Results and Discussion

4-RESULTS AND DISCUSSION

4.1. Chemical composition of flax meal:

The chemical composition of flax meal **(Table, 1)** show high protein (36.9%) content, which confirms the view that flax meal by-product is excellent source of protein. These results are similar to those mentioned by **Tolba (1999) and El-Kady *et al.* (2001).**

The antinutritive factor trypsin inhibitor, total cyanogenic, total phenolic compounds and phytic acid in the flax meal were 2.5mg/g, 0.42 mg/g, 196 mg/100g and 2.9%, respectively.

These data are in agreement with those reported by **El-Kady *et al.* (2001).**

4.2. Extraction of flax meal protein isolate

4.2.1. Effect of extracting solvent pH on protein:

This experiment was carried out in order to establish the proper pH values required for flax meal protein extraction.

The obtained results are presented in **Table (2)** and illustrated in **Fig. (1)**. From these results it is shown that the maximum flax meal protein extraction was achieved at pH 12. Also, results show that on the acidic pH range, the percentage of the extracted protein was very low and reached its lowest amount at pH 4 (isoelectric point). However, at basic pH (10.0) the percentage of the extracted protein was found to be 88.9%.

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-ions 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 isoelectric point. **Hassan (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.

4.2.2. Chemical composition of flax meal protein isolate:

The data in **Table (3)** show the chemical composition of flax meal protein isolates. It is clear that protein isolate has high protein content (90.1%), low ash (2.1%) with free trypsin inhibitor activity and cyanoginic glycosides but, it contains little amounts of total phenolic compounds (23.2 mg/100g) and phytic acid (0.24%). Similar results were obtained by **Taha and Mohamed (2003) and Abd El-Aleem and Soltan (2005).**

Table (1): Chemical composition of flax meal (on dry weight basis)

Variety	Carbohydrates %	Moisture %	Fat %	Protein %	Ash %	Fiber %	Trypsin Inhibitor (mg/g)	Total Cyanogenic (mg/g)	Total phenols (mg/100g)	Phytic Acid %
Viking	29.572	9.7	5.4	36.9	6.14	8.9	2.5	0.42	196	2.9

Table (2): Effect of extracting solvent pH on % protein isolation.

pH of extracting solvent	% protein isolation
1	54.0
2	40.5
3	36.7
4	25.1
5	55.3
6	65.2
7	70.7
8	77.3
9	79.8
10	88.9
11	89.2
12	90.1

Fig. (1): Effect of extracting solvent pH on protein isolation from flax meal.

