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Factors affecting the reproducibility of fermentation of grape juice and of the aroma composition of wines

I. Grape maturity, sugar, inoculum concentration, aeration, juice turbidity and ergosterol

by

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Maßgebliche Faktoren für die Reproduzierbarkeit der Vergärung von Traubenmost und die Aromazusammensetzung von Wein

I. Traubenreife, Zucker, Konzentration des Inokulums, Luft, Klarheit des Mostes und Ergosterin

Zusammenfassung. — Einige Faktoren, welche die Bildung flüchtiger Weininhaltsstoffe bei der Gärung beeinflussen, wurden gaschromatographisch untersucht. Besonders die Wirkung „heterogener" Faktoren im Traubenmost, wie zum Beispiel Trübung und Luftgehalt, auf die Gärungsgeschwindigkeit und die Bildung von Fettsäuren, Fuselalkoholen und Estern wurde untersucht. Es wurde gezeigt, daß die Gärungsverzögerung bei klarfiltrierten und entlüfteten Traubensäften durch die Zufügung kleiner Mengen von Ergosterin vor der Inokulation verhindert werden kann. Wurde die Gärung von geklärtem Most durch Luft oder durch Ergosterin angeregt, so zeigten die Chromatogramme der Weine eine starke Ähnlichkeit. Die Traubenreife erwies sich als der wichtigste Faktor, der die Bildung von flüchtigen Säuren, Alkoholen und Estern bei der Gärung beeinflußt.
Die Vergärung von entlüftetem und klarfiltriertem Most mit Zusatz von 0,5 g Bentonit/l ergab Weine, die bei der Weinprobe die höchsten Punktezahlen erhielten.

Introduction

The application of gas chromatography has over the last fifteen years contributed considerably to the increase in the knowledge of wine aroma components. Up to the present stage, research has been largely limited to identification of new components and relatively little has been done on the practical application of such findings. It is revealing that the routine analysis of wine is still mainly based on determination of traditional compounds, such as alcohol, acid, sugar and SO_2, whereas no specific attention has been given to possibilities for routine analysis of flavour compounds.

Practical application possibilities for present-day knowledge of wine aroma components

A degree of succes has been achieved in cultivar identification by means of specific aroma components, especially in the case of wines of strongly aromatic cultivars such as Gewürztraminer, Buketttraube, Riesling and typical muscat wines (RAPP *et al.* 1977). Characterisation of such wines by means of computer programmes is based on their terpene contents, but this is usually ineffective in differentiating these cultivars in blends (Schreier *et al.* 1976).

Ribéreau-Gayon (1971) predicted at the O.I.V. congress in Mendoza in 1971 that planned control of aroma composition during fermentation would be an important aspect in the future development of oenology. Several negative factors, such as H_2S, SO_2, lactic acid, aldehydes and mercaptans are used in aroma judgment, but very little positive quality factors are similarly applied to date (Wagener and Wagener 1968, du Plessis 1975).

The following fields of study can be considered as research possibilities in the application of the latest findings on aroma components:

1. The relationship of chromatogram peaks and peak ratios and sensory evaluation data of wines,
2. the control of the formation of such components during fermentation by cellar technological practices and must composition,
3. fully reproducible vinification using the gas chromatogram as criterium.

Such a control of aroma formation can on the one hand find application in making a greater variety of wines and on the other be useful in making wines of consistently high quality. Very little indeed is known of the factors responsible for the quality aspects of top quality wines. In many cases the attainment of high quality in wines appears to be a matter of chance. In effect, the essential variables responsible for the high quality in these wines are generally unknown and consequently it is often not possible to reproduce wines of exceptionally high quality. It appears that the gas chromatogram can serve as a fingerprint of a specific wine even if all the components responsible for peaks on the chromatogram have not been identified It can be argued that if 16 identical points are sufficient to prove that two fingerprints are identical, it can be supposed that two wines will be identical if a certain number of randomly chosen components show precisely corresponding peak heights and ratios in their respective gas chromatograms. For practical purposes it would be logical to utilize those components which give clear and readily measureable peaks such as fusel alcohols, volatile acids and esters. It is not a pre-requisite that these components are in themselves contributors to the aroma as long as their fingerprint chromatograms are representative for the wines. Most of these components nevertheless have recognizable odours and since their concentrations in wine are normally above their threshold values, they should contribute to the aroma (Suomalainen 1971).

The control of aroma formation

With the aim of controlling and directing fermentation and aroma formation, a study of variables which can either retard or promote the production of individual volatiles is under investigation at the O.V.R.I. Factors affecting the formation of volatile fermentation products can be roughly distinguished in two groups. On the one hand there are the internal homogenous must factors i.e. the concentration of dissolved must components. On the other hand "external" factors are distinguished, such as yeast species, grape properties, cultivar and degree of maturation, physical and cellar technological factors and heterogenous juice factors, such as suspended grape particles (turbidity) and air. Ester formation mainly takes place at the beginning of the exponential phase of fermentation and is coupled to the yeast proliferation process. Factors affecting the activity of this process therefore also do affect aroma production (NORDSTRÖM 1965).

