Effect of Tortilla Production on Proteins in Sorghum and Maize[1]

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ABSTRACT

The solubility and molecular weight distribution of sorghum and maize proteins were determined during tortilla preparation. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis patterns were obtained for four protein fractions (albumin and globulin, prolamins, alcohol-soluble reduced glutenins, and glutenins) from raw grain, *nixtamal*, masa, and tortilla samples of sorghum and maize. Alkaline processing decreased the solubility of proteins in salt water and alcohol and increased the amount of unextractable proteins. The number and intensity of protein bands in the electrophoretic patterns changed after processing for both grains, with more changes observed in sorghum than in maize samples. Prolamins were more efficiently extracted with 60% *tert*-butyl alcohol than with 70% isopropyl alcohol. Pepsin digestibility of protein in sorghum and maize samples decreased after processing, with sorghum having slightly lower digestibilities than maize. Processing sorghum and maize into tortillas significantly affected protein solubility and structure.

Sorghum is a staple food grain in many regions of Africa (Vogel and Graham 1978, Rooney et al 1986). In Central America, sorghum is used to prepare tortillas, which are traditionally prepared from maize (Futrell and Jones 1982, Murty et al 1982). Ortega et al (1986) studied the effect of alkaline cooking on maize proteins during tortilla preparation. However, the effect of alkaline processing on sorghum proteins is poorly understood. Sorghum proteins, as in other cereals, are characterized by their solubility in water (albumin), salt solution (globulins), alcohol (prolamins), alcohol with reducing agent (alcohol-soluble reduced glutenins), and alkali detergents (glutenins) (Virupaksha and Sastry 1968). The major storage proteins of sorghum were more difficult to extract than similar maize proteins because of their many disulfide cross-linkages and their more hydrophobic nature (Paulis and Wall 1979, Taylor et al 1984). Hamaker et al (1986) reported that solubility of sorghum and maize albumins, globulins, and prolamins decreased after cooking the flour in water (neutral pH), and the amount of prolamins solubilized was less for sorghum than for maize. They also reported similar pepsin digestibility values for uncooked sorghum and maize proteins; however, after cooking, pepsin solubilized fewer proteins from sorghum than from maize. When sorghum flour was cooked under acidic conditions (pH 3.0; Kirleis 1985), more prolamin and less glutenin proteins were solubilized. As a result, the pepsin digestibility of acidic gruel was 65% compared with 51% for neutral gruel from sorghum.

The objectives of this research were to determine the effects of alkaline cooking (nixtamalization) on sorghum and maize proteins by solubility fractionation, electrophoresis, and pepsin hydrolysis. Proteins of processed sorghum and maize (*nixtamal*, masa, and tortillas) were characterized.

MATERIALS AND METHODS

Sorghum (ATx623× CS3541 with white pericarp, no pigmented testa, and about 20% floury endosperm) and white maize (Asgrow

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405W with white pericarp, no testa, and about 20% floury endosperm) were used to make tortillas. The raw grains and their products from tortilla preparation (*nixtamal*, masa, and tortillas) were used to study protein changes during processing (cooking in alkali, grinding, and baking). All samples, including raw grain, were lyophilized for 48 hr, milled into flour in a Udy laboratory cyclone mill with a 1.0-mm screen mesh, and stored frozen in plastic bags.

The conditions for sorghum *nixtamal* preparation in the laboratory were as follows: a ratio of grain to water of 1:2.5 (w/v), 1.0% calcium oxide (CaO), cooking time of 20 min after placing the grain into boiling water, and a steep time of 4 hr. *Nixtamal* from maize was prepared in a commercial plant in Houston, TX, using the following conditions: a ratio of grain to water of 1:1.85 (w/v), 6.25% CaO, steam injected into cook tank until a temperature of 83° C was reached (about 0.5 hr), 0.5 hr cooling time to 65° C, and overnight steeping. Sorghum and maize *nixtamals* were washed and ground into masa in a stone grinder, then the masa was shaped and baked into tortillas.

