NOTE

Effects on Proteins in Sorghum, Maize, and Pearl Millet When Processed Into Acidic and Basic *Tô*[1]

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Sorghum, maize, and pearl millet are processed into many traditional foods and are consumed by people in regions of Africa, Asia, and Latin America (Rooney et al 1986). The retention of cereal protein quality and quantity during processing is important for dietary reasons. Improved cultivars are being developed and should be evaluated for their processing characteristics and food quality. Hence, techniques to evaluate the effects of food preparation need to be established and used.

Thick porridges are popular in many semiarid regions and are usually made under neutral conditions in eastern and southern Africa, under acidic conditions in Burkina Faso, and under basic conditions in Mali (Rooney et al 1986). When neutral conditions were used to prepare 10% (w/v) gruels of sorghum or pearl millet, the amounts of albumins, globulins, and prolamins were drastically decreased (Hamaker et al 1986, Ejeta et al 1987, Rom et al 1992). Similar results were observed when sorghum or maize was processed into tortillas (basic conditions) (Ortega et al 1986, Vivas et al 1987, Vivas et al 1990). Residue proteins increased after all processes.

Molecular weight changes of proteins were revealed after processing into gruels (neutral condition) and tortillas (basic condition) (Hamaker et al 1986, Ortega et al 1986, Ejeta et al 1987, Vivas et al 1987, Vivas et al 1990). Protein digestibility (in vitro and in vivo) decreased slightly or substantially after processing sorghum into thick porridges under neutral, acidic, or basic conditions (Bach-Knudsen and Munck 1985, Hamaker et al 1986, Vivas et al 1987, Bach-Knudsen and Munck 1988, Bach-Knudsen et al 1988). Protein digestibilities of maize and pearl millet were less affected by processing than were those of sorghum.

Hence, stiff porridge preparation (25% solids) using traditional acidic and basic conditions was conducted to further characterize protein solubility (fractionation), molecular weight (electrophoresis), and in vitro pepsin digestibility.

MATERIALS AND METHODS

Materials and *Tô* Preparation

Sorghum (SC265 with a thick, white pericarp, nonpigmented testa, and hard endosperm texture) grown in Lubbock, TX, in 1984; a market sample of pearl millet (Sanio Sinebougou with a thick, grey pericarp and intermediate endosperm texture) purchased in Mali in 1984; and a food-quality yellow maize (Asgrow 404 with a hard endosperm texture) grown in southern Texas in 1986 were evaluated.

Grain was decorticated with a tangential abrasive dehulling device (model 4E-115, Venables Machine Works Ltd., Saskatoon, Saskatchewan, Canada) equipped with an eight-sample cup plate (Reichert et al 1982) until 16% of sorghum, 20% of maize, and 18% of pearl millet was removed. Raw grain (control) and *tô* (acidic and basic) were lyophilized for 72 hr, ground in a Udy laboratory cyclone mill (Udy Corp., Ft. Collins, CO) using a 1.0-mm-mesh screen, and stored frozen (−7°C) in sealed plastic bags.

Tô (a stiff porridge) was prepared under acidic (pH 4.6) and basic (pH 8.8) conditions as described by Da et al (1982). Flour (9.5 g) of decorticated sorghum, maize, and pearl millet was added to distilled water (25.0 ml) to make a slurry. Acidic *tô* samples were prepared by mixing 19.0 ml of distilled water and 1.0 ml of lemon juice in a 100-ml beaker and boiling on an electric hot plate (Thermodyme Sybran Corp., Dubuque, IA) set on high temperature. The flour-water slurry was added to the boiling mixture and cooked for 5 min with continuous stirring to prevent lump formation. The final paste was poured into 10-ml beakers. Basic *tô* was prepared similarly except that 20 ml of a basic solution (0.45 g of KOH in 200 ml of water [0.070*M*]) was the boiling solution.

Preparation of Protein Extracts

Proteins were sequentially extracted from flour and lyophilized *tô* prepared from sorghum, maize, and pearl millet. Proteins were fractionated using albumins plus globulins and nonprotein nitrogen (0.1*M* NaCl); prolamins (60% *tert*-butyl alcohol); alcohol-soluble reduced glutelins (ASG, 60% *tert*-butyl alcohol containing 2% β-mercaptoethanol [βME]); glutelins (2% sodium dodecylsulfate [SDS] with 5% βME and 0.0625*M* Tris [pH 6.8]); and residue proteins (Vivas et al 1987).

Protein Content and In Vitro Digestibility

The micro-Kjeldahl method (Technicon Instruments 1976) was used to determine total nitrogen content. Protein content was calculated by multiplying the nitrogen value by 6.25. In vitro digestibility of protein by pepsin was determined (Mertz et al 1984, Bookwalter et al 1987).

