Evolution of Chlorpyrifos, Fenarimol, Metalaxyl, Penconazole, and Vinclozolin in Red Wines Elaborated by Carbonic Maceration of Monastrell Grapes

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The influence of the different steps involved in the wine-making process on the disappearance of chlorpyrifos, fenarimol, metalaxyl, penconazole, and vinclozolin in red wines elaborated by carbonic maceration of Monastrell grapes was studied. The initial levels of the residues in grapes ranged from 0.28 mg/kg (penconazole) to 1 mg/kg (chlorpyrifos). Ten days after the beginning of maceration, the compound that had decreased least was chlorpyrifos (83% of initial value remaining), whereas metalaxyl, the least persistent of the residues, had decreased to 49% of its initial value. In the free-run juice, on the other hand, the highest percentage remaining corresponded to metalaxyl (10%) and the lowest to chlorpyrifos (0.1%). After pressing, the percentages of initial values eliminated in pomace varied from 82.7% for chlorpyrifos to 17.7% for metalaxyl, whereas in the press juice the opposite was the case (37% metalaxyl and 2% chlorpyrifos remaining). In finished wine, there were residues of all the pesticides, with the exception of chlorpyrifos, metalaxyl (21%) being the most persistent. The percentages eliminated in the lees varied from 1.5 to 2.5% of the initial value.

Keywords: *Carbonic maceration; red wine; pesticide residues; wine-making procedures*

INTRODUCTION

Recent years have seen a change in people's taste for wine, and consumers increasingly demand greater quality. Vintners have responded by manufacturing new types of wine and by generally widening the range of products they offer. One such novelty is to produce wine by carbonic maceration, although the technique itself is not new, having been introduced in France >60 years ago (Flanzy, 1935).

The system is based on the phenomena that take place naturally inside grape berries when they are kept intact in an anaerobic environment. The result is a richer wine of greater aromatic quality having a greater content of phenolic compounds, all of which lend personality to the product (Jackson et al., 1978; Flanzy et al., 1987; Tesnier et al., 1989; La Notte et al., 1992; Fondville-Bagnol, 1996; Valerio, 1997; Lorincz and Vas, 1998).

There has been much research into determining the best conditions for obtaining quality wines by carbonic maceration. Among the parameters and variables studied have been the following: harvesting and conditions of transporting the grapes (Flanzy et al., 1986); temperature and length of the anaerobic step (Zuñel, 1994; Lorincz et al., 1997); and length of the aging process in casks (Salinas et al., 1996; Jimeno, 1997). However, there are no data available concerning the influence of pesticide residues on the kinetics of the fermentation process or the changes in the levels of these compounds during the carbonic maceration vinification process, although numerous studies have been made of their evolution during traditional wine-making methods (Cabras et al., 1987, 1997, 1998; Goodwin and Ahmand, 1998; Navarro et al., 1997, 1999).

For the reason set out above, we studied the time course changes in four fungicides (fenarimol, metalaxyl, penconazole, and vinclozolin) and one insecticide (chlorpyrifos) frequently used on vines during the fermentation process using carbonic maceration and the effect of the different wine-making steps on their elimination.

MATERIALS AND METHODS

Phytosanitary Treatments and Sampling. In September 1998, the phytosanitary treatments (three replications) specified in a previous paper (Navarro et al., 1999) were carried out under the same conditions with the exception of Ventine MZ (80% mancozeb). The trial was carried out in an experimental plot located in Jumilla, Murcia (southeastern Spain) in a 15-year-old plantation of *Vitis vinifera* (cv. Monastrell). The samples were taken 24 h after application.

