Food Chemistry 188 (2015) 71–76

Contents lists available at ScienceDirect

Food Chemistry

journal homepage: www.elsevier.com/locate/foodchem

Analytical Methods

Carbon disulfide formation in papaya under conditions of dithiocarbamate residue analysis

R.B. Abakerli [a], R. Sparrapan [a], A.C.H.F. Sawaya [a,*], M.N. Eberlin [a], J.L.P. Jara [a], N.R. Rodrigues [a], E.F. Fay [b], A.J.B. Luiz [b], T.D.L. Galvão [c], D. dos S. Martins [d], O.K. Yamanishi [e], H.H.B. Toledo [f]

[a] Institute of Chemistry – University of Campinas, IB-Unicamp, CPQBA-Unicamp, Campinas, SP, Brazil
[b] Embrapa Environment, Jaguariúna, SP, Brazil
[c] EBDA, Teixeira de Freitas, BA, Brazil
[d] INCAPER, Vitória, ES, Brazil
[e] FMAV-UnB, Brasília, DF, Brazil
[f] IAL, São Paulo, SP, Brazil

ARTICLE INFO

Article history:
Received 13 January 2013
Received in revised form 27 January 2015
Accepted 17 April 2015
Available online 22 April 2015

Keywords:
Dithiocarbamate residue analysis
Papaya
Carbon disulfide
Benzylisothiocyanate

ABSTRACT

Golden, *Sunrise Solo* and *Tainung* cultivars of papaya were found to release CS_2 when submitted to experimental conditions of dithiocarbamate residue analysis. Three common analytical methods were used to quantitate CS_2; one spectrophotometric method and two chromatographic methods. All three methods gave positive CS_2 results for all three papaya varieties. Other endogenous compounds present in isooctane extracts of papaya fractions detected via gas chromatography (GC/ITD) using electron ionization (EI) were: carbonyl sulfide, dimethyl sulfide, carbon disulfide, 2-methylthiophene, 3-methylthiophene, 2-ethylthiophene, 3-ethylthiophene, benzylisothiocyanate, benzylthiocyanate and benzonitrile. Control samples were obtained from papaya plantations cultivated in experimental areas, in which no treatment with fungicides of the dithiocarbamate group was applied. Endogenous CS_2 levels were compared with true dithiocarbamate residues measured in papaya samples from the field trials following applications of the mancozeb fungicide. Three days after application, true dithiocarbamate residues, measured by the procedure with isooctane partitioning and GC–ITD, were at the average level of 2 mg kg^{-1}.

1. Introduction

Papaya (*Carica papaya* L.) is an important food crop. According to FAOSTAT (2010), papaya production represents nearly 10% of world production of tropical fruits. The main producer is Brazil (supplying 25% of the world demand) followed by Mexico, Nigeria, Indonesia and India. The European Community and the United States are the main importers of Brazilian papaya (Oliveira & Vitória, 2011). Although the amount of exported papaya grew 620% between 1996 and 2008, Brazil still exports less than 2% of its production (FAOSTAT, 2010). With an eye on the international market, papaya producers in Brazil have invested into improve fruit quality and meet the requirements of importing countries, such as limited amounts of fungicide residues.

The papaya tree is very sensitive to environmental and climatic variations, mainly when is still young, and demands a sanitary control in all the phenological phases (Tatagiba, Liberato, Zambolim, Ventura, & Costa, 2002; Trindade, 2000). The main post harvest disease of papaya fruits, anthracnose (*Colletotrichum gloeosporioides*), is responsible for up to 40% of fruit loss. Although this fungus infects unripe fruit, the disease only appears when fruit are ripe, mainly during transport and storage. Another very common fungal disease is caused by *Asperisporium caricae*, characterized by the appearance of black spots on the fruit surface and resulting in commodity devaluation (Barreto, Savan, de Lima, & Loso, 2011). Other fungal diseases cause the stem and peduncle to rot as well as the fruit during the storage and ripening (Pereira, Martins, Michereff, da Silva, & Câmara, 2012). Packinghouses also wash fruits and apply a hydrothermal treatment (48 ± 1 °C, for 20 min, followed by cooling at 16 °C for 20 min) to prevent the severity of post-harvest diseases.

Prevention and control of fungal diseases is therefore necessary for papaya production and fungicides are routinely applied every fifteen days at doses of 2 g/L (Ahuja & Mohapatra, 2010) with a half-life of approximately 4 days. One of the most important fungicides is a polymeric complex of zinc and manganese ethylenebis dithiocarbamate (EBDC) known as mancozeb. As EBDC compounds are insoluble in organic solvents or water, the main analytical

* Corresponding author.

http://dx.doi.org/10.1016/j.foodchem.2015.04.059
0308-8146/

methods used to measure its residues in papaya are indirect, since they simply measure the amount of carbon disulfide (CS_2) formed during acid hydrolysis (Cicotti, 2003). All the analytical methods for EBDC residue determination are variations of the method developed by Keppel (1969, 1971), in which CS_2 is absorbed in a solution containing amine to form a complex which is then spectrophotometrically measured. The CS_2 in the headspace of the hydrolysis bottle can also be measured by gas chromatography (GC) (McLeod & McCully, 1969). A variation of this method involves the dissolution of the CS_2 in an organic solvent layer (isooctane) with subsequent GC analysis (Harrington, Horner, Hird, Griffiths, & Reynolds, 1998).

