Improved Sample Pretreatment of the Carbon Disulfide Evolution Method for the Determination of Dithiocarbamate Residues in Lettuce

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In this study the sample pretreatment of the carbon disulfide evolution method for the determination of dithiocarbamate residues in lettuce, described by Keppel, was examined and improved. The following observations were made. The distribution of dithiocarbamates in lettuce heads is uneven. By deheading the lettuce and mixing the leaves, the sample taken for analysis is insufficiently homogeneous. The relative standard deviation varies from 10 to 34%. Thiram (a widely used dithiocarbamate) decomposes rapidly in chopped lettuce (50% within half an hour). When hydrochloric acid (2.4 M) containing stannous chloride is added immediately after chopping, decomposition does not occur. The existing method (deheading and mixing of lettuce leaves) is compared with the modified method (chopping and acid addition) for samples from the field treated with thiram as well as zineb. The mean results of both methods correspond well, with a much higher reproducibility for the modified method. The relative standard deviation of the modified method varies from 1 to 4%.

INTRODUCTION

The dithiocarbamates form an important class of pesticides for broad-spectrum control of a variety of fungal diseases on growing crops. Structures of the two important dithiocarbamates used in Dutch lettuce culture are depicted in Figure 1. In fact, thiram is not a dithiocarbamate, but in pesticide chemistry it is always mentioned together with dithiocarbamates. The analysis of the intact and individual dithiocarbamates is complex. Most dithiocarbamates decompose rapidly under the influence of plant juice (Ministry of Welfare, Health and Cultural Affairs, 1988). Moreover, the polymeric dithiocarbamates do not dissolve in any solvent. Finally, dithiocarbamates form strong complexes with a variety of metal ions (Irth et al., 1986).

Various methods have been developed for the analysis of the dithiocarbamates, such as spectrophotometry (Keppel, 1969, 1971), gas chromatography (Spiegelenberg et al., 1979; Newsome, 1974), and liquid chromatography (Gustafsson and Thompson, 1981; Smith et al., 1980). Because of the chemical properties mentioned, most of these methods are based on degradation of the dithiocarbamates prior to detection. The most commonly used method is still that of Keppel (1969). In this method the dithiocarbamates are hydrolyzed with hydrochloric acid and stannous chloride to carbon disulfide, which is subsequently determined spectrophotometrically as cupric complexes of *N*,*N*-bis(2-hydroxyethyl)dithiocarbamic acid. However, the Keppel method is not very reproducible (Keppel, 1971; Thier, 1977). This was also experienced when our laboratory was engaged in the analysis of dithiocarbamates in lettuce.

The purpose of this study was to modify the Keppel method to improve the reproducibility. It was assumed that sample pretreatment could be the critical step, because samples are not chopped to avoid decomposition of dithiocarbamates. In this study, the distribution of dithiocarbamates in lettuce heads and in mixed lettuce leaves was examined first. Next, the rate of decomposition of dithiocarbamates in chopped lettuce under different conditions was determined. Finally, the Keppel method and a modified method were compared.

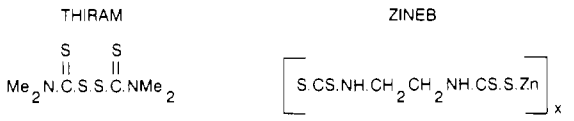

Figure 1. Structures of fungicidal dithiocarbamates.

EXPERIMENTAL PROCEDURES

The existing method used in our laboratory is in accordance with the method described by Keppel (1969). For a better understanding of this study, the method will be described briefly.

Reagents. *Stannous Chloride in Hydrochloric Acid.* Dissolve 31.3 g of $SnCl_2 \cdot 2H_2O$ (BDH, 97%) in 0.5 L of HCl (Merck, 37–38%) and dilute with water to 2.5 L.

NaOH (Merck, 97%) was used as a solution in water (6.5% w/v).

Color Reagent. Dissolve 36 mg of copper(II) acetate·H_2O (BDH, 98%) in 100 mL of diethanolamine (Baker, p.a.) and dilute to 1 L with ethanol (96%).

