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FRACTIONATION AND CHARACTERIZATION OF PROTEINS OF SOME EGYPTIAN FIELD CROPS

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ABSTRACT: The proteins considered a true expression of genetic characteristics in plant crops and can be used for classifying of many varieties of different crops by using protein electrophoreses SDS PAGE, to fractionate and characterize proteins. Results showed that barley crop whose varieties had a protein C-hordein with molecular weight 21KDa which characterized in each variety but differ in its percentage. Corn crop varieties were characterized by presence of zien protein with molecular weight 19, 17 and 11KDa. While in rice crop, varieties were differed according to protein bands especially in tublin protein with molecular weight 48KDa. Also, the varieties of wheat crop were differed by percentage of some essential protein gliadin: gamma-beta-alpha, with molecular weight 58,42 and 35KDa, respectively. On the other hand, legumes crops: chickpea, faba bean and lentil proteins could be characterized with a lot of protein bands and had been noticed greet difference in kinds of protein or in its percentage of different varieties through molecular weight from 245KDa (Gamma globulin protein) to 11KDa Vicilins proteins. While in fiber crops proteins: cotton and flax were had a less protein bands which were appeared in some varieties of flax protein whose had one protein band (conlinin) with molecular weight 18KD, also cotton protein varieties had different percentages of 7S vicilins proteins with molecular weight 49 and 46KDa. Although oil crops: peanuts, soybean and sunflower proteins had clear differences in all varieties as number of protein bands and/or percentages. Peanuts varieties had the same protein bands their weights from 88KDa (glutenin) to 19KDa (Conarachin) but as a different percentages in each variety. In soybean proteins varieties there were differences in number of protein bands and percentages, sunflower varieties had almost the same protein bands but differed in their percentages and their weights were between 245KDa to 11KDa. Results confirmed that protein electrophoreses could be used as a simple method of classification of filed crop varieties.

Key words: Field corps, electrophoreses, SDS PAGE, cereals, legumes, fibers, oils, proteins.

INTRODUCTION

The proteins, lipid and carbohydrates are the main component in the cell of plant crops. Each one of them can be used for classifying of many varieties of different crops with lot of tests especially protein. As it usually presented in any crop, which gives the ability to classify the crops using protein electrophoreses SDS PAGE as revealed by Laemmli (1970). This method was considered one of a lot of methods for

determination of protein but it had priority as it compares the proteins content differences, appearance of bands, molecular weights and their percentages. That easily could be used as classification of many varieties in the same crops and in different crops. This method of classification could be applied in cereals crops as in presence of some essential protein in all varieties with different percentage and disappearance bands of non essential proteins in some varieties as studied by Southan and

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Macritchie (1999), Bobalova *et al.* (2008) and Ayad (2010) and reported by **FAO (2018)**. While in legumes crops such as: chickpea, faba bean and lentil whose known as the mother of plant protein were found lot of protein bands and had been noticed great differences in kinds of protein fractions or in its percentage and in molecular weights as 245KDa (Gamma globulin protein) to 11KDa (Vicilins proteins) of different varieties and this could be a point of view in their protein content and its percentage in each variety where these were stated by **Alajaji and El-Adawy (2006), Klupšaitė and Juodeikiene (2015) and Wallace *et al.* (2016)**. Meanwhile fiber crops were taken as for difference of percentage for main proteins like: Conlinin (18KDa) and Vicilins (46-49KDa) **Ayad (2010)** and **Gandhi *et al.* (2017)**. Although Oil crops had also clear difference in all varieties at protein content percentage and number of proteins **Miroljub *et al.* (2004), Zhou *et al.* (2013) and Mueller *et al.* (2014)**, this means that protein electrophoreses can be easily used in classification not only Egyptian crops but also their varieties, so this study was conducted to fractionate and characterize different proteins of some Egyptian field crops.

MATERIALS AND METHODS

Source of Materials

The new varieties of the four crop groups: cereal crops: barley, corn, rice and wheat, legume crops: chickpea, fababean and lentil, fiber crops: cotton and flax and oil crops: peanut, soybean and sunflower were obtained kindly from Agricultural Research Center, Giza, Egypt, (yield of 2017 season). All varieties of each field crops groups are shown in Table 1.

Methods

Proteins of different crops were analyzed by Proteins Electrophoreses SDS PAGE method as recommended by **Laemmli (1970)** as follows:

- Prepare the separating gel 15% 2.4 ml dH₂O, 5 ml (29.2% acrylamide and 0.8% bis-acrylamid), 2.5 ml 1.5M Tris pH8.8, 100ul 10% SDS, 100μl 10%APS and 100μl TEMED.
- Prepare the Stacking gel 4% 6.1 ml dH₂O, 1.3 ml (29.2% acrylamide and 0.8% bis-acrylamid),

2.5 ml 0.5M Tris pH6.8, 100μl 10% SDS, 100μl 10%APS and 100μl TEMED.

