McMillan, M.; Thompson, J. C. "An Outbreak of Suspected Solanine Poisoning in School Boys". *Q. J. Med.* **1979**, *190*, 227.
Mulder, E. G. "Importance of Molybdenum in the Nitrogen Metabolism of Microorganisms and Higher Plants". *Plant Soil* **1948**, *1*, 94.
Mulder, E. G. "Molydenum in Relation to Growth of Higher Plants and Microorganisms". *Plant Soil* **1954**, *5*, 368.
Nair, P. M.; Behere, A. G.; Ramaswamy, N. K. "Glycoalkaloids of *Solanum tuberosum*". *J. Sci. Ind. Res.* **1981**, *40*, 529.
Patil, B. C.; Sharma, R. P.; Salunkhe, D. K.; Salunkhe, K. "Evaluation of Solanine Toxicity". *Food Cosmet. Toxicol.* **1972**, *10*, 395.
Phillips, W. E. "Naturally Occurring Nitrate and Nitrite in Foods in Relation to Infant Methemoglobinemia". *Food Cosmet. Toxicol.* **1971**, *9*, 219.
Possingham, J. V. "The Effect of Molybdenum on the Organic and Inorganic Phosphorus of Plants". *Austr. J. Biol. Sci.* **1954**, *7*, 221.
Possingham, J. V. "The Effect of Mineral Nutrition on the Content of Free Amino Acids and Amides in Tomato Plants. I. A Comparison of Effects of Deficiencies of Copper, Zinc, Manganese, Iron and Molybdenum". *Austr. J. Biol. Sci.* **1956**, *9*, 539.
Sinden, S. L.; Deahl, K. L.; Aulenbach, B. B. "Effect of Glycoalkaloids and Phenolics on Potato Flavor". *J. Food Sci.* **1976**, *41*, 520.
Sinden, S. L.; Sanford, L. L.; Webb, R. E. "Genetic and Environmental Control of Potato Glycoalkaloids". *Am. Potato J.* **1984**, *61*, 141.
Steel, R. G. D.; Torrie, J. H. *Principles and Procedures of Statistics*; McGraw-Hill: New York, 1980.
Steinberg, R. A. "Metabolism of Inorganic Nitrogen by Plants". In *Organic Nitrogen Metabolism*; McElroy, W. D., Glass, B., Eds.; 1956, Johns Hopkins University: Baltimore, MD, 1956; p 153.
Timashov, N. D. "Effect of Foliar Nutrition of Potato Plant by Molybdenum and Nickel on Some Metabolic Processes". *Fiziol. Rast. (Moscow)* **1959**, *6*, 354.
Ulrich, A.; Rieri, D.; Hills, F. J.; George, A. G.; Moore, M. D.; Johnson, C. M. "Analytical Methods for Use in Plant Analysis". *Bull.—Calif. Agric. Exp. Stn.* **1959**, *No. 766*.
Walters, C. L.; Carr, F. P. A.; Dyke, C. S.; Saxby, M. J.; Smith, P. L. R. "Nitrite Sources and Nitrosamine Formation in Vitro and in Vivo". *Food Cosmet. Toxicol.* **1979**, *17*, 473.
White, J. W., Jr. "Relative Significance of Dietary Sources of Nitrate and Nitrite". *J. Agric. Food Chem.* **1975**, *23*, 886.
Zitnak, A. "Photoinduction of Glycoalkaloids in Cured Potatoes". *Am. Potato J.* **1981**, *58*, 415.

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Effect of Autofermentation on the Physicochemical Properties of Proteins of Sorghum–Groundnut Composite Flour

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The effect of autofermentation on the physicochemical properties of proteins of sorghum–groundnut (70:30 by weight) composite flour is studied by sedimentation velocity measurements, gel filtration on ACA-44, polyacrylamide gel electrophoresis at pH 9.2, spectral measurements, fluorescence spectra, and circular dichroism measurements. Sedimentation velocity measurements, gel filtration, and PAGE suggest a decrease in the molecular size of the proteins due to autofermentation. Both groundnut and sorghum proteins are equally susceptible to proteolysis during fermentation. The spectral measurements indicate increased polyphenol–protein interactions, conformational changes in the proteins resulting in an altered chromophoric environment of aromatic amino acids, and a decrease in the content of the limited ordered structure.

