Influence of Nitrogen Fertilization on the Physicochemical and Functional Properties of Bread Wheats

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ABSTRACT

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Soft (Salamanca) and semihard (Anáhuac) red wheat cultivars were grown with three levels of N fertilizer (0, 110, and 220 kg N/ha). Commercial urea and urea labeled with ^{15}N were used. Emission spectrometry was used to detect ^{15}N in the grain incorporated from the fertilizer. The ratio of N from fertilizer to total N in the grain ranged from 22.5 to 23.8% for the lower level of fertilization, and from 29.5 to 39.5% at the higher level. Grain N for each cultivar increased slightly at each level of application. N application caused a reduction in test weight and 1,000-kernel weight, whereas no change was observed in falling number. Sedimentation values, elasticity, and deformation energy measured by Chopin alveograph increased with N application. Farinograph water absorption and mixing stability were significantly increased with N treatments only in the Anáhuac wheat. With both the soft and semihard wheats, the mixograph peak time exhibited a general tendency to decrease with increasing protein content. Surprisingly, at the high fertilization level the proportion of essential amino acids of both cultivars went down. Functionality properties of Salamanca and Anáhuac wheat flours, as assessed with the baking test, were improved with N application.

Nitrogen is essential for plant growth and production of plant protein. A variety of elements of plant nitrogen metabolism significantly affect the productivity of wheat crops, suggesting that the quantity and quality of grain production may be influenced by manipulating these elements. The growth environment may be altered, or the plant genotype may be improved to achieve higher grain productivity (Abrol et al 1984, Cregan and van Berkum 1984). Detailed studies published on the interrelationships between nitrogen uptake, grain yield, and nitrogen concentration in the wheat kernel (Benzian and Lane 1981, Benzian et al 1983, Gales 1983) reported that loss of yield could be caused by a deficiency of nitrogen in the soil, or by conditions which prevent nitrogen from being absorbed at the rate required for maximum growth. If the factors which control the availability and absorption of nitrogen were better understood, some yield loss might be prevented and efficiency of fertilizer use improved.

Negative grain protein to grain yield correlations have been obtained in wheat studies (Benzian and Lane 1981, Benzian et al 1983, McNeal et al 1972), and yet some of these same workers have observed protein and yield increases simultaneously (Benzian and Lane 1981, Johnson et al 1969, McNeal et al 1971). Studies by Johnson and Mattern (1978), using a large number of wheat genotypes, indicated that elevated grain nitrogen content in wheat results both from increased uptake of nitrogen and from more complete translocation of this nutrient from the foliage to the developing grain. In addition, isotope-labeled fertilizers have been used in field experiments to measure the efficiency of fertilizer utilization, as affected by time of application and various interactions (Blacklow 1982, Fried et al 1975). These experiments, by directly measuring uptake of nitrogen from the fertilizer, have gone a long way in the quantitative separation of the two major sources of nutrient supply to the plant, i.e., the soil and the fertilizer. Much information on the relationship of nitrogen uptake to grain yield/grain composition has been produced in the recent past. However, there is still a lack of information about the effects of nitrogen uptake on the physicochemical and functional properties of bread wheats. Our objective in this investigation was to study the influence of nitrogen fertilization on these properties of two important wheat cultivars.

MATERIALS AND METHODS

Field Experiments

The two cultivars used were Salamanca and Anáhuac, which were selected on the basis of their importance in the local wheat market. Salamanca and Anáhuac are graded as soft and semihard red wheats, respectively; both with medium baking properties (INIA 1982). The trials were conducted at the farm of the Instituto Nacional de Investigaciones Nucleares, Irapuato, México, during 1982–1983. When commercial urea was applied, plot size consisted of four rows of 10 m with 30 cm between rows; this gave an area of 15 m^2. The size of the isotopic plot consisted of two rows of 0.8 m with the same spacing, covering an area of 0.48 m^2. The levels of nitrogen used were 0, 110, and 220 kg/ha, which were applied as granular urea. The nitrogen application was split: one half of the total amount, 55 or 110 kg/ha, was applied 5 cm deep at sowing, and the remainder was topdressed onto the soil surface 35 days after sowing. Commercial urea and urea labeled with ^{15}N (0.84%

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excess abundance) were used as fertilizers. All plots were fertilized at sowing with 40 kg/ha of P_2O_5; K was not required. Seed density was 120 kg/ha. All treatments were replicated four times in a randomized block design. The crop was grown under irrigation, and normal agronomic practices were followed.

