

Table V. Solubilization of Nitrogen in Protein Fractions with Water

material	pH	nitrogen solubility, ^a %
fraction I protein spray-dried ^b	7.4	5.05
freeze-dried ^b	7.4	12.81
fraction I protein (salt added) spray-dried	7.4	6.12
freeze-dried	7.4	12.64
freeze-dried	8.5	42.45

^a Average for two values. ^b Dried as suspension; other samples were dried as solution.

solved in salt solution at pH 8.5 before drying.

The studies reported in this paper show that tobacco can be processed on a large scale to yield crystalline fraction I protein and other protein concentrates suitable for feed or food. Improved equipment for collection and recrystallization of fraction I should enable even greater quantities of tobacco to be processed.

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LITERATURE CITED

- Anderson, J. W., Rowan, K. S., *Phytochemistry* **6**, 1047 (1967).
 Association of Official Analytical Chemists, "Official Methods of Analysis", 12th ed, Washington, DC, 1975.
 Bahr, J. R., Bourque, D. P., Smith, H. J., *J. Agric. Food Chem.* **25**, 783 (1977).
 Bickoff, E. M., Kohler, G. O., U.S. Patent 3823 128, July 9, 1974.
 Bickoff, E. M., Booth, A. N., de Fremery, D., Edwards, R. H., Knuckles, B. E., Miller, R. E., Saunders, R. M., Kohler, G. O., in "Protein Nutritional Quality", Friedman, M., Ed., Marcel Dekker, New York, 1975, p 319.
 Chan, P. H., Sakano, K., Singh, S., Wildman, S. G., *Science* **176**, 1145 (1972).
 Cohen, M., Ginoza, W., Dorner, R. W., Hudson, W. R., Wildman, S. G., *Science* **124**, 1081 (1956).
 de Fremery, D., Miller, R. E., Edwards, R. H., Knuckles, B. E., Bickoff, E. M., Kohler, G. O., *J. Agric. Food Chem.* **21**, 886 (1973).
 Edwards, R. H., de Fremery, D., Mackey, B. E., Kohler, G. O., *Trans ASAE* **20**, 423 (1977).
 Edwards, R. H., Miller, R. E., de Fremery, D., Knuckles, B. E., Bickoff, E. M., Kohler, G. O., *J. Agric. Food Chem.* **23**, 620 (1975).
 FAO Nutritional Meeting Report Series No 52; WHO Technical Report Series No. 522, Energy and Protein Requirements, 1973.
 Haisman, D. R., *J. Sci. Food Agric.* **25**, 803 (1974).
 Henry, K. M., Ford, J. E., *J. Sci. Food Agric.* **16**, 425 (1965).
 Hollo, J., Koch, L., *Process Biochem.* **37** (Oct. 1970).
 Janson, J.-C., *J. Agric. Food Chem.* **19**, 581 (1971).
 Kawashima, N., Wildman, S. G., *Biochem. Biophys. Acta* **229**, 749 (1971).
 Kawashima, N., Wildman, S. G., *Ann. Rev. Plant Physiol.* **21**, 325 (1970).
 Knuckles, B. E., de Fremery, D., Bickoff, E. M., Kohler, G. O., *J. Agric. Food Chem.* **23**, 209 (1975).
 Kohler, G. O., Knuckles, B. E., *Food Technol.* **13**, 191 (1977).
 Kohler, G. O., Bickoff, E. M., Spencer, R. R., Witt, S. C., Knuckles, B. E., Proceedings of the 10th Technical Alfalfa Conference, ARS-79-46, 1968, p 71.
 Kohler, G. O., Palter, R., *Cereal Chem.* **44**, 512 (1967).
 Loomis, W. D., Battaile, J., *Phytochemistry* **5**, 423 (1966).
 Lowe, R. H., *FEBS Lett.* **78**, 98 (1977).
 Pierpoint, W. S., *Biochem. J.* **112**, 609 (1969a).
 Pierpoint, W. S., *Biochem. J.* **112**, 619 (1969b).
 Pirie, N. W., in "Leaf Protein: Its Agronomy, Preparation, Quality, and Use", IBP Handbook No. 20, Pirie, N. W., Ed., Blackwell Scientific, Oxford, 1971, Chapter 5.
 Schroeter, L. C., "Sulfur Dioxide, Applications in Foods, Beverages, and Pharmaceuticals", Pergamon Press, Elmsford, N.Y., 1966, pp 191-220.
 Smith, A. K., Johnson, V. L., *Cereal Chem.* **25**, 399 (1948).

