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Fractionation and electrophoresis of tomato waste seed proteins

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Abstract

Proteins of deoiled meal and alkali-extracted concentrate of tomato seeds were classified by successive extractions with sodium chloride (0.5 M), aqueous ethanol (70%) and acetic acid (0.05 M) into albumin, globulin, gliadin and glutenin. The globulin was the major protein in both meal and alkali-extracted concentrate. Native and sodium dodecylsulphate polyacrylamide gel electrophoresis of various fractions of seed meal and alkali extracted concentrate revealed that the proteins were made up of sub-units of lower molecular weights. It also indicated that different solvents extracted proteins having different molecular weights.

Keywords: Tomato seed; Protein fractionation; Native; SDS; Gel electrophoresis; Molecular weight

1. Introduction

Tomato paste manufacturing units generate 7.0–7.5% solid waste of raw material and 71–72% of this waste is pomace (Sogi, 2001; Sogi & Bawa, 1998). Seeds, the major constituent of the pomace, contain 22.2–33.9% protein and 20.5–29.6% lipids (Carlson, 1981; Geisman, 1981; Latief, 1982; Sogi, & Bawa, 1998; Tsatsaonis & Bosku, 1975). The seed protein could be extracted to produce protein concentrate/isolate (Kramer & Kwee, 1977a; Latlief & Knorr, 1983; Liadakis, Tzia, Oreopoulou, & Thomopoulos, 1995; Liadakis, Tzia, Oreopoulou, & Thomopoulos, 1998; Sogi, 2001; Tchorbanov, Ushanova, & Litchev, 1986). Functional properties of tomato seed meal and protein concentrates have been evaluated and found to be comparable with other plant proteins (Doxastakis, Kiosseoglou, Bosku, 1988; Liadakis et al., 1998; Rahma, Moharram, & Mostafa, 1986; Sogi, 2001). The nutritional quality of tomato seed proteins is equivalent to commonly used plant proteins as is evident from amino acid score, growth of micro-organism (*Tetrahymena pyriformis*), proteolytic activity and rat feeding trials (Kramer & Kwee 1977a; Rahma et al., 1986; Sogi, 2001). The seed protein is rich in lysine, i.e. 80–100 g/kg N (Cantarella, Palma, & Caruso, 1989; Rymal, Smit, & Nakayama, 1974) and can supplement products that are deficient in this amino acid, such as cereals (Brodowski & Geisman, 1980; Carlson et al., 1981; Yaseen et al., 1991). No anti-nutritional factors or harmful constituents have been reported in tomato seeds (Rahma et al., 1986) and that makes it a better source of protein than other non-conventional sources.

Tomato seed proteins have also been fractionated and globulin has been observed to be the major fraction, comprising 70% of total proteins (Moharram & Messallam, 1980). Characterisation of tomato seed proteins has revealed 4–5 fractions (Egger, 1975); however, Moharram & Messallam (1980) reported 3 fractions out of which one was major, having high mobility and two minor with low mobility. The present study was undertaken to fractionate the proteins of tomato seed meal as well as alkali-extracted protein concentrate using different solvents and also to characterise these fractions using native (N) and sodium dodecylsulphate (SDS) polyacrylamide gel electrophoresis (PAGE).

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2. Materials and methods

2.1. Materials

Tomato seeds were separated from the pomace, collected from a tomato paste manufacturing plant located at Amritsar (India), by a sedimentation technique (Sogi, Bawa, & Garg, 2000) and dehydrated at 70 °C for 5 h in a cabinet dryer (Sogi & Bawa, 1998). Seeds were ground using a hammer mill (M/S Narang Scientific works, New Delhi), extracted with hexane, desolventized and ground again to pass through a 85 mesh sieve to obtain fine powder, termed deoiled meal.