Preparation of Protein Extracts

Proteins were fractionated using the method reported by Paulis et al (1975) and Paulis and Wall (1979) with slight modifications. Four sequential fractions or extracts were obtained from flours (1.5 g) for all samples. Three grams of glass beads (1.0 mm diam) were added to each tube before protein extraction. Each fraction was extracted two times, the first for 2 hr and the second for 1 hr, using the same ratio of solvent to flour (6:1, w/v) each time. All extracts were centrifuged at 10,000 × *g* for 20 min, and the clear supernatant was analyzed. The albumins and globulins (fraction I) were extracted with 0.5*M* NaCl by shaking at 4° C. Prolamins (kafirins or zein, fraction II) were extracted with 60% *tert*-butyl alcohol by shaking at room temperature. The prolaminlike fraction (fraction III; or alcohol-soluble reduced glutenins, ASG) was extracted with a mixture of 60% *tert*-butyl alcohol or 70% isopropyl alcohol and 2% β-mercaptoethanol (β-ME) by shaking at room temperature. The glutenins or sodium dodecyl sulfate (SDS) fraction (fraction IV) was obtained by extracting in a water bath at 50° C with a buffer containing 0.0625*M* Tris (pH 6.8), 2% SDS, and 5% β-ME.

Protein Determination of Extracts

Crude protein (N × 6.25) of sorghum and maize flours and extracts was determined using the micro-Kjeldahl method with an autoanalyzer system (Technicon 1976). Pepsin hydrolysis was determined according to Axtell et al (1981) using 200 mg of flour suspended in 100 ml of 0.1*N* phosphate buffer (pH 2.0) containing 50 mg of pepsin. The solution was incubated at 37° C for 2 hr and centrifuged at 10,000 × *g* for 15 min at 4° C. The supernatant (15 ml) was removed and assayed for solubilized nitrogen. Protein digestibility was calculated by dividing the solubilized nitrogen (after substracting enzyme blank) by the total amount of initial nitrogen.

Dilution of Extracts

A 1-ml sample of each extract from fraction I was placed in a vial with 0.33 ml of 0.25*M* Tris (pH 6.8) containing 8% SDS, 20% β-ME, and 40% glycerol. Fraction I extracts from raw grain flour contained more proteins, hence they were subsequently diluted threefold with a 0.0625*M* Tris (pH 6.8) containing 2% SDS, 5% β-ME, and 10% glycerol. Aliquots of 0.020 ml of extracts from cooked grain flours and 0.015 ml for extracts of raw grain were injected into the gels for electrophoresis.

One-milliliter of extracts from fractions II and III was evaporated before being prepared for electrophoresis. Fraction II proteins were resuspended with 2 ml of 0.0625*M* Tris (pH 6.8) containing 2% SDS, 5% β-ME, and 10% glycerol for raw grain and 1.0 ml of this buffer for cooked grains. Fraction III proteins were resuspended in 3.0 ml of this buffer. Aliquots of fraction II proteins of 0.005 ml for sorghum and 0.015 ml for maize were used for electrophoresis, whereas 0.005-ml aliquots were used for fraction III. Extracts from fraction IV were diluted eightfold with the Tris-SDS buffer and 0.015 ml was used for electrophoresis.

Electrophoresis of Protein Extracts

Discontinuous SDS-polyacrylamide gel electrophoresis (SDS-PAGE) was performed following the procedure of Laemmli (1970). The original method was modified for use in a vertical slab, twin-gel system. Slab gels (14 × 20 cm) with a 12.5% acrylamide concentration were cast to a thickness of 0.75 mm. Between 0.005 and 0.015 mg of protein was injected into 5.0-mm wide wells that could contain up to 0.025 ml of solution. Bromophenol blue was incorporated to the electrode buffer as a marker for electrophoresis. Electrophoresis was conducted in a cold room (4° C), starting with a constant current of 50 mA for the stacking gel and 65 mA constant current for the resolving gel. Electophoresis was completed after 6–8 hr.

