

Isolation and Characterization of Protein Isolated from Sesame Seeds (*Sesamum indicum*) Meal.

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Abstract

Sesame protein was isolated from sesame seeds meal with alkaline solvent. The optimum scheme of isolation was obtained using NaOH 0.5 N as extraction solvent; solvent to sample ratio of (1:30) W/V at pH 10; temperature of 45°C and isolation time 60min. Protein isolate contained 92.43% crude protein as the major constituent. The major essential amino acids in sesame seeds meal protein isolate were leucine, phenylalanine, valine and tyrosine, while the predominant non-essential ones were glutamic, arginine, aspartic and glycine. Tryptophan was the first limiting amino acid in sesame seeds meal. Water and oil holding capacities of sesame seeds meal protein were 1.30 gm water/ gm protein and 3.07 gm oil/ gm protein, respectively. The minimum solubility of sesame seeds meal protein was found at pH 4 while, maximum emulsifying activity and emulsion stability values were obtained at pH 10.

Keywords: Sesame seeds meal protein, optimum conditions, protein isolation, functional properties

1. Introduction

Sesame (*Sesamum indicum* L.) is an important oilseed which is cultivated in many tropical countries. In 2009, the world production of sesame seed was 3,976,968 tons and the major production areas were Asia (2,489,518 tons) and Africa (1,316,690 tons), constituting about 62.6 and 33.1% of the total world production (FAO, 2009).

A side from being an important source of edible oil, sesame seed is an essential ingredient for traditional Asian food and desserts. Sesame seed contains 40-50% oil, 20-25% protein, 20-25% carbohydrate and 5-6% ash (Onsaard, 2012).

In the sesame oil industry, sesame seed is commonly used as the raw material for oil extraction, either using organic solvents or by mechanical pressing. The sesame meal is considered a by-product after oil extraction. It is usually fed to animals as a protein source. The meal is good source of nutrition, containing approximately 55.69% (Hassan, 2013).

This meal has high potential for use as a protein source or as an ingredient in the food industry. Sesame protein extracts or concentrates normally are prepared by isoelectric precipitation and salt precipitation (Gandhi and Srivastava, 2007). If a sesame protein ingredient is going to find widespread application as an ingredient in the food industry, it is important to know its functional properties, for example solubility, water holding capacity, fat absorption

capacity and emulsifying properties (Jimoh and Aroyehun, 2011).

2. Objectives/Purpose of the study:

Therefore, the first target of this work is to figure out the optimum conditions for maximum isolation of protein from sesame seeds meal, where the second one is to study the functional properties of this protein.

3. Methodology and sub headings:

3. Materials and Methods

Materials

Sesame seeds meal was used as a source of sesame protein. Throughout this research it was obtained from Abo Eiad company, Almahala El-Kobra city, Egypt. All chemicals used in this study were of HPLC spectral grade and purchased from El-Gomhoria Company for chemicals and drugs, Tanta city, Egypt.

Methods

Preparation of sesame seeds meal:

Sesame seeds meal was prepared by subjecting sesame seeds to oil extraction using mixture of chloroform and methanol (2:1 v/v) for 24 hours, then filtrated, dried and ground up to pass through 100 mesh screen sieve. The powdered samples were kept in polyethylene bags at room temperature until used.

Chemical composition of sesame seeds meal and protein isolate (moisture; crude protein; ether extract; crude fiber and ash) was estimated using the A.O.A.C (2000) methods, where total carbohydrates were calculated by difference. According to the methods described by Chapman and Pratt (1978) magnesium, iron, manganese, zinc and copper were determined using atomic absorption spectrophotometer (Zeiss FMD3), where flame photometer was used for the determination of potassium, sodium and calcium.

Isolation of protein sesame seeds meal:

Protein was isolated from sesame seeds meal using different solvents (distilled water, NaOH 0.5N, NaOH 0.1N, NaCl 1%

and NaCl 0.5 %) at different pH values (from 2 to 13) and different sample to solvent ratios (1:10, 1:20, 1:30 and 1:40) at different temperatures (25, 30, 35, 40, 45 and 50°C) for different periods (30, 45, 60, 75 and 90 minutes). Protein content recovered from each step was determined using the A.O.A.C (2000) methods.

Optimum conditions for maximum production were applied. The recovered protein were participated based on its isoelectric point, centrifuged, dried at low temperature (40°C), then ground up to pass through 100 mesh screen sieve. The powdered samples were kept in polyethylene bags at room temperature until use.