Protein concentrate was prepared by mixing deoiled meal with 1.2% NaOH using a 70/1 solvent to meal ratio, stirred for 5 min, centrifuged at 2600×***g***, acidified to pH 3.8 using HCl solution, centrifuged at 2600×***g*** and vacuum-dried (100 mm Hg, 50 °C). Samples were kept in airtight containers until used.

2.2. Methods

A modified Osborne fractionation procedure was followed to separate proteins form deoiled meal and its alkali-extracted protein concentrate (Chen & Bushuk, 1970) to obtain albumin (water-soluble), globulin (salt-soluble), gliadin (ethanol-soluble), soluble (acetic acid-soluble) and insoluble (residual fraction) glutenin. A sample (10 g, dry basis) was extracted successively with sodium chloride (0.5M), aqueous ethanol (70%) and acetic acid (0.05 M). The salt-soluble fraction was dialysed overnight in a dialysing tube (Pore size 2.4 nm, Hi-Media Laboratories Ltd., Mumbai) at 15 °C and centrifuged at 2600×***g*** for 15 min to separate salt and water soluble fractions. The remaining fractions were dialysed similarly to remove ethanol and acetic acid. Different fractions were freeze dried and nitrogen content was determined by the micro-Kjeldahl procedure (AOAC, 1990).

Electrophoresis was performed in a vertical slab (Mini-PROTEAN 3, Bio-Rad Laboratories, Hercules, USA) to determine molecular weight of various protein fractions obtained by Osborne classification (Laemmli, 1970). The resolving (10%, pH 8.8) and stacking (5%, pH 8.6) gels were prepared using acrylamidebisacrylamide solution, Tris–HCI buffer, ammonium-persulphate solution and tetramethyl-ethylene-diamine (TEMED) according to Bollag, Rozycki, and Edelstein, (1996).

Each of the Osborne fractions (100 mg) was dissolved in distilled water (10 ml) and their protein contents were determined by the Lowry method (Lowry et al., 1951). The protein solution (150 μl) of each fraction was mixed with 150 μl of Native as well as SDS-PAGE sample buffers. The samples for SDS-PAGE were heated in a boiling water bath for 3 min and allowed to cool to room temperature.

The above samples, equilibrated for protein content (50 μg), were loaded and allowed to run at 100 volts until the tracking dye reached the bottom of the gel. The gel was removed, stained overnight with Coomassie stain solution, extra dye was removed by repeated washings using Coomassie destain solution, and preserved in 3% acetic acid.

Standard protein mixture (M/S Bangalore Genei Pvt Ltd., Bangalore) was used as reference and developed along with each electrophoretic analysis. The molecular weights of proteins in test samples were computed employing a regression equation using Excel 9.8 (Microsoft Inc; Fig 1).