The gels were then removed from the slabs and placed in a solution containing acetic acid, methanol, and water (12:50:38, v/v) overnight. Proteins were visualized by the silver-staining method of Goldman (1981). The proteins used as molecular weight (MW) standards were: cytochrome C (12,400), carbonic anhydrase (29,000), bovine serum albumin (66,000), alcohol dehydrogenase (150,000), and α-amylase (200,000). The molecular weight of proteins was estimated from the log-log plot of relative mobility versus molecular weight of protein standards.

RESULTS AND DISCUSSION

Protein solubility of sorghum and maize was affected by alkaline processing into tortillas (Table I). The amounts of protein in fractions I (albumin and globulin) and II (prolamins) were reduced after processing (cooking in alkali to obtain *nixtamal*, grinding into masa, and baking into tortillas). Quantities of protein fraction III decreased for sorghum and increased for maize, whereas values of fraction IV proteins remained constant for both grains after processing. An increased amount of protein was observed in the residue after processing, apparently because of losses in solubility

TABLE I
Protein Solubility Distribution of Sorghum and Maize During Tortilla Preparation

Sample	Total Nitrogen[a] (%)	Percent of Total Nitrogen Extracted					Protein Recovery
		Fraction I	Fraction II	Fraction III	Fraction IV	Residue	
Sorghum							
Raw	1.40	24.3	15.2	36.8	15.7	7.9	99.2
Nixtamal	1.49	7.1	6.1	38.5	16.1	20.8	88.6
Masa	1.47	6.9	6.3	37.7	15.6	20.3	86.8
Tortilla	1.44	7.2	4.8	26.5	17.3	33.4	89.2
Maize							
Raw	1.50	23.9	33.1	19.6	15.3	2.4	94.3
Nixtamal	1.53	8.4	15.2	29.9	16.3	15.4	85.2
Masa	1.56	8.2	20.0	30.5	15.7	15.7	90.1
Tortilla	1.55	7.9	13.8	31.3	15.4	28.9	97.3

[a] Milligrams of nitrogen per 100 mg sample on dry weight basis.

of fractions I and II proteins. Extractability of these fractions in sorghum was more affected (lower) than the same fractions in maize.

Changes in solubility of sorghum and maize proteins during nixtamalization were generally similar to data reported by Hamaker et al (1986) and Ortega et al (1986). However, more protein was extracted in this study using *tert*-butyl alcohol in fraction III, and less protein was extracted in fraction IV (Table I). This was observed at all stages of processing. Hence *tert*-butyl alcohol was a better solvent for fraction III proteins in sorghum and maize. Extraction rates of protein from sorghum and maize ranged from 91 to 92% and from 57 to 74% for raw and processed samples, respectively (Table I). Comparable protein extraction rates were reported for raw sorghum (87 to 93%) and raw maize (84 to 95%) (Paulis and Wall 1977a, 1979 Hamaker et al 1986).

The number of bands of fraction I proteins ranged from 18 to 19 for raw and 11 to 13 for processed grain (Fig. 1). The silver-staining method yielded dark bands for relatively small quantities of protein (Fig. 1 and Table II). The combination of 12.5% acrylamide gels and the silver-staining method yielded clearer, more resolved protein bands than previous studies on sorghum and maize proteins (Paulis and Wall 1977b, 1979; Landry et al 1983; Taylor and Schussler 1984). Protein extracts in this study showed a wide molecular mass range, i.e., 13.5 to 620 kDa. The molecular mass of proteins soluble in salt water was reported to be from 22 to 70 kDa (Schechter and deWet 1975, Paulis et al 1975).

The SDS-PAGE patterns of fraction I, II, III, and IV proteins of raw and processed sorghum and maize illustrate the effects of processing on protein solubility (Figs. 1–5). The amount of protein injected into the gels for electrophoresis was similar for each protein fraction (Table II).

Albumins and Globulins

The electrophoretic pattern of fraction I proteins from sorghum and maize and the molecular weights of the major protein bands are presented in Figure 1 and Table III. Losses of proteins soluble in salt water were observed for both sorghum and maize after nixtamalization (Table I). These losses were reflected by the disappearance or loss of intensity of several bands, or both, especially in the low molecular weight region (below 12 kDa). Proteins were probably solubilized and extracted during cooking and steeping in alkaline solution and washing of the *nixtamal*, or the proteins became insoluble in salt water. Hamaker et al (1986) also reported a decrease in albumins and globulins after cooking of sorghum and maize. Some losses of these proteins also have been reported during tortilla preparation from maize (Ortega et al 1986).