Amino acids determination:

Amino acids of isolated protein were determined in agricultural Res., Center, Cairo, based to the method described by Pellet and Young (1980). Tryptophan was determined calorimetrically in the alkaline hydrolyzate,

4. Result/Findings:

Table (1):Gross chemical composition of sesame seeds meal(%on dry weight basis)

Components Sample	Moisture	Crude protein	Ether extract	Ash	Crude fiber	Total carbohydrates*
Sesame seeds meal	8.79	51.05	1.16	6.05	18.26	41.74

* Total carbohydrates were determined by difference.

Table (2): Minerals content of sesame seeds meal (% on dry weight bases).

Components Sample	Mg	Ca	K	Na	Zn	Mn	Fe	Cu
Sesame seeds meal	2.58	1.91	0.53	0.31	0.03	0.05	0.13	0.00

Table (3): Gross chemical composition of protein isolated from sesame seeds meal(%on dry weight basis).

Component Sample	Moisture	Crude protein	Ether extract	Ash	Total carbohydrates*
Sesame seeds meal	7.79	92.43	0.26	2.80	4.50

*Total carbohydrates were calculated by difference

using P-dimethyl-amino-benaldehyde according to the method of Miller (1967).

Functional properties of protein isolate:

pH-solubility profile was performed using the method of A.O.A.C. (2000), where water and oil holding capacities were conducted following Gandhi and Srivastava (2007) procedures. Emulsifying activity and emulsion stability were carried out based on the methods of Neto *et al.* (2001) using the following equations:

$$\text{Emulsifying activity (\%)} = \frac{\text{Height of emulsified layer in the tube}}{\text{Height of the total contents in the tube}} \times 100$$

$$\text{Emulsifying stability (\%)} = \frac{\text{Height of emulsified layer after heating}}{\text{Height of emulsified layer before heating}} \times 100$$

Table (4). Amino acids composition (gm/100gm protien) of sesame seeds meal protein isolate.

Amino acids	Sesame seeds meal protein	Casein*
Essential amino acids		
Leucine	6.92	9.20
Isoleucine	3.85	5.01
Lysine	2.95	7.51
Cysteine	3.11	-
Valine	4.89	5.42
Methionine	2.95	2.96
Phenylalanine	5.22	9.81
Threonine	3.45	3.43
Tryptophan**	1.03	-
Tyrosine	4.63	-
Total essential amino acids	39,00	43.34
None essential amino acids		
Proline	3.83	5.92
Aspartic acid	8.36	5.97
Serine	3.83	5.59
Glutamic acid	18.49	17.53
Glycine	5.22	1.72
Alanine	5.20	2.65
Arginine	13.16	4.22
Histidine	2.91	2.63
Total non essential amino acids	61.00	46.23

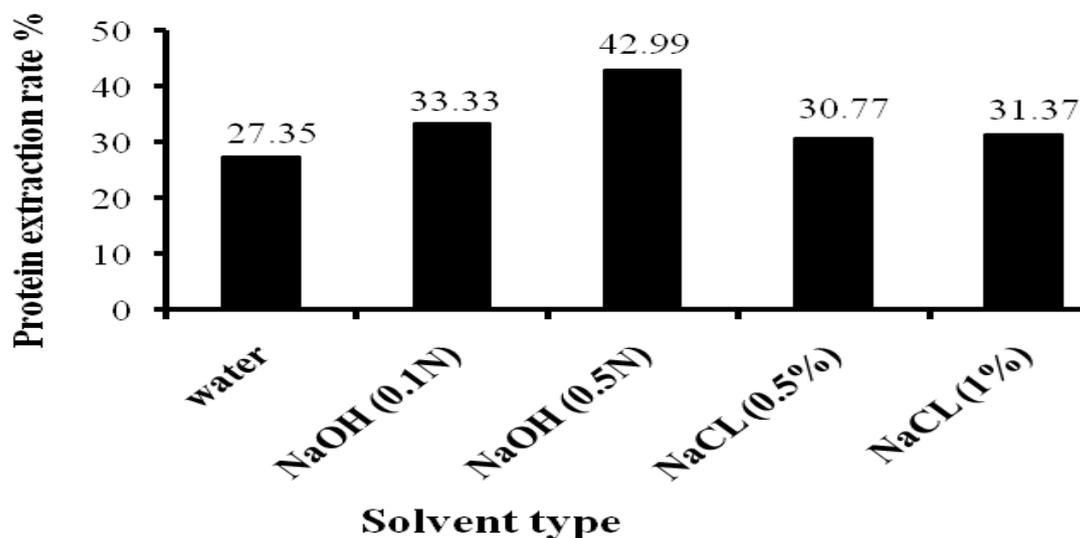
*FAO/WHO 1989

**Tryptophan was determined calorimetrically method according to Miller (1967).

Table (5): Water and oil holding capacities of sesame seeds meal protein

Type of protein	Water holding capacity	oil holding capacity
Sesame meal protein	1.30	3.07
Soy protein*	6.06	2.94

