to, e.g., six or eight, and a quadruple determination should therefore generally be applied.

The total standard deviation on the three types of hydrolysate has been calculated as functions of h, and selected values are given in Table III. It appears that the differences in magnitude of $s(h)$ (standard deviation on h) are relatively small. The standard deviations shown correspond to a standard deviation on DH of 0.2–0.3% absolute, which should be satisfactory for all purposes (in this calculation the *systematic* error arising from the uncertainty on h_{tot} is neglected).

CONCLUSION

The present work has shown that an accurate, reproducible, and generally applicable determination of DH (the degree of hydrolysis) can be based on a modification of the TNBS reaction, as described in detail in the Experimental Section.

It is our experience from the use of the TNBS assay that now and then suddenly a large spreading of the results occur. This spreading can generally be traced back to inhomogeneities in the $NaDodSO_4$ sample solution. Certain protein and protein hydrolysate products are difficult to disperse in $NaDodSO_4$ without having been reduced by a disulfide reducing agent. Unfortunately, mercaptoethanol and other reducing agents containing sulfhydryl groups ruin the assay, as stated previously. Often it is possible, however, to disperse the protein without the use of mercaptoethanol by *homogenizing* the material in hot $NaDodSO_4$ and this procedure is therefore recommended.

Another potential source of error is deterioration of the leucine standard. Fresh standards should therefore be prepared regularly, and for accurate work it is recommended to use an additional standard as control, e.g., glycylglycine.

Except for the TNBS, the reagents used are relatively harmless. No particular safety precautions need to be taken except that gloves should be worn when making the TNBS solution.

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LITERATURE CITED

Adler-Nissen, J., *J. Agric. Food Chem.* **24**, 1090 (1976).
Adler-Nissen, J., *Process Biochem.* **12**(6), 18 (1977).
Adler-Nissen, J., Sejr Olsen, H., *ACS Symp. Ser.* **No. 92**, 125 (1979).
Burger, W. C., *Anal. Biochem.* **57**, 306 (1974).
Castellan, G. W., "Physical Chemistry", Addison-Wesley, Reading, MA, 1964, pp 215–217.
Eastoe, J. E., Leach, A. A., in "The Science and Technology of Gelatin", Ward, A. G., Courts, A., Ed., Academic Press, New York, 1977, p 93.
Fields, R., *Biochem. J.* **124**, 581 (1971).
Freedman, R. B., Radda, G. K., *Biochem. J.* **108**, 383 (1968).
Goldfarb, A. R., *Biochemistry* **5**, 2570 (1966).
Habeeb, A. F. S. A., *Anal. Biochem.* **14**, 328 (1966).
Hald, A., "Statistical Theory with Engineering Applications", Wiley, New York, 1951, pp 579–583.
Hennrich, N., *Kontakte (Merck)* **1973**(2), 3 (1973).
Jones, D. B., *U.S. Dep. Agric. Circ.*, 183 (1931).
Kotaki, A., Harada, M., Yagi, K., *J. Biochem.* **55**, 553 (1964).
Novo Industri, "Crystalline Porcine Trypsin Novo", IB 051, Bagsvaerd, Denmark, 1971.
Novo, Industri, "Subtilisin A", IB 169, Bagsvaerd, Denmark, 1978a.
Novo Industri, "Hydrolysis of Food Proteins in the Laboratory", IB 102, Bagsvaerd, Denmark, 1978b.
Provansal, M. M. P., Cuq, J.-L. A., Cheftel, J.-C., *J. Agric. Food Chem.* **23**, 938 (1975).
Satake, K., Okuyama, T., Ohashi, M., Shinoda, T., *J. Biochem.* **47**, 654 (1960).
Smith, A. K., Circle, S. J., "Soybeans: Chemistry and Technology", Vol. 1, Avi Publishing Co., Westport, CT, 1972, pp 62–63.
Steinhardt, J., Beychock, S., "The Proteins", Vol. II, 2nd ed, Neurath, H., Ed., Academic Press, New York, 1964, pp 160–171.

