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Changes in Gross Chemical Composition with Emphasis on Lipid and Protein Fractions During Germination of Fenugreek Seeds

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ABSTRACT

Germination of fenugreek seeds for 3 and 5 days increased moisture, crude protein, crude fibre, non-protein nitrogen and ash contents, but total lipids and carbohydrates were decreased. Marked increases of Na and P, as well as Mg and Zn were observed. Triacylglycerols decreased continuously and this was accompanied by an increase in free fatty acids, monoglycerides, sterolesters and polar lipids. Albumin was the major protein fraction followed by globulin, glutelin and prolamin. Germination increased glutelin and non-protein nitrogen; the other fractions were decreased. The protein in vitro *digestibility was improved after germination. PAGE showed a marked dissociation of albumin and globulin fractions after germination.*

INTRODUCTION

Fenugreek seed (*Trigonella foenum graecum* L.) is an annual legume cultivated as a hay crop in Mediterranean countries (Rosengarten, 1969).

There are several reports about the chemical composition, mineral content, fatty acid composition and amino acids of fenugreek seeds (Shankaracharya & Natarajan, 1972, 1973; El-Madfa, 1975). In Egypt, about 12 475 ha are cultivated with fenugreek, producing 22 126 tons of seeds during the 1979 season (Ministry of Agriculture, 1981). The ground seeds are used as a supplement to wheat and corn flours for bread-making. They are also added as a flavouring agent to concentrated can syrup to produce the well known local food *hulba makoda* and fenugreek seed extract is used as a soft drink either hot or cold. The sprouted seeds are also consumed and known as germinated *hulba*.

In general, germination improves the nutritive value of seeds (Kakade & Evans, 1966), increasing their vitamin contents (Chattopadhyay & Banerjee, 1954; Banerjee *et al.*, 1955) and reducing some of the antinutritional factors, i.e. trypsin inhibitor (Collins & Saunders, 1976), phytate content (Reddy *et al.*, 1978) and haemagglutinin activity (Subbulakshmi *et al.*, 1976).

There is a lot of information available about trypsin inhibitor and haemagglutinin activities, phytic and ascorbic acid contents, *in vitro* protein digestibility, nitrogenous constituents (El-Mahdy & El-Sebaiy, 1982; El-Shimi & Damir, 1984) and carbohydrate fractions (El-Mahdy & El-Sebaiy, 1983) in germinated fenugreek seeds, but not throughout germination. Yet, there are no available data about the types of change in the lipid fractions and protein patterns during germination of fenugreek seeds.

Therefore, this work was aimed at studying the effect of germination time on the changes in chemical composition, minerals, lipid fractions, fatty acid constituents and electrophoretic patterns of proteins of fenugreek seeds.

MATERIALS AND METHODS

Materials

Fenugreek seeds

The seeds (*Trigonella foenum graecum* L.) var. Giza-2 were obtained from the local market of the Kena Governorate, upper Egypt, during 1985. They were cleaned by hand to remove foreign materials and used for germination.

Standard lipids

A lipid extract from cottonseed var. Giza70 (60 days after flowering) was used as a reference, containing all known fractions of the neutral and polar lipids (Abd El-Aal, 1981).

Germination procedure

About 500 g of cleaned seeds were first sterilized by soaking in absolute ethanol for 1 min, then washed with distilled water and steeped in 2 litres of distilled water for 6 h at room temperature (~28°C). The steeped seeds were kept between thick layers of cotton cloth and allowed to germinate in the dark at room temperature for 3 and 5 days. The cloth and seeds were moistened during the germination period by sprinkling with water once a day. The germinated seeds, at the particular time, were dried in an electric air draught oven at 35°C for 24 h.

After drying, the seeds were ground in a laboratory hammer mill to pass through a 40 mesh (British Standard screen) sieve. Defatted samples, as previously described (Abd El-Aal *et al.*, 1986*a*) were used to study the effect of germination on the protein properties.

