Sodium Dodecyl Sulphate-Polyacrylamide Gel Electrophoresis (SDS-PAGE) Characterization of protein fractions in ten peanut bunch type varieties

ABSTRACT

Aim: To isolate these fractions, a progressive centrifugation technique was employed, utilizing water, sodium chloride, phosphate buffer, and alcohol.

Place and Duration of Study: A study conducted in department of biochemistry Junagadh Agricultural University, Junagadh for analysis seeds of the bunch-type groundnut were supplied by the Main Oilseeds Research Station in Junagadh during 2020-2022.

The methodology used: Using SDS-PAGE separation method, four key protein fractions: albumin, prolamin, globulin, and glutelin were analyzed.

Results: Theresults revealed the highest albumin and globulin contents ranging from 15.05-20.81% and 69.91-77.99%, respectively. The globulin fraction was found to be significantly higher in proportion compared to the other three fractions. Glutelin and prolamin were found to be very low percentage-wise in bunch varieties but it was found to be in the range of 1.49-3.42 % in glutelin as well as prolamins were found to be in the range of 1.5-3.95%. The SDS-PAGE analysis of the ten-bunch type varieties revealed significant variations in protein profiles, as evidenced by the total number of bands and their respective molecular weight-retention factor (MW-Rf) values. The albumin and globulin fractions exhibited the highest MW-Rf values collectively, whereas glutelin and prolamin demonstrated minimal banding patterns.

Conclusion: This study concludes that the observed qualitative and quantitative differences in seed protein profiles are invaluable for varietal identification.

Keywords: protein fraction, globulin, bunch, groundnut

1. INTRODUCTION:

The Groundnut (*Arachis hypogaea* L.) is an annual legume and is also known as peanut, earthnut, goober pea, jack nut, pygmy nut, monkey-nut, manila nut and ground bean. The groundnut is often called "The King of Oilseeds" and is botanically known as Arachis hypogaea and belongs to the family Leguminosae. It is the world's fourth most important source of edible vegetable oil and the third most important source of vegetable protein. As it is a tropical legume mainly grown to produce oil and for human and animal consumption.

Several health advantages are there with the consumption of groundnut. [11,4]. Groundnut seed can be eaten raw (uncooked), boiled, or roasted in India, and its flour is used to make confections and baked foods. When fed to cattle, groundnut haulm had a higher nutrient digestibility of around 53% and crude protein digestibility of 88%. Consumption of regular groundnut reduces the risk of developing Type II diabetes [5], cardiovascular disease[7], colon, prostate, and breast cancer[2]. It also appears to prevent osteoporosis and protein deficiency [10]. In our study [3], we found that palmitic acid was the highest among the saturated fatty acids in all ten bunch-type peanut varieties, which is crucial for determining their flavour, stability, shelf life, and nutritional value. Additionally, oleic acid, which lowers LDL cholesterol [6], is beneficial for heart health, making higher oleic acid content desirable for reducing heart disease risk.

Protein fraction analysis in peanut grown in India especially in Gujarat has dearth of data or information. The present study, focuses the comparative analysis of protein fractions using SDS-PAGE of four fractions, including albumins globulins, glutelin and prolamin provides new and advantageous information regarding the evolution, diversification and genomic constitution of the varieties belonging to ten-bunch type peanuts one more benefit was to understand the seed storage protein variation among the different types of varieties. It can also be used to identify the genetic variation in groundnut.

2. MATERIALS AND METHODS

Seeds of the bunch-type groundnut were supplied by the Main Oilseeds Research Station in Junagadh. The defatted powder was obtained using a Soxhlet apparatus. The protein fractions of the peanut seeds—albumin, globulin, glutelin, and prolamin—were extracted through progressive centrifugation of the defatted powder with water, sodium chloride, phosphate buffer, and alcohol, respectively. For the extraction and estimation of all protein fractions, 0.5 g of defatted samples underwent sequential centrifugation with different solvents. The supernatants were used to estimate the respective protein fractions by the Folin-Lowry method [9], and the loading values were calculated for PAGE analysis. SDS-PAGE for seed protein profiling was performed as described by Laemmli,1970.Relative molecular weight (M) 255-10 kDa, for each standard used SDS-PAGE gels results. Data analysis of the Rf values for individual protein fraction bands was conducted using GEL ANALYZER (19.1) software.

