CHEMICAL ANALYSIS OF CONCORD GRAPE ESSENCE AS RELATED TO CONCORD GRAPE FLAVOR

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As Related to Concord Grape Flavor

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ABSTRACT

CHEMICAL ANALYSIS OF CONCORD GRAPE ESSENCE
AS RELATED TO CONCORD GRAPE FLAVOR

By

Theodore William Moeller

Twelve 150-fold grape essences were obtained from three Michigan essence manufacturers for chemical analysis and flavor evaluation. Methyl anthranilate concentrations of volatile essence fractions ranged from 4 to 126 ppm, total esters ranged from 100 to 11,400 ppm (as ethyl acetate), total carbonyls ranged from 250 to 6600 ppm (as acetone), and chemical oxygen demands ranged from 20,000 to 200,000 ppm. Acetaldehyde and acetone levels were present from 18 to 2811 ppm and 0 to 440 ppm respectively. Similar variations were evident in ultraviolet absorption spectra and gas-solid chromatographic analyses.

Acetaldehyde was the single most abundant contributor to total carbonyl values. Ultraviolet absorption spectra indicated that unsaturated aldehydes were also present. Although compound concentrations in headspace vapors were not necessarily related to their respective solution concentrations, highly significant correlations between headspace and liquid acetaldehyde levels and

headspace ethyl acetate and total ester levels were present. Correlations between chemical and flavor panel data were generally poor.

Flavor panel results showed that juices prepared from the essences were generally lower in flavor quality than commercial juices available to the consumer. Juices prepared from several essences, however, were acceptable to the panelists and were of the same overall quality as commercial juices.

Using the methods described, no single component of Concord grape essence can be measured quantitatively for use as an absolute index of essence flavor quality. Thus, an intricate balance of components within the essence seems necessary for high flavor quality. This may best be measured by headspace vapor analysis via gas chromatography.

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By

Theodore William Moeller

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INTRODUCTION

In many cases, the manufacturers of Concord grape juice and juice products have found it desirable to strip single-strength juice or puree of its essence and concentrate the remaining portion to approximately 70° Brix. By utilizing this method of concentration, shipping weights and costs, container costs, refrigeration loads, man-hour requirements, and the possibility of "chance" fermentation are all reduced (Murch and Ziemba, 1958). When these essences are added back to the final product in proper amounts, manufacturers are able to produce full flavored products possessing all the natural Concord grape flavors and aromas (Roger, 1961).

In the manufacture of such essences, the degree of concentration or essence strength is designated by the producer as being of a particular "fold". Thus, an essence of X fold theoretically has had each volatile component present in the original juice concentrated X times. This same figure is the value utilized by the end user of the essence to determine the amount of essence to be added to the final product. This value is actually derived by establishing a ratio of the rate of volumetric flow of input juice to the rate of volumetric flow of essence

output of the concentration unit. It is assumed that uniform concentration and 100 percent recovery of each chemical compound present in the original juice has been achieved.

A flaw in utilizing this method, however, is the assumption that uniform concentration and 100 per cent recovery of each chemical compound present in the original juice has been achieved; obviously, this is not necessarily the case.

The purpose of this study was to develop a simple, fast, and accurate test procedure which could be used to quantitate a single component and/or group of components present in various Concord grape essences, and to determine if this test may be used as a measurement of true essence strength.

REVIEW OF LITERATURE

Several investigators have attempted to characterize and elucidate the composition of not only Concord grape juice, but many other common juices. Methyl anthranilate was implicated in a study by Power and Chesnut (1921) as being a natural and apparently constant constituent of Concord grape juice. This compound possessed a decidedly grape-like odor in dilute aqueous solutions and was suspected to improve grape flavor when added to grape juices. It was found in other fruit juices by Power (1921).

Methyl anthranilate was first discovered in 1895, in neroli oil. It has also been found in the oils of tuberose, ylang-ylang, Spanish orange blossoms, sweet orange rind, bergamot leaves, jasmin flowers, gardenia and certain varieties of apples. It was pointed out that even though methyl anthranilate has been found in all of the above, the compound is chiefly associated to the flavor in grape-type products (Scott, 1923). In a study involving varietal differences of grapes, it was found that pure-bred Vitis labrusca varieties (including Concord) contained relatively large amounts of methyl anthranilate. Varying amounts of the compound were found in hybrids of this species and tended to be present in higher concentrations

when Vitis labrusca was the predominant genotype. On the other hand, Vitis vinifera (European-type grapes) and Vitis rotundifolia (Southern grapes) were found to be completely devoid of methyl anthranilate. As a result, it was generalized the methyl anthranilate imparted a distinctive Concord-type odor to those varieties in which it occured, although several exceptions were noted. For example, Catawba, a hybrid of Vitis labrusca-Vitis vinifera, was found to contain none of the compound, but had an odor characteristic of those grapes having relatively large amounts of methyl anthranilate (Power and Chesnut, 1923). A similar observation was made by Sale and Wilson (1926) on the variety Campbell, another Vitis labrusca-Vitis vinifera cross. It was noted, however, that Campbell juices did contain exceptionally large quantities of volatile esters and that the total volatile ester content varied directly with the flavor and fragrance of juices of the Concord variety.

No additional work was published on Concord grape flavor until volatile constituents present in aqueous solutions of Concord grape essences were examined by Holley et al. (1955). Water-insoluble derivatives of the various compounds were prepared and examined chromatographically. Seven compounds were identified and quantitated. Ethanol and ethyl acetate were the most abundant compounds present, while the methyl anthranilate concentration was three orders

of magnitude less than that of the former two compounds. A synthetic essence was prepared utilizing the concentrations of the seven compounds identified and quantitated. Comparing the ultraviolet absorption spectra of both essences, it was found that a weak maximum absorption at 280 nm in the natural essence was replaced by a minimum at 270 nm in the synthetic essence. The odor of the synthetic essence was lacking in components essential to natural Concord odor. However, when a chloroform extract, washed with acid to remove any methyl anthranilate present and concentrated to obtain a small amount of oil, was added to the synthetic essence at the rate of 0.02 mg/ml, the resultant mixture had an odor which closely resembled that of the natural essence. The effect of this addition upon ultraviolet absorption was not discussed.

In the more recent research, emphasis has been placed on essence composition. Most of the studies have been made using gas chromatography and mass spectrometry for compound separation and identification. Such an approach was used in a study of diethyl ether extracts of 100 "fold" Welch Concord essence. Sixteen volatiles were identified in that fraction of the original extract vaporized via bubbling nitrogen gas through the extract and subsequently collected in a dry ice-acetone trap at atmospheric pressure (Stevens et al., 1965). Ethanol and ethyl acetate again were reported as being most abundant of all

compounds identified while dimethyl vinyl carbinol, having a cresol-like odor, was the third most prevalent compound present. A small amount of material collected in a room temperature trap possessed a pungent odor, but was not examined.

In a study of flavor variations of various Concord juices, methyl anthranilate was indicated as being essential to characteristic Concord flavor (Clore et al., 1965). The study showed the methyl anthranilate concentration in the grape itself increased steadily during the grape growing season until maturity. Total volatile esters as well as the total volatile ester-methyl anthranilate ratio fluctuated considerably during maturation and from season to season. Methyl anthranilate concentrations above a certain level did not increase the Concord flavor of the juices.

Among compounds other than methyl anthranilate present in Concord essence, n-valeraldehyde was detected as an undesirable anomoly present in essences from atypical Canadian Concord grapes (Neudoerffer et al., 1965). A flavor described as "sweet and fruity" was evident in essences produced from grapes having ten times the normal amounts of this compound. Although n-valeraldehyde was the compound of primary interest, 31 additional compounds were identified when further analyses were conducted on ethyl chloride extracts of such essences.

In the most recent report on Concord grape essence, 60 compounds were separated and identified in isopentane extracts of 150-fold Seneca essence (Stern et al., 1967). Relatively large amounts of ethyl acetate were found. However, comparatively small concentrations of ethanol and dimethyl vinyl carbinol were found, in contrast to the results of Steven et al. (1965). This was probably due to their relative insolubility in isopentane. These extracts contained unusually large amounts of crotonates, particularly the ethyl ester, and were reported to comprise a large percentage of the oil.

Chemical compositions of the essences of grape varieties other than Concord have been reported. Seventy-seven compounds were identified as being present in isopentane extracts of Muscat of Alexandria essence (Stevens et al., 1966). A relatively large percentage of this oil was made up of terpene alcohols with linaloöl and geraniol most abundant. Although several esters were present, they made up only a small percentage of the oil and no methyl anthranilate was found. Fifty-seven compounds were identified by Stevens et al. (1967) in isopentane extracts of an essence from another Vitis vinifera variety, Grenache. Unlike the Muscat variety, a significantly larger amount of esters and a much smaller amount of terpene alcohols were present in this essence. The bulk of this essence was made up of alcohols. Trans-2-hexenal and hexanal were

two of six aldehydes identified and represented a large percentage of the essence. Ketones were virtually absent and no methyl anthranilate was reported. This work was substantiated by Stevens et al. (1969) comparing composition of Grenache juices and Rosé wines. Analysis of trichloromonofluoromethane (Freon 11) extracts, showed 1-hexanol the most abundant of all the compounds present while large amounts of ethyl acetate and aldehydes were also present. In a study of White Riesling, another Vitis vinifera variety, diethyl ether extracts of the essence were shown to have alcohols as their major constituent. There was no methyl anthranilate found and ethyl and isoamyl acetates were the only esters reported (Van Wyk et al., 1967).

As mentioned above, virtually all recent grape flavor research has been focused on the separation and isolation of the compounds present in essences. No serious attempt has been made to quantitatively correlate the presence, absence, or concentration of any one compound or group of compounds to the actual flavor potency of a given grape essence or its resultant product. Quantitation of various compounds and groups of compounds and their correlation to flavor has been attempted in this study.

MATERIALS AND METHODS

Materials

Essences: Grape essences labelled 150-fold were obtained from three Michigan essence manufacturers and coded as follows:

Manufacturer: A, B, or C.

Type: P, Concord puree; J, Concord juice; N, Niagara juice.

Year or date of production: 68, 1968, etc.; 10/8, October 8; UK, date unknown. An essence coded AP6910/6 for example, was manufactured by manufacturer A on October 6, 1969. Unless a specific date was given, it was assumed the essence was a representative composite of the entire year's production for that type.

If the sample was stored at -23C until August 10, 1970, F was added to the code.

Essences of 1968 grapes were obtained on August 12, 1969 and labelled AJ68, AP68, and BJ68. Each essence was transferred to one-pint glass bottles and stored at -23C until April 10, 1970. At that time, five bottles of each essence were removed from frozen storage and stored at 2C until analyzed. Two additional bottles of each essence

were removed from -23C storage on August 10, 1970, and stored at 2C as above.

Essences of 1969 grapes were obtained on March 13, 1970, and labelled AJ69, AP69, AN69, AJ6910/2, AJ6910/7, AJ6910/8, AJ69UK, BJ69, CJ69. Each essence was transferred to glass bottles as above. Where essence quantities were sufficient, half the bottles of each essence were stored at -23C until August 10, 1970, when they were placed in 2C storage until analyzed. The remaining bottles were placed directly in 2C storage until analyzed.

Concentrate: Two-gallons of Concord grape concentrate, stripped of its essence, was obtained on March 13, 1970, and was of type AJ69; it was labelled by the manufacturer as being 70° Brix but was measured by refactometry as 66.5° Brix.

Juices: Three heat-processed and two frozen commercial Concord grape juices were purchased from a local supermarket for potential use as reference juices in flavor panel evaluations of the test essences. The following code was used to disguise the origin of each juice:

Manufacturer: W, X, Y, or Z.

Type: F, frozen; H, heat-processed.

Methods of Analysis

Steam distillation: Five ml of each cold essence (2C) was steam distilled in an all glass distillation

apparatus of conventional design. A glass tube affixed to the end of the water cooled Graham condenser was immersed in ca. 30 ml of distilled water in an iced 250 ml volumetric flask. Approximately 200 ml of distillate were collected in about 10 minutes. The non-distillable residue of each essence was quantitatively transferred to a second 250 ml volumetric flask. Both flasks were made to volume with distilled water, labelled as volatile and non-volatile respectively, and stored at 2C until analyzed. Where essence quantities were sufficient, triplicate distillations were performed, otherwise, only one distillation was performed.

Methyl anthranilate determination: A simple modification of the method by White (1966) was used on both the volatile and non-volatile portions of each essence.

Reagents:
- A. Hydrochloric acid (81 ml/100 ml)
- B. Sodium nitrite (3 g/200 ml H_2O)
- C. Hydrazine sulfate (5 g/200 ml H_2O)
- D. Potassium-1-naphthol-2-sulfonate (5.6 g (90% practical)/100 ml H_2O)
- E. Sodium carbonate (50 g/200 ml H_2O)

Duplicate 10.0 ml aliquots of each essence fraction were transferred to 50 ml volumetric flasks and the above reagents were added in the following sequence:

1. Add 0.5 ml of A and 0.5 ml of B. Let stand exactly 2 minutes.

2. Add 1.5 ml of C. Let stand exactly 1 minute.
3. Add 1.0 ml of D. Mix well.
4. Immediately add 1.5 ml of E.
5. Dilute to volume with distilled water and mix. After standing for 10 minutes at room temperature, the absorbance of each solution was read at 490 nm in 10 mm colorimeter tubes with a Bausch & Lomb Spectronic 70.
6. Results are expressed as ppm methyl anthranilate. The standard solution was prepared by dissolving 500 mg of methyl anthranilate in 50 ml of 95% ethanol. This solution was quantitatively transferred to a 100 ml volumetric flask and diluted to volume with distilled water, yielding a 5 mg/ml solution. A 10 µg/ml solution was prepared from this solution by proper dilution; 1.0, 2.0, ... 7.0 ml aliquots of this solution were transferred to individual 50 ml volumetric flasks where the proper reagents were added as described above. The absorbance of each solution was measured and plotted as a function of methyl anthranilate concentration.

Total ester determination: The method of Thompson (1950), as described by Clore et al. (1965), was applied to 2.0 ml duplicates of the volatile and non-volatile portions (or appropriate dilution thereof) of each essence.

After adding the usual reagents to the essences in 16 mm colorimeter tubes and thoroughly mixing, the absorbance of each was measured at 540 nm with a Bausch & Lomb Spectronic 70. Results are expressed as ppm ethyl acetate. The standard solution was prepared by dissolving 2.5 g of ethyl acetate in 50 ml of 95% ethanol. This solution was quantitatively transferred to a 100 ml volumetric flask and made to volume with distilled water, yielding a 25 mg/ml solution. From this solution, 25, 50, ... 200 µg/ml solutions were prepared via proper dilutions; 2.0 ml of each of these solutions were then treated as described above. The absorbance of each solution was measured and plotted as a function of ppm ethyl acetate.

Chemical oxygen demand (COD): The colorimetric method of McNary et al. (1957) was applied to 50.0 ml duplicate samples of the volatile and non-volatile portions (or appropriate dilution thereof) of each essence. The absorbance of each sample was measured in 16 mm colorimeter tubes at 650 nm with a Bausch & Lomb Spectronic 70. Results are expressed as ppm COD. The standard solution was prepared by dissolving 2.0 g of reagent grade glucose, dried overnight at 105C, in distilled water and diluting to 100 ml in a volumetric flask. 10.0, 20.0, ..., 90.0 ml aliquots of this solution were each diluted to 200 ml in volumetric flasks, thus giving 106.7, 113.4, ..., 960.3 ppm COD respectively (1 g of glucose is equivalent to

1.067 g COD). Fifty ml of each solution were treated in the usual manner. The absorbance of each solution was measured and plotted as a function of ppm COD.

Total carbonyl: The colorimetric method of Peleg and Mannheim (1970) was used to measure carbonyl levels of each essence. One change in the method was in carbonyl-free methanol preparation. Stock methanol was treated with 1 g of 2,4-dinitrophenylhydrazine and 4 g of trichloro-acetic acid per 500 ml, distilled through a ten-plate Oldershaw column, and stored in glass bottles. The authors reported the use of Girard P reagent to accomplish the same end. The test was applied to triplicate 1.0 ml aliquots of the volatile and non-volatile portions (or appropriate dilution thereof) of each essence. The results are expressed as ppm acetone. The standard solution was prepared by weighting 1.25 g of acetone into 475 ml of cold water in a 500 ml volumetric flask. After diluting to volume with distilled water, aliquots of the solution were diluted to prepare 0, 5, 10, ..., 45 ppm solutions. One ml of each solution was treated in the usual manner. The absorbance for each solution was determined and plotted as a function of ppm acetone.

Thin layer chromatographic separation and quantitation of acetone and acetaldehyde 2,4-dinitrophenylhydrazones: A modification of the method of Neuberg et al. (1952) was used to prepare the DNPH derivatives. Triplicate 5.0 ml

aliquots of each essence were directly treated in 250 ml separatory funnels with 2.0 to 4.0 ml of the 2,4-dinitrophenylhydrazine reagent described by the authors. The amount of reagent used for each sample was determined by the results of the total carbonyl test. After standing at room temperature for 15 minutes, each solution was extracted with 5 X 15 ml of carbonyl-free chloroform (distilled through a ten-plate Oldershaw column after treatment with 1 g of 2,4-dinitrophenylhydrazine and 4 g of trichloroacetic acid per 500 ml). The chloroform layers were combined in 250 ml standard taper Erlenmeyer flasks and evaporated to approximately 30 ml with a Büchi rotary evaporator. Full aspirator vacuum was maintained in the evaporator while the bottom edge of the Erlenmeyer was placed in a 30C water bath. The concentrated extracts were then quantitatively transferred to 50 ml volumetric flasks and diluted to volume with carbonyl-free chloroform.