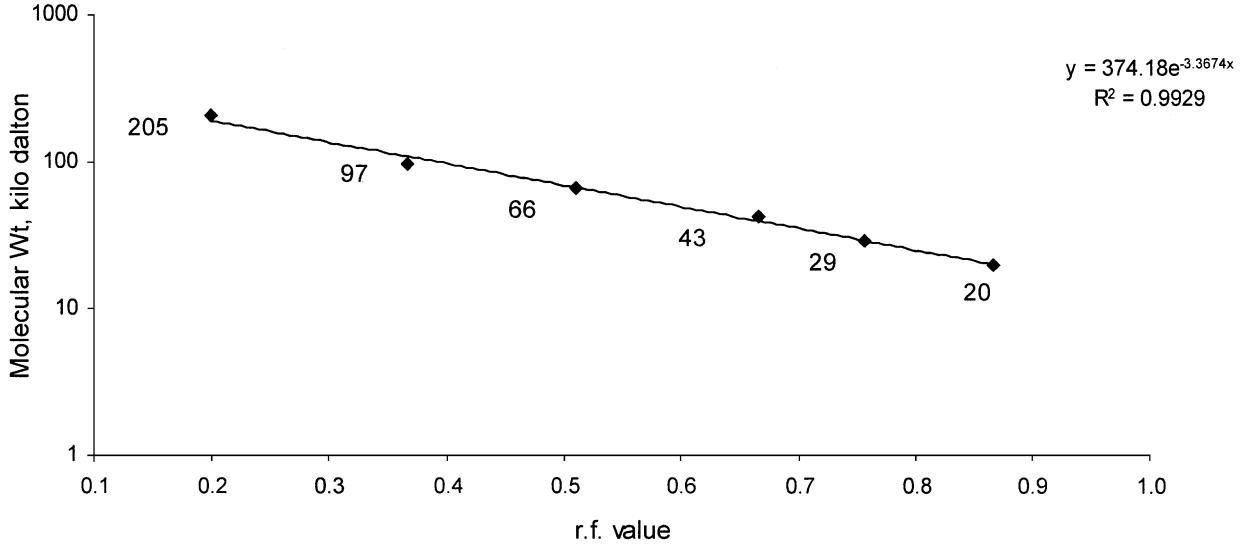

Fig. 1. N-PAGE (10%) of standard proteins.

3. Results and discussion

Table 1 reveals that the successive extractions of deoiled meal and alkali-extracted concentrate from tomato seeds with distilled water, NaCl, ethyl alcohol and acetic acid solutions yielded 23.0 and 10.8 g of total extractable protein/100 g crude protein, respectively. The major fraction in deoiled meal and alkali-extracted protein concentrate was globulin, constituting 61.0 and 37.1% while the minor fractions were soluble glutenin and gliadin in descending order, respectively. The insoluble residues from deoiled meal and alkali-extracted concentrate consisted mainly of glutenin, i.e. 77.0 and 89.2 g /100g of crude protein respectively.

Solubility, structure and functionality are common criteria for classification of proteins, the first of these has been used for a long time (Rakosky, 1989). Present studies on solubility of deoiled meal and concentrate reveal that the former has a higher proportion of soluble proteins than the later one. The lower extractable proteins in alkali concentrates may be attributed to the denaturation caused by alkaline conditions during protein isolation and subsequent drying of precipitates. The relative proportions of different fractions from deoiled meal and alkali concentrate indicate that the alkali

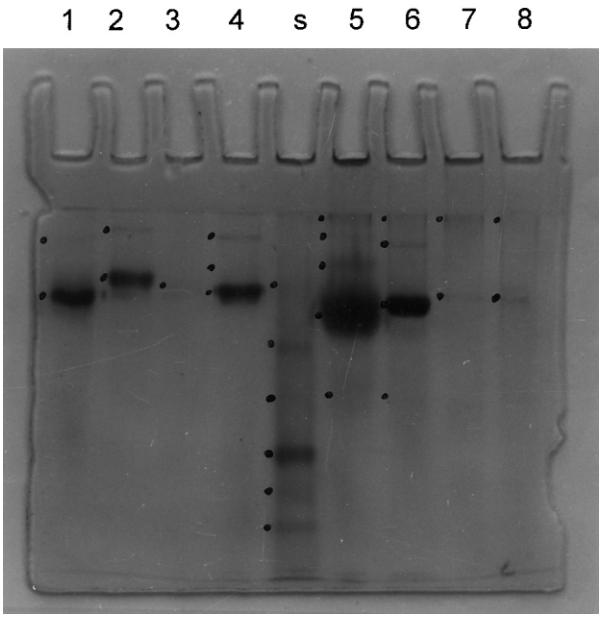

Fig. 2. Native-PAGE (10%) of fractions obtained by successive extraction of deoiled meal [water (1), salt (2), alcohol (3), acetic acid (4)] and alkali-extracted protein concentrate [water (5), salt (6), alcohol (7), acetic acid (8)] of tomato seeds. Standard protein (S).

