## Summary

Factors affecting the rate of oxidation of nitric oxide myoglobin to metmyoglobin have been studied. Three distinct pseudo-first-order mechanisms were observed: an autoxidation, an oxidation by nitrous acid, and a photo-oxidation.

The autoxidation was not affected by pH within the range 5.0 to 7.5 or by nitrite in the absence of nitrous acid— i.e., above pH 6.3. The $Q_{10}$ of autoxidation was 5.0.

The rate of oxidation by nitrous acid was directly proportional to the square of the nitrous acid concentration, and was therefore markedly influenced by nitrite concentration and by pH.

The rate of photo-oxidation was dependent on light intensity and temperature ($Q_{10} = 1.6$), and decreased slightly with increasing acidity. Nitrite reduced the rate slightly at pH levels above pH 6.3, but increased it progressively with increasing acidity from pH 6.3 to 5.4. Evidence is presented that the photo-oxidation proceeds by an active molecule mechanism and not by a light-catalyzed dissociation.

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## WINE CONSTITUENTS

# Comparative Analysis of Fusel Oils from Thompson Seedless, Emperor, and Muscat of Alexandria Wines

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The compositions of fusel oils from three varieties of *Vitis vinifera*—Thompson seedless, Emperor, and Muscat of Alexandria—are reported. The differences in composition are evaluated with respect to the possible effects of variations in fermentation conditions, distillation conditions, treatment of the fusel oil after removal from the column, and differences due primarily to the variety of grape fermented. Consideration of the low boiling alcohols found and the amino acids that would be required to form them by the Ehrlich mechanism indicates that the over-all formation of fusel oils must be extremely complex. Further studies of the varietal effect upon fusel oil composition would be of value in selecting the best grape variety for any particular type of brandy.

FUSEL OILS GENERALLY have been considered to be mixtures of alcohols with boiling points in the range extending from a temperature slightly above that of the ethyl alcohol boiling point to a temperature slightly higher than that of the boiling point of *n*-hexyl alcohol. Investigations of fusel oils from many different source materials have demonstrated that the principal components always consist of these simple alcohols (*6, 8, 9, 11, 14, 19*). The substances other than the alcohols through *n*-hexyl alcohol present in fusel oils to the extent of 5 to 15% have been investigated less thoroughly and reported to consist mainly of very high boiling esters (*12, 15, 16, 19*), alcohols with boiling points higher than that of *n*-hexyl alcohol (*12, 15, 16*), pyrazine derivatives (*12, 15, 16*), and, in certain cases, terpenes (*10, 12, 17*).

The composition of a fusel oil is determined by the nature of the substance fermented, the fermentation conditions (yeast type, temperature, aeration, agitation, sulfur dioxide content), the distillation procedure, and the washing treatment of the fusel oil after removal from the distilling column. The investigation of Schicktanz, Etienne, and Steele (*14*) has demonstrated that the fermentation, distillation, and washing procedures employed by two different distilleries definitely can influence the composition of the fusel oil from a common source material. More recently Jensen and Rinne (*6*) have reported differences not only in composition but also in constituents in fusel oils obtained from fermentation of paper mill sulfite waste liquors from 15 mills in Finland.

This paper reports the compositions of three fusel oils obtained from distillations of wines from three different varieties of grapes (*Vitis vinifera*). Two of the samples, the Thompson Seedless and the Emperor, were obtained from a large winery-distillery located in the San Joaquin Valley in California, and presumably were fermented, distilled, and washed under identical plant operating conditions. The fusel oil sample from Muscat of Alexandria wine was obtained by use of pilot plant scale equipment in these laboratories. An attempt was made to duplicate in the pilot plant as nearly as possible the fermentation and subsequent processing conditions used in the large distillery.
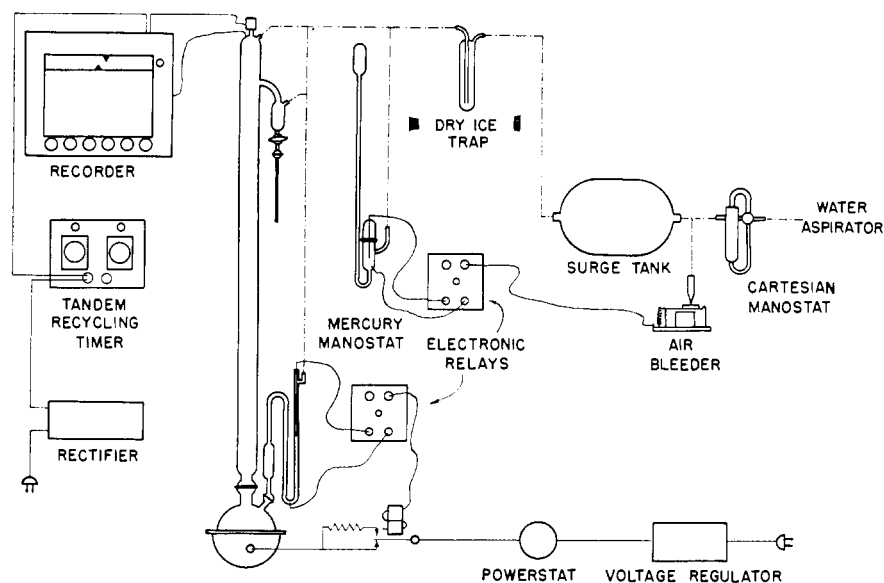
Figure 1. Schematic diagram of distillation apparatus

The fusel oils were analyzed by careful fractional distillation of rigorously dried aliquots of each sample. The composition of each distillation fraction was investigated by determining the physical and chemical properties of each fraction and converting the components into characteristic derivatives which could be identified.

## Apparatus

**Distillation Apparatus** The first laboratory distillation for each of the fusel oils was carried out in a Heli-grid Podbielniak column 48 inches long and 8 mm. in diameter, set up as shown schematically in Figure 1. The distillations were carried out at a constant pressure of 740 mm. of mercury to eliminate the variations in head temperature which would result from changes in atmospheric pressure. The pressure was maintained at 740 mm. of mercury by two competing manostats and a water aspirator. The Cartesian diver manostat was set to maintain the pressure slightly below 740 mm. of mercury, while the mercury manostat operated a bleeder to allow air to enter the system through a capillary tube when the pressure dropped below 740 mm. of mercury. When functioning properly, the bleeder opened several times per minute.

The manometer which measured the pressure drop through the column also regulated the heat input to the boiling pot. Thus, assuming that the viscosity and density of the distilling fluid did not vary excessively, the rate of boil-up was maintained constant. The back-pressure manometer was filled with diethylene glycol, in which was dissolved a small amount of sodium nitrite in order to increase the conductivity sufficiently to activate the electronic relay. It was

found desirable to provide a two-way stopcock which permitted the bleeding in of air at daily intervals on the boiler side of the back-pressure manometer to prevent alcohol vapors from filling the high pressure side of the manometer and dissolving in the manometer fluid.

The head temperature was recorded on a Brown variable span and variable span shift recorder, equipped with control switches which allowed the distillation to be continued without attention as long as the head temperature remained constant. If the head temperature rose above a desired set value, the take-off valve on the fractionating column was automatically closed, thus putting the column on total reflux.

Further fractionation of the samples obtained using the equipment described above was usually accomplished by the use of a Wheeler center-rod column, a micro-Podbielniak column, or an atmospheric pressure Piros-Glover spinning band column.

**Liquid-Liquid Extractor** The liquid-liquid extractor used in the isolation of the Muscat fusel oil was a variation of the Kutscher-Steudel extractor as shown in Figure 2. An open tube, sintered-glass, fractionation column with the equivalent of eight theoretical plates was used in the setup to minimize the recirculation of the extracted fusel oil. The apparatus was also modified by the introduction of a sintered-glass bubbler at the end of the delivery tube to give better dispersion of pentane in the aqueous-alcohol phase.

