Determination of Citral in Lemongrass and Citrus Oils by Condensation with Barbituric Acid

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Citral is one of the basic raw materials of the essential oil, flavor, and cosmetic industry. Its quantitative determination is therefore of great importance to both producers and processors of aromatic chemicals and related products. When the α,β-unsaturated aldehyde is treated with barbituric acid in aqueous ethyl alcohol, condensation takes place to form citrylidenebarbituric acid. The reaction product displays strong ultraviolet absorption at 336 mμ, and the intensity of the band observed at this wave length, under controlled conditions, is utilized for quantitative measurements. The method should prove useful to the essential oil, flavor, and cosmetic industry for process control and quality evaluation of raw materials and consumer goods.

Citral is one of the most important raw materials of the essential oil, flavor, and cosmetic industry. It is used extensively for the compounding of synthetic flavors, for the scenting of soaps and toiletries, and for the preparation of numerous perfume compositions. Considerable quantities serve as starting material for the manufacture of such valuable products as the ionones and methyl ionones, of vitamin A, and other polyene compounds. United States production of citral more than tripled during the last decade, amounting to 109,000 pounds in 1957 (*23*).

Recently a total synthesis from readily available basic raw materials, such as calcium carbide and acetylene, has been achieved (*3*, *16*), but commercially the α,β-unsaturated aldehyde is still obtained almost exclusively from lemongrass oils whose quality is judged primarily on the basis of their citral content. Other essential oils highly esteemed on account of their typical fragrant aroma, because of the presence of the terpene aldehyde, are the citrus oils—e.g., oil of grapefruit, oil of lemon, oil of lime, oil of orange, and oil of tangerine. Standards and specifications based upon the important flavoring principle—total aldehydes, which include citral—have been established for most of these products and are recognized by the industry (*6*).

Since the aldehyde was first isolated, by Bertram in 1888, from the oil of *Backhousia citriodora*, analysts have sought procedures for its quantitative determination in both natural and synthetic preparations. All methods reported before this investigation was begun suffer from lack of specificity, and other shortcomings as discussed by Guenther and Langenau (*8*). The procedure described here is based on extensive prior work on the use of malonylurea to prepare Knoevenagel condensation products of aldehydes. It involves reaction of citral with barbituric acid in dilute ethyl alcohol, and spectrophotometric examination of the derivative, citrylidenebarbituric acid, generated under controlled conditions. The method is superior in one or more ways to those previously reported, in that it is highly specific, rapid, and easy to execute, requiring only a small sample and no special reagents or equipment. It is also sufficiently reproducible to satisfy the analytical chemist.

While this work was in progress, Stenlake and Williams published a method for determining α,β-unsaturated aldehydes and ketones by reaction with Girard-T reagent. They reported analytical data for citral in lemon and lemongrass oil, cinnamaldehyde in cinnamon oil, and carvone in dill and caraway oil (*21*). More recently, these authors presented another method based on the reducing action of sodium borohydride on α,β-unsaturated carbonyl compounds (*20*).

While this study was under way, Stanley, Lindwall, and Vannier were at work on a procedure for determining citral in lemon oil based on the development of a green coloration when the aldehyde reacts with vanillin and piperidine in an ethyl alcohol solution (*17*).

Experimental

Materials. Barbituric acid (Eastman Kodak, White Label) recrystallized from water; melting point 248° to 251° C. (decomposition).

Citral, experimental sample, freshly distilled and stored under nitrogen (The Trubek Laboratories, Inc., East Rutherford, N. J.), hydroxylamine assay, 99.56%; $\epsilon^{EtOH}_{max\ 237.5\ m\mu} = 14.975$.

Ethyl alcohol, anhydrous.

Apparatus. Cary Model 11 recording spectrophotometer, matched silica cells of 1-cm. path length.

Preparation of Reagent. Weigh out 1.0 gram of barbituric acid and transfer to a dry 100-ml. volumetric flask. Add 20 ml. of distilled water from a pipet, rinsing down any material which may adhere to the neck of the flask. Stopper lightly and dissolve by warming gently on a hot plate. Dilute to 100 ml. with ethyl alcohol and place the flask in a water bath maintained at 25° C. Make up to volume with alcohol after temperature equilibration and mix thoroughly.

The solution prepared easily from readily available and inexpensive chemicals may be kept for about 2 weeks, whereafter it will gradually develop a yellowish coloration, unless stored in a refrigerator when not in use.

Construction of Calibration Graph. Weigh accurately into six 25-ml. volumetric flasks quantities of about 10, 15, 20, 25, 30, and 35 mg. of citral, respectively. Make up to volume with the reagent, mix thoroughly, and place the vessels in a water bath kept at 25° C. After 40 minutes, withdraw 1-ml. aliquots into 200-ml. volumetric flasks containing approximately 100 ml. of ethyl alcohol, dilute to the mark with the solvent, mix well, and measure the absorption of the preparations at 336 mμ using as blank a solution of 1 ml. of the reagent in 200 ml. of ethyl alcohol.

Because of the small quantities of material required for computation of the calibration graph it is advisable to determine further reference points in the following manner. Weigh accurately into a 25-ml. volumetric flask about 300 mg. of citral and dilute to the mark with 20% aqueous ethyl alcohol. Add carefully from a microburet about 0.5-, 1.0-, 1.5-, 2.0-, and 2.5-ml. aliquots into 25-ml. volumetric flasks, make up each flask to the mark with the reagent, and process further as described.

Construct the calibration graph by plotting determined absorbance values

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vs. weights of samples used (Figure 1).

Analytical Procedure. Weigh a suitable amount of the oil into a volumetric flask of appropriate size, make up to volume with the reagent, and mix thoroughly. Place the reaction vessel in a water bath kept at 25° C. and withdraw aliquots after 40 minutes for dilution with ethyl alcohol and spectrophotometric examination at 336 mμ, using as blank a similarly diluted aliquot of the reagent.

From the analytical value recorded, subtract the corresponding absorbance of the oil at 336 mμ when dissolved in ethyl alcohol.

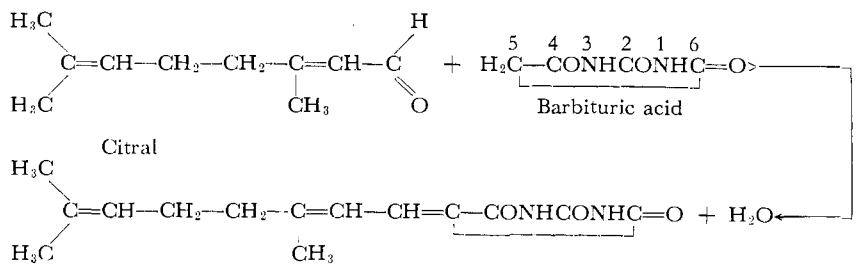
The experimental conditions found to be most satisfactory with regard to sample weights and volumes for the oils investigated are given in Table I.

