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Separation, Diagnosis and Evaluation of Functional Properties of Peanut and Soybean Proteins

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ABSTRACT

Peanuts and soybeans contain all essential amino acids and a high amount of protein and minerals which provide nutritious for normal health, so the purpose of our research was to study the chemical composition of soybeans and peanuts, by separating proteins from there, and diagnose these proteins by the electro-migration technique, soybeans protein ccontain 7 bundles, while 11 protein bundles were shown in peanuts. Some functional properties of the separated proteins which including the solubility were studied by using three solutions (distilled water, 6 M sodium chloride, sodium phosphate buffer solution pH 4). The highest solubility 73% for peanuts proteins was obtained by using calcium chloride and 52% for soybeans proteins. Whereas, the gelation characteristic was studied at concentrations of 6, 8, 10 % for proteins and two concentrations of calcium chloride 20, 30 mM at temperatures of 80, 100 °C. The concentration of 10% of protein and calcium chloride 30 mM at 100 °C gave the best results.

Keywords: Separation, Peanut Proteins, Soybean Proteins, Solubility, Gelation, electrophoresis.

INTRODUCTION

The meat, fish , chicken, dairy and other animal products are It contains proteins in abundance. Animal sources contain all the essential amino acids which provide the proteins that the human body needs. Regardless of meat the beans, and peanuts also contained enough amounts protein (Schaafsma, 2000). The body must take adequate amount of amino acids to complement natural and active growth, and out of twenty of the eleven amino acids are unnecessary and constantly synthesized in the body through various reactions. While the other nine amino acids cannot be created in our body, therefore it is required to have them by the diet. Until now, we knew that proteins that include every the essential amino acids in required proportions are complete proteins, such casein, egg white proteins. Peanuts are another vital source of protein (Settaluri et al., 2012). Peanuts contain all amino acids that the body needs for normal growth and metabolism (Hoffmann

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and Falvo, 2004), a lack of amino acids in the diet lead to different disease, for example, lack of Trypto-Van can lead to pellagra (skin disorder very severe, and sometimes fatal (Seal, 2007).

Peanut is a legume crop that belongs to the Fabaceae family, Arachis genus,. Peanuts are consumed in many forms, such as peanut butter, roasted peanuts, boiled peanuts, peanut oil, energy bars, and desserts (Settaluri, 2012). Peanuts are a spirited source nutrient that play a main function in the growth and energy gain living things, contain many nutrients such as antioxidants, minerals and vitamins that are necessary the pumping of vital nutrients into the human body to maintain normal health (Settaluri et al, 2012). Peanut is called poor man protein because it is available at an affordable price, its main oilseeds and it contains calcium, phosphorus, iron, zinc, boron and they are a good source of protein (Kalpana et al, 2013). Although the importance of peanut proteins, some people are allergic to some of its proteins, such as Aar h1 and Aar h3 (Yusnawan et al, 2012). Soybeans (Glycine max) were distinguished for their protein content (35- 42%) and a high_fat (16 - 27%), which makes soybeans one of the most valuable and popular crops (Kumar et al, 2006). Soybeans are known as oilseeds because of their high oil content (19%) with many beneficial nutrients, including proteins (36%), carbohydrates (35%) (17% of which are dietary fiber), minerals (5%) (Liu, 1997). Soybean protein is a good alternative to animal protein, it contains the same sulfuric amino acids (methionine and cysteine) as animal proteins and most of the essential amino acids needed for animal and human nutrition (Sacks et al, 2006 ; Hajos, 1996). Because of high levels of protein in peanuts and soybeans which an added nutritional value, this study aimed to separate their proteins by electrophoresis technique and to study some of their functional properties.

MATERIALS AND METHODS

Samples preparation

Samples of peanuts and soybeans were evacuated from the markets of Basrah-Iraq. they were cleaned and milled to obtain a powder. Hexane adding to them at a rate of (15 ml/g) at 20 °C for a whole night. The defatted meals were collected by centrifugation 10,000 g, 10 min at 4 °C, dried in air, and stored at -20 °C until use (Senakoon et al, 2012). The chemical composition of powder was being performed according to AOAC (2010).

