Protein Composition of Proso Millet

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Dehulled white proso millet was ground to a flour preparatory to studying its protein composition. The flour was extracted successively with water, 1% sodium chloride, and alcohol. Both 60% *t*-butyl alcohol and 60% ethanol were used. Electrophoretic patterns and amino acid composition of each extract were obtained. Protein bodies were identified by both light and electron microscopy. They are composed mainly of prolamines which dissolve in hot *t*-butyl alcohol.

Millet is a minor cereal crop in the United States. In many parts of the world, however, it is grown in large quantities and is an important component of the diet. An estimated 16.9 million short tons of millet were produced worldwide in 1965 (FAO, 1966). Most of it was used for food. Cereal grain can be mixed with oilseed meals to provide optimum amounts of essential amino acids as well as other nutrients required in the diet (Inglett *et al.*, 1969).

Knowledge of the protein composition of millet is important in determining the adequacy of this cereal for food uses. The amino acid composition of millets has been investigated by Mangay *et al.* (1957), Rakhimbaev (1967), and Wilkinson *et al.* (1968). The purpose of this study is to gain more detailed information about the composition, microscopic structure, and physical-chemical properties of proso millet protein.

MATERIALS AND METHODS

White proso millet dehulled with a tooth grinder was supplied by Excello Commodities, Inc. of Denver, Colo., and was reduced to a flour in a cyclone hammer mill.

Starch content of the grain was determined by a polarimetric method (Earle and Milner, 1944). Nitrogen was measured by semimicro-Kjeldahl and crude fiber by the official AOAC method (1965).

First, the flour was defatted with *n*-butyl alcohol (Jones and Dimler, 1962). No protein was removed during lipid extraction. The defatted flour was then extracted successively with water, 1% sodium chloride, and alcohol in the manner described by Jones and Beckwith (1970). The alcohols used were 60% *t*-butyl alcohol and 60% ethanol both at 60° C.

Electrophoresis was carried out in acrylamide gel with aluminum lactate-lactic acid buffer at pH 3.1 (Jones and Beckwith, 1969).

Kernels were prepared by light microscopy by soaking in water for several hours before fixing in Lewitzsky's fixative. Six micron transections were cut in a cryostat. Starch was removed by α-amylase treatment. Sections were then stained with iodine vapor.

For electron microscopy, small pieces of transections approximately 0.5 mm. thick were fixed first in glutaraldehyde and then in osmium tetroxide. They were dehydrated with acetone and embedded in Epon 812. Thin sections were cut and mounted on grids for examination in an RCA EMU-3F microscope.

Samples for amino acid analyses were hydrolyzed in refluxing constant boiling HCl. Sample-to-acid ratio was approximately 1 mg. to 2 ml. Hydrolysates were evaporated to dryness, and the residue was dissolved in pH 2.2 citrate buffer for analysis.

The 3-hour analysis procedure of Benson and Patterson (1965) was used on a Beckman Spinco Model 120 amino acid analyzer. Integration of peaks was accomplished electronically with an Infotronics-Integrator, and the amino acid analysis data were automatically computed (Cavins and Friedman, 1968).

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RESULTS

Microscopic examination of destarched endosperm sections shows that the protein consists mainly of globular bodies measuring up to 2.5 μ in diameter (Figure 1). In the outer endosperm cells, some of the globular protein is embedded in an amorphous matrix protein, but further into the endosperm there is little matrix protein. The protein bodies are mainly prolamines which dissolve in hot *t*-butyl alcohol.

A small area of protein from the outer endosperm as seen in the electron microscope is shown in Figure 2. At a magnifica-

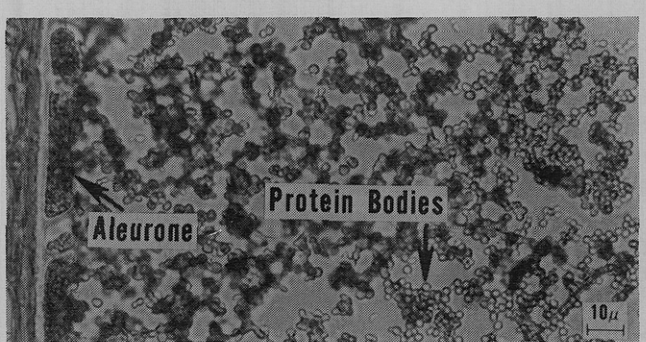

Figure 1. Amylase-treated section of white proso millet

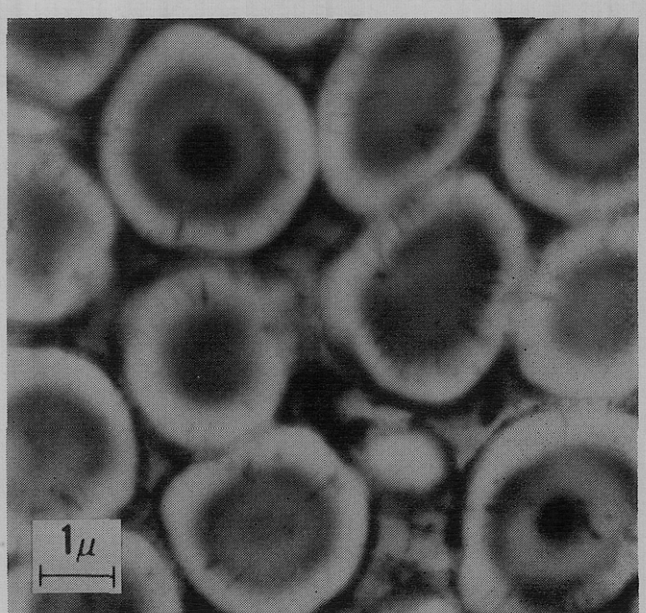

Figure 2. Electron micrograph of proso millet showing protein bodies and matrix protein. × 8500

