Procedure. Using infrared lamps, take 100-ml. samples of milk to dryness, ash at 500° to 550° C., digest on water bath with 5 ml. of approximately 6*N* hydrochloric acid, together with a little water, and filter. Take the filtrate to dryness to remove most of the hydrochloric acid, dissolve in about 5 ml. of water, transfer to a 50-ml. centrifuge tube, and wash with about 10 ml. of water. Add 3 ml. of 10% HEEDTA and two drops of bromothymol blue, and titrate slowly with approximately 2*N* ammonium hydroxide to the blue-green color. (The volume prior to addition of ammonium hydroxide is about 20 ml.) Centrifuge and transfer the supernatant to a 50-ml. beaker and set aside.

Dissolve the precipitate in about 10 drops of nitric acid, add another 3 ml. of 10% HEEDTA, and repeat the precipitation of calcium phosphate. Centrifuge, add the second supernatant to the first, and take the whole to dryness under an infrared lamp. To the residue, add 15 ml. of nitric acid, cover with a Speedyvap, and take to dryness on the hot plate to remove ammonia. Add 10 ml. of the acid mixture and, when most of the nitric acid has been removed, raise the hot plate temperature to ensure efficient oxidation by the perchloric acid. When the vigorous oxidation reaction is complete, allow the beaker to cool, wash down the Speedyvap and the sides of the beaker with distilled water and return to the hot plate at low temperature to evaporate off the water.

When fuming starts, raise the temperature to fume off the bulk of the sulfuric acid, and finally flame the beaker. Cool beaker contents, add about 2 ml. of water, bring to near boiling on the hot plate, and transfer to a 10-ml. graduated centrifuge tube. Add 3 ml. of the phosphoric-periodate solution, mix, immerse in boiling water, and keep just boiling for about 2 hours. The initial volume of about 10 ml. is reduced to about 6 ml. over this period. After cooling, centrifuge to remove water from the upper parts of the tube and to bring down the small amount of calcium sulfate, which usually occupies about 0.3 ml. Make the volume up to 6.3 ml.—i.e., 6 ml. over the volume occupied by the calcium sulfate, mix thoroughly, recentrifuge, and transfer to a 5-cm. cell for density determinations at 525 mμ.

Conclusions

Recoveries of manganese by this method appear satisfactory, taking into account the levels involved. Although not investigated, DTPA would probably behave in much the same way as HEEDTA. This method may well have application to other materials high in calcium and/or phosphorus, such as bone, teeth, phosphate rock, and limestone.

Acknowledgment

The author is indebted to Dow Chemical Co. for samples of HEEDTA and DTPA.

Literature Cited

(1) Kroll, H., Kuykendall, J. R., Powers, J. A., "Chelation," Wiley, New York (in press), quoted by Brown, J. C., *Ann. Rev. Plant Physiol.* **7,** 185 (1956).
(2) Richards, M. B., *Analyst* **55,** 554 (1930).
(3) Vallee, B. K., *Anal. Chem.* **25,** 986 (1953).

Received for review July 9, 1957. Accepted March 17, 1958. Soil Bureau Publ. No. 120.

POTATO EXTRACTION

Determination of End Point in Extraction of Free Amino Acids from Potatoes

EUGENE A. TALLEY, FAIRIE LYN CARTER, and WILLIAM L. PORTER

Eastern Utilization Research and Development Division, Agricultural Research Service, U. S. Department of Agriculture, Philadelphia 18, Pa.

A satisfactory batchwise procedure for the extraction of the free amino acids from potatoes has been developed. Lysine and arginine are the last of the amino acids to be extracted by 70% aqueous ethyl alcohol using this procedure. The order of extraction of the amino acids in potatoes was very different from that reported in young corn shoots. Methods used for the extraction of plant constituents should be tested on each plant material for which they are to be used.

Aqueous ethyl alcohol, 70 to 80%, has been recommended and used (*1–6, 10, 13*) to extract the free amino acids from plant materials; however, the workers were not very specific as to when the extraction was complete. Woodward and Rabideau (*17*) have reported on the completeness of extraction of amino acids and other components in corn. They used hot 80% ethyl alcohol in a Soxhlet apparatus, which is known to cause destruction of glutamine (*14*), and as the authors wished to obtain this compound essentially unchanged, their procedure was unsatisfactory.

In the case of sugars, Williams and Potter (*15*) found, "that the sugar solution entrapped in the spongy plant material is of the same concentration as the remainder of the solution" and "that the alcohol-insoluble material does not occupy a significant volume." This procedure did not work in extracting amino acids from potatoes.

