RESULTS AND DISCUSSION

4- RESULTS AND DISCUSSION

4.1. Chemical composition of sunflower seed, kernels and by-products:

The preliminary work carried on the sunflower seed, kernels, and by – products included the proximate analysis and determination of the chemical composition and evaluate the quality of the protein concentrate and isolate.

Chemical composition of sunflower seeds, kernels and by-products are presented in Table (1). The moisture content of sunflower seeds, kernels and by – products is usually determined for commerical considerations. Beside the high content of moisture affects the keeping quality of polysaccharides. The results shown in Table (1) are similar to those mentioned by **Hind (1998) and Alaa (2002).** The results show that fat content is higher in sunflower kernels and seeds than in by-products since their ratios were 55.4%, 41.85% and 5.45%, respectively. These results are in agreement with the data obtained by **El-Amry (1995) and Hind (1998).**

The abovementioned results indicate that dehulled sunflower meal (kernels) contains lower crude fibers content (9.3%) when compared with sunflower seeds and by-products (20.9 and 29.96%). The crude total protein was found to be higher in by-products (52.8%) than in sunflower seeds and kernels (22.55 and 26.68%). These results are in agreement with those obtained by **Shamanthaka and Subramanian (1984) and Abu-El-Seoud (1989)** who found that the removal of hulls, seed coat and lipids increased the total protein content between 46.8% and 57.6% in sunflower meal. They also, found that the separation of seed coat decrease fiber content.

Total soluble sugars and ash content were determined. The results are shown in Table (1). The results recorded that the values of total soluble sugars content were 5.27, 0.50 and 3.22% and these ash content are 4.15, 4.04 and 6.45% in seeds, kernels and by-products, respectively. The results obtained are little different than those reported by **Hind (1998).**

Table (1): Chemical composition of sunflower seeds, kernels and by-products (g/100g dry weight).

Components	Sunflower seeds	Kernels	By-products
Moisture	5.37	4.15	9.12
Fat	41.85	55.41	5.45
Total protein	22.55	26.68	52.80
Ash	4.15	4.04	6.45
Total soluble sugars	5.27	0.50	3.22
Fiber	20.91	9.3	29.96

4.2. Factor affecting the extraction of sunflower polyphenols.

Sunflower proteins have unique organoleptic and functional properties that would make them useful in processed foods **(Sosulski, 1979 b)**. There are no known toxic constituents and antinutritional factors in sunflower meal. The major difficulty in the utilization of sunflower meal in human diets is the presence of polyphenols in the seed. Phenolic compounds have been a major deterrent to the use of sunflower protein in food products, due to their chromophoric properities. If the pH of sunflower flour or meal is raised above nutrality, its color progresses from a cream yellow to light green, to dark green and finally, to brown **(Cater *et al.*, 1972)**. Polyphenoloxidase catalyzes the oxidation of polyphenols to O- quinones which may bind covalently with thiol or amino groups of proteins **(Loomis, 1974)**.

The oxidation of polyphenols not only reduce the protein quality since this condenstaion compound cannot be metabolized by humans. **(Deshpande *et al.*, 1984).**

4.2.1. Effect of pH :

An experiment was carried in order to established the proper pH values required for sunflower polyphenol extraction.

Data presented in Table (2) and illustrated in Fig. (4) show that the maximum polyphenol extraction was achieved at pH 5.0.

This result is in agreement with that obtained by **Giancarlo and Marco (1977)** who noticed the ability of acidic butanol (pH 5) to remove the polyphenols and oligosaccharides from sunflower meal without detectable protein denaturation.

4.2.2. Effect of number of extraction:

The effect of number of extraction on the percentage of sunflower polyphenols extraction (kernels and by-products) was studied. The obtained data are presented in Table (3) and illustrated in Fig. (5).

The obtained results show that 7 times extraction was sufficient for extraction of most polyphenols. The percentage removal of polyphenolic compounds increased by increasing numbers of extraction. After seven times extration removal of polyphenole compounds from kernels and by-products were 90.78% and 93.40%, respectively. This result is in agreement with that obtained by **Sabir *et al.* (1974)** who found that the complete removal of polyphenol compounds from sunflower required long periods of shaking with polar organic solvents.

Table (2) Effect of pH on the extraction of polyphenol from sunflower kernels and by – product meals.

pH	Polyphenolic extraction %	
	Kernels	By-products
3	78.83	78.90
5	90.87	93.53
7	52.97	60.89
9	46.00	51.56

Table (3): Effect of numbers of polyphenol extraction of from sunflower kernels and by-product meals.

Numbers of extraction	Polyphenolic extraction %	
	Kernels	By-products
1	36.95	48.00
2	44.96	56.44
3	54.00	36.55
4	64.85	73.55
5	79.00	88.22
6	88.37	92.88
7	90.78	93.40
8	90.87	93.50

