

The influence of copper-containing fungicides on the copper content of grape juice and on hydrogen sulphide formation

by

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Der Einfluß von kupferhaltigen Fungiziden auf den Kupfergehalt von Traubenmost und auf die Schwefelwasserstoffbildung

Zusammenfassung. — Es wird eine Methode zur direkten Veraschung unvergorener Mostproben beschrieben. Damit wird gezeigt, daß die Fungizide Kupferoxychlorid und Bordeaux den Kupfergehalt unvergorener Moste zwei- bis vierfach erhöhen können. Die erhöhten Kupferkonzentrationen verursachen eine beträchtlich höhere H_2S -Bildung während der Gärung. Der Wert von Kupferabschlußspritzungen wird im Hinblick auf die H_2S -Bildung in Frage gestellt.

Introduction

Over the past decade or 15 years there has been an increasing use of fungicides against *Plasmopara*, *Botrytis* and *Oidium* in South African vineyards. Similarly increasing incidence of H_2S production during fermentation is often discussed among wine makers but has never been surveyed and substantiated. Although there may be several factors which can account for the higher incidence of H_2S formation it also seems possible that it may be related to the use of these fungicides, some of which are known to contain ionic copper.

Copper ions are reported to increase production of hydrogen sulphide in synthetic media (ASHIDA *et al.* 1963, KIKUCHI 1965 a, b). Preliminary tests demonstrated the influence of the fungicide "Copperoxychloride" on H_2S formation (ESCHENBRUCH 1971) which have been confirmed by DITTRICH and STAUDENMAYER (1972) using cupric sulphate and copper (II) acetate.

It was the objective of this investigation to assess the influence of several copper-containing fungicides on the concentration of copper in the grape juice and the subsequent formation of H_2S during fermentation of such juice.

Materials and Methods

Spray programme and fungicides

Vitis vinifera cv. Pinotage was treated with one normal copper spray (2 kg Copperoxychloride per 460 litre, wettable powder, 50% copper) before blossom. After blossom different treatments were given as indicated in Table 1. Dosage levels of the various fungicides are given in Table 2.

Harvest and fermentation conditions

Grapes were collected at random from 32 vines, crushed by hand, kept for one hour in a container and then filtered and pressed through cheese cloth. The juice was kept frozen until the fermentations (in triplicate) were started. Five hundred

Table 1
Spray programmes for vineyard
Spritzplan der Rebanlage

Treatment		Time of spraying (weeks before harvest)				
		12	8	6	3	1
Control		—	—	—	—	—
Programme	I	+	+	+	—	—
	II	+	+	+	+	—
	III	+	+	+	+	+

— Not sprayed,
+ Sprayed.

Table 2
Dosage levels of fungicides
Anwendungsmengen der Fungizide

Trade names	Chemical names	% active ingredient	Concentration (kg/450 l)
Bordeaux	Copperoxychloride	10	7
Copperoxychloride	Copperoxychloride	50	2
Copperoxychloride + Zineb	Copperoxychloride + Zn-ethylene-bis-dithiocarbamate	37 + 20	1

ml quantities were measured into 1 litre Erlenmeyer flasks, the latter fitted with fermentation caps, sterilized at 120 °C for 5 minutes, inoculated with 1% of the pure cultured yeast strain WE I (*Saccharomyces cerevisiae*, O.V.R.I. collection) and kept at 25 °C. As soon as fermentations had finished H₂S was determined according to BRENNER *et al.* (1955). In cases where H₂S formation had to be determined during the course of fermentation (Fig. 1) 5 l quantities of juice (*V. vinifera* cv. Steen) in 10 l aspirators were used. Samples for analysis were drawn daily.

