Chlozolinate Fate during Vinification Process

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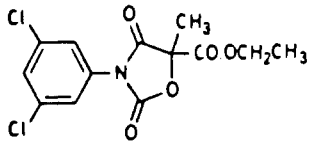
The influence of two types of vinification processes (with and without maceration) on the residual behavior of chlozolinate [ethyl (*R,S*)-3-(3,5-dichlorophenyl)-5-methyl-2,4-dioxooxazolidine-5-carboxylate] and its metabolite [3-(3,5-dichlorophenyl)-5-methyloxazolidine-2,4-dione] was studied. No chlozolinate was found in either case after the second racking. Racking greatly reduced also the metabolite content which seems not to be affected by fermentation. The metabolite was almost completely eliminated (97–100%) by clarifying with charcoal, whereas a mixture of bentonite and gelatine reduced its content slightly (26–36%). A mixed bentonite–charcoal–gelatin system led to partial elimination of the metabolite (82–90%). On bottling, the metabolite was absent only in the wine treated with 1 g/L charcoal, whereas its concentration ranged between 0.01 and 0.27 ppm in the other cases. No change in metabolite concentration was found after 6 months of storage.

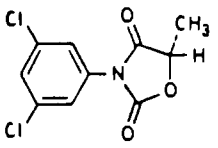
INTRODUCTION

Chlozolinate [ethyl (*R,S*)-3-(3,5-dichlorophenyl)-5-methyl-2,4-dioxooxazolidine-5-carboxylate] is a fungicide used in Italy on grapes, against *Botrytis cinerea*, *Sclerotinia* spp., and *Monilia* spp. (Garibaldi et al., 1982; Parducci, 1990).

Cabras et al. (1984) reported the kinetics of degradation of chlozolinate in white wine at two different pH values. They observed a rapid breakdown of the fungicide in wine with half-lives of 0.35 and 0.14 day at pH 3.00 and 4.00, respectively. The degradation of chlozolinate was accompanied by the formation of a new compound, which was identified in a subsequent work as 3-(3,5-dichlorophenyl)-5-methyloxazolidine-2,4-dione (metabolite I) (Pirisi



chlozolinate



metabolite I

et al., 1986). Flori et al. (1982) reported a reduction of chlozolinate residue from 2.66 to 0.13 ppm after vinification of white grapes. Since there is no information in the literature describing the fate of metabolite I during the vinification process, the behavior of residues of chlozolinate and metabolite I during two types of vinification process of Moscato grapes is reported here.

MATERIALS AND METHODS

Chemicals. All commercial solvent and standard chemicals were used as supplied. Authentic standard of chlozolinate was purchased from Erhenstorfer (Augsburg, FRG), and its metabolite I was kindly supplied by Agrimont (Italy).

Grapes. Grapes (Moscato) were collected in two local vineyards in Piedmont, Italy. The plots were treated with a 50% chlozolinate formulation (Serinal; Agrimont, Italy) at 1.6 kg/ha, 34 days before harvest. Random 100-kg samples of grapes from each vineyard were collected and immediately processed.