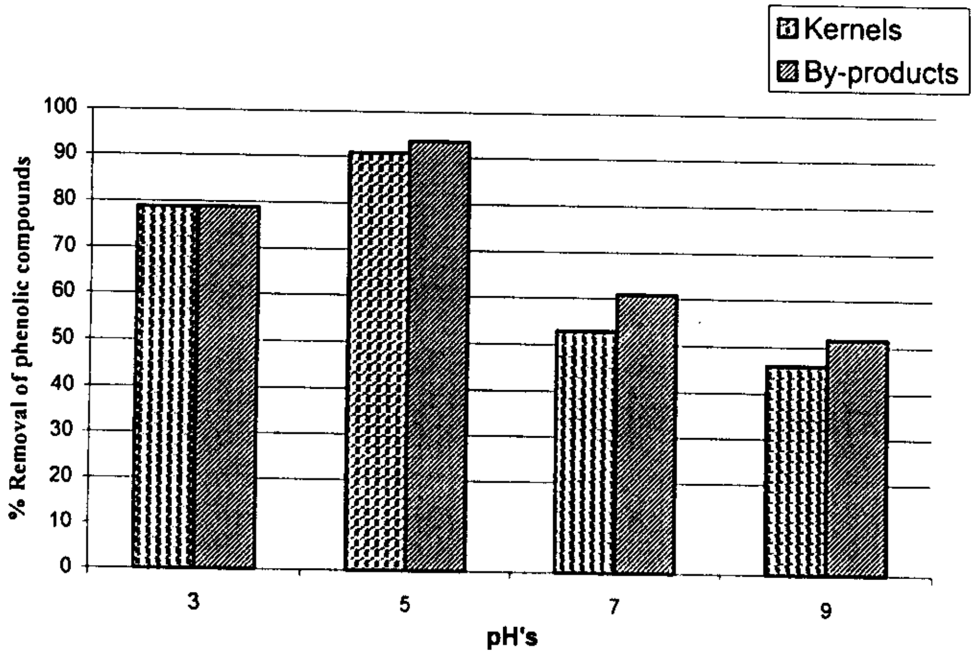


Fig. (4): Effect of pH on the extraction of polyphenol from sunflower kernels and by-products meal.

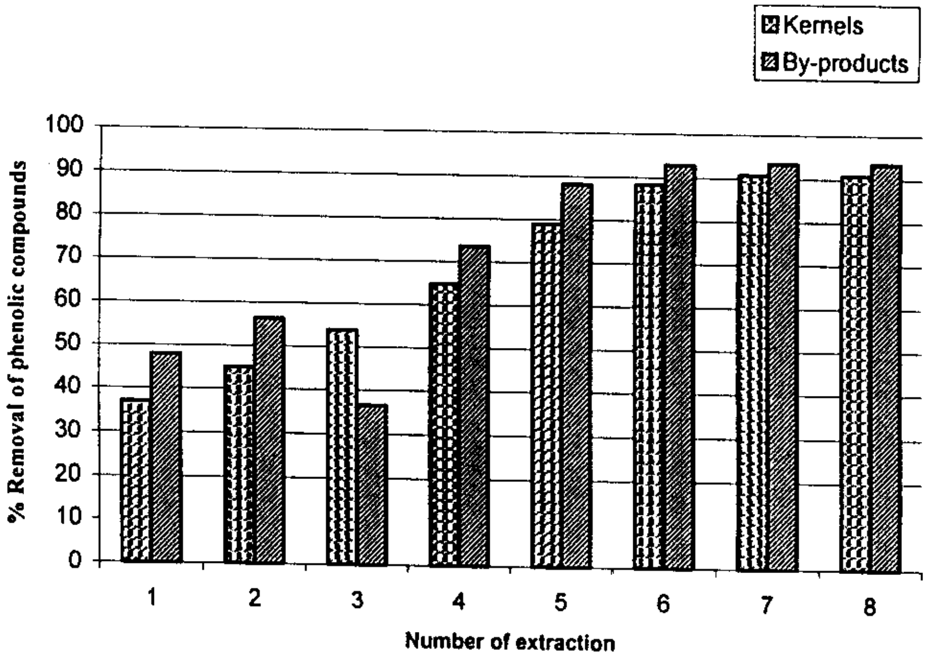


Fig. (5): Effect of number extraction on polyphenol from kernels and by-products meal.

4.3. Preparation of colorless sunflower protein concentrates:

Sunflower meals contained several polyphenolic compounds such as chlorogenic acid, caffeic and quinic acids. The major difficulty in the utilization of sunflowe meals in human diet is the presence of hulls and polyphenolic compounds in the seeds. The hulls contribute to high crude fiber content of the meal. Polyphenolic compounds may be binded with protein and gave an undesirable taste and color of the sunflower meal. Therefore removal of polyphenolic substances from sunflower meals has been attempted with different orgainc solvents and reducing agents in acidic media.

4.3.1. Effect of different solvents on the removal of polyphenolic compounds form sunflower kernels and by-products meal in acidic medium:

The total polyphenols content and its removal percent from sunflower kernels meal and by-products were determined after extraction with different organic solvents such as ethanol, methanol, acetone and butanol in acidic medium (pH 5.0). The obtained results are shown in Table (4).

The results in Table (4) indicate that the polyphenol contents of sunflower kernel and by-products meals were 3.87 and 4.50 g/100g, respectively in control treatments. These results are in agreement with those reported by **Sosulski *et al.* (1977)**.

From the results recorded in Tables (4 and 6) it is shown that treatment with methanol solution at acidic pH gave the highest removal of polyphenols about 92.14 and 95.4% from kernel and by-products meals, respectively. **Rahma and Rao (1981)** mentioned similar results in their previous study.

The results in Table (6) indicate that treatments with methanol solution at acidic pH gave the highest removal of polyphenols fractions free, bound and chlorogenic acid about (92.5, 97.1 and 91.6 and 91.9, 97.9 and 96.6%) from kernel and by-products meals, respectively.

4.3.2. Reducing agents (salts).

The total polyphenols content and its removal percent from sunflower kernels and by-products meals were determined after extraction. Different reducing salts such as sodium sulfite, ascorbate and dithionate were used in acidic medium (pH 5.0). The obtained results are shown in Table (5 and 6).

From the abovementioned results the ascrobate as a reducing salt was found be the suitable for the removal of polyphenols from sunflower kernels and by-product meals. The percentage removal were 68.32 and 67.77% for the above materials, respectively.

Table (4): Total polyphenols content and its removal percent from sunflower kernels and by- product meals using acidified organic solvent.

