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GC-ITMS Determination and Degradation of Captan during Winemaking

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Captan and its metabolite tetrahydrophthalimide (THPI) were determined in grapes, must, and wine by GC-ITMS. Pesticides were extracted with acetone/petroleum ether (50:50 v/v). Because of the high selectivity of the ITMS detector, no interferent was found and cleanup was not necessary. Recoveries from fortified grapes, must, and wines ranged between 90 and 113% with a maximum coefficient of variation of 11%. Limits of quantitation were 0.01 mg/kg for both compounds. In model systems, captan and its metabolites, THPI, *cis*-4-cyclohexene-1,2-dicarboxylic acid, and 1,2,3,6-tetrahydrophthalamic acid, were determined by HPLC. The degradation of captan during winemaking was studied. Captan degraded in must, giving 100% THPI, and at the end of fermentation, only THPI was found in wine. The degradation of captan to THPI was due to the acidity in must and wine. This metabolite was present at low levels on grapes, and, unlike captan, it had no negative effect on the fermentative process. Model systems showed that the mechanism of disappearance of captan in grapes was due to photodegradation and codistillation.

KEYWORDS: Captan; tetrahydrophthalimide; metabolites; winemaking; residues

Captan [*N*-(trichloromethylthio)cyclohex-4-ene-1,2-dicarboximide] and folpet are two fungicides belonging to *N*-trihalomethylthio derivatives. The degradative behavior of folpet from vine to wine was previously studied (*1*). During winemaking this active ingredient degraded completely, and at the end of fermentation, phthalimide was the main product in wine. The disappearance of captan in grapes after field treatment is well documented, whereas the fate of the active ingredient during the wine-making process is not described in the literature (*2*, *3*). The degradation of captan during apple processing (125 °C for 20 min and pH 4.0) has been described (*4*), and tetrahydrophthalimide (THPI; 1,2,3,6-tetrahydrophthalimide) was the main product formed (96.5%). Other minor products, such as tetrahydrophthalic acid (0.3%) (THPA; *cis/trans*-1,2,3,6-tetrahydrophthalic acid) and tetrahydrophthalamic acid (0.2%) (THPAM; *cis/trans*-1,2,3,6-tetrahydrophthalamic acid), have been identified (**Figure 1**). This behavior is similar to that of folpet in must, where acidity is responsible for the degradation of the fungicide.

Because captan and folpet are two structurally similar fungicides, their degradation behavior could be supposed to be the same. Despite these preliminary remarks, the degradation behavior of captan during winemaking has never been studied, and many residue-monitoring programs in wine are limited to only the active ingredient without considering any of the degradation products. In this work, the fate of captan during winemaking in must has been studied. This paper also describes a simple, rapid method to determine captan and THPI in grapes, must, and wine by GC-ITMS and captan and its metabolites in model systems by HPLC.

EXPERIMENTAL PROCEDURES

Field Trials. The experiment was carried out on cv. Nuragus grapes in a vineyard at Ussana (Ca, Italy) with a 2.5 × 1.5 m plant density. Each block consisted of 60 plants with four replications for each experiment. Orthocide 50 wettable powder at 50% active ingredient (ai) was the commercial formulation. It was used at the dose recommended by the manufacturer (250 g/hL of water). The compound was sprayed with an AM 150 (Oleo-Mac, Regio Emilia, Italy) manual sprayer. Treatment was carried out on September 4, 2001, and samplings were performed at 21, 25, and 28 days after treatment. During the experiment it did not rain, and the maximum and minimum average temperatures were, respectively, 33.1 and 17.1 °C.

Winemaking. The wine-making process was carried out as described previously (*1*, *5*). Briefly, grape samples were pressed and stemmed together; sodium metabisulfite (200 mg/L) was added, and the mixed sample was divided into two equal parts. One part was allowed to ferment with the skins (vinification with maceration); the other was dripped, and the resulting must was allowed to ferment (vinification without maceration). Because captan has an antiseptic activity on the

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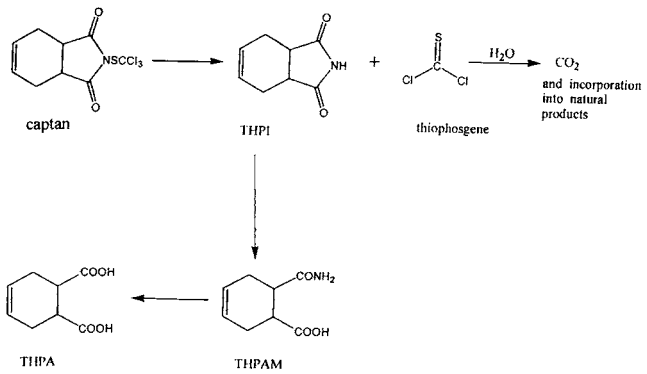

Figure 1. Captan and its main metabolites: tetrahydrophthalimide (THPI), tetrahydrophthalamic acid (THPAM), and tetrahydrophthalic acid (THPA).

fermentative microflora, 500 mg of dry yeast per kilogram of grapes was added to each sample. Fermentation had a regular course in all samples, and after 15 days, the obtained wines were filtered and analyzed for fungicide residues.

