AGENCY FOR INTERNATIONAL DEVELOPMENT
WASHINGTON, D. C. 20523

BIBLIOGRAPHIC INPUT SHEET

FOR AID USE ONLY

1. SUBJECT CLASSIFICATION

A. PRIMARY: Agriculture — AF30-0180-0000

B. SECONDARY: Plant breeding--Cereals--Wheat

2. TITLE AND SUBTITLE

Screening for high lysine content in wheat

3. AUTHOR(S)

Mattern,P.J.; Schmidt,J.W.; Johnson,V.A.

4. DOCUMENT DATE	5. NUMBER OF PAGES	6. ARC NUMBER
1970	3p.	ARC 633.11.M435

7. REFERENCE ORGANIZATION NAME AND ADDRESS

Neb.

8. SUPPLEMENTARY NOTES *(Sponsoring Organization, Publishers, Availability)*

(In Cereal science today,v.15,no.12,p.409-411)

9. ABSTRACT

10. CONTROL NUMBER	11. PRICE OF DOCUMENT
PN-RAA- 266	
12. DESCRIPTORS	**13. PROJECT NUMBER**
Lysine Tests Wheat	**14. CONTRACT NUMBER** CSD-1208 Res.
	15. TYPE OF DOCUMENT

AID 590-1 (4-74)

Screening for High Lysine Content in Wheat[1]

P. J. Mattern, J. W. Schmidt, and V. A. Johnson[2]
Department of Agronomy
University of Nebraska
Lincoln

RAPIDLY INCREASING WORLD population and associated stresses on available food supplies make it essential that the nutritional value of food stocks be maximized.

The nutritional potential of maize has been improved with the recognition of genes for higher lysine contents (1,2,3,4). The success in improving protein quality of maize suggests the possibility of similar improvement in other cereal species.

The essential amino acids, lysine, methionine, threonine, and tryptophan, are the first nutritionally limiting amino acids in wheat (5). Varieties higher in these limiting amino acids, particularly lysine, would make a significant contribution to the nutrition of people on a wheat diet or of populations in developing countries where wheat could be important in the diet. A genetic source of high lysine could be incorporated into new wheat varieties to provide better protein nutrition.

The soluble protein fractions in typical wheat endosperm are composed of higher levels of the basic amino acids, lysine, arginine, and histidine, than the insoluble fractions. These basic amino acids increase as the protein content decreases (6,7,8,9,10,11). Therefore, as would be expected, it has been reported that soft endosperm portions of the wheat kernel have less total nitrogen but a greater amount of the basic amino acids than the hard endosperm portion (12).

Wheats with hard-milling properties, long mixing requirement, and high loaf volume tend to have glutens which are more water-insoluble than the softer short-mixing wheats. Therefore, selection for high-quality bread wheats over the years may have resulted in wheat varieties with low lysine content. Conversely, soft endosperm, low-protein wheats can be expected to contain higher percentages of the water-soluble proteins than the hard wheats if the protein quality characteristics differ from those of bread wheats.

Although a tremendous amount of research has been reported on the nutritional aspects of wheat, no study heretofore has been systematically organized to carry out a wide-scale evaluation of large numbers of wheat samples to find varieties or mutants which might have improved nutritional characteristics. This paper reports such a study and the analytical methods utilized.

The World Wheat Collection maintained by the USDA, and which contains over 16,000 entries, was made available for a study to identify genetic sources of higher lysine content in wheat. The first portion of the collection that we screened consisted of the softer endosperm types of common wheats. The work is being supported by the Agency for International Development, Department of State, Washington, D.C.

---

[1]Contribution from the Department of Agronomy, Nebraska Agricultural Experiment Station, Lincoln, and Crops Research Division, Agricultural Research Service, U.S. Department of Agriculture. Published with the approval of the Director as Paper No. 2137, Journal Series, Nebraska Agricultural Experiment Station. The research was supported by a grant provided by AID, Department of State, Washington, D.C. Presented at the 52nd Annual Meeting, Los Angeles, Calif., 1967.

Mention of firm names or trade products does not imply that they are endorsed or recommended by the U.S. Department of Agriculture over other firms or similar products not mentioned.

[2]Associate Professor of Agronomy, University of Nebraska; Professor of Agronomy, University of Nebraska; Research Agronomist, Crops Research Division, Agricultural Research Service, U.S. Department of Agriculture and Professor of Agronomy, University of Nebraska.

Copyright © 1970 American Association of Cereal Chemists, Inc., 1821 University Avenue, St. Paul, Minnesota 55104. All rights reserved.

