The Effect of Grape Cultivar and Yeast Strain on Fermentation Rate and Concentration of Volatile Components in Wine

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Wines were made from five grape cultivars using eight strains of *Saccharomyces cerevisiae*. The mean fermentation rate was determined for the completed fermentation as well as for the yeast proliferation phase. The concentrations of thirty volatile wine components were gas chromatographically determined using a packed column. Fermentation rate and wine volatile production are largely affected by a juice turbidity fraction. It was indicated that cultivar juices differed in the filterability of that fraction in that filtration of Colombar and Chenin blanc juices caused serious lagging fermentation problems whereas the filtrates of Cape Riesling and Muscat d'Alexandrie juices readily fermented dry. Although seven of the eight yeast strains required the turbidity fraction for active yeast proliferation and completion of fermentation, the requirements of these yeast strains for this fraction differed markedly. Three groups of volatiles could be distinguished in the wines, namely those whose concentrations were:

- **mainly related to the grape juice,**
- **dependent on precursors in the juice and the chemical transformation capacity of the yeast strain, and**
- **dependent on the yeast strain.**

A basic fermentation bouquet concept was proposed.

Over the last two decades many research results have been published, reporting new wine aroma quality compounds which are often found in concentrations as low as 10^{-9}g.l^{-1}. With the rapid development of analytical technology, the number of quantitatively determinable components will undoubtedly increase possibilities to investigate the origin of wine volatiles and the ways to manipulate their individual concentrations and thus wine quality. The three primary factors which can affect wine aroma are yeast strain or species, vinification procedure and grape cultivar.

Recent investigations regarding the effect of yeast strain on wine aroma confirmed the diversity of yeasts with respect to the production of esters and higher alcohols (Soufleros & Bertrand, 1979; Di Stefano, Ciolfi & Delfini, 1981; Soles, Ough & Kunkee, 1982). Chen (1978) evaluated the production of higher alcohols from a biochemical viewpoint whereas Nordström (1964, 1965, 1966a, 1966b) approached the biosyntheses of fatty acids and esters from co-enzyme-A activated precursors on a biomathematical basis.

Vinification procedures to promote wider taste and aroma spectra in wines have received little attention. Results obtained to date indicate limited possibilities in this field (Wucherpfennig & Bretthauer, 1970; Ribéreau-Gayon, Lafon-Lafourcade & Bertrand, 1975; Bertrand, Marly-Brugerolle & Sarre, 1978; Killian & Ough, 1979; Houtman, Marais & Du Plessis, 1980 a, 1980 b; Houtman & Du Plessis, 1981).

To date the interaction between yeast strain and cultivar has not been investigated and its possible contribution towards improved vinification procedures should be studied with regard to the steering of aroma production and the variability of the aroma composition of wines.

In this study several yeast strains and grape cultivars will be compared in respect of practical criteria such as fermentation rate, the ability to complete fermentation and the production of wines with varying aroma characteristics.

MATERIALS AND METHODS

Grape cultivars, wine making:

Wines were made from the cultivars Chenin blanc, Sémillon, Muscat d'Alexandrie, Cape Riesling and Colombar. All juices were from the 1982 vintage except for the juices noted in Table 4 (1981 vintage). Since cultivar replicates were not done, the results must be regarded as indicative only.

Free run juice from a Bucher static separator was used. After overnight settling at approximately 3°C the juice was filtered through No. 3 porosity filter sheets and EK-filtration was applied as indicated in the relevant Tables. The juices were de-aerated by rapid sparging with carbon dioxide for five minutes.

Wines were made in 0,4 (laboratory) and in 70 litre (cellar) quantities by standard procedures as applied at the V.O.R.I. at a fermentation temperature of 15°C. All juices received 0,3 g.l^{-1} di-ammonium phosphate which is the maximum permitted by the European Economic Community (Anon., 1985). Fermentations which were not complete by the 35th day after inoculation were terminated to prevent possible microbial infection and air diffusion into the wine.

