Table I

	1.5% OV-17–1.95% QF-1	3% OV-1
Instrument	Micro-Tek	Micro-Tek
Column	6 ft, glass	6 ft, glass
Support	Supelcoport (100–120 mesh)	Chromosorb W (100–120 mesh)
Carrier gas	5% methane–argon	5% methane–argon
Flow rate	50 ml/min	75 ml/min
Tank pressure	50 psi	50 psi
Temperature column	160°	160°
Detector	200°	200°
Injection port	240°	240°
Chart speed	15 in./hr	15 in./hr
Sensitivity	$(1.8 \times 10^{-8}) \times 32$	$(1.8 \times 10^{-8})^2 \times 16$

Table II. Recovery of 2,4-D from Wheat Grain

Amt fortified, ppm	% recovery	Amt fortified, ppm	% recovery
0.05	88.0	0.5	92.1
0.1	94.7	1.0	89.8

shown in Table I. For the parameters listed, the retention times of the 2,4-D standard on the mixed and OV-1 columns are 15 and 22 min, respectively.

RESULTS AND DISCUSSION

Recovery experiments were performed by adding 0.05 to 1.0 ppm of standard 2,4-D to wheat samples before extraction was begun. The efficiency of the overall procedure was determined by comparing the peak heights of the standards with those from fortified samples. Unfortified samples were processed in an identical manner and were used as controls. The recoveries were determined by comparing the differences in peak heights between the fortified sample and the control with the standard.

The entire process, including the column cleanup which removes interfering chromatographic peaks, yielded recoveries between 77 and 95%. The results of this recovery study are summarized in Table II, and the values for repetitive runs are listed in Table III. The lower limit of detection for the procedure is about 0.05 ppm of 2,4-D. Some batches of ether, when run through the entire procedure as a solvent check, show interfering peaks on the chromatograms. A purification step, consisting of passing ether through a column of Woelm basic alumina (W-200, activity I, prewashed with ether), removes the interfering substances. Approximately 50 g of alumina will purify about 1000 ml of ether.

Table III. Recovery of 2,4-D from Wheat Grain, Repetitive Runs

Sample no.	Amt 2,4-D fortified, ppm	% Recovery	% Av
6071	1.0	86.5; 80.7; 90.0; 87.8; 77.4	84.5
6031	0.1	89.8; 81.5; 90.3; 85.4; 94.7	88.4

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Aubry E. Dupuy, Jr.*
Terrell J. Forehand
Han Tai

Pesticide Monitoring Laboratory
Technical Services Division
Office of Pesticide Programs
Environmental Protection Agency
Bay St. Louis, Mississippi 39520

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A Kinetic Study of the Cyclization of L-Glutamine to 2-Pyrrolidone-5-carboxylic Acid in a Model System

The pseudo-first-order kinetics of the cyclization of glutamine to 2-pyrrolidone-5-carboxylic acid (PCA) and the one-half-order catalysis of this reaction by acetic acid were demonstrated in a model system.

The conversion of L-glutamine to the bitter tasting 2-pyrrolidone-5-carboxylic acid (PCA) in foods has been well documented (Foreman, 1914; Shallenberger et al., 1959; Mahdi et al., 1961; Clydesdale et al., 1972). Some of the effects of thermal processing, storage, and nitrogen fertilization on the formation of PCA in beets have also been studied by Lee et al. (1971). In addition, Archibald (1945) reported that complete conversion of glutamine to PCA was catalyzed by both acid and base. Wilson and Cannan (1937) examined the equilibrium between glutamic acid and PCA at pH values above 10 and below 4 using an indirect method of analysis for glutamic acid. The deamination and cyclization of glutamine to PCA are different from the glutamic acid–PCA equilibrium. Therefore, this study was undertaken to determine the effect of acid on the kinetics of the transformation of glutamine to PCA in the pH ranges of most foods (3–6). Acetic acid and sodium hydroxide were chosen as the buffer system for this study.

EXPERIMENTAL SECTION

Materials. All chemicals were reagent grade and obtained from commercial sources. The L-glutamine used had a specific optical rotation of $[\alpha]^{22}D$ +6.2° (1% H_2O).

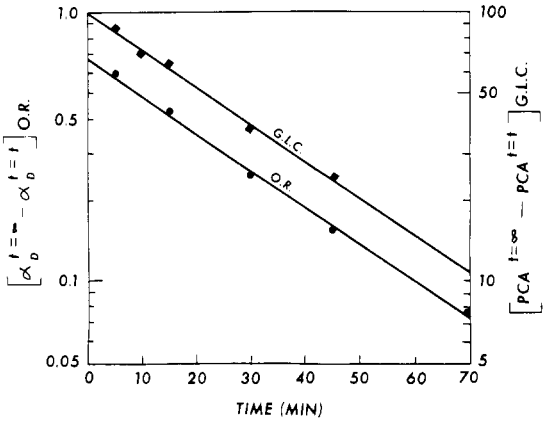

Figure 1. First-order kinetic plot of cyclization of glutamine to PCA using gas–liquid chromatography (GLC) and optical rotatory technique (OR), at pH 5.

