

The Possibilities of applying present-day Knowledge of Wine Aroma Components: Influence of several Juice Factors on Fermentation rate and Ester Production during Fermentation

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ABSTRACT

The influence of several juice factors on fermentation and ester production was investigated to achieve a more satisfactory reproducibility of wine aroma composition. The contents of two acetates and three ethyl esters in the wine were used as the main analytical criteria. The factors which exerted the most marked influence on ester composition and fermentation were grape maturity, sugar content, fermentation temperature, and juice clarity. The cultivars examined exerted no noticeable differences.

INTRODUCTION

Esters are important aroma components of wine, and several studies have shown their correlation with wine quality (Wagener & Wagener, 1968; Suomalainen, 1971; Du Plessis, 1975; Malik, 1975). Grapes contain very small quantities of them; they have their major origin in the fermentation process (Bezzubow, Pisarnitskii, Egorov & Rodopulo, 1971; Suomalainen, 1971; Engan, 1974; Malik, 1975).

In pilot studies carried out at this Institute, large differences were observed in ester concentrations in representative and randomly chosen wines of each of six white cultivars. Furthermore, no significant differences could be established between average specific ester concentrations on a cultivar basis. These specific findings indicated that the cultivar, as such, exercises an insignificant effect on the pattern of ester synthesis during fermentation and is, in fact, secondary to known relevant factors such as i.a. juice composition and turbidity, oxygen, yeast strain and proliferation rate, as well as cellar technological conditions. The relevant literature in this field reveals apparent contradictions in respect of the influence of the abovementioned factors. Contradictions are to be expected if the effects of some of the pertinent variables are not taken into consideration. In view of these apparent anomalies it is necessary that individual variables be studied under less complex conditions and systems defined, which could lead to more reproduceable results.

Since the major portion of the aroma is formed during fermentation, it appears logical that the perceptibility of the cultivar character of a wine might be strongly dependent upon the composition of the fermentation aroma, which often dominates the grape bouquet. Ester concentrations and concentration ratios in the wine could be meaningfully used as sensitive indicators of the reproducibility of the fermentation process. A control of the

process in such a manner that various gaschromatographic patterns can be pursued, will be useful in a standardisation of fermentation conditions and lead to greater diversity and consistently higher quality of wines. This study was aimed at the determination of must and technological factors which control ester production, and at the effect of these factors on the concentration of individual esters or groups of esters in the wine.

MATERIALS AND METHODS

Materials

Wines: To study the cultivar effect representative wine samples from the Cape wine show were obtained and analysed.

Juices:

Sequential series of 24 fermentations were carried out with free-run juice of grapes from one specific vineyard. The white wine cultivar most planted in South Africa, i.e. Chenin blanc, was chosen for this study. The grapes were harvested at different stages of maturity, crushed and SO₂ added to 100 mg/l and the juice partly de-acidified where necessary by the standard Acidex procedure to approximately 6 g/l total acidity (Table 1). The juice was settled for 24 hours under cooling at 5 °C, the supernatant drawn off and held under nitrogen at -12 °C until required. Thawing and homogenisation were also carried out under nitrogen.

To study the effect of clarity the following procedures were applied to obtain juice in 4 degrees of clarity, viz. C₁ - C₄.

- (a) The supernatant of settled juice was taken as such (C₁).
- (b) Juice C₁ was coarsely filtered through a No. 3 cellulose-asbestos sheet (C₂).
- (c) Juice C₁ was centrifuged for 10 min at 8 000 × g (C₃).
- (d) Juice C₁ was sterile filtered (C₄).

Extraction of finely suspended grape particulate matter: Four litres of settled juice (C₁) were centrifuged for 10 min. at 8 000 × g. The wet grape particulate deposit (18g) was macerated under nitrogen for one day at room temperature with 75 ml methanol. Subsequently 300 ml ether were added to the macerated mixture and again held for one day. The solid phase was filtered off and washed with 50 ml ether which was added to the filtrate.