Table 1
Extractable tomato seed proteins using the Osborne fractionation technique, ($n=3$)

Extraction solvent	Protein type	Protein yield, g/100g crude protein[a]	
		Deoiled meal	Alkali extracted concentrate
Distilled water	Albumin	5.41±1.74 (23.5±7.54)	3.52±0.86 (32.6±7.91)
NaCl, 0.5 M	Globulin	14.1±1.09 (61.0±4.73)	4.01±0.53 (37.1±4.94)
Ethanol, 70%	Gliadin	1.63±0.51 (7.06±2.20)	1.57±0.34 (14.4±3.11)
Acetic acid, 0.05 M	Soluble glutenin	1.98±0.50 (8.62±2.18)	1.72±0.60 (15.92±5.53)
Residue (N×6.25)	Insoluble glutenin	77.0±4.06	89.2±1.97

[a] Mean±S.D. Values in parentheses indicate percent extractable proteins.

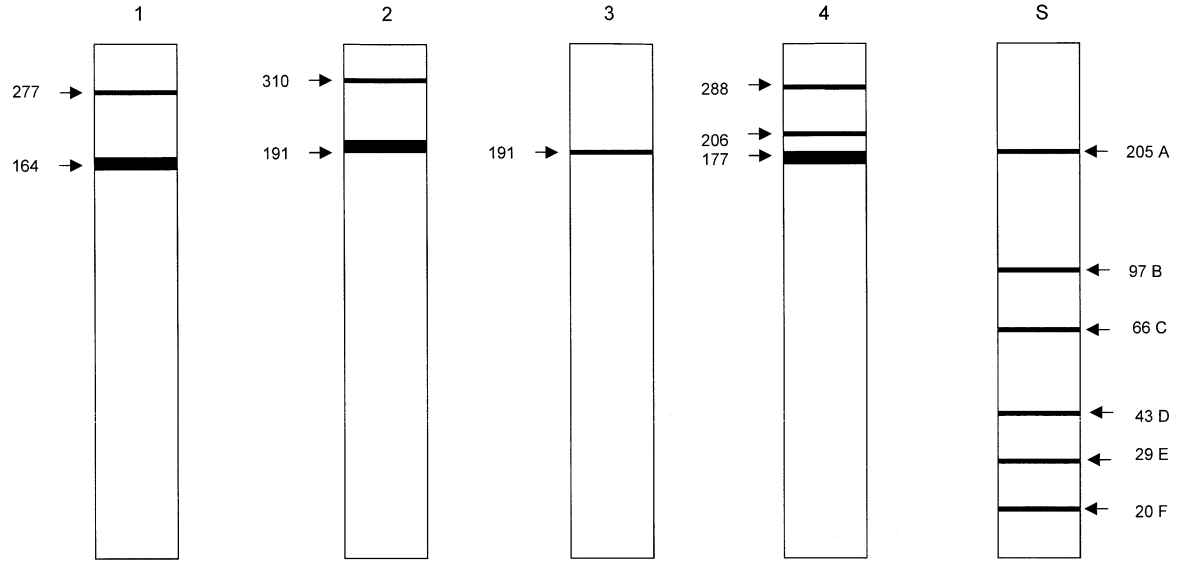

Fig. 3. Native-PAGE (10%) of fractions obtained by successive extraction of deoiled meal [water (1), salt (2), alcohol (3) and acetic acid (4)] of tomato seeds. Standard protein (S).

