Kinetics of Maneb Degradation during Thermal Treatment of Tomatoes

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The kinetics of maneb degradation in tomato homogenates at high temperatures and at two pH values (4 and 9) and the rate of formation of the toxic metabolite, ethylenethiourea (ETU), were studied. Maneb was measured as carbon disulfide by headspace gas-chromatography and ETU by high-performance liquid chromatography with photodiode array detection. First-order kinetics adequately described the degradation of maneb in tomato homogenates. The degradation rate constants exhibited an Arrhenius temperature dependence in the range from 50 to 90 °C and the apparent activation energy (E_a) was calculated to be 36 KJ mol^{-1} in homogenates with natural pH (4). Raising temperature from 60 to 75 and to 90 °C, ETU formation was significantly increased. Interestingly, the selectivity toward ETU showed a downward trend when the total conversion increased at longer heating times. When the pH of the tomato homogenates was adjusted to 9, the degradation of maneb proceeded faster at both 60 and 90 °C. The combination of alkaline pH and the highest temperature (90 °C) resulted in the maximum ETU conversion rates. The results of the present study on the fate of maneb and ETU residues during tomato processing, may prove valuable in estimating potential risk from dietary exposure.

KEYWORDS: Pesticide; maneb; ethylenethiourea; degradation; kinetics; tomatoes; processing

INTRODUCTION

Ethylene-bis-dithiocarbamates (EBDCs: maneb, zineb, mancozeb, metiram) are organic, nonsystemic fungicides used on a wide variety of agricultural crops.

The EBDCs are considered to have low short-term toxicity. A major toxicological concern, however, is ethylenethiourea (ETU), a degradation product and metabolite common to all EBDCs. Laboratory animals have developed cancer, thyroid disorders, and birth defects after being fed with high levels of ETU (*1*). In estimating the potential risk posed from dietary exposure to this group of pesticides, the breakdown of EBDC residues to ETU prior to the consumption of treated food commodities should be taken into consideration.

Food processing is the major pathway through which exposure to ETU occurs. In particular, thermal treatments are associated with the higher conversion factors of EBDCs to ETU (*2*−*5*). Several types of thermal processes, for which the important parameters of temperature, time, and pH vary widely, are applied in industrial practice. Modeling the degradation of EBDCs during processing on the basis of kinetic studies could alleviate the need for specific sets of data.
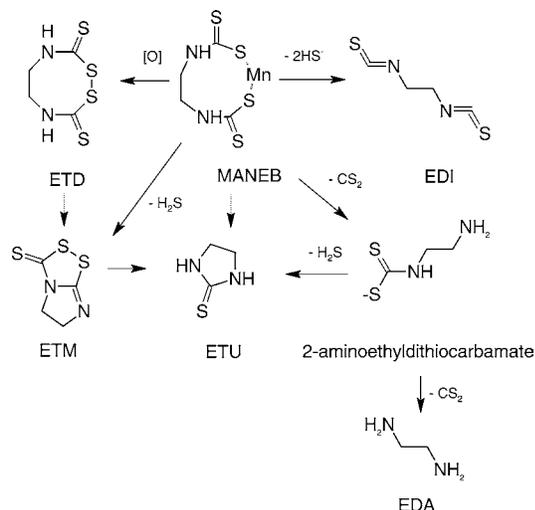
In literature, there are few kinetic studies involving complex food substrates; examples include the case of veterinary drug residues degradation (*6*, *7*) or carcinogenic/mutagenic compound (heterocyclic amines) formation (*8*) in meat muscle. In contrast, the hydrolysis kinetics of pesticides in aqueous buffer solutions at room (*9*−*11*) or elevated temperatures (*12*−*13*) has been the subject of many studies.

The degradation of the EBDCs is complex, and the exact reaction mechanisms still remain not well understood. At the same time, the kinetic studies that have been published so far have investigated the decomposition of disodium EBDC (nabam), a water soluble compound sharing the same ligand with the group of the EBDCs but not actually used as pesticide, in buffers (*14*−*16*). Limited information on the degradation kinetics of the manganese and zinc complexes of ethylenebis-dithiocarbamic acid (maneb, zineb, mancozeb) is available (*17*−*19*), although several efforts are recently devoted to the development of oxidative treatments for the efficient degradation of EBDCs and ETU as a means of decontamination (*20*−*23*). Notwithstanding, the results obtained with nabam can be extended qualitatively to other EBDCs. It was found that temperature and pH play an important role in the decomposition of nabam. Marshall (*15*) reported that under acidic conditions, the main products are ethylenediamine (EDA) and carbon disulfide, while in neutral and alkaline conditions, the formation

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Scheme 1. Chemical Degradation of Maneb[a]

ETD   MANEB   EDI

ETM   ETU   2-aminoethyldithiocarbamate

EDA

[a] Refs 1, 15, 34, and 42.

of ETU is promoted. A possible degradation scheme was proposed although the postulated intermediates have not been isolated (**Scheme 1**). In a recent report (*16*), the acid decomposition of nabam was studied in aqueous solutions at 25 °C in the range of H_0 −5 to pH 5, where H_0 is the Hammett acidity function. It was concluded that acid decomposition toward EDA takes place through the dithiocarbamate anion and a zwitterion intermediate. No general acid catalysis was observed.