Table I. Weights and Dilutions Recommended for Citral Analysis of Essential Oils by Barbituric Acid Condensation Method

Essential Oil	Weight, Mg.	Final Volume, Ml.	Dilution of Aliquot with Ethyl Alcohol, Ml.	Dilution Factor, D
Lemongrass	20–30	25	1 to 200	5000
Lime				
Persian	25–35	25	2 to 50	625
Mexican	45–55	25	2 to 50	625
Lemon				
Italian	50–60	25	2 to 50	625
Californian	70–100	25	2 to 50	625
Grapefruit	100–125	10	2 to 50	250
Orange				
Bitter	160–180	10	2 to 50	250
Sweet	200–400	10	2 to 50	250

Results and Discussion

Nature of Reaction. When citral is reacted with barbituric acid in dilute aqueous ethyl alcohol, condensation takes place in accordance with the equation:



Citrylidenebarbituric acid

Under suitable conditions the reaction product, citrylidenebarbituric acid, precipitates in the form of slender, yellowish needles which can readily be isolated and purified for physicochemical characterization. Melting point after recrystallization from 20% aqueous ethyl alcohol 172.0–3.5° C. (decomposition).

Strong experimental evidence that the reaction proceeds as shown via the hydrogens attached to carbon atom 5 of the barbituric acid molecule stems from the observation that the pH of the medium increases as the condensation process takes place. Malonylurea is a relatively strong acid ($K_{25°\,C.} = 1.05 \times 10^{-4}$), because of the presence of an active methylene group flanked by two carbonyl linkages (*11*, *12*). A 0.075 *M* solution in 20% aqueous ethyl alcohol exhibits a pH of 2.68. When to 400 ml. of such a solution is added excess citral (6 grams), precipitation of the reaction product begins within 10 minutes and appears to be complete after about 45 minutes, the pH of the system having risen to 3.54. Product yield, 7.2 grams or 91.5% of theoretical.

Also in accordance with the mechanism postulated no reaction takes place when instead of barbituric acid its 5,5-dimethyl derivative is used. Similarly, other 5,5-disubstituted malonyl ureas—e.g., 5,5-diethylbarbituric acid (Veronal) and 5-ethyl-5-phenylbarbituric acid (Luminal)—fail to interact.

However, 1,3-dimethylbarbituric acid condenses smoothly with the aldehyde to form citrylidene-1,3-dimethylbarbituric acid. A solution of 1.5 grams of the malonylurea compound (*2*), melting point 122.4–2.9° C., in 50 ml. of 20% aqueous ethyl alcohol, exhibits a pH of 3.02. On addition of excess citral (2.5 grams) the derivative gradually precipitates, the pH of the system climbing to 3.79 within 45 minutes. Yield, 2.3 grams or 82.4% of theoretical. The melting point of the product after recrystallization from 20% aqueous ethyl alcohol was 102.3 – 102.8° C.

Nature of Reaction Products. Further experimental support regarding the course of the reaction may be deduced from the physicochemical properties of the condensation product. Microchemical analyses agreed closely with the assigned formula as shown in Table II.

The compound gave a negative Schiff test, indicating that the aldehydic function of the reactant, citral, was lacking. Hydrogenation experiments carried out in glacial acetic acid over Adams' platinum oxide catalyst showed that 3 moles of hydrogen were consumed per mole of product in accordance with its three sites of unsaturation. Barbituric acid was found to be unreactive when similarly assayed.

Additional evidence for the structure of the condensation product may be obtained from an inspection of the infrared absorption spectrum (Figure 2) along with the spectra of the reactants, as recorded by a Perkin Elmer Model 21 double-beam spectrophotometer equipped with rock salt optics. Citral was examined in a sodium chloride cell of 0.0155-mm. path length and the solids were analyzed by the potassium bromide pellet technique as described by Chatten and Levi (*4*).

Barbituric acid displays characteristic N—H stretching vibrations at 3180 and 3060 cm.$^{-1}$, respectively, and its three

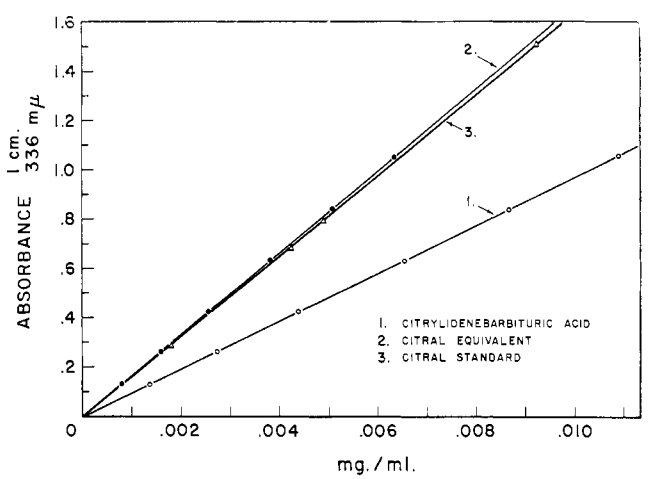


Figure 1. Calibration graphs

Table II. Microchemical Analysis of Citrylidenebarbituric Acid, $C_{14}H_{18}O_3N_2$

Carbon, %		Hydrogen, %		Oxygen, %		Nitrogen, %	
Found	Theoret.	Found	Theoret.	Found	Theoret.	Found	Theoret.
64.27	64.10	7.03	6.92	18.21	18.30	10.74	10.68

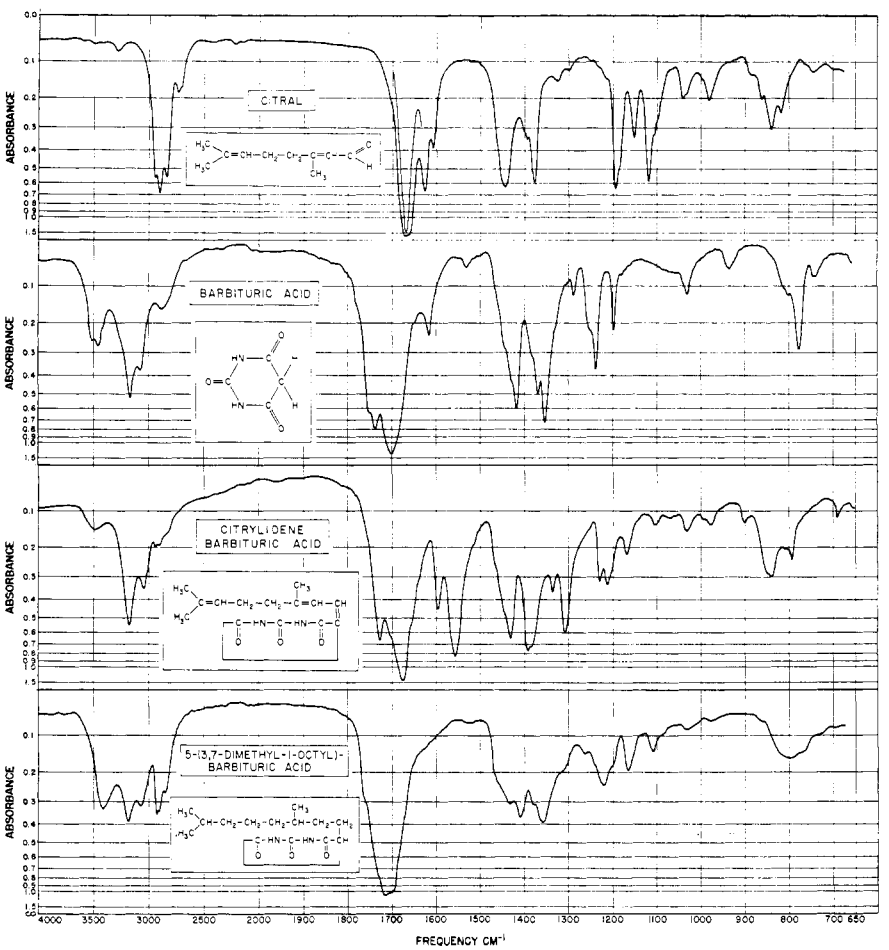


Figure 2. Infrared spectra of citral, barbituric acid, citrylidenebarbituric acid and 5-(3,7-dimethyl-1-octyl)-barbituric acid

