Fate of Some Common Pesticides during Vinification Process

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This paper discusses the fate of the concentration of some of the most widely used pesticides (parathion-methyl, fenitrothion, dichlofluanid, chlorpyrifos, vinclozolin, chlozolinate, procymidone, iprodione, copper oxychloride) during the vinification process. The vines were treated 2 days before harvest to find the maximum levels of these pesticides possible and to make it easier to study the influence of the different enological operations on their dissipation. All organic pesticides showed a continuous decrease throughout the wine making process. Dichlofluanid, chlorpyrifos, chlozolinate, parathion-methyl, fenitrothion were nearly undetectable at the end of the vinification. Procymidone, vinclozolin, and iprodione were the most persistent pesticides. The fate of copper and of other metabolically related metals (iron, zinc, manganese) was also studied. In the treated must, the initially high levels of copper decrease drastically during the alcoholic fermentation, reaching levels similar to the controls.

Keywords: *Pesticides; parathion-methyl; fenitrothion; dichlofluanid; chlorpyrifos; vinclozolin; chlozolinate; procymidone; iprodione; copper oxychloride; vinification*

INTRODUCTION

Grapes that are to be used to make wine need to be treated with pesticides to protect the vines and grapes against attack by several blights (Carbonel-Grimbaum, 1989; Dominguez, 1993). However, these pesticides may then be present in wine and affect its suitability as a food (Gnaegi *et al.*, 1983; Cabanis and Cooper, 1991; Otero *et al.*, 1994). The presence of pesticides has also been associated with stuck and sluggish fermentations (Girond *et al.*, 1989; Larue, 1991; Otero *et al.*, 1993) and with problems in malolactic fermentation (Sarpis-Domercq, 1980). In the particular case of treatments with cupric salts, the additional problem of the appearance of cupric precipitates in wine must be considered (Hsia *et al.*, 1975). Generally it is accepted that the correct use of pesticides does not cause problems because pesticide levels in wine are much lower than the limits that can produce problems of this kind (Garcia-Cazorla and Xirau-Vayeda, 1994). Nevertheless, when the preharvest interval between treatments and harvest are not respected by the grape growers, the risk of having higher pesticide levels is not negligible. In this case, the higher levels of some pesticides can involve considerable economic losses if the maximum residue limits established by law are surpassed (Cabanis and Cooper, 1991).

Nowadays, the lack of international laws concerning pesticide residue limits in wine has prompted the Sub-Comission for the Unification of Analysis Methods and Appreciation of Wines (Office International de la Vigne et du Vin, OIV) to study the possibility of establishing maximum residue limits for wines (OIV, 1995). The aim of this work was to study, in must and wine, the levels of some of the most widely used pesticides in viticulture, when the preharvest intervals were not respected. The influence of the different enological operations on the fate of the pesticides has also been studied. The organic pesticides used in this study were parathion-methyl, fenitrothion, dichlofluanid, chlorpyrifos, vinclozolin, chlozolinate, procymidone iprodione, and copper oxychloride because they are the usual treatments against the most frequent blights such as grape berry moth *(Lobesia botrana)*, vine pyralid caterpillar *(Sparganothis pilleriana)*, downy mildew *(Plasmopara viticola)*, grey rot *(Botrytis cinerea)*, and oidium *(Uncinula necator)*.

The fate of the copper concentration, as an indicator of the presence of copper oxychloride, and of some metabolically related metals (iron, zinc, manganese) (Underwood, 1977; Brady *et al.*, 1994) were also studied to find out how this treatment affects the concentration of these metals during the vinification process.

MATERIALS AND METHODS

Chemicals. All the products were of high purity and suitable for gas chromatography and atomic absorption analysis. The standards for organic pesticide analysis were purchased from Ehrenstorfer (Augsburg, Germany). Internal standard (dieldrin) was obtained from the Institute of Organic Industrial Chemistry (Annopol, Poland).

