GAS CHROMATOGRAPHIC DETERMINATION OF ETHYL ESTERS OF FATTY ACIDS IN BRANDY OR WINE DISTILLATES

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ABSTRACT.

The ethyl esters of fatty acids containing even numbers of carbon atoms, particularly caprylic, capric, caprylic, lauric, myristic, and palmitic acids, are characteristic components of distilled alcoholic beverages such as brandy, rum, and whisky. The most abundant esters in brandy are usually those of caprylic (C8), capric (C10), and lauric (C12) acids. A method was developed for quantitative determination of these three esters by gas chromatography. Sample preparation consisted of taking 100 ml of sample, diluting with water to 20 vol % alcohol, extracting four times with methylene chloride, and removing solvent from the combined extracts. Two microliters of a known volume [5 ml] of the concentrated ester extract, with ethyl pellargonic acid as an internal standard, was chromatographed on a 6-ft x 1/8-inch FFAP column programmed from 100 to 225°C at 7.5°C per minute. Ratios of individual esters to pellargonic peak height were compared with those for known standard ester solutions for quantitative determinations.

Typical continuous-still beverage brandies distilled from fresh wines with suspended yeast cells contained 2 to 3 mg of caprylic, 5 to 10 mg of capric, and 5 to 8 mg of lauric per 100 ml. Comparable distillates obtained by continuous low-temperature (vacuum) distillation or by simple batch distillation contained less of these fatty acid esters in brandies. The levels of these fatty acid esters in brandies were markedly reduced when yeast cells were removed from the wine prior to distillation. The formation of esters by yeasts during fermentation, possible distillation variables affecting the concentrations, and their probable significance in brandy are discussed.

Gas chromatography, coupled with increasingly sensitive detection systems, has permitted many reports of the aroma compounds contained in alcoholic beverages and in the raw materials from which they are derived. Fatty acid esters have been identified in rum (11, 18), bourbon and Scotch whiskies (5, 6, 11, 12), beer (7, 9), brandy and cognac (1, 3, 13), and grape and berry wines (14, 19). Recent reviews of the aroma compounds in beer and wine have been published by Lawrence (8), Stevens (17), Suomalainen (16), and Webb (19).

The same predominant fatty acid esters found in alcoholic beverages derived from grain are, in general, the major esters found in wines and in sugar-based substrates. Although nearly all of the reported studies of aroma compounds in alcoholic beverages have contained only qualitative results of composition, the relative proportions of the major components are readily apparent from the peak sizes of published gas chromatograms. The striking feature observed is the similarity of the ester composition of various alcoholic beverages, in spite of the dissimilarity of raw materials used and the easily recognizable aroma differences.

Nordström studied various aspects of the formation of ethyl acetate and other fatty acid esters by yeasts and has reviewed (10) his series of eleven reports published between 1961 and 1964 in the Journal of the Institute of Brewing. Esters are formed primarily as a part of the bio- synthetic process, and their formation requires the activation of the fatty acid moiety of acyl-CoA compounds, which then combine with alcohols of the medium, of which, of course, ethyl alcohol predominates. The formation of esters does not appear to result from direct esterification reactions between alcohol and free acids of the fermenting medium. Consequently, the levels of esters may be decreased by means of the chemical equilibrium for corresponding alcohol and acid. It is well known that the even-numbered fatty acids predominated in natural products, apparently because the acid precursors are synthesized by successive couplings of the acyl-CoA ester with acetyl-CoA (a C2 addition to the molecule).

Although the ester composition of a beverage would include any esters in the raw material not subsequently destroyed in processing as by heat, the evidence is clear that yeast growth is the primary source of ester formation. This fact accounts for the general similarity of ester patterns in gas chromatographic analysis of diverse alcoholic beverages.

The fatty acid ester contribution to the total aroma of alcoholic beverages does not appear to depend on the presence of any single ester or group of esters but rather on the relative composition of a group of esters always present. However, in a few cases with wines, compounds have been isolated which are more or less unique to a grape variety or species and account, in part at least, for its recognizable aroma, e.g., methyl anthranilate in certain varieties of Vitis labrusca (4) and linalool in muscat grapes (20). Distilled beverages before aging are not as complex as wines, owing to elimination of non-volatile or weakly volatile compounds by distillation. Furthermore, the levels of the volatile aroma materials in brandies or any distilled alcoholic beverage can be affected markedly by the nature and conditions of the distillation process. Certain compounds may be present in significant quantities or entirely absent as a result of the distillation method or conditions.