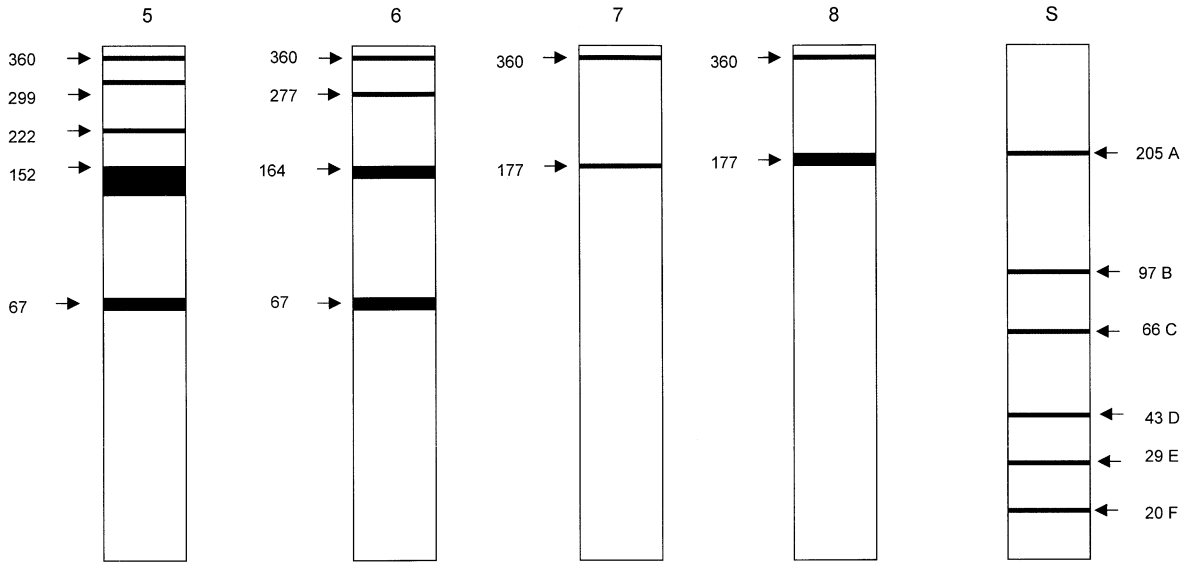

Fig. 4. Native-PAGE (10%) of fractions obtained by successive extraction of alkali-extracted protein concentrate [water (5), salt (6), alcohol (7), acetic acid (8)] of tomato seeds. Standard protein (S).

concentrate preparation, involving solublisation of protein under alkaline conditions, followed by precipitation at isoelectric pH, gave a lower globulin fraction than the deoiled meal. Earlier, Moharram and Messallam (1980) reported that globulin was the major fraction of tomato seed proteins, forming 70% of the extractable proteins. Later, Rahma (1986) revealed that the nitrogen solubility indices of deoiled tomato seed meal in water and NaCI solution (5%) were 30.7 and 58.2%, respectively which are similar to the results reported in the present study.

The protein fractionation studies by other researchers have revealed that the albumin, globulin, gliadin, soluble and insoluble glutenin contents were 7.9, 4.1, 43.7, 11.6 and 26.7 percent for wheat (Hwang & Bushuk, 1973), 19.6, 21.7, 3.9, 16.1 and 38.7 percent for deoiled tamarind kernel (Bhattacharya, Bal, & Mukherjee, 1994), and 10–22.7, 4.7–15.2, 35–67.9, 9.4–51.1 and 8.1–11.9% for sorghum (Elkhalifa & Tinay, 1999), respectively. These studies support the present findings, i.e. different plant materials have varying proportiors of the various protein fractions, based on their solubility.

Native as well as SDS PAGE of various extracts of deoiled meal and alkali concentrate of tomato seeds were carried out to find out their molecular weights. Results of N-PAGE (10%) of the water-soluble fraction of deoiled meal revealed two proteins having molecular weights of about 277 and 164 kDa (Figs. 2 and 3). The fraction with high mobility was predominant. A similar pattern was observed in the salt-soluble fraction; however, the molecular weights of the two bands were ~ 310 and 191 kDa. The alcohol-soluble fraction resolved into a minor band of about 191 kDa. The acetic acid fraction exhibited 3 bands of proteins with approximate