1. Juice turbidity, air, ergosterol and inoculum concentration

It seems logical to launch an investigation into the formation of the individual aroma components in homogenous, filtered and de-aerated juice, especially because of the experience that high quality white wines are generally made from the physically cleanest juices (WUCHERPFENNIG and BRETTHAUER 1970, RIBÉREAU-GAYON *et al.* 1975, BERTRAND *et al.* 1978). However, under those circumstances fermentation is often retarded or even incomplete (WUCHERPFENNIG and BRETTHAUER 1970, RIBÉREAU-GAYON *et al.* 1975, SINGLETON *et al.* 1975, BERTRAND *et al.* 1978). However, under those circumstances fermentation is often retarded or even incomplete (WUCHERPFENNIG and BRETTHAUER 1970, RIBÉREAU-GAYON *et al.* 1975). In order to standardize the optimal conditions for complete and reproducible fermentations, the effect of the "external" factors on fermentation of homogenous and de-aerated juice had to be investigated first. In this connection recent observations on the function of ergosterol in yeast proliferation are of great importance. LAFON-LAFOURCADE *et al.* (1977) recorded the essential activity of suspended grape particles and steroids in keeping up the viability of the yeast. ARIES and KIRSOP (1977) provided convincing data on the relationship between air, ergosterol formation by yeast, yeast inoculum concentration and proliferation in beer brewing. Yeast requires ergosterol for proliferation, but under anaerobic conditions it nearly completely utilizes its own ergosterol. In this case proliferation stops after a few yeast generations. Under aerobic conditions the yeast can produce ergosterol, but this is limited to approximately 10 mg/g of dry yeast. The quantity of ergosterol formed in the earliest phase of fermentation is consequently dependent upon the quantity of yeast inoculum and of oxidation potential in the must. Where massive inoculation, e.g. 15 %, is used under anaerobic conditions, the yeast contains sufficient ergosterol to effect a growth which will ensure a complete fermentation or alternatively it may still produce ergosterol during one generation (6 h). Conversely with a low inoculation, e.g. 0.5 %, the yeast will contain too little ergosterol, even in the presence of air during the first hours, to effect satisfactory proliferation. Only if oxygen is present in sufficient concentration, for some successive yeast generations will enough ergosterol and yeast cells be produced to ensure a complete fermentation.

2. Yeast species

The yeast species is definitely the factor which can exert the most marked effect on the production of volatiles (SUOMALAINEN 1971). ROSET and MARGULIS (1972) reported on yeasts differing in their capabilities of producing ethanol as well as higher alcohols, fatty acids and esters. However, in the experiments reported in this paper the yeast species will not be investigated as a variable.

3. Cultivar

It is generally accepted that the cultivar plays an important role in aroma formation during fermentation, but this generalisation has only been confirmed in a very few cases. VAN WYK *et al.* (1977) e.g. indicated that iso-amyl acetate formation is stimulated by the juice of Pinotage grapes, but apart from this case, there is no clear relationship between cultivar and production of volatiles during fermentation.

Material and methods

1. Material

Free run juice of Chenin blanc grapes of varying degrees of maturity was used after either clearing by settling or filtration through sterilising E.K. sheets.

A fermentation stimulating lipid fraction was extracted from the grape particulate material present in settled juice. For this purpose the juice was centrifuged and the sediment (4.5 g/l) macerated with 4 volumes of methanol followed by 20 volumes of ether. The combined extracts were evaporated and dried at 40 °C under vacuum. The lipid residue (0.4 g/l of juice) together with 0.5 g Tween 80 was dissolved in 20 ml ethanol. This solution was added to samples of sterile filtered grape juice (5 ml/l) for yeast growth stimulation.

In other cases ergosterol (1 % in hot ethanol) was added to the clear juice.

2. Fermentation

Fermentations were initially carried out at 15 °C in 0.4 l lots in the laboratory. The experiments were repeated in 65 l volumes for sensory evaluation of the wines. Yeast inoculations were done with *Saccharomyces cerevisiae* strain WE 14 of the O.V.R.I. collection, usually at a 2.5 % addition.

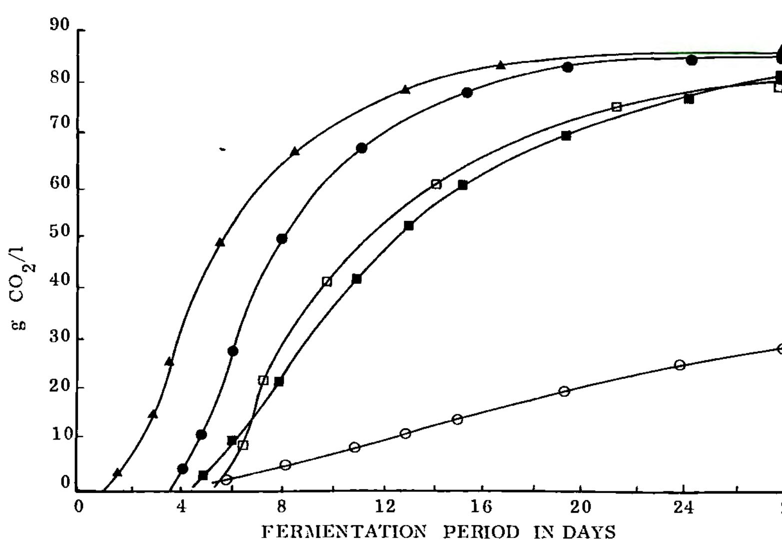


Fig. 1: Effect of juice clarity, aeration and yeast inoculum concentration on the fermentation rate of de-aerated grape juice. ——○ = Sterile filtered juice (SF). ——● = Settled juice (S); 90 % SF + 10 % S; SF juice, inoculated under air in headspace. ——■ = S juice, centrifuged (C). ——□ = SF juice + 1/4 part of the extracted lipid fraction from the centrifuged sediment from C juice. ——▲ = SF juice, inoculum concentration 15 %.

Einfluß von Klarheit des Mostes, Belüftung und Konzentration der zugesetzten Hefe auf die Gärungsgeschwindigkeit von entlüftetem Traubenmost. ——○ = Sterilgefilterter Most (SF). ——● = Vorgeklärter Most (S); 90 % SF + 10 % S; SF-Most bei Anwesenheit von Luft im Leerraum beimpft. ——■ = S-Most, zentrifugiert (C), ——□ = SF-Most + 1/4 der Lipidfraktion, die aus dem Zentrifugensediment von C-Most extrahiert wurde. ——▲ = SF-Most, Konzentration der zugesetzten Hefe 15 %.

In this work the effects of the following variables were studied: maturity of the grapes, juice clarity, aerobic and anaerobic conditions, inoculum percentage and additions of bentonite and ergosterol. The effects of these variables on fermentation were determined by measuring the fermentation rate and the production of fusel alcohols, volatile acids and esters as criteria for reproducibility.

3. Analysis

The method described by MARAIS and HOUTMAN (1979) was applied for the quantitative gaschromatographic determination of esters and alcohols in the wines. The concentrations of volatile acids were determined according to CROWELL and GUYMON (1969).

4. Wine quality evaluation

Wine qualities from the 65 l fermentations were sensorily evaluated by a taste panel of O.V.R.I. (TROMP 1977), with special emphasis on aroma quality. The wine score is given in percent and specified remarks of panel members were tabulated.