Treatments	Kernels meal			By-products meal		
	Total polyphenol (g/100g)	% removal of polyphenols	Color	Total polyphenol (g/100g)	% removal of polyphenols	Color
Control	3.87	-	Creamy	4.50	-	Brown
Ethanol	0.422	89.09	Yellow - white	0.370	91.7	Grey
Methanol	0.304	92.14	Yellow - white	0.207	95.4	Grey
Acetone	0.332	91.42	Yellow - white	0.284	93.68	Grey
Butanol	0.353	90.87	White	0.291	93.53	Grey

Table (5): Effect of different reducing salts on the removal of polyphenolic compounds from sunflower kernels and by-product meal in acidic medium

Treatments	Kernels meal			By-products meal		
	Total polyphenol (g/100g)	% removal	Color	Total polyphenol (g/100g)	% removal	Color
Control	3.87	-	Creamy	4.50	-	Brown
Sodium sulfite	1.34	64.08	Creamy	1.86	58.66	Grey
Ascorbate	1.23	68.21	White	1.45	67.77	Grey
Dithionate	1.81	53.22	White	2.09	53.55	Grey

Table (6): Effect of different treatments on the removal of polyphenols fractions from sunflwoer kernel and by-products meals

Treatments	Kernel meals						By- product meals					
	Polyphenols				Chlorogenic acid		Polyphenols				Chlorogenic acid	
	% Free phenols	% Removal	% Bound phenols	% Removal	% Chlorogenic acid	% Removal	% Free phenols	% Removal	% Bound phenols	% Removal	% Chlorogenic acid	% Removal
Control	2.29	-	1.58	-	2.72	-	2.99	-	1.51	-	3.39	-
Ethanol	0.172	92.5	0.132	91.6	0.117	95.6	0.230	92.3	0.140	90.7	0.176	94.8
Methanol	0.172	92.5	0.132	91.6	0.065	97.9	0.086	97.1	0.121	91.9	0.114	96.6
Acetone	0.224	90.2	0.108	93.2	0.100	96.32	0.089	97.1	0.147	90.3	0.158	95.3
Butanol	0.184	91.96	0.169	89.3	0.078	97.13	0.138	95.4	0.153	89.9	0.137	95.9
Na_2So_3	0.82	94.14	0.56	94.6	0.362	86.6	1.11	52.9	0.56	62.9	0.421	87.5
Ascorbate	0.72	98.6	0.51	97.7	0.454	83.3	0.96	96.8	0.49	67.5	0.513	84.3
Dithionste	1.07	53.27	0.74	53.2	0.562	79.3	1.38	53.5	0.71	52.9	0.621	81.6

* Fractionation of chlorogenic acid by using HPLC.

4.3.3. The chemical composition of protein concentrate after treatment with several acidic organic solvents and reducing salts:

The results concerning the effect of different treatments (organic solvents and reducing salts) on the chemical composition of protein concentrate from sunflower kernel and by-products meals are tabulated in Table (7).

The data presented in Table (7) indicate that protein content of all samples increased after treatments. It could be noticed that sunflower kernels and by-products meals contain higher amount of protein in all treatments compared with the content of sunflower seed meal. The increase of protein may be due to the solubility of major amount of carbohydrates especially when using the above solvents. These results are in agreement with those reported by **Hind (1998).**

On the other hand, the ash content was decreased in all investigated samples. These results are confirmed with the results stated by **Shamanthaka and Subramanian (1984)** who reported that three times extraction of kernels with those solvents reduced the ash content from 8.3 to 2.3%.

However, crude fiber content was also affected by the abovementioned treatments as shown in the same Table (7). The percentage of crude fiber was relatively increased after treating of sunflower by-products meals. On the contrary, it was found that crude fiber was reduced in sunflower kernels meal after treatments. This reduction in crude fiber may be to the separation of translucent layer or testa from kernels during the treatment as explained by **Sosulski and Sarwar (1973).**

Table (7): Chemical composition of protein concentrate after treatment with several organic solvents and reducing salts.

Samples	Kernels meal					By-products meal				
Components / Treatments	Protein %	Ash %	Fiber %	Carbohydrates %	T. phenols %	Protein %	Ash %	Fiber %	Carbohydrates %	T. phenols %
Untreated	60.67	4.04	9.3	21.99	3.87	52.80	6.45	29.96	33.18	4.5
Methanol	67.90	3.06	7.3	13.07	0.422	61.10	5.72	31.96	22.8	0.370
Ethanol	66.18	3.91	7.5	15.80	0.304	61.90	5.53	32.33	24.9	0.207
Acetone	65.51	3.10	8.3	16.20	0.332	60.31	5.60	31.63	24.7	0.284
Butanol	66.34	3.90	8.1	14.90	0.353	61.50	5.91	31.89	23.90	0.291
Sodium sulfite	63.54	3.07	8.9	16.69	1.34	58.10	5.37	30.79	27.17	1.86
Dithionate	61.08	3.33	8.8	19.2	1.23	55.39	6.10	29.93	30.17	1.45
Ascorbate	63.39	3.17	8.5	16.5	1.81	57.90	6.27	29.91	28.95	2.09

4.4. Extraction of colorless sunflower protein isolate:

4.4.1. Effect of pH of the extracting solvent on sunflower protein:

These experiments were carried out in order to establish the proper pH values required for sunflower protein extraction.

The obtained results are presented in Table (8) and illustrated in Fig. (6). From these results it is shown that the maximum sunflower protein extraction was achieved at pH 10. On the other hand, results show that on the acidic pH range, the percentage of the extracted protein was very low and reached its lowest amount at pH 5 (isoelectric point). However, at basic pH (10.0) the percentage of the protein extracted was found to be 96.15 and 92.6% for kernel and by-products meals, respectively.