Grapes. The work was carried out in the vineyard of the Escola d'Enologia de Tarragona (Universitat Rovira i Virgili) in the village of Constantí (Tarragona, Spain). Two plots were used: one planted with Macabeo grapes for white wine vinification and the other planted with Cabernet Sauvignon for red wine vinification. Both Macabeo and Cabernet Sauvignon vines were trained with goblet pruning. Both plots were divided into four subplots with one file of vines between subplots in order to prevent cross contamination. The first subplot was the control; the second was treated with four pesticides: parathion-methyl (Metil Parafene, Rhône-Poulenc, 100 mL/hL), fenitrothion (Sunithion, Argos, 150 mL/hL), dichlofluanid (Euparen, Bayer, 200 g/hL), and chlorpyrifos (Dursban, Sandoz, 200 mL/hL). The third subplot was treated with copper oxychloride (Cupagrex 50 PM, Sadisa, 3 kg/ha), and the fourth subplot was treated with vinclozolin (Ronilan, Basf, 150 g/hL), chlozolinate (Serinal, Agrimont, 150 g/hL), procymidone (Subimoto, Agrocos, 100 g/hL), and iprodione (Rovral, Rhône-Poulenc, 1.5 L/ha). Two days after treatment, the grapes were harvested and used for wine making.

Methods of Vinification. Laboratory-scale vinifications of the Macabeo grapes and the Cabernet Sauvignon grapes

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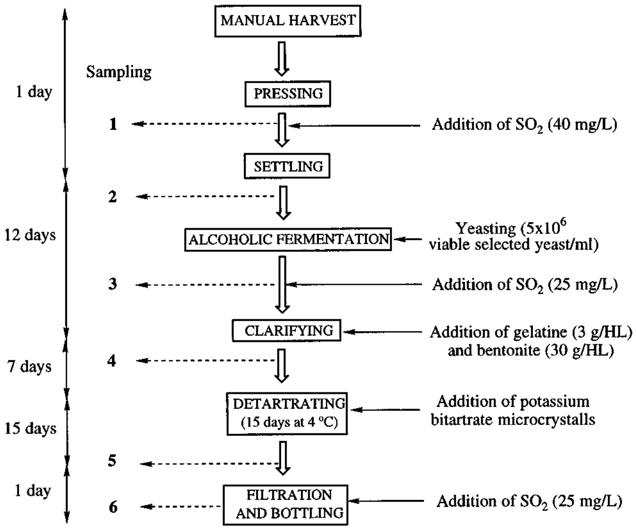

Figure 1. Flowsheet and sampling for white wine vinification.

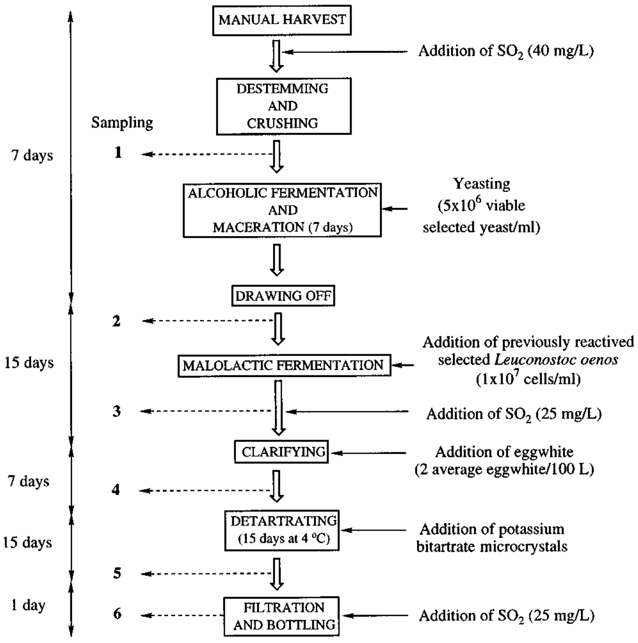

Figure 2. Flowsheet and sampling for red wine vinification.

were carried out with the usual white and red wine methods, respectively. A detailed outline of sampling and methods of vinification is given in Figures 1 and 2, respectively. For each vinification, five replicates of 25 kg of grapes were used. Both kinds of vinification process were performed at room temperature.

All the samples were conserved at −20 °C until the end of the vinification process and then used for analytical determinations. All the data are expressed as the arithmetic average of the five vinifications ± SEM.

Analytical Procedures. Parathion-methyl, fenitrothion, dichlofluanid, chlorpyrifos, vinclozolin, chlozolinate, procymidone, and iprodione were determined by gas chromatography, according to Sala *et al.* (1996). Samples of must and wine were extracted with *n*-hexane using dieldrin as the internal standard. Chromatographic analyses were performed with a Hewlett-Packard 5890 II gas chromatograph equipped with an electron capture detector.