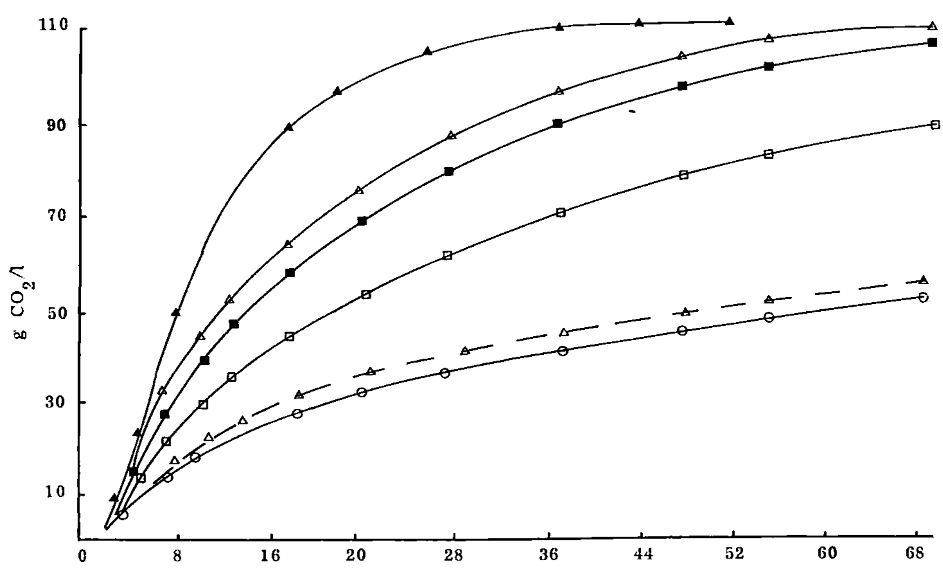


Fig. 2: Effect of air and of ergosterol, added as a 1 % solution in hot ethanol, on the fermentation rate of sterile filtered and de-aerated grape juice. ——O = Juice inoculated and fermented under strictly anaerobic conditions. ——▲ = Nitrogen headspace (0.8 l/l juice) replaced by air immediately after inoculation. ——□= 12,5 mg ergosterol/l juice. ——■ = 25 mg ergosterol/l juice. ——△ = 50 mg ergsterol/l juice. — —△ = 50 mg ergosterol/l juice, applied at the 5th d of fermentation.

Einfluß von Luft und Ergosterin (1 %ige Lösung in heißem Äthanol) auf die Gärungsgeschwindigkeit von sterilgefiltertem und entlüftetem Traubenmost. ——O = Unter strengem Sauerstoffausschluß beimpfter und vergorener Most. ——▲ = Stickstoff im Leerraum (0,8 l/l Most) unmittelbar nach der Hefezugabe durch Luft ersetzt. ——□ = 12,5 mg Ergosterin/l Most. ——■ = 25 mg Ergosterin/l Most. ——△ = 50 mg Ergosterin/l Most. — —△ = 50 mg Ergosterin/l Most, am 5. d der Gärung zugesetzt.

Results and discussion

1. Juice clarity, ergosterol, air and percentage yeast inoculum: Effect on fermentation rate

The fermentation rate was controlled to a large degree by the above-noted seemingly unrelated factors (Fig. 1). The fermentation of completely clear juice (sterile filtered) which had been de-aerated with nitrogen, sometimes showed lengthy lag periods before fermentation commenced and fermentation was always slow and often ceased long before all the sugar had been metabolized. This phenomenon can be prevented or overcome by massive yeast inoculations or aeration of the juice. The minimal turbidity remaining in a juice after settling is also efficient in overcoming a retarded fermentation of completely clear juice. Addition of as little as 10 % of a settled juice to sterile filtered juice or inoculation under aerobic conditions, were sufficient to cause fermentation to proceed as effectively as that in settled juice as revealed by the relevant plots in Fig. 1.

The addition of an ether extract of the centrifuged precipitate of settled juice, after the removal of ether and addition of Tween 80, to completely clear juice (sterile filtered) promoted fermentation under anaerobic conditions (Fig. 1). Approximately the same effect was obtained with additions of small amounts of ergosterol to the clear juice (Fig. 2). Apparently, juice turbidity particles contain ergosterol or similar acting components which promote yeast growth, in a readily available form. The yeast growth and fermentation promoting effects were optimal with additions of 50 mg ergosterol/l of juice. The ergosterol should be added before inoculation; additions in a later stage of fermentation exerted far less effect (Fig. 2).

Table 1

Effect of juice clarity on fermentation rate, yeast mass and ester concentrations in wine fermented under anaerobic conditions

Einfluß der Klarheit des Mostes auf Gärungsgeschwindigkeit, Hefemasse und Esterkonzentrationen des Weines bei Vergärung unter Sauerstoffausschluß

Parameters measured	100 % Sterile filtered juice	90 % Sterile filtered + 10 % settled juice	100 % Settled juice	F (column 1 vs. 2)
Fermentation rate (% CO_2/d)[1]	3.8	11.9	11.1	**
Fresh yeast mass (g/l)	5.1	10.4	11.0	**
i-Amyl acetate (mg/l)	2.65	8.1	9.1	**
2-Phenyl ethyl acetate (mg/l)	0.30	0.85	1.00	**
Ethyl hexanoate (mg/l)	1.38	1.42	1.35	NS
Ethyl octanoate (mg/l)	0.51	0.86	0.91	*
Ethyl decanoate (mg/l)	0.17	0.34	0.34	**

[1]) The average fermentation rate is given over the period from 0 to 50 % sugar consumption.
* = Significant at 95 % confidence level.
** = Significant at 99 % confidence level.
NS = Not significant.

2. Effect on production of volatile wine components

Ester concentrations in wines often correlate with yeast mass and fermentation rate and are consequently also often indirectly dependant on juice clarity and aeration.

