

Figure 2. Protein efficiency ratios (PER) of rats fed different levels of dietary available methionine plus cystine. The PER of the three experiments were adjusted to be comparable. Bean diet (—). Soybean diet (- - -).

indicate that methionine added to the soybean meal diet was more fully utilized for growth than that added to the bean diet using either rat growth or rat PER as criteria. The curves indicate the presence of an as yet unknown substance which interfered with the utilization of dietary me-

thionine.

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Lemon Juice Particulates: Comparison of Some Fresh Juices and a Commercial Concentrate

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Techniques were developed for characterizing size distributions of lemon juice particles. Precision was sufficient to reveal patterns that may typify fresh, unpasteurized juice prepared in the

laboratory. A commercial concentrate differed markedly from the fresh juices. Results of two sedimentation experiments are discussed.

The natural turbidity and some of the organoleptic properties of citrus juices are due to the presence of suspended particles. Stevens *et al.* (1950) stabilized the suspensions by heating. Baker and Bruemmer (1971) devised a treatment for orange juice that produced suspensions of enhanced stability and turbidity. Mizrahi and Berk (1970) reported some properties of orange juice particulates. The present work was directed toward the development of methods to facilitate characterization of a variety of lemon juice products, primarily from the standpoint of particle size.

EXPERIMENTAL SECTION

Fruit specimens were selected for similarity of size and color and for low incidence of visible defects such as scars, bruises, and infections. Each specimen was washed before juice was extracted; a glass reamer was used and care was taken to minimize contamination of the juice with peel oil. Seeds revealed by slicing were removed prior to juicing. Nearly all coarse solids were removed by straining through cheesecloth, and the rest were removed by filtration through fritted glass disks. C-Porosity frits were used for samples that were to be centrifuged. All other samples

were filtered with EC-porosity frits. Frit filtration did not visibly alter the turbidity of lemon juice.

Particle Sizing. Particle size was estimated with a Model A Coulter Counter using apertures having nominal diameters of 30, 50, and 200 μm . To facilitate accurate counting, juice specimens were diluted fourfold or more with a particle-counting electrolyte. This electrolyte was prepared from reagent grade chemicals and distilled deionized water; the stock solution was 0.125 M NaCl with 1 g/l. of Na_2SO_3 added as preservative. This solution was vacuum filtered through a sintered-glass disk of UF-porosity and boiled briefly. Electrolyte was discarded within 2 days of boiling. Dilutions were made gravimetrically.

In the counting procedure adopted, each range of particle sizes was scanned in one direction and then back with the electrode polarity control set for automatic reversal. The result was a pair of counts for each particle size with both polarities represented in each pair. Counts were replicated further because an overlap was needed when there was (a) a sample dilution or (b) an aperture change. These replicates consisted of an extra pair of counts at each of two adjacent particle sizes. There were two overlaps within the range of the 50- μm aperture and one for the 200- μm aperture.

To minimize error from aperture plugging, erratic or excessively long counts were discarded and apertures were

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Table I. Aperture Calibration^a

Nominal diameter	Matthews and Rhodes (1970)	Present work
1.099	1.20	1.18
1.305	1.27	1.27
1.947		2.01
3.49	3.40	3.46
12-13		11.13

^a Particle diameters in micrometers.

Table II. Lemon Juice Concentrate

Citric acid, g/l.	392.6
Citric acid, %	30.7
Brix	58.05
SO ₂ , ppm	175
Pulp content, %	8-10
Pasteurization temp, °F	170-176
Pasteurization time, min	1.00-1.25

examined microscopically immediately before each use. Aperture resistance was measured each time a freshly diluted suspension was placed in the sample beaker. The jacket of the sample beaker was provided with a copious flow of water from a thermostat set for 25°.

The 50- and 200- μm apertures were calibrated with the particles referred to in Table I. The particles having a nominal 12-13 μm diameter were paper mulberry pollen; all other particles were latexes obtained from Coulter Electronics, Inc., Hialeah, Fla. Calibration was started by using the 50- μm aperture to estimate the half-count threshold for each lot of particles. Then, using the three diameters found by Matthews and Rhodes (1970), the corresponding values for the instrument constant, k , were calculated using $k = Td^{-3}$, where T is half-count threshold and d is particle diameter. The mean of the three estimates of k was adopted as the instrument constant for use with the 50- μm aperture. Particle diameters obtained using the latter k estimate are given in the last column of Table I.