- Prepare the seed samples (100 mg sample +300 ul saline) after centrifuging at 13,000 xg for 5 min 4°C take 100 μl +500 μl+ acetone for overnight -20°C after that centrifuging at 13,000 xg for 3 min at 4°C Mix (1:4) sample: sample buffer (10% SDS, 20% Glycerol, 0.2M Tris pH6.8, 10mM beta-mercapto-ethanol and 0.05% bromophenolblue) and heat them on 95°C for 5 min
- Prepare running buffer 25mM Tris-HCl, 200mM glycine and 0.1% (W/V) SDS, start runs (80V for 4 hr.)
- After run stain gel in staining solution (50% dH₂O, 40% methanol, 10% glacial acetic acid and 0.1% coomassie brilliant blue for 20 min with gentle agitation
- Destain gel in destaining solution (50% dH₂O, 40% methanol and 10% glacial acetic acid).

RESULTS AND DISCUSSION

The varieties of cereal crops were composited in the proteins electrophoreses SDS Page with lot of different protein bands which differ in: appearance, thickness and percentage as every crop and its varieties had special protein bands refer to it, that can be easily used for classifying of these varieties using protein content as in Table 2 and Figures 1, 2 and 3 as follows:

In barley whose varieties were Named: (B1, B2 and B3) had three protein bands with molecular weight 33KDa, 21KDa and 11KDa in B1 variety, the protein bands were 49%, 33% and 18%, respectively. While in B2 variety bands were 57%, 22% and 21%, respectively. But in B3 variety bands were 48%, 26% and 26%, receptively. These results indicated that three new varieties had the same protein bands but differed in the percentage of each other which may easily classify these varieties. B2 have the highest percentage of protein C-Hordin with molecular weight 33KDa (57%) while B1 variety and the B3 have less percentage (49% and 48%). The other protein bands also differed from each other in all varieties. These results were agreed with those obtained by **Bobalova *et al.* (2008)**.

Table 1. Crops and their varieties

Group of crops	Field of crop	Variety	Code of Variety
Cereal crops	Barley	Giza 125	B1
		Giza 130	B2
		Giza 134	B3
	Corn	Yellow (186 M.H.)	C1
		White (131M.H.)	C2
		White (Giza 2)	C3
	Rice	Skha 104	R1
		Giza 178	R2
		Giza 182	R3
	Wheat	Gimaza 680	W1
Giza 11		W2	
Shandwel 94		W3	
Chickpeas	B 2 (magwhar)	CP1	
	B 2 (Giza 195)	CP2	
	B 3 (Giza 1)	CP3	
Legume crops	Faba bean	Giza 716	FB1
		Giza 3	FB2
		Egypt 1	FB3
	Lentil	Giza 370	L1
		Sinai 1	L2
Fiber crops	Cotton	Giza 51	L3
		Giza95	CO1
	Flax	Giza 90	CO2
		Skha 6	F1
		Giza 12	F2
Seed oil crops	Peanuts	Giza 9	F3
		Giza 5(Red Strain)	PE1
		Giza 6	PE2
	Soybean	Giza 4	PE3
		Giza 22	SB1
		Giza 111	SB2
Sunflower	Carford	SB3	
	Giza 102	SF1	
		Skha 53	SF2

Table 2. Proteins electrophoreses SDS PAGE of some cereal crops

Protein molecular weight (KDa)	RF	Cereal crop											
		Barley			Corn			Rice			Wheat		
		B 1	B 2	B 3	C 1	C 2	C 3	R 1	R 2	R 3	W 1	W 2	W 3
245	0.009	-	-	-	-	-	+	-	-	-	-	-	-
65	0.245	-	-	-	-	-	-	-	-	-	+	+	+
63	0.288	-	-	-	-	-	-	+	-	+	-	-	-
58	0.333	-	-	-	-	-	-	-	-	-	+	+	+
48	0.392	-	-	-	-	-	-	+	+	-	-	-	-
42	0.486	-	-	-	-	-	-	-	-	-	+	+	-
35	0.542	-	-	-	-	-	-	-	-	-	+	-	-
33	0.559	+	+	+	-	-	-	-	-	-	-	-	-
28	0.592	-	-	-	-	-	-	-	-	-	-	+	+
25	0.628	-	-	-	+	+	+	-	-	-	+	+	+
24	0.652	-	-	-	-	-	-	+	+	+	-	-	-
21	0.718	+	+	+	-	-	-	-	-	-	-	-	-
19	0.893	-	-	-	-	+	-	-	-	-	-	-	-
17	0.900	-	-	-	+	+	+	-	-	-	-	-	-
11	0.971	+	+	+	+	+	+	+	+	+	-	+	+
Total of bands		3	3	3	3	4	4	4	3	3	5	6	5