Preparation of thin layer plates: A silica gel GF-254 (Merck)/distilled water slurry (1:2) was blended at high speed in a Waring blendor for 1 minute and immediately spread on 10 cm X 20 cm glass plates; a Desaga/Brinkman spreader set at 0.5 mm was used. After setting overnight at room temperature, the plates were activated at 105C for 1 hour and stored in a desiccator at room temperature until used. Depending upon the derivative concentration, 5 to 20 µl of each extract was spotted on a single plate with

lambda pipettes. Triplicate plates were developed at room temperature to 10 cm in two consecutive solvent systems:

System 1: Petroleum ether (75-92C)-diethyl ether-chloroform, 50:30:20 (v:v:v).

System 2: Ethyl acetate-chloroform-hexane-methanol, 10:20:60:2.5 (v:v:v:v).

Each developing tank was lined with filter paper to ensure tank saturation.

The spots were visualized with ultraviolet light, removed with a Brinkman spot collector (cat. no. 0410139-8), and eluted into the collection flask with carbonyl-free chloroform. This solution was then quantitatively transferred to a 5 ml graduate cylinder and adjusted to 3 ml either by addition of carbonyl-free chloroform or by solvent evaporation with nitrogen. After transferring to 10 mm colorimeter tubes, each solution was measured for absorbance with a Bausch & Lomb Spectronic 70; the acetaldehyde derivative was measured at 352 nm while the acetone derivative was measured at 358 nm. A blank value was obtained for each plate by removing absorbant from an unused portion of each plate at the same distance from the origin as the spot of interest. Identification was made by spotting known derivatives. Results are expressed as ppm acetaldehyde and acetone, respectively. The standard solution was prepared by weighing 400 mg of each derivative into a 50 ml beaker and dissolving in carbonyl-free

chloroform. After quantitatively transferring to a 100 ml volumetric flask and diluting to volume with carbonyl-free chloroform yielding a 0.4 µg/µl solution, 5, 10, ..., 40 µl aliquots of each were spotted on plates (6 replicates), developed, and treated in the usual manner. The absorbance of each resultant solution was determined and plotted as a function of ppm derivative. The conversion of ppm derivative to ppm acetaldehyde or acetone was made by multiplying ppm (derivative basis) by 0.1965 or 0.2438 respectively, the ratio of the molecular weight of each compound to that of its derivative.

Ultraviolet absorption: Each essence was diluted, 1.0 ml to 25.0 ml, in a volumetric flask with distilled water. The absorbance of each solution was determined in 1 cm silica glass cuvettes with a Beckman DB spectrophotometer. Water was used as the reference. If the absorbance was above 1.00 for any essence solution, a 1.0 ml to 50.0 ml dilution of the essence was used. A Beckman strip chart recorder was used in the log mode to record absorbance directly. Results are expressed as absorbance at wavelengths where absorption peaks occurred.

Gas chromatography: Gas-solid chromatography was utilized to separate various compounds present in the headspace over each essence. Chromatographic conditions were as follows:

Instrument:	Hewlett Packard model 5750 research gas chromatograph equipped with a Mosley model 7127A recorder with Disc integrator.
Detectors:	Dual flame ionization
Columns:	1/8 in. X 10 ft. stainless steel packed with 80-100 mesh Chromosorb 101 (3.2 g/column). Columns were conditioned overnight at 250C with 10 cc/min helium flow.
Carrier gas:	Helium Tank gauge pressure: 60 psig; flow rates adjusted via rotometers
Flow rates:	Column A: 35 cc/min Column B: Adjusted to provide proper base-line compensation during temperature programming. Hydrogen gauge pressure: 9 psig; flow rate: 28 cc/min Air gauge pressure: 25 psig; flow rate: 370 cc/min
Temperatures:	Injection port: 250C Detectors: 250C Collection vent: 285C Oven program: 80 to 200C Post injection interval: 2 min Linear program rate: 4C/min Upper limit interval: 10 min

Range: 10^2

Attenuation: Adjusted between 4 and 128 to obtain maximum peak height less than 100 per cent recorder response.

An effluent splitter was inserted between the end of column A and detector A, thus diverting 1/6 of the flow emerging from column A through the heated collection vent. This was necessary to maintain proper baseline compensation during temperature programming.

Headspace sampling and chromatographic analysis: Duplicate 10.0 ml samples of each essence (at 2C) were pipetted into each of two cold 30 ml serum bottles. Single 10.0 ml samples were treated similarly for those essences where quantities were insufficient for duplicates. Each bottle was stoppered with a puncturable, resealable rubber stopper and heated in a vigorously stirred 50 ± 0.2C water bath. After heating exactly 30 minutes, a 1.0 ml headspace vapor sample was withdrawn from the bottle headspace via a gas-tight syringe and injected into the chromatograph. After the oven program was begun, the baseline was adjusted as needed to provide zero recorder response.

Peak identification: Where possible, peaks were tentatively identified by injecting known compounds and observing their retention times. When sufficient quantities of given compounds were present, mass spectroscopic examination was used to provide positive identification.

This was accomplished by installing a single column in a LKB gas chromatograph coupled with a LKB 9000 magnetic deflection mass spectrometer. As compounds emerged from the column, they were ionized. The m/e was monitored via a Honeywell ultraviolet recorder. All data was recorded on magnetic tape and fed into a Digital 8/1 computer, which performed all calculations necessary to derive per cents abundance and total ionization. These results were plotted as bar graphs by a computer-controlled plotter.

Peak quantitation: Identifiable peaks were quantitated using a Disc integrator; integrator counts were corrected for baseline drift with a drift corrector and adjusted for attenuation differences among peaks. Results are expressed as percentage total peak area.

Flavor evaluation: Flavor panels were utilized to examine the typicalness of Concord grape flavor (relative to a reference juice), acceptability, and relative preference of each essence; each essence was added to Concord grape concentrate diluted with cold tap water to yield single strength juice.

Two sources of panelists were utilized. Initial flavor panels were each composed of twenty, randomly selected individuals from the Michigan State University Department of Food Science. A final "consumer panel" of fifty-two panelists was solicited from the author's apartment complex to examine consumer preference of juices prepared from two selected essences.

The soluble solids content, pH, and total titratable acidity were determined for each juice. Frozen commercial juices were diluted directly to 15.7-16.0° Brix with cold tap water. When necessary, citric acid was added to achieve a level of 0.70 per cent acid (as citric).

In every case, each panelist was given approximately 15 ml of each juice in a small plastic cup. Each juice was kept iced until serving and was assigned a two-digit random number.

Reference juice: Four of the juices purchased from a local supermarket were prepared as described above. Each panelist was given three pairs of juices, one pair at a time, at each of two sittings on successive days to determine juice acceptability and typicalness of Concord flavor. All possible pair combinations were presented to each panelist. The commercial juice possessing the most typical Concord flavor while still being acceptable was used as the reference juice in panels involving essences. The ballot accompanying each pair of juices is described as follows.

Concord Grape Juice Sensory Evaluation

Before you are two samples of Concord grape juice.

1). Indicate which sample has the more typical grape flavor by placing an "X" in the appropriate box below.

2). Indicate whether each sample is acceptable or not acceptable by placing an "X" on the appropriate line below.

Please rinse your mouth with water before tasting each sample.

	Sample (Code)		Sample (Code)
	☐		☐
Acceptable	____	Acceptable	____
Not Acceptable	____	Not Acceptable	____

Essence-containing juices: Single strength, reconstituted juices were prepared using Concord grape concentrate and each essence according to the following formula:

76.9 g Concord grape concentrate.

2.0 ml grape essence.

Make to 319.8 g with cold tap water, yielding 300 ml volume.

In using this formulation, it was assumed that all essences were 150-fold, as was indicated by the manufacturer.

For each flavor panel, three reconstituted juices and the reference juice were judged together. At one panel sitting, panelists judged as to typicalness and

acceptability. At another sitting, panelists judged the relative preference of the juices by ranking. Ballots accompanying the juices in these panels are illustrated as follows:

Typicalness and acceptability ballot:

Flavor Difference Evaluation

Instructions

1. Please make your evaluations based on your concept of typical grape flavor.
2. Determine the degree of flavor difference between each numbered sample and the reference sample R.
   a. If you do not detect any flavor difference, place a check opposite the words No Difference.
   b. If, in your judgment, any flavor difference does exist, place a check in one of the other four boxes opposite the term which best describes the degree of flavor difference.
3. After rating the flavor difference, place a check on one of the lines at the bottom of each column, indicating whether the flavor of the numbered sample is acceptable or not acceptable to you.

Degree of Flavor Difference	Sample Number		
	___	___	___
Much More Typical	☐	☐	☐
Slightly More Typical	☐	☐	☐
No Difference	☐	☐	☐
Slightly Less Typical	☐	☐	☐
Much Less Typical	☐	☐	☐
Acceptable	___	___	___
Not Acceptable	___	___	___

Relative preference ballot:

Ranking Method of Flavor Evaluation

Rank the samples in the order of how well you like them, giving the best sample or the one you like best a rank of 1 and rank the others below. You may use your own judgment whether to swallow or not to swallow the product, and the time to wait between samples.

Ranking

1._____

2._____

3._____

4._____

Statistical analysis of data: Analysis of variance (Amerine et al., 1965a) was used to statistically analyze all chemical data. Since the number of replications for distillations, extractions, and headspace vapor analysis were not equal for all essences, a randomized block design was used. Data showing significant sample differences were further subjected to Duncan's multiple range test (LeClerg, 1966). Where appropriate, coefficients of correlation were also determined (Amerine et al., 1965b).

Tukey's one-factor range test (Tukey, 1953) was used to test for differences of typicalness data obtained from flavor panels for the various juices. In each case, the reference juice was assigned a score of 3; The remainder of the scores were assigned as follows: much more

typical, 1; slightly more typical, 2; no difference, 3; slightly less typical, 4; much less typical, 5. The analysis was conducted on the basis of two samples, ie. the test sample vs the reference.

The binomial test was used for acceptability scores (Amerine et al., 1965c). All rank tests were analyzed by using normal score transformation followed by analysis of variance (Li, 1957). Data showing significant sample differences were further subjected to Duncan's multiple range test (LeClerg, 1966).

RESULTS AND DISCUSSIONS

Steam distillation: The results of recovery studies of volatiles using the all-glass steam distillation apparatus are listed in Tables 1 through 3. Five ml each of a) 200 µg/ml methyl anthranilate solution, b) 25 mg/ml ethyl acetate solution, and c) 200 µg/ml methyl anthranilate-25 mg/ml ethyl acetate solutions were distilled and quantitated. These solution concentrations were extrapolated (Holley et al., 1955) as estimates of methyl anthranilate and total ester levels in 150-fold essences.

Table 1. Distillation recovery of methyl anthranilate.

	Solutions		
	I	II	III
Distillation	µg MA/10 ml distillate		
1	38.0	42.5	40.0
2	38.5	42.5	41.0
3	39.0	39.0	41.0
4	38.0	38.5	41.0
5	37.5	41.0	40.0
Mean	38.2	40.7	40.6
Reference 1	40.0	43.0	41.0
Reference 2	40.0	43.0	41.0
Mean	40.0	43.0	41.0
% Recovery	95.5	94.7	99.0
Mean %			95.7

Table 2. Distillation recovery of ethyl acetate.

Distillation	Solutions I	II	III
	µg EA/2 ml distillate		
1	910	850	900
2	910	900	950
3	920	920	920
4	960	920	980
5	920	900	940
Mean	924	898	938
Reference 1	980	950	980
Reference 2	960	950	1000
Mean	970	950	990
% Recovery	95.3	94.5	94.8
Mean %			94.8

Table 3. Distillation recovery of methyl anthranilate/ ethyl acetate.

Distillation	Solutions I	II	III
	(µg MA/10 ml dist.)/(µg EA/2 ml dist.)		
1	39/1000	41/950	41/980
2	40/1010	41/980	42/1010
3	40/1010	42/960	44/1020
4	42/1000	44/970	39/1000
5	42/1010	42/970	45/1020
Mean	40.6/1006	42.0/966	42.2/1006
Reference 1	41/1030	41/1000	42/1030
Reference 2	44/1040	45/1020	41/1040
Reference 3	41/1030	42/1030	41/1030
Mean	42.0/1033	42.7/1017	41.4/1033
% Recovery	96.7/97.4	98.5/95.0	102.0/97.4
Mean %			99.0/96.6

Recoveries were based on solution concentrations before distillation (labelled reference). Recoveries in excess of 95% could be consistently obtained if all glass joints were kept wet with water. No significant amount of methyl anthranilate or ethyl acetate was found in the residue of each distillation. Therefore, it was concluded that losses which did occur were not the result of incomplete or inefficient distillation, but from leakage through glass joints and/or failure to condense.

Methyl anthranilate: Mean methyl anthranilate levels for each essence fraction are listed in Table 4. There were significant differences among the various essences at the 1% level. There were no significant differences among replicates. Complete data are listed in the Appendix in Table 19.

Methyl anthranilate levels ranged from zero to 126 ppm in the volatile essence fractions. The levels in many essences were not significantly different from each other and there were no consistant trends as to manufacturer, type, or year of the essence. Daily production samples of essence AJ69 showed highly significant differences in methyl anthranilate levels. Frozen storage had no apparent effect upon the retention of methyl anthranilate in the essences.

No methyl anthranilate was found in non-volatile fractions of the essences.

Table 4. Methyl anthranilate concentrations for the volatile fraction of essences.

Essence	MA ppm	Statistical Significance[1] 5%	1%	Essence	MA ppm	Statistical Significance[1] 5%	1%
AJ68F	0[3]	a	a	AP69F	29[3]	fg	fg
AJ68	4[2]	b	a	CJ69	32[2]	gh	g
BJ69	9[2]	c	c	BJ68F	33[3]	h	gh
AP68	10[2]	c	cd	CJ69F	34[3]	h	h
AP68F	11[3]	cd	cd	AJ69UKF	35[3]	h	h
BJ69F	14[3]	d	d	BJ68	47[2]	i	i
AJ69F	22[3]	e	e	AJ6910/8	47[3]	i	i
AJ69	22[2]	e	e	AJ6910/7	54[3]	j	j
AP69	27[2]	f	f	AJ6910/2	78[3]	k	k
AJ69UK	28[3]	f	f	AN69	126[2]	l	l

[1]Like letters denote no significant difference among essences.

[2]Mean value of 6 determinations.

[3]Mean value of 2 determinations.

Holley et al. (1955) reported a methyl anthranilate concentration of 33 ppm for 83-fold Concord essence prepared in their laboratory. If this value is adjusted to a 150-fold level, a theoretical value of 60 ppm is obtained. This level, when coupled with the 0.80-1.49 ppm value for single strength juice reported by Scott (1923), is generally greater than methyl anthranilate concentrations in essences

examined in this study. This was possibly due to the relatively long storage and handling during commercial preparation. Essence AN69, having the highest methyl anthranilate content, was an essence stripped from the juice of Niagara grapes, a white labrusca variety, and was included in the study primarily for methyl anthranilate comparisons.

Total esters: Mean total ester levels for each essence examined are given in Table 5. Total volatile ester levels of the essences ranged from 100 to 11,400 ppm and indicated very little similarity among samples. At the 5% level, only two pairs of essences (BJ68F, BJ68 and BJ69, AJ69F) were not significantly different. As with methyl anthranilate levels, there was no relationship between manufacturer, year, or freezing storage, and the total ester level. Total ester levels were significantly different (1% level) among daily production samples of essence AJ69. Essences prepared from puree, however, did contain generally greater total ester levels in their volatile fractions than those essences prepared from juices.

Although several of the non-volatile fractions showed trace amounts of esters, the values obtained were within the experimental error of the procedure and may be considered insignificant.

If, as above, the 83-fold essence examined by Holley et al. (1955) is converted via calculations to a

Table 5. Total ester concentrations for the volatile fraction of essences.

Essence	EA ppm	Statistical Significance[1] 5%	1%	Essence	EA ppm	Statistical Significance[1] 5%	1%
BJ68F	100[3]	a	a	AJ69UK	4600[3]	j	i
BJ68	100[2]	a	a	AJ69	5108[2]	k	j
AJ6910/2	250[3]	b	b	BJ69	5333[2]	l	k
AJ6910/7	700[3]	c	c	AJ69F	5400[3]	l	k
AJ68	925[2]	d	d	BJ69F	5675[3]	m	l
AJ68F	1000[3]	e	d	AP68	5800[2]	n	m
AN69	1683[2]	f	e	AP68F	6285[3]	o	n
CJ69	2317[2]	g	f	AP69F	8850[3]	p	o
AJ69UKF	2600[3]	h	g	AP69	9142[2]	q	p
CJ69F	4100[3]	i	h	AJ6910/8	11,400[3]	r	q

[1]Like letters denote no significant difference among essences.

[2]Mean value of 6 determinations.

[3]Mean value of 2 determinations.

150-fold base, a theoretical ethyl acetate value of approximately 6300 ppm is obtained. This essence therefore contained a relatively high total ester concentration when compared to those of essences examined here. Again, this difference is possibly due to essence age and method of preparation.

Chemical oxygen demand: Mean chemical oxygen demand (COD) levels for the essences examined ranged from 20,000 to 205,000 ppm and are listed in Table 6. No significant differences were present among replicates. Samples, however, were significantly different at the 1% level and suggested gross differences between essence folds. Puree essences, for example, exhibited significantly lower COD values than their juice counterparts. Thus, it was apparent that some component or series of components present in Concord puree and not present in juice was a cause of less efficient volatile recoveries for essences from puree than from juice. There were no apparent trends in COD as to the year or manufacturer of essences. However, as with methyl anthranilate and total volatile esters, COD values were significantly different (1% level) for daily production samples of essence AJ69. Complete data are given in the Appendix in Table 23.

No oxidizable organic material remained in the nonvolatile portion of any essence other than AJ68 and AJ68F. The levels were 2500 and 3000 ppm respectively and were probably due to a small amount of debris observed in these samples.

Several authors have reported the use of chemical oxygen demand to indicate the concentration of organic volatiles present in fruit juices. Jensen (1961) reported a positive relationship between "oxidation number" (COD)

Table 6. Chemical oxygen demand for the volatile fraction of essences.