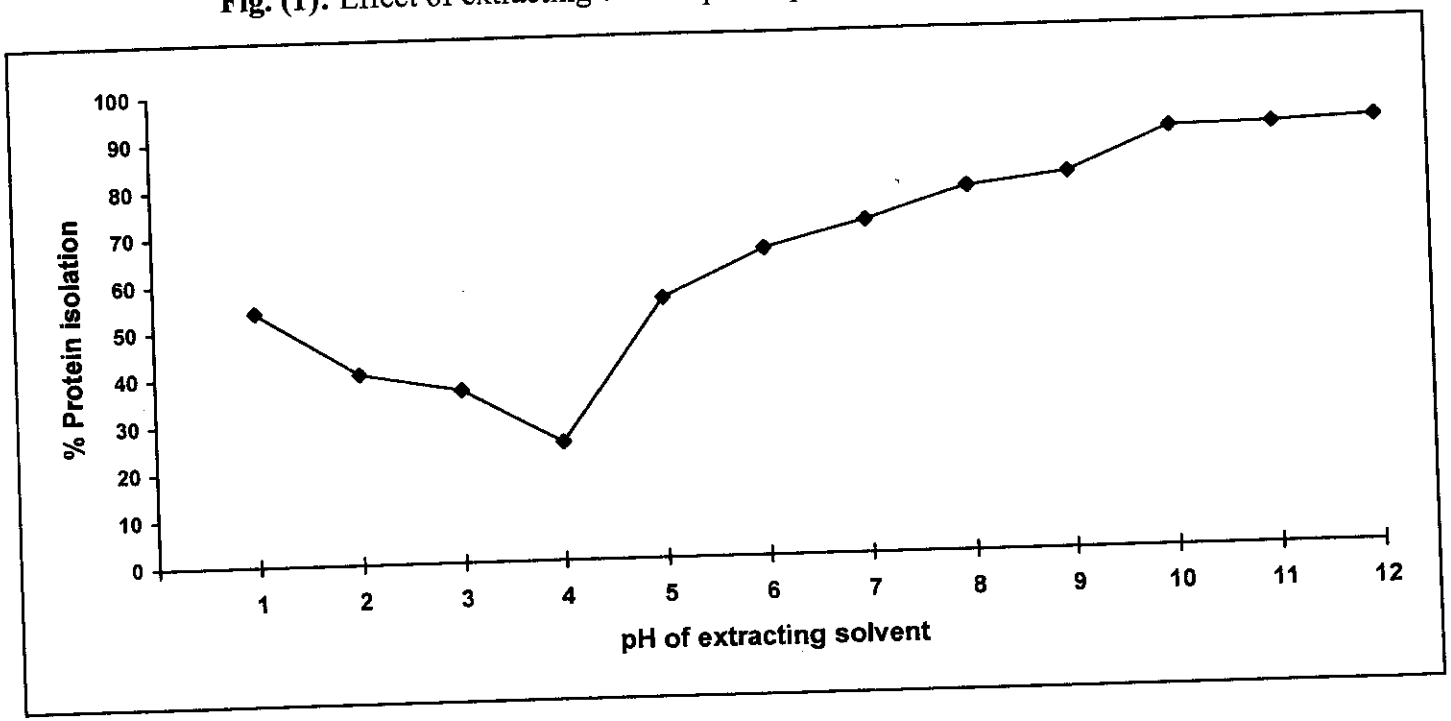


Table (3): Chemical composition of flax meal protein isolate (on dry weight basis).

Sample	Moisture %	Fat %	Protein %	Ash %	Fiber %	Trypsin Inhibitor mg/g	Total Cyanogenic mg/g	Total Phenolic Compounds (mg/100g)	Phytic Acid %
Protein isolate	8.4	0.0	90.1	2.1	0.97	0.0	0.0	23.2	0.24

4.3. Effect of different treatments on the removal of antinutritional factors:

4.3.1. Effect of different treatments on the removal of phytic acid, trypsin inhibitor, cyanogenic glycosides and total phenolic compounds from flax meal:

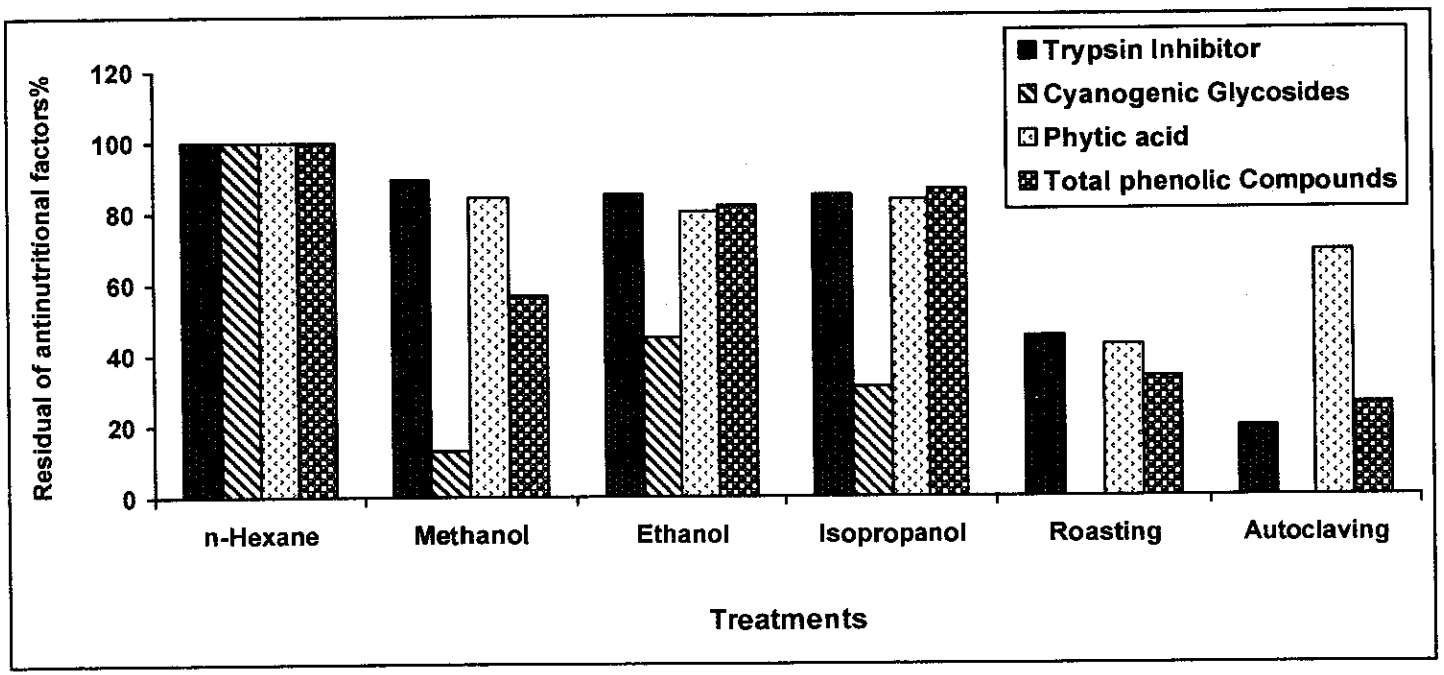
Phytic acid or its salt (phytate) is a cyclic derivative of inositol containing six phosphate radicals. Its physiological significance lies in the facts that it readily chelates di- and tri-valent metal ions as calcium, magnesium, zinc and iron the poorly soluble compounds that are not readily absorbed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