Chemicals. Captan [*N*-(trichloromethylthio)cyclohex-4-ene-1,2-dicarboximide] (Riedel de Haën, Hannover, Germany), THPI (Fluka, Milan, Italy), and THPA (*cis*-4-cyclohexene-1,2-dicarboxylic acid, Lancaster Synthesis, Morecambe, U.K.) were all analytical standards. THPAM was synthesized according to the method of Arrizabalaga et al. (*6*). Acetone, acetonitrile, and methanol were HPLC grade solvents (Carlo Erba, Milan, Italy), whereas petroleum ether (Carlo Erba) was a special reagent for pesticide determination. Anhydrous sodium sulfate and sodium chloride were of analytical grade (Carlo Erba).

Stock standard solutions for pesticide determinations (~700 mg/kg each) were prepared in acetone. For degradation experiments in must and in model systems, stock solutions of ca. 300 and 1000 mg/kg were prepared in methanol. Working standard solutions were obtained by dilution with the extract solution from untreated grapes, must, and wine for GC-ITMS determinations and with the mobile phase for HPLC determinations.

Residue Analyses. *GC-ITMS Determination.* The GC-ITMS method was used for the determination of captan and THPI in grapes, must, and wine.

A Varian 3800 GC, coupled with a Saturn 2000 ITMS and a Varian 7800 autosampler, was used. The ion trap was equipped with a chemical liquid ionization system. The gas chromatograph was fitted with a carbofrit inserted into the glass liner. The column was a fused silica capillary DB 5 MS (30 m, 0.25 mm; 0.25 μm). The operating conditions for GC-ITMS were as follows: 4 μL injection volume into a model septum injector programmable from 60 to 150°C (30 °C/s), manifold temperature, 100 °C; transfer line, 200 °C; ion trap temperature, 150 °C. The oven was programmed as follows: 60 °C (1 min), raised to 240 °C (3 °C/min). The ion trap MS conditions were as follows: ionization mode from 9 to 12 min; liquid CI μSIS (methanol) from 12 to 18 EI SIS range mode (*m*/*z* 70−310). Data handling was performed via measurement type area; quantitation ions were 152 for THPI and 79, 149, and 264 for captan.

HPLC Determination. The HPLC method was used for the determination of captan and its metabolites (THPI, THPAM, and THPA) during the degradation experiments on model systems. An Agilent 1100 series liquid chromatograph (Agilent Technologies, Waldbronn, Germany) was used, consisting of a G1322A degasser, a G1311A quaternary pump, a G1313A autosampler, a G1316A column oven, and a G1315A diode array detector, controlled by HP ChemStation software. An S_5 C_8 column (250 × 4.6 mm i.d., 5 μm) (Waters Spherisorb, S.p.A., Milan, Italy) was employed. The flow rate was 1 mL/min. The gradient run used was as follows: time 0, acetonitrile/10^{-3} N H_2SO_4 (45:55); time 12, acetonitrile/10^{-3} N H_2SO_4 (60:40); and equilibrated to the initial conditions for 6 min. The wavelength was 200 nm and the injection volume 100 μL.

Extraction Procedure. A 5 g aliquot of homogenized sample (grapes, must, or wine) was weighed in a 30 mL screw-capped flask; 4 g of NaCl and 10 mL of acetone/petroleum ether (50:50 v/v) were added, and the tube was agitated in a rotatory stirrer for 15 min. The phases were allowed to separate, and an aliquot of the organic layer was injected for GC analysis.

Degradation Experiments on Grapes, Must, and a Model System. (A) Must. Must samples were treated with aliquots of sulfuric acid and sodium hydroxide solutions to adjust the pH to 3 and 4, respectively. Portions of 100 g of the sample were weighed in a 125 mL screw-capped flask, and 2 mL of ethanolic solution of captan was added. At prefixed intervals an aliquot of grape must was analyzed to determine the remaining residues of captan and those formed of tetrahydrophthalimide. The tests were carried out in four replicates.