Essence	COD ppm	Statistical Significance[1] 5%	1%	Essence	COD ppm	Statistical Significance[1] 5%	1%
AP68F	20,650[3]	a	a	AJ6910/7	74,050[3]	j	i
AP68	22,125[2]	b	a	AJ68F	77,050[3]	k	j
AP69F	26,525[3]	c	b	AJ68	77,100[2]	k	j
AP69	27,375[2]	c	b	BJ69	85,933[2]	l	k
BJ68F	33,750[3]	d	c	BJ69F	89,150[3]	m	l
BJ68	38,125[2]	e	d	CJ69	99,800[2]	n	m
AN69	47,050[2]	f	e	AJ69UKF	105,000[3]	o	n
AJ69F	66,000[3]	g	f	AJ69UK	107,200[3]	p	o
AJ69	68,167[2]	h	g	CJ69F	109,600[3]	q	p
AJ6910/2	71,350[3]	i	h	AJ6910/8	204,800[3]	r	q

[1]Like letters denote no significant difference among essences.

[2]Mean value of 6 determinations.

[3]Mean value of 2 determinations.

and the "fold" of volatiles in apple concentrates. This value, however, was not necessarily related to the flavor enhancing quality of essences and ethanol was suggested as a contributor to this discrepancy. Charley (1962) made a correction in oxidation number for ethanol and derived the term "aroma number", a value which more closely correlated with the true flavor contribution of the essence. He also

suggested that carbonyls and esters be determined separately to help better understand the relationship between oxidation number, aroma number, and the flavor enhancing quality of essences.

Total carbonyl: The mean total carbonyl level for each essence is listed in Table 7. Total carbonyl levels

Table 7. Total carbonyl concentrations for the volatile fraction of essences.

Essence	Acetone ppm	Statistical Significance[1] 5%	1%	Essence	Acetone ppm	Statistical Significance[1] 5%	1%
BJ69F	213[3]	a	a	AP68F	620[3]	e	fg
BJ69	257[2]	ab	ab	AP68	718[2]	f	g
BJ68F	282[3]	ab	ab	AJ69F	958[3]	g	h
CJ69	298[2]	abc	ab	AJ69	1103[2]	h	i
AJ6910/2	308[3]	abc	ab	AN69	1129[2]	hi	i
BJ68	338[2]	bc	abc	AJ69UKF	1218[3]	i	i
CJ69F	398[3]	cd	bcd	AJ69UK	1508[3]	j	j
AJ6910/7	462[3]	d	cd	AJ68	2264[2]	k	k
AP69F	492[3]	d	de	AJ68F	2347[3]	k	k
AP69	596[2]	e	ef	AJ6910/8	6613[3]	l	l

[1]Like letters denote no significant difference among essences.

[2]Mean value of 9 determinations.

[3]Mean value of 3 determinations.

for volatile fractions ranged from approximately 200 to 6600 ppm and indicated definite trends as to the essence type and manufacturer. None of the essences from purees had high carbonyl levels while the essences from juices made by the same manufacturer (A) had relatively high levels of carbonyls. There were no significant differences among distillates or replicates. Complete data are listed in the Appendix in Table 25.

Although several non-volatile fractions showed trace amounts of carbonyls, the values obtained were well within the experimental error of the procedure and may be considered insignificant.

The presence of carbonyls in grape essence has been reported in the literature by several authors. Stern et al. (1967) mentioned no fewer than six such compounds were present in isopentane extracts of Concord essence but made no effort to quantitate or correlate any of these compounds to their relative importance of flavor contribution.

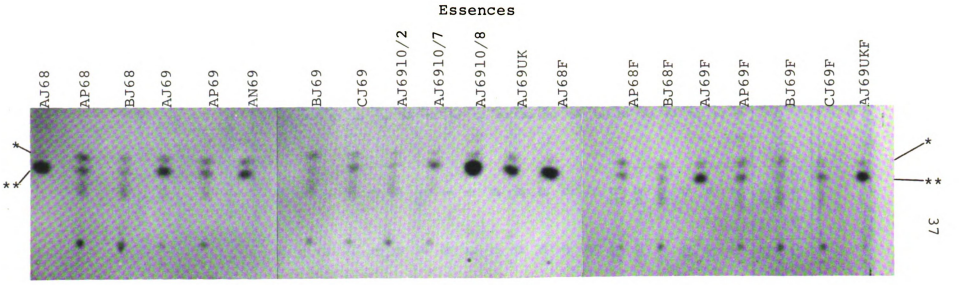
Thirteen carbonyls, eight of them aldehydes, were separated and identified from ethyl chloride extracts of Concord grape juices by Neudoreffer et al. (1965). These compounds were present in relative amounts ranging from large to trace. n-Valeraldehyde was correlated with one group of essences having an undesirable flavor anomoly.

This study exemplified the importance of carbonyls as a contributor to Concord grape flavor.

Acetone and acetaldehyde 2,4-dinitrophenylhydrazones: Typical separations of acetone and acetaldehyde 2,4-dinitrophenylhydrazones via thin layer chromatography are illustrated in Figure 1. (To facilitate photography, plates were sprayed with 0.5% Rhodamine B in 95% ethanol.) The two derivatives were identified by spotting recrystallized knowns prepared from stock laboratory reagent. Upon spraying with 10% potassium hydroxide in 95% ehtanol, the acetone derivative turned dark brown while the acetaldehyde derivative showed a distinctive red-brown; both colors were short lived. When plates were developed exactly 10 cm in each solvent system, R_f values of 0.62 and 0.58 were obtained for acetone and acetaldehyde 2,4-dinitrophenylhydrazones respectively.

Acetone and acetaldehyde concentrations obtained by this method of separation are listed in Table 8. The acetaldehyde values ranged from 18 to 2811 ppm. There were no statistically significant differences among plates. Complete data are listed in the Appendix in Table 28.

It was found necessary to recrystallize the 2,4 DNPH before use since some lots gave positive acetaldehyde values. For example, one lot gave values of 127 ppm acetaldehyde for each ml of reagent used in derivative preparation. No acetone derivative was obtained with the reagent.



*Denotes acetone 2,4-dinitrophenylhydrazone.

**Denotes acetaldehyde 2,4-dinitrophenylhydrazone.

Figure 1. Thin layer chromatographic separation of essence 2,4-dinitrophenylhydrazones.

Table 8. Acetaldehyde and acetone concentrations of the essences.

Essence	Acetaldehyde ppm	Statistical Significance[1] 5%	1%	Acetone ppm	Statistical Significance[1] 5%	1%
BJ68	18[2]	a	a	274[2]	hi	gh
BJ69	35[2]	a	ab	136[2]	d	de
BJ69F	43[2]	a	ab	211[2]	fg	f
AP68	54[2]	ab	ab	440[2]	k	i
CJ69F	55[2]	ab	ab	0[2]	a	a
BJ68F	87[2]	bc	ab	59[2]	b	abc
AJ6910/2	105[3]	c	b	47[3]	ab	ab
AP69	205[2]	d	c	384[2]	j	i
CJ69	210[2]	d	c	84[2]	bc	bcd
AP68F	253[2]	d	c	252[2]	gh	gh
AP69F	255[2]	d	c	136[2]	d	de
AJ6910/7	309[3]	e	d	111[3]	cd	cd
AJ69F	378[2]	f	e	147[2]	de	de
AJ69	419[2]	fg	e	184[2]	ef	ef
AN69	435[2]	g	e	309[2]	i	h
AJ69UKF	807[3]	h	f	192[3]	ef	ef
AJ69UK	815[3]	h	f	214[3]	fg	fg
AJ68	1288[2]	i	g	10[2]	a	a
AJ68F	1488[2]	j	h	0[2]	a	a
AJ6910/8	2811[3]	k	i	3[3]	a	a

[1]Like letters denote no significant difference among essences.

[2]Mean value of 9 determinations.

[3]Mean value of 3 determinations.

The acetone values ranged from 0 to 440 ppm. Significant differences at the 5% level were found between the acetone extractions. There were no significant differences between the plates. Further analysis showed sample X extraction interaction significance at the 1% level. Extractions 1 and 3 were significantly different but not extractions 1 and 2 or 2 and 3. These differences may be due to variations in the time delay between derivative preparation and spotting and/or to the method of concentration. They were not considered extremely important as sample differences for acetone and acetaldehyde levels were quite large between essences. It is felt, however, a method of separation, eg. column chromatography, eliminating concentration after extraction, would be more desirable than the method used.

Holley et al. (1955) reported that their 83-fold Concord essence contained 300 and 30 ppm for acetone and acetaldehyde respectively. Placing these values on a 150-fold basis yields theoretical levels of 542 ppm acetone and 54 ppm acetaldehyde. The acetone levels for the essences examined in the current study were generally lower than those of this essence. The acetaldehyde levels, however were considerably greater. Relative essence age, methods of essence production, condition of grapes and/or juice prior to essence preparation, and methods of derivative preparation could have contributed to these differences.

Carbonyl concentrations obtained by analysis of each essence were converted to μ moles/ml essence by dividing each respective value by the proper molecular weight. These conversions are listed in Table 9 and indicate the relative contributions of acetone, acetaldehyde, and other carbonyls to the total carbonyl level of each essence.

Ultraviolet absorbance: Mean absorbancies of each essence at absorption wavelengths of the ultraviolet spectrum from 200 to 300 nm are listed in Table 10. There were no statistically significant differences among replicates. Complete data are listed in the Appendix in Table 29.

Figures 2 and 3 illustrate the typical ultraviolet absorption spectrum for each essence. Each essence absorbed very sharply in the region of 205-215 nm. At 243 nm, however, not all essences exhibited a distinct absorption peak. The ratio of absorbance at 210-213 nm to the absorbance at 243 nm are also listed in Table 10.

Holley et al. (1955) reported ultraviolet absorbance spectra for natural and synthetic essences. The spectra were identical except a maximum at 280 nm in the natural essence was replaced by a minimum at 270 nm in the synthetic essence. Although absorption peaks at these wavelengths were not observed for essences examined in

Table 9. Carbonyl data.

Column Essence	1 Total Carbonyls μ moles / ml	2 Acetaldehyde μ moles / ml	3 Acetone μ moles / ml	4 Total (2+3) μ moles / ml
BJ69F	3.67	0.98	3.64	4.62
BJ69	4.43	0.80	2.35	3.15
BJ68F	4.86	1.98	1.02	3.00
CJ69	5.14	4.77	1.45	6.22
AJ6910/2	5.31	2.39	0.81	3.20
BJ68	5.83	0.41	4.72	5.13
CJ69F	6.86	1.25	0.00	1.25
AJ6910/7	7.97	7.02	1.92	8.94
AP69F	8.49	5.79	2.35	8.14
AP69	10.29	4.66	6.62	11.28
AP68F	10.69	5.75	4.35	10.10
AP68	12.38	1.23	7.59	8.82
AJ69F	16.51	8.59	2.54	11.13
AJ69	19.04	9.52	3.18	12.70
AN69	19.44	9.89	5.33	15.22
AJ69UKF	20.99	18.35	3.31	21.66
AJ69UK	25.99	18.51	3.69	22.20
AJ68	39.02	29.25	0.17	29.42
AJ68F	40.50	33.80	0.00	33.80
AJ6910/8	114.00	63.85	0.05	63.90

Table 10. Ultraviolet absorbancies of essences.

Essence[1]	Absorbance[2] at 205nm	at 213nm	Statistical Significance[3] 5%	1%	at 243nm	Statistical Significance[3] 5%	1%	Ratio 210-213nm / 243nm
AJ68	0.435	-----	--	--	-----	--	--	----
AJ68F	0.447	-----	--	--	-----	--	--	----
CJ69	-----	0.312	a	a	0.118	a	ab	2.64
BJ69	-----	0.315	a	ab	0.100	a	a	3.15
BJ69F	-----	0.320	a	ab	0.110	a	a	2.91
CJ69F	-----	0.322	ab	ab	0.125	a	abc	2.68
BJ68F	-----	0.430	ab	abc	0.172	b	cd	2.50
AP68	-----	0.442	bc	bcd	0.155[4]	--	--	2.85
BJ68	-----	0.457	c	cd	0.177	b	d	2.58
AP68F	-----	0.462	c	de	0.193[4]	--	--	2.39
AJ69	-----	0.485	d	ef	0.170[4]	--	--	2.85
AJ69F	-----	0.489	de	ef	0.170[4]	--	--	2.88
AP69	-----	0.504	de	f	0.160[4]	--	--	3.15
AP69F	-----	0.509	e	f	0.163[4]	--	--	3.12
AJ6910/7	-----	0.557	f	g	0.205	c	de	2.77
AJ69UK	-----	0.579	g	gh	0.165	b	bcd	3.51
AJ69UKF	-----	0.602	h	h	0.195	b	d	5.04
AJ6910/2	-----	0.699	i	i	0.248	d	e	2.79
AJ6910/8	-----	0.876	j	j	0.287[4]	--	--	3.05
AN69	-----	1.300	k	k	0.463	e	f	2.67

[1]Essences were diluted 1.0 ml to 25.0 ml with water.

[2]Mean values of 3 determinations.

[3]Like letters denote no significant difference among essences.

[4]No point of inflection or peak was present. This value was used only to compute the absorbance ratio and was not included in the statistical analysis.

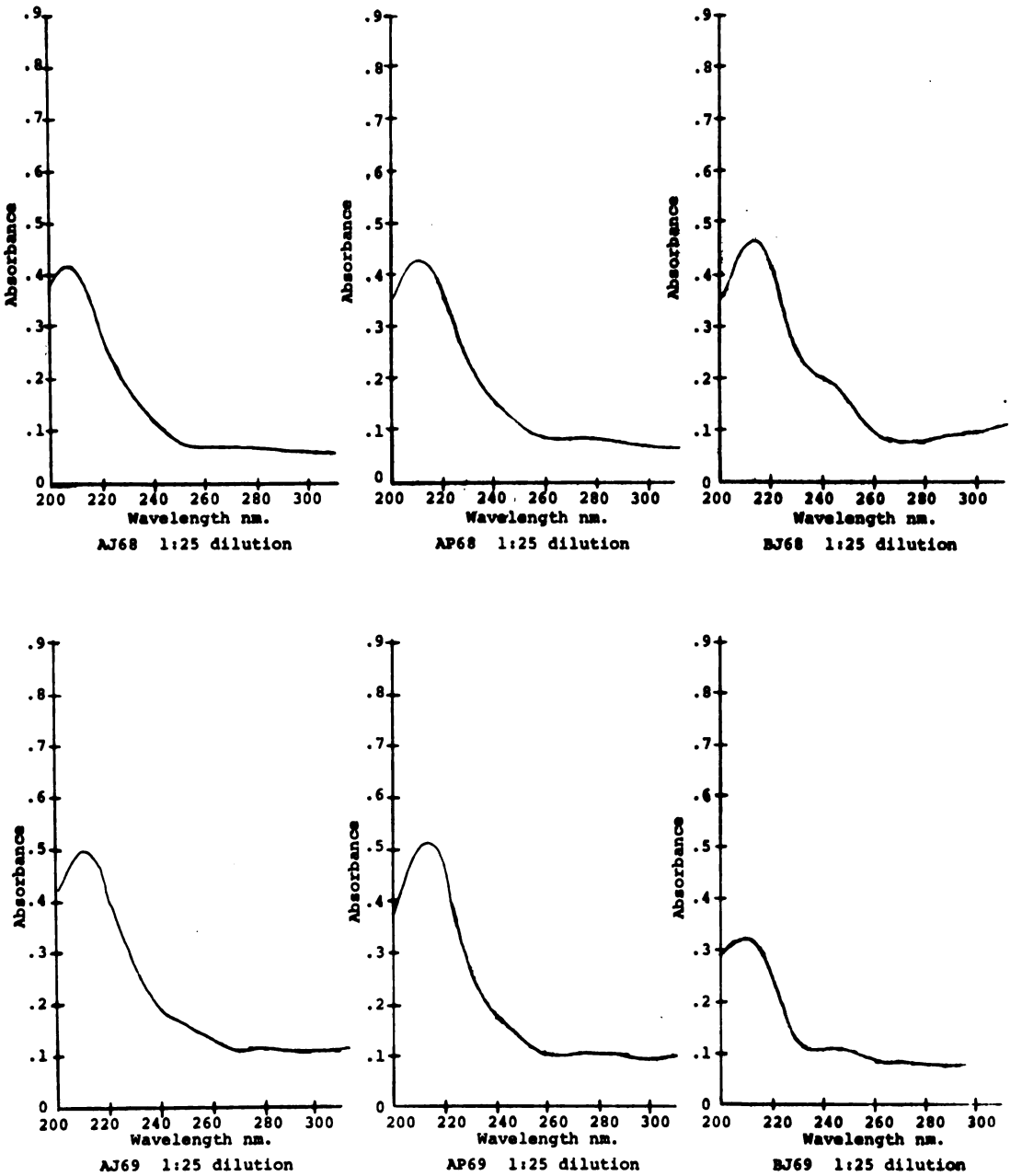


Figure 2. Ultraviolet absorption spectra of 6 dilute essences.