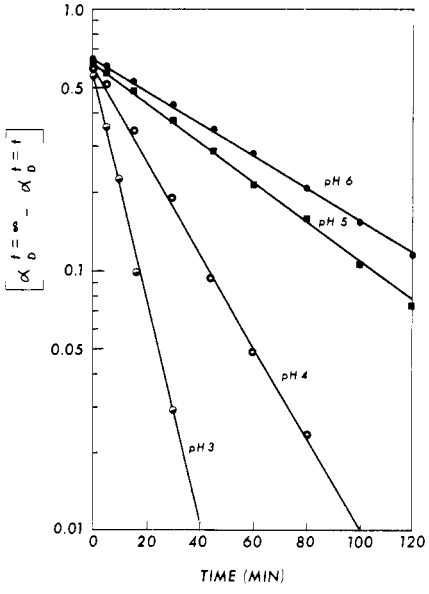

Figure 2. First-order kinetic plot of cyclization of glutamine to PCA in sodium acetate buffer solutions with an acetate ion concentration of 0.073 mol/l. and pH 3, 4, 5, and 6.

Buffer Solutions. A series of buffer solutions was prepared using acetic acid and sodium hydroxide at pH values of 3, 4, 5, and 6 with a constant acetate ion concentration (0.073 *M*), and another series was prepared at pH 4 with acetate ion concentrations of 0.3, 0.15, 0.073, and 0.014 *M*. All acetic acid and acetate ion concentrations were calculated using the equilibrium constant of acetic acid (1.75×10^{-5} at 25°).

Reaction Conditions. The buffer solutions containing 1% L-glutamine were heated to 100° in a water bath to initiate the reaction. Aliquots were removed at various times and cooled in ice water to stop the reaction, and measured for PCA content, optical rotation, and pH.

Optical Rotation. Optical rotations were determined in a 2-dm tube at 22° with a Rudolf polarimeter Model 80 equipped with an oscillating polarizer and a photoelectric readout.

Thin-Layer Chromatography. Samples were spotted directly on precoated 250-μ thick cellulose plates and developed with phenol–water (1:4). Glutamine and glutamic acid were visualized with a 1% ninhydrin spray and PCA was detected with a spray of Methyl Orange (saturated in ethanol–ethyl acetate, 1:1) according to Rowlands and Young (1952).

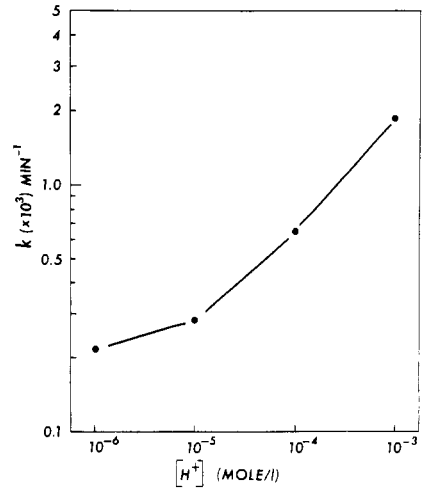

Figure 3. Plot of the log of rate constants for the formation of PCA from glutamine vs. the log of hydrogen ion concentration.

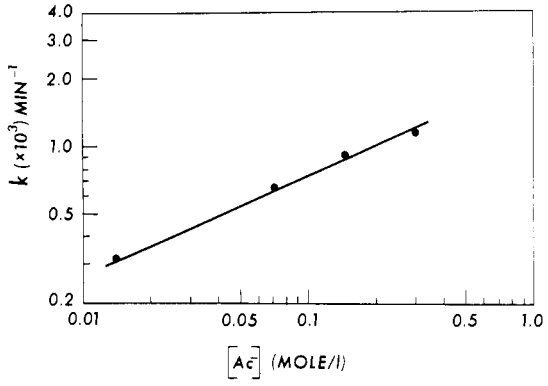

Figure 4. Plot of the log of rate constants for the formation of PCA from glutamine vs. the log of acetate ion concentration, at pH 4.

PCA Analysis. PCA was extracted from the reaction mixture with ethyl acetate (Pucher and Vickery, 1939) and the bistrimethylsilyl derivative was prepared and quantitated using gas–liquid chromatography (Lee, 1970).

RESULTS AND DISCUSSION

Figure 1 shows the pseudo-first-order kinetic plot of the formation of PCA in a 1% glutamine solution and the pseudo-first-order kinetic change in the optical rotation of the same solution. The fact that these two lines are parallel indicates that the change in optical rotation is entirely due to the transformation of glutamine to PCA. Two possible side reactions would be the formation of glutamic acid and γ-poly(glutamic acid). Thin-layer chromatography of the reaction mixture showed a continuous increase in the PCA with a decrease in glutamine. Neither glutamic acid nor any other ninhydrin positive spot was detected during the reaction, which further supports the conclusion that the sole product is PCA. Also, there were no significant changes in pH during the reaction.