The methanol and ether in the filtrate were evaporated at 40 °C and the fatty residue finally dried under vacuum.

TABLE I
Analytical data of Chenin blanc grape juices

Sample No.	°Balling	Reducing Sugar (g/l)	pH	Total* acidity (g/l)	SO ₂ (mg/l)		α-amino nitrogen (mg N/l)
					free	total	
74/1	14,7	140	3,36	6,9	36	91	—
74/44	21,5	210	3,40	6,8	26	63	—
75/1	13,6	126	3,85	5,4	34	88	125
75/75	19,3	184	3,29	7,6	30	85	205
75/125	22,5	220	3,46	5,8	24	88	240
76/1	13,5	124	3,95	4,7	30	86	—
76/7	20,0	176	3,51	6,3	36	74	—
76/25	20,3	180	3,45	6,7	42	72	—
76/122	22,7	220	3,30	6,0	41	88	—
77/2	16,3	143	3,07	12,5	28	84	—
77/24	17,7	168	3,15	9,1	28	70	—
77/76	18,9	174	3,30	8,9	23	64	—
77/145	20,6	195	3,20	8,0	18	46	—
77/205	21,1	193	3,30	7,7	20	66	—

*High acidity in relatively unripe grapes was partially neutralised.

This residue (1,60 g) was dissolved in 80 ml 96 vol % ethanol and 2,0 g Tween 80 added as emulsifier. Of this solution 5 ml were added to 1 l of the sterile filtered juice C₄.

Juice lees additions: Free-run juice was settled and the lees separated from the supernatant. The supernatant was sterile filtered (C₄) and the separated lees added to this limpid juice on a volumetric basis.

Nutrilite and amino acid additions: The following nutritives were added individually and in combination to samples at two to tenfold the concentrations (mg/l) reported for grapes (Ribéreau-Gayon & Peynaud, 1964): Biotin (0,06); thiamine (0,4); nicotinic acid (4,0); pyridoxine (1,0); inositol (10,0) and pantothenic acid (2,5).

Amino acid combinations were also added to individual samples as follows:

(Amino acid and NH₄C1 additions are expressed in mg N/l).

- Leucine (16) + i-leucine (16) + threonine (16) + valine (16) + methionine (16).
- Arginine (60) + lysine (20).
- Tryptophane (25) + phenylalanine (30) + tyrosine (25).
- Asparagine (40) + glutamine (40).
- Ammonium chloride (80).

Yeast: A pure culture of *Saccharomyces cerevisiae* (WE 14) was used throughout. Unless otherwise noted propagation of the yeast culture was carried out in sterilized juice at 15 °C for 8 days.

Inoculation: Inoculations were carried out under standard aerobic conditions or under nitrogen, which entailed the displacing of air with nitrogen immediately before and after inoculation. Apart from the initial displacement of air no further nitrogen was applied. Unless otherwise stated a 2,5% inoculation was carried out.

Fermentations: Fermentations were carried out at 11° or 15 °C in 400 ml juice lots in 750cm³ containers with double glass bulb fermentation caps.

Fermentation rate: The fermentation rate was expressed as the average daily CO₂ mass loss (%) from the first determinable loss (0,5 g/l) to the point where a mass loss of 50% of the completed fermentation occurred.

Time of sample analysis: Since ester formation occurs at a relatively early stage of fermentation viz. the exponential phase (Nordström, 1965), samples were analysed on a comparative time basis. Samples exhibiting lengthy fermentation were analysed at the same time as faster fermenting samples i.e. on completion of the latter fermentations, except in the cases where the influence of time on ester formation was studied.

Yeast mass: Yeast mass was determined by weighing the moist yeast after centrifuging the 400 ml samples at 8 000 × g for 10 min. and decanting the supernatant.

Ester determination: The following esters were determined: iso-amylacetate (AA), n-hexyl acetate (HA), ethyl caproate (C₆E), ethyl caprylate (C₈E), ethyl caprate (C₁₀E), ethyl laurate (C₁₂E) and 2-phenyl ethyl acetate (PEA).

