Our results indicated that there existed a very good correlation between the protein content of a soy product and its selenium content. This tendency of selenium to follow protein in foods may possibly explain the low selenium levels found in the whole blood and plasma of Guatemalan children suffering from kwashiorkor, a protein deficiency disease (Burk et al., 1967).

The soy products that contained nutritionally adequate levels of selenium, such as the concentrates and isolates, could contribute significantly to meeting the nutritional need for the element. Cantor et al. (1975) found that the biological availability of selenium from plant materials was generally greater than that from materials of animal origin. For example, they reported that the nutritional availability of the selenium in soybean meal was about 60% whereas that of fish meal was only 23%.

The variation that was found in the selenium content of the soy products used in this study could be due to several factors: (1) differences in the selenium content of the soils on which the soybeans were grown; (2) losses of selenium due to the processing techniques used in the initial preparation of the soybeans, and (3) addition of other ingredients that may or may not contain substantial amounts of selenium.

The overall conclusion from this study is that although some soybean analogues contain levels of selenium that are comparable to those in the corresponding ordinary foodstuffs, others do not; therefore, all soybean-based products cannot be considered reliable sources of this essential trace element.

LITERATURE CITED

Arthur, D., *Can. Inst. Food Sci. Technol. J.* **5**, 165 (1972).
Burk, R. F., Jr., Pearson, W. N., Wood, R. P., II, Viteri, F., *Am. J. Clin. Nutr.* **20**, 723 (1967).
Cantor, A. H., Scott, M. L., Noguchi, T., *J. Nutr.* **105**, 96 (1975).
Coppock, J., "World Soy Protein Conference", *J. Am. Oil Chem. Soc.* **51**, 59A (1974).
Ferretti, R. J., Levander, O. A., *J. Agric. Food Chem.* **22**, 1049 (1974).
Ganther, H. E., Goudie, C., Sunde, M. L., Kopecky, M. J., Wagner, P., Oh, S. H., Hoekstra, W. G., *Science* **175** 1122 (1972).
Higgs, D. J., Morris, V. C., Levander, O. A., *J. Agric. Food Chem.* **20**, 678 (1972).
Hoffman, I., Westerby, R. J., Hidiroglou, M., *J. Assoc. Off. Anal. Chem.* **51**, 1039 (1968).
Kubota, J., Allaway, W. H., Carter, D. L., Cary, E. E., LaZar, V. A., *J. Agric. Food Chem.* **15**, 448 (1967).
Morris, V. C., Levander, O. A., *J. Nutr.* **100**, 1383 (1970).
Odell, A. D., *J. Inst. Can. Technol. Aliment.* **2**, A69 (1969).
Oldfield, J. E., Allaway, W. H., Draper, H. H., Frost, D. V., Jensen, L. S., Scott, M. L., Wright, P. L., in "Selenium in Nutrition", National Academy of Sciences, Washington, D.C., 1971.
Rosenfeld, I., Beath, O., "Selenium", Academic Press, New York, N.Y., 1964.
Schwarz, K., Foltz, C. M., *J. Am. Chem. Soc.* **79**, 3292 (1957).

Received for review July 15, 1975. Accepted October 1, 1975.

Simple, Rapid Quantitative Determination of Lysine and Arginine by Thin-Layer Chromatography

Saroj Bahl, Saida Naqvi, and T. A. Venkitasubramanian*

A simple, rapid method for the quantitative determination of lysine and arginine in wheat and tissue samples by thin-layer chromatography is described. Lysine and arginine are separated on thin layers of silica gel G; the chromatograms are sprayed with ninhydrin and developed under controlled conditions. Ethanol with copper sulfate is used as an eluting solvent and the optical density read at 510 mμ. As many as 12 chromatograms can be run and eluted in 2 days. Continuous attention is not required and the recovery is consistently above 96%.

The determination of amino acid composition of food proteins has been a subject of research for more than a century. Increased attention was given to this question in the last few years because of the recognition of the specific nutritive roles played by certain amino acids for growth, reproduction, lactation, and maintenance. The numerous and immense difficulties in developing reliable analytical procedures are actually best attested to by the vast literature on the subject. Considerable progress was made after the introduction of microbiological and chromatographic methods during the last two decades (Menden and Cremer, 1970).

Thin-layer chromatography on silica gel, developed especially for separating lipophilic substances, at first seemed less suitable in the case of hydrophilic substances. However, silica gel like cellulose contains a considerable amount of water, depending on its state of hydration. Its outstanding suitability for the chromatography of amino acids is therefore not too astonishing. Quantitative analysis of mixtures of amino acids by this technique is, however, beset with several difficulties. Lysine and arginine show very close values for most of the solvent systems reported and this is the major hurdle in the quantitative separation of these amino acids.

