Preparation of Protein Concentrate from Pearl Millet

Procedures are presented for separating about 80% of the protein in pearl millet grain as a concentrate. These procedures involve extraction of lipid-free whole millet flour with 70% aqueous isopropyl alcohol, followed by extraction with aqueous sodium hydroxide (pH 11), or the reverse of this sequence; and finally, extraction with dilute mineral acid (pH 2). The extracts are dialyzed and lyophilized to yield three protein-rich fractions. About 70% of the pearl millet protein is concentrated in two fractions that, as a composite, assay 78% protein. The protein has essentially the same amino acid composition as the protein of the original lipid-free flour. This composite should be useful as a protein ingredient in foods.

Pearl millet is a staple cereal food grain in many parts of the world. In addition to having quality protein, compared to most other cereals, pearl millet has excellent fat, starch, vitamin, and mineral compositions. It is one of the most nutritive cereals consumed (U.S. Agency for International Development, 1971). Millet, as one of the coarse grains, has a high fiber content (Desikachar, 1975). It also has a high oil content (4–6%) when compared to most cereals, resulting in rapid rancidification of the whole milled flour. Consequently, the keeping quality of the milled product is limited (Casey and Lorenz, 1977).

Protein concentrates have been prepared from other cereal grains such as wheat (Wu and Sexson, 1975), sorghum (Wu, 1978), and rice (Connor et al., 1976). Pearl millet cultivars having a protein content of 20% or more are not uncommon, with lysine being the single limiting amino acid (U.S. Agency for International Development, 1971; Burton et al., 1972). Protein concentrates are finding increased usage as ingredients in foods. We could find no published procedure for obtaining the nutritious protein of the millets in the form of a concentrate. This paper describes such a procedure.

MATERIALS AND METHODS

Millet. Clean pearl millet grain [*Pennisetum Americanum* (L.) Leake] was used as received from the Georgia Coastal Plain Experiment Station, Tifton, GA. The grain was homogenized in petroleum ether with a tissue homogenizer, and the lipids were removed by decanting the supernatant. The flour was air-dried and ground with a mortar and pestle to pass through a 60-mesh screen. The protein contents of the lipid-free whole flour and of the fractions of the concentrate were estimated by the macro-Kjeldahl procedure, with 6.25 as the conversion factor. Amino acid analyses were made by ABC Laboratories, Inc., Columbia, MO, by gas chromatography by the procedure described by Kaiser et al. (1974). The chemical scores for protein quality were calculated from amino acid composition and the amino acid scoring pattern suggested by the FAO/WHO expert committee (1973).

Protein Solubility. A protein solubility curve was constructed for whole millet flour over a wide range of pH values. One-gram portions of the flour, contained in separate test tubes, were treated with 50-mL volumes of deionized water containing sufficient sodium hydroxide or mineral acid to give the final pH desired. The suspension was magnetically stirred for 15 min and the supernatant recovered by centrifugation. The pH was determined by means of a glass electrode and the total nitrogen determined on duplicate aliquots.

Protein Concentrate. The schematic diagram for preparing protein concentrate from pearl millet is shown in Figure 1. Approximately 20 g of the whole millet flour was extracted overnight with 200 mL of 70% aqueous isopropyl alcohol. The flour was recovered by centrifugation and washed two times with 200-mL volumes of 70% aqueous isopropyl alcohol. The combined extracts were dialyzed overnight against deionized water at ambient temperature, and lyophilized to give a white powder. The recovered flour was extracted three times for 30 min each with 200-mL volumes of water adjusted to pH 11 with 0.5 M sodium hydroxide. At the end of each extraction the supernatant was recovered by centrifugation and adjusted to pH 4 with concentrated HCl, and the precipitated proteins were recovered by centrifugation. The combined fractions were suspended in a neutral solution (pH 7) and freeze-dried to give an off-white powder. The supernatant was adjusted to pH 2 with concentrated HCl and the flour once more extracted three additional times with equal volumes of the pH 2 supernatant. The combined extracts were neutralized (pH 7) with dilute NaOH, dialyzed overnight against deionized water at ambient temperature, and freeze-dried to give a tan grainy material.