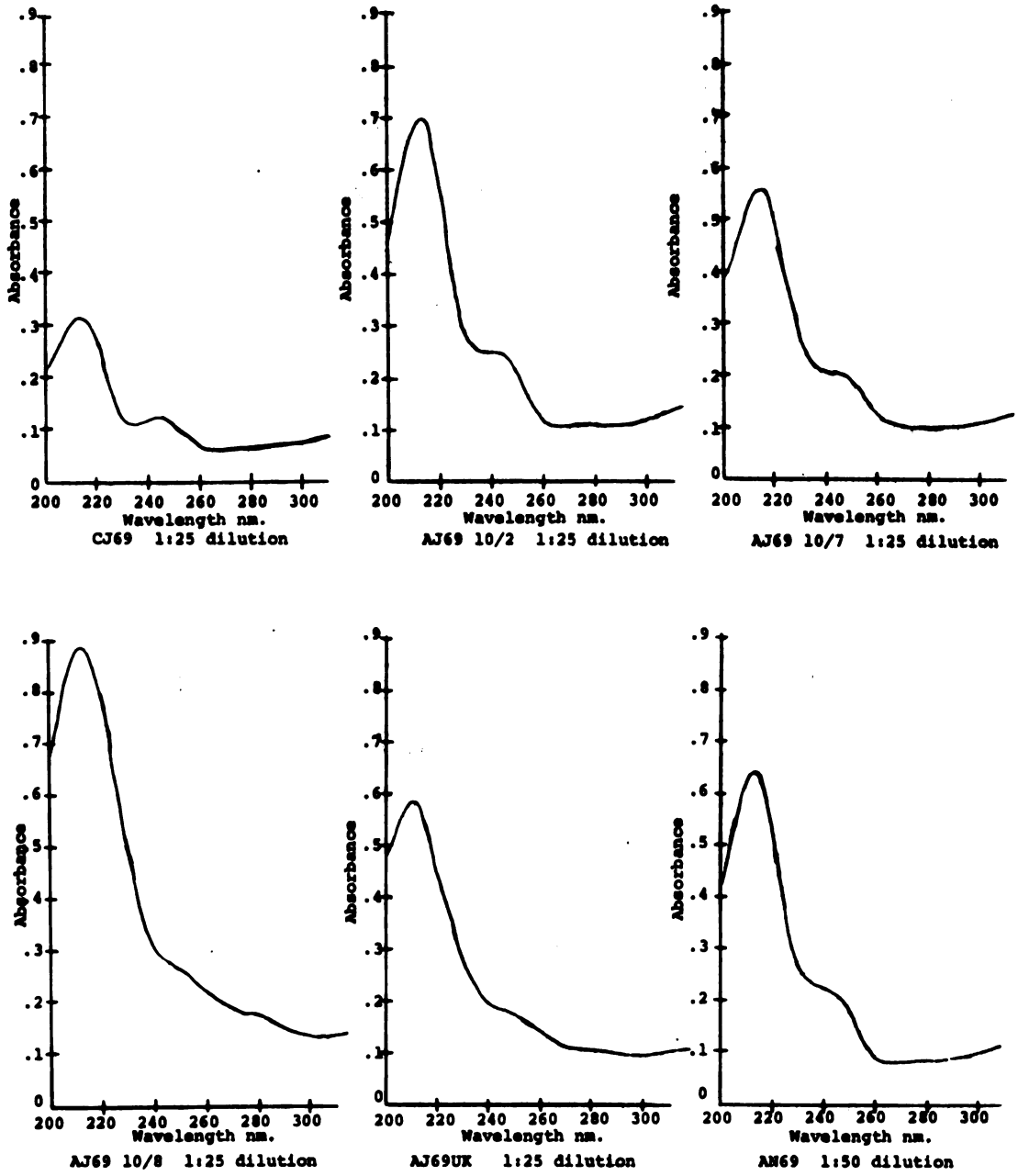


Figure 3. Ultraviolet absorption spectra of 6 dilute essences.

this study, the slight absorption at 243 nm was reported by Holley et al.

Absorption in the 200-300 nm region is one of very strong absorbance by conjugated unsaturation and/or unsaturated aldehydes (Roberts and Caserio, 1965). Neudoreffer (1965) reported the presence of acetoin and crotonal in his study of Concord grape essence. Thus it is possible that peaks in the 200-300 nm region could be attributed to the presence of one or both of these compounds or to compounds of similar structure.

Gas-solid chromatography: Compounds in the headspace over each essence which were separated and quantitated using gas-solid chromatography are listed in Table 11. In all, 7 peaks, ie. peaks A-D, F, H, and I, were positively identified via mass spectrometry, retention times, and essence enrichment. Formaldehyde and n-propanol, peaks E and G respectively, were not present over the essences in quantities sufficient to permit proper m/e analysis and were identified using retention times and essence enrichment only. Peaks K and L were not identified but were due to compounds of known masses. Peak K represented a compound of mass 70 while peak L represented a mixture of compounds of masses 88 and 116. Further identification was not possible because of instrument limitations. Figures 4 and 5 illustrate typical mass spectra obtained.

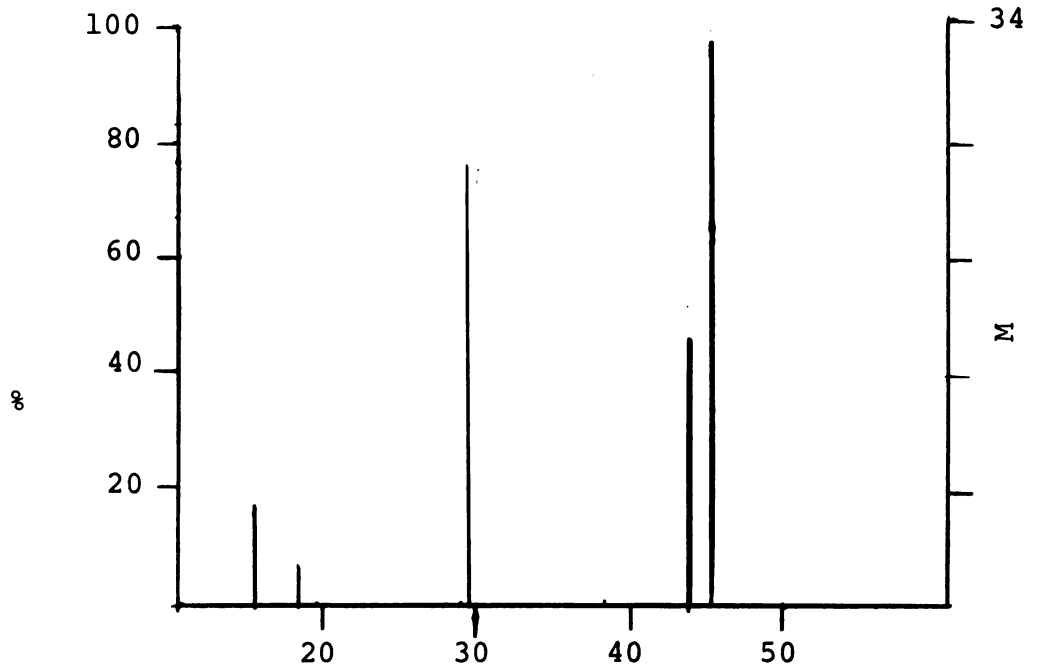


Figure 4. Mass spectrum of gas-solid chromatographic peak B, acetaldehyde.

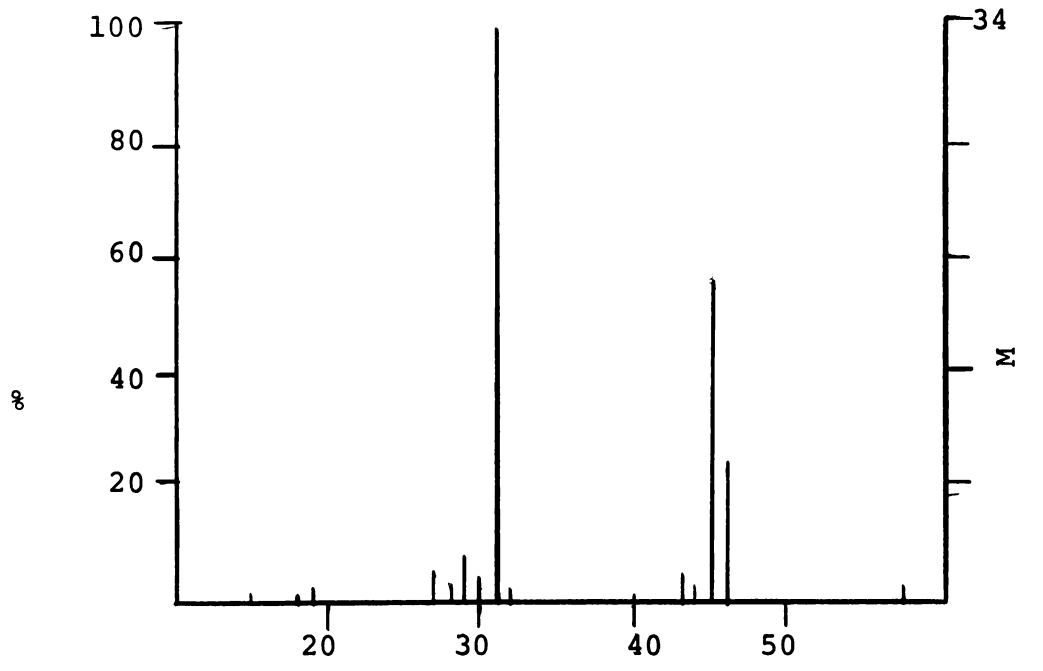


Figure 5. Mass spectrum of gas-solid chromatographic peak C, ethanol.

Table 11. Gas-solid chromatographic peak identities.

Peak	Compound	Peak	Compound
A	Methanol	G	n-Propanol
B	Acetaldehyde	H	Ethyl acetate
C	Ethanol	I	Iso-butanol
D	Acetone	J	*
E	Formaldehyde	K	*
F	Methyl acetate	L	*

*Not identified.

Figures 6 through 17 illustrate gas chromatographic separations for each essence headspace. Numbers at the tip of many peaks indicate the factor (attenuation) by which the area of that peak must be multiplied to normalize its area with that of the non-numbered peaks; the greatest instrument sensitivity used for any given essence analysis was range 10^2 and attenuation 4. Each peak is expressed as mean percentage total Disc integrator count in Table 12. No adjustments were made to account for sensitivity differences the flame detectors have for the various compounds quantitated.

Headspace vapor samples are the simplest, and most precise method of sampling a food aroma for the chemical analysis. The aroma of a particular food product depends not only upon the qualitative nature of the compounds in

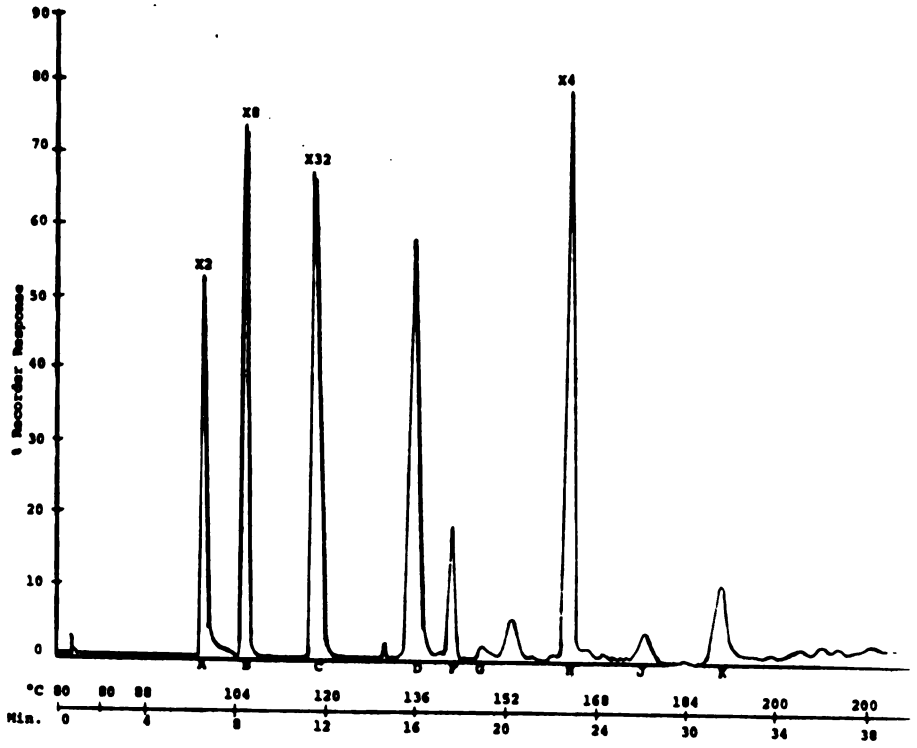


Figure 6. Gas-chromatographic separation of headspace volatiles over essence AJ68.

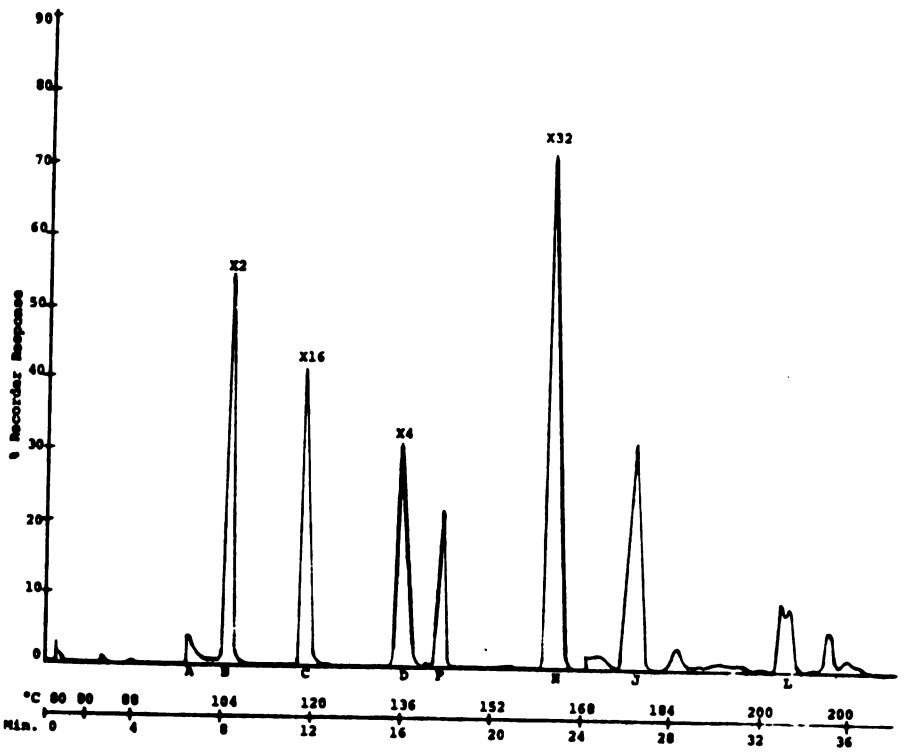


Figure 7. Gas-solid chromatographic separation of headspace volatiles over essence AP68.

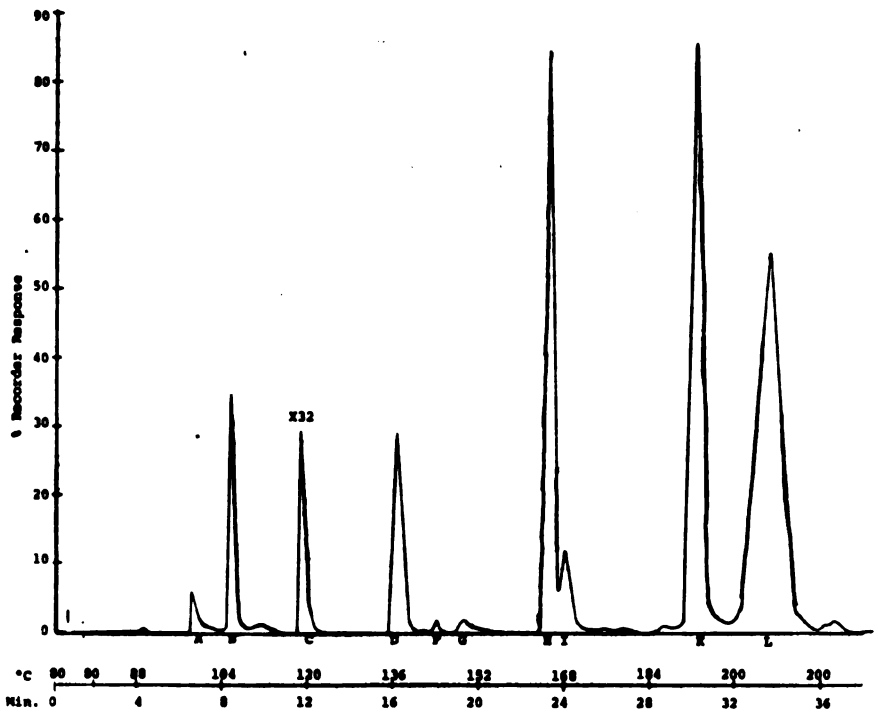


Figure 8. Gas-solid chromatographic separation of headspace volatiles over essence BJ68.

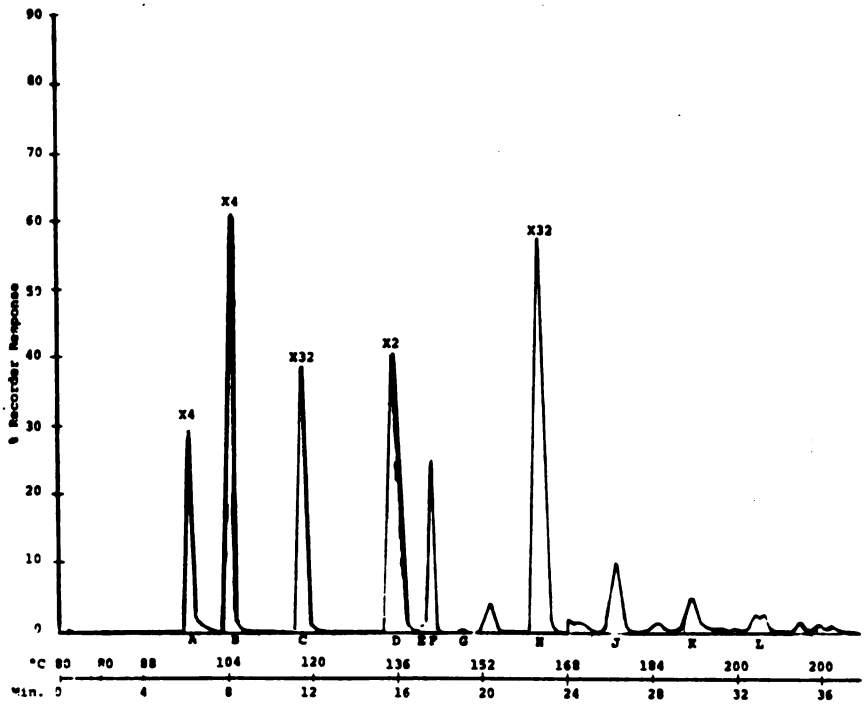


Figure 9. Gas-solid chromatographic separation of headspace volatiles over essence AJ69.