Analytical and Rheological Tests

Whole wheat flour was obtained with a Udy cyclone mill using an 80-mesh screen. Flour of approximately 70% extraction was obtained with a Brabender Quadrumat Junior mill. A Kjeldahl method was used to determine N content. Protein content was determined by multiplying N values by 5.7. Kjeldahl digests were kept to measure the enrichment of the nitrogen content with ^{15}N by emission spectrometry. The procedure for emission spectrometry has been described in detail by Faust (1981).

The sedimentation test was performed by the modified procedure described by Preston et al (1982). Falling number was determined on 7 g of ground whole wheat flour following AACC method 56-81B (AACC 1976). The equation of Lorenz and Wolt (1981) was used to calculate falling numbers at sea level. The 35-g mixogram procedure was used according to AACC method 54-40; farinograms were produced in a Brabender farinograph (method 54-20; AACC 1976). Alveograph characteristics of the flours were determined keeping the temperature at 25°C and using the method reported by the equipment supplier (M. Chopin, Boulogne, France).

For amino acid analysis an automatic analyzer (Beckman model 121) was used, following the procedure of Spackman et al (1958). Results are based on an average of two determinations per sample. All the amino acid analyses were adjusted to an N recovery of 95%.

Baking Studies

Flours were baked in duplicate using a conventional sponge-dough procedure (Paredes-López and Covarrubias-Alvarez 1984). Baking absorption was estimated from the farinograph absorption. The resulting bread was scored as described previously (Paredes-López and Bushuk 1983).

RESULTS AND DISCUSSION

It was found that N taken up by the grain from the fertilizer was generally higher after the second application. For Salamanca and Anáhuac cultivars, the proportion of kernel N from fertilizer was higher at 220 than at 110 kg N/ha. These levels are lower than those obtained by Fried et al (1975) and within the range of those reported by FAO (1980). Interestingly, it has been shown that the split application of fertilizer incorporates 10–25% more N into the grain than the common procedure of a single application (FAO 1980, INIA 1982, López-Martínez 1980); similar experiences have been reported by other workers (Badzhov and Ikonomova 1971). In conclusion, in spite of the benefits of the split application, the experimental results showed low nitrogen proportions taken up by the soft (Salamanca) and semihard (Anáhuac) wheat cultivars. As reviewed by Abrol et al (1984), Cregan and van Berkum (1984), Desai and Bhatia (1978), and Gales (1983), it is necessary to develop genotypes in which a greater proportion of N is used directly by the developing seed, leaving lower amounts of N in the straw and other parts of the plant.

At the levels of N fertilizer tested, increases of protein content were higher for Anáhuac than for Salamanca wheat (Table I). On average, different experiments with these cultivars have increased grain yields by about 12% by applying 110 or 220 kg N/ha (not shown). McNeal et al (1972) and Bhatt et al (1981) reported that test weight was not affected by N fertilization. Puri et al (1980), however, found lower test weights for wheat genotypes with higher levels of N fertilization; according to Kosmolak and Crowle (1980), the same trend was followed by 1,000-kernel weight and test weight of five hard red spring cultivars. In this study, both wheats exhibited a decrease of test weight and 1,000-kernel weight in relation to the control sample (Table I). Flour yields of the two cultivars did not change (not shown). Sedimentation values increased for the N treatments described for both wheats (Table I); and, as expected, differences in falling number were not significantly correlated with N level within each cultivar. Data summarizing the effects of N fertilization on farinogram characteristics are given in Table I and Figure 1. While differences in farinograph mixing stability were not significant for Salamanca samples, Anáhuac showed a remarkable enhancement of this parameter; for both cultivars, water absorption followed the same trend as mixing stability, corroborating the results of Kosmolak and Crowle (1980).

Figure 2 illustrates the type of curve produced with the alveograph. Some selected experiments are reported here. The resistance of the dough to expansion results in a steep vertical rise of the curve to an optimum point P (maximum pressure). For both cultivars, changes in N fertilization produced only slight changes in tenacity, but elasticity (L) was notably enhanced. Means of the deformation energy of the dough (W) are given in Table I; the Anáhuac wheat dough showed a remarkable increase of deformation energy with increased levels of N application. Figure 3 presents only some of the mixograph evaluations carried out; however, mixogram peak height, mixing stability, and peak time were analyzed for all the experiments. The first two variables did not present a definite trend; but for both cultivars, peak time showed a general tendency to decrease with increasing protein content of the samples (Fig. 3). The constant water absorption of