Several fraction I proteins were present in raw sorghum and maize that were not present in processed samples (Table III). However, processed sorghum samples also contained several bands not present in the raw grain (Fig. 1 and Table III). Similar electrophoretic patterns were observed among proteins from processed sorghum or maize, except for tortilla samples, which had less intense bands than *nixtamal* or masa.

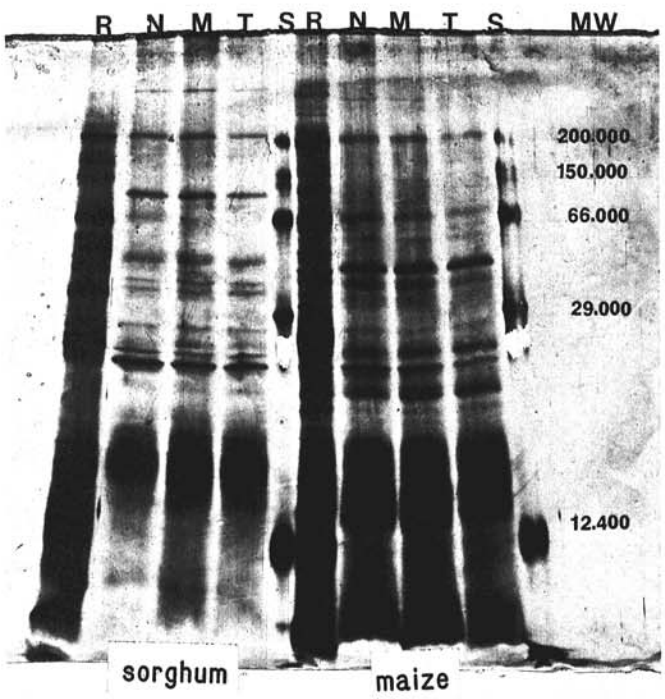

Fig. 1. Sodium dodecyl sulfate-polyacrylamide gel electrophoretic pattern of albumins and globulins (fraction I) from raw grain (**R**), *nixtamal* (**N**), masa (**M**), and tortilla (**T**) samples of sorghum and maize, and standard proteins (**S**) with known molecular weight (**MW**).

Prolamins and Alcohol-Soluble Reduced Glutenins

The molecular weight distribution of prolamins (fraction II) is presented in Figures 2 and 3 and Table IV. Reductions in intensity of fraction II proteins were observed in processed samples compared with raw grain. Raw and processed maize samples contained fraction II proteins with similar molecular weights, whereas processed sorghum had lower bands, e.g., the 14 kDa protein was missing, Hence, alkaline processing not only decreased the solubility of fraction II proteins, it also altered the molecular weight distribution of alcohol-soluble proteins in sorghum samples. Hamaker et al (1986) also reported that a smaller quantity of alcohol-soluble proteins was extracted from sorghum than from maize.

The molecular weight distribution of prolamins extracted with 70% isopropyl alcohol had fewer bands than when 60% *tert*-butyl alcohol was used (Figs. 2 and 3, Table IV). Electrophoretic patterns of raw sorghum fraction II proteins extracted with 70% isopropyl alcohol were similar to those reported by Hamaker et al (1986). However, after cooking sorghum flour, they observed that fraction II proteins were not extractable using 70% isopropyl alcohol. Apparently, *tert*-butyl alcohol is a better solvent than isopropyl alcohol for extracting fraction II proteins.

An increased intensity and a greater number of bands were observed for sorghum and maize fraction III proteins compared with fraction II after alkaline cooking and steeping, and after grinding into masa and baking into tortillas (Fig. 4 and Table V). Several fraction III proteins from sorghum and maize were also less intense in the tortilla sample compared with other processed samples. However, the molecular weight distribution of fraction III proteins in raw sorghum extracted with 70% isopropyl alcohol (Hamaker et al 1986) was different from fraction III proteins extracted with *tert*-butyl alcohol (Fig. 4). Fewer and less intense bands were observed in the isopropyl alcohol extracts.