As mentioned previously, nearly all gas chromatographic studies of the fatty acid esters in alcoholic beverages have been essentially qualitative in nature for obvious reasons, considering the complexity of the system involved. However, Keppet et al. (7), using the technique of headspace analysis, reported the quantitative levels of the ethyl esters of acetic, caprylic, and caprylic acids, isoamyl acetate, and 2-phenethyl acetate in beer. Also, de Bocca et al. (2) analyzed various whiskies, spirits, and distilling samples by GC and reported quantitative levels for ethyl formate, ethyl acetate, ethyl lactate, and ethyl caprylate (octanoate). We have developed a method for low-concentration levels to be determined by gas chromatography of the esters of high-boiling fatty acids. Quantitative estimation was made for ethyl esters of caprylic, capric, and lauric acids, with ethyl pellargonic used as an internal standard.

MATERIALS AND METHODS

Equipment: The chromatograph used was an F & M Model 700-12 having dual columns and dual hydrogen flame ionization detectors.

Gas chromatographic operating conditions: Two liquid substrates were used for peak separation and identification, FFAP, 10%, w/w coated on 60/80-mesh Chromosorb GAW DMCS, was used for quantitative analysis. Neopentyl glycol adipate (NPGA), 10%, w/w on 60/80-mesh Chromosorb WAW; also gave good separations. But the FFAP was preferred because under the programmed-temperature conditions, it gave less baseline drift. Both columns were 6-ft x 1/8-in.-OD stainless-steel tubing.

The oven temperature was programmed...
from 100 to 225°C at 7.5°C/min and held at the upper temperature limit until all peaks were off the column. Detector and injection ports were maintained at 250°C. The helium carrier-gas flow was 60 ml/min.

Reference materials: For peak identification and as standards for quantitative analysis, the ethyl esters of caprylic (octanoic), pelargonic acid (nonanoic), lauric acid (dodecanoic), and myristic acid (tetradecanoic) were purchased from Aldrich Chemical Company. The ethyl ester of capric (docanocic) was supplied by Eastman Organic Chemicals. Ethyl caproate (hexanoic) and ethyl palmitate (hexadecanoic) were synthesized by reacting excess ethyl alcohol with caproic (hexanoic) or palmitic (hexadecanoic) acids in the presence of a mineral acid catalyst.

GC assay of the purchased esters showed that only one (ethyl caprylate) was completely pure, as indicated by a single peak at the appropriate retention time. All others contained trace amounts of neighboring esters. It was necessary to utilize these assays in adjusting the amounts of each reagent ester weighed out in preparing solutions of known concentrations for quantitative analysis.

Concentration of wine distillates for GC: In contrast to commonly used techniques for more abundant components of brandy, such as fusel oil, the higher fatty acid esters are not present in sufficient concentration to permit direct sample injection. Therefore, the following concentration procedure was used. One hundred ml of a brandy distillate or brandy was placed in a separatory funnel and diluted with water to lower the ethyl alcohol content to about 20% by volume. The diluted sample was extracted 4 times with 100-ml aliquots of methylene chloride. The combined methylene chloride layers were dried with anhydrous MgSO4, filtered, and concentrated by distillation on a Vigeaux column to about 1 ml of residue. The residue was transferred quantitatively to a 12-ml graduated centrifuge tube and accurately diluted to 5 ml with methylene chloride. This concentrate was used for GC analysis.

Quantitative GC analysis: A stock solution containing 1500 ppm of each of the three most abundant esters — ethyl caprylate, ethyl caprate, and ethyl laurate — was prepared in absolute ethyl alcohol. Dilutions of the stock solution were made so as to prepare working standards containing levels of each ester appropriate for the samples to be analyzed. Generally, a range of 0-750 ppm of ester was adequate for analysis of all samples tested. Usually 3 to 5 levels in this range were used.

Selection of an internal standard for these analyses was difficult because of the crowded peaks shown on the chromatograms. An ideal internal standard should be similar in properties to the compounds to be analyzed but be absent from the test material. The near absence of odd-numbered carbon fatty acid ethyl esters from wine distillates permitted the choice of ethyl pelargonate as the internal standard. Generally, the test sample was first chromatographed without addition of internal standard to ensure that no appreciable peaks eluted at the same retention time as ethyl pelargonate, which emerges midway between ethyl caprylate and ethyl caprate.

For quantitative analysis, 1 part of ethyl pelargonate (1 g/100 ml absolute ethyl alcohol) was added to 10 parts of the ester working standard solution or the test sample, and 2 μl of the mixture was injected onto the GC column. The peak height of each ester in each working standard divided by the peak height of internal standard was plotted versus the concentration of ester in the working standard. The standard curve of peak-height ratios versus concentration of each ester was linear up to 1500 ppm, although for most samples analyzed by this procedure an upper limit of 750 ppm was sufficient. For test samples, the same ratios were calculated and converted to concentration units by reading from the standard curve for each ester.