Standard Solution of Carbon Disulfide (Merck, p.a., 99%). Dilute 1 mL of a stock standard solution (containing 7.5 mg of carbon disulfide/mL of ethanol) to 100 mL with ethanol.

Standard Solution of Thiram (Ehrenstorfer, 99%). Weigh 24 mg of thiram in a 100-mL volumetric flask. Dissolve thiram in acetone (Merck, p.a.) and make up to the mark with acetone.

Apparatus. Place a two-neck, round-bottom 1-L boiling flask in a heating mantle controlled by a variable transformer. Through one flask neck pass an air inlet tube reaching nearly to the flask bottom. Connect the other neck to a reflux condenser. Connect the top condenser outlet to two traps in series, one (containing NaOH solution) for the removal of H_2S and one (with a 25-mL calibration mark, containing color reagent) for the reaction of CS_2 and the color reagent. Connect the outlet of this second trap via a stopcock to a vacuum pump (Leybold-Hereaus) to draw air through the system.

Standard Curve. To a series of 25-mL volumetric flasks add amounts of standard CS_2 solution varying from 0 to 5 mL. To each flask add 20 mL of color reagent. Dilute to the mark with ethanol and mix. Let stand for 15 min and read absorbances at 430 nm against a mixture of 20 mL of color reagent and 5 mL of ethanol as reference. Plot absorbances against micrograms of CS_2 per gram of lettuce.

Procedure. Dehead the lettuce heads (a laboratory sample contains 10 lettuce heads) and mix the separate leaves well. Weigh 75 g into the flask. Place the flask into the heating mantle and connect the condenser and traps. Start the vacuum pump and apply gentle vacuum to the outlet of the CS_2 trap. Add 130 mL of the solution of $SnCl_2$ in HCl to the flask and apply the air inlet

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Table I. Distribution of Dithiocarbamates Determined as CS_2 in Lettuce Heads

sample	lettuce heads mean content (n = 4), mg of CS_2/kg	content, inner leaves, mg of CS_2/kg	content, outer leaves, mg of CS_2/kg
lettuce 1	1.9	<0.4	2.9
lettuce 2	1.7	<0.4	2.3

tube. Switch on the heating and reflux the contents of the flask for 1.5 h. Shut off the heating, disconnect the vacuum, and take off the CS_2 trap. Dilute to the mark with ethanol and mix. Measure absorbance at 430 nm against the reference. Measure the CS_2 content (micrograms per gram) present with the aid of the standard curve.

Experiments. To determine the distribution of dithiocarbamates in lettuce heads and in mixed lettuce leaves and the rate of decomposition of dithiocarbamates in chopped lettuce, the following experiments were carried out.

Experiment I. From two laboratory samples (each containing 10 lettuce heads) known to contain dithiocarbamates, outer and inner leaves were analyzed separately according to the method described above. Next the remaining leaves of each sample were mixed well and analyzed in quadruplicate according to the method described above.

Experiment II. A laboratory sample (containing 10 lettuce heads) known to contain no dithiocarbamates was chopped fine in a cutting machine (Stephan, capacity 12 L), and six portions of 75 g of chopped lettuce were weighed in the boiling flasks. Next, a standard solution of thiram in acetone, corresponding to 2 mg of CS_2/kg, was added and mixed with the chopped lettuce. Two portions were analyzed immediately. Two portions were analyzed after they were allowed to stay in the air for 0.5 h, and two portions were analyzed after 1 h in the air.

Experiment III. A laboratory sample known to be free of dithiocarbamates was chopped fine in the cutting machine, and six portions of 75 g of chopped lettuce were weighed in the boiling flasks. Next, a standard solution of thiram in acetone, corresponding to 2 mg of CS_2/kg, was added and mixed with the chopped lettuce. Two portions were analyzed immediately. The other four portions were immediately placed into the apparatus and vacuum was installed. However, no acid was added and the heat supply was not switched on. Two portions were further analyzed after 0.5 h, and two portions were further analyzed after 1 h.