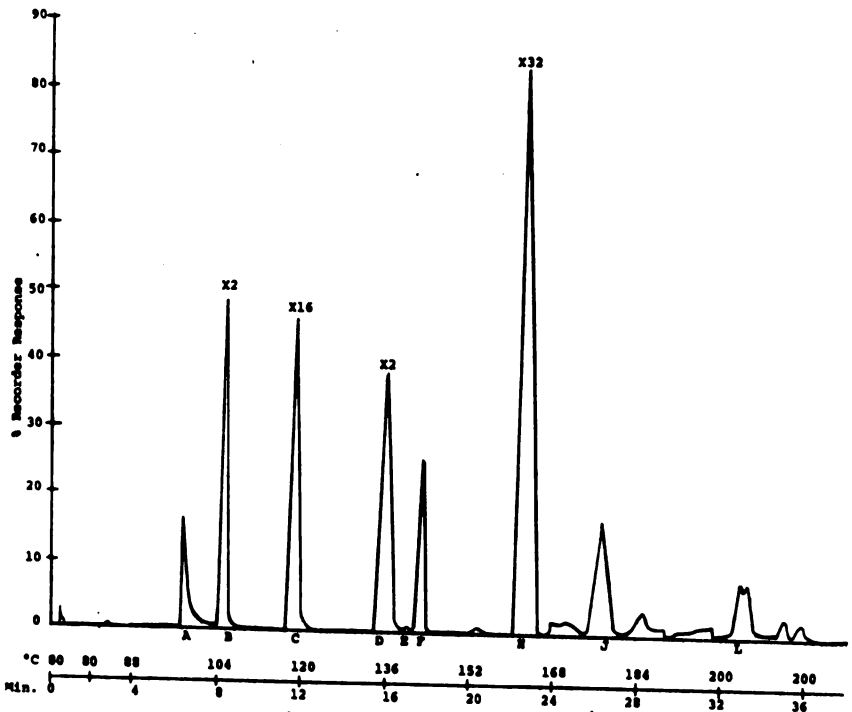


Figure 10. Gas-solid chromatographic separation of headspace volatiles over essence AP69.

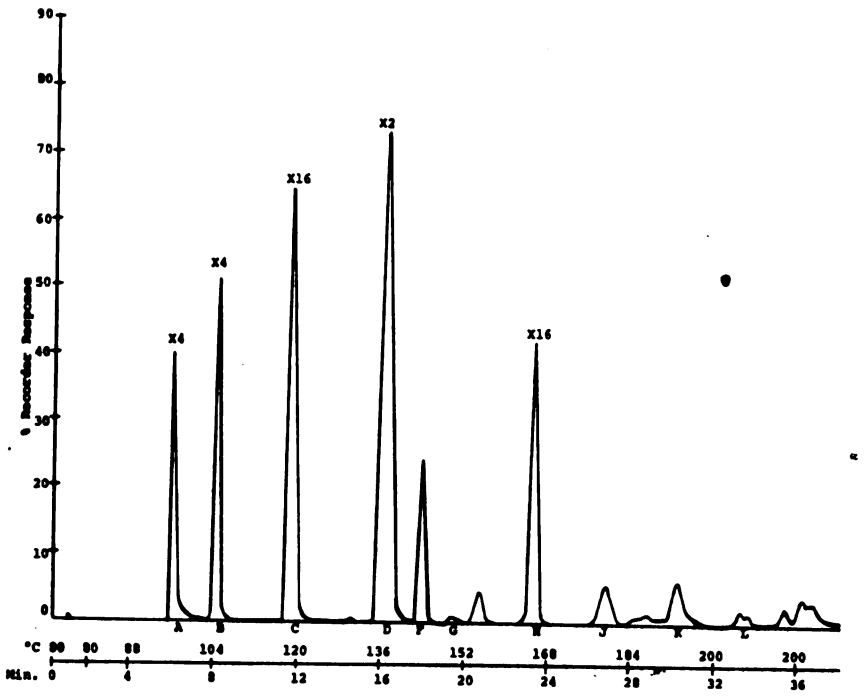


Figure 11. Gas-solid chromatographic separation of headspace volatiles over essence AN69.

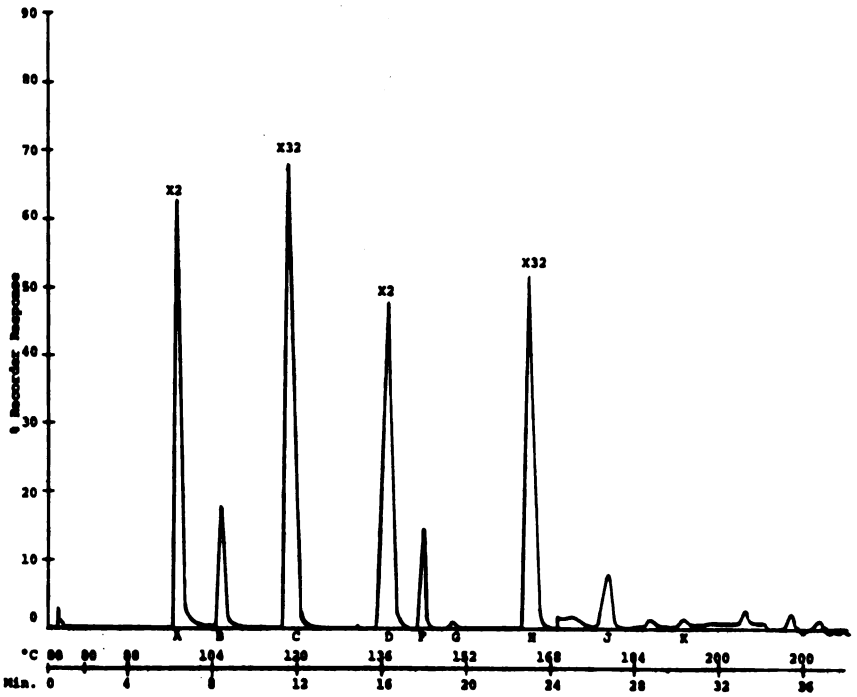


Figure 12. Gas-solid chromatographic separation of headspace volatiles over essence BJ69.

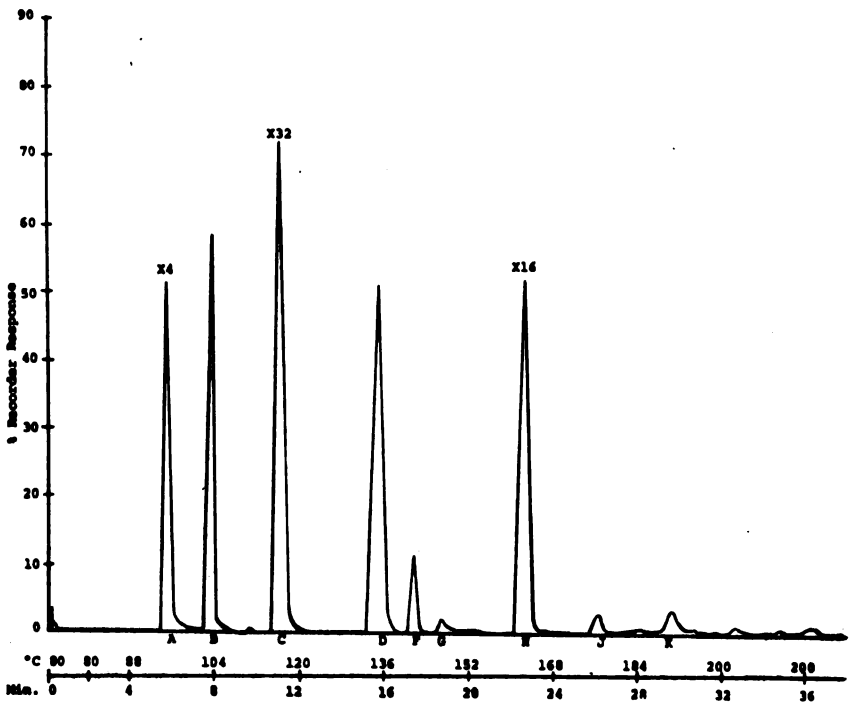


Figure 13. Gas-chromatographic separation of headspace volatiles over essence CJ69.

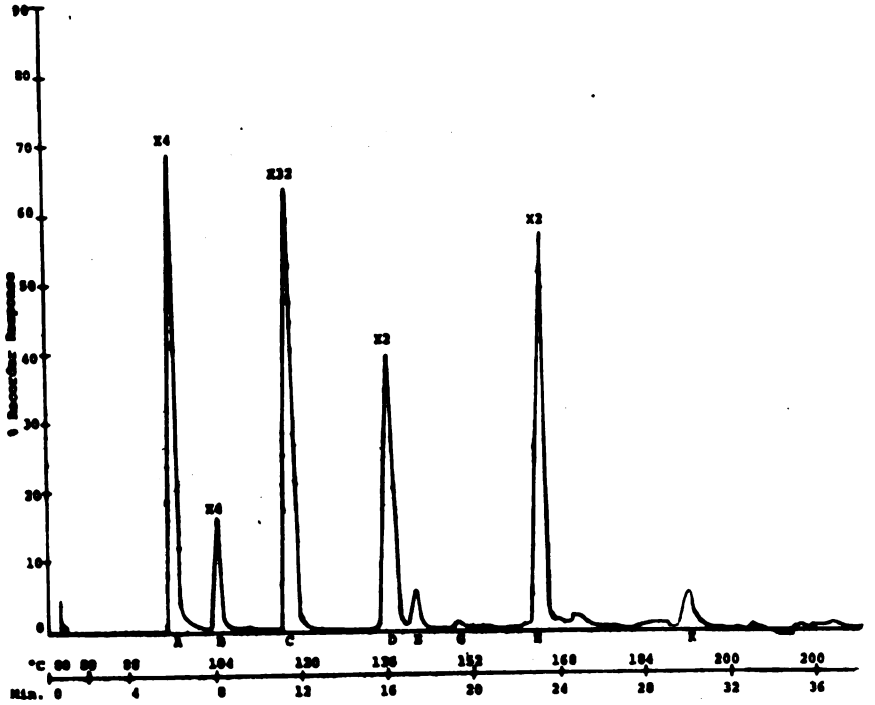


Figure 14. Gas-chromatographic separation of headspace volatiles over essence AJ6910/2.

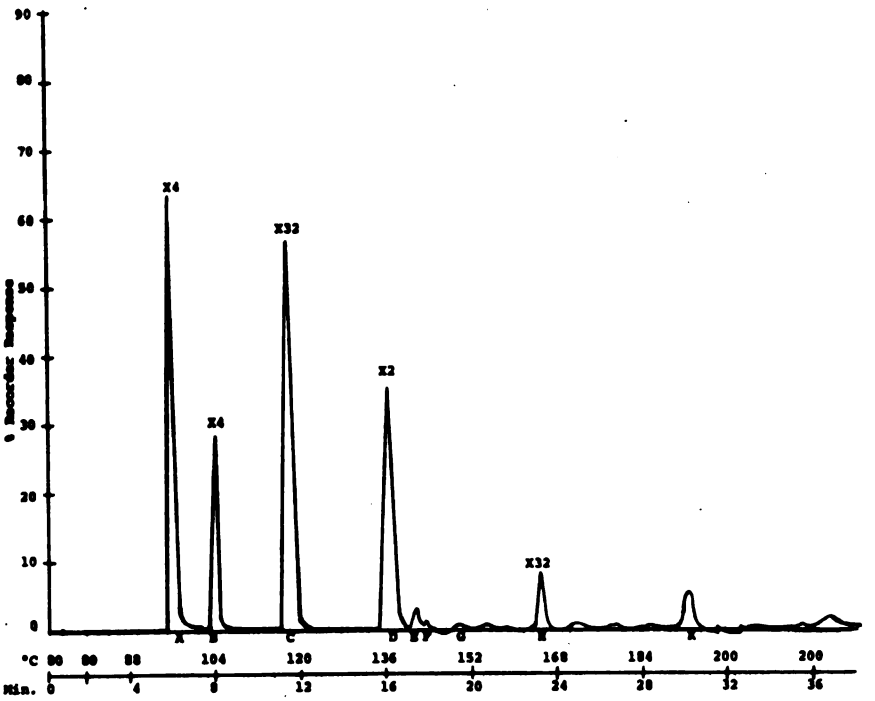


Figure 15. Gas-chromatographic separation of headspace volatiles over essence AJ6910/7.

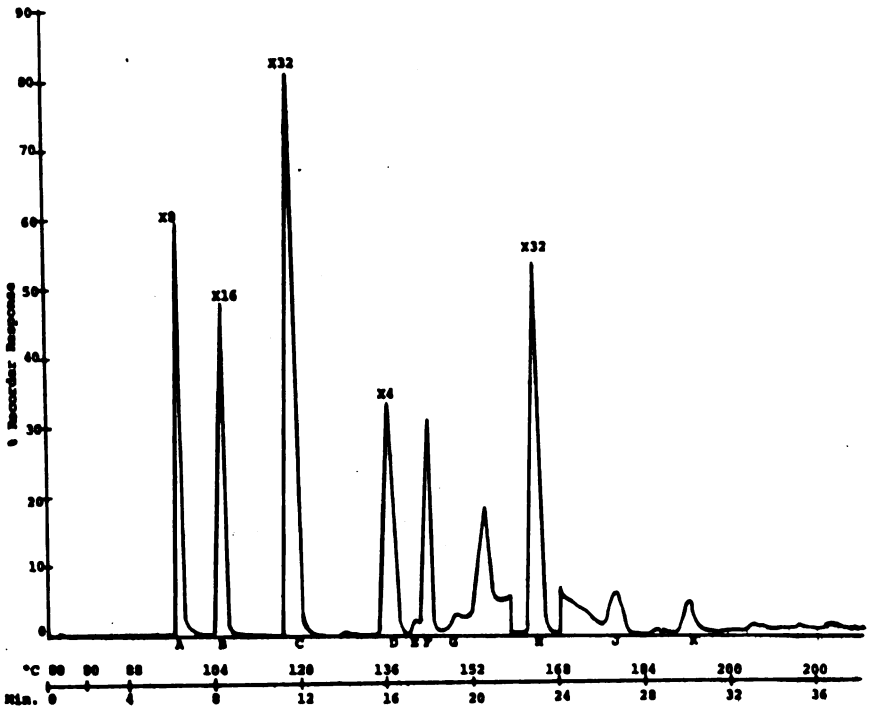


Figure 16. Gas-chromatographic separation of headspace volatiles over essence AJ6910/8.

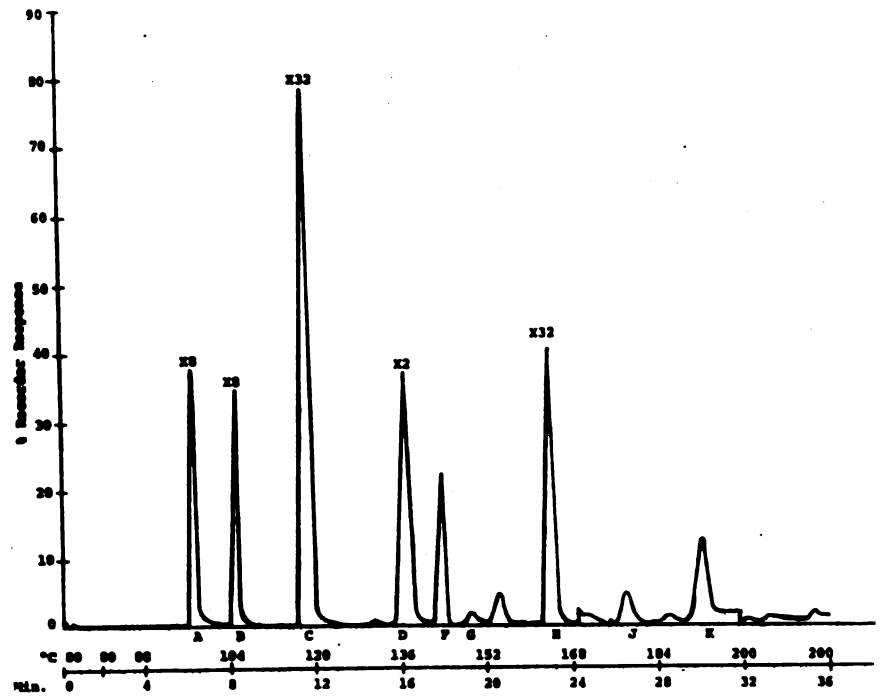


Figure 17. Gas-solid chromatographic separation of headspace volatiles over essence AJ69UK.

Table 12. Integrator count percentages for gas chromatography.