Wines

Esters were determined quantitatively by gas chromatography. To 200 ml centrifuged wine a solution of 2,00 ml ethyl nonanoate (0,050 g/l 96% ethanol) was added as internal standard. The esters were extracted with 3 × 50 ml ether, the combined ether extract was washed with 1 × 40 ml 0,5 N NaOH, followed by 4 × 40 ml H₂O and subsequently dried overnight over anhydrous magnesium sulphate. The filtrate was concentrated to ca 0,5 ml in a waterbath at 40 °C and injected as such. The individual ester concentrations were calculated on a peak area basis (height × width at half height). The gas chromatography parameters were as follows:

Columns: 3 m × 3 mm stainless steel.

Carrier phase: Chromosorb G. DMCS 60/80 mesh.

Stationary phase: Apiezon M, 5%.

Tailing reducer: purified manoxol O.T. (3% of stationary phase).

Carrier gas: N₂, 30 ml min⁻¹.

Temperature program: 60 °C for 15 min., raised at 6 °C per minute to 120 °C, 120 °C for 8 min., raised at 7,5 °C per min. to 180 °C.

Detector: flame ionization.

Yeast

Subsequent to the mass determination of the freshly centrifuged yeast, it was mixed with a 60 ml methanol 15 ml ether mixture and kept for 3 days at -12 °C. After filtering off the yeast through a fine glass filter and

washing with 20 ml fresh methanol/ether mixture, two further 3-day extractions at -12°C were made, once with 60 ml methanol/ether (1:1) mixture and finally with 60 ml ether. Subsequently 2,00 ml of ethyl nonanoate internal standard solution (1,00 g/l 96% ethanol) were added to the extracts and washings. The solution was washed with 1×50 ml 0,5 N NaOH and 4×70 ml water to remove the methanol and subsequently concentrated to ca 10 ml in a waterbath at 40°C and washed with 5 ml water. The extract was used as such and quantitation carried out as before.

RESULTS AND DISCUSSION

Variation in ester concentrations in wine and yeast during fermentation: The final concentration of an ester in a wine is dependent i.a. on its formation and destruction during the fermentation process and on its distribution between the wine and the yeast as shown in Fig. 1 and Table 2. The stage of maximum ester content under the conditions of this study (11°C) occurred 2–3 weeks after the onset of fermentation. Subsequent to this stage a decrease occurred which was most marked in the cases of AA and C₆E. Decreases in especially PEA, C₈E and C₁₀E were not as high. In addition to chemical stability the relatively high concentrations of the latter two esters in the yeast cell itself (Table 2) are possibly contributory to this phenomenon in the stabilising effect which they exert on the relatively constant values of these esters in the wine. In this respect it must be noted that due to the increasing apolarity of the higher esters their relative concentration increases in the yeast cell and decreases in the wine. The C₁₂E ester e.g. is present in the cell in amounts up to 50 times more than in the wine (Table 2). Similar findings in beer have been reported (Nykänen, Nykänen & Suomalainen, 1977).

Cultivar effect: The influence of white cultivars on ester formation is shown in Table 3. It is clear that marked variations occurred in the ester concentrations within each cultivar and, furthermore, that these concentration ranges of the cultivar overlap to such an extent that the cultivar effects could not be differentiated by visual examination of standard deviations.