carbonyl linkages give rise to a strong band at 1700 cm.$^{-1}$, a band of medium intensity at 1735 cm.$^{-1}$, and an inflection at 1750 cm.$^{-1}$. The derivative shows analogous absorption throughout the 3200 to 3100-cm.$^{-1}$ region which observation indicates that the N—H groups of the barbituric acid moiety have remained intact. It also exhibits marked absorption throughout the carbonyl stretching frequency region, but at distinctly longer wave lengths. Two sharp bands occur at 1670 and 1720 cm.$^{-1}$, respectively, and an inflection is observed at 1700 cm.$^{-1}$. The phenomenon is in accord with the establishment of a more enhanced system of conjugated double bonds by the reaction. The intense absorptions seen at 1550 and 1590 cm.$^{-1}$, where both citral and barbituric acid are practically transparent, further confirms the structure assigned to the compound.

The infrared absorption spectrum of the hydrogenated condensation product, isolated from glacial acetic acid solution and recrystallized from dilute aqueous ethyl alcohol—melting point 125–8° C. —shows considerably less detail than the parent compound. A broad band in the 1715-cm.$^{-1}$ region and two inflections (1730 and 1760 cm.$^{-1}$) are indicative of the three carbonyl linkages in the molecule, but carbon-carbon double bond frequencies are no longer present.

The spectra of 1,3-dimethylbarbituric acid and its citrylidene derivative (Figure 3) lack structural features in the 3200 to 3100 cm.$^{-1}$ wave-length range (absence of N—H groups). However, marked absorption occurs throughout the carbonyl stretching frequency region. The acid displays strong bands at 1655 and 1685 cm.$^{-1}$ and an inflection at 1735 cm.$^{-1}$ Its derivative exhibits intense absorption at 1665 and 1720 cm.$^{-1}$ as well as an inflection at 1650 cm.$^{-1}$ Thus, in the spectra of both citrylidenebarbituric acid and its 1,3-dimethyl analog the major carbonyl absorptions occur at comparable positions and at lower frequencies than in the corresponding malonylureas (1665 ± 5 cm.$^{-1}$ and 1720 ± 5 cm.$^{-1}$, respectively). In both instances the molar infrared intensities of these bands are considerably enhanced over those of the barbituric acids—a phenomenon also due to the sequence of unsaturations established by the reaction. The presence of two sharp bands in both spectra near 1550 and 1600 cm.$^{-1}$, not observed in the spectra of the reactants, further confirms the existence of a similarly constituted system of double bonds in both molecules.

Finally, the ultraviolet absorption characteristics of both derivatives also support the postulated reaction mechanism and structural features of the molecules. Citral, an α,β-unsaturated aldehyde, exhibits maximal absorbance at 237.5 mμ (ϵ_{max}^{EtOH} = 14,975). Citrylidenebarbituric acid as well as the 1,3-dimethyl analog displays similar and considerably enhanced absorption at longer wave lengths, because of comparable conjugations established in these molecules by the condensation reaction. Citrylidenebarbituric acid, $\epsilon_{max\ 336\ m\mu}^{EtOH}$ = 25,325; citrylidene-1,3-dimethylbarbituric acid, $\epsilon_{max\ 339\ m\mu}^{EtOH}$ = 25,950.

Utilization of Reaction for Quantitative Analysis

Attempts to determine citral gravimetrically in accordance with the procedure described proved unsuccessful, because of the limited solubility of barbituric acid in dilute aqueous ethyl alcohol and incomplete precipitation of the condensation product. Although results were improved when using dilute dioxane as solvent system, product yields could not be made to exceed 90% of theoretical. Measurement of the ultraviolet absorption of the derivative at 336 mμ (Figure 4) proved a much more reliable parameter for gaging citral recoveries and determining the aldehyde in essential oils and related products.

Preliminary studies showed that acid or base catalysis, generally believed to govern the mechanisms of classical Knoevenagel condensations, was not required for reaction to take place. Product formation was extremely sluggish in anhydrous ethyl alcohol, but increased considerably with water and/or barbituric acid concentration (Figure 5). When the experiments were performed at 25° C. and a 1% solution of barbituric acid in 20% aqueous ethyl alcohol was used as reactant, maximum product yields were obtained after 40 minutes. These conditions were considered optimal as they allowed for the analysis of several samples within a reasonable period of time and with a satisfactory degree of precision.

A calibration graph using citrylidenebarbituric acid (CBA) purified by re-

peated recrystallization from ethyl alcohol was constructed and the aldehyde equivalent plotted (Figure 1). When assaying the citral reference standard—99.56% by hydroxylamine titration—in accordance with the procedure described, recoveries amounted to 97.7 ± 0.9% Taking these factors into consideration, the experimental results were calculated from the equation:

$$\text{Per cent citral content} = \frac{\text{slope}_{\text{CBA Calibration graph}} \times \text{molecular weight}_{\text{Citral}} \times \text{absorbance}_{\text{Reaction-sample}} \times 100 \times \text{dilution factor} \times 100}{\text{molecular weight} \times \text{weight sample (mg.)} \times \text{per cent citral recovered}}$$

$$= \frac{0.010358 \times 152.23 \times \text{absorbance}_{\text{corr.}} \times 10{,}000 \times D}{262.30 \times \text{sample weight (mg.)} \times 97.7}$$

$$= \frac{0.6153 \times \text{absorbance}_{\text{corr.}} \times D}{\text{sample (mg.)}}$$

where D = dilution factor as recorded in Table I.

As far as the authors are aware, none of the spectrophotometric methods for citral reported to date are based on the successful isolation of a specific reaction product and its physicochemical characterization as illustrated in this study. Such data make for a fuller understanding of the nature and scope of an analytical method, although for practical purposes they need not be known as long as it has been established that the property being measured is proportional to the concentration of the material being examined. Routine application of the method, therefore, requires construction of one calibration graph only based on an authenticated specimen of citral (Figure 1, No. 3). It is imperative to use a genuine product for this purpose, as the accuracy of the method depends on the purity of the reference standard.

Reaction Kinetics

Kinetic measurements to establish optimum analytical conditions were carried out for both the formation and decomposition reaction of the derivative.