Although recently LC–MS methods have been developed which identify diverse dithiocarbamate fungicides, it is known that the spontaneously decompose into CS_2 when in contact with acidic plant juice. Therefore the quantification of CS_2 is still the routine method used and Maximum Residue Limits (MRL) are specified in mg CS_2 per kg of food (Crnogorac & Schwack, 2009).

But this indirect residue analysis may suffer from the interference of endogenous CS_2 which is measured along with the CS_2 from fungicides. This interference is well known for the *Brassicaceae* family in which the CS_2 background levels may reach the established Maximum Residue Limits (Perz, van Lishaut, & Schwack, 2000). The endogenous formation of CS_2 and of other volatile sulfur compounds such as carbonyl sulfide is due to the degradation of natural isothiocyanates which, in turn, derive from an enzymatic degradation of glucosinolates, present in dicotyledonous plants. Glucosinolates are ionic organic compounds with a thioglucose linked to the carbon of a sulfonated oxime containing a variable side group (R). Glucosinolates occur in 16 botanic families including the *Caricaceae* (Rodman, 1991; Rodman, Soltis, Soltis, Sytsma, & Karol, 1998). In the *Caricaceae* family, benzylglucosinolate and benzylisothiocyanate are found in all parts of the plant (Tang, 1971, 1973; Tang, Syed, & Hamilton, 1972).

Generally glucosinolate levels are higher in the seeds and ten times lower in the roots, leaves and stems but their concentrations vary with tissue type, plant health, nutrition and age. When plant tissues are damaged, isothiocyanates are released by the hydrolytic action of an enzyme (myrosinase). The chain of reactions involves initial cleavage of the thioglucoside linkages yielding D-glucose and the unstable thiohydroxymate-*O*-sulfonate that spontaneously loses sulfate and rearranges to isothiocyanate, thiocyanate or nitrile. Isothiocyanates may suffer further degradation forming CS_2 among other products (Pecháček, Velíšek, & Hrabcová, 1997).

The determination of EBDC residues by simply quantifying CS_2 is therefore susceptible to false positive results or overestimates in species with endogenous CS_2. The purpose of this work was to investigate the background level of CS_2 in *C. papaya* L. using three commercially important cultivars: *Golden*, *Sunrise Solo*, and *Tainung*, which were grown without contact with sulfur agrochemicals, either in the form of pesticides or fertilizers. Methods for the analysis of CS_2 were also compared. The levels of CS_2 in fruit which were treated with EBDC were also evaluated after periods ranging from zero to 14 days, as the clearance period is considered to be 3 days (ANVISA, 2010).

2. Material and methods

2.1. Control papaya samples

Field experiments intended to produce Control papaya fruits of *Sunrise Solo*, *Golden* and *Tainung* cultivars were conducted in areas of papaya production, without contact with sulfur agrochemicals. The experimental areas were virgin fields of papaya production or experimental fields at Research Institutions, located in Cruz das Almas (CA), Teixeira de Freitas (TF) and Barreiras (B) located in the State of Bahia; and in Linhares (L) in the State of Espírito Santo. The seedlings of *Sunrise Solo* (generation F2) and *Golden* cultivars were obtained from Embrapa Mandioca and Fruticultura Research Center, from papaya seeds that never received any chemical treatment. Seedlings production was under 75% lightening. After 50 days of sowing, they were transplanted to the experimental fields at CA and TF. In CA papaya plants were also cultivated in a greenhouse.

The seeds of *Tainung* cultivar were obtained from Taiwan; seedlings were produced in small plastic bags with soil collected at the farm and mixed with chemical and organic fertilizers. The papaya fruits were cultivated under normal conditions of papaya production.

The samples from Linhares (L) were divided in two batches and one of them was submitted to the hydrothermal treatment at 48 ± 1 °C, for 20 min., followed by cooling at 16 °C for 20 min., since this is a common practice to prevent the severity of post harvest diseases. Therefore this treatment was carried out to verify if it also affects the CS concentrations.

All fruit samples were taken according to the commercial maturation stages (1 and 2) or unripe (0).

2.2. Field application of EBDC

In all the experimental papaya fields, some plots were selected for EBDC applications. Manzate® was applied at 15 day intervals using the normal field dose (200 g/100 L) and some plots were kept as control. At TF four applications were made and in all the other field trials six applications were made. Fruit samples were taken from plants in the central area of the plot, discarding the border plants, considering the commercial maturation stages (1 and 2) or unripe (0); collected at 0, 3, 7 and 14 days after the last application of the fungicide. The L samples were divided in two batches and one of them was submitted to the hydrothermal treatment at 48 ± 1 °C, for 20 min, followed by cooling at 16 °C for 20 min.