Ashing procedure

Since no direct method for the determination of trace elements in unfermented must was available a technique with reference to ZEEMAN and BUTLER (1962) was developed. 30 ml of must were measured into silica crucibles containing 10 ml of conc. H₂SO₄, thoroughly mixed, allowed to stand for one hour, subsequently heated on a waterbath until a syrup was formed and dried completely under an infra-red lamp. The crucible was then placed in a muffle furnace and the sample ashed at 500 °C for one hour. Upon cooling 4 ml of 1 N HNO₃ was added and the sample dried on a sandbath (≈ 150 °C). It was then ashed again at 500 °C for 30 minutes. The ashing was repeated until a white ash was obtained, which was then dissolved in 10 ml of 1 N HNO₃ + 0.15% lanthanum, transferred quantitatively into a 25 ml volumetric flask, made up to volume with the same solution, mixed, filtered and finally placed in a polyethylene bottle with screw cap. A blanc was prepared in a similar way.

Measurement of the trace elements

A Techtron AA5 Atomic Absorption Spectrophotometer with recorder and sample changer was used. To improve signals the scale was expanded 8×. A stand-

ard was prepared from commercially available standard solutions (Titrisol, Merck) with 0.15% lanthanum and 1 N HNO_3 diluent.

Results and Discussion

Data of recovery tests with the new technique showed that losses of up to 10% occurred during dry ashing. This agrees with the computed coefficient of variation of 10%. Losses of this extent are generally experienced when using similar methods to destruct organic matter (GORSUCH 1970). Normally methods applied for the determination of trace elements in must and sweet wine require pretreatment, namely fermentation, in order to remove organic material (BERGNER and LANG 1971, LIPIS *et al.* 1969). This, however, is a very time consuming process. In the described method no pretreatment is necessary and the sample can be analysed directly, giving a final result within 8 hours. When only relative comparisons are made losses of the order of magnitude mentioned are acceptable. The time saving factor seems sufficiently advantageous to justify this method.

Table 3 gives the concentrations of the trace element copper in the unfermented grape juice. The copper content was increased 2—4 times after treatment with Copperoxychloride, but had only doubled with programme III using Bordeaux and was not influenced by Copperoxychloride plus Zineb. This seems to be related to the much lower content of copper of the last two fungicides (Table 2).

The last column of Table 3 shows the H_2S concentrations of these must samples after they had been fermented. Spray programme III with Bordeaux and all three with Copperoxychloride caused higher H_2S formation.

Statistical evaluation of these results show the following: No significant difference, at the 5% probability level, in the Cu^{++} as well as the H_2S content was found between the control, Bordeaux and Copperoxychloride + Zineb treatments. The Copperoxychloride treatment, however, yielded significantly higher Cu^{++} as

Table 3

Copper content of unfermented¹⁾ and the H_2S concentration of fermented samples²⁾
Kupfergehalt unvergorener und H_2S -Konzentration vergorener Proben

Fungicides	Spray programme	Cu^{++} (mg/l)	H_2S ($\mu\text{g/l}$)
Control	—	0,98	8,5
Bordeaux	I	0,98	5,1
	II	1,00	6,3
	III	1,98	17,0
Copperoxychloride	I	2,56	16,3
	II	2,93	31,6
	III	3,91	52,7
Copperoxychloride + Zineb	I	—	10,6
	II	—	8,6
	III	1,23	8,2
Standard error of mean		0,137	2,02

¹⁾ Mean values of duplicate determinations.

²⁾ Mean values of three fermentations.

