Vitis 8, 23—37 (1969)

Australian Wine Research Institute, Adelaide, South Australia

β-Phenethanol and n-hexanol in wines: Influence of yeast starin, grape variety and other factors; and taste thresholds

by

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Introduction

A study of the influence of yeasts and bacteria on wine composition and quality is under way at the Australian Wine Research Institute, and the influence of yeasts on higher alcohol formation has recently been reported (1). Two alcohols belonging to this general group, β-phenethanol (β-phenyl ethyl alcohol) and n-hexanol, have come into prominence as possible quality constituents in wines. β-Phenethanol has a pleasant fruity aromatic aroma and is a major component in synthetic rose oil, and n-hexanol also has a pronounced aroma.

Both β-phenethanol and n-hexanol have been reported in beverages, and more attention has been directed to β-phenethanol in beer (11—17) and wine (3, 18—22), since it is present in greater amounts than n-hexanol. The concentration of β-phenethanol in beer is usually between 5 and 40 ppm, and Sihto and Arkima (17) considered it to be a negative quality factor. Wines contain between 10 and 140 ppm (3, 18, 22) and 7 to 102 ppm have been reported in cider (2). n-Hexanol has been reported in small amounts in grapes and wine by various workers [reviewed by Webb (10) and Drawert and Rapp (21)] as part of the volatile constituents detected, but quantitative data on amounts present in various wines could not be found in the literature.

In general, detailed data on the various factors responsible for variations in amounts of both β-phenethanol and n-hexanol in wines are not available, and their significance in wine quality is not known. This paper reports the results of an investigation of these two compounds in experimental and commercial wines of various types, and includes a study of some factors which effect the amounts present, the taste thresholds of the two compounds and possible pathways of formation.

Materials and Methods

1. Measurement of β-phenethanol and n-hexanol

Both of these compounds were measured gas chromatographically. A rapid quantitative method suitable for many samples could not be found in the literature, so a method was developed, based on our previous gas chromatographic experience and the methods used by Kieser *et al.* (2) and Usseglio-Tomasset (3). The method avoided prolonged continuous extraction and proved to be very workable. Details are given below.

Duplicate samples of 100 ml of each wine were distilled without fractionation, with several additions of distilled water, until 200 ml of distillate were collected. Each sample was transferred to a 250 ml glass-stoppered separating funnel, 11.9 g sodium chloride A. R. (3 molar) added, and extracted with four only 20 ml lots of redistilled methylene chloride. The four extracts were combined and the methylene

chloride removed by distillation at less than 40° C. The distillation was terminated when approximately 2 ml of extract remained in the 100 ml distillation flask used. This was transferred quantitatively to a 5 ml glass-stoppered volumetric flask, and the distillation flask rinsed with several 0.5 ml lots of methylene chloride which were added to the volumetric flask. An aliquot of 1.0 ml of freshly prepared internal standard (0.049 ml redistilled 2-phenyl-2-propanol [Fluka] in 25 ml methylene-chloride, corresponding to 400 ppm final concentration) was added and the flask made to volume with methylene chloride. 5 μl were injected into a Perkin Elmer 801 gas chromatograph operating under the following conditions:

column	— diisodecylphthalate 15% on chromosorb W 60—80 mesh, 6 ft × 1/8 in
injector	— 280° C
oven	— 135° C isothermal
detector	— 200 ° C — flame ionisation
nitrogen	— 30 ml/min
hydrogen	— 30 ml/min
air	— 350 ml/min
attenuation	— 10

The internal standard had a retention time of 12 minutes under these conditions and β-phenethanol and n-hexanol had relative retention times of 1.41 and 0.21 respectively. A typical chromatogram is shown in Fig. 1.

Peak heights and retention times of the peaks corresponding to β-phenethanol, n-hexanol and the internal standard were measured, and the product of peak height by retention time was used to calculate peak areas (4). The ratio of peak area of each alcohol to the peak area of the internal standard was calculated, and related to a previously prepared calibration curve obtained with a range of concentrations of the pure alcohols carried through the same procedure. These calibration curves were linear for the amounts of the two alcohols found in the wines examined.

Recoveries of 92 to 108% (mean 98%) were obtained with this method, and the reproducability was of the order of ±5—7%. Recoveries at the distillation and extraction stages were checked and found to be quantitative.