Yeasts:

The following *Saccharomyces cerevisiae* strains from the V.O.R.I. collection were studied: WE 14, WE 372, WE 392, WE 432, WE 452, WE 457, WE 459 and WE 460. In the laboratory, juices blanketed with CO_2 were inoculated with 2,5% (v/v) of six day old yeast cultures propagated in sterilised grape juice. In the cellar fermentations were carried out only with rehydrated dried

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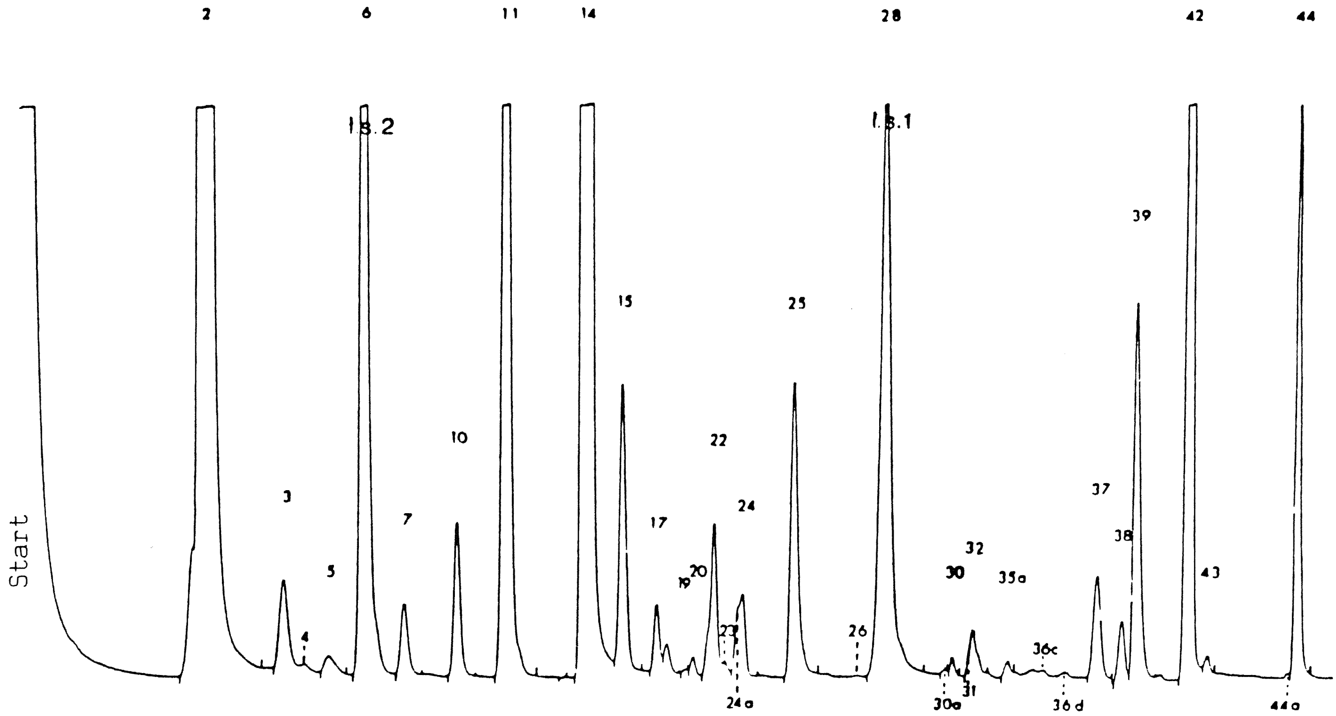

FIG. 1
Peak numbering on a gas chromatogram of the Freon extract from Muscat d'Alexandrie. (2 = ethyl acetate, 7 = ethyl butyrate, 10 = i-butanol, 11 = i-amyl acetate, 14 = i-amyl alcohol, 15 = ethyl hexanoate, 17 = hexyl acetate, 22 = hexanol, 25 = ethyl octanoate, 32 = ethyl decanoate, 38 = 2-phenyl ethyl acetate, 39 = hexanoic acid, 42 = 2-phenyl ethyl alcohol, 44 = octanoic acid, IS 1 = internal standard, IS 2 = internal standard. (Balance of components were not identified.)

WE 14 and WE 452 yeast preparations at $0{,}5g.l^{-1}$.

Fermentation time and rate:

Fermentation times were determined over two periods, viz. the fermentation period covering the sugar consumption from 5-50% of the sugar concentration of the juice (Ft 5-50) and the period from zero to 99% sugar utilisation (Ft 0-99). The corresponding fermentation rates are expressed as the mean percentage sugar utilised per day over these periods, viz.

$$Fr\ 5\text{-}50 = \frac{45}{Ft\ 5\text{-}50}\%.\ d^{-1} \text{ and}$$

$$Fr\ 0\text{-}99 = \frac{99}{Ft\ 0\text{-}99}\%.d^{-1} \text{respectively.}$$

To determine the Ft 0-99 in the case of lagging fermentations, the fermentation curve was extrapolated to the 99% sugar utilisation level.