Nutrilitite and amino acid effect: The influence of amino acids on yeast growth and ester formation has been reported by several authorities. Studies concerning this aspect were carried out on juices 75/1, 75/75 and 75/125, of which the α -amino nitrogen contents are given in Table 1. Apart from the known effects, such as the direct

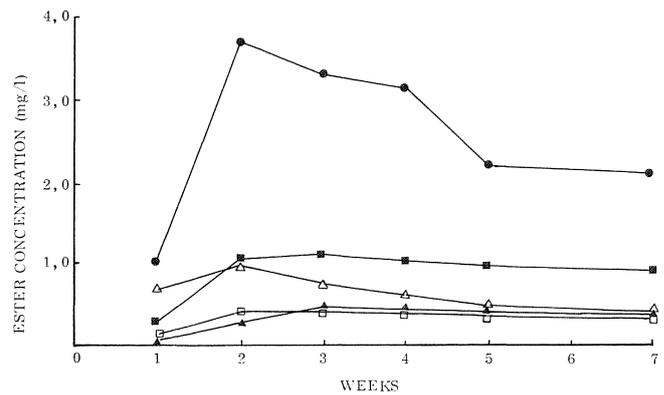


FIG. 1

Development of ester concentrations in Chenin blanc must during a seven week period of fermentation and yeast contact. (Grape juice 74/44, C₁, inoculated with 5% yeast culture under anaerobic conditions, was fermented at 11°C .)

- △ Ethyl caproate
- Ethyl caprylate
- ▲ Ethyl caprylate
- i-Amyl acetate
- 2-Phenyl ethyl acetate

stimulation of ester formation by addition of precursors, for instance the production of PEA from phenylalanine, no indirect effect was observed on the production of other esters such as the fatty acid ethyl esters. It was found that the juice material studied obviously contained sufficient of these nutrilites and nutrients for optimal ester production. These findings confirm those of Harvalia (1976) viz. that ester composition of wines was not significantly related to the amino acid composition of musts in general fermentation practice since musts normally contain adequate concentrations of these components.

Grape maturity and sugar content of juice: The influence of grape maturity on ester formation was studied on juices 74/1 and 74/44 whose sugar contents were 140 and 210 g/l respectively (Table 1). The influence of sugar as a prominent factor in maturity on this facet was investigated by the addition of glucose as indicated in Table 4A. Increasing the sugar content of juice 74/1 to that of 74/44 effects significant increases in the concentration of both ethyl and acetate esters (Table 4A, columns 1 and 2 and Table 4B). Sugar addition to juice 74/1 (immature) had the same effect on ethyl ester formation as grape maturation. Their concentrations in the wine were to all intents identical with those formed by fermentation of juice 74/44; in fact, no significant differences were shown (Table 4A, columns 2 and 3).

TABLE 2
Distribution of esters between the wine and yeast phases at three stages of fermentation* of Chenin blanc juice

Ester concentrations (mg/litre fermenting liquid)	Weeks after inoculation								
	2			4			7		
	in wine	in yeast	total	in wine	in yeast	total	in wine	in yeast	total
AA	3,70	0,0	3,70	3,15	0,0	3,15	2,10	0,0	2,10
PEA	0,41	0,0	0,41	0,39	0,0	0,39	0,36	0,0	0,36
C ₆ E	1,00	0,0	1,00	0,62	0,0	0,62	0,44	0,0	0,44
C ₈ E	1,00	0,95	1,95	1,00	1,25	2,25	0,90	1,10	2,00
C ₁₀ E	0,30	2,70	3,00	0,40	7,40	7,80	0,40	7,70	8,10
C ₁₂ E	0,06	1,05	1,11	0,03	1,65	1,68	0,04	2,00	2,04

*Anaerobic inoculation and fermentation at 11°C .

TABLE 3
*Range of ester concentrations in six white cultivar wines

Ester	Cultivar					
	Chenin blanc (mg/l)	Riesling (mg/l)	Palomino (mg/l)	Semillon (mg/l)	Clairette blanche (mg/l)	Colombard (mg/l)
AA	2,0-9,2	2,7-7,4	3,6-7,5	0,8-8,0	4,5-15,9	3,0-10,2
HA	0,10-0,42	0,14-0,32	0,05-0,34	0,04-0,25	0,28-0,65	0,16-0,39
PEA	0,09-0,30	0,15-0,33	0,13-0,39	0,05-0,30	0,23-0,48	0,17-0,39
C ₈ E	0,65-1,05	0,70-1,25	0,85-1,60	0,55-1,20	0,90-1,45	0,70-1,30
C ₈ E	1,50-2,30	1,75-2,60	1,95-2,65	1,45-2,45	2,00-2,70	1,95-2,40
C ₁₀ E	0,37-0,65	0,42-0,60	0,39-0,73	0,35-0,73	0,45-0,80	0,43-0,60

*Six samples of each cultivar.