Prolamins and ASG in raw sorghum (Figs. 2 and 4) had very similar molecular mass and electrophoretic patterns especially between 14 and 93 kDa. Electrophoretic patterns of these fractions

TABLE II
Micrograms of Protein Added to Gels for Electrophoresis

	Fraction				
Sample	**I**	**II[b]**	**II[c]**	**III**	**IV**
Sorghum					
Raw	13.2	8.2	14.7	4.4	2.1
Nixtamal	10.8	6.9	3.8	4.9	2.3
Masa	10.8	7.1	6.6	4.8	2.2
Tortilla	10.6	5.3	4.5	3.3	2.4
Maize					
Raw	13.9	12.8	13.7	2.5	2.2
Nixtamal	13.3	6.0	7.2	3.9	2.4
Masa	13.1	8.0	8.8	4.1	2.3
Tortilla	12.6	5.5	6.5	4.2	2.3

[a] Protein content expressed as N × 6.25.
[b] Fraction extracted with *tert*-butyl alcohol.
[c] Fraction extracted with isopropyl alcohol.

TABLE III
Molecular Weights[a] of Albumins and Globulins (Fraction I) from Sorghum and Maize During Tortilla Preparation

Protein Bands	Sorghum		Maize	
	Raw	Processed[b]	Raw	Processed[b]
1	...	620	450	450[c]
2	260	260	270	...
3	170	...	170	170
4	160	...	160	...
5	130	...	150	...
6	...	87	87	...
7	70	...	67	67
8	...	67	55	55
9	58	...	51	51
10	51	...	47	40
11	47	47	40	40
12	43	...	37	37
13	40	40	31	...
14	36	36	27	27
15	32	...	24	24
16	28	28	21	21[c]
17	...	27	19	19
18	...	26	17	17
19	25	...	15	15
20	24	...	...	...
21	23	23	...	...
22	...	15[c]	...	...
23	14	14[c]	...	...
24	13	...	...	...

[a] Molecular mass in kilodaltons.
[b] Processed samples refers to nixtamal, masa and tortillas.
[c] These proteins were not present in all processed samples.

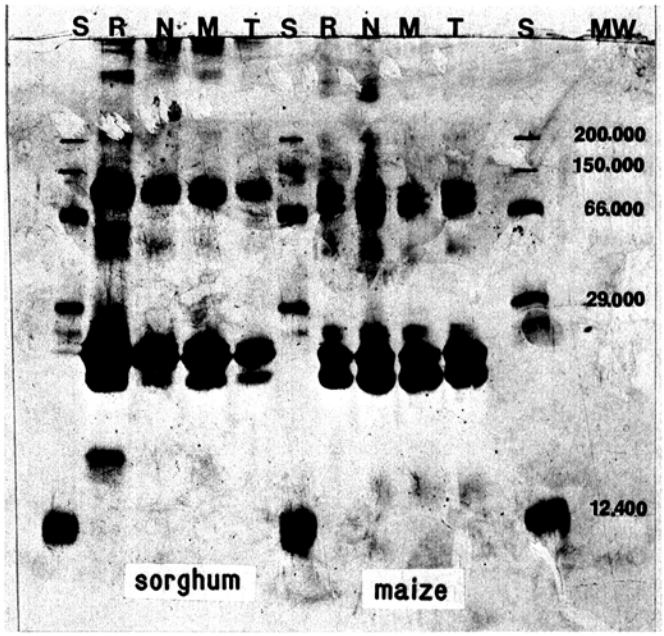

Fig. 2. Sodium dodecyl sulfate-polyacrylamide gel electrophoretic pattern of prolamins (fraction II) extracted from raw grain (**R**), *nixtamal* (**N**), masa (**M**), and tortilla (**T**) of sorghum and maize with 60% *tert*-butanyl alcohol, and standard (**S**) proteins with known molecular weight (**MW**).