Gas chromatographic analyses:

Wine volatiles were determined by the method of Marais & Houtman (1979) and peaks numbered as shown in Fig. 1.

Relative concentrations of unidentified components were obtained by comparing their peak heights with that of ethyl octanoate (Fig. 1 peak 25) using the following formula:

$$C_x = \frac{h_x}{h_{eo}}.C_{eo} \text{ where}$$

C_x = concentration of unidentified component
h_x = peak height of unidentified component
h_{eo} = peak height of ethyl octanoate
C_{eo} = concentration of ethyl octanoate in the wine in $\mu g.l^{-1}$ as found according to Marais & Houtman (1979).

The data obtained from laboratory fermented samples agreed satisfactorily with counterpart cellar data and these were consequently combined.

RESULTS AND DISCUSSION

Fermentation rate:

Effect of juice clarification: The fermentation rates in three juices were compared after clarification by settling, filtration and EK-filtration (Table 1). The Fr 0-99 was considerably decreased as a result of the filtrations i.e. in relation to settled juice and serious lagging fermentation was observed in the filtered Colombar and Chenin blanc juices, where fermentation times ranged from 53 to 77 days. Evidently an essential fermentation promoting fraction was removed from the juice by the filtration process and conditions in the highly clarified juices were not conducive to fermentation. This insoluble fraction was even more strongly affected by EK-filtration demonstrating that at least part of this fraction can pass through a filter sheet with porosity 3. These results indicate that this fraction is present in the free run grape juice in a finely dispersed form.

TABLE 1
Effect of juice clarification on the fermentation rate (Fr 0-99) in % sugar.d^{-1} of three juices fermented with yeast strain WE 14.

Cultivar	Clarification method		
	Settling	Filtration (No. 3)	EK-filtration
Colombar	2,3	1,3	1,0
Chenin blanc	4,4	1,9	1,2
Cape Riesling	6,6	3,6	2,5

Effect of grape cultivar: Results in Table 1 indicate that out of three cultivars the composition of the filtered Cape Riesling juice samples was such that fermentation rate was higher than that of Colombar and Chenin blanc and was completed albeit at a lower rate than normal. Apparently a yeast growth promoting juice turbidity fraction was present in a highly dispersed form in the Cape Riesling samples consequently sufficient amounts could pass through the filter.

Effect of yeast strain: The fermentation rates of all eight investigated yeast strains were in relation to settled juice retarded considerably by juice filtration but some strains were more sensitive to this treatment than others (Table 2). Strain WE 432, for instance, fermented well in the filtered juice and completed fermentation in 33 days. Fermentations with the seven other yeast strains, however, showed lagging shortly after the yeast proliferation phase.

TABLE 2

Effect of yeast strain on the fermentation rate (% sugar.d^{-1}) of filtered and settled Chenin blanc juice.

Clarification	Yeast strain							
	WE 14	WE 372	WE 392	WE 432	WE 452	WE 457	WE 459	WE 460
Fr 5-50								
Filtered	6,4	5,1	5,6	9,0	9,0	5,6	7,5	5,7
Settled	9,0	9,0	11,2	11,2	14,1	11,8	12,9	12,5
Fr 0-99								
Filtered	1,4	0,7	0,5	3,0	2,2	0,8	1,1	0,8
Settled	3,6	3,1	2,2	3,6	4,4	4,4	4,5	4,4

Fig. 2 illustrates that two generally used yeast strains, WE 14 and WE 452, notwithstanding differences in their counterpart Fr 5-50 values, follow a similar pattern over the cultivar series in a nutrient deficient medium (filtered juice) with WE 452 utilizing this medium more efficiently than WE 14. It should also be kept in mind that degree of filtration cannot simply be defined by the number of the filter sheet for, as a result of clogging of the No. 3 filters during filtration, the effect of the latter sheets on fermentation properties, can under these conditions be very similar to that of EK-filters.

