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# Diacetyl in Australian dry red wines and its significance in wine quality

by

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## Introduction

Diacetyl is becoming recognised as an important flavour compound in dry red table wines, and when present in sufficient concentration, can impart a butter-like aroma to wine. Diacetyl is known to be an important component of the aroma of butter (1, 2), and its presence in wine is associated with the presence of lactic acid bacteria which promote a malo-lactic fermentation.

The study of diacetyl in wine is part of a continuing investigation into the influence of micro-organisms on the composition and quality of wines. Studies on the influence of wine yeasts have recently been reported (3), together with their effect on the growth of bacteria which bring about the malo-lactic fermentation (4), and considerable attention has been given to the role of these bacteria (5, 6). The study of diacetyl has arisen directly from this work.

This paper reports the results of a survey of the diacetyl content of Australian dry red wines. In the past, diacetyl has sometimes been measured together with acetoin (acetyl methyl carbinol) as one value, but since diacetyl is the important flavour compound, this has been measured directly. As far as we are aware, the results herein represent the most comprehensive survey yet reported of diacetyl in wine.

Taste thresholds of diacetyl were measured in two dry red table wines made from different grape varieties, by a panel of tasters, to assess the significance of the diacetyl values obtained.

## Materials and Methods

In 1968 a total of 466 dry red table wines of current vintage was obtained from wineries in South Australia, New South Wales and Victoria, and represented all the major wine growing areas in these three States, which together produce over 95 per cent of the total Australian production.

The wines were received at the Institute from 3 to 5 months after making, and were stored at 15° C and analysed as soon as possible. As the chemical method for diacetyl measurement described previously (6) was too slow for the number of wines involved, a more rapid gas-chromatographic method was developed. This method was based on headspace sampling, of which one of the authors had previous experience (8), and the use of the electron capture detector. The use of this detector had been reported for diacetyl measurements in beer (9, 10), but as far as could be ascertained, its use in wine analysis has not been reported. Accordingly, the method developed is described below.

10 ml wine, 1.0 ml of 10 per cent acetone in water (internal standard) and 2.0 ml water were pipetted into a 60 ml bottle, which was closed with a rubber Suba seal and completely immersed in a 35° C water bath for 30 minutes. The bottle was then raised so that the rubber seal was exposed, the seal was dried and 2 to 5 ml

(depending on diacetyl content) of head-space vapour were withdrawn with a 10 ml Hamilton gas-tight syringe after repeated aspiration, and injected into the gas chromatograph (Perkin-Elmer 801).

Operational parameters were as follows:

Column: Porapak Q 80—100 mesh 6 ft × 1/8 in stainless steel.

Temperatures: Injection port 175°, column oven 150° (isothermal), detector 110° C.

Carrier gas: Nitrogen 30 ml per minute through the column and 140 ml per minute through a diluent bypass leading to the detector.

Attenuation: 100.

Recorder: Leeds and Northrup Speedomax G 1 millivolt full-scale deflection, chart speed 15 in per hour.

Peaks appeared in the following order and retention time, and a typical analysis is shown in Fig. 1.

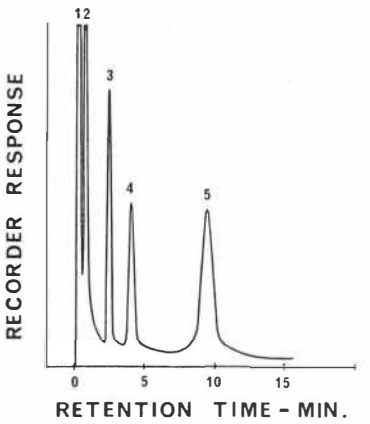


Fig. 1

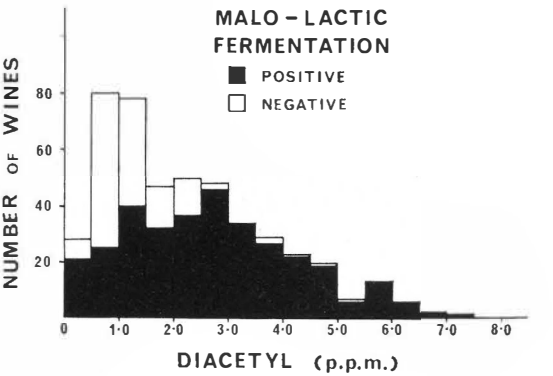


Fig. 2

Fig. 1: Typical gas-chromatographic trace of diacetyl analysis.