(B) Model System. Aqueous solutions at pH 3 and 4 with 10^{-3} and 10^{-4} N H_2SO_4, respectively, were prepared, and an aliquot of captan solution was added. At prefixed intervals the solutions were injected for HPLC analysis for the determination of captan, THPI, THPAM, and THPA. All of the tests were carried out in four replicates.

Evaporation, thermodegradation, codistillation, and photodegradation experiments on model systems were conducted according to the procedure described in a previous study *(7)*. All tests were carried out in four replicates using captan in the commercial formulation prepared in acetone.

(a) Evaporation and Thermodegradation Experiments. An aliquot of captan solution was placed on Teflon membrane filters and placed inside a 10 mL dark vial with a screw-closed cap. After 24 h in the oven at 50 °C, the vials were placed in a freezer at −20 °C to allow the evaporated active ingredient residues to condense on the walls. The residues in the filter and on the walls were extracted and analyzed by HPLC. The residues present on the walls show that the active iingredient was evaporated from the filter. A difference between the residue found in the vial submitted to 50 °C and that determined in the control filter indicates loss by thermodegradation.

(b) Codistillation Experiment. A membrane filter added with a solution of pesticide was placed on top of a 10 mL vial containing 5 mL of water with a screw cap with a center hole to let the water vapor pass through the filter as through the fruit surface. After 24 h in the oven at 50 °C, the filter was removed and the vial weighed to determine the amount of water lost by evaporation. The residue on the filter was extracted and analyzed by HPLC. The comparison between the amount of residue on the filter and that present on the control filter shows the amount of active ingredient that codistills. To obtain a correct evaluation of these data, we must consider the amount of residue lost by evaporation and by thermodegradation.

(c) Sunlight Photodegradation Experiments. For each studied compound, aliquots of captan solution were poured into Petri dishes and evaporated at room temperature. The dishes were exposed to direct sunlight and removed from the sunlight at prefixed intervals. The residues contained in the dishes were analyzed by HPLC.

Statistical Analysis. Variance analysis (ANOVA) and comparisons between average values were performed with the Duncan test at $P < 0.05$.

RESULTS AND DISCUSSION

Analytical Methods. GC methods are usually used for the determination of captan and THPI. A DB5 column allows the separation of the two compounds. Because captan possesses three chlorine atoms in its molecule, ECD detectors provide high sensitivity. Loss of these atoms during captan degradation to THPI determines a significant decrease in sensitivity. Therefore, THPI cannot be determined with this detector at a low concentration. To obtain a good sensitivity for THPI, we had to use an NPD detector, which, however, showed a low response for captan. Because a low detection level cannot be obtained with the same detector for both compounds, the sample should be analyzed separately by ECD and NPD or with the two detectors in line. A good sensitivity for the two compounds together was achieved with an ITMS detector coupled with a GC arranged for large volume injection, fitted with a carbofrit inserted in the glass liner (*8*−*10*). Using this technique it was

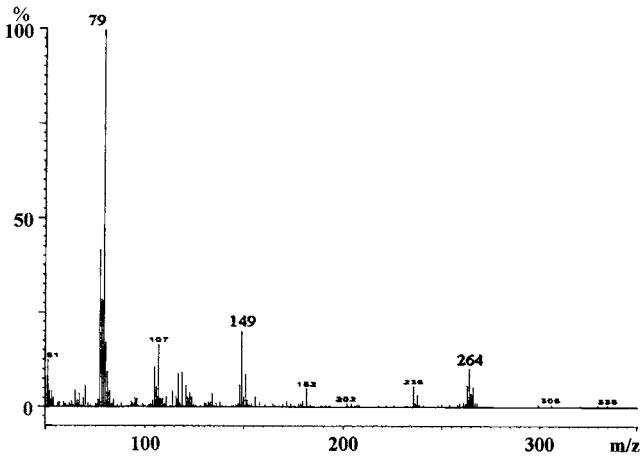

Figure 2. Mass spectrum of captan obtained by GC-ITMS in the EI mode.

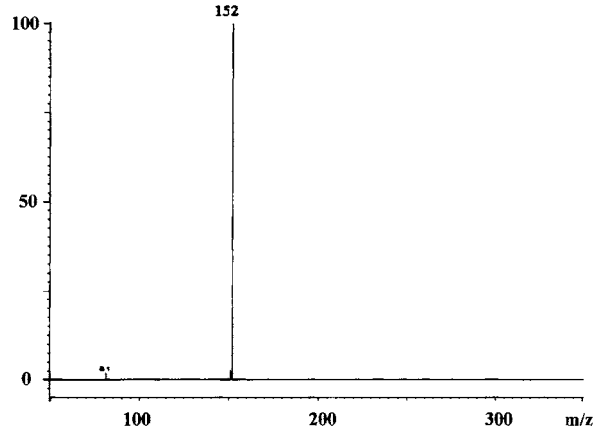

Figure 3. Mass spectrum of THPI, obtained by GC-ITMS in the CI mode using methanol.