RESULTS

Ester separation and identification: Figure 1 shows a chromatogram of a concentrate of a beverage brandy with the
FFAP column. This brandy distillate had been drawn as a side-stream product from the distillation below the top of a 24-plate 12-in.-diameter continuous pilot column. Simultaneously, a 5% heads cut was drawn from the condenser and a low oils cut (about 10%) was taken from 2 plates below the product plate. The distilling wine was freshly fermented from the St. Emilion variety.

The chromatogram clearly shows that ethyl caprylate, caprate, and laurate are major ester components of the concentrate. For the brandy used for this illustrative chromatogram, the first two esters are more concentrated at peak width on scale, so it either must be diluted or the instrument sensitivity decreased prior to analysis for peak-height measurements. A chromatogram with the FFAP column is quite similar to that with FFAP with two exceptions. The retention time for 2-phenethyl alcohol is affected by the kind of packing, so that with FFAP it is eluted before ethyl laurate, whereas with FFAP it follows this ester. A similar effect was found with furfural. On the NPG column it is eluted 1.5 min before ethyl caprylate, but on FFAP it follows the ester peak by 0.5 min. Neither compound is present in the brandy shown in figure 1. Since the brandy used is a product from a continuous still, no furfural is normally formed, and under the operating conditions employed 2-phenethyl alcohol is very low at the product plate.

Peak identities were established by comparison of retention times of known compounds on the two GC columns used. In addition, all ester peaks were effectively removed from the wine-distillate chromatogram simply by refluxing the distillate mixture at 40°C prior to the extraction with methylene chloride. A chromatogram of a brandy hydrolyzed in this manner showed that the loss of ethyl ester peaks was accompanied by an increase of the poorly separated broad peaks labeled "Free Fatty Acids" in figure 1.

Some of the peaks were collected for odor examination and confirmation of identity by infrared spectrophotometry. For these purposes, the hydrogen flame detector assembly was replaced with a thermocoupling detector and the chromatographic columns were replaced with 6-ft x 1/8-in.-OD Carbopax 20M columns in order to be able to inject larger samples onto the larger-diameter column. Carbopax 20M also affects a good separation of the esters. The peaks labeled ethyl caprylate, ethyl caprate, and ethyl laurate were collected separately and were shown by infrared spectrophotometric analysis to be ethyl esters of straight-chain saturated fatty acids. The identities of ethyl caproate (the small peak just prior to n-hexyl alcohol in figure 1) and n-hexyl alcohol could easily be confirmed by their characteristic colors at the exit port of the detector.

The identity of 2-phenethyl alcohol was established by its retention time and also by observing its characteristic rose-like odor at the exit port during the analysis of another type of brandy (pot still) containing a lower level of this compound than the samples represented by figures 1 and 2.

Tests of the analytical method: To test the efficiency of the extraction procedure, a brandy distillate known to be high in esters was extracted 4 times with 100-ml portions of methylene chloride, and the solvent fractions were concentrated as previously described. The remaining aqueous layer was re-extracted 4 additional times and concentrated in the same way. Chromatography of the two concentrates showed that no esters remained in the aqueous solutions after the first extraction procedure.

The precision of the quantitative GC analysis for the three most abundant esters was tested by extracting and concentrating 5 separate 1-ml samples of the same brandy. The five concentrates were then analyzed for ethyl caprylate, caprate, and laurate. Table 1 lists the values found. While the results are considered good, the low values for sample number 4 indicated that losses of some of the esters can occur during the manipulations required in this analytical procedure.

Applications of the method for analysis of wine distillates: Several factors are reported to affect the level of fatty acid esters formed during fermentation, i.e., temperature of fermentation, pH, and nutrient composition of the medium (10). In addition, the higher ester content of the brandy product may be controlled by taking advantage of another particularly interesting phenomenon. Nordström (10) showed that ethyl esters with molecular weights greater than ethyl caprylate are rather securely bound in some way to the yeast cell, and even ethyl caprylate is mostly not free in solution. The esters, however, released from the cell upon heating during the distillation process. The possibility is readily apparent of controlling the level of fatty acid esters in the brandy distillate by limiting the mass of yeast cells present.