In respect of the acetate ester concentrations in the wine, AA and PEA show a significant decrease as a result of increased grape maturity (Table 4A, columns 1 and 3). In the case of these acetates this maturation effect cannot be attributed to the increase in sugar content since elevated sugar content and grape maturity have the opposite effect in this case (Table 4A, columns 1, 2 and 3). Increasing the sugar content of the more mature grape juice 74/44 to 230 g/l induced a further increase in the concentration of both acetate and ethyl esters in the wine, although significance was only shown with respect to AA, C₈E and C₁₀E (Table 4A, columns 3 and 4). The influence of sugar on ester formation was re-examined on juice 76/1. Relevant data are given in Table 5. With sugar content as the only variable an increase in this component confirmed the former data, viz. both acetate and ethyl esters increased significantly in the wine with increasing must sugar content (Table 5). As a point of interest it was noted here that, under these conditions, the sugar content had no effect on the rate of fermentation even at the high concentration of 274 g/l.

A possible explanation for the increase in the more apolar esters e.g. C₈E and C₁₀E, may be found in the increased wine alcohol content with the higher sugar concentration in the juice. Under these conditions the higher ethanol concentrations could promote formation of the ethyl esters as well as raise the solubility of this specific group of esters in the wine. Especially in view of the large concentrations of these esters in the yeast itself, their partition coefficients in the wine phase/yeast lipid phase are important. In the case of more elevated ethanol concentrations these coefficients will alter in favour of the wine phase. Although grape sugar content has been shown to influence ester formation, the stage of maturity of the grape, as seen apart from its sugar content, has also been indicated as influencing acetate but not ethyl ester formation. It is clear that there are unknown factors whose influence, especially upon the acetate ester production, varies during the ripening of the grape.

Juice clarity, yeast growth and rate of fermentation in relation to ester production: Several authors, especially Nordström (1965), have emphasised the close

TABLE 4A
*Influence of grape maturity and sugar level on concentration of esters in the Chenin blanc wine

Column No.	Immature grape juice 74/1 (C ₁)		Mature grape juice 77/44 (C ₁)	
	1	2	3	4
Glucose addition (g/l)	0	70	0	30
Final sugar concentration (g/l)	140	210	210	240
Ester concentration (mg/l)				
AA	3,02	3,96	1,87	2,13
PEA	0,36	0,55	0,27	0,29
C ₈ E	0,56	0,69	0,67	0,71
C ₈ E	0,73	1,07	1,07	1,15
C ₁₀ E	0,32	0,46	0,42	0,50

*5% yeast inoculation under nitrogen, fermented at 11 °C. Average ester values of 6 replicates.

TABLE 4B
Statistical significance of the differences between the ester concentrations in Chenin blanc wines, due to grape maturity and sugar content variations^a

Esters	Sugar level 140 vs 210 g/l		Sugar level 210 g/l Immature vs mature grapes col. 2 vs 3	Sugar level 210 vs 240 g/l Mature grapes col. 3 vs 4
	Immature vs mature grapes col. 1 vs 3	Immature grapes col. 1 vs 2		
AA	**	**	**	*
PEA	**	**	**	NS
C ₈ E	*	**	NS	NS
C ₈ E	**	**	NS	*
C ₁₀ E	**	**	NS	**

^a Data from Table 4A.

* Significant at 95% confidence level.

** Significant at 99% confidence level.

NS Not significant.

