Kepner, R. E., Webb, A. D., Maggiora, L., *Am. J. Enol. Viticult.* **20**, 25 (1969).
Kepner, R. E., Webb, A. D., Muller, C. J., *Am. J. Enol. Viticult.* **23**, 103 (1972).
Leppänen, O., Ronkainen, P., Koivisto, T., Denslow, J., *J. Inst. Brew.* **85**, 278 (1979).
Liu, M. T. H., Banjoko, O., Yamamoto, Y., Moritani, I., *Tetrahedron* **31**, 1645 (1975).
Makoto, M., Hoshino, M., *Jikeikai Med. J.* **23**, 189 (1976).
Mattioda, G. D., Ger. Offen. Patent 1 903 076 (1969).
Maw, G. A., Coyne, C. M., *Arch. Biochem. Biophys.* **117**, 499 (1966).
Mayer, D., in "Houben/Weyl, Methoden der Organischen Chemie", Vol. VII/2c, 4th ed., Müller, E., Ed., Thieme, Stuttgart, 1977, p 2232.
Meunier, J. M., Bott, E. W., *Chem. Mikrobiol. Technol. Lebensm.* **6**, 92 (1979).
Muller, C. J., Kepner, R. E., Webb, A. D., *Am. J. Enol. Viticult.* **22**, 156 (1971).
Muller, C. J., Kepner, R. E., Webb, A. D., J. Agric. Food Chem. **20**, 193 (1972).
Nikiforov, A., Schmidt, U., *Monatsh. Chem.* **105**, 1044 (1974).
Nordmann, J., Mattioda, G. D., Fr. M. Patent 7593 (1970).
Oser, B. L., Ford, R. A., *Food Technol.* **32**(2), 60 (1978).
Piloty, M., Baltes, W., *Z. Lebensm.-Unters.-Forsch.* **168**, 374 (1979).
Prochazka, M., Palecek, M., *Collect. Czech. Chem. Commun.* **35**, 1399 (1970).
Schreier, P., *CRC Crit. Rev. Food Sci. Nutr.* **12**, 59 (1979).
Schreier, P., Drawert, F., Winkler, F., *J. Agric. Food Chem.* **27**, 365 (1979).
Schreier, P., Drawert, F., Abraham, K. O., *Lebensm.-Wiss. Technol.* in press (1980).
Van Straten, S., Ed., "Volatile Compounds in Food", 4th ed, CIVO, TNO, Zeist, 1977.
Webb, A. D., Kepner, R. E., Maggiora, L., *Am. J. Enol. Viticult.* **20**, 16 (1969).
Webb, A. D., Muller, C. J., *Adv. Appl. Microbiol.* **15**, 75 (1972a).
Webb, A. D., Muller, C. J., Kepner, R. E., Eriksson, K., Nährli, M., *Am. J. Enol. Viticult.* **23**, 121 (1972b).
Webb, A. D., Eriksson, K., Muller, C. J., Kepner, R. E., *Am. J. Enol. Viticult.* **27**, 27 (1976).

Received for review January 9, 1980. Accepted March 31, 1980.

Volatile Ester Hydrolysis or Formation during Storage of Model Solutions and Wines

David D. Ramey and Cornelius S. Ough*

The effects of temperature, ethanol concentration, and pH on the rate of hydrolysis of common volatile esters of wine (ethyl butanoate, ethyl hexanoate, ethyl octanoate, ethyl decanoate, isobutyl acetate, isoamyl acetate, hexyl acetate, and 2-phenylethyl acetate) were investigated in model situations. The pseudo-first-order rate constants for temperature and pH effects were calculated as well as the second-order rate constants for $[H^+]$ effect on model solution ester hydrolysis. The effect of acid catalysis of species other than H^+ was calculated and found to be minor. Ethanol concentration differences in amounts of 10–14% v/v had little effect on the rates. Activation energies and thermodynamic activation constants were calculated for the esters. Several wines were also analyzed for changes in ester concentration with time at several different temperatures.

Volatile esters are introduced into wine primarily by yeast during fermentation. Although small quantities of esters are present in grapes prior to fermentation, the amounts are negligible compared to those introduced enzymatically by the yeast (Schreier et al., 1976; Stevens et al., 1969; Usseglio-Tomasset and Bosia, 1978; Van Wyk et al., 1967; Webb, 1973). Ethyl esters of straight-chain, saturated fatty acids and acetate esters of higher alcohols predominate, since these compounds are present in high concentrations in the fermenting medium and within the yeast cell (Majaama, 1978; Nelson and Wheeler, 1939; Nordstrom, 1963, 1964a). These esters have pleasant, fruitlike aromas which are particularly pronounced in new wines.

While the enzymatic mechanism of formation of esters and the factors affecting the quantities synthesized have been studied extensively in wine, beer, and synthetic media, little work has been done on their fates following fermentation or on the rates at which they are hydrolyzed or formed. Studies have been made of the rates of hydrolysis of various esters in strongly acidic media (>85% H_2SO_4), but this is of limited applicability to a buffered, weakly acidic solution such as wine, since the reaction mechanism differs.