## Experimental

**Thompson Seedless Fusel Oil** The fusel oil obtained from the distillation of wine made entirely from Thompson Seedless grapes was dried by the following

procedure. Excess solid sodium chloride was added to the fusel oil and the saturated brine solution which separated as a second phase was removed and discarded. The organic phase was dried for 1 month over four successive portions of anhydrous magnesium sulfate.

Then 1201 grams of the dried Thompson Seedless fusel oil were fractionally distilled, after the addition of 157 grams of absolute ethyl alcohol to remove any remaining traces of water as the water–ethyl alcohol azeotrope. Analyses of the initial fractions for the presence of
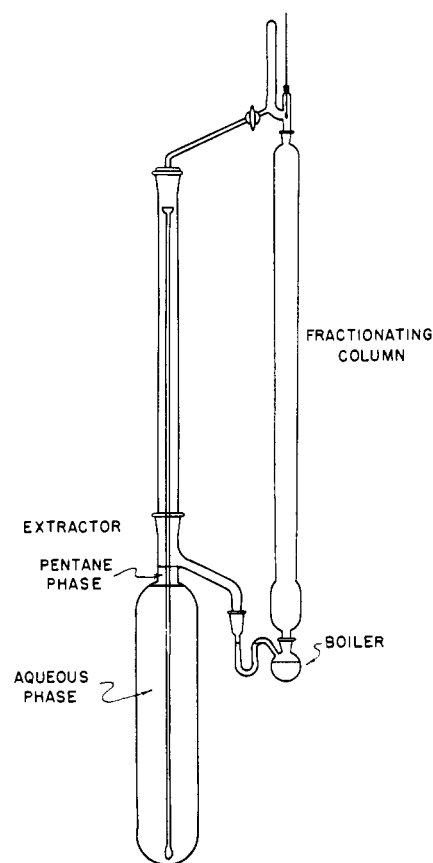


Figure 2. Liquid-liquid extractor used in isolation of Muscat of Alexandria fusel oil

water in ethyl alcohol by the method described by Robertson (13) indicated that the material in the boiler was not completely free of water. An additional 217 grams of absolute ethyl alcohol were required to remove all the residual water. Each fraction was numbered and preceded by the letters TS (Thompson Seedless).

With the collection of fraction TS-25 the head temperature rose erratically and continued to fluctuate through the collection of fraction TS-28. This erratic behavior of the head temperature was believed to be due to the slow decomposition of some high boiling material, possibly some complex tertiary alcohol. To separate the high boiling

## Table I. Properties of Fractions from Thompson Seedless Fusel Oil

| TS Fraction No. | B.P., 740 Mm., °C | Weight, G. | $n_D^{25}$ | $d^{25}$ | $\alpha_{obsd.}$ 2 Dm. | Sap. Eq. |
|---|---|---|---|---|---|---|
| 1–24 | <77.8 | ... | ... | ... | ... | ... |
| 25–8 | See experimental section | | | | | |
| 29 | 75.6–77.5 | 3.47 | 1.3623 | ... | ... | ... |
| 30–32 | 77.5–77.7 | 58.65 | 1.3594 | ... | ... | ... |
| 33 | 77.7–79.5 | 6.12 | 1.3595 | ... | ... | ... |
| 34 | 79.5–96.5 | 2.70 | 1.3785 | ... | ... | 5,100 |
| 35 | 96.5–96.8 | 3.60 | 1.3830 | ... | ... | 5,800 |
| 36 | 96.8–102.4 | 1.22 | 1.3858 | ... | ... | 5,500 |
| 37 | 102.4–106.6 | 1.82 | 1.3913 | ... | ... | 5,900 |
| 38 | 106.6–107.1 | 1.70 | 1.3930 | ... | ... | 6,400 |
| 39 | 107.1–107.2 | 7.86 | 1.3933 | ... | ... | 11,000 |
| 40–43 | 107.2 | 52.19 | 1.3935 | ... | ... | ... |
| 44 | 107.2–108.1 | 3.93 | 1.3935 | ... | ... | ... |
| 45 | 108.1–116.8 | 3.32 | 1.3960 | 0.8049 | ... | 13,000 |
| 46 | 116.8–117.2 | 3.90 | 1.3972 | 0.8062 | 0 | 5,100 |
| 47 | 117.2–127.3 | 4.02 | 1.4017 | ... | −3.65 | 11,000 |
| 48 | 127.3–128.5 | 6.41 | 1.4071 | ... | −8.00 | 6,900 |
| 49 | 128.5–129.0 | 18.42 | 1.4081 | 0.8141 | −8.70 | ... |
| 50 | 129.0 | 19.29 | 1.4082 | 0.8145 | −8.42 | ... |
| 51 | 129.0–129.2 | 16.94 | 1.4088 | 0.8145 | −8.72 | ... |
| 52–76 | 129.5–131.6 | 420.24 | 1.4053 | 0.8079 | −2.43 | ... |
| 77–98 | 131.6 | 376.43 | 1.4047 | ... | ... | ... |
| 99 | 131.6–133.2 | 10.67 | 1.4046 | ... | ... | ... |
| 100 | 133.2–156.2 | 2.54 | 1.4145 | ... | ... | ... |
| 101 | 156.2–158.1 | 3.67 | 1.4180 | ... | ... | ... |
| 102 | 158.1–158.5 | 3.40 | 1.4164 | ... | ... | ... |
| | B.P., Atm. | | | | | |
| 103 | 146.0–148.0 | 2.05 | 1.4101 | ... | ... | ... |
| | B.P., 45 Mm. Hg | | | | | |
| 104 | 78.0–87.0 | 1.29 | 1.4164 | ... | ... | ... |
| 105 | 87.0–90.0 | 2.71 | 1.4181 | ... | ... | ... |
| 106 | Combined with FR TS-105 | | | | | |
| 107 | 90.0–133.0 | 1.16 | ... | ... | ... | ... |
| 108 | Residue | ... | ... | ... | ... | 250 |

material from the remainder of the fusel oil, the material remaining in the boiler was distilled at a pressure of 19 mm. of mercury, 966 grams of distillate boiling up to 130° C. at 19 mm. of mercury and 73.3 grams of residue designated as TS-residue A were obtained.

The fractionation of the Thompson Seedless fusel oil was again started after fractions TS-25 to TS-28 had been added to the 966 grams of distillate obtained above, along with 48.5 grams of absolute ethyl alcohol to remove any moisture that might have been absorbed from the atmosphere. The distillation was continued for 75 days, during which time 102 fractions and a residue, TS-residue B, were collected.

TS-residue A from the first vacuum distillation was redistilled at 10 mm. of mercury to give 65.3 grams of material boiling up to 175° C. and 9.0 grams of residue. The distillate was combined with TS-residue B and fractionated in a Wheeler center-rod column to give five additional fractions, TS-103 to TS-107.

The distillation curve for the fractionation of the Thompson Seedless fusel oil is plotted in Figure 3, and the weight and physical constants for each fraction are listed in Table I.

**Analysis of Fractions.** Fractions containing only one alcohol were identified by their physical properties, melting points of their 3,5-dinitrobenzoates prepared by the pyridine method (19), and mixed melting points with known derivatives. The chromatographic methods of White and Dryden (20) were used to analyze fractions containing a mixture of alcohols whose 3,5-dinitrobenzoates were reported to be separable. The materials isolated from the separated bands were weighed and identified from melting points and mixed melting points with known derivatives. The composition of the fraction was calculated from the weights of material isolated from the separated bands.

Compositions of fractions containing mixtures of alcohols whose 3,5-dinitrobenzoates were not chromatographically separable were calculated from their physical constants, which were assumed to have a linear relationship with concentration. The composition of fractions which contained a mixture of active amyl and isoamyl alcohols were determined from measurements of the optical rotation, because this physical property yielded the most accurate results.

The presence of esters in the various fractions was observed by determining saponification equivalents on 65- to 300-mg. samples by the diethylene glycol method (18). Whenever the saponification equivalent indicated the presence of a sufficient quantity of esters, a larger amount of the fraction was saponified to permit identification of the esters present.