The present paper provides a method which is particularly appropriate for the estimation of these two amino acids in wheat and tissue samples by the technique of thin-layer chromatography.

EXPERIMENTAL SECTION

Reagents used included: (1) ethanol–water, freshly distilled ethanol was mixed with distilled water in the proportion of 70:30 (v/v); (2) phenol–water, freshly distilled phenol was mixed with distilled water in the proportion of 75:25 (v/v); (3) ninhydrin–spray reagent, 0.3 g of ninhydrin was dissolved in 100 ml of 1-butanol and mixed

Department of Biochemistry, Vallabhbhai Patel Chest Institute, University of Delhi, Delhi-110007, India.

with 3 ml of glacial acetic acid; (4) eluting solvent consisted of 5 mg of copper sulfate in 100 ml of 80% ethanol.

Procedure. (A) *Preparation of the Sample.* Samples of wheat flour containing 10 mg of protein were hydrolyzed in a test tube with 6 *N* HCl distilled from an all-glass apparatus. Each sample was dissolved in 6 ml of acid contained in a 25-ml borosilicate glass tube. The tubes were evacuated and sealed and the hydrolysis was carried out in an autoclave held at 110 ± 2°C for 24 hr. The tubes were cooled and the HCl removed by repeated evacuation on a rotary evaporator at 40°C (Woychik et al., 1961). Samples were then washed three times with 5-ml portions of 10% propanol and the supernatants pooled. The pH of the pooled samples was adjusted to 7–7.2. The solution was decolorized with activated charcoal and centrifuged. The samples were concentrated on a water bath (45°C) in vacuo. The final volume was made up to 5 ml.

(B) *Thin-Layer Chromatography.* Silica gel G (30 g) (E. Merck) was vigorously shaken with 63 ml of water for 30 sec in a closed erlenmeyer flask and applied on 20 × 20 cm glass plates by means of Stahl's applicator (Shandon Scientific Co., London, England). A layer thickness of 0.2 mm was used. The plates were dried in air overnight in a horizontal position. Suitable aliquots of amino acid (0.5–2 μg) hydrolysates were applied on TLC plates with the help of micropipets in horizontal streaks. Rectangular chambers designed by the Shandon Co. were used for the development of the plates.

The plates were first developed in ethanol–water solvent system, taken out, and air dried. They were then redeveloped in a phenol–water solvent system using the same ascending technique. The chromatograms were dried and sprayed with ninhydrin–spray reagent. Color was developed by keeping the plates in an oven at 110°C for 20 min. Lysine and arginine streaks were identified by suitable standards co-chromatographed with each run.

(C) *Estimation of Amino Acids.* The red bands corresponding to lysine and arginine were scraped into clean, dry test tubes. The color was eluted with 4 ml of eluting solvent. Samples were centrifuged and the optical density of the supernatants was read at 510 mμ. The amount of amino acid was calculated by comparison with the results obtained with a standard amount of amino acid subjected to an identical procedure. This procedure was employed for the analysis of some standard varieties of wheat obtained from Indian Agricultural Research Institute, New Delhi, India.

Tissue Samples. Liver and plasma samples of male, weanling, Wistar rats fed 10% casein diets for 6 weeks (served as a control group of animals in another study) were also analyzed for lysine and arginine by the same technique. The tissue samples were prepared for analysis by the method of Tallan et al. (1954).

The protein-free filtrate was collected, adjusted to pH 7–7.2, and decolorized if necessary. The procedure for chromatographic separation and analysis of amino acids was the same as that described for wheat samples.

RESULTS AND DISCUSSION

The separation of a mixture of amino acids on silica gel G is shown in Figure 1. Lysine remained at the point of application and arginine separated as a distinct band above it. It appeared from the results obtained that a particular pH range of 6.8–7.2 was required to achieve the best separation of lysine from arginine. A more acidic or alkaline pH caused overlapping of these two amino acids. It was also found essential to decolorize the samples before subjecting them to the chromatographic procedure in order to obtain two distinct bands of lysine and arginine. Figure 2 shows the separation of lysine and arginine from two wheat varieties subjected to this technique.