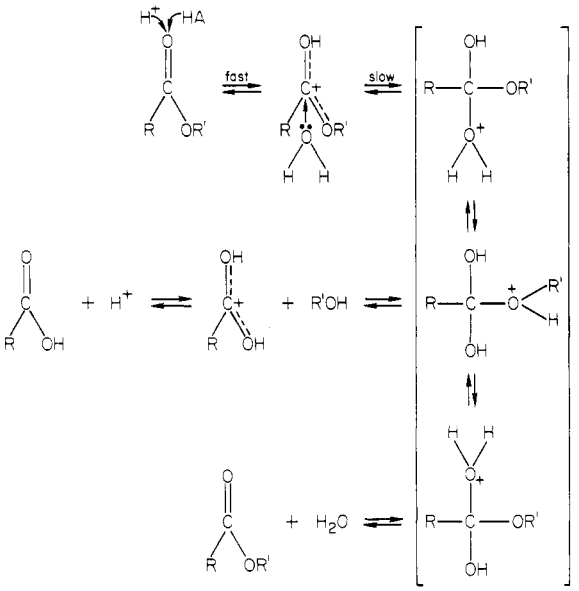
The probable mechanism of hydrolysis of esters in wine or solutions modeled after wine is that of Bamford and Tipper (1972) and Isaacs (1974) and is given below. The reaction is entirely reversible, one direction resulting in ester hydrolysis and the other in esterification of the component acid and alcohol, so that factors affecting the reaction rate in one direction will affect the reverse reaction similarily. The catalyst may be either a free hydrogen ion or an undissociated proton of an organic acid.

The first studies of esterification rates in model solutions approximating wine were completed by Ribéreau-Gayon and Peynaud (1936). Polyprotic acids such as tartaric, malic, and succinic esterified more rapidly than the monoprotic acids acetic, propanoic, and butanoic. Generally, the more complicated the acid within each category, that is, the higher its molecular weight, the more slowly it was esterified. Using the equilibrium constant of 4 calculated for esterification by Berthelot (Berthelot and Saint-Gilles, 1962–1963) $[K_e = [\text{ester}][H_2O]/([\text{acid}][\text{alcohol}])]$, they calculated the theoretical limit of esterification and found that none of the acids studied were esterified to that limit.

Nordstrom (1963, 1964a,b) provided a kinetic analysis of ester formation by yeast in synthetic media but employed enzyme kinetics.

Department of Viticulture and Enology, University of California, Davis, California 95616.

0021-8561/80/1428-0928$01.00/0



Spirov and Goranov (1977) analyzed white table wines after 8 months of storage and found increased concentrations of ethyl formate, isobutyl acetate, hexyl acetate, ethyl octanoate, and ethyl lactate.

Simpson (1978) subjected Australian white table wines to bottled storage at 15 and 50 °C. Both 15 and 50 °C treatments showed increases in ethyl hexanoate, ethyl octanoate, ethyl decanoate, ethyl furoate, and diethyl succinate concentrations by headspace analysis. Hexyl acetate concentration decreased.

Marais (1978) examined the effects of pH and temperature on South African Colombard wine over a 16-week period. Isoamyl, hexyl, and 2-phenylethyl acetates all decreased in concentration with time, more so at higher temperature and at lower pH. Ethyl hexanoate, ethyl octanoate, and ethyl decanoate concentrations decreased only slightly, if at all. Diethyl succinate concentration increased more rapidly at lower pH and higher temperature.

This research was designed to investigate the effects of temperature, pH, and ethanol concentration on the rates of hydrolysis of the common odorous esters synthesized by yeast. The hypothesis was that these esters are introduced enzymatically into wine in excess of their equilibrium concentrations and that they are gradually hydrolyzed until they approach equilibrium with their component acids and alcohols.

MATERIALS AND METHODS

Model Solutions. Model solutions were prepared by using distilled water, acidulated with tartaric acid to 0.75 g/100 mL and adjusted with NaOH to the desired pH. Alcohol levels were adjusted with 95% ethanol to 10, 12, and 14%, v/v, following which an aliquot of stock ester solution was introduced. The stock ester solution was prepared by pipetting each of the eight esters into 95% ethanol in such quantities that, based on their densities and the size of the aliquot introduced into the model solutions, the concentration of each ester in the model solution would be about 50 mg/L. The model solutions were filtered with diatomaceous earth through an air pressure filter to adsorb excess, insoluble esters—those of higher molecular weight. After filtration, initial ester concentrations varied from approximately 3 to 30 ppm. Solutions were bottled in 375-mL screw-cap bottles and sealed with paraffin. Two bottles of each series were analyzed for ester concentrations immediately, and the rest were stored in temperature-controlled water baths.

Wines. The red wine was a Pinot noir from Oakville, CA. At grape crushing, 75 mg/L SO_2 was added and the must was fermented at 21 °C (70 °F) by using *Saccharomyces cerevisiae* strain Montrachet yeast. It was pressed at 5° brix and, after finishing fermentation, was cold stabilized for 2 weeks at 0 °C, when it was filtered into 750-mL screw-cap bottles and sealed with paraffin. The pH was 3.36, the titratable acidity 0.76 g/100 mL, and the ethanol 13.6% v/v.

The white wine was a Chardonnay from Oakville, CA. At grape crushing, 75 mg/L SO_2 was added, the grapes were pressed, and the juice settled overnight at 0 °C. The clear juice was fermented to dryness at 16 °C (60 °F) with the same strain of yeast (Montrachet) and cold stabilized for 2 weeks at 0 °C and then filtered and bottled. The pH was 2.94, the titratable acidity 1.09 g/100 mL, and the ethanol 11.2%.

Both red and white wines were analyzed immediately after bottling for ester concentrations and then stored in temperature-controlled water baths.