2.1. Juice clarity

Results are given in Table 1 on the concentration of five esters in wines made from settled juice (S), sterile filtered juice (SF) and a 1 : 9 mixture of S and SF juice under anaerobic conditions. The SF juice did not complete fermentation and the ester concentrations of the wines were significantly lower than those in the wine of the S juice. The addition of 10 % S juice to SF juice was sufficient to increase the ester concentrations to the levels of that in the wines of the S juices.

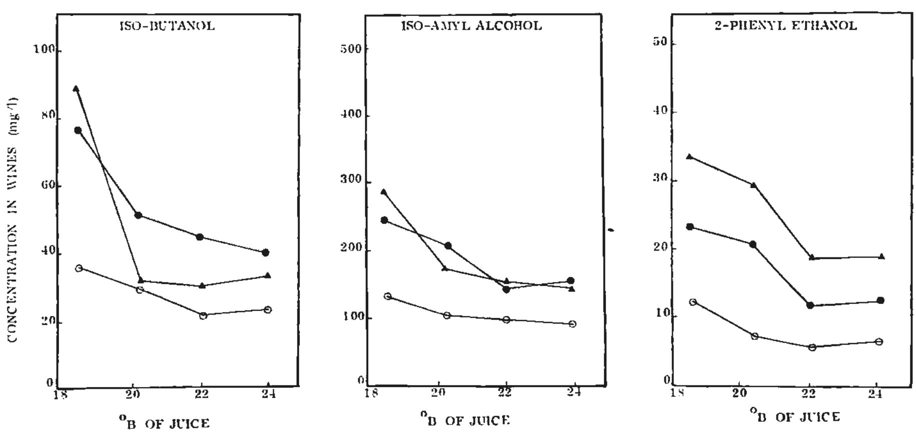


Fig. 3: Effect of juice clarity and air on fusel alcohol production during fermentation of juices of grapes of varying degrees of maturity. ——○ = Sterile filtered juice, de-aerated. ——▲ = Sterile filtered juice, aerated 6 × 15 min during first 3 d after inoculation. ——● Settled juice.

Einfluß von Klarheit des Mostes und Luft auf die Bildung von Fuselalkohol bei der Vergärung von Traubenmosten unterschiedlichen Reifegrades ——○ = Sterilgefilterter Most, entlüftet. ——▲ = Sterilgefilterter Most, in den ersten 3 d nach der Hefezugabe 6 × 15 min belüftet. ——● = Vorgeklärter Most.

As indicated in Fig. 3, the production of fusel alcohols was markedly increased by turbid material in the juice, in conformation with the studies of Wucherpfennig and Bretthauer (1970), Ribéreau-Gayon *et al.* (1975) and Bertrand *et al.* (1978). It is generally accepted that aroma becomes less clean with increasing juice turbidity. Juice turbidity particles have a direct and harmful effect on wine aroma. The presence of large quantities of grape juice sediment (lees) causes not only malodours in wine, but also retards production of esters by the yeast. Juice turbidity can therefore affect the production of volatiles in two ways. Firstly, a small residual turbidity can promote a good and complete fermentation as well as ester formation (Table 1). Secondly, large quantities of lees suppress ester formation (Fig. 4), cause development of malodorous components and stimulate fusel alcohol production.

2.2. Air

The influence of air upon ester formation was dependant on juice clarity. Yeast growth as well as ester production in completely clear juice were enhanced by air (Table 2). If the yeast had no need of oxygen, such as in slightly turbid settled juice, then the presence of air caused a decrease in ester production (Table 3). A rapid

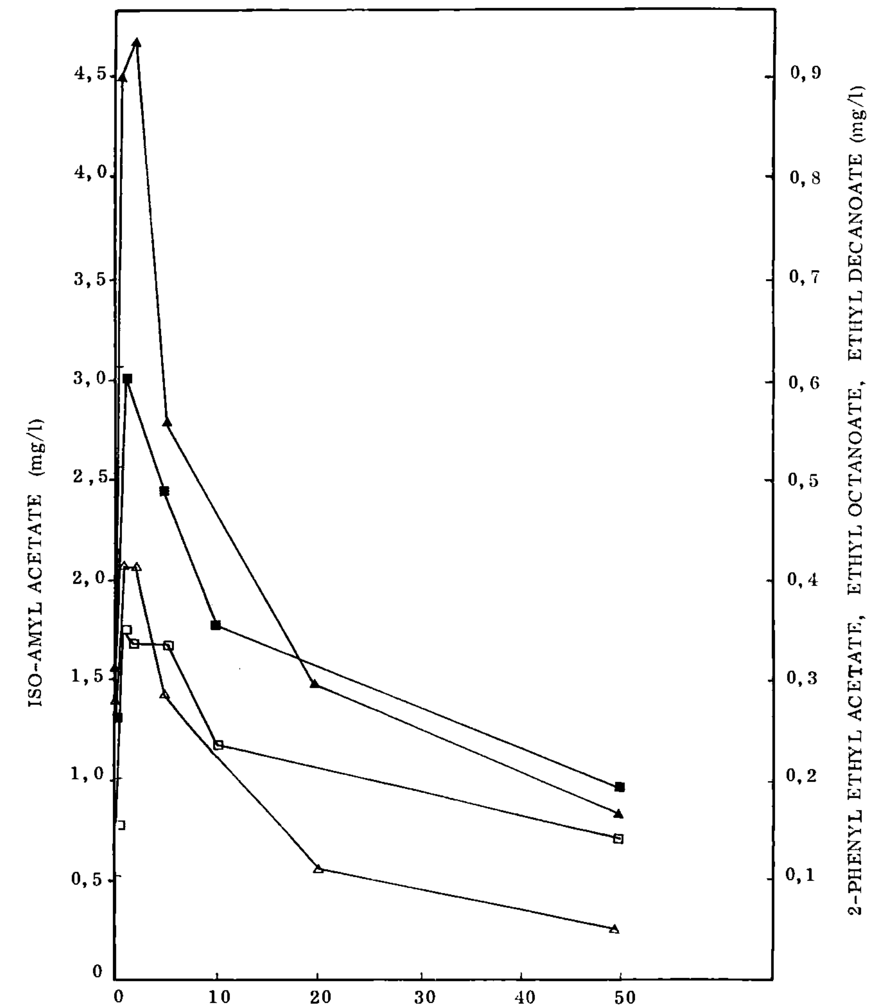


Fig. 4: Effect of the addition of juice lees to sterile filtered juice on ester formation under anaerobic conditions ——▲ = i-Amyl acetate. ——△ = 2-Phenyl ethyl acetate. ——■ = Ethyl octanoate. ——□ = Ethyl decanoate.