2.3. Analysis of papaya samples

Papaya samples were analyzed by three different laboratories, each one using their established method for EBDC residue analysis. The isooctane partition method was applied to samples from all field trials. Headspace method for CS_2 determination was performed on samples from L and TF field trials. The spectrophotometric method for CS_2 determination was applied to samples from L and B; since this method requires a larger amount of sample, not all papaya fractions could be analyzed. At least three determinations were made for whole and divided fruit samples.

2.4. Carbon disulfide analysis

2.4.1. Sample processing

Fruit samples were maintained at a temperature <10 °C in order to delay ripening throughout sample processing. Half of the fruit samples were divided into peel, pulp and seeds; peel and pulp were homogenized in presence of dry ice, using a food processor. The remaining fruit samples were divided in half lengthwise; one half of the fruit was homogenized as described, the other half was just chopped into small pieces. The processed material was kept frozen at temperatures between −19 and −22 °C until analysis.

2.4.2. Isooctane partition method for CS_2 determination

Determination was carried out according to Harrington et al. (1998). 50 g portions of each papaya fraction plus 25 mL isooctane and 150 mL of 1.5% stannous chloride solution in 12% hydrochloric acid were placed in hydrolysis flasks and incubated for two hours

at 80 °C. CS_2 was quantitated injecting 10 μL of the isooctane extract in a HP-6890 gas chromatograph (Hewellet-Packard) with a flame photometric detector operating in sulfur mode and a HP-Plot Q column (30 m × 0.53 mm × 40 μm); carrier N_2 gas flow 7.0 mL min^{-1}, injector temperature 150 °C, detector temperature 200 °C, oven programming 80 °C for 3 min., then heating at a rate of 10 °C min^{-1} until 250 °C. CS_2 in samples was quantitated by comparison to a calibration curve of CS_2 in isooctane ranging between 0.03 and 0.8 μg mL^{-1}. The limit of detection (LOD) was 0.02 mg kg^{-1} of fruit.

2.4.3. CS_2 confirmation and compounds identification by GC–MS

An aliquot of 10 μL of the isooctane extracts of seeds and peel were analyzed in a CP 3900 Varian gas chromatograph with a Saturn 2000 mass spectrometer, using a RTX-1 column (30 m × 0.32 mm × 4 μm) with carrier gas (N_2) flow 2.6 mL min^{-1}, injector temperature 150 °C; splitless mode, oven programming 50 °C for 7 min; then heating at a rate of 5 °C min^{-1} to 150 °C. Compounds were identified by comparison to standards.

2.4.4. Headspace method for CS_2 determination

Determination was carried out according to McLeod and McCully (1969) with modifications. 4 g portions of each papaya fraction were added to 15 mL of 3.0% stannous chloride solution in 8 M hydrochloric acid and 6 mL of EDTA 10% solution. Hydrolysis flasks were sealed and incubated for two hours at 90 °C. Volumes of 100–1000 μL of the *headspace* of samples were injected. Carbon disulfide was quantified in a Varian 3700 gas chromatographer with flame photometric detector operating in sulfur mode, using a 2 m/3 mm, PT 28% Alltech 223 + 4% KOH on Chromosorb R 80/100 mesh column. Carrier N_2 gas flow 40.0 mL min^{-1}; injector temperature 210 °C; detector temperature 210 °C; oven temperature 115 °C. Quantifications were made by comparison to a calibration curve of CS_2 in isooctane between 0.75 and 30 ng mL^{-1}. The limit of detection (LOD) was 0.02 mg kg^{-1} of fruit.

2.4.5. Spectrophotometric method for CS_2 determination

The S 15 method (Germany, 1998) based on the classical method of Keppel (1969), was used. Portions (300 g) of each papaya fraction were used. CS_2 was trapped in a solution of diethanolamine and absorbance of the complex formed was measured at 435 nm using a B-382 Micronal spectrophotometer. The LOD is accepted to be 0.1 mg/kg for samples of 500 g with mean recoveries of 74–103% (Germany, 1987).