Electrophoresis of Protein Extracts

The molecular weight distributions of the four protein extracts were determined using discontinuous SDS-polyacrylamide gel electrophoresis (Laemmli 1970, Vivas et al 1987). Proteins were visualized by the silver staining procedure (Merrill et al 1981). Cytochome C (12 kilodaltons [kDa]), carbonic anhydrase (29 kDa), bovine serum albumin (66 kDa), alcohol dehydrogenase (150 kDa), and α-amylase (200 kDa) were used as molecular weight standards.

Protein extracts were stabilized with SDS and βME before electrophoresis. Albumin plus globulin extracts (1.0 ml) were mixed with 0.33 ml of a concentrated sample buffer containing 0.25*M* Tris (pH 6.8), 8% SDS, 2% βME, and 40% glycerol. Aliquots containing 12–15 μg of albumins plus globulins were injected into the wells of the stacking gel. Prolamin and ASG extracts (1.0 ml) were heated in tubes (75 ml) in a water bath (100°C) until the solvent was evaporated. The proteins were resuspended in buffer containing 0.0625*M* Tris (pH 6.8), 2% SDS, 5% βME, and 10% glycerol. Aliquots containing 6–9 μg of prolamins and 5–7 μg of ASG were injected into the wells of the stacking gel. Glycerol (10% [v/v]) was added to extracts, and 5–9 μg of glutelins was injected into the wells of the stacking gel.

Statistical Analysis

Three observations of protein fractions and pepsin digestibility were conducted from each of two replicates. Differences among means were determined using analysis of variance and least significant differences in a completely randomized block design.

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RESULTS AND DISCUSSION

Protein Solubility

Processing into *tô* affected the solubility of proteins in sorghum, maize, and pearl millet (Table I). Albumins plus globulins, prolamins, and glutelins decreased during the preparation of acidic and basic *tô*. ASG and residue proteins generally increased during processing.

The amounts of albumins plus globulins decreased about 50% during processing into *tô* in the cereals evaluated (Table I). Pearl millet contained more extracted proteins in the albumins plus globulins fraction, before and after processing, than did sorghum or maize. This was attributed to a larger germ in pearl millet (Okoh et al 1985, Ejeta et al 1987, Subramanian et al 1990).

The amount of prolamins from pearl millet flour (48%) was comparable to that from maize (45%) and was about twice as much as the amount from sorghum (20%) (Table I). More maize prolamins were observed in *tô* than in pearl millet or sorghum. However, the amount of prolamin was lower in basic than in acidic *tô* in all cereals.

The amount of ASG increased more during the processing of pearl millet and maize into *tô* than during the processing of sorghum (Table I). The amount of ASG in sorghum increased only in acidic *tô*. The amount of ASG in maize and pearl millet to increased more in the basic than in the acidic condition.

The amount of glutelin decreased during processing into *tô* (Table I); however, each cereal had similar amounts of soluble glutelin.

Residue proteins increased during processing into *tô* for all cereals (Table I). More residue proteins were observed in *tô* prepared from pearl millet than in that prepared from sorghum, which was higher than maize *tô*. More residue proteins were observed in basic *tô* than in acidic *tô*.

Thus, large proportions of albumins plus globulins, prolamins, and glutelins become insoluble during processing. Apparently, the proteins that became insoluble in these fractions appeared in the ASG and residue fractions. Similar decreases in albumins plus globulins and prolamins were observed in 10% (w/v) gruels or tortillas prepared from sorghum, maize, or pearl millet (Hamaker et al 1986, Ortega et al 1986, Ejeta et al 1987, Vivas et al 1987).

Albumins Plus Globulins

Albumins plus globulins separated into many bands, with molecular weights ranging from 9 to 270 kDa (Fig. 1A). Similar molecular weight proteins were observed earlier (Misra et al 1976, Guiragossian et al 1978, Galyean et al 1980). The germ of sorghum contains similar molecular weights of albumins plus globulins (Taylor and Schussler 1986). This suggests that the germ was the primary source of proteins extracted using NaCl.

Processing into *tô* either changed the intensity or the molecular weight of proteins after processing (Fig. 1A). Notable are the increases in proteins with molecular weights of 12 and 24 kDa from sorghum: 11, 23, 26, and 33 kDa from pearl millet; and 12 and 34 kDa from maize after processing. Acidic *tô* contained fewer and more weakly stained proteins than did basic *tô*, which had a larger number of intense protein bands (Fig. 1). Variation in staining and protease activity may have contributed to these results because the same amount of protein was injected into each well for analysis.

Prolamins

Prolamins from the three cereals responded differently during preparation into acidic and basic *tô* (Fig. 1B). Major bands were observed between 21 and 26 kDa for all cereals, and their molecular weights remained the same after processing. Sorghum prolamins in acidic *tô* were unchanged, whereas those in basic *tô* had less intense protein bands. Pearl millet prolamins decreased in intensity after processing into acidic or basic *tô*. Maize prolamins were not affected by processing into acidic or basic *tô*. Molecular weights of prolamins in tortillas prepared using sorghum or maize were not affected by processing (Vivas et al 1987).