A SPB-5 (30 m × 0.25 mm i.d., 0.25 μm ft) fused-silica capillary column was used. The carrier gas was high-purity helium (head pressure, 140 kPa; split flow, 80 mL/min; purge flow, 5 mL/min). The make-up gas was nitrogen (45 mL/min). The injector and detector were held at 250 and 350 °C, respectively. Oven temperature was isothermal (210 °C).

Copper, iron, zinc, and manganese from the different samples were analyzed by atomic absorption spectrophotometry after digestion with ultrapure nitric acid (Merck, Darmstadt, Germany) for 24 h at 120 °C (Akirida-Demertzi *et al.*, 1988).

RESULTS AND DISCUSSION

There were no important differences between the kinetics of the fermentations of the differently treated musts and the controls, indicating that the high initial levels of the pesticides in treated musts do not inhibit yeast metabolism under these vinification conditions. The L-malic acid in all the experimental groups used during red wine vinification disappeared completely with no problems, which showed that the presence of these pesticides did not affect malolactic fermentation.

White Wine Vinification. The fate of parathion-methyl, fenitrothion, dichlofluanid, chlorpyrifos, vinclozolin, chlozolinate, procymidone, and iprodione during white wine vinification is shown in Table 1. All these pesticides continuously decreased throughout the process, and it was the operations of settling and alcoholic fermentation which most affected this decrease. All the percentages of decrease of the pesticides described below are expressed as the decrease during the indicated vinification step referred to the initial concentration.

The settling caused chlorpyrifos to completely disappear and the levels of dichlofluanid and chlozolinate to fall quite considerably (decreases of 85 and 70%, respectively). The levels of fenitrothion and parathion-methyl also fell (decreases of 40 and 25%, respectively). However, all the antibotrytis pesticides were only slightly affected by settling: iprodione (15%), vinclozolin (13%), and procymidone (4%).

Alcoholic fermentation also had a considerable effect on pesticide concentrations. Dichlofluanide and chlozolinate were below the detection limit by the end of alcoholic fermentation. The rest of the pesticides also dropped considerably by the end of the alcoholic fermentation: parathion-methyl (77%), fenitrothion (69%), vinclozolin (52%), procymidone (36%), and iprodione (35%).

The amounts of the pesticides continuously decreased throughout the rest of the wine-making process (the clarifying, the detartrating, and the sterilizing filtration steps). Dichlofluanid, chlorpyrifos, chlozolinate, parathion-methyl, and fenitrothion were nearly undetectable by the end of the vinification. Procymidone, vinclozolin, and iprodione, on the other hand, were the most persistent pesticides and appeared in bottled wines at levels of close to 1 ppm. It is necessary to remark that these results were obtained when preharvest intervals were not respected and cannot be extrapolated to conditions of correct use of these pesticides.

The fate of copper, iron, zinc, and manganese is shown in the Table 2. At the beginning of white wine vinification, the copper concentration in the treated must was very much higher than in the controls. Settling does not result in a very important decrease in the amount of copper (67%). During the fermentation step, the initially very high levels of copper in the treated must decreased drastically (92%) and at the end of the alcoholic fermentation reached levels similar to the controls. This substantial decrease in copper levels may be due to the capacity of yeast to bind and take up this metal and to the capacity of copper to combine with sulfurous compounds, produced by yeast metabolism, and then to precipitate (Bidan and Collon, 1985; Cabras *et al.*, 1995). The fate of iron, zinc, and manganese from treated musts was not very different from the controls,

Table 1. Fate of Pesticides during White Wine Vinification[a]

VS[b]	PM	FT	DF	CP	CZ	VC	PC	IP
1	60 ± 7	170 ± 32	629 ± 91	93 ± 20	890 ± 76	2218 ± 99	2043 ± 102	2510 ± 138
2	45 ± 5	103 ± 6	93 ± 22	nd[c]	266 ± 9	1920 ± 48	1955 ± 29	2138 ± 118
3	14 ± 2	53 ± 7	nd	nd	nd	1071 ± 32	1316 ± 79	1619 ± 227
4	10 ± 1	42 ± 3	nd	nd	nd	830 ± 33	896 ± 36	1298 ± 123
5	8 ± 1	38 ± 4	nd	nd	nd	705 ± 25	776 ± 27	1202 ± 132
6	3 ± 1	21 ± 4	nd	nd	nd	585 ± 38	701 ± 39	1164 ± 111