These results could be explained on the basis of the exhibited role of ionogenic groups in protein molecules in lyophilic colloidal systems of protein solutions. These proteins might be positively or negatively charged depending on the hydrogen ion concentration of the medium. The amount of NaOH bounds by the protein molecules depends on the equilibrium of hydrogen, **Wu and Sexson (1979).**

The ionogenic groups of proteins are present largely as zweitter-ions at isoelectric point. Thus at the alkaline pH values the base displace the hydrogen from ammonium groups $(-NH_3)^+$ of the zweitter-ion giving negative charges to the protein molecule that would increases as the normality of the base is increased **Samir (1976).**

Solubility of a specific protein reached to its minimum at the isolectric point. **Sania (1980)** noticed that the solubility increased with increasing the acidity or alkalinity which might be attributed to the increase of repulsive electric forces induced by charges of same sign that might exist on protein molecules.

Table (8): Effect of extracting solvent pH on protein isolation.

pH of extracting solvent	% protein isolation	
	Kernel	By-product
1	65.10	43.20
2	52.20	31.20
3	43.3	23.30
4	29.9	19.10
5	25.2	18.20
6	27.9	19.90
7	51.4	22.50
8	66.3	33.40
9	95.9	70.50
10	96.1	92.50
11	96.2	91.90

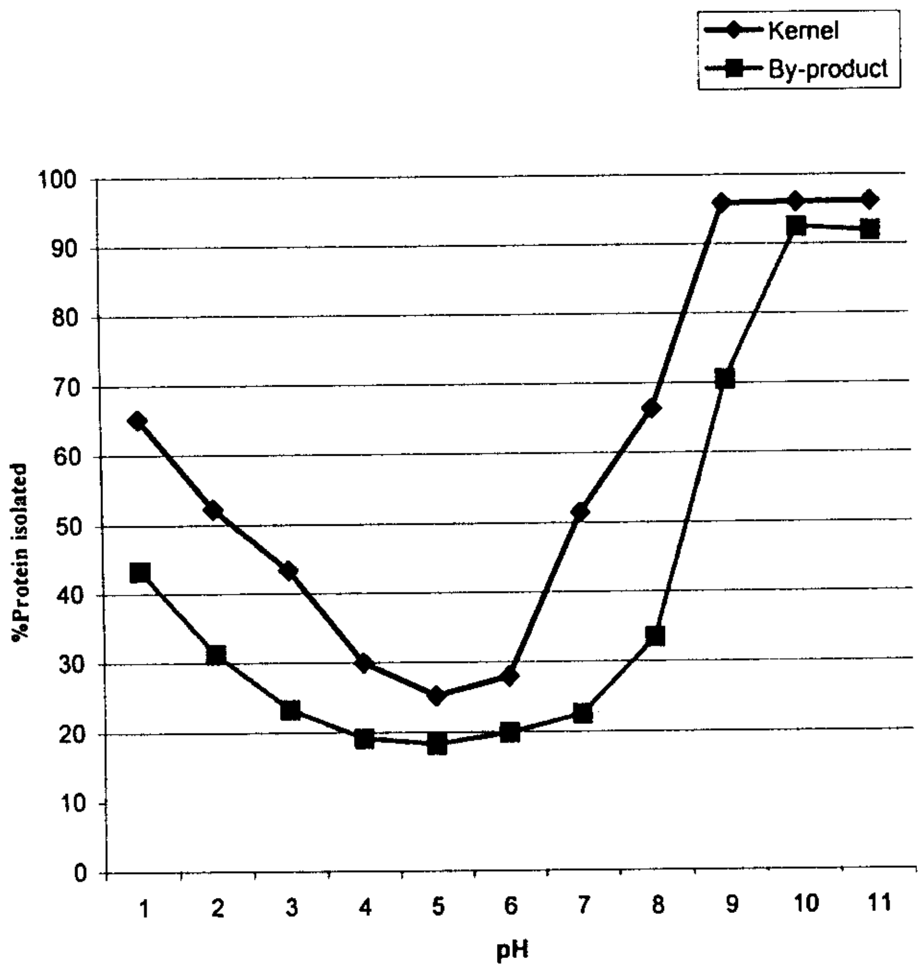


Fig. (6): Effect of pH on protein isolation from sunflower kernels and by-products meal.

4.3.4. The chemical composition of protein isolate from sunflower kernel and by-products meal:

The chemical composition of sunflower protein isolate (SPI) was determined.

The obtained results are tabulated in Table (9). From these results, it is indicated that total protein content was 92.10 and 92.05 % for kernel meals after treated with NaOH or Na_2SO_3 while, these values for by – products were 89.17 and 90.35% under the abovementioned treatments.

These results are slightly coincide with the data reported by **Canella *et al.* (1977)** who found that protein isolate of sunflower meal was 93.5%.

However, ash, fiber, and total soluble sugars contents were found to be 1.93 and 1.96, 0.79 and 0.75 and 1.90 and 1.86 for kernel meals after treatments by NaOH or Na_2SO_3 respectively. But these values were 2.15 and 2.13, 0.97 and 0.99 and 3.34 and 3.43 for by-product meals treated by the above reagents.

Table (9): Chemical compositions of protein isolates from sunflower kernel and by-product.

Components	Kernel		By - products	
	NaOH	Na_2SO_3	NaOH	Na_2SO_3
Total protein	92.10	92.05	89.17	90.35
Ash	1.93	1.96	2.15	2.13
Fiber	0.79	0.75	0.97	0.99
Total soluble sugars	1.90	1.86	3.34	3.43

4.4. Effect of alkaline medium on the removal of polyphenolic compounds from sunflower kernels and by-product meals.

The total polyphenols of sunflower kernels meal and by-products were extracted with sodium hydroxide and different reducing salts such as sodium sulfite, ascorbate, dithionate and sodium sulfite with iso-propanol in alkaline medium (pH 10.0). The obtained results are shown in Table (10).