Alcohol-Soluble Reduced Glutelins

Molecular weights of ASG and prolamins were similar in each cereal, i.e., between 21 and 28 kDa (Fig. 1B and C). Processing into *tô* had less effect on ASG than on prolamins. Sorghum ASG had less intense bands in acidic *tô* than in basic *tô*. Intensity of ASG from raw pearl millet was less than expected in both replicates. Maize ASG were not affected by processing into acidic or basic *tô*. Molecular weights of prolamins and ASG from sorghum, maize, and pearl millet were similar to those reported earlier (Paulis and Wall 1979, Lasztity 1984, Taylor et al 1984, Lagudah and Hanna 1990).

Glutelins

Molecular weights of glutelins were heterogeneous (Fig. 1D), as was reported earlier for sorghum and maize (Lasztity 1984). Molecular weights of several glutelins and salt-soluble proteins (albumins plus globulins) were similar in each cereal evaluated. High-MW glutelins decreased whereas low-MW glutelins increased after processing into *tô*. Pearl millet and maize glutelins had increased intensity in acidic and basic *tô*, especially in the 20- to 29-kDa range. Intensities of glutelins from sorghum gruel (pH 7) increased (Hamaker et al 1986) because some prolamins became more disulfide cross-linked during cooking.

In Vitro Pepsin Digestibility

In vitro pepsin digestibility decreased after preparation of *tô*

TABLE I
Fractionation and In Vitro Digestibility of Proteins in Acidic and Basic *Tô* Prepared Using Sorghum, Pearl Millet, and Maize

Sample	Nitrogen[a] (%)	Protein Fraction, %					Protein Recovered (%)	Pepsin Digestibility (%)[c]
		Albumins + Globulins	Prolamin	ASG[b]	Glutelin	Residue		
Sorghum								
Raw	1.91	16.1	20.0	44.0	14.7	8.9	103.7	73
Acidic	1.89	7.0	7.0	52.1	7.9	20.8	94.8	59
Basic	1.95	7.0	4.0	46.2	4.0	29.0	90.2	51
Maize								
Raw	1.69	19.1	45.0	21.8	15.3	2.9	104.1	78
Acidic	1.73	11.0	34.0	29.1	5.3	10.7	90.1	70
Basic	1.70	12.2	29.1	32.4	5.0	12.3	91.0	68
Pearl Millet								
Raw	1.57	25.4	47.9	11.4	12.9	3.3	100.9	93
Acidic	1.55	14.0	17.0	24.0	6.0	29.4	90.0	84
Basic	1.56	13.0	11.0	28.3	6.6	34.2	93.1	81
LSD ($P < 0.01$)	0.09	2.6	3.0	2.7	3.7	3.3		4.0

[a] Milligrams of nitrogen per 100-mg sample on dry-weight basis.
[b] Alcohol-soluble glutelins.
[c] Pepsin digestibility values were calculated by dividing the solubilized nitrogen by total nitrogen in sample × 100.

(Table I). The highest digestibility values were observed for pearl millet (flour and *tô*), whereas sorghum had the lowest digestibility values. Lower digestibilities were observed in basic *tô* than in acidic *tô*. Ejeta et al (1987) also observed higher digestibility of protein from pearl millet than from sorghum. The significant reduction in sorghum digestibility is attributed to prolamins and ASG complexing with starch during cooking (Hamaker et al 1986; Bach-Knudsen and Munck 1985, 1988; Bach-Knudsen et al 1988; Rom et al 1992). In vivo protein digestibility of sorghum decreased while the biological value increased after cooking in neutral and acidic conditions (Bach-Knudsen and Munck 1985, 1988; Bach-Knudsen et al 1988).

CONCLUSIONS

The distributions of proteins in pearl millet and maize were similar, e.g., prolamins between 45 and 48% and ASG between 11 and 22%. Sorghum had 20% prolamins and 44% ASG. Albumins plus globulins, prolamins, and glutelins decreased during preparation into acidic or basic *tô*. ASG and residue proteins generally increased during processing. Basic *tô* samples prepared from all cereals had more ASG and residue proteins than did acidic *tô*.

Proteins in sorghum and pearl millet were more affected by processing than were maize proteins. Processing did not change the molecular weights of most proteins that were retained within each fraction. Some proteins became increasingly cross-linked via disulfide bonds during processing into *tô*. More shifting of intensity and molecular weight was observed in albumins plus globulins than in other protein extracts. Prolamins from sorghum (basic *tô*) and from pearl millet (acidic and basic *tô*) decreased in intensity during processing. Some glutelin proteins increased in intensity after processing.