Fermented sorghum-based foods are prepared traditionally in Africa for human consumption (Vogel and Graham, 1979). Studies on Kisra bread, one of the traditional sorghum-based foods of Sudan, revealed that Kisra is nutritionally inadequate in terms of promoting growth or maintaining nitrogen balance (Eggum et al., 1983; Ahmed et al., 1987). An addition of 30% edible groundnut flour to sorghum meal was found to produce significant improvement in conventional Kisra bread (Ahmed et al., 1987). The proteins of the fermented composite flour differ from that of fermented sorghum flour in their functional characteristics (Ahmed and Ramanatham, 1987b) as well as their digestibility (Ahmed et al., 1987; Ahmed and Ramanatham, 1987c). In the present investigation the changes that occur in the physicochemical properties of the proteins during autofermentation of sorghum–groundnut composite flour were studied.

MATERIALS AND METHODS

Locally purchased sorghum grain was cleaned, dried, and milled to pass through 60-mesh sieve to obtain sorghum flour. The edible groundnut flour (EGF) was prepared by further defatting of the expeller-pressed groundnut cake (TGL, Adoni, A.P., India) with hexane and removing traces of solvent by hot air (48 ± 2 °C). The dried material was ground into fine powder.

Ultrogel ACA44 (ACA44) was a product of LKB Chemicals; acrylamide, amido black, and ammonium persulfate were from E. Merck; *N,N,N′,N′*-tetramethylethylenediamine (TEMED) was from Fluka; bisacrylamide was from Koch-Light Laboratories Ltd. All other chemicals and reagents were analytical grade (BDH; Sarabhai M. Chemicals).

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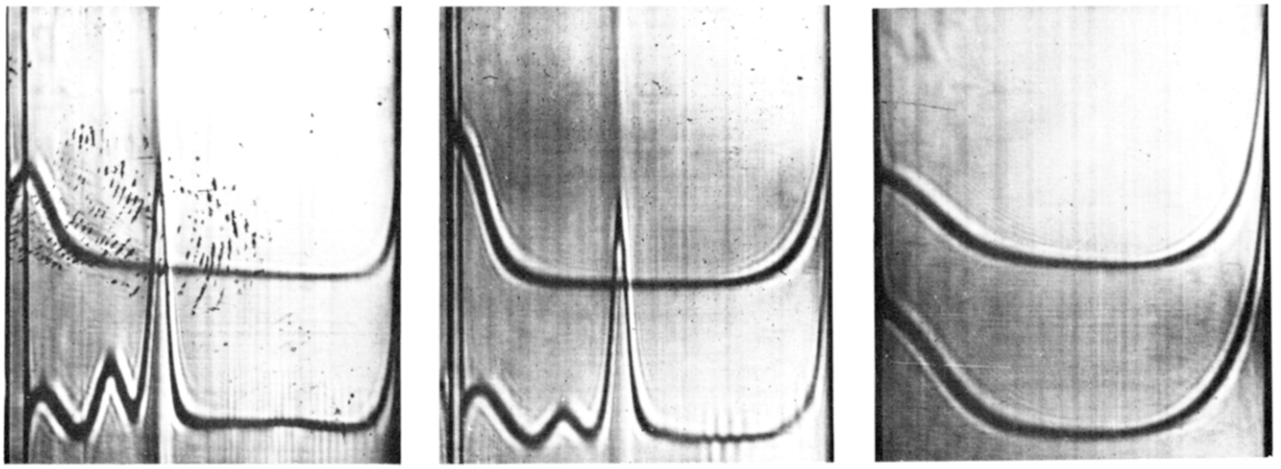

Figure 1. Sedimentation velocity pattern of total proteins (0.02 M glycine/NaOH buffer, pH 9.2): (a) nonfermented sorghum proteins (top) and groundnut proteins (bottom); (b) nonfermented composite flour proteins (bottom) and fermented composite flour proteins (24 h) (top); (c) fermented sorghum proteins (24 h) (top) and fermented sorghum proteins (45 h) (bottom). Sedimentation proceeds from left to right.

