due to the lower dose mass used.

A significant amount of labeled carbon dioxide was expired by the intact rat which was orally dosed. However, very little radioactive carbon dioxide was produced when hesperetin-3-^{14}C was incubated with rat cecal flora. These data suggest that the β oxidation of the propyl chain of the phenylpropanoic acid metabolites is mediated by mammalian and not bacterial enzymes. This has been reported previously by Griffiths and Smith (1972).

Intraperitoneal injection of hesperetin-3-^{14}C to a bile duct cannulated rat resulted in excretion of 100% of the administered radioactivity in the bile. These data indicate that no radioactive carbon dioxide was generated because the flavanone did not come into contact with intestinal microflora and undergo metabolism to phenylpropanoic acids. If, as suggested, no phenylpropanoic acids were produced, it appears that the mammalian systems are not capable of carrying out the required acyl (i.e., carbonyl to phloroglucinol ring) cleavage. Scheline (1973) has recently reported similar results for closely related compounds.

For the orally dosed rats, nearly 40% of the radioactivity administered as hesperetin-3-^{14}C was expired as carbon dioxide. This means that a minimum of 40% of the dose was metabolized by acyl cleavage and that these products were in turn further metabolized by β oxidation to benzoic acids. This must be considered a minimum value because the degradation of phenylpropanoic acids to benzoic acids will afford labeled acetate that would be included in biochemical pathways other than those resulting in carbon dioxide. At the dose levels employed in these experiments, it is apparent that benzoic acid derivatives, including *m*-hydroxyhippuric acids (Booth et al., 1957), are quantitatively the most significant metabolites of hesperetin-3-^{14}C.

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Functional Characteristics of Starches from Proso and Foxtail Millets

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The functional characteristics of proso and foxtail millet starches were determined and compared with those of wheat and rye starches. The millet starches showed higher water binding capacity values and gelatinization temperatures than the wheat starch. With two exceptions, the millet starches produced swelling power values at 90 °C which were similar to those of the wheat starch, but lower than those of the rye starch. Solubilities of the millet starches were lower than those of the wheat starch, except for the starch from one millet variety. Amylograph viscosities of millet starches were higher than those of the wheat starch at all reference points.

Millet is a very important food plant in many parts of the world. In the United States, however, it is a minor cereal crop. Proso (*Panicum milaceum* L.) is the common millet which has been grown since prehistoric times for human use. Foxtail millet (*Setaria italica* (L.) Beauv.) is generally grown for hay or forage (Hinze, 1972).

In the U.S. the proso type is used in feeding rations, as birdseed, and also as a human food. Dehulled proso can be consumed as a puffed cereal or cooked as a hot breakfast cereal. Millet flour can be used as a partial substitute in formulations, which call for wheat flour, to impart a distinct nutlike flavor.

Both proso and foxtail millet are somewhat higher in protein than rice, sorghum, corn, and oats (Matz, 1959). Amino acid compositions of millet varieties have been determined by Mangay et al. (1957), Wilkinson et al. (1968), and Jones et al. (1970). The cereal is higher in ash and fiber compared to other cereals used for human consumption (Hinze, 1972). Very little, however, has been published about the properties of millet starches.

It was the purpose of this investigation to study the functional characteristics of starches isolated from proso and foxtail millet varieties.

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MATERIALS AND METHODS

(A) Sample Identification. Samples selected for this study included six varieties of proso millet: Abarr and

Table I. Water Binding Capacities and Gelatinization Temperature Ranges of Millet Starches

	Starch	Water binding[a] capacity, %	Gelatinization temp[a] range, °C		
			Initial	Midpoint	Final
Proso millets	Abarr	116.3	53	57	60
	Leonard	124.1	55	57	60
	Big Red	122.9	59	61	64
	Black Russian	93.3	56	58	60
	Akron Proso (73-21-1)	95.8	56	59	62
	Akron Proso (73-1055)	99.7	58	59	61
Foxtail millets	Golden German	122.1	55	59	61
	Chinese	135.3	52	54	58
HRS wheat	Chris	71.8	47	50	53
Rye	Prolific	88.1	47	51	54

[a] Averages of four separate determinations.