Distillation/Extraction. An apparatus similar to that described by Likens and Nickerson (1964) and Schultz et al. (1977) was used to simultaneously distill the samples and extract the esters. Samples were adjusted to the same alcohol concentration prior to analysis by addition of 100% ethanol [as Killian and Ough (1979)] since variations in alcoholic strength alter the vapor–liquid partition coefficient of the volatile solutes and hence the efficiency of their extraction.

Three hundred and fifty milliliters of model solution (750 mL of wine) was used. Boiling chips and internal standards were added. A 250-mL solvent flask was filled with 60 mL of redistilled pentane and connected to the distillation/extraction apparatus. Distilled water and redistilled pentane were used to fill the return tubes at the bottom of the apparatus.

Samples were refluxed in the distillation/extraction apparatus for exactly 30 min. The pentane was then transferred to a 250-mL separatory funnel, and 20 mL of 1 N aqueous NaCl was added to extract any ethanol. The salt solution was extracted twice more with 25-mL portions of pentane, and these were combined with the extract. Anhydrous Na_2SO_4 was added to remove any water, and the extract was stored overnight at −8 °C.

Concentrations. The extract was transferred into a 250-mL round-bottom flask, and the Na_2SO_4 was rinsed twice with small portions of pentane. The pentane extract was distilled through a 40-cm Vigreux-type fractionating column until only 5 mL of extract remained. The residue was transferred with two small pentane rinses to a 10-mL Kontes concentrator tube. A glass ebullator was added, a 10-cm micro Snyder column was attached, and the pentane was further refluxed and distilled off, using a heating block, until approximately 0.5 mL of extract remained. This residual was transferred to a 2-mL glass vial, sealed with a silicon-coated, red-rubber septum, and stored at −8 °C until GC analysis.

Gas Chromatography. Ester separation was accomplished with a Hewlett-Packard Model 5720A gas chromatograph fitted with a flame ionization detector. The column used was a 30-m glass capillary column with an internal diameter of 0.25 mm, coated with SE-30.

The operating conditions for gas chromatography were as follows: injection temperature, 220 °C; detector temperature, 270 °C; split ratio, 50:1; carrier gas (N_2) flow, 13.4 cm/s; hydrogen, 34 mg/min; air, 230 mL/min; makeup gas (N_2), 30 mL/min; oven temperature, 55 °C (10 min), 3 °C/min to 155 °C; range and attenuation, 1 × 4 or 1 × 2.

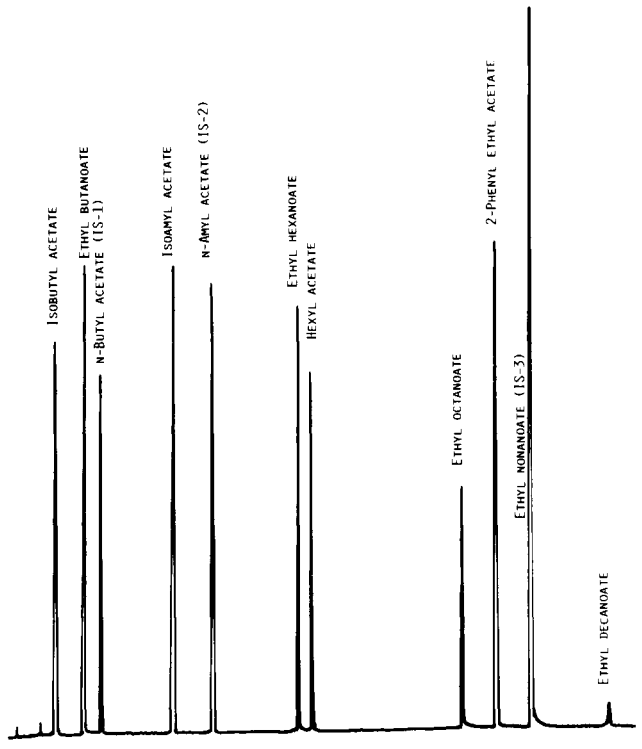


Figure 1. Model solution extract chromatogram.

Esters were identified routinely from retention times but had been previously identified by means of dissimilar liquid-phase gas chromatography, Kovats retention indices, and gas chromatography–mass spectrometry (Killian and Ough, 1979).

The mass spectra verification was made with a Finnigan Quadrapole 3200 mass spectrometer operated at 68 eV interfaced to a Finnigan Model 9500 gas chromatograph and a Finnigan 6000 MS data system. The same SE-30 column was used for separation. The temperature programming and other conditions were similar to those described above. Scans were made every 3 s from 30 to 300 mass units. Comparisons of spectra to that of knowns were done.

Quantification. Three internal standards were *n*-butyl acetate, *n*-amyl acetate, and ethyl nonanoate. They were weighed cold and transferred quantitatively by rinsing with 95% ethanol into 250-mL volumetric flasks.

Quantities were weighed so that when introduced into the distilling flask with a 1-mL pipet a concentration of 25 mg/mL would be achieved in the model solutions. For the wine analyses, dilutions were made from the model solution internal standards to obtain a concentration of 0.5 mg/mL in the distilling flask.

Peak areas were measured by a Varian Aerograph CDS 101 electronic integrator.

