Determination of Citral in Food and Drug Products by the Barbituric Acid Condensation Method

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The determination of citral in agricultural and food products is a problem of considerable industrial importance. Data reported herein demonstrate the extension of the barbituric acid–citral condensation (BAC) method of analysis to a wide range of such preparations containing only small amounts of the terpene aldehyde and further illustrate the specificity of this reaction. The assay should therefore be valuable in quality control of citrus products and related compositions marketed by the food, drug, and cosmetic industries.

During the summer of 1958, while developing the barbituric acid condensation method of citral analysis for essential oils, a request for experimental details from the physiology department of Oxford University (*10*) stimulated the authors to extend their study to a variety of food and drug products. At the time, Leach and Lloyd had observed that single, subcutaneous doses of the α,β-unsaturated aldehyde (5 μg. per kg.) raised the ocular tension of rabbits and caused damage to vascular endothelia. Accordingly, food and drug preparations containing citral were considered a potential contributory cause of glaucoma and cardiovascular diseases in man (*11*). Analogous biological investigations carried out subsequently at the laboratories of the Canadian Food and Drug Directorate failed to confirm the occurrence of these symptoms. Ocular tension was observed to remain unchanged and histopathological examination of autopsied animals revealed no abnormal conditions (*6*).

Meanwhile, the authors had demonstrated the applicability of their assay procedure to numerous commercial preparations containing minute amounts of the terpene aldehyde. The results obtained, although now stripped of any clinical significance, are reported to illustrate the application of the method to a series of flavoring extracts for which legal standards and specifications have been established and to present, for the first time, citral data for a variety of widely used food and drug products, including jams, marmalades, juices, conserves, jellies, puddings, and soft drink powders.

Experimental

The analytical procedure as adopted for the examination of essential oils has previously been reported in detail (*12*).

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Table I. Analysis of Natural and Artificial Lemon Extracts for Citral by the BAC Method

Product (Label Information)	Supplier	Weight Analytical Sample,[a] Gram/10 Ml.	Citral, % Weight by Weight: Bac method	Citral, % Weight by Weight: A.O.A.C. method
Lemon, pure	A	0.083	0.89	1.18
Lemon, pure	A	0.087	0.90	0.92
Lemon extract, true	B	0.299	0.26	0.32
Lemon extract, true	B	0.303	0.25	0.25
Lemon extract, true[b]	B	0.515	0.15	0.25
Lemon extract, pure	C	0.573	0.13	0.18
Lemon extract, pure	D	0.478	0.16	0.18
Lemon extract, pure	E	0.769	0.10	0.18
Lemon extract, pure	J	0.363	0.21	0.27
Lemon essence	F	0.237	0.33	0.47
Lemon, artificial	A	[c]	0.0018	0.008
Lemon, artificial	I	[c]	0.0009	0.014
Lemon extract, artificial	G	[c]	0.0015	0.028
Lemon extract, artificial	G	[c]	0.0062	...
Lemon extract, artificial	H	[c]	0.0001	0.017

[a] Yielding an absorbance reading of approximately 0.5 at 336 mμ following 2 to 50 ml. dilution.
[b] Stored under normal conditions of use in a kitchen cupboard for 7 years.
[c] Approximately 2 grams were used for analysis and 2-ml. aliquots diluted to 25 ml., subjected to ultraviolet examination at 336 mμ. Because of the low absorbance of these products at 336 mμ, use of samples displaying absorbances of 0.5 at this wave length is not recommended.

Table II. Analysis of Official Citrus Flavorings by BAC Method

Product	Analytical Sample, G.ams	Citral Content, Mg /100 Ml.: Estimated[a]	Citral Content, Mg /100 Ml.: Found
Compound Orange Spirit, U. S. P. (Spiritus Aurantii Compositus)	0.2[b]	110–240	182
Spirit of Orange, C. F. (Spiritus Aurantii)	1	10–30	17.4
Lemon Tincture, B. P.; U. S. P. (Tinctura Limonis)	3	5–10	6.3
Sweet Orange Peel Tincture, U. S. P.	50	0.2–0.7	0.41
Orange Tincture, B. P. (Tinctura Aurantii)			
Lemon Syrup, B. P. (Syrupus Limonis)	50	0.2–0.5	0.27
Tincture Sweet Orange, C. F. (Tinctura Aurantii Dulcis)	75	0.1–0.3	0.18
Orange Syrup, B. P.; U. S. P. (Syrupus Aurantii)	100	0.02–0.04	0.02
		Mg /100 Grams	
Lemon Peel Dried, B. P. (Limonis Cortex Siccatus)	0.3	50–90	60.3
Lemon Peel Fresh, B. P. (Limonis Cortex Recens)	1	10–20	16.1

[a] Tentative values, based on product composition and average citral content of essential oils present (*2, 12, 15*).
[b] Samples may be analyzed without pretreatment (pentane extraction) in accordance with the procedure described.