Table II. Swelling Power of Millet Starches[a]

	Starch	Swelling power at °C			
		60	70	80	90
Proso millets	Abarr	2.49	4.06	8.84	10.76
	Leonard	2.55	3.16	7.98	12.07
	Big Red	2.47	4.29	9.34	14.52
	Black Russian	2.47	4.07	8.64	11.29
	Akron Proso (73-21-1)	2.33	3.56	8.63	10.52
	Akron Proso (73-1055)	2.47	5.07	9.12	13.04
Foxtail millets	Golden German	2.57	5.14	9.33	11.12
	Chinese	2.92	5.84	8.39	11.37
HRS wheat	Chris	4.77	6.13	8.28	11.80
Rye	Prolific	5.47	6.64	9.54	15.16

[a] Average of four separate determinations.

Leonard are named varieties grown in the area; experimental lines are identified as Black Russian (P.I. 346-937), Big Red (P.I. 346-946), Akron Proso 73-21-1 (a selection of Common White), and Akron Proso 73-1055, a white type of unknown origin. The foxtail millets used were: Golden German, a variety grown in Colorado, and P.I. 391-638, a Chinese line. All varieties were grown at the Central Great Plains Field Station of the Colorado State University Agricultural Experiment Station at Akron, Colo. during the 1975 crop year.

(B) Preparation of Starches. Millet starches were isolated by steeping the grain at 6 °C for 24 h in distilled water buffered at pH 6.5 (0.02 M acetate) and rendered 0.01 M in mercuric chloride as suggested by Adkins and Greenwood (1966). After steeping, the excess water was decanted, and the grain washed several times with distilled water and wet-milled in a Waring Blendor for 3 min. The grain suspension was screened through a bolting cloth. The starch was recovered from the filtrate by centrifugation (15 min at 5000 rpm), washed repeatedly by resuspending in distilled water, and centrifuging followed by air drying.

Wheat and rye starches were prepared as described by Lorenz (1976).

(C) Methods for Measurements of Functional Characteristics. Water binding capacity was measured using the method of Medcalf and Gilles (1965). Swelling power and solubility determinations were carried out for the temperature range of 60–90 °C by the procedure of Leach et al. (1959).

Gelatinization temperature ranges were followed by observing loss of birefringence. A polarizing microscope equipped with a Kofler hot stage as described by Schoch and Maywald (1968) was used.

Pasting properties were determined by means of the Brabender Visco-Amylograph. Nine parts of starch solids per 100 parts (420 ml) of distilled water were heated from 30 to 92 °C, kept at this temperature for 30 min, then cooled to 35 °C and held at 35 °C for 60 min. The following reference viscosities (Brabender units, B.U.) are reported: viscosity at 92 °C; after 30 min at 92 °C; at 35 °C; and 60 min after reaching 35 °C.

The water binding capacity data, final gelatinization temperatures, and swelling power and solubility data at 90 °C were analyzed statistically.

RESULTS AND DISCUSSION

(A) Water Binding Capacity. The results of determinations of water binding capacity of the starches are shown in Table I. The millet starches had higher water binding capacities than the starches of HRS wheat and rye. The Chinese foxtail millet starch produced the highest and Black Russian proso starch the lowest value. The differences in values between the millet starches and the HRS wheat starch were significant ($\alpha = 0.05$). The foxtail millet starches Golden German and Chinese showed significantly higher ($\alpha = 0.05$) water binding capacity values than the proso starches Black Russian, Akron 73-21-1, and Akron 73-1055.

(B) Gelatinization Temperature Ranges. Values for gelatinization temperatures of the wheat and rye starches are within the range of values reported in the literature. Values for the millet starches, shown in Table I, were significantly ($\alpha = 0.05$) higher than those observed for the wheat and rye starches. Among the millet starches, Big Red proso exhibited the highest final gelatinization temperature while the starch from the Chinese foxtail millet had the lowest.

The gelatinization temperature reflects the degree of orderly arrangement of the molecules in the starch granule. The higher gelatinization temperatures of the millet starches would indicate a higher degree of association.

(C) Swelling Power and Solubility. The swelling power and solubility of the starches over a range of temperatures were determined to provide evidence of the associative bonding within the granules. Swelling power data are presented in Table II. The values increased with

Table III. Solubilities of Millet Starches (%)[a]

	Starch	% solubility at °C			
		60	70	80	90
Proso millets	Abarr	0.75	2.07	3.26	5.37
	Leonard	1.19	1.78	3.68	5.47
	Big Red	1.04	1.18	10.77	13.59
	Black Russian	0.66	1.33	3.45	6.20
	Akron Proso (73-21-1)	0.30	1.32	3.71	4.99
	Akron Proso (73-1055)	0.49	2.40	3.68	5.73
Foxtail millets	Golden German	0.83	2.25	2.51	3.90
	Chinese	0.55	2.94	3.10	5.39
HRS wheat	Chris	1.67	2.35	2.48	8.21
Rye	Prolific	3.65	4.05	4.29	4.80

[a] Average of four separate determinations.