3. Results

3.1. Endogenous CS_2 in papaya grown without use of pesticides or fertilizers containing sulfur

The partition method in isooctane was applied to 65 samples of three varieties of papaya obtained at the four sites of field experimentation. The headspace analysis was performed on 51 samples of the varieties *Golden* and *Sunrise Solo*. The spectrophotometric method was applied to 16 samples of varieties *Golden* and *Tainung*. Table 1 summarizes the results of the average concentrations of CS_2 in the varieties *Golden*, *Sunrise Solo* and *Tainung*. As Table 1 shows, low levels of CS_2 were observed in all cultivars independently of the analytical method used. Results are expressed as mean concentrations of CS_2 with standard deviations for samples of *Golden*, *Sunrise Solo* and *Tainung* cultivars. To estimate the average values of CS_2 concentration, half the limit of detection (LOD) was attributed to samples with CS_2 concentration below the LOD (U.S. Environmental Protection Agency, 2000). For each method of analysis used, the probability of observing CS_2 concentrations higher than 0.05 mg kg^{-1} (twice the LOD) were calculated. The empirical cumulative probability distribution from the observed data was calculated. In this case, it can be stated that the probability of finding a sample of papaya with CS_2 concentration equal to or greater than 0.05 mg kg^{-1} is 55% by the method of headspace, 94% for the spectrophotometric method and 12% for the isooctane method. From the 16 samples analyzed by the spectrophotometric method only one result was <0.05 mg kg^{-1} which represents only 6% of the analyzed samples.

Using the isooctane partition method (Table 2), peel and seeds presented the highest endogenous CS_2 concentrations. CS_2 was not detected in most of the pulp samples. The highest endogenous CS_2 concentrations were in papaya samples from B field trial, which might be related to cultivation under the commercial system of papaya production. Field samples of the *Golden* cultivar from CA also showed high concentrations of carbon disulfide, which might be related to the occurrence of *Phytophthora* in the plantation. It is known that a self-protection process occurs, liberating natural isothiocyanates, when the health of the plant is affected (Nascimento, Frighetto, Malavasi, & Habibe, 2003). For the samples from the other locations, the CS_2 concentrations were in the same order of magnitude, independently of the cultivar evaluated.

The results of the headspace method presented endogenous CS_2 concentrations in the same range as the isooctane method (Table 3); but both L and TF samples presented higher results by the headspace method. Again, the peel and seeds presented higher concentrations than the pulp or whole fruit.

The highest concentrations of endogenous CS_2 were found using the spectrophotometric method (Table 4). For example, the whole chopped normal sample from L presented a CS_2 concentration of 0.93 ± 0.18 mg kg^{-1} using the spectrophotometric method compared to 0.20 ± 0.17 mg kg^{-1} using the headspace method and 0.03 ± 0.02 mg kg^{-1} using the isooctane partition method. Clearly, the results of all three methods cannot be compared directly, and differences in CS_2 concentrations are accounted by the particular experimental conditions applied in each method. According to Schwack and Nyanzi (1993), the use of diethanolamine in the spectrophotometric analytical procedure (such as in the method used herein) provides no discrimination between CS_2 and COS. This failure might be the reason for the much higher CS_2 levels observed using the spectrophotometric method compared to the chromatographic methods. Perz et al., 2000 also suggest that H_2S formed in the digestion/distillation apparatus may meet volatile isothiocyanates, increasing artificially the final CS_2 concentration. Other parameters, such as the hydrolysis temperature, or ratio of sample to acid, could explain the higher CS_2 concentrations observed using the headspace method (90 °C) than the isooctane method (80 °C).

3.2. Identification of sulfur compounds by GC–MS

During the chromatographic analysis of the isooctane extracts, several peaks were observed, which could be attributed to sulfur

Table 1
Average concentration of endogenous CS_2 (mg kg^{-1}) in whole chopped papaya fruit measured by three methods.

Cultivar	Field trial	Isooctane	Headspace	Spectro photometric
Golden	B	0.03 ± 0.03	–	0.50 ± 0.66
	L	0.03 ± 0.02	0.21 ± 0.25	0.79 ± 0.31
	CA	0.21 ± 0.15	–	–
	TF	0.02 ± 0.02	0.06 ± 0.03	–
Tainung	B	0.01 ± 0.02	–	0.24 ± 0.25
Sunrise Solo	CA	0.02 ± 0.01	–	–
	TF	0.02 ± 0.01	0.03 ± 0.02	–

n = 3–18 determinations.

Table 2
Average concentration of CS_2 (mg kg^{-1}) in papaya fractions using the isooctane partition method; n = 3–6 determinations.