This study agrees with the Woodward and Rabideau (*17*) and Oland and Yemm (*9*) findings that the amino acids are extracted at different rates. Hence, the extraction must be complete or the relative amounts of the different amino acids in an extract will depend on the extent of extraction. Woodward and Rabideau found that the last amino acids to be removed from young corn shoots were aspartic and glutamic acids. Oland and Yemm found arginine to be the last amino acid to be extracted from apple twigs. Arginine and lysine—with a slight emphasis on the latter—were the last amino acids to be removed from potatoes, according to the present work. Methods used for the extraction of constituents from materials derived from different plant species should be tested on each plant material for which they are used.

Experimental

The equilibrium procedure of Williams and Potter (*15*) was compared with a batchwise procedure using Wisconsin Russet potatoes of specific gravity 1.076, which corresponds to a solids content of approximately 19%. The potatoes were hand-peeled and slurried in ethyl alcohol in an electric blender at high speed (10,000 r.p.m.) for 3 minutes. The slurry was transferred to a sampling blender with sufficient alcohol to make the final concentration 70% by weight, taking into consideration the water originally in the potatoes. Samples were taken for the extraction procedures and for nitrogen analyses with the blender running at low speed. The actual amount taken was determined by weight.

Table I. Progressive Changes in the Nitrogen Content of Potato Extracts during Extraction

	Wisconsin Russets			Maine Russets
	Mg. Kjeldahl N / G. fresh wt.	% of total N	μMole leucine equiv.[a] / G. fresh wt.	μMole leucine equiv.[a] / G. fresh wt.
Slurry	2.917	100	...	...
Extracts				
1	1.226	42.0	45.19	52.13
2	0.245	8.40	8.51	12.65
3	0.0867	2.97	2.50	2.65
4	0.0318	1.09	0.724	0.596
5	0.0184	0.63	0.328	0.344
(Soxhlet)				
6	0.0407	1.40	0.645	0.307
7	0.0181	0.62	0.307	0.380
8	0.0086	0.30	0.098	lost
9	...	...	0.061	0.173

[a] Ninhydrin-reactive material as μmoles of leucine equivalents per gram fresh weight (*8*).

Table II. Progress of Extraction of Lysine and Arginine from Wisconsin Russet Potatoes

	$\frac{\text{Lysine N in Extract}}{\text{Total N in Potatoes}} \times 100$	$\frac{\text{Lysine}^a \text{ per Extract}}{\text{Total Extractable Lysine}} \times 100$	$\frac{\text{Arginine N in Extract}}{\text{Total N in Potatoes}} \times 100$	$\frac{\text{Arginine}^a \text{ per Extract}}{\text{Total Extractable Arginine}} \times 100$
Extract No.[b]				
1	0.48	25	2.04	32
3	0.17	8.7	0.54	8.5
5	0.10	5.2	0.25	3.9
(Soxhlet)				
6	0.24	12.5	0.44	6.9
8	0.04	2.1	0.07	1.1
9	0.00	0.0	0.00	0.0
Totals	1.92 (max.) 1.34 (min.)		6.36 (max.) 4.20 (min.)	

[a] Minimum values, using maximum value as total (see text).
[b] Extracts 2, 4, and 7 not analyzed by Moore-Stein technique in interest of time.

Table III. Amino Acid Content of Maine Russet Potatoes

Amino Acid	First Extract, μMole / G. fresh wt.	Combined Extracts, μMole / G. fresh wt.
1	0.18[a]	0.26[a]
2	0.04[a]	0.07[a]
2a	...	0.03[a]
3	0.07[a]	0.02[a]
4	0.05[a]	0.05[a]
5 Aspartic acid	3.17	5.32
6 Threonine	1.59	1.51
7 Serine, glutamine, asparagine[b]	21.78	31.85
8 Glutamic acid	3.78	5.66
9 Proline	0.76	0.90
10 Glycine	0.23	0.33
11 Alanine	0.50	0.55
12 Cystine	0.05	0.09
13 Valine	2.66	2.74
14	0.03[a]	0.04[a]
15 Methionine	0.35	0.43
16 Isoleucine	0.70	0.77
17 Leucine	0.46	0.44
18 Tyrosine	1.03	1.01
19 Phenylalanine	0.93	1.14
20 β-Alanine Tyrosine + phenylalanine + β-alanine	2.10	2.44
21 γ-Aminobutyric acid	3.50	3.67
22 Tryptophan	0.25	0.17
23 Histidine	0.50	0.68
24 Lysine	0.58	1.32
25 Ammonia	5.93	4.17
26 Arginine	1.03	2.88

[a] Calculated as micromoles of leucine equivalents as their identity has not been determined. [b] Calculated as asparagine.