Analytical methods

Moisture, total nitrogen, ash, crude fibre and non-protein nitrogen contents were determined according to the AOAC (1975) method. The total soluble sugars were determined in the 80% ethanol extract by the method of Dubois *et al.* (1956). Glucose was used to prepare the calibration curve. Total lipids were determined gravimetrically using a chloroform:methanol mixture (2:1 v/v), and purified according to the procedure of Folch *et al.* (1957).

Determination of minerals

Na, K, Ca, Mg, Zn, Cu, Fe and Co were determined using a Shimadzu A.A. 630-2 atomic absorption spectrophotometer. Phosphorus content was determined by the method of the AOAC (1975). The total ash of each sample was dissolved in hydrochloric acid (1 + 1, v/v) and the total volume made up to 50 ml with redistilled water.

Total lipid analysis

This was carried out on a thin-layer of silica gel H plate with hexane–diethylether–acetic acid (80:20:1, by volume) as the developing system (Mangold & Malins, 1960). The spots were detected by iodine vapour and identified by comparing the R_f values with that reported in the literature (Christie, 1976; Abd El-Aal, 1981; Abd El-Aal *et al.*, 1986*b*). Further confirmation was achieved by co-chromatography using the standard cottonseed lipids. For quantitative determination the chromatograms were sprayed with 50% sulfuric acid solution and scanned using the charring densitometry technique (Blank *et al.*, 1964). The area under each peak representing lipid components was calculated in relation to the total peak area.

Fatty acid analysis

Fatty acid compositions of the total lipid, the isolated triacylglycerol and polar lipid fractions were determined by gas chromatography. The methyl esters were prepared according to Chalvardjian (1964) using 1% of H_2SO_4 in absolute methanol. A Perkin-Elmer Sigma-2 instrument with flame ionization detector was used. It was fitted with a (2 m × 2 mm) glass column packed with 6% Silar 5 CP on Chromosorb WHP 100/120 mesh at a temperature of 200°C. The flow rate was 20 ml/min with nitrogen gas as carrier. Peak integration was calculated using a spectrophysics SP-4000. Reported fatty acids are percentages of the total peak area. Standard fatty acid methylesters were used for identification. Further identification was done by plotting the log retention time against equivalent carbon chain length.

Fractionation of total protein on the basis of solubility

This was determined as previously described by Abd El-Aal *et al.* (1986*a*) for apricot kernel protein. The sequential solvents used to extract each protein fraction were distilled water of pH 6·8 (albumins), 1·0M NaCl (globulins), 70% ethanol (prolamins) and 0·2M NaOH (glutelins). The residue after these extractions was also digested for protein estimation and designated residual protein.

Protein solubility

This was done for the defatted flour samples in 1M NaCl solution according to Rahma & Narasinga Rao (1979), using a flour to solvent ratio of 1:10 w/v.

In vitro *digestibility of fenugreek flours*

This was determined by pepsin followed by pancreatin according to the method of Akeson & Stahmann (1964). Digestibility by the other proteolytic enzymes such as pepsin, trypsin and pancreatin in single systems was done under the same conditions as described by Abd El-Aal *et al.* (1986*a*).

Polyacrylamide Gel Electrophoresis (PAGE)

This was carried out by the procedure of Davis (1964) using 7·5% gel. The experiments were done in 0·01M sodium phosphate buffer of pH 7·8 at a constant current rate of 3 milliamps per tube for 3 h. The gels were stained with 0·5% amido black in 7·5% acetic acid for 1 h and then destained in 7·5% acetic acid. Electrophoresis was conducted for the dialyzed total protein as well as water, 1M NaCl, 70% ethanol and 0·2M NaOH extracts. The dialysis was conducted overnight with several changes of buffer solution.