The alcohol and acid portions from the esters were separated and converted to 3,5-dinitrobenzoate and p-phenylphenacyl or p-phenylazophenacyl derivatives, respectively. Chromatographic investigation of the acid derivatives (5, 7) showed the presence of small amounts of acetate esters in fractions TS-35 to TS-38, inclusive. The p-phenylphenacyl derivative melted at 110.5–11.0° C. and the p-phenylazophenacyl derivative at 125–7° C., each melting with no depression when mixed with a known derivative. Chromatographic investigation of the

Figure 3. Distillation curve for Thompson Seedless fusel oil
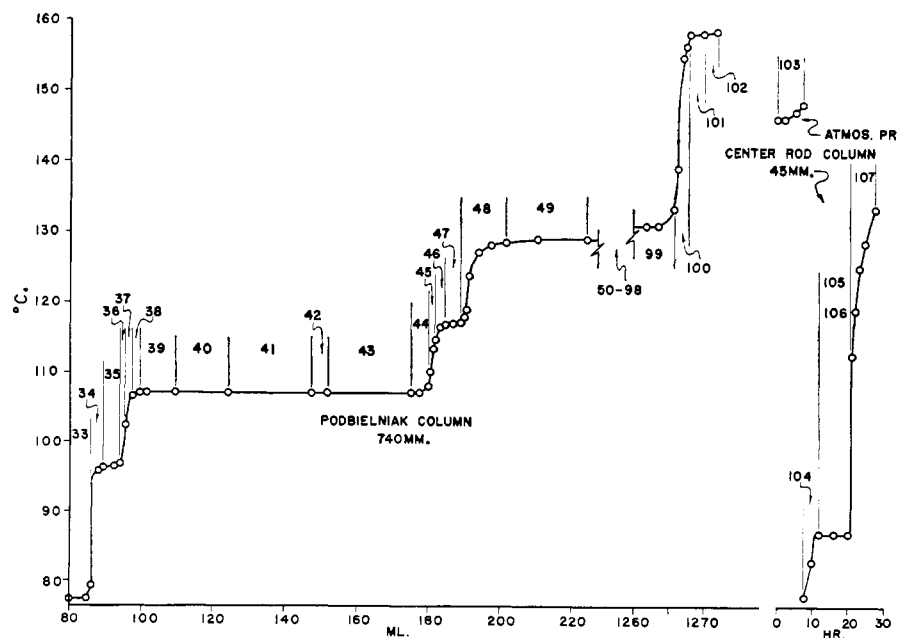
## Table II. Analysis of Thompson Seedless Fusel Oil

| Distilln. Fraction No. | Chromatographic Analyses of 3,5-Dinitrobenzoate Derivatives | | | | Composition of Fractions, Wt. % | | | | | | Calcd. Wt. of Component, G. |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| | Position of band | Material in band, mg. | M.p. of deriv., °C. | Mixed M.P. with 3,5-Dinitrobenzoate | Alcohol constituent of deriv. | Chromatographic | $\alpha$ | $d^{25}$ | $n_D^{25}$ | Value used | |
| TS-1 to 33 | | | | | Ethyl | | | | | | |
| TS-34 | Upper | 5.2 | 89.5–90.5 | Ethyl 91.0–92.0 | Ethyl | 20.8 | | | | 20.8 | |
| | Lower | 16.1 | 65.5–66.0a | | Propyl | 79.2 | | | | 79.2 | 2.14 |
| TS-35 | One band | ... | 66.0–75.0a | Propyl 73.0–74.8 | Propyl | 100 | | | | 100 | 3.60 |
| TS-36 | Upper | 15.2 | 65.7–75.0a | Propyl 73.0–75.1 | Propyl | 71.6 | | | | 71.6 | 0.87 |
| | Lower | 4.8 | 84.5–85.5 | Isobutyl 85.0–87.0 | Isobutyl | 28.4 | | | | 28.4 | 0.35 |
| TS-37 | Upper | 4.0 | 66.0–67.0a | | Propyl | 18.3 | | | | 18.3 | 0.33 |
| | Lower | 15.2 | 85.0–86.5 | Isobutyl 85.5–87.0 | Isobutyl | 81.7 | | | | 81.7 | 1.49 |
| TS-38 | One band | ... | 86.0–87.0 | Isobutyl 86.0–87.0 | Isobutyl | 100 | | | | 100 | 1.70 |
| TS-39 to 43 | | | | | Isobutyl | | | 100 | 100 | 100 | 60.05 |
| TS-44 | One band | ... | 86.2–87.5 | Isobutyl 86.0–87.0 | Isobutyl | 100 | | | | 100 | 3.93 |
| TS-45 | One band | ... | 52.0–57.0b | | Isobutyl | | | 10 | 29 | 19.5 | 0.65 |
| | | | | | n-Butyl | | | 90 | 71 | 80.5 | 2.67 |
| TS-46 | One band | ... | 61.8–64.8 | n-Butyl 62.0–63.8 | n-Butyl | 100 | | | | 100 | 3.90 |
| TS-47 | Upper | 15.3 | 58.0–61.0 | n-Butyl 61.0–63.0 | n-Butyl | 63.6 | 57.4 | | 55.8 | 58.9 | 2.37 |
| | Lower | 7.7 | 79.0–81.0 | Active amyl 79.0–82.0 | Active amyl | 36.4 | 42.6 | | 44.2 | 41.1 | 1.65 |
| TS-48 | Upper | 5.7 | 54.0–63.0 | n-Butyl 55.0–63.0 | n-Butyl | 21.3 | 13.5 | | 12.4 | 15.8 | 1.01 |
| | Lower | 18.6 | 80.0–82.3 | Active amyl 80.5–82.5 | Active amyl | 78.7 | 86.5 | | 87.6 | 84.2 | 5.40 |
| TS-49 | One band | ... | 81.8–83.0 | Active amyl 81.8–82.9 | Active amyl | | 91.5 | | | 91.5 | 16.85 |
| | | | | | Isoamyl | | 8.5 | | | 8.5 | 1.57 |
| TS-50 | | | | | Active amyl | | 88.5 | | | 88.5 | 17.07 |
| | | | | | Isoamyl | | 11.5 | | | 11.5 | 2.22 |
| TS-51 | One band | ... | 80.0–82.3 | Active amyl 81.0–82.5 | Active amyl | | 91.6 | | | 91.6 | 15.51 |
| | | | | | Isoamyl | | 8.4 | | | 8.4 | 1.43 |
| TS-52 to 76 | | | | | Active amyl | | 24.68 | | | 24.68 | 103.7 |
| | | | | | Isoamyl | | 75.32 | | | 75.32 | 316.5 |
| TS-77 to 99 | One band | ... | 63.0–64.2 | Isoamyl 63.5–64.5 | Isoamyl | 100 | | 100 | 100 | 100 | 387.10 |
| TS-100c | | | | Isoamyl | Isoamyl | | | | | 50 | 1.27 |
| | | | | n-Hexyl | n-Hexyl | | | | | 50 | 1.27 |
| TS-101 to 106 | One band | ... | 57.0–58.5 | n-Hexyl 57.0–59.0 | n-Hexyl | 100 | | | | 100 | 13.12 |
| TS-107 | Upper | ... | 106.0–108.0d | Phenyl ethyl 107.0–108.0 | Phenyl ethyl | | | | | Trace | |
| | Middle | | | | | | | | | | |
| | Bottom | ... | 53.0–55.0e | n-Hexyl 55.0–58.5 | n-Hexyl | | | | | 25 | 0.29 |
| | | | | | Residue | | | | | 75 | 0.87 |
| TS-108 plus residue from distillation of TS-residue A | | | | | Residue | | | | | | 75.0 |

a Recrystallized from ethyl alcohol, melting point was raised to 73.0–74.5 ° and showed no depression with n-propyl 3,5-dinitrobenzoate.

b Mixture of n-butyl and isobutyl 3,5-dinitrobenzoate reported to be inseparable (20).

c Break fraction assumed to contain 50% isoamyl and 50% n-hexyl alcohols.

d Carbon-hydrogen analysis: calculated for $C_{15}H_{12}O_6N_2$: C, 56.96; H, 3.83. Found: C, 56.99; H, 3.83.

e From chromatographic evidence this fraction was assumed to contain 25% n-hexyl alcohol and 75% residue with a trace of β-phenyl ethyl alcohol.