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Determination of the Total Pepsin–Pancreatin Indigestible Content (Dietary Fiber) of Soybean Products, Wheat Bran, and Corn Bran

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Successive pepsin and pancreatin digestions were used to determine indigestible content (IDC) of various soybean products and cereal brans. IDC included insoluble material as well as solubilized carbohydrate and protein separated by ultrafiltration (molecular weight above 5000). Total IDC as percent of dry matter was: corn bran, 97; soybean hulls, 86; wheat bran, 52; whole soybean, 23; soy protein concentrate, 40; and defatted soy flakes, 16. The IDC values include 3–25% soluble material recovered by ultrafiltration. Chemical analyses of the insoluble nondigestible fraction from soybean hulls indicated a composition of 71% cellulose, 20% hemicellulose, 9% lignin plus ash. The percent protein digestibility was estimated as: whole soybean, 68; defatted soy flakes, 81; soybean hulls, 60; soy protein concentrate, 61; corn bran, 43; and wheat bran, 60. The large values for undigested protein in soy protein products were unexpected.

Recent reports on the nutritional significance of indigestible components of human food have stimulated new interest in the characterization of these components and their functionality, particularly the plant polysaccharides. Trowell (1977) discusses the significance of fiber in the human diet and defines dietary fiber as "the remnants of plant cells resistant to the alimentary enzymes of man". Other discussions on the definition and application of the term "dietary fiber" include those of Van Soest and Robertson (1977), Southgate (1977), and Meyer and Calloway (1977).

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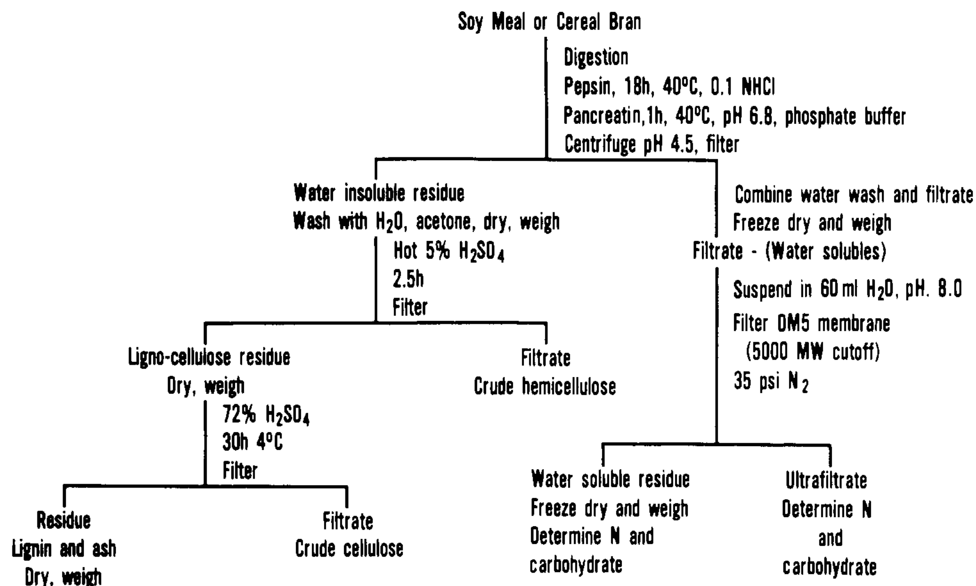

Figure 1. Fractionation and analysis scheme for dietary fiber determination in soy products or cereal brans.

The biochemical methods of Southgate (1969) and Van Soest (1966) are generally accepted for determining dietary fiber and characterizing its composition. Hellendoorn et al. (1975) report on an in vitro, mammalian enzyme, digestion procedure which they feel more nearly reflects the physiological behavior in the digestive tract of man than do the biochemical methods, especially the crude fiber methods. This approach, however, does not measure soluble polysaccharides and oligosaccharides, which together with insoluble polysaccharides are available for fermentation by intestinal microorganisms (Van Soest and Robertson, 1977).

Kawamura (1967), Aspinall et al. (1967), Delente and Ladenburg (1972), and Eldridge et al. (1979) have reported on the content of various carbohydrates present in soybeans and soybean products. These studies, however, did not measure dietary fiber content.

