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Proteins of acha (*Digitaria exilis* Stapf): Solubility fractionation, gel filtration, and electrophoresis of protein fractions

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Proteins from *Digitaria exilis* (acha) and durum wheat were characterised by gel filtration (GF), sodium dodecyl sulphate polyacrylamide-gel electrophoresis (SDS-PAGE), and acid-PAGE. The proteins of acha show compositional and solubility differences from those of durum studied under the same conditions. From the acid-PAGE results, 1M urea extracted the gliadins of wheat. However, a urea concentration of up to 6M was necessary to solubilise acha proteins corresponding to α-gliadins.

The proportion of glutelins and residue proteins in acha was higher than all other Osborne fractions. The amino-acid composition for acha-protein fractions showed significantly greater amounts of hydrophobic and sulphur amino acids than those in durum. The enhanced solubility of acha proteins in urea or propanol (in the presence of a reducing agent) suggests that hydrophobic as well as covalent disulphide interactions are responsible for the relative insolubility of acha proteins in conventional solvents.

SDS-PAGE analysis of wholemeal acha flour revealed that a major component with molecular weight 25·2 kDa, which was absent in durum wheat, forms a basic structural component of acha storage proteins. GF and SDS-PAGE of the four soluble-protein factions for acha proteins (albumin, globulin, prolamin, and glutelin) showed that similar-molecular-weight components (obtained by GF) were relatively heterogeneous by SDS-PAGE.

INTRODUCTION

Cereal proteins can be separated into pure fractions by methods such as, gel-permeation chromatography, pH and salt precipitation, and ultracentrifugation (Bietz & Wall, 1973; Huebner & Wall, 1976; Graveland *et al.*, 1982). A large number of studies have concentrated on the fractionation of wheat, barley, and oats proteins (Kasarda *et al.*, 1976; Schofield & Booth, 1983). The proportion of protein fractions can give insights into the technological uses of cereals.

D. exilis (Stapf) is a cereal indigenous to The Sahel region in Africa (Dalziel, 1937; Anngers, 1973). Its common name is acha, fonio, or hungry rice. It belongs to the grasses family, *Poaceae*, and grows under conditions of low rainfall with relatively high yields (500–800 kg/ha). The thousand-grain size is 0·4–0·5 g. *D. exilis* is relatively less well known than sorghum, which grows in the same arid regions (Rooney *et al.*, 1986. Hulse *et al.*, 1990). The use of *D. exilis* as food has already been reviewed (Jideani, 1990, 1993; Lanza *et al.*, 1962).

Here we report the fractionation of *D. exilis* proteins by using three solvent systems: (I) sequential solvents of Osborne (1924), (II) those of Landry and Moureaux (1970), and (III) a solvent system involving the alkylation of glutelins. Solubility fractions from Osborne solvent fractionation were separated by gel filtration on Sephacryl S-200 HR. Components separated by gel filtration were analysed by electrophoresis. The amino-acid compositions of *D. exilis* protein fractions were also determined and are compared with amino-acid composition of durum-wheat proteins.

MATERIALS AND METHODS

Materials

Samples of acha (Jos, Nigeria) and durum wheat (Quaker Oats Ltd, UK) were stored at 4°C under nitrogen until use. Sodium dodecyl sulphate (SDS), Sephacryl S-200 HR, gel-filtration molecular-weight markers (MW-GF-200KIT), and SDS molecular-weight markers (MW-SDS-200KIT) were from Sigma,

Table 1. Solvent systems used for protein sequential fractionation

Solvent system	Protein fraction	Composition[a]
I[b]	Albumin/globulin	(a) 0·5M NaCl
	Prolamin	(b) 70% (v/v) Ethanol
	Glutelin	(c) 0·1M NaOH
II	Albumin/globulin	(a) 0·5M NaCl
	Prolamin	(b) 55% (v/v) Propanol (4°C)
	Glutelin-like	(c) Borate buffer (pH 10) + 2% (v/v) 2-ME
	True-Glutelin	(d) 0·5% (w/v) SDS + 2% v/v 2-ME
III	Albumin/globulin	(a) 0·5M NaCl
	Prolamin	(b) 55% (v/v) Propanol + 2% (v/v) 2-ME
	Alkylated glutelin	(c) 4-Vinyl pyridine, Tris, HNO_3, 2-ME, KCl, Urea, disodium EDTA and acetic acid
		(d) 0·1M Phosphate buffer (pH 7·0) + 1% (w/v) SDS + 8 M Urea.

[a] 2-ME = 2-mercaptoethanol; Tris = tris (hydroxymethyl) amino methane.
[b] In Solvent system I, 2-ME was added to propanol at concentrations of 2% and 5% (v/v) in separate extractions.

UK. Analytical-grade reagents were used unless otherwise stated.

Solubility fractionation of proteins

Cereal grains were ground to pass a 212-μm sieve by using a hammer mill. Wholemeal flours (10 g) were sequentially extracted according to the procedure of Osborne (1924) by stirring with 50 ml of 0·5M sodium chloride, 70% (v/v) ethanol, or 0·1M sodium hydroxide at 4°C for 3 h, followed by centrifugation at 6000 × g for 30 min. Supernatants were pooled, dialysed against distilled water, and freeze-dried. The compositions of other solvent systems adopted (solvents II–III) are given in Table 1. All fractions and the final residues were freeze-dried. The protein content of wholemeal flours and fractionation products was determined for acha (N × 6·25) and for durum wheat (N × 5·7) by using the micro-Kjeldahl method (AOAC 47·023) of the Association of Official Analytical Chemists (1980).

Acid-PAGE analysis of acha and durum prolamins

Acha (weight equivalent to that of a single durum kernel) or durum wholemeal was extracted with 1–6M urea (Table 2) for 1 h and centrifuged. The clarified extracts were analysed by acid-PAGE by using a 6% polyacrylamide gel with a continuous acetic acid/glycine buffer system as described by Salmon and Burbridge (1985). Gels were stained overnight in a solution of 48% (w/v) trichloroacetic acid/water/(0·25%, w/v) PAGE blue G-90 (19 : 57 : 4). No destaining was required, though gels were rinsed in distilled water prior to photography.