TABLE IV
Molecular Weights[a] of Prolamins (Fraction II) from Sorghum and Maize Proteins During Tortilla Preparation

Protein Bands	Extracted with *tert*-Butyl Alcohol				Extracted with Isopropyl Alcohol			
	Sorghum		Maize		Sorghum		Maize	
	Raw	Processed[b]	Raw	Processed[b]	Raw	Processed[b]	Raw	Processed[b]
1	850	850[c]	98	98	...	...	98	...
2	290	...	87	87	...	...	87	87
3	93	93	78	78	93	...	78	78
4	64	...	28	28	...	...	28	...
5	25	25	27	27	25	25	27	...
6	24	24	24	24	24	24	24	24
7	23	...	21	21	...	...	21	21
8	21	...	...	...	21	...	14	...
9	15	...	...	...	14	...	10	...

[a] Molecular mass in kilodaltons.
[b] Processed samples refers to *nixtamal*, masa, and tortilla.
[c] These proteins were not present in all processed samples.

TABLE V
Molecular Weights[a] of Alcohol-Soluble Reduced Glutenins (Fraction III) and Glutenins (Fraction IV) from Sorghum and Maize Proteins During Tortilla Preparation

Protein Bands	Sorghum		Maize		Sorghum		Maize	
	Raw	Processed[b]	Raw	Processed[b]	Raw	Processed[b]	Raw	Processed[b]
1	...	850	98	98	450	450[c]	400	400[c]
2	...	290[c]	87	87	350	350[c]	360	360[c]
3	93	98	...	78	64	64	98	98
4	64	64	...	53	25	25	87	87
5	25	25	...	47	24	24	78	78[c]
6	24	24	28	28	15	15	28	28
7	23	23	27	27	12	12	27	27
8	21	21	24	24	10	10	24	24
9	15	15	21	21	...	...	...	15
10	...	...	15	15	...	...	10	10
11	...	...	10	10	...	...		

[a] Molecular mass in kilodaltons.
[b] Processed samples refers to *nixtamal*, masa, and tortilla.
[c] These proteins were not present in all processed samples.

of raw maize were not as similar, because two low molecular mass bands (10 and 15 kDa) were observed for ASG but were not present in the prolamin fraction. In general the ASG fraction had more bands than prolamins. The ASG gels had more low molecular weight bands and fewer or fainter high molecular weight bands than prolamins. Apparently the reducing agent (β-ME) used to extract ASG dissolved some of the large protein complexes and produced lower molecular weight proteins.

Glutenins

The SDS-PAGE patterns of glutenins from sorghum and maize differed from the those of the other fractions (Fig. 5 and Table V). The molecular weight distribution of glutenins in raw sorghum and maize shifted to higher molecular weights during alkaline processing. Higher molecular weight proteins were observed in *nixtamal* and masa samples compared to the raw grains, whereas fewer and less intense protein bands were generally observed in tortilla samples. This is different than what was observed for fraction I proteins, where a dark smear was observed on the low molecular weight region; for fraction IV proteins the dark smear was observed in the high molecular weight region. Apparently, the protein distributions of fractions I and IV were inversely affected by alkaline cooking.

TABLE VI
In Vitro Pepsin Protein Disgestibility of Sorghum and Maize Flours

Sample	Pepsin Digestibility (%)
Sorghum	
Raw	40.7
Nixtamal	19.7
Masa	22.3
Tortilla	16.3
Maize	
Raw	42.8
Nixtamal	30.6
Masa	30.5
Tortilla	25.0
LSD (0.05)	7.39

[a] Pepsin values were calculated by dividing the solubilized nitrogen by total nitrogen in sample × 100.