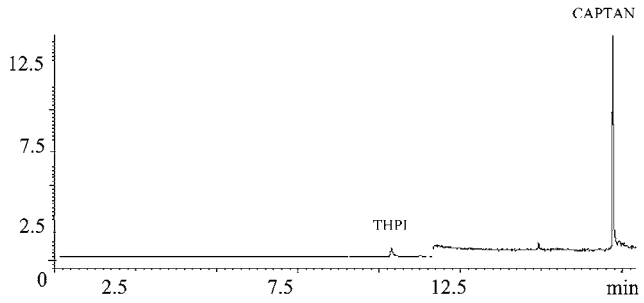

Figure 4. GC-MS chromatogram of THPI (0.13 mg/kg) and captan (0.76 mg/kg) in grapes. For GC-MS conditions see text.

possible to inject 4 μL together with good response linearity. The best conditions in ITMS were, for captan, electron impact (EI) in the selected ion storage (SIS) range mode and, for THPI, chemical ionization (CI) (MeOH) in μSIS, as reported in the GC-ITMS determination session (**Figures 2** and **3**). Because this technique is highly selective and no interference peak was detected, no clean up was necessary (**Figures 4** and **5**). The matrix effect was evaluated by comparing the response of the two pesticides solubilized in an organic solvent and in a matched matrix of grapes, must, and wine. An increase in response (~20%) was noticed at low concentrations due to the matrix effect. Therefore, working standard solutions were prepared in blanks from untreated grapes, must, and wine. Calibration graphs for captan and THPI were constructed measuring peak area versus concentration. Good linearities were achieved in the range

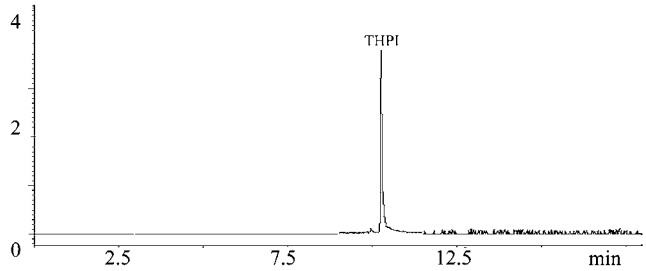

Figure 5. GC-MS chromatogram of THPI (0.43 mg/kg) in wine. For GC-MS conditions see text

of 0−5 mg/kg for captan and for THPI, with correlation coefficients between 0.9997 and 0.9999. The found limit of quantitation (LOQ) was 0.01 mg/kg for both compounds.

The HPLC method was used to study the degradation of captan in model systems because it allows the simultaneous determination of captan, THPI, and the acid metabolites, THPAM and THPA, which are not detectable by GC. The separation of all compounds was obtained with a gradient as reported in the HPLC determination session (**Figure 6**). Calibration graphs (external standard mode) were constructed for all of the studied compounds, plotting peak heights versus concentrations. Good linearities were achieved in the range of 0−10 mg/kg, with correlation coefficients between 0.9996 and 0.9998.

Recovery Assays. Untreated grapes, must, and wine samples were fortified with 0.01, 0.1, 1.0, and 5.0 mg/kg captan and THPI and processed according to the above-described procedure. Recovery assays showed values obtained from four replicates ranging between 90 and 113% with a maximum coefficient of variation (CV) of 11%.

Residues. Samplings started 21 days after treatment, and during the week three samples were collected. At harvest, captan and THPI residues were found in all samples of grapes, at concentrations from 0.35 to 0.76 mg/kg for captan and ~0.1 mg/kg for THPI (**Table 5**). Many trials were performed in different countries to evaluate the degradation rate of captan, and all showed similar THPI residue levels (*2*). In these trials, residues were determined just after treatment and at prefixed intervals. The reported data indicated that captan residues decreased during the experiment, even by several grams, whereas small amounts of THPI (0.1−0.2 mg/kg) were always present, and these levels were not correlated with the degraded amounts of captan, the coefficient stoichiometric of transformation of captan into THPI being 0.5. Therefore, 0.5 g of THPI, or proportional quantities depending on its degradation rate, should be formed for each gram of degraded captan. Found THPI residues were always of the same magnitude, and it was difficult to understand how this metabolite was formed and why it was always present in grapes. Captan is a nonsystemic fungicide, and its degradative mechanism could mainly depend on evaporation, thermodegradation, photodegradation, and codistillation processes. To verify the disappearance mechanism of captan on grapes and the processes that were responsible for the formation of THPI, we carried out experiments on model systems. All of these experiments were repeated in the presence of the epicuticular wax of grapes, as it could affect both the degradation kinetics of captan and its degradation products (*11*−*13*).