Table I. Yields in Fractions of Pearl Millet Protein Concentrates

fraction	material	fraction wt, g	protein content, %	% of original protein
A	70% aq *i*-PrOH extract	2.40	81.4	48.5
B	aq NaOH extract, pH 11 to pH 4	1.37	70.6	24.2
C	mineral acid extract, pH 4 to pH 2	1.83	19.8	9.1
D	residual meal	10.5	4.0	10.5
B′	aq NaOH extract, pH 11 to pH 4	1.70	67.3	28.5
A′	70% aq *i*-PrOH extract	1.98	86.2	42.8
C′	mineral acid extract, pH 4 to pH 2	1.81	20.0	9.0
D′	residual meal	9.0	4.9	11.0

RESULTS AND DISCUSSION

The solubility of the protein in pearl millet as a function of pH is shown in Figure 2. The solubility curve is typical of that of most seed proteins (Fontaine et al., 1946). Minimum solubility for millet proteins occurs between pH 4 and 4.5. Protein solubility increases sharply below 4 down to pH 1. The greatest increase in protein solubility at the higher pH values is between 4.5 and 8.5, increasing less rapidly thereafter and reaching a value of 98% at pH 11.

Aqueous isopropyl alcohol (70%), sodium hydroxide, and hydrochloric acid solution at various pH values were used to obtain three protein concentrate fractions. A solvent-to-solute ratio of 10 to 1 was used for each extraction. The extraction was carried out by two procedures. Figure 1 illustrates the approach in which the first extraction solvent was aqueous isopropyl alcohol. In the second approach, aqueous extraction at pH 11 is used as the initial step. The results of a typical run by each

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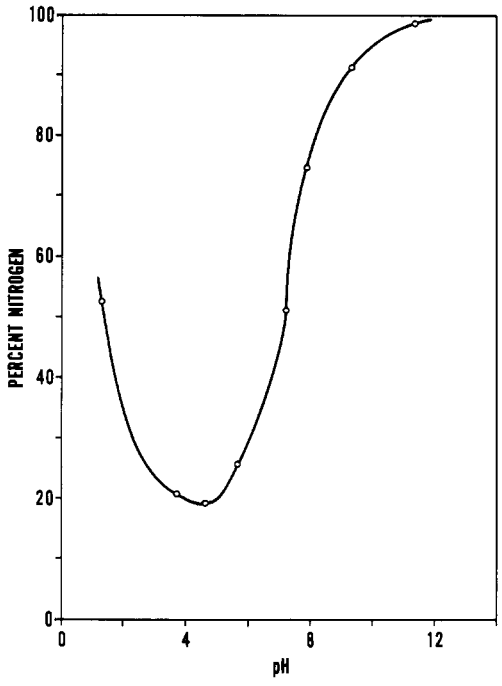

Figure 1. Schematic diagram for preparing protein concentrate from pearl millet.

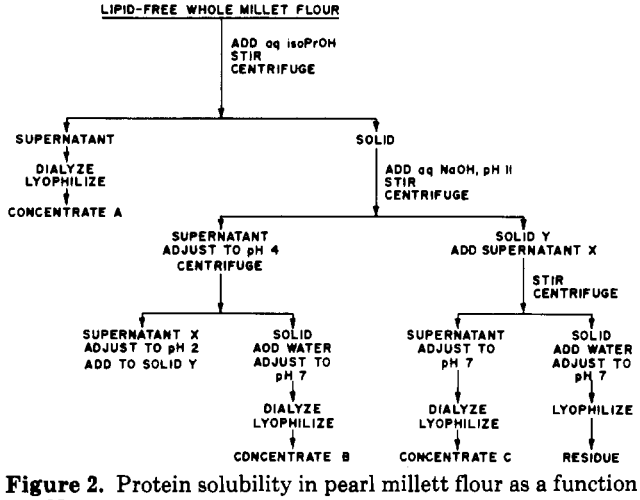

Figure 2. Protein solubility in pearl millett flour as a function of pH.

procedure are presented in Table I. .