Suomalainen and Nylén (15) distilled a synthetic nitrogen-free medium fermented by baker's yeast in a pilot-plant-scale pot still. One lot of medium was distilled with yeast cell present, and the other was distilled after clarification by centrifuging. Their experiments showed little difference in the aroma components in the two distillates except for ethyl esters of caprylic, capric, and caprilic acids were present when the yeast cells were included in the medium distilled. However, their two lots of media distilled were

### Table 1

<table>
<thead>
<tr>
<th>Sample no.</th>
<th>Ethyl ester (mg/100 ml)</th>
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<tbody>
<tr>
<td></td>
<td>Caprylate</td>
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<tr>
<td>1</td>
<td>3.2</td>
</tr>
<tr>
<td>2</td>
<td>3.1</td>
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<td>3</td>
<td>3.1</td>
</tr>
<tr>
<td>4</td>
<td>3.9</td>
</tr>
<tr>
<td>5</td>
<td>3.0</td>
</tr>
</tbody>
</table>

Mean: 3.06, 7.40, 6.06
Std. Dev.: 0.11, 0.37, 0.28
fermented separately and had other slight differences in addition to yeast cell population. We have conducted similar experiments on both large- and small-scale distillations.

For one experiment, 1000 gal. of grape must (from a mixture of the Talmag and Folle blanche varieties) was fermented into a wine containing 10.7% by volume ethyl alcohol. The wine was divided into 2 equal lots; one was treated with 500 ppm bentonite to promote settling of yeast cells. The two resultant 500-gal. lots, one clarified and one with yeast cells still in suspension, were distilled in the 26 plate single-column continuous still described previously. The 160° proof beverage-brandy type distillates were collected as side-stream products from a point 6 plates below the top. A 5% heads fraction was drawn from the condenser, but no low cuts were taken. Figure 2 shows chromatograms of concentrates prepared from methylene chloride extractions of the two brandy distillates using the FFAP column.

It is apparent that the esters of fatty acids were mostly removed from the distilling wine by bentonite fining. Ethyl caprylate appears to be partially bound to the yeast cell and partially free in the solution.

In a small scale experiment, 3 liters of a well mixed turbid fresh wine was distilled in a 5 liter all glass simple pot still. A second 3 liter lot of the same wine was filtered brilliant with an Ertel Model 10 laboratory pressure filter and distilled in the same manner as the turbid lot. The distillates contained 56% ethyl alcohol by volume. One-hundred ml samples of each distillate were extracted and concentrated and analyzed as before on the FFAP column. The quantitative results are shown in Table 2.

The results further confirm that the esters are partially contained within or are bound to the yeast cell but are volatilized from the intact cell by the distillation process. We found little change in the peak areas of n-hexyl alcohol or of 2-phenethyl alcohol as affected by the presence or absence of yeast cells; but both ethyl myristate and ethyl palmitate were much lower in the distillate of clarified wine.

As previously stated, several factors influence the concentration of fatty acid esters in a fermented alcoholic beverage, but for distilled beverages the distillation process itself is a major factor. Comparative analyses were made of brandy distillates produced in 3 different still types. Distillates of the same wine lot (St. Emilion) were made in the 26-plate continuous still described before, in an all-copper pot still of 350-gallon capacity by the Charente method (two successive simple distillations), and in a Pyrex glass 4-inch packed column operated as a continuous vacuum still. No effort was made to control the yeast cell density in the distilling wine, but it was considered essentially the same in all three lots distilled. The results for the three major fatty acid esters are shown in Table 2.

3. The distillate from the 26-plate continuous still analyzed is the same product shown chromatographically in figure 1. However, since the peaks for ethyl caprylate and ethyl laurate are off scale, and since the internal standard is absent in the chromatogram of figure 1, it is obvious that it is not the chromatogram used for quantitative measurements. The sensitivity of the electrometer was reduced by a factor of 4 in order to obtain suitable peak heights for measurement. The higher concentration of esters in the continuous still product than in both the pot still and the vacuum still distillate is characteristic, based on these and similar results for comparable samples.

**DISCUSSION**

The gas chromatographic method presented here for quantitative estimation of the ethyl esters of fatty acids in wine distillates is reasonably precise and relatively simple to carry out. The esters are effectively removed from alcohol-water solutions by methylene chloride extraction, and the subsequent concentration is rapid and simple owing to the easy removal of methylene chloride by volatilization. The three esters measured are well separated on either the NPQ or FFAP columns, although the latter substrate is preferred for its greater stability during programmed temperature runs. There are at least two points of caution, however. The internal standard used for these analyses, ethyl palmitate, has the same retention time as linoleate on either GC column.

Since the latter compound is present in wines from muscat varieties and in distillates thereof, one must allow for the increased peak height contributed by linoleate when using the GC method for esters with ethyl palmitate as internal standard. At best, this is a serious limitation to the accuracy expected with linoleate-containing samples.