Gel filtration (GF) on Sephacryl S-200 HR

The albumin, globulin, prolamin, and glutelin fractions obtained from Osborne (solvent I) fractionation were analysed by gel filtration on a Sephacryl S-200 HR column (90 cm × cm^2), essentially as described by Chen and Bushuk (1970). Protein samples (1–2 % w/v) in 0·05 M Tris–HCl buffer (pH 7·5) and 70mM SDS were eluted with the Tris-HCl buffer containing 35mM SDS as dissociating agent (Bottomley *et al.*, 1982). Fractions (5 ml) were collected at the eluent-flow rate of 0·5 ml/min, with monitoring at 280 nm (LKB Uvicord S monitor). Peaks from the GF profiles were pooled, dialysed overnight against distilled water, and then freeze-dried. The gel-filtration column was calibrated by using 12·4–200-kDa protein standards (MW-GF-200; Sigma, UK).

SDS-Polyacrylamide-gel electrophoresis of acha proteins

Freeze-dried Osborne fractions (see above) were subjected to electrophoresis on a 7·5–18% linear-gradient gel by using the discontinuous-buffer system of Laemmli (1970). Proteins were suspended for 2 h in a sample buffer containing 1% (w/v) dithiothreitol, with occasional mixing, and then heated for 3 min at 100°C before SDS-PAGE fractionation. Gels were stained for 6 h by using 0·05% (w/v) Coomassie blue R-250 dissolved in methanol : acetic acid : water (25 : 7 : 68) and destained in the same solvent. Protein molecular weights were determined from a standard curve of mobility plotted against MW of SDS protein MW marker (MW-SDS-200 KIT).

Table 2. Specification of materials used in sample preparation for acid-PAGE shown in Fig. 1

	Wheat lanes				Acha lanes					
	1	2	3	4	5	6	7	8	9	10
Sample ratio	1	1	1	1	4	4	4	4	4	4
Extracting solvent (μl)	130	130	130	130	390	390	390	390	390	390
Concentration:										
Urea (M)	1	2	3	4	1	2	3	4	5	6
2-ME (M)	0	0.1	0.1	0.1	0	0.1	0.1	0.1	0.1	0.1
Extract applied (μl)	30	30	30	30	90	90	90	90	90	90

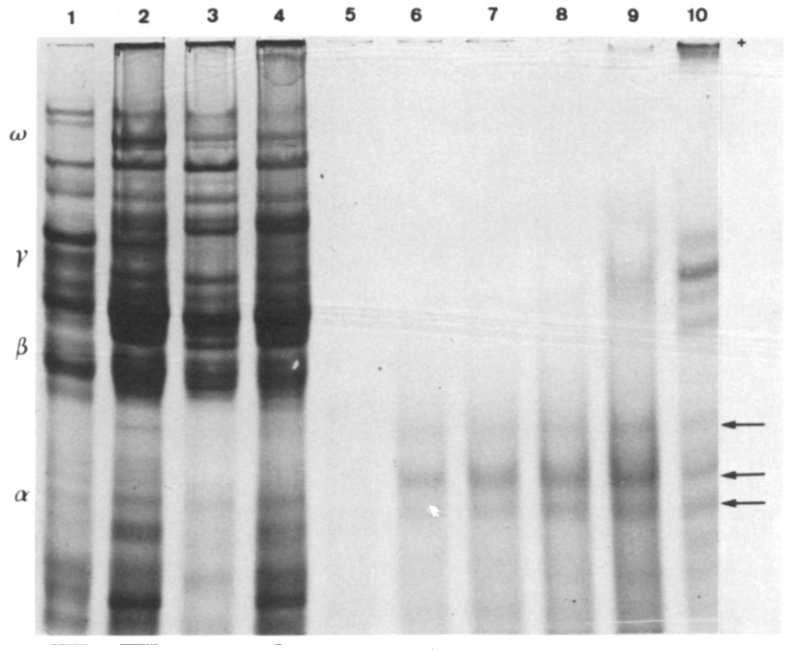


Fig. 1. Acid-PAGE analysis of polypeptides extracted from wholemeal flour in 0·1M 2-ME by using increasing concentrations of urea (see Table 2), without further reduction before electrophoresis. Lanes 1–4 (wheat) and 5–10 (acha).

Amino-acid analysis

Amino-acid analyses of wholemeal flour and the protein fractions were performed by the method of Bidlingmeyer *et al.* (1984). Samples were hydrolysed by using 6N HCl/1% (w/v) phenol vapour at 110°C for 24 h *in vacuo*. Protein hydrolysates were treated with phenylisothiocyanate to form phenylthiocarbamyl-derivatives of the amino acids, which were then analysed by using a Waters HPLC system (Millipore Ltd) fitted with a reverse-phase C-18 column. For cysteine estimation, aliquots each containing 100 μg of protein were first S-carboxymethylated and then analysed as above.

RESULTS AND DISCUSSION

Acid-PAGE analysis of wholemeal flours

Results for acha and wheat wholemeal flours by acid-PAGE analysis are shown in Fig. 1. The concentrations of urea used in the extraction of samples for the acid-PAGE analysis are shown in Table 2. For wheat samples, there was no significant change in the band patterns at 2M, 3M, and 4M urea concentrations (Fig. 1, lanes 2–4). Acid-PAGE results clearly show ω, γ, β, and α-gliadins (Bushuk & Zillman, 1978). For acha, the protein-band intensity increased with increasing concentration of (1–5M) urea (Fig. 1, lanes 5–10). At ≥6 M urea there was evidence of denaturation as seen by the appearance of a gel.

Three protein bands (Fig 1, arrows) for acha had a similar size : charge ratio to the α-gliadins in durum wheat (MW 33–44 kDa). Thus there appear to be α-gliadin-like prolamins in acha. At urea concentrations of 5M and 6M (lanes 9 and 10, respectively) bands having low electrophoretic mobility were also seen at the regions corresponding to those of γ and β-gliadin fractions. Altogether, about twelve protein components were extracted at 6M urea concentration. Some prolamin components (aggregates) were too large to enter the gel and could be seen as stained proteins at the point of application. The generally less intense acid-PAGE bands for acha (Fig. 1, lanes 5–7) are due to the lower solubility and/or lower initial levels of prolamin in this cereal (see later).

Solubility of acha proteins

The percentage total protein in various Osborne fractions are shown in Table 3. Acetic acid extraction gave a lower yield (1·3%) than sodium hydroxide extraction (14·0%) for acha glutelins. Acha glutelins, referred to as 'digitenins', were present at a higher level (14·0%) than the prolamin fraction (Table 3). The low level of prolamins in acha explains the less intense protein bands in acid-PAGE (Fig 1, see above). The prolamin content of acha (5·5%) compares with a rice-prolamin content of about 5% (Padhye & Salunkhe, 1979). However, in cereals such as maize, wheat, and barley, prolamin is present in greater amounts (Lasztity, 1986).