[a] All data are the mean ± SEM of five different vinifications. Pesticide concentration is expressed in micrograms per liter. PM, parathion-methyl; FT, fenitrothion; DF, diclofluanid; CP, chlorpyrifos; CZ, chlozolinate; VC, vinclozolin; PC, procimydone; IP, iprodione. [b] VS, vinification steps: (1) the must after pressing; (2) the must after settling; (3) the wine at the end of alcoholic fermentation; (4) the wine after the clarifying process; (5) the wine after the detartrating process; (6) the wine after sterilizing, filtering, and bottling. [c] nd, nondetectable.

Table 2. Fate of Copper, Iron, Manganese, and Zinc during White Wine Vinification[a]

	Cu (mg/L)		Fe (mg/L)		Mn (mg/L)		Zn (mg/L)	
VS[b]	C	T	C	T	C	T	C	T
1	1.45 ± 0.16	46.00 ± 2.64	1.89 ± 0.10	1.67 ± 0.49	0.26 ± 0.01	0.35 ± 0.01	0.59 ± 0.02	0.38 ± 0.03
2	1.20 ± 0.11	43.00 ± 2.51	2.71 ± 0.17	1.32 ± 0.11	0.28 ± 0.01	0.32 ± 0.01	0.46 ± 0.04	0.33 ± 0.01
3	0.17 ± 0.07	0.46 ± 0.15	2.05 ± 0.14	1.78 ± 0.28	0.29 ± 0.02	0.34 ± 0.01	0.38 ± 0.03	0.25 ± 0.02
4	0.17 ± 0.08	0.35 ± 0.11	2.99 ± 0.25	3.37 ± 0.33	0.38 ± 0.01	0.45 ± 0.02	0.52 ± 0.02	0.30 ± 0.01
5	0.16 ± 0.08	0.28 ± 0.10	2.76 ± 0.23	2.54 ± 0.28	0.39 ± 0.01	0.44 ± 0.01	0.23 ± 0.02	0.17 ± 0.01
6	0.17 ± 0.09	0.37 ± 0.14	2.45 ± 0.24	3.26 ± 0.40	0.36 ± 0.01	0.49 ± 0.02	0.35 ± 0.02	0.24 ± 0.02

[a] All data are the mean ± SEM of five different vinifications. Concentration in micrograms per liter. C, control; T, treated. [b] VS, vinification steps: (1) the must after pressing; (2) the must after settling; (3) the wine at the end of alcoholic fermentation; (4) the wine after the clarifying process; (5) the wine after the detartrating process; (6) the wine after sterilizing, filtration, and bottling.

Table 3. Fate of Pesticides during Red Wine Vinification[a]

VS[b]	PM	FT	DF	CP	CZ	VC	PC	IP
1	281 ± 11	1372 ± 130	1474 ± 176	1866 ± 299	3507 ± 228	4600 ± 207	3553 ± 266	1963 ± 79
2	33 ± 1	146 ± 7	nd	6 ± 1	nd	912 ± 41	1442 ± 123	1632 ± 122
3	21 ± 3	122 ± 16	nd	4 ± 1	nd	796 ± 20	1369 ± 110	1656 ± 141
4	10 ± 1	68 ± 4	nd	nd	nd	445 ± 13	720 ± 54	942 ± 75
5	9 ± 1	69 ± 8	nd	nd	nd	398 ± 14	702 ± 21	714 ± 57
6	4 ± 1	39 ± 6	nd	nd	nd	293 ± 19	588 ± 35	642 ± 74

[a] All data are the mean ± SEM of five different vinifications. Pesticide concentration is expressed in micrograms per liter. PM, parathion-methyl; FT, fenitrothion; DF, diclofluanid; CP, chlorpyrifos; CZ, chlozolinate; VC, vinclozolin; PC, procimydone; IP, iprodione. [b] VS, vinification steps: (1) the must after destemming and crushing; (2) the wine after drawing off; (3) the wine at the end of malolactic fermentation; (4) the wine after the clarifying process; (5) the wine after the detartrating process; and (6) wine after sterilizing, filtration, and bottling.