*These values were cited from Onsaard et al. (2010)

**Figure (1):** Effect of using different solvents on protein isolation from sesame seeds meal.

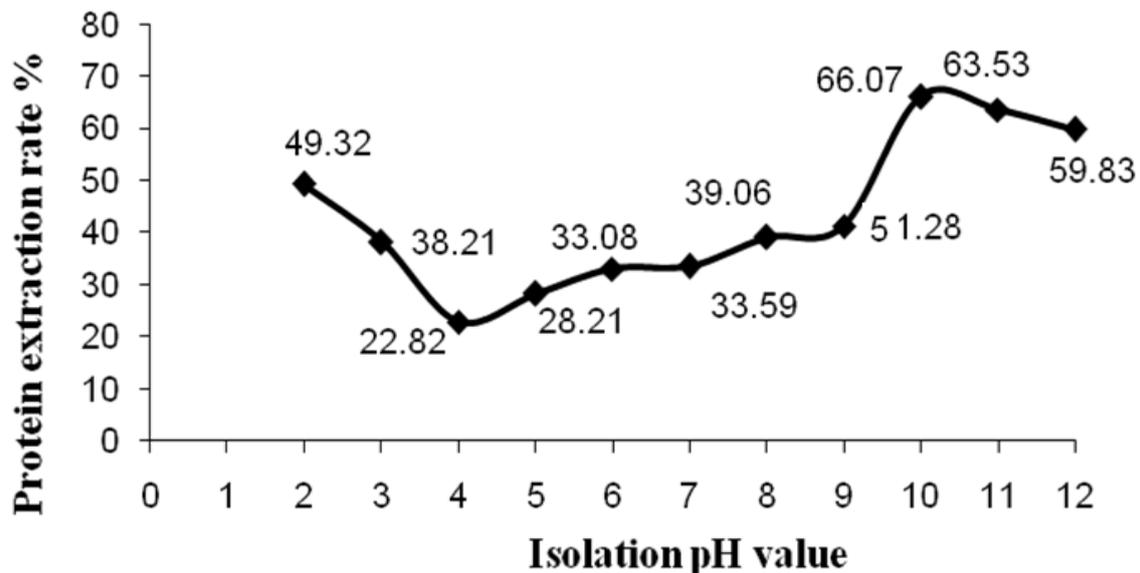


Figure (2): Effect of different pH values on protein isolation rate (%) from sesame seeds meal.

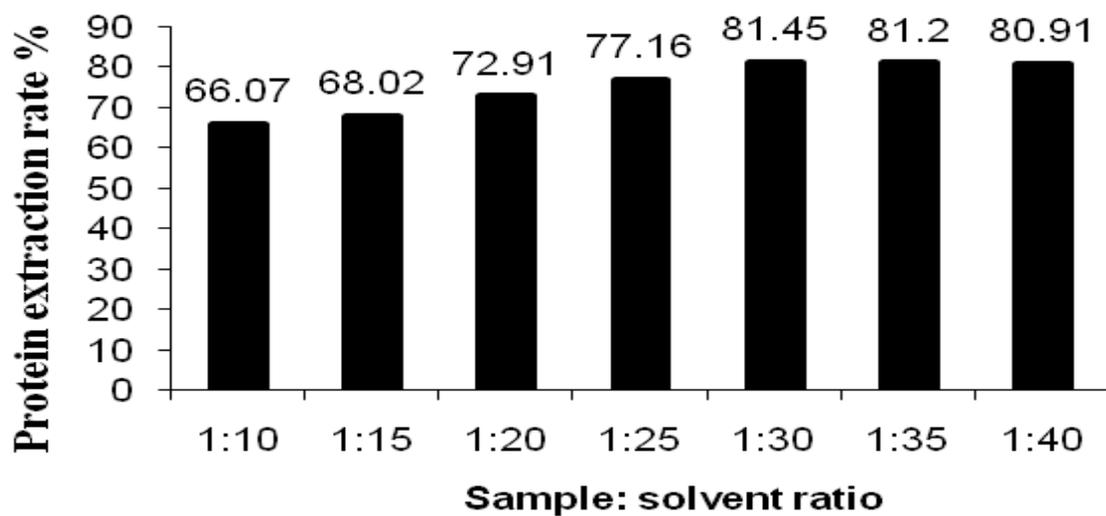


Figure (3): Effect of using different sample: solvent ratios on isolation rate % of protein from sesame seeds meal.