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alcohol derivatives showed no additional alcohols besides those that were originally present as free alcohols in the distillation fractions.

The results of analyses of Thompson Seedless fusel oil are given in Table II.

**Emperor Fusel Oil** The sample of fusel oil obtained from the distillation of wine made entirely from Emperor grapes was dried in a manner similar to that used for the Thompson Seedless fusel oil prior to distilling.

Vacuum distillation of 1200 grams of the dried Emperor fusel oil gave 1145 grams of material boiling up to 100° C. at 45 mm. of mercury and 50.8 grams of residue (E-residue A). The distillate, together with 257 grams of absolute ethyl alcohol, was then fractionated in the equipment shown in Figure 1. An additional 271 grams of absolute ethyl alcohol were required to remove all the residual water in the fusel oil. The fractionation was carried out over a period of 44 days, during which time 100 fractions were collected. The 29.1 grams of residue recovered from the boiler was designated as E-residue B.

E-residue A was again vacuum-distilled at a presssure of 8 mm. of mercury to give 28.2 grams of distillate boiling up to 135° C. and 16.5 grams of residue. The distillate was combined with E-residue B and fractionated in the Wheeler center-rod column at 50 mm. of mercury. Eight fractions were collected over a period of 5 days, and 42.5 grams of residue were recovered from the boiler.

The distillation curve for the fractionation of the Emperor fusel oil is plotted in Figure 4, while weights and physical

Figure 4. Distillation curve for Emperor fusel oil
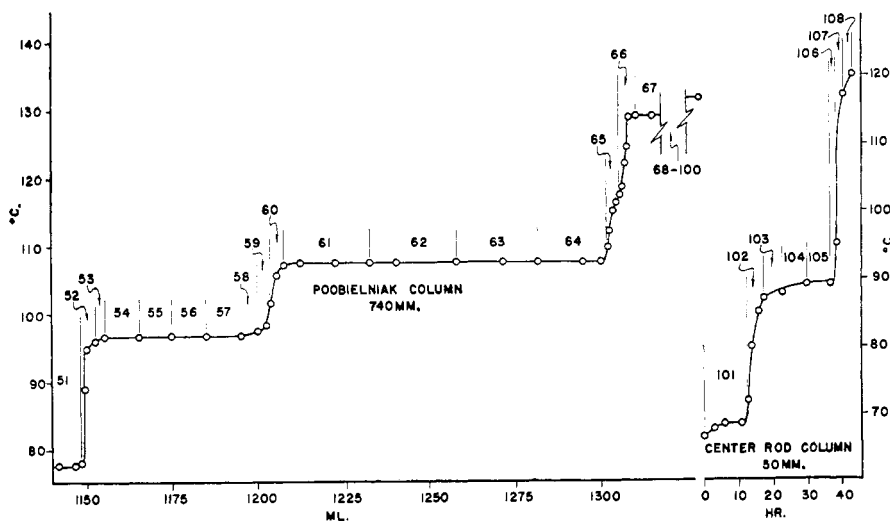
## Table III. Properties of Fractions from Emperor Fusel Oil

| E Fraction No. | B.P., 740 Mm., °C. | Weight, G. | $n_D^{25}$ | $d^{25}$ | $\alpha_{obsd.},$ 2 Dm. | Sap. Eq. |
|---|---|---|---|---|---|---|
| 1–50 | <77.6 | 905.72 | ... | ... | ... | ... |
| 51 | 77.6–78.0 | 11.96 | 1.3952 | ... | ... | ... |
| 52 | 78.0–96.1 | 3.17 | 1.3750 | 0.7950 | ... | 25,000 |
| 53 | 96.1–96.7 | 2.30 | 1.3827 | 0.7998 | ... | 350,000 |
| 54–57 | 96.7–96.8 | 31.89 | 1.3830 | 0.7997 | − 0.02 | ... |
| 58 | 96.8–99.5 | 3.85 | 1.3845 | 0.8006 | − 2.43 | 70,000 |
| 59 | 99.5–101.2 | 2.99 | 1.3895 | 0.8014 | −10.20 | 57,000 |
| 60 | 101.2–107.2 | 3.35 | 1.3930 | 0.7995 | − 4.50 | 35,000 |
| 61 | 107.2–107.5 | 19.91 | 1.3936 | 0.7980 | − 0.03 | 35,000 |
| 62–63 | 107.5–107.6 | 38.90 | 1.3936 | ... | ... | ... |
| 64 | 107.6–109.7 | 16.36 | 1.3936 | 0.7980 | ... | 18,000 |
| 65 | 109.7–117.4 | 3.09 | 1.3963 | 0.8045 | − 0.06 | 17,000 |
| 66 | 117.4–128.3 | 4.31 | 1.4040 | 0.8119 | − 5.77 | 15,000 |
| 67 | 128.3–129.1 | 12.63 | 1.4080 | 0.8142 | − 8.59 | 12,000 |
| 68–94 | 129.1–131.6 | 478.77 | 1.4053 | 0.8064 | − 2.01 | ... |
| 95–100 | 131.6 | 103.65 | 1.4047 | ... | 0 | ... |
| | **B.P., 50 Mm. Hg.** | | | | | |
| 101 | 66.5–72.0 | 3.37 | 1.4053 | ... | ... | ... |
| 102 | 72.0–87.0 | 1.24 | 1.4140 | ... | ... | ... |
| 103 | 87.0–87.8 | 1.73 | 1.4180 | ... | ... | ... |
| 104 | 87.8–89.0 | 3.43 | 1.4171 | ... | ... | ... |
| 105 | 89.0 | 1.86 | 1.4163 | ... | ... | ... |
| 106 | 89.0–103.0 | 0.47 | 1.4164 | ... | ... | ... |
| 107 | 103.0–120.0 | 1.42 | 1.4222 | ... | ... | ... |
| 108 | 120.0 | 0.93 | 1.4185 | ... | ... | ... |
| 109 | Residue | 42.50 | ... | ... | ... | 229 |

properties for the distilled fractions are recorded in Table III. These fractions were analyzed by the same procedures as the Thompson Seedless fusel oil. The results are given in Table IV.

**Muscat Fusel Oil** Three tons of Muscat of Alexandria grapes from the experimental plot of the University of California at Davis were fermented to wine. The wine was then distilled in the 24-plate experimental still, 12 inches in diameter, of the Viticulture and Enology Department to separate the fusel oil fraction. During distillation the accumulating fusel oil was slowly withdrawn from the column along with a considerable quantity of ethyl alcohol. As the fusel oil fraction thus recovered did not separate into two phases, the material was diluted with water to 50% alcohol content and extracted with $n$-pentane for 53 days in the continuous extractor shown in Figure 2. During this time the $n$-pentane in the boiler was changed three times.