RESULTS AND DISCUSSION

Proximate composition

Table 1 shows the chemical composition of the fenugreek seeds at various times of germination. The moisture content of ungerminated seeds increased from 11·2% to 66·1% and 72·2%, while dry weight decreased by about 14·6% and 16·3% of the ungerminated seeds after 3 and 5 days of germination, respectively. These values are less than those reported by El-Mahdy & El-Sebaiy (1982) which may be due to a difference in the germination procedure used. After 3 days of germination both the total

TABLE 1
Effect of Germination on the Changes in Chemical Composition of Fenugreek Seeds.[a] (%)

Constituents[c]	*Germination time (days)*		
	0	*3*[b]	*5*[b]
Total nitrogen	5·41 ± 0·23	5·62 ± 0·12	5·98 ± 0·28
Crude protein (N × 6·25)	33·8 ± 0·23	35·1 ± 0·12	37·4 ± 0·28
Total lipids	7·51 ± 0·12	4·71 ± 0·25	6·24 ± 0·24
Total soluble sugars	8·78 ± 0·20	6·35 ± 0·32	16·5 ± 0·41
Crude fibre	5·76 ± 0·21	8·25 ± 0·33	13·7 ± 0·80
Total ash	3·56 ± 0·10	3·04 ± 0·18	3·70 ± 0·30
Total carbohydrates[d] (other than soluble sugars)	40·6 ± 0·69	42·5 ± 0·82	22·5 ± 0·96

[a] Moisture content of the seeds at 0, 3 and 5 days of germination was 11·15 ± 0·02%, 66·10 ± 0·15% and 72·24 ± 0·56%, respectively.
[b] Loss in dry weight at 3 and 5 days of germination was 14·58 ± 1·51% and 16·34 ± 1·72%, respectively.
[c] Means of three determinations ± SD, on dry weight basis.
[d] By difference.

lipids and total soluble sugars showed a more moderate reduction in their percentages than that of the ungerminated seeds. The reduction accounted for 37·3% and 27·7% of their original amounts, respectively, probably because of utilization of lipids and soluble sugars as energy sources to start germination. These changes agree with those reported by Walt & Merrill (1963) for germinated mung beans and soybeans and by El-Mahdy & El-Sebaiy (1983) for germinated fenugreek seeds.

Fenugreek seeds contained 33·8% crude protein. El-Madfa (1975) and El-Mahdy & El-Sebaiy (1983) have reported a value of 24·5% for Egyptian fenugreek seeds. Germination of fenugreek seeds for 5 days caused an

increase in protein content. Crude fibre and ash were also increased as germination proceeded. On the other hand, total carbohydrates showed a marked reduction after 5 days of germination, which accounted for 44·4% of the original content. The changes in the polysaccharides of the fenugreek seed during germination agree well with those previously noted by El-Shimi & Damir (1984). Koller *et al*, (1962) stated that the breakdown of starch during germination of seeds may be attributed to the increase in amylase and phosphorylase activities in respiratory metabolism and utilization by the growing sprouts.

Mineral composition

Table 2 gives the changes in individual minerals during the germination of fenugreek seeds. There was a marked increase in both Na and P with time of germination. A moderate increase was also observed for Mg and Zn. The remaining minerals did not show any marked changes compared with ungerminated seeds. Aminah & Ruth (1984) have reported that the germination had a consistent effect on the mineral contents of soybean and mungbean. They observed an increase in both Na and P after germination. The forementioned data were more or less in accordance with those recently reported by El-Shimi & Damir (1984). However, the 5-days germinated seeds are generally better sources of magnesium, phosphorus and zinc than ungerminated seeds. Walker *et al.* (1948) and Van Den Berry *et al.* (1972) showed that phytic acid has a negative effect on the absorption of Ca, Fe, Mg, Zn and other trace essential elements. Phytic acid is found

TABLE 2
Effect of Germination on the Changes in Mineral Content of Fenugreek Seeds.[a] (mg/100 g dry matter)

Element	*Germination time (days)*		
	0	*3*	*5*
Na	130	218	627
K	32·3	18·9	30·4
Ca	186	158	185
Mg	152	140	178
P	348	402	540
Zn	4·11	4·96	5·62
Cu	2·93	2·18	2·42
Fe	21·6	17·8	21·1
Co	0·61	0·92	0·54

[a] Means of two determinations.

in dry fenugreek seeds and is reported to decrease due to the increase of phytase activity during germination of fenugreek seeds (El-Shimi & Damir, 1984). Hence, the availability of mineral nutrients may be increased due to the decrease of phytic acid content in germinated fenugreek seeds.