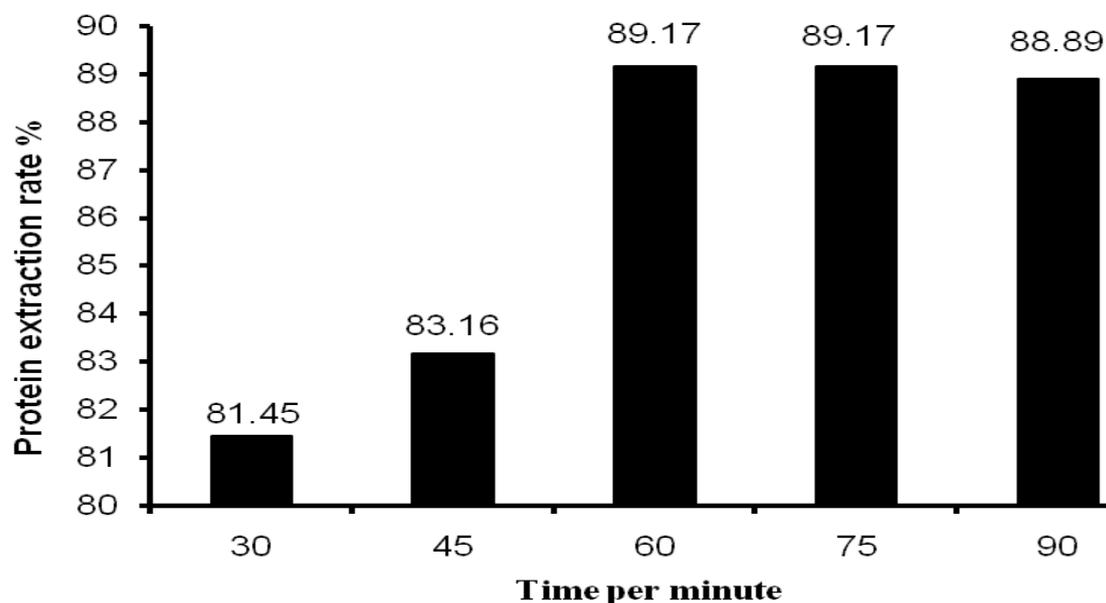


Figure (4): Effect of using different isolation periods (min) on protein extraction rate % from sesame seeds meal.

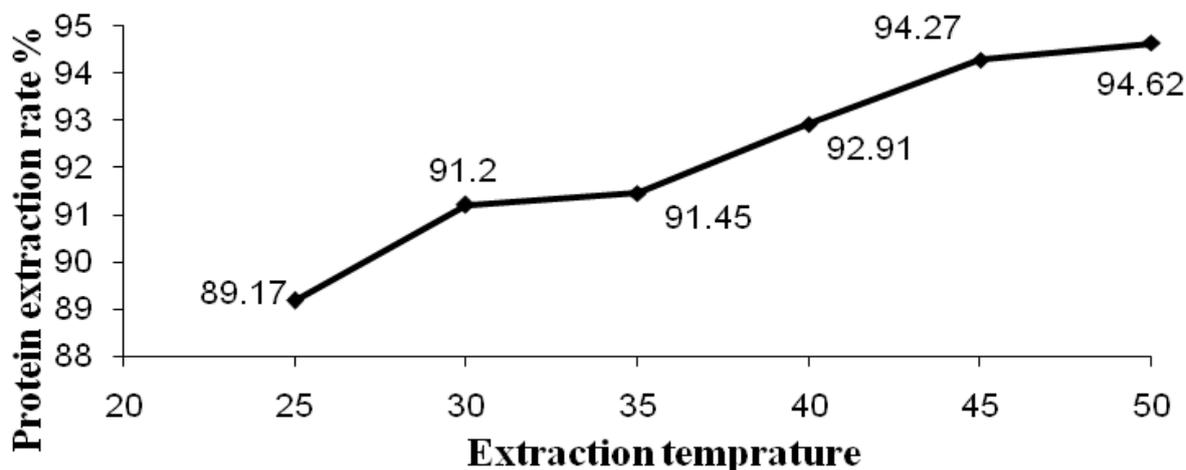


Figure (5): Effect of using different temperatures on protein extraction rate % from sesame seeds meal.