For this reason the presence of phytate in many seeds like flax seed 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 flax seeds. These inhibitors are capable of combining with the enzyme to from 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 disulfide bridges. The other family is the Bowman-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)**.

Fig. (2): Effect of different treatments on the removal of antinutritional factors from flax meal.



Toxicity of cyanogenic glycosides is due to the release of hydrogen cyanide by the action of β-glycosidease, which acts as a potent respiratory inhibitor by complexing with metalloporphyin-containing enzymes.

Polyphenolic compounds tannins are known to decrease protein digestibility either by binding with digestive enzymes such as trypsin and chymotrypsin or binding directly to dietary proteins.

The effect of different treatments on the removal of antinutritional factors (trypsin inhibitor, cyanogenic glycosides, phytic acid and total phenolic compounds of flax meal are shown in **Table (4)** and **Fig. (2)**. The obtained results indicate that roasting and autoclaving of flax meal for 20 min. inactivated trypsin inhibitor to 54.8 and 80.4%, respectively.

The cyanogenic glycosides were totally destroyed after 20 min autoclaving and roasting. As shown in **Fig. (2)** autoclaving seems more effective on the destruction of total phenolic compounds compared with roasting and solvent treatments but less effective on the destruction of phytic acid compared with roasting similar observation was reported by **Vijayakumari *et al.* (1998)**.

4.4. Amino acids of flax meal and flax meal protein isolate:

The results of amino acids content of flax meal and protein isolate are presented in **Table (5)**. The provisional amino acid scored pattern proposed by **FAO/WHO (1973)** qualified an Ideal Protein as one in which 36% of the total residues are essential amino acids.

The flax meal protein isolate had higher essential amino acid contents than proposed 36% for an Ideal Protein. The data in **Table (5)**

indicate that flax meal protein isolate is lower in sulphur containing amino acids, *i.e.,* systine and methionine but, had high quantities of Leu, Phenyl Alanine, Valine and Lysine (6.1, 5.7, 5.7 and 4.1 g/100g protein), respectively. Also, protein isolate contains high level of Glu, Asp and Arg. Since Lysine is the first limiting essential amino acid in most cereal protein **(Tsen *et al.*, 1974).** Consequently addition of flax meal protein isolate to food would certainly be very fruitful in fortifying foods and may be considered as a potential source of high quality plant protein for incorporation into food products **(Madhusudan and Singh, 1983).**

Table (5): Amino acids content of flax meal and flax meal protein isolate (g/100g protein).