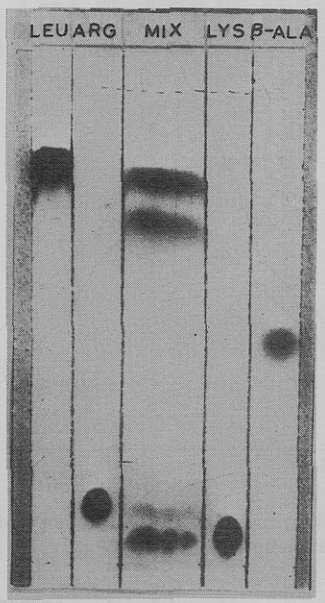

Figure 1. Separation of lysine and arginine from a mixture of amino acids on silica gel G.

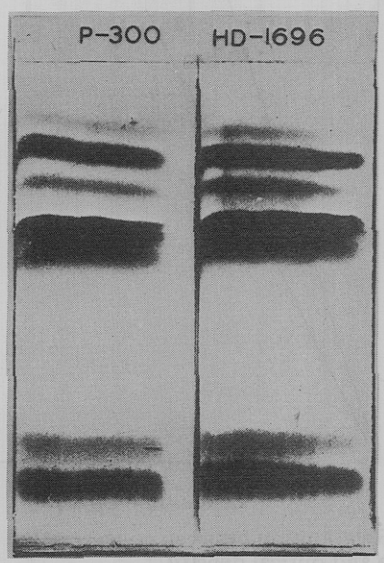

Figure 2. Separation of lysine and arginine from two wheat varieties (P-300 and HD-1696) on silica gel G.

A minimum temperature of 100°C was required for the development of the color of the amino acid streaks. Intensity of the color was maximum at a temperature of 110 ± 2°C. Similar temperatures for detection of amino acids have been suggested by Pataki (1968).

It was also observed that the addition of copper sulfate to the eluting solvent intensified the color obtained with both the amino acids. Concentrations exceeding 5 mg/100 ml of eluting solvent had no additional intensifying effect. Various solvents such as methanol, propanol, and butanol were also tried for the elution of lysine but ethanol gave maximum recovery. The color was found to be stable for a time period of 24 hr after which a gradual reduction in the intensity followed.

The relationship of optical density of lysine and arginine samples to wavelength is graphically presented in Figure 3. As is evident from the results obtained, the optical density of both the amino acid samples was at its maximum at a wavelength of 510 mμ. The color intensity of amino acids was therefore measured at this wavelength in

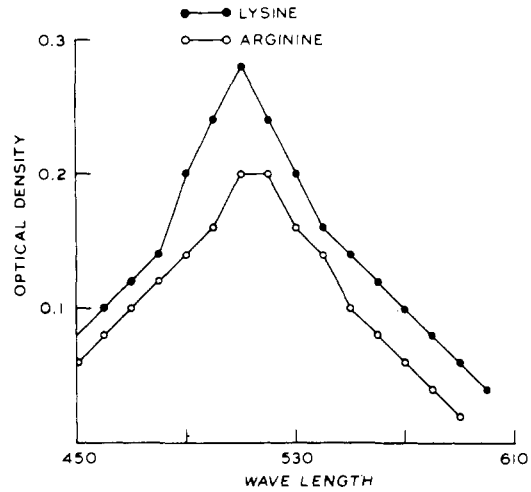

Figure 3. Relationship of optical density of lysine and arginine to wavelength.

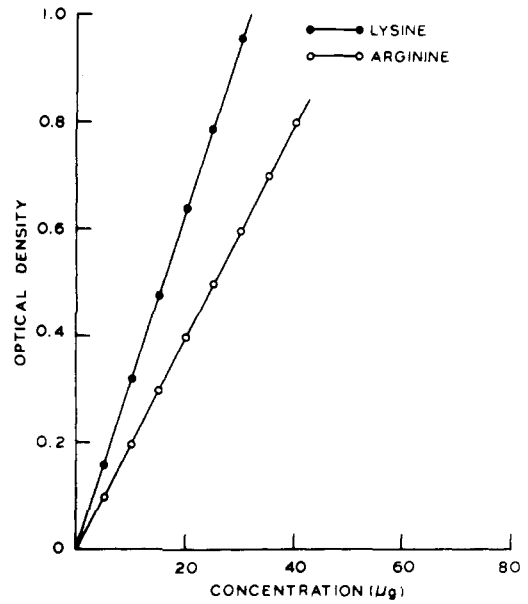

Figure 4. Relationship between concentration of lysine and arginine and optical density.