Essence	Peak A Total Count %	A Statistical Significance[1] 5%	A 1%	Peak B Total Count %	B Statistical Significance[1] 5%	B 1%	Peak C Total Count %	C Statistical Significance[1] 5%	C 1%	Peak D Total Count %	D Statistical Significance[1] 5%	D 1%	Peak E Total Count %	E Statistical Significance[1] 5%	E 1%	Peak F Total Count %	F Statistical Significance[1] 5%	F 1%
AJ68[2]	2.35	b	b	12.96	h	h	71.01	hi	g	2.31	a	a	0.00	a	a	0.48	fg	f
AP68[2]	0.32	a	a	1.65	b	b	21.25	b	b	3.86	k	j	0.00	a	a	0.41	ef	def
BJ68[2]	0.61	a	a	1.64	b	b	68.17	h	fg	2.50	e	e	0.00	a	a	0.09	ab	ab
AJ69[2]	3.17	c	c	4.09	de	de	38.44	c	c	2.63	g	g	0.00	a	a	0.41	ef	def
AP69[2]	0.35	a	a	1.46	b	b	15.52	a	ab	2.23	c	c	0.00	a	a	0.43	fg	def
AN69[2]	5.78	e	e	7.06	f	f	45.17	d	d	8.50	l	k	0.00	a	a	0.90	h	g
BJ69[2]	2.00	b	b	0.34	a	a	50.97	e	de	2.70	h	h	0.00	a	a	0.27	cd	c
CJ69[2]	3.84	d	cd	1.43	b	b	62.19	g	f	1.85	a	a	0.00	a	a	0.29	d	cd
AJ6910/2[3]	7.15	f	f	2.33	c	c	81.06	j	h	3.72	j	i	0.18	b	b	0.00	a	a
AJ6910/7[3]	7.06	f	f	3.52	d	d	74.55	i	gh	3.70	j	i	0.14	c	c	0.03	a	a
AJ6910/8[3]	5.45	e	e	9.52	g	g	47.59	de	d	2.57	f	f	0.00	a	a	0.48	fg	f
AJ69UK[3]	4.26	d	d	4.63	e	e	55.59	f	e	2.03	b	b	0.00	a	a	0.32	de	cde
AJ68F[3]	2.28	b	b	13.43	h	h	67.46	h	fg	2.32	d	d	0.00	a	a	0.52	g	f
AP68F[3]	0.17	a	a	1.77	bc	bc	14.88	a	ab	2.71	h	h	0.00	a	a	0.46	fg	ef
AJ69F[3]	2.31	b	b	4.44	e	de	34.97	c	c	2.24	c	c	0.00	a	a	0.30	d	cd
AP69F[3]	0.34	a	a	1.50	b	b	14.00	a	a	2.01	b	b	0.00	a	a	0.46	fg	ef
BJ69F[3]	2.06	b	b	0.39	a	a	50.79	e	de	2.75	i	h	0.00	a	a	0.24	cd	c
CJ69F[3]	4.22	d	d	1.18	b	ab	69.03	h	fg	1.85	a	a	0.00	a	a	0.18	bc	bc

[1]Like letters denote no significant difference among essences.

[2]Values are means of 2 determinations, replicates "a" only.

[3]Values are single determinations, replicate "a" only.

Table 12. (cont.)

Essence	Peak G Total Count %	G Statistical Significance[1] 5%	G 1%	Peak H Total Count %	H Statistical Significance[1] 5%	H 1%	Peak I Total Count %	I Statistical Significance[1] 5%	I 1%	Peak J Total Count %	J Statistical Significance[1] 5%	J 1%	Peak K Total Count %	K Statistical Significance[1] 5%	K 1%	Peak L Total Count %	L Statistical Significance[1] 5%	L 1%
AJ68[2]	0.06	c	b	9.67	ab	a	0.00	a	a	0.19	cd	bcde	0.55	a	d	0.00	a	a
AP68[2]	0.00	a	a	70.48	h	f	0.00	a	a	1.13	g	h	0.00	a	a	0.43	f	e
BJ68[2]	0.30	d	c	4.57	a	a	1.19	b	b	0.00	a	a	8.45	h	e	12.11	g	f
AJ69[2]	0.02	ab	ab	50.27	g	de	0.00	a	a	0.38	e	f	0.20	ef	bc	0.09	c	b
AP69[2]	0.00	a	a	78.83	i	f	0.00	a	a	0.54	f	g	0.00	a	a	0.33	e	d
AN69[2]	0.03	abc	ab	30.69	de	b	0.00	a	a	0.42	e	f	0.30	f	c	0.18	d	c
BJ69[2]	0.03	abc	ab	43.09	f	cd	0.00	a	a	0.24	d	de	0.04	abc	a	0.00	a	a
CJ69[2]	0.05	bc	ab	24.89	cd	b	0.00	a	a	0.12	b	bc	0.12	bcde	ab	0.00	a	a
AJ6910/2[3]	0.03	abc	ab	5.05	a	a	0.00	a	a	0.00	a	a	0.15	de	abc	0.00	a	a
AJ6910/7[3]	0.03	abc	ab	10.35	ab	a	0.00	a	a	0.00	a	a	0.15	de	abc	0.00	a	a
AJ6910/8[3]	0.04	bc	ab	33.68	e	bc	0.00	a	a	0.14	bc	bcd	0.06	abcd	a	0.00	a	a
AJ69UK[3]	0.03	abc	ab	32.37	e	b	0.00	a	a	0.15	bc	bcde	0.13	cde	ab	0.02	ab	ab
AJ68F[3]	0.05	bc	ab	12.80	b	a	0.00	a	a	0.22	d	cde	0.27	f	bc	0.00	a	a
AP68F[3]	0.00	a	a	77.23	i	f	0.00	a	a	1.10	g	h	0.05	abcd	a	0.44	f	e
AJ69F[3]	0.02	ab	ab	55.79	g	e	0.00	a	a	0.39	e	f	0.08	abcd	a	0.06	bc	ab
AP69F[3]	0.00	a	a	80.58	i	f	0.00	a	a	0.54	f	g	0.00	a	a	0.31	e	d
BJ69F[3]	0.05	bc	ab	43.08	f	cd	0.00	a	a	0.25	d	e	0.02	ab	a	0.00	a	a
CJ69F[3]	0.02	ab	ab	23.16	c	b	0.00	a	a	0.09	b	ab	0.05	abcd	a	0.00	a	a

[1]Like letters denote no significant difference among essences.

[2]Values are means of 2 determinations, replicates "a" only.

[3]Values are single determinations, replicate "a" only.

the product, but also upon their concentration and relative ratios. There is a relationship between the concentration of a specific compound in the vapor phase at a given temperature and the vapor pressure of the compound, the type of medium in which it is distributed, its degree of solubility in the medium, its concentration in the medium, and its miscibility with other organic compounds in the mixture (Nawar, 1966). Kepner et al. (1964) reported a method of quantitation using direct headspace sampling and standard curves prepared from predetermined solvent systems closely resembling the product being examined. However, because of the complexity of the essence system examined in this study, integrator counts for each peak were adjusted for instrument attenuation and expressed as a percentage of the sum total integrator counts for all peaks. The resultant percentages were used as a means of quantitation.

Inspection of Table 12 and Figures 6 through 17 indicate each essence headspace contains virtually the same compounds. Quantities of each do vary considerably among essences although each was supposedly a 150-fold product.

The headspace over essence BJ68 (Figure 6) was rather unusual in respect to the number of major peaks it produced. Peak I was present in this sample while not present in any other. Peaks K and L (two compounds) were

present in other essence headspaces, but in levels several orders of magnitude less than that of BJ68. These two peaks represent unidentified compounds.

The headspace over essence AJ6910/8 (Figure 14) contained something causing a broad "peak" to elute from the column at approximately 160C. This "peak" was not present in any other essence and has been neither identified nor quantitated.

To place the gas-solid chromatographic data in proper perspective, it should be noted that human olfactory sensitivities for many compounds are often much greater than that of a flame ionization detector. Thus, an organoleptically important compound may not even be detected with the flame ionization detector (Flath et al. 1969). Olfactory thresholds are of primary concern in the determination of the relative contribution of any given compound to a products flavor (Guadagni et al. 1963). Guadagni et al. (1966) reported that some of the smaller peaks in gas chromatograms represent compounds giving the greatest odor intensity. Headspace vapor analysis does tend to overemphasize more volatile components present within the system, thus a low threshold compound responsible for the primary flavor of an essence may not be sampled at all (Forss et al., 1967).

Flavor panels: Flavor panel data for selection of a commercially available juice to be used as the reference

in flavor panel evaluation of juices prepared from essences are listed in Table 13. Emphasis for the juice selected as the reference was placed on acceptability as opposed to preference; the preference of any given juice is, by definition, dependent upon the juice with which it is paired in flavor panels. The acceptability test gives a more accurate indication of the intrinsic quality of a juice and is independent of other juices.

Of the juices examined for reference juice use, two possessed highly acceptable flavors and were both produced by manufacturer W. Juice WH, heat processed and bottled, was significantly acceptable on each of three presentations to semi-trained panelists. Similarly, juice WF, a frozen concentrate, was significantly acceptable on each of six presentations to the same panelists. When compared against each other in a paired comparison test, there was no significant difference between the two, thus indicating they were fully equivalent. Because of the ease of preparation of the heat-processed juice for flavor panels, it was chosen as the reference juice. Other juices were significantly inferior than these two in preference and acceptability.

Flavor panel data for flavor difference (relative to the reference juice) and acceptability of juices prepared from each essence are listed in Table 14. The method of scoring used for flavor difference panels was

Table 13. Flavor panel data for reference juice determination.

Juice	Number of Panelists	Number Preferred	Number Acceptable	Number Not Acceptable
Test 1				
XH	18	14*	15*	3
YH		4	9	9
WF	17	13*	13*	4
YH		4	10	7
YH	18	7	9	9
ZF		11	11	7
Test 2				
ZF	19	10	17**	2
WF		9	17**	2
WF	20	11	18**	2
XH		9	11	9
ZF	20	11	13	7
XH		9	7	9
Test 3				
WH	20	15*	19**	1
XH		5	11	9
WF	19	11	14*	5
XH		8	12	7
XH	19	11	10	9
ZF		8	9	10
Test 4				
WF	20	11	16*	4
WH		9	15*	4
ZF	20	6	13	7
WH		14	16*	3
WF	20	14	17**	3
ZF		6	16**	2

*Indicates significance at the 5% level.

**Indicates significance at the 1% level.

Table 14. Flavor panel data for flavor difference and acceptability.

Essence	Number of Panelists	Flavor Difference Score[1]	Number Acceptable	Number Not Acceptable
Panel 1	20			
BJ68		77*	14	6
AJ68		84*	8	12
AP68		87*	12	8
Panel 2	20			
BJ69		64	15*	5
AJ69		77*	13	7
AP69		92*	6	14
Panel 3	20			
None		72*	16*	4
CJ69		67*	17*	3
AN69		82*	7	13
Panel 4	20			
AJ6910/2		77*	15*	5
AJ6910/8		84*	6	14
AJ69UK		79*	16*	4
Panel 5	19			
AJ6910/7		75*	12	7
CJ69F		72*	14	5
AJ69UKF		82*	11	8
Panel 6	20			
AJ68F		88*	6	14
AP68F		75*	14	6
AP69F		89*	7	13
Panel 7	17			
BJ68		67*	10	7
BJ69		63	16**	1
CJ69		60	14*	3
Panel 8	19			
BJ68		72*	14*	5
CJ69		69*	18**	1
BJ69		75*	15*	4

*Indicates significance at the 5% level.

**Indicates significance at the 1% level.

[1]Relative to reference juice WH which was assigned a score of 60.

such that juices having Concord flavor less typical than the reference juice received greater numerical scores than did juices having more typical Concord flavor. For statistical purposes, the reference juice was assigned a flavor difference total of 60 (20 member panel). No attempt was made to determine if sample differences existed between flavor difference scores for juices prepared from essences; it is possible that two juices, one being much less typical than the other, could have maximum flavor difference scores of 100 (20 member panel).

Flavor difference totals indicated that juices prepared from essences were generally less typical in Concord flavor than the reference juice. There were, however, two exceptions from this generalization; essences BJ69 and CJ69 were the only essences tested not significantly different from the reference juice on each occasion presented to the semi-trained panel. These juices were certainly two of the better prepared from essences in this panel series.

The acceptability of these juices, measured in the same series of panels for typicalness of Concord flavor, indicated essences BJ69 and CJ69 acceptable on every occasion they were presented to the panel. These results substantiated the relatively good quality of juices prepared from essences BJ69 and CJ69. Other acceptable juices were made from essences AJ6910/2, AJ69UK, BJ68, and concentrate diluted as usual but with no essence.

The acceptability of juice prepared using no essence whatsoever indicated the concentrate used had greater than minimum threshold concentrations of flavor compounds essential to acceptable Concord grape flavor. Analysis of the stripped concentrate diluted to 15° Brix indicated virtually no methyl anthranilate and approximately 7 ppm total volatile esters (as ethyl acetate). The reference juice contained 2 ppm and 12 ppm methyl anthranilate and total volatile esters respectively. Methyl anthranilate (Scott, 1923, and Clore, 1965) and total esters (Sale and Wilson, 1926) have been implicated as making valuable contributions to Concord grape flavor. However, if these compounds did make valuable contributions to the flavor of the reference juice in their respective concentrations, it is doubtful whether the acceptability of the juice made with stripped concentrate only could be attributed to such low concentrations of these two compounds. This statement can be made only if amounts of these compounds in the stripped concentrate were below threshold levels.

Although juices made from essences were not significantly unacceptable to the flavor panels, neither were most significantly acceptable. This would indicate the presence of compounds in the various essences which, when the essence was added to the concentrate in the usual amounts, was related to a reduction of juice acceptability.

Results of ranking to determine juice preference are listed in Table 15. Juices prepared from essences BJ69 and CJ69 were not significantly different from the reference juice on each of four occasions while juices prepared from essences BJ68 and AJ6910/2 were not significantly different on two occasions. These latter essences made juices having relatively high preference, but were not placed so highly when considered for typicalness of Concord flavor and acceptability. Juices made from essence AJ69 were not significantly different in preference from the reference juice on only one of three occasions.

Although no coefficient of correlation was determined, it was noticed that a trend was developing between the total carbonyl levels of essences and flavor panel results. In general, juices made from essences having relatively high flavor difference totals (low typicalness), low acceptability, and high rank totals (low preference) contained relatively high total carbonyl levels. Thus, an essence containing these high total carbonyl levels was added to the concentrate in amounts such that the essence would contain approximately 300 ppm total carbonyls (as acetone) on a 150-fold basis. Juices were prepared from essence AJ69 in this manner for ranking. Duplicate panels indicated a definite flavor improvement of the juices prepared in this manner; these juices were preferred less in the second panel than in the first (see Table 15).

Table 15. Flavor panel data for preference by ranking.

Juice or Essence	Number of Panelists	Rank		Data Transformation		Statistical Significance[1]	
		Total	Mean	Total	Mean	5%	1%
Panel 1	20						
WH		36	1.80	9.40	0.470	a	a
BJ68		46	2.30	2.66	0.133	ab	ab
AJ68		55	2.75	-3.39	-0.170	bc	ab
AP68		63	3.15	-8.97	-0.449	c	b
Panel 2	20						
WH		36	1.80	9.57	0.479	a	a
BJ69		43	2.15	4.72	0.236	ab	a
AJ69		52	2.60	-1.20	-0.060	b	ab
AP69		69	3.45	-13.09	-0.655	c	b
Panel 3	20						
WH		38	1.90	8.24	0.412	a	a
CJ69		42	2.10	5.45	0.273	a	a
AJ6910/2		51	2.55	-0.60	-0.030	a	ab
AN69		69	3.45	-13.09	-0.655	b	b
Panel 4	20						
WH		31	1.55	12.96	0.648	a	a
AJ6910/7		47	2.35	2.06	0.103	b	ab
AJ69UK		53	2.65	-2.19	-0.110	b	bc
AJ6910/8		69	3.45	-12.53	-0.627	c	c
Panel 5	20						
WH		31	1.55	12.96	0.648	a	a
BJ69		41	2.05	5.79	0.290	b	b
AP68F		61	3.05	-7.98	-0.399	c	c
AJ68F		67	3.35	-11.50	-0.575	c	c
Panel 6	20						
WH		45	2.25	3.52	0.176	a	a
CJ69		47	2.35	1.86	0.093	a	a
BJ69		53	2.65	-1.93	-0.097	a	a
AJ69		55	2.75	-3.39	-0.170	a	a
Panel 7	20						
WH		44	2.20	3.99	0.200	a	a
No essence		45	2.25	3.26	0.163	a	a
BJ68		48	2.40	1.46	0.073	a	a
AJ6910/2		63	3.15	-8.71	-0.436	a	a

[1]Like letters denote no significant difference among samples within panels.

Table 15. (cont.)

Juice or Essence	Number of Panelists	Rank Total	Rank Mean	Data Transformation Total	Data Transformation Mean	Statistical Significance[1] 5%	Statistical Significance[1] 1%
Panel 8	20						
WH		38	1.90	7.34	0.367	a	a
BJ69		45	2.25	3.13	0.157	a	a
CJ69		50	2.50	0.00	0.000	a	ab
AJ69		67	3.35	-11.50	-0.575	b	b
Panel 9	20						
WH		41	2.05	6.05	0.303	a	a
BJ69		45	2.50	3.26	0.163	a	a
CJ69		48	2.40	1.46	0.073	a	a
AJ69		66	3.30	-12.83	-0.642	b	b
Panel 10	20						
WH		39	1.95	7.38	0.369	a	a
0.55 ml AJ69		51	2.55	-0.34	-0.017	a	a
1.10 ml AJ69		54	2.70	-2.79	-0.140	a	a
0.00 ml AJ69		56	2.80	-4.25	-0.213	a	a
Panel 11	20						
WH		28	1.40	15.02	0.751	a	a
0.55 ml AJ69		52	2.60	-1.20	-0.060	b	b
0.00 ml AJ69		53	2.65	-2.19	-0.110	b	b
1.10 ml AJ69		67	3.35	-11.63	-0.582	c	b
Panel 12	20						
0.53 ml AN69		39	1.95	7.38	0.369	a	a
CJ69		43	2.15	4.72	0.236	ab	a
0.23 ml AJ68		49	2.45	0.60	0.030	b	a
1.00 ml AP69		69	3.45	-12.70	-0.635	c	b
Panel 13 (Consumer panel)	52						
CJ69		111	2.13	13.09	0.267	a	a
YH		132	2.54	-1.59	-0.039	a	a
0.55 ml AJ69		135	2.65	-5.84	-0.112	a	a
AJ69		139	2.57	-5.66	-0.109	a	a

[1]Like letters denote no significant difference among samples within panels.

A similar experiment was conducted by preparing juices from essences AJ68, AN69, and AP69 as described above; juice prepared from essence CJ69 was used as the reference in a ranking panel. The juices of essences AJ68 and AN69 were not significantly different from the CJ69 juice while the juice of essence AP69 was different. Some factor other than total carbonyl level, adversely related to flavor preference, was evidently present in essence AP69. This essence did contain exceptionally high total ester and ethyl acetate levels, a possible explanation of its juice having relatively low preference.