2-Phenethyl acetate, having a retention time on the FFAP column of only about 0.2 minute after ethyl laurate, can, if present in appreciable amounts, interfere with the determination of the laurate ester. Although 2-phenethyl alcohol and 2-phenethyl acetate are present in wine, neither appears to be a significant component in continuous still beverage brandies, owing to their low volatilities.

Generally, the gas chromatograms of a typical beverage-brandy distillate will show peaks for the ethyl esters of myristic and palmitic acids, the latter being the larger of the two. Also some fatty acids emerge toward the end of the chromatogram under the conditions used herein, the peaks for which are generally tailing.

As was indicated, the levels of fatty acid esters are higher in the continuous still product than in either the continuous vacuum (low-temperature) product or the pot still product (involving prolonged heating at atmospheric pressure, so at a relatively high temperature). At this stage of our investigation we believe that some fatty acid esters are formed in the low-oils plate area of the rectifying section of the high-temperature still by esterification.

<table>
<thead>
<tr>
<th><strong>TABLE 2</strong></th>
<th>Effect of Suspended Yeast in Wines on Ethyl Esters in Their Wine Distillates</th>
</tr>
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<tbody>
<tr>
<td><strong>Experiment</strong></td>
<td><strong>Wine treatment</strong></td>
</tr>
<tr>
<td>Pilot-scale continuous still</td>
<td>None</td>
</tr>
<tr>
<td></td>
<td>Yeast removed by bentonite fining</td>
</tr>
<tr>
<td>Lab-scale pot still</td>
<td>None</td>
</tr>
<tr>
<td></td>
<td>Filtered</td>
</tr>
</tbody>
</table>

<table>
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<tr>
<th><strong>TABLE 3</strong></th>
<th>Fatty Acid Esters in Distillates of a Single Wine from Three Distilling Processes</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Still Type</strong></td>
<td><strong>Proof of distillation</strong></td>
</tr>
<tr>
<td>26-plate continuous</td>
<td>110</td>
</tr>
<tr>
<td>Pot</td>
<td>141</td>
</tr>
<tr>
<td>Continuous vacuum</td>
<td>134</td>
</tr>
</tbody>
</table>
of fatty acids released from suspended yeast cells, whereas in the vacuum column the temperature (about 10 to 115°F) in the exhaust section is too low to cause either free fatty acid release or appreciable esterification. In the simple pot distillation, on the other hand, there would be a sufficiently high temperature for esterification but perhaps too little concentration of alcohol at any time to permit significant ester formation.

More information will be given in a subsequent paper on the distribution of fatty acid esters in the plate liquids of a continuous fractionating column and in periodic samples of a batch distillation. The question naturally arises as to the significance of the ethyl fatty acid esters in brandy or other beverages from the sensory viewpoint. The ethyl esters of capric, caprylic, capric, and lauric acids exhibit fairly distinct or characteristic odors. Caproate is rather fragrant and intense, suggestive of banana oil; caprylate is less fragrant and more pungent, but also intense and suggestive of crude grape fusel oil; caprate is milder, less intense, more fatty or tallowy in tone; laurate is the least aromatic with a waxy candle-like odor. Combined, these four esters suggest a commercial "cognac oil." The only sample of "cognac oil" which we have examined chromatographically consisted mainly of ethyl laurate, with small amounts of the other even-numbered esters.

The general odor of new beverage brandies with appreciable higher ester levels is usually described in our laboratory as "pungent" or "yeast-like." Samples with low levels of these esters are milder in smell and typically somewhat smoother. The observed pungency may well be caused by free fatty acids as much as or more than by the esters. While it is impossible to make any conclusions as to optimum amounts of higher esters, it seems fair to say that they are regular components of brandies, whiskies, rums, etc., and contribute to the whole of the sensory character of the product. It is our opinion that they are very important contributors to the aged "bouquet" of aged spirit, especially the odors clinging to a glass after swirling above the level of liquid or after the glass is emptied.

Distillers have the means of decreasing or enhancing the higher ester content in accordance with their policies of production and desired sensory quality or character. The density of suspended yeast cells in the distilling material is obviously a major factor for control. We would point out that the use of fortified wines for distilling material will almost certainly lead to low levels of fatty acid esters, not only from the alcohol-diluting effect of the neutral wine spirits, but also because fortification will inevitably precipitate most of the yeast in a short time. In the same vein, one major purpose or advantage of using fortified wine is for preservation against bacterial spoilage until the distillation is carried out. Certainly any appreciable storage period would produce nearly complete sedimentation of yeast.

LITERATURE CITED