Product Formation. Solutions of barbituric acid in dilute aqueous ethyl alcohol were added at accurately measured temperatures to known weights of citral in 25-ml. volumetric flasks and the vessels were kept in a water bath regulated to ± 0.02° C. by means of a Beckman contact thermometer. Aliquots were pipetted at definite time intervals into anhydrous ethyl alcohol to quench the reaction, and absorbancies of the preparations were measured at 336 mμ. The experimental data are recorded in Table III and Figure 6, which illustrate that the reaction proceeds via a first-order mechanism with regard to both citral and barbituric acid.

An Arrhenius plot illustrating the effect of temperature on the rate of formation of citrylidenebarbituric acid revealed a linear relationship between log k and $1/T$. It showed that the activation energy, E_a of the reaction is small (7.6 kcal. mole^{-1} degree^{-1}) and the corresponding frequency factor ($B = \frac{k}{e^{-Ea/RT}}$) is equal to 1.2×10^4. The significance of these constants is, unfortunately, obscured as they include parameters of any pre-equilibria processes. However, the low concentrations used in this study and the absence of extraneous catalysts may prove to be conditions more suitable for mechanistic interpretation than previous kinetic investigations of the Knoevenagel reaction (*5, 13, 14*),

Product Decomposition. This reaction was studied by dissolving accurately weighed amounts of citrylidenebarbituric acid in 50-ml. volumes of aqueous ethyl alcohol at constant temperature, removing aliquots of the preparations into anhydrous ethyl alcohol at given time intervals, and measuring ultraviolet absorbancies of the solutions at 336 mμ for quantitative interpretations. Experimental data illustrate the increased rate of reaction with temperature and water concentration (Table IV). No marked effects were observed in the presence of excess barbituric acid which on the basis of the ultraviolet data assembled evidently did not constitute a major breakdown product.

Specificity of Reaction. The selectivity of the method was established by application to synthetic preparations of known composition and to a series of recovery experiments on natural products as recorded in Tables V and VI.

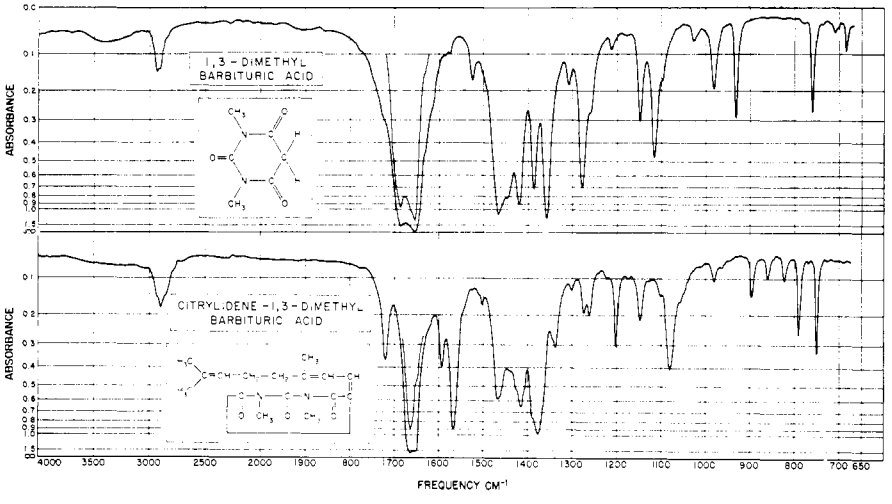


Figure 3. Infrared spectra of 1,3-dimethylbarbituric acid and citrylidene-1,3-dimethylbarbituric acid

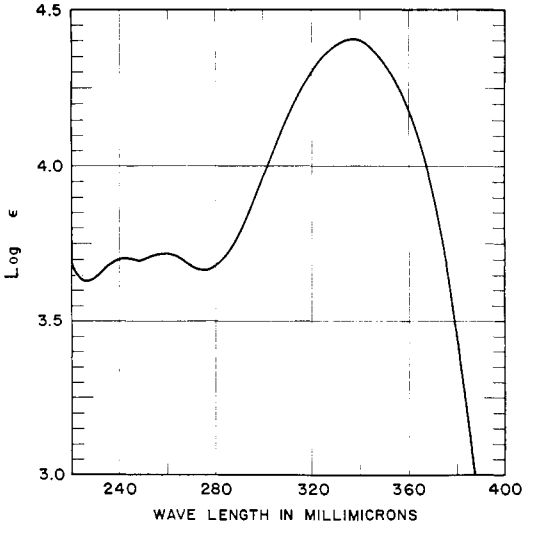


Figure 4. Ultraviolet spectrum of citrylidenebarbituric acid

Precision and Accuracy of Method

The precision obtainable in accordance with the procedure described is determined primarily by the precision of the volumetric operations, which include pipetting and diluting of sample aliquots, while the accuracy of the method is governed by the authenticity of the reference standard and general performance of the assays under conditions similar to those followed for the construction of the calibration graph.

Derivatization was maximal after 40 minutes at which time samples should be withdrawn for analysis. But the time-product curve reaches its maximum gradually and recoveries accurate to within 2.5% may still be realized when assaying samples reacted for 35 or 45 minutes. The condensation product was found to be fairly stable in anhydrous ethyl alcohol and satisfactory results were still obtained after the preparations had been left for about 30 minutes prior to ultraviolet examination (Table VII). However, in order to ascertain reliable analyses for citral and essential oils containing high percentages of the aldehyde, all variables involved should be as carefully controlled as possible.

Some of the oils investigated—e.g., oil of orange and grapefruit, containing only small amounts of citral and other oxygenated constituents in the presence of more than 90% of terpenes—did not completely dissolve on addition of the reagent and appeared opalescent to turbid following reaction. Reproducible data were, nevertheless, obtained in all instances, because clear solutions formed immediately on diluting sample aliquots with ethyl alcohol prior to ultraviolet examination. Results were, furthermore, accurate as determined by recovery experiments (Table VI).

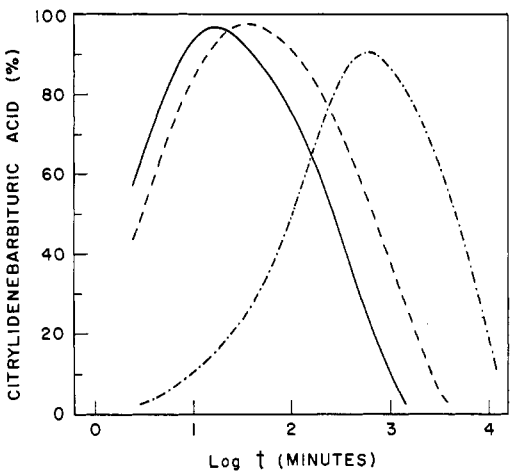


Figure 5. Effect of reagent composition on formation of citrylidenebarbituric acid

——Barbituric acid 1%; water 40%
- - - -Barbituric acid 1%; water 20%
—·—·Barbituric acid 0.25%; water 0%