Fermentation. A composite flour containing 70 parts of sorghum meal (SM) and 30 parts of EGF was naturally fermented (natural flora present in the meal) according to standardized conditions of Ahmed and Ramanatham (1987a). The ratio of water to flour in the blend was 3:1 and in the control (SM) was 2:1 due to differences in water absorption capacities of the two systems. The conditions briefly involve autofermentation at 37 °C of sorghum meal and composite flour for 45 and 24 h, respectively. For comparative reasons a batch of sorghum meal was fermented for 24 h. Fermented slurries were freeze-dried and ground into fine material. All measurements in this work were carried out on defatted nonfermented and fermented materials.

Extraction of Proteins. Proteins were extracted from fermented and nonfermented material in 0.02 M glycine/NaOH buffer of pH 9.2 with a solvent to solute ratio of 5:1. The suspension was centrifuged at 6000 rpm for 30 min and supernatant dialyzed against the same buffer containing 0.002% NaN_3. The dialyzed material was lyophylized and stored in a desiccator for all experiments.

Protein Concentration. Absorbance of different dilutions at 280 nm was measured against their protein content (N × 6.25), and hence $E^{1\%}_{1cm}$ of each protein was established.

Ultracentrifugation. Analytical ultracentrifugation was carried out on a Beckman Model E analytical ultracentrifuge fitted with phase plate Schlieren optics and rotor temperature indicator and control (RTIC) unit. Runs were performed at 59 780 rpm using a Kel F cell centerpiece (4°, 12 mm) at 27 °C with 1% protein solution in 0.02 M glycine/sodium hydroxide buffer (pH 9.2). Photographs were taken at regular intervals. Plates were read on a Gaertner microcomparator, and $S_{20,w}$ values were calculated by the standard procedure (Schachman, 1959).

Gel Filtration. A 2-mL aliquot of protein (40–60 mg) in glycine/sodium hydroxide buffer (0.02 M, pH 9.2) was fractionated on a column (1.8 × 88 cm) of Ultrogel ACA44 equilibrated with 0.02 M glycine/sodium hydroxide buffer (pH 9.2) containing 0.02% sodium azide. Fractions of 3 mL were collected in an automatic fraction collector (Fraction 300), and the absorbance of the fractions was monitored at 280 nm. The void volume (V_0) of the column was determined with blue dextran (MW 20 × 10^5), and the internal volume (V_i) was determined with ε-DNP-lysine. The apparent MW of fractions was measured from a graph relating log MW versus V_e/V_0 of the MW markers bovine serum albumin (66 000), ovalbumin (45 000), trypsin (23 300), and β-lactoglobulin (18 400).

Polyacrylamide Gel Electrophoresis (PAGE). PAGE was carried out according to Davis (1964) using 7.5% gels in 0.02 M glycine/sodium hydroxide buffer (pH 9.2). Proteins (90–150 μg) dissolved in the same buffer with sucrose and tracking dye were loaded on the gels. Electrophoreses were carried out in the same buffer. Gels were stained with amido black solution and destained in acetic acid solution (7.5%). The gels were later scanned in a Beckman Model 26 scanner.

Absorption Spectra. Ultraviolet (UV) absorption spectra of proteins (0.8–1.2 OD) in glycine/sodium hydroxide buffer (0.02 M, pH 9.2) were recorded at room temperature (~26 °C) in the range 220–420 nm in a Beckman DU-7 spectrophotometer.