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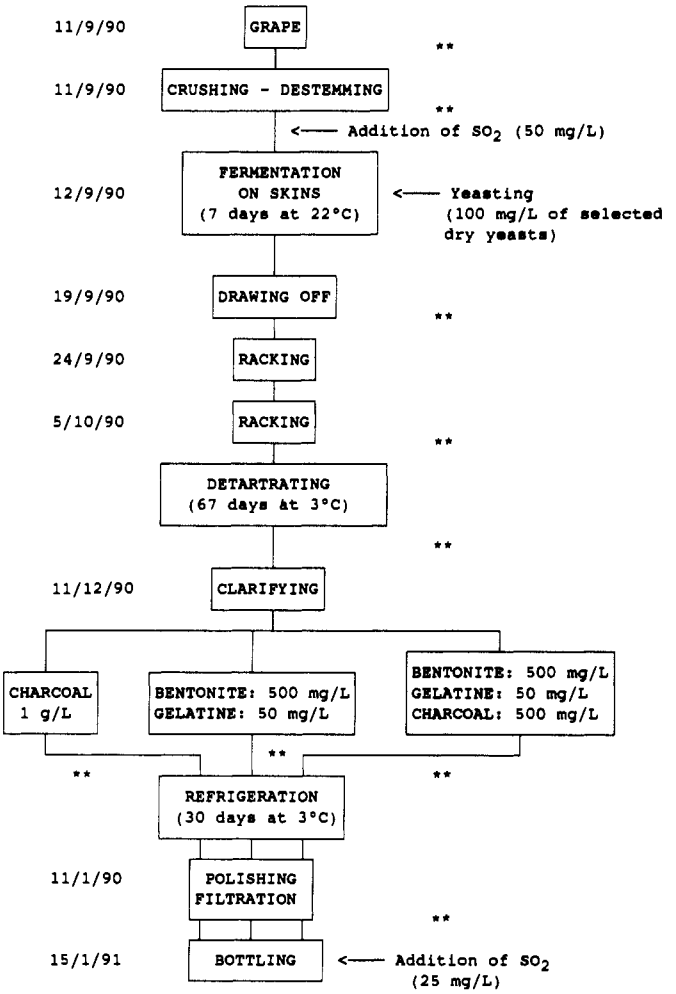

Figure 1. Flow sheet for the vinification process with maceration (* *, sampling for residues analysis).

Methods of Vinification. Two methods of vinification were compared: (a) with maceration; (b) without maceration according to the traditional winemaking process for sweet sparkling Asti wine. Vinification procedures were performed at laboratory scale. A detailed outline of these methods is given in Figures 1 and 2. For each method, two replicates of 50 kg of grapes harvested in two different vineyards were used.

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Table I. Residues (Parts per Million) of Chlozolinate and Metabolite I during Vinification Process with Maceration[a]

operation step		chlozolinate		metabolite I as chlozolinate	
		grapes from vineyard 1	grapes from vineyard 2	grapes from vineyard 1	grapes from vineyard 2
grape		1.31	0.73	0.18	0.10
after crushing and destemming		1.29	0.51	0.23	0.15
after fermentation and drawing off	wine	0.02	0.02	0.72	0.20
	lees	0.06	0.02	0.88	0.35
	vinasse	0.31	0.04	2.72	0.89
after second racking	wine	0.01	0.01	0.33	0.11
	lees	0.03	0.02	0.34	0.15
before clarifying		nd[b]	nd	0.33	0.11
after clarifying with charcoal (A)	wine	nd	nd	0.01	nd
	lees	0.01	nd	0.15	0.06
after clarifying with bentonite–gelatin (B)	wine	nd	nd	0.24	0.07
	lees	0.01	nd	0.25	0.08
after clarifying with bentonite–gelatin–charcoal (C)	wine	nd	nd	0.06	0.01
	lees	0.01	nd	0.23	0.06
after polishing filtration	A	nd	nd	nd	nd
	B	nd	nd	0.27	0.06
	C	nd	nd	0.05	0.01
6 months after bottling	A	nd	nd	nd	nd
	B	nd	nd	0.20	0.07
	C	nd	nd	0.05	0.01

[a] Mean of three replications. SD < 3.5%. [b] nd, below detection limit (0.01 ppm).

Table II. Residues (Parts per Million) of Chlozolinate and Metabolite I during Vinification Process without Maceration[a]

operation step		chlozolinate		metabolite I as chlozolinate	
		grapes from vineyard 1	grapes from vineyard 2	grapes from vineyard 1	grapes from vineyard 2
grape		1.31	0.73	0.18	0.10
after crushing		1.25	0.76	0.17	0.10
after pressing defecation					
first racking	must	0.05	0.03	0.27	0.19
	lees	6.96	2.85	0.84	0.67
after second racking	must	nd[b]	nd	0.25	0.14
	lees	0.02	0.02	0.32	0.24
after fermentation					
before filtration		nd	nd	0.16	0.10
after filtration		nd	nd	0.15	0.12
after clarifying with bentonite–gelatin–charcoal	wine	nd	nd	0.02	0.02
	lees	0.01	0.01	0.40	0.34
after filtration		nd	nd	0.02	0.01
after pasteurization		nd	nd	0.01	0.01

[a] Mean of three replications. SD < 3.5%. [b] nd, below detection limit (0.01 ppm).

