Chlorcholine Chloride Residues in Grapes and Their Fate in Winemaking

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Five concentrations of chlorcholine chloride aqueous solutions were applied at the specified rate: 400, 800, 1200, 1600, and 2000 ppm (750 ml per plant). Residues ranging from 0.3 to 0.6 ppm of chlorcholine chloride were observed in mature grapes at time of harvest following a single application at the beginning of flowering. These results were obtained using two analytical techniques: colorimetry and gas chromatography. Both of these methods evaluated for reproducibility and accuracy have given good results. At the end of fermentation the chlorcholine chloride residues are recovered unmodified; the main part is collected in the wine. The balance of fermentation products is not substantially modified.

The widespread use of plant-growth regulant chlorcholine chloride (2-chloroethyltrimethylammonium chloride: CCC, Cycocel) (Tolbert, 1960ab; Wittwer and Tolbert, 1960) necessitated the development of sensitive, reliable methods for determination of residues in vegetable foods.

Petrosini *et al.* (1969) have determined CCC residues in strawberries by a chemical method that consists of extraction, preliminary purification of the quaternary ammonium compounds by cation exchange resin chromatography, further purification by adsorption with an alumina column, and absorptivity measurement of a dipicrylamine-chlorcholine chloride complex at 415 mμ. This method is practically the same as Businelli *et al.* (1969) have used for the determination of CCC residues in tomatoes and grapes.

Tafuri *et al.* (1970) have recently reported the gas chromatographic estimation of the CCC at residue levels. The technique is based on prior reaction with sodium benzenethiolate. This procedure requires some prepurifications and concentration prior to the reaction with the benzenethiolate salt.

A recent report (Businelli *et al.*, 1969) indicates that two treatments carried out on grapes at 200 and 300 ppm CCC concentration induced internode and shoot shortening, greater setting, and more compact clusters. The CCC residues in grapes were below the limit of detectability of the colorimetric method used (0.2 ppm). In other countries the CCC treatments are made with more concentrated solutions (from 300 to 2000 ppm).

The purpose of this paper is to describe the amount of CCC residues found in grapes with treatments at higher concentrations and the fate of CCC in winemaking. An additional objective is to present a comparison of the accuracy and precision of the colorimetric and gas chromatographic methods used.

EXPERIMENTAL

Field Experiment. In the Azienda Maccarese in Rome (Italy), the grape plants of the white Malvasia received a single treatment with CCC on June 9, 1969, at the beginning of flowering.

Five concentrations of CCC aqueous solutions were applied at the specified rate: 400, 800, 1200, 1600, and 2000 ppm (750 ml per plant). Every plot (10 plants) had four replications. A control plot was added. The experimental design was randomized blocks.

The salient effects of these CCC treatments concerning vegetative and reproductive characters were like those obtained with 200 and 300 ppm applications (Businelli *et al.*, 1969; Pugliano, 1967).

The grapes collected on Sept. 30 were in good condition and ripe. Immediately after being picked, they were put in polythene bags and brought to the Institute.

From date of application to harvest the minimum average temperature was 16.8° C; the maximum average temperature was 26.5° C. The first rain occurred 20 days after the CCC application. There were 12 days of rain, giving a total of 100.5 mm of precipitation.

Aliquots of berries deriving from lot E and the control plot were separately pressed and the musts were collected in 10-l. glass bottles and fermented under conditions simulating winemaking in casks. The bottles were provided with two tubes: the first one for admission of compressed air for mixing; the other for the exit of fermentation gases connected to a Drechsel washing bottle containing H_2O.

When fermentation came to an end, the wines were then drawn off from the lees, which represented 12% of the total volume.

CCC Residues Determination. 250 g of berries macerated in a blender, must, or lees were transferred to a 1-l. flask with 250 ml of distilled water and were boiled under reflux for 3 hr. After cooling and centrifugation, the supernatant was decanted and the residue was washed with 50 ml of water. It was again centrifuged and decanted. The washing procedure was repeated three times. The supernatants were filtered into a 1-l. separatory funnel, 300 ml of diethyl ether were added, and this was allowed to stand for 24 hr with occasional mixing.

For wine, 250 ml of wine were directly transferred to a 1-l. separatory funnel, 300 ml of diethyl ether were added, and this was allowed to stand for 24 hr with occasional mixing.

Proceeding with the method used for the colorimetric determination of CCC residues in strawberries (Petrosini *et al.*, 1969), the only differences were the following:

The effluent of the cation exchange resin column was collected in a 100 ml volumetric flask and was made up to volume with distilled water. Five milliliters of the resin column eluate, if the colorimetric method was used, or 90 ml, if gas chromatographic method was employed, were taken to dryness and transferred to the second alumina column. The eluate (100 ml) of the second column was evaporated in a beaker to dryness for colorimetric procedure or to near dryness for gas chromatographic estimation.