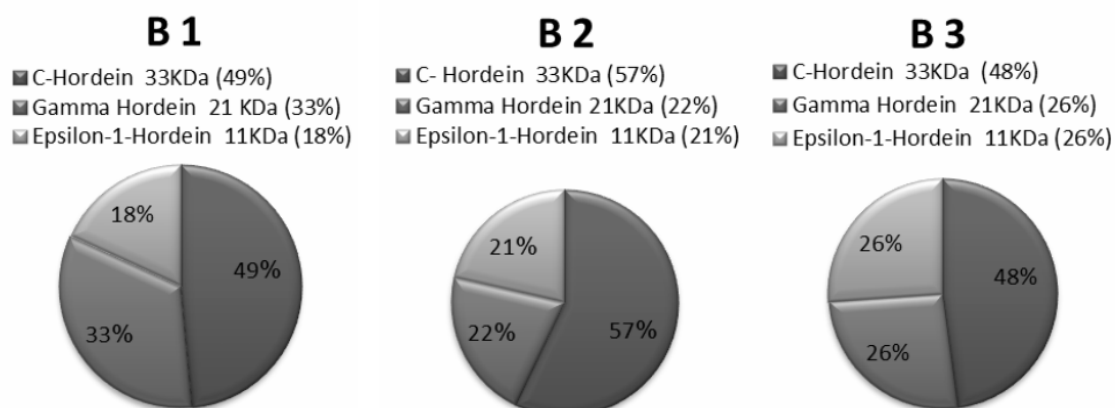


Fig. 1. The protein composited in barley varieties by electrophoreses SDS PAGE

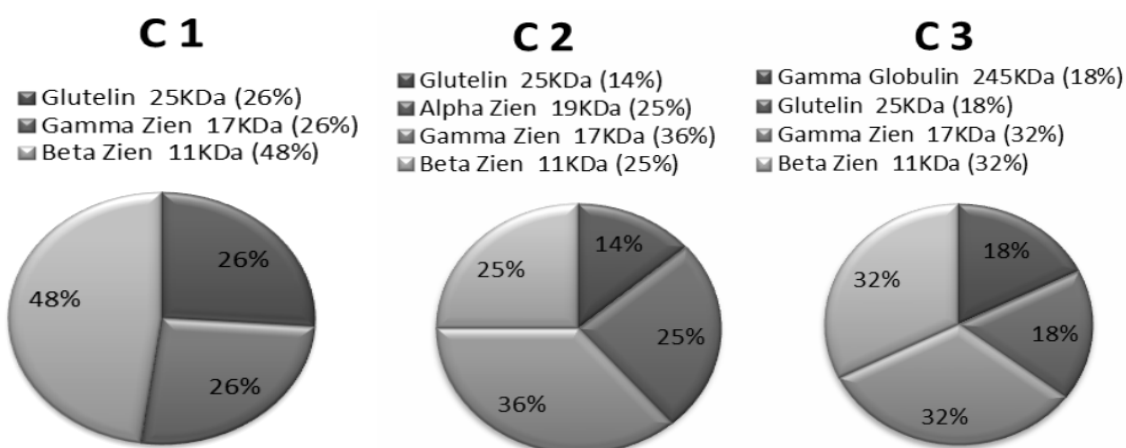


Fig. 2. The protein composited in corn varieties by electrophoreses SDS PAGE

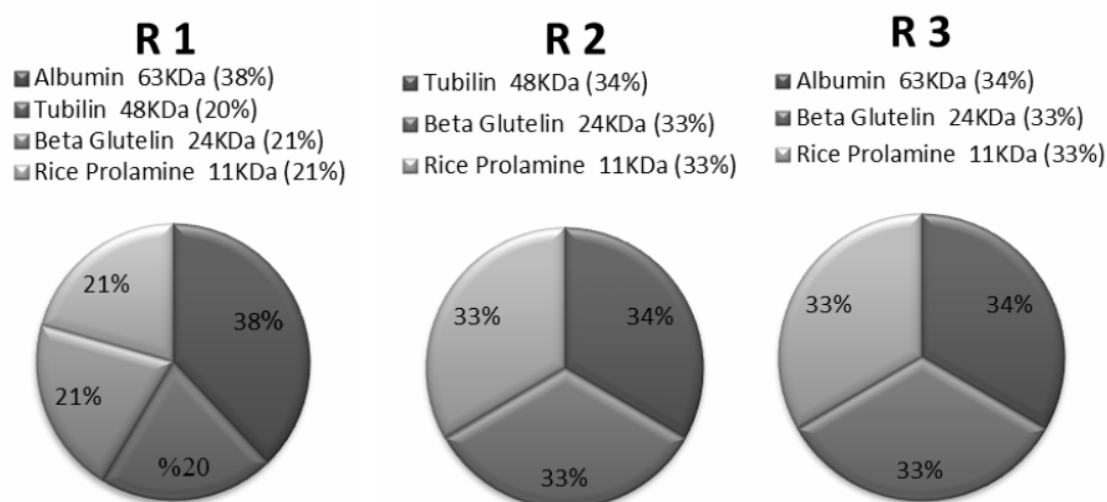


Fig. 3. The protein composited in rice varieties by electrophoreses SDS PAGE

The three corn varieties: C1, C2 and C3 had the same protein bands: glutelin (25KDa), gamma zien (17KDa) and beta zien (11KDa) but in different percentages (Fig. 2). The variety C2 contain alpha zien protein (19KDa), also variety C3 contain gamma globulin (245KDa) the results obviously showed that each variety had differed protein band fingers which can be used in classification of corn varieties. That was verified as revealed by **Koc et al. (2012)** and **FAO (2018)**.

The Rice varieties R1, R2 and R3 had about 3- 4 protein bands as in Table 2 and Fig. 3. The R1 variety had four protein bands: albumin

(63KDa) 38%, tubilin (48KDa) 20%, beta glutelin (24KDa) 21% and rice prolamine (11KDa) 21%. R2 variety had a three protein bands which were: tubilin (48KDa) 34%, beta glutelin (24KDa) 33% and rice prolamine (11KDa) 33%. While R3 variety had also three protein bands: albumin (63KDa) 34%, beta glutelin (24KDa) 33% and rice prolamine (11KDa) 33%. Results indicated that rice varieties can be classified as R1 variety which had the higher protein bands (4 bands). Meanwhile R2 variety and R3 variety had protein bands differed in molecular weight and percentage, which were noticed by **Hui Bai et al. (2011)** and **Yang et al. (2013)**.