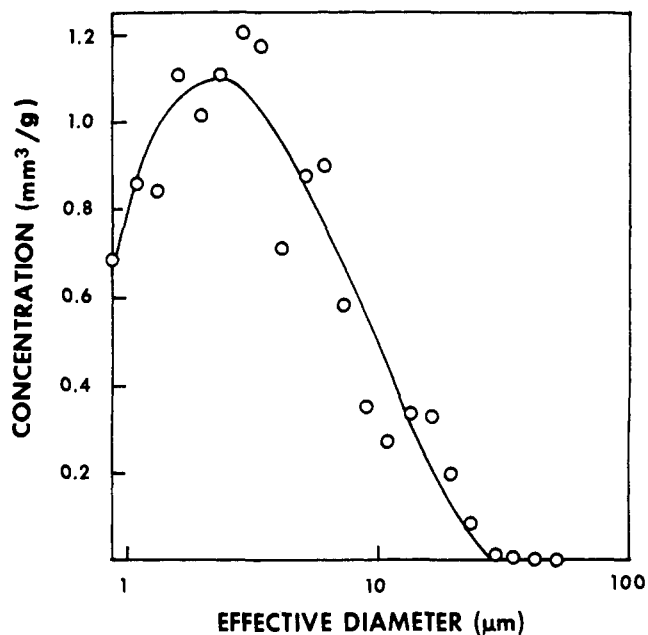
Only the 3.49- μm latex and the pollen were large enough to be useful in calibrating the 200- μm aperture. The mean of the k values for these two lots of particles was adopted as the instrument constant for the 200- μm aperture. The 30- μm aperture was calibrated with the 3.49- μm latex alone.

The manometer that meters aliquots of suspension through the calibrated apertures has three switch-selected volumes designated 50, 500, and 2000 μl . The 2000- μl volume was tentatively accepted as correct. The volume ratios have been measured with good precision by repeatedly counting suitable suspensions using neighboring pairs of manometer volumes.

The coincidence correction of Princen (1966) was used. Background corrections were applied to coincidence-corrected counts as required. The background for each lot of particle-counting electrolyte was determined daily. Observed backgrounds were never large enough to significantly affect results. The lower limit for particle detection was set at 0.8 μm for the 50- μm aperture and 0.7 μm for the 30- μm aperture.

In acquiring particle size distribution data, counts were accumulated at 23 particle diameters that increased logarithmically from 0.8 to 55.0 μm . It was assumed that particles could be treated as spheres and that the total particle volume, V , in the size interval d_1 to d_u is $V = N\pi(d_1^3 + d_u^3)/12$, where N is the corrected particle count for the designated size interval.

Description of Size Distributions. To facilitate comparison of particle size distributions, a cubic curve was fitted to each set of data using a least-squares method. Examples showing experimental counting results and the fitted cubics are given in the figures. While the fit was not

**Figure 1.** Particle-size distribution of Upland lemons.

always good at the large particle extremity, useful estimates of distribution widths were obtained. Modal diameters taken from the fitted cubic curves do not necessarily represent true modal particle diameters; they are simply regarded as approximate indicators of the central tendency of the observed distributions. Furthermore, since the actual shapes of the particles dealt with in this work are not known, the diameters reported are regarded as dimensions that are approached as the particles become more nearly spherical in shape. Future refinement of the instrument k values would systematically alter particle diameter estimates.

Tabulated particle concentrations express the number of cubic millimeters of particulate solids per gram of juice. These estimates of concentration were obtained by summing the experimental data and are thus independent of the fitted cubic equation.

Turbidity Evaluation. Turbidity was determined on samples of juice that had been prepared for counting as described. The dilutions needed to closely bracket the reference optical absorbance of 0.5 were performed gravimetrically using particle-counting electrolyte. Each turbidity estimate was based upon four aliquots that were examined at 600 nm with a Cary 14 spectrophotometer using 1-cm cuvettes. Linear interpolation provided a precise estimate of the dilution needed to attain the reference absorbance of 0.5. This dilution, found by interpolation, is the turbidity index referred to in the present work.

Particle Sedimentation. Lemons used in the differential sedimentation experiment were stored at about 3°. Serum density was adjusted by dissolving reagent grade KBr in the juice. The run with 6.0% KBr employed lemons from Yuma, Ariz. Fruit for the other three runs came from Ventura, Calif. A Sorvall refrigerated centrifuge with an SS-34 rotor was used to pellet particles. Centrifuge temperature was 4°, and the speed was held at 17,500 rpm for 10 min; g -max 37,000. Supernates were removed by decantation, and densities were measured at 20° with calibrated volumetric flasks. Pellets were washed three times with distilled, deionized water; each portion of water was equal to the volume of the original juice sample. In the washing process, each pellet was gently redispersed with a glass tissue homogenizer prior to repelleting. Washed pellets were freeze dried and weighed for comparison with original juice weight.