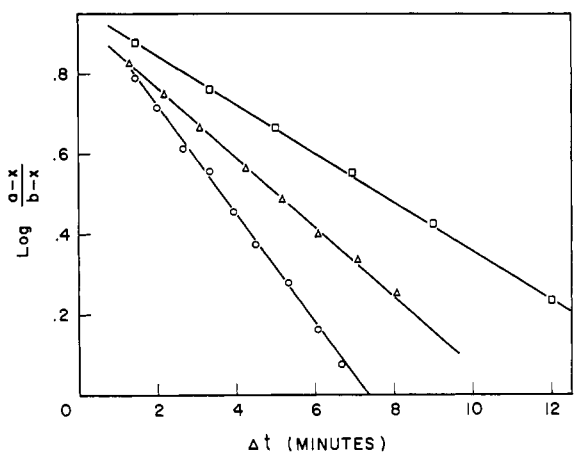


Figure 6. Rate of formation of citrylidenebarbituric acid

□ T = 25° C.; 46.2 mg. citral/25 ml. reagent
△ T = 35° C.; 23.0 mg. citral/25 ml. reagent
○ T = 45° C.; 17.9 mg. citral/25 ml. reagent

Ordinate units arbitrary to accommodate their graphs

Table III. Rate of Formation of Citrylidenebarbituric Acid in Aqueous Ethyl Alcohol

Citral, Mg./25 Ml.	Barbituric Acid, G./100 Ml.	Water, Ml./100 Ml.	Temp., ° C.	L Mole^{-1} Sec.$^{-1}$
24.4	1.000	20	24.75	0.0324
46.2	1.000	20	25.00	0.0348
9.0	1.000	20	25.00	0.0318
21.2	0.500	20	25.00	0.0330
			Av. 0.0330 ± 0.012	
23.6	1.000	20	19.75	0.0255
23.3	1.000	40	19.75	0.0378
20.5	1.000	20	35.00	0.0470
17.9	1.000	20	45.00	0.0671

Table IV. Rate of Decomposition of Citrylidenebarbituric Acid in 50 Ml. of Alcoholic Barbituric Acid

Citrylidene Barbituric Acid, Mg.	Barbituric Acid, G./50 Ml.	Water, Ml./50 Ml.	Temp., ° C.	k, Sec.$^{-1}$ × 10^5
35.0	0.47	10	25	1.34
35.3	0.47	10	35	2.45
19.4	0.47	10	45	4.02
18.8	0.47	20	25	2.55
18.8	0.47	20	45	7.90

Application of Method to Essential Oils

Results obtained on genuine lemongrass and citrus oils are shown in Tables VIII and IX along with citral values secured by conventional methods of analysis. Comparison of the data given in columns of 2 and 3 illustrate the degree specificity of the method, because the differences observed must be attributed to the presence of other carbonyl compounds in these products. Lemongrass oils are known to contain small amounts of citronellal, isovaleraldehyde, decylaldehyde, methylheptenone, acetone, and α,β-dihydropseudoionone (*10*), while the occurrence of a number of aliphatic aldehydes—e.g., octanal, nonanal, decanal, lauraldehyde as well as citronellal—in citrus oils has been established (*9*).

When assaying these products by the official method of analysis (oximation by hydroxylamine hydrochloride) or semi-official procedures (phenylhydrazine titration, sodium sulfite or bisulfite absorption techniques) all carbonyl compounds would be affected, although not necessarily to the same extent. Yet experimental results are expressed in terms of one species only—e.g., citral. These assays, although yielding a good deal of useful criteria, cannot by themselves provide any specific information regarding the true citral content of essential oils. The official method of analysis suffers from a still more serious shortcoming in that it cannot even be conveniently utilized for demonstrating the presence of citral in a particular product. Furthermore, no method has as yet been reported which

would permit quantitative recovery of the aldehyde once it has been subjected to oximation. To a considerable extent this drawback applies to all other methods for citral analysis of essential oils published so far. Large samples and tedious separations would always be necessary to afford even semiquantitative determinations. The barbituric acid condensation method allows, however, for the isolation of a crystalline reaction product from a single drop of oil containing only 2 to 3% of citral. A modification of the procedure permitting the detection of trace quantities of the aldehyde in essential oils and its microchemical characterization is now being developed (*15*).

Recently, a method for the quantitative estimation of citral in lemongrass oil based on the intense ultraviolet absorption of the aldehyde at 237 ± 1 mμ was published by Surve, Chakravarti, and Bhattacharyya (*22*). Results obtained by their procedure are shown in Table VIII column 5 for three East Indian and five West Indian lemongrass oils. Good agreement with the results secured by the barbituric acid condensation method was realized only for the East Indian oils. The West Indian products contain variable amounts of myrcene (*10*), and the possibility that they might not submit to accurate analysis by direct ultraviolet measurements was correctly foreseen by Surve, Chakravarti, and Bhattacharyya (*22*). A sample of myrcene [ex *Picea mariana* (Mill.)] dissolved in ethyl alcohol displayed maximal absorbance at 225 mμ (ϵ = 16,340). In accordance with this observation, peak absorbances of the West Indian oils were more intense and occurred at lower wave lengths (235 mμ) than those of the East Indian Oils (237.5 mμ). Hence, citral values computed from ultraviolet absorbances of the West Indian oils were markedly higher than those obtained by the barbituric acid condensation method. When selecting 237.5 mμ as reference wave length, the citral contents of these oils would be about only 2% lower than the values recorded for 235 mμ and therefore still too high.

Citral–total carbonyl ratios as derived from the experimental data are shown in column 4 of Tables VIII and IX. They evidently constitute very characteristic compositional parameters and should prove valuable criteria for establishing the identity as well as judging the quality of essential oils and related products.

Terpeneless Products. Analytical data for terpeneless citrus oils are presented in Table X. These products vary considerably in composition, depending on their mode of preparation (*7*) and the analytical procedure described should prove particularly useful in the quality control of these important raw materials, whose manufacture requires a great deal of knowledge and experience.

Table V. Analysis of Synthetic Blends of Essential Oils by Barbituric Acid Condensation Method

Preparation	Citral Content, %	Total Carbonyl Content Expressed as Citral, %	Recovery, % Citral by barbituric acid condensation method	Recovery, % Total carbonyl expressed as citral by hydoxylamine titration (*7*)
Lemongrass oil[a]	79.93	91.43	80.21 79.90	88.96
Lemon oil[b]	4.36	8.35	4.29 4.35	8.05

[a] % composition. Caprylaldehyde 3.06, citronellal 3.55, decylaldehyde 1.75, heptaldehyde 1.99, Δ^3-carene 0.80, dipentene 1.51, myrcene 1.22, β-pinene 1.09, citronellol 1.44, isopulegol 1.99, linaloöl 1.67.

[b] % composition. Caprylaldehyde 0.76, citronellal 0.77, decylaldehyde 1.11, heptaldehyde 0.93, camphene 1.47, *d*-limonene 85.98, α-pinene 1.13, geraniol 0.84, linaloöl 0.67, α-terpineol 0.96, linalyl acetate 1.02.