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Influence of Nitrogen Fertilization on Potato Discoloration in Relation to Chemical Composition. 2. Phenols and Ascorbic Acid

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The effect of nitrogen fertilization on enzymatic darkening of Katahdin potatoes was examined with respect to phenolic and ascorbic acid content. Ammonium nitrate was applied at rates of 100, 150, 200, and 250 lb/acre. Enzymatic darkening and phenolic content increased significantly ($p < 0.01$) as the level of nitrogen increased. A significant positive correlation ($r = +0.97$) was found between phenolic content and enzymatic discoloration. Ascorbic acid increased significantly ($p < 0.01$) with increased nitrogen levels.

Previous work from our laboratory has indicated the importance of nitrogen fertilization and its effect on tuber discoloration with regard to lipids, potassium, and dry matter content of Katahdin potatoes (Mondy and Koch,

1978). Potatoes receiving higher amounts of nitrogen exhibited greater enzymatic discoloration, increased dry matter, and lowered lipid and potassium content than control tubers. A significant negative correlation ($r = -0.95$) was established between the degree of enzymatic discoloration and lipid content of potato tubers. Other important chemical constituents in the potato tuber related to enzymatic discoloration are phenols and ascorbic acid.

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Early studies by Van Middlelem (1952) showed that potato discoloration was positively correlated with tyrosine, a monophenol, and dihydroxy phenyl alanine, a diphenol, in potato tissue. Mapson et al. (1963) also found a direct correlation between tyrosine content and tuber susceptibility to enzymatic darkening. Chlorogenic acid, a polyphenol, has been shown to be the chief phenolic constituent of potatoes, and Mondy et al. (1967) found a significant positive correlation ($r = +0.83$) between potato discoloration and total phenolic content. A survey of the literature revealed a lack of information on the effect of nitrogen fertilization on the total phenolic content of potatoes. Mulder (1949), however, observed that a high dressing of nitrogen increased the tyrosine content of tubers.

Ascorbic acid is important to the nutritive value and possibly black spot susceptibility of potato tissue. Several studies have examined tuber ascorbate content with respect to nitrogen fertilization but no consistent trends have been observed. Karikka et al. (1944) failed to establish a relationship between different fertilization regimes and tuber ascorbic acid content. Augustin (1975) concluded that nitrogen fertilization decreased ascorbic acid levels in Russet Burbank potatoes. In contrast to these findings, Sinha (1955) reported an increase in ascorbic acid with nitrogen fertilization. Ascorbic acid is recognized as an antioxidant and can be used to prevent enzymatic browning of cut surfaces of fruits and vegetables. Anderson and Zapsalis (1957) found 1–3% solutions of ascorbic acid to be effective in preventing discoloration of vacuum packaged prepeeled potatoes. Ascorbic acid which is more concentrated at the bud end of the tuber (Baird and Howatt, 1948) may inhibit discoloration by reducing *o*-quinones back to the *o*-diphenol form. Johnson and Schall (1957) observed that the rapid accumulation of chlorogenic acid and other diphenols in potato slices immediately after cutting was paralleled by immediate increases in ascorbic acid levels but the ascorbic acid then decreased after 24–48 h. Farkas et al. (1960) observed significant interactions between phenolic compounds and ascorbic acid in viral-infected plants and suggested that ascorbic acid reduced quinones back to their corresponding phenols as part of a defense mechanism to control necrosis.

This study was undertaken in order to determine the effect of different levels of nitrogen fertilization on tuber discoloration and to study further the interrelationships of total phenols and ascorbic acid on black spot susceptibility of potatoes.

MATERIALS AND METHODS

Katahdin potatoes grown at the Cornell Vegetable Research Farm in Riverhead, Long Island, were used in this study. Inorganic nitrogen in the form of ammonium nitrate was banded at planting with rates of 0, 100, 150, 200, 250 lb per acre. The randomized block design contained two replicated plots per treatment. Tubers were harvested 24 weeks after planting and stored at 5 °C until analyzed. The tubers were separated into cortex (including the peel) and pith sections, and the cortex tissues were used since the cortex area is more susceptible to discoloration.

Determination of Discoloration. Color measurements were made on potato tissue using the Hunter Color Difference Meter as previously described by Mondy et al. (1967). Cortex tissue from four potatoes was used for each determination. Duplicate determinations were made on each lot of potatoes.