Fig. 2: Diacetyl content and occurrence of malo-lactic fermentation in 466 Australian dry red table wines.

| | | |
|---|---|---|
| 1 | air | 0.1 min, |
| 2 | water | 0.5 min, |
| 3 | ethanol | 2.2 min, |
| 4 | acetone | 4.0 min, |
| 5 | diacetyl | 9.5 min. |

The identity of the diacetyl peak was confirmed on two other stationary phases of differing polarity.

The product of peak height by retention time was measured for diacetyl and acetone, and the ratio calculated and calibrated for diacetyl concentration. The calibration was carried out with redistilled diacetyl analysed by the dimethyl-glyoxime method (6), both as pure solutions and added to wine in place of the 2 ml distilled water. Pure diacetyl was very difficult to prepare and the redistilled product never assayed higher than 85 per cent on a weight basis.

Two syringes were used alternately. After use they were dried in a hot air oven at 60° C and returned to a compartment in the water bath to attemperate. By this means an analysis could be carried out every 15 minutes, and duplicate determinations usually agreed to within ±5 per cent.

The chemical dimethyl-glyoxime method and the gas chromatographic method were compared using a range of wines differing widely in amounts of diacetyl, and the two methods gave results which agreed closely, e. g. for six wines with diacetyl content ranging from 0.4 to 7 ppm the mean values were 3.8 ppm (dimethyl-glyoxime) and 3.9 ppm (gas chromatography). Above about 8 ppm the gas chromatographic method gave somewhat lower values than the chemical method, but these levels did not occur naturally in the wines examined. Recoveries of added diacetyl in the range of 0 to 8 ppm were accurate by both methods.

The occurrence of malo-lactic fermentation was measured by paper chromatography using the method of RIBÉREAU-GAYON (11).

Triangular taste tests (12) were used for the taste threshold measurements, and statistical evaluation of the results was measured by $\chi^2$ tests. Solutions of purified and assayed diacetyl were prepared in two dry red table wines of 1967 vintage, which differed in the amounts of diacetyl which they contained, and presented in a range of concentrations to 11 tasters on a minimum of five different days for each concentration. Tasting was continued until the threshold level at a statistical significance of $P < 0.01$ could be obtained for each taster.

## Results

The results of the analyses are shown in Fig. 2, which presents in histogram form the diacetyl content of the wines in class intervals of 0.5 ppm, together with the occurrence of malo-lactic fermentation.

The diacetyl values ranged from 0 to 7.5 ppm with a mean of 2.4 ppm. Malo-lactic fermentation had occurred in 71 per cent of the wines, and the mean diacetyl value for these wines was 2.8 ppm. The fermentation had not taken place in 29 per cent of the wines, and the mean diacetyl value for these wines was 1.3 ppm.

The change in diacetyl content of red table wines with time was measured with a range of these wines made from *Vitis vinifera,* variety Shiraz (the Syrah of the Rhone Valley and the Petite Sirah of California) in the Institute's experimental winery, and stored at 15° C during the course of the measurements. The diacetyl content at the end of malo-lactic fermentation was taken as zero time, and the mean loss of diacetyl as a percentage of that present at zero time was 19 per cent at 4 months (3 wines), 22 per cent at 8 months (8 wines), 26 per cent at 12 months (7 wines) and 28 per cent at 18 months (5 wines).

The diacetyl content of a range of dry white table wines made in the Institute's experimental winery from the grape varieties *V. vinifera* vars. Riesling, Clare Riesling and Semillon by different yeasts was measured, but no diacetyl was detected in any of the wines. (The minimum detectable amount measurable by the method was 0.1 ppm).

The results of the taste threshold measurements with diacetyl added to two dry red table wines are given in Table 1, which shows the minimum statistically detectable addition of diacetyl to the two wines. For the wine containing 0.3 ppm the lowest detectable addition was 1 ppm, and for the wine containing 3 ppm the lowest detectable addition was 4 ppm. The first wine was an Australian "flagon-style" red wine and was lighter in colour and had less aroma than the second wine, which was of higher quality.