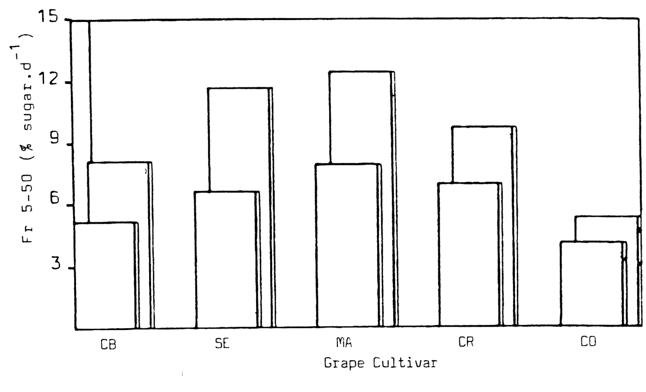

FIG. 2

Fermentation rates of yeast strains WE 14 and WE 452 in the proliferation stage (Fr 5-50) in filtrates of five grape cultivar juices. The data from WE 14 yeast are given by the front bar of the counterpart pairs and that of WE 452 by the rear bar (CB = Chenin blanc, SE = Sémillon, MA = Muscat d'Alexandrie, CR = Cape Riesling, CO = Colombar).

The Fr 5-50 values give a clear and comparable image of the fermentation rates during the proliferation phase because it does not include the lag phase before start of fermentation. These lag phases were more or less specific for the different yeast strains e.g. for WE 14 and WE 452 one and two days respectively (data not shown).

Volatile wine components:

Reproducibility of fermentation: The average values of the individual volatile compounds of laboratory wines are shown together with those of their counterpart cellar produced wines in Fig. 3. With the exception of peaks 18 and 24a no appreciable differences between the two peak ranges were observed.

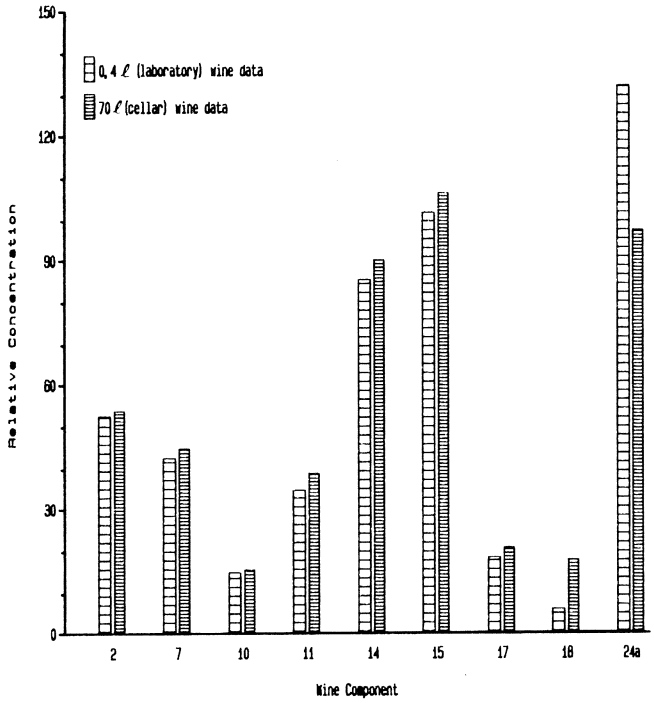

FIG. 3

Comparison of concentrations of volatiles in wines made in 0,4 *l* (laboratory) and 70 *l* (cellar) quantities.

Effects of grape cultivar and yeast strain: The concentrations of volatile wine components are determined on the one hand by the grape juice as supplier of varying concentrations of basic components and precursors and on the other by the yeast strain in its role as producer of the final volatiles. Twenty-nine wine components could be classified in three main groups.

Group 1: These component concentrations are apparently determined by the cultivar. The component represented by peak No. 23 for instance (Fig. 1), was present in the wines of all five cultivars but in very different concentrations with the highest level in Cape Riesling (Fig. 4.a). The concentration ranges for the WE 14 and WE 452 yeasts were of much the same order of magnitude per cultivar and indicate that this component was affected in only a small degree by the yeast strain. In some cases the wine components appear to be extremely cultivar specific. For example, peak No. 43 occurred exclusively in Muscat d'Alexandrie wines and was not markedly affected by the yeast strain being present at 43 and 45 $\mu g.l^{-1}$ in the WE 14 and WE452 wines respectively (data not shown). Components re-