In the zonal rotor work, a Beckman L2-65B centrifuge equipped with a Ti-14 rotor was loaded at 3000 rpm. Set

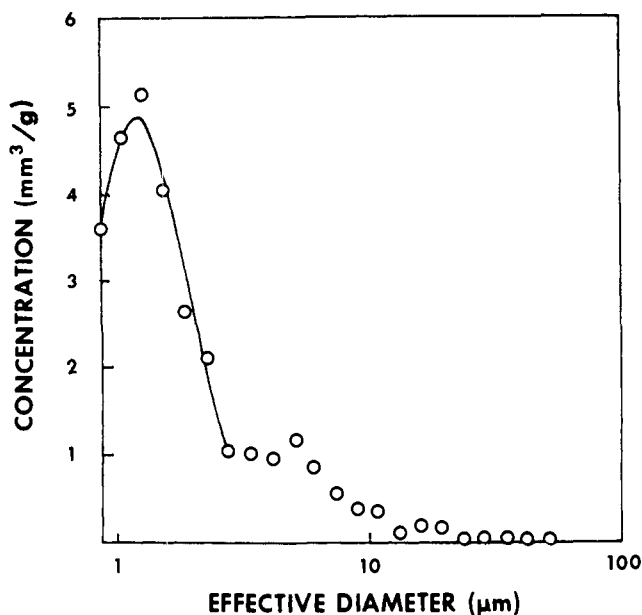


Figure 2. Particle-size distribution of a commercial concentrate.

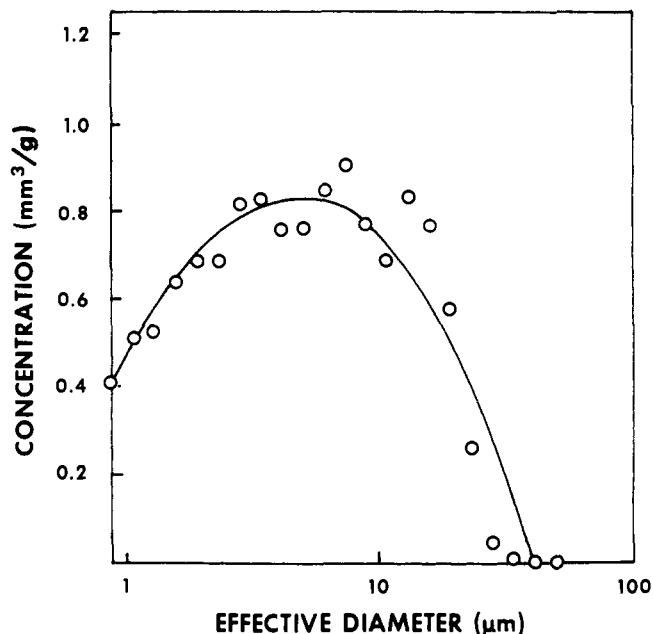


Figure 4. Particle-size distribution of SK lemons.

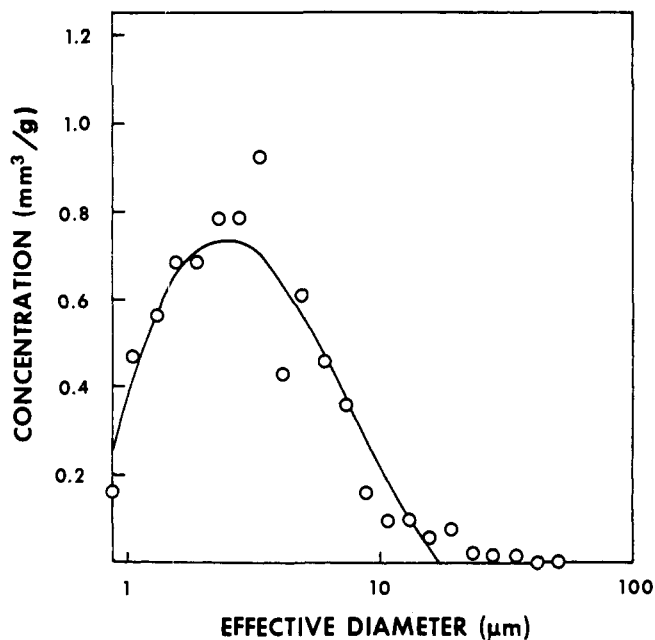


Figure 3. Particle-size distribution of Corona lemons.