EBDCs are widely used to protect tomato crops against various diseases and a maximum residue limit of 3 mg/kg (expressed as CS_2) has been set in the European Community (*24*). Tomatoes are commonly used in domestic cooking as well as industrially processed to a large extent. Although most of the industrial processes involve heating of the substrate, peeling the fruits by 18 to 20% hot lye causes a substantial increase in the pH also (*25*). Preliminary treatments, such as washing tomatoes for 10 min in water, reduced the EBDC residues by 30−55%, while washes with sodium hypochlorite have been shown to remove residues even more efficiently (*26*).

Maneb is an important representative of this group of pesticides. Maneb residues on field-sprayed tomatoes were significantly higher than those corresponding to treatment with other EBDC containing formulations (*5, 27*). At the same time, maneb products exhibited higher conversion rates to ETU (*28*).

An adequate kinetic analysis of a degradation process should include the identification of a kinetic model and the estimation of the Arrhenius parameters (activation energy and preexponential factor) so that accurate extrapolations of kinetic behavior can be made. The available data from previous research on EBDC degradation can only give information applying to the conditions of time and temperature used in the original experiments.

The aim of this work was to study the kinetics of maneb degradation and ETU formation in tomato homogenates at elevated temperatures, with the view to predict the fate of the residues under the conditions prevailing in the manufacture of tomato products. Experiments were carried out in pulps with natural pH (4.2) and in pulps with alkaline pH (9.0). Degradation pathways that can account for the results obtained are also discussed; as well as their significance for the estimation of risk posed by maneb residues in food systems.

MATERIALS AND METHODS

Materials. Maneb and ETU standards were obtained from Dr. Ehrenstorfer (Augsburg, Germany). A maneb solid solution of 1000 mg/kg in lactose was used for spiking. This standard was stored at −20 °C for one month. ETU stock and working standard solutions were prepared in ultrapure water.

Carbon disulfide (CS_2) was analytical grade. Fresh standard solutions in acetone (25 mg/mL, 10 mg/mL, and 1 mg/mL) were used for the preparation of the calibration standards by injecting an appropriate amount into the crimp sealed digestion bottles containing blank samples.

The methanol was HPLC grade and the water was obtained by a Nanopure UV purification instrument (Barnstead, Dubuque, IA) supplied by demineralized water. Dichloromethane and acetone were pesticide grade (Labscan, Dublin, Ireland). Hydrochloric acid 37%, sodium hydroxide, and stannous chloride dihydrate ($SnCl_2 \cdot 2H_2O$) were analytical grade. D(+)-Lactose monohydrate powder extra pure was from Riedel de Haën (Seelze, Germany).

Filters with a pore size of 0.45 μm and a GHP (polypropylene) membrane were from Gelman Sciences Inc. (Ann Arbor, MI). Glass microfiber filters were from Whatman (Kent, UK).

Extrelut NT 20 column fillings (part no. 1.15093.0001) were obtained from Merck (Darmstadt, Germany).

Organically grown tomatoes were purchased from the local market.

Thermal Treatments. Tomato homogenates spiked with maneb were treated at different temperatures. At each temperature, remaining maneb and ETU formed were determined at six different heat treatment times.

To prepare the samples, tomatoes were manually washed and homogenized using a Waring blender. Individual samples, corresponding to the weight specified for the analysis method, were spiked with the maneb spiking solution. By analyzing each sample as a whole, after the heat treatment, errors that might result from nonhomogeneous distribution of the active substance in a large starting quantity of test material were eliminated.

For the determination of maneb, 50-g portions of tomato homogenates were introduced to 250-mL glass bottles and spiked at a concentration of 5 mg/kg. The bottles were placed in a constant temperature water bath held at 50, 60, 75, or 90 °C (accuracy, ± 0.1 °C). At appropriate time intervals, bottles were removed and the samples analyzed for the remaining maneb. The experiments were repeated five to seven times.

ETU formation was studied at 60, 75, and 90 °C. For this purpose, 20-g portions of tomato homogenates were placed in glass beakers with watch glasses on top and spiked at 5 mg/kg. After heating for prefixed time intervals, the samples were removed, immediately cooled, and analyzed for ETU. Control samples, which consisted of spiked with maneb samples not subjected to thermal treatments, were also analyzed for ETU to account for its presence in the maneb standard and/or its formation during the analysis. The experiments were repeated four times.

The natural pH of the tomato homogenates ranged between 4.0 and 4.2. After adjusting the pH of the homogenates to 9 with 8 N NaOH, remaining maneb and ETU formed at 60 and 90 °C were evaluated by the same procedure as described previously.

The initial °Brix of the tomato homogenates was 4.5° and remained constant during the thermal treatments; water vapors condensed in the interior of the sample bottles. No visible change in the color of the tomato homogenates was observed when samples were heated. A reversible change occurred though, toward a darker red shade, when the pH was adjusted to 9.