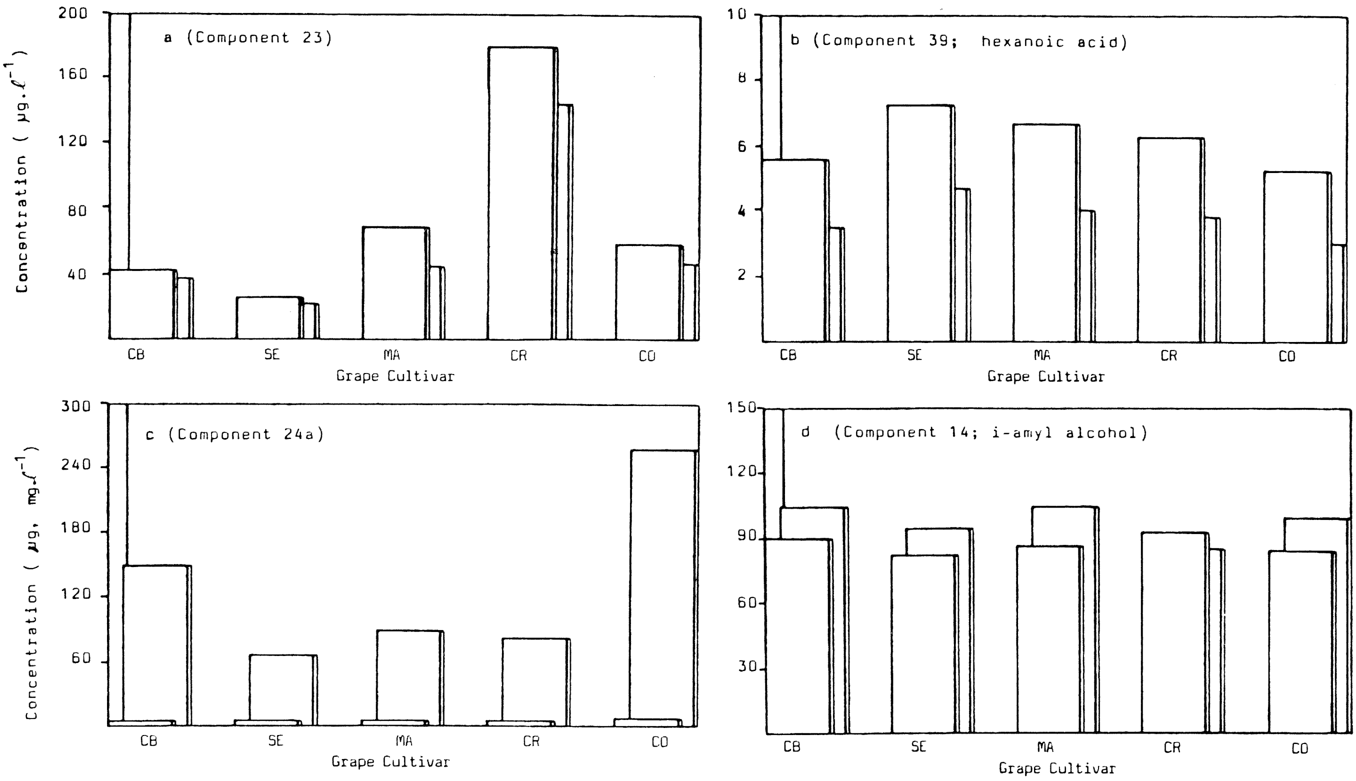

FIG. 4

The effect of cultivar and yeast strain on the concentration of wine volatiles. The data from WE 14 yeast are given by the front bar of the counterpart pairs. WE 452 data are given by the rear bar.

a. Cultivar effect as indicated by component 23.
b. Concentration of component 39 as affected by yeast strain and juice.
c. Concentration of component 24a as affected by yeast strain.
d. Concentration of component 14 as affected by cultivar.

(CB = Chenin blanc, SE = Sémillon, MA = Muscat d'Alexandrie, CR = Cape Riesling, CO = Colombar).