3.Result and discussion

Albumin content of ten different varieties of bunch types was found significantly different. The maximum albumin content was observed in TG-26 (20.81) which was at par with TPG-45 (20.26%) and TG-37A (20.09%) (Table 1). whereas minimum albumin content was observed in variety GG-5 (15.05%) (Figure 1). The results for albumin content in varieties of bunch type showed significant variation. These results are in agreement with [1], they studied the effect of γ-radiation on total protein solubility, albumin, globulin and SDS-ME(SDS-sodium dodecyl sulfate; ME-2-mercapto ethanol,) fractions by using SDS-polyacrylamide gel electrophoresis. They found albumin content in ranged between 14.5% (7.5 KGy) to 19.5% (0.0 KGy). The values for globulin content of bunch-type varieties were found to be significantly different in salt soluble protein fraction content and it was ranged from 69.91 to 77.99 % in these ten varieties. (Table 1 and figure 2). The percent values of glutelin content in bunch type varieties recorded differently in alkali-soluble seed protein content and it was ranged in 1.49 % (GJG-31) to 3.42 % (GG-2) with an average value of 2.33 % (Figure 3 and table 1). These data are also in line with [12] four groundnut varieties (Massriya, Sinya, Chounfakhi and Trabilsia) used in determining the peanut seed proteins with three known classes of storage proteins and their results indicated that glutelin content was in ranged of 0.5(Chounfakhi) to 1% (Massriya and Sinya). Prolamin content of ten different varieties of bunch types was found to be in the range of 1.5 to 3.95 %.

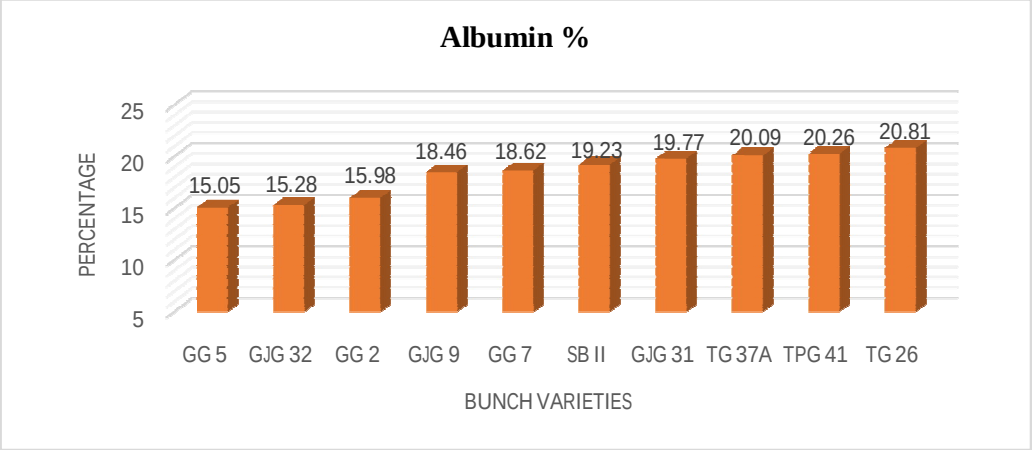

Figure 1: Comparative analysis for albumin fraction (%) in seed kernel of ten groundnut varieties of bunch type