Wheat varieties: W1, W2 and W3 had about 5- 6 protein bands with molecular weight: 65 KDa, 58 KDa, 42 KDa, 35 KDa, 28 KDa, 25 KDa and 11KDa. The W1 variety had five protein bands: omega gliadin (65 KDa) 13%, gamma gliadin (58KDa) 36%, beta gliadin (42 KDa) 13%, alpha gliadin (35 KDa) with percentage 13% and beta glutelin (25 KDa) 25%.

Although W2 variety had six protein bands: omega gliadin (65 KDa) 11%, gamma gliadin (58 KDa) 28%, beta gliadin (42 KDa) 11%, alpha glutelin (28KDa) 11% and beta glutelin (25KDa) 19% and prolamine (11 KDa) 20%, W3 variety had also five protein bands: omega gliadin (65 KDa) 12%, gamma gliadin (58 KDa) 32%, alpha glutelin (28KDa) 12%, beta glutelin (25 KDa) 22% and prolamine (11 KDa) 22%. These varieties can be classified as W2 the higher protein bands (6 bands) than the other varieties (5 bands) which were differed in protein band molecular weight and protein band percentage and could be used in classification of these varieties. These results were revealed by *Southan and Macritchie (1999)* and *Hong et al. (2000)*.

Table 3 and Figures 6, 7 and 8 declared the Legumes crops and their varieties using proteins electrophoreses SDS PAGE to classify according to molecular weight, RF, protein bands as well as their percentage as follows:

Chickpea varieties: CP1, CP2 and CP3 can be classified by protein electrophoreses analysis according to percentage of each protein bands. Legumins with molecular weight: 50, 48 and 46KDa which recorded in the varieties CP1, CP2 and CP3 with 39%, 42% and 50%, respectively. While vicilins with molecular weight: 25, 23, 17, 15 and 11KDa which recorded in varieties CP1, CP2 and CP3 with 61%, 58% and 50%, respectively. Results indicated that CP3 variety had perfect equal percentage of fraction but in differed some molecular weight of vicilins: 23 and 17KDa which differ for the other varieties.

The faba bean varieties: FB1, FB2 and FB3 had a lot of protein bands (9-10 bands) which have molecular weights from 245KDa to 17KDa and RF from 0.009 to 0.971 with different percentages in each variety (Fig. 6). This showed the probability of classification of the

varieties according to protein bands or their percentages.

Lentil varieties: L1, L2 and L3 stated differences in RF, molecular weights and percentages of protein bands. Bands with molecular weight 75 KDa for conglutin, bands with molecular weight of 65, 59, 58, 42 and 35 KDa for legumins. Where bands with molecular weight 34, 29, 25, 22, 20, 19 and 17KDa for vicilins. It was noticed that each variety had one more molecular weight of legumins and vicilins which differ from each other as well as the percentages. This showed the probability of classification of these varieties according to protein bands and its percentage.

All results in Legume crops were in accordance with these reported by *Alajaji and El-Adawy (2006)*, *Hefnawy (2011)*, *Hameed et al. (2012)* and *Wallace et al. (2016)*.

In Table 4 and Figs. 8 and 9, results showed that the fiber crops and their varieties were classified and determined with proteins electrophoreses SDS PAGE as follows:

Table 4 and Fig. 8 showed that cotton varieties CO1 and CO2 had mostly the same protein bands with molecular weight: 245KDa, 49KDa, 46KDa, 20KDa and 17KDa but they were differed in their percentage as CO2 had higher in protein band 7S vicilins with molecular weight: 49 and 46KDa while the other variety CO1 was higher in 245KDa (gamma globulin) which meant to be a classification factor for these varieties, these results were confirmed with those reported by *Gandhi et al. (2017)*.

On the other hand flax varieties: F1, F2 and F3 had about 1-2 protein bands with molecular weights: 28KDa and 18KDa. Variety F1 had two proteins: conlinin (18KDa) 61% and glutelin (28KDa) 39%. While variety F2 had only Conlinin (18KDa) 100%. Although F3 variety had Conlinin (18KDa) 72% and Glutelin (28KDa) 28%. It could classify these varieties according presence and percentages of protein bands. The obvious results were agreed with those stated by *Ayad (2010)*.