ETU Stability. A separate experiment to investigate ETU stability toward heating at 90 °C in tomato homogenates was carried out. Portions (20 g) of tomato homogenates were placed in glass beakers and spiked with ETU at 5 mg/kg. At different time intervals up to a time period of 80 min, samples were removed from the water bath, and the percentage of ETU recovered was determined. Two trials were run on different days.

Maneb Residue Analysis. *Sample Preparation.* Maneb residues were determined as carbon disulfide by a modification of the official

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EN 12396-2 method applying the GC-headspace technique (*29*). To the 50 g of spiked tomato homogenates that have been subjected to the thermal treatment, 100 mL of 1.5% stannous chloride solution in hydrochloric acid (4 N) was added. The bottles were immediately sealed with a crimped septum and were placed in a water bath at 90 °C for 2 h. The bottles were then removed and left to acquire room temperature before 200 μL from the headspace were withdrawn with a gastight syringe and injected to the GC system. CS_2 evolved from the acid digestion of the samples was quantified by an external standard method.

GC Analysis. A Fisons 8530 gas chromatograph equipped with a flame photometric detector (FPD) operating in the sulfur mode (emission, 394 nm) and with a fused capillary column (DB-5 J & W Scientific, 30-m × 0.53-mm, 1.5-μm film thickness) was used. The oven temperature program was as follows: a 60 °C initial temperature was held for 5 min and then was increased to 200 °C by a rate of 20 °C min^{-1}. The injection port and detector temperatures were 150 °C and 230 °C, respectively. Carrier gas (helium) flow through the column was 10 mL min^{-1}. A split ratio of 1:5 was used.

ETU Residue Analysis. *Sample Preparation.* ETU residues were determined according to the HPLC method already described (*30*).

Briefly, the 20-g sample portion was transferred to a 500-mL Waring blender, 160 mL of methanol/water (3:1, v/v) were added and the mixture was homogenized for 2 min. The homogenate was filtered through a glassfiber filter in a Buchner funnel. The blender cup was rinsed with an additional portion of 40 mL of the extraction solvent. The filtrate was transferred to a flask and concentrated to 20 mL on a rotary evaporator. The pH of the concentrate was adjusted at 7−9 with 2% NaOH, loaded to the Extrelut column, left 10−20 min for equilibration and eluted with 200 mL of dichloromethane. The eluate obtained was removed on a rotary evaporator. The residue was transferred quantitatively with water to a 5-mL volumetric flask.

HPLC Analysis. The final aqueous solution was filtered through 0.45-μm pore size membrane filters. Samples (20 μL) were injected to the HPLC system. The HPLC apparatus consisted of a Model 600E pump, a Model 996 PDA detector, and a Model 717plus autosampler (Waters, U.S.A). Data were processed using the Millenium 32 software (Version 3.05.01). A Nucleosil 100 C18, 240-mm × 4.6-mm, column was used (Phase Separations Chromatography). The mobile phase was water/methanol (95:5, v/v) at a flow rate of 1 mL min^{-1} (isocratic) and the eluate was monitored at 240 nm. The mobile phase was degassed with helium constantly.

Statistical Analysis. Maneb degradation rate constants were estimated using linear regression analysis. The data on ETU formation were subjected to analysis of variance while comparisons of means were carried out using the least-significance difference (LSD) test at a significance level of 0.05. Statistical analyses were performed using the Excel 97 software (Microsoft Co., U.S.A).

RESULTS AND DISCUSSION

Residue Analytical Methodology. Maneb residues were measured as CS_2 by headspace gas chromatography with flame photometric detection (GC/FPD). The FPD response for sulfur containing compounds is proportional to the concentration of sulfur raised to an exponential factor (*n*), which can be determined experimentally by plotting the log of the response versus the log of the concentration. The value of *n* can be then introduced to a linearizing system connected to the detector to correct for non linearity. For CS_2, the curve thus obtained was $y = 1.90x + 6.92$ ($R^2 = 0.997$) for the concentration range from 0.05 to 4 mg/kg. The exponential factor set to the system was consequently $n = 1.9$. Linear calibration graphs, within 1 order of magnitude, were subsequently obtained ($R^2 \geq 0.99$). Maneb recovery values for spiked tomato homogenates exceeded the acceptable upper limit of 120%, for concentrations of the analyte less than 0.2 mg/kg, when aqueous CS_2 calibration standards were used. Ahmad et al. (*31*) have also observed matrix effects in the determination of EBDC residues by headspace GC, leading to the overestimation of the actual residues in various

Table 1. Maneb Method Accuracy and Precision Statistics Using Tomato Homogenates as Substrate

spiking level (mg/kg)	recovery (%)	RSD_r (%)	RSD_R (%)
4–5[a]	99.9	8.2	12.6
4.4–5.2[b]	96.1		9.8
2.6–3.8[c]	99.8		9.4
0.6–1.8[d]	82.8	2.8	7.5
0.13–0.38[d]	88.7	9.2	11.6

[a] Analysis performed on 14 different days in duplicate ($n = 14 \times 2$). [b] Analysis performed on 13 different days ($n = 13 \times 1$). Substrate pH adjusted to 9.0 ± 0.1. [c] Analysis performed on 5 different days ($n = 5 \times 1$). [d] Analysis performed on 6 different days in duplicate ($n = 6 \times 2$).