Table I. Composition of Proso Dehulled Millet

Component	Per Cent of Total Weight (Dry Basis)
Protein	12.5
Starch	80.1
Fat	4.9
Fiber	0.7

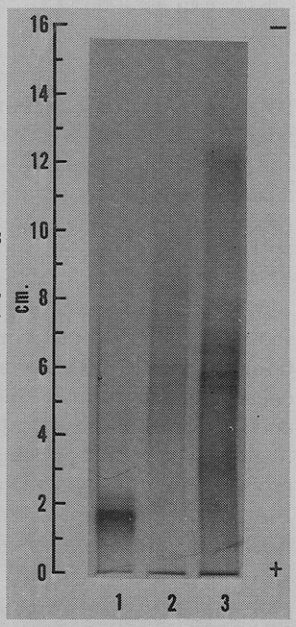

Figure 3. Electrophoretic patterns of proteins of proso millet
Aluminum lactate-lactic acid buffer, 8*M* urea, pH 3.1, 400 V, 2.5 hours.
1. *t*-Butyl alcohol solubles
2 Water solubles
3. Sodium chloride solubles

tion of approximately 8500, some protein bodies are seen to have a central core surrounded by two or more bands. The matrix protein appears to be somewhat heterogeneous as shown by the different stain intensities.

An analysis of dehulled millet is given in Table I. Per cent protein was calculated by multiplying Kjeldahl nitrogen by 6.25.

Of the total nitrogen in millet, 5% can be extracted with water and another 4% by 1% sodium chloride. After extraction of the flour with water and 1% sodium chloride, an additional 52% of the millet nitrogen can be dissolved at 60° C. with either 60% *t*-butyl alcohol or 60% ethanol. At room temperature these alcohols dissolve only 3% of the total nitrogen. *t*-Butyl alcohol is preferred to ethanol as an extractant since it can be freeze-dried directly. Ethanol solutions must be dialyzed against water before freeze-drying. During dialysis protein precipitates and is difficult to redissolve.

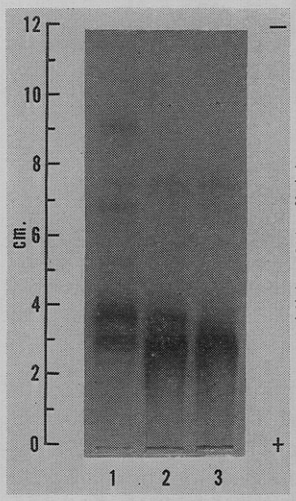

Figure 4. Electrophoretic patterns of alcohol solubles of proso millet
Aluminum lactate-lactic acid buffer, 8*M*, urea, pH 3.1, 400 V, 4 hours.
1. *t*-Butyl alcohol at room temperature
2. *t*-Butyl alcohol at 60° C.
3. Ethyl alcohol at 60° C.

The *t*-butyl alcohol-soluble fraction is nearly all protein based on a factor of 6.25. Water solubles contain only 35% protein and salt solubles only 38%. Figure 3 illustrates the difference in electrophoretic mobilities of the prolamines, albumins, and globulins. The alcohol solubles move much more slowly than do the water and salt solubles. The slow mobility is a result of the small amount of the basic amino acids—lysine, arginine, and histidine—in the prolamine fraction.

Figure 4 shows the electrophoretic patterns of the prolamines. There is no essential difference between the patterns of *t*-butyl alcohol extracts prepared at room temperature and at 60° C. The ethanol solubles contain less of the component 3.7 cm. from the origin than does the butanol extract. A similar difference is found between the butanol and ethanol extracts of grain sorghum (Jones and Beckwith, 1970).

The amino acid composition of protein extracted from

Table II. Amino Acids of Proso Millet Protein

g./16 g. Nitrogen

Amino Acid	Dehulled Grain	H_2O Solubles	NaCl Solubles	*t*-BuOH Solubles	Residue
Lysine	1.5	6.5	4.4	0.1	2.0
Histidine	2.1	2.6	2.6	1.6	4.1
Ammonia	2.9	1.5	1.1	3.0	2.9
Arginine	3.2	9.1	13.3	1.3	4.8
Aspartic	6.2	4.6	7.4	5.3	7.3
Threonine	3.0	4.6	3.2	2.7	4.0
Serine	6.3	4.5	5.9	7.3	4.9
Glutamic	21.3	21.2	20.3	25.0	18.2
Proline	7.3	4.9	4.6	6.6	6.1
Glycine	2.1	6.3	5.4	1.0	4.3
Alanine	10.9	6.9	6.7	12.8	8.6
½ Cystine	0.5	2.4	4.1	1.2	0.2
Valine	5.4	5.1	5.0	5.0	5.4
Methionine	2.2	1.7	0.7	2.4	0.7
Isoleucine	4.1	3.1	2.7	4.5	3.5
Leucine	12.2	6.0	5.2	14.6	9.3
Tyrosine	4.0	3.1	3.0	4.3	5.1
Phenylalanine	5.5	3.3	3.3	6.5	4.1
Tryptophan	0.8	...	...	...	...

millet flour is given in Table II. The amino acid content of dehulled grain is also included. The prolamines have less lysine, arginine, and glycine and more alanine, methionine, and leucine than do the albumins or globulins. Arginine content of the globulins was high. Residue protein had less cystine than the soluble fractions. We assume that all the ammonia arises from the hydrolysis of the amide group of asparagine or glutamine. Therefore, about 90% of all the acidic amino acids of prolamine and the insoluble residues are in the amide form. Only about 40% of the acids of the water and salt solubles is made up of amides.

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