Analytical Procedures. Wine samples, lees, or homogenized grapes (20 g) were added to 25 mL of *n*-hexane, shaken for 10 min on a mechanical shaker and then centrifuged for 5 min at 3000 rpm. The organic phase was transferred to a round-bottom flask. The aqueous phase was re-extracted twice more with 25 mL of *n*-hexane following the same shake–centrifuge procedure used for the original extraction. The organic phases pooled from the three extractions were evaporated to dryness at 35 °C on a rotary evaporator; the residues were redissolved in 5 mL of water/acetonitrile 1:1 (v/v) and then analyzed by HPLC. HPLC analyses were performed with a Varian HPLC 5020 system using a LiChrospher RP18 column and an UV–vis detector operating at a wavelength of 210 nm. The mobile phase (1 mL/min) was sodium lauryl sulfate (0.1%) in water acidified to pH 3 with orthophosphoric acid/acetonitrile. The column was eluted using a linear gradient of 63–80% acetonitrile over 6 min. All analyses were performed in duplicate. The reliability of the analytical method was tested by adding known amounts of chlozolinate and metabolite I to untreated samples of grapes, lees, or wine 10 min before extraction. The average recovery for chlozolinate was 98 ± 3% and for metabolite I 83 ± 3.5%. The detection limits were 0.01 ppm for both compounds. Parts A and B of Figure 3 show typical HPLC chromatograms of control nonfortified wine sample and wine sample fortified with chlozolinate and metabolite I at 0.2 ppm.

RESULTS AND DISCUSSION

Chlozolinate and metabolite I residues on grapes, must, wine, and lees during vinification processing with or without maceration are reported in Tables I and II, respectively.

Grapes from the field-treated plots contained both chlozolinate and metabolite I. Chlozolinate was found to be unstable in must in all trials. Degradation of chlozolinate was accompanied by formation of metabolite I. The increase of metabolite I concentration was higher in samples subjected to maceration because of transformation of the parent compound during fermentation. In winemaking without maceration, because of retarded fermentation, the chlozolinate was removed from the must with the lees during racking before its transformation in metabolite I. After the second racking, chlozolinate residues in must were below the detection limits. Traces of the fungicide (0.01 ppm) were found in lees after clarification in winemaking without maceration as a consequence of concentration. In both vinification procedures, racking provides an important route of residue reduction as shown by decreasing concentration of both chlozolinate and metabolite I residues in wine and the high residue content in lees after racking. Flori and Zironi (1984) showed similar results for chlozolinate during vinification process without maceration of Trebbiano grapes.

Racking was less effective on metabolite I reduction than on reduction of chlozolinate residues. After the second racking, the residue concentration of metabolite

