Table I. Effects of Dichlorovos on Growth and Ochratoxin Production in Yeast Extract–Sucrose Medium

Strain	Dichlorvos, mg/100 ml of broth	Dry mycelia, g/100 ml of broth	% control	Ochratoxin A, mg/100 ml of broth	% control	Ochratoxin B, mg/100 ml of broth	% control
UGa no. 40	0	3.54 ± 0.13[a]		3.77 ± 0.24		2.59 ± 0.09	
	0.1	3.49 ± 0.10	99	3.69 ± 0.29	98	2.64 ± 0.11	102
	1	3.57 ± 0.16	101	2.68 ± 0.20	71	1.35 ± 0.10	52
	10	3.24 ± 0.09	91	1.05 ± 0.08	28	0.99 ± 0.07	38
	30	2.80 ± 0.10	80	0.81 ± 0.11	21	0.29 ± 0.05	11
NRRL 3174	0	2.86 ± 0.12		5.15 ± 0.22		1.72 ± 0.07	
	0.1	2.91 ± 0.14	102	5.19 ± 0.28	101	1.68 ± 0.09	98
	1	2.78 ± 0.10	97	4.05 ± 0.19	79	1.25 ± 0.09	73
	10	2.54 ± 0.08	89	2.55 ± 0.26	50	0.88 ± 0.07	51
	30	2.34 ± 0.11	82	1.34 ± 0.16	26	0.38 ± 0.05	22

[a] Average of three replications.

Table II. Effects of Dichlorvos on Ochratoxin Production on Corn

Strain	Dichlorvos, mg/100 g of corn	Ochratoxin A, mg/100 g of corn	% control	Ochratoxin B, mg/100 g of corn	% control
UGa no. 40	0	3.65 ± 0.30[a]		1.84 ± 0.17	
	0.1	3.77 ± 0.24	103	1.77 ± 0.11	96
	1	2.88 ± 0.28	79	1.37 ± 0.09	74
	10	1.63 ± 0.19	45	0.84 ± 0.09	46
	30	0.94 ± 0.08	26	0.32 ± 0.05	17
NRRL 3174	0	4.86 ± 0.35		2.33 ± 0.19	
	0.1	4.69 ± 0.31	97	2.27 ± 0.21	97
	1	3.68 ± 0.27	76	1.15 ± 0.11	49
	10	2.83 ± 0.19	58	0.63 ± 0.08	27
	30	0.67 ± 0.06	14	0.19 ± 0.03	8

[a] Average of three replications.

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Use of a Simplified Method for Lysine by Gas Chromatography

Gas chromatography with a 12-ft column, higher internal standard, and lower temperature and carrier gas rate improved accuracy and precision of lysine determination in 50% longer time than an earlier simplified method

In 1972 we described a short method (Zscheile and Brannaman, 1972) for quantitative determination of lysine in crude acid hydrolysates of wheat and rice seeds. Derivatization was simple and the gas chromatography brief (13 min). Our subsequent use of this method (Ruckman *et al.*, 1973) led to further evaluation of factors affecting reproducibility and to the allowance of more time for chromatography when brevity is not essential.

Following our custom of adding lysine standard [step 5 (Zscheile and Brannaman, 1972) (all steps referred to in this communication are from this reference)] to one sample of each day's run (12–24 samples) and chromatographing with and without lysine standard on both columns, used alternately, we noted occasional fairly large calibration differences between columns. During 17 runs on wheat with 2 sets of columns extending over 1 year, these

differences ranged from −7.0 to +6.6% (in 9 of these runs the differences were less than 1%). Peak areas due to added lysine were about equal to the areas under the phenanthrene internal standard peaks (these reached a height of *ca.* 0.15–0.30 mV, with 1 μl injected volume). To make the phenanthrene peak larger (*ca.* 0.7–0.9 mV) and the determination more accurate we recommend the use of 1.8 ml of dry ethanol containing 2.0 mg/ml of phenanthrene, making the total phenanthrene per sample 3.6 mg (step 9).