## Discussion

The amount of diacetyl present in some of the wines was surprisingly high, and this was closely related to the occurrence of malo-lactic fermentation, and to the

Table 1

Taste thresholds of diacetyl added to two dry red table wines

| Wine | Grenache 1967 | Shiraz 1967 |
|---|---|---|
| Diacetyl content | 0.3 | 3.0 |
| Taster | Minimum detectable added diacetyl ppm | |
| A | 6 | 10 |
| B | 1 | 6 |
| C | 1 | 4 |
| D | 6 | 10 |
| E | 4 | 4 |
| F | 4 | 4 |
| G | >10 | >10 |
| H | >10 | >10 |
| I | >10 | >10 |
| J | >10 | 10 |
| K | — | >10 |

L.S.D. ($P < 0.01$).

presence of a butter-like aroma in these wines. The results in Fig. 2 are of considerable interest in that they appear to be the summation of two separate sets of data, depending on whether or not the wines had undergone a malo-lactic fermentation. As expected, the wines which had undergone malo-lactic fermentation contained a wide range of diacetyl, the amounts probably being related to the type of bacteria present in the various wines, as it is known that diacetyl formation is influenced by the strain of bacteria (6, 7). However, an appreciable number of wines, which had not undergone malo-lactic fermentation, contained up to 2.5 ppm diacetyl and a few contained up to 5.5 ppm. We infer that the diacetyl in these wines was produced by yeast, and it is known that yeasts differ in this regard (13), but the range of values was surprisingly large.

A greater range of diacetyl concentration was present than was generally reported in the literature. PEYNAUD (15) and PEYNAUD and LAFON (16) found amounts up to 3 ppm in a range of red Bordeaux wines, and up to 6 ppm in six Algerian wines, whilst KIELHÖFER and WÜRDIG (17) and DITTRICH and KERNER (18) found that normal German wines, mainly white, contained less than 1 ppm. DITTRICH and KERNER considered that wines containing above 0.9 ppm were faulty, and their highest value for such wines was 4.3 ppm. KUNKEE *et al.* (19) found diacetyl levels in 9 Southern California wines ranging from less than 2 to 8 ppm.

Various factors have been reported in the literature as influencing the amount of diacetyl formed by micro-organisms. These include the strain of micro-organism, amounts of citric acid, pyruvic acid and amino acids (particularly valine) in the medium, aeration, agitation, and temperature of fermentation. Further work is in progress in our laboratory on the influence of some of these factors on the diacetyl content of wine.

The reduction in diacetyl content of wines stored at constant temperature indicates an initial decrease, amounting to 19 per cent reduction in the first four months following the termination of malo-lactic fermentation, when diacetyl con-

tent is at its maximum level, and a gradual decrease of 28 per cent after 18 months. Considering the chemical reactivity of diacetyl, its relative stability in dry red wine is surprising. Possibly the anthocyanins and tannins present have a protective function, because we have found that diacetyl added to white wines disappears rapidly.

The taste threshold tests showed that ability to detect a difference in diacetyl level depended on the type of wine and the original diacetyl content. Tasters differed widely in their ability to detect differences in diacetyl levels, which was expected from the results of previous threshold measurements with other compounds (8). It has been our experience that a small amount of diacetyl, 1 to 4 ppm depending on the wine, added complexity to the aroma and improved quality. Amounts above 3 to 4 ppm (again depending on the wine), became increasingly evident as diacetyl and at higher levels such as 5 to 7 ppm the wines had a distinct butter-like aroma, which was considered objectionable. It is interesting to note that 0.5 ppm is regarded as a high value for beer (20).

## Summary

The diacetyl content of 466 Australian dry red table wines ranged from less than 0.1 ppm to 7.5 ppm with a mean of 2.4 ppm. Malo-lactic fermentation had occurred in 71 per cent of the wines, which had a mean diacetyl level of 2.8 ppm. In wines which had not undergone malo-lactic fermentation the mean diacetyl level 1.3 ppm.

Taste threshold tests showed that a difference of as little as 1 ppm could be detected in a light dry red wine containing 0.3 ppm diacetyl. In a full flavoured darker wine of higher quality containing 3 ppm the minimum detectable addition was 1.3 ppm.

It is considered that diacetyl in amounts up to 2 to 4 ppm, depending on the wine, improved quality by adding complexity to the flavour. Above these levels the aroma of diacetyl became identifiable as such and resulted in a reduction in quality. The diacetyl content of a range of red table wines stored at 15° C showed a mean decrease of 19 per cent in diacetyl content in 4 months, 22 per cent in 8 months, 26 per cent in 12 months and 28 per cent in 18 months.

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