Wine-Making Procedure. Intact bunches of freshly harvested grapes were weighed into three replicates of 10 kg and allowed to ferment in carbonic anaerobiosis in separate fermentation vessels, and the atmosphere of each one was immediately saturated with carbon dioxide. This operation was carried out by injecting the gas very slowly through a valve located in the lower part of the vessel to ensure total displacement of the oxygen. The vessels were maintained at 28−30 °C for 10 days. After this time, the free-run juice and the bunches were devatted and the mixture was crushed and pressed. The press must obtained was introduced in new vessels, which were maintained at 20−22 °C until the end of fermentation. Five days later, racking was carried out to separate the wine from the lees. The weights recorded (kilograms ± SD, $n = 3$) after each of the wine-making steps were

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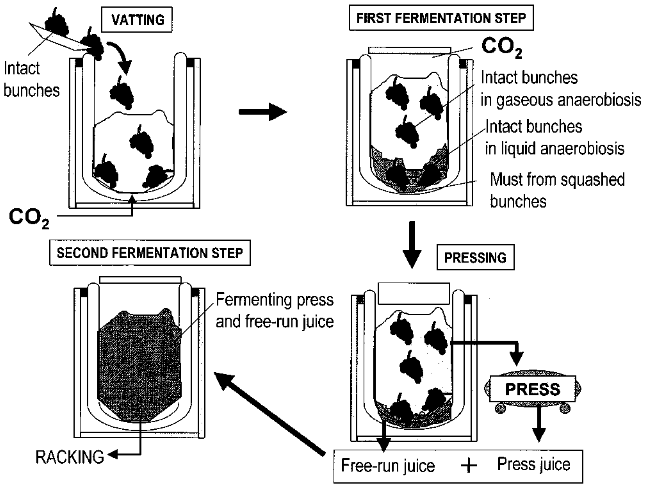

Figure 1. Scheme for carbonic maceration wine-making.

as follows: grapes after 10 days, 8.81 ± 0.11; free-run juice at 10 days, 1.18 ± 0.10; grapes after crushing, 8.49 ± 0.11; after pressing, 2.55 ± 0.13 for pomace and 6.22 ± 0.13 for must; and after racking, 0.46 ± 0.01 for lees and 4.67 ± 0.15 for wine. Several times during the whole process samples of grapes, must (free-run and press juice), wine, pomace and lees were taken to study the levels of pesticide residues. Figure 1 shows the procedure followed for wine-making by carbonic maceration.

Extraction and Analysis of Pesticide Residues. A micro on-line method was used to extract the pesticides in grapes, must, and wine. Chlorpyrifos, fenarimol, penconazole, and vinclozolin were determined by GC-ECD, and metalaxyl was determined by GC-MSD in the conditions mentioned in a previous paper (Oliva et al., 1999).

RESULTS AND DISCUSSION

Evolution of Residue Levels. Table 1 shows the residual concentrations found during the wine-making process from harvest to racking. By taking into account the measurements made after crushing, pressing, and racking, the total quantities of residues (milligrams) present in each vessel were calculated (see Table 2) to ascertain changes in residue levels during the wine-making process as a whole. The total quantity present in the recently harvested grapes was considered the 100% value in all cases and was used to calculate the disappearance of the pesticides studied.

As can be seen from Table 2, remaining quantities at the end of sampling in the racked wine did not exceed 2.5% for any product except metalaxyl (21%).

The residual level of chlorpyrifos in the must wine one separated from the pomace was 0.03 ppm (1.8% of that in the freshly harvested grapes) and less than the maximum residue limit (MRL) in grapes (0.5 ppm). After racking of the wine, no residues of this insecticide were found.

With regard to fenarimol, 85% of the values found in the must were eliminated during the final phase of fermentation. After racking, 1.9% of the initial quantity found in freshly harvested grapes remained in the wine.

Of the five products studied, metalaxyl was the most persistent. The residual level in must was still high (0.36 ppm) and very close to the MRL in grapes (0.5 ppm), and even the levels detected in the racked wine (0.26 ppm) were much higher than those of the other products, The losses metalaxyl undergoes during fermentation are low (28%), and 42% of the initial level found in treated grapes remains in the finished wine, confirming its low degradation kinetic. Kakalikova (1994), too, found it in wine produced 4 years previously from grapes that had been treated with the recommended doses 14, 28, 35, and 42 days before harvesting.