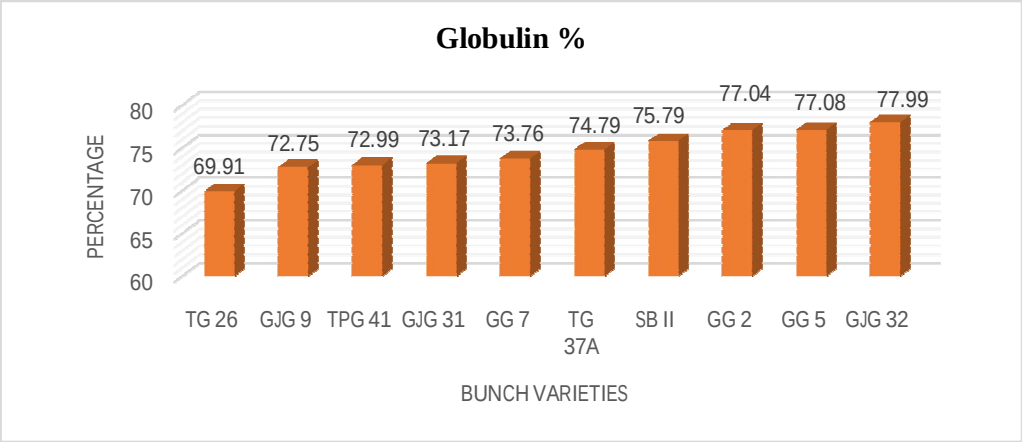

Figure 2: Comparative analysis for globulin fraction (%) in seed kernel of ten groundnut varieties of bunch type

The maximum prolamin content was recorded in GJG-32 (3.95 %) which was at par with GG-7 (3.65 %) and whereas minimum prolamin content was observed in variety GG-5 (1.5 %) (Figure 4). Results for prolamin content in varieties of bunch type showed significant variation. These results are in agreement with the [12] studied four Tunisian Groundnut seed cultivars. Their results found that prolamins content ranged between 1%(Massriya) to 2%(Sinya).

Table 1: Mean % of four protein fractions

Serial number	Bunch type peanut varieties	Albumin Mean %	Globulin Mean %	Glutelin Mean %	Prolamin Mean %
1	GG-2	15.98	77.04	3.42	1.88
2	GG-5	15.05	77.08	1.63	1.50
3	GG-7	18.62	73.76	2.33	3.65
4	GJG-9	18.46	72.75	2.75	2.41
5	GJG-31	19.77	73.17	1.49	2.43
6	GJG-32	15.28	77.99	2.63	3.95

7	**SB-11**	19.23	75.79	2.47	2.29
8	**TG-26**	20.81	69.91	2.64	3.92
9	**TG-37A**	20.09	74.79	1.55	2.18
10	**TPG-41**	20.26	72.99	2.41	1.71

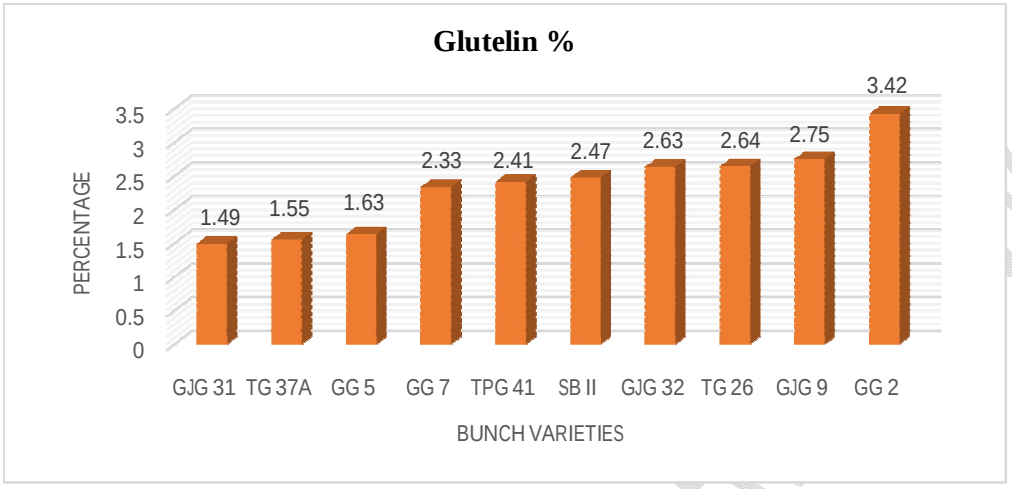

Figure 3: Comparative analysis for glutelin fraction (%) in seed kernel of ten groundnut varieties of bunch type