Table 5 and Figures 10, 11 and 12 showed three oils crops and their varieties according to the proteins bands using proteins electrophorese SDS PAGE as follows:

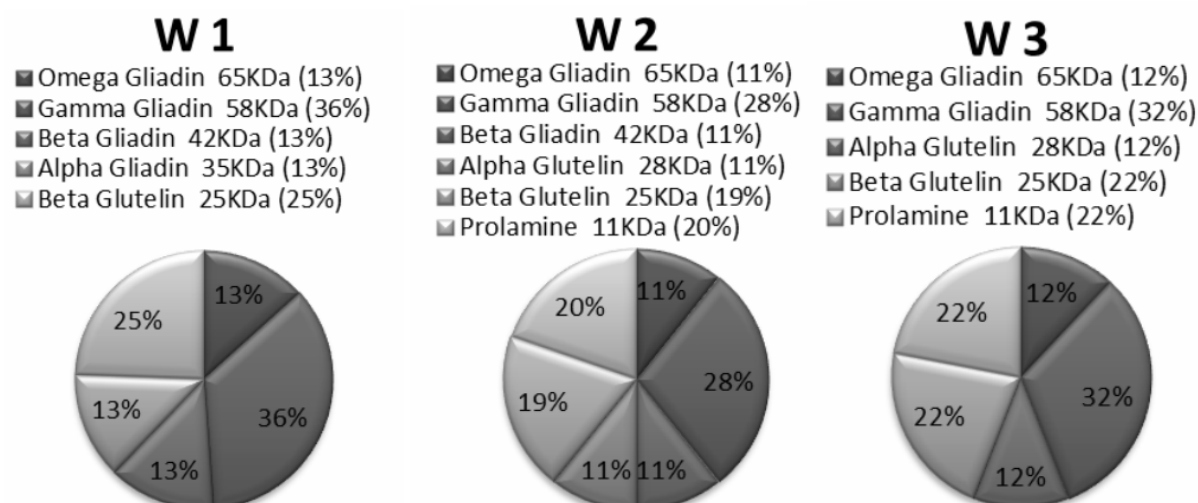


Fig. 4. The protein composited in wheat varieties by electrophoreses SDS PAGE

Table 3. Proteins electrophoreses SDS PAGE of some legume crops

Protein molecular weight (KDa)	RF	Legume crop								
		Chickpea			Faba bean			Lentil		
		CP 1	CP 2	CP 3	FB 1	FB 2	FB 3	L 1	L 2	L 3
245	0.009	-	-	-	+	+	+	-	-	-
75	0.213	-	-	-	+	+	+	+	+	+
65	0.245	-	-	-	-	-	-	-	+	+
63	0.288	-	-	-	+	+	+	-	-	-
59	0.326	-	-	-	-	-	-	+	+	-
58	0.333	-	-	-	-	-	-	-	-	+
50	0.345	+	+	+	+	+	+	-	-	-
48	0.392	+	+	+	-	-	-	-	-	-
46	0.412	+	+	+	+	+	+	-	-	-
42	0.486	-	-	-	-	-	-	+	+	+
35	0.542	-	-	-	+	+	+	+	+	-
34	0.553	-	-	-	-	-	-	-	-	+
30	0.565	-	-	-	+	+	+	-	-	-
29	0.578	-	-	-	-	-	-	-	+	-
26	0.614	-	-	-	+	+	+	-	-	-
25	0.628	+	+	+	-	-	-	+	-	-
23	0.695	+	+	-	-	-	-	-	-	-
22	0.716	-	-	-	-	-	-	-	+	+
20	0.721	-	-	-	-	-	-	+	-	-
19	0.893	-	-	-	+	+	+	-	+	+
17	0.900	+	+	-	+	+	+	+	+	+
15	0.949	+	+	+	-	-	-	-	-	-
11	0.971	+	+	+	-	-	-	+	-	-
Total of bands		8	8	6	10	10	10	8	9	8