Each sample for the extraction tests contained about 100 grams of fresh potato. The samples were made to volume with 70% aqueous ethyl alcohol by weight and mixed thoroughly by shaking. The equilibrium samples were made up to 2, 4, and 8 liters and allowed to stand with occasional shaking. Equivalent aliquots of the supernatant liquid were removed after 2 hours and 1, 2, and 5 days. The aliquots were filtered to remove small amounts of suspended material.

The batchwise sample was made up to about 800 to 900 ml. and swirled more or less continuously for 15 minutes. The mixture was filtered by suction on paper, and the cake was rinsed with 70% alcohol to give combined filtrate and washings of approximately 1 liter. The filter cake was immediately redispersed in 70% alcohol by shaking for 15 minutes. (Preliminary experiments indicated that dispersal in a high-speed blender might cause breakdown of higher molecular weight materials to the point where they would be partly extracted.) The first extract was made up to a 1-liter volume before aliquots were removed for analysis. The later extracts were concentrated under reduced pressure at a temperature below 40° C. and then made up to convenient volumes.

After five extractions were carried out as above, the cake was transferred to a Soxhlet extraction thimble and extracted in the Soxhlet apparatus using about 300 ml. of 70% ethyl alcohol. The apparatus siphoned about four times per hour. The alcohol was changed at the end of each 8-hour period. Four Soxhlet extracts were obtained. The batchwise procedure was repeated later using some sprouted Maine Russet potatoes reported to contain 20.2% solids.

Total nitrogen in the slurry and in the various extracts was estimated by Kjeldahl nitrogen determinations (*16*). Ninhydrin-reactive material in the extracts was estimated by a photometric procedure (*8*). The amino acid composition of the extracts was checked by the Moore and Stein ion exchange procedure (*7*) as modified in this laboratory (*11*).

Results and Discussions

In the equilibrium procedure, as the volume of alcohol used per 100 grams of comminuted fresh potato was increased, the amount of nitrogen extracted approached, but did not reach, a limiting value even when 8 liters of alcohol were used. Only 53.1% of the original nitrogen [estimated by Kjeldahl method (*16*)] was extracted as compared with 57.4% obtained with the batchwise method described above. The figure for 4 liters was 49.5% and for 2 liters was 47.2%. This discrepancy was not due to a time factor as aliquots separated from the potato slurry in less than 2 hours gave the same results as later aliquots that had remained in contact with the potato solids for periods up to 5 days.

Table I compares the nitrogen contents of the two batchwise extracts obtained. These results, however, show only part of the story, because the composition of the individual extracts changes during extraction. Data obtained from the Wisconsin Russets (Table II) show how these changes occur during the extraction of lysine and arginine, the last two amino acids to be removed. Even though not all the extracts were analyzed, one can set maximum and minimum limits for the total extractable compounds by assuming that those extracts not reported would contain not more than the immediately preceding extract or less

than the immediately following extract. Although the ninth extract did show ninhydrin activity when tested directly against the ninhydrin reagent (*8*), ion exchange chromatography experiments, using the Moore and Stein procedure (*7*), showed no definite peaks in the effluent curve.

The fourth and fifth extracts were estimated to contain very few acid and neutral amino acids, these having been essentially removed in the first three extracts. Only four peaks caused by this group were found in the fourth extract. The largest of these, the asparagine peak (which may contain glutamine and possibly serine) accounted for only 0.43% of the original nitrogen in the potatoes or about 0.75% of the extracted nitrogen. (As seen from Table III, this peak accounts for most of the extractable nitrogen.) In terms of leucine equivalents, the other three peaks amounted to only 22% of the asparagine peak. In the fifth extract, the asparagine peak amounted to only 0.006% of the original nitrogen and only two other smaller peaks were found in this group. The sixth (first Soxhlet) extract contained only slightly more of the acid and neutral amino acids than did the fifth. Thus essentially all of the acid and neutral amino acids are removed when 40 to 50% of the lysine and 30 to 40% of the arginine are still unextracted.

Fortunately, the acid and neutral amino acids are easily removed, as one of these, glutamine, would be converted by heat into pyroglutamic acid (*14*) if it were not essentially removed before Soxhlet extraction is begun. While asparagine is not completely stable to heat (*12*), small amounts of it remaining until the Soxhlet extraction would not significantly change the results. Other members of the acid and neutral fractions are probably more stable than asparagine.