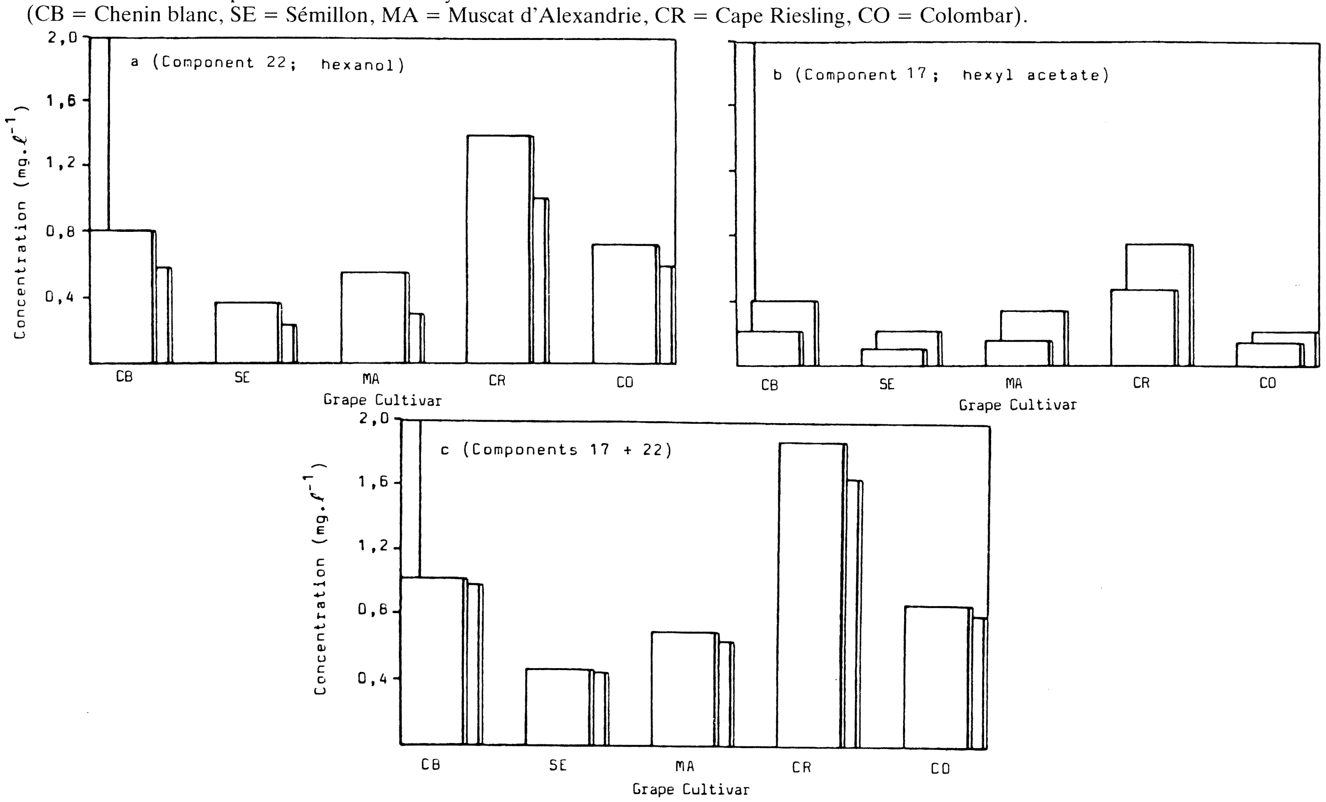

FIG. 5

The effect of cultivar and yeast strain on hexanol and hexyl acetate formation and on their ratio in wines. The data from WE 14 yeast are given by the front bar of the counterpart pairs. WE 452 data are given by the rear bar.

a. Concentration of hexanol (component 22) as affected by cultivar and yeast strain.
b. Concentration of hexyl acetate (component 17) as affected by cultivar and yeast strain.
c. Variation in the sum of hexanol and hexyl acetate as affected by cultivar.

(CB = Chenin blanc, SE = Sémillon, MA = Muscat d'Alexandrie, CR = Cape Riesling, CO = Colombar.)

TABLE 3

Effect of yeast strain on the concentration of aroma components in the wines made from settled Chenin blanc juice

Peak No.	Volatile wine component (mg.l^{-1})	Yeast strain							
		WE 14	WE 372	WE 392	WE 432	WE 452	WE 457	WE 459	WE 460
39	Hexanoic acid	5,8	4,9	6,4	2,1	3,7	5,5	6,0	5,8
44	Octanoic acid	7,4	7,1	9,3	4,4	5,6	8,0	7,8	8,1
25	Ethyl octanoate	1,35	1,35	1,40	0,83	1,10	1,45	1,30	1,40
32	Ethyl decanoate	0,45	0,40	0,47	0,24	0,34	0,43	0,46	0,47
10	i-Butanol	25	15	25	31	9	21	20	32
42	2-Phenyl ethanol	7,4	7,7	11,4	6,5	8,8	10,1	13,4	12,5
38	2-Phenyl ethyl acetate	0,12	0,17	0,22	0,10	0,29	0,37	0,38	0,43
24a*	Unknown	<0,01	0,08	0,03	0,03	0,25	0,02	0,19	0,19

*Relative values; calibration factor not determined

presented by peaks 23, 24, 30a, 31, 44a, 35a, 36c, 36d and 43 fall into this group with the last four specific for Muscat d'Alexandrie.