Table 3. Percentage total protein in various Osborne fractions from acha and durum[a]

Fraction	Acha	Durum
Albumin	3·5	11
Globulin	1·8	7·0
Prolamin	5·5	42
Glutelin	14·0	30
Residue	55·2	7·8

[a] Data are means of three replicates, CV = 10%. Total protein contents of wholemeal acha and durum flour are 7·5% and 14%, respectively.

Table 4. Amino-acid composition of durum and acha protein fractions (mol %)[a]

Amino acids	Protein fraction of durum				Protein fraction of acha			
	Albumin	Globulin	Prolamin	Glutelin	Albumin	Globulin	Prolamin	Glutelin
Asp	7·8	7·9	3·3	3·3	6·8	8·1	6·5	8·1
Glu	15·8	11·8	24·1	24·8	8·7	14·2	17·0	14·8
Cys-cm	4·4	1·6	1·5	1·4	4·8	2·2	0·5	1·8
Ser	7·1	7·4	7·8	8·0	9·7	7·2	6·7	7·0
Gly	9·5	11·3	3·9	9·7	10·4	11·8	5·9	11·2
His	2·2	3·1	2·2	2·0	1·2	2·8	1·2	2·4
Arg	3·7	8·2	2·2	2·9	4·6	9·1	2·7	4·5
Thr	4·8	4·5	3·0	3·7	5·5	4·2	4·9	5·4
Ala	7·8	8·7	3·8	6·9	19·4	9·0	17·8	11·0
Pro	10·6	5·7	18·4	13·7	6·3	4·6	7·6	7·3
Try	2·3	2·4	2·4	2·2	1·5	2·7	2·3	2·8
Val	6·0	6·5	4·9	4·3	5·3	5·8	5·8	5·6
Met	1·0	1·2	0·7	1·4	1·2	0·9	1·1	1·5
ILe	3·5	3·8	4·9	3·1	3·4	3·0	3·7	3·4
Leu	7·6	7·7	9·1	6·9	5·8	6·8	10·7	7·8
Phe	3·2	3·6	5·7	3·7	1·3	3·5	4·0	2·9
Lys	2·6	4·9	0·6	1·8	4·0	4·1	2·0	3·0
Classified Distribution of Amino acids								
1. Hydrophobic	31·9	28·5	43·7	33·1	23·4	24·6	32·4	28·5
2. Uncharged polar	28·1	27·2	18·6	25·0	31·8	28·1	20·3	28·3
3. Basic	8·5	16·2	5·0	6·7	9·8	16·0	5·9	9·9
4. Acidic	23·6	19·7	27·4	28·1	15·5	22·3	23·5	22·9

[a] Tryptophan not determined; Cysteine determined as carboxymethylated derivative (Cys-cm); Classified distribution of amino acids after Padhye and Salunkhe (1979) and Rupnow (1992); Alanine was not included in the hydrophobic grouping (see text).

In Table 3, the level of protein in the acha residue (55·2%) was significantly higher than that observed for durum wheat (7·8%). Residual-protein levels of 34–56% have been reported in some wheat varieties (Chen & Bushuk, 1970; Bietz & Wall, 1975). The solubilisation of residue protein by solvents II and III (Table 1) will be discussed below. The relatively low protein recovery in acha (80%) may be due to the loss of low-molecular-weight-protein components during dialysis (Visking tubing M_r cut-off 12 000) of the various fractions.

Amino-acid composition of acha and durum fractions

The amino-acid profiles of protein fractions in acha (Table 4) have not been reported previously. Levels of glutamic acid and proline were high in the glutelin and prolamin fractions of both acha and durum wheat. The levels of lysine, arginine, and threonine were low, as expected for cereal-storage proteins. The higher contents of lysine and arginine in the albumin and globulin fractions imply that these fractions probably contain mostly metabolic proteins (Lasztity, 1986). The relatively high value of alanine obtained was due to interference from the nearby ammonia peak in the amino-acid analysis. Both peaks appeared at the same point on the chromatogram.

From Table 4, the order of hydrophobicity of acha fractions was prolamin (32·4) > glutelin (28·5) > albumin (23·4) ≈ globulin (24·6). The same order of hydrophobicity is observed for durum-wheat fractions. The relatively high level of hydrophobic residues in prolamin of acha may account for the solubility of this fraction in alcohols. There was no significant difference in the level of hydrophobic or sulphur residues in soluble proteins from acha and durum.

SDS-PAGE of wholemeal flours

Figure 2 shows the electrophoresis pattern of wholemeal flour of acha and durum samples. The SDS-extractable proteins of both acha and durum appear within a similar molecular-weight range in the region from approximately 12 to 116 kDa. Electrophoresis bands for durum wheat were more intense owing to a greater protein content (14%) and to the relatively greater solubility of durum proteins. High-MW-protein bands (66–116 kDa) were present in both cereals. Altogether, about 21 protein components were identified in acha (lanes 5 and 6), with estimated molecular weights in the range from 14 to 104 kDa. The most predominant protein component in acha (see arrow in Fig. 2), with a molecular weight of 25·2 kDa, was not present in durum.

SDS-PAGE of acha protein-solubility fractions

The electrophoresis pattern for proteins extracted by using solvent II is shown in Fig. 3. The detailed compositions of solvents are given in Table 1. Figure 3, lane 1, shows the proteins of the water-soluble (albumin) fraction with from ten to twelve protein components (MW = 14–116 kDa). Lane 2 shows the proteins of the salt-soluble (globulin) fraction with from six to eight protein components (MW = 14–45 kDa). The

