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Effects of isolation technique and conditions on the extractability, physicochemical and functional properties of pigeonpea (*Cajanus cajan*) and cowpea (*Vigna unguiculata*) protein isolates. I. Physicochemical properties

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Abstract

Physicochemical properties of pigeonpea and cowpea protein isolates were determined as a function of extraction technique and pH of the extracting medium. Protein extractability by the isoelectric point precipitation (IP) technique was positively correlated within the pH of the NaOH solution used in the pH range 8.5–12.5. The micellization (MP) technique extracted significantly ($P < 0.05$) less protein than the IP technique when extraction pH of the NaOH was 9.5 or higher, and 10.5 or higher from cowpea and pigeonpea, respectively. The subunit composition and electrical mobility of the isolates were not affected by extraction technique and pH conditions. However, it was observed that the IP isolate extracted at pH 12.5 had the lowest proportion of hydrophilic amino acids, suggesting that the pH of the extracting medium exerted a major influence on the hydrophilicity of the isolates. Pigeonpea MP isolate exhibited significantly ($P < 0.05$) higher exposed hydrophobicity than the IP isolates except for those extracted at pH 9.5 and 10.5. However, the cowpea MP isolate exhibited significantly lower exposed hydrophobicity than the IP isolate extracted at pH 8.5 but this was higher than the rest of the IP isolates. For IP isolates, an inverse relationship was apparent between the extraction pH and hydrophobicity. The MP isolates from both legume seeds were significantly lighter in colour than the corresponding IP isolates and, for the latter, the lightness value (L) was inversely correlated with extraction pH. Differential scanning calorimetry showed that the MP isolates exhibited higher transition enthalpy (ΔH) than the IP. For the IP isolates, ΔH decreased with increasing extraction pH.

1. Introduction

Tropical legumes such as pigeonpea and cowpea are crops that are well adapted to the semi-arid zones of Kenya because of their drought tolerance. Over the last 30 years, the usage of concentrated proteins from plant seeds has increased tremendously because of greater knowledge of their functional properties, processing and nutritive value. While soybeans have had a competitive advantage over other legume seeds, there is a need to develop other sources of concentrated plant proteins (Vose, 1980) which ideally should be crops that are widely grown in tropical countries. Although the chemical composition of pigeonpea and cowpea seeds has been reported in several publications (Jorg & Klein, 1989; Longe, 1980; Mnembuka & Eggum, 1993, 1995; Mosse & Baudet, 1983; Singh, Rao & Subrahmanyam, 1993), little information is available on the effects of extraction conditions on the physicochemical and functional properties of their protein isolates. A review of available literature reveals that more effort has been invested in the nutritional and chemical evaluation of these legumes than the studies of those physicochemical and biochemical properties that bear upon the technological transformation and postharvest stability (Hulse, 1991). As pointed out by Mitchell and Ledward (1986), the modern food processing industry is becoming increasingly dependent on the manufacture of fabricated foods rather than the preservation of commodities grown or reared on the farm. Since many past developments of fabricated foods have been as a result of inspired creativity and trial and error manipulation of ingredients

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with little understanding of the underlying science, there is now a need for the food technologist to understand the behaviour of individual ingredients in the formulated foods.

The present study was thus aimed at determining the effects of extraction techniques and conditions on the extractability and the physicochemical properties of protein isolates from commercial pigeonpea and cowpea samples from Kenya with a view to assessing the potential of these local resources as alternative protein sources for commercial exploitation.

2. Materials and methods

Commercial varieties of pigeonpea and cowpea were purchased from a dealer in Nairobi, Kenya. The pigeonpea and cowpea seeds were stored at 10°C until used. Suproplus 651 soy protein isolate (Protein Technologies International, Zwaanhofweig, Belgium) was used for comparison whenever applicable. The legume seeds were ground to pass a 250 μm sieve prior to extraction of protein.

2.1. Preparation of protein isolates

Two protein extraction techniques described by Paredes-Lopez and Ordorica-Falomir (1986) were applied with some modifications.

Isoelectric protein (IP) isolates were obtained by extracting legume meal with 0.1 M NaOH (1:10 meal: solvent, w/v) adjusted to pH values 8.5, 9.5, 10.5, 11.5 and 12.5. The suspension was homogenized (Ultra Turrax T25, Janke and Kunkel GmbH & Co., KG., Stauffen, Germany) at 8500 rpm for 30 min followed by centrifugation at 5000 *g* for 15 min. The supernatant was filtered and protein precipitated by adjusting the pH to 4.5 using 0.1 M HCl. The precipitated protein was recovered by centrifuging, followed by washing three times in excess water and centrifuging. The isolate was dried at 50°C for 48 h. The isoelectric isolates were designated the code IP followed by the pH of extraction.