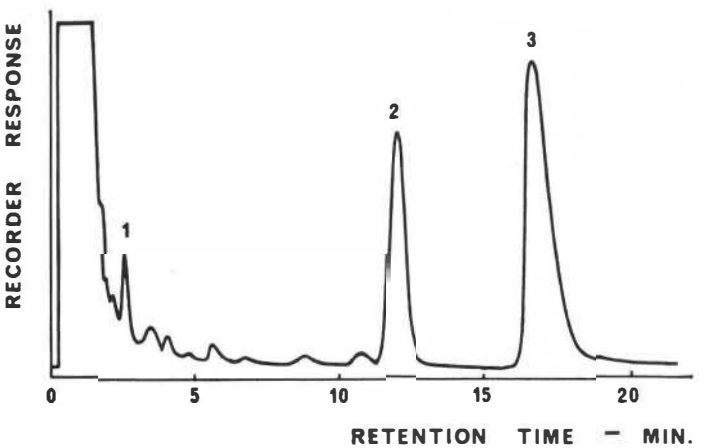

Fig. 1: Gaschromatographic trace of n-hexanol (1), 2-phenyl-2-propanol (internal standard) (2), and β-phenethanol (3).

Identity of the peaks was checked by comparison of retention times with those of the pure alcohols with three columns of differing polarity, and, in the case of β-phenethanol, confirmed by trapping the peak and comparing its identity with that of authentic β-phenethanol by mass-spectrometry. It was not possible to trap sufficient n-hexanol to check its mass spectrum.

2. Yeasts and fermentation procedures

Details of the *Saccharomyces* yeasts used and the fermentation procedures have already been reported (5). The yeasts included those used commercially in Australian wine making, and also included a strain used commercially in California (*S. cerevisiae* No. 727 "Montrachet" strain) and one used in cider making in southern England and cider research at the Long Ashton Cider Research Station of the University of Bristol (*S. cerevisiae* No. 350).

3. Wines examined

The wines analysed in this investigation were from three sources.

(1) Experimental wines made with pure yeasts on laboratory scale from filter-sterilised sulphited grape juices in quantities ranging from 300 ml to 3 l.
(2) Wines made in 120 and 240 l lots under controlled conditions in the Institute's experimental winery, from various authentic grape varieties grown in different viticultural regions. The juices were sulphited, but not filter-sterilised.
(3) Commercial wines from various Australian viticultural areas, which were exhibited for awards in the 1967 Adelaide Championship Wine Show, conducted by the Royal Agricultural and Horticultural Society of South Australia.

Results

1. Formation by different wine yeasts

Eight yeasts representing three species of *Saccharomyces* were inoculated into triplicate 3 l lots of filter-sterilised grape juices from *Vitis vinifera* varieties Riesling, Semillon and Ugni Blanc (syn. Trebbiano, White Hermitage). The resulting wines were analysed and the results are shown in Table 1. It can be seen that the yeast strains differed considerably in the amounts of β-phenethanol produced under comparable conditions, there being about a four fold difference between yeasts. *S. cerevisiae* No. 275 formed considerably more β-phenethanol than any other yeast examined. Yeasts also differed in the amounts of n-hexanol produced under the same conditions, but the range of values was less than for β-phenethanol. More n-hexanol was formed from Semillon juice than the other two varieties.

Table 2 reports results obtained in fermentations on pilot-plant scale with four yeasts, two of which (Nos. 138 and 350) were included in Table 1, showing that the yeast strain influenced the amounts of β-phenethanol formed under wine-making conditions with non-sterilised must, but had little effect on the amounts of n-hexanol in the wines.

2. Influence of grape variety

It is evident from Tables 1 and 2 that the grape variety influenced the amounts of the two alcohols in the wines irrespective of the yeast strain. The amount of β-phenethanol was significantly higher in wines made from Riesling grapes than from Semillon and Ugni Blanc (Table 1) and higher in Pedro than Ugni Blanc and

Table 1

Formation of β-phenethanol and n-hexanol by wine yeasts in three filter-sterilised grape juices

(Means of triplicates)

Yeast species	No.	β-Phenethanol (ppm)				n-Hexanol (ppm)			
		Riesling	Semillon	Ugni Blanc	Mean	Riesling	Semillon	Ugni Blanc	Mean
S. fructuum	138	7.8	6.7	8.7	7.8	3.0	3.7	2.8	3.1
S. cerevisiae	161	6.2	4.8	5.7	5.6	3.2	4.3	2.5	3.3
S. cerevisiae	275	29.0	16.0	14.0	20.0	2.5	3.6	2.5	2.9
S. cerevisiae	348	6.2	6.5	8.5	7.6	2.9	3.8	2.5	3.1
S. cerevisiae	350	9.1	7.6	10.0	8.9	2.7	3.9	2.2	2.9
S. species	719	9.1	6.3	7.7	7.7	3.4	3.9	3.0	3.4
S. oviformis	723	9.4	7.8	9.3	8.9	3.5	4.2	3.2	3.6
S. cerevisiae	729	12.0	8.3	8.7	9.5	3.5	3.8	3.0	3.4
Mean		11.0	7.9	9.1	9.4	3.1	3.9	2.7	3.2