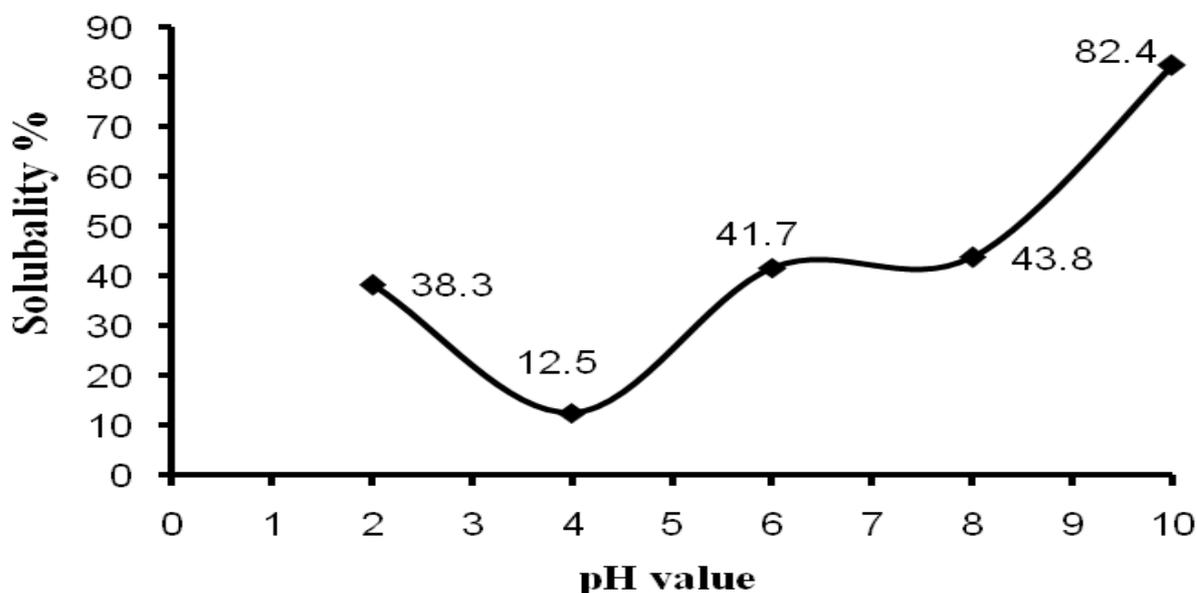


Figure (6): Effect of pH value on protein solubility

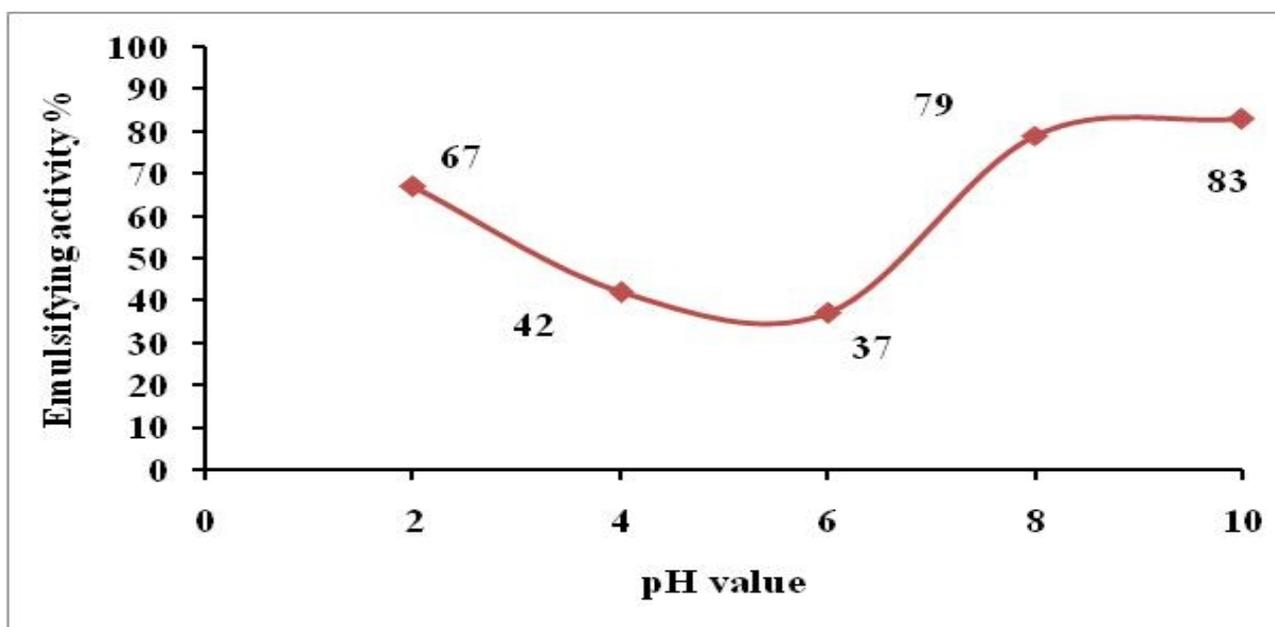


Figure (7): Effect of pH value on sesame seeds meal protein emulsifying activity

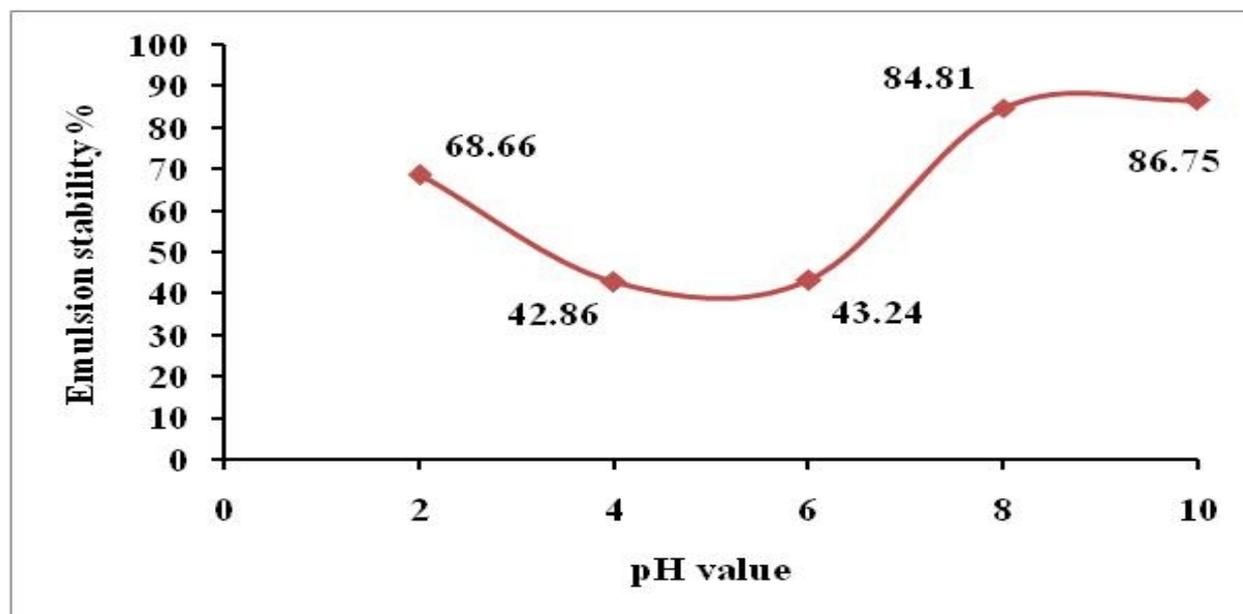


Figure (8): Effect of pH value on sesame seeds meal protein emulsion stability

5. Discussion:

Gross chemical composition of sesame seeds meal:

The obtained data are presented in table (1). It could be noticed from the results that, the moisture, crude protein, ether extract, ash, crude fiber, and total carbohydrates contents were 8.79, 51.05, 1.16, 6.05, 18.26, and 41.74%, respectively. These results are in accordance with those reported by (Gandhi and Taimini, 2009; Onsaard *et al.*, 2010; Jimoh and Aroyehun, 2011 and Hassan, 2013).

Minerals composition of sesame seeds meal:

Results of minerals composition of sesame seeds meal are summarized in table (2). The data indicate that, magnesium, calcium, potassium and sodium were the major elements in the investigated sample where their values were 2.58, 1.91, 0.53 and 0.31%, respectively. On the other hand, zinc, manganese and iron are found in fewer values in the same sample as shown in the same table.