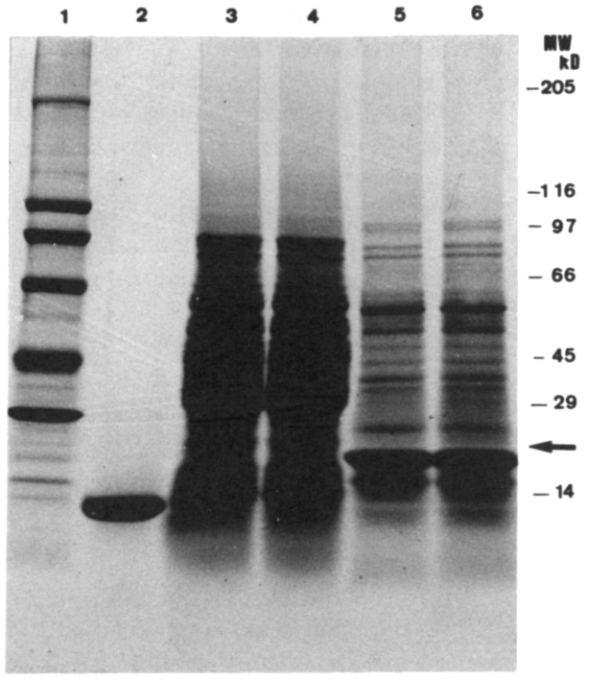


Fig. 2. SDS-polyacrylamide-gel electrophoresis of total SDS-extractable proteins from acha and durum in 7·5–18% linear-gradient gel. Lanes 1–2 Protein standards, 3–4 durum, and 5–6 acha. Standards used were: myosin (205 kDa), β-galactosidase (116 kDa), phosphorylase (97 kDa), albumin-bovine (66 kDa), albumin-egg (45 kDa), carbonic anhydrase (29 kDa), and α-lactalbumin (14·2 kDa).

14-kDa component is more prominent than the others. Lanes 4–6 show proteins of the alcohol-soluble (prolamin) fraction extracted with increasing concentrations of 2-ME (in solvent II). At 55% propanol, increasing the 2-ME concentration from 0% to 2% and 5% resulted in solubilisation of 21·4 ± 5·9, 30·3 ± 2·9, and 31·0 ± 4·5% protein, respectively in the extract. The two or three major prolamin components extracted have MW of 14–45 kDa. Lanes 7 and 8 show glutelin-like and true glutelin (Landry & Moureaux, 1970) proteins, respectively. Both fractions contain several components with molecular weights of 12–116 kDa. The above observations are similar to the reports on Italian millet (Monteiro *et al.*, 1982), finger millet (Ramachandra *et al.*, 1978), and maize (Misra *et al.*, 1976).

The electrophoresis pattern of the proteins sequentially extracted by using solvent system III (Table 1) is shown in Fig. 4 (lanes 1–4). The extraction technique is considered a more severe solubilising technique (Shewry *et al.*, 1977). Lane 1 shows that the albumin/globulin fractions contain a wide MW range of protein components. However, the 25·2-kDa protein component obtained by SDS extraction (Fig. 2, lanes 5 and 6) was absent. The prolamins extracted at 60°C with solvent III-b (lane 2), with solvent II-b at 4°C (lane 7), or with 70% (v/v) ethanol (solvent I-b) at 4°C (lane 8) appear to consist of three components, including a major one at 25·2 kDa. The presence of 2-ME and the use of propanol (compared with ethanol) enhanced the solubilisation of prolamins in acha. Hence both non-covalent (hydrophobic) and covalent (e.g. S–S bonds) contribute to the low solubility and high residual protein of acha in conventional solvents. The greater prolamin extractions with 2-ME (Fig. 4, lanes 2 and 7 compared with lane 8) show that the major 25·2-kDa component forms a part of a disulphide-linked protein aggregate within acha. On SDS-PAGE (15% gel), two sulphur-amino-acid-rich acha proteins have been reported recently to have MW of 17·5 and 19 kDa (de Lumen *et al.*, 1993).

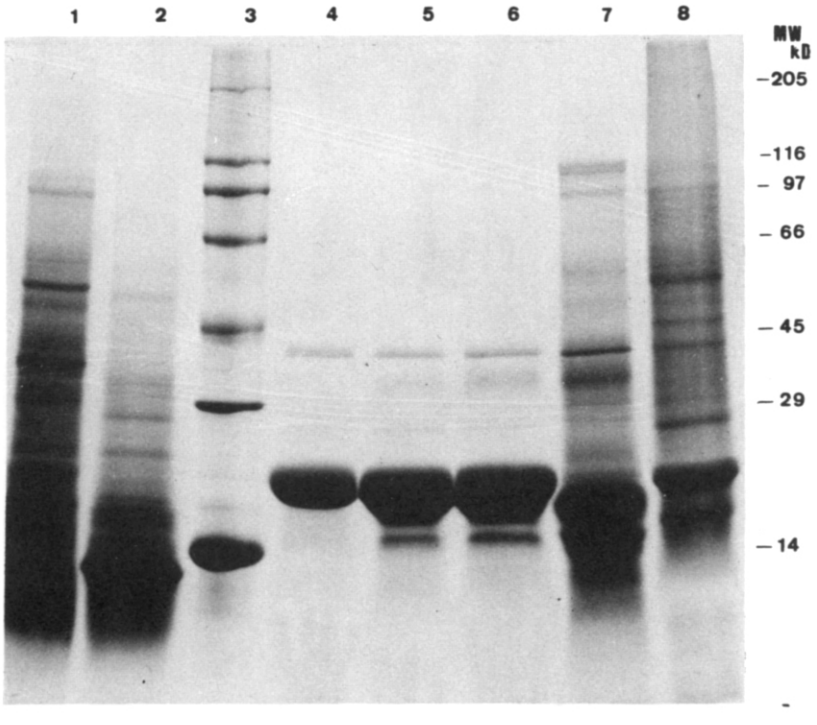


Fig. 3. SDS-polyacrylamide-gel electrophoresis of solubility fractions from acha by using solvent system II (see Table 1). Left to right: 1, albumins; 2, globulins; 3, standard proteins; 4, prolamin; 5, prolamin (2% 2-ME); 6, prolamin (5% 2-ME); 7, glutelin-like; 8, true glutelin.