Its application to flavoring extracts was realized as follows.

Samples ranging from 0.1 to 2.0 grams were weighed accurately into 10-ml. volumetric flasks, diluted with the reagent (1% of barbituric acid solution in 20% of aqueous ethyl alcohol) and mixed thoroughly. The reaction vessels were placed in a water bath kept at 25° C. and aliquots withdrawn after 40 minutes for dilution with the solvent and subsequent spectrophotometric examination at 336 mμ. Similarly diluted aliquots of the reagent were used as blanks. Corresponding absorbances displayed by ethanolic solutions of the products at 336 mμ were also determined and from the analytical data, citral values were obtained by means of Equation 1, where 0.6153 represents the analytical factor for purity and recovery of the citral sample used as reference standard (*12*).

Products containing more than 0.1% of citral were analyzed in accordance with this procedure. If a preparation was suspected or found to contain a smaller amount, it was pretreated by continuous extraction with reagent grade pentane which had been purified by fractional distillation and passage through a column of silica gel. The extract was gently evaporated by means of an indirect stream of nitrogen and the residue taken up in 2 ml. of ethyl alcohol. A 0.5-ml. aliquot was made up to 5 ml. with the solvent and 4 ml. of this solution diluted to 25 ml. prior to ultraviolet examination. For analysis a 1-ml. aliquot of the extract was made up to 5 ml. with the reagent, the solution kept at 25° C. for 40 minutes, diluted comparably thereafter with ethyl alcohol (2 to 25 ml.), and its absorbance measured at 336 mμ, using as blank a solution of 2 ml. of reagent in 25 ml. of of ethyl alcohol.

Difference curves in terms of $E^{1\%}_{1cm.}$ for both reacted and unreacted sample were constructed in some instances to demonstrate the presence of citral in the product examined and conversion of the terpene aldehyde to citrylidene barbituric acid (*12*).

Jams, marmalades, conserves, citrus peels, jellies, puddings, and soft drink powders were similarly processed following dilution and trituration with distilled water to a suitable consistency.

$$\text{Per cent citral (w./w.)} = \frac{0.6153 \times [E^{1\%}_{1cm.336m\mu\ \text{(sample following reaction)}} - E^{1\%}_{1cm.336\ m\mu\text{(sample prior to reaction)}}]}{10} \quad (1)$$

$$\text{Citral (\% w./w.)} = \frac{0.6153 \times [\text{absorbance}_{\text{Extracted sample following reaction}} - \text{absorbance}_{\text{Extracted sample prior to reaction}}] \times 62.5}{\text{weight of original sample (grams)} \times 0.5 \times 1000} \quad (2)$$

where

0.6153 = analytical factor (*12*)
0.5 = portion of total weight of extracted material contained in volume of extract used for analysis
62.5 = dilution factor

$$= \frac{0.0769 \times \text{absorbance of extracted sample}_{\text{corrected}}}{\text{weight of original sample (grams)}}$$

Table III. Analysis of Citrus Juices by the BAC Method

Product (Label Information)	Supplier	Citral, % (w./w. × 10^4)[a]
Limeade, frozen	N	8.50
Lemon juice, frozen, unsweetened, California	L	0.08
Lemonade, frozen, concentrate, California	L	0.10
Citrus-orange juice	P	0.00
Orange juice		
Frozen, concentrate	B	0.86
Frozen, concentrate, unsweetened	M	0.09
Unsweetened	B	0.10
Sweetened, Florida	O	0.11

[a] Values equivalent to milligrams of citral/kg. of juice.

Table IV. Analysis of Food Products by the BAC Method

Product (Label Information)	Supplier	Analytical Sample, Grams[a]	Citral, % (w./w. × 10^4)[b]
Conserves			
Orange peel, oil of orange and sugar added	R	56.5	9.2
Lemon peel, oil of lemon and sugar added	R	45.5	5.2
Marmalades			
Orange chip	S	233.2	0.08
Lemon chip	S	213.5	0.10
Pineapple–grapefruit–lemon–orange	U	50.0	0.18
Lime	T	212.4	0.27
Orange–lemon–grapefruit	B	94.1	0.64
Jellies			
Lime	B	85.0	3.9
Lime	Y	85.3	0.13
Lemon	B	95.4	5.8
Lemon	Y	85.2	0.22
Orange	B	84.9	1.9
Orange	Y	85.2	0.34
Puddings			
Lemon	B	95.4	10.1
Orange	B	84.9	3.5
Soft Drink Powders			
Lemon	V	6.103	51.0
Lemon	M	8.650	0.2
Lemon	W	7.310	19.0
Lime	V	6.178	11.1
Lime	M	9.175	6.8
Lime	W	7.185	0.4
Orange	V	6.044	3.0
Orange	M	8.855	0.3
Orange	W	7.258	0.2
Orange	X	5.459	0.2

[a] Because of compositional variations of these products sample weights for obtaining optimal conditions of analysis may differ appreciably.
[b] Values equivalent to milligrams of citral/kg. of product.