Experiment IV. A laboratory sample known to be free of dithiocarbamates was chopped fine in the cutting machine, and 12 portions of 75 g of chopped lettuce each were weighed. To six portions was added a standard solution of thiram in acetone, corresponding to 2 mg of CS_2/kg, and mixed with the chopped lettuce. To the other six portions was added a standard solution of thiram in acetone, corresponding to 0.4 mg of CS_2/kg, and mixed with the chopped lettuce. To all portions was immediately added 130 mL of HCl containing $SnCl_2$. Two portions of both concentrations were immediately analyzed. Two portions of both concentrations were analyzed after they were allowed to stay in the air for 0.5 h, and two portions of both concentrations were analyzed after 2.5 h in the air.

RESULTS AND DISCUSSION

Distribution of Dithiocarbamates in Lettuce Samples. To determine the distribution of dithiocarbamates in lettuce heads, experiment I was carried out. The results of this experiment are given in Table I. The outer leaves were found to contain a higher concentration of dithiocarbamates than the whole heads. In the inner leaves no dithiocarbamates could be detected.

Next, from laboratory samples known to contain dithiocarbamates, four or five subsamples (75 g) were analyzed according to the Keppel method (Table II). The relative standard deviations varied from 10 to 34%. These high values are in agreement with values given in the literature (Keppel, 1971; Thier, 1977). Deheading and mixing of leaves probably makes the samples insufficiently homogeneous. This is understandable in view of the uneven distribution of dithiocarbamates in lettuce heads as demonstrated in Table I.

Table II. Homogeneity of Dithiocarbamates Determined as CS_2 in Mixed Lettuce Leaves

sample	individual measurements, mg of CS_2/kg	mean, mg of CS_2/kg	RSD, %
lettuce 1	1.5, 1.6, 2.6, 1.7	1.9	28
lettuce 2	1.4, 1.5, 1.6, 2.1	1.7	19
lettuce 3	4.7, 11.7, 6.4, 8.6, 10.3	8.3	34
lettuce 4	1.1, 0.65, 0.62, 0.84, 0.68	0.78	27
lettuce 5	5.5, 4.7, 5.9, 5.3, 6.0	5.5	10
lettuce 6	3.5, 3.3, 3.2, 3.6, 4.2	3.6	11

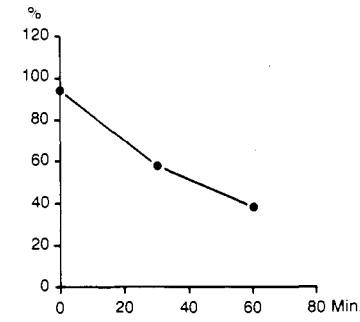

Figure 2. Decomposition of thiram in chopped lettuce in the air (expressed as percent of theory), as a function of time. Starting concentration of thiram in lettuce corresponds to 2 mg of CS_2/kg; each data point represents the mean of two readings.

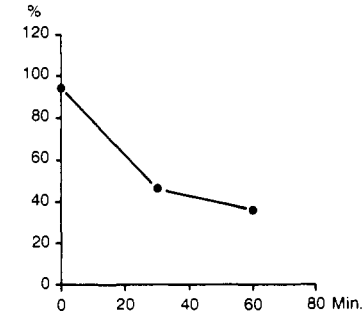

Figure 3. Decomposition of thiram in chopped lettuce, placed into the apparatus with installed vacuum (expressed as percent of theory), as a function of time. Starting concentration of thiram in lettuce corresponds to 2 mg of CS_2/kg; each data point represents the mean of two readings.

Decomposition of Dithiocarbamates in Chopped Lettuce. To study the rate of decomposition of dithiocarbamates in chopped lettuce, some modeling was applied. Thiram was chosen as the model compound because it is easily soluble, is often used in lettuce culture, and is unstable in contact with plant juices. Details are described under Experimental Procedures. The results of experiment II are depicted in Figure 2, which shows that almost half of the amount of thiram has decomposed after 0.5 h.