Standard curves were prepared from duplicate analyses of four ester concentrations. From plots of (ester area)/(internal standard area) vs. (ester concentration)/(internal standard concentration) for each ester the concentrations of the unknown esters were calculated. The bases of quantification were as follows:

internal standard	ester concn determined
n-butyl acetate	isobutyl acetate
	ethyl butanoate
	isoamyl acetate
n-amyl acetate	ethyl hexanoate
	hexyl acetate
ethyl nonanoate	ethyl octanoate
	2-phenylethyl acetate
	ethyl decanoate

Table I. Pseudo-First-Order Rate Constants for Hydrolysis of Esters in Model Solutions: Temperature Effect

ester and temp, °C	k_{obsd}, s^{-1} × 10^9	SD (× 10^9)	coeff of correlation (r)
ethyl butanoate			
4.4	2.692	0.4531	−0.917
12.8	8.316	0.4265	−0.992
21.1	18.10	1.093	−0.989
29.4	40.62	1.519	−0.996
37.8	71.20	3.119	−0.995
ethyl hexanoate			
4.4	2.639	3.945	−0.262
12.8	15.25	4.665	−0.800
21.1	25.78	2.612	−0.971
29.4	51.79	2.745	−0.992
37.8	89.59	5.091	−0.991
ethyl octanoate			
4.4	13.11	8.023	−0.555
12.8	28.84	10.37	−0.751
21.1	44.54	8.716	−0.902
29.4	67.30	9.436	−0.946
37.8	107.2	11.97	−0.965
ethyl decanoate[a]			
4.4	41.53	3.518	−0.983
12.8	(145)	(38.6)	−0.967
21.1	(205)	(35.1)	−0.986
29.4	(251)	(42.7)	−0.986
37.8	(272)	(113)	−0.924
isobutyl acetate			
4.4	9.063	1.839	−0.894
12.8	15.43	1.786	−0.962
21.1	39.98	1.866	−0.994
29.4	76.21	1.919	−0.999
37.8	147.5	6.557	−0.995
isoamyl acetate			
4.4	1.439	3.332	−0.171
12.8	14.58	2.159	−0.940
21.1	32.60	1.173	−0.997
29.4	73.59	1.946	−0.998
37.8	148.9	3.119	−0.999
hexyl acetate			
4.4	4.212	4.878	−0.332
12.8	20.55	5.944	−0.816
21.1	39.26	3.172	−0.981
29.4	84.15	2.772	−0.997
37.8	164.5	4.212	−0.999
2-phenylethyl acetate			
4.4	11.99	4.771	−0.715
12.8	28.89	8.263	−0.842
21.1	46.59	6.957	−0.939
29.4	75.09	11.54	−0.956
37.8	136.3	6.850	−0.993

[a] Values in parentheses are approximate only; see the text.

RESULTS AND DISCUSSION

A sample chromatogram of the model solution extract is shown in Figure 1.

Eight esters of the model solution samples were followed at five temperatures, three ethanol concentrations, and three pH values for a period of 200 days. The effect of temperature on hydrolysis can be demonstrated by Figure 2, which shows the log of the isoamyl acetate concentration plotted vs. time. Data on the other seven esters were plotted in a similar manner. The pseudo-first-order rate constants (temperature effects) for the eight esters of the model solutions were summarized in Table I along with the standard deviations and the coefficients of correlation. These rate constants also depend on the catalyst present. This will be discussed under the effects of pH.

The ethyl esters hydrolyzed more slowly than the acetate esters, with the exception of ethyl decanoate. This is logical considering the high concentration of ethanol, a

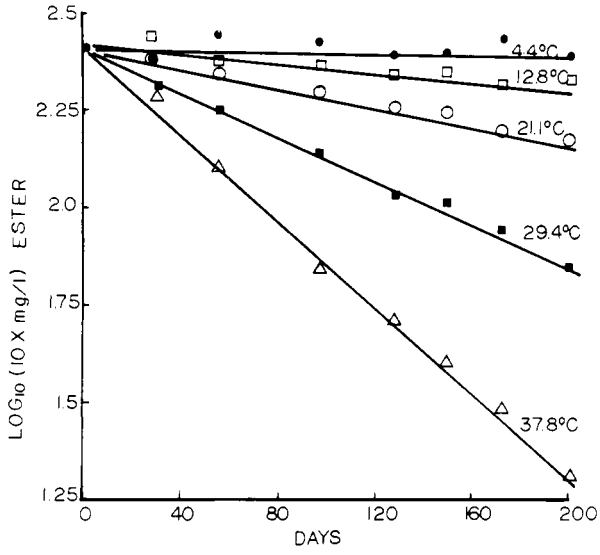


Figure 2. Effect of temperature on the hydrolysis of isoamyl acetate.

Table II. Activation Energies of Esters in Model Solutions

ester	activation energy (E_a), kcal mol^{-1}	SD	coeff of correlation (r)	preexponential factor (A), s^{-1}
ethyl butanoate	16.8	0.845	−0.997	52100
ethyl hexanoate	12.7	0.574	−0.998	80.0
ethyl octanoate	10.4	0.679	−0.994	2.38
isobutyl acetate	14.8	0.719	−0.997	3530
isoamyl acetate	16.5	0.190	−1.00	61000
hexyl acetate	14.8	0.463	−1.00	4380
2-phenylethyl acetate	12.0	0.710	−0.995	37.3

hydrolysis product of ethyl esters. These esters begin reacting at levels much nearer their equilibrium concentrations, and since rate declines logarithmically, they would not be expected to achieve the velocity of acetate esters or hydrolyze to as great an extent. In addition, whatever role ethanol plays in transesterification would be apparent only for acetate esters, where it would augment their rate of disappearance. Transesterification of an ethyl ester would result in products identical with reactants and would not contribute positively to reaction rate.

The ethyl esters also hydrolyzed more rapidly as their molecular weight increased, so much so that reliable data could not be obtained for ethyl decanoate except at the lowest temperature; the reaction must be monitered each week rather than monthly. Approximate rate constants based on three readings only are included in parentheses in Table I for comparative purposes.