The levels of penconazole remaining in must after pressing were already below the MRL of 0.2 ppm. These fell to 0.01 ppm after racking, which represents 33% of the concentration measured in the must after pressing and 2.5% of the initial value in grapes. Other fungicides of the triazole family such as propiconazole also decrease by as much as 90% of the initial value in red wines (FAO/WHO, 1988), which is similar to our results with penconazole. In the case of tebuconazole, initial levels fell by ~50% by the time the finished wine was obtained (Cabras et al., 1997). The levels of tetraconazole, a member of the triazole family and widely used in vine, fell by 100% of the initial value found in grapes (Cabras et al., 1998).

Finally, the remaining concentration of vinclozolin (which has a relatively high MRL of 5 ppm) was also below that recorded in the harvested grapes. After fermentation of the must and following racking, ~20% of the initial concentration found in the must remained, whereas the final level in the finished wine represented ~1% of the value observed in grapes.

Influence of Oenological Procedures. Figures 2−4 show the levels of the five products studied that remained after the different wine-making steps.

One of the key steps in producing a wine by carbonic maceration and one which is largely responsible for the characteristics of the wine thus obtained is the anaerobic step in a CO_2 atmosphere. Halfway through this stage, the values of the phytosanitary products remaining are practically the same as the initial values, indicating that the lack of oxygen prevents their degradation during the first days of the process.

Table 1. Residual Concentrations (n = 3, Milligrams per Kilogram) Found in the Different Control Stages

stage	$\bar{x}$ ± SD				
	vinclozolin	metalaxyl	penconazole	chlorpyrifos	fenarimol
grapes at harvest	0.974 ± 0.110	0.609 ± 0.035	0.284 ± 0.037	1.002 ± 0.0144	0.370 ± 0.080
maceration, 5 days	0.965 ± 0.182	0.597 ± 0.041	0.291 ± 0.003	1.338 ± 0.023	0.356 ± 0.032
bunches	0.826 ± 0.027	0.338 ± 0.015	0.176 ± 0.009	0.946 ± 0.011	0.254 ± 0.008
free-run juice	0.079 ± 0.008	0.509 ± 0.022	0.040 ± 0.011	0.006 ± 0.001	0.059 ± 0.008
crushed grapes	0.969 ± 0.112	0.415 ± 0.035	0.197 ± 0.027	0.993 ± 0.134	0.252 ± 0.028
pomace	2.343 ± 0.428	0.399 ± 0.016	0.507 ± 0.089	3.072 ± 0.391	0.483 ± 0.028
press must	0.094 ± 0.024	0.357 ± 0.013	0.045 ± 0.006	0.028 ± 0.003	0.077 ± 0.009
must, 2 days	0.036 ±0.004	0.320 ± 0.007	0.023 ± 0.004	0.011 ± 0.002	0.044 ± 0.007
must, 4 days	0.023 ± 0.001	0.287 ± 0.025	0.023 ± 0.004	0.008 ± 0.001	0.027 ± 0.004
racked wine	0.019 ± 0.006	0.258 ± 0.014	0.015 ± 0.001	BDL[a]	0.015 ± 0.003
lees	0.325 ± 0.064	0.287 ± 0.011	0.152 ± 0.009	0.405 ± 0.018	0.125 ± 0.004

[a] Below detection limit (0.005 ng/μL).

