Rica during 2002–2003 was investigated. In 11 samples, thiabendazole was found at concentrations ranging from 0.050 to 2.510 mg/kg. In five samples, carbendazim was found at concentrations ranging from 0.140 to 1.100 mg/kg, whereas thiophanate-methyl was not detected in any sample. Analysis was carried out using HPLC-DAD and positive samples were confirmed by GC–MS. Recoveries of carbendazim, thiabendazole and thiophanate-methyl at four fortification levels (0.5, 1.0, 2.5 and 4.0 mg/kg) were in the range 81–96% for carbendazim and thiabendazole and 63.2–69.8% for thiophanate-methyl. Only two samples contained carbendazim that exceeded the FAO/WHO Codex Alimentarius and the Italian Department of Health maximum residue limits.

Keywords: Banana; Maritime Authority; Carbendazim; Thiabendazole; Thiophanate-methyl; HPLC-DAD

1. Introduction

Banana is the fruit with the highest consumption in the European market. It is an excellent tropical fruit, has an agreeable flavour and a high nutritional value. The contribution to the intake of sugars, fibre, vitamins, and minerals from the consumption of bananas is high, with a very low contribution to the intake of fat. Approximately 70% of the total production of bananas in the world occurs in Latin America. The main banana suppliers are Ecuador, Costa Rica, Panama, Brazil, Honduras and India. Ecuador is the main producer of bananas, producing about 30% of all the bananas from Latin America.

There are many variables that influence the chemical composition of bananas, such as methods of cultivation, fertilizers and pesticides used, quality of water for irrigation, or storage and commercialization conditions. Benzimidazole fungicides are systemic pesticides, widely used in agriculture for pre- and post-harvest treatment for the control of a wide range of fruit and vegetable pathogens (Papadopoulou-Mourkidou, 1991). They are some of the most detected pesticides during monitoring programmes and it is crucial to assess consumers' exposure to those fungicides through foods. The main compounds of the benzimidazolic family are benomyl, carbendazim (MBC), thiabendazole (TBZ) and thiophanate-methyl (TM). Benomyl is a systemic fungicide, applied to the soil to control a variety of fruit diseases. Benomyl rapidly degrades to carbendazim, its main degradation product, and this is also a systemic fungicide, used to combat a wide range of diseases. It is believed that the fungicidal activity of benomyl is due to the presence of carbendazim (Clemens & Sisler, 1969; Guan, Davis, Jin, & Baillie, 1994; Itak, Selisker, Jourdan, Fleeker, & Herzog, 1993; Liu, Mattern, Yu, & Rosen, 1990).

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0308-8146/$ - see front matter
doi:10.1016/j.foodchem.2003.12.009

Because benzimidazole fungicides in food constitute a significant health risk (Banks & Soliman, 1997; Urani et al., 1995) and bananas undergo a high consumption rate, the continuous control of these pesticide residues in imported fruits is of great importance.

Fifty banana samples imported to Italy were collected from the Maritime Authority of Salerno Customs Port (Italian Department of Health), during 2002–2003 and were analysed in our laboratory. The simultaneous determination of benomyl (as total MBC), TBZ and TM residues was carried out by HPLC-DAD and positive samples were confirmed by GC–MS. The results of this monitoring of banana samples imported from Latin America are reported.

2. Materials and methods

2.1. Samples

Fifty imported banana samples from Ecuador (32), Panama (10) and Costa Rica (8) were collected from the Maritime Authority of Salerno Customs Port (Italian Department of Health), during 2002 (30 samples) and 2003 (20 samples). The samples were transported to the laboratory and kept under frozen conditions until analysis.

2.2. Materials and reagents

All organic solvents were products of Carlo Erba, Milano (Italy). Compounds of purity 97–99% were products of Dr. Ehrenstorfer (Augsburg, Germany) and provided by Labservice Analytica, Bologna (Italy). Stock standard solutions of MBC, TBZ and TM were prepared by diluting 100 mg of the standards in 100 ml of ethyl acetate to obtain a concentration of 1 mg/ml. Ready-to-use Extrelut-20 columns (Merck, Darmstadt, Germany) were used as received for the on column partition.

2.3. Sample preparation

Fifty grammes of product taken from homogeneous samples of banana were homogenised with 100 ml of 0.02 N HCl/MeOH (80:20) for at least 5 min, centrifuged at 5000 rpm for 15 min and filtered through a glass microfibre filter under vacuum. An aliquot of this solution (20 ml) was brought to pH 7.5 with diluted NaOH and loaded on top of an Extrelut-20 column (Merck). After adsorption for 20 min, elution was performed with 100 ml of dichloromethane. The eluate was evaporated to dryness with a rotary evaporator at a bath temperature of 30 °C and the residue was finally taken up in ethyl acetate (2 ml). An aliquot (20 μl) was injected into the HPLC-DAD.