		β-Phenethanol	n-Hexanol
L.S.D. ($P < 0.05$) between	means of triplicates	1.4	0.5
	means of yeasts	1.3	0.2
	means of varieties	0.8	0.1

Table 2

Formation of β-phenethanol and n-hexanol in experimental wines, 1966 vintage, made on pilot-plant scale with 4 yeasts and 3 grape varieties

(Means of duplicates)

Species	No.	β-Phenethanol (ppm)				n-Hexanol (ppm)			
		Pedro	Tokay	Ugni Blanc	Mean	Pedro	Tokay	Ugni Blanc	Mean
S. fructuum	138	32	18	25	25	3.3	5.3	2.3	3.6
S. cerevisiae	213	21	8	14	14	3.4	4.9	2.0	3.4
S. cerevisiae	350	27	18	24	23	3.6	5.0	2.3	3.6
S. cerevisiae	727	34	20	30	28	3.4	4.8	2.1	3.4
Mean		28	16	23	22	3.4	5.0	2.2	3.5

		β-Phenethanol	n-Hexanol
L.S.D. ($P < 0.05$)	means of duplicates	4	0.3
	means of yeasts	2	0.2
	means of varieties	2	0.2

Tokay (Table 2). n-Hexanol was higher in Tokay wines than in wines made from the other two varieties in Table 2. (Australian Tokay is possibly the Hungarian Harslevelü.)

In addition to the results shown in Tables 1 and 2 the influence of grape variety was examined in detail in wines made in the experimental winery from authentic grape varieties grown in different viticultural areas and soil types over a period of six years. Representative results have been brought together in Table 3

Table 3

Influence of grape variety, viticultural area and soil type on the amounts of β-phenethanol and n-hexanol in table wines made under comparable conditions

Area	Soil	Riesling							Clare Riesling							Shiraz						
		1959	1960	1961	1962	1963	1964	Mean	1959	1960	1961	1962	1963	1964	Mean	1959	1960	1961	1962	1963	1964	Mean
		β-Phenethanol (ppm)																				
Barossa Valley	A	55	31	10	16	15	17	24	46	18	15	13	12	8	19	60	50	49	56	50	50	53
	B	49	44	11	13	15	19	25	55	28	20	16	16	17	25	55	52	49	56	50	59	55
Eden Valley	C	60	28	13	12	15	10	23	30	11	9	10	15	9	14	53	35	37	25	26	23	33
	D	48	39	7	16	13	11	22	45	20	9	10	12	10	18	43	37	51	25	21	23	33
River Murray		—	46	16	12	15	22	22	—	24	21	14	23	27	22	29	67	45	52	46	57	49
Mean		53	38	11	14	15	16	23	44	20	15	13	16	14	20	48	48	46	43	39	43	44
		n-Hexanol (ppm)																				
Barossa Valley	A	2.5	3.0	2.1	2.4	2.6	3.1	2.6	3.2	4.2	2.0	3.2	4.0	3.0	3.3	3.3	4.4	3.7	2.6	2.9	3.2	3.4
	B	3.6	3.0	2.7	3.3	3.3	3.4	3.2	3.1	3.7	3.0	3.1	2.8	2.4	3.0	3.2	4.7	3.2	3.0	2.9	3.3	3.4
Eden Valley	C	3.4	4.3	3.2	2.8	3.0	2.9	3.3	3.1	3.9	2.9	3.9	4.7	3.4	3.7	3.8	4.7	3.4	3.4	4.2	4.1	3.9
	D	3.7	3.4	3.4	3.0	3.3	2.7	3.3	4.0	2.6	3.4	3.8	4.7	3.7	3.7	3.9	5.8	3.8	3.8	5.2	3.6	4.4
River Murray		—	3.2	2.4	1.8	2.2	3.1	2.5	—	3.2	2.4	1.5	2.7	3.0	2.6	3.4	3.2	3.0	2.7	3.2	3.5	3.2
Mean		3.3	3.4	2.8	2.7	2.9	3.0	3.0	3.4	3.5	2.7	3.1	3.8	3.1	3.3	3.5	4.6	3.4	3.1	3.7	3.5	3.6