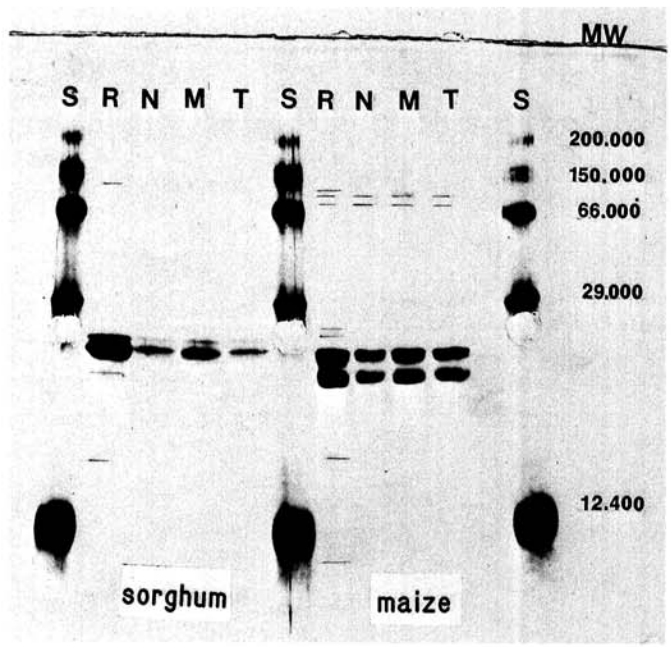

Fig. 3. Sodium dodecyl sulfate-polyacrylamide gel electrophoretic pattern of prolamins (fraction II) extracted from raw grain (**R**), *nixtamal* (**N**), masa (**M**), and tortilla (**T**) samples of sorghum and maize with 70% isopropyl alcohol, and standard (**S**) proteins with known molecular weight (**MW**).

Hamaker et al (1986) reported an increase in intensity of bands between 20 and 24 kDa for fractions IV and V (combined) after cooking sorghum flour in water; whereas similar amounts of these proteins were observed at each stage of processing in the study (Fig. 5).

The residue fraction contained significantly greater amounts of protein during nixtamalization (and grinding) and during baking into tortillas (Table I). The residue proteins were not separated by

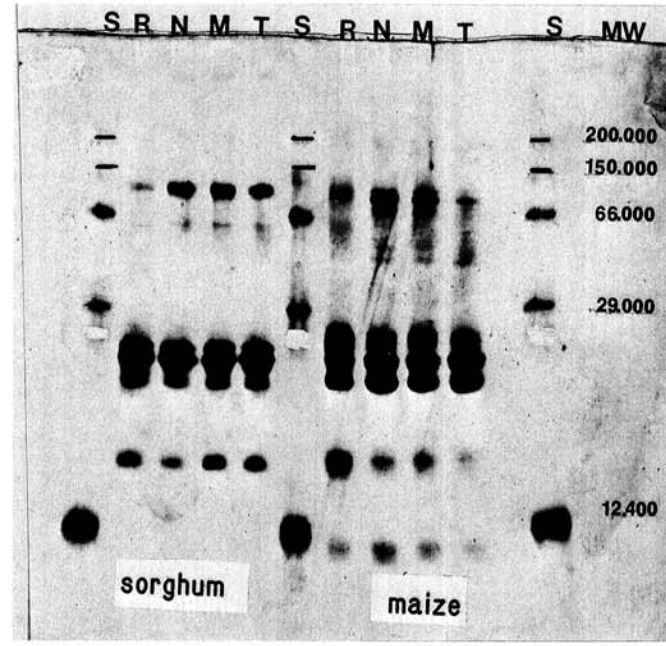

Fig. 4. Sodium dodecyl sulfate-polyacrylamide gel electrophoretic pattern of alcohol-soluble reduced glutenins (fraction III) from raw grain (**R**), *nixtamal* (**N**), masa (**M**), and tortilla (**T**) samples of sorghum and maize, and standard (**S**) proteins with known molecular weight (**MW**).