In our present studies we also utilized mammalian enzyme digestion to isolate insoluble nondigestible residue from whole soybeans and various soybean products as well as from wheat and corn brans which have been analyzed by other methods. These fractions were then analyzed for hemicellulose, cellulose, and lignin plus ash, as well as protein and total carbohydrate. Ultrafiltration of the soluble digestates was used to isolate material above 5000 mol wt, which was then analyzed for carbohydrate and protein content. This soluble indigestible residue was further analyzed by gas and thin-layer chromatography (TLC) to determine the composition of the carbohydrates present.

MATERIALS AND METHODS

Preparation of Soy Products. Certified seed-grade Amsoy soybeans were frozen in liquid nitrogen and ground in a Wiley mill through a 20-mesh screen. Other soy products were ground through a 40-mesh screen. Similar Amsoy beans were used to prepare dehulled defatted soybean meal according to previously described procedures (Sessa et al., 1969). The recovered soybean hulls were passed through an air classifier to remove fines and meal particles. Soy protein concentrates were prepared by extraction of dehulled defatted flakes at pH 4.4, procedure B, as described by Rackis et al. (1971), then steamed in a preheated autoclave for 10 min at 100 °C. Milled soybean hulls (A. E. Staley Co., Decatur, IL) were used as is. A sieve analysis showed 85% of the particles had a distribution of 25–40 mesh, and the remaining 15% were held on a 60 mesh U.S. standard sieve.

Cereal Products. A soft white winter (SWW) wheat bran, designated as certified food grade wheat bran, was purchased from the American Association of Cereal Chemists. A hard red spring (HRS) wheat bran, Waldron variety, was supplied by the Spring and Durum Wheat Quality Laboratory, Agricultural Research, Science and Education Administration, U.S. Department of Agriculture, Fargo, ND, and a dry-milled corn bran was obtained from Lauhoff Grain Company, Danville, IL. The wheat and corn brans were sieved to pass a no. 18 U.S. standard sieve and remain on a no. 30 U.S. standard sieve.

Preparation and Isolation of Nondigestible Residue. Insoluble and soluble nondigestible residues were prepared by a modification of the Hellendoorn et al. (1975) procedure as outlined in Figure 1. Triplicate samples of about 1 g were weighed out and suspended in 100 mL of 0.1 N HCl, stirred with 100–120 mg of porcine pepsin (2755 units/mg, Sigma Chemical Co., St. Louis, MO), plus thymol and isoamyl alcohol, then covered and heated in a 40 °C water bath for 18 h. The digests were next brought to pH 6.8 with 4 N NaOH, and 50 mL of pH 6.8 phosphate buffer plus 300 mg of sodium dodecyl sulfate and 100–120 mg of porcine pancreatin (4 X NF grade) were added, then stirred in a 40 °C water bath for 1 h. After bringing to pH 4.2–4.5 with 4 N HCl, the samples were centrifuged at 5000 rpm and filtered through tared 50-mL Gooch filters medium frit. The residues were washed with water and acetone, then dried overnight at 105 °C before reweighing. The combined filtrate and water washes were freeze-dried.

A portion of the filtrate fraction was resuspended in 60 mL of water, brought to pH 8.0 with 4 N NaOH with a few crystals of thymol added as preservatives, then filtered in an Amicon Model 212 ultrafiltration apparatus through a DM5 membrane (5000 mol wt cutoff) at a pressure of 35 psi nitrogen. The UM series of amicon membranes has a lower cutoff but cannot be used in the presence of sodium dodecyl sulfate in the digestate. After concentration of the sample, diafiltration was continued overnight until 500–1000 mL of water had been flushed through. The soluble residue remaining in the filter cell was recovered and freeze-dried, then analyzed for nitrogen and carbohydrate. The ultrafiltrate was also freeze-dried and analyzed for nitrogen and carbohydrate to determine the digestible carbohydrate and protein. A reagent blank containing similar amounts of enzyme and other reagents was sub-