TABLE I
Effect of Nitrogen Fertilization on Some Quality Parameters of Salamanca and Anáhuac Cultivars

Treatment[a]	Protein (14% mb)	Test Weight (kg/hl)	1,000-Kernel Weight (g)	Sedimentation Test (ml)	Falling Number (sec)	Farinogram Absorption (%)	Farinogram Stability (min)	Chopin Alveograph Deformation Energy, W (10^{-4} J)
Salamanca								
Control	10.5	82.4	38.8	69.5	334	54.1	4.1	120
1S-2S[b]	10.8	81.2	35.2	76.2	330	54.2	3.6	125
3S-4S[c]	11.5	80.2	33.7	73.0	333	54.5	4.4	135
F Test[d]	S	S	S	S	NS	NS	NS	S
Anáhuac								
Control	9.3	81.5	27.8	50.5	329	57.6	3.2	130
6A-7A[b]	10.4	78.6	22.9	58.8	332	58.1	9.0	145
8A-9A[c]	11.6	75.7	20.0	65.5	327	61.3	9.9	215
F Test[d]	S	S	S	S	NS	S	S	S

[a] Treatments with same N fertilization were combined. S = Salamanca, A = Anáhuac.
[b] Treatments with 110 kg N/ha.
[c] Treatments with 220 kg N/ha.
[d] S = Means significantly different at $P < 0.01$; NS = means not significantly different at $P < 0.05$.

the mixograph test may yield erroneous results for the mixograph development time. This observation is also in agreement with the findings of Kosmolak and Crowle (1980).

The amino acid analyses in Table II were the only determinations of these components carried out for this study; the rest of the samples were not analyzed. The analytical results of the flour samples revealed that N application (220 kg N/ha) produced a slight reduction in the proportion of the essential amino acids in Salamanca wheat. This reduction was greater for Anáhuac wheat; it decreased from 24.6 to 23.08 g/100 g protein. As indicated before, fertilization augmented the protein content of wheat flour but at the same time reduced the quality of the protein as judged solely by the amino acid composition. For both wheats, isoleucine, leucine, and proline decreased more than 0.1 g/100 g protein, whereas glutamic acid showed an increase with increased N fertilizer. In earlier studies to determine the effect of N fertilization on the amino acid composition of wheat (Abrol et al 1971, Dubetz and Gardiner 1979), it was found that glutamic acid, proline, and phenylalanine increased and that lysine, threonine, and valine decreased. Our results are generally in disagreement with these findings. However, it is interesting to note that the decrease of the proportion of essential amino acids with N fertilizer is in agreement with results calculated from the study of Dubetz and Gardiner (1979). Dubetz et al (1979) found that N fertilization changed the proportions of the soluble protein fractions of wheat but did not change the proportions of the amino acids in the individual fractions. According to Mitra et al (1979), of the major endosperm protein fractions, prolamins are the least costly to the plant in terms of energy expenditure. It is also more energetically advantageous for the plant to store the available N in the form of glutamic acid and its amide than in the eight essential amino acids. These observations might partially explain the amino acid results found in this study.

The effects of N fertilizer on some of the baking properties of the cultivars under study are shown in Table III. Loaf volume and crumb structure were improved in both wheats, whereas the score for crumb color remained almost the same. The N treatments produced a greater increase in loaf volume for Anáhuac than for Salamanca wheat. For both wheats, the general appearance of the control samples was graded as unsatisfactory; after the N treatments this appearance improved considerably. As expected, the loaf volumes for the two cultivars increased with increasing protein content. Interestingly, loaf-volume response appears highly

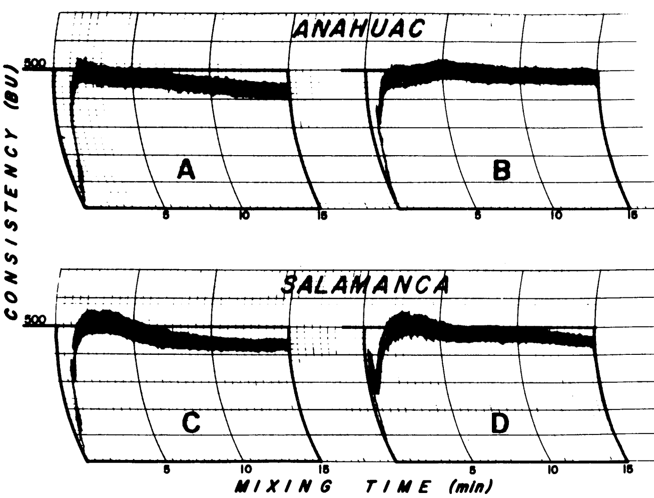

Fig. 1. Farinogram curves of the flour samples: **A**, **C** = control samples; **B**, **D** = samples fertilized with 220 kg N/ha.