Fluorescence Spectra. The fluorescence spectra of proteins (0.18 OD) in glycine/sodium hydroxide buffer (0.02 M, pH 9.2) were measured in a Perkin-Elmer Hitachi spectrofluorophotometer, Model 203, at 26 °C. The emission spectra were measured in the range 290–380 nm after excitation at 285 nm.

Far-UV CD. The far-ultraviolet circular dichroism measurements were made at room temperature (27 °C) in a Jasco J20C automatic recording spectropolarimeter calibrated with camphor-d_{10}-sulfonic acid. Solutions of proteins (0.2–0.3 mg/mL) were used with a cell of 1.0-mm path length, and measurements were taken in the wavelength range 200–260 nm. Mean residue ellipticities (θ_{MRW}, deg·cm^2/dmol) were calculated by the method of Adler et al. (1973).

RESULTS AND DISCUSSION

The sedimentation velocity patterns of proteins appear in Figure 1. Sorghum proteins (Figure 1a, top) sedimented as one peak with $S_{20,w}$ value of 1.5 S. The proteins of edible groundnut flour sedimented as three components with $S_{20,w}$ values of 12.4, 8.3, and 1.9 S (Figure 1a, bottom). The proteins of sorghum-ground composite flour sedimented as three components with $S_{20,w}$ values of 12, 8, and 1.8 S (Figure 1b, bottom). The $S_{20,w}$ values for the proteins in composite flour did not change, suggesting the lack of protein–protein interactions among sorghum and groundnut proteins. After fermentation, a sedimentation coefficient of <1S for both composite flour proteins (Figure 1b, top) and sorghum proteins (Figure 1c, top and bottom) was observed. The considerable reduction in molecular