Table V. Recovery of Citral from Food and Drug Products by the BAC Method of Analysis

Product	Citral, Mg./Kg.	Citral Added, Mg.	Total Citral Content, Mg / G. Theoretical	Found
Lemon juice, frozen, 200 grams	0.18	0.080	0.58	0.72
Orange juice, single strength, 500 grams	0.11	0.160	0.43	0.30
Tincture of orange, B. P., 50 grams	4.51	0.080	6.12	5.90

Citral data for the various products were calculated by means of Equation 2.

Results and Discussion

Experimental data summarizing the analysis of natural and artificial lemon extracts are given in Table I. They illustrate that of nine genuine samples received from seven different manufacturers, three showed citral contents of less than 0.2% and hence failed to meet the requirements of the Canadian Food and Drugs Act (*5*). Similar analyses of five artificial preparations obtained from four different suppliers revealed that without exception these products contained, at best, only trace amounts of the flavomatic.

Examination of both types of compositions by the Association of Official Agricultural Chemists' procedure yielded invariably higher results because of the inferior specificity of the *m*-phenylenediamine reaction (*14*).

Citral data for official flavorings are reproduced in Table II. Standards and criteria of identity for these products have been recognized by the British and United States Pharmacopeia, respectively, as well as other reference compendia (*1–5*, *13*). However, because of the lack of suitable assay procedures, requirements for citral content have not yet been established. Displaying a high degree of sensitivity and selectivity, the present method should prove of value for this purpose. Experimental results are reproducible, and their comparison with citral values as deduced from the composition of these products will readily demonstrate whether or not the citral content of a given preparation falls within the anticipated range (Table II, columns 3 and 4). None of the products tabulated, if genuine, contain any constituents capable of significantly interfering in the reaction.

Experimental data illustrating the analysis of single-strength and concentrated canned citrus juices are given in Table III. Extensive researches on the composition of these products have been carried out during the past quarter of a century. Wilson and Hall, processing a total of 10,000 gallons of California Valencia orange juice demonstrated the presence of three carbonyl compounds, acetone, acetaldehyde, and citronellal in the volatile oil (*7*). More recently, Kirchner and Miller, in continuation of their long-term studies of citrus products, combined vacuum fractionation, extraction, and column chromatographic techniques to determine no less than 29 constituents, nine of which they identified as carbonyl components (*9*). Three thousand gallons of freshly reamed, 2500 gallons of freshly canned, and 1520 gallons of stored, canned juice, respectively, were used for this work. Citral was not detected in any of the products examined, although its occurrence in grapefruit juice—approximately 0.1 mg. per kg. as based on the analysis of a 2500-gallon sample—had previously been established (*8*).

Experimental data on jams, marmalades, conserves, jellies, puddings, and soft drink powders are given in Table IV. These products were found to differ in many respects and slight procedural variations—e.g., solvent ratio, period of extraction, and size of analytical sample—had to be adopted to secure meaningful results. Some measure of the efficiency and selectivity of the assays was established by means of recovery experiments (Table V). As far as the authors are aware no data which could be used for comparison purposes have as yet been reported in the literature.

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PEA PROTEIN EVALUATION

Correlation between Alcohol-Insoluble Substances and Lysine Availability in Canned Peas

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APPROXIMATELY 47% of the protein in the average Israeli diet is derived from grain products (*9*), mainly wheat, which is poor in lysine. A popular vegetable protein source relatively rich in lysine (*16*) is canned peas (*Pisum sativum*). The variety grown for canning is Perfection.

Canned peas are commonly evaluated on acceptability criteria related to the organoleptic properties of the product, such as alcohol-insoluble substances (AIS) (*10*). No relationship, however, has hitherto been established between these criteria and a chemically determinable protein quality value (*13*).

The nutritive value of the protein in a food may be reduced by procedures involving heat (*24*). This reduction can often be compensated for to a large extent by supplementing the processed material with lysine, although analysis by conventional means shows little actual change in lysine concentration as a