TABLE 5
Sugar contents in juice and correlations with ester concentrations in Chenin blanc wines

Glucose addition (g/l) Final must sugar content (g/l)	0 124	30 154	60 184	120 244	150 274	Correlation coefficient (r)
Ester concentrations in wines (mg/l)						
AA	2,00	2,85	3,50	4,15	5,15	0,98**
PEA	0,23	0,31	0,31	0,40	0,42	0,97**
C ₆ E	0,44	0,39	0,50	0,53	0,64	0,90**
C ₈ E	0,35	0,32	0,42	0,54	0,47	0,85*
C ₁₀ E	0,13	0,15	0,15	0,24	0,28	0,97**

Grape juice 76/1 (95% C₄ + 5% C₁).

Inoculated with 5% yeast under aerobic conditions; fermentation temperature 11 °C.

*Significant at 95% confidence level.

**Significant at 99% confidence level.

relationship between yeast mass, fermentation rate and ester production. These relationships were consequently studied on juice 76/7, and relevant data are given in Tables 6A and B. It is clear that a highly significant correlation existed between yeast mass and fermentation rate. Furthermore, except for the C₆E ester the concentrations of both acetate and ethyl esters correlate highly significantly with both fermentation rate and yeast mass.

The latter parameters were, as a result of these findings, also incorporated into a study on the effect of juice clarity on ester formation. The influence on fermentation tempo, effected by different degrees of juice turbidity under anaerobic conditions, is shown in Fig. 2. The fermentation of sterile filtered juice (C₄) is, relative to the settled juice (C₁), markedly slower. The fermentation curves of coarse filtered (C₂) and centrifuged (C₃) juice were approximately the same and located between C₁ and C₄. The strong

retardation of the fermentation of the C₄ juice was completely cancelled out by the addition of 10% C₁ juice.

The addition of a small amount of turbidity to the C₄ juice, which was scarcely visible, was evidently sufficient to promote a considerable yeast activity. Subsequent repeat studies carried out with juice from grapes of different stages of maturity confirmed these results.

Data on the influence of juice clarity on ester formation together with relevant fermentation rates and yeast mass are given in Table 6A. It is clear that the addition of the very small amount of turbidity contained in the relatively clear settled juice (C₁) to the limpid filtered juice (C₄) effects a significant increase in the two acetate esters and C₈E and C₁₀E. The C₆E ester does not appear to be affected. The exceptional effectiveness of the small turbidity particles in the juice in causing ester increments is further illustrated in the comparison between the data of

TABLE 6A
Influence of juice clarity on fermentation rate, yeast mass and ester formation in Chenin blanc wines^a

Column No. Juice clarities	1 C ₄	2 90% C ₄ + 10% C ₁	3 C ₁	F	
				col. 1 vs col. 2	col. 2 vs col. 3
Fermentation rate (% CO ₂ /day)	3,75	11,1	11,1	415**	NS
^b Yeast mass (g/l)	5,1	10,4	11,0	25**	NS
Ester concentrations (mg/l)					
AA	2,65	8,05	9,05	94**	NS
PEA	0,30	0,85	1,00	43**	NS
C ₆ E	1,38	1,42	1,35	NS	NS
C ₈ E	0,51	0,86	0,91	7,2*	NS
C ₁₀ E	0,17	0,34	0,34	30**	NS

^a Averaged values of 8 replicates; grape juice 76/7. Inoculated under anaerobic conditions. Fermentation temperature 15 °C.

^b Yeast masses corrected for suspended grape material.

* Significant at 95% confidence level (F = 5,59).

** Significant at 99% confidence level (F = 12,25).

NS Not Significant.