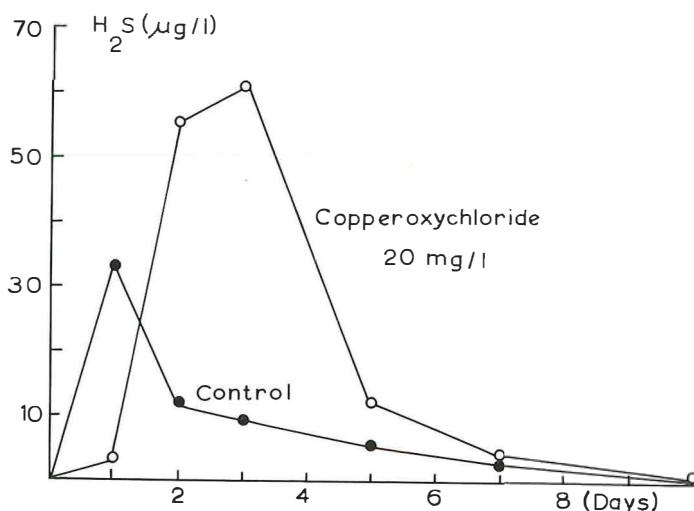


Fig. 1: Hydrogen sulphide formation during fermentation of grape must by strain WE I. (Must composition and treatment: Steen, var. *V. vinifera*, vintage 1972, sugar content 20%, total acid 6.7 g/l, pH 3.3, sterilisation at 120 °C for 5 minutes, inoculum 2%). Mean values of three parallel fermentations.

Schwefelwasserstoffbildung während der Mostgärung durch den Hefestamm WE I. (Mostzusammensetzung und Behandlung: *Vitis vinifera*, Sorte Steen, Jahrgang 1972, Zuckergehalt 20%, Gesamtsäure 6,7 g/l, pH 3,3, Sterilisierung 5 Minuten bei 120 °C, Inokulum 2%). Durchschnittswerte aus 3 parallelen Gärversuchen.

well as H₂S readings. Here a regular significant increase in H₂S content was found in the spray programmes I, II and III. It also could be shown that the Copperoxychloride I programme did not yield a significantly higher H₂S reading than the control. The copper content was, however, significantly higher than that of the control. This difference in the differences could be due to the fact that Cu⁺⁺ is measured more accurately, having a coefficient of variation of 10%, than H₂S with a coefficient of variation of 21%. A correlation of 0,95 was found between the copper content of the must and the H₂S concentration at the end of fermentation.

To complete these results fermentations with deliberately added Copperoxychloride were carried out. The H₂S formation by strain WE I, as shown in Fig. 1, nearly doubled.

The question arises as to how copper can stimulate H₂S formation. On the one hand copper ions are constituents of many enzymes, viz. oxidases. On the other hand copper is well known for its inhibitory effect on respiration — one of the main reasons for its wide and successful application as fungicide. ASHIDA *et al.* (1963) reported that by culturing a yeast strain in a copper medium, a copper resistant strain was obtained, which produced much more hydrogen sulphide than the parent strain. They supposed that these yeasts through adaptation protect themselves from injury by copper by forming H₂S and consequently copper sulphide. A change in yeast type promoted by copper was demonstrated by HOGGAN and COMPSON (1963). If the pure cultured yeast Type W was allowed to come into contact with copper surfaces, or when this metal was added as a salt, then the presence of Type S was found even in the first generation. This Type S caused impairment of the fining ability, production of undesirable flavours and unpleasant odours in the final beer.

In some wine producing countries, e.g. Germany or Switzerland, a very late copper spray of the grapes has been considered (DITTRICH 1973) with the objective of precipitating the H_2S formed during fermentation as copper sulphide. Taking the available information into account, the advantage of a late copper spray with regard to H_2S formation must be strongly questioned. The use of copper cooling coils during fermentation is still practised in some South African wineries. This practise has to be reconsidered as well since grape juice is highly corrosive. Relatively small concentrations of copper in the grape juice (Table 3) considerably stimulate the production of H_2S thus not preventing but rather inducing off-flavours. The influence of deliberately added copper salts to wines on off-flavours of such beverages is of purely chemical nature since they are added after fermentation.

Summary

A technique for direct ashing of unfermented grape juice samples is described. With this method it is demonstrated that fungicides such as Copperoxychloride and Bordeaux, when used in vineyards, can cause a two to four-fold increase in copper content of unfermented musts. Increased copper levels considerably stimulate hydrogen sulphide formation during fermentation. For this reason the application of late copper sprays to vines is to be reconsidered.

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