Table (10) Effect of alkaline medium on the removal of polyphenolic compounds from sunflower kernels and by- product meals

Treatments	Kernel meal			By-product meal		
	Total polyphenol (g/100g)	% removal	Color	Total polyphenol (g/100g)	% removal	Color
Control	3.87	-	Yellow	4.50	-	Brown
Sodium hydroxide	0.34	91.20	Green	0.31	93.1	Dark green
Sodium sulfite	0.36	90.70	White creamy	0.34	92.4	Dark creamy
Ascorbate	0.51	86.80	Creamy	0.48	89.4	Dark yellow
Dithionate	0.52	86.60	Creamy	0.48	89.4	Dark yellow
Sodium sulfite + isopropanol	0.35	90.96	White	0.34	92.4	Dark yellow

Data reported in Table (10) indicate that treatment with NaOH at pH 10.0 gave the highest removal of polyphenols (91.20 and 93.1%) from kernel meals and by-product meals. But, the color of the isolated protein after treatment was found to be green and dark green. This color due to the oxidation of chlorogenic acid in alkaline medium and limits the use of sunflower seeds protein in the food industry.

From the abovementioned results it is indicated that treatment of sunflower kernel and by-products with above reducing salts removed most polyphenols 86.6-90.7 and 89.4-92.4%. These reducing agents prevents the accumulation of quinones which may be participate in reactions leading to colored products (**Gheyasuddin *et al.*, 1970**).

On the other hand, when using sodium sulfite with isopropanol for extraction produced good white isolate protein because the alcohol is able to break H-bonds as it is known that polyphenols at acidic pH remain combined with protein in unusually strong H-bonds, (**Pomenta and Burns, 1971**).

4.5. Determination of sunflower protein subunits molecular weight by using SDS-PAGE:

Polyacrylamide Gel Electrophoresis in the presence of detergent Sodium Dodecyl Sulphate (SDS-PAGE) was used for determining the subunit molecular weights (M.W.) of protein extracted by alkaline solutions from both sunflower kernel and by-products after different treatments.

The Pharmacia Low Molecular Weight (LMW) calibration kit provides six protein standards covering subunits M.W. ranging from 14.4 to 94 KD was used for constructing the calibration curve. The SDS-PAGE polypeptide patterns of the overall polypeptides in sunflower kernel and by-products together with protein standards are shown in Fig. (8 & 9).

The relative mobility (Rm) of each protein standard calculated from Fig. (7) and its molecular weights are shown in Table (11). A graph of log M.W. against Rm gave a straight line as shown in Fig. (7). From which the molecular weight of the subunits dissociation from both samples protein were determined (Table, 13). The obtained results show that both sunflower kernel and by-products protein dissociate into 11 subunits with molecular weight (MW) ranging from 95.499 to 14.125 KD.

Data in Fig. (8) and Table (12) showed that kernel protein having strong intensity bands than by-products in the all treatments.

These results are in agreement with those reported by **Sodini and Canella (1977)** who described the use of acidic butanol reagent for removing phenolics from sunflower meal without causing detectable protein denaturation.

Table (11): Molecular weights of standard proteins and their relative mobilities.

Standard proteins	Molecular weight M.W	Log M.W	Relative mobility (Rm)
Phosphorylase	94000	4.973	0.18
Albumin	67000	4.826	0.26
Pvalbumin	43000	4.633	0.45
Carbonic anhydrase	30000	4.477	0.56
Trypsin inhibitor	20100	4.303	0.74
Lactalbumin	14400	4.158	0.84

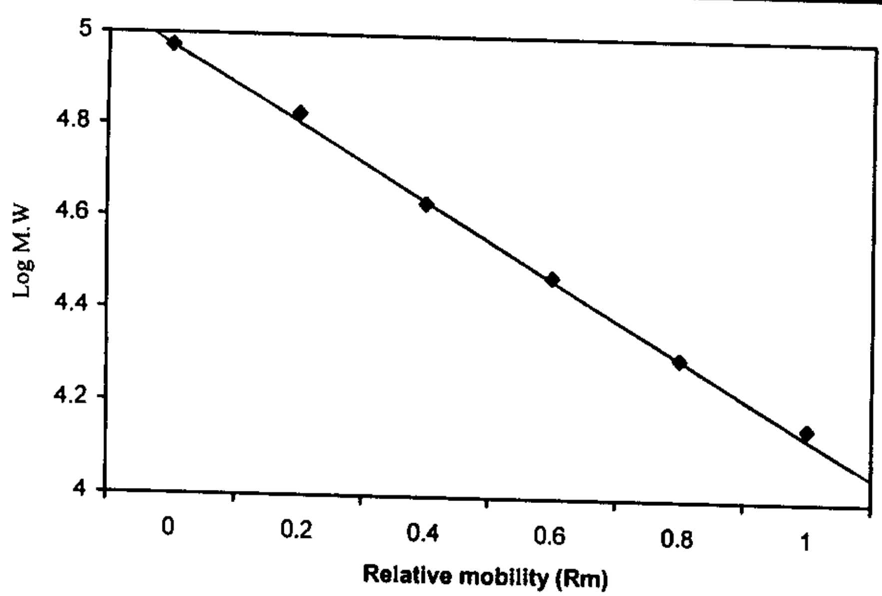


Fig. (7): Calibration curve for M.W. determination of subunits sunflower protein by SDS-PAGE.

Table (12): Molecular weights of sunflower kernel meal.