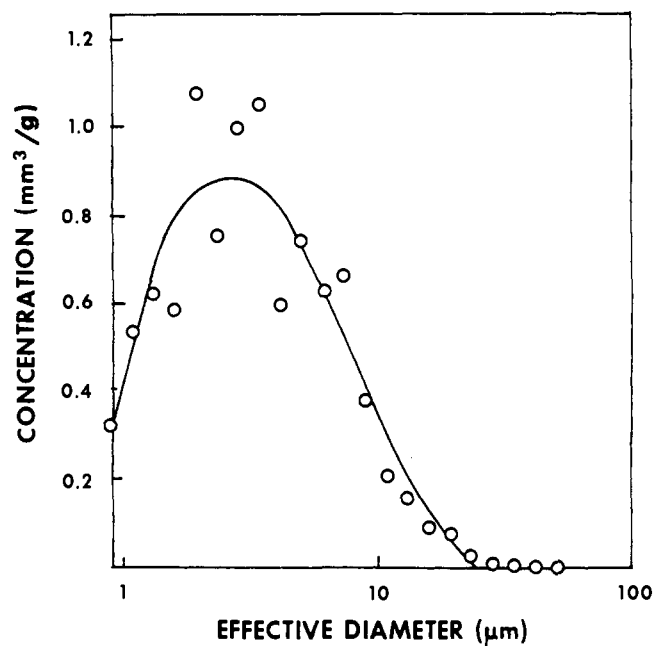


Figure 5. Particle-size distribution of S.J.C. lemons.

temperature was 4°. A Beckman Model 141 pump was used to load the rotor. The gradient ranged linearly from 10 to 60% sucrose over a volume of 500 ml. The sample injected onto this gradient was 10 ml of fresh, unpasteurized juice mixed with 40 ml of particle-counting electrolyte. Before the rotor was sealed, 50 ml of the electrolyte was injected to provide an overlay. The cushion, consisting of 60% sucrose, had a nominal volume of 50 ml. The rotor was brought to full speed and held there for 4 hr; g-max 171,800. Rotor speed was 4000 rpm during fraction collection. Drop-counting was used to collect the rotor contents in 39 approximately equal fractions. Densities of selected fractions were measured with ASTM D 941 pycnometers.

Selected fractions from the zonal run were examined with the Coulter Counter using a 30- μ m aperture. Approximately 100 mg of a given fraction was weighed out with a Cahn electrobalance and then made up to 100 ml with a particle-counting electrolyte. Observed counts were corrected by subtracting electrolyte background; no coincidence correction was applied. After the background corrections were made, all results were converted to the basis

of 100 mg of gradient fraction per sample.

Origin of Plotted Distributions. The lot of lemons from a Corona, Calif., grove was stored for 81 days under simulated commercial conditions: 11-12°, 85-90% relative humidity.

Lemons from an Upland, Calif., grove were held at laboratory ambient temperature until they became light-yellow. A flow of high humidity air was provided during the holding period. The plot shown in Figure 1 and the results identified with Upland elsewhere are based upon three single lemon distributions.

Lemons designated "S.J.C." were from a grove in San Juan Capistrano, Calif. The results represent a single sample of juice from ten S.J.C. lemons.

Lemons designated "SK" were purchased at a retail market. These lemons were trademarked SK; the skins were free of blemishes, but their thinness and color indicated that senescence was near. SK results are the average of three distributions; each distribution represented a juice specimen prepared from two lemons.

Table III. Size Distribution Characteristics of Juice Particles from a Group of Corona Lemons

Color ^a	Stand dev ^b	No. of lemons	Storage, days	Particle concn, ^c mm ³ /g	Modal diam, μ m	Width at half-height, μ m
YG-Y	0	14	8	8.5	2.42	6.52
LY-DY	0	14	24	7.5	2.41	6.64
DY	1.5	1	18	7.5	3.06	10.71
Y	0.9	1	16	8.8	2.78	8.34
Y	-0.8	1	17	9.5	2.49	6.99
DY	-1.8	1	22	7.3	2.08	6.62
LY-Y	0	14	73	8.8	2.56	7.63
Y	0	1	78	7.0	2.54	7.75
Y	0	1	79	8.7	2.75	8.70
Y	0	1	80	6.4	2.52	7.54
Y	0	1	81	9.1	3.13	12.79

^a YG: yellow-green, LY: light-yellow, Y: yellow, DY: dark-yellow. ^b The original lot of lemons had a mean weight of 120.3 g, standard deviation 11.8 g. Tabulated standard deviations are in units of 11.8 g. Zero indicates a deviation from the mean weight of less than 3%. The weight distributions of the groups of 14 lemons represented the parent distribution as closely as possible. ^c Particles with less than 0.8 μ m effective diameter not detected; see text.