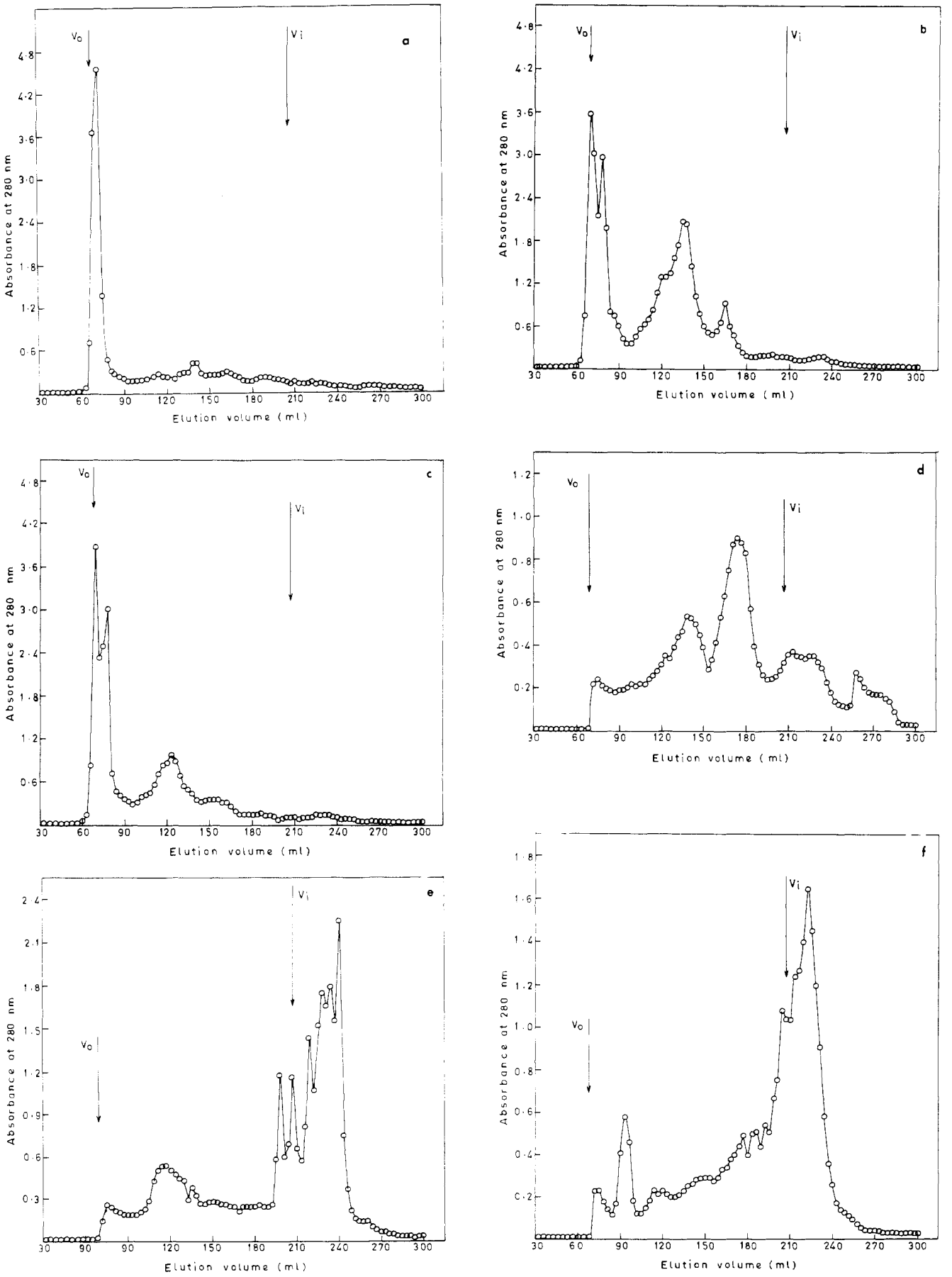

Figure 2. Gel filtration pattern of total proteins (0.02 M glycine/NaOH buffer, pH 9.2): (a) nonfermented sorghum proteins; (b) groundnut proteins; (c) composite flour proteins; (d) fermented sorghum proteins (24 h); (e) fermented sorghum proteins (45 h); (f) fermented composite flour proteins (24 h).

Table I. Apparent Molecular Weights of Degradation Products

	highest	lowest
sorghum (24-h fermentation)		
V_e/V_0	1.09	2.52
% area	4.8	33.2
MW	43100	11200
sorghum (45-h fermentation)		
V_e/V_0	1.04	2.96
% area	2.1	33.3
MW	46200	7100
composite flour (24-h fermentation)		
V_e/V_0	1.08	3.0
% area	5.2	14.2
MW	44600	7100

size of proteins of fermentation may well indicate that sorghum proteins as well as proteins derived from groundnut-like arachin and conarachin II are equally susceptible to proteolysis.

Gel Filtration. Sorghum proteins were excluded from the column as one major component with a V_e/V_0 of 1.0. The groundnut proteins were resolved into four major components with V_e/V_0 of 1.0, 1.13, 1.74, and 1.96 (Figure 2b). The composite flour proteins were eluted at V_e/V_0 values of 1.0, 1.13, 1.8, and 2.1 (Figure 2c). On fermentation for 24 h, sorghum proteins eluted into five fractions with V_e/V_0 of 1.0, 1.43, 1.78, 2.0, and 2.52 (Figure 2d). Extending the fermentation time of sorghum meal to 45 h resulted in further degradation of the proteins with their V_e/V_0 being 1.0, 1.35, 1.65, 1.74, 2.13, 2.56, 2.69, 2.78, and 2.96 (Figure 2e). Similar degradation of proteins of the composite flour was observed on fermentation for 24 h. The V_e/V_0 values of the degradation products were 1.0, 1.65, 1.96, 2.13, 2.48, 2.65, 2.87, and 3.0 (Figure 2f). The apparent molecular weights of the degradation products (Table I) may indicate that groundnut proteins appeared more susceptible to proteolysis than sorghum proteins when both are fermented under similar conditions. The presence of polyphenols in sorghum has influenced the elution pattern after fermentation. This was observed with degradation products occurring beyond the internal volume of the gel ($V_e > V_i$).