Table 2. Kinetics Data for Maneb Degradation in Tomato Homogenates (pH 4) Obtained by Linear Regression[a]

temp (deg C)	$k_{obs} \times 10^{-2}$ (min^{-1})	R^2	$t_{1/2}$ (min)
50	0.40 ± 0.02	0.94 ± 0.03	173
60	0.68 ± 0.06	0.94 ± 0.01	102
75	1.1 ± 0.1	0.96 ± 0.01	62
90	1.8 ± 0.3	0.96 ± 0.009	39

[a] Values are means ± SE

commodities, while their approach to use thiophene as internal standard did not efficiently address the problem. In the present work, to compensate for the matrix effects, matrix-matched calibration was applied in assessing method performance (**Table 1**) and in the degradation experiments. The method detection limit was 0.03 mg/kg expressed as CS_2.

ETU residues were determined by an HPLC/PDA method. The accuracy (>74%), precision (RSD < 9%), and detection limit of the method (0.002 mg/kg) for tomato samples have been reported in a previous work (*30*). The analysis of control samples, spiked with maneb at a concentration level of 5 mg/kg and analyzed for ETU at t_0, showed that, using the ETU methodology, the percentage of the conversion of existing maneb residues to ETU during the analysis is 1.6 ± 0.6% mol/mol (mean ± SD, $n = 15$).

Kinetics of Maneb Degradation. *Effect of Temperature.*
Maneb degradation in tomato homogenates was found to follow 1st-order kinetics

$$\ln(c_t/c_0) = - k_{obs} \times t \qquad (1)$$

where c_t is the concentration of maneb at various time intervals, c_0 the initial concentration, and k_{obs} the degradation rate constant.

Regression analysis was used to calculate the rate constants at different temperatures, from the slopes of the curves (eq 1). **Table 2** summarizes the maneb degradation rate constants, half-lives ($t_{1/2}$), and regression coefficients obtained.

EBDCs as nonsystemic pesticides consist entirely of surface residues, and as expected no conjugates have been found in the interior of plants (*32*). It is noted that the behavior of residues in spiked samples does not differ from the behavior of field-incurred residues when the latter are in a free unconjugated form (*33*).

The effect of temperature on degradation rate was studied in the range from 50 to 90 °C. As heating generally inactivates enzymes present in the substrate, the contribution of enzymatic mechanisms in the degradation should be insignificant under the conditions studied. It was found that the higher the temperature, the fastest the degradation of maneb. At 50 °C,
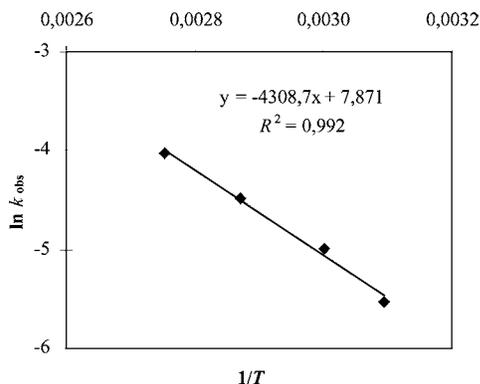
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Figure 1. Arrhenius plot for maneb degradation in tomato homogenates.

Table 3. Predictions, Based on the Kinetic Parameters Calculated, for the Percentage of the Maneb Degraded Under Various Heat Treatments of Tomato Substrates

process	temp (deg C)	time (min)	% maneb degraded
sterilization	120	15	50
cooking	100	20	40
canning	96	20	36
canning	93	15	26

Table 4. Percentage (% mol/mol) Conversion of Maneb to ETU at 60, 75, and 90 °C in Tomato Homogenates (pH 4)[a]

time (min)	temp (deg C)		
	60	75	90
8			14.6 ± 0.3
15	5.9 ± 1.0^b	12.0 ± 1.6^c	18.0 ± 1.3^d
30	8.7 ± 1.0^b	15.8 ± 1.7^c	19.9 ± 1.6^c
45	12.2 ± 1.3^b	18.3 ± 1.3^c	22.0 ± 1.2^c
60	13.8 ± 1.2^b	21.7 ± 1.4^c	23.6 ± 1.9^c
75	15.1 ± 0.5^b	23.8 ± 1.7^c	27.1 ± 3.3^c
90	15.2 ± 0.7^b	24.2 ± 0.4^c	

[a] Values are means $\pm$ SE. [b–d] Means in the same row marked by different letters are significantly different according to LSD test ($P < 0.05$).

the degradation rate was slow, and 47% of the initial amount still remained after heating for 180 min. At 90 °C, maximum degradation was observed with only 34% maneb remaining after 60 min.