Fifty-two untrained and inexperienced panelists were solicited from the author's apartment complex for participation in a consumer flavor panel. They were given commercial juice YH and juices prepared from essences CJ69 and AJ69 and asked to indicate their preference by ranking. This combination of juices was chosen for several reasons. Juice YH, previously examined for reference use, was a commercial juice of relatively low acceptability, juice prepared from essence CJ69 was one of the better juices examined while juice prepared from essence AJ69 was not particularly favorable to semi-trained panelists. The latter essence was served to the untrained panelists as juices prepared using 2.0 ml and 0.55 ml essence per 300 ml final volume single strength juice respectively. The

results indicated that consumer preference for juices prepared from these essences is well within the range of products presently available to the consumer in supermarkets.

Chemical test interrelationships: Chemical test data were paired for each essence and inspected for possible interrelationships.

Coefficients of correlation (Amerine, 1965b) were calculated only for those pairings which appeared related. These coefficients are listed in Table 16.

Although many coefficients of correlation were significantly different from r=0 ($t=[1-r^2]/[n-2]$, n-2 degrees of freedom, where n=the number of data pairs, [Amerine, 1965b]), no practical significance should be placed on many, particularly those between r=-0.6 and r=0.6. Least squares regression lines were calculated for data with coefficients significantly different from r=0. The contribution of data to the linear portion of the line may be represented by r^2 (Mendenhall, 1967b). Any r approaching zero from $\pm$ 0.7, for example, would indicate less than 50 per cent of data points significantly contributing to the linear portion of the line. Thus, the standard error of estimate (Little, 1966) for the regression line was calculated and included in Table 16. Significant correlation coefficients do not necessarily indicate cause/effect relationships.

Table 16. Chemical vs chemical correlations for the essences.

Correlation	n Pairs	Coefficient of Correlation	Regression Equation
Total carbonyl vs Ultraviolet absorbance (210-213 nm)	16	0.474*	Y=1646X-157.7±676.5
Total carbonyl vs TLC acetaldehyde[1]	19	0.904**	Y=0.636X-1.560±4.927
Total carbonyl vs TLC acetone[1]	19	-0.195	
Total carbonyl vs TLC acetaldehyde+TLC acetone[1]	19	0.880**	Y=0.598X+2.272±6.517
Total carbonyl vs GSC acetaldehyde	17	0.947**	Y=56.5X-91.0±1.84
TLC acetaldehyde vs GSC acetalcehyde	17	0.862**	Y=0.010X+0.440±2.90
TLC acetone vs GSC acetone	18	0.353	
Total esters vs GSC ethyl acetate	18	0.773**	Y=0.00607X+11.62±30.79
Total esters vs GSC ethyl acetate+GSC methyl acetate	18	0.771**	Y=0.00609X+11.86±31.13

[1]Indicates values converted to μ moles/ml to facilitate calculations.

*Indicates significance at the 5% level using Student's t test, one tailed.

**Indicates significance at the 1% level using Student's t test, one tailed.

A significant coefficient (r=0.474) was calculated for total carbonyl vs ultraviolet absorbance at 210-213 nm. This indicated a possible contribution of unsaturated aldehydes to total carbonyl levels (Roberts and Caserio, 1965). Total carbonyl levels were closely related to acetaldehyde levels as measured by both thin layer (r=0.904) and gas-solid (r=0.947) chromatography. Acetone was not related to total carbonyl levels (r=-0.195) and thus made no major total carbonyl contributions.

A good correlation existed between thin layer and gas-solid chromatographic determinations of acetaldehyde (r=0.862). This indicated that either method could be used to determine acetaldehyde levels of essences as those levels in essence headspaces were positively related to levels within the respective essences. This relationship did not exist, however, for acetone (r=0.353). Acetone volatility was evidently affected by a factor or factors within the essence causing inconsistencies between headspace and liquid essence concentrations.

Liquid essence total ester levels were related to headspace ethyl acetate (r=0.773) and methyl acetate + ethyl acetate levels (r=0.771). This was reasonable as ethyl acetate has been reported as the single most abundant ester in Concord grape essence (Holley et al., 1955).

No other significant correlations between the various chemical determinations were apparent.

Flavor panel vs chemical relationships: Flavor difference totals and per cent acceptability scores were inspected for possible significant correlations with chemical test data. Where apparent relationships existed, coefficients of correlation were calculated and listed in Table 17. Coefficients were treated similar to those of chemical interrelationships. Rank total scores were not considered for correlation since scores are not independent of other juices judged in the same panel. There was a general lack of significant correlation between flavor panel data and chemical data as only one coefficient (% acceptable vs ultraviolet absorbance at 243 nm) exceeded $r=0.8$.

Even though no apparent relationship existed, the coefficient for flavor panel results vs methyl anthranilate was determined; considerable attention has been given to connecting this compound with Concord grape flavor. The concentration of this compound when paired with flavor difference totals and per cent acceptability scores yielded coefficients of $r=-0.073$ and $r=-0.104$ respectively. This, however, did not necessarily conflict with reports in the literature relating this compound to Concord grape flavor. Clore (1965), for example, pointed out that methyl anthranilate is a threshold factor in Concord flavor. Thus, threshold levels could be reached at very low methyl anthranilate concentrations; amounts exceeding the

Table 17. Flavor vs chemical correlations for the essences.

Correlation	n Pairs	Coefficient of Correlation	Regression Equation
% Acceptable vs Methyl anthranilate	17	-0.104	
Flavor difference score vs Methyl anthranilate	17	-0.073	
% Acceptable vs Total esters	17	-0.319	
Flavor difference score vs Total esters	17	0.348	
% Acceptable vs Ultraviolet absorbance (210-213 nm)	14	-0.535*	Y=-0.00437X+0.785±0.483
Flavor difference score vs Ultraviolet absorbance	14	0.485*	Y=0.00961X-0.243±0.500
% Acceptable vs Ultraviolet absorbance (243 nm)	8	-0.854**	Y=0.00407X-0.131±0.104
Flavor difference score vs Ultraviolet absorbance (243 nm)	8	0.712**	Y=0.00637X-0.302±0.141
% Acceptable vs Ultraviolet absorbance (210-213 nm/243 nm)	14	-0.168	
Flavor difference score vs Ultraviolet absorbance (210-213 nm/243 nm)	14	0.316	

% Acceptable vs Chemical oxygen demand	16	0.447*	Y=728X+22,727±69,704
Flavor difference score vs Chemical oxygen demand	16	-0.451*	Y=-1870X+213,913±69,547
% Acceptable vs Total carbonyl	16	-0.256	
Flavor difference score vs Total carbonyl	16	0.423	
% Acceptable vs TLC acetaldehyde	15	-0.393	
Flavor difference score vs TLC acetaldehyde	15	0.250	
% Acceptable vs GSC acetaldehyde	16	-0.609**	Y=-0.123X+11.39±5.02
Flavor difference total vs GSC acetaldehyde	16	0.436*	Y=0.238X-17.26±5.70
% Acceptable vs GSC methyl acetate	16	-0.627**	Y=-0.00644X+0.742±0.347
Flavor difference score vs GSC methyl acetate	16	0.513	Y=0.0147X+0.806±0.383

*Indicates significance at the 5% level using Student's t test, one tailed.

**Indicates significance at the 1% level using Student's t test, one tailed.

threshold concentration do not add to and possibly even degrade the flavor of these essences. Thresholds were not examined in this study.

No significant correlations were found between flavor panel and total ester results. However, a trend indicated that greater total ester levels in essences tended to produce juices poorer in flavor quality.

Absorbance in the 210-213 nm range of the ultraviolet spectrum correlated significantly with both acceptability ($r=-0.535$) and flavor difference data ($r=0.485$). Although the regression line attached to this data had a relatively large error term, the line indicated a general depletion of flavor quality with increased absorption at this wavelength. Ultraviolet absorption at 243 nm correlated with flavor panel results better than other chemical data (acceptability, $r=-0.854$; flavor difference, $r=0.712$). Again, an inverse relationship between absorption and overall flavor quality existed, indicating the possible importance of unsaturated aldehydes.

The ratio of the absorptions of each essence at the above wavelengths was determined and when paired with acceptability and flavor difference data of flavor panels, no significant correlations existed ($r=-0.168$ and $r=0.316$ respectively).

Significant correlation coefficients resulted when chemical oxygen demands were paired with flavor difference

totals (r=-0.451) and acceptability scores (r=0.447). Unlike the trend of other flavor/chemical correlations, chemical oxygen demand showed a general increase with essence flavor quality. This indicated a positive, but not necessarily absolute, relationship between total oxidizable organics and general essence flavor quality. This trend was in agreement with Charley (1962), who reported a very good relationship between "fold" and chemical oxygen demand. It was mentioned, however, that this relationship is not necessarily related on a flavor quality basis.

Although an inverse trend did exist between total carbonyl levels and general flavor quality, no significant correlations were present (acceptability, r=-0.256; flavor difference, r=0.423). Those essences with relatively high total carbonyl levels were generally inferior in flavor quality to those having relatively low total carbonyl levels.

Thin layer acetaldehyde data did not correlate significantly with flavor analysis (acceptability r=-0.393; flavor difference r=0.250). However, gas chromatographic acetaldehyde data did yield significant coefficients with flavor difference totals (r=0.436) and acceptability scores (r=-0.609). Both of these analyses showed the same trend as total carbonyl data.

Correlation of gas chromatographic methyl acetate data with flavor panel results showed significant

coefficients (acceptability, r=-0.627; flavor difference, r=-0.513). Similar to other individual compound data, an inverse relationship existed between methyl acetate levels in essence headspace and overall flavor quality.

SUMMARY AND CONCLUSIONS

The range of compound quantities found in the essences of this study was truly remarkable if one considers that each essence was labelled as 150-fold by its respective manufacturer. For example, methyl anthranilate was found in concentrations ranging from 4 to 126 ppm. Total esters were present in amounts ranging from 100 to 11,400 ppm (as ethyl acetate); total carbonyls ranged from 250 to 6600 ppm (as acetone). Any or all of these variations could be possible and total organic carbon count, ie. chemical oxygen demand, could remain relatively constant. However, in these essences, even chemical oxygen demands ranged from 20,000 to in excess of 200,000 ppm.

Volatile fractions of daily production samples taken within the same week for essence AJ69 yielded chemical oxygen demands ranging from 70,000 to more than 200,000 ppm, total ester levels from 250 to 11,400 ppm, total carbonyl levels from 300 to 6600 ppm, and methyl anthranilate levels from 28 to 78 ppm. For these series of samples, total ester, total carbonyl, acetaldehyde, and chemical oxygen demand increased together, showing a positive relationship among these factors.

Considering these variations, it is not difficult to understand essence add-back problems encountered by the end users of these products. Grapes themselves, the raw material of essence manufacture, undoubtedly contributed to these variations. But certainly the largest variations were introduced by non-standardized production practices.

Chemical-chemical interrelationships indicated acetaldehyde as the single most abundant contributor to total carbonyl values. The generally high acetaldehyde levels found in the lower flavor quality essences could have been derived from products of spontaneous fermentation of grapes and/or juices prior to essence stripping. Neudoerffer et al. (1965) reported relatively high levels of acetone and acetaldehyde in Concord essences having undesirable flavor anomolies.

Many of the more prominent recorder responses in gas chromatograms of headspace vapors over the essences were from the presence of ethyl acetate. This compound was correlated (1% level of significance) with total volatile ester levels in the liquid portion of the essences and was reported by Holley et al. (1955) as being the single most predominent ester present in Concord grape essence. Neudoerffer et al. (1965) also reported relatively large amounts of ethyl acetate in the Concord essences they studied.

Correlations of chemical data with flavor panel results were generally not very good. Of all correlations, ultraviolet absorbance at 243 nm had the highest coefficient ($r=-0.854$) with acceptability scores. Absorbance at this wavelength was only prevalent in those essences ranking relatively well in taste panels. Thus, absorbance at this wavelength would appear mandatory for an essence to be of high flavor quality.

Flavor panel analysis of juices tested indicated stripped concentrate diluted to 15.5-16.0° Brix was quite acceptable to flavor panelists. This juice was not as typical in Concord flavor as the reference juice but ranked with it in preference. Therefore, in general, the addition of essences to the stripped concentrate caused a degradation of the flavor quality of the diluted concentrate. This conclusion is quite reasonable, particularly if the essences possessing poorer flavor qualities were prepared from juices or purees which had undergone various degrees of spontaneous fermentation. Undesirable fermentation products could have been stripped from the juice and into the essence, thus making the essences poor and the concentrate good in flavor quality.

Several trends were evident in the chemical-flavor comparisons. These are as follows:

1. The level of methyl anthranilate in the essences apparently did not affect flavor typicalness,

acceptability, or preference of juices made from these essences.

2. As essence total volatile esters increased, general flavor quality in terms of flavor difference scores, acceptability, and preference for juices made from these essences decreased.

3. Chemical oxygen demand gave a general indication of flavor quality, in a positive, but not absolute, manner.

4. As acetaldehyde and/or total carbonyl levels increased, general flavor quality decreased.

The basic conclusion of this study was that no single component of Concord grape essence, using methods described, can be measured quantitatively and used to determine essence quality in terms of flavor enhancement capacity for Concord grape products. An intricate balance of components within the essence seemed necessary for high flavor quality and may best be measured by headspace vapor analysis via gas chromatography. Similar conclusions were reported for other food products by Wolford et al. (1963), Heinz et al. (1964) and Powers (1968).

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APPENDIX

Table 18. Methyl anthranilate standard curve.

Methyl Anthranilate µg/50 ml	Solution/Replicate				
	1/a	1/b	2/a	3/a	3/b
	Absorbance at 490 nm*				
0	0.000	0.000	0.000	0.000	0.000
10	0.036	0.034	0.030	0.034	0.035
20	0.068	0.066	0.068	0.069	0.072
30	0.102	0.102	0.101	0.098	0.102
40	0.138	0.134	0.143	---	0.141
50	0.174	0.157	0.163	0.172	0.169
60	0.201	0.206	0.204	0.196	0.208
70	0.246	0.237	0.238	0.246	0.246

$r = 0.999$ $\quad Y = 0.0035 X - 0.0032 \pm 0.0066$

*10 mm colorimeter tubes

Table 19. Methyl anthranilate data for the volatile fraction of the essences.

Essence	Replicate	Distillate 1	2	3	Essence	Replicate	Distillate 1
		ppm MA					ppm MA
AJ68	a	4	5	5	AJ6910/8	a	46
	b	4	4	4		b	47
AP68	a	11	11	9	AJ69UK	a	28
	b	8	12	11		b	27
BJ68	a	42	50	53	AJ68F	a	0
	b	46	43	47		b	0
AJ69	a	20	20	23	AP68F	a	11
	b	24	23	20		b	11
AP69	a	28	25	28	BJ68F	a	36
	b	25	27	27		b	29
AN69	a	124	119	127	AJ69F	a	21
	b	128	130	128		b	23
BJ69	a	9	9	9	AP69F	a	28
	b	9	9	9		b	30
CJ69	a	31	32	31	BJ69F	a	14
	b	32	33	32		b	14
AJ6910/2	a	78	--	--	CJ69F	a	31
	b	78	--	--		b	36
AJ6910/7	a	52	--	--	AJ69UKF	a	35
	b	56	--	--		b	35

Table 20. Total ester standard curve.

Ethyl Acetate µg/ml distillate	Solution/Replicate					
	1/a	1/b	2/a	2/b	3/a	3/b
	Absorbance at 540 nm*					
0	0.000	0.000	0.000	0.000	0.000	0.000
25	0.078	0.074	0.076	0.071	0.080	0.080
50	0.160	0.153	0.157	0.143	0.153	0.160
75	0.250	0.234	0.242	0.229	0.233	0.238
100	0.323	0.317	0.321	0.308	0.325	0.330
125	0.409	0.392	0.403	0.385	0.409	0.423
150	0.478	0.459	0.491	0.469	0.485	0.498
175	0.561	0.549	0.577	0.542	0.561	0.569
200	0.648	0.624	0.653	0.634	0.624	0.643

$r = 0.999$ $\quad Y = 0.0032 X - 0.001 \pm 0.017$

*16 mm colorimeter tubes

Table 21. Total ester data for the volatile fraction of the essences.

Essence	Replicate	Distillate 1	2	3	Essence	Replicate	Distillate 1
		ppm EA					ppm EA
AJ68	a	900	950	900	AJ6910/8	a	11,400
	b	900	950	900		b	11,400
AP68	a	5850	5850	5800	AJ69UK	a	4550
	b	5750	5750	5800		b	4650
BJ68	a	150	150	150	AJ68F	a	1000
	b	100	100	150		b	1000
AJ69	a	5100	5100	5050	AP68F	a	6250
	b	5100	5150	5150		b	6300
AP69	a	9100	9150	9300	BJ68F	a	100
	b	9150	9050	9100		b	100
AN69	a	1650	1650	1700	AJ69F	a	5450
	b	1700	1700	1700		b	5350
BJ69	a	5350	5350	5400	AP69F	a	8900
	b	5400	5300	5300		b	8800
CJ69	a	2400	2300	2300	BJ69F	a	5650
	b	2300	2300	2300		b	5700
AJ6910/2	a	250	--	--	CJ69F	a	4050
	b	250	--	--		b	4150
AJ6910/7	a	700	--	--	AJ69UKF	a	2600
	b	700	--	--		b	2600

Table 22. Chemical oxygen demand standard curve.

COD ppm	Solution/Replicate			
	1/a	1/b	2/a	2/b
	Absorbance at 650 nm*			
0	0.000	0.000	0.000	0.000
107	0.036	0.036	0.040	0.040
214	0.076	0.076	0.078	0.077
320	0.112	0.118	0.115	0.114
427	0.153	0.152	0.155	0.152
533	0.192	0.191	0.192	0.189
640	0.231	0.231	0.227	0.231
746	0.266	0.270	0.264	0.264
853	0.308	0.305	0.303	0.301
960	0.344	0.342	0.337	0.337

$r = 0.999$ $\quad Y = 0.000355\ X + 0.002 \pm 0.0093$

*16 mm colorimeter tubes

Table 23. Chemical oxygen demand data for the volatile fraction of the essences.