Determination of Total Phenols. The methods used for phenol determinations was the same as that reported

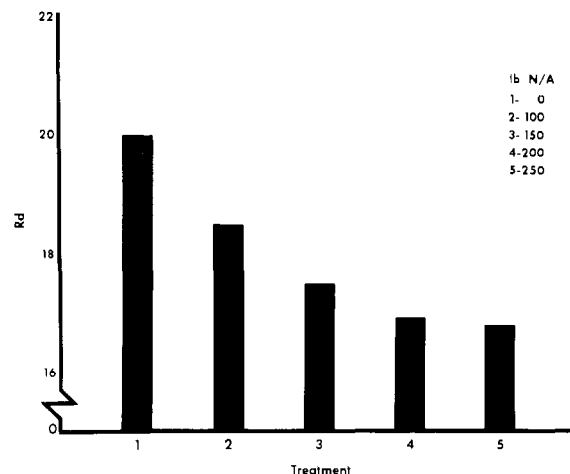


Figure 1. Effect of nitrogen fertilization on potato discoloration. R_d values decrease as blackening increases. lb N/A = pounds of nitrogen per acre.

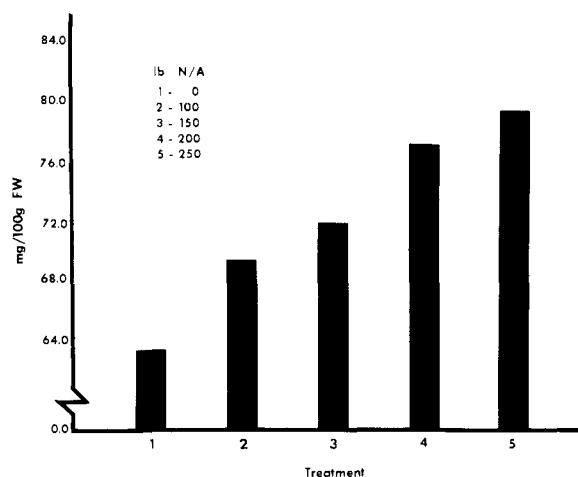


Figure 2. Phenolic content of cortex tissue from Katahdin potatoes treated with different levels of nitrogen fertilization.

by Mondy et al. (1966). Three tubers were selected for each extract and two extracts were made for each treatment.

Determination of Ascorbic Acid. The L-ascorbic acid content was determined on cortex tissue using the indophenol method of Horwitz (1970). Cortex tissue of three potatoes from each treatment was used for each extract. Duplicate determinations were made on each treatment.

Statistical Analysis. A one factor analysis of variance and the Dunnett test which compares every treatment with the control was used to determine the statistical significance of the effect of nitrogen fertilization on discoloration and phenol and ascorbic acid content. The Pearson product-moment correlation coefficient was used to determine the relationships between discoloration, phenolic and ascorbic acid content, and levels of fertilization (Steel and Torrie, 1960).

RESULTS AND DISCUSSION

Discoloration and Phenols. Tubers from plants receiving high levels of nitrogen fertilizer discolored significantly more ($p < 0.01$) than control tubers (Figure 1). The phenol content also increased with higher levels of nitrogen fertilization (Figure 2). A significant ($p < 0.01$) positive correlation was observed between phenolic content and enzymic discoloration. Koblet (1947, 1948) also found that enzymatic discoloration increased with increased nitrogen fertilization, although he did not relate the

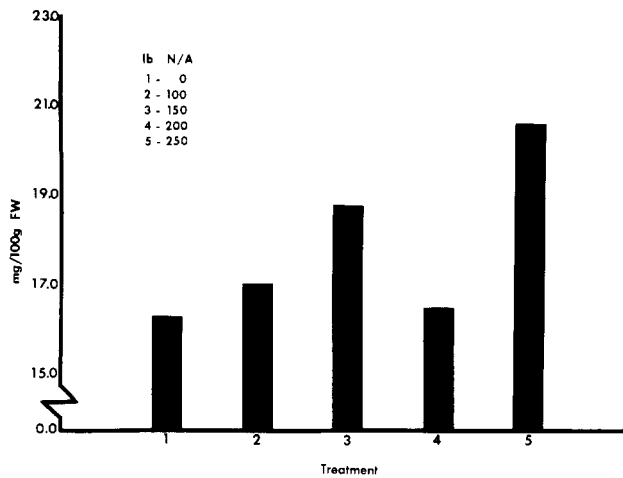


Figure 3. Ascorbic acid content of cortex tissue from Katahdin potatoes treated with different levels of nitrogen fertilization.

discoloration to phenolic content. High phenolic content has been associated with bitterness and astringency in cooked potatoes (Mondy et al., 1971). Although he did not report the phenolic content, Dimitrov (1975) reported that the flavor of cooked potatoes was adversely affected by nitrogen fertilization. It is possible that increased phenolic content accompanying nitrogen fertilization may have been responsible for the poor flavor he observed.