M.W. of bands K.D.	Samples								
	1	2	3	4	5	6	7	8	9
1	95	95	95	95	95	95	95	95	95
2	91	91	91	91	91	91	91	91	91
3	79	79	79	79	79	79	79	79	79
4	71	71	71	71	71	71	71	71	71
5	56	56	56	56	56	56	56	56	56
6	43	43	43	43	43	43	43	43	43
7	32	32	32	32	32	32	32	32	32
8	28	28	28	28	28	28	28	28	28
9	21	21	21	21	21	21	21	21	21
10	18	18	18	18	18	18	18	18	18
11	14	14	14	14	14	14	14	14	14

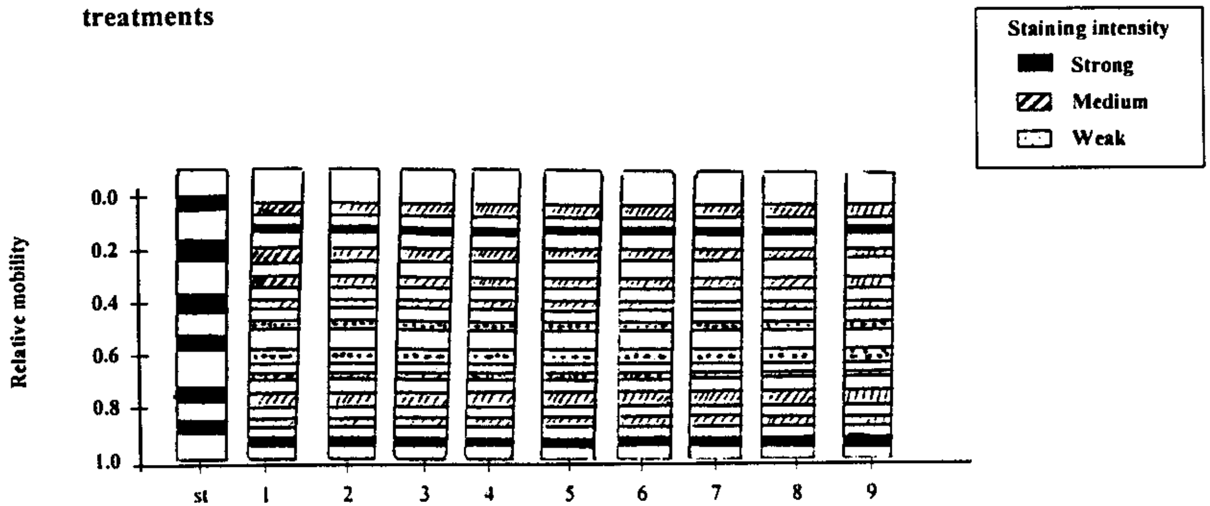
1- Untreated
2- Treated with Methanol
3- Treated with Ethanol
4- Treated with Butanol
5- Treated with Acetone
6- Treated with Sodium sulfite
7- Treated with Dithionate
8- Treated with Ascorbate
9- Treated with NaOH

Table (13): Molecular weights of sunflower by-products meal.

M.W. of bands K.D.	Samples								
	1	2	3	4	5	6	7	8	9
1	95	95	95	95	95	95	95	95	95
2	91	91	91	91	91	91	91	91	91
3	79	79	79	79	79	79	79	79	79
4	71	71	71	71	71	71	71	71	71
5	56	56	56	56	56	56	56	56	56
6	43	43	43	43	43	43	43	43	43
7	32	32	32	32	32	32	32	32	32
8	28	28	28	28	28	28	28	28	28
9	21	21	21	21	21	21	21	21	21
10	18	18	18	18	18	18	18	18	18
11	14	14	14	14	14	14	14	14	14

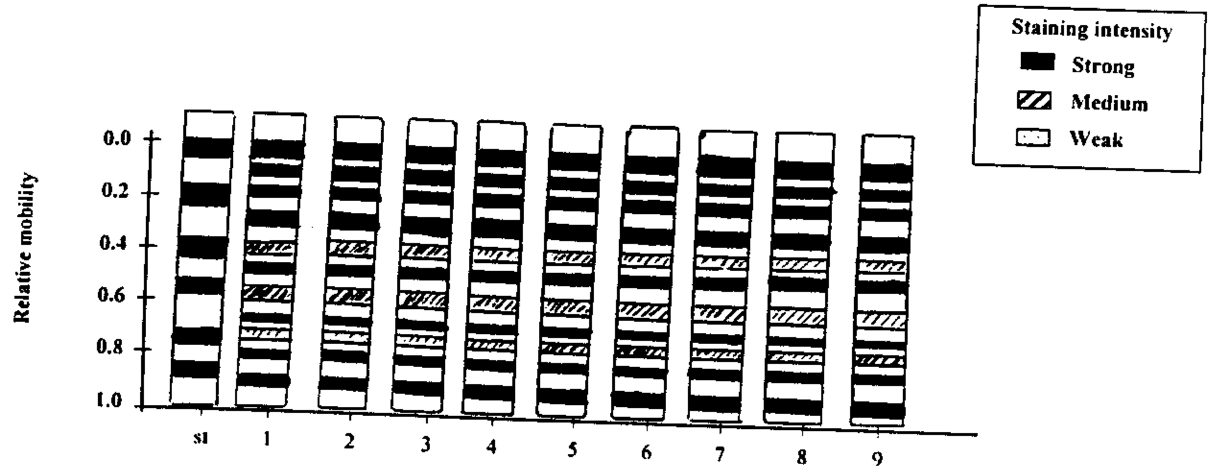
1- Untreated
2- Treated with Methanol
3- Treated with Ethanol
4- Treated with Butanol
5- Treated with Acetone
6- Treated with Sodium sulfite
7- Treated with Dithionate
8- Treated with Ascorbate
9- Treated with NaOH

Fig (8): SDS- PAGE pattern of sunflower (by- products meal) protein extracted after different treatments



1- Untreated
2- Treated with Methanol
3- Treated with Ethanol
4- Treated with Butanol
5- Treated with Acetone
6- Treated with Sodium Sulfite
7- Treated with Dithionate
8- Treated with Ascorbate
9- Treated with NaOH

Fig (9) : SDS- PAGE pattern of sunflower (kernel meal) protein extracted after different treatments



1- Untreated
2- Treated with Methanol
3- Treated with Ethanol
4- Treated with Butanol
5- Treated with Acetone
6- Treated with Sodium Sulfite
7- Treated with Dithionate
8- Treated with Ascorbate
9- Treated with NaOH

4.6. Increasing the bioavailability of sunflower meal :

4.6.1. Effect of different treatments on the removal of phytic acid and trypsin inhibitor activity from sunflower meals:

Phytic acid or its salt (phytate) is a cyclic derevative of inositol containing six phosphate redicals. Its physiological significance lies in the facts that it readily chelates such di- and Tri- valent metal ions as calcium, magnesium, zinc and iron the poorly soluble compounds that are not readily absorbed from the intestines.