Micelle protein (MP) isolates were prepared by homogenizing 10% (w/v) legume meal suspensions in 0.25 M NaCl, pH 6.5 for 30 min at room temperature. The extract was centrifuged as described above and the filtered supernatant was diluted with distilled water at 4°C (1:3 protein extract:water). After standing for 6 h, protein was recovered by centrifugation and treated as specified for the isoelectric isolates above. The micelle isolates were designated by the code MP.

2.2. Chemical composition

Moisture, crude protein (N%×6.25), fat, ash, crude fibre and phytate were determined according to AOAC (Association of Official Analytical Chemists, 1990), trypsin inhibitor activity according to AACC (American Association of Cereal Chemists, 1983), and oligosaccharides according to the method described by Knudsen (1986). Amino acid composition was determined according to the Pico-Tag Amino Acid Analysis System (Waters Chromatography Div., Millipore Co., Milford, MA) and the amino acids were classified according to the nature of the side chain viz. hydrophilic, hydrophobic, cyclic and sulfur and expressed as % of the total.

2.3. Protein hydrophobicity

Protein exposed hydrophobicity was determined according to a fluorescence method described by Townsend and Nakai (1983) using 6,9,11,13,15-octadecatetranoic acid (*cis*-parinaric acid) (Sigma Chemical Co., St. Louis, MO) as a fluorescence probe and measured on a Perkin–Elmer LS-5 Luminescence Spectrometer (Perkin–Elmer, Oak Brook, IL).

2.4. Sodium dodecyl sulfate–polyacrylamide gel electro-phoresis (SDS–PAGE)

SDS–PAGE was performed according to the method described by Smith (1994). Samples were prepared in a buffer containing Tris/HCl, SDS, β-mercaptoethanol, glycerol and bromophenol. Calibration was by MW-SDS-100B molecular weight standards kit (Sigma Chemical Co., St. Louis, MO).

2.5. Isoelectric focusing

The isoelectric points were determined according to the PhastSystem Separation Technique File No. 100 using PhastGel IEF 3-9 media as described in the PhastSystem Users Information Manual (Pharmacia LKB Biotechnology, Uppsala, Sweden; PhastSystem, 1990).

2.6. Colour

Colour was determined with a Minolta Chroma Meter CR-300 (Minolta Camera Co., Osaka, Japan). Measured values were expressed as L, a, b colour units where L = lightness, $+a$ = redness, $-a$ = greenness, $+b$ = yellowness, $-b$ = blueness, and $\Delta E = (\Delta L^2 + \Delta a^2 + \Delta b^2)^{1/2}$ refers to the total colour difference between the sample and Suproplus 651 soy isolate. The L, a, b values of the standard tile were 97.95, −0.07 and +1.66, respectively.

2.7. Differential scanning calorimetry

Differential scanning calorimetry was performed according to the method described by Paredes-Lopez,

Ordorica-Falomir & Olivares-Vasquez (1991) using a DuPont 2910 Thermal Analyzer (DuPont Co., Wilmington, DE). Computation of the thermograms was by DuPont Thermal Analyst 2000 System software.

2.8. Statistical analysis

Analysis of variance, Duncans multiple range test and linear correlation were done using the Statistical Analysis System package (SAS, 1987).