Table 2. Amount (Milligrams) of Pesticide and Percentage Remaining ($n = 3$) in the Whole Weight of Sample (Kilograms) for Each Control Stage

	vinclozolin		metalaxyl		penconazole		chlorpyrifos		fenarimol	
stage	mg	%	mg	%	mg	%	mg	%	mg	%
grapes at harvest	9.74	100	6.09	100	2.84	100	10	100	3.7	100
maceration, 5 days	9.65	99.1	5.97	98.03	2.91	102	10.4	103	3.56	96.2
bunches	7.27	74.6	2.97	48.8	1.55	54.6	8.32	83	2.28	61.6
free-run juice	0.44	9.65	0.61	10	0.05	1.76	0.01	0.1	0.07	1.89
crushed grapes	8.24	84.6	3.53	57.9	1.67	58.8	8.44	84.2	2.4	64.9
pomace	6.34	65.1	1.08	17.7	1.37	48.2	8.29	82.7	1.3	35.1
press must	0.59	6.06	2.25	36.9	0.28	9.86	0.18	1.8	0.48	12.9
must, 2 days	0.22	1.87	1.89	31	0.14	4.93	0.07	0.7	0.28	7.57
must, 4 days	0.14	1.44	1.81	29.7	0.14	4.93	0.05	0.5	0.17	4.59
racked wine	0.1	0.76	1.29	21.2	0.07	2.46	BDL[a]	BDL	0.07	1.89
lees	0.15	1.54	0.13	2.13	0.07	2.46	0.18	1.8	0.06	1.62

[a] Below detection limit.

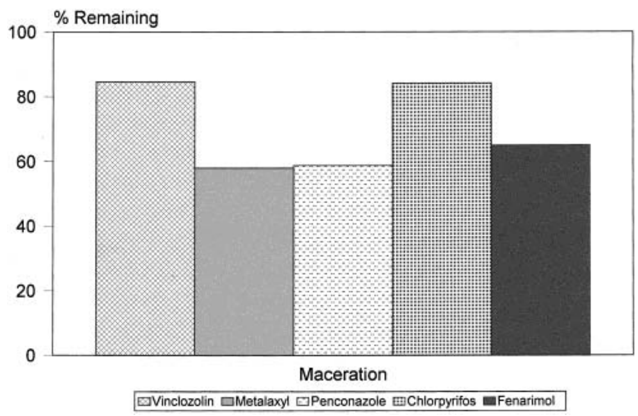

Figure 2. Percentage of pesticide residues remaining after maceration.

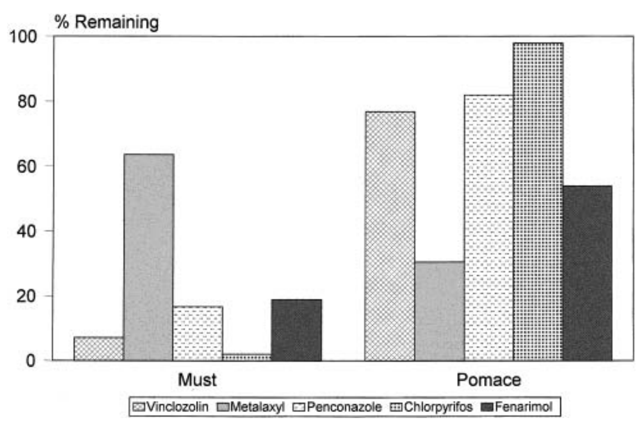

Figure 3. Percentage of pesticide residues remaining after pressing.

At the end of this anaerobic maceration step and after crushing of the mixture formed by bunches and free-run juice, the remaining levels vary from 58% for metalaxyl to 85% for vinclozolin. We found no bibliographical references to the reduction of residue levels during carbonic maceration, although many exist (albeit contradictory at times) for the traditional maceration procedures. For example, Flori et al. (1982) and Cabras et al. (1986) pointed to decreases in vinclozolin and other dicarboximidic fungicides, metalaxyl and other phenylamides, penconazole, and fenarimol, whereas other studies (Miller et al., 1985; Cabras et al., 1995) concluded that there were no significant differences in the levels of methiocarb and some organophosphorus insecticides (chorpyrifos, methidathion, quinalphos, and parathion-methyl) whether or not maceration was carried out. In the case of cyprodinil, pyrimethanil, and tebuconazole, the maceration step had no influence, whereas fludioxonil was eliminated to a greater extent (Cabras et al., 1997).