Table I. Indigestible Residue Content of Soybean and Cereal Products[a]

sample	insoluble residue,[b] %	soluble residue[b] carbohydrate, %	soluble residue[b] protein, %	total indigestible residue, %	dietary fiber, %
whole soybean flour	15.7 ± 1.1	3.3	5.8	24.8	5.1[c]
dehulled, defatted flour	10.3 ± 0.6	3.6	2.4	16.3	11.9[c]
laboratory-prepared soy hulls	72.8 ± 1.5				
commercial soy hulls	78.8 ± 0.2	3.7	3.6	86.1	
soy protein concentrate	15.4 ± 0.85	7.2	17.4	40.0	
HRS wheat bran	45.5 ± 0.7	2.9	3.1	51.6	56,[c] 48[d]
SWW wheat bran	46.8 ± 1.4				36[e]
corn bran	93.7 ± 1.3	3.1		96.8	89[e]

[a] Percent of dry starting material. Insoluble residue, triplicate determinations; soluble residue, single determination. [b] See Figure 1. [c] Hellendoorn et al. (1975). [d] Southgate (1977). [e] Schaller (1977).

Table II. Percent Carbohydrate and Protein Digestibility in Various Cereal Brans and Soybean Products

sample	total carbohydrate	total protein[a]	carbohydrate[b] digestibility	protein[b] digestibility
whole soybeans	24	38.0	40.7	68.2
dehulled, defatted soy flour	30	50.4	58.4	81
soybean hulls	80	10.1	2.0	59.6
soy protein concentrate	18.5	70.5	18.4	61.0
red spring wheat bran	57	16.2	18.3	60.2
corn bran	77.9	3.5	3.7	43.4

[a] Nitrogen (dry basis) × 6.25. [b] Percent sample carbohydrate or nitrogen in ultrafiltrate fraction divided by total carbohydrate and nitrogen in the sample.

jected to the same digestion and ultrafiltration treatment in order to correct for the proportion of enzyme protein in each of the digested and undigested fractions.

The hemicellulose, cellulose, and lignin plus ash composition of the insoluble, nondigestible residue was determined by extraction with hot 5% H_2SO_4 and with 72% H_2SO_4 at 4 °C according to the procedure of Southgate (1969) as modified by Elchazly and Thomas (1976). Samples were filtered with 50-mL Gooch filters medium-to-coarse frit.

Analytical. Total carbohydrates were determined as glucose by the Dubois et al. (1951) procedure after hydrolysis of the nondigestible fractions according to the procedure of Sloneker (1971) as modified by Eldridge et al. (1979). The procedures of Sloneker (1971) were also used to determine the sugar components by GC analysis of their alditol acetates.

Thin-layer chromatography (TLC) of the soluble carbohydrates was accomplished by elution with 1-butanol/acetic acid/diethyl ether/water (9:6:3:1) on Brinkmann, F254, silica gel G plates along with standards for the major sugar components.

RESULTS AND DISCUSSION

Indigestible Residue Content of Soy and Cereal Products. Based on the modified enzymatic digestion procedure illustrated in Figure 1, both soluble and insoluble indigestible fractions were isolated to determine the total indigestible residue content of soybean and cereal products. Values are given in Table I. Insoluble residue values for laboratory-prepared soy hulls approach those for the commercial preparation, and the insoluble residue value for the SWW wheat bran is similar to that of the HRS bran. The soluble residue values for the laboratory hulls and the SWW wheat bran were not determined at this time. Except for the protein concentrate, most of the indigestible material is in the insoluble residue. The soluble indigestible protein value for soy protein concentrate was 17.4% out of a total of 40% indigestible residue. The large amount of indigestible protein was unexpected and needs to be investigated further. Total indigestible residues from whole and defatted soy flour were 23 and 16.3%, respectively. These values are much greater than the 5.1% indigestible residue for soybeans and 11.9% for defatted soy flour reported by Hellendoorn et al. (1975). The Hellendoorn values do not include soluble indigestible protein and carbohydrate. The soluble carbohydrates and proteins above 5000 mol wt increase total indigestibles by over 60% in the soy flours and protein concentrate.