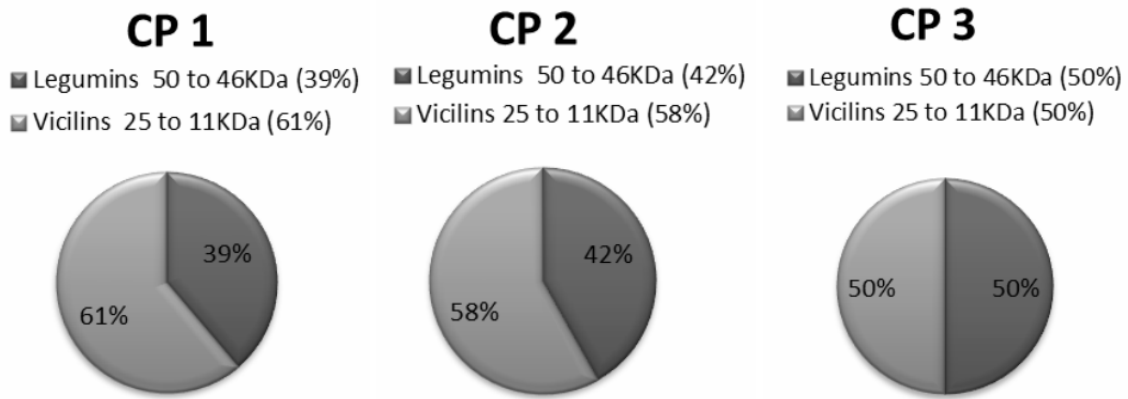


Fig. 5. The protein composited in chickpea varieties by electrophoreses SDS PAGE

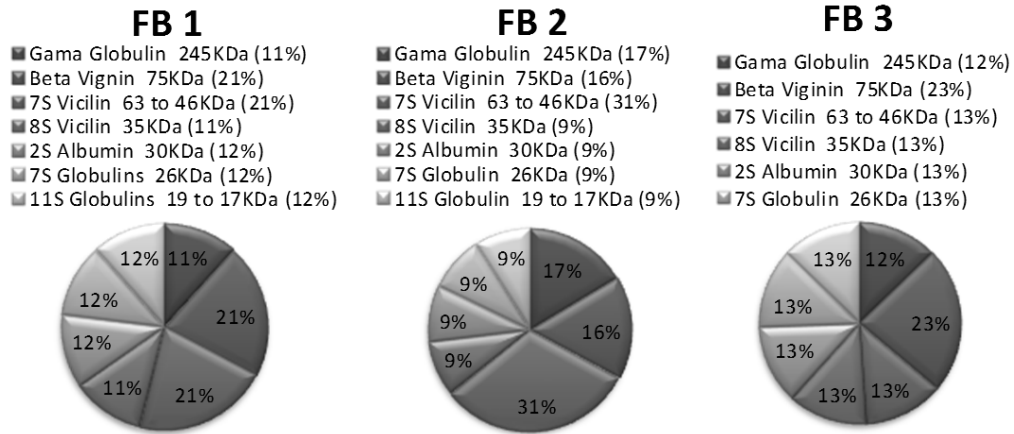


Fig. 6. The protein composited in faba bean varieties by electrophoreses SDS PAGE

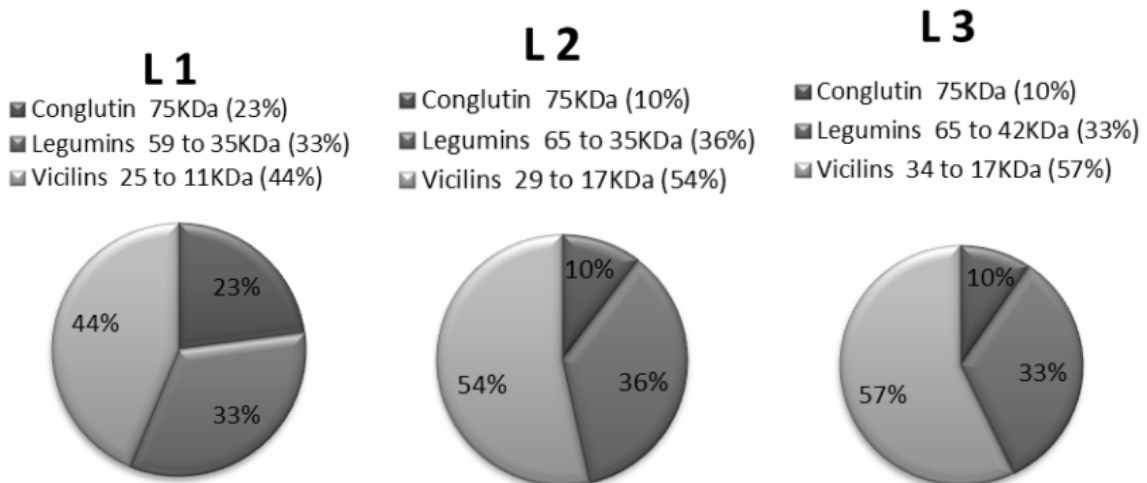


Fig. 7. The protein composited in lentil varieties by electrophoreses SDS PAGE

Table 4. Proteins electrophoreses SDS PAGE of some fiber crops

Protein molecular weight (KDa)	RF	Fiber Crop				
		Cotton		Flax		
		CO 1	CO2	F 1	F2	F3
245	0.009	+	+	-	-	-
49	0.224	+	+	-	-	-
46	0.376	+	+	-	-	-
25	0.628	-	-	+	-	+
20	0.721	+	+	-	-	-
18	0.649	-	-	+	+	+
17	0.900	+	+	-	-	-
Total of bands		5	5	2	1	2

