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Volumetric Determination of Total Aldehydes in Citrus Oils

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A titrimetric method for determining citral has been developed; it is based on the quantitative reaction of the citral with semicarbazide hydrochloride to form a semicarbazone. The semicarbazone is removed and the excess unreacted semicarbazide determined iodometrically using chloramine-T reagent. Two moles of chloramine-T are required per mole of semicarbazide. The method has been applied for the determination of the total aldehydes in citrus oils (expressed as citral in lemon and lime and as decanal in orange). The present method can be used in the routine quality evaluation of citrus oils, and the sample required is in the range of 0.5 to 1.0 g.

Citrus oils are a major by-product of the citrus processing industry. The commercially important peel oils include lime, lemon, orange, grapefruit, bergamot, etc. These are used as flavoring ingredients in a variety of foods like soft drinks, ice cream, frozen desserts, confectionery, baked goods, and also in perfumery industry. The oils chiefly consist of terpene hydrocarbons (80–95%) and oxygenated terpenes such as citral, decanal, nootkatone linalyl acetate, terpineol, geranyl acetate, etc. The hydrocarbons are unstable and susceptible to photochemical and oxidation reactions and slowly contribute to the deterioration in the quality of the oils. They act largely as the carriers for the oxygenated compounds which are mainly responsible for the characteristic citrus flavors. With a view to obtaining stable and concentrated citrus oils, they are either partly or completely deterpenated. This is achieved by employing a variety of techniques like solvent partition (Merory, 1968; Ruys, 1957), column chromatography (Anandaraman et al., 1976; Braverman and Solomiansky, 1957; Kirchner and Miller, 1952; Rovesti and Rovesti, 1967; van der Lijn and Lifshitz, 1969), fractional distillation, (Lifshitz et al., 1969), etc.

Among the various oxygenated constituents, the aldehydes have been considered to have the most profound influence on the flavor quality of the citrus oils. For example, the characteristic odor of lemon and lime is mainly due to citral. Several methods have been described in literature for the determination of the aldehyde(s) content. As early as 1909, Hiltner described a colorimetric method for the determination of α,β-unsaturated aldehydes using *m*-phenylenediamine reagent. The accuracy of the method has been improved by using the photoelectric spectrophotometer (Kleber, 1912). The volumetric method, employing phenylhydrazine reagent, was adopted as the USP procedure (1965) for sometime for the determination of citral in lemon oil. Stanley et al. (1958) described a specific method for the determination of citral in lemon oil based on its reaction with vanillin–piperidine reagent giving a green color. Levi and Laughton (1959) reported an ultraviolet absorption spectrophotometric method wherein the citral is converted into its barbituric acid derivative (λ_{max} 336 nm). The colorimetric method of Ismail and Wolford (1970) employs *N*-hydroxybenzenesulfonamide reagent which determines the total aldehydes. This method has undergone some modifications in the hands of Dougherty and Petrus (1971) and Petrus et al. (1970). Surve et al. (1958) have described an ultraviolet spectrophotometric method for citral based on its characteristic absorption peak around 238 nm. The most widely used method for the determination of the aldehydes in citrus oils is the volumetric procedure employing hydroxylamine hydrochloride reagent (Bennett and Salamon, 1927, 1930; AOAC, 1970).

During our work on the quality evaluation of citrus oils, it was necessary to determine the aldehyde content in comparatively small samples (0.5 to 1 g) of the citrus oils. Therefore, a titrimetric method has been developed using chloramine-T (sodium derivative of *N*-chloro-*p*-toluenesulfonamide) reagent. The aqueous solution of this reacts as if it were a hypochlorite (Bishop and Jennings, 1958):

$$RNCl{\cdot}Na + H_2O \rightarrow RNH_2 + NaOCl \quad (1)$$

where R = $CH_3C_6H_4SO_2$-. Chloramine-T has been recently employed for the determination of flavor strength in mustard (Shankaranarayana et al., 1972), asafetida (Abraham et al., 1973), and radish (Damodaran, 1975), and also for the determination of the derivatives of dithiocarbamic acid (Abraham et al., 1975). The present method consists of the quantitative conversion of the aldehydes into their semicarbazone derivatives (eq 2) and then determining the unreacted semicarbazide oxidimetrically using chloramine-T reagent (eq 3):

$$RCHO + NH_2CONHNH_2 \rightarrow RCH{=}NNHCONH_2 + H_2O \quad (2)$$

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where R = $(CH_3)_2C{=}CH(CH_2)_2C(CH_3){=}CH$-, $CH_3(CH_2)_8$-, $CH_3(CH_2)_{10}$-, etc.

$$NH_2CONHNH_2{\cdot}HCl + 2(O) \rightarrow N_2 + CO_2 + NH_4Cl + H_2O \quad (3)$$

The excess chloramine-T is determined iodometrically.