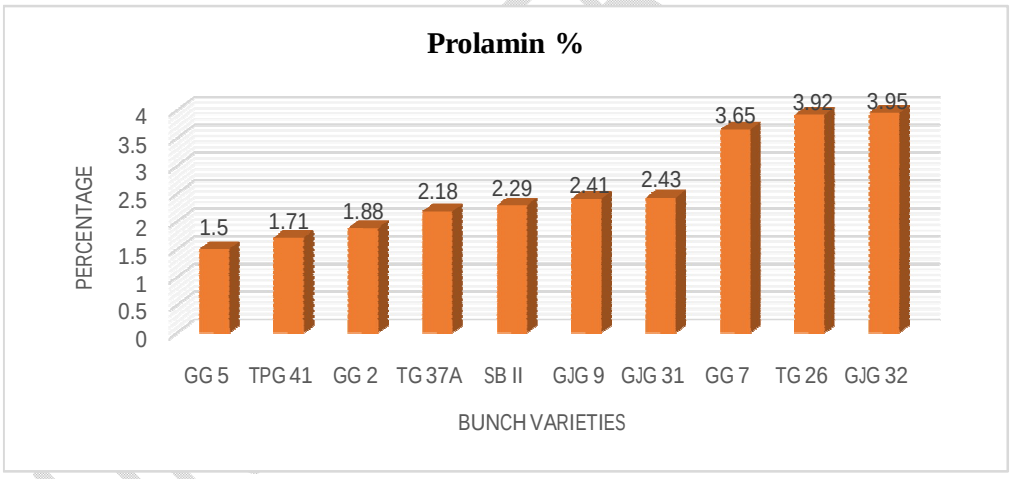

Figure 4: Comparative analysis for prolamin fraction (%) in seed kernel of ten groundnut varieties of bunch type

3.1 SDS-PAGE analysis:

3.1.1 Albumin

The results of albumin profiling are illustrated in figure 5 of ten bunch variety. The number bands noticed in the ten-bunch type varieties ranged from 12 to 18. The maximum protein bands (18) were found to be in TPG-41 variety whereas lowest bands (12) were observed in GJG-31. Total nine monomorphic band were found with band numbers viz., 6, 7, 11, 15, 16, 17, 18, 19, and 20 whereas eleven polymorphic bands were detected in SDS PAGE gel viz, 1, 2, 3, 4, 5, 8, 9, 10, 12, 13 and 14(table 2). The MW-Rf value of different protein bands in bunch varieties of groundnut based on SDS-PAGE was found to range from 0.012 to 0.991. GG-7, GJG-32, SB-11, TG-26 and TG-37A were resolved the protein band of MW-Rf value of 0.012(Table 2). Out of ten bunch-type groundnut

varieties only GG-2, GG-7, GJG-9, TG-26 and TPG-41 were observed for the protein band of MW-Rf value of 0.047. Similar findings also suggested by Singh*et al*.,2018 found that 15-21 bands were resolved.