Activation energies and preexponential factors (A) in Table II were obtained by plotting the logarithm of the rate constants at five temperatures against the reciprocal of the absolute temperature. Activation energy varies inversely with molecular weight in the ethyl series, which is reflected in the more rapid hydrolysis of the higher boiling esters; that is, a smaller energy requirement will allow more rapid reaction rates at the same temperature. The same inverse relationship exists between the preexponential factor, A, and molecular weight. This may be at least partially explained by a decrease in vibrational frequency between the acyl carbon and oxygen of the transition state. As the fatty acid chain grows in length, the increased mass results in slower and fewer vibrations.

Table III. Thermodynamic Activation Quantities of Esters in Model Solutions

ester	free energy of activation ($\Delta G^{\ddagger}$), kcal mol^{-1}	enthalpy of activation ($\Delta H^{\ddagger}$), kcal mol^{-1}	entropy of activation ($\Delta S^{\ddagger}$), cal mol^{-1} K^{-1}
ethyl butanoate	27.1	16.2	−36.9
ethyl hexanoate	26.8	12.2	−49.8
ethyl octanoate	26.6	9.85	−56.8
isobutyl acetate	26.6	14.2	−42.3
isoamyl acetate	26.7	15.9	−36.6
hexyl acetate	26.6	14.3	−41.8
2-phenylethyl acetate	26.5	11.4	−51.3

No trends emerge within the acetate esters, probably because the structural dissimilarity of the alcohol moieties, two branched chains, one straight chain, and one aromatic substituent, confounds tendencies which would be otherwise apparent.

Somewhat anomalous results were obtained for k_{obsd} at 4.4 °C for four of the eight esters; rates were slower than expected, which appears on the Arrhenius plots of isoamyl acetate, ethyl hexanoate, and hexyl acetate. This behavior was observed for ethyl decanoate also, though the data have not been included due to poor quantification. There are no apparent structural similarities to explain these results. Bender et al. (1958) has examined and recalculated much of the published data on ester hydrolysis and found no "unequivocal evidence" supporting temperature dependency of the activation energies of hydrolysis of any carboxylic esters. For these reasons the anomalous low points have been eliminated from activation energy calculations. Further experiments in the 0–15 °C range may clarify these results.

The entropies ($\Delta S^{\ddagger}$), enthalpies ($\Delta H^{\ddagger}$), and free energies ($\Delta G^{\ddagger}$) of activation were calculated and are reported in Table III. Of these quantities, $\Delta S^{\ddagger}$ is of most interest as it confirms aspects of the mechanism of hydrolysis. It represents a measure of the increase in randomness which occurs when water adds to the protonated ester to form the transition state. Positive values of $\Delta S^{\ddagger}$ thus indicate a transition state with more internal degrees of freedom, both rotational and vibrational, than the reactants. The values of $\Delta S^{\ddagger}$ for the esters studied are all negative, indicating an increase in order upon formation of the transition state and a consequent decrease in degrees of freedom.

Entropies of activation also serve to confirm the bimolecular formation of the transition state. Long et al. (1957) has surveyed the literature and proposed that negative $\Delta S^{\ddagger}$ values correspond to A-2 mechanisms of hydrolysis, according to Ingold's (1969) classification (A = acid catalyzed, 2 = bimolecular formation of transition complex). Positive $\Delta S^{\ddagger}$ values result from A-1 (unimolecular) mechanisms. These results support Long's observation.

Results of the calculations for the pseudo-first-order rate constants for the ethanol effect are given in Table IV. Differences found due to ethanol are slight and often not significant. No consistent trend exits. It is probable that over a wider range of ethanol concentration a correlation would be found between that and the hydrolysis rate of ethyl esters, as reported by Onishi et al. (1977) in brandy.

Rate constants for three pH values are reported in Table V. Each ester hydrolyzed more rapidly at lower pH, with the exception of ethyl octanoate, for which the pH 3.58 and 4.10 rate constants did not differ significantly due to data scatter. Hydrolysis obeys a pseudo-first-order rate law in ester concentration in which the effect of the catalyst is

Table IV. Pseudo-First-Order Rate Constants for Hydrolysis of Esters in Model Solutions: Ethanol Effect

ester and % ethanol	k_{obsd}, $s^{-1} \times 10^9$	SD ($\times 10^9$)	coeff of correlation (r)
ethyl butanoate			
10	24.71	0.6131	−0.999
12	18.18	1.093	−0.990
14	18.79	0.9596	−0.992
ethyl hexanoate			
10	31.61	4.398	−0.946
12	25.78	2.612	−0.971
14	35.29	3.199	−0.977
ethyl octanoate			
10	47.95	6.797	−0.945
12	44.54	8.716	−0.902
14	43.39	7.090	−0.929
isobutyl acetate			
10	43.47	2.559	−0.990
12	39.98	1.866	−0.994
14	34.36	1.359	−0.996
isoamyl acetate			
10	41.56	1.066	−0.999
12	32.60	1.173	−0.997
14	36.81	0.9329	−0.999
hexyl acetate			
10	48.89	5.384	−0.966
12	39.26	3.172	−0.981
14	51.58	3.492	−0.987
2-phenylethyl acetate			
10	53.47	6.451	−0.959
12	46.59	6.957	−0.939
14	31.24	8.983	−0.841