Lower in vitro protein digestibility was observed for *tô* prepared from sorghum than for *tô* from maize or pearl millet. Pearl millet samples had higher protein digestibilities than did maize samples. These results support the postulate that prolamins and ASG in sorghum complex with starch, forming a network that is not accessible for enzyme attack.

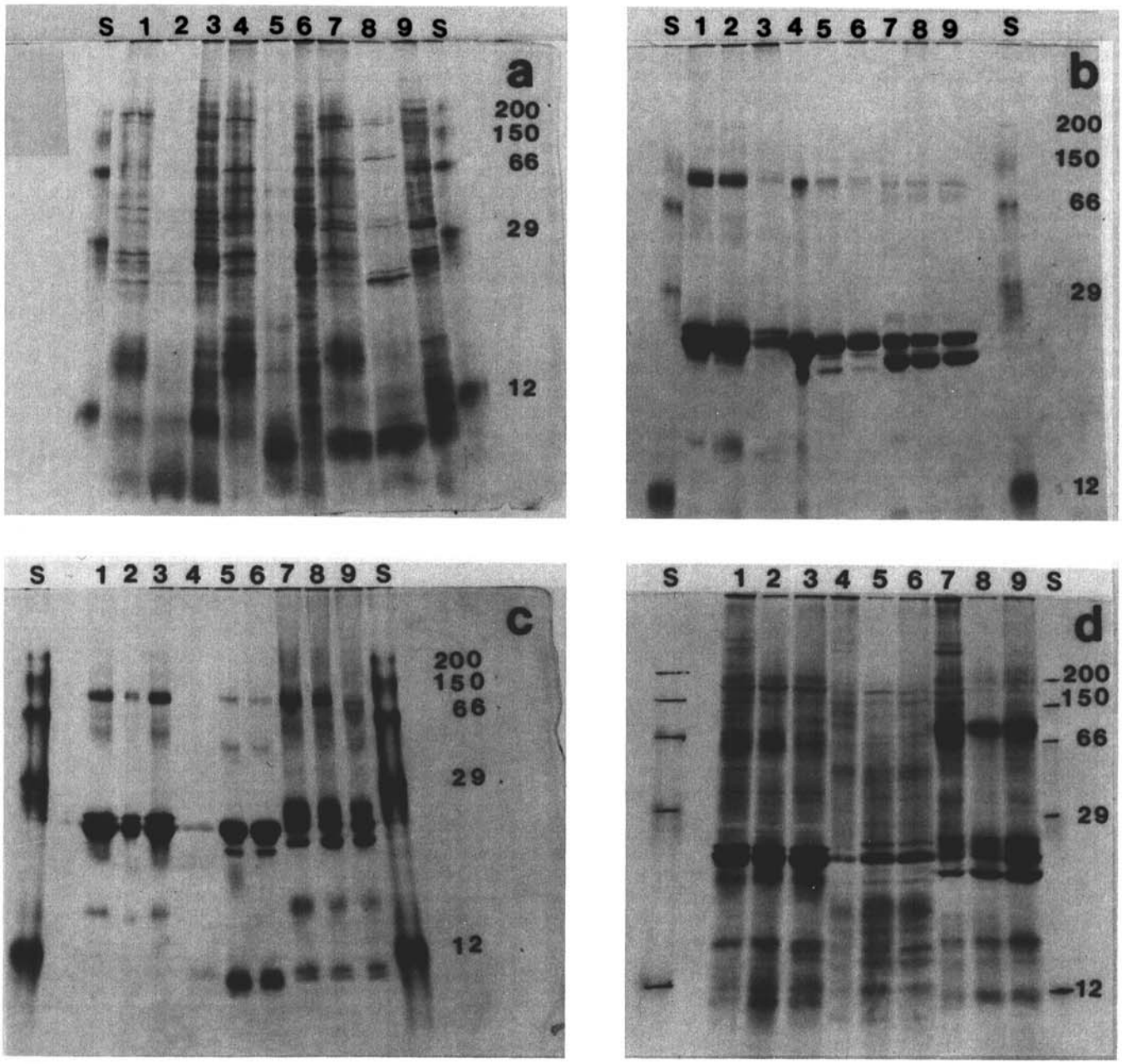

Fig. 1. Sodium dodecylsulfate-polyacrylamide electrophoresis separations of albumins plus globulins **(A)**, prolamins **(B)**, alcohol soluble glutelins **(C)**, and glutelins **(D)** from raw grain (well labels = 1, 4, 7), acidic *tô* (2, 5, 8), basic *tô* (3, 6, 9) samples from sorghum (1–3), pearl millet (4–6), and maize (7–9), and proteins with known molecular weight (S, numbers beside gels, kiloDaltons).

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