As is apparent from the results, there are five solubility classes of proteins: (1) those soluble in aqueous isopropyl alcohol, (2) those soluble in aqueous alkali, (3) those soluble in aqueous acids, (4) those soluble in both aqueous isopropyl alcohol and aqueous alkali, and (5) those that did not dissolve in any of the solvent systems. The largest fraction was obtained when the flour was extracted first with 70% aqueous isopropyl alcohol. This fraction contained the largest amount of material (11.9% of the original flour) and contained the highest percentage of proteins (48.5% based on the total amount of proteins present in the flour). The amount of material recovered in the alcohol-soluble fraction and the protein content were somewhat reduced (9.9% of material and 42.8% of total protein) when 70% aqueous isopropyl alcohol was not used as the initial extraction agent. However, the yield and protein content of the alkali-soluble fraction are greater (8.5% of material, vs. 6.8%; and 28.5% of total protein, vs. 24.2%) when aqueous sodium hydroxide is the primary extraction medium. This difference can be assumed to represent those proteins that have good solubility in both

Table II. Amino Acid Composition of Main Concentrate Fractions and of Composite (g/16 g of Nitrogen Recovered)[a]

amino acid	original meal[b]	fractions from Table I					
		A	B	A + B[b]	B'	A'	A' + B'[b]
Ala	8.5	10.3	6.4	8.6	7.3	9.8	8.6
Val	5.4	5.8	5.5	5.7	5.8	5.7	5.8
Gly	2.7	0.6	5.2	2.3	4.8	0.7	2.6
Ile	4.7	5.5	3.6	4.8	4.2	6.0	5.1
Leu	11.2	14.2	7.6	11.8	8.6	13.4	11.2
Pro	7.1	7.5	5.6	6.8	5.2	7.9	6.6
Thr	4.1	3.5	4.1	3.8	3.9	3.6	3.8
Ser	5.6	5.5	4.8	5.2	5.3	5.4	5.3
Met	1.9	1.4	2.3	1.8	1.8	2.3	2.1
Phe	5.5	6.3	4.3	5.6	4.7	6.1	5.5
Asp	8.4	7.3	7.7	7.4	7.8	7.0	7.4
Glu	21.0	24.8	14.7	21.1	15.3	24.5	20.4
Tyr	3.5	3.2	4.3	3.6	3.7	3.6	3.6
Lys	2.5	0.3	5.7	2.4	4.9	0.2	2.4
His	2.3	1.6	3.6	2.4	3.2	1.7	2.4
Arg	5.3	1.2	12.0	5.1	10.9	1.2	5.7
1/2-Cys	1.7	1.3	2.4	1.8	1.9	1.7	1.8
recov %	86	76	62	75	57	78	76

[a] The composites (A + B) and (A' + B') were prepared by combining fractions from separate but identical runs to those for which data are given in Table I. [b] Protein content of lipid-free meal, 20.9%; of composite (A + B), 78.0%; of composite (A' + B'), 77.5%.

Table III. Chemical Scores of Pearl Millet Concentrates and Composites[a]

amino acid	suggested protein level, mg/g of protein	chemical scores						
		original meal	A	B	(A + B)	A'	B'	(A' + B')
Lys	55	45	5	104	44	4	89	44
Thr	40	103	88	100	95	98	90	95
Met-Cys	35	103	77	135	103	114	106	111
Leu	70	160	203	109	169	191	123	160
Ile	40	118	138	90	120	150	105	128
Val	50	108	116	110	114	114	116	116
Phe-Tyr	60	150	158	143	153	140	162	152

[a] Chemical score = mg of amino acid in 1 g of protein × 100/mg of amino acid suggested.

solvent systems. About 80% of the total proteins present in the flour were obtained in the three fractions by either procedure.

Concentrate fractions A and B (or A' and B', see Table I), containing approximately 70% of the total protein from the grain, were analyzed for amino acid composition, along with composites A + B and A' + B' (Table II). There are substantial differences in a number of the amino acids in the proteins in fractions A and B. The greatest difference was in lysine, with over 90% of that present in the two fractions turning up in the concentrate extracted into aqueous sodium hydroxide (fraction B and B'). The composites of A + B and A' + B', however, were nearly identical in amino acid composition, and this composition was little different from that of the original meal.

As an estimate of protein quality, the chemical scores (without tryptophan) were determined for the lipid-free meal, the main fractions, and composites A + B and A' + B' by the amino acid scoring pattern suggested by the joint FAO/WHO expert committee (1973) (Table III). Lysine seems to be the only deficient amino acid in the original meal and in both composites, although there seems also to have been a slight loss of threonine in both composites.