Table V. Pseudo-First-Order Rate Constants for Hydrolysis of Esters in Model Solutions: pH Effect

ester and pH	k_{obsd}, $s^{-1} \times 10^9$	SD ($\times 10^9$)	coeff of correlation (r)
ethyl butanoate			
2.95	64.11	2.106	−0.997
3.58	18.18	1.093	−0.990
4.10	9.516	0.7463	−0.982
ethyl hexanoate			
2.95	82.12	5.758	−0.986
3.58	25.78	2.612	−0.971
4.10	4.145	4.798	−0.723
ethyl octanoate			
2.95	74.47	6.850	−0.976
3.58	44.54	8.716	−0.902
4.10	48.78	10.45	−0.886
isobutyl acetate			
2.95	135.9	8.050	−0.990
3.58	39.98	1.866	−0.994
4.10	19.70	2.346	−0.960
isoamyl acetate			
2.95	129.2	3.199	−0.999
3.58	32.60	1.173	−0.997
4.10	14.10	2.692	−0.906
hexyl acetate			
2.95	153.3	5.704	−0.996
3.58	39.26	3.172	−0.981
4.10	18.34	4.691	−0.847
2-phenylethyl acetate			
2.95	118.9	9.809	−0.981
3.58	46.59	5.224	−0.979
4.10	35.16	7.650	−0.899

accounted for, not in the overall rate equation, since it is regenerated, but is included in k_{obsd}:

$$k_{obsd} = \sum k_{cat}[\text{catalyst}]$$

where the summation indicates that more than one catalyst may be present. In this case apparent vaues of k_{H^+} may be obtained by plotting k_{obsd} against $[H^+]$ and taking the slope as k_{H^+}:

$$k_{obsd} = k_{H^+}[H^+]$$

Table VI. Graphic Second-Order Rate Constants in $[H^+]$ for Hydrolysis of Esters in Model Solutions

ester	k_{H^+}, L mol^{-1} $s^{-1} \times 10^4$	SD ($\times 10^4$)	coeff of correlation (r)
ethyl butanoate	0.527	0.009	0.999
ethyl hexanoate	0.665	0.011	0.999
isobutyl acetate	1.115	0.001	0.999
isoamyl acetate	1.111	0.017	0.999
hexyl acetate	1.305	0.027	0.999
2-phenylethyl acetate	0.815	0.032	0.999

Table VII. Matrix Solutions of Second-Order Rate Constants in Hydrogen Ion, Undissociated Tartaric Acid, and Bitartrate for Hydrolysis of Esters in Model Solutions (L mol^{-1} s^{-1})

ester	k_{H^+}	k_{H_2T}	k_{HT^-}
ethyl butanoate	0.613×10^{-4}	-2.33×10^{-7}	1.67×10^{-7}
ethyl hexanoate	0.646×10^{-4}	-2.01×10^{-7}	1.82×10^{-7}
isobutyl acetate	1.19×10^{-4}	-1.09×10^{-7}	3.12×10^{-7}
isoamyl acetate	1.24×10^{-4}	-4.05×10^{-7}	1.78×10^{-7}
hexyl acetate	1.51×10^{-4}	-6.47×10^{-7}	2.71×10^{-7}
2-phenylethyl acetate	1.18×10^{-4}	-8.82×10^{-7}	8.69×10^{-7}

The apparent second-order rate constants in $[H^+]$ are given in Table VI. The graphs are very nearly linear, indicating hydrolytic rates to be a direct function of $[H^+]$ in the ranges found in wine. The acetate esters are more sensitive to $[H^+]$ than the two listed ethyl esters.

None of the plots for the effect of $[H^+]$ on hydrolysis rate pass through the origin; that is, at an extrapolated $[H^+]$ of zero, hydrolysis would still continue. This may be taken as a measure of the amount of the reaction catalyzed by species other than hydrogen ion, in this case, undissociated tartaric acid and bitartrate. The complete rate constant for this system is

$$k_{obsd} = k_0 + k_{H^+}[H^+] + k_{OH^-}[OH^-] + k_{H_2T}[H_2T] + k_{HT^-}[HT^-] + k_{T^{2-}}[T^{2-}]$$

where k_0 is a water constant and represents the rate of the uncatalyzed reaction. This term and the OH^- term may be eliminated as being negligible in an acid system. In a more complex system such as wine many other catalytic species would enter into the equation.

The second-order rate constants in $[H^+]$ determined graphically and reported in Table VI are empirical constants but are not accurate. As the pH changes the distribution of acid species varies, which affects the rate. These effects are confounded in the graphs of k_{obsd} vs. $[H^+]$. The equation for k_{obsd} was simplified to include the most probable catalytic species in order to separate these mingled results:

$$k_{obsd} = k_{H^+}[H^+] + k_{H_2T}[H_2T] + k_{HT^-}[HT^-]$$

This equation exists for each ester at three different pH values, with k_{obsd} and $[H^+]$ determined experimentally and the concentrations of the acid species at each pH in 12% ethanol calculated from the dissociation constants of Usseglio-Tomasset and Bosia (1978). These three equations, each with three unknown constants, may be solved for each ester as a 3×3 matrix. The solutions were presented in Table VII.