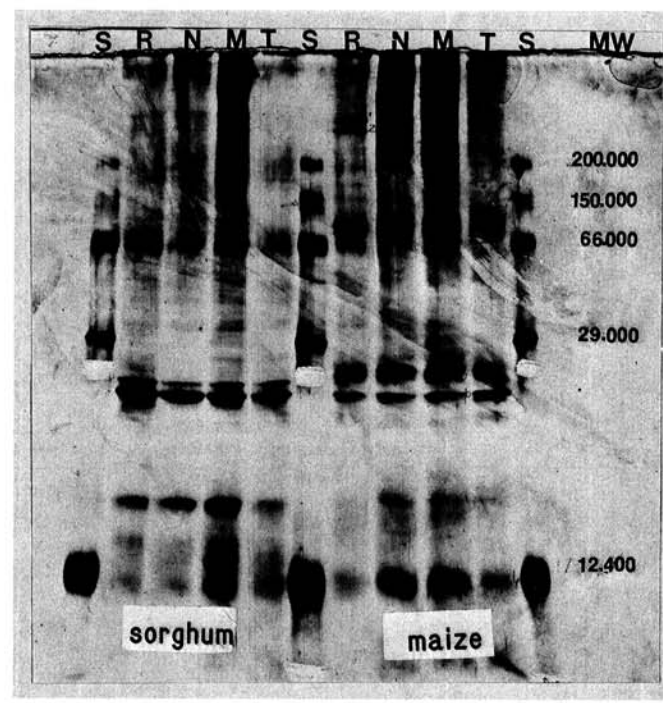

Fig. 5. Sodium dodecyl sulfate polyacrylamide gel electrophoretic pattern of glutenins (fraction IV) from raw grain (**R**), *nixtamal* (**N**), masa (**M**), and tortilla (**T**) samples of sorghum and maize, and standard (**S**) proteins with known molecular weight (**MW**).

SDS-PAGE, as they were not soluble in the gel or sample buffers. However, these proteins had a higher molecular weight or they were more chemically crosslinked than fraction IV proteins.

Sorghum Versus Maize

The protein solubility distributions of raw and processed sorghum samples were different from those of maize (Table I). However, both sorghum and maize had decreased amounts of fraction I and II proteins and increased residue proteins after nixtamalization, grinding, and baking into tortillas. The SDS-PAGE patterns of sorghum and maize were different for each solubility fraction. For example, a low molecular mass protein (15 kDa) was observed in fraction II of raw sorghum but not of raw maize. Protein patterns for fractions II and III of raw sorghum were similar as were the patterns for raw maize. Paulis and Wall (1979) also found similarities between the SDS-PAGE patterns for fractions II and III of raw sorghum when they extracted with 60% *tert*-butyl alcohol. They concluded that these two fractions have similar polypeptide constituents.

Pepsin Digestibility of Protein

Protein digestiblity of raw and processed sorghum and maize flours were assessed using pepsin. The values for in vitro protein digestibility revealed that processing (alkali cooking, grinding, and baking) significantly reduced the digestibility of sorghum and maize proteins (Table IV). Digestibility of sorghum proteins after processing was apparently more affected than maize proteins. The formation of protein complexes during cooking probably made the proteins less soluble and less available for enzyme attack. Hamaker and co-workers (1986) also reported reductions in pepsin digestibility of sorghum and maize after cooking under neutral conditions. They also found decreased digestibilities using a multiple enzyme method and related this reduction of protein digestibility to losses of protein solubility.

CONCLUSIONS

Solubility of sorghum and maize proteins was affected by cooking in alkali, steeping, grinding, and baking into tortillas. The solubilily of fraction I and II proteins decreased during processing, while the amounts of protein in the residue of processed samples increased. In vitro protein digestibility (using pepsin) of sorghum and maize decreased after alkali cooking.

Alkali processing also affected the molecular weight distribution of raw sorghum and maize proteins. The number and intensity of protein bands decreased after processing. Also, higher molecular weight glutenin proteins were observed for some of the processed sorghum and maize samples. Hence, alkali cooking of sorghum and maize produced structural molecular weight and solubility changes in sorghum and maize proteins. This includes the formation of cross-linkages and the disruption of tertiary structures stabilized by disulfide bonds or noncovalent attractive forces (Paradez and Saharopulas 1982). Apparently, alkaline processing had a more pronounced effect on the molecular weight and solubility of sorghum proteins than of maize proteins.

Prolamins of sorghum and maize were more completely extracted with *tert*-butyl alcohol than with isopropyl alcohol, especially after processing.

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