Table IV. Amylograph Viscosities of Millet Starches (Brabender Units)

	Starch	Viscosity at 92 °C	Viscosity after 30 min at 92 °C	Viscosity cooling to 35 °C	Viscosity after 60 min at 35 °C
Proso millet	Abarr	760	630	950	1340
	Leonard	795	650	910	1260
	Big Red	620	480	580	670
	Black Russian	550	380	740	1030
	Akron Proso(73-21-1)	695	480	840	1580
	Akron Proso(73-1055)	710	500	940	1340
Foxtail millet	Golden German	840	620	1100	1220
HRS wheat	Chris	270	340	510	810
Rye	Prolific	390	470	760	1150

temperature as expected. The wheat and rye starches exhibited a more rapid and less restricted swelling at 60 °C compared to all millet starches. At 70 °C these differences, however, were not quite as pronounced. At 90 °C the Akron 73-21-1 starch showed the lowest while the Big Red starch produced the highest value, which, however, was still below that of the rye starch. The swelling power values at 90 °C for the Big Red starch were significantly ($\alpha = 0.05$) higher than those obtained with the wheat starch. The rye starch values at 90 °C were significantly higher than those of the millet starches, except for the Big Red starch.

Leach et al. (1959) postulated that the bonding forces within the starch granule would influence the manner of swelling. Thus, a highly associated starch with an extensive and strongly bonded micellar structure should be relatively resistant toward swelling. The 60 and 70 °C data indicate the greater resistance toward swelling of the millet starches compared to those of the wheat and rye starches. The values for swelling power at higher temperatures represent progressive relaxation of the bonding forces within the granules.

Solubility data of the starches are presented in Table III. The values increased as temperature increased as expected. The tremendous increase in solubility of the Big Red starch at 80 °C, however, stands out. A statistical evaluation of the 90 °C values indicated significant ($\alpha = 0.05$) differences between the wheat starch and all millet starches, the Big Red starch having significantly higher and all other millet starches significantly lower values than the wheat starch.

Plotting swelling power against percentage solubles provides a comparison of the solubilization of different starches at equal levels of swelling as shown in Figure 1. Percentage solubility increases as swelling power increases. This increase, however, is not necessarily a straight line relationship.

(D) Amylograph Viscosities. There is little or no relation between swelling power and Brabender pasting curves (Miller et al., 1973). Therefore, both swelling power and Amylograph viscosities are usually determined when

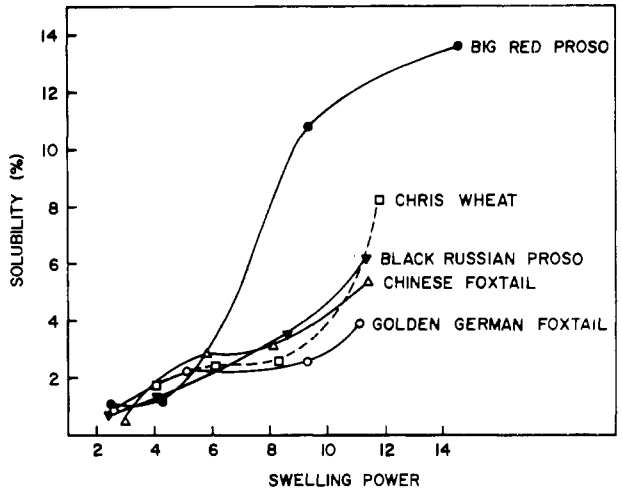

Figure 1. Relationship between swelling power and solubility of millet starches.

functional characteristics of starches are studied.

The viscosity values obtained at the different reference points are presented in Table IV. Brabender viscosities of all millet starches at 92 °C were considerably higher than those of the wheat and rye starches. Holding the temperature of the pastes at 92 °C produced increased viscosities of the wheat and rye starches. Viscosities of the millet starches, however, decreased. Cooling to 35 °C and holding the temperature of the paste at 35 °C for 1 h caused the viscosity of all starches to increase. This increase in viscosity on cooling reflects the retrogradation tendency of a starch. Except for the Big Red starch, viscosity values of millet starches at the final reference point were considerably higher than that of the wheat starch.