in which several interesting features are apparent. In the white wines made from Riesling and Clare Riesling (a quality white grape which may have originated from Austria, but is not Riesling) the amounts of β-phenethanol in 1959 and 1960 were higher than in 1961 to 1964, whilst the amounts in Shiraz (the Syrah of the Rhone Valley and the Petite Sirah of California) were uniformly high. Wines from other red varieties were not examined. The wines made from Shiraz grown in Eden Valley on yellow or grey brown podzolic soils (soils C and D) were consistently lower than from Shiraz grown in the nearby Barossa Valley on red-brown earth and solodised solonetz (soils A and B). More β-phenethanol was formed in wines made from Clare Riesling grown on soil B than on soil A.

Smaller differences were apparent in the amounts of n-hexanol present, and the most notable feature was the lower level in wines from all three varieties grown in the River Murray irrigation area. In 1961 and 1962 the amounts of n-hexanol present were generally lower than in the other years examined.

The amounts of β-phenethanol and n-hexanol were measured in 24 further white table wines made under controlled conditions in 130 l lots in the Institute's

Table 4

Amounts of β-phenethanol and n-hexanol in varietal wines picked from grapes at different stages of ripeness and made under controlled conditions on pilot plant scale

Grape variety		Year	Yeast No.	Grape juice			Wine	
				Specific gravity	pH	Acid g/l	β-Phenethanol ppm	n-Hexanol ppm
Clare Riesling	A	1965	723	1.071	3.34	11.8	14	2.8
Clare Riesling	B	1965	723	1.080	3.47	7.6	18	3.2
Clare Riesling	C	1965	723	1.092	3.69	5.2	21	2.7
Clare Riesling	D	1965	723	1.097	3.86	4.1	24	2.5
Pedro	A	1965	723	1.062	3.59	5.0	19	3.6
Pedro	B	1965	723	1.080	3.52	5.5	24	2.0
Pedro	C	1965	723	1.081	3.95	3.8	20	3.4
Tokay	A	1965	723	1.078	3.45	7.7	24	2.9
Tokay	B	1965	723	1.088	3.76	5.4	20	3.0
Ugni Blanc	A	1965	723	1.078	3.49	6.9	16	1.8
Ugni Blanc	B	1965	723	1.095	3.86	4.8	22	1.3
Madiera	A	1965	723	1.068	3.27	9.0	21	1.7
Madiera	B	1965	723	1.095	3.54	5.9	24	1.5
Semillon	A	1965	723	1.077	3.26	9.1	18	1.8
Semillon	B	1965	723	1.083	3.57	7.2	10	1.6
Clare Riesling	A	1966	729	1.088	3.55	6.4	15	2.8
Clare Riesling	B	1966	729	1.088	3.60	6.8	16	2.6
Pedro	A	1966	729	1.089	3.56	5.5	19	2.1
Pedro	B	1966	729	1.090	3.62	5.7	23	1.9
Tokay	A	1966	729	1.083	3.67	7.1	18	3.1
Tokay	B	1966	729	1.088	3.77	5.3	17	3.1
Ugni Blanc	A	1966	729	1.070	3.30	8.1	13	1.9
Ugni Blanc	B	1966	729	1.083	3.48	7.2	14	1.5
Ugni Blanc	C	1966	729	1.092	3.59	4.6	19	1.2

experimental winery. The wines were made from authentic white grape varieties grown in the experimental vineyard of the South Australian State Department of Agriculture at Loxton in the River Murray irrigation area.

Wines were made in 1965 and 1966 and in both years grapes were picked from the same vines at several stages of maturity and made into wine under comparable conditions. The results (Table 4) show the amounts of β-phenethanol and n-hexanol in the wines in relation to the grape variety and the specific gravity and acidity of the grape juice. There appears to be a correlation in some varieties between stage of ripeness of the grapes and the amounts of both alcohols present in the wines. In general, the more mature grapes from the same variety had more β-phenethanol and less n-hexanol. The correlation is not exact and when all varieties are pooled it is not significant, but for some individual varieties the trend is apparent.

3. Influence of contact time of grape juice with grape skins during fermentation

Results with the grape varieties Shiraz, Riesling and Clare Riesling (Table 3) showed that the highest amounts of β-phenethanol were obtained with Shiraz grapes fermented in contact with grape skins. It was reasoned that these high values could be due either to the grape variety or the presence of grape skins during the fermentation, on the supposition that more of the β-phenethanol precurser may be in the unbroken cells of the skin of the berry than in the pulp.