Table 1 shows the data obtained from the model systems to determine the effects of evaporation, thermodegradation, and codistillation. Because no captan residues were found on the walls of the vials, it can be deduced that it did not evaporate, whereas the difference between the residues in the filter treated

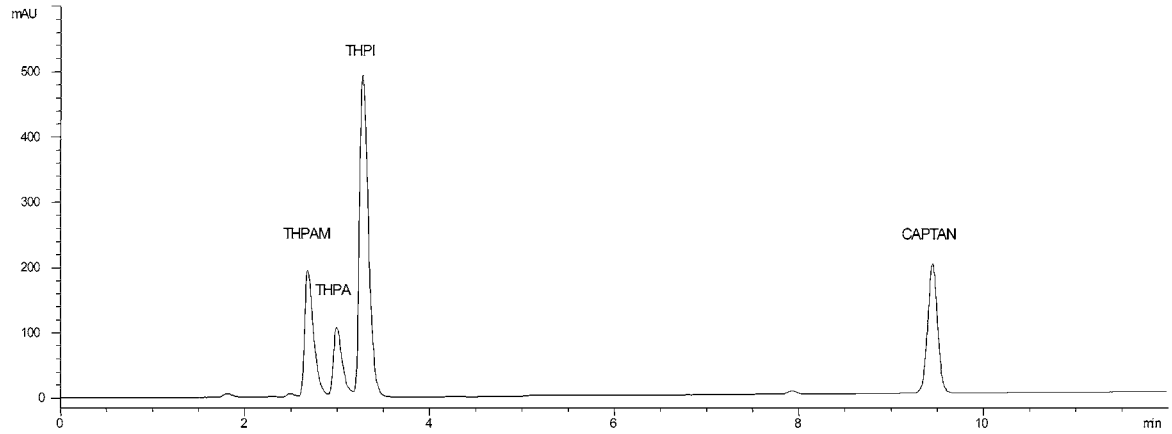

Figure 6. HPLC chromatogram of captan and its metabolites: THPI, THPAM, and THPA. For HPLC conditions see text.

Table 1. Determination of the Effect of Codistillation, Evaporation, and Thermodegradation on Captan in the Commercial Formulation and THPI

Evaporation and Thermodegradation

pesticide	wax	control (*c*) (μg/cm^2)	vial (*v*) (μg/cm^2)	filter (*f*) (μg/cm^2)	difference [$c - (v + f)$] (μg/cm^2)
captan	without	3.30 ± 0.19	nd[a]	2.55 ± 0.12	0.75
	with	3.30 ± 0.09	nd	1.83 ± 0.36	1.47
THPI	without	3.14 ± 0.22	0.59 ± 0.01	2.75 ± 0.19	ns[b]
	with	3.14 ± 0.17	0.45 ± 0.03	2.75 ± 0.16	ns

Codistillation

pesticide	wax	control (*c*) (μg/cm^2)	vial (*v*) (μg/cm^2)	filter (*f*) (μg/cm^2)	difference ($c - f$) (μg/cm^2)
captan	without	1.87 ± 0.13		1.01 ± 0.26	0.86
	with	1.59 ± 0.23		0.67 ± 0.13	0.92
THPI	without	4.21 ± 0.18		2.15 ± 0.14	2.06
	with	4.01 ± 0.49		2.44 ± 0.08	1.57

[a] nd, not detectable. [b] ns, not significant.

Table 2. Residues (Milligrams per Kilogram ± SD) of Captan, THPI, THPAM, and THPA after Exposure to Direct Sunlight

time (h)	captan	time (h)	THPI	time (h)	THPAM	time (h)	THPA
0	5.7 ± 0.2	0	4.9 ± 0.3	0	7.5 ± 0.4	0	13.3 ± 0.3
1	1.7 ± 0.1	0.5	1.6 ± 0.2	1	5.0 ± 0.3	1	10.0 ± 0.7
2	0.6 ± 0.1	1	1.3 ± 0.3	3	4.1 ± 0.4	7	9.0 ± 1.0
5	0.3± 0.1	2	1.0 ± 0.2	6	3.4 ± 0.5	14	7.8 ± 2.4
9	0.3 ± 0.0	4	0.6 ± 0.1	9	2.4 ± 0.2	24	5.6 ± 0.6
				15	1.8 ± 0.1	38	2.6 ± 0.5
$t_{1/2}$	5.1		1.5		8.1		43.2
r	–0.865		–0.884		–0.955		–0.970

and the control filter (0.75 and 1.47 μg/cm^2) showed that the decrease in captan was due to thermodegradation. In the presence of wax a decrease of 45% in captan residues was observed versus 23% when the experiments were performed without wax. Analysis of the chromatograms obtained during these experiments showed that THPI and the other metabolites (THPAM and THPA) did not form. THPI showed a tendency to evaporate (~15% in both cases, with and without wax), but it did not show any thermodegradation. This indicates that if THPI were formed during the captan degradation process, it would evaporate only partially.