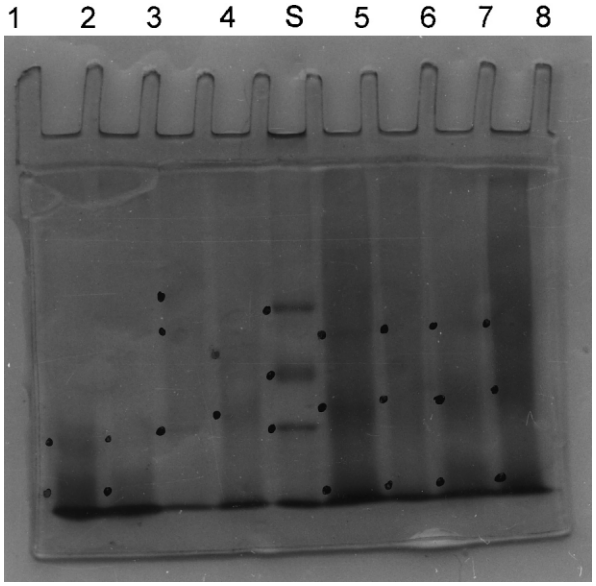

Fig. 5. SDS-PAGE (10%) of fractions obtained by successive extraction of deoiled meal [water (1), salt (2), alcohol (3), acetic acid (4)] and alkali-extracted protein concentrate [water (5), salt (6), alcohol (7), acetic acid (8)] of tomato seeds. Standard protein (S).

molecular weights of 288, 206 and 177 kDa. The N-PAGE of water, salt, alcohol and acetic acid treatment of alkali extracted protein concentrate, revealed 5 (360, 299, 222, 152 and 67 kDa), 4 (360, 277, 164 and 67 kDa) and 2 (360 and 177 kDa) bands each for the latter ones, respectively (Figs. 2 and 4). The results of the present study are in accordance with Rahma et al. (1986) who studied the electrophoretic pattern of tomato seed proteins and reported three bands of proteins, out of which one with high mobility was the major while two with

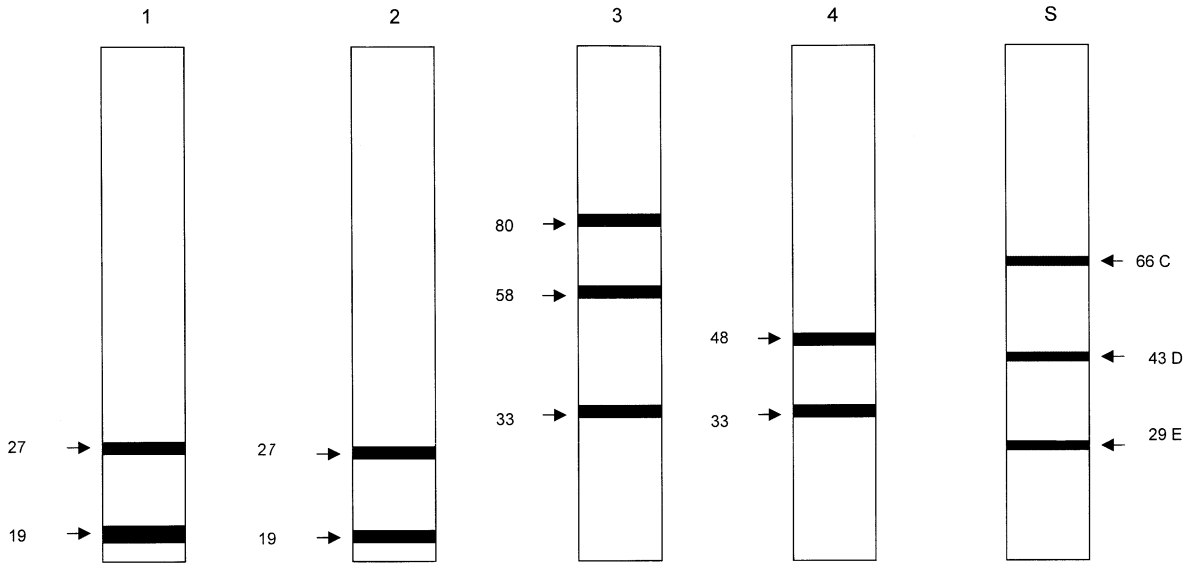

Fig. 6. SDS-PAGE (10%) of fractions obtained by successive extraction of deoiled meal [water (1), salt (2), alcohol (3) and acetic acid (4)] of tomato seeds. Standard protein (S).