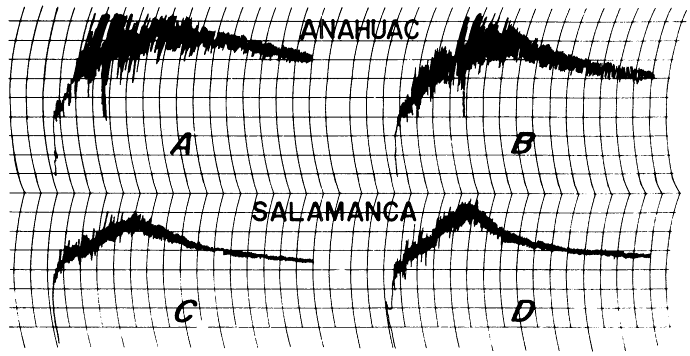

Fig. 3. Mixogram curves of the flour samples: **A**, **C** = control samples; **B**, **D** = samples fertilized with 220 kg N/ha.

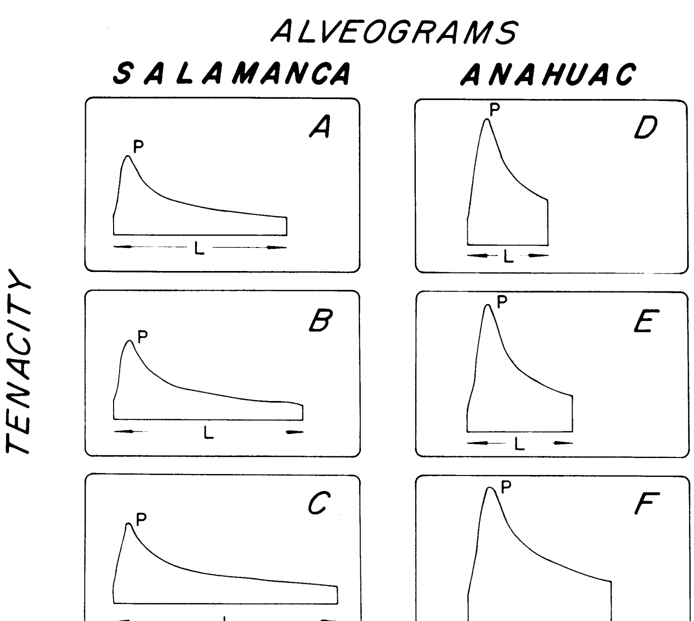

Fig. 2. Alveogram curves of the flour samples: **A**, **D** = control samples; **B**, **E** = samples fertilized with 110 kg N/ha; **C**, **F** = samples fertilized with 220 kg N/ha.

TABLE II
Effect of Nitrogen Fertilization on Amino Acid Composition of Wheat Flour from Selected Treatments

	Treatment			
	Salamanca		**Anáhuac**	
Amino Acid[a]	**Control**	**220 kg N/ha**	**Control**	**220 kg N/ha**
Essential				
Lysine	1.40	1.50	1.29	1.37
Threonine	2.71	2.71	2.72	2.77
Valine	3.88	3.97	3.40	3.57
Methionine	0.30	0.23	0.39	0.30
Isoleucine	3.28	2.89	4.40	3.15
Leucine	6.13	6.02	6.85	6.32
Phenylalanine	5.23	5.28	5.55	5.60
Subtotal	22.93	22.60	24.60	23.08
Nonessential				
Histidine	1.54	1.48	1.66	1.68
Arginine	4.01	3.68	2.85	3.58
Aspartic acid	4.14	7.57	4.49	4.48
Serine	4.56	4.49	4.77	4.52
Glutamic acid	29.90	30.77	29.96	31.04
Proline	14.78	12.02	15.18	12.67
Glycine	3.42	3.30	3.49	3.41
Alanine	2.69	2.49	2.70	2.63
Tyrosine	3.28	3.27	3.62	4.49
Ammonia	3.75	3.33	1.68	3.42
Subtotal	72.07	72.40	70.40	71.92

[a] Expressed as g/100 g protein. Cysteine and tryptophan were not determined.