Table VI. Recovery of Citral from Essential Oils

Product	Citral Content, %	Sample, Weight, Mg.	Citral Added, Mg.	Citral Content of Analytical Sample, % Theoret.	Citral Content of Analytical Sample, % Found
Lemongrass oil	70.20				
		24.15	8.40	77.87	77.60
		24.00	11.02	79.58	80.18
		20.10	11.38	80.98	80.86
Lemon oil	3.29	36.40	1.68	7.56	7.65
Lime oil	3.63	45.45	1.68	7.07	7.16
Orange oil	0.23_3	208.93	0.84	0.63_3	0.62_3
Grapefruit oil	0.12_5	81.30	0.84	1.14_6	1.11_4

Effects of Ultraviolet Characteristics of Essential Oils on Analytical Method

As described in the experimental section, the procedure requires measurement of the ultraviolet absorption of the sample at 336 mμ as a separate operation. This is hardly a shortcoming of the method, because ultraviolet analyses are carried out routinely in all industrial and research laboratories concerned with the processing of essential oils and aromatic chemicals. In fact, ultraviolet examination of these products has come to be recognized as an important general technique for establishing their quality and authenticity.

Molar absorbances displayed at 336 mμ by the materials studied were found to range all the way from low to relatively high intensities, and in order to save the analyst time in the preparation of solutions exhibiting meaningful absorptions at this wave length, concentrations yielding absorbance readings close to 0.5 were computed from the experimental data as shown in Table XI. Positions of maxima observed in the neighborhood of the critical wave length and related $E_{1\,cm.}^{1\%}$ values are also given. The magnitude of the corrections involved becomes evident from an inspection of column 6. It illustrates that background absorption is relatively small for lemongrass oils, fairly marked for lemon oils, and intense for orange, lime, and grapefruit oils. The ultraviolet characteristics of citrus oils beyond 300 mμ are largely due to the presence of coumarin compounds (*18*, *19*).

Table VII. Stability of Citrylidenebarbituric Acid under Standard Experimental Conditions

Product	Citral, % Initially	Citral, % After 30 min.
Citral	99.32	98.64
Lemongrass oil	70.20	70.03
Lemon oil	3.29	3.26
Lime oil	3.63	3.59
Orange oil	0.23_3	0.23_2
Grapefruit oil	0.12_5	0.11_7

For analytical purposes it is necessary only to measure the absorbance of the reacted sample at 336 mμ, where the citral–barbituric acid condensation product shows its maximum. Yet maximal absorption of the reacted material does not always occur at this wave length. If the oil displays a maximum in the neighborhood of 336 mμ and, moreover, contains but little citral, the peak of the reaction will be displaced. These conditions apply to oil of orange, lime, and grapefruit. Lemon oils generally show analytical peak absorbances at 335 ± 2 mμ, because their ultraviolet absorption at the maximum 310 to 315 mμ and throughout the 330- to 340-mμ range is considerably less intense. Lemongrass

oils display weak absorption only at 336 mμ and because they contain relatively high percentages of citral, reaction peaks are always observed at this wave length also. Background corrections are, accordingly, small for these products.

Strong evidence that the corrected absorptions are attributable to citral present in the sample and the formation of citrylidenebarbituric acid when analytical peak absorptions are not observed at 336 mμ, may be obtained from the experimental data by plotting $E^{1\%}_{1cm.}$ curves for both the reaction and the oil. Subtracting the latter from the former will produce a difference curve exhibiting its absorption maximum at or close to 336 mμ. The phenomenon is illustrated in Figure 7 for California sweet orange oil, Dominican bitter orange oil, Mexican lime oil, N.F., and Israeli grapefruit oil.

Application of Reaction to Essential Oil Constituents and Aromatic Chemicals

In order to appraise possible interferences by other compounds and determine the scope of the reaction, the method was applied to a number of commercial products of interest to the essential oils, flavor, and cosmetic industry (Table XII).

The experimental data show that dihydrocitral reacts with barbituric acid similar to citral, the condensation product exhibiting peak absorption at 334 mμ. This compound would, therefore, interfere in the assay. As far as the authors are aware, its presence in essential oils has, however, not yet been established. Other substances condensing with the reagent, but displaying maxima at quite different wave lengths are benzaldehyde, cinnamaldehyde and furfural. The intense absorptions shown by benzaldehyde (325.5 mμ) and cinnamaldehyde (369.5 mμ) might well permit the quantitative determination of these aromatics in essential oils—e.g., oil of bitter almond, peach, and apricot kernel, oil of cassia, cinnamon bark—and related products, such as essences, flavorings, and pharmaceutical preparations. Vanillin and ethylvanillin exhibit intense absorption maxima, but, unfortunately too close together (400 to 402 mμ) to make the reaction the basis of a specific quantitative method of analysis.

Additional commercial products similarly analyzed, but found to be unreactive were methylheptenone, methylnonyl ketone and biacetyl (aliphatic ketones), mesityl oxide, α,β-pseudoionone (α,β-unsaturated ketones), citronellol, linaloöl, menthol and isopulegol (aliphatic and cyclic terpene alcohols), heptanal, octanal, and decanal (aliphatic aldehydes). Some of the compounds displayed apparent citral contents of up to 0.5%, but it was established by microcrystal tests (*15*) that the interferences were caused by the

Table VIII. Analysis of Lemongrass Oils by Barbituric Acid Condensation Method

Lemongrass Oil	Citral by Barbituric Acid Condensation Method, %	Total Carbonyl Expressed as Citral, %	Citral in Carbonyl Fraction, %	Citral Method of Surve, Chakravarti, and Bhattacharyya (22), %[a]
East Indian	74.4[b]	80.5[c]	92.4	75.3
	74.1			
	68.6[d]	72.3[c]	94.9	69.1
	68.2			
	70.2	75.4[c]	93.1	72.0
	70.9			
West Indian Guatemala	67.3	75.0[e]	89.7	77.1
	67.0			
	67.4	74.0[e]	91.1	79.4
	67.9			
	67.5	75.2[e]	89.8	79.0
	68.1			
Puerto Rico	61.3	67.6[f]	91.1	71.5
	61.8			
	62.9	68.0[f]	92.1	69.9
	62.3			

[a] Direct measurement of peak absorbance in ultraviolet region.
[b] Sample analyzed after 8 months' storage at about 5° C. Original citral content by hydroxylamine titration, 84%. Courtesy Dr. Sadgopal, Indian Standards Institution, New Delhi, India.
[c] Hydroxylamine titration.
[d] Sample analyzed after 8 months' storage at about 5° C. Original citral content by hydroxylamine titration, 76.5%. Courtesy Dr. Sadgopal, Indian Standards Institution, New Delhi, India.
[e] Neutral sulfite method; courtesy Fritzsche Brothers, New York, U.S.A.
[f] Neutral sulfite method; courtesy Dr. Nadal, University of Puerto Rico, Research Center, Mayaguez, Puerto Rico.