TABLE 6B
Correlations between rate of fermentation, yeast mass and concentrations of esters^a

	Correlation coefficients (r)	
	Fermentation rate	Yeast mass
Yeast mass	0,84**	—
<i>Esters</i>		
AA	0,90**	0,83**
PEA	0,91**	0,83**
C ₆ E	NS	NS
C ₈ E	0,71**	0,58**
C ₁₀ E	0,84**	0,72**

^a Data from Table 6A.

columns 2 and 3 of Table 6A. The differences between ester concentrations are in this case insignificant, and consequently a small amount of this material appears to be sufficient to bring about the maximum changes in yeast growth, fermentation rate and ester formation. It appears that the settled must (C₁) contained at least a ten-fold surplus of the critical quantities of this material. Confirmatory results were obtained in subsequent repeat studies on juices from the same vineyard but different years, viz. 76/1, 25 and 122 and 77/2, 76 and 205. Enhanced ester production can be attributed to the stimulated yeast proliferation and fermentation, especially during the exponential phase of fermentation, and is therefore in all probability an indirect result of the influence of the suspended grape material.

In the light of the recent investigations of Aries & Kirsop (1977) on the relations between ergosterol and yeast proliferation, a connection between the growth promoting activity of these suspended grape particles and ergosterol seems probable. On this basis it appeared likely that an active fraction should be extractable from the solid particles. Grape particulate was consequently extracted for an apolar fraction and its effect noted on the fermentation rate. These findings are given in Fig. 3. It is clear that the extract from the suspended material in 0,25 l C₁ juice, had a marked influence on raising the fermentation rate of 1 litre of C₄ juice to a rate very close to that of the settled juice (C₁). It was considerably more effective than the residue remaining after extraction. These initial findings demonstrate that at least one causal factor in the positive influence of turbidity on fermentation is based on extractable components contained in or adsorbed by the grape particulate of settled juice (C₁).

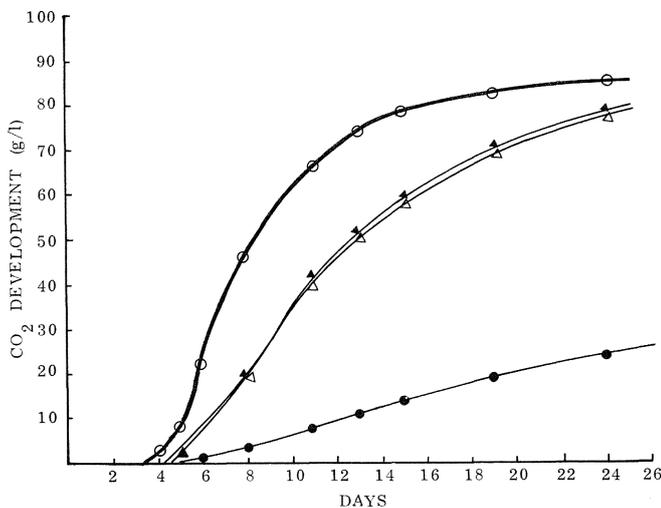


FIG. 2

Influence of varying degrees of Chenin blanc juice clarity on fermentation pattern. (Juice 76/7, inoculated under anaerobic conditions with 0,5% yeast culture. Fermentation temperature 15 °C).

- juice clarity C₄
- ▲ juice clarity C₃
- ▲ juice clarity C₂
- juice clarity C₁ as well as 90% C₄ + 10% C₁

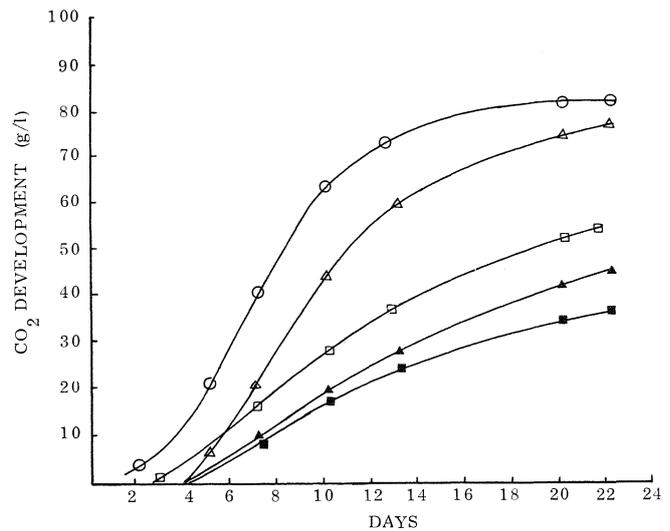


FIG. 3

Fermentation promoting influence of grape particulate extract (GPE) of juice 77/24. (Inoculated under anaerobic conditions with 2,5% yeast culture. Fermentation temperature 15 °C).