The dependence of rate constants from temperature was found to be well described ($R^2 = 0.992$) by the Arrhenius equation (**Figure 1**)

$$\ln k_{\text{obs}} = \ln A - (E_a/RT) \qquad (2)$$

where E_a is the activation energy, R is the ideal gas constant ($8.314 \text{ J mol}^{-1} \text{ K}^{-1}$), A is the frequency factor (time^{-1}) and T is the absolute temperature.

The apparent activation energy was calculated to be 36 KJ mol^{-1}. On the basis of the estimated Arrhenius parameters, predictions about the amount of maneb degraded under various thermal treatments are possible. It should be stressed that the need to establish processing factors to refine residue estimates is increasing, as the safety assessment methodologies evolve and more stringent safety standards are introduced. EBDCs have a considerable economical impact as efficient fungicides with extensive use. In light of the toxicological concerns that have raised though, this group of pesticides is continuously under scrutiny and periodical reassessments are undertaken. **Table 3** shows the predicted amount (%) of maneb degraded in tomato homogenates under thermal processing conditions different from those tested experimentally. According to these estimates, a substantial decrease in the levels of maneb residues is expected under conventional industrial or household thermal treatments. The maximum percentage decrease of 50% would correspond to sterilization, which represents the most severe process. Although the reduction in the levels of the residues is generally desirable, in terms of their presence in the processed product, in the case of the EBDCs, an important issue is to which extent conversion to the more toxic degradation product, ETU, occurs.

Conversion to ETU. Several experimental works have indicated that the degradation of the EBDCs gives rise to various products. The major degradation pathways in acidic and neutral media lead to the formation of ETU and EDA (*15*). Possible

intermediate products include 2-aminoethyldithiocarbamate, ethylenethiuram monosulfide (ETM), ethylenethiuram disulfide (ETD), (*15*) and ethylenediisothiocyanate (EDI), (*34, 35*) (**Scheme 1**). Less than 10% of ETM is recovered as CS$_2$ by the acid disgestion method (*36*), while the acid digestion of isothiocyanates, and consequently of EDI, leads to the liberation of carbonyl sulfide instead (*37*). On the other hand, 2-amino-ethyldithiocarbamate can contribute 1 equiv of CS$_2$ when the analysis for dithiocarbamates is carried out.

ETU is the degradation product of importance due to health safety issues. In fact, the toxical effects exhibited by the EBDCs, among which the most critical is thyroid toxicity, are related to their biotransfornation to ETU (*38*).

In the tomato homogenates, the formation of ETU was measured at 60, 75, and 90 °C. The percentage of maneb degradation to other products was calculated as the difference $100 - $ (% maneb remaining $+$ % conversion to ETU (mol/mol)).

The percentages of maneb conversion to ETU as affected by temperature and time of heating are presented in **Table 4**. The highest conversions were found at 90 °C. In addition, the rate of ETU formation is higher initially; more than 40% of the total amount is produced within the first 15 min. Interestingly, at 90 °C, the conversion toward ETU within the first 15 min was 18% with only a further 9% conversion in the remaining 1h. The conversion factors obtained at 75 °C are significantly higher ($P < 0.05$) than those obtained at 60 °C when compared at each time interval. Similarly, when the temperature is raised from 75 to 90 °C, the amount of the parent compound converted to ETU in the first 15 min increased significantly. However, this difference tends to become less pronounced after prolonged heating. These results indicate that while temperature has an important impact on the conversion of the parent compound to ETU, the effect of the duration of heating is less prominent.

In **Figure 2,** the observed yields of ETU at different temperatures versus time of reaction are plotted, as well as the estimated combined yields to other products. It can be seen that at 90 °C, the conversion to ETU is dominant at the initial steps of the reaction. An induction period before the appearance of other products is evident. On the other hand, degradation at 60 °C is characterized by the dominance of the alternative degradation pathway at later stages. These results suggest that the reaction leading to ETU exhibits a stronger temperature dependence due to a higher activation energy (E_a). Thus, at a high enough temperature, the reaction becomes dominant.

The change in the selectivity during the course of the reaction is more explicitly illustrated in **Figure 3**, where the fractional yield of ETU is plotted as function of the total maneb conversion. At low conversions, formation of ETU is favored. As the amount of maneb degraded increases, the reaction toward
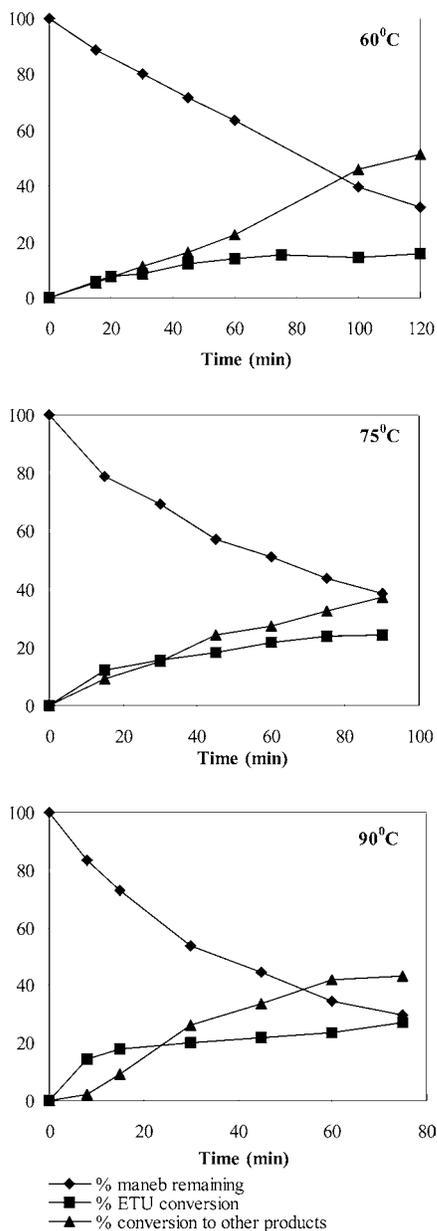
Figure 2. Maneb degradation and product distribution at 60, 75, and 90 °C. Substrate, tomato homogenates (pH 4).