Codistillation experiments showed that 46% of captan was lost when the experiment was performed without wax and that 58% was lost in the presence of wax. An inverse behavior was observed for THPI, with 49 and 40% losses, respectively. Considering that captan thermodegrades by 23 and 45%, respectively, in the absence and presence of wax and that 15% of THPI evaporates in both cases, we can assume codistillation losses of 23 and 14%, respectively, without and with wax for captan and a codistillation loss of ~30% for THPI.

The data reported for photodegradation experiments (**Table 2**) showed that THPI (1.5 h) degraded with a lower half-time than that observed for captan (5.1 h). This means that if the photodegradation process of captan yields THPI, it cannot be determined because its degradation rate is higher than its formation rate. On the contrary, it is possible to determine THPAM and THPA because their degradation rates are lower than those observed for captan. The chromatograms obtained for the captan photodegradation experiment did not indicate the presence of any of the studied metabolites, so the hypothesis that in grapes captan follows the same degradation pathway observed for apples can be excluded (*4*).

The model systems showed distinctly that captan does not degrade with formation of THPI in the processes of evaporation, codistillation, photodegradation, and thermodegradation, and even if it were formed, it would not be possible to determine because THPI disappeared at a rate faster than that of captan. Therefore, we supposed that the formation of THPI could be due to the acidic activity of must, as has been observed for folpet. To evaluate the effect of pH on the formation rate of THPI, captan degradation was studied in must and in a model system at two different pH levels.

The data reported in **Table 3** show the degradation of captan in must. At the beginning of the experiment its degradation in THPI is not quantitative but tends to become so in time. The amount of THPI found in must after 2 h corresponds to ~20% of the degraded captan, and the transformation percentage increases progressively until it reaches a quantitative yield in 3 days. This indicates that THPI is not formed directly from captan. Therefore, we can assume that captan degrades with the formation of an intermediate compound before being transformed in THPI. This metabolite can catalyze the process, and when THPI reaches a concentration of 2 g/L, the transformation becomes quantitative.

The decay rate of captan at pH 4 is higher than that observed at pH 3, with half-time values of 24.4 and 30.5 h, respectively. These data do not agree with those reported in the literature, which show that captan was readily hydrolyzed in water and the reaction was independent of pH in the pH range of 2−6

Table 3. Degradation of Captan (Milligrams per Kilogram ± SD) in Must at pH 4 and 3

	pH 4			pH 3		
time (h)	captan	THPI	THPI formed[a] (%)	captan	THPI	THPI formed[a] (%)
0	5.63 ± 0.27	0.16 ± 0.02	0	5.43 ± 0.69	0	0
2	4.61 ± 0.52	0.27 ± 0.02	21 ± 2	4.67 ± 0.22	0.17 ± 0.01	18 ± 1
7	3.77 ± 0.19	0.76 ± 0.03	64 ± 3	4.11 ± 0.20	0.24 ± 0.02	53 ± 3
24	2.25 ± 0.03	1.37 ± 0.10	81 ± 5	3.01 ± 0.39	0.83 ± 0.01	69 ± 11
33	2.21 ± 0.50	1.54 ± 0.10	90 ± 10	2.43 ± 0.19	1.22 ± 0.07	77 ± 6
48	0.90 ± 0.04	2.20 ± 0.05	93 ± 3	1.30 ± 0.25	1.77 ± 0.06	86 ± 13
72	0.76 ± 0.04	2.69 ± 0.10	103 ± 3	1.12 ± 0.18	2.53 ± 0.18	109 ± 11
$t_{1/2}$	24.4			30.5		
r	–0.9728			–0.9788		

[a] Transformation yield of captan in THPI.