Shiraz and Muscat Gordo Blanco were selected for study because they are normally fermented in contact with grape skins for some period. The grapes were destemmed and crushed in the laboratory, inoculated with yeast No. 729 and allowed to ferment at 25° C in replicated wide-mouth glass jars of 1 l capacity plugged with cotton wool. At intervals the juice was separated from the skins and allowed to continue to ferment to dryness. After fermentation, the wines were filtered and stored at 4° C until analysed. The results are shown in Table 5.

It can be seen that the contact time of grape skins with the juice during fermentation has little influence on the amounts of β-phenethanol and n-hexanol formed in these two juices. With Shiraz grapes a reduction in the amount of β-phenethanol and an increase in the amount of n-hexanol occurred with prolonged contact with skins.

Table 5

Influence of contact time of juice with grape skins on formation of β-phenethanol and n-hexanol during fermentation by *S. cerevisiae* No. 729

(Means of duplicates)

Grape variety	Time of contact (h)	β-Phenethanol (ppm)	n-Hexanol (ppm)
Muscat Gordo	0	43	2.2
Muscat Gordo	5	47	1.9
Muscat Gordo	24	45	1.8
Muscat Gordo	70	47	2.3
Muscat Gordo*)	140	64	7.6
Shiraz	0	53	2.4
Shiraz	5	44	3.3
Shiraz	24	44	3.0
Shiraz	70	38	3.6

*) Homogenised.

Table 6

Influence of fermentation temperature on formation of β-phenethanol and n-hexanol by three yeasts in filter-sterilised Riesling grape juice

(Means of duplicates)

Yeast species	No.	β-Phenethanol (ppm) 15°	25°	35°	mean	n-Hexanol (ppm) 15°	25°	35°	mean
S. cerevisiae	161	9	12	8	10	2.0	1.5	1.7	1.8
S. cerevisiae	275	22	18	8	16	2.4	2.3	2.3	2.3
S. cerevisiae	729	11	16	9	12	2.7	2.6	2.8	2.7
Mean		14	15	8	13	2.4	2.2	2.4	2.3

		β-Phenethanol	n-Hexanol
L.S.D. ($P < 0.05$)	between means of duplicates	4	0.4
	beween means of yeasts	2	0.2
	beween means of temperatures	2	0.2

A portion of the Muscat Gordo Blanco juice and skins after 70 hours contact was homogenised with a laboratory vitamizer and the purée allowed to ferment to dryness and then filtered. Considerably more of each constituent was formed under these conditions, indicating release of either the alcohols or their precursers bound in the cell walls of the berries.

4. Influence of temperature of fermentation

This was examined with three yeasts (Nos. 161, 275, and 729) at three temperatures in duplicate fermentations, and the results are shown in Table 6.

An increase in temperature of fermentation from 15 to 35° C resulted in a mean decrease in the amount of β-phenethanol formed, but had no significant effect on n-hexanol. The reduction in β-phenethanol content was largely due to yeast 275, which formed the highest amount at 15° C, and this amount was reduced by 63% at 35° C.

Table 7

Influence of pH on formation of β-phenethanol and n-hexanol in filter-sterilised Riesling juice by three yeasts at 15° C

(Means of duplicates)

Yeast species	No.	β-Phenethanol (ppm) pH 3.0	pH 3.5	pH 4.0	Means	n-Hexanol (ppm) pH 3.0	pH 3.5	pH 4.0	Means
S. cerevisiae	161	8	11	11	11	2.1	2.2	1.9	2.1
S. cerevisiae	275	10	20	20	17	1.9	2.0	2.1	2.0
S. cerevisiae	729	5	7	11	8	2.3	2.4	2.4	2.4
Mean		8	13	14	12	2.1	2.2	2.1	2.1

		β-Phenethanol	n-Hexanol
L.S.D. ($P < 0.05$)	between means of duplicates	4	0.5
	between means of yeasts	3	0.3
	between means of pH	3	0.3

5. Influence of pH

This was examined with the same three yeasts and grape juices as were used for the experiment with different temperatures. The grape juice was adjusted to pH values of 3.0, 3.5 and 4.0 with either hydrochloric acid or sodium hydroxide, then sterile filtered and inoculated in duplicate with the yeasts. The results are shown in Table 7.

The amounts of β-phenethanol formed by the three yeasts at pH 3.5 and 4.0 were approximately 70% more than at pH 3.0. The three yeasts behaved somewhat differently in that yeast 729 produced the highest amount at pH 4.0 whereas the amounts produced by the other two yeasts were the same at pH 3.5 and 4.0. The amounts of n-hexanol formed by the three yeasts were not influenced by differences in the pH of the medium.