Einfluß des Zusatzes von Trub aus Most zu sterilgefiltertem Most auf die Esterbildung unter Sauerstoffausschluß. ——▲ = i-Amylacetat. ——△ = 2-Phenyläthylacetat. ——■ = Äthyloctanoat. ——□ = Äthyldecanoat.

fermentation, induced by air, increased the fusel alcohol content in the wine (Fig. 3), as was the case with juice turbidity.

2.3. Ergosterol

Ergosterol enhanced the formation of acetates in wines from sterile filtered and de-aerated juice. The concentrations of other volatiles were nearly identical in the cases of fermentation stimulated by either ergosterol or air (Table 4). Non stimulated fermentations and fermentations stimulated by a threefold increase in inoculum dosage gave rise to wines with slightly different gas chromatograms.

In view of both the positive and negative effects of suspended grape solids in wine making it is of practical importance that the causes of both effects be identified. It appears highly probable that the negative effect of juice turbidity, i.e. formation of malodours in the wine, may be circumvented by stimulation of fermentation of clear juice by air or ergosterol addition before inoculation.

Table 2

Effect of de-aerating clear filtered juice with nitrogen prior to inoculation on ester concentration of wine

Einfluß der Entlüftung eines klargefilterten Mostes mit Stickstoff vor der Hefezugabe auf die Esterkonzentration des Weines

Esters	Ester concentration (mg/l) Juice de-aerated	Juice not de-aerated
i-Amyl acetate	1.15	2.35
2-Phenyl ethyl acetate	0.14	0.31
Ethyl hexanoate	0.94	1.41
Ethyl octanoate	0.38	0.70
Ethyl decanoate	0.16	0.36

Table 3

Effect of de-aerating a grape juice mixture, containing 95 % sterile filtered juice and 5 % settled juice, with nitrogen prior to inoculation on ester concentration of wine

Einfluß der Entlüftung eines Traubenmostgemisches (95 % sterilgefilterter Most + 5 % vorgeklärter Most) mit Stickstoff vor der Hefezugabe auf die Esterkonzentration des Weines

Esters	Ester concentration (mg/l) Juice de-aerated	Juice not de-aerated
i-Amyl acetate	4.95	3.55
2-Phenyl ethyl acetate	0.42	0.33
Ethyl hexanoate	1.08	0.50
Ethyl octanoate	0.84	0.42
Ethyl decanoate	0.41	0.19

2.4. Percentage yeast inoculation

If conditions are favourable for yeast growth and complete fermentation, inoculations of 2.5 % or 10 % cause no significant differences between the ester concentrations of wines (data not shown).

2.5. Grape maturity; juice sugar content

Production of esters, volatile acids and fusel alcohols were studied on musts from grapes from the same vineyard, which were harvested at weekly intervals and the concentrations were plotted against °Balling of the grapes (Figs. 3, 5, 6). These results show that grape maturity has a very pronounced effect on the production of volatiles during fermentation. In many cases, this effect surpasses that of must clarity or air. In the case of clear filtered de-aerated juice this phenomenon shows that the "homogenous juice factors", i.e. the dissolved juice components, can exert a great effect on the composition of wine volatiles.

Table 4

Concentration of volatile components in wines (mg/l) from stimulated and non-stimulated fermentations of sterile-filtered and de-aerated grape juice

Konzentration der Aromakomponenten in Wein (mg/l) aus sterilgefiltertem und entlüftetem Traubenmost mit und ohne Stimulation der Gärung

Parameters measured	Non-stimulated fermentation	Stimulation (applied before inoculation)		
		Threefold inoculation dosage (7,5 %)	Nitrogen in head-space replaced by air	Addition of 50 mg ergosterol/l in 5 ml of ethanol
Fermentation rate (% CO_2/d)[1]	1.6	3.6	6.1	5.0
Fermentation time (d)	80	80	45	44
Residual sugar content in wine (g/l)	40[2]	10.5[2]	1.5	3.0
i-Butanol	15	15.2	19.2	16.5
i-Amyl alcohol	91	84	78	81
n-Hexanol	0.53	0.61	0.60	0.57
2-Phenyl ethanol	6.0	6.7	6.6	6.9
Hexanoic acid	4.30	4.15	3.95	3.80
Octanoic acid	7.40	5.85	5.95	5.25
Ethyl acetate	27	33	41	42
n-Hexyl acetate	0.10	0.08	0.10	0.10
i-Amyl acetate	1.40	1.22	1.45	1.55
2-Phenyl ethyl acetate	0.07	0.06	0.10	0.11
Ethyl butyrate	0.26	0.31	0.31	0.32
Ethyl hexanoate	0.90	0.80	0.88	0.87
Ethyl octanoate	0.85	0.92	1.05	1.08
Ethyl decanoate	0.35	0.41	0.37	0.37

[1]) The average fermentation rate is given over the period from 0 to 50 % sugar consumption.
[2]) The fermentation ceased before completion.

Increasing maturity coincides with decreased production of fusel alcohols i.e. i-butanol, i-amyl alcohol and 2-phenyl ethyl alcohol (Fig. 3). The investigated variables had similar effects on the fermentation of juice from grapes of different grades of maturity.

With regard to the effect of increasing grape maturity on the production of fatty acids during fermentation a clear tendency of increasing concentration was observed with acetic acid, propionic acid, dodecanoic acid and 3-hydroxybutyric acid (Fig. 5). These increases were more or less independent of the applied fermentation technique. On the other hand, no distinct relationship was observed between grape maturity and the fermentative production of butyric acid, hexanoic acid (caproic acid), octanoic acid (caprylic acid) and decanoic acid (caprinic acid). The data of 3 of these fatty acids show a remarkably similar pattern (Fig. 5).