Total lipid analysis

The changes in lipid components during fenugreek seed germination are illustrated in Fig. 1 and Table 3; it can be seen that dry and germinated seeds contain at least nine lipid components. The dry fenugreek seeds contain higher proportions of free fatty acids and partial glycerides. This may be attributed to the partial hydrolysis of the reserve triglycerides in

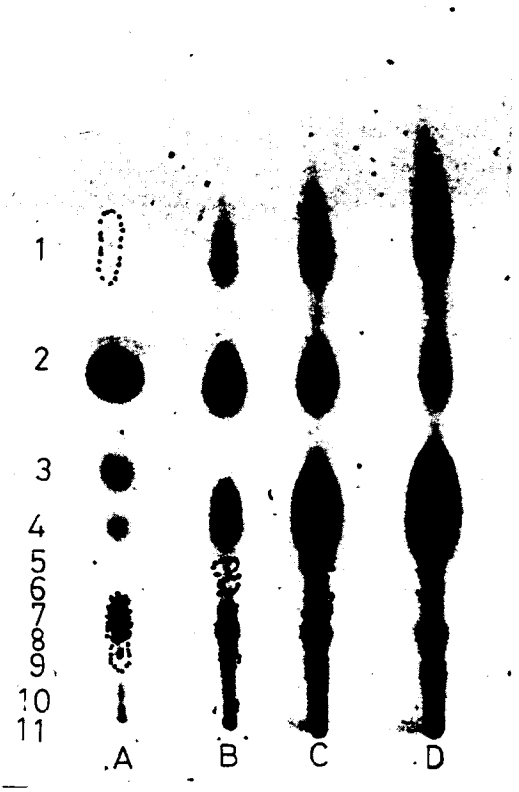

Fig. 1. Thin-layer chromatogram of total lipids fractions of fenugreek seed during germination. Silica gel H plate in hexane: diethylether: acetic acid (80:20:1 by volume). Detection: charring with 50% aqueous sulfuric acid. (A) Standard cotton seed oil. (B) Ungerminated fenugreek seed. (C) and (D) Fenugreek seed germinated for 3 and 5 days, respectively. 1 = sterolesters, 2 = triacylglycerols, 3 = tocopherols (?), 4 = free fatty acids, 5 and 6 = unknowns, 7 = 1,3-diacylglycerols, 8 = sterols, 9 = 1,2 (2,3)-diacylglycerols, 10 = monoglycerides and 11 = polar lipids.

TABLE 3
Effect of Germination on the Changes in Total Lipid Components of Fenugreek Seeds, (Weight %)

Component[a]	*Germination time (days)*		
	0	*3*	*5*
Sterolesters	2·86 ± 0·41	3·38 ± 0·45	4·88 ± 0·40
Triacylglycerols	70·1 ± 2·81	19·0 ± 1·66	14·8 ± 1·31
Free fatty acids	16·5 ± 2·61	61·3 ± 2·33	63·8 ± 2·35
1,3-Diacylglycerols	0·50 ± 0·10	0·45 ± 0·06	0·42 ± 0·10
Sterols	4·46 ± 0·64	4·14 ± 0·60	4·69 ± 0·91
1,2 (2,3)-Diacylglycerols	2·59 ± 0·80	2·35 ± 0·30	2·56 ± 0·10
Monoglycerides	0·13 ± 0·02	0·46 ± 0·13	1·45 ± 0·11
Polar lipids	1·54 ± 0·23	5·59 ± 0·95	6·34 ± 0·83
Unknown (s)	1·27 ± 0·55	3·35 ± 0·91	1·09 ± 0·14

[a] Means of three determinations ± SD.

the seeds due to increase in lipase activity. As the seeds germinated, a further breakdown, at a faster rate, occurred in the triglyceride component. The increase in the free fatty acids of oleaginous seeds through germination was reported by El-Nochrashy *et al.* (1974).