The constants for tartaric acid are predominantly negative, which could be true if it inhibited hydrolysis. This is not likely, which suggests that the simplified cataytic model is not exact and that other species such as tartrate may play a catalytic role in the reaction. However, there is very close agreement between the graphically determined k_{H^+} values of Table VI and the matrix solutions in Table

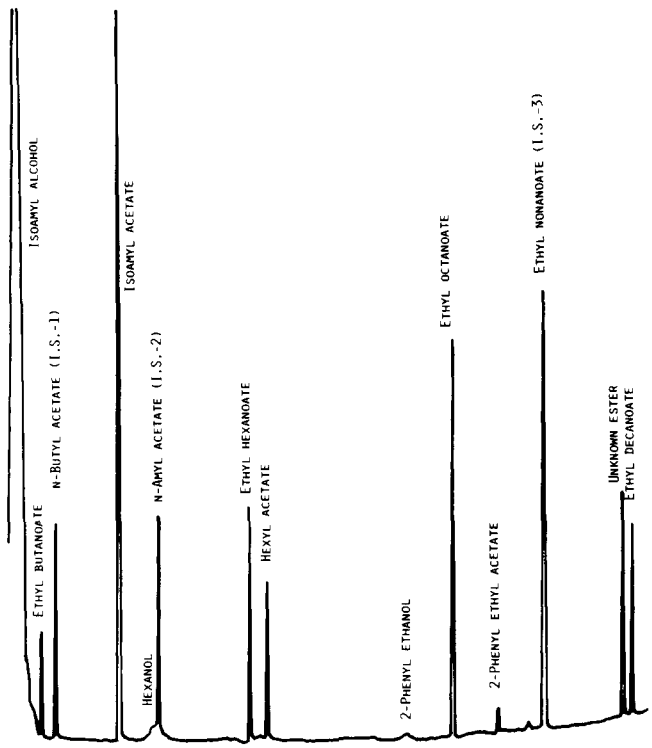


Figure 3. Chardonnay wine extract chromatogram.

Table VIII. Pseudo-First-Order Rate Constants for Hydrolysis of Esters in Chardonnay: Temperature Effect

ester and temp, °C	k_{obsd}, $s^{-1} \times 10^9$	SD ($\times 10^9$)	coeff of correlation (r)
ethyl octanoate			
4.4	3.145	4.611	−0.266
12.8	−1.573	6.317	0.101
21.1	−2.719	6.451	0.170
29.4	1.048	7.490	−0.495
37.8	7.250	11.30	−0.253
ethyl decanoate			
4.4	12.79	4.878	−0.731
12.8	10.64	6.690	−0.543
21.1	16.29	5.491	−0.771
29.4	7.010	12.02	−0.232
37.8	6.371	13.27	−0.192
isoamyl acetate (+active amyl)			
4.4	23.83	5.144	−0.884
12.8	41.26	9.622	−0.869
21.1	98.73	4.763	−0.994
29.4	220.5	4.371	−1.00
37.8	422.6	13.17	−1.00
2-phenylethyl acetate			
4.4	−1.279	16.69	0.031
12.8	21.03	13.33	−0.541
21.1	34.94	22.98	−0.528
29.4	184.8	30.76	−0.926
37.8	167.7	32.63	−0.932

VII, indicating that the matrix solutions are substantially correct. Given this, the matrix-determined k_{H^+} values are 100–300 times the order of magnitude of the combined constants for tartaric acid and bitartrate, which means that pH is far more important in determining rates of ester hydrolysis than is total acidity.

A sample wine chromatograph of the extracted esters is shown in Figure 3. The esters followed were ethyl octanoate, combined isoamyl and active amyl acetates, and 2-phenylethyl acetate.

The pseudo-first-order rate constants obtained graphically are given in Tables VIII and IX. In both wines the acetate esters behaved as in model solutions, disappearing

Table IX. Pseudo-First-Order Rate Constants for Hydrolysis of Esters in Pinot Noir: Temperature Effect

ester and temp, °C	k_{obsd}, $s^{-1} \times 10^9$	SD ($\times 10^9$)	coeff of correlation (r)
ethyl octanoate			
4.4	24.23	9.089	−0.736
12.8	32.41	8.156	−0.851
21.1	20.87	8.983	−0.687
29.4	41.40	7.757	−0.909
37.8	15.14	12.10	−0.455
ethyl decanoate			
4.4	−18.95	10.05	0.609
12.8	−31.56	7.010	0.878
21.1	−24.98	11.83	0.652
29.4	−30.12	13.19	0.681
37.8	−9.942	9.649	0.387
isoamyl acetate (+active amyl)			
4.4	13.01	10.85	−0.439
12.8	26.18	3.439	−0.952
21.1	54.72	6.317	−0.963
29.4	103.2	3.785	−0.996
37.8	199.1	6.344	−0.998
2-phenylethyl acetate			
4.4	15.70	21.40	−0.287
12.8	45.53	15.70	−0.764
21.1	58.93	21.00	−0.753
29.4	91.43	27.48	−0.830
37.8	117.4	23.27	−0.900

Table X. Activation Energies of Esters in Wines

ester	activation energy (E_a), kcal mol^{-1}	SD	coeff of correlation (r)	preexponential factor (A), s^{-1}
isoamyl acetate (+active amyl)				
Chardonnay	15.3	0.732	−0.997	22600
Pinot noir	14.1	0.150	−1.00	1510
2-phenylethyl acetate				
Pinot noir	6.96	0.566	−0.994	0.00920

with time and more rapidly at higher temperatures. Changes in ethyl ester concentrations were slight, with ethyl octanoate concentration remaining approximately constant at all temperatures in the Chardonnay and decreasing slightly in the Pinot noir. Ethyl decanoate concentration dropped slightly in the Chardonnay but increased in the Pinot noir. Of the two esters followed visually but not quantitatively, ethyl hexanoate concentration did not change appreciably, while hexyl acetate concentration declined sharply, to near zero at the higher temperatures. These results correspond to those of the model solutions, in which the acetate esters hydrolyzed more rapidly and to a greater extent than did the ethyl esters, and also to those of Marais (1978), who found that acetate esters had diminished more than ethyl esters in wine after 4 months storage.