EXPERIMENTAL SECTION

Materials and Reagents. *Citral* (Kelkar and Co., Bombay). This was purified (Surve et al., 1958) by converting it (20 g) into an adduct by reacting with saturated sodium sulfite solution (260 mL). The unreacted impurities were removed by washing with ether. The citral was regenerated by treating the adduct with sodium hydroxide (10% aqueous solution, 390 mL), extracted with ether, and distilled [90 °C (5 mmHg); yield, 15 g]. The purity of this sample was ascertained by measuring its absorbance values in the ultraviolet spectral region using Beckman DU spectrophotometer (solvent: ethanol, λ_{max} 238 nm, ϵ 14 500; λ_{max} 325 nm, ϵ 70). The infrared spectrum of the above sample showed the following characteristic absorption bands: 2900 (strong), 1680 (very strong), 1450 (strong), 1380 (strong), 1190 (medium), and 1130 cm^{-1} (strong).

Citrus Oils. Natural and deterpenated citrus oils such as lime, lemon, and orange were obtained both from within the country and abroad.

Methyl Cyanide (British Drug House, Bombay). About 1 L of this was refluxed with chloramine-T (10 g) for 1 h and the fraction distilling at 77 °C was collected and used.

Chloroform (Reechem, Hyderabad). This was once distilled and used.

Semicarbazide Hydrochloride Solution. (I) A stock solution was prepared by dissolving 5 g of semicarbazide hydrochloride (A.R. Grade, Koch-Light) in 100 mL of distilled water. (II) An aliquot of the above (10 mL) was diluted to 100 mL.

Sodium Acetate Solution. A 12% solution (w/v) of sodium acetate (Reechem, Hyderabad) was prepared using distilled water.

Chloramine-T (British Drug House, England). An approximately 0.1 M solution was prepared by dissolving 28 g of chloramine-T in 1 L of water. The solution was kept overnight, filtered, and then used.

Sodium Thiosulfate Solution. 0.05 N.

Procedure. (*a*) *Determination of Semicarbazide Hydrochloride.* An aliquot of the semicarbazide solution II containing 10 to 50 mg of semicarbazide hydrochloride was transferred into an Erlenmeyer flask (250 mL), containing a mixture of 20 mL of chloramine-T and 20 mL of 2 N sulfuric acid. The reaction mixture was kept aside for 45 min. The unreacted chloramine-T was determined by the addition of potassium iodide (15% solution, 10 mL), the liberated iodine titrated against sodium thiosulfate solution using starch as indicator. A suitable chloramine-T blank was carried out. The difference in the titer values corresponds to the amount of chloramine-T consumed by the semicarbazide.

(*b*) *Determination of Citral.* An accurately weighed quantity of citral (0.1 to 0.4 g) was dissolved in methyl cyanide and the volume made up to 100 mL. An aliquot (10 mL) of the above was treated with 1 mL of semicarbazide hydrochloride (I) and 0.5 mL of sodium acetate solutions and refluxed on a boiling water bath for 1 h. The reaction mixture was cooled, transferred to a separating funnel, and diluted using 50 mL of water. The semicarbazone derivative formed was extracted repeatedly with chloroform (15 mL × 4). The aqueous layer was transferred into an Erlenmeyer flask, containing a mixture of 20 mL of chloramine-T and 10 mL of 10 N sulfuric acid. After a period of 45 min, the unreacted chloramine-T was determined iodometrically. A suitable blank was carried out using methyl cyanide. The amount of citral was calculated from the titer using the following relationship: 1 mL of N sodium thiosulfate = 0.038 g of citral.

(*c*) *Recovery of Added Citral in Lime Oil.* Stock solutions of lime oil (20 g/100 mL) and citral (1 g/100 mL) were prepared using methyl cyanide. An aliquot of the lime oil (5 mL) was mixed with varying volumes of the citral solution (0.5 to 3.0 mL) and the volume made up to 10 mL with methyl cyanide. The aldehyde content in these was determined as above.

(*d*) *Determination of Total Aldehydes in Citrus Oils.* An aliquot of citrus oil stock solution in methyl cyanide (10 mL containing 0.5 to 1.0 g of natural oil/0.02 to 0.50 g of deterpenated oil) was used for the determination of the aldehyde content as described for citral.