It was also observed that the decrease in the triacylglycerols was accompanied by an increase of polar lipids, monoglycerides and sterol ester components. Free sterols were fairly stable throughout the germination period (Table 3).

Fatty acid composition

More than seven saturated fatty acids were found in the total lipid, triacylglycerols and polar lipids either in the dry or germinated fenugreek seeds. Palmitic acid represents the major saturated one followed by stearic acid (Table 4). Also, appreciable quantities of arachidic and behenic acids are found. Palmitic acid was nearly double, in the polar lipid fraction, compared to that found in total lipids and the triacylglycerol components, while stearic acid content was nearly the same either in dry or germinated seeds. During germination these acids showed a continuous gradual decrease in the case of the polar lipid fraction while it was fairly stable during germination in the total lipids and its triacylglycerol component.

Of the total unsaturated fatty acid present in the fenugreek seed, linoleic acid was the predominant constituent, followed by linolenic and oleic acids. The polar lipid fraction contains higher amounts of oleic acid and less of linolenic acid compared to that present in the total lipid and triacylglycerol. During germination the oleic acid was stable in the case of

TABLE 4
Effect of Germination on Fatty Acid Constituents of Total Lipids, Triacylglycerols and Polar lipids of Fenugreek Seeds (%)

Fatty acid[a]	*Germination times (days)*								
	Total lipids			*Triacylglycerols*			*Polar lipids*		
	0	*3*	*5*	*0*	*3*	*5*	*0*	*3*	*5*
14:0[b]	0·15	0·15	0·13	0·13	0·13	0·16	0·64	0·49	1·82
16:0	11·7	11·7	11·7	9·62	10·0	10·3	19·3	19·8	17·4
17:0	0·10	0·13	0·23	0·35	0·35	0·33	0·26	0·35	0·63
18:0	4·71	3·80	4·20	4·10	4·52	4·60	6·92	4·47	4·40
20:0	1·10	1·20	0·70	0·93	1·31	1·45	2·20	0·62	0·60
22:0	0·82	0·81	0·91	0·51	0·66	0·83	0·10	0·43	0·54
24:0	0·11	0·33	0·14	0·25	0·56	0·28	0·56	0·81	0·98
16:1	0·28	0·28	0·30	0·26	0·32	0·55	0·60	0·41	0·32
18:1	16·0	15·3	15·2	12·1	15·1	17·3	20·7	20·4	20·6
18:2	38·4	40·0	40·3	38·0	38·5	38·6	36·8	40·0	39·4
18:3	25·1	24·8	23·6	29·2	27·3	23·8	9·48	10·2	10·4
20:1	0·20	0·44	0·44	0·36	0·36	0·36	0·36	0·83	0·37
20:2	0·18	0·13	0·10	0·20	0·10	Tr[c]	0·30	0·30	0·20
22:1	0·42	0·61	0·50	0·45	0·59	0·20	1·70	0·85	2·00
Others[d]	0·84	1·39	1·39	3·08	0·79	1·19	0·13	0·04	0·39
Total saturated	18·7	18·0	18·1	15·9	17·5	17·9	30·0	27·0	26·3
Total unsaturated	80·5	80·6	80·6	81·0	81·7	80·9	69·9	73·0	73·3

[a] Means of two determinations.
[b] Fatty acid chain length: Number of double bonds.
[c] Tr: Trace.
[d] These include 12:0, 14:1, 15:0, 16:2, 17:1, 17:2, 20:3 and 24:1.

the total lipid and polar lipid fractions, while linoleic acid increased slightly as germination proceeded. Triacylglycerols contain higher amounts of linolenic acid compared to that in the polar lipid fraction. This acid showed a continuous gradual decrease with increase in germination time. The results showed that the net changes in the fatty acid constituents were only slight except for that of the polar lipid fraction. This general observation has also been noted by other investigators in their studies on the germination of some oleaginous seeds (El-Nochrashy *et al.*, 1974).