Table I. Recovery Experiments by Adding Known Amounts of Lysine and Arginine to Protein Hydrolysates

Amount of amino acid added, mg	Added amount of Lys found, mg	Added amount of Arg found, mg	Recovery, % of Lys	Recovery, % of Arg
0.6	0.579	0.581	96.50	96.83
0.8	0.770	0.780	96.25	97.50
1.0	0.965	0.975	96.50	97.50
1.5	1.450	1.462	96.67	97.47
2.0	1.930	1.944	96.50	97.20
	Mean recovery		96.48	97.30

this method. The linear relationship between the concentrations of these amino acids and the respective optical densities is shown in Figure 4. Concentrations as low as 1 μg can be estimated by this method with sufficient accuracy and reproducibility.

Recovery experiments were also carried out by adding known amounts of pure amino acids to the protein hydrolysates and the results are given in Table I. Mean recovery for both amino acids was found to be consistently above 96%.

Table II. Lysine and Arginine Content of Some Wheat Varieties

Wheat varieties	Lys, g/100 g of protein: Present method	Lys, g/100 g of protein: Automatic AA anal. technique	Arg, g/100 g of protein: Present method	Arg, g/100 g of protein: Automatic AA anal. technique
1. P-300	2.97	2.66	4.89	4.77
2. Kalyan Sona	2.23	2.10	4.93	4.89
3. HD-1696	2.80	2.82	5.28	5.15
4. Norteno	2.86	2.71	5.31	5.29
5. Sonalika	2.63	2.57	5.19	5.21

Table III. Tissue[a] Lysine and Arginine Contents of Rats Fed Casein Diets

	Plasma, μmol/100 ml	Liver, μmol/g of tissue
Lysine	41.8	48.5
Arginine	9.8	12.2

[a] Tissue samples from six male rats were pooled and analyzed.

The results of the analysis of some standard wheat varieties obtained from Indian Agricultural Research Institute, New Delhi, are presented in Table II. The values for the two amino acids reported by Indian Agricultural Research Institute using the automatic amino acid analysis (Spackman et al., 1958) technique are also given. Comparison of the two sets of data reveals that the values compare well with each other. Results of the analysis of liver and plasma samples of male rats fed casein diets are summarized in Table III.

Thin-layer chromatography, though a very valuable analytical tool in the hands of the researcher, has not been successfully employed for the estimation of lysine and arginine in protein samples owing to certain limitations. The major problem in the separation of lysine from a mixture of other amino acids is that above a certain concentration, lysine and arginine spots overlap with each other. This difficulty was also encountered by Clark (1968) in her technique of quantitative determination of amino acids by thin-layer chromatography. It was with the object of overcoming this problem that this technique was developed and every effort was made to conserve the sensitivity, reproducibility, and rapidity of the method.

The sensitivity and reproducibility of the method are of the same order as that of established standard procedures. It is less time consuming as compared to several other methods reported for the estimation of lysine, and does not require continuous attention from the researcher except in the last stage when the spots are eluted and read. The entire procedure requires only 2 days for completion and 12 or more chromatograms can be run in a day.

The method can be successfully employed for the determination of lysine and arginine levels in cereals as well as in the tissue samples. Values for tissue lysine and arginine of rats fed casein diets obtained by this technique compare well with those reported for animals under similar experimental conditions using the automatic amino acid analysis procedure (Swendseid et al., 1962; Harker et al., 1968; Jacobs and Crandall, 1972). Tissue amino acid levels bear an important relationship to the dietary and nutritional status of the animal. An investigation of changes in the concentration of amino acids in muscle, liver, and plasma assumes significant value in several experiments where the objective is to evaluate the quality of dietary protein (Harker et al., 1968; Munro, 1970). This technique

can therefore be used either alone, when it is desirable to estimate the lysine and arginine content of a given protein sample, or in conjunction with other procedures when the complete amino acid composition of protein is in question, but an accurate assessment of the lysine level is important such as in animal experiments related to the study of lysine deficiency. The method may also find use in agricultural research laboratories devoted to the development of high lysine wheat and rice varieties.

ACKNOWLEDGMENT

The authors gratefully acknowledge the cooperation extended by A. Austin, Indian Agricultural Research Institute, New Delhi, India, for providing the wheat varieties for analysis and the data obtained by automatic analysis for the sake of comparison.