With regard to the presence of residues in the free-run juice liberated during maceration, metalaxyl and vinclozolin were the compounds that remained in the highest concentrations (10 and 9.6%, respectively, of the initial levels). The great difference between the levels of these products and those of the other product studied (0.1−1.9%) may be related with their greater solubility in hydroalcoholic solutions, as has been observed in studies of traditional wine-making processes (Navarro et al., 1997). This is confirmed by the data referring to metalaxyl levels in must.

Pressing eliminates most of the residues initially found in grapes because they are mostly on the skin. Figure 3 shows the relative proportions of the residues of the five products studied in the two fractions obtained during wine-making (must and pomace). The data were calculated by considering the levels in crushed grapes as 100%. As can be seen, chlorpyrifos (98%), penconazole (82%), and vinclozolin (77%) maintained high levels in the pomace, whereas the other two products fell to <55%. According to several authors (Flori and Cabras, 1990; Scarponi and Martinetti, 1992; Lanari et al., 1993), some products such as penconazole, fenarimol, vinclozolin, and metalaxyl are easily adsorbed by the pomace so that during pressing they are more likely to be lost than other products, findings that coincide with ours.

The levels found in must after pressing vary from 2 to 20% with the exception of metalaxyl (64%), which probably remains dissolved in the must after maceration due to its high water solubility (7.1 g/L).

Fermentation, too, plays an important part in reducing pesticide levels. In a racked wine, for example, some of the losses are due to metabolic degradation and some pesticide resudies are retained in the lees. Figure 4 shows the distribution on the residues in lees and racked wine, the value remaining in the must being taken as the 100% value.

Fenarimol levels fell substantially during fermentation (72.9%), whereas the amount eliminated with the racking was 12.5%, that remaining in the wine representing 14.6% of the initial value in must.

The elimination of chlorpyrifos followed a different course, this substance being totally (100%) trapped in the lees after racking.

At the end of the wine-making process, the amount of pesticides remaining in the racked wine varied from

Table 3. Percentage Remaining in Traditional (T) and Carbonic Maceration (CM) Wine-Making: Comparative Study

	vinclozolin		metalaxyl		penconazole		chlorpyrifos		fenarimol	
stage	T	CM	T	CM	T	CM	T	CM	T	CM
grapes	100	100	100	100	100	100	100	100	100	100
midmaceration	71	99	95	98	88	100	93	100	67	96
must	27	6	69	37	24	10	13	2	30	13
pomace	27	65	24	18	38	48	34	83	21	35
wine	17	1	51	21	13	2	1	0	13	2
lees	8	1	17	2	10	2	11	2	6	2

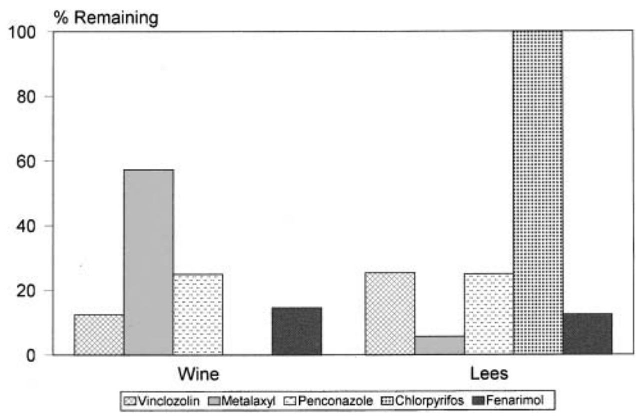

Figure 4. Percentage of pesticide residues remaining after racking.

0% (chlorpyrifos) to 57.3% (metalaxyl) of the initial values present in the must. The respective values of the three other products were 24.9% (penconazole), 14.6% (fenarimol), and 12.5% (vinclozolin).