Electrophoresis (PAGE). The PAGE patterns at pH 9.2 0.02 M glycine/sodium hydroxide buffer are shown with their respective densitometric tracings in Figure 3 (migration from right to left). The nonfermented sorghum proteins (Figure 3a) could be resolved in two major bands along with some basic proteins that did not enter the gel. The groundnut proteins were resolved into five components (Figure 3b). Due to overlapping of protein components, the composite flour proteins could be resolved into four components (Figure 3c). Concentration differences could have caused the apparent elimination of one band. After 24-h fermentation, sorghum proteins gave one faint band (Figure 3d). Extending the fermentation time of sorghum meal to 45 h resulted in complete degradation of proteins (Figure 3e). At the end of 24-h fermentation of composite flour, there is very little unhydrolyzed native protein (Figure 3f). Both sorghum and groundnut proteins are equally hydrolyzed, resulting in fast-moving not well-resolved components. The degradation process in proteins as a result of fermentation has been reported in some food systems (Padhye and Salunkhe, 1978; Canella et al., 1985).

UV Spectra. The UV spectra of the proteins were recorded between 420 and 220 nm (Figure 4-1). The sorghum proteins exhibit two maxima, one around 340 nm and another around 260 nm, suggesting the presence of phenolic compounds and nucleic acids along with proteins.

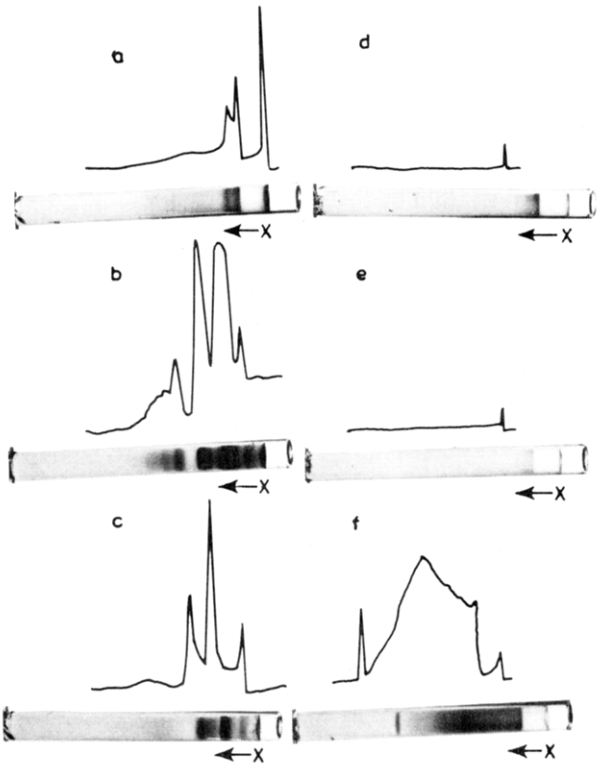

Figure 3. Polyacrylamide gel electrophoresis of total proteins (0.02 M glycine/NaOH, pH 9.2): (a) nonfermented sorghum proteins; (b) groundnut proteins; (c) composite flour proteins; (d) fermented sorghum proteins (24 h); (e) fermented sorghum proteins (45 h); (f) fermented composite flour proteins (24 h).