Fractionation of crude protein

Table 5 shows the protein fractions of ungerminated and 3- and 5-days germinated fenugreek seeds. Albumin was the highest, 43·8%, followed by globulin, 27·2, then glutelin, 17·2%. Prolamin was found to be the lowest

TABLE 5
Protein Fractions of Fenugreek Seeds at Different Times of Germination (%)[a]

Protein fraction	*Germination time (days)*		
	0	*3*	*5*
Albumins	43·8 ± 1·2	38·6 ± 1·5	29·9 ± 1·4
Globulins	27·2 ± 0·6	22·7 ± 0·4	17·7 ± 0·2
Prolamins	7·37 ± 0·2	6·48 ± 0·4	5·25 ± 0·2
Glutelins	17·2 ± 0·8	21·0 ± 0·8	26·9 ± 0·7
Non-protein nitrogen	3·78 ± 0·2	10·8 ± 0·3	19·9 ± 0·5
Residual protein	0·65 ± 0·02	0·42 ± 0·01	0·40 ± 0·03

[a] Means of three determinations ± SD.

fraction (7·37%). These data are in good agreement with those reported by El-Mahdy & El-Sebaiy (1982) for ungerminated fenugreek seed. The germination process, for different times, showed drastic changes in these fractions. There was an observed decrease in albumin, globulin and prolamin of 31·8%, 35·1% and 28·8%, respectively, after 5 days of germination, below their original values in the ungerminated seed. However, the glutelin fraction increased by 56·7% due to germination. Also, non-protein nitrogen showed a marked increase after germination (Table 5).

This was due to hydrolysis of these protein fractions, by proteolytic enzymes, to their constituent amino acids during germination (Hegazi, 1974; Chen & Thacker, 1978).

Protein digestibility

The *in vitro* protein digestibility of ungerminated and germinated fenugreek seed by different enzyme systems and the protein solubility index are shown in Table 6. The results show that the digestibility was improved by germination. The rates of increase in digestibility after 3 and 5 days of germination were: 26·2% and 31·6% for pepsin; 33·1% and 71·8% for trypsin; 30·7% and 62·5% for pancreatin and 11·4% and 13·9% for pepsin followed by pancreatin. The increase of trypsin digestibility of germinated seeds could be due to the decrease in trypsin inhibitor content during germination. El-Hag *et al*, (1978) found that germination of red kidney beans decreased the trypsin inhibitor activity. Also, Venkataraman *et al*, (1976) found an increase in the *in vivo* digestibility of green gram after 72 h of germination. Our findings agree well with their results. The hydrolysis of protein molecules by proteolytic enzymes during germination

TABLE 6
Effect of Germination for Different Periods on the *In vitro* Digestibility and Protein Solubility Index of Fenugreek Seeds[a]

Enzyme used	*Casein*	*Germination time (days)* 0	3	5
		Digested protein (%)		
Pepsin	33·6	55·1	69·5	72·5
Trypsin	73·5	42·5	56·6	73·1
Pancreatin	91·8	46·5	60·8	75·6
Pepsin–pancreatin	98·4	72·3	80·6	82·4
Soluble protein	—	56·7	59·7	72·7

[a] Means of two determinations.

may also be responsible for the increase in digestibility of germinated seed over the original seed.

Solubility of the protein also increased with the time of germination. It increased by 28·1% after 5 days of germination over that of ungerminated seed. The increase in protein solubility after germination may be due to the hydrolysis of the protein molecules to low molecular weight and high soluble protein fractions by the protein-hydrolyzing enzymes, which are more active during germination. Suberbie *et al.* (1981) found that the solubility of soybean increased after germination; this confirms our data.

