AOAC Official Method 965.31
Lemon Juice

First Action 1965
Final Action 1980

A. Preparation of Sample

Mix ca 5–6 g Celite analytical filter aid with 175 mL lemon juice (ca 80–100 milliequivalent acid/100 mL juice). Filter with suction (if not completely clear, refilter through fresh Celite pad) and store in glass-stoppered flask.

B. Total Amino Acids

Pipet 25 mL prepared sample into 150 mL beaker. (If test sample is suspected of containing SO_2, boil exactly 1 min and cool.) Add NaOH (1 + 1) dropwise to pH 6–7. Titrate potentiometrically with 0.1M NaOH to pH 8.4. Add 10 mL neutralized 37% HCHO (titrated potentiometrically with 0.1M NaOH to pH 8.4 ≤1 h before use) and titrate resulting acidity back to pH 8.4 with 0.1M NaOH. Total amino acids (milliequivalent/100 mL juice) = 0.4 ×mL 0.1M NaOH for second titration.

C. Total Polyphenolics

(**a**) *Calibration of spectrophotometer.*—Accurately weigh two 1.4–1.5 g portions KNO_3. Transfer to 250 mL volumetric flasks, dissolve in H_2O, and dilute to volume. Zero instrument at 302 nm and measure *A* of each solution in 1 cm cell. Calculate standard *A* for each solution, $A' = a \times \text{molarity } KNO_3 = (6.99 \times \text{g } KNO_3)/(101.11 \times 0.25) = 0.2765 \times \text{g } KNO_3$. Divide average *A′* by average measured A at 302 nm to obtain correction factor.

(**b**) *Determination.*—Pipet 0.5 mL prepared sample into 10 mL volumetric flask, and dilute to volume with alcohol. Transfer to centrifuge tube, cover with Al foil to prevent evaporation, and centrifuge. Measure UV spectrum of supernate with recording spectrophotometer from 300 to 400 nm or with manual spectrophotometer at 2 nm intervals from 325 to 335 nm. Multiply *A* of 325–335 nm peak by correction factor, (**a**), and report as *A* of total polyphenolics.

D. Recoverable Oil

See **968.20** (*see* 37.1.56).

References: *JAOAC* **46,** 353, 359(1963); **48,** 530(1965); **51,** 6, 464(1968).

l-Malic Acid
First Action 1966

E. Standard Rotation for l-Malic Acid

Accurately weigh ca 15, 25, and 35 mg *l*-malic acid (*see* **968.19** [*see* 37.1.44]) into 25 mL volumetric flasks. Add ca 0.4 g citric acid to each flask. Dissolve in 5 mL H_2O, add 1 drop phenolphthalein, and neutralize with NaOH (1 + 1). Add CH_3COOH until phenolphthalein color disappears, then 2 drops excess. Dilute to volume with H_2O. Measure initial optical rotation (α_1) of each standard solution. (Exercise great care in measuring optical rotation, since small uncertainty in measurement will cause large error in final result.) Saturate 10 mL of each standard solution with 1.3 g uranyl acetate·$2H_2O$. Keep in dark 30 min with occasional shaking. (Exposure of uranyl complex to strong light causes it to become insoluble; therefore conduct operations in semidarkness.) Filter off excess uranyl acetate and measure optical rotation (α_u) within 5 min after filtering. Calculate standard rotation ($R_{standard}$) for each solution as follows:

$$C_{malic} = (\text{mg malic acid}/67.04) \times 4$$
$$= \text{milliequivalent malic acid}/100 \text{ mL}$$

$$\Delta\alpha = \alpha_1 - \alpha_u$$

$$R_{standard} = C_{malic}/\Delta\alpha$$

Use average standard rotation for subsequent calculations.

F. Determination

In graduate mix 15 mL test sample, **A**, with 45 mL alcohol and let stand 10 min. Centrifuge pectin precipitate. Evaporate alcoholic juice to thick syrup (ca 1–2 mL) in rotary vacuum evaporator (≤50°C). Add 13–14 mL H_2O to syrup and mix thoroughly. Pipet 2 mL pectin-free test sample into 100 mL beaker, and add 25 mL H_2O. Titrate potentiometrically to pH 8.4 with standardized 0.1M NaOH. Acidity (milliequivalent/100 mL pectin-free test sample) = 5 ×(mL alkali). Pipet 10 mL pectin-free test sample into 25 mL volumetric flask, add 1 drop phenolphthalein, and proceed as in determination of standard rotation. *l*-Malic acid concentration in diluted, neutralized test sample, $[MA]_D = R_{standard} \times (\alpha_1 - \alpha_u)$. Calculate citric acid:malic acid ratio by dividing 0.4 times acidity of pectin-free test sample by $[MA]_D$.

References: *JAOAC* **46,** 353(1963); **48,** 530(1965); **49,** 621(1966).

CAS-97-67-6 (levo-malic acid)