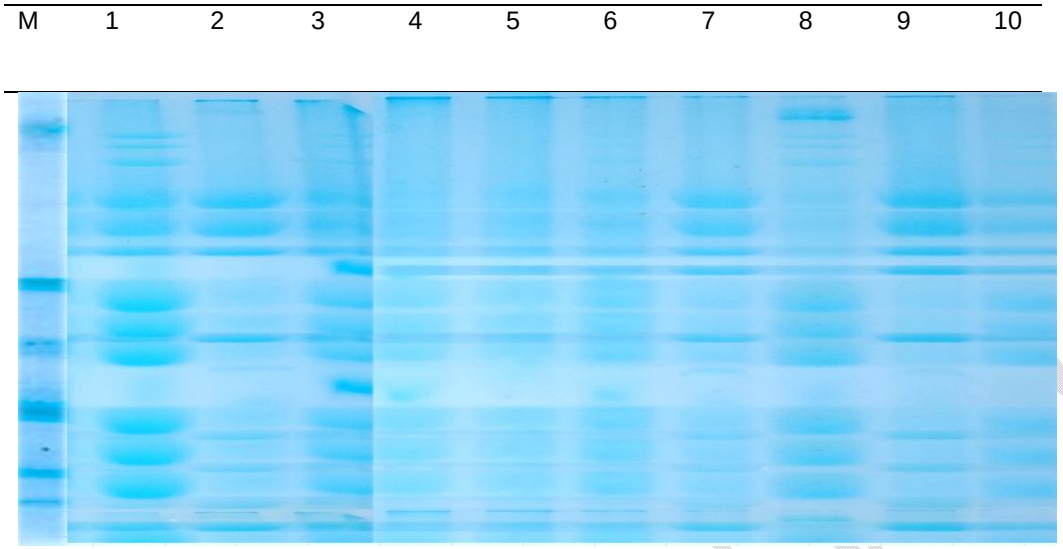

Figure 5: SDS PAGE showing banding patterns for albumin fraction of bunch type varieties{M=275-10kDa}.

3.1.2 Globulin:

Comparison of ten bunch variety by SDS-PAGE analysis for globulin fraction protein profiling as shown in figure 6. The number of bands in ten bunch type groundnuts in globulin fraction found to range from 13 to 21. The bands with MW-Rf value of 0.092, 0.227, 0.406, 0.456, 0.559, 0.590 and 0.695 were present in all the bunch whereas the remaining 16 were polymorphic innature (table 3).

Out of the ten varieties, the maximum protein bands (21) were found to be in TG-26 variety while a minimum number of bands (13) were detected in SB-11 of bunch type (Figure 6). Protein with MW-Rf value of 0.035 was only present in all the bunch type of varieties except GG-2.GG-5 and GJG-32 bunch type of varieties were found absent in the protein band with MW-Rf value of 0. 067. Protein bands with MW-Rf value 0.367, 0.504 and 0.637 were found absent in verities viz., GG-5, GJG-9, GJG-32 and SB-11 of bunch type (table 3). Similar findings for protein bands in groundnut seed were also observed by Singh*et al*.,2018 studied 50 groundnut cultivars and analyzed for globulin protein fraction on SDS-PAGE. Their results showed that globulin protein fraction resolved 22 protein bands.