Amylograph curves of some of the starches are shown in Figure 2. The Big Red starch showed a very high peak viscosity, which is not apparent from the data shown in Table IV. The viscosity then decreased considerably. Increases in viscosity upon cooling were only slight

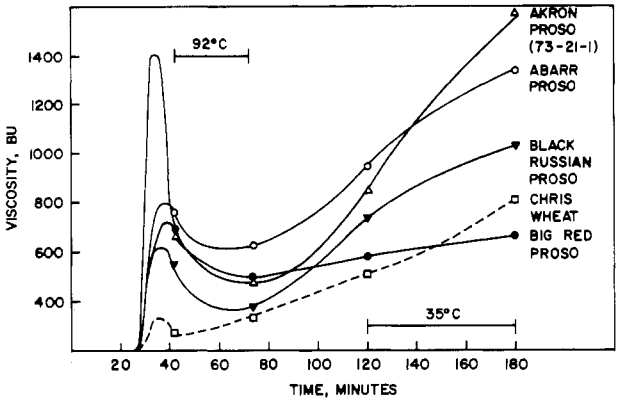

Figure 2. Amylograph curves of millet starches.

compared to all other starches. The final viscosity reading after 60 min at 35 °C was the lowest of all starches studied.

CONCLUSIONS

Proso and foxtail millet starches had higher water binding capacities and higher gelatinization temperatures than the wheat and rye starches. Swelling power values at 60 °C were higher for the wheat and rye starches than for the millet starches. With two exceptions, swelling power at 90 °C showed comparable values for wheat and millet starches, but lower values than observed for rye starch.

Millet starches were less soluble than wheat or rye starch at 60 °C. At 90 °C, solubilities of the millet starches were lower than those of the wheat starch with one exception.

Amylograph viscosities of both proso and foxtail millet starches were higher than those of wheat starch at all reference points.

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Functional Properties of Succinylated and Acetylated Leaf Protein

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Succinylation of 84% of the ϵ-amino groups of lysine increased bulk density, improved flavor, and increased solubility of protein isolated from alfalfa leaves over tenfold. The succinylated leaf protein demonstrated a typical pH solubility curve except that it did not resolubilize below the isoelectric pH. The emulsifying activity was enhanced by 32% and the foaming capacity threefold. Succinylation had no effect on the viscosity of dilute solutions of leaf proteins nor on the amino acid composition. Acetylation of leaf protein improved solubility and foaming capacity to a much lesser extent.

The potential of leaves to provide significant amounts of protein for food use has been discussed by several authors (Pirie, 1970; Stahmann, 1968; Kinsella, 1970; Kohler et al., 1974). The amino acid compositions of several leaf protein preparations have been reviewed by Betschart and Kinsella (1974) and Wang and Kinsella (1976a,b). Furthermore, several researchers have demonstrated the effectiveness of properly prepared leaf protein as a protein supplement for protein deficient foods (Subba-Rau et al., 1972; Oke, 1971; Woodham, 1972; Kawatra et al., 1974). Unfortunately, varied and contradictory results concerning the composition and biological value of leaf proteins frequently emanate from the different methods used in their preparation, e.g., failure to remove saponins by washing the crude protein results in poor nutritional value.

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Sufficient information has been accumulated so that it is now possible to prepare high quality protein concentrates from various leaves especially alfalfa (Pirie, 1971; Kohler et al., 1974; Betschart and Kinsella, 1974; Wang and Kinsella, 1976a,b).

However, for adoption by food manufacturers the isolated protein should display a range of critical functional properties, e.g., solubility, surface activity, gelation, etc. These will determine the successful application of novel proteins for the supplementation of foods and for the fabrication of new foods. Hence, research on novel food proteins should include evaluation of the functional properties of these proteins. The available information on functional properties of leaf proteins, e.g., solubility, gelation, foaming, and emulsifying properties, has been reported (Lu and Kinsella, 1972; Betschart and Kinsella, 1974; Wang and Kinsella, 1976a,b).

These studies indicated that methods used in preparation of leaf proteins cause denaturation which results in poor solubility, with subsequent deterioration in functionality. Hence, as with several other novel proteins, methods for improving functional properties are needed