Analysis of the residual aqueous ethyl alcohol solution after pentane extraction, by the method described by Guymon and Nakagiri ([4]), showed that approximately 40 grams of fusel oil remained unextracted. Most of this resid-

## Table IV. Analysis of Emperor Fusel Oil

| Distilln. Fraction No. | Chromatographic Analyses of 3,5-Dinitrobenzoate Derivatives | | | | | Composition of Fractions, Wt. % | | | | | | Calcd. Wt. of Component, G. |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Position of band | Material in band, mg. | M.p. of deriv., °C. | Mixed M.P. with 3,5-Dinitrobenzoate | | Alcohol constituent of deriv. | Chromatographic | $\alpha$ | $d^{25}$ | $n_D^{25}$ | Value used | |
| E-1 to 51 | | | | | | Ethyl | | | | | | |
| E-52 | Upper | 11.2 | 92.5–93.0 | Ethyl | 92.5–93.5 | Ethyl | 35.7 | | | | 35.7 | |
| | Lower | 16.4 | 72.2–74.0 | Propyl | 73.5–74.5 | Propyl | 64.3 | | | | 64.3 | 2.04 |
| E-53 | One band | | 65.6–66.0[a] | | | Propyl | 100 | | 100 | 100 | 100 | 2.30 |
| E-54 to 57 | | | | | | Propyl | | | 100 | 100 | 100 | 31.89 |
| E-58 | Upper | 26.7 | 72.5–74.0 | Propyl | 73.0–74.5 | Propyl | 88.2 | | | | 88.2 | 3.39 |
| | Lower | 3.2 | 87.0–89.0[b] | | | ( − )sec-Butyl | 11.8 | | | | 11.8 | 0.46 |
| E-59 | Upper | 15.3 | 64.0–66.0[a] | | | Propyl | 45.5 | | | | 45.5 | 1.36 |
| | Lower | 15.7 | 86.0–88.0[b] | | | ( − )sec-Butyl | 54.5 | | | | 54.5 | 1.63 |
| E-60 | One band[c] | | | | | ( − )sec-Butyl | | 34 | | | 34 | 1.14 |
| | | | | | | Isobutyl | | 66 | | | 66 | 2.21 |
| E-61 | | | 85.5–87.0[d] | Isobutyl | 85.7–86.8 | Isobutyl | 100 | | 100 | 100 | 100 | 19.91 |
| E-62 to 63 | | | 86.0–86.7[d] | Isobutyl | 85.8–86.2 | Isobutyl | | | | 100 | 100 | 38.90 |
| E-64 | | | 86.0–86.7[d] | Isobutyl | 85.8–86.2 | Isobutyl | 100 | | 100 | 100 | 100 | 16.36 |
| E-65 | One band | | | | | Isobutyl | | 84.5 | | 80 | 82.2 | 2.54 |
| | | | | | | $n$-Butyl | | 15.5 | | 20 | 17.8 | 0.55 |
| E-66 | Upper | 15.3 | 61.0–63.0 | $n$-Butyl | 60.5–64.0 | $n$-Butyl | 48.6 | 35.2 | | | 41.9 | 1.81 |
| | Lower | 14.3 | 80.0–82.8 | Active amyl | 82.0–84.8 | Active amyl | 51.4 | 64.8 | | | 58.1 | 2.50 |
| E-67 | | | | | | Active amyl | | 90.0 | | | 90.0 | 11.37 |
| | | | | | | Isoamyl | | 10.0 | | | 10.0 | 1.26 |
| E-68 to 94 | | | | | | Active amyl | | 20.43 | | | 20.43 | 97.8 |
| | | | | | | Isoamyl | | 79.57 | | | 79.57 | 381.0 |
| E-95 to 100 | | | | | | Isoamyl | | | | 100 | 100 | 103.65 |
| E-101 | One band | | 62.5–63.7 | Isoamyl | 62.5–63.6 | Isoamyl | 100 | | | | 100 | 3.37 |
| E-102 | Upper ¼ | | 53.0–57.0 | Isoamyl | 53.0–61.0 | Isoamyl | 25 | | | | 25 | 0.31 |
| | Lower ¾ | | 56.0–57.5 | Hexyl | 56.5–57.5 | Hexyl | 75 | | | | 75 | 0.93 |
| E-103 to 106 | | | 57.5–58.5[d] | Hexyl | 57.0–58.5 | Hexyl | 100 | | | | 100 | 7.49 |
| E-107 | Upper | | | | | | | | | | | |
| | Middle | | | | | }Residue | 50 | | | | 50 | 0.71 |
| | Lower | | 55.5–57.0[e] | Hexyl | 55.5–58.0 | Hexyl | 50 | | | | 50 | 0.71 |
| E 108 | 3 bands | | | Not identified | | Residue | | | | | | 0.93 |
| E 109 plus residue from distillation of E residue A | | | | | | Residue | | | | | | 59.05 |

[a] Recrystallized from ethyl alcohol, melting point increased to 73.0–74.5° and showed no depression with $n$-propyl 3,5-dinitrobenzoate.

[b] Further evidence for presence of sec-butyl alcohol is shown by optical rotation of these fractions listed in Table IV.

[c] As mixture of isobutyl and sec-butyl 3,5-dinitrobenzoates is reported inseparable ([20]), composition of this fraction was calculated from density.

[d] Melting points for 3,5-dinitrobenzoates prepared from distillation fraction without subsequent chromatographing.

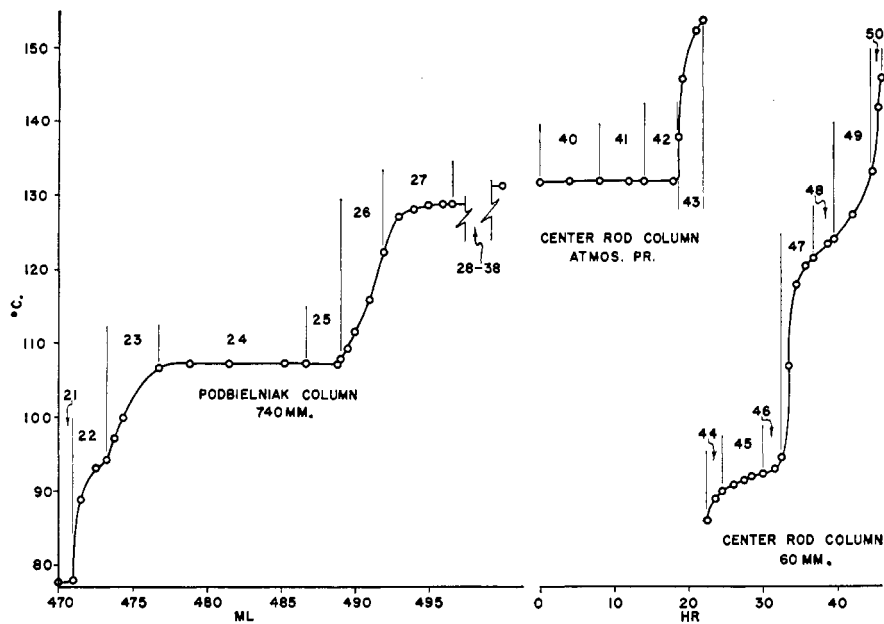[e] From chromatographic evidence this fraction was assumed to be 50% $n$-hexyl alcohol and 50% residue.