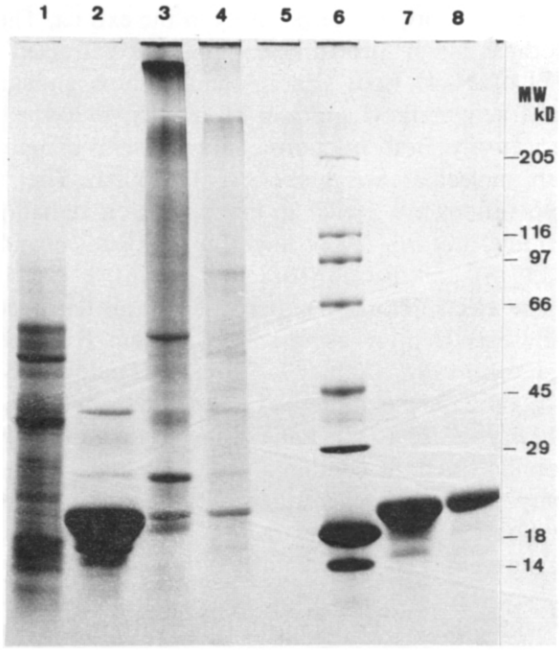


Fig. 4. SDS-polyacrylamide-gel electrophoresis of solubility fractions from acha by using solvent system III (see Table 1). Left to right: 1, albumin and globulin; 2, prolamins (propanol + 2-ME at 60°C); 3, alkylated glutelins; 4, glutelin-like; 5, residual proteins; 6, standard proteins; 7, prolamins (propanol + 2-ME at 4°C); 8, prolamin (ethanol at 4°C).

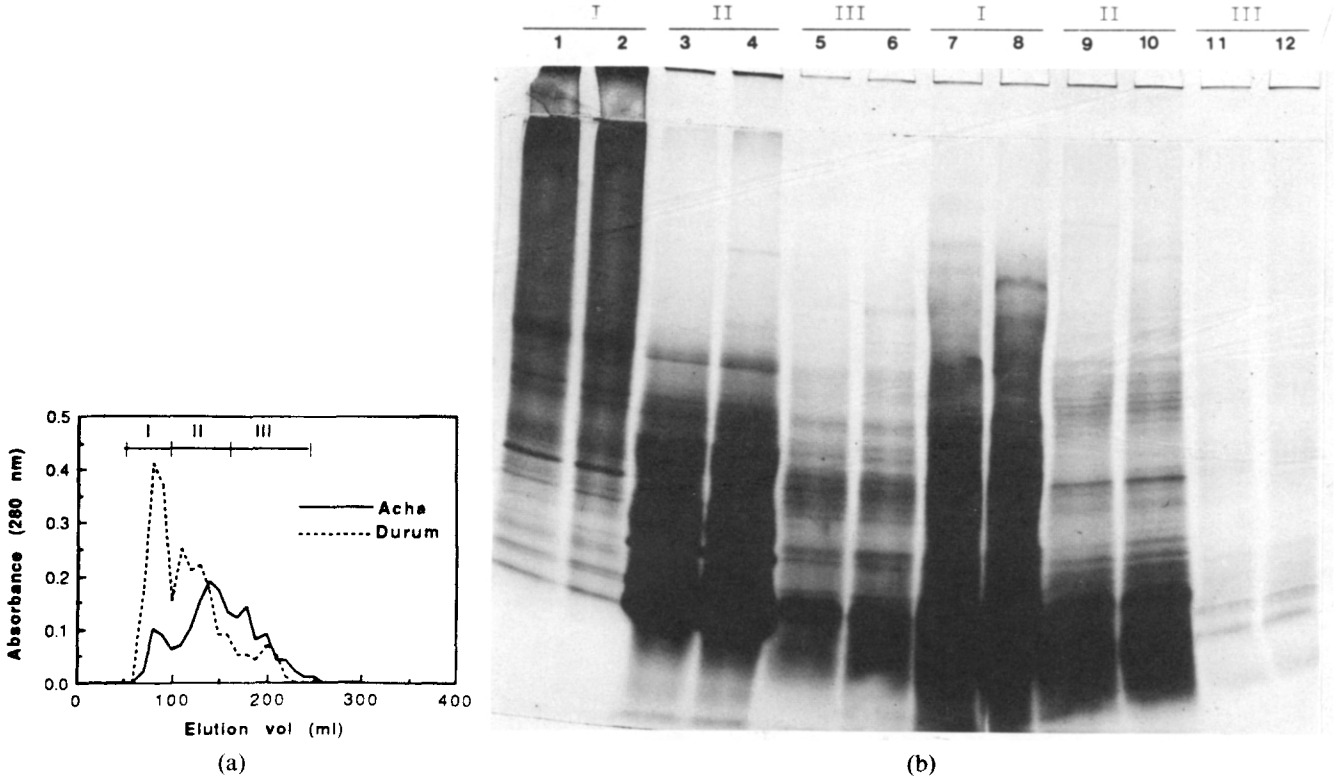
An alkylated glutelin fraction in acha (Fig. 4, lane 3) showed higher MW proteins including components with MW of >205 kDa visible in the stacking gel zone. The 25·2 kDa component of acha protein is also present in the glutelin fraction, though in a very small quantity. Lane 4 shows the pattern of the fraction extracted with a solution containing 8 M urea and 1% (w/v) SDS (Table 1). After sequential extraction with solvent III (Table 1), some proteins of molecular weight 25·2 kDa and those that could not enter the stacking gel were unextracted (lane 5). The presence of a 25·2-kDa protein component in most fractions (lanes 2, 4, and 6) except the albumin/globulin fraction (lane 1) suggests that the 25·2 kDa subunit forms the basic structural component of storage proteins of acha. It is notable that this component appears to be absent in durum wheat.