Among the bran samples the total of 86.1% indigestible residue in soy hulls approaches the 96.8% total for corn bran, but includes 3.6% soluble indigestible protein. The corn bran did not have enough soluble indigestible protein for a good estimate. The total indigestible residue of 51.6% in HRS wheat bran, which includes 3.1% soluble indigestible protein, is comparable to a reported value of 48% unavailable carbohydrate (Southgate, 1977). Schaller (1977) reports values of 89% dietary fiber in corn bran and 36% for a standard SWW wheat bran as determined by a modified Van Soest procedure that includes α-amylase digestion.

Protein and Carbohydrate Digestibility. Each of the samples and their digestible and indigestible fractions were analyzed for total carbohydrate as glucose and for Kjeldahl nitrogen. Protein and carbohydrate digestibility were calculated as the net nitrogen and carbohydrate in the ultrafiltrate divided by nitrogen and carbohydrate in the sample. Digestibilities are summarized in Table II. Digestibilities of 58.4% carbohydrate and 81% protein are shown for defatted soy flour. These values decline to digestibilities of 40.7% for carbohydrate and 68.2% for protein in whole soybeans. Digestibilities in toasted protein concentrate were only 18.4% carbohydrate and 61.0% protein. Preparation of soy protein concentrates removes nearly all oligosaccharides (Rackis, 1976) and some nitrogen. To what extent extraction or the toasting process decreases digestible protein requires further investigation. Protein digestibility in the soy hulls is 59.6%, close to that for protein concentrate, but the 2% carbohydrate digestibility is less than that for corn bran. Protein digestibility in the HRS wheat bran also is about 60%. Saunders et al. (1972) report protein digestibility of 72% for an enzy-

Table III. Carbohydrate Content and Carbohydrate Caloric Value of Dehulled, Defatted Soybean Meal

constituent	meal, %
polysaccharide content, total[a]	15-18
acidic polysaccharides	8-10
arabinogalactan	5
cellulosic material	1-2
starch[b]	0.5
oligosaccharide content, total[c]	15
sucrose	6-8
stachyose	4-5
raffinose	1-2
verbascose	Tr
caloric value[d]	1.68 cal/g

[a] Aspinall et al. (1967). [b] Boonvisut and Whitaker (1976). [c] Kawamura (1967). [d] Liener (1972).

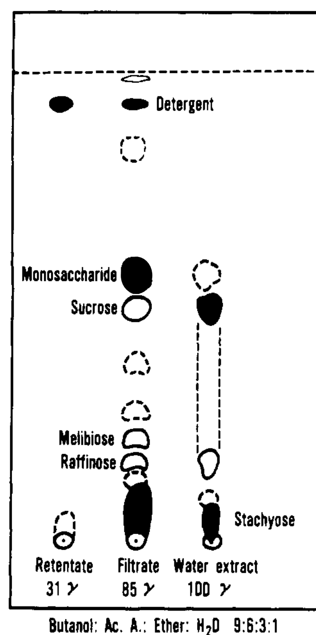

Figure 2. Thin-layer chromatogram of retentate, ultrafiltrate, and water extract carbohydrates from whole soybeans.

matic in vitro digestion and 73% in vivo protein digestibility in rats, with 37% total digestibility for HRS wheat bran. Hellendoorn et al. (1975) report 98.5% protein digestibility in soybeans and 93% in wheat bran; however, they included only the insoluble residue with the indigestible material.

As shown in Table III, the carbohydrate content of dehulled, defatted soy flour is close to 30%, almost equally divided between polysaccharides and oligosaccharides. The actual percentage of total carbohydrate that is available for energy ranges from 14% in chicks to 40% in rats (Liener, 1972). The degree of digestibility in the human gastrointestinal tract remains to be determined. In the absence of data in humans, the available energy value for rats is taken by the Foreign Agricultural Organization of the United Nations to be the digestibility value for carbohydrates in soybeans. On this basis, the caloric value becomes 1.68 cal/g of carbohydrate rather than a value of about 4 for starch and other highly digestible carbohydrates.