Group 2: These component concentrations depend on interaction between grape juice components and yeast strain. For instance, although component 39 showed some concentration variations over the cultivar range (Fig. 4 b), differences between yeasts were much more pronounced. This component was consistently approximately 40% lower in the wines produced with yeast WE 452 compared to those produced with WE 14. The concentration of wine volatiles in this group apparently depends on the concentration of specific basic components in the juices as well as on the production capacity of the yeast strains. The function of the juice as supplier of basic components and the yeast as the producer of volatiles is shown clearly in the case of components 22 (hexanol) and 17 (hexyl acetate) (Fig. 5a, b, c). The content of the hexanol precursor varied markedly in the five juices as shown by their hexanol concentrations in the wines (Fig. 5a). On the other hand the degree of acetylation, and consequently the hexanol: hexyl acetate ratio, is determined entirely by the yeast strain where WE 452 produces, more hexyl acetate (Fig. 5b) and WE 14 more hexanol (Fig. 5a). However, the sum of the alcohol and the acetate concentrations showed little difference between the two yeast strains and it was indicated to be related to the cultivar (Fig. 5c). Component 24a shows this tendency very clearly being present in the wines from yeast strain WE 14 in traces whereas relatively high concentrations occurred in the WE 452 wines from the same cultivars (Fig. 4c). Furthermore, large differences in concentration occurred especially in WE 452 wines of Chenin blanc, Colombar and Sémillon.

The effect of the yeast on component 24a is clearly shown in Table 3. Chenin blanc fermented with WE 452, WE 459 and WE 460 show relatively high concentrations of component 24a, whereas only traces were noted in the wines of the same juice fermented with yeasts WE 14, WE 392, WE 432, and WE 457.

Thirteen components indicated by peaks 4, 11, 15, 17, 19, 20, 22, 24a, 26, 30a, 37, 38 and 42 could be classified in this group.

Group 3: In this group wine component concentrations were apparently not markedly affected by the cultivar. Component 14 (i-amyl alcohol) is a typical example of the group and shows that it appears to be only slightly affected by the cultivar (Fig. 4d).

This component is an example of the fermentation bouquet components, a group of simple alcohols, acids and esters which are mainly products of sugar metabolism (Nordström, 1964, 1966a, 1966b; Chen, 1978) (See Table 4). In wines made from filtered juices the concentrations of these fermentation bouquet volatiles were to a large degree independent of the grape cultivar. The quantitative composition of the fermentation bouquets of five cultivar wines made from EK filtrates were remarkably similar (Table 4). Because of these low concentrations and the similarity of all wines from

TABLE 4

Fermentation bouquet components in the wines of EK-filtered juices of five grape cultivars*

Peak No.	Volatile wine component	Cultivar				
		Chenin blanc	Sémillon	Muscat d' Alexandrie	Cape Riesling	Colombar
39	Hexanoic acid	4,0	4,3	4,3	4,6	4,4
44	Octanoic acid	3,2	4,1	4,7	4,6	3,7
7	Ethyl butyrate	0,32	0,26	0,34	0,34	0,30
15	Ethyl hexanoate	0,65	0,60	0,59	0,63	0,63
25	Ethyl octanoate	1,23	0,98	1,01	0,93	0,94
10	i-Butanol	25	23	31	25	23
14	i-Amyl alcohol	81	77	98	90	79
42	2-Phenyl ethanol	5,1	4,2	5,5	5,0	4,8
2	Ethyl acetate	48	45	46	43	48
11	i-Amyl acetate	1,3	1,0	2,2	2,2	1,0
38	20Phenethyl acetate	0,06	0,05	0,09	0,07	0,05

*Data from 1981 vintage. Fermented with yeast strain WE 14. Concentration in mg.l^{-1}

EK-filtered juices in this respect the designation "basic fermentation bouquet" is proposed for the fermentation bouquet of such wines.