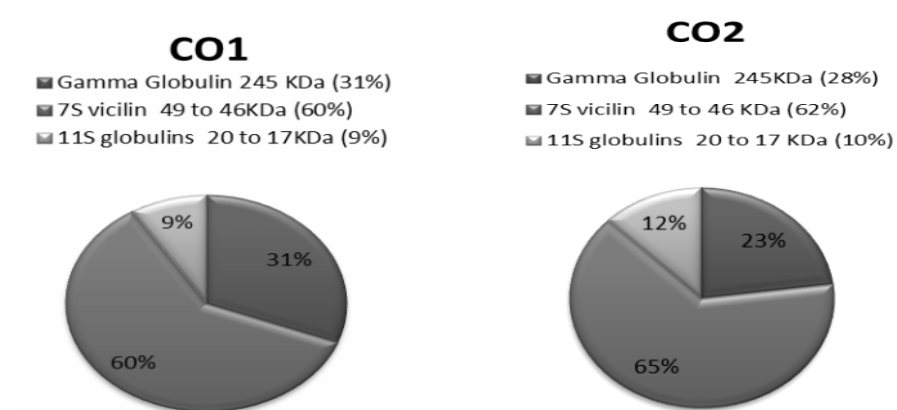


Fig. 8. The protein composited in cotton varieties by electrophoreses SDS PAGE

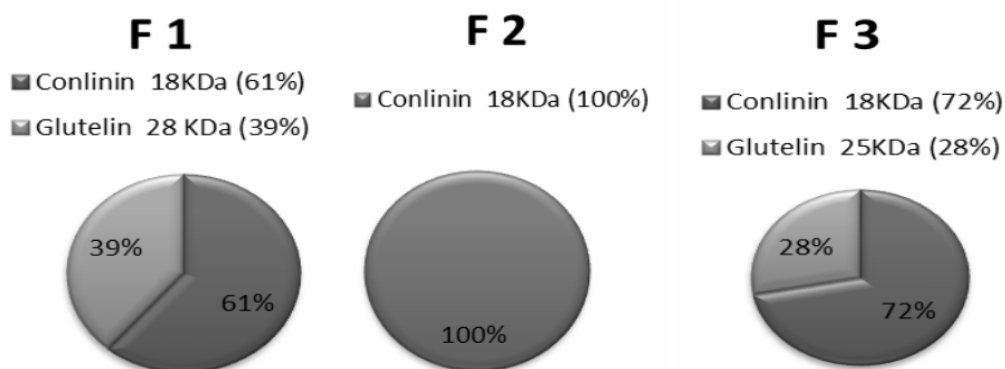


Fig. 9. The protein composited in flax varieties by electrophoreses SDS PAGE

Table 5. Proteins electrophoreses SDS PAGE of some seed oil crops

Protein molecular weight (KDa)	RF	Seed oil crops							
		Peanut			Soybean			Sunflower	
		PE 1	PE 2	PE 3	SB 1	SB 2	SB 3	SF 1	SF 2
245	0.009	-	-	-	+	+	-	+	+
158	0.091	-	-	-	+	+	-	-	-
135	0.10	-	-	-	-	-	-	+	+
100	0.141	-	-	-	+	-	-	-	-
88	0.992	+	+	+	-	-	-	-	-
75	0.213	-	-	-	+	+	-	-	-
63	0.288	+	+	+	+	+	+	-	-
56	0.339	+	+	+	-	-	-	-	-
47	0.400	-	-	-	+	+	+	+	+
38	0.512	+	+	+	-	-	-	-	-
35	0.542	-	-	-	+	+	+	-	-
30	0.565	+	+	+	-	-	-	+	+
20	0.721	-	-	-	+	+	+	+	+
19	0.893	+	+	+	-	-	-	+	+
17	0.900	-	-	-	+	+	+	+	+
11	0.971	-	-	-	-	-	-	+	+
Total of bands		6	6	6	9	8	5	8	8