For this reason the presence of phytate in many seeds like sunflower has come to be regarded as an antinutritional factor which interferes with the bioavailability of minerals essential for optimal health.

On the other hand, trypsin inhibitors are active against proteolytic enzyme, especially trypsin, which are distributed widely in sunflower seeds. These inhibitors are capable of combining with the enzyme to form enzyme-inhibitor-complex, which is usually completely devoid to proteolytic activity. Also, these inhibitors have been classified into two families on the basis of their molecular weights and cystine contents.

First family is the **kunitz** family that has molecular weights about 20 KD and two di sulfide bridges. The other family is the **Bawman-** Birk family that has molecular weights of about 8 KD and is characterized by a high cystine content (seven disulfite bridges), both inhibitors were characterized by **Birk *et al.* (1963).**

Effect of different treatments (acidic and alkaline medium) on the removal of phytic acid and trypsin inhibitor activity from kernel and by-products meal are shown in Table (14).

The obtained results indicate that dehulling of sunflower seed led to increase the relative concentration of phytate. This suggests that phytate might be characteristically present in the kernel and in very little in the hull since the hull contributes a substantial protein of the whole seed weight (about 21% of the whole seed is hull) and removing the hull will lead to an increase in the concentration of phytate on a unit weight basis.

However dehulling may affect the assay of phytate by improving its extraction from the sunflower seed during the assay.

The amount of antinutritive factors in the sunflower kernel and by-products meal (untreated) were determined to be 4.32 and 3.45% phytic acid and 1.5 and 1.1 mg/g trypsin inhibitor.

The results presented in Table (14) indicate that treatments of the kernel and by-products with different alkaline solutions were more effective in removing phytic acid and trypsin inhibitor (antinutritional factors) than different acidic media.

These results are in agreement with those reported by **Saeed and Cheryan, (1988) and Abu El-Seoud (1989).**

Table (14): Effect of different treatments on phytic acid and trypsin inhibitor from kernels and by-products meal.

Treatments	Phytic acid (%)		Trypsin inhibitor (mg/g)	
	Kernels	By-product	Kernels	By-product
Untreated	4.32	3.45	1.5	1.1
Acidic medium				
Ethanol	1.31	1.12	0.521	0.50
Methanol	1.15	0.92	0.53	0.42
Butanol	0.88	0.71	0.54	0.41
Acetone	0.99	0.84	0.56	0.44
Sodium sulfite	1.62	1.29	0.98	0.83
Dithionate	1.71	1.51	0.96	0.85
Ascorbate	1.72	1.49	0.96	0.81
Alkaline medium				
Sodium hydroxide	0.32	0.25	0.0	0.0
Sodium sulfite	0.29	0.21	0.0	0.0
Dithionate	0.34	0.27	0.0	0.0
Ascorbate	0.33	0.27	0.0	0.0
Sodium sulfite+Isopropanol	0.22	0.18	0.0	0.0

4.6.2. Effect of different treatments on sunflower in vitro protein digestability:

To evaluate the effect of different treatments on sunflower meal, the digestability index for all treatments was determined.

The obtained results are recorded in Table (15). The results show that the digestability index for sunflower kernel and by-products before treatments was found to be 80.47 and 78.46%, respectively. While, after treatment of sunflower kernel and by- products with different organic solvents in acidic medium (pH.5.0) protein digestibility index increased comparing with untreated materials.

On the other hand, alkaline solution at pH, 10.0 of sunflower kernel and by- products meals, the protein digestibility index (%) for kernel and by- products ranged from (79.42 to 87.84%) and (79.12 to 85.21%) respectively at different solutions.

However, **Prasad (1988)** showed that chlorogenic acid has the ability to interact with protein and also to inhibit in- vitro the activity of some digestive enzymes such as trypsin and Lipase, the protein- binding and inhibitory effects of chlorogenic acid which may be of nutritional signficance.

These contents under treatments of the raw flour was improved by different alkaline treatments giving the higher digestibility index than other treatments.

The highest amount of protein digesti-bility index (%) was found to be 93.65% for kernel and 93.70% for by-products when treated by sodium hydroxide.

This increment due to decrease the amount of polyphenols and trypsin inhibitor activity under these conditions are in agreement with those reported by **Nuria *et al.* (1999)** who found that polyphenols can affect the quality of sunflower protein in several ways such as reducing the digestibility, prolongoing or shortening the storage life and stability and adversely altring the functional properties and behaviour of sunflower protein in food systems.

Table (15): Effect of different treatments on sunflower meal in- vitro protein digestibility.

Treatments	Protein digestibility index %	
	Kernel	By-product
Untreated	80.47	78.46
Acidic medium		
Methanol	87.54	84.01
Ethanol	87.84	85.21
Butanol	85.45	84.89
Acetone	84.53	83.95
Sodium sulfite	79.90	79.43
Dithionate	79.42	79.12
Ascorbate	80.83	79.39
Alkaline medium		
Sodium hydroxide	93.65	93.70
Sodium sulfite	93.52	93.53
Dithionate	93.11	93.14
Ascorbate	93.12	93.15
Sodium sulfite + Isopropanol	93.57	93.62

4.6.3. Effect of different treatments on the amino acid components:

The amino acids component of kernel meals, by-products meal, protein concentrate and protein isolate of sunflower are determined and presented in Table (16).

The obtained results indicate that glutamic and aspartic acids are the most abundant amino acids followed by argnine, and leucine in kernel meals, by-product meals, protein concentrate and protein isolate.