6. Influence of L-phenylalanine on β-phenethanol formation

Sterile-filtered Sultana grape juice, containing 19% sugar (refractometer) and 60 meq/l titratable acid with pH 3.8, was enriched with graded amounts of L-β-phenylalanine, and duplicate lots were each fermented with three yeasts. When the fermentations were complete the wines were filtered and stored in full containers at 4° C until analysed. The results are given in Table 8.

The presence of added L-phenylalanine at 100 ppm increased the amount of β-phenethanol formed by the three yeasts, corresponding on a molar basis to conversion of 41, 30 and 45% respectively. The yeast which produced the highest yield of β-phenethanol in the absence of added β-phenylalanine (No. 275) showed the least increase in its presence. The addition of 1 g/l of ammonium sulphate had no noticable effect on the yield of β-phenethanol.

7. Analysis of commercial wines

Samples of commercial wines submitted for awards in the Adelaide Championship Wine Show were obtained and analysed. The wines were made in wineries in various States of Australia from a range of grape varieties and as such could be taken as being representative of quality Australian wines.

Table 8

Influence of added L-β-phenyl alanine and ammonium sulphate on formation of β-phenethanol by three yeasts in filter-sterilised Sultana grape juice

(Means of duplicates)

Phenyl alanine ppm	*S. cerevisiae* No. 161 ppm	*S. cerevisiae* No. 275 ppm	*S. cerevisiae* No. 729 ppm	Mean
nil	9.5	16.5	9.0	11.7
10	9.5	17.0	8.0	11.5
30	11.5	18.0	12.5	14.0
100	40.0	39.0	42.0	40.3
Mean	17.6	22.6	17.9	19.4
$(NH_4)_2 SO_4$ 1 g/l	10.0	13.0	9.5	10.8

L.S.D. ($P < 0.05$) between means of duplicates 2.6
between means of yeasts 1.4
between means of phenyl alanine 1.7

Table 9

Amounts of β-phenethanol and n-hexanol in commercial wines submitted for awards in the Adelaide Wine Show 1967

Wine Type	Class No.	No. of wines	β-Phenethanol (ppm)		n-Hexanol (ppm)	
			Range ppm	Mean ppm	Range ppm	Mean ppm
Dry red table — Claret style	25	22	34—74	54	1.5— 7.8	4.9
Dry white table — Hock style	21	17	10—36	21	1.3— 3.9	2.4
Sherry, pale dry fino style	27	12	21—70	46	1.8— 5.2	3.2
Tawny port	32	16	15—47	28	3.5—12.0	5.7
Muscat	31	12	5—30	14	2.7—10.0	5.1

The results (Table 9) show that the dry red wines had a higher content of both β-phenethanol and n-hexanol than the dry white wines, which agreed with the results for Shiraz experimental wines reported above. Of the fortified wine types, the fino sherries had the greatest amount of β-phenethanol and the least n-hexanol. Overall, the dry white table wines and muscats had the least β-phenethanol, while the dry red table wines and sherries had the most. Sherries and dry white table wines had the least n-hexanol — approximately half the amount of the other classes. The results were examined in relation to the tasting scores made by the judges in awarding prizes, but no apparent correlations were evident between the amounts of either β-phenethanol or n-hexanol and the tasting scores.

8. Taste thresholds of β-phenethanol and n-hexanol

Taste thresholds were measured as minimum detectable differences by addition of known amounts of the redistilled alcohols to a dry white table wine made in 1964 in the Institute's experimental winery from Riesling grapes grown in the Barossa Valley of South Australia. Some details of the composition of the wine were as follows: — ethanol 13.7% by volume, pH 3.1, titratable acidity 115 meq/l, β-phenethanol 24 ppm and n-hexanol 3.5 ppm. The wine was selected as being a high quality wine typical of the grape variety and the viticultural region.

A total of 11 tasters participated. They included both sexes and covered a wide range in ages, and were drawn from the staffs of the Australian Wine Research Institute and the CSIRO Division of Horticultural Research. Two of the tasters were wine judges of some years' experience (A and B) and two others (J and K) had little or no previous contact with wine.

The threshold values were obtained by triangular taste tests (6) along the lines recently reported for higher alcohols (1). Tastings were held two or three times daily on successive days. The alcohols were tasted separately and each concentration was only tasted once per day. The tasters were asked to record which coded glass of wine of the three presented on each occasion was different and whether it contained more or less of the constituent under test. The tastings were statistically controlled and a significance level of $P < 0.01$ was adopted as the minimum significant difference. The results are shown in Table 10.