Gel filtration and SDS-PAGE analysis of protein solubility fractions

Albumin and globulin fractions

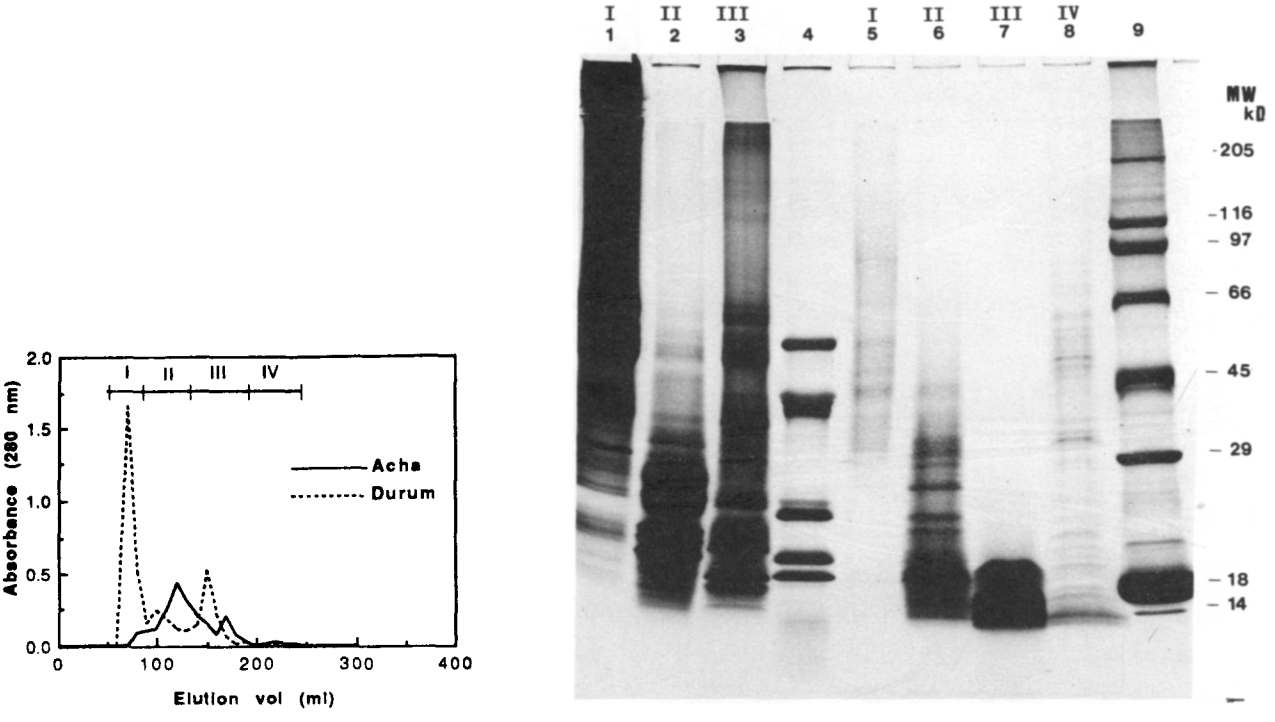
Figures 5 and 6 show the gel-filtration spectra and the SDS-PAGE for acha and durum albumin (Fig. 5) and globulin (Fig. 6). Elution peaks were collected as indicated. The peaks (I–III) identify gel-filtration fractions with SDS-PAGE patterns for each cereal. In Fig. 5, elution peaks (I, II, and III) were heated or not heated at 100°C for 3 min prior to gel electrophoresis. Each group of protein treated thus was run side by side. There was no significant difference between the SDS-PAGE patterns for heated and unheated samples for all the groups (lanes 1–12).

Fraction I (Figs 5(a) and 6(a)) appeared close to the exclusion volume (determined with blue dextran). Quantitatively, this group was less in acha than durum. When fraction I from durum wheat was subjected to



(a) (b)

Fig. 5. Albumin fraction: (a) gel filtration and (b) SDS-PAGE patterns. In (b), lanes 1–6 durum and 7–12 acha. Fraction numbers (I–III) indicate corresponding elution cuts. Proteins applied to lanes with odd numbers are not heat-treated and those with even numbers are heat-treated (see text for details).



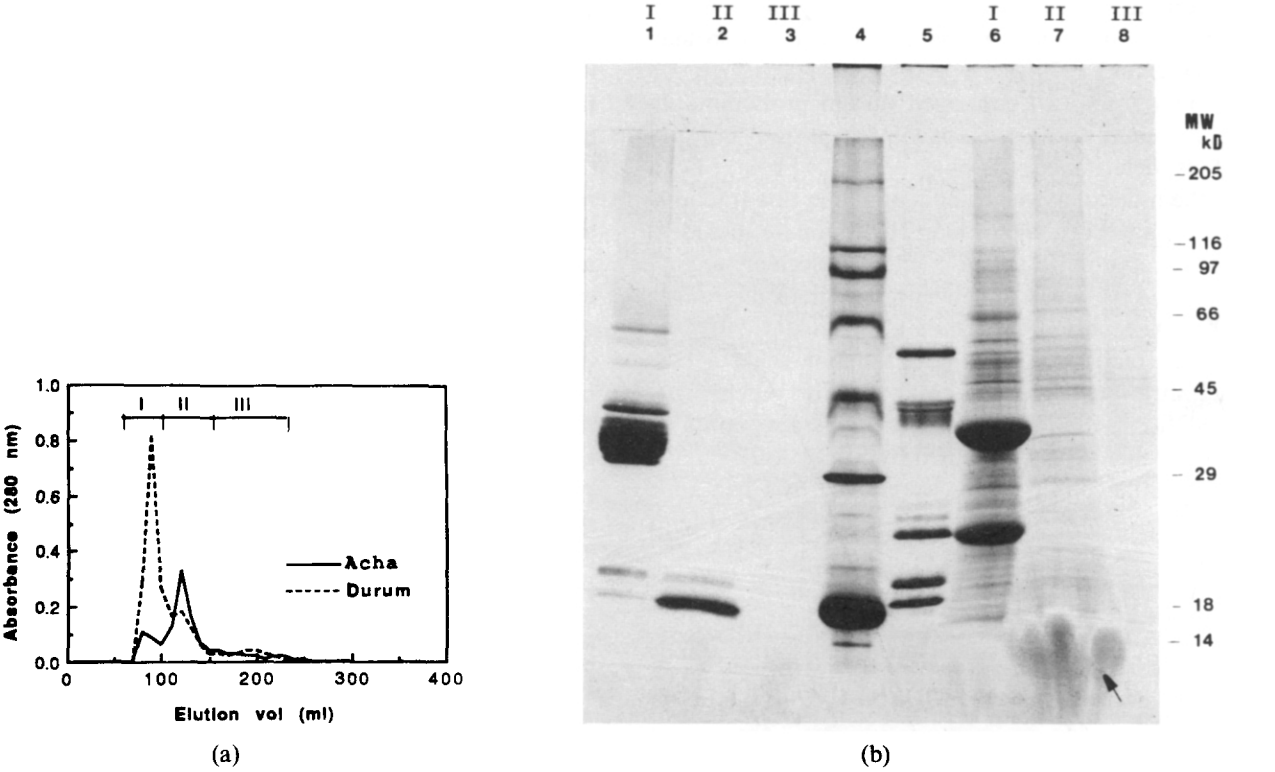
(a) (b)

Fig. 6. Globulin fraction: (a) gel filtration and (b) SDS-PAGE patterns. In (b) lanes 1–3 durum; 4 and 9 protein standards; 5–8 acha. Fraction numbers (I–IV) indicate corresponding elution peaks in Fig 6(a).