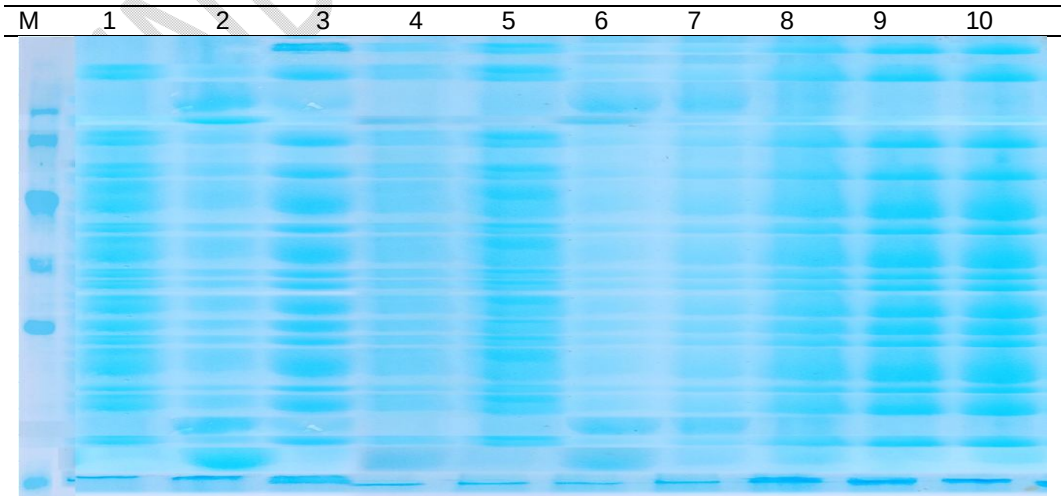

Figure 6: SDS PAGE showing banding pattern for Globulin fraction of bunch type varieties{M=255-10kDa}

3.1.3 Glutelin:

Evaluation of ten bunch variety analyzed for SDS-PAGE and the results of glutelin fraction protein profiling as presented in figure 7.Total 10 protein bands were resolved by the ten-bunch type in SDS-PAAGE analysis of glutelin protein fraction. The bands with MW-Rf value of 0.321, 0.374, 0.845 and 0.937 were present in all bunch type. six protein bands were polymorphic appearance viz., MW-Rf value 0.052, 0.193, 0.257, 0.489, 0.593 and 0.762 in bunch type varieties (table 4). In variety GG-2 (10) and GG-7 (4) of bunch type the maximum and minimum protein bands were found, respectively. The MW-Rf value of 0.257 was expressed in only GG-2. The protein band with MW-Rf value of 0.193 was present in the all varieties except GG-7 verity. Protein band with MW-Rf value of 0.052 was present in the GG-2, GJG-31, SB-11, TG-26, TG-37A and TPG-41 but not found in the GG-5, GG-7, GJG-9 and GJG-32 of bunch type groundnut. The GG-5 and GG-7 varieties not resolve protein bands with the MW-Rf values of 0.489, 0.593 and 0.762.

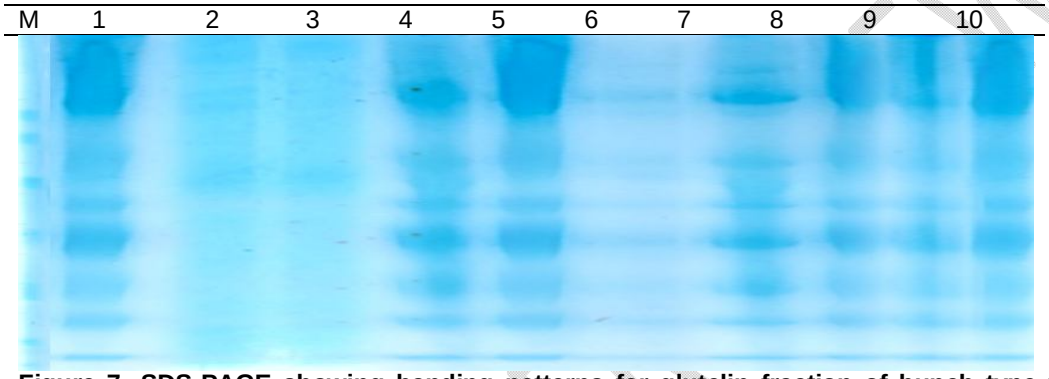

Figure 7: SDS-PAGE showing banding patterns for glutelin fraction of bunch type varieties {M=255-10kDa}

3.1.4. Prolamin:

Comparison of ten bunch variety analysed for SDS-PAGE and the results of prolamin fraction protein profiling as presented in figure 8. The number of bands were found in the ten bunch type varieties i.e., 7 bands. The monomorphic bands with MW-Rf value of 0.381, 0.522, 0.625, 0.741 and 0.911 were present in all the ten-semi spreading. two protein bands were polymorphic appearance viz., MW-Rf value 0.164 and 0.303 in ten bunch type varieties (Table 5).Among the varieties, the maximum protein bands (7) whereas minimum bands (6) found in the bunch type (table 5). Band with MW-Rf value 0.164 was absent in variety TG-26, TG-37A and TPG-41 whereas bands with MW-Rf value 0.303 were present in all varieties except GJG-31 and GJG-32.