The screening of a large number of samples poses a challenge. It is expected that a high lysine wheat would have higher levels of water-soluble protein. One could either determine the actual levels of water-soluble protein or employ an indirect approach, such as measuring the change in pH of an extracting solution brought about by the buffering action of the soluble proteins. A protein determination is desirable because it may be possible to identify genetic sources of high protein while simultaneously screening for lysine. New genetic sources of high protein would be useful because they would increase the potential nutritional value of wheat.

Methods for protein (nitrogen) considered included dye binding, ninhydrin reaction with the hydrolysate (colorimetric), conventional Kjeldahl, an automatic nitrogen gas analyzer, and automatic analyzers for either processing Kjeldahl digests from wheat or acid hydrolysates.

For lysine screening, ion-exchange chromatography, enzymatic and colorimetric methods were considered.

Our present scheme uses the Udy dye-binding method for protein determination. Lysine was evaluated on an aliquot of an acid hydrolysate with the Beckman Spinco amino acid analyzer which had been modified with four short resin columns and an additional buffer pump for cleanup and regeneration of columns.

After our work was started, Villegas et al. (13) reported a screening method for lysine using an automatic amino acid analyzer. White and Gauger (14) employed an automated simultaneous scheme for lysine and total amino acid (for protein) as a screening technique for lysine in seed hydrolysates.

Methods

Grinding. Wheat samples were ground with a Udy Cyclone hammer mill through a 0.024-in. screen. Samples were sifted through two special stacked sieves, blended, and placed in a controlled humidity cabinet at 48% r.h. to maintain the moisture levels of samples at approximately 11.25%. Only control samples were routinely checked daily for moisture, thereby conserving samples and work effort.

Hydrolysis. Ground wheat samples (0.1 g. dry weight basis) were dispersed in 10 ml. 6N HCl in a 25-ml. Pyrex test tube. The contents were sealed and the hydrolysis was completed in an oven held at 110° ± 2°C. for 24 hr. The tube contents were filtered, evaporated to dryness, and placed in a vacuum desiccator over NaOH for 24 hr. Hydrolysates were dissolved in 50 ml. of pH 2.2 citrate buffer and refiltered. Palter and Kohler (15,16) report a comprehensive study of factors affecting acid hydrolysis of seed proteins.

Protein. Protein values were determined by a modification of the shaker method, AACC method 46-14 (17). Sample size was reduced to 0.5 g. (dry weight basis) and dye volume to 25 ml.

Lysine. An aliquot of the hydrolysate was analyzed on the short column of a Beckman Spinco Model 120C amino acid analyzer using the method of Spackman et al. (18). The amino acid analyzer was modified with a total of four short columns. Two buffer pumps and a nitrogen pressure system for equilibrating buffer were required to operate four columns in a sequential manner. Samples were applied about every 8 min. The process was programmed so that only the lysine peak was recorded. Chromatograms were integrated with a Beckman Automatic Digital Integrator Model 125. The lysine peak area was converted to percent lysine content using commercial standards.

Data Processing. Percent protein and lysine peak area were punched on data cards. The computer print-out consisted of the laboratory sample number, the wheat C.I. number, the percent lysine and percent protein (dry weight basis), lysine percent of protein (g. lysine per 100 g. of protein), and a lysine index which took into consideration the protein and a weighted lysine figure. This index was calculated by multiplying percent protein by (percent lysine)^2.

Results and Discussion

One hundred determinations on a control sample analyzed over several months were evaluated to check the precision of the Udy protein method. The mean protein content on a dry weight basis was 18.38% with a standard deviation of 0.13 and a coefficient of variation of 0.7%.

Twenty-two samples were prepared and evaluated to determine the precision of the amino acid analyzer for lysine. The mean lysine content on a dry matter basis was 0.51% with a standard deviation of 0.01 and a coefficient of variation of 2.0%.

Standards of flour, wheat, and lysine were run at frequent intervals to control the accuracy of the screening work.

Analyses of the first 679 samples of the World Wheat Collection provide the following data. The percent protein ranged from 5.55 to 21.10%; the percent lysine from 0.25 to 0.59% (dry weight basis); and the lysine percent of total protein (or g. lysine per 100 g. of protein) from 1.77 to 3.76. The averages for the above were 13.41, 0.40, and 3.10% for percent protein, percent lysine, and lysine percent of the total protein, respectively.