3. Results and discussion

3.1. Chemical composition of pigeonpea and cowpea seeds

The chemical composition of pigeonpea and cowpea seeds is presented in Table 1. Cowpea was significantly ($P < 0.05$) higher in crude protein content than pigeonpea, and the values obtained in the present study were similar to those previously reported by Mnembuka and Eggum (1993, 1995). From the context of viable commercial exploitation, the protein levels of 22.6 and 29.3% for pigeonpea and cowpea, respectively, imply that extraction of protein may have to be carried out concomitantly with the other major macromolecule component (starch) in order to ensure economic feasibility. The crude fibre, lipid, and ash contents of the two legumes were within the ranges previously reported (Chavan, Kadam & Salunkhe, 1989a, 1989b; Mnembuka and Eggum, 1993; Reddy, Pierson, Sathe & Salunkhe, 1984); pigeonpea was significantly ($P < 0.05$) higher in lipid content than cowpea. No significant differences were observed in trypsin inhibitor activities of the two legumes. Pigeonpea was higher in sucrose and raffinose than cowpea but the latter was higher in stachyose. The oligosaccharide contents observed in these legumes in the present study were lower than those reported previously (Chavan et al., 1989a, 1989b; Liew & Buckle, 1990; Longe, 1980; Reddy et al., 1984), probably due to differences in variety and analytical techniques. Raffinose, stachyose and verbascose have been identified as flatulence-inducers and when ingested cause accumulation of gas, discomfort, diarrhea, pain and cramps (Deshpande & Deshpande, 1991; Liew & Buckle, 1990; Phillips, 1993); a factor which has tended to render legumes less acceptable. Cowpea was significantly ($P < 0.05$) higher in phytate content than pigeonpea and the values recorded in this study were within the ranges previously reported (Deshpande & Deshpande; Reddy et al., 1984). Presence of phytate has been reported to influence the nutritional and functional properties of cereals and legumes and their derived foods by complexing with proteins, amino acids (Reddy et al., 1989) and trace minerals, especially zinc (Erdman, 1981).

Table 1
Chemical composition of pigeon pea and cowpea seeds[a]

Component	Pigeonpea	Cowpea
Moisture (%)	13.12 ± 0.06a	13.22 ± 0.05a
Crude protein (%)	22.6 ± 0.57b	29.3 ± 0.01a
Crude fibre (%)	6.27 ± 0.27a	6.66 ± 0.11a
Crude lipid (%)	1.84 ± 0.02a	0.62 ± 0.02b
Ash (%)	4.10 ± 0.03a	4.26 ± 0.05a
Starch (%)	45.73 ± 0.06a	34.21 ± 0.07b
Trypsin inhibitor (TIU/g)	386 ± 1.12a	373 ± 0.10a
Phytate (mg/g)	0.65 ± 0.04b	1.58 ± 0.04a
Sucrose (%)	0.72 ± 0.02a	0.17 ± 0.00b
Raffinose (%)	0.35 ± 0.00a	0.00 ± 0.00b
Stachyose (%)	0.65 ± 0.04b	1.58 ± 0.04b

[a] Means in a row followed by the same letter are not significantly different ($P < 0.05$). Values given are means of duplicate determinations.

3.2. Protein extractability

The % recovery of protein isolates from pigeonpea and cowpea by micellization and isoelectric precipitation techniques is presented in Table 2. The % of total seed protein extracted ranged from 35.1 to 58.1%. For the isoelectric precipitation technique, a significantly ($P < 0.05$) higher % of the protein was recovered as the pH of the alkaline extraction solvent was increased from 8.5–12.5. At lower alkaline pH (8.5–10.5), a higher % of protein was extracted from cowpea than from pigeonpea; at pH 11.5 there was no significant difference in extractability but at pH 12.5 more protein was extracted from pigeonpea than from cowpea. Protein extractability (Y) was positively correlated to extraction pH (X) for pigeonpea ($Y = 5.6790X–14.926$, $R^2 = 0.92$) and cowpea ($Y = 4.3160X + 0.226$, $R^2 = 0.98$). The pH-dependency of protein recovery from leguminous seeds has been observed previously (Kazazis & Kalaissakis, 1979; Molina, Arguetta & Bressani, 1976; Pant & Tulsiani, 1969; Sefa-Dedeh & Stanley, 1979). Application of the micellization technique resulted in significantly ($P < 0.05$) higher protein extractability from pigeonpea than cowpea.

Table 2
Extractability of protein from pigeonpea and cowpea seeds by micellization and isoelectric precipitation techniques[a]

Isolate[b]	% Total seed protein	
	Pigeonpea	Cowpea
MP	40.2 ± 0.51f	36.7 ± 0.67g
IP 8.5	35.1 ± 0.28h	36.4 ± 0.32g
IP 9.5	39.7 ± 0.52f	42.0 ± 0.57e
IP 10.5	40.2 ± 0.03f	44.9 ± 0.25d
IP 11.5	50.5 ± 0.25c	51.0 ± 0.69c
IP12.5	58.1 ± 0.65a	53.5 ± 0.61b

[a] Means followed by the same letter are not significantly different ($P < 0.05$). Values given are means of duplicate determinations.