Table IV. Standard Deviations of Selected Mean Values for Corona Lemons^a

Fruit sample	Particle concn, ^b	Modal di- ameter	Width at half- height
A. Singles, broad weight range	13	16	23
B. Singles, narrow weight range	17	10	27
C. Groups of 14	8	3	9

^a Values shown were calculated from Table III data and are expressed as percentages of the mean values. See note appended to Table III concerning weights of lemons in Group C. ^b Particles with less than 0.8 μ m effective diameter not detected; see text.

The juice concentrate (Table II) was provided by the Lemon Products Division of Sunkist Growers, Corona, Calif. It did not require filtration. After being reconstituted with distilled, deionized water, it was examined as if it were filtered, fresh juice. The particle-size distribution was measured after 87 days of storage at about 0°. Figure 2 shows that the cubic equation was fitted to a subset of the experimental data. To permit satisfactory estimation of the distribution characteristics, data for the larger particles were excluded.

RESULTS AND DISCUSSION

Particle-size distributions of laboratory-extracted, un-pasteurized lemon juices varied appreciably but showed, nevertheless, a relatively constant pattern. Large particles generally made a small contribution to the total volume of detectable particles. For example, the rather typical distribution in Figure 3 has 92% of its observed volume below the effective diameter of 12 μ m and 75% below 6.6 μ m. In most of the distributions, the size of the particle that appeared to account for most of the volume was in the range 2-3 μ m. The particle size that corresponds to the greatest volume contribution is the effective modal diameter. For the Figure 3 distribution, the effective modal diameter is 2.41 μ m.

Experimental constraints led to truncation of particle-size distributions at 0.8 μ m. The shapes of observed distributions suggest, however, that particles smaller than 0.8 μ m were present in laboratory preparations of un-pasteurized juice. Because the character of the particle-size distribution below 0.8 μ m is unknown, the mean particle diameter is not readily estimated. This led to selection of the mode of the fitted curve as a practical index of the central tendency of the distribution. Distribution widths were taken from the fitted curves at half the modal height. Most of the distribution widths were found without extrapolation. Figures 1 and 2 show examples that required extrapolation. Distribution width varied considerably.

Corona Lemons. Table III gives results for Corona lemons and Table IV gives standard deviations of selected categories of means from Table III. Table III data were arranged to facilitate interpretation; consequently storage times are not consecutive throughout. The results fall into two major groups separated by the storage interval of 24 to 73 days that was provided to help reveal possible changes due to aging of the fruit.

The one contrast in Table IV that is statistically significant occurs between the modal diameter results for groups A and C, where the variance ratio is significant at the 4% level. The variance ratio for width results of groups B and C was nearly significant (6% level). This suggests that if juice samples are composited from a suitably large number of carefully selected lemons, the precise estimation of particle modal diameter and distribution width will be facilitated. The data indicate that a comparable conclusion regarding the concentration of particles larger than 0.8 μ m is unwarranted.

Perhaps the most important conclusions to be drawn from this experiment are that (1) storage could be extended over a substantial period without gross alteration of particle concentration, modal diameter, or distribution width, and (2) individual lemons were liable to have particle properties that departed substantially from the mean values of the group from which they are selected.

General Comparison. Table V gives results for five

Table V. Comparison of Turbidity and Particle-Size Characteristics of Several Lemon Juices

Source	Color ^a	Turbidity index	Particle concn, ^d mm ³ /g	Modal diam, μ m	Width at half-height, μ m
Corona	LY-DY	3.4	7.5	2.41	6.64
Upland	LY	2.6	12.7	2.20	8.13
S.J.C. ^b	LY	4.1	9.6	2.53	7.63
SK ^b	DY	3.3	12.4	4.94	21.76
Concentrate ^c		8.9	4.2	1.20	1.37

^a LY: light-yellow, DY: dark-yellow. ^b See text. ^c Reconstituted to 57.0 g/l. from 392.6 g/l. of citric acid; properties of concentrate are shown in Table II. ^d Particles with less than 0.8 μ m effective diameter not detected; see text.

lemon juices. Their particle size distributions are plotted in Figures 1 through 5; note that the scale of the ordinate for juice concentrate differs substantially from the ordinates of the fresh juice plots. The plots of particle-size distribution show that the fresh, unpasteurized juices varied considerably, and that the properties of the reconstituted juice prepared from commercial concentrate were strikingly different. Although the turbidity of the reconstituted juice was higher than that of the unpasteurized fresh juices, its concentration of detectable particles was the smallest found. This is the clearest case yet encountered of the correlation of high turbidity with small modal diameter and narrow particle-size distribution.