Hydrolysates of samples exhibiting lysine peak values 20% higher than the mean are being rerun. Samples low in lysine are also being rechecked.

The lysine values found are in good agreement with published values which range from 2.87 to 3.67% (5). Samples which appear to offer a potential as new genetic sources for lysine are being evaluated for all the amino acids in order that the best possible over-all protein nutritional potential is realized.

During recent years wheat breeders have made advances in obtaining wheats with high yield potential. Currently there is much effort to incorporate genes for higher protein in these higher yielding types. As varieties with higher lysine contents are identified, they will be crossed with existing high yielding, high-protein wheats. This will maximize the potential nutritional value of wheat protein.

The screening techniques which have been described are identifying samples which exhibit a substantial range in lysine contents. These samples will be the basis for research to investigate the potential for even faster screening techniques.

Literature Cited

1. ROBLES, P. C. E. The microbiological assay of lysine in different varieties of corn genetically controlled. Escuela Farm., Guatemala 13: 15 (1951).
2. MERTZ, E. T., BATES, L. S., and NELSON, O. E. Mutant gene that changes protein composition and increases lysine content of maize endosperm. Science 145: 279 (1964).
3. MERTZ, E. T., VERNON, OLIVIA A., BATES, L. S., and NELSON, O. E. Growth of rats fed on opaque-2 maize. Science 148: 1741 (1965).
4. NELSON, O. E., MERTZ, E. T., and BATES, L. S. Second mutant gene affecting the amino acid pattern of maize endosperm proteins. Science 150: 1469 (1965).
5. AMERICAN ASSOCIATION OF CEREAL CHEMISTS. Wheat:

Chemistry and technology, Table III, p. 262. The Association: St. Paul, Minn. (1964).
6. HEPBURN, F. N., and BRADLEY, W. B. The amino acid compostion of hard wheat varieties as a function of nitrogen content. Cereal Chem. 42: 140 (1965).
7. McELROY, L. W., CLANDININ, D. R., LOBAY, W., and PETHYBRIDGE, S. I. Nine essential amino acids in pure varieties of wheat, barley, and oats. J. Nutr. 37: 329 (1949).
8. NIMMO, C. C., O'SULLIVAN, MARY T., MOHAMMAD, A., and PENCE, J. W. Fractionation and zone electrophoresis of proteins of water-soluble materials of flour. Cereal Chem. 40: 390 (1963).
9. PRICE, S. A. The amino acid composition of whole wheat in relation to its protein content. Cereal Chem. 27: 73 (1950).
10. SIMMONDS, D. H. Variations in the amino acid composition of Australian wheats and flours. Cereal Chem. 39: 445 (1962).
11. WOYCHIK, J. H., BOUNDY, JOYCE A., and DIMLER, R. J. Amino acid composition of proteins in wheat gluten. J. Agr. Food Chem. 9: 307 (1961).
12. McDERMOTT, E. E., and PACE, J. Comparison of the amino acid composition of the protein in flour and endosperm from different types of wheat, with particular reference to variation in lysine content. J. Sci. Food Agr. 11: 109 (1960).
13. VILLEGAS, EVA, McDONALD, C. E., and GILLES, K. A. Determination of lysine on the automated amino acid analyzer by a triplicate-sample method. Cereal Chem. 45: 432 (1968).
14. WHITE, L. M., and GAUGER, MONA A. Simultaneous automated determination of L-lysine and total amino acids in seed hydrolyzates. In: Automation in analytical chemistry, Vol. 1, p. 213, Technicon Symposium, 1967. Mediad: White Plains, N.Y. (1968).
15. KOHLER, G. O., and PALTER, RHODA. Studies on methods for amino acid analysis of wheat products. Cereal Chem. 44: 512 (1967).
16. PALTER, RHODA, and KOHLER, G. O. Survey hydrolysis procedure for lysine analysis. Cereal Chem. 46: 22 (1969).
17. AMERICAN ASSOCIATION OF CEREAL CHEMISTS. AACC Approved methods (formerly Cereal laboratory methods, 7th ed.). The Association: St. Paul, Minn. (1962).
18. SPACKMAN, D. H., STEIN, W. H., and MOORE, S. Automatic recording apparatus for use in the chromatography of amino acids. Anal. Chem. 30: 1190 (1958).

[Received February 26, 1970. Accepted July 6, 1970]

Reprinted from CEREAL SCIENCE TODAY, December 1970, Vol. 15, No. 12
Published by the American Association of Cereal Chemists, Inc.
1821 University Avenue, St. Paul, Minnesota 55104