Table I. Analysis of Semicarbazide Hydrochloride with Chloramine-T

Weight of semicarbazide hydrochloride taken, mg	Volume of chloramine-T consumed, expressed as thiosulfate (0.05 N), mL	Moles of chloramine-T per mole of semicarbazide hydrochloride
10	7.10	1.98
20	14.15	1.97
30	21.40	1.99
40	28.00	1.95
50	36.00	2.01

Table II. Analysis of Citral by Chloramine-T Method

Citral taken, g	Citral found,[a] g
0.0105	0.0103
0.0202	0.0194
0.0245	0.0229
0.0310	0.0297
0.0346	0.0336
0.0398	0.0376

[a] Average of triplicate values.

RESULTS AND DISCUSSION

Table I gives the data on the oxidation of semicarbazide hydrochloride using chloramine-T. It can be seen from Table I and eq 3 that 1 mol of semicarbazide reacts with 2 mol of chloramine-T. Based on this, the amount of semicarbazide hydrochloride can be calculated using the following expression: 1 mL of N chloramine-T = 0.02788 g of semicarbazide hydrochloride. Table II shows the results of analyses of pure citral. The determination of citral is based on two reactions, namely, quantitative conversion of the citral into its semicarbazone (Shriner and Turner, 1930) and determining the excess unreacted semicarbazide by oxidation with chloramine-T. The removal of semicarbazone derivative is necessary as it was found to undergo oxidation with chloramine-T. This was confirmed by independent experiments wherein the citral semicarbazone was found to react with chloramine-T under similar conditions. The removal of semicarbazone was achieved by using solvents such as ethyl acetate, ether, and chloroform. However, chloroform was found to be the best solvent in view of its efficient extraction and easy removal, after extraction, from the separating funnel. Table II reveals a close agreement between the weight of citral taken and found. Using this method, one can determine citral samples as small as 10 mg with a fair degree of accuracy. It can be seen from Table III that the recovery data are in agreement with the added amounts of citral

Table III. Data on the Recovery of Added Citral from the Lime Oil

Weight of oil taken, g	Amount of citral Added, mg	Found, mg	% recov of added citral
1.0057		5.23	
1.0057	5	10.25	100.40
1.0057	10	15.10	98.70
1.0057	15	19.95	98.13
1.0057	20	24.98	98.75
1.0057	25	30.05	99.53
1.0057	30	35.02	99.30

even in a natural system like that of a lime oil.

The hydroxylamine hydrochloride procedure is the widely used method for the determination of the total aldehydes in citrus oils. However, the main drawbacks of this method are the requirement of larger quantities of the samples (10 g) and the difficulty of detection of the end point during titration. The chloramine-T method can, however, analyze as small a sample as 0.5 g of natural oil or 0.02 g of deterpenated oil (Table IV). Since this is an iodometric method, the end point is sharp, and, further, the initial removal of the organic impurities by chloroform eliminates the formation of emulsion. Table IV includes data on the aldehyde content of the citrus oils and their physical properties such as specific gravity, refractive index, and specific rotation, determined according to standard procedures (AOAC, 1970). However, no correlation was found between the aldehyde content and the physical properties.

The present method can be used as a routine procedure in the quality evaluation of citrus oils for determining the