The concentrations of the important ethyl esters of octanoic acid and decanoic acid in wines increase with increased sugar contents of the juice. This relationship is observed in the case of increasing grape maturity and also when increasing amounts of glucose were added to a juice of low sugar content. The dotted lines in Fig. 6 show the average values of the ester concentrations in the wines of 6 musts enriched with glucose from 18 to 20, 22 and 24 °B.

With esters of the acetate group quite opposite effects of grape maturity and of increasing sugar contents by glucose addition were shown. Increasing of sugar content by the addition of glucose caused increasing production of i-amyl acetate and 2-phenyl ethyl acetate during fermentation, but the increasing maturity of the grapes had a markedly opposite effect (Fig. 6). The effect of sugar concentration on the formation of these acetate esters is apparently dominated by a contra-effect which is also related to grape maturity.

3. Partition of esters between wine and yeast

During fermentation, part of the ester material is retained in the lipid phase of the yeast, especially in the case of the apolar long chain esters (Nykänen *et al.* 1977). High ethanol content of the wines, due to greater grape maturity, could be respon-

Table 5

Distribution of esters between wine and yeast after fermentation of juice with and without addition of 70 g glucose/l

Verteilung der Ester auf Wein und Hefe nach der Vergärung von Most mit und ohne Zusatz von 70 g Glucose/l

Esters	mg Ester concentration/l fermented juice			
	Juice at 15 °B		Juice at 15 °B + 70 g glucose/l	
	In wine	In yeast	In wine	In yeast
i-Amyl acetate	3.15	0	4.05	0
2-Phenyl ethyl acetate	0.35	0	0.55	0
Ethyl hexanoate	0.50	0	0.65	0
Ethyl octanoate	0.70	0.90	1.05	1.50
Ethyl decanoate	0.32	6.70	0.43	8.70
Ethyl dodecanoate	0.02	1.30	0.04	2.10

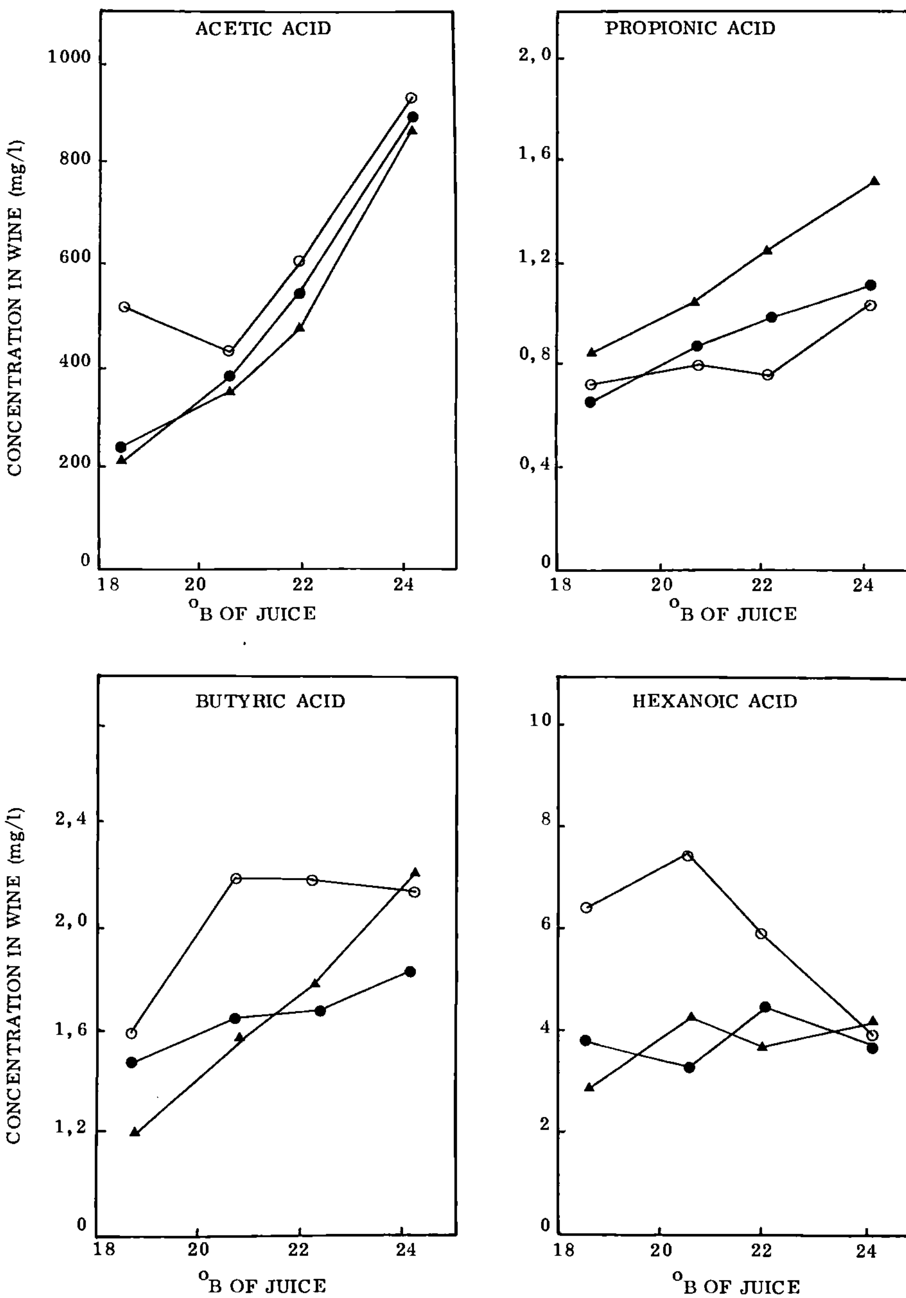
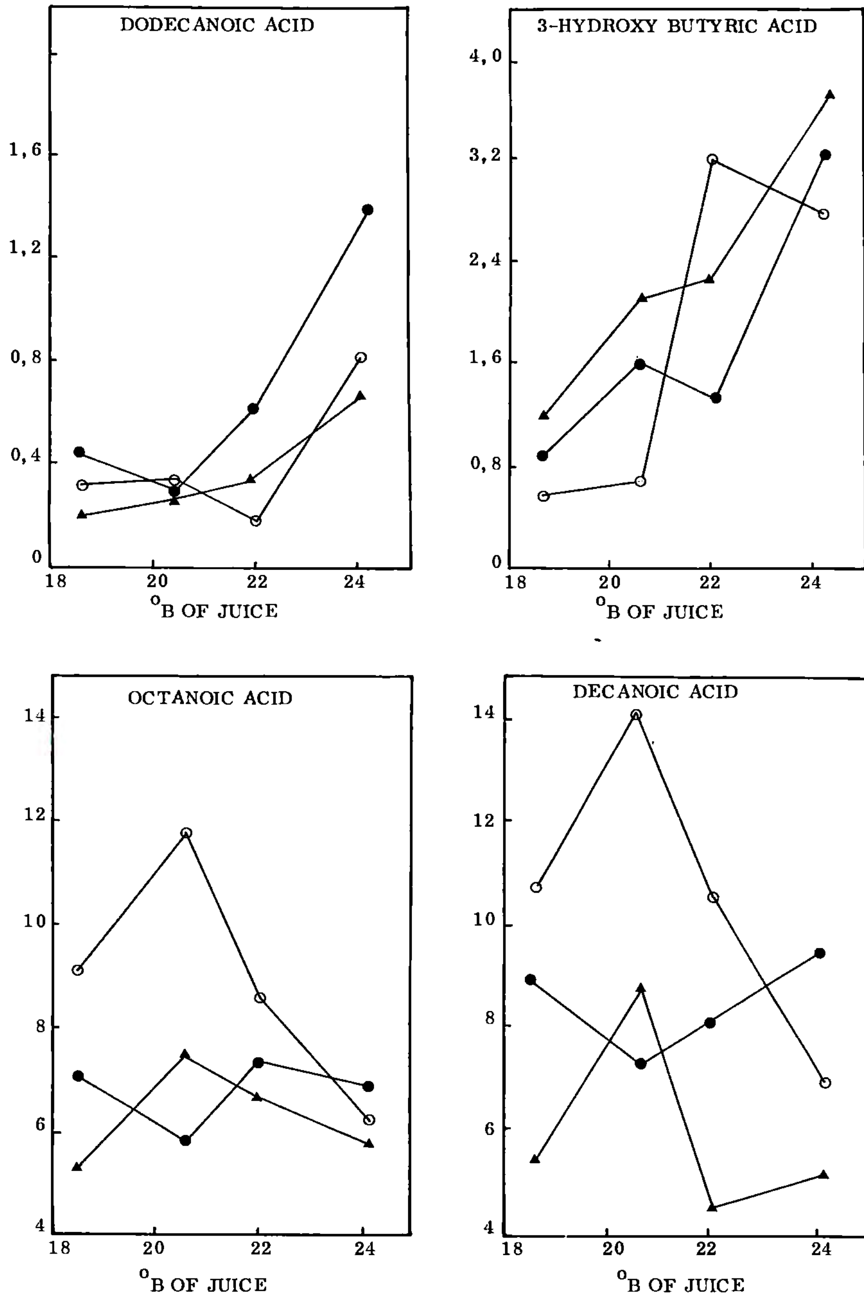