The lowest threshold level which could be detected was 30 ppm for β-phenethanol, with a background of 24 ppm, and 4 ppm for n-hexanol, with a background of 3.5 ppm. The addition of small amounts of β-phenethanol, of the order of 50 ppm was considered by those tasters who could detect this amount to improve the quality of the wine. The addition of n-hexanol was not considered to improve the quality.

Table 10

Taste thresholds of β-phenethanol and n-hexanol added to a Riesling dry white table wine, containing 24 and 3.5 ppm respectively

Taster	β-Phenethanol (ppm)	n-Hexanol (ppm)
A	40	4
B	50	6
C	>200*	>30*
D	100	8
E	>200*	15
F	—	10
G	40	8
H	30	—
I	>200*	>30*
J	>200*	—
K	>200*	>30*

Significance level $P < 0.01$

* Maximum amount added.

— Taster not available.

It can be seen that the tasters varied widely in their ability to detect differences in the amounts of both alcohols. In order to keep the tastings within workable limits, the highest level of each component tested was 200 ppm for β-phenethanol and 30 ppm for n-hexanol. These amounts were several times greater than the highest levels found in wines. Some tasters could not detect even higher levels, such as 500 ppm β-phenethanol and 100 ppm n-hexanol.

Discussion

β-Phenethanol

Formation of β-phenethanol by bakers' yeast was demonstrated as early as 1907 by Ehrlich (7) in his classical work on the formation of fusel alcohols, and confirmed with brewers' yeast by Thorne (8) in a synthetic medium with phenylalanine as sole nitrogen source. β-Phenethanol was demonstrated in *Vitis rotundifolia* grapes in 1956 by Kepner and Webb (9) who considered at that time that it may be responsible for the characteristic flavour of these grapes, but has subsequently been shown to be present in various fruits and fermented beverages (10).

Our results show that yeasts differ considerably in the amounts of β-phenethanol formed during alcoholic fermentation of grape juice, and it is apparent that observed differences in the β-phenethanol content of various wines is explained, at least in part, by the yeast strain.

Formation of β-phenethanol by yeasts would be expected to follow a similar pathway to that of other higher alcohols, such as the amyl alcohols and iso-butanol, in which their pathway of formation parallels that of the synthesis of the corresponding amino acids from sugar to the keto-acid stage (24). In β-phenethanol synthesis the keto acid phenyl pyruvic acid (25) would by analogy be decarboxylated and reduced to β-phenethanol or transaminated to phenylalanine.

β-Phenethanol could also be formed by the classical Ehrlich mechanism from phenylalanine by decarboxylation and reductive deamination (7), but it is unlikely

that this is either a major or even a minor pathway unless excess phenylalanine is present, since STEVENS (12) has demonstrated the presence of β-phenethanol in fermented substrates free from amino acids.

The influence of grape variety on amount of β-phenethanol formed indicates compositional differences between varieties, presumably in nitrogenous constituents, but this was not examined. ÄYRÄPÄÄ (15) has shown that β-phenethanol formation is reduced by nitrogen content above 100 ppm in the medium, and increased by phenylalanine above about 75 ppm. The phenylalanine content of musts in the Bordeaux area is 0 to 18 ppm (27). CASTOR's values for California musts (28) of 30—70 ppm are suspect because of the unexplained "drifting" results he obtained.

Stimulation of β-phenethanol formation in the presence of 100 ppm phenylalanine (Table 8) is in keeping with ÄYRÄPÄÄ's findings. The grape juices used contained 500 ppm total nitrogen. We may assume that the yeasts had adequate nitrogen for protein synthesis and deamination of phenylalanine was not necessary to provide further nitrogen. Only when a high level of phenylalanine was added more β-phenethanol was formed, and the conversion was only 30 to 45% of that theoretically obtainable.

The tasting results indicate the importance of β-phenethanol in the aroma of wines. Differences in amounts present in different wines are of the magnitude detected by some tasters, and the addition of small amounts of β-phenethanol of the order of 50 ppm appeared to improve the quality of the wine.