Table IV. Data on the Physical Properties and Aldehyde Content of Citrus Oils

Sample particulars	Physical properties: Specific gravity 26°/26 °C	Refractive index at 26 °C	Specific rotation $[\alpha]^{26}_D$	Determination of aldehyde[a]: Weight taken	Aldehyde (%) found by chloramine-T method
Lime oil					
Natural					
A (centrifuged)	0.8758	1.4824	38.70	1.0321	4.14
B (centrifuged)	0.8833	1.4852	38.77	1.0150	4.82
C (distilled)	0.8660	1.4750	39.39	1.0118	0.65
D (distilled)	0.8722	1.4762	57.57	1.9886	0.35
E (distilled)	0.8710	1.4754	42.18	1.0057	0.52
F (distilled)	0.8604	1.4725	43.24	1.0047	2.60
G	0.8718	1.4765	42.75	1.0087	0.58
H	0.8636	1.4744	49.64	1.0097	0.64
I (distilled)	0.8634	1.4735	43.05	1.0089	0.47
J	0.8628	1.4748	40.81	1.0125	0.72
Deterpenated					
G_1	0.9475	1.4638	13.97	0.2320	1.96
H_1	0.9022	1.4750	17.98	0.5269	1.69
I_1	0.9264	1.4800	−10.87	0.2131	1.89
L(CFTRI)	0.9729	1.5042	−2.00	0.0510	28.69
Lemon oil					
Natural					
P (cold pressed)	0.8553	1.4722	68.14	1.0709	2.87
Q	0.8543	1.4770	33.21	1.2082	6.06
R	0.8541	1.4714	65.89	1.0078	4.10
S	0.8537	1.4735	66.10	1.0152	3.80
T	0.8547	1.4758	46.90	1.0026	3.74
U	0.8557	1.4725	68.63	1.0135	3.78
V	0.8517	1.4720	66.19	1.0300	2.34
X	0.8954	1.4772	−5.83	1.0177	4.45
Deterpenated					
K	0.8960	1.4800	19.95	0.0213	41.10
Y	0.8561	1.4770	64.80	0.2072	5.76
S_1	0.8935	1.4818	−3.98	0.0489	38.93
T_1	0.8706	1.4750	6.25	0.1472	17.98
V_1	0.9471	1.4795	19.07	0.2038	19.60
Z (CFTRI)	0.9378	1.4795	−4.98	0.0514	36.00
Orange oil					
Natural					
AA (cold pressed)	0.8467	1.4728	98.40	0.4754	0.89
AB (cold pressed)	0.8486	1.4705	108.20	0.5008	1.17
AC (cold pressed)	0.8465	1.4715	10.74	0.5088	1.92
AD	0.8476	1.4718	106.50	1.0113	1.77
AE	0.8461	1.4720	107.40	1.0093	2.27
AF	0.8482	1.4730	110.40	1.0048	1.52
AG	0.8455	1.4710	108.10	1.0036	1.93
AH	0.8450	1.4706	107.80	1.0124	1.02
Deterpenated					
AI	0.9152	1.4788		0.2053	7.60
AJ	0.8511	1.4705		0.2110	5.11
AE_1	0.8736	1.4680	23.85	0.1456	21.91
AF_1	0.8682	1.4730	80.37	0.2075	5.17
AG_1	0.8890	1.4620	1.99	0.2070	22.99
AH_1	0.8712	1.4754	52.12	0.2070	24.79

[a] Expressed as citral for lime and lemon; decanal in the case of orange.

total aldehydes. Although ketones also react in the above method, they are present in negligible concentrations and do not contribute much to the flavor.

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Moniliformin, a Mycotoxin from *Fusarium fusarioides*

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Fusarium fusarioides was found to be highly toxic to ducklings and rats. The toxic metabolite, moniliformin (sodium or potassium salt of 1-hydroxycyclobut-1-ene-3,4-dione) was isolated as the sole toxin from *F. fusarioides*. Isolates obtained from peanuts, sorghum, millet, peaches, soil, and dried fish were all toxic to ducklings. The chronic toxicity of an isolate from millet was determined, and 10, 25, and 50% moldy meal incorporated into commercial rat mash all proved toxic. The effect of different incubation temperatures and periods was investigated. Material produced at three different temperatures was of approximately equal toxicity, although less of the material produced at higher temperature was consumed. Analysis showed that the material incubated at 31 °C contained the most moniliformin. Self-heating to a maximum of 6–8 °C took place at all incubation temperatures. The thin-layer chromatographic analytical method was applied to four randomly selected isolates, all of which produced moniliformin, ranging in quantities from 200–840 mg/kg. Methods are described for the analyses and isolation of moniliformin from yellow corn inoculated with cultures of *F. fusarioides*. The analytical methodology involves either thin-layer chromatography of the extracts or purification of these on Dowex-1 (Cl^-) resin; 0.2 M sodium chloride elutes the moniliformin which is subjected to quantitation by UV spectroscopy.

Toxin production is frequently encountered amongst many species and isolates of the genus *Fusarium*, the most common being zearalenone (Pathre and Mirocha, 1976; Mirocha and Christensen, 1974), moniliformin (Cole et al., 1973; Kriek et al., 1977), and the chemically related group of trichothecenes (Bamburg, 1976; Smalley and Strong, 1974; Saito and Ohtsubo, 1974). The associations of these toxins, and the species of *Fusarium* that produce them, with mycotoxicosis have been reviewed in detail (Joffe, 1974; Saito and Ohtsubo, 1974).

Samples of millet, *Pennisetum typhoides* (Burm.) Staph and Hubb., obtained from the households of patients in South West Africa suffering from the hemorrhagic disease Onyalai were mycologically investigated. Amongst others, *Fusarium fusarioides* (Frag. and Cif.) Booth could readily be isolated and proved to be toxic in feeding trials with ducklings (Rabie et al., 1975; Steyn and Rabie, 1976). Although the occurrence of *F. fusarioides* appeared to be less widespread than *Phoma sorghina* (Sacc.), it could nevertheless be isolated quite frequently from millet, which

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