Amino acids	Flax meal	Protein isolate
EAA		
Lys	4.1	4.1
Leu	5.7	6.1
Isoleu	4.4	4.6
Cys	1.2	1.4
Met	1.6	1.7
Phe	5.9	5.7
Tyr	3.4	3.3
Thr	3.8	4.0
Val	5.6	5.7
TEAA	35.7	36.6
NEAA		
His	2.6	2.5
Arg	9.4	9.8
Asp	10.5	10.3
Glu	17.2	16.1
Ser	5.2	5.3
Pro	4.4	4.5
Gly	4.5	4.3
Ala	4.3	4.27
TNEAA	58.1	57.07
TAA	93.80	93.67

Table (6): Total amino acid, essential amino acid, non-essential amino acid, E:N, E:T, EAAT of flax meal and protein isolate (g/100g protein).

	TAA	EAA	NEAA	E/N	E/T	EAAI
Flax meal	**93.80**	**35.70**	**58.10**	**61.45**	**38.06**	**76.17**
Protein isolate	**93.67**	**36.60**	**57.07**	**64.66**	**39.27**	**78.73**

E:N = Ratio of essential amino acids to non-essential amino acids

E:T = Ratio of essential amino acids to Total amino acids

EAAI = Essential amino acids index

This based upon ratios of the amounts of essential amino acids in protein relative to their amount in whole egg protein (46.87).

Table (8): The physical and chemical properties of flax seed oil.

Flax seed oil variety	Physical properties		Chemical properties					
	Refractive Index At 25°C	Specific gravity At 25°C	Acid value mg/g	Peroxide value	Saponification value	Iodine Value g/100g	Ester value	Unsaponifiable Matter (%)
Viking	1.473	0.93	1.47	2.135	193.41	187	191.94	0.97

4.6.1. Fatty acids composition of flax oil

The fatty acids composition of flax oil extracted by n-hexane was qualitatively and quantitatively determined by gas liquid chromatography through their methyl esters according to **AOAC (1995).** The obtained results in **Table (9).** The GLC sheets identified the presence of five fatty acids between C_{16} and C_{18}. Two fatty acids are saturated and three unsaturated. The saturated fatty acids were identified as palmetic and stearic acids, while the unsaturated were oleic, lenoleic, lenolenic acids. The saturated fatty acids represented 8.34 and 7.92% with total amount of 16.26%, while the unsaturated fatty acids were 33.77, 14.25 and 35.72% with total amount of 83.74%.

From **Table (9)** it is quite clear that lenolenic acid (the pre- dominant fatty acid) had the highest amount of unsaturated fatty acids (35.72%) followed by oleic acid (33.77%) and lenoleic acid (14.25%). The ratio of saturated to unsaturated was 1 : 5. Therefore, flax seed oil can be classified as dry oil. The obtained results are in agreement with those reported by **Gill (1987).** Also, **Ibrahim (2000)** who reported the same ratio of total saturated (13.86 – 17.11%) and unsaturated (82.89 – 86.14%) fatty acids of linseed oil Giza 6, Giza 7 and Giza 8 varieties.

4.6.2. Unsaponifiable matter composition:

The unsaponifiable matter of vegetable oils consists of substances not related in structure to oils or fats such as saturated hydrocarbons, squalene, sterols, aliphatic alcohols, terpene alcohols and other phenolic compounds. In the present work, the unsaponifiable matter was identified and determined by gas liquid chromatography against authentic compounds. The standard chromatogram used to characterize the hydrocarbons and sterols in flax seed oil sample. **Table (10)** indicate the retention times (RT) and relative retention times (RRT) of standard hydrocarbons and sterols.

Table (9): Fatty acids composition of flax seed oil determined by gas liquid chromatography.

Fatty acids	R.T	R.R.T	%
C_{16} : 0	16.42	0.87	8.34
C_{18} : 0	18.33	0.97	7.92
C_{18} : 1	18.85	1.00	33.77
C_{18} : 2	19.65	1.04	14.25
C_{18} : 3	20.63	1.09	35.72
T.S	--	--	16.26
T.US	--	--	83.74

Table (11) present the composition of unsaponifiable matter of linseed oil under investigation. The standard chromatogram of the unsaponifiable could be divided into two parts. The first part corresponded to the saturated and unsaturated hydrocarbons. The second part comprised the sterol compounds. GLC analysis of the unsaponifiable matter show the presence of 27 peak corresponding to 27 fractions of hydrocarbons and sterols, which varied in their retention time (RT). The relative retention times (RRT) of the different fractions were calculated in relation to β-sitosterol (29.60), which was given a value 1.00. The fractions were identified by comparing their RRT with those of the authentic samples (standard) chromatography under the same conditions. The identified fractions could be deduced from **Table (10)** as follows:

Table (10): Retention times (RT) and relative retention times (RRT) for the authentic hydrocarbons and sterols.