Table IX. Analysis of Citrus Oils by Hydroxylamine Titration and Barbituric Acid Condensation Method

Citrus Oil	Citral by Barbituric Acid Condensation Method, %	Total Carbonyl Expressed as Citral by Hydroxylamine Titration (7), %	Citral in Carbonyl Fraction, %
LEMON			
California			
Cold pressed, U.S.P.	2.49	2.83	88.0
Hand pressed, U.S.P.	2.82	3.13	90.1
	2.65		
	2.82		
Cold pressed, Fullerton Foothills near Coast	2.64	3.35[a]	78.8
Cold pressed, Ventura Coastal growing area	2.50	3.16[a]	79.1
Cold pressed, San Joaquin Valley, Calif., inland, arid growing area	1.97	2.28[a]	86.4
Cold pressed, Yuma, Ariz., desert growing area	1.87	2.74[a]	68.2
Cold pressed, blend of growing areas to meet U.S.P. requirements	2.42	2.86	84.6
Cold pressed, representative blend current production, 1958	2.09	2.61	80.1
Steam distilled from pulp	2.20	2.67[a]	82.4
Steam distilled from peel	2.04	2.67[a]	76.4
Steam distilled from juice	0.86	1.89[a]	45.5
Cyprus			
Hand pressed, B.P.	3.34	3.74	89.3
	3.22		
Cold pressed, B.P.	3.46	3.96	87.4
Italian			
Reggio Calabria	3.30	4.40	75.0
	3.26		
Sicily			
Machine pressed	3.47	4.12	84.2
Machine pressed	3.63	3.85	94.3
Machine pressed	3.39	3.93	86.3
Sicily, Messina, machine cold pressed without distillation	3.16	3.60	87.8
Sicily			
Messina	3.19	3.74	85.3
Palermo	3.53	3.73	94.6
Palermo	3.16	3.36	94.0

Table IX. Analysis of Citrus Oils by Hydroxylamine Titration and Barbituric Acid Condensation Method (Continued)

Citrus Oil	Citral by Barbituic Acid Condensation Method, %	Total Carbonyl Expressed as Citral by Hydroxylamine Titration (7), %	Citral in Carbonyl Fraction, %
LIME			
Persian, cold pressed, Fla.	5.25	6.46[a]	81.3
	4.70	5.38	87.4
Mexican			
Cold pressed, grown in Calif.	3.53	5.32[a]	66.4
Cold pressed, Mexico	3.08	3.42[a]	90.1
Cold pressed, Mexican or seedling	3.21	3.78	84.9
Expressed, extra	3.42	4.53	75.5
Distilled, Mexican extra	0.07	0.74	9.5
Distilled	0.29	0.91[a]	31.9
ORANGE[b]			
Sweet			
Fla., cold pressed, Valencia	0.25	1.81	13.8
	0.27	2.01	13.4
	0.20	1.45	13.8
	0.22		
California,			
Cold pressed, Valencia	0.11	1.49	7.4
Cold Pressed, U.S.P. exchange brand	0.12	1.52	7.9
Cyprus, hand pressed, B.P.C.	0.28	1.65	17.0
Sicily	0.21	1.22	17.2
Israel, cold pressed, Shamuti, Jaffa variety	0.23	1.59	14.5
	0.22	1.63	13.5
Bitter			
Dominican Republic, hand pressed, N.F.	0.10	1.90	5.3
Dominican Republic, cold pressed, N.F.	0.01	0.74	13.5
	0.11		
Seville	0.10	1.48[a]	6.8
GRAPEFRUIT[b]			
Calif., cold pressed	0.043	0.64	6.7
	0.047		
Florida			
Cold pressed, 1 year old, produced during 1956–57 season	0.11	1.40	7.9
Cold pressed, produced during 1957–58 season	0.11	1.26	8.7
Israel, cold pressed, marsh seedless variety	0.12	0.77	15.6
	0.09	0.77	11.7

[a] Analyses by phenylhydrazine method carried out at laboratories of Exchange Lemon Products Co., Corona, Calif., and received through the courtesy of W. L. Stanley, U. S. Dept. of Agr., Agricultural Research Service, Pasadena, Calif.

[b] It is customary to report the total carbonyl content of this oil as decylaldehyde which compound is believed to be the main carbonyl constituent present. The experimental data recorded in the table are expressed in terms of citral, however, to permit uniform comparison between the two sets of values. Actually, the molecular weights of citral and decylaldehyde are sufficiently close and the citral content of the oil so small that the mode of expression of the titrimetric measurements is of little or no importance.

presence of minute amounts of the α,β-unsaturated aldehyde in these preparations prior to reaction. Only at high concentrations (Table XII) did the aliphatic aldehydes produce an ephemeral peak in the ultraviolet region causing slight absorbance at 336 mμ.

A number of geraniol samples of both natural and synthetic origin were also examined. In all instances peak absorbances at 336 mμ could be detected, corresponding citral values ranging from 0.05% for a synthetic product to 1.2% for one isolated from oil of palmarosa. The reaction proved, furthermore, a simple means of gaging the rate of citral formation in these preparations under different conditions of storage. A sample of citronellal was found to contain 0.11% of the α,β-unsaturated aldehyde, an observation confirmed qualitatively by distilling the product through a Mini-Cal Podbielniak high temperature fractionation column using diphenylmethane as booster. Citral was identified in the last of the distillate fractions following reaction with the reagent and examination of the resulting crystal formations under the microscope. The spectrophotometric assay required approximately 200 mg. of sample and was carried out within 1 hour. In the distillation experiment 30 grams of material were used and practically a day's attention was given to an analysis yielding only qualitative results (recovery 0.07%).

An example illustrating the interference of furfural is shown graphically in Figure 8 which records the analysis of a genuine sample of distilled lime oil. The product was found to display no maximum throughout the 300 to 370 mμ wavelength range when dissolved in ethyl alcohol. Following reaction with barbituric acid a broad peak was observed at 353 mμ and the difference spectrum exhibited maximum absorbance at a still longer wave length (359 mμ). The occurrence of furfural in Mexican lime oil distilled from the juice of green, crushed fruits was established by Guenther and Langenau (*9*), who isolated the aldehyde as the oxime from the fraction boiling from 160° to 170° C. The present authors used a modified Akabori

Table X. Analysis of Terpeneless Citrus Oils by Barbituric Acid Condensation Method

Terpeneless Oil	Sample,[a] Mg./25 Ml.	Dilution,[a] Ml.	Citral by Barbituric Acid Condensation Method, %	Total Carbonyl Expressed as Citral by Hydroxylamine Titration (7), %	Citral in Carbonyl Fraction, %
Lemon	29	1 to 200	53.7	80.4	66.8
Lime					
Expressed	48	1 to 200	26.3	30.1	87.4
Distilled	700	2 to 50	0.26	1.96	13.3
Orange, sweet	16	2 to 50	11.3	40.1	28.2
	17	2 to 50	12.2	23.5	51.9
Grapefruit	14	2 to 50	1.83	2.93	62.4

[a] Yielding absorbance readings of approximately 0.5 at 336 mμ.