TABLE III
Baking Properties of Salamanca and Anáhuac Cultivars

Treatment[a]	Loaf Volume (cc)	Crumb Structure[b]	Crumb Color[b,c]	General Appearance
Salamanca				
Control	568	7.0	9.0	Unsatisfactory
1S-2S[d]	631	9.0	9.0	Satisfactory
3S-4S[e]	643	9.5	9.0	Satisfactory
F Test	S	···	···	
Anáhuac				
Control	514	6.5	9.0	Unsatisfactory
6A-7A[d]	568	8.0	9.0	Satisfactory
8A-9A[e]	643	9.0	9.5	Satisfactory
F Test[f]	S	···	···	

[a]Treatments with same N fertilization were combined. S = Salamanca, A = Anáhuac.
[b]Values are based on a scale of 1–10, with 10 being the best score.
[c]Yellow-cream in all cases.
[d]Treatments with 110 kg/ha.
[e]Treatments with 220 kg/ha.
[f]S = Means significantly different at $P<0.01$.

correlated with protein content for both Salamanca and Anáhuac wheats.

In general, N fertilization produced more profound changes in Anáhuac than in Salamanca wheat, as shown by the test weight, 1,000-kernel weight, sedimentation test, and farinograph absorption and stability. The same was true for the deformation energy of the alveograph and amino acid composition. The mixograph characteristics were less affected by the changes in protein content. For both cultivars, breadmaking experiments also showed the effects of the N fertilization. In other words, N fertilization not only produced different changes in the quantity of the protein from both wheats, but also different effects on protein quality. It is highly likely that the functional changes exhibited by the dough reflect profound modifications in the molecular composition of the gluten. Prugar and Šašek (1970) reported that applications of N fertilizer consistently led to a large increase in gliadins, with slight changes in the rest of the protein fractions. Doekes and Wennekes (1982) found that increasing N fertilization, using five different cultivars, increased the ratio of gliadin to glutenin correspondingly. Further studies are needed at the molecular level to explain the functional changes induced by N fertilization in Salamanca and Anáhuac cultivars.

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LITERATURE CITED

ABROL, Y. P., UPRETY, D. C., AHUJA, V. P., and NAIK, M. S. 1971. Soil fertilizer levels and protein quality of wheat grains. Aust. J. Agric. Res. 22:195.

ABROL, Y. P., KUMAR, P. A., and NAIR, T. V. R. 1984. Nitrate uptake and assimilation and grain nitrogen accumulation. Page 1 in: Advances in Cereal Science and Technology. Vol. 6. Y. Pomeranz, ed. Am. Assoc. Cereal Chem.: St. Paul, MN.

AMERICAN ASSOCIATION OF CEREAL CHEMISTS. 1976. Approved Methods of the AACC. Methods 54-20, 54-40, and 56-81B; approved April 1961. The Association: St. Paul, MN.

BADZHOV, K., and IKONOMOVA, E. 1971. ^{15}N for studying nitrogen tranformation in soil, nitrogen nutrition of plants, and in assessing available nitrogen in soil. Page 21 in: N-15 in Soil-Plant Studies. IAEA: Vienna.

BENZIAN, B., and LANE, P. 1981. Interrelationship between nitrogen concentration in grain, grain yield and added fertilizer nitrogen in wheat experiments of south-east England. J. Sci. Food Agric. 32:35.

BENZIAN, B., DARBY, R. J., LANE, P., WIDDOWSON, F. V., and VERSTRAETEN, M. J. 1983. Relationship between N concentration of grain and grain yield in recent winter-wheat experiments in England and Belgium, some with large yields. J. Sci. Food Agric. 34:685.

BHATT, G. M., PAULSEN, G. M., PULP, K., and HEYNE, E. G. 1981. Preharvest sprouting in hard winter wheats: Assessment of methods to detect genotypic and nitrogen effects and interaction. Cereal Chem. 58:300.

BLACKLOW, W. M. 1982 ^{15}N moved to the grain of winter wheat when applied as nitrate to senescing flag leaves. Aust. J. Plant Physiol. 9:641.

CREGAN, P. B., and van BERKUM, P. 1984. Genetics of nitrogen metabolism and physiological/biochemical selection for increased grain crop productivity. Theor. Appl. Genet. 67:97.

DESAI, R. M., and BHATIA, C. R. 1978. Nitrogen uptake and nitrogen harvest index in durum wheat cultivars varying in their grain protein concentration. Euphytica 27:561.