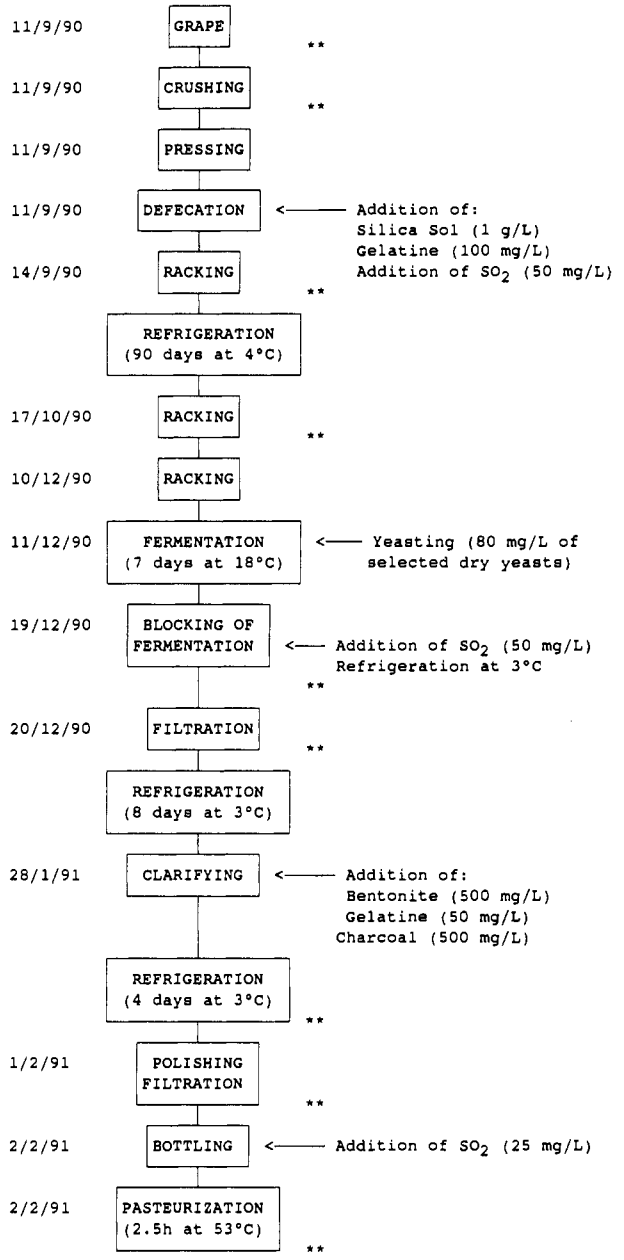

Figure 2. Flow sheet for the vinification process without maceration (* *, sampling for residues analysis).

I ranged from 0.11 to 0.33 ppm. Fermentation appeared to have slight effect on metabolite I degradation (Table II). In winemaking without maceration the average residue reduction after fermentation was 32%. Among the tested clarifying substances, only charcoal produced a decrease of metabolite I concentration. The highest efficiency in removing residues was observed with treatment with pure charcoal (1 g/L). Bentonite and gelatin alone did not affect appreciably metabolite I concentration in wine. These results are in agreement with those reported by Cabras et al. (1983, 1987), who found that charcoal induced a 93% decrease of vinclozolin (another dicarboximide fungicide) in wine, while bentonite had no detectable effect. Also, Flori et al. (1984) observed that bentonite had no effect on dicarboximide residue reduction. Pasteurization did not affect metabolite I concentration in wine. On bottling, only in the wine subjected to clarification with 1 g/L charcoal were metabolite I residues below the detection limit. Six months after bottling, the concentration of metabolite I residues was not appreciably changed (Table I). The stability of metabolite I in wine precludes the use of a holding storage time as an effective decontamination technique for this compound.

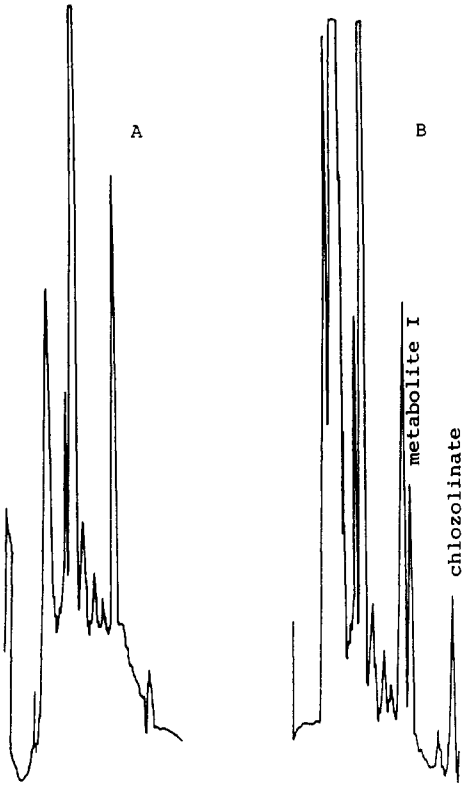

Figure 3. Typical HPLC chromatograms from analysis of chlozolinate and its metabolite I residues. (A) Untreated wine; (B) treated wine containing 0.2 ppm of both chlozolinate and metabolite I.

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