The thin-layer chromatogram (Figure 2) compares soluble carbohydrates from the retentate and ultrafiltrate fractions of a whole soybean digestate with the carbohydrate composition from a water-soluble extract of soybeans. In the retentate sample no low-molecular-weight carbohydrate spots are visible. For the ultrafiltrate fraction, the spots with the R_f of stachyose, raffinose, melibiose, or monosaccharides increase or remain unchanged, while the sucrose spot decreases compared to carbohydrates in the soybean water extract. This indicates that some polysaccharide and much sucrose but only small amounts of raffinose and stachyose have been hydrolyzed to simpler sugars. Ultrafiltration has effectively separated out lower molecular weight carbohydrates and so resulted in a more complete estimate of the amount and kind of material available for fermentation and other physiological activity in the gastrointestinal tract.

Table IV. Composition of Insoluble-Indigestible Residue of Soybean Products[a]

component	hulls, %	whole soybeans, %	protein concentrate, %
crude hemicellulose	19.8	41.8	62.0
crude cellulose	70.9	52.8	37.3
lignin and ash	9.4	5.4	0.6
protein[b]	2.8	3.4	3.5

[a] Percent of dry matter, single determinations. [b] Included in values for other fractions—so adds up to more than 100%.

Table V. Component Sugars of Soluble Indigestible Residues (Mole Percent)

sugar	soy protein concentrate	soy hulls	corn bran	wheat bran	whole soybean
rhamnose	3.1	2.6	1.3	0.7	5.5
fucose	2.0	1.2	1.3		0.9
ribose	4.4	9.3	9.2	10.8	5.5
arabinose	24.5	18.7	14.0	19.4	26.5
xylose	5.1	3.5	6.2	26.6	8.6
mannose	4.7	3.3	4.4	1.4	5.7
galactose	49.6	55.5	13.6	9.6	40.1
glucose	6.6	6.0	50.1	31.5	7.2

Insoluble Indigestible Residue. The composition of the insoluble indigestible residue, in terms of crude hemicellulose, cellulose, and lignin (see Figure 1) as defined by Elchazly and Thomas (1976), is shown in Table IV for various soy products.

Crude cellulose is the major component of the insoluble, indigestible residue fraction from soybean hulls, whereas crude hemicellulose is the major fraction in the protein concentrate residue. Whole soybeans, reflecting the composition of both hulls and protein concentrate, have more equal proportions of cellulose and hemicellulose. Each of the residue samples had around 3% protein. Less than 1% lignin plus ash was found in the protein concentrate residue. Toasting of the protein concentrate did not result in more unavailable protein in the insoluble residue compared to insoluble residue in whole beans.

Sugar Composition of Soluble Indigestible Residues. The water-soluble indigestible carbohydrates from the ultrafiltration retentate were analyzed for their sugar components by gas chromatography of the alditol acetates after hydrolysis. As shown in Table V, the major sugars identified in the soy products were arabinose and galactose, suggesting that much of the soluble polysaccharide residue from the soy products may be the arabino galactan characterized by Aspinall (1967). The major sugar in the cereal brans appear to be glucose, possibly from residual starch residues. The wheat bran residue also has major amounts of xylose and arabinose. The ribose content may suggest the presence of RNA. In the water-soluble residues of

soybean hulls and cereal brans, 9–11 mol % of the sugar was ribose.