Figure 5. Distillation curve for Muscat fusel oil

Figure 6. Redistillation of FR.M-26 + 27 and FR.M-43 to -45 on spinning band column ▶
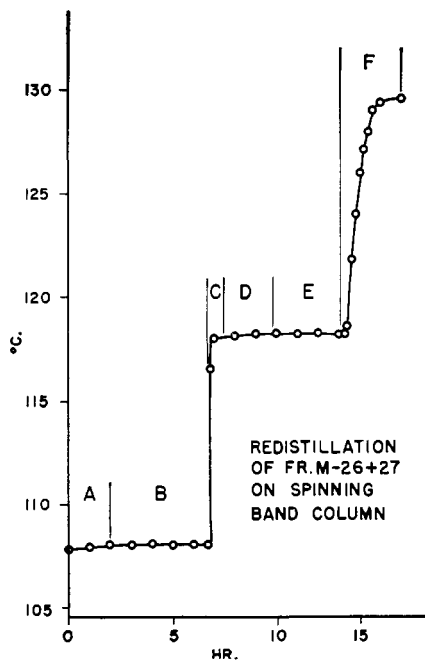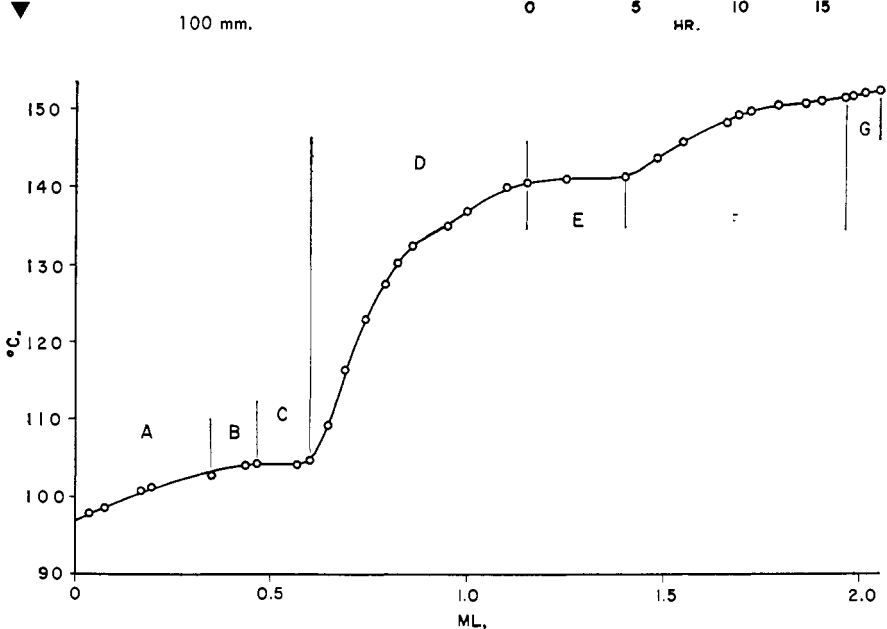
Figure 7. Redistillation of M-47 to M-50 on spinning band column ▼

ual fusel oil was recovered by distilling the extracted residue in a 1-inch diameter 40-plate continuous Oldershaw column and allowing the fusel oil to accumulate in the column. The distillate was kept as high in proof as possible in order to minimize the loss of fusel oil at the head, and the feed and take-off rates were adjusted to maintain the fusel oil below the 25th plate. As the fusel oil separated into a second phase inside the distilling column, its position could be observed through the unsilvered slit in the distilling column. When the last of the 50% ethyl alcohol solution containing the fusel oil had been fed into the distilling column, the feed was changed to water, which caused the fusel oil within the column to move upward as the water azeotropes. When the phase containing the fusel oil reached the take-off point, the distillate receiver was changed, permitting collection of a fusel oil-rich
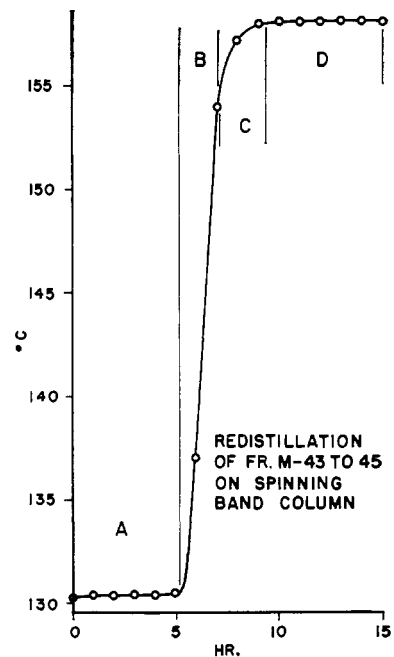
fraction which separated into two phases. The aqueous phase was saturated with sodium chloride, the two phases were separated, and the aqueous phase was extracted with pentane to recover the last traces of fusel oil.

The pentane extracts from the above extraction and the continuous extractions and the fusel oil phase were combined and dried over anhydrous magnesium sulfate. After the drying agent was removed by filtration, essentially all the pentane was distilled out using a micro-Podbielniak column to give 289 grams of Muscat fusel oil. This material was then vacuum-distilled to give 268.2 grams of distillate boiling up to 101° C. at 33 mm. of mercury and 6.2 grams of residue (M-residue A). The 14.6 grams of material that were lost were probably pentane, as some material in the liquid

oxygen trap volatilized when warmed slightly above room temperature. To the 268.2 grams of material obtained above were added 342.3 grams of absolute ethyl alcohol, and the mixture was fractioned in the equipment shown in Figure 1. The fractional distillation was continued for 22 days, during which time 38 fractions and 13.7 grams of residue (M-39) were obtained. Fractions M-40 to M-43 were obtained when residue M-39 was fractionated further in the Wheeler center-rod column at atmospheric pressure. After M-residue A had been added to the boiler, the fractionation was continued at 60 mm. of mercury to give fractions M-44 to M-50.

A better separation of *n*-butyl alcohol from isobutyl and active amyl alcohols was obtained by combining and redistilling fractions M-26 and M-27 through the atmospheric pressure Piros-Glover spinning band column. Fractions M-27A to M-27F and a residue (M-27G) were collected.

Fractions M-43, M-44, and M-45 were combined and refractionated through the atmospheric pressure Piros-Glover spinning band column to obtain a cleaner separation of isoamyl from *n*-hexyl alcohol and to check for the presence of *n*-amyl alcohol. Four fractions (M-45A to M-45D) and a residue (M-45E) were collected. As the distillation curve in Figure 5 shows no break at the boiling point of *n*-amyl alcohol and fraction 45-B gave no chemical evidence of the presence of *n*-amyl alcohol, this alcohol must be considered to be absent.

Fractions M-47 to M-50 were also combined and refractionated through the reduced pressure Piros-Glover spinning band column. During the distillation seven fractions (M-50A to M-50G) and a residue (M-50H) were obtained.

The saponification equivalents of fractions M-50D to M-50F, inclusive, indicated the presence of large amounts of esters. Investigation of these esters by the methods described established that they were ethyl caproate and ethyl caprylate.

The distillation curves for the fractionation and refractionations of the Muscat of Alexandria fusel oil are plotted in Figures 5, 6, and 7, and the data for the fractions are recorded in Table V. The results of the analysis of the Muscat fusel oil are given in Table VI.

### Results and Discussions

The results of the analyses of the Thompson Seedless, Emperor, and Muscat of Alexandria fusel oils are summarized in Table VII. The compositions are listed in weight per cent on an ethyl alcohol- and water-free basis. Also included for comparison is the com-

### Table V. Properties of Fractions from Muscat of Alexandria Fusel Oil

| M Fraction No. | B.P., 740 Mm., °C. | Weight, G. | $n_D^{25}$ | $d^{25}$ | $\alpha_{obsd.}$, 2 Dm. | Sap. Eq. | |
|---|---|---|---|---|---|---|---|
| 1–20 | <77.8 | 349.59 | | | | | |
| 21 | 77.8–78.0 | 16.65 | 1.3595 | | | | |
| 22 | 78.0–96.2 | 1.74 | 1.3741 | | | 44,000 | |
| 23 | 96.2–106.8 | 2.86 | 1.3883 | | 0 | >100,000 | |
| 24 | 106.8–107.3 | 7.89 | 1.3936 | 0.7977 | | 41,000 | |
| 25 | 107.3–107.4 | 1.82 | 1.3939 | | | >100,000 | |
| 26 | 107.4–124.2 | 2.43 | 1.3970 | 0.8035 | | >100,000 | combined and |
| 27 | 124.2–128.9 | 3.79 | 1.4072 | 0.8142 | −8.40 | 35,000 | refractionated |
| 28–33 | 128.9–131.6 | 108.27 | 1.4060 | 0.8076 | −2.85 | | |
| 34–38 | 131.6 | 84.96 | 1.4047 | | 0 | | |
| 39 | Residue | 13.69 | | | | | |
| B.P., Atmos. Press. | | | | | | | |
| 40–42 | 132.0 | 6.76 | 1.4047 | | | | |
| 43 | 132.0–154.0 | 0.71 | 1.4101 | | | | combined and refractionated |
| B.P., 60 Mm. Hg | | | | | | | |
| 44 | <86.0 | 0.34 | 1.4141 | | | | |
| 45 | 86.0–92.5 | 1.79 | 1.4178 | | | | |
| 46 | 92.5–94.0 | 0.51 | 1.4178 | | | | |
| 47 | 94.0–121.5 | 0.97 | 1.4338 | | | 1,790 | |
| 48 | 121.5–124.0 | 0.46 | 1.4482 | | | | combined and |
| 49 | 124.0–132.0 | 1.04 | 1.4468 | | | 360 | refractionated |
| 50 | 132.0–146.0 | 0.44 | 1.4512 | | | | |
| 51 | Residue | 6.85 | | | | 273 | |