The composition of the commercial wines examined is of considerable interest (Table 9). The high levels of β-phenethanol in the dry red table wine would be due in large part to the influence of the Shiraz grape which forms the basis of many of the Australian red table wines. Flor sherry is also high in β-phenethanol and this supports the prediction of WEBB and KEPNER (19) that β-phenethanol must contribute significantly to the overall aroma of flor sherry. Although the tasting results indicated that a high β-phenethanol content improved the quality of the wine used in the tasting, it is not surprising that no correlation existed between β-phenethanol content and judging scores for the commercial wines exhibited for awards. Quality ratings for the various wine types are given for a range of features which would not be related to the content of β-phenethanol, such as wood age, oak extract, muscat aroma, "port" character, freedom from oxidation and other faults.

n-Hexanol

Our results have shown that n-hexanol is normally present in wines, but in lower concentrations than β-phenethanol. The amounts present are not markedly influenced by yeast strain, pH or temperature of fermentation, but are influenced by grape variety, year of vintage and the time of contact of the grape skins with the fermenting juice.

It is noteworthy that the commercial wines examined which contained relatively high levels of n-hexanol were all derived from fermentation in the presence of grape skins for some period, further indicating that higher levels are correlated with a longer period of extraction of the grape skins. When this is considered with our other findings it suggests that fermentation *per se* is not as important in influencing the amounts of n-hexanol in wines as the grape variety and contact of grape skins during fermentation. This would tend to support the contention of DRAWERT, RAPP and ULRICH (29), based on a study of model systems, that n-hexanol is derived from hexen-2-al-1 formed from linolenic acid in the grape berry. The final reduction of hexen-2-al-1 to n-hexanol appears to be the only step brought about by yeast, and n-hexanol has been demonstrated in must (21, 30).

The tasting results show that differences in amounts of n-hexanol present in various wines can be distinguished organoleptically by certain tasters. The lowest detectable difference for the wine used in the tastings was 4 ppm, and this may be compared with the findings of Drawert *et al.* (29) that 1 ppm could be detected in a neutral wine, and that this produced a woody taste. We did not find that n-hexanol added to wine imparted a woody taste as such, but it tended to give the wine a foreign aroma which we regarded as a reduction in quality. The results must be regarded with some reservation because they do not take into account synergism and complex interactions with other aroma compounds, but they do give some quantitative data on taste thresholds of the pure compounds.

Summary

β-Phenethanol and n-hexanol were measured by gas chromatography in a wide range of commercial and experimental wines. Commercial wines ranged from 5 to 74 ppm β-phenethanol and 1.3 to 12 ppm n-hexanol. Dry red wines and fino sherries were high in β-phenethanol, and ports, muscats (both sweet dessert wines) and dry red wines were high in n-hexanol.

The strain of yeast strongly influenced formation of β-phenethanol. Mean yields by eight yeasts *(Saccharomyces)* during fermentation of grape juices from three varieties of *Vitis vinifera* under comparable conditions ranged from 5.6 to 20 ppm. The strain of yeast had little effect on amounts of n-hexanol in the wines.

Varieties of *V. vinifera* differed in amounts of β-phenethanol and n-hexanol formed in the wines made therefrom, irrespective of the yeast strain used. Wines made from Shiraz (Syrah) grapes were characterised by high levels of β-phenethanol in comparison with white grape varieties examined. The year of vintage influenced amounts of β-phenethanol in wines, and the soil type and viticultural area also effected the amounts of both β-phenethanol and n-hexanol formed.

More β-phenethanol was formed by fermentation at 15° C and 25° C than at 35° C, and more was formed at pH 3.5 and 4.0 than at pH 3.0. n-Hexanol content was not influenced significantly by either temperature of fermentation or pH of the must.

Addition of β-phenylalanine to the must resulted in formation of more β-phenethanol by fermentation, and the mechanism of formation of both β-phenethanol and n-hexanol is discussed.

Taste thresholds were measured as minimum detectable differences in a Riesling dry white wine. Values for different tasters ranged from 30 to > 200 ppm for β-phenethanol (10 tasters) and 4 to > 30 ppm for n-hexanol (9 tasters). Added β-phenethanol, but not n-hexanol, was considered to improve the quality of the wine.

Acknowledgements

The authors are indebted to the late J. C. M. Fornachon, former Director of the Australian Wine Research Institute, for constructive critism during the course of the investigation. They are also indebted to Miss D. A. Bridson for organising the tastings and to the tasters who willingly gave their time. Thanks are due to the Managers of the Nuriootpa Viticultural Station and the Loxton Research Station of the State Department of Agriculture of South Australia for permission to use grapes from the experimental vineyards, and for assistance with their collection. Mr. M. W. Hughes of the CSIRO Division of Soils kindly identified β-phenethanol by mass-spectrography. The senior author is indebted to Professor A. D. Webb and Prof. Dr. F. Drawert for stimulating discussions.

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Eingegangen am 12. 11. 1968

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