Moreover, data in table (2) indicated that, copper is not predominant in the sample. These results are mostly in harmony with those of (Gandhi and Srivastava, 2007; Adegunwa *et al.*, 2012 and Hassan, 2013) who studied mineral composition of sesame seeds meal. It was found that, the most predominant elements were magnesium, calcium, potassium and sodium, but the minor ones were zinc, manganese, iron and copper.

The optimum conditions for maximum protein isolation:

Effect of using different solvents on the protein isolation from sesame seeds meal:

Figure (1) shows the effect of using different solvents (distilled water, NaOH 0.1N, NaOH 0.5N, NaCl 0.5% and NaCl 1%) in isolation of protein from sesame seeds (*Sesamum indicum*) meal at 25°C for 30 minutes and the meal to solvent ratio was 1:10. It could be observed that, the highest amount of isolated protein was obtained with NaOH 0.5N as a solvent. This may be due to the effect of high pH value of the

solvent, (about 9.5). The solubility of protein increased with increasing pH value above 7.5 (Attia *et al.*, 2000). It was mentioned by (Schut, 1976) that, suggested NaOH causes a shift in the isoelectric point to move away of the acidic PH as a result of specific ion binding effects. Since inorganic anions are bound to protein more strongly than inorganic cations due to their smaller hydrated, anions are able to attain a closer proximity to the protein molecule and are able to screen the charged groups of the protein more effectively than cations.