Figure 3. Selectivity to ETU formation expressed as the percentage fractional yield versus total maneb conversion at 60, 75, and 90 °C. Substrate, tomato homogenates (pH 4).

Figure 4. ETU stability in tomato homogenates at 90 °C.

other products is promoted. The pattern of the degradation at different temperatures is qualitatively similar, with the ETU selectivity following the same trend, but there are marked quantitative differences. Initial ETU fractional yields range from 53% at 60 °C to 88% at 90 °C, corresponding to total maneb conversion of 11 and 17%, respectively. From these findings, it can be assumed that direct formation of ETU from the parent compound or via an extremely unstable precursor should be taking place. The EBDC oxidation products, ETM, and ETD form ETU under elevated temperatures (*15*, *32*, *34*). Nevertheless, their accumulation in the system studied does not seem probable, because an upward trend in ETU selectivity would be expected after prolonged heating. As most thermal processes apply heating to substrates for relatively short time periods (**Table 3**), the fact that ETU formation is initially favored over other degradation pathways is important in estimating risk.

To rule out the possibility that the decrease observed in the toxicant fractional yield is due to its own further degradation, the stability 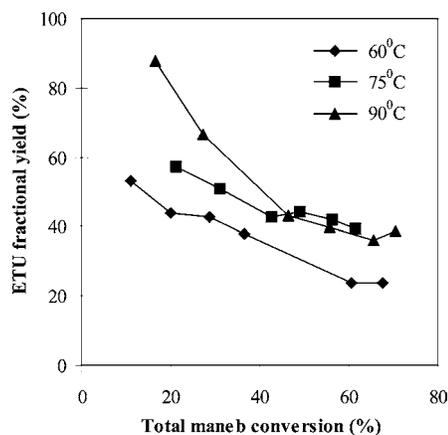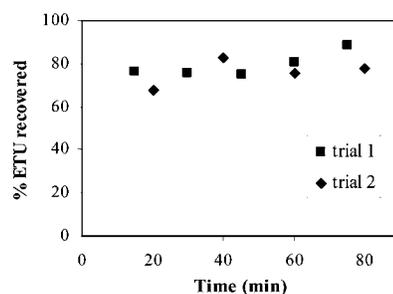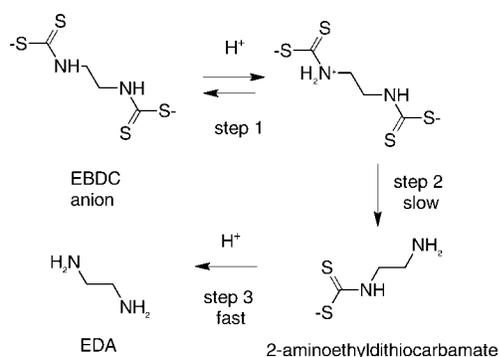of ETU in tomato homogenates at 90 °C was studied. **Figure 4** presents the percentage of ETU recovered versus time. Regression analysis revealed no decline during the time period examined (the slope confidence intervals of each trial ranged from −0.06 to 0.45 and from −0.52 to 0.77). It is concluded that ETU is a stable terminal product in the matrix studied under the most intense heating conditions applied. These results are in agreement with previous reports, according to which ETU was found extremely stable toward hydrolysis and heating. ETU IR spectra showed no changes when heated at temperatures ranging from 20 to 240 °C (*35*). Changes in concentration were also not observed in aqueous solutions held at 90 °C for 3 months over the pH range 5.0−9.0 (*39*).

The experimental data obtained are consistent with two degradation mechanisms. Either parallel degradation pathways operate simultaneously, and the reactions leading to other products seem to start with delay because they involve intermediates releasing carbon disulfide upon acid digestion or two consecutive reactions are taking place, the first toward ETU formation and the second toward other products.