Cultivar	Field trial and treatment	Peel	Pulp	Seeds	Whole fruit	
					Homogenized	Chopped
Golden	B	0.28 ± 0.15	0.10 ± 0.07	0.19 ± 0.18	0.07 ± 0.06	0.03 ± 0.03
	L-normal	0.07 ± 0.06	0.02 ± 0.01	0.05 ± 0.02	0.06 ± 0.03	0.03 ± 0.02
	L-hydrothermal treatment	0.03 ± 0.01	0.01 ± 0.00	0.03 ± 0.01	0.05 ± 0.04	0.03 ± 0.02
	TF ripeness (0)	0.03 ± 0.01	0.01 ± 0.01	0.04 ± 0.01	0.03 ± 0.01	0.02 ± 0.01
	TF ripeness (1)	0.04 ± 0.02	0.01 ± 0.01	0.05 ± 0.02	0.03 ± 0.01	0.02 ± 0.01
	TF ripeness (2)	0.10 ± 0.06	0.02 ± 0.01	0.06 ± 0.04	0.03 ± 0.01	0.01 ± 0.01
	CA greenhouse	–	–	–	0.24 ± 0.16	–
	CA field	0.29[a]	0.03[a]	1.19[a]	0.31 ± 0.04	0.21 ± 0.15
Tainung	B	0.10 ± 0.04	0.07 ± 0.04	0.15 ± 0.07	0.03 ± 0.01	0.02 ± 0.01
Sunrise Solo	TF ripeness (0)	0.04 ± 0.01	0.02 ± 0.01	0.05 ± 0.01	0.03 ± 0.01	0.02 ± 0.02
	TF ripeness (1)	0.06 ± 0.07	0.02 ± 0.02	0.04 ± 0.01	0.04 ± 0.01	0.02 ± 0.01
	TF ripeness (2)	0.05 ± 0.03	0.01 ± 0.01	0.05 ± 0.02	0.02 ± 0.01	0.01 ± 0.01
	CA greenhouse	0.10[a]	0.05[a]	0.30[a]	0.08 + 0.01	–
	CA field	0.02[a]	0.02[a]	1.31[a]	0.02 + 0.01	0.02 + 0.01

[a] Only one determination, samples analyzed after one year storage.

Table 3
Average concentration of CS_2 (mg kg^{-1}) in papaya fractions using the headspace method; n = 4–6 determinations.

Cultivar	Field trial and treatment	Peel	Pulp	Seeds	Whole fruit	
					Homogenized	Chopped
Golden	L-normal	0.35 ± 0.20	0.10 ± 0.05	0.52 ± 0.23	0.21 ± 0.13	0.20 ± 0.17
	L-hydrothermal treatment	0.25 ± 0.19	0.05 ± 0.03	0.53 ± 0.36	0.10 ± 0.05	0.22 ± 0.32
	TF ripeness (0)	0.06 ± 0.01	0.02 ± 0.03	0.13 ± 0.03	0.06 ± 0.02	0.04 + 0.01
	TF ripeness (1)	0.06 ± 0.03	0.03 ± 0.03	0.14 ± 0.02	0.08 ± 0.06	0.07 + 0.06
	TF ripeness (2)	0.11 ± 0.01	0.06 ± 0.03	0.33 ± 0.20	0.10 ± 0.01	0.07 + 0.01
Sunrise Solo	TF ripeness (0)	0.06 ± 0.01	0.04 ± 0.02	0.11 ± 0.02	0.10 ± 0.02	0.03 ± 0.03
	TF ripeness (1)	0.05 ± 0.03	0.01 ± 0.01	0.09 ± 0.04	0.04 ± 0.01	0.02 ± 0.02
	TF ripeness (2)	0.03 ± 0.02	0.01 ± 0.02	0.16 ± 0.10	0.05 ± 0.02	0.03 ± 0.02

Table 4
Average concentration of CS_2 (mg kg^{-1}) in papaya fractions using the spectrophotometric method; n = 4 determinations.

Cultivar	Field trial location	Peel	Pulp	Seeds	Whole fruit chopped
Golden	B	0.55 ± 0.34	0.15 ± 0.04	0.27 ± 0.14	0.50 ± 0.66
	L-normal	0.86 ± 0.93	0.33 ± 0.19	–	0.93 ± 0.18
	L-hydrothermal treatment	0.27 ± 0.31	0.24 ± 0.29	–	0.70 ± 0.35
Tainung	B	0.34 ± 0.18	0.15 ± 0.07	0.11 ± 0.03	0.24 ± 0.25

compounds. Besides the CS_2 peak, occurring at a retention time of 8.78 min, other peaks attributed to characteristic compounds were observed for each fraction. Fig. 1(A–E) shows example chromatograms of isooctane extracts of peel, seeds, pulp and whole fruits obtained with the papaya cultivars. In the pulp extract (C), most of these compounds were not observed, so further studies focused on the extracts of peel and seeds.

The sulfur compounds present in the papaya isooctane extracts were identified by GC–MS, with a specific column for sulfur compounds (RTX-1) Fig. 1(F–I). In this column, the retention time of CS_2 was 3.6 min and an inversion of the elution order of CS_2 and dimethyl sulfide order was observed in comparison with the previous elution order. Duplicates of each sample were analyzed and the compounds identified were: carbonyl sulfide, dimethyl sulfide, carbon disulfide, 2-methylthiophene, 3-methylthiophene, 2-ethylthiophene, 3-ethylthiophene, benzonitrile, benzylisothiocyanate and benzylthiocyanate. Although benzonitrile was found in all the samples, it is not a sulfurous compound. The drop in the baseline observed between 12 and 15 min in the chromatograms in Fig. 1 is caused by the interference of the elution of the solvent (isooctane) on the detector.