Refractionation of M-26 + M-27

| M-27 | B.P., Atmos. Press. | | | | |
|---|---|---|---|---|---|
| A | 107.5–108.1 | 0.09 | 1.3935 | |
| B | 108.1–117.0 | 0.66 | 1.3932 | |
| C | 117.0–118.1 | 0.19 | 1.3960 | |
| D | 118.1–118.3 | 0.29 | 1.3975 | |
| E | 118.3 | 0.53 | 1.3972 | |
| F | 118.3–129.6 | 0.29 | 1.4009 | −2.92 |
| G | Residue | 2.70 | | −9.30 |

Refractionation of M-43 to M-45

| M-45 | B.P., Atmos. Press. | | | |
|---|---|---|---|---|
| A | 131.0–133.0 | 0.50 | 1.4023 |
| B | 133.0–156.0 | 0.24 | 1.4105 |
| C | 156.0–158.1 | 0.41 | 1.4225 |
| D | 158.1 | 0.60 | 1.4190 |
| E | Residue | 0.79 | |

Refractionation of M-47 to M-50

| M-50 | B.P., 100 Mm. Hg | | | |
|---|---|---|---|---|
| A | 96.4–102.8 | 0.29 | 1.4500 | |
| B | 102.8–104.4 | 0.11 | 1.4425 | |
| C | 104.4–104.8 | 0.09 | 1.4282 | |
| D | 104.8–140.8 | 0.47 | 1.4411 | 612 |
| E | 140.8–141.6 | 0.23 | 1.4273 | 287 |
| F | 141.6–152.1 | 0.47 | 1.4455 | 269 |
| G | 152.1–153.0 | 0.13 | 1.4524 | |
| H | Residue | 0.43 | | |

position of a mixed varietal grape fusel oil, previously reported from these laboratories (*19*). In the present investigation the three fusel oils were analyzed rigorously for all constituents with boiling points up to and including that of *n*-hexyl alcohol. The small amount of high boiling residue in each case was not investigated, except to show by the determination of saponification equivalents that the material consisted almost entirely of esters. In the previous investigation of the mixed varietal grape fusel oil (*19*) this high boiling ester residue was rigorously analyzed. The alcohol portions of the esters were essentially those alcohols which were most abundant in the free state in the fusel oil. This suggested the probable formation

of these high boiling esters in the brandy rectifying column. Consequently, in the present investigation a detailed analysis of the ester residue portion of each of the fusel oils was not considered likely to furnish any appreciable amount of information relative to possible varietal differences in the fusel oil compositions.

The compositions of fusel oils may be expected to be determined by the four factors mentioned above. In the work reported here the Thompson Seedless and the Emperor fusel oils were obtained from the same distillery and were presumably fermented, distilled, and washed under identical plant operating conditions, so variations in composition were expected to reflect differences due to the grape varieties used as starting materials.

## Table VI. Analysis of Muscat Fusel Oil

| Distilln. Fraction No. | Position of band | Material in band, mg. | M.P. of deriv., °C. | Mixed M.P. with 3,5-Dinitrobenzoate | | Alcohol constituent of deriv. | Chromatographic | α | $d^{25}$ | $n_D^{25}$ | Value used | Calcd. Wt. of Component, G. |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| M-1 to 21[a] | | | | | | Ethyl | | | | | | |
| M-22 | Upper | 18.4 | 90.0–91.0 | Ethyl | 90.0–92.5 | Ethyl | 43.0 | | | | 43.0 | |
| | Lower | 19.8 | 65.5–66.5[b] | | | n-Propyl | 57.0 | | | | 57.0 | 0.99 |
| M-23 | Upper | 22.2 | 69.7–70.5 | n-Propyl | 70.0–71.0 | n-Propyl | 52.6 | | | | 52.6 | 1.50 |
| | Lower | 17.1 | 86.2–87.2 | Isobutyl | 85.5–86.0 | Isobutyl | 47.4 | | | | 47.4 | 1.36 |
| M-24 | | | 86.5–87.0[c] | Isobutyl | 86.5–87.5 | Isobutyl | | | 100 | 100 | 100 | 7.89 |
| M-25 | | | 85.8–87.0 | Isobutyl | 86.0–87.0 | Isobutyl | | | | 100 | 100 | 1.82 |
| M-26 and 27[d] | | | | | | Isobutyl | | | | | 16.8 | 1.04 |
| | | | | | | n-Butyl | | | | | 24.2 | 1.51 |
| | | | | | | Active amyl | | | | | 58.9 | 3.66 |
| M-28 to 33 | | | | | | Active amyl | 28.92 | | | | 28.92 | 31.31 |
| | | | | | | Isoamyl | 71.08 | | | | 71.08 | 76.96 |
| M-34 to 42 (less M-39) | | | 61.0–62.7[c] | Isoamyl | 62.0–63.5 | Isoamyl | | | | 100 | 100 | 84.96 |
| M-43 to 45[d] | | | | | | Isoamyl | | | | | 24.4 | 0.69 |
| | | | | | | n-Hexyl | | | | | 75.6 | 2.15 |
| M-46 | One band | | 58.0–59.0 | n-Hexyl | 58.5–59.6 | n-Hexyl | 100 | | | | 100 | 0.51 |
| M-47 to 50[d] | | | | | | n-Hexyl | | | | | 27.5 | 0.80 |
| | | | | | | Residue | | | | | 72.5 | 2.11 |
| | | | | | | Phenylethyl | | | | | Traces | |
| M-51 | | | | | | Residue | | | | | 100 | 6.85 |
| M-27A, B | | | | | | Isobutyl | | | | 100 | 100 | 0.75 |
| M-27C | | | | | | Isobutyl | | | | 28 | 28 | 0.05 |
| | | | | | | n-Butyl | | | | 72 | 72 | 0.14 |
| M-27D and E | One band | | 61.0–62.0 | n-Butyl | 63.2–64.7 | n-Butyl | 100 | | | | 100 | 0.82 |
| M-27F | | | | | | n-Butyl | | | 65.5 | 63.0 | 64.3 | 0.19 |
| | | | | | | Active amyl | | | 34.5 | 37.0 | 35.7 | 0.10 |
| M-27G | | | | | | Active amyl | | 100 | | | 100 | 2.70 |
| M-45A | One band | | 61.5–63.5 | Isoamyl | 62.0–64.0 | Isoamyl | 100 | | | | 100 | 0.50 |
| M-45B[e] | | | | | | Isoamyl | | | | | 50 | 0.12 |
| | | | | | | n-Hexyl | | | | | 50 | 0.12 |
| M-45C to E | One band | | 55.0–56.0 | n-Hexyl | 54.5–57.5 | n-Hexyl | 100 | | | | 100 | 1.80 |
| M-50A to C | One band | | 57.0–58.0 | n-Hexyl | 57.0–58.5 | n-Hexyl | 100 | | | | 100 | 0.49 |
| M-50D | Lower[f] | | 53.0–56.0 | n-Hexyl | 54.5–57.5 | n-Hexyl | | | | | 25 | 0.12 |
| | | | | | | Residue | | | | | 75 | 0.35 |
| M-50E | Two bands | | Oils | | | Residue | | | | | | |
| M-50F | Upper | | 106.5–108.0 | β-Phenyl-ethyl | 107.0–108.0 | Residue | | | | | Traces | 1.26 |
| | Middle | | Oil | | | Residue | | | | | | |
| | Lower | | Oil | | | Residue | | | | | | |
| M-50G | | | | | | Residue | | | | | | |
| M-50H | Residue | | | | | Residue | | | | | | |

[a] Included approximately 21 grams of extraction solvent, n-pentane, with ethyl alcohol.