- C₄ juice + 5 ml ethanol
- ▲ C₄ juice + 0,20 g/l Tween 80 in 5 ml ethanol
- △ C₄ juice + 0,2;0 g/l Tween 80 + 0,16 g/l GPE in 5 ml ethanol
- C₄ juice + the residue of the extraction mentioned under Δ + 5 ml ethanol
- C₁ juice

Juice lees in relation to ester production: The presence of small amounts of finely suspended grape particulate has been shown to be critical in relation to fermentation and ester production. In the course of wine making a considerable amount of coarse material is precipitated during the settling process. On the basis of the foregoing it appeared important that the influence of this material should also be studied in the context of ester formation. Data on this study are presented in Table 7. It will be noted, and as could be expected from the previous findings, that 1 to 2% addition of juice lees effected a clear increase in ester production. However, from 5% progressively larger additions caused significant and related decreases in ester concentrations. Relevant literature concerning the production of esters and higher alcohols often reported apparently contradictory findings. This study has indicated that such anomalies could be influenced by

TABLE 7
Influence of varying lees admixtures with limpid juice upon ester formation in Chenin blanc wines^a

Esters (mg/l)	Lees admixture (%)								^b Correlation coefficient (r)
	0	1	2	5	10	20	50	100	
AA	1,50	4,45	4,60	2,75	2,95	1,45	0,83	0,78	-0,76*
PEA	0,27	0,41	0,41	0,28	0,23	0,11	0,05	0,06	-0,75*
C ₆ E	0,54	0,82	1,03	0,72	0,57	0,55	0,32	0,26	-0,81*
C ₈ E	0,25	0,59	0,56	0,48	0,35	0,44	0,19	0,09	-0,92**
C ₁₀ E	0,16	0,34	0,33	0,33	0,23	0,21	0,14	0,13	-0,83*

^a Grape juice 76/1, C₄ admixed with the lees of free run juice 76/1 settled for 24 hours (resulting in 11 vol. % lees + 89 vol. % juice C₁). Inoculated with 5% yeast under anaerobic conditions. Fermentation temperature 11 °C.

^b Correlation coefficients are calculated over the 2–100% lees admixtures (98–0% juice C₄) between percentage admixture and ester concentration.

* Significant at 95% confidence level (P 0,05).

** Significant at 99% confidence level (P 0,01).

factors operative in systems which are not sufficiently clearly defined. It has been shown that factors such as grape maturity, sugar content of the juice and, especially, grape particulate material are important in this respect. Very little work has been published on the turbidity factor. In view of the marked effects which it can bring about on wine aroma components it is seen as an aspect which needs further study, especially in connection with ester production and organoleptically determined wine quality.

Fermentation temperature: The influence of temperature on ester formation in sterile filtered juices (74/1.C₄, 74/44.C₄ and 75/1.C₄) inoculated under nitrogen is shown in Table 8. It is clear that the concentration of the acetate esters AA and PEA increase when the fermentation temperature was increased from 11 °C–15 °C. The formation of C₆E, C₈E, and C₁₀E also manifests the effect of temperature and production thereof attains a maximum in the 15 °C series.

TABLE 8

Ester concentrations in wines in relation to the fermentation temperatures

Esters (mg/l)	Fermentation temperature (°C)	
	11	15
AA	1,70	5,20
PEA	0,22	0,46
C ₆ E	0,68	1,15
C ₈ E	0,42	0,70
C ₁₀ E	0,38	0,52
Fermentation time (days)	60	27
Fermented sugar (%)	84	98

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