As mentioned above, a postulated degradation intermediate that can release CS_2 upon acid digestion is 2-aminoethyldithiocarbamate. Humeres et al. (*16*), reported that first-order plots for nabam degradation at room temperature in buffers between pH 2.0 and 4.3 were found biphasic, the rate constant of the second plot similar to that obtained from the decomposition of 2- aminoethyldithiocarbamate. Although 2- aminoethyldithiocarbamate has been synthesized and isolated by the reaction of equimolar amounts of EDA and CS_2 at low temperatures (*40*), it is extremely unstable at high temperatures. Efforts to detect this compound during the thermal decomposition of nabam in acidic buffers were unsuccessful, and it was thus assumed that its own further decomposition, producing EDA as a principal species (92%) and ETU as a minor product (8%) at 90 °C and at a pH value of 4, is more rapid than its formation (*15*). On

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Scheme 2. Maneb Acid Decomposition toward EDA, Rate Determining Step 2

Table 5. Kinetics Data for Maneb Degradation in Tomato Homogenates after pH Adjustment to 9, Obtained by Linear Regression[a]

temp (deg C)	$k_{obs} \times 10^{-2}$ (min^{-1})	R^2	$t_{1/2}$ (min)
60	1.4 ± 0.2	0.96 ± 0.02	48
90	7.4 ± 0.7	0.98 ± 0.006	9

[a] Values are means ± SE.

Table 6. Percentage (% mol/mol) Conversion of Maneb to ETU at 60 and 90 °C in Tomato Homogenates (pH Adjusted to 9)[a]

time (min)	temp (deg C)	
	60	90
15	3.4 ± 0.1	32.3 ± 5.0
30	5.0 ± 1.4	47.0 ± 1.1
45	4.7 ± 0.6	51.5 ± 3.7
60	8.2 ± 4.4	47.6 ± 1.8
75	6.0 ± 2.4	47.4 ± 6.1
90		55.7 ± 1.9

[a] Values are means ± SE.

the basis of these reports, this postulated intermediate is too unstable to explain the induction period observed in the experimental data for maneb degradation toward other products (**Figure 2**). A degradation pathway, consistent with the findings of the present study, may proceed through a fast protonation of the amine-group of one dithiocarbamic moiety, followed by a rate-determining slow cleavage of the C–N bond leading to the formation of 2-aminoethyldithiocarbamate, which is subsequently degraded fast (**Scheme 2**). The observed rate of maneb degradation might then be a combination of the rate-determining step 2 and of the cyclization rate toward ETU.

If the second hypothesis is valid, ETU formation can be regarded as the initiation reaction, the acidic byproducts of which, namely hydrogen sulfide, promote degradation through another pathway. Following the dissociation of the metal complex under elevated temperatures, successive protonations of the released EBDC anion may take place (EBDC free acid $pK_a = 2.87$) (*16*). The ratio of neutral/anionic form of the fungicide and presumably the acid decomposition rate, which lead to EDA and CS_2, would be affected by small changes in pH, particularly near its pK_a. Therefore, it is assumed that a shift in the relevant equilibrium may induce a change in selectivity. Preferable to this hypothesis is the observation that at 90 °C, where the highest conversion levels to ETU are obtained, the most abrupt drop in ETU selectivity with concurrent acceleration of the alternative path is also noted as the degradation proceeds (**Figure 3**). However, the variation of the pH of the substrate during the thermal treatments was less than ± 0.2.

The rate constants of the reactions involved in the degradation of maneb in the substrate studied should be of the same magnitude approximately because 1st-order kinetics are maintained, and no break in the plots of $\ln(c_t/c_0)$ versus time have been observed. At the same time, when a process involves several steps with different activation energies E_a, the relative contributions may vary with both temperature and extent of conversion. Nevertheless, in the present case, despite the complexity of the reaction, the observed rate constants exhibit an apparent Arrhenius dependence.

The Effect of pH on Maneb Degradation and on ETU Conversion. The experimental data for maneb degradation in tomato homogenates after pH adjustment to 9 fit the equation of a reversible 1st-order decay

$$\ln((c_t - c_\infty)/(c_0 - c_\infty)) = -k_{obs} \times t \qquad (3)$$

where c_t is the concentration of maneb at various time intervals, c_0 the initial concentration, c_∞ the equilibrium concentration, and k_{obs} the degradation rate constant.

The degradation under alkaline conditions proceeds fast until a maximum conversion of approximately 85% is reached. The degradation rate increased substantially with the increase in temperature (**Table 5**). The estimated half-lives ($t_{1/2}$) were 48 min at 60 °C and 9 min at 90 °C. Because the respective estimates at pH 4 were 102 and 39 min, it is obvious that degradation is accelerated by alkaline pH.

The temperature increase, at pH 9, has a significant effect on ETU formation (**Table 6**). As temperature is raised by 30 °C, the conversion to ETU is about 10 times higher. Moreover, at 60 °C, there is no significant difference ($P > 0.05$) between the percentages of ETU formation at pH 4 and at pH 9 (**Table 6** vs **Table 4**). In contrast, at 90 °C, the conversion to ETU in the alkaline pH is much higher than the conversion in the acidic physiological pH of tomato homogenates. The industrial lye peeling procedure, which is performed at high pH values and high temperatures, should be regarded as critical for the possible contamination with ETU of processed products. Although most of the toxicant is expected to dissolve in the aqueous washes, a significant amount may still remain in the processed food if the initial concentration of the parent compound is high.