[b] When recrystallized from ethyl alcohol melting points were raised to 73.0–74.5° and showed no depression with n-propyl 3,5-dinitrobenzoate.

[c] For 3,5-dinitrobenzoate prepared from fraction without chromatography.

[d] Fractions combined and refractionated. Compositions listed are composites obtained from analyses of fractions from redistillations. See analyses of these redistillation fractions in table.

[e] Fraction assumed to be 50% isoamyl alcohol and 50% hexyl alcohol.

[f] Fraction assumed to be 25% n-hexyl alcohol and 75% residue.

The relative amounts of ethyl alcohol in the dried Emperor and Thompson Seedless fusel oils, 32.5 and 10.5 weight %, respectively, indicated that there was considerably less efficient washing out of the ethyl alcohol in the Emperor fusel oil. Those fusel oils which had been most thoroughly washed with water, as indicated by the relatively lower ethyl alcohol contents, would also be expected to be lower in propyl and butyl alcohol contents, because of the water solubilities of these alcohols. Examination of the data in Table VII indicates that this may be the case, as smaller concentrations of n-propyl, sec-butyl, and isobutyl alcohols are found in the Thompson Seedless fusel oil than in the Emperor fusel oil. The n-butyl alcohol content of the Thompson Seedless fusel

oil is, however, approximately twice that of the Emperor fusel oil. Consequently, it is reasonable to conclude that the Thompson Seedless fusel oil, before the washing process, contained a relatively greater concentration of n-butyl alcohol than the Emperor fusel oil.

The dried Muscat of Alexandria fusel oil contained only a very small amount of ethyl alcohol, 1.3 weight %, because of the method of isolation which was necessary. As the fermentation and distillation were carried out on a relatively small amount of starting material, the fusel oil would not separate as a separate phase in the 12-inch-diameter experimental still and had to be isolated by a sequence of further distillations and extractions with n-pentane. Trial experiments under the conditions used had shown that n-pentane

would extract only very small amounts of ethyl alcohol. The relatively smaller amounts of n-propyl and isobutyl alcohols in the Muscat fusel oil as contrasted to the Emperor fusel oil thus might also be explained as due to incomplete extraction of these low molecular weight alcohols by the n-pentane. The complete absence of sec-butyl alcohol in the muscat fusel oil is undoubtedly correct, however, as the pentane extraction isolated reasonable amounts of the other low boiling alcohols and thus should have extracted at least a small amount of sec-butyl alcohol if it were present. On the other hand, the complete absence of sec-butyl alcohol in the Thompson Seedless fusel oil is not so certain. If the relatively lower amounts of n-propyl and isobutyl alcohols in the Thompson Seedless fusel oil

than in the Emperor fusel oil are actually the result of the more thorough water-washing to which the Thompson Seedless fusel oil was subjected, it is conceivable that any sec-butyl alcohol might have been completely removed from the Thompson Seedless fusel oil during washing.

The somewhat greater amounts of active amyl and isoamyl alcohols in the Muscat and Thompson Seedless fusel oils, as contrasted to the Emperor fusel oil, may also be an indirect result of the compositions due to differences in varieties of grapes fermented or to differing fermentation conditions do occur.

There is no significant variation in the amounts of n-hexyl alcohol in the four fusel oils, particularly when the varying degree of the water-washing is considered. The occurrence of acetate esters in the low boiling fractions of only the Thompson Seedless fusel oil is believed to be of significance. The caproate and caprylate esters identified in the Muscat fusel oil are not of significance, common fusel oil alcohols, if formed according to the Ehrlich mechanism, would require amino acids not known to occur naturally, serves to emphasize once again that the over-all formation of fusel oils is considerably more complex than the mechanism originally proposed by Ehrlich.

## Acknowledgment

## Table VII. Compositions of Fusel Oils

| Component | Thompson Seedless, Wt. % | Emperor, Wt. % | Muscat of Alexandria, Wt. % | Mixed Varietal (19), Wt. % |
|---|---|---|---|---|
| n-Propyl alcohol | 0.66 | 5.14 | 1.07 | 4.1 |
| (−)sec-Butyl alcohol | 0.00[a] | 0.40 | 0.00 | 4.9 |
| Isobutyl alcohol | 6.52 | 9.77 | 5.20 | 18.3 |
| n-Butyl alcohol | 0.95 | 0.55 | 0.65 | 1.9 |
| Active amyl alcohol | 15.31 | 14.00 | 15.02 | 9.6 |
| Isoamyl alcohol | 67.89 | 61.38 | 72.73 | 54.0 |
| n-Hexyl alcohol | 1.40 | 1.14 | 1.49 | 1.50 |
| β-Phenyl ethyl alcohol | Trace | [b] | Trace | 0.00 |
| Acetate ester | Trace | 0.00 | 0.00 | ... |
| Residue[c] | 7.25 | 7.61 | 3.85 | 5.6 |

[a] May have been removed during washing process.
[b] Distillation discontinued below boiling point of β-phenyl ethyl alcohol.
[c] Residue consists mainly of esters.

treatment of the first two fusel oils, which possibly led to the isolation of smaller amounts of the lower boiling alcohols in each case. The relative amounts of isoamyl to active amyl alcohol isolated from the Thompson Seedless, Emperor, Muscat of Alexandria, and mixed varietal fusel oils were 4.43, 4.38, 4.84, and 5.83 to 1, respectively. The relative amounts were essentially constant in the case of the Thompson Seedless and Emperor fusel oils, while definitely different ratios were observed from the other two fusel oils. As these first two fusel oils were obtained from the same distillery, where the fermentation conditions were presumably identical, it is difficult to say definitely whether the differences in the last two actually reflect varietal differences or are the result of fermentation differences. It is not likely that the extent of the water washing would appreciably affect the relative amounts of the isoamyl and active amyl alcohols. The relatively high ratio of isoamyl to active amyl alcohol in the mixed varietal fusel oil, which contained 5.7 weight % ethyl alcohol in the dried material, may possibly be the result of different fermentation conditions, but it may also be an indication that definite varietal differences exist. The relatively higher amounts of the three- and four-carbon alcohols in the mixed varietal fusel oil, which was the most thoroughly water-washed of the fusel oils investigated, also indicate that differences in however, as they occurred only in the highest boiling fractions which were investigated and should actually be considered as part of the high boiling ester residue.

The lack of β-phenyl ethyl alcohol in the Emperor fusel oil is also not significant since the distillation was discontinued in this case at a temperature below the boiling point of β-phenyl ethyl alcohol. It is possible that this alcohol might have been found in the Emperor fusel oil if the distillation had been continued to a sufficiently high temperature. However, a rigorous analysis of all the high boiling material from the mixed varietal fusel oil failed to show the presence of β-phenyl ethyl alcohol, which leads to the conclusion that the presence or absence of this alcohol may be a varietal factor. According to the scheme for the formation of fusel oil alcohols established by Ehrlich (3), β-phenyl ethyl alcohol would be derived from phenylalanine. The amino acid content of Thompson Seedless grapes grown in the experimental plots of the University of California at Davis during the same season the Thompson Seedless fusel oil was isolated has been reported (7). A comparison of the relative amounts of valine, isoleucine, leucine, and phenylalanine and the isobutyl, active amyl, isoamyl, and β-phenyl ethyl alcohols which would be formed by the Ehrlich mechanism has shown little or no correlation. This and the fact that several of the

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