The observation of carbonyl sulfide in the papaya isooctane extracts corroborates previous work on degradation of isothiocyanates under neutral or acid hydrolysis (Pechácek et al., 1997). Similarly, the observation of benzylisothiocyanate and benzonitrile in the extracts corroborates with the fact of these compounds originate from benzylglucozinolate present in papaya.

To confirm the presence of these identified compounds in the other samples analyzed by GC/FPD, standards of Dimethyl sulfide, 2-Methyl Thiophene, 3-Methyl Thiophene and Benzyl Isothiocyanate were injected to confirm their retention time (Fig. 1F–I). Benzyl Isothiocyanate has a retention time around 37 min under the GC/FPD conditions, and it was not seen in the chromatograms of papaya extracts.

3.3. True EBDC residues

To compare concentrations of endogenous CS_2 with those resulting from the use of dithiocarbamate, field applications of the Manzate® fungicide in the papaya plantations were made and samples were collected and analyzed to check for dithiocarbamate residues with the isooctane partition method.

Table 5 presents the average residues of mancozeb in homogenized and chopped fruits after 0, 3 (normal clearance interval), 7 and 14 days after last treatment, expressed as CS_2 concentration. No statistical differences between the results for chopped and

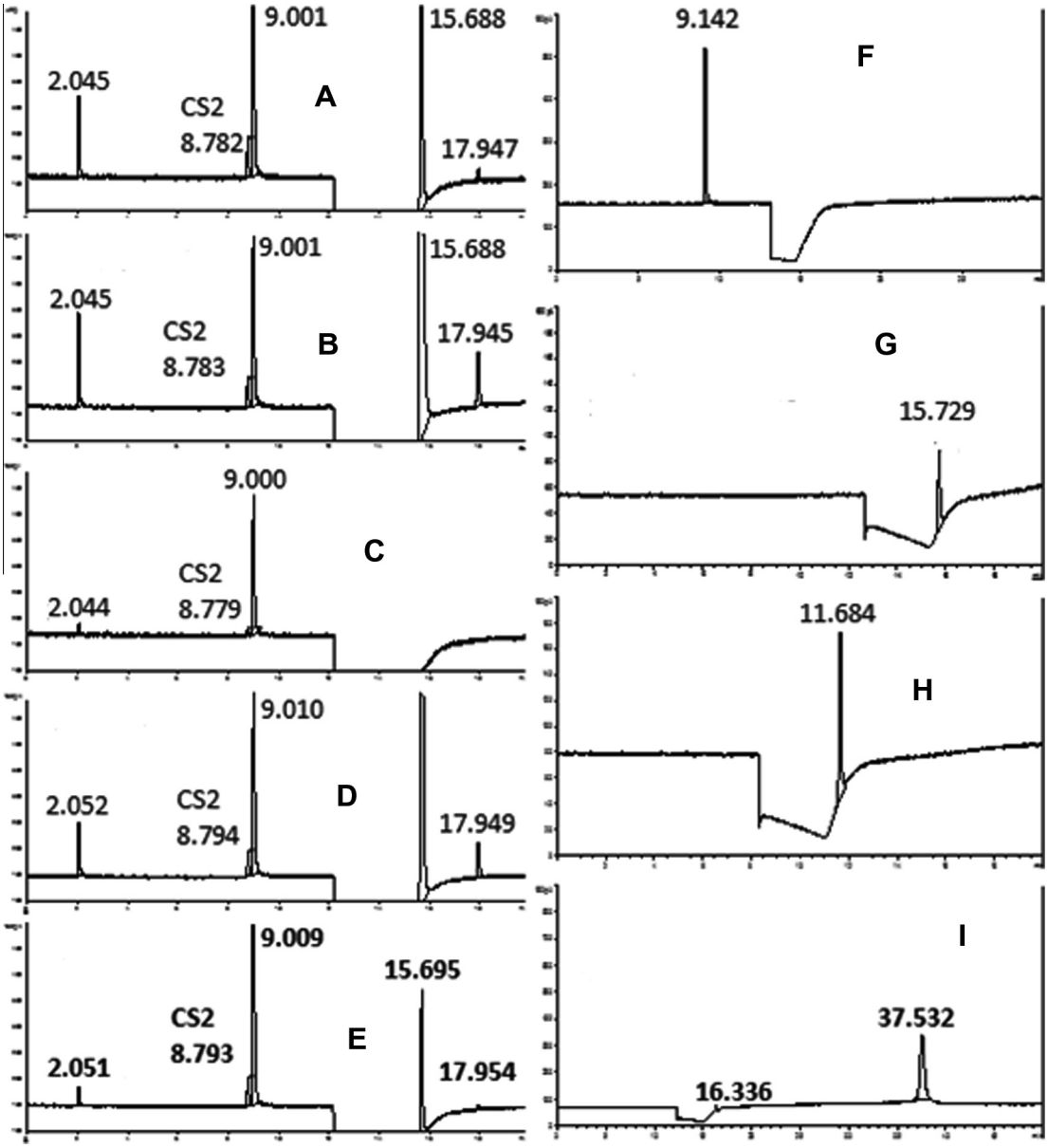

Fig. 1. Flame photometric detector chromatograms of isooctane extracts of peel (A), seeds (B), pulp (C), homogenized whole fruits (D), chopped whole fruit (E) of the Golden cultivar from the field trial at TF. CS_2 peak at 8.78 min. Flame photometric detector chromatograms of standards of: Dimethylsulfide (F), 2-Methyl Thiophene (G), 3-Methyl Thiophene (H) and Benzyl Isothiocyanate (I).