SDS-PAGE (Fig 5(a)), most of the proteins did not enter or migrate far or form sharp bands within the polyacrylamide gel (lanes 1 and 2). That is, the relatively soluble albumin and globulin fractions contain highly aggregated proteins. In Fig. 6, peak IV for acha when analysed by SDS-PAGE (lane 8) showed protein bands that seem to overlap with bands in peaks I–III (lanes 5–7).

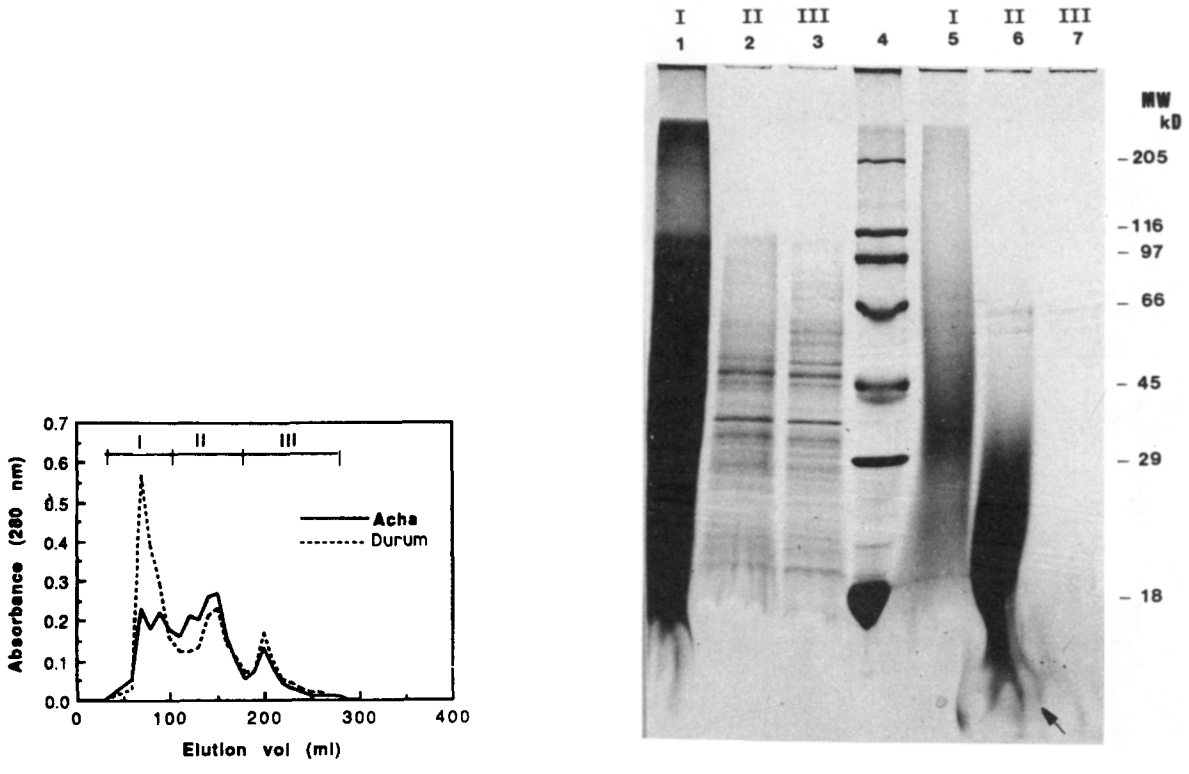
Prolamin and glutelin fractions

Figure 7 shows the gel-filtration chromatogram and corresponding SDS-PAGE-band patterns for acha and



(a) (b)

Fig. 7. Prolamin fraction: (a) gel filtration and (b) SDS-PAGE patterns. In (b), lanes 1–3 durum; 4 and 5, protein standard; 6–8 acha. Fraction numbers (I–III) indicate corresponding elution peaks in Fig. 7(a).



(a) (b)

Fig. 8. Glutelin fraction: (a) gel filtration and (b) SDS-PAGE patterns. In (b) lanes 1–3 durum; 4 protein standard; 5–7 acha. Fraction numbers (I–III) indicate corresponding elution peaks in Fig. 8(a).

durum prolamins. Three elution peaks were collected as indicated (Fig. 7(a)). Relatively small peaks were obtained in the gel-filtration spectrum for acha owing to the lower proportion of prolamin in the cereal (Table 3). Acha prolamins were observed to be readily soluble in SDS–Tris buffer, whereas a low solubility of alcohol-soluble protein extracts in dimethylformamide, an alternative dissociating agent, has been reported (Chen & Bushuk, 1970).

Fraction I (Fig 7(a)) contained durum prolamins of molecular weight above the apparent exclusion limit of the gel as determined by blue dextran. From SDS-PAGE, the molecular weight of subunits in this fraction was in the range of 24–66 kDa. This estimate includes the 50 kDa reported to be the average molecular weight of wheat gliadins but is somewhat lower than the range of 80–90 kDa reported by Chen and Bushuk (1970).

Prolamin fraction II (Fig 7(a)), a major peak in the acha gel-filtration spectrum, did not show much protein in SDS-PAGE. Fraction III proteins also contain very little protein when examined by SDS-PAGE (lanes 3 and 8). Similar observations were made by Chen and Bushuk (1970). A low-molecular-weight protein-like component also consistently appeared in the SDS-PAGE profile of the ethanol-soluble and NaOH-soluble fractions (Figs 7(b) and 8(b), indicated by an arrow). This component, which produced serrated and poorly defined bands, may represent small polypeptides or non-protein constituents (Hu & Esen, 1981).

Three major glutelin peaks (Fig. 8(a), I–III) from the gel-filtration analysis were pooled and analysed by SDS-PAGE. Previous GF results on durum glutenin with a P-150 gel matrix (BIO RAD) showed no fractionation (Chen & Bushuk, 1970). Here fractionations were obtained because of the use of a wider-MW-range gel matrix. Fraction I (Fig 8(a)) contained proteins of molecular weight above the exclusion limit of the gel support used. SDS-PAGE resolution of glutelin fractions I and II (lanes 5 and 6) was consistently poor owing to non-dialysable non-protein impurities in these fractions. These non-protein substances are thought to be nucleic acids (DNA, RNA) on the basis of absorbance measurements at 260 as compared with 280 nm (Hu & Esen, 1981).

There appears to be some overlap of components in fractions II and III for both durum (lanes 2 and 3) and acha (lanes 6 and 7). These overlaps could possibly indicate different levels of aggregation of the protein components affected.