Isolated Proteins from Peanuts and Soybeans

The methods was conducted by (Speroni, 2010) taking 1gm of the defatted samples and adding 10 ml of distilled water at a ratio of (10:1) w/v, pH is adjusted to 8.5 for peanuts and to 8 for soybeans by 1 M NaOH and left for 90 min with stirring. The centrifugation was done at a speed of 3200 cyc/min for a period of 30 min at a temperature of 4°C, and pH was regulate to 4.5 by 1M HCl.

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Centrifugation was carried out at 3200 cyc/min for a period of 20 min at 4°C and the proteins were collected. process of lyophilization carried out and the proteins were stored in sealed packages.

electrophoresis

According to (Jiang *et al*, 2014) with some modifications, electrophoresis was being carried out by using acrylamide gel with nitrous-acrylamide (15% separation gel and 4% stacking gel), electrode circulating solution was prepared from (4% SDS, 20% glycerol, and 0.125M Tris-HCl buffer solution) at pH 6.8 plus bromophenol blue stain 0.02%), 80 µl of protein samples (2 mg/ml) were taken and mixed with 20 µl of 10% SDS and 2 µl of 2-comptonethanol with 1 µl of bromophenol blue stain 1% (w/v). The samples were placed at a temperature of 100°C for a period of 5 min, they were separated by centrifugation of 15,000 cyc/min for 10 min at room temperature. After the migration was completed, the gel was placed in a 0.05% Coomassie brilliant blue dye solution (dissolved in 15% methanol and 5% acetic acid and left for 24 hr. The gel extracted and put in the washing solution (methanol 30% and 10% acetic acid) and limitation the protein bundles, calculate the molecular weight through a marker (standard molecular weights of 14.4 - 100 kDa), and calculating the relative motion (Rm) value.

Rm = Traveled distance by the protein\ Traveled distance by the stain (Laemmli, 1970)

Solubility

The solubility of proteins was estimated according to (Mirhosseini and Amid, 2013) by mixing 1 g of samples with 25 ml (distilled water, 6 M sodium chloride, sodium phosphate buffer solution pH 4) on a magnetic stirrer at laboratory temperature, and gradually raised the temperature to 80 °C for 30 min, after the solution is centrifuged at a speed of 6000 cyc/min for a period of 30 min to get rid of the insoluble substance, the sample transferred to a petri dish of known weight, leaving it to dry at 105°C until the weight is stable. The percentage of solubility was estimated using the following equation:

Solubility% = C1 / C2 × 100

Whereas ; C1 = weight of the concentrated filtrate ; C2 = weight of the crude

Gelation

The strength of the gel for peanut and soybean proteins was measured the method described by (Kuhn *et al*, 2010) with simple modifications. 6, 8, 10%, and two concentrations of calcium chloride 20, 30 mM of peanut and soybean proteins were used. Placed by a water bath during 30 min in temperatures of 80,100 °C, it was fast cooled into 10°C using an ice bath. salts were removed through dialysis bags for 48 hr at 10 °C.

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STATISTICAL ANALYSIS

Completely random design (CRD) was used with 3 replicates for 0.05 significance with L.S.D test to determine substantial differences between the means according to the SPSS program.

RESULTS AND DISCUSSION

Chemical composition

shows Figure (1) the chemical composition raw materials used in the study. Statistical analysis of the results showed a statistically significant variations ($p<0.05$) between the results of the peanut and soybean compositions. Soybeans moisture is 3.92%, protein 41.78%, fat 26.12%, ash 5.12%, and carbohydrates 23.06%, while peanuts contain 6.08%, 32%, 51.87%, 2.56%, 7.49% for moisture, protein, fat, ash and carbohydrates, respectively. The results show a higher percentage of protein and carbohydrates of soybeans compared to peanuts, on the other hand, the percentage of moisture, fat, and ash was higher for peanuts compared to soybeans. These results were in agreement with (Özcan and Seven, 2003) who mentioned that the moisture content of peanuts was 6.07%, protein 36.93%, fat 44.09%, and ash was 2.05%. Our results were also closed to (Kumar *et al*, 2006) who studied the chemical composition of different types of soybeans, and found the protein content ranged between 35 - 42%, while the fat percentage was between 17-27%. Among the main factors that affect the quality of the seeds were their low moisture content, a low percentage of impurities, ability to break, discoloration, heat damage (internal cracks), fungi and insects damage, high density, the ratio of protein and oils, as well as the biological effectiveness of seeds, environmental conditions, the harvest season, drying methods, storage techniques, transportation, species, and variety (Sinha and Muir, 1973).