In **Figure 5**, the product distribution during the degradation is presented. It can be observed that at 60 °C the ETU conversion levels are low, while other unidentified products constitute the majority of the reaction output. Conversely, at 90 °C, ETU is the major degradation product. The selectivity toward ETU formation remained almost constant during the course of the reaction: $14.4 \pm 2.6\%$ at 60 °C and $59.5 \pm 3.8\%$ at 90 °C, although a gradual shift of pH from 9 to 7 was observed.

From these findings, it is evident that a different degradation mechanism should operate in alkaline pH. This mechanism may involve the formation of EDI as an intermediate because monosubstituted dithiocarbamates in alkaline surroundings are known to decompose into isothiocyanates by elimination of hydrogen sulfide (*41*). In fact, the fungitoxicity of the EBDCs has been attributed partly to the formation of isothiocyanates, which interact with sulfhydryl proteinaceous groups, causing deactivation/inhibition of enzymes and cellular functions (*42*). Isothiocyanates are stable in strong alkaline media but decompose rapidly at high temperatures (*43*). Additionally, degradation
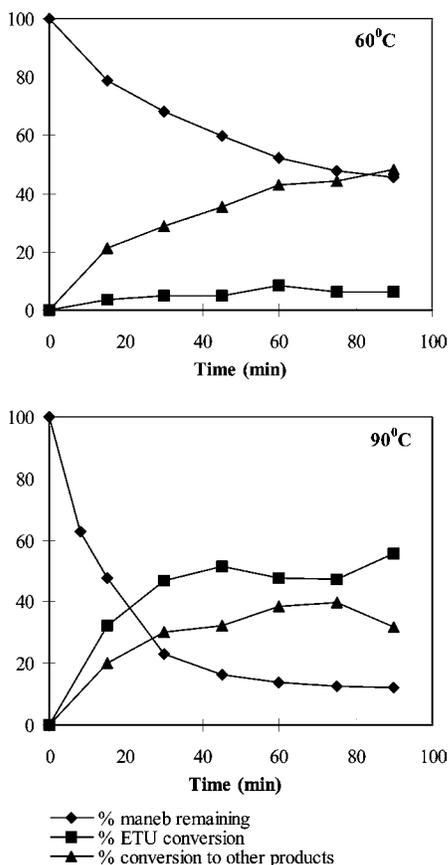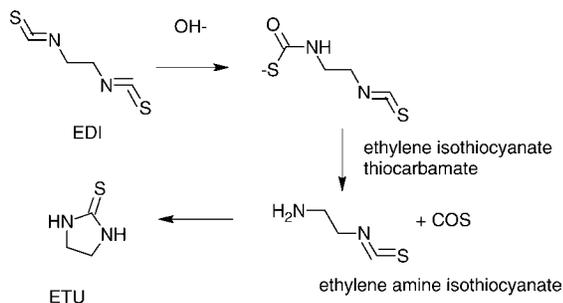
Figure 5. Maneb degradation and product distribution at 60 and 90 °C. Substrate, tomato homogenates, pH adjusted to 9.

Scheme 3. EDI Conversion to ETU in Alkaline pH at Elevated Temperatures

of EDI can lead to ETU formation (*34*). A proposed mechanism proceeds through the irreversible addition of hydroxyl ions to the carbon atom of one isothiocyanate group and formation of monothiocarbamate. After the release of carbonyl sulfide, the amine formed can react further with the second isothiocyanate group producing ETU (**Scheme 3**).

Therefore, from literature data, EDI is the most probable degradation product observed in excess in alkaline tomato homogenates at 60 °C (**Figure 5**). Its instability at elevated temperatures and its ability to convert to ETU can account for the product profile observed at 90 °C.

The present study shows that despite the complex nature of a food substrate, it is possible to create acceptably accurate kinetic models of maneb degradation in food systems that could assist in estimating consumer dietary exposures. Obviously, the stability of maneb may be variable in different food systems; the pH of the substrate, for example, should play an important

role. It was also found that a significant fraction of the parent compound degraded is converted to ETU. However, to model the formation of the toxicant, further research on the chemistry of the EBDC degradation is needed.

ABBREVIATIONS USED

EBDCs, ethylene-bis-dithiocarbamates; EDA, ethylene-diamine; EDI, ethylenediisothiocyanate; ETD, ethylenethiuram disulfide; ETM, ethylenethiuram monosulfide; ETU, ethylene-thiourea; FPD, flame-photometric detector; HPLC, high-performance liquid chromatography; PDA, photodiode array detector; P, probability; R^2, square of the Pearson correlation coefficient; RSD_r, relative standard deviation calculated from results generated under repeatability conditions; RSD_R, relative standard deviation calculated from results generated under reproducibility conditions; SD, standard deviation; SE, standard error of mean; c_t, concentration at various time intervals; c_0, initial concentration; c_∞, equilibrium concentration; A, frequency factor; E_a, activation energy; k_{obs}, degradation rate constant; $t_{1/2}$, degradation half-life; R, ideal gas constant.

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