DOEKES, G. I., and WENNEKES, L. M. J. 1982. Effect of nitrogen fertilization on quantity and composition of wheat flour protein. Cereal Chem. 59:276.

DUBETZ, S., and GARDINER, E. E. 1979. Effect of nitrogen fertilizer treatments on the amino acid composition of Neepawa wheat. Cereal Chem. 56:166.

DUBETZ, S., GARDINER, E. E., FLYNN, D., and De La ROCHE, I. A. 1979. Changes in soluble protein fractions and amino acid composition of spring wheat as influenced by nitrogen fertilizer. Can. J. Plant Sci. 59:506.

FAO. 1980. Maximizing the Efficiency of Fertilizer Use by Grain Crops. FAO Fertilizer Bulletin 3. FAO: Rome.

FAUST, H. 1981. FAO/IAEA Interregional Training Course on the Use of N-15 in Soil Science and Plant Nutrition. Training Manual. Zfl-Mitteilungen, nr. 38. Zentral Institut für Isotopen und Strahlenforshung von Akademie der Wissenschaften der DDR: Leipzig.

FRIED, M., SOPER, R. J., and BROESCHART, H. 1975. ^{15}N-labeled single-treatment fertility experiments. Agron. J. 67:393.

GALES, K. 1983. Yield variation of wheat and barley in relation to crop growth and soil conditions—A review. J. Sci. Food Agric. 34:1085.

INIA. 1982. Guía para Cultivar Trigo en El Bajío. INIA-Centro de Investigaciones Agrícolas de El Bajío: Celaya, Gto., México.

JOHNSON, V. A., and MATTERN, P. J. 1978. Improvement of wheat protein quality and quantity by breeding. Exp. Med. Biol. 105:301.

JOHNSON, V. A., MATTERN, P. J., WHITED, D. A., and SCHMIDT, J. W. 1969. Breeding for high protein content and quality in wheat. Page 29 in: New Approaches to Breeding for Improved Plant Protein. IAEA: Vienna.

KOSMOLAK, F. G., and CROWLE, W. L. 1980. An effect of nitrogen fertilization on the agronomic traits and dough mixing strength of five Canadian hard red spring wheat cultivars. Can. J. Plant Sci. 60:1071.

LÓPEZ-MARTÍNEZ DE A., L. 1980. Eficiencia en el Uso de Fertilizantes Nitrogenados y Fosfóricos. UIADB-ININ: Irapuato, Gto., México.

LORENZ, K., and WOLT, M. 1981. Effect of altitude on falling number values of flours. Cereal Chem. 58:80.

McNEAL, F. H., BERG, M. A., BROWN, P. L., and McGUIRE, C. F. 1971. Productivity and quality response of five spring wheat genotypes, *T. aestivum* L., to nitrogen fertilizer. Agron. J. 63:908.

McNEAL, F. H., BERG, M. A., McGUIRE, C. F., STEWART, V. R., and BALDRIDGE, D. E. 1972. Grain and plant nitrogen relationships in eight spring wheat crosses, *T. aestivum* L. Crop. Sci. 12:599.

MITRA, R. K., BHATIA, C. R., and RABSON, R. 1979. Bioenergetic cost of altering the amino acid composition of cereal grains. Cereal Chem. 56:249.

PAREDES-LÓPEZ, O., and BUSHUK, W. 1983. Development and "undevelopment" of wheat dough by mixing: physicochemical studies. Cereal Chem. 60:19.

PAREDES-LÓPEZ, O., and COVARRUBIAS-ALVAREZ, M. M. 1984. Influence of gamma radiation on the rheological and functional properties of bread wheats. J. Food Technol. 19:225.

PRESTON, K. R., MARCH, P. R., and TIPPLES, K. H. 1982. An assessment of the SDS-sedimentation test for the prediction of Canadian bread wheat quality. Can. J. Plant Sci. 62:545.

PRUGAR, J., and ŠAŠEK, A. 1970. Einfluss der organischen und Mineraldüngung auf die Vertretung der Eiweissfraktionen im Weizenkorn. Getreide Mehl Brot 20(4):27.

PURI, Y. P., QUALSET, C. O., JAN, C. C., and BAGHOTT, K. G. 1980. Durum and bread-wheat response to nitrogen for yield physical and chemical characteristics. Φyton 39:127.

SPACKMAN, D. H., STEIN, W. H., and MOORE, S. 1958. Automatic recording apparatus for use in the chromatography of amino acids. Anal. Chem. 30:1190.

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