Component	RT	RRT
C_{12}	4.39	0.15
C_{14}	7.96	0.27
C_{15}	9.84	0.33
C_{16}	11.46	0.39
C_{17}	12.22	0.41
C_{18}	14.35	0.48
C_{19}	15.10	0.51
C_{20}	16.47	0.56
C_{21}	18.20	0.61
C_{22}	19.15	0.65
Squalene	24.05	0.81
Cholesterol	26.11	0.88
Stigma sterol	27.48	0.93
β-sitosterol	29.60	1.00

Relative retention time for β-sitosterol was given a value of 1.0

Table (11): Unsaponifiable matter component of linseed oil the relative percentages of the unsaponifiable matter fractions expressed in terms of total peak area.

Peak number	RT	RRT	Identification	Fraction%
1	4.46	0.15	C_{12}	0.93
2	4.81	0.16	Unknown	1.65
3	6.37	0.22	Unknown	1.58
4	7.24	0.25	Unknown	1.36
5	7.99	0.27	C_{14}	1.08
6	9.36	0.34	C_{15}	0.36
7	10.48	0.36	Unknown	1.37
8	11.01	0.38	C_{16}	1.73
9	11.88	0.41	C_{17}	1.65
10	12.43	0.43	Unknown	1.15
11	12.90	0.44	Unknown	1.58
12	13.83	0.47	Unknown	1.66
13	14.61	0.50	C_{18}	6.78
14	15.10	0.52	C_{19}	1.80
15	15.55	0.53	Unknown	1.30
16	16.36	0.56	C_{20}	4.91
17	16.80	0.58	Unknown	7.79
18	17.14	0.59	C_{21}	7.88
19	20.67	0.71	C_{22}	0.79
20	22.47	0.77	Unknown	1.15
21	23.38	0.80	Squalene	3.25
22	24.99	0.86	Unknown	1.80
23	25.66	0.88	Cholesterol	3.54
24	26.72	0.92	Campsterol	3.61
25	27.07	0.93	Stigmasterol	7.86
26	29.19	1.00	β-sitosterol	15.15
27	30.66	1.05	Unknown	16.81

Total hydrocarbons 48.51%

Total sterols 52.02%

4.6.2.1. Hydrocarbon composition:

The hydrocarbons of linseed unsaponifiable matter were fractionated by GLC into twenty different compounds of which ten were characterized, while the others were unknowns. The unknowns might have one or more double bonds (or same other functional groups) that influence their retention times. The identified hydrocarbons were C_{12}, C_{14}, C_{15}, C_{16}, C_{17}, C_{18}, C_{19}, C_{20}, C_{21} and C_{22}.

The hydrocarbons content of the sample was 48.51%

4.6.2.2. Sterol composition:

The spectrum of sterol compounds of flax seed is much simpler than that of hydrocarbons. Only five sterols were identified and two unknowns. The identified sterols, which had relative retention times (RRT) 0.80, 0.88, 0.92, 0.93 and 1.00 can be identified as squalene, cholesterol, campesterol, stigmasterol and β-sitosterol, while the other two compounds of RRT 0.86 and 1.05 were unknowns.

The data indicate that sterol content was 52.02%. β-sitosterol and the unknown compound of RRT 1.00 and 1.05 had the highest ratios (15.15 and 16.81%), while squalene had the lowest amount (3.25%).

β-sitosterol has inhibitory effect on cholesterol absorption as stated by **Montgomry *et al.* (1974)**. Cholesterol synthesizes by plants as well as animals, and it is an important intermediate during the synthesis of steroids **(Luckner, 1972)**.

The ratio of total hydrocarbons to total sterols was 1.00 : 1.07.