Table 4. Degradation of Captan in THPI (Milligrams per Kilogram ± SD) in Aqueous Solution at pH 4 and 3

	pH 4			pH 3		
time (h)	captan	THPI	THPI (%)	captan	THPI	THPI (%)
0	5.78 ± 0.12	0	0	8.64 ± 0.80	0	0
3	4.77 ± 0.45	0.25 ± 0.03	49 ± 4	6.60 ± 1.08	0.28 ± 0.06	28 ± 5
6	4.61 ± 0.25	0.45 ± 0.04	76 ± 4	6.03 ± 1.23	0.49 ± 0.09	38 ± 7
12	4.08 ± 0.21	0.81 ± 0.08	94 ± 5	5.53 ± 1.40	0.88 ± 0.15	58 ± 14
18	3.30 ± 0.11	1.13 ± 0.07	90 ± 3	4.59 ± 0.44	1.21 ± 0.17	61 ± 6
24	2.78 ± 0.20	1.41 ± 0.05	93 ± 7	4.09 ± 0.22	1.54 ± 0.23	70 ± 4
48	1.30 ± 0.52	2.30 ± 0.08	102 ± 4	2.63 ± 0.28	2.57 ± 0.46	92 ± 9
72	0.43 ± 0.31	2.81 ± 0.27	104 ± 5	1.30 ± 0.32	3.34 ± 0.79	101 ± 11
$t_{1/2}$	20.1			29.2		
r	–0.9945			–0.9911		

Table 5. Degradation of Captan during Winemaking

		vinification with maceration		vinification without maceration		
sample		grapes (mg/kg)	wine (mg/kg)	grapes (mg/kg)	must (mg/kg)	wine (mg/kg)
1	captan	0.76 ± 0.21	<0.01	0.76 ± 0.21	0.54 ± 0.08	<0.01
	THPI	0.13 ± 0.03	0.48 ± 0.09	0.13 ± 0.03	0.16 ± 0.04	0.43 ± 0.13
2	captan	0.49 ± 0.10	<0.01	0.49 ± 0.10	0.39 ± 0.08	<0.01
	THPI	0.10 ± 0.01	0.28 ± 0.07	0.10 ± 0.01	0.14 ± 0.03	0.31 ± 0.05
3	captan	0.35 ± 0.19	<0.01	0.35 ± 0.19	0.31 ±0.10	<0.01
	THPI	0.08 ± 0.02	0.30 ± 0.09	0.08 ± 0.02	0.15 ± 0.02	0.30 ± 0.06

(*14*). Other data (*15*) show that the degradation of captan in a soil−water mixture was pH dependent.

To verify whether the degradative action of must is due to acidity, the degradation of captan was studied in a model system made up of an aqueous solution at the same pH previously considered for must. From the data reported in **Table 4**, we can see that captan had the same degradative behavior in must and in the model system with similar half-time values for both pH values studied. The obtained data indicate that the acidic activity of the must is responsible for the kinetic degradation of captan into THPI. Therefore, we can suppose that the detection of small amounts of THPI residues in grapes could be due to the degradation of captan during analysis or sampling, when the residues come into contact with the must leaking from crushed grapes. The analysis of pesticide residues usually requires the following steps: sample grinding, residue extraction, followed by purification if necessary, and chromatographic determination.

Because all samples are submitted to grinding before extraction, depending on the number of samples a significant interval of time could pass between grinding, when the captan comes into contact with the acid must, and extraction. This could explain the presence of small amounts of THPI assigned to the grapes. To verify this hypothesis, grape samples were treated, collected, and submitted to extraction without grinding to avoid the active ingredient contacting the acid must. First, the treatment solution was checked and no THPI residues were detected. The results obtained from this test, replicated five times, always showed a THPI residue of 0.05 mg/kg on grapes. These data showed that only a very low level of THPI was formed on the grapes. Therefore, the higher levels reported in the literature (0.1-0.3 mg/kg) can be attributed partly to residue formation in grapes and mainly to acid degradation.

Vinification. Before the vinification experiments were carried out, tests were performed to check whether the presence of THPI could have a negative effect on the fermentative microflora. Therefore, fermentation tests were carried out with the addition of a known quantity of THPI (4 mg/kg) on untreated grapes. After pressing, the must was left to ferment without any yeast addition. Fermentation had a regular course, and no significant difference was observed between the samples with THPI and the control samples. This indicates that unlike other thiophthalimide compounds, THPI does not show an antiseptic activity. Each sample was submitted to two vinification processes: vinification without maceration (the sample was dripped and the resulting must left to ferment) and vinification with maceration (the sample was allowed to ferment with skins). The data reported in **Table 5** for the vinification without maceration showed that in the must, mean values of captan residues are

lower than those present on grapes, whereas THPI residues were higher. This could be attributed to the action of acidity during pressing.