The similarity of the basic fermentation bouquet components formed from EK-filtered juices is striking especially when the wide ranges of concentrations of these volatiles in wines produced by standard V.O.R.I. procedures are considered. For instance concentration ranges for i-butanol, i-amyl alcohol, i-amyl acetate and 2-phenyl ethyl acetate are 8-36, 39-160, 2-16 and 0,01-0,55 mg.*l* respectively (Houtman, Marais & Du Plessis, 1980 b; Houtman & Du Plessis, 1981). During normal cellar practice concentrations of these volatiles are raised above the basic fermentation bouquet values, mainly as a result of the differences of juice turbidity properties and variation of yeast strains. The concentrations of five of the fermentation bouquet components (Table 5, numbers 7, 14, 15, 25 and 44) were found to be generally independent of the yeast strain whereas concentrations of components 10, 11 and 39 (Table 5) were altered by the yeast strain. This property could possibly be helpful in characterization of yeast strains.

The effect of the grape cultivar on the composition of the fermentation bouquet of cellar wines appears to be small. In Table 5 the mean concentrations of eleven volatiles of the cultivars Colombar, Chenin blanc, Sémillon, Muscat d'Alexandrie and Cape Riesling fermented with two yeast strains are given with their coefficients of variation. These coefficient of variation values are relatively small and probably indicate relative unimportance of the effect of cultivar on fermentation bouquet.

TABLE 5

The effect of two yeast strains on the concentration of basic fermentation bouquet components in the wines of five grape cultivars*

Peak No.	Volatile wine component	Yeast strain			
		WE 14		WE 452	
		Mean concentration (mg.l^{-1})	CV (%)	Mean concentration (mg.l^{-1})	CV (%)
7	Ethyl butyrate	0,43	7	0,41	19
15	Ethyl hexanoate	0,91	14	0,93	29
25	Ethyl octanoate	1,16	15	0,99	16
32	Ethyl decanoate	0,36	12	0,27	9
2	Ethyl acetate	43	14	94	21
11	i-Amyl acetate	2,9	38	7,0	39
39	Hexanoic acid	6,2	14	3,8	17
44	Octanoic acid	6,8	18	5,6	22
10	i-Butanol	15,6	12	8,4	20
14	i-Amyl alcohol	88	5	98	9
42	2-Phenyl ethanol	5,6	23	9,1	15

* Chenin blanc, Sémillon, Muscat d'Alexandrie, Cape Riesling and Colombar

CV : Coefficient of variation.

CONCLUSIONS

A finely dispersed fermentation promoting juice turbidity fraction is essential for a smooth fermentation of grape juices and the production of dry white wines under anaerobic conditions. Under the conditions of study the grape cultivars differed with respect to this turbidity fraction. In the case of settled as well as highly clarified juices fermentation of the cultivars Cape Riesling and Muscat d'Alexandrie was generally far less prone to lagging than that of Chenin blanc and Colombar.

The main volatiles in the wines could, on the basis of their origin, be divided into three main groups which differed in their dependency on basic or precursor material in the juice and the production capacity of the yeast strain as well as a combination of the two factors.

The wine volatiles are almost all strongly affected by the fermentation promoting juice turbidity fraction. Grape aroma volatiles are diminished in the juice as a result of rigorous clarification which could be due to the removal of aromatic oils. The production of fermentation bouquet components decreases under such conditions.

The composition of the basic fermentation bouquet was very constant and, in fact, it appeared to be largely independent of cultivar. However, as noted, three of the components could be affected by yeast strain. Hence, an effective but not too drastic, juice clarification is a major wine quality determining factor in the cellar. However, turbidity can also be detrimental in that unacceptably high fusel alcohol contents and malodours can arise from high concentration of juice solids.

Grape cultivar dependent bouquet was indicated to differ but slightly between wines of Chenin blanc, Sémillon, Cape Riesling and Colombar. However, the Muscat d'Alexandrie wine can be easily distinguished from these latter cultivars on the basis of at least four specific peaks.

Fermentation rate and concentration of wine volatiles can be affected by yeast strain. This aspect should be considered when selecting yeast strains to produce specific components and effect rapid fermentation. Fermentation rate can be increased by specific turbidity fractions which can possibly also contain grape aroma components.

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