To check if CS_2 is formed during the decomposition of thiram, experiment III was carried out. If in this experiment CS_2 is formed during the keeping period, it will be collected in the CS_2 trap instead of disappearing into the air. The results of this experiment are given in Figure 3. The values in Figures 2 and 3 do not differ significantly. It is therefore evident that CS_2 is not formed during decomposition of thiram in lettuce juice. In view of these results, chopped lettuce samples have to be analyzed immediately. For large series of samples this is not practical, however.

Decomposition of dithiocarbamates in plant juice is probably due to enzymes released after chopping (Ministry of Welfare, Health and Cultural Affairs, 1988). For this

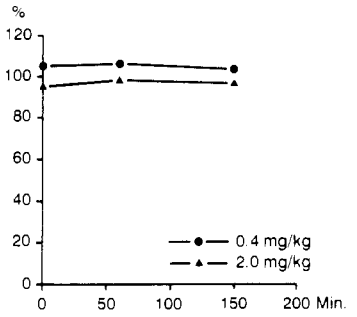

Figure 4. Decomposition of thiram in chopped lettuce in the air, with addition of acid (expressed as percent of theory), as a function of time. Each data point represents the mean of two readings.

Table III. Comparison Study of the Two Pretreatment Methods for Field-Treated Samples

sample	pretreatment	measurements, mg of CS_2/kg	mean, mg of CS_2/kg	RSD, %
lettuce 1	mixing leaves	1.8, 1.9, 3.0	2.2	30
lettuce 1	chopping	2.2, 2.1, 2.2	2.2	3
lettuce 2	mixing leaves	1.3, 1.0, 1.5	1.3	19
lettuce 2	chopping	1.4, 1.4, 1.5	1.4	4
lettuce 3	mixing leaves	7.7, 6.0, 11.5	8.4	34
lettuce 3	chopping	9.1, 9.3, 9.7	9.4	3

reason experiment IV was carried out. Immediately after the lettuce had been chopped and thiram had been added and mixed in, HCl containing $SnCl_2$ was added. It was expected that the acid would block the action of the enzymes. Figure 4 shows that thiram is stable for at least 2.5 h when the acid is added and the heat supply is not switched on. The hypothesis stated above seems to be valid. Series of samples can be pretreated (including acid addition) before further analyses have to be carried out.

Comparison of the Existing and the Modified Method. The model study described above had to be checked for its applicability to field-treated samples known to contain dithiocarbamates. For this reason the existing method (deheading and mixing) was compared with the modified method (chopping and acid addition) for some field-treated samples. Table III shows that the relative standard deviation is 5–10 times lower in the modified method than in the existing method. The reason for this is a drastic increase of the homogeneity of the sample when it is chopped. Moreover Table III shows that the mean CS_2 contents obtained for both methods agreed well. As a result, it is plausible that also in field-treated samples no decomposition of dithiocarbamates occurs if acid is added immediately.

Finally, both methods were compared for samples treated with zineb, because zineb is, next to thiram, the dithiocarbamate most frequently used in lettuce culture. Moreover, zineb has a totally different chemical structure (see Figure 1) and hence chemical properties different from those of thiram. The results of this study are given in Table IV. From this table it appears again that the mean contents obtained with both methods agree well but with a much lower relative standard deviation for the modified method.

Table IV. Comparison Study of the Two Pretreatment Methods for Samples Treated with Zineb

sample	pretreatment	measurements, mg of CS_2/kg	mean, mg of CS_2/kg	RSD, %
lettuce	mixing leaves	49, 27, 48, 40, 39	41	22
lettuce	chopping	45, 44, 45, 44, 44	44	1

Conclusions. The distribution of dithiocarbamates in lettuce heads is inhomogeneous. Deheading the lettuce and mixing the leaves make the sample taken for analysis insufficiently homogeneous.

Thiram decomposes rapidly in chopped lettuce. During decomposition no CS_2 is formed. When HCl containing $SnCl_2$ is added, decomposition is blocked.

The existing method (deheading and mixing) and the modified method (chopping and acid addition) correspond well as to mean results, but the modified method has a much higher reproducibility. This holds for both thiram and zineb in lettuce. Given the differences in chemical properties between thiram and zineb, it is conceivable that the modified method will also be applicable to other dithiocarbamates.

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