Table XI. Ultraviolet Absorption of Essential Oils in Ethyl Alcohol[a]

Essential Oil	Weight,[b] Mg./100 Ml.	$E^{1\%}_{1cm.}$, 336 mμ	λ_{max}, mμ	$E^{1\%}_{1cm.}$, max.	Analytical Correction, %
Lemongrass					
East Indian	80–110	4.7–6.3	237.5	680–740	0.4–0.6
West Indian	75–95	5.2–6.8	234.5	700–780	0.5–0.6
Lemon					
Italy	150–200	2.5–3.3	311–313	4.5–5.9	4.1–5.3
Sicily	115–150	3.3–4.3	312–314	5.1–7.1	6.2–7.0
Cyprus	145–155	3.2–3.4	313	5.6–5.8	5.6–5.7
U.S.A.	165–285	1.8–3.0	310–315	3.1–4.7	4.4–8.6
Lime					
Mexican	18–34	15–27	314–316	22–39	22–33
Persian	15–18	28–33	315–318	40–46	25–30
Distilled	500	1.1	274	11.4	18
	2000	0.25	265	35.1	18
Orange					
Sweet	235–400	1.3–2.1	326–330	1.3–2.3	11–41
Bitter	80–90	5.6–6.0	268–269	4.4–4.8	66–79
			320	8.3–8.8	
Grapefruit	45–60	8.3–11.0	267–269	6.9–8.9	82–94
			317–320	12.3–16.5	
Terpeneless					
Lemon	200	2.5	237	595	0.3
			321	2.8	
Lime	4.7	106	226	482	20.0
			240	444	
			313	174	
Lime, distilled	1600	.31	272.5	13.5	6.9
Orange, sweet	343	1.5	231	213	0.8
	1420	.35	235	116	0.2
Grapefruit	2.6	189	268	153	86.5
			318	274	

[a] Data based on examination of samples comprised in this study. [b] Yielding absorbance readings of approximately 0.5 at 336 mμ.

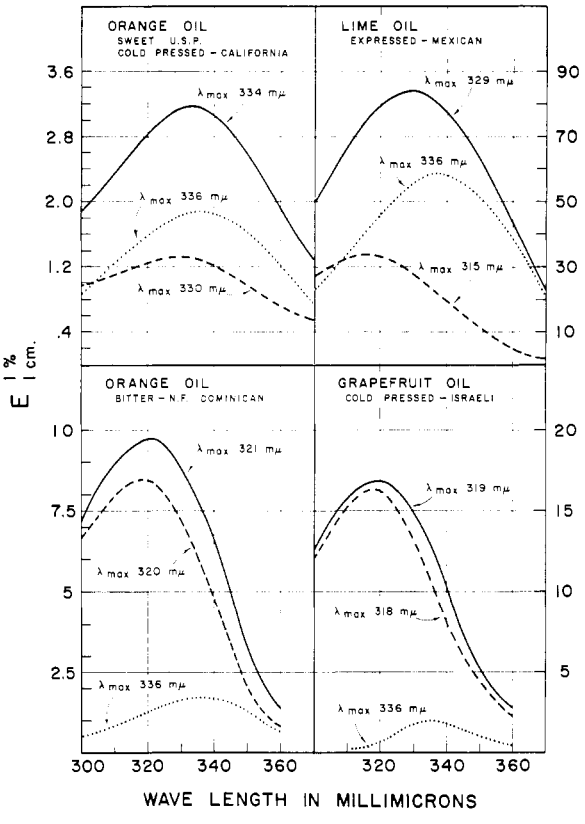


Figure 7. Analysis of orange, lime, and grapefruit oil by barbituric acid condensation method

– – – –Ultraviolet absorption curve of essential oil
————Ultraviolet absorption curve of essential oil following reaction
. . . . . Difference curve

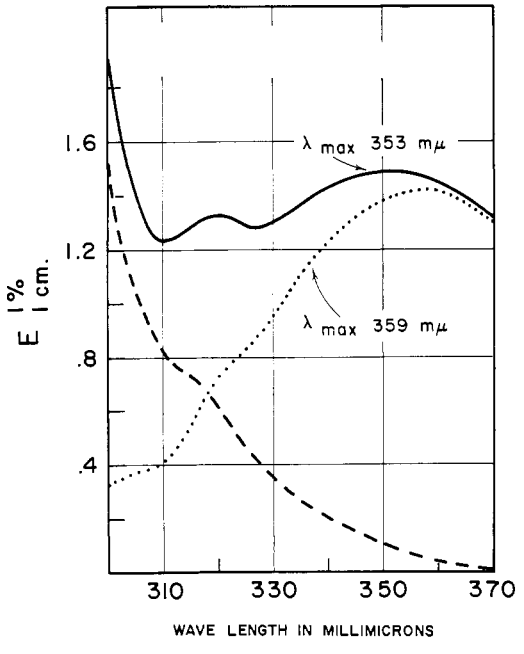


Figure 8. Analysis of distilled Mexican lime oil by barbituric acid condensation method

– – – –Ultraviolet absorption curve of essential oil
————Ultraviolet absorption curve of essential oil following reaction
. . . . . Difference curve

color test (*1*) to confirm their assumption. A few drops of the oil were heated with a slight excess of barbituric acid in about 1 ml. of 50% aqueous ethyl alcohol for 5 minutes on a water bath. After cooling, one drop of aniline was added and the test tube was gently shaken. The upper layer became gradually blue-violet, the lower purple-red and after standing for about 30 minutes the blue-violet coloration of the upper layer turned yellow. Based on this identification a comparison of the analytical peak absorptions displayed by furfural at 366 mμ and citral at 336 mμ would indicate that the sample contained about 0.03% of furfural and 0.04% of citral. Thus the ultraviolet data recorded in Table XI for essential oils and in Table XII for essential oil constituents and aromatic chemicals may be utilized to obtain valuable information regarding product composition. It should be mentioned in this connection that the second sample of distilled lime oil examined and showing a citral content of 0.29% (Table IX) displayed different ultraviolet absorption characteristics, both the reacted specimen and the difference curve exhibiting distinct maxima throughout the 336 mμ region. It gave in accordance with these observations no color test for furfural. However, the characteristic colorations were readily obtained when trace quantities of the aldehyde were added to the experimental sample prior to reaction.

The compositional differences of distilled lime oils thus observed should, however, not be astounding in view of the drastic conditions under which these products are obtained. Their prolonged exposure to the action of heat and an acidic medium reduces markedly the total carbonyl content while practically destroying the very fragile aldehydic components, furfural and citral.

Extension of Method to Other Essential Oils and Related Products. The method has been applied to a number of essential oils and quantitative data have been obtained for the first time on products in which citral is known to be present as a minor component, in which it has been suspected but as yet not definitely identified, and in which it has never been thought to occur. The aldehyde has also been determined by the procedure described in many pharmaceutical preparations and flavoring compositions. Results of these studies are to form the subject matter of forthcoming publications from this laboratory.

Table XII. Reaction of Essential Oil Constituents and Related Compounds with Barbituric Acid

Compound	λ_{max}, mμ	$\frac{Absorbance_{max}}{Absorbance_{336m\mu}}$	Apparent Citral Content, %
Benzaldehyde	325.5	1.1	61.4
Butyraldehyde[a]	403	5.1[b]	0.13
Cinnamaldehyde[a]	369.5	2.1[b]	69.0
Crotonaldehyde[a]	308	2.8	45.1
Dihydrocitral	334	1.0	98.1
Ethylvanillin,[a] (Bourbonal)	402	3.9[b]	18.1
Furfural[a]	366	2.3	59.9
Vanillin[a]	401	4.3[b]	21.4

[a] Products purified by fractionation or recrystallization.
[b] Peak absorbances not reached within 45 minutes.

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