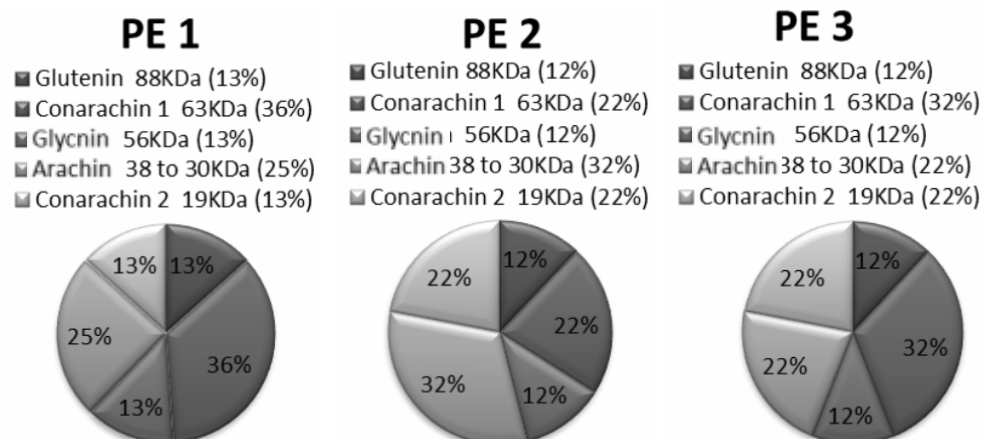


Fig. 10. The protein composited in peanuts varieties by electrophoreses SDS PAGE

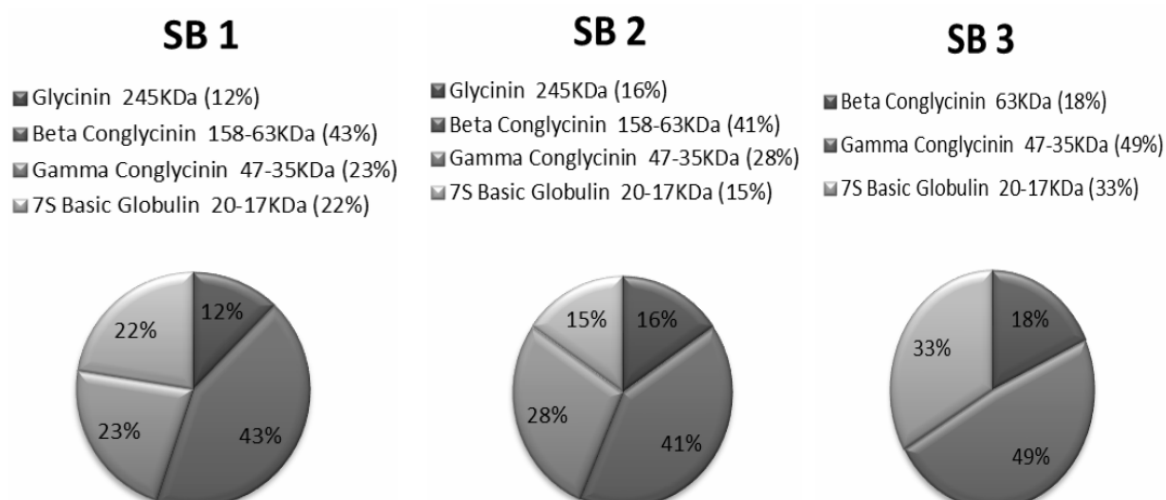


Fig. 11. The protein composited in soybean varieties by electrophoreses SDS PAGE

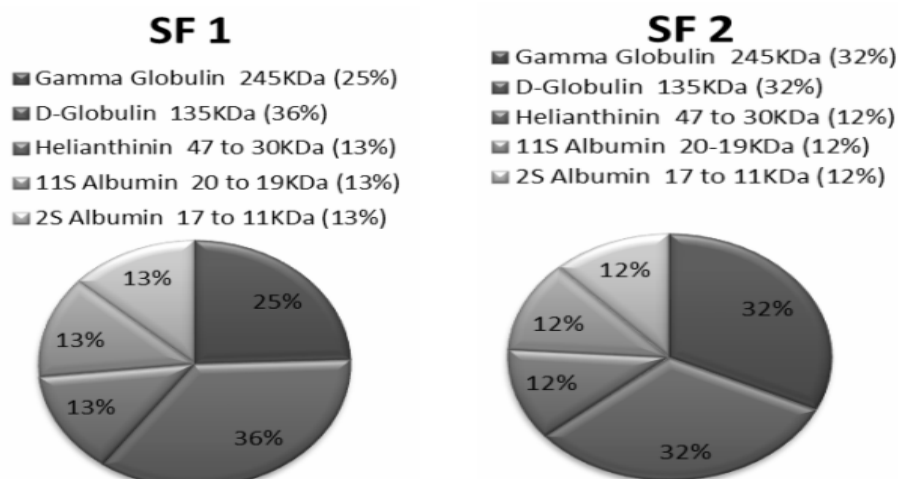


Fig. 12. The protein composited in sunflower varieties by electrophoreses SDS PAGE

As in Fig. 10, peanut varieties: PE1, PE2 and PE3 had about six protein bands: 88KDa, 63KDa, 56KDa, 38KDa, 30KDa and 19KDa. As in PE1 variety which had a higher percentage of conarachin 1 (63KDa) 36%. While PE2 variety had higher percentage of arachin (38 to 30 KDa) 32%. Meanwhile PE3 variety had higher percentage of conarachin 1 than PE2 but less than PE1, this makes easily to classify these varieties due to differences in protein percentages.

The soybean varieties: SB1, SB2 and SB3 as showed in Fig. 11 were easily classified by means of protein bands and its percentages as

SB3 variety had less protein bands than others, while the two other varieties were completely differed in percentage of protein bands.

In the last Fig. 12 showed that the sunflower varieties: SF1 and SF2 with five kind of protein bands with different molecular weights: 245KDa, 135KDa, 47KDa, 30KDa, 20KDa, 19KDa, 17KDa and 11KDa. The two varieties had the same protein bands but differed in the percentage which consider as a classification factor for this varieties.

These results were in agreement with those were reported by *Miroljub et al. (2004)*, *Zhou et al. (2013)* and *Mueller et al. (2014)*.

Conclusion

It could be concluded that using proteins Electrophoreses SDS PAGE assay could be used for classifying the varieties of different crops according to proteins factions and its characteristics.

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تفريد وتمييز بروتينات بعض المحاصيل الحقلية المصرية

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يعتبر البروتين، الكربوهيدرات والدهون من أهم المحتويات التي على أساسها تحدد أهمية المادة الغذائية سواء كانت نباتية (محاصيل حقلية،....) أو حيوانية، ويمكن استخدام أحد هذه المحتويات وخاصة المحتوى البروتيني في تصنيف المحاصيل بجميع أنواعها حيث يختلف المحتوى البروتيني طبقاً لنوعها وتركيز البروتين فيها من هنا أصبح تقدير أنواع البروتين من الأولويات التي يمكن أن تستخدم في التصنيف النوعي لكل أصناف المحاصيل الحقلية، حيث أمكن إجراء ذلك بطرق مختلفة منها: تفريد بروتينات المحاصيل الحقلية تحت الدراسة باستخدام خاصية الهجرة الكهربائية Electrophoresis للبروتينات المختلفة بواسطة SDS- PAGE وتم تصنيفها طبقاً لنوع البروتينات المفصولة من حيث دليل الهجرة (RF) والوزن الجزيئي والنسبة المئوية لكل منها، وتم تطبيق ذلك لدراسة ثلاث أصناف من محاصيل الحقل (محاصيل الحبوب- محاصيل البقوليات- محاصيل الألياف- محاصيل الزيوت)، وقد أوضح فصل وتمييز بروتينات أصناف من هذه المحاصيل وجود إختلاف واضح في المحتوى البروتيني من حيث نوع البروتين أو تسميته طبقاً للوزن الجزيئي ودليل الهجرة (RF) وأمن النسبة المئوية لكل جزء بروتيني من بروتينات كل محصول حقل، وبذلك يمكن استخدام نتائج تقدير المحتوي البروتيني ونوعيته كيميائياً في تصنيف المحاصيل الحقلية

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