Table 4. Fate of Copper, Iron, Manganese, and Zinc during Red Wine Vinification[a]

	Cu (mg/L)		Fe (mg/L)		Mn (mg/L)		Zn (mg/L)	
VS[b]	C	T	C	T	C	T	C	T
1	0.39 ± 0.06	10.6 ± 0.44	4.40 ± 0.32	4.20 ± 0.51	1.01 ± 0.04	1.50 ± 0.07	0.94 ± 0.06	0.80 ± 0.07
2	0.35 ± 0.03	0.12 ± 0.06	5.57 ± 0.28	4.87 ± 0.17	0.62 ± 0.02	0.80 ± 0.03	0.06 ± 0.03	0.09 ± 0.05
3	0.17 ± 0.04	0.02 ± 0.01	6.54 ± 0.24	5.57 ± 0.12	0.67 ± 0.02	0.82 ± 0.03	0.14 ± 0.05	0.11 ± 0.05
4	0.05 ± 0.02	0.02 ± 0.01	7.61 ± 0.32	5.88 ± 0.14	0.62 ± 0.02	0.75 ± 0.01	0.05 ± 0.03	0.04 ± 0.02
5	0.02 ± 0.01	0.01 ± 0.01	6.39 ± 0.29	5.39 ± 0.07	0.61 ± 0.02	0.78 ± 0.02	0.05 ± 0.03	0.02 ± 0.01
6	0.03 ± 0.02	0.01 ± 0.01	5.36 ± 0.28	5.74 ± 0.10	0.68 ± 0.03	0.91 ± 0.04	0.09 ± 0.05	0.02 ± 0.01

[a] All data are the mean ± SEM of five different vinifications. Concentrations in microgams per liter. C, control; T, treated. [b] Vinification steps: (1) the must after destemming and crushing; (2) the wine after drawing off; (3) the wine at the end of malolactic fermentation; (4) the wine after the clarifying process; (5) the wine after the detartrating process; (6) wine after sterilizing, filtering, and bottling.

which shows that the initially very high levels of copper did not affect the concentration of these metabolically related metals.

Red Wine Vinification. In general terms, at the beginning of the wine-making process, the levels of the pesticides were higher in red wine vinification than in white wine vinification, probably due to the skin maceration that takes place in red wine fermentation.

The fate of parathion-methyl, fenitrothion, dichlofluanid, chlorpyrifos, vinclozolin, chlozolinate, procymidone, and iprodione during red wine vinification is shown in Table 3. All these pesticides showed a continuous decrease throughout the red wine-making process, and it was the alcoholic fermentation that most affected their decrease.

The alcoholic fermentation caused chlorpyrifos, dichlofluanid, and chlozolinate to completely disappear and also considerably reduced the amounts of parathion-methyl (88%), fenitrothion (89%), vinclozolin (80%), and procymidone (59%). Iprodione was less affected by alcoholic fermentation (decrease of 17%).

The malolactic fermentation, clarifying, detartrating, and sterilizing filtration steps considerably reduced the pesticides present and was specially significant in the case of iprodione and procymidone (decreases of 50 and 24%, respectively).

Dichlofluanid, chlorpyrifos, chlozolinate, parathion-methyl, and fenitrothion were virtually undetectable by the end of the red wine vinification. Procymidone, vinclozolin, and iprodione were, as in the white wine vinification, the most persistent pesticides and appeared in the bottled wine at levels of close to 1 ppm.

The fates of copper, iron, zinc, and manganese of control and treated must (Table 4) were very similar to that in white wine vinification. Therefore, it may be attributed to the same reasons as in white wine vinification.

All these results seem to indicate that dichlofluanid, chlorpyrifos, chlozolinate, parathion-methyl, fenitrothion, and copper oxychloride disappear completely during white and red wine vinification, even when the safety periods between treatment and harvest are not respected. However the three specific antibotrytis pesticides (procymidone, vinclozolin, iprodione) were much more persistent and their presence in bottled wine was in some cases higher than the maximal levels established by law. These results agree, in general terms, with the results obtained from other studies (Cabras *et al.*, 1983; Flori and Cabras, 1990). Therefore, the treatment of vines with procymidone, vinclozolin, or iprodione must rigorously respect the safety periods if they are not to appear in bottled wine. This is really quite a major problem since at the end of the grape maturation process, the risk of grey rot is at its greatest because of the usual rains in September and October. In this situation, grape growers may be tempted to treat their vines with these products in order to protect their production.

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