If the colorimetric determination was used, the residue was dissolved in 0.03*N* sodium hydroxide solution, the color was

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Table I. Recovery, Percent, of CCC[a]

Added, ppm	Berries		Must		Wine		Lees	
	Colorimetric method	Gas chromatographic method	Colorimetric method	Gas chromatographic method	Colorimetric method	Gas chromatographic method	Colorimetric method	Gas chromatographic method
0.2[b]	82	79	81	80	81	83	81	80
0.5	80	80	83	82	77	80	85	84
1.0	83	81	80	75	79	81	80	79
1.5	80	78	79	78	82	79	78	79

[a] Average of triplicate analyses. [b] A peak height of 23% of full scale deflection is obtained injecting 1 μl in chloroformic solution (attenuation: × 1; Leeds & Northrup 1mV Speedomax recorder) under the conditions described for the gas chromatographic method.

Table II. CCC Residue in Treated Berries[a] (All Figures are Ppm on Fresh Weight)

Lots	CCC concentration used for the Treatment	Colorimetric method	Gas chromatographic method
Control	0	0.05	<0.005
A	400	0.30	0.27
B	800	0.39	0.35
C	1200	0.44	0.38
D	1600	0.30	0.25
E	2000	0.61	0.53
E_1 (fortified)[b]	...	1.48	1.46

[a] Mean of three determinations. [b] Lot E_1 was prepared by adding directly to the berries of lot E an amount of the Cycocel corresponding to about 1 ppm of chlorcholine chloride.

Table III. Composition of Just Pressed Musts

Lots	% Reducing sugars as glucose (w/w)	Titratable acidity as tartaric (g/l)
Control	17.4	6.3
A	18.6	6.4
B	17.3	6.0
C	16.6	6.9
D	16.0	7.4
E	14.5	7.1

Table IV. CCC Residues in Must, Wine, and Lees[a] (All Figures are in Ppm on Fresh Weight)

Samples	Colorimetric method		Gas chromatographic method	
	Control	Lot E	Control	Lot E
Must	0.05	0.61	<0.005	0.55
Wine	0.03	0.56	<0.005	0.50
Lees	0.05	0.20	<0.005	0.18

[a] Mean of three determinations.

developed with dipicrylamine reagent, and the absorbance was measured at 415 mμ.

For gas chromatographic detection the residue was transferred into a test tube with 50% methanol in acetone and was evaporated to dryness with a stream of dry nitrogen. Proceeding with the benzenethiolate salt for the conversion of CCC to volatile 1-phenylthio-2-dimethylaminoethane, it was determined gas chromatographically as already reported (Tafuri *et al.*, 1970).

Reducing Sugars and Titratable Acidity of Musts. Reducing sugars have been determined according to Lane and Eynon (1923); titratable acidity with 0.1*N* NaOH, phenol red (Brémond, 1937).

RESULTS

Average recoveries of chlorcholine chloride are about 80% (Table I). While average control values are of the order of 0.05 ppm by the colorimetric method, no peak corresponding to CCC is observed with the gas chromatographic procedure. The average control value of 0.2 ppm reported for grapes in a previous work (Businelli *et al.*, 1969) concerns the CCC determination on the berries with the grape stalks.

The amounts of residues determined in treated berries are presented in Table II. The determinations on berries revealed a very low amount of residues. For this reason it was thought desirable to prepare a new lot by adding directly an amount of the Cycocel corresponding to about 1 ppm of chlorcholine chloride to the berries of lot E that had been just macerated (lot E_1).

The amount of residue recovered on lot E_1 proves the efficiency of both procedures in determining CCC residues.

The results of the chemical analysis carried out on the various lots of must are reported in Table III. This table shows that the composition of musts coming from the lots of differently treated berries has noticeable differences only at higher dosages with CCC.

CCC residues on must, wine, and lees derived from lot E grapes are reported in Table IV. These results give a sufficiently detailed picture of the fate of CCC during the course of winemaking. Of the CCC found on must, about 95% passes into the filtered wine, but in the lees, as a consequence of reduced volume, there is a relatively high amount of the chlorcholine chloride. However the amounts of residues recovered, calculated on the basis of the ratio wine:lees (88 to 12), are approximately quantitative with respect to the level of these residues in the berries.

The transformation of sugar into alcohol is completed (8.6 vol. % of alcohol) and the analytical data (density, fixed acids, volatile acids, extract, ash) of the wine composition are in close agreement with those of the corresponding must.

DISCUSSION

The amounts of CCC recoverable from grapes, when the treatments are made with more concentrated solutions (from 300 to 2000 ppm), are at an acceptably low level (American Cyanamid Company, 1966).

The results obtained also show that the main part of chlorcholine chloride present in grapes is not modified during fermentation and is recoverable in wine. The balance of fermentation products is substantially unmodified.

Separation of CCC from aqueous extracts by a two column

chromatographic system, with a cation exchange resin in the first column and alumina in the second, has enabled us to obtain reproducible results for CCC residue determinations whether CCC is measured colorimetrically as dipicrylamine-CCC complex at 415 mμ, or gas chromatographically after conversion of CCC to volatile 1-phenylthio-2-dimethylaminoethane with benzenethiolate salt.

It has not been determined whether the entire purification required for the colorimetric method is also needed for gas chromatography. The gas chromatographic method may be shortened if some steps can be eliminated or simplified.

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