Table 5
Average dithiocarbamate residues in papaya expressed as CS_2 (mg kg^{-1}) after Manzate® applications; n = 3–6 determinations.

Cultivar	Field trial and treatment	DAY 0		DAY 3 (clearance security)		DAY 7		DAY 14	
		Homog.	Chopped	Homog.	Chopped	Homog.	Chopped	Homog.	Chopped
Golden	B	2.3 ± 0.8	1.7 ± 0.5	2.2 ± 1.3	1.4 ± 0.6	1.9 ± 0.7	1.0 ± 0.4	1.4 ± 0.4	1.1 ± 0.4
	L-normal	4.7 ± 1.1	3.7 ± 1.3	2.0 ± 0.9	1.7 ± 1.2	3.8 ± 1.3	3.4 ± 3.4	2.0 ± 0.4	4.4 ± 5.5
	L-hydrothermal treatment	2.7 ± 0.8	1.8 ± 0.5	1.3 ± 0.3	1.0 ± 0.6	0.8 ± 0.3	0.6 ± 0.1	0.5 ± 0.2	0.5 ± 0.1
	TF	3.5 ± 0.9	4.6 ± 1.3	3.0 ± 0.5	3.6 ± 1.5	2.4 ± 0.8	2.8 ± 1.4	1.7 ± 0.8	2.0 ± 1.1
Tainung	B	1.0 ± 0.6	0.9 ± 0.7	1.8 ± 0.1	0.9 ± 0.4	1.5 ± 0.3	1.6 ± 1.5	1.0 ± 0.2	2.3 ± 3.3
Sunrise Solo	TF	5.1 ± 2.0	4.9 ± 1.0	2.8 ± 1.1	3.5 ± 1.1	2.8 ± 0.6	2.9 ± 0.6	1.6 ± 0.6	1.3 ± 0.5

homogenized samples were noted, although the variability of the residues is much higher for the chopped samples than for the homogenized samples. This variability might be due to the heterogeneous deposition of the fungicide on the fruit surface and the way that the samples are taken for the analysis. Three days after the last treatment, the CS_2 concentration varied in the

chopped samples ($n = 36$) from 0.39 to 6.10 mg kg^{-1} and in the homogenized samples from 0.56 to 4.39 mg kg^{-1}.

Other studies (Caldas, Miranda, Cinceição, & de Souza, 2004) found similar CS_2 concentrations in papaya consumed in Brazil, average residues of 0.52 mg/kg of fruit. Even after 14 days, all the true residue values were well above those for endogenous CS_2 (0.07 mg kg^{-1}) in papaya samples obtained with the isooctane partition method. Ahuja and Mohapatra (2010) measured CS_2 concentration of 8.1 mg/kg of fruit directly after application but this was reduced to 1.87 mg/kg of fruit after 1 week. These true mancozeb residues present CS_2 concentrations well above the endogenous levels.

4. Conclusions

C. papaya L. generates CS_2 from endogenous compounds which might be mistaken, when applying analytical methods, as being originated from exogenous dithiocarbamate residues. When papaya fruit are treated with EBDCs, however, the residue level, even after 14 days, is much higher than that of endogenous compounds. Three days after application, true dithiocarbamate residues, measured by the procedure with isooctane partitioning and GC/FPD, were at the average level of 2 mg kg^{-1}. Other methods, such as the headspace method and the spectrophotometric method tested herein, result in higher endogenous CS_2 concentration, due to method particularities.

References

Ahuja, A. K., & Mohapatra, S. (2010). Persistence of mancozeb residues in papaya (*Carica papaya*). *Indian Journal of Agricultural Sciences, 80*, 532–533.

Barreto, L. F., Savan, P. A. L., de Lima, L. L., & Loso, B. N. (2011). Evaluation of fungicides to control *Asperisporuium caricae* in Papaya crop. *Revista Brasileira de Fruticultura, E*, 399–403.

BRASIL. Agência Nacional de Vigilância Sanitária. Resolução RE n. 2250, de 18 de maio de 2010.

Caldas, E. D., Miranda, M. C. C., Cinceição, M. H., & de Souza, L. C. K. R. (2004). Dithiocarbamates residues in Brazilian food and potential risk for consumers. *Food and Chemical Toxicology, 42*, 1877–1883.

Cicotti, M. (2003). Alkylenebis(dithiocarbamates). In P. W. Lee (Ed.), *Handbook of residue analytical methods for agrochemicals* (pp. 1089–1098). Chichester: John Wiley.