[b] MP = micelle protein, IP = isoelectric protein.

The isoelectric precipitation technique solubilised more protein when extraction pH was 9.5 and higher for cowpea, and 11.5 and higher for pigeonpea than the micellization technique. These results demonstrated that the extractability of protein from these legumes was influenced by extraction conditions and species.

3.3. Chemical composition of protein isolates

The chemical composition of pigeonpea and cowpea isolates is shown in Table 3. Generally, cowpea isolates showed significantly ($P < 0.05$) higher protein contents than the corresponding pigeonpea isolates, probably because cowpea seed had a higher protein content than pigeonpea seed. The highest protein content was recorded in cowpea IP 8.5 (92.9%) and the lowest in pigeonpea IP 12.5 (78.1%) but no significant difference was observed between the protein contents of pigeonpea and cowpea MP. The protein content of the pigeonpea MP was similar to those of IP 8.5–10.5 and significantly higher than those of IP 11.5 and 12.5 but, for cowpea, MP was lower in protein content than IP 8.5–11.5. For the isoelectrically-precipitated isolates, an inverse relationship was apparent between the pH of extraction and the protein content of the resulting isolates. No trypsin inhibitor activity, phytate or oligosaccharides were detected in any of the protein isolates, indicative of the complete elimination of these antinutrients during the extraction process. Similar elimination or reduction of these antinutrients has been previously observed in faba bean micelle isolate (Arntfield, Ismond & Murray, 1985), great northern bean isolate (Sathe & Salunkhe, 1981) and in cowpea concentrates (Molina, Bressani & Elias, 1977). The lipid content of the pigeonpea isolates was significantly ($P < 0.05$) higher than that of the corresponding cowpea isolates. The concentration of lipids in the pigeonpea isolates was notably high in the isoelectric isolates extracted at pH 10.5 and above, a phenomenon that had been observed previously in isolates obtained from pea (Sumner, Nielsen & Youngs, 1981) and faba bean (McCurdy & Knipfel, 1990). The crude fibre and ash contents of pigeonpea and cowpea isolates did not display any consistent trends vis à vis either the extraction technique or the pH of the alkaline extraction solvent.

3.4. Amino acid composition

The amino acid composition of pigeonpea and cowpea isolates, expressed as a function of the nature of the side chains, is presented in Table 4. In general, cowpea isolates were significantly ($P < 0.05$) higher in hydrophilic amino acid residues than the pigeonpea isolates and the reverse was true for the hydrophobic amino acids. For pigeonpea, IP 8.5 and 11.5 showed the highest % of hydrophilic amino acids whereas the lowest % was recorded in IP 12.5. Cowpea IP 10.5 had the highest % of hydrophilic amino acids and IP 12.5 the lowest; an observation that implied that extraction at pH 12.5 significantly affected the hydrophilicity of the isolates. The pigeonpea and cowpea IP 12.5 isolates exhibited the highest hydrophobic/hydrophilic ratios of 0.64 and 0.61, respectively; the ratio for pigeonpea MP was similar to those of IP 8.5–11.5 but that of the cowpea micelle isolate was significantly ($P < 0.05$) different from those of IP 8.5, 10.5 and 12.5. The hydrophobicity/hydrophilicity ratio has been identified as one of the factors known to influence some functional properties of proteins such as solubility, emulsification, fat absorption and elasticity (Damodaran, 1994; Kinsella, Damodaran & German, 1985; Wright, 1983). The raw data of amino acid composition also indicated that all isolates were devoid of cysteine due either to its destruction during extraction or the exclusion of the protein fraction containing it. It has been reported previously (Coffmann & Garcia, 1977; Nashef, Osuga, Lee, Ahmed, Whitaker & Feeney, 1977) that alkali treatment of protein resulted in the loss of cysteine. However, the micelle isolates in the current study that did not involve alkali/acid treatments were also devoid of cysteine, which suggests that the explanation related to the exclusion of the cysteine-rich fraction during extraction is more tenable than the destruction theory.