Fractions A and A' were extremely deficient in lysine, and fractions B and B', in which the lysine accumulated, have a much improved amino acid balance over the original meal.

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Received for review July 16, 1979. Accepted November 7, 1979. Names of companies or commercial products are given solely for the purpose of providing specific information; their mention does not imply recommendation or endorsement by the U.S. Department of Agriculture over others not mentioned.

Partial Characterization of Tannin–Protein Complexes in Five Varieties of Grain Sorghum by Automated Gel Filtration Chromatography

First-moment apparent molecular weights (M_{A_1}) for the glutelin fraction of five varieties of grain sorghum were determined by gel chromatography with detection by UV absorbance at 254 and 206 nm. For the low-tannin varieties, M_{A_1} based on absorbance at either wavelength was about 1.7×10^3. For an intermediate-tannin variety, M_{A_1} was 1.82×10^3 when based on detection at 206 nm and 2.21×10^3 when based on 254 nm, whereas the high-tannin variety had values of 0.90×10^3 and 2.36×10^3 at 206 and 254 nm, respectively. The higher values of M_{A_1} at 254 nm for intermediate- and high-tannin varieties compared to low-tannin ones were attributed to higher levels of protein–tannin complexes which have higher extinction coefficient at 254 than at 206 nm for molecular weights in excess of 2500. This conclusion was supported by measurement of tannin and protein content in the glutelin fraction.

Sorghum is an important food and feed grain in the United States and the world. High-tannin (polyphenol) varieties give higher acre yields of processed grain than low-tannin varieties. Unfortunately, however, tannin content and nutritional value in sorghum are inversely related (Jambunathan and Mertz, 1973).

Recently, Chibber et al. (1978) have postulated that tannin–prolamin interactions have caused many of these proteins to appear in the glutelin fraction when high-tannin sorghum grain was subjected to a classical fractionation scheme such as that devised by Landry and Moreaux (1970). Since protein–tannin interactions may adversely affect the nutritional value of grain sorghum through diminished protein availability, it was our purpose in this communication to examine the glutelin fraction from high-, intermediate-, and low-tannin varieties of sorghum for protein–tannin complexes.

MATERIAL AND METHODS

Seeds. Five varieties of whole grain sorghum were tested, and had been produced in 1970 at College Station, TX, and stored under refrigeration. Three of the varieties, SC 301, CK 60, and TX 615 have been designated low in tannin; one variety, NSA 740 as intermediate; and one variety, GA 615, as high (see Table I). Morphological descriptions of these varieties have been reported (Sullins, 1972; Sullins and Rooney, 1974).

Milling. The whole grain was ground in a Wiley Mill to pass a 40-mesh screen. The ground meal was defatted by suspension in stirred hexane for 1.5 h (1 L/250 g of meal) and then removal of the solvent by filtration. The particle size was further reduced by grinding in a ball mill for three 10-min intervals with periods of air cooling in between.

Protein Extraction. The glutelin fraction of each variety was obtained by a modified procedure of earlier methods (Landry and Moureax, 1970; Jambunathan and Mertz, 1973) according to Neucere and Sumrell (1979). Defatted meal was extracted with deionized water. Albumins were separated as supernatant from the insoluble residue by centrifugation. The residue was extracted with 0.5 M NaCl and centrifuged again, and globulins were removed with the supernatant. Residue from the salt extraction was extracted with aqueous *tert*-butyl alcohol (60% butyl alcohol by volume) that contained 0.1 M guanidine hydrochloride, and centrifuged to yield a supernatant containing prolamins. The residue of the previous step was extracted with 0.16 M borax–0.048 M NaOH buffer, buffer, pH 10, which was also 0.5% in sodium dodecylsulfate. After centrifugation, the supernatant glutelin fraction was separated from the residue, dialyzed against deionized water, and freeze-dried.

Protein Content. Crude protein (N × 6.25) in each variety was determined for the whole seed and the glutelin fraction by the microKjeldahl nitrogen method (Horowitz, 1975). Protein in the glutelin fraction was dissolved in 0.8 N NaCl and determined by the Lowry method (Lowry et al., 1951).

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