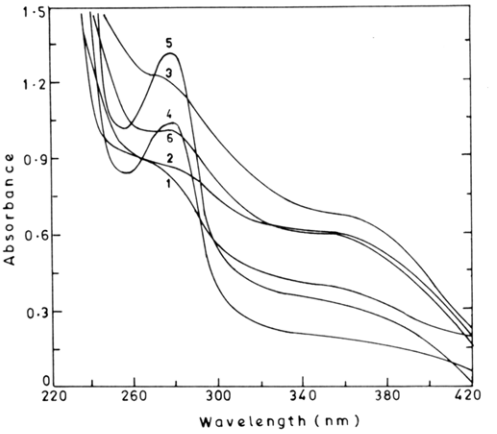

Figure 4. Ultraviolet absorption spectra of total proteins (0.02 M glycine/NaOH buffer, pH 9.2): (a) sorghum proteins; (2) fermented sorghum proteins (24 h); (3) fermented sorghum proteins (45 h); (4) groundnut proteins; (5) composite flour proteins; (6) fermented composite flour proteins (24 h).

The groundnut proteins (Figure 4-4) exhibit λ_{max} around 277 nm, with absorption around 340 nm suggesting little contamination by nucleic acids and phenolic compounds. The blend of the two proteins (composite flour) gave λ_{max} around 277 nm with increased absorption at 340 nm (Figure 4-5). Fermentation of sorghum meal for 24 h caused a shift in λ_{max} of peak 2 to longer wavelength, and the absorption at 340 nm increased (Figure 4-2). Extending the fermentation time to 45 h has further increased the absorption at 340 nm (Figure 4-3). Similarly by fermentation of composite flour to 24 h, the λ_{max} of its proteins shifted toward longer wavelengths and showed an increased absorption at 340 nm (Figure 4-6). The increase in absorption around 340 nm of all proteins after fer-

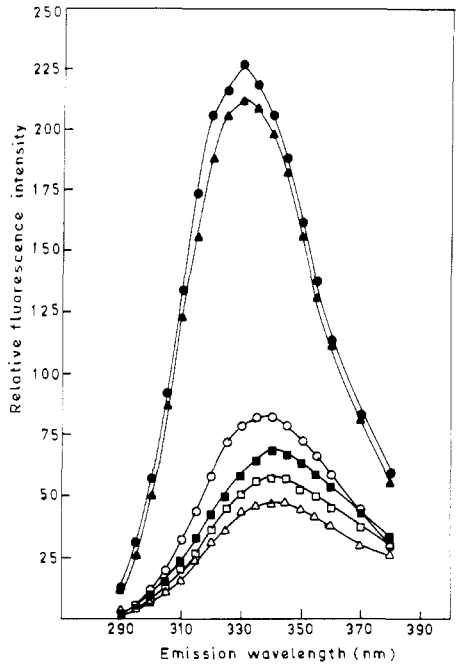

Figure 5. Fluorescence emission spectra of total proteins (0.02 M glycine/NaOH buffer, pH 9.2): sorghum proteins, ○; groundnut proteins, ●; composite flour proteins, ▲; sorghum fermented for 24 h, △; sorghum fermented for 45 h, □; composite flour fermented for 24 h, ■.

mentation could well suggest an increased favored polyphenol-protein interaction. The shift in λ_{max} toward longer wavelengths may be explained by the more polar environment for aromatic amino acids due to fermentation.

Fluorescence Emission. The fluorescence emission spectra of proteins after excitation at 285 nm are shown in Figure 5. Sorghum proteins gave emission maximum at 335 nm, while both groundnut proteins and proteins of composite flour exhibited a maxima at 330 nm. Due to fermentation there is a quench in fluorescence and shift in the emission maxima toward longer wavelengths, suggesting an altered chromophoric environment due to either dissociation, a decrease in molecular size, or conformational changes in proteins.

Circular Dichroism. The conformational changes in proteins due to fermentation were recorded by the CD measurements in the range 200–260 nm (Figure 6). The sorghum proteins have a θ_{MRW} value of −4000° cm^2/dmol at 208 nm, suggesting the occurrence of very little ordered structure. The composite flour proteins have greater ellipticity values compared to sorghum proteins, suggesting more ordered structure (higher content of α-helix and β-structure). The little-ordered structure in these proteins was lost by fermentation. The characteristic decrease in the little-ordered structure of these proteins on fermentation was a sign of conformational changes. From θ_{MRW} values of fermented proteins it appears as if both sorghum and groundnut proteins assumed similar conformations after fermentation.