3.5. Protein hydrophobicity

The exposed hydrophobicity of pigeonpea and cowpea isolates, presented in Table 5, indicated that pigeonpea isolates exhibited significantly ($P < 0.05$) higher hydrophobicity values than the corresponding cowpea isolates. This observation is in agreement with earlier results (Table 4) which showed that the former was higher in hydrophobic amino acids, than the latter. For pigeonpea, MP was significantly ($P < 0.05$) lower in hydrophobicity than IP 9.5 and 10.5 and higher than IP 8.5, 11.5 and 12.5. However, for cowpea, MP was lower in hydrophobicity than IP 8.5 and higher than IP 9.5–12.5. This observation indicated that the response of exposed hydrophobicity to isolation technique was species-dependent. As the pH of extraction was increased, the hydrophobicity values of the pigeonpea IP isolates increased, reaching a maximum at pH 10.5 and then decreased markedly beyond this point. For cowpea IP isolates, there was a progressive decrease in hydrophobicity with increasing pH of extraction. Thus, while the pigeonpea and cowpea IP 12.5 showed the highest % of hydrophobic amino acids (Table 4) their exposed hydrophobocities, as determined by the fluorescence probe method, were lower than the other IP isolates. This observation strongly suggested that higher extraction pH probably affected the conformation of the protein through electrostatic interactions. As pH is increased above the isoelectric point, the protein acquires a

Table 3
Chemical composition of pigeonpea and cowpea protein isolates[a]

Component	Pigeonpea						Cowpea					
	Isolates[b] MP	IP 8.5	IP 9.5	IP 10.5	IP 11.5	IP 12.5	MP	IP 8.5	IP 9.5	IP 10.5	IP 11.5	IP 12.5
Moisture %	5.40 ± 0.17e	5.59 ± 0.13e	5.46 ± 0.16e	6.38 ± 0.10cd	6.76 ± 0.06c	8.01 ± 0.25b	5.21 ± 0.46e	6.73 ± 0.23cd	6.41 ± 0.07c	6.26 ± 0.20d	6.26 ± 0.42d	8.45 ± 0.085a
Crude protein %	82.8 ± 0.34ef	83.4 ± 0.43ef	82.4 ± 0.33f	82.4 ± 0.29f	81.2 ± 0.37g	78.1 ± 0.56h	83.2 ± 0.81ef	92.9 ± 0.58a	91.3 ± 0.22b	89.5 ± 0.51c	85.4 ± 0.28d	83.7 ± 0.57e
Crude lipid %	1.81 ± 0.04g	2.44 ± 0.04e	2.81 ± 0.02d	4.04 ± 0.01c	4.40 ± 0.01b	5.28 ± 0.00a	1.16 ± 0.03i	0.33 ± 0.02k	0.57 ± 0.04j	1.35 ± 0.05h	1.38 ± 0.01h	1.90 ± 0.04f
Crude fibre %	1.83 ± 0.08a	1.25 ± 0.08e	1.63 ± 0.00cd	1.65 ± 0.07cd	1.54 ± 0.04d	1.57 ± 0.06d	1.81 ± 0.11ab	1.78 ± 0.11abc	1.79 ± 0.08abc	1.64 ± 0.06cd	1.54 ± 0.03d	1.57 ± 0.06d
Ash %	0.94 ± 0.03d	1.40 ± 0.12c	1.50 ± 0.01c	0.81 ± 0.08de	0.73 ± 0.00e	1.80 ± 0.08b	0.85 ± 0.06de	2.02 ± 0.10a	1.97 ± 0.09ab	1.35 ± 0.05c	2.05 ± 0.14a	1.79 ± 0.06b
NFE[c] %	12.7 ± 0.69ab	11.6 ± 0.81abc	11.7 ± 0.51abc	11.1 ± 0.64bc	12.1 ± 0.59ab	13.2 ± 0.99a	13.5 ± 1.43a	2.95 ± 1.15e	4.40 ± 0.57de	6.13 ± 0.93d	9.59 ± 0.64c	11.1 ± 1.03bc
Trypsin inhibitor TIU/g	ND[d]	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Phytate mg/g	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Sucrose %	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Raffinose %	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Stachyose %	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND

[a] Means in a row followed by the same letter are not significantly different ($P < 0.05$). Values given are means of duplicate determinations.
[b] MP = micelle protein; IP = isoelectric protein.
[c] NFE = nitrogen free extract (by difference).
[d] ND = not detected.