Ascorbic Acid. Ascorbic acid content of cortex tissues increased significantly ($p < 0.01$) with increased levels of nitrogen (Figure 3), with the exception of treatment 4 (200 lb of N/acre). A nonsignificant increase in ascorbic acid with treatment 4 could not be explained, while a significant increase in ascorbic acid with other treatments is in agreement with Ishevskaya (1965) and Sinha (1955) who reported an increase in ascorbic acid with nitrogen fertilization. Augustin (1975) reported changes in ascorbic acid with nitrogen fertilization as varietal characteristics. However, Teich and Menzies (1964) reported decrease in ascorbic acid with increasing rates of nitrogen, but these workers used 0, 30, and 60 lb/acre nitrogen applications, while in the present study 0, 100, 150, 200, and 250 lb/acre nitrogen levels were applied. Secondly, Teich and Menzies reported their ascorbic acid values on the whole potato tuber basis, while in our study cortex tissue, the area of high metabolic activities, was used for analysis.

Although ascorbic acid acts as an antioxidant and inhibits enzymatic darkening in vacuum-packaged prepeeled potatoes (Anderson and Zapsalis, 1957), it did not seem to play a major role in preventing nitrogen-induced enzymatic darkening of potatoes. Similar conclusions about the effect of ascorbic acid on enzymatic darkening were reached by Mondy et al. (1960), who found that Ontario potatoes, a variety susceptible to darkening contained higher levels of ascorbic acid than Pontiac potatoes, a

variety resistant to darkening.

This study indicates that heavy nitrogen fertilization raises the phenolic content of potato cortex tissue which contributes to increased enzymatic discoloration. Ascorbic acid content is also positively associated with level of nitrogen, but apparently ascorbic acid is not present in sufficient amounts to inhibit enzymatic discoloration. Although lowering the amount of nitrogen fertilizer applied to potatoes may result in a somewhat lower ascorbic acid content, the benefits obtained in reducing tuber discoloration, with it resulting waste, would make the practice desirable. From this study it appears that high levels of nitrogen fertilization are unnecessary. High levels of nitrogen fertilization for potatoes not only increases the cost of production and increases nitrate-nitrite pollution in run-off water but also decreases the quality of potatoes.

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LITERATURE CITED

- Anderson, E. E., Zapsalis, C., *Food Eng.* **29**, 114 (1957).
 Augustin, J., *J. Food Sci.* **40**, 1295 (1975).
 Baird, E., Howatt, J. C., *Can. J. Res.* **26**, 433 (1948).
 Dimitrov, S., *Rastenievud. Nauki* **12**, 108 (1975).
 Farkas, G. L., Kiraly, S., Solymosy, F., *Virology* **12**, 408 (1960).
 Horwitz, W., Ed., "Official Methods of Analysis", 11th ed, Association of Official Analytical Chemists, Washington, DC, 1970.
 Ishevskaya, I. M., *Dokl. TSKhA* **108**, 161 (1965).
 Johnson, G., Schall, L. A., *Am. Potato J.* **34**, 200 (1957).
 Karikka, K. J., Dudgeon, L. T., Hauck, H. M., *J. Agric. Res.* **68**, 49 (1944).
 Koblet, R., *Landwirtsch. Jahrb. Schweiz* **61**, 665 (1947).
 Koblet, R., *Landwirtsch. Jahrb. Schweiz* **62**, 827 (1948).
 Mapson, L. W., Swain, T., Tomalin, A. W., *J. Sci. Food Agric.* **14**, 673-84 (1963).
 Mondy, N. I., Klein, B. P., Smith, L. I., *Food Res.* **25**, 693 (1960).
 Mondy, N. I., Gedde-Dahl, S. B., Mobley, E. O., *J. Food Sci.* **31**, 33 (1966).
 Mondy, N. I., Mobley, E. O., Gedde-Dahl, S. B., *J. Food Sci.* **32**, 378 (1967).
 Mondy, N. I., Metcalf, C., Plaisted, R. L., *J. Food Sci.* **36**, 459 (1971).
 Mondy, N. I., Koch, R. L., *J. Agric. Food Chem.* **26**, 666 (1978).
 Mulder, E. G., *Plant Soil* **2**, 59 (1949).
 Sinha, K. P., *Proc. Bihar. Acad. Agric. Sci.* **4**, 103 (1955).
 Steel, R. G. D., Torrie, J. H., "Principles and Procedures of Statistics", McGraw-Hill, New York, 1960.
 Teich, A. H., Menzies, J. A., *Am. Potato J.* **41**, 169 (1964).
 Van Middlelem, C. H., Ph.D. Thesis, Cornell University. Ithaca, NY, 1952.

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