The lack of correlation (Table V) between concentration of particles larger than $0.8 \mu\text{m}$ and turbidity index is not surprising when the distribution widths are considered. The two lowest turbidities correspond not only with the highest particle concentrations but also with the greatest distribution widths. Since the small particle portions of the particle-size distributions differ only slightly, these large widths indicate higher proportions of large particles and hence lower overall light-scattering capability. In juice from the SK lemons (Figure 4), modal diameter and distribution width were considerably greater than for any of the other juices. More work is needed to indicate how common such broad distributions may be.

Sedimentation Experiments. The differential sedimentation work with KBr indicated that the density of most of the juice particles ranged from 1.10 to 1.20 g/ml, and the median was about 1.17 g/ml. These results suggested that a gradient ranging from 10 to 60% sucrose would be suitable for isopycnic fractionation of lemon juice particles. The zonal rotor experiment, using such a sucrose gradient, provided evidence of the heterogeneity of the juice particles. Particles larger than $1.1 \mu\text{m}$ displayed a dominant band that spread from about 1.09 to about 1.21 g/ml. Most of the detectable particles were smaller than $1.1 \mu\text{m}$. When all particles larger than $0.7 \mu\text{m}$ were counted,

the band for particles larger than $1.1 \mu\text{m}$ was completely obscured by the bands of smaller particles, which were spread over all the fractions with no band dominating. The apparent density range of the smaller particles was about 1.01 to 1.26 g/ml. These results with particles from fresh, unpasteurized juice suggest that, in general, a narrow band of effective diameters includes a wide variety of densities. Such density variability is consistent both with the biological origin of the juice and with certain aspects of juice production. The bulk of the juice sac cell consists of the vacuole, whose contents are mixed with the cell sap during extraction. It is not known if the organelles originally present in the cell sap survive or if growth processes such as agglomeration occur. Nevertheless, it is probable that material originally present as organelles contributes significantly to juice particulates. The fragility of juice particles may be responsible for the contrast between Figure 2 and the other particle-size distributions. Apparently the concentration process led to the loss of large particles. This manifestation of particle fragility could be plausibly attributed to organelles that had remained intact prior to juice concentration but subsequently suffered membrane rupture or loss.

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Comparison of Extraction Methods for Triazine Herbicides in Root Crops Using Electrolytic Conductivity Detection

James F. Lawrence

A number of extraction methods for the analysis of triazine herbicides (atrazine, propazine, simazine, sencor, prometone) in foods, soil, and water were compared for gas chromatographic electrolytic conductivity determination in root crops (potato, carrot, turnip, beet, and parsnip). All of the methods produced similar results although they differed in the use of reagents and cleanup techniques. The use of the nitrogen-specific detection system made the hexane partition and

column cleanup steps unnecessary, thus shortening analysis time considerably. The method of choice consisted of methanol extraction, followed by partitioning between water and chloroform. The chloroform extract was concentrated for direct glc analysis. Interferences appeared only as irregular changes in baseline. No interfering peaks were observed for any of the triazines in the crops studied at 0.02 ppm or greater.

The use of the Coulson electrolytic conductivity detector (CCD) for the determination of residues of nitrogen-containing pesticides has increased greatly in recent years. The utility of the CCD for the selective gas chromatographic analysis of S, Cl, or N-containing pesticides has been investigated by a number of workers (Cochrane and

Greenhalgh, 1973; Cochrane and Wilson, 1971; Cochrane *et al.*, 1972; Greenhalgh and Cochrane, 1972; Laski and Watts, 1973; Lawrence, 1973; Patchett, 1970). These authors examined the response of a variety of pesticides as well as the effects of a number of operating parameters in the oxidative, reductive, and pyrolytic modes. As little as 0.1 ng of organic nitrogen may be detected in the reductive mode (Patchett, 1970). The determination of triazine herbicides in samples by CCD has been carried out by a few workers. Westlake *et al.* (1970) analyzed for ACD 15M

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