Table 4
Amino acid composition of pigeonpea and cowpea protein isolates according to the nature of side chains[a]

Isolate	Nature of side chain (% of total)				
	Hydrophilic	Hydropphobic	Cyclic	Sulphur	Hydrophobic/Hygrophilic
Pigeonpea					
MP[b]	58.4 ± 0.16de	34.8 ± 0.03bc	5.24 ± 0.26a	1.40 ± 0.25f	0.60 ± 0.01bc
IP 8.5[c]	59.2 ± 0.03d	34.7 ± 0.14bc	4.69 ± 0.09bcde	1.40 ± 0.09f	0.59 ± 0.01cd
IP 9.5	58.7 ± 0.17de	35.1 ± 0.13bc	4.86 ± 0.12bcd	1.36 ± 0.07f	0.60 ± 0.00bc
IP 10.5	58.2 ± 0.18e	35.0 ± 0.06bc	5.39 ± 0.55a	1.49 ± 0.11def	0.60 ± 0.01bc
IP 11.5	59.2 ± 0.18d	34.4 ± 0.13ab	5.10 ± 0.17ab	1.38 ± 0.06f	0.59 ± 0.01cd
IP 12.5	57.0 ± 0.08f	36.5 ± 0.13a	5.06 ± 0.04abc	1.43 ± 0.00	0.64 ± 0.00a
Cowpea					
MP	61.6 ± 0.07ab	32.9 ± 0.13fg	4.48 ± 0.16de	1.46 ± 0.05ef	0.54 ± 0.00f
IP 8.5	60.3 ± 0.94c	33.8 ± 0.74de	4.20 ± 0.04e	1.69 ± 0.25cde	0.57 ± 0.02de
IP 9.5	60.8 ± 0.12bc	33.5 ± 0.43ef	4.26 ± 0.13e	1.82 ± 0.01abc	0.55 ± 0.01ef
IP 10.5	61.7 ± 0.24a	32.2 ± 0.11g	4.20 ± 0.13e	1.92 ± 0.01ab	0.52 ± 0.01g
IP 11.5	60.2 ± 0.03c	33.2 ± 0.02ef	4.58 ± 0.03cde	1.99 ± 0.01a	0.55 ± 0.00g
IP 12.5	58.2 ± 0.66e	35.4 ± 0.67b	4.65 ± 0.13bcde	1.71 ± 0.15bcd	0.61 ± 0.01b

[a] Means in a column followed by the same letter are not significantly different ($P < 0.05$). Values given are means of duplicate determinations.
[b] MP = micelle protein.
[c] IP = isoelectric protein.

Table 5
Exposed hydrophobicity of pigeonpea and cowpea protein isolates[a]

Isolate[b]	Pigeonpea	Cowpea
MP	3089b	2076e
IP 8.5	3053c	2669d
IP 9.5	3465a	1742g
IP 10.5	3479a	1310k
IP 11.5	1826f	1463i
IP12.5	1673h	1352j

[a] Means followed by the same letter are not significantly different ($P < 0.05$). Values given are means of duplicate determinations.
[b] MP = micelle protein; IP = isoelectric protein.

net negative charge and this conceivably caused the hydrophobic groups to be buried into the interior of the protein molecule, as previously reported (Aluko & Yada, 1993).

3.6. SDS–PAGE and isoelectric focusing

SDS–PAGE profiles, shown in Fig. 1 indicated that, while distinct differences existed between the subunit profiles of pigeonpea and cowpea isolates, all components were similar for isolates extracted using the micellization and isoelectric precipitation techniques for the respective legumes. These results demonstrated that extraction technique and conditions had no effect on the subunit composition of the protein isolates. Paredes-Lopez et al. (1991) had reported slight differences between the SDS–PAGE patterns of chickpea micelle and isoelectric isolates. As shown in Table 6, all pigeonpea and cowpea isolates presented similar isoelectric focusing patterns, consisting of 4 bands corresponding to pIs in the range 5.46–7.44 and 5.54–7.49, respectively. These results indicated that isolates from the two legumes exhibited similar electrical mobility, regardless of the extraction technique and extraction pH conditions.