Effect of pH on the isolation of protein:

Figure (2) shows the protein extracted from sesame seeds meal by 0.5N NaOH at different pH values (2-12), 25°C for 30 min using 1:10 of sample to solvent ratio.

The results illustrated in Figure (2) indicate that, the extraction rate of protein from sesame seeds meal at pH 2 was 49.32. Increasing pH to 4, decreased the extraction rate. This may be due to the minimum protein solubility occurred between pH3.5 and 4.5, the apparent isoelectric pH region for both sources (Esmat, 1991). Damodaran (1997) states that, the minimum solubility occurs at about the isoelectric point (pI) of proteins, and the majority of food proteins are acidic proteins, thus exhibit minimum solubility at pH 4–5, and maximum solubility at alkaline pH. According to Sorgentini and Wagner (2002), the occurrence of minimum solubility near the isoelectric point is due primarily to both the net charge of peptides, which increase as pH moves away from the isoelectric point, and surface hydrophobicity that promotes the aggregation and precipitation via hydrophobic interactions.

Thereafter, the rate increased again until reaching to 66.07% at pH 10. These increments in protein extraction rate may be attributed to the increases in protein solubility at pH values on each side away of the apparent isoelectric pH region (Chavan *et al.*, 2001). At pH higher than 10, the amount of isolated protein was slightly decreased.

This means that, the optimum pH value for maximum isolation of protein from sesame seeds meal was pH 10. In

conclusion, the results in Figure (2) could be also pointed out that, the pH value of the isolation media has a great effect on the protein extracted from sesame seeds meal. Attia *et al.* (2000) reported that, the solubility of protein increased by increasing pH above 7.5.

Effect of using different sample to solvent ratios on the isolation of protein:

Isolation process was carried out at 25°C for 30 min and pH 10 for sesame seeds meal using different sample to solvent ratios (1:10, 1:15, 1:20, 1:25, 1:30, 1:35 and 1:40 w/v) where, 0.5N of sodium hydroxide was used as a solvent.

The results illustrated in Figure (3) indicate that, sample: solvent ratio (w/v) 1:10 was too low to be used. This may be due to the swelling of the investigated samples by solvent adsorption, extracting solution concentration became dense, causing increase of the solution viscosity which hampered molecular diffusion. The extraction speed decreased accordingly. Although the extracting concentrate was dense, the obtained amount of protein was low, hence, extraction was the lowest (Di *et al.*, 2006).

Isolation rate increased with increasing sample to solvent ratio. Upon reaching ratios ranged from (1:30) to (1:40), the extraction rate did not significantly differ. As mentioned by Feng *et al.* (2004) this may be due to the adverse effect of high solvent to sample ratio which could cause low protein content of the extraction and interfered with further chemical modification such as alkali degradation.

From such results, it could be noticed that, the highest amount of isolated protein was obtained at 1:30 of sample: solvent ratio. Where at this ratio, the percent of the recovered protein was 81.45. Similar results were found by Khedr and Mohamed (2004).

Effect of using different periods on the isolation of protein: In this experiment, the isolation process was carried out using 0.5N NaOH as a solvent in a ratio of 1:30 (w/v) at 25°C for different periods (30, 45, 60, 75 and 90 min) at pH10 and the results are illustrated in Figure (4).

The results indicated that, the amount of isolated protein increased gradually with extending the isolation time from 30 to 60min. Then, constant rate was recorded thereafter. This means that, the optimum period for maximum protein isolation was 60 min. the previous results may be explained as follow: solvent had enough time to penetrate the cells and extract protein at suitable pH. Similar results were obtained elsewhere (Liadakis *et al.*, 1995 and Perez, 2003) who reported that, prolonging the extraction time after reaching the optimum time for protein extraction did not influence the protein extractability.

Effect of using different temperatures on the isolation of protein:

The isolation process was carried out using 0.5N NaOH as a solvent using different temperature (25, 30, 35, 40, 45, 50°C) for 60 min at pH10 and sample: solvent ratio of 1:30. The results were illustrated in Figure (5). The results revealed that, isolation rate of protein increased with increasing temperature. The increment of protein extraction rate with

increasing temperature may be due to fast moving of molecules which develop increased mass transfer rate of interface between sample and solvent. Therefore, elevating temperature could promote mass transfer and solubility, reduce viscosity of solution and thus increase the extraction rate (Li *et al.*, 2005).

However, higher temperature than 50°C could cause a reduction of protein activity and thermal denaturation of protein. Additionally, with the elevating of temperature, colour of the extracted protein became dark. These results are in agreement with these reported by Onsaard *et al.* (2010), who mentioned that, temperature of isolation process has a little effect on the amount of protein isolation.