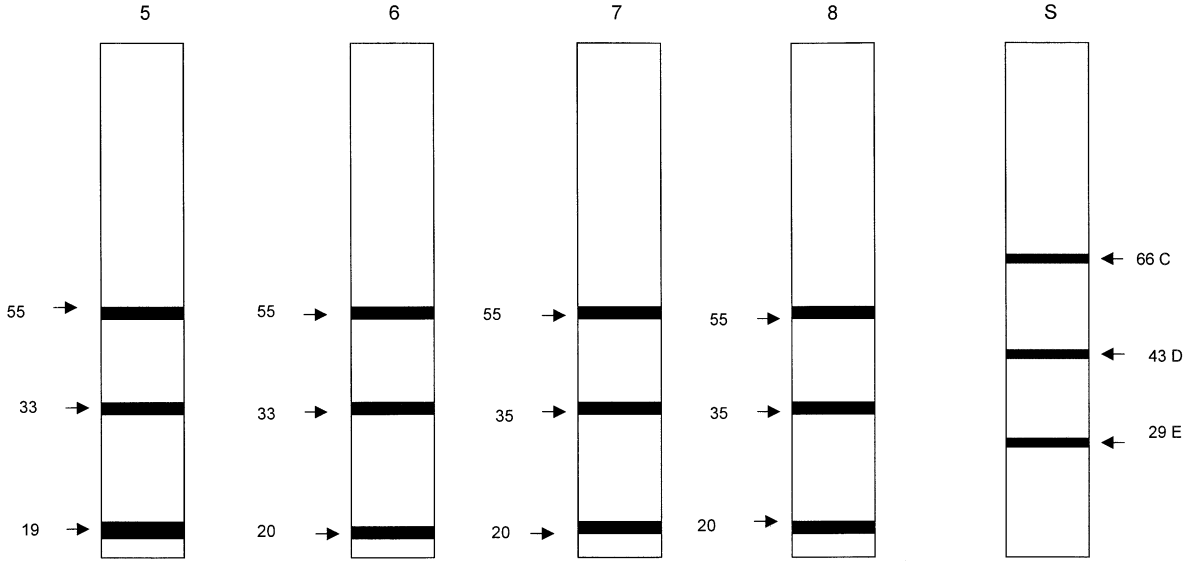

Fig. 7. SDS-PAGE (10%) of fractions obtained by successive extraction of alkali-extracted protein concentrate [water (5), salt (6), alcohol (7), acetic acid (8)] of tomato seeds. Standard protein (S).

low mobility were minor ones, except that one band of low mobility was missing in the present study. The difference observed might be due to the different extraction procedures followed in these studies.

The SDS-PAGE (10%) of deoiled meal of tomato seeds resolved into proteins of high mobility (Figs. 5 and 6). Water-and salt-soluble fractions revealed two bands, each with molecular weights of about 27 and 19 kDa. The alcohol fraction showed three bands of approximately 80, 58 and 33 kDa, whereas two bands of 48 and 33 kDa were observed in the acetic acid treated fraction. The fractions of alkali concentrates (Figs. 5 and 7) exhibited three bands each; however, water (~55, 33 and 19 kDa), salt (~55, 33 and 20 kDa), alcohol and acetic acid (~55, 35 and 20 kDa) showed slightly different proteins.

The present study shows that tomato seed meal and concentrate contain a specific proportion of various proteins, different from other protein sources. Characterisation of the fractions revealed that these are composed of sub-units having different molecular weights. The functional properties of the proteins in a given food system could be co-related to the nature of the proteins.

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