Table 1
Mean percent recovery ± RSD of carbendazim, thiabendazole and methyl thiophanate in banana samples at 0.1, 1.0, 2.5 and 4.0 mg/kg fortification levels ($n = 4$)

Pesticide	Fortification level			
	0.1 mg/kg	1.0 mg/kg	2.5 mg/kg	4.0 mg/kg
Carbendazim	85.3 ± 4	87.0 ± 5	88.2 ± 4	87.2 ± 7
Thiabendazole	81.0 ± 8	96.0 ± 6	91.1 ± 6	89.1 ± 5
Methyl-thiophanate	65.0 ± 4	63.2 ± 3	69.8 ± 5	69.7 ± 3

2.4. HPLC analysis

Quantitative HPLC analyses were performed on an HP 1100 series HPLC, equipped with a photodiode array detector, from Agilent Technologies (Palo Alto, CA). The column used was a C18 μ-Bondapack (300 × 4.0 mm i.d.). Chromatographic separation was carried out using a methanol/water–0.6% NH_3 (50:50) mixture. The flow-rate was of 1.0 ml/min and the injection volume was 20 μl. Peaks were detected at 288 nm.

2.5. GC–MS analysis

A Finnigan GCQ (Trace GC 2000 coupled with a Polaris MSD) gas chromatography–mass spectrometer, with a split/splitless capillary injection port was used. Column was a 30 m × 0.25 mm i.d., 0.25 μm capillary SPB-608 (Supelco). The temperature programme applied was as follows: 110 °C for 2 min, 110–250 °C at 6 °C/min, and 250 °C for 15 min. The injection volume was 1 μl.

2.6. Recoveries experiments, detection limits and quantitative evaluation

Untreated banana samples (reference materials certified for their purity) were fortified, on average at 0.5, 1.0, 2.5 and 4.0 mg/kg, by adding standard solutions in ethyl acetate. Samples were allowed to equilibrate for 30 min prior to extraction, and were processed according to the above procedure. The recovery assays were replicated four times. The recovery values were not related to the spiking level. Data derived from these experiments are presented in Table 1. The detection limits (DLs) calculated by using a signal-to-noise (S/N) ratio of 3, were in the range 0.001–0.02 mg/kg. The quantity of each pesticide was calculated by external standard method.

3. Results and discussion

A study of the possible contamination of banana (*Musa acuminata*) samples imported in Italy from Ec-

Table 2
Carbendazim, thiabendazole and methyl-thiophanate in 50 samples of banana imported to Italy from Latin America during 2002/2003[a]

Pesticide	Mean value (mg/kg)	Concentration range (mg/kg)	Number of positive samples
Ecuador (32 samples)			
Carbendazim	0.350	0.350	1
Thiabendazole	0.798	0.090–2.500	9
Methyl-thiophanate		ND[b]	–
Panama (10 samples)			
Carbendazim	0.926	0.680–1.100	3
Thiabendazole	0.050	0.050–0.070	2
Methyl-thiophanate		ND[b]	–
Costa Rica (8 samples)			
Carbendazim	0.140	0.140	1
Thiabendazole		ND[b]	–
Methyl-thiophanate		ND[b]	–

[a] Determination made in duplicate. Number of samples without residues: 34. Number of samples with residues greater than the Codex MRL: 2.
[b] ND = not detectable.

uador, Panama and Costa Rica during years 2002–2003 was carried out. Fifty samples received from the Maritime Authority of Salerno Port (Italian Department of Health), were analysed. MBC, TBZ and TM were determined by HPLC-DAD and positive samples were confirmed by GC–MS. Benomyl is almost completely converted into MBC, both directly on fruits and during the analysis. On the other hand, TM is only partially degraded to MBC under the same conditions (Bicchi et al., 1989). As a consequence of this, in Italy, benomyl and TM tolerance limits must be expressed as MBC. Italian legislation (2000) establishes the maximum residue limits (MRLs) in this matrix of 1 mg/kg for MBC and of 5 mg/kg for TBZ.

The calculation of the amount of the pesticides present was carried out by comparing the peak areas for unknown samples with the corresponding peaks for standards, according to established procedures. Recovery tests were performed by fortifying banana samples certified for their purity and which were found negative when analysed for the same active substances. As Table 1 shows, the average TM recoveries were lower than 70% for all levels, in accordance with the results obtained by Bicchi et al. (1989) for apple and pear products. In fact, under the acidic conditions used in the extraction, 15% of TM was converted into MBC.

The levels of carbendazim, thiabendazole and thiophanate-methyl in banana samples are presented in Table 2. Thirty-four samples contained no detectable residues. Carbendazim was detected in 5 samples (10% of samples studied), at concentrations ranging from 0.140 to 1.100 mg/kg. Thiabendazole was detected in 11 samples (22% of samples studied), at concentrations ranging from 0.050 to 2.510 mg/kg. Thiophanate-methyl was not detected in any sample.

A further confirmatory analysis of positive samples was carried out by means of GC–MSD. MSD can be employed to achieve selective detection, by full scan or selective ion monitoring (SIM), of target pesticides in the presence of the complex matrix. The masses of the selected ions for carbendazim, thiabendazole and thiophanate-methyl were 191, 201 and 342, corresponding to the ions M^+, respectively. The selectivity was enhanced by the use of GC–MS–MS techniques.

The results of our monitoring indicate that, among 50 samples of imported bananas that were examined, only two samples contained carbendazim that exceeded the FAO/WHO Codex Alimentarius maximum residue limits (MRLs) (Codex Alimentarius Commission, 1996) and the maximum permissible levels recorded by the Italian Department of Health. Therefore, the results appear to be reassuring as to contamination of imported bananas, probably because of controlled pesticide treatments.

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