Electrophoresis patterns

Total protein of ungerminated seed showed six bands in the gel electrophoresis pattern (Fig. 2). There was an aggregate on the top of the gel followed by three bands of high molecular weight fractions in the middle region of the gel; there was a diffuse band and albumin with high relative mobility located in the lower portion. After 3 and 5 days of germination there was a drastic change in the PAGE pattern. The aggregates disappeared, the slow moving bands dissociated to slightly faster moving fractions. The relative mobility increased from 0·145 and 0·181 to 0·259 and 0·306 after 3 days and to 0·256 and 0·314 after 5 days of germination. The diffused band in the middle of the gel also disappeared. The albumin fraction showed a very slight increase in relative mobility from 0·651 to 0·658 after germination; also, the intensity of the dye showed a gradual decrease, indicating a higher rate of dissociation of this fraction. This was confirmed by the decrease in the albumin fraction during germination (Table 5).

The water-extracted protein showed three major bands; two were in upper portion and one in the lower part of the gel. Also, some minor

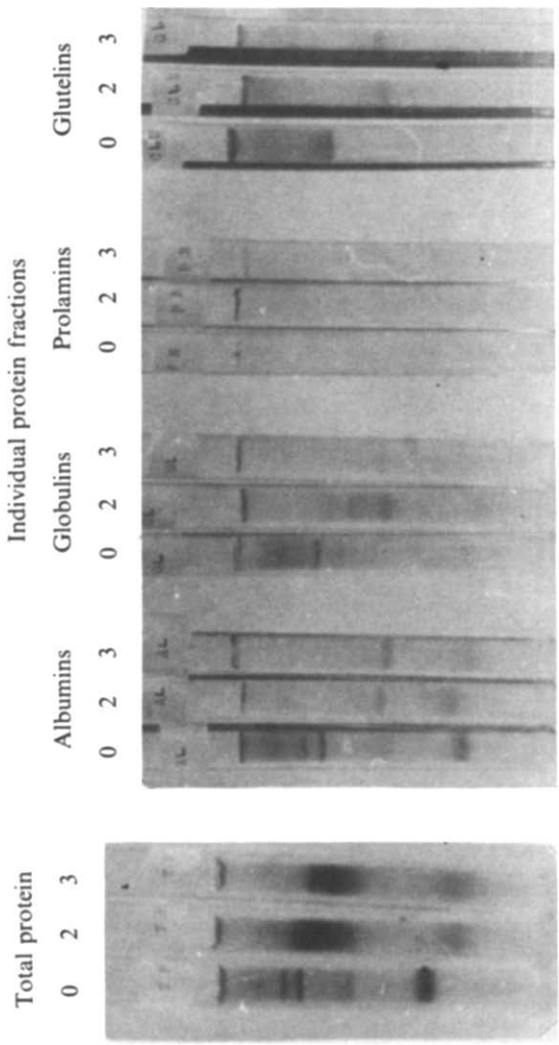

Fig. 2. Polyacrylamide gel electrophoresis of germinated and ungerminated fenugreek seed proteins in 0·01M phosphate buffer of pH 7·8 and 7·5% gel.

bands were observed in the upper and middle parts of the gel for ungerminated seed. Germination showed a decrease in the major bands and the minors disappeared. The rate of dissociation increased with the time of germination. The globulin fraction showed the same trend of decrease as the germination time increased. The decrease was highest after 5 days of germination. The major bands had the same relative mobility as that in the original and germinated seeds. Prolamins were not detected by electrophoresis even when higher concentrations were loaded. This could be due to their low molecular weight which should have been extracted with water along with albumin. The glutelin fraction showed only one band in the germinated samples with a relative mobility of 0·422 corresponding to 0·288 for that of the ungerminated sample. The increase in the relative mobility for the glutelin fraction indicates the dissociation of this protein to lower molecular weight due to germination.

The results of PAGE indicated that germination caused a dissociation of globulin and glutelin fractions and a decrease in their contents.

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