LITERATURE CITED

Clark, M. E., *Analyst* **93**, 810 (1968).
Harker, C. S., Allen, P. E., Clark, H. E., *J. Nutr.* **94**, 495 (1968).
Jacobs, F. A., Crandall, J. C., *Nutr. Rep. Int.* **5**, 27 (1972).
Menden, E., Cremer, H., "Newer Methods of Nutritional Biochemistry", Vol. IV, Albanese, A. A., Ed., Academic Press, New York, N.Y., 1970, p 125.
Munro, H. N., "Mammalian Protein Metabolism", Vol. IV, Munro, H. N., Ed., Academic Press, New York, N.Y., 1970, p 299.
Pataki, G., "Techniques of Thin-Layer Chromatography in Amino Acid and Peptide Chemistry", Ann Arbor Science Publishers, Ann Arbor, Mich., 1968, p 90.
Spackman, D. H., Stein, W. H., Moore, S., *Anal. Chem.* **30**, 411 (1958).
Swendseid, M. E., Hickson, J. B., Friedrich, B. W., *J. Nutr.* **78**, 115 (1962).
Tallan, H. H., Moore, S., Stein, W. H., *J. Biol. Chem.* **211**, 927 (1954).
Woychik, J. H., Boundy, J. A., Dimler, R. J., *J. Agric. Food Chem.* **9**, 307 (1961).

Received for review August 8, 1973. Resubmitted May 20, 1975. Accepted August 19, 1975.

Light-Dependent Carotenoid Synthesis in the Tomato Fruit

Leoncio C. Raymundo,[1] Clinton O. Chichester, and Kenneth L. Simpson*

(1) Light was excluded from growing fruits of normal red, high-beta, apricot, and tangerine tomato genotypes while attached to the vine in order to study the effect of light on the biosynthesis of carotenoids. (2) Pigment formation in the immature fruit of the normal red, high-beta, and apricot genotypes was inhibited in darkness. The carotenoids of dark-ripened, dark-grown fruits of the three genotypes are qualitatively similar to those found in either the light-grown control fruits or the dark-grown fruit which was further ripened in light. (3) β-Carotene was not detected in the immature dark-grown tangerine tomato and only a small amount of the pigment was present in the ripe dark-grown fruit. Lycopene and neurosporene did not accumulate in the dark-grown, dark-ripened fruit but appeared when the fruit was exposed to light probably due to the photoconversion of the poly-cis carotenoids to their corresponding all-trans isomer. The carotenoid composition of dark-grown, light-ripened tangerine tomato is identical with that of the light-grown control fruit. (4) Biosynthetic autonomy of chloroplast and choromoplast carotenoids was suggested.

The carotenoid composition of the tomato fruit undergoes extensive modification during ripening, quantitatively as well as qualitatively. The predominantly cyclic nature of the carotene of the chloroplast changes to the more diverse constitution characteristic of the chromoplast.

The synthesis of chromoplast carotenoids in the tomato fruit is inhibited at high temperature. Light, on the other hand, has a more profound effect than temperature on the biosynthesis of carotenoids in the chloroplast.

Light is necessary in the induction of chloroplast replication and in the control of certain phases of plastid transformation in higher plants (Boasson et al., 1972). Etiolated plants accumulate protochlorophyll and small amounts of carotenoids (Valadon and Mummery, 1969). Synthesis of the carotenoid component and rapid conversion of protochlorophyll to chlorophyll are initiated on exposure to light (Smith and Benitez, 1954; Goodwin and Phagpolngarm, 1960; Virgin, 1967).

Department of Food and Resource Chemistry, University of Rhode Island, Kingston, Rhode Island 02881.

[1]Present address: Department of Food Science and Technology, College of Agriculture, University of the Philippines at Los Baños, College, Laguna, Philippines.

In addition, many plant tissue cultures will synthesize chloroplast pigments when grown in light (Powell, 1925; Stobart et al., 1967). Roots, whether excised or attached to the plant, can be induced to form chloroplasts by exposure to light (Powell, 1925; Bjorn, 1963; Heltne and Bonnett, 1970; Bajaj and McAllan, 1969). However, light is reported to be not essential for the synthesis of carotenoids in ripening tomato fruit (Smith, 1936; Vogele, 1937). The effect of light on pigment biosynthesis in higher plants as well as in nonphotosynthetic, photochromogenic microorganisms has been reviewed by Kirk and Tilney-Bassett (1967).

In the present study, changes in the carotenoid composition of tomato fruits grown in the absence of light were investigated.

EXPERIMENTAL SECTION

Fruits. The tangerine tomato fruits were obtained from plants grown in the greenhouse from August to May as well as from plants grown in the field during the following summer. Summer Sunrise, apricot, and high-beta tomatoes were all field grown.

Flowers at anthesis or fruits less than 10 mm in diameter were wrapped with a black polyethylene bag or a bag made from carbon paper. Each bag was then enclosed in an