These physicochemical measurements suggest that due to autofermentation the proteins of sorghum and composite flour are dissociated, degraded, and denatured. Both sorghum and groundnut proteins are equally susceptible to proteolysis during autofermentation. These changes in physicochemical properties of proteins influence the functionality of composite flour proteins in their food formulations.

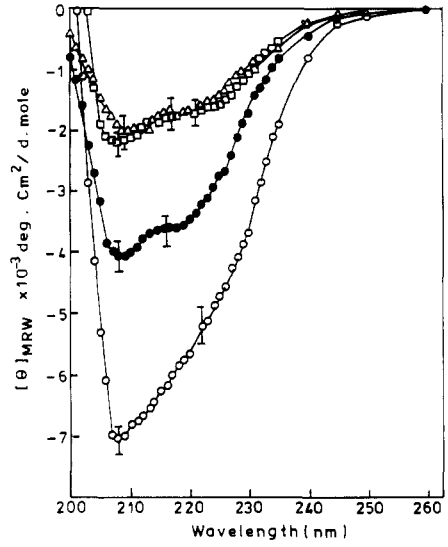

Figure 6. Far-UV-CD spectra of total proteins (0.02 M glycine/NaOH buffer, pH 9.2): sorghum proteins, ●; composite flour proteins, ○; fermented sorghum proteins, △; fermented composite flour proteins, □.

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LITERATURE CITED

Adler, A. J.; Greenfield, N. J.; Fasman, G. D. *Methods Enzymol.* **1973**, *27*, 675–767.

Ahmed, A. R.; Ramanatham, G. "Essential Amino Acid Composition and In Vitro Digestibility of Protein of Sorghum Bread "Kisra" Enriched with Edible Defatted Groundnut Flour". *Nutr. Rep. Int.* **1987a**, *35*, 487–495.

Ahmed, A. R.; Ramanatham, G. "Effect of Natural Fermentation on the Functional Properties of Protein-Enriched Composite Flour". *J. Food Sci.* **1987b**, in press.

Ahmed, A. R.; Chandrasekhara, H. N.; Ramanatham, G. "The Protein Quality of Sorghum Bread "Kisra" Enriched with Edible Defatted Groundnut Flour". *Nutr. Rep. Int.* **1987**, *35*, 205–210.

Canella, M.; Bernardi, A.; Castriotta, G.; Russomanna, G. "Functional Properties of Fermented Sunflower Meal". *Lebensm.-Wiss.-Technol.* **1984**, *17*, 146.

Canella, M.; Castriotta, G.; Bernardi, A. "Functional Characteristics of Germinated Sunflower Meal and Sunflower Fermented after Germination". *Lebensm-Wiss.-Technol.* **1985**, *18*, 24–27.

Davis, B. J. "DISC Electrophoresis II. Methods and Application to Human Serum Proteins". *Ann. N.Y. Acad. Sci.* **1964**, *121*, 404–427.

Eggum, B. O.; Monowar. L.; Backkundsen, K. E.; Munck, L.; Axtell J. "Nutritional Quality of Sorghum and Sorghum Foods from Sudan". *J. Cereal Sci.* **1983**, *1*, 127.

Padhye, V. W.; Salunkhe, D. K. "Biochemical Studies on Black Gram III. Fermentation of the Black Gram and Rice Blend and Its Influence on the *in Vitro* Digestibility of the Proteins". *J. Food Biochem.* **1978**, *2*, 327–347.

Schachman, H. K. In *Ultracentrifugation in Biochemistry*; Academic: New York, 1959; p 242.

Vogel, S.; Graham, M. "Sorghum and Millet; Food Production and Use". Report on workshop held in Nairobi, Kenya, July 1978; IDRC Publication 1979.

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