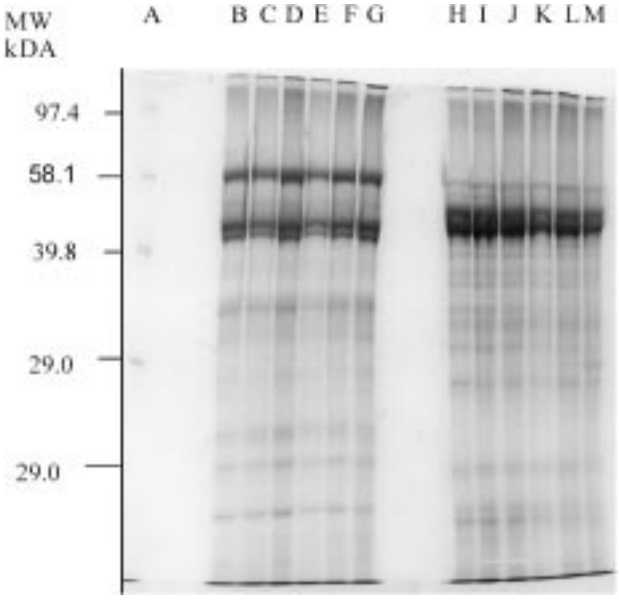

Fig. 1. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis profiles of pigeonpea and cowpea protein isolates. A = standard protein MW markers, B = pigeonpea micelle, C–G = pigeonpea isoelectric, H = cowpea micelle, I–M = cowpea isoelectric.

3.7. Colour

The Hunter Lab colour values presented in Table 7 show that pigeonpea isolates were significantly ($P < 0.05$) lighter in colour (L) and lower in total colour difference (ΔE) than the corresponding cowpea isolates. The L values ranged from 82.27 for pigeonpea MP to 56.91 for cowpea IP 12.5 and the ΔE values from 15.68 to 40.97, respectively. For both legumes, MP isolates were significantly ($P < 0.05$) lighter in colour than the respective IP isolates, an observation consistent with that reported for chickpea isolates (Paredes-Lopez et al., 1991). For the isoelectric isolates, an inverse relationship was observed between the L value (Y) and extraction pH (X) for pigeonpea ($Y = 84.878–2.8840X$, $R^2 = 0.76$) and cowpea ($Y = 77.153–3.9710X$, $R^2 = 0.77$), and a positive relationship was evident between ΔE (Y) and extraction pH (X) for pigeonpea ($Y = 2.7470X + 13.141$, $R^2 = 0.72$) and cowpea ($Y = 4.0650X + 20.339$, $R^2 = 0.77$). Visually the isolates appeared reddish-yellow to dark brown, an observation similar to that of Kazazis and Kalaissakis (1979) who reported that oven-drying yielded isolates brown in colour with a gelatinized hard texture. The intensity of browning of cowpea seeds in boiling water has been reported to be pH-dependent with maxima in the ranges 5–6 and 9–10, ranges which are consistent with the release of acid- and alkali-labile phenolic compounds, respectively (Onigbinde & Onobun, 1993). This probably accounted for the observed general increase in isolate pigmentation with increasing extraction pH in the current study. The cowpea seeds used in the present study were the brown type and the presence of tannins in this type has been reported in other studies (Akinyele, Onigbinde, Hussain & Omololu, 1986; Mnembuka & Eggurn, 1995), but the pigeonpea material used was cream coloured and lower in tannins (Mnembuka and Eggum, 1995). This difference was manifested by the lighter colour of the isolates obtained from the latter. Pigmentation in legume protein isolates can be controlled by carrying out an aqueous preextraction at pH 5.5 (Paredes-Lopez et al., 1991), addition of sodium metabisulfite to the extracting medium (Mansour, Peredi & Dworschak, 1992) and by dehulling the raw material prior to extraction (Onigbinde & Onobun, 1993).

Table 6
Isoelectric points of pigeonpea and cowpea protein isolates

Isolates[a]	Isoelectric pH
Pigeonpea MP and IP	5.46, 5.62, 6.25, 7.44
Cowpea MP and IP	5.54, 5.71, 6.44, 7.49

[a] MP = micelle protein; IP = isoelectric protein.

3.8. Differential scanning calorimetry

As shown in Table 8, significant ($P < 0.05$) differences were observed in the transition enthalpies (ΔH) and denaturation temperatures (T_d) among selected pigeonpea and cowpea isolates. For both legumes, MP isolates exhibited significantly ($P < 0.05$) higher ΔH than the IP isolates, an observation that was consistent with previous studies on isolates from chickpea (Paredes-Lopez et al., 1991), canola, soybean, field pea and faba bean (Murray, Arntfield & Ismond, 1985). These differences have been attributed to partial denaturation of the IP isolates. It has been reported (Murray et al.) that the micellization extraction technique exposes faba bean protein isolate to conditions of minimum harshness compared to those encountered during isoelectric precipitation and, therefore, yields a protein with the least conformational and structural modifications. This was manifested by the higher ΔH values of the micelle isolate compared to the isoelectric isolate. For IP isolates, ΔH decreased with increasing extraction pH, indicating that