Fig. 5: Effect of juice clarity and air on volatile acid production during fermentation of juice from grapes of varying degrees of maturity. ——○ = Sterile filtered juice, de-aerated. ——▲ = Sterile filtered juice, aerated 6 × 15 min during first 3 d after inoculation. ——● = Settled juice.



Einfluß von Klarheit des Mostes und Luft auf die Bildung flüchtiger Säuren bei der Vergärung von Traubenmosten unterschiedlichen Reifegrades. ——○ = Sterilgefilterter Most, entlüftet. ——▲ = Sterilgefilterter Most, in den ersten 3 d nach der Hefezugabe 6 × 15 min belüftet. ——● = Vorgeklärter Most.

sible for a shift in the partition equilibria of these esters between the yeast lipid phase and the wine phase. In Table 5, however, it is shown that these equilibria for ethyl octanoate and decanoate in the cases of fermentations of juice of 15 and 22 °B were not markedly affected by the elevated alcohol content of the wine from the latter juice.

4. Wine quality

The results of wine quality evaluation on 5 fermentation variations are given in Table 6. Strictly anaerobic fermentation of clear-filtered juice with an addition

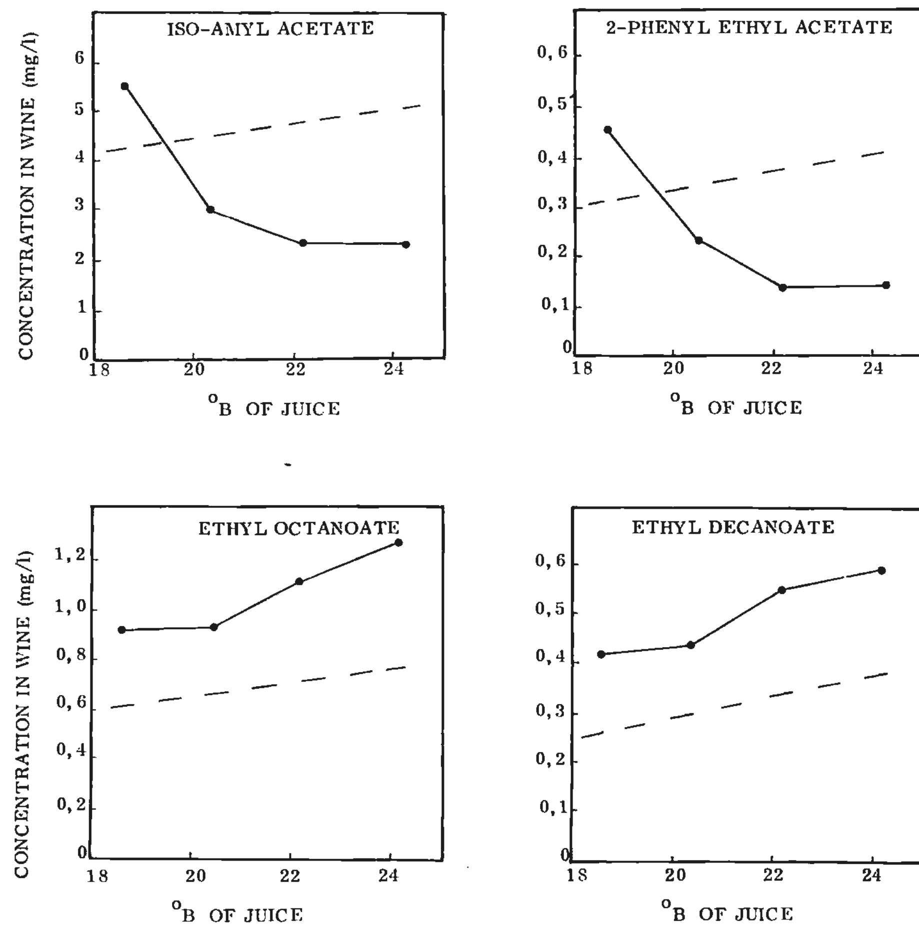


Fig. 6: Ester concentrations in wines made from grapes of varying degrees of maturity and from juices with artificially increased sugar contents. ——● = Wines from juices of 4 degrees of maturity. — — — = Average slope of ester concentrations in wines from juices which were artificially enriched by additions of glucose.

Esterkonzentrationen in Weinen aus Trauben unterschiedlichen Reifegrades und aus mit Zucker angereicherten Mosten. ——● = Weine aus Mosten 4 verschiedener Reifegrade. — — — = Durchschnittlicher Anstieg der Esterkonzentration in Weinen aus mit Glucose angereicherten Mosten.

Table 6

Sensory evaluation of wines obtained under five fermentative conditions

Sensorische Beurteilung von Weinen, die unter fünf verschiedenen Gärungsbedingungen hergestellt wurden

Series No.	Conditions			Fermentation time (d)	Unfermented sugar in wine (g/l)	Wine score (%)	Sensory aroma evaluation (remarks)[2]			
							Negative			Positive
	Juice clarity	Aeration[1])	Bentonite addition (g/l)				H_2S Mercaptan Malodour	Impure Grass Yeast Skin-odour	Oxidation	Clean Agreeable Flavoury
1	Settled	N_2	0	51	2.0	51	11	11	7	0
2	Sterile filtered	N_2 (air)	0	76	9.3	51	5	17	13	0
3	Sterile filtered	N_2 (air)	0.5	76	5.7	63	7	5	3	20
4	Sterile filtered	Air (6×)	0	41	1.7	48	27	5	3	0
5	Sterile filtered	Air (6×)	0.5	39	1.8	50	29	10	2	0

[1]) N_2 = Juice de-aerated with nitrogen and maintained under anaerobic conditions.
N_2 (air) = Treated as under N_2: 5 min aeration after stoppage of fermentation in order to revive the yeast.
Air (6×) = Juice aerated 6 × 15 min, twice daily during first 3 d of fermentation.

[2]) Percentage of panel members remarking on a particular flavour property.

Data: 4 replicates with juices of different grades of maturity.

of 0.5 g bentonite/l (series No. 3) gave the best wines and flavour. The fermentations of the series 2 and 3 ceased before all the sugar was consumed. A slight aeration, applied in a late stage of fermentation in order to revive the yeast, caused oxydation of the wines in No. 2 series. The bentonite added to No. 3 series evidently protected these wines against this oxydation. The difference in sensory evaluation between series 3 and the other series was highly significant. The wine scores of the series 1, 2, 4 and 5 were about equal, but their aroma properties were markedly different. According to the remarks of the panel the 8 wines of the series 4 and 5, which were aerated in the first days of fermentation, developed mercaptan-like odours.

Conclusions

It is most surprising in a science as old as wine making that so few systematic studies have been made to define which juice factors control the formation of aroma components during fermentation. Up to the present, research on the effect of individual must components on wine aroma has been limited to amino acids, glucose and several acids and cations. The relation between e.g. amino acids and the production of higher alcohols via the so-called "Ehrlich pathway" as well as other pathways e.g. from glucose to fatty acids, fusel alcohols and esters is well known, but in a practical sense, the relationship between juice components and wine flavour is factually still unknown terrain (HARVALIA 1976).

As far as incompletely defined factors such as grape maturity and juice turbidity are concerned, it is important that the active chemical agents be determined. In this respect the recent findings on ergosterol activity have enhanced the possibility of working under exactly defined, though not strictly homogenous conditions.

In making high quality white wines it is usually the aim to achieve absolutely clear juice, strictly anaerobic conditions and slow fermentation at low temperatures. By addition of pure ergosterol, which is a natural grape component, to the clear juice instead of juice turbidity particles, the fermentation stimulating effect of juice turbidity may be attained without the danger of formation of malodours.

To achieve systematic and good reproducible results in wine research, especially with regard to the relationship between juice composition, aroma formation during fermentation and sensorily evaluated wine quality, it is essential to exclude the imponderable effects on fermentation and aroma exerted by heterogenous factors such as must turbidity and oxygen. Completely clear and de-aerated juice should serve as primary material, employing the lowest possible amount of yeast inoculum. Exact additions of air or ergosterol can be used in order to give sufficient stimulation to the yeast to complete fermentation. Above mentioned additions should be applied to the juice before inoculation.

With such additions of air and of ergosterol almost identical wine aromagrams were obtained from the same juice. Apparently the influence of these agents on aroma formation is an indirect one connected with their yeast growth promoting facility.

The possibility for a systematic approach to the relationship between juice components and wine aroma has become a reality due to the relatively small number of variables and the advanced aroma analytical techniques.

Summary

Gas chromatographic analysis was used for the investigation of some factors which determine the production of volatile wine components during fermentation. Especially the effect of "heterogenous" juice factors such as turbidity and air content were investigated in respect of fermentation rate and the production of fatty acids, fusel alcohols and esters. It was shown that the fermentation lag of clear filtered and de-aerated grape juices could be prevented by small ergosterol additions before inoculation. A marked similarity was observed in the chromatograms of wines from fermentations of clear juices which were stimulated either by air or by ergosterol. Grape maturity proved to be a major factor affecting the production of volatile acids, alcohols and esters during fermentation.

Fermentations of de-aerated and clear filtered juice with an addition of 0.5 g bentonite/l yielded wines with the best scores on sensory evaluation.

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