LITERATURE CITED

Aspinall, G. O., Begbie, R., McKay, J. E., *Cereal Sci. Today* **12**, 223 (1967).
Boonvisut, S., Whitaker, J. R., *J. Agric. Food Chem.* **24**, 1130 (1976).
Delente, J., Ladenburg, K., *J. Food Sci.* **37**, 372 (1972).
Dubois, M., Gilles, K. A., Hamilton, J. K., Rebers, P. A., Smith, F., *Anal. Chem.* **23**, 25 (1951).
Elchazly, M., Thomas, B., *Z. Lebensm. Unters. Forsch.* **162**, 329 (1976).
Eldridge, A. C., Black, L. T., Wolf, W. J., *J. Agric. Food Chem.* **27**, 799 (1979).
Hellendoorn, E. W., Noordhoff, M. G., Slagman, J., *J. Sci. Food Agric.* **26**, 1461 (1975).
Kawamura, S., *Tech. Bull. Fac. Agric. Kagawa Univ.* **15**, 117 (1967).
Liener, I. E., in "Soybeans: Chemistry and Technology", Smith, A. K., Circle, S. J., Ed., Avi Publishing Co., Inc., Westport, CT, 1972, pp 203–277.
Meyer, S., Calloway, D. H., *Cereal Chem.* **54**, 110 (1977).
Rackis, J. J., in "World Soybean Research", Hill, L. D., Ed., Interstate Printers and Publishers, Inc., Danville, IL, 1976, pp 892–903.
Rackis, J. J., Honig, D. H., Sessa, D. J., Cavins, J. F., *J. Food Sci.* **36**, 10 (1971).
Saunders, R. M., Connor, M. A., Edwards, R. H., Kohler, G. O., *Cereal Chem.* **49**, 436 (1972).
Schaller, D., *Food Prod. Devel.* **11**, 70 (1977).
Sessa, D. J., Honig, D. H., Rackis, J. J., *Cereal Chem.* **46**, 676 (1969).
Sloneker, J. H., *Anal. Biochem.* **43**, 539 (1971).
Southgate, D. A. T., *J. Sci. Food Agric.* **20**, 331 (1969).
Southgate, D. A. T., *Nutr. Rev.* **35**, 31 (1977).
Trowell, H., *Nutr. Rev.* **35**, 6 (1977).
Van Soest, P. J., *J. Assoc. Off. Anal. Chem.* **49**, 546 (1966).
Van Soest, P. J., Robertson, J. B., *Nutr. Rev.* **35**, 12 (1977).

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An Evaluation of Three Methods for the Selection of High Lysine Genotypes of Maize

Anthony P. Rhodes,* Magni Bjarnason, and W. Gerhard Pollmer

The prolamin turbidity test, the ninhydrin color test, and a method based on butanol extraction for the measurement of zein have been evaluated for the selection of high lysine maize genotypes derived from the mutant *opaque-2*. For preliminary screening of large populations the ninhydrin color test and the prolamin turbidity test were sufficiently sensitive to differentiate between high lysine and normal genotypes. In addition these were rapid and simple techniques whereby one person could screen about 100 samples/day. However, for further selection within a high lysine population using the method based on butanol extraction a good correlation was obtained between the protein content after butanol extraction and lysine expressed as a proportion of grain dry weight. The output of this method is 60 samples per person per day.

A simple and rapid technique for the selection of "modified endosperm" high lysine genotypes is required in maize breeding programs using the *opaque-2* mutant. Quite frequently breeders are now able to grow two generations per year by using sites in both the southern and northern hemispheres. Consequently the period available for lysine selection prior to resowing may be only a few weeks. In addition, such preliminary screening is often carried out in field stations where laboratory facilities are limited, so that screening methods should not be dependent upon complex autoanalysis equipment.

A number of such techniques are currently in use in maize breeding programs. These include: measurement of basic amino acids by dye binding capacity (Fornasari et al., 1975), measurement of tryptophan by glyoxylic acid reagent method and subsequent correlation with lysine content (Hernandez and Bates, 1969), and measurement of lysine content by reaction with 2-chloro-3,5-dinitropyridine (Villegas and Mertz, 1971).

Two of the methods investigated in this study are based on the negative correlation between the content of zein and lysine in maize derived from the *opaque-2* mutant (Salamini and Baldi, 1969). Frömberg et al. (1971) found that after butanol extraction of zein from 48 selfed lines of "modified endosperm" *opaque-2* maize the ratio of butanol-insoluble nitrogen to total kernel nitrogen was closely correlated ($r = 0.817$) with lysine content of the protein (g of lysine/100 g of protein) and this was designated the "nitrogen–butanol–nitrogen" (NBN) method. In addition, the content of insoluble nitrogen was closely correlated ($r = 0.847$) with the lysine content on a whole kernel basis (g of lysine/100 g of dry matter) and this was designated the BN method. The second method studied here, based on estimation of zein, involves extraction with 70% (v/v) ethanol, followed by addition of a salt solution to the ethanolic extract and measurement of the resulting turbidity. This technique has been developed for maize (Paulis et al., 1974a,b) and barley (Rhodes, 1975) and in this paper is referred to as the prolamin turbidity test.

The third method studied here depends upon the three–four-fold increase in free amino acids found in the grains of *opaque-2* maize (Mertz et al., 1974). The presence

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