Column lengths of 12 ft provided better resolution. Twelve-foot columns, lower carrier gas rates, and a column temperature of 190° extended the time for lysine emergence to 10–11 min, providing a better base line for lysine and better separation from the small peak immediately preceding lysine. Carrier gas pressure was varied to make lysine passage time equal for two columns. We also adapted a weekly treatment of columns with 15 μl of Freon R11 to clean the flame detectors. Ten runs on wheat over 6 weeks on one pair of columns had differences of −2.0 to +5.2%, a considerable decrease from the earlier group. Likewise, ten runs on rice over 8 weeks on one pair of columns had differences of −1.0 to +5.0%. Columns have performed satisfactorily for 200 samples. Usually the first few inches of column next to the injector become caked or discolored and only this portion needs repacking.

Since the small pre-lysine peak was sometimes not completely separate on the sloping base line, we employed a slow program of temperature change to resolve these peaks more completely. A rice sample gave ratios of lysine/phenanthrene of 1.022 and 1.025 on column A and 1.001 and 1.032 on column B by the short isothermal method. It was then programmed, after 5 min at 100°, at 2°/min to 210°. Lysine emerged at 45 min with a horizontal base line and phenanthrene at 51 min; after the phenanthrene peak, five other small peaks appeared, which would normally emerge while the alternate column was in use. Two such programmed runs gave ratios of 1.071 on column A and 1.060 on column B; thus, ratios were higher by 4.5% on column A and 4.3% on column B. We, therefore, consider our results on rice by the usual short method to be *ca.* 4.4% low because of failure to integrate lysine completely with no correction for the sloping base line. Wheat often has a larger pre-lysine peak than rice; a similar study on a wheat with a large pre-lysine peak (0.06 mV) gave an estimated 6% low value for the rapid method.

When short analysis time is not of prime importance, we employ 12-ft columns (isothermal column at 190°, with injector and detector temperatures of 245°) with carrier gas pressures adjusted to deliver lysine to the detector at 10 min and phenanthrene at 18–20 min for complete chromatography. The base line is nearly horizontal for lysine. With 0.5-g samples of seed meal, 15 ml of the 25-ml hydrolysate (step 4) gives good quantitative peaks. For calibration with known lysine (step 5) a 1.0-g sample of meal is hydrolyzed with 20 ml of 6 *N* HCl and the lysine is added to a second 15-ml aliquot from the 50-ml hydrolysate (step 4).

Dried leaves and immature heads of wheat produced curves similar to those of grain and were analyzed in the same manner.

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A Modern Approach to the Isolation and Characterization of L-Ascorbic Acid (Vitamin C)

Vitamin C was isolated from lemons in 55% yield by a new method utilizing ion exchange techniques. The substance was characterized by modern physical chemical methods.

Classical methods (Zilva, 1924, 1930; Szent-Györgyi, 1928; Waugh and King, 1932) of isolating L-ascorbic acid involve cumbersome fractional precipitations and employ conditions toward which the compound is not stable. As a result, the recovery of pure material from most natural sources is often low. We have, therefore, devised a general isolation procedure in which ion exchange techniques are employed. When specifically applied to the isolation of vitamin C from lemons, an overall recovery of at least 55% of crystalline material was obtained.

In essence the isolation procedure involves precipitation of citric acid and other strong organic acids as Pb^{2+} salts, removal of the excess Pb^{2+} from the supernatant by cation exchange chromatography, removal of acetic acid by evaporation, and separation of ascorbic acid by displacement chromatography.

A somewhat similar anion exchange method was used (Klose *et al.*, 1950) to obtain ascorbic acid from immature walnut hulls in which the vitamin is stated to be present to the extent of 16–20% of the dry weight. The anion exchange procedure was not, however, used in conjunction with Pb precipitation and cation exchange techniques. Ion exchange was also used for the partial purification of vitamin C from citrus rinds (Mottern and Buck, 1948).

METHODS

Juice was expressed from 40 lemons and particulate matter was removed by filtration through glass wool. The filtrate, 1.4 l., contained about 600 mg of ascorbic acid in 180 g of nonvolatile material. The filtrate (pH 2.40) was passed through 100 ml of AG 50W-X4 (50–100 mesh, H^+ form) cation exchange resin and the resin was washed with 300 ml of H_2O. This step was included to give generality to the procedure. It is designed to remove heavy metal ions, convert all anions to free acids, and inactivate ascorbic acid oxidase. It probably could be omitted with lemon juice. The combined effluent and wash (pH 2.15) contained 600 mg (3.4 mequiv) of ascorbic acid in 1200