No detectable captan residues were found in wine obtained with the latter process, and only THPI was present in wine. THPI residues in wine are due to captan transformation caused by the acidity of must. Actually, summing up the amounts of THPI present in must with those of captan expressed as THPI, we can see that the results are not significantly different from the levels of THPI determined in wine. Analogous considerations can be made for vinification with maceration. THPI residues determined at 6 months and 1 year after fermentation were unchanged in wine.

Conclusions. The data reported in this study showed that the THPI residues determined in grapes were mainly due to captan degradation due to the acidic activity of the must. Only a low level was formed on grapes. The transformation can occur during either sampling or analysis. Model systems showed that the mechanism of disappearance of captan in grapes was mainly due to photodegradation and codistillation.

During winemaking captan degraded quantitatively in THPI, and at the end of fermentation only THPI residues were present in wine. This degradation product is very stable, and after 1 year, its concentration was unchanged. Therefore, the determination of the active ingredient in wine is unnecessary and only its metabolite should be considered.

LITERATURE CITED

(1) Cabras, P.; Angioni, A.; Garau, V. L.; Melis, M.; Pirisi, F. M.; Farris, G. A.; Sotgiu, C.; Minelli, E. V. Persistence and metabolism of folpet in grapes and wine. *J. Agric. Food Chem*. **1997**, *45*, 476−479.

(2) FAO/WHO. *Pesticide Residues in Food—2000. Evaluations Part I—Residues*; Paper 165; FAO: Rome, Italy, 2001; p 5−110.

(3) Frank, R.; Northover, J.; Braun, H. E. Persistence of captan on apples, grapes, and pears in Ontario, Canada, 1981−1983. *J. Agric. Food Chem*. **1985**, *33*, 514−518.

(4) Alary, J.; Bescos, D.; Monge, M. C.; Debrauwer, L.; Bories, G. F. Laboratory simulation of captan residues degradation during apple processing. *Food Chem.* **1995**, *54*, 205−211.

(5) Cabras, P.; Garau, V. L.; Pirisi, F. M.; Cubeddu, M.; Cabitza, F.; Spanedda, L. Fate of some insecticides from vine to wine. *J. Agric. Food Chem.* **1995**, *43*, 2613−2615.

(6) Arrizabalaga, P.; Castan, P.; Laurent, J. P. Intramolecular influence of a carboxylic function on platinum blue synthesis. A systematic study of complexes originating from acid amides. *J. Am. Chem. Soc.* **1984**, *106*, 4814−4818.

(7) Cabras, P.; Caboni, P. L.; Cabras, M.; Angioni, A.; Russo, M. F. M. Persitence of azadiracthin residues on olives after field treatment. *J. Agric. Food Chem*. **2002**, *50*, 3491−3494.

(8) Martinez Vidal, J. L.; Arrebola, F. J.; Mateu-Sànchez, M. Appliction to routine analysis of a method to determine multiclass pesticide residues in fresh vegetables by gas chromatography/tandem mass spectrometry. *Rapid Commun. Mass Spectrom.* **2002**, *16*, 1106−1115.

(9) Mocholì, F. *New Trends on Pesticide Residue Analysis by GC/MS and GC/MS/MS*; Varian Iberica: Valencia, Spain, 2001.

(10) Vargo, C.; Rightnour, B.; Linton, C. *An Alternative to Glass Wool Packings for GC Injection Liners*; Pittcon: Atlanta, GA, 1997.

(11) Cabras, P.; Angioni, A.; Garau, V. L.; Melis, M.; Pirisi, F. M.; Minelli, E. V. The effect of epicuticular waxes of fruits on the photodegradation of fenthion. *J. Agric. Food Chem*. **1997**, *45*, 3681−3683.

(12) Pirisi, F. M.; Angioni, A.; Cabizza, M.; Cabras, P.; Maccioni, E. Influence of epicuticular waxes on the photolysis of pirimicarb in solid phase. *J. Agric. Food Chem*. **1998**, *46*, 762−765.

(13) Pirisi, F. M.; Angioni, A.; Cabizza, M.; Cabras, P.; Falqui Cao, C. Photolysis of pesticides: influence of epicuticular waxes from *Persica laevis* DC on the photodegradation in the solid phase of aminocarb, methiocarb and fenthion. *Pest. Manag. Sci.* **2001**, *57*, 522−526.

(14) *Captan in Metabolic Pathways of Agrochemicals*; Roberts, T., Hutson, D., Eds.; The Royal Society of Chemistry, Cambridge, U.K., 1999; Part 2, pp 1351−1358.

(15) FAO/WHO. *Pesticide Residues in Food—2000. Evaluations Part I—Residues*; Paper 165; FAO: Rome, Italy, 2001; p 19.

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