The results of the second batch extraction (Table I, column 5 closely duplicated the first except that the Soxhlet extraction was not quite as efficient as before. Table III gives the amino acid estimations (*7*, *8*) for the first extract and for the combined aliquots of all the extracts except number eight, which was lost. Some of the other individual extracts were also checked, though the results are not included in the table. Expressed as percentage of amino acids found in the combined extracts, the seventh and ninth extracts contained 9.5 and 3.8%, respectively, of the lysine, and 5.4 and 2.0% of the arginine, while the fourth and fifth extracts contained 0.14 and 0.10% of the acid and neutral amino acids. In Table III, the relative amounts of the amino acids do not differ greatly in the two columns except in the case of lysine and arginine. In a few cases—i.e., threonine—the amount estimated in the combined aliquots was less than in the first extract—probably because of poor separation between peaks and the resultant error in deciding the correct amount to allot to each peak.

Literature Cited

(1) Coulson, C. B., *J. Sci. Food Agr.* **6,** 674 (1955).
(2) Cruickshank, I. A. M., *Ibid.*, **8** 26 (1957).
(3) Dent, C. E., Stepka, W., Steward, F. C., *Nature* **160,** 682 (1947).
(4) Hulme, A. C., *Food Sci. Abstr.* **28,** 345 (1956).
(5) Le Tourneau, Duane, *Botan. Gaz.* **117,** 238 (1956).
(6) McKee, H. S., Nestel, Lydia, Robertson, R. N., *Australian J. Biol. Sci.* **8,** 467 (1955).
(7) Moore, Stanford, Stein, William H., *J. Biol. Chem.* **192,** 663 (1951).
(8) *Ibid.*, **211,** 907 (1954).
(9) Oland, K., Yemm, E. W., *Nature* **178,** 219 (1956).
(10) Possingham, J. V., *Australian J. Biol. Sci.* **9,** 539 (1956).
(11) Talley, E. A., Carter, F. L., Porter, W. L., Eastern Regional Research Laboratory, Philadelphia 18, Pa., unpublished work, 1956.
(12) Talley, E. A., Fitzpatrick, T. J., Porter W. L., *J. Am. Chem. Soc.* **78,** 5836 (1956).
(13) Thompson, John F., Steward, F. C., *Plant Physiol.* **26,** 421 (1951).
(14) Vickery, H. B., Pucher, G. W., Clark, H. E., Chibnall, A. C., Westall, R. G., *Biochem. J.* **29,** 2710 (1935).
(15) Williams, K. T., Potter, E. F., *Ibid.*, **38,** 401 (1955); **39,** 427 (1956).
(16) Willits, C. O., Ogg, C. L., *J. Assoc. Offic. Agr. Chemists* **33,** 181 (1950).
(17) Woodward, C. C., Rabideau, G. S., *Plant Physiol.* **28,** 535 (1953).

Received for review September 18, 1957. Accepted March 6, 1958. Second Delaware Valley Regional Meeting, ACS, Philadelphia, Pa., February 1958.

ASCORBIC ACID OXIDATION

Hydrogen Peroxide–Induced Oxidation of Ascorbic Acid in Passion Fruit Juice

EDWARD ROSS

Horticulture Department, State College of Washington, Pullman, Wash.

ANNIE TOM CHANG

Food Processing Laboratory, University of Hawaii, Honolulu, T. H.

Passion fruit juice is particularly suited for study of the hydrogen peroxide–induced oxidation of ascorbic acid. Although hydrogen peroxide is decomposed in the fresh juice, concurrent oxidation of ascorbic acid occurs. The catalatic activity is destroyed by heat. The aerobic, nonenzymic, peroxidatic-like oxidation is first order with respect to ascorbic acid, exhibiting a rate constant between 0.048 and 0.060 min.$^{-1}$ in natural juice at pH 3.0. The rate of the anaerobic reaction decreases rapidly after 15 minutes. In this reaction, the peroxide is apparently consumed stoichiometrically, while, in the aerobic reaction, it acts as a catalyst for the autoxidation of ascorbic acid. Significance of the results is discussed on the basis of induced reactions of hydrogen peroxide in the presence of ferrous ions.

Ascorbic acid is catalytically oxidized in the presence of hydrogen peroxide by the peroxidase system and by metal ions and their nonenzymic compounds. Both systems are present in the higher plants and have been individually demonstrated in several fruit juices. Neither has been investigated in passion fruit (*Passiflora edulis*). Routine analyses at the Food Processing Laboratory indicated that juice of the yellow passion fruit (*38*) variety (*P. edulis f. flavicarpa*) is comparable to citrus juices (*37*), as regards stability of ascorbic acid. Thus, hydrogen peroxide–induced oxidation of ascorbic acid in passion fruit juice may be measured with negligible interference from concurrent aerobic oxidation.