Table 7
Hunter (L, a, b) colour of soybean, pigeonpea and cowpea protein isolates[a]

Isolate[b]	L	a	b	ΔE[c]
Soybean	97.16 ± 0.06a	0.03 ± 0.01i	2.68 ± 0.10f	–
Pigeonpea MP	82.27 ± 0.34b	−0.77 ± 0.24j	6.77 ± 0.38c	15.68 ± 0.28h
Pigeonpea IP 8.5	80.80 ± 0.49c	0.66 ± 0.08h	7.48 ± 0.31b	16.97 ± 0.51g
Pigeonpea IP 9.5	77.67 ± 0.47d	1.41 ± 0.06g	8.02 ± 0.73a	20.36 ± 0.45f
Pigeonpea IP 10.5	80.40 ± 0.33c	1.29 ± 0.03g	5.26 ± 0.27d	17.00 ± 0.40g
Pigeonpea IP 11.5	74.09 ± 0.60f	2.83 ± 0.12f	5.33 ± 0.10d	23.39 ± 0.62e
Pigeonpea IP 12.5	68.17 ± 0.64g	3.26 ± 0.22de	1.53 ± 0.21g	29.19 ± 0.69d
Cowpea MP	76.14 ± 1.00e	3.15 ± 0.25ef	4.40 ± 0.20e	21.32 ± 0.94f
Cowpea IP 8.5	74.92 ± 1.38f	3.59 ± 0.10d	2.00 ± 0.10g	22.53 ± 1.39e
Cowpea IP 9.5	69.05 ± 0.21g	6.03 ± 0.54b	1.80 ± 0.08g	28.75 ± 0.23d
Cowpea IP 10.5	59.96 ± 0.77i	6.48 ± 0.11a	−0.61 ± 0.08i	37.90 ± 0.70b
Cowpea IP 11.5	65.36 ± 0.33h	6.47 ± 0.04a	0.34 ± 0.08h	32.52 ± 0.35c
Cowpea IP 12.5	56.91 ± 0.27j	5.58 ± 0.03c	−2.59 ± 0.17j	40.97 ± 0.37a

[a] Means in a column followed by the same letter are not significantly different ($P < 0.05$). Values given are means of duplicate determinations.
[b] MP = micelle proteins; IP = isoelectric protein.
[c] ΔE was calculated using soybean protein isolate as reference.

Table 8
Differential scanning calorimetry (DSC) thermogram values of pigeonpea and cowpea protein isolates[a]

Isolate[b]	Denaturation temperature (T_d, °C)	Transition enthalpy (ΔH, J/g protein)
Pigeonpea MP	93.78 ± 0.03a	16.22 ± 0.02a
Pigeonpea IP 8.5	92.00 ± 0.04c	11.21 ± 0.04b
Pigeonpea IP 9.5	92.70 ± 0.10b	4.78 ± 0.04e
Pigeonpea IP 11.5	91.22 ± 0.01e	2.66 ± 0.03g
Cowpea MP	90.14 ± 0.06g	8.14 ± 0.01c
Cowpea IP 8.5	91.35 ± 0.03d	5.01 ± 0.01d
Cowpea IP 9.5	91.28 ± 0.04de	3.45 ± 0.02f
Cowpea IP 11.5	90.53 ± 0.04f	2.18 ± 0.03h

[a] Means in a column followed by the same letter are not significantly different ($P < 0.05$). Values given are means of duplicate determinations.
[b] MP = micelle protein; IP = isoelectric protein.

the degree of protein denaturation increased with increasing extraction pH as previously reported for faba bean isolates (Arntfield & Murray, 1981). ΔH values represent a useful parameter for assessing the degree of denaturation of plant proteins, despite the complexities involved in their interpretation. Thus, if a protein is partially denatured, the magnitude of ΔH is decreased and is zero if the protein is completely denatured (Arntfield and Murray; Hermansson, 1979). Pigeonpea MP exhibited higher T_d than the IP isolates but the reverse was true for cowpea isolates. An inverse relationship between T_d and extraction pH was evident for both pigeonpea and cowpea IP isolates. Previous studies have shown that T_d of canola MP isolate was higher than that of the IP isolate but the reverse was true for soybean, faba bean and field pea isolates (Murray et al., 1985).

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