Since the initial, postfermentation concentrations vary from wine to wine, it is not surprising that individual ester behavior should differ in each wine. Some generalizations can be made: acetate esters of fusel oils tend to diminish during aging more than ethyl esters of fatty acids, and the ethyl esters of polyprotic acids such as tartaric and succinic, which are not appreciably synthesized by yeast, or of lactic fermentation may be chemically esterified during the course of aging. Whichever direction the reaction proceeds, its velocity will be speeded by lower pH and higher temperature.

The activation energies for isoamyl (plus active amyl) acetate in Chardonnay and Pinot noir and for 2-phenyl-

Table XI. Thermodynamic Activation Quantities of Esters in Wines

ester	free energy of activation ($\Delta G^{\ddagger}$) kcal mol^{-1}	enthalpy of activation ($\Delta H^{\ddagger}$) kcal mol^{-1}	entropy of activation ($\Delta S^{\ddagger}$), cal mol^{-1} K^{-1}
isoamyl acetate (+active amyl)			
Chardonnay	26.1	14.7	−38.6
Pinot noir	26.4	13.5	−44.0
2-phenylethyl acetate			
Pinot noir	28.3	6.38	−74.5

ethyl acetate in Pinot noir are reported in Table X. The values for isoamyl acetate differ slightly between the two wines and from the activation energy obtained in model solutions.

Thermodynamic quantities of activation are given in Table XI. As with the values obtained from model solutions, $\Delta S^{\ddagger}$ for isoamyl acetate is negative, suggesting the crowded tetrahedral transition state, and is more negative for 2-phenylethyl acetate with its bulky phenyl group.

SUMMARY

If present in greater than equilibrium amounts, volatile wine esters will hydrolyze at characteristic rates.

Acetate esters of higher alcohols generally hydrolyze more rapidly than ethyl fatty acid esters in both wine and model solutions.

Reaction velocity increases with temperature and varies directly with [H$^+$] in a linear manner. Hydrogen ions are roughly 100 times more active as reaction catalysts than tartaric acid. Ethanol concentration has no effect on hydrolysis rates of ethyl or acetate esters in the range found in table wines.

Ethyl esters of fatty acids hydrolyze more rapidly in model solutions as molecular weight increases.

The entropies of activation of ester hydrolysis in both model solutions and wines suggest an increase in intramolecular order upon formation of the transition complex, which corresponds with the supposed reaction mechanism involving a tetrahedral intermediate formed by addition of water to the protonated ester.

In maturing wine, esters may hydrolyze, be formed through chemical esterification, or remain at constant equilibrium concentrations depending on their initial, postfermentation levels.

LITERATURE CITED

Bamford, C. H., Tipper, C. F. H., *Compr. Chem. Kinet.* **10**, 57 (1972).
Bender, M. L., Ginger, R. D., Unik, J. P., *J. Am. Chem. Soc.* **80**, 1044 (1958).
Berthelot, M., Saint-Gilles, Pêan de, *Ann. Chim. Phys.*, 65 (1962–1963).
Ingold, C. K., "Structure and Mechanism in Organic Chemistry", Cornell University Press, Ithaca, NY, 1969.
Isaacs, N. S., "Reactive Intermediates in Organic Chemistry", Wiley, New York, 1974, pp 427–428.
Killian, E. E., Ough, C. S., *Am. J. Enol. Vitic.* **30**, 4 (1979).
Likens, S. T., Nickerson, G. B., *Proc. Am. Soc. Brew. Chem.*, 5 (1964).
Long, F. A., Pritchard, J. G., Stafford, F. E., *J. Am. Chem. Soc.* **79**, 2362 (1957).
Majaama, E., *Mallas Olut*, 189 (1978).
Marais, J., *Vitis* **17**, 396 (1978).
Nelson, E. K., Wheeler, D. H., *Ind. Eng. Chem.* **31**, 1279 (1939).
Nordstrom, K., *J. Inst. Brew.* **69**, 310 (1963).
Nordstrom, K., *J. Inst. Brew.* **70**, 42, 226, 328 (1964a).
Nordstrom, K., *J. Inst. Brew.* **70**, 233 (1964b).
Onishi, M., Guymon, J. F., Crowell, E. A., *Am. J. Enol. Vitic.* **28**, 152 (1977).
Riberêau-Gayon, J., Peynaud, E., *Bull. Soc. Chim. Fr.* **5**, 2325 (1936).
Schreier, P., Drawert, F., Junker, A., *J. Agric. Food Chem.* **24**, 331 (1976).
Schultz, T. H., Flath, R. A., Mon, T. R., Eggling, S. G., Teranishi, R., *J. Agric. Food Chem.* **25**, 446 (1977).
Simpson, R. F., *Vitis* **17**, 274 (1978).
Spirov, N., Goranov, N., *Lozar. Vinar.* **26**(2), 22 (1977).
Stevens, K. L., Flath, R. A., Lee, A., Stern, D. J., *J. Agric. Food Chem.* **17**, 1102 (1969).
Usseglio-Tomasset, L., Bosia, P. D., *Riv. Vitic. Enol.* **31**, 380 (1978).
Van Wyk, C. J., Webb, A. D., Kepner, R. E., *J. Food Sci.* **32**, 660 (1967).
Webb, A. D., *Proc. Int. Symp. Yeasts, 3rd*, Part II, 297 (1973).

Received for review December 13, 1979. Accepted June 3, 1980.