Gross chemical composition of protein isolate:

Table (3) show that, the protein content of the protein extracted from sesame seeds meal was 92.43%. It had a low percentages of ether extract 0.26%, ash 2.80% and 4.50 carbohydrates, respectively. Similar results were found by (Orliac *et al.*, 2002; Khalil *et al.*, 2008 and Horax *et al.*, 2011) who isolated protein from sunflower seed, legumes and cottonseeds. It was found that, crude protein content of protein extracted from these sources ranged between 77.49 to 92.50 %. In addition, all protein isolate contained a low percentage of ether extract, ash and total carbohydrates.

Amino acid composition of protein isolate:

Table (4) shows the amino acid composition of sesame seeds meal protein isolate. It could be noticed that, sesame seeds meal protein contain almost all types of amino acids. It can be also found that, in sesame seeds meal protein, glutamic and arginine were the most predominant amino acids. The major essential amino acids in sesame seeds meal protein were leucine, phenylalanine, valine and tyrosine, while the most predominant none essential amino acids were glutamic, arginine, aspartic and glycine. Tryptophan was the least abundant amino acid in sesame seeds meal. These results were in the same line with Olagunju *et al.* (2013) who stated that, defatted sesame seed flours contained an appreciable amount of amino acids.

Functional properties of sesame seeds meal protein isolate:

pH-solubility profile:

Solubility is one of the most important characteristics of proteins because it is not only important by itself, but also influences other functional properties. Good solubility of proteins is required in many functional applications, especially for emulsions, foams and gels, because soluble proteins provide a homogenous dispensability of the molecules in colloidal systems and enhance the interfacial properties (Zayas, 1979). The pH-solubility curve of sesame seeds meal protein is presented in Figure (6).

The solubility profile of sesame seeds meal protein indicated that, protein solubility reduced as the pH increased from 2 to 4, which corresponding to its isoelectric point, after which subsequent increases in pH increased protein solubility progressively. The minimum solubility for native sesame seeds meal protein (12.50%) was at pH 4 which corresponds

to its isoelectric point. The highest protein solubility (82.40%) was observed at pH 10. These results were in the same line with Kanu *et al.* (2007) who reported that, sesame seeds meal protein isolate had a minimum solubility in the pH region between 4.5 to 5, the same findings was recorded in case of commercial soy protein.

Water and oil holding capacities:

Water holding capacity is likely due to the fact that protein has a great ability to swell, dissociate and unfold exposing additional binding sites (Perez, 2003). Meanwhile, several authors have related the oil absorption capacity to interaction of non polar side chain of the protein as well as to the conformation features of the proteins (Horax *et al.*, 2011). The data presented in Table (5) shows that, water and oil holding capacities of sesame seeds meal protein were 1.30 gm water/ gm protein and 3.07 gm oil/ gm protein. These results were in agreement with those of Onsaard *et al.* (2010) and Zhao *et al.* (2012) who found that, water and oil holding capacities of sesame seeds meal protein were ranged from 1.29 to 3.30 gm water/ gm protein and from 1.19 to 3.08 gm oil/ gm protein.

Emulsifying activity:

Figure (7) shows that, maximum emulsifying activity of sesame seeds meal protein (83%) was obtained at pH 10 of the protein solution. Emulsifying activity decreased with increasing pH until reaching the minimum value (37%) at pH 6. The same table also shows that, increasing pH more than 6 helped to increase emulsifying activity. These results were in agreement with Zhao *et al.* (2012) who found that, the maximum emulsifying activity of sesame seeds meal protein was obtained at pH 10 and its minimum value was at pH 6

Emulsion stability:

Figure (8) shows that, minimum emulsion stability of native sesame seeds meal protein was recorded at pH 4 (42.86%), followed by subsequent increase in emulsion stability as the pH increased. The maximum emulsion stability of sesame seeds meal protein (86.75%) was obtained at pH10. These results were in agreement with those of Zhao *et al.* (2012) who found that, the maximum emulsifying stability of sesame seeds meal protein was obtained at pH 10.

This research has revealed that, sesame seeds meal seem to be a potential source of proteins, to supplement the dietary requirements of humans. The optimum extracting parameters of solvent, solvent to sample ratio, temperature, pH value and extraction period were 0.5 N NaOH, 1:30, 45 , 10 and 60 minutes, respectively. Under these optimum conditions, the protein extraction rate was above 94%. Protein extract solubility, emulsifying activity and emulsion stability are greatly affected by pH. Results given in this work showed that, sesame protein may be potential ingredients for future feed supplements.

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