However, the total essential amino acids contents were 37.39, 37.08, 36.01 and 37.12 for kernel meals, by- product meals without treatment, protein concentrate after butanol treatment and protein isolated after Na_2SO_3 treatment respectively. These results are in agreement with those reported by **Bodwell and Hopkins (1985).** They found that essential amino acids of oil seed protein ranged from 35 to 45% of their total amino acids. Also they reported that these levels of essential amino acids equal or exceed reference patterns that are based on human requirements.

On the other hand, the total amino acids components of kernel meals amounted to 94.89 while, these values for by-product meal without treatment, protein concentrate after butanol treatment, and protein isolated treated with Na_2SO_3 were 93.08, 92.91 and 94.47 g /100g protein respectively. These results are in agreement with those **Bau *et al.* (1983) and Alaa (2002).** They reported that the amino acid components of proteins from extracted sunflower meal at acidic butanol medium was similar to that of the unextraxted meal. Also, they reported that no significant differences were observed in dehulled and defatted sunflower meals in essential amino acid content except for lysine which was slightly reduced.

Table (16): Effect of different treatments on the amino acids components of kernel and by – product meals (g / 100 g protein)

Components of amino acids	Kernel meals	By- product meals		
		Without treatment	Protein concentrate after Butanol treatments	Protein isolate after Na_2SO_3 treatments
E. A.A				
Lys	3.90	3.83	3.51	3.82
Leu	7.50	7.40	7.32	7.36
Iso- Leu	4.20	4.40	4.38	4.43
Cys+ Met	1.50+2.34	1.30+2.30	1.2+2.27	1.3+2.3
Phe + Tyr	4.80+3.30	4.90+3.10	4.98+3.20	5.11+3.20
Try	1.44	1.40	1.42	1.40
Thr	3.65	3.70	3.50	3.60
Val	4.76	4.75	4.23	4.60
T.E.A.A.	**37.39**	**37.08**	**36.01**	**37.12**
N.E.AA				
His	2.70	2.60	2.50	2.62
Arg	8.10	8.20	8.10	7.93
Asp	8.30	8.10	7.90	7.80
Glu	19.50	18.70	19.80	20.20
Ser	4.40	4.20	4.30	20.40
Pro	4.70	4.60	4.80	4.80
Gly	5.40	5.20	5.30	5.20
Ala	4.40	4.40	4.20	4.40
T.N.E.A.A.	**57.50**	**56.00**	**56.90**	**57.35**
T.A.A	**94.89**	**93.08**	**92.91**	**94.47**

Table (17) : Amino Acid groups of kernel and by – products before and after treatments.

Samples	TAA	EAA	NEAA	E/N %	E/T %	EAAI %	Sulfur Amino acid %	Aromatic amino acids %	Acidic amino acids %	Basic amino acids%	Aliphatic amino acids %	OH-amino acid %	Imino acid %
Kernel meals	94.89	37.39	57.50	65.02	39.40	79.43	4.04	12.89	29.29	15.49	27.67	11.96	4.95
Without treatment (By- product)	93.08	37.08	56.00	66.20	39.83	78.77	3.86	12.89	28.79	15.71	28.09	11.81	4.94
Protein concentrat after treatment with Butanol	92.91	36.01	56.90	63.20	39.75	76.50	3.73	13.02	29.81	15.18	27.37	11.83	5.16
Protein isolate After treatment with NO_2SO_3	94.47	37.12	57.35	64.70	39.29	78.86	3.81	13.05	29.63	15.21	27.51	11.85	5.08

Table (18): Essential amino acids components and amino acids score of sunflower kernel and by- Products.

Amino acids	FAO/WHO 1973 pattern (g/100g) protein	Amino acids content (g/100 g protein)				Amino acids score			
		Kerenl meals	By- produet meals			Kernel meals	By- product meals		
			Without treatment	Protein concentrate	Protein isolate		Without treatment	Protein concentrate	Protein isolate
Isoleucine	4	4.2	4.4	4.38	4.43	105	110	110	111
Leueine	7	7.5	7.4	7.32	7.36	107	106	105	105
Lysine	5.5	3.9	3.83	3.51	3.82	71	70	64	69
Total sulfur amino acid	3.5	3.84	3.60	3.47	3.60	110	103	99	103
Total aromatic amino acid	6	8.1	8.0	8.18	8.31	135	133	136	139
Threionine	4	3.65	3.70	3.50	3.60	91	93	87	90
Tryptophan	1	1.44	1.40	1.42	1.40	144	140	142	140
Valine	5	4.67	4.75	4.23	4.60	95	95	85	92

Total amino acid components of kernel and by-products meal under investigation, Essential amino acids / None essential amino acids (E/N), Essential amino acids / Total amino acids (E/T), Essential amino acid index (EAAI) and percentage groups of amino acids / total amino acids were determined and the data are shown in Table (17).

Table (17) indicate that there is no difference in total amounts of aromatic and basic amino acids. On the other hand, kernel protein and by- products without treatment contains higher amount of essential amino acids. The nutritional quality of food protein depends on the relative proportions of the essential amino acids, WHO 1973, qualified an ideal protein in which 36% of the total residues are essential amino acids.

However, both sunflower kernel meals, by- product meals, protein concentrate and protein isolate had higher essential ratios than the proposed 36% for an ideal protein.

The data presented in Table (18) show that the essential amino acids component and amino acids score of sunflowers kernel and by-products meals, compared to FAO / WHO pattern. The obtained results indicate that lysine was the first limiting essential amino acids. The values recorded were 71%, 70%, 64% and 69% in kernel, by- products meals, protein concentrate and protein isolate, respectively. On the other hand, tryptophane and aromatic amino acids (phenylalanine and tyrosin) were the most abandant amino acids score. These results are in agreement with those reported by **Alaa (2002).**