Comparison between Pesticide Dissipation in Traditional and Carbonic Maceration Wine-Making. In this last section we compare the dissipation of the five products studied during a series of steps common to both wine-making processes, using the data obtained in the first part of this study and the results of a similar study of a traditional process (Navarro et al., 1999). Table 3 shows the comparative results.

From this table, it can be appreciated that the values remaining halfway through the process of maceration are higher in the carbonic method. This can be explained by taking into account the fact that the degradation reactions mostly occur in aqueous phase and that in the first 5 days of carbonic maceration most of the grape berries remain intact so that there is no great transfer of pesticide from the solid to the liquid phase.

Hardly any degradation, therefore, takes place during this time, whereas in the maceration step of the traditional process there is close contact between both phases from the very beginnig, and so residues are eliminated more readily.

However, after pressing, the quantities remaining in the must of the carbonic method are clearly lower. This change in the pattern of elimination is most probably due to the fact that the solid phase which, of course, contains the greatest quantity of pesticide, after devatting and crushing of the intact bunches and free-run juice, does not remain in contact with the must wine for very long so that the transfer of active ingredients to the hydroalcoholic phase is very limited. Consequently, the residual values in pomace for carbonic wine-making are higher except in the case of metalaxyl.

With regard to the racking step, the quantities remaining in both lees and wine of the carbonic method are 5−10 times lower except in the case of metalaxyl, for which reduction is only half of that observed for the other products. This is due to the fact that the residual values in must are lower in the carbonic method.

Table 4. Statistical Parameters (Dissipation Rate Constant and Half-Life Time in Days) Derived from the Lineal Fit Carried out during the Fermentation of the Must in Traditional (T) and Carbonic Maceration Wine-Making (CM)

	T		CM	
compound	K	t_2	K	t_2
vinclozolin	−0.0298	23.2	−0.2188	3.2
metalaxyl	−0.0135	51.3	−0.0061	11.4
penconazole	−0.0331	20.9	−0.1415	4.9
chlorpyrifos	−0.1578	4.4	−0.3132	2.2
fenarimol	−0.0321	21.6	−0.2325	2.9

Finally, to illustrate the rate of residue dissipation in must wine, Table 4 shows the statistical parameters derived from fitting the experimental data according to a pseudo-first-order kinetic (Timme and Freshe, 1980). The period covered is that between pressing and the moment when the finished wine is obtained, which is 17 days in the case of the traditional method and 6 days in the case of the carbonic maceration method.

The values show the good correlation existing between ln R_t and time ($r > 0.93$), which is confirmed when the real initial concentrations are compared with those calculated theoretically (R_0), small variations of between 0.003 (for chlorpyrifos) and 0.048 (for metalaxyl) being obtained. In all cases the greater dissipation rate was observed with the carbonic method, which means a considerable decrease in their half-life time.

On the basis of the findings, the following order of dissipation can be established:

carbonic maceration: chlorpyrifos > fenarimol > vinclozolin > penconazole > metalaxyl

traditional: chlorpyrifos > penconazole > fenarimol > vinclozolin > metalaxyl

As can be seen, chlorpyrifos dissipates rapidly in both methods, whereas metalaxyl disappears very slowly.

Conclusions. The residual values of all the compounds studied were higher halfway through maceration in the carbonic method. After pressing, on the other hand, the residual concentrations in must were lower in the carbonic method. For this reason, with the exception of metalaxyl, the residual values in pomace were higher in the carbonic method, which must be taken into account if this byproduct is to be used for animal feed. In the finished wine, the residual values were always lower for the wine produced by carbonic maceration. With regard to the dissipation of residues during the fermentation stage, the product that disappears most rapidly is chlorpyrifos in both methods, whereas metalaxyl has the slowest kinetics of dissipation. As in traditional wine-making processes, the importance of correctly carrying out the different wine-making stages of the carbonic method so that residues are properly reduced is manifest.

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