Figure 2 shows the pseudo-first-order kinetic plot of the optical rotation change of glutamine–acetate buffer solutions at different hydrogen ion concentrations. Clearly, the first-order transformation of glutamine to PCA is catalyzed by acetic acid. However, this catalysis is not first order in hydrogen ion, as can be seen in Figure 3. Between pH 3 and 4 the catalytic effect of hydrogen ion is approximately one-half order, then decreasing to almost zero between pH 5 and 6. This decrease in the order of the catalysis by hydrogen ion must occur if the reaction is to be catalyzed by both acid and base as observed by Archibald (1945).

A similar result is obtained when the hydrogen ion concentration is held constant (pH 4) and the acetate ion concentration is varied. In Figure 4 a plot of the rate constant vs. the acetate ion concentration shows a half-order dependence on acetate ion. Furthermore, a plot of the undissociated acetic acid concentration in both sets of experiments vs. the rate constant shows approximately one-half-order dependence on acetic acid. In all experiments the results were the same whether the rate was measured by the optical rotation of the total solution or by gas–liquid chromatographic analysis of the formation of PCA.

CONCLUSION

The data presented here indicate that the cyclization of glutamine to PCA at 100° is first order in glutamine and catalyzed by acid. However, the role of acetic acid in this reaction is complicated by its half-order kinetics. Therefore, any mechanism proposed for this reaction must consider the unusual kinetics observed for acetic acid catalysis. The rate can be expressed either in terms of the hydrogen ion or acetate ion concentrations as: rate = $k[H^+]^{1/2}$. $[Ac^-]^{1/2}$[glutamine].

Although this work was done with a well-defined model system it does imply some facts about the role of glutamine in the formation of PCA during the thermal processing and storage of foods. For example, when a standard thermal process does not result in complete conversion of free glutamine to PCA, the final level of PCA will be affected by the concentration of any catalyst which may be present. This study indicates that the greater the acidity of a food the greater the rate of PCA formation from glutamine.

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Terry E. Acree
Chang Y. Lee*

Department of Food Science and Technology
Cornell University
New York State Agricultural Experiment Station
Geneva, New York 14456

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Correction

SEASONAL VARIATION IN THE COMPOSITION OF CALIFORNIA AVOCADOS

In this article by Grant G. Slater, Solomon Shankman, John S. Shepherd, and Roslyn B. Alfin-Slater [*J. Agric. Food Chem.* **23**(3), 468 (1975)] some of the data in Table II, p 469, were not accurate. The correct information appears in the following table.

Table II. Fatty Acids of California Avocados[a]

Component	Fuerte					Hass		
	10/22/68[b]	2/13/69	12/7/68	4/5/68	Av	4/5/68	6/4/68	Av
	As Percent of Total Fatty Acids							
Saturated[c]								
Palmitic	16.5	12.1	10.0	10.6	12.3	16.1	4.1	10.1
Stearic	0.0	0.0	0.0	0.0	0.0	0.1	0.1	0.1
Subtotal	16.5	12.1	10.0	10.6	12.3	16.2	4.1	10.2
Unsaturated[c]								
Palmitoleic	5.9	1.9	1.3	4.8	3.5	7.4	0.5	4.0
Oleic	71.8	73.9	82.2	72.5	75.1	63.5	93.3	78.4
Linoleic	6.0	10.5	6.6	11.2	8.6	12.2	2.2	7.2
Linolenic[d]	0.0	0.7	0.1	0.8	0.4	0.8	0.0	0.4
Arachidonic	0.1	0.1	0.0	0.0	0.1	0.0	0.0	0.0
Subtotal	83.7	87.1	90.2	89.3	87.7	83.9	96.0	90.0
Total	100.2	99.2	100.2	99.9	100.0	100.1	100.1	100.2
Ratio unsat./sat.[e]	5.1	7.2	9.0	8.4	7.4	5.2	23.4	14.3
Ratio P/S[f]	0.36	0.93	0.67	1.13	0.73	0.80	0.53	0.75
	As Percent of Pulp[g]							
Saturated								
Palmitic	1.2	1.3	1.5	2.2	1.7	2.6	0.8	1.8
Stearic	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Unsaturated								
Palmitoleic	0.4	0.2	0.2	1.0	0.5	1.2	0.1	0.7
Oleic	5.4	8.1	12.7	14.9	10.2	10.1	18.3	13.8
Linoleic	0.4	1.1	1.0	2.3	1.2	1.9	0.4	1.3
Linolenic	0.0	0.1	0.0	0.2	0.1	0.1	0.0	0.1
Arachidonic	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0

[a] Average of two determinations on the ripe fruit from a composite sample of 15 avocados. [b] Date fruit arrived at Shankman Laboratories. [c] The range of error estimated by Dr. Shankman as ±0.7%. [d] Plus related acids. [e] Unsaturated fatty acids/saturated fatty acids. [f] Polyunsaturated fatty acids/saturated fatty acids. [g] Assumptions used were: unsaponifiable fraction 2%, glycerol 7.6%, phospholipid residues, etc, 0.4%, for a total of 10% non-fatty acids.