In general, gel-filtration profiles of the albumin, globulin, prolamin, and glutelin fractions showed a similar number of peaks for acha and durum wheat. No attempt was made to study the protein component of each GF peak. The identification of such protein components may provide information essential for an understanding of the molecular structure of acha proteins. The differences in results obtained from solubility fractionation reflect intrinsic differences in protein organisation of acha compared with that of durum wheat.

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REFERENCES

AOAC (1980). *Association of Official Analytical Chemist* In 'Official Methods of Analysis', 13th ed., Washington D.C., pp. 858.

Annegers, J. F. (1973). Ecology of dietary patterns and nutritional status West Africa. 1. Distribution of starchy staples. *Ecology Food Nutr.*, **2**, 107–19.

Bidlingmeyer, B. A., Cohen, S. A. & Tarvin, T. L. (1984). Rapid analysis of amino acids using pre-column derivatization. *J. Chromatogr.*, **336**, 93–104.

Bietz, J. A. & Wall, J. S. (1973). Isolation and characterisation of gliadin-like subunits from glutenin. *Cereal Chem.*, **50**, 537–47.

Bietz, J. A. & Wall, J. S. (1975). The effect of various extractants on the subunit composition and associations of wheat glutenin. *Cereal Chem.*, **52**, 145–55.

Bottomley, R. C., Kearns, H. F. & Schofield, J. D. (1982). Characterisation of wheat flour and gluten proteins using buffers containing sodium dodecyl sulphate. *J. Sci. Food Agric.*, **33**, 481–91.

Bushuk, W. Zillman, R. R. (1978). Wheat cultivar identification by gliadin electrophoregrams. 1; Apparatus, method and nomenclature. *Can. J. Plant Sci.*, **58**, 505–15.

Chen, C. H. & Bushuk, W. (1970). Nature of proteins in triticale and its parental species. 1: Solubility characterisation and amino acid composition of endosperm proteins. *Can. J. Plant Sci.*, **50**, 9–14.

Dalziel, J. M. (1937). *The Useful Plants of West Tropical Africa*, Crown Agents, London, pp. 535–6.

de Lumen, B. O., Thompson, S. & Odegard, J. W. (1993). Sulphur amino acid-rich proteins in acha (*Digitaria exilis*), a promising underutilized African cereal. *J. Agric. Food Chem.*, **41**, 1045–7.

Graveland, A., Bosveld, P., Lichtendonk, W. J., Moonen, H. E. & Scheepstra, A. (1982). Extraction and fractionation of wheat flour proteins. *J. Sci. Food Agric.*, **33**, 1117–28.

Hu, B. & Esen, A. (1981). Heterogeneity of soybean seed proteins: one-dimensional electrophoretic profiles of six different solubility fractions. *J. Agric. Food Chem.*, **29**, 497–501.

Huebner, F. R. & Wall, J. S. (1976). Fractionation and quantitative differences of glutenin from wheat varieties varying in baking quality. *Cereal Chem.* **53**, 258–69.

Hulse, J. H., Laing, E. M. & Pearson, O. E. (1990). *Sorghum and millets: Their Composition and Nutritive Value*. Academic Press, New York, NY, USA.

Jideani, I. A. (1990). Acha—*Digitaria exilis*: the neglected cereal. *Agric. Int.*, **42**, 132–134, 143.

Jideani, I. A., & Akingbala, J. O. (1993). Some physicochemical properties of acha (*Digitaria exilis*) and iburu (*Digitaria iburua*). *J. Sci. Food Agric.*, **63**, 369–74.

Kasarda, D. D., Bernardin, J. E. & Nimmo, C. C. (1976). Wheat proteins. *Adv. Cereal Sci. Technol.*, **1**, 158–236.

Laemmli, U. K. (1970) Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature*, **227**, 680–5.

Landry, J. & Moureaux, T. (1970). Heterogeneite des glutelines du grain de mais; extraction selective at composition en acides amines des trois fractions isolees. *Bull. Soc. Chim. Biol.*, **52**, 1021–37.

Lanza, M., Regli, P. & Busson, F. (1962). Etude chimique du grain de *Digitaria iburua*. *Med. Trop.*, **22**, 471–6.

Lasztity, R. (1986). *The Chemistry of Cereal Proteins*. CRC Press, Boca Raton, FL, USA.

Misra, P. S., Mertz, E. T. & Glover, D. V. (1976). Studies on corn proteins. X: Polypeptide molecular weight distribution in Landry-Moureaux fractions of normal and mutant endosperms. *Cereal Chem.*, **53**, 705–10.

Monteiro, P. V., Virupaksha, T. K. & Rao, D. R. (1982). Proteins of Italian millet; amino acid composition, solubility fractionation and electrophoresis of protein fractions. *J. Sci. Food Agric.*, **33**, 1072–9.

Osborne, T. B. (1924). The Vegetable Proteins (2nd edition) Longman, Green, New York, NY, USA.

Padhye, V. W. & Salunkhe (1979). Extraction and characterization of rice proteins. *Cereal Chem.*, **56**, 389–93.

Ramachandra, G., Virupaksha, T. K. & Shadaksharaswamy, M. (1978). Comparison of the protein fractions of finger millet. *Phytochemistry*, **17**, 1487–90.

Rooney, L. W., Kirleis, A. W. & Murty, D. S. (1986). Traditional foods from sorghum: their production, evaluation and nutritional value. *Adv. Cereal Sci. Technol.*, **8**, 317–53.

Rupnow, J. H. (1992). Proteins: biochemistry and applications. In *Encyclopedia of Food Science and Technology*, ed. Y. H. Hui. Wiley-Interscience, New York, NY, USA, pp. 2182–91.

Salmon, S. E. & Burbridge, K. M. (1985). *Wheat variety identification by polyacrylamide gel electrophoresis.* Flour Milling and Baking Research Association Bulletin, No. 2 (FMBA) Chorleywood, U.K., pp. 78–88.

Schofield, J. D. & Booth, M. R. (1983). In *Developments in Food Proteins* (2nd edition), ed. B. J. F. Hudson. Applied Science Publishers, London, pp. 1–66.

Shewry, P. R., Pratt, H. M. & Miflin, B. J. (1977). In *Techniques for the Separation of Barley and Maize Proteins* (EUR 5687e) ed. B. J. Miflin and P. R. Shewry. Commission of the European Communities, Luxembourg, pp. 37–47.