Essence	Replicate	Distillate			Essence	Replicate	Distillate
		1	2	3			1
		ppm COD					ppm COD
AJ68	a	76,700	77,500	76,700	AJ6910/8	a	208,000
	b	77,500	76,700	77,500		b	201,600
AP68	a	22,500	22,250	21,750	AJ69UK	a	107,200
	b	22,150	21,600	22,500		b	107,200
BJ68	a	38,000	38,000	37,750	AJ68F	a	77,600
	b	38,750	37,750	38,500		b	76,500
AJ69	a	68,500	67,500	68,000	AP68F	a	21,300
	b	67,500	69,000	68,500		b	20,000
AP69	a	27,250	28,000	27,000	BJ69F	a	33,800
	b	27,500	27,250	27,250		b	33,700
AN69	a	47,200	47,500	47,500	AJ69F	a	66,000
	b	46,000	47,500	46,600		b	66,000
BJ69	a	85,000	86,600	85,800	AP69F	a	26,400
	b	85,800	85,800	86,600		b	26,650
CJ69	a	102,000	99,400	98,000	BJ69F	a	89,800
	b	99,000	101,000	99,400		b	88,500
AJ6910/2	a	71,300	---	---	CJ69F	a	110,200
	b	71,300	---	---		b	109,000
AJ6910/7	a	72,600	---	---	AJ69UKF	a	105,000
	b	75,500	---	---		b	105,000

Table 24. Total carbonyl standard curve.

Acetone ppm	Solution/Replicate					
	1/a	1/b	2/a	2/b	3/a	3/b
	Absorbance at 480 nm*					
0	0.000	0.000	0.000	0.000	0.000	0.000
5	0.077	0.112	0.088	0.085	0.104	0.063
10	0.116	0.163	0.166	0.137	---	---
15	0.202	0.240	0.235	0.216	0.192	0.206
20	0.305	0.305	0.314	0.297	0.290	0.301
25	0.310	0.367	---	0.364	0.364	0.367
30	0.444	0.435	0.447	0.435	0.406	0.435
35	0.512	0.475	0.505	0.502	0.498	0.505
40	0.573	0.577	0.569	0.542	0.569	0.538

$r = 0.996$ $\quad Y = 0.0139\ X + 0.012 \pm 0.032$

*16 mm colorimeter tubes

Table 25. Total carbonyl data for the volatile fraction of the essences.

Essence	Replicate	Distillate 1	Distillate 2	Distillate 3	Essence	Replicate	Distillate 1
		ppm acetone					ppm acetone
AJ68	a	2150	2360	2240	AJ68F	a	2370
	b	2160	2480	2260		b	2300
	c	2140	2150	2440		c	2370
AP68	a	725	750	750	AP68F	a	590
	b	725	625	625		b	575
	c	760	750	750		c	695
BJ68	a	325	325	375	BJ68F	a	260
	b	300	350	375		b	260
	c	340	340	375		c	325
AJ69	a	1175	1090	1165	AJ69F	a	875
	b	1015	1070	1140		b	955
	c	1150	1070	1050		c	1045
AP69	a	575	640	555	AP69F	a	460
	b	560	610	620		b	535
	c	570	645	590		c	480
AN69	a	1170	1150	1075	BJ69F	a	225
	b	1055	1130	1190		b	230
	c	1165	1055	1175		c	185
BJ69	a	260	235	235	AJ6910/2	a	310
	b	250	260	290		b	350
	c	255	235	290		c	265
CJ69	a	240	285	340	AJ6910/7	a	490
	b	320	325	285		b	425
	c	215	325	350		c	470
AJ6910/8	a	6800	--	--	CJ69F	a	305
	b	6240	--	--		b	610
	c	6800	--	--		c	280
AJ69UK	a	1425	--	--	AJ69UKF	a	1190
	b	1575	--	--		b	1235
	c	1525	--	--		c	1230

Table 26. Acetone 2,4-dinitrophenylhydrazone standard curve.

Derivative µg/3 ml	Solution/Replicate				
	1/b	2/a	2/b	3/a	3/b
	Absorbance at 358 nm*				
0	0.000	0.000	0.000	0.000	0.000
2	0.069	0.056	0.066	0.086	0.072
4	0.097	0.112	0.202	0.176	0.084
6	0.146	0.166	0.161	0.197	0.149
8	0.208	0.224	0.240	0.238	0.199
10	0.262	0.286	0.301	0.299	0.264
12	0.308	0.342	0.325	0.349	0.330
14	0.330	0.409	0.429	0.429	0.347
16	0.516	0.523	0.509	0.465	0.423

$r = 0.979$ $\quad Y = 0.028\ X + 0.009 \pm 0.057$

*10 mm colorimeter tubes

Table 27. Acetaldehyde 2,4-dinitrophenylhydrazone standard curve.

Derivative µg/3 ml	Solution/Replicate			
	1/a	1/b	2/a	2/b
	Absorbance at 352 nm*			
0	0.000	0.000	0.000	0.000
2	0.056	0.054	0.058	0.058
4	0.111	0.112	0.152	0.153
6	0.220	0.184	0.199	0.177
8	0.237	0.252	0.290	0.240
10	0.305	0.319	0.328	0.301
12	0.390	0.380	0.398	0.380
14	0.432	0.429	0.505	0.453
16	0.542	0.545	0.488	0.505

$r = 0.994$ $\quad Y = 0.033\ X - 0.007 \pm 0.035$

*10 mm colorimeter tubes

Table 28. Thin layer chromatographic data for the essences.

Essence	Plate	Extraction			Extraction		
		1	2	3	1	2	3
		ppm acetone			ppm acetaldehyde		
AJ68	1	24	20	24	1271	1236	1106
	2	0	0	24	1495	1240	1298
	3	0	0	0	1361	1298	1291
AP68	1	428	545	378	11	28	54
	2	451	389	347	82	90	1
	3	567	451	402	92	72	53
BJ68	1	338	219	219	19	5	19
	2	414	173	217	39	1	31
	3	451	173	205	19	3	29
AJ69	1	170	175	166	461	467	394
	2	183	197	166	442	391	422
	3	244	183	170	369	414	414
AP69	1	451	424	334	178	257	206
	2	401	377	341	178	225	192
	3	341	334	451	170	237	206
BJ69	1	195	137	144	21	53	31
	2	119	144	149	31	39	58
	3	112	112	112	58	1	27
CJ69	1	49	71	37	220	243	225
	2	95	117	85	170	182	243
	3	110	112	76	196	194	214
AN69	1	254	244	293	394	408	451
	2	312	400	332	408	477	394
	3	332	300	315	451	451	477
AJ6910/2	1	41	--	--	119	--	--
	2	41	--	--	121	--	--
	3	63	--	--	175	--	--
AJ6910/7	1	117	--	--	328	--	--
	2	95	--	--	292	--	--
	3	112	--	--	308	--	--
AJ6910/8	1	0	--	--	2579	--	--
	2	0	--	--	2854	--	--
	3	10	--	--	2999	--	--
AJ68F	1	0	0	0	827	725	662
	2	0	0	0	756	764	725
	3	0	0	0	764	709	764
AP68F	1	244	219	204	200	257	298
	2	229	292	295	255	259	210
	3	254	260	275	308	235	259
BJ68F	1	61	46	61	100	90	64
	2	59	83	37	100	60	129
	3	88	46	49	68	111	60
AJ69F	1	141	144	124	337	420	373
	2	129	180	158	336	394	355
	3	158	129	158	394	355	434
AP69F	1	122	139	168	227	225	259
	2	158	141	119	245	284	253
	3	122	124	129	275	273	253
BJ69F	1	129	197	144	51	39	72
	2	176	202	127	21	82	0
	3	129	202	129	53	0	66
CJ69F	1	0	0	0	19	37	25
	2	0	0	0	64	82	82
	3	0	0	0	64	60	60
AJ69UK	1	222	--	--	844	--	--
	2	176	--	--	844	--	--
	3	244	--	--	758	--	--
AJ69UKF	1	219	--	--	797	--	--
	2	176	--	--	828	--	--
	3	180	--	--	797	--	--

Table 29. Ultraviolet absorbance data for the essences.

Essence[1]	Replicate	Wavelength		
		205 nm	210-213 nm Absorbance	243 nm
AJ68	a	0.420	---	0.100[2]
	b	0.450	---	0.160[2]
	c	0.435	---	0.110[2]
AP68	a	---	0.455	0.185[2]
	b	---	0.425	0.135[2]
	c	---	0.445	0.145[2]
BJ68	a	---	0.460	0.185
	b	---	0.465	0.190
	c	---	0.445	0.155[2]
AJ69	a	---	0.495	0.190[2]
	b	---	0.485	0.180[2]
	c	---	0.475	0.140[2]
AP69	a	---	0.510	0.185[2]
	b	---	0.510	0.170[2]
	c	---	0.490	0.125[2]
BJ69	a	---	0.320	0.120
	b	---	0.320	0.110
	c	---	0.305	0.070
CJ69	a	---	0.315	0.130
	b	---	0.310	0.125
	c	---	0.310	0.100
AJ6910/2	a	---	0.695	0.245
	b	---	0.710	0.275
	c	---	0.690	0.225
AJ6910/7	a	---	0.555	0.205
	b	---	0.545	0.180
	c	---	0.570	0.230[2]
AJ6910/8	a	---	0.880	0.310[2]
	b	---	0.880	0.285[2]
	c	---	0.865	0.265[2]
AJ69UK	a	---	0.580	0.160
	b	---	0.585	0.180
	c	---	0.570	0.155[2]
AJ68F	a	0.440	---	0.110[2]
	b	0.455	---	0.110[2]
	c	0.445	---	0.170[2]
AP68F	a	---	0.455	0.155[2]
	b	---	0.470	0.230[2]
	c	---	0.460	0.195[2]
BJ68F	a	---	0.420	0.165
	b	---	0.435	0.190
	c	---	0.435	0.160[2]
AJ69F	a	---	0.480	0.155[2]
	b	---	0.490	0.165[2]
	c	---	0.495	0.190[2]
AP69F	a	---	0.500	0.145[2]
	b	---	0.505	0.155[2]
	c	---	0.520	0.190[2]
BJ69F	a	---	0.310	0.090
	b	---	0.330	0.140
	c	---	0.320	0.100
CJ69F	a	---	0.315	0.115
	b	---	0.330	0.150
	c	---	0.320	0.110
AJ69UKF	a	---	0.585	0.180
	b	---	0.600	0.215
	c	---	0.620	0.190
AN69	a	---	1.280	0.430
	b	---	1.330	0.520
	c	---	1.290	0.440

[1]Essences were diluted 1.0 ml to 25.0 ml with water.

[2]No point of inflection or peak was present.

Table 30. Gas-solid chromatographic data for the essences.

Essence	Bottle	Replicate	Peak* A	B	C	D	E	F	G	H	I	J	K	L
			Percentage total integrator count											
AJ68	1	a	2.41	13.31	70.35	2.33	0.00	0.49	0.05	9.87	0.00	0.20	0.50	0.00
	1	b	2.59	11.60	73.63	2.18	0.00	0.43	0.04	8.45	0.00	0.16	0.50	0.00
	2	a	2.28	12.61	71.66	2.28	0.00	0.47	0.06	9.46	0.00	0.17	0.59	0.00
	2	b	2.69	9.95	76.59	2.09	0.00	0.35	0.04	7.29	0.00	0.15	0.49	0.00
AP68	1	a	0.39	1.41	23.26	3.71	0.00	0.41	0.00	68.79	0.00	1.09	0.00	0.40
	1	b	0.27	1.36	22.75	4.51	0.00	0.40	0.00	68.82	0.00	1.05	0.00	0.45
	2	a	0.25	1.88	19.24	4.00	0.00	0.41	0.00	72.16	0.00	1.17	0.00	0.45
	2	b	0.22	1.86	18.90	4.25	0.00	0.41	0.00	72.31	0.00	1.14	0.00	0.46
BJ68	1	a	0.59	1.66	67.95	2.60	0.00	0.09	0.27	4.75	1.15	0.00	8.50	12.08
	1	b	0.64	1.53	71.20	2.26	0.00	0.10	0.35	4.81	0.96	0.00	8.24	9.44
	2	a	0.63	1.62	68.38	2.39	0.00	0.09	0.33	4.38	1.22	0.00	8.39	12.14
	2	b	0.80	0.94	71.10	1.86	0.00	0.04	0.28	2.58	1.03	0.00	7.94	9.17
AJ69	1	a	3.04	4.23	37.30	2.63	0.00	0.36	0.01	51.40	0.00	0.38	0.20	0.09
	1	b	4.47	3.26	49.76	2.82	0.00	0.32	0.01	38.54	0.00	0.28	0.19	0.06
	2	a	3.30	3.94	39.57	2.62	0.00	0.46	0.02	49.13	0.00	0.38	0.20	0.08
	2	b	3.49	3.87	40.42	2.56	0.00	0.45	0.05	48.21	0.00	0.36	0.18	0.08
AP69	1	a	0.35	1.47	15.41	2.31	0.00	0.44	0.00	78.83	0.00	0.53	0.00	0.32
	1	b	0.27	1.43	16.79	2.24	0.00	0.43	0.00	77.58	0.00	0.53	0.00	0.32
	2	a	0.34	1.45	15.62	2.14	0.00	0.42	0.00	78.82	0.00	0.54	0.00	0.34
	2	b	0.36	1.47	15.81	2.14	0.00	0.47	0.00	78.69	0.00	0.53	0.00	0.32
AN69	1	a	5.63	7.07	45.12	8.50	0.00	0.91	0.03	30.85	0.00	0.43	0.29	0.19
	1	b	5.68	7.30	44.01	8.45	0.00	0.94	0.03	31.78	0.00	0.44	0.29	0.17
	2	a	5.83	7.04	45.21	8.50	0.00	0.88	0.03	30.53	0.00	0.40	0.30	0.17
	2	b	6.87	6.42	48.80	8.35	0.00	0.80	0.03	27.16	0.00	0.38	0.29	0.15
BJ69	1	a	2.16	0.31	52.83	2.71	0.00	0.25	0.03	41.12	0.00	0.22	0.03	0.00
	1	b	1.92	0.34	49.54	3.26	0.00	0.31	0.03	43.95	0.00	0.28	0.03	0.00
	2	a	1.84	0.36	49.10	2.69	0.00	0.29	0.02	45.05	0.00	0.26	0.04	0.00
	2	b	2.04	0.35	50.53	2.76	0.00	0.27	0.03	43.44	0.00	0.25	0.03	0.00
CJ69	1	a	4.16	1.32	68.84	1.84	0.00	0.26	0.05	20.94	0.00	0.12	0.13	0.00
	1	b	3.78	1.37	65.12	1.93	0.00	0.32	0.05	27.05	0.00	0.11	0.11	0.00
	2	a	3.51	1.53	63.54	1.85	0.00	0.32	0.05	28.84	0.00	0.11	0.11	0.00
	2	b	4.07	1.19	68.18	1.84	0.00	0.27	0.04	24.18	0.00	0.10	0.10	0.00
AJ6910/2	1	a	7.15	2.33	81.06	3.72	0.18	0.00	0.03	5.05	0.00	0.00	0.29	0.00
	1	b	7.53	2.03	80.80	3.87	0.20	0.00	0.04	4.99	0.00	0.00	0.31	0.00
AJ6910/7	1	a	7.06	3.52	74.55	3.70	0.14	0.03	0.03	10.35	0.00	0.00	0.29	0.00
	2	b	6.71	4.26	72.18	3.90	0.18	0.03	0.04	12.03	0.00	0.00	0.31	0.00
AJ6910/8	1	a	5.45	9.52	47.59	2.57	0.00	0.48	0.04	33.68	0.00	0.14	0.11	0.00
	1	b	5.65	9.30	45.00	2.05	0.00	0.47	0.04	33.05	0.00	0.13	0.10	0.00
AJ69UK	1	a	4.26	4.63	55.59	2.03	0.00	0.32	0.03	32.37	0.00	0.15	0.26	0.02
	1	b	4.46	4.32	58.12	1.99	0.00	0.38	0.03	29.90	0.00	0.16	0.37	0.02
AJ68F	1	a	2.28	13.43	67.46	2.32	0.00	0.52	0.05	12.80	0.00	0.22	0.55	0.00
	1	b	2.49	12.78	69.32	2.18	0.00	0.51	0.05	11.73	0.00	0.20	0.41	0.00
AP68F	1	a	0.17	1.77	14.88	2.71	0.00	0.46	0.00	77.23	0.00	0.16	0.10	0.44
	1	b	0.18	1.81	14.63	3.52	0.00	0.48	0.00	77.33	0.00	1.19	0.10	0.48
AJ69F	1	a	2.31	4.44	34.97	2.24	0.00	0.30	0.02	55.79	0.00	0.39	0.16	0.06
	1	b	2.96	4.19	35.29	2.49	0.00	0.52	0.02	53.55	0.00	0.39	0.15	0.14
AP69F	1	a	0.34	1.50	14.00	2.01	0.00	0.46	0.00	80.58	0.00	0.54	0.00	0.31
	1	b	0.32	1.47	14.19	2.03	0.00	0.47	0.00	80.38	0.00	0.53	0.00	0.32
BJ69F	1	a	2.06	0.39	50.78	2.75	0.00	0.24	0.05	43.08	0.00	0.25	0.04	0.00
	1	b	1.95	0.39	48.06	2.66	0.00	0.25	0.04	45.97	0.00	0.28	0.05	0.00
CJ69F	1	a	4.22	1.18	69.03	1.85	0.00	0.18	0.02	23.16	0.00	0.09	0.10	0.00
	1	b	3.91	1.20	67.90	1.90	0.00	0.20	0.07	24.44	0.00	0.10	0.14	0.00

*See Table 11 for peak identity.