Crnogorac, G., & Schwack, W. (2009). Residue analysis of dithiocarbamate fungicides. *Trends in Analytical Chemistry, 28*, 40–50.

FAOSTAT (2010). *Papayas: U.S. import-eligible countries; world production and exports.* URL <http://faostat.fao.or/default.aspx>. Data Base results from FAO website. Food and Agriculture Organization of the United Nations.

Germany, *Manual of pesticide residue analysis*, vol. 1, 1987. In: Their, H. & Zeumer, H. Working Group 8 – dithiocarbamate and thiram, disulfide fungicide S15. WCH, Weinheim, pp. 353.

Germany, *Non-fatty foods – Determination of dithiocarbamate and thiuram disulfide residues*. Part 1, S method. Beuth. Berlin, 1998.

Harrington, P., Horner, J., Hird, S., Griffiths, T., & Reynolds, S.L. (1998). *Modification of the method for measurement of dithiocarbamate residues as carbon disulfide in fruit and vegetables*, Final Report FD 98/46, 34 pages, CSL, York.

Keppel, G. E. (1969). Modification of the carbon disulfide evolution method for dithiocarbamate residues. *Journal of the Association of Official Analytical Chemistry, 52*, 162–167.

Keppel, G. E. (1971). Collaborative study of the determination of dithiocarbamate residues by a modified carbon disulfide evolution method. *Journal of the Association of Official Analytical Chemistry, 54*, 528–532.

McLeod, H. A., & McCully, K. A. (1969). Headspace gas procedure for screening food samples for dithiocarbamate pesticides residues. *Journal of the Association of Official Analytical Chemistry, 52*, 1226–1230.

Nascimento, A. S., Frighetto, R. T. S., Malavasi, A. & Habibe, T. C. (2003) Avaliação dos teores de benzil isotiocianato (BITC) em mamoeiros sadios e infectados pela meleira em condições de campo e telado. In: *Papaya Brasil: Qualidade do mamão para o mercado interno*. Martins, D dos S (Ed.). Vitória, ES: Incaper.

Oliveira, J. G., & Vitória, A. P. (2011). Papaya: Nutritional and pharmacological characterization, and quality loss due to physiological disorders. An overview. *Food Research International, 44*, 1306–1313.

Pecháček, R., Velíšek, J., & Hrabcová, H. (1997). Decomposition products of allyl isothiocyanate in aqueous solutions. *Journal of Agricultural and Food Chemistry, 45*, 4584–4588.

Pereira, A. V. S., Martins, R. B., Michereff, S., da Silva, M. B., & Câmara, M. P. S. (2012). Sensitivity of *Lasiodiplodia theobromae* from Brazilian papaya orchards to MBC and DMI fungicides. *European Journal of Plant Pathology, 132*, 489–498.

Perz, R. C., van Lishaut, H., & Schwack, W. (2000). CS_2 blinds in Brassica crops: False positive results in the dithiocarbamate residue analysis by the acid digestion method. *Journal of Agricultural and Food Chemistry, 48*, 792–796.

Rodman, J. E. (1991). A taxonomic analysis of glucosinolate-producing plants. Part 1: Phenetics. *Systematic Botany, 16*, 598–618. Kent.

Rodman, J. E., Soltis, P. S., Soltis, D. E., Sytsma, K. J., & Karol, K. G. (1998). Parallel evolution of glucosinolate biosynthesis inferred from congruent nuclear and plasmid gene phylogenies. *American Journal of Botany, 85*, 997–1006.

Schwack, W., & Nyanzi, S. (1993). Simultaneous UV–spectrophotometric determination of CS_2 and COS using the piperidine, pyrrolidine, ethylenediamine or morpholine reagent. *Fresenius Journal of Analytical Chemistry, 345*, 705–711.

Tang, C. S. (1971). Benzylisothiocyanate of papaya fruit. *Phytochemistry, 10*, 117–121.

Tang, C. S. (1973). Localization of benzylglucosinolate and thioglucosidase in Carica papaya fruit. *Phytochemistry, 12*, 769–773.

Tang, C. S., Syed, M. M., & Hamilton, R. A. (1972). Benzyl isothiocyanate in the *Caricaceae*. *Phytochemistry, 11*, 2531–2533.

Tatagiba, J. S., Liberato, J. R., Zambolim, L., Ventura, J. A., & Costa, H. (2002). Controle e condições climáticas favoráveis à antracnose do mamoeiro. *Fitopatologia Brasileira, 27*, 186–192.

Trindade, A.V. (2000) Org. Mamão Produção: Aspectos Técnicos Embrapa Mandioca e Fruticultura, Cruz das Almas BA Serie Frutas do Brazil, 3.

U.S. Environmental Protection Agency (2000). *Assigning values to nondetected/non-quantified pesticide residues in human health food exposure assessments*, Office of Pesticide Programs, Washington, DC.