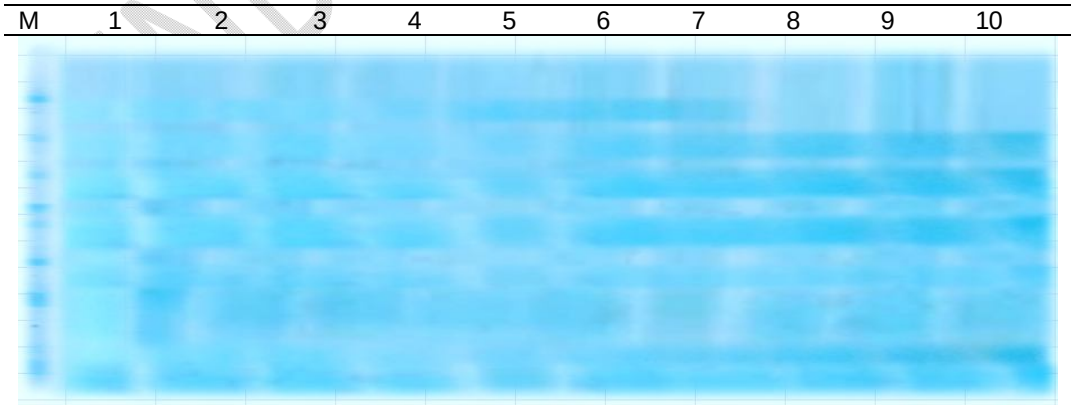

Figure 8: SDS PAGE showing banding patterns for prolamin fraction of bunch type varieties {M=255-9kDa}

Similar findings for protein bands in groundnut seed were also observed by [13] examining the seed prolamins obtained by alcohol extraction and subsequent acetone precipitation analysed by SDS–PAGE. The profile contained polypeptides of small sizes to almost 66 kDa. Four major bands were unique at 22.4 kDa to below 14.4 kDa.

4. CONCLUSION:

The scientist working in peanuts for proteome analysis may also benefit from this data produced by experiments. To find quality features in the groundnut genotypes from this investigation, variability in protein profiling in different kinds might be helpful. In conclusion, the SDS-PAGE analysis in our work considerably improves our understanding of the protein fractions in peanuts. We were able to separate and identify several protein fractions, which gave us important new information on the complexity and variety of proteins found in peanuts. As the proteins found potentially contribute to both the nutritional and allergic aspects of peanuts, the results highlight the potential advantages of more thorough protein fraction study.

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REFERENCE:

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Band number	MW-Rf	GG 2	GG 5	GG 7	GJG 9	GJG 31	GJG 32	SB-11	TG 26	TG 37A	TPG 41
1	0.012	0	0	1	0	0	1	1	1	1	0
2	0.047	1	0	1	1	0	0	0	1	0	1
3	0.102	1	0	1	0	0	0	0	1	1	1
4	0.125	1	0	1	1	0	0	0	1	1	1
5	0.161	1	1	1	1	0	1	1	1	1	1
6	0.233	1	1	1	1	1	1	1	1	1	1
7	0.310	1	1	1	1	1	1	1	1	1	1
8	0.351	0	0	1	1	1	1	1	1	1	1
9	0.404	0	0	0	1	1	1	1	1	1	1
10	0.469	1	1	1	0	1	0	0	1	0	1
11	0.506	1	1	1	1	1	1	1	1	1	1
12	0.547	0	0	1	1	0	0	0	1	1	1
13	0.600	1	1	1	1	0	1	1	1	1	1
14	0.670	1	1	0	1	0	0	0	0	0	0
15	0.723	1	1	1	1	1	1	1	1	1	1
16	0.761	1	1	1	1	1	1	1	1	1	1
17	0.817	1	1	1	1	1	1	1	1	1	1
18	0.906	1	1	1	1	1	1	1	1	1	1
19	0.942	1	1	1	1	1	1	1	1	1	1
20	0.991	1	1	1	1	1	1	1	1	1	1

Table 2: MW-Rf value on different albumin protein fraction bands of bunch type groundnut varieties based on SDS-PAGE

Table 3: MW-Rf value of different globulin protein fraction bands of bunch type groundnut varieties based on SDS-PAGE

Band number	MW-Rf	GG 2	GG 5	GG 7	GJG 9	GJG 31	GJG 32	SB-11	TG 26	TG 37A	TPG 41
1	0.035	0	1	1	1	1	1	1	1	1	1
2	0.067	1	0	1	1	1	0	1	1	1	1
3	0.092	1	1	1	1	1	1	1	1	1	1
4	0.126	1	1	0	0	0	1	0	1	0	0
5	0.227	1	1	1	1	1	1	1	1	1	1
6	0.266	1	0	1	0	1	0	1	1	1	1
7	0.338	1	1	1	1	1	1	0	1	1	1
8	0.367	1	0	1	0	1	0	0	1	1	1
9	0.406	1	1	1	1	1	1	1	1	1	1
10	0.456	1	1	1	1	1	1	1	1	1	1
11	0.504	1	0	1	0	1	0	0	1	1	1
12	0.559	1	1	1	1	1	1	1	1	1	1
13	0.590	1	1	1	1	1	1	1	1	1	1
14	0.637	1	0	1	0	1	0	0	1	1	1
15	0.695	1	1	1	1	1	1	1	1	1	1
16	0.729	1	0	1	1	1	1	1	1	1	1
17	0.769	1	1	1	1	1	0	0	1	1	1
18	0.800	1	1	1	1	1	0	0	1	1	1
19	0.825	0	1	1	1	1	0	0	1	1	1
20	0.850	0	0	0	0	0	0	0	0	0	0
21	0.892	0	1	1	1	1	1	1	1	1	1
22	0.923	0	1	1	1	1	1	1	1	1	1
23	0.974	0	1	0	1	0	1	0	0	0	0

Table 4: MW-Rf value of different glutelin protein fraction bands of bunch type groundnut varieties based on SDS-PAGE

Band number	MW-Rf	GG 2	GG 5	GG 7	GJG 9	GJG 31	GJG 32	SB-11	TG 26	TG 37A	TPG 41
1	0.052	1	0	0	0	1	0	1	1	1	1
2	0.193	1	1	0	1	1	1	1	1	1	1
3	0.257	1	0	0	0	0	0	0	0	0	0
4	0.321	1	1	1	1	1	1	1	1	1	1
5	0.374	1	1	1	1	1	1	1	1	1	1
6	0.489	1	0	0	1	1	0	1	1	1	1
7	0.593	1	0	0	1	1	0	1	1	1	1
8	0.762	1	0	0	1	1	1	1	1	1	1
9	0.845	1	1	1	1	1	1	1	1	1	1
10	0.937	1	1	1	1	1	1	1	1	1	1

Table 5: MW-Rf value of different prolamin protein fraction bands of bunch type groundnut varieties based on SDS-PAGE

Band number	MW-Rf	GG 2	GG 5	GG 7	GJG 9	GJG 31	GJG 32	SB-11	TG 26	TG 37A	TPG 41
1	0.164	1	1	1	1	1	1	1	0	1	0
2	0.303	1	1	1	1	0	0	1	1	1	1
3	0.381	1	1	1	1	1	1	1	1	1	1
4	0.522	1	1	1	1	1	1	1	1	1	1
5	0.625	1	1	1	1	1	1	1	1	1	1
6	0.741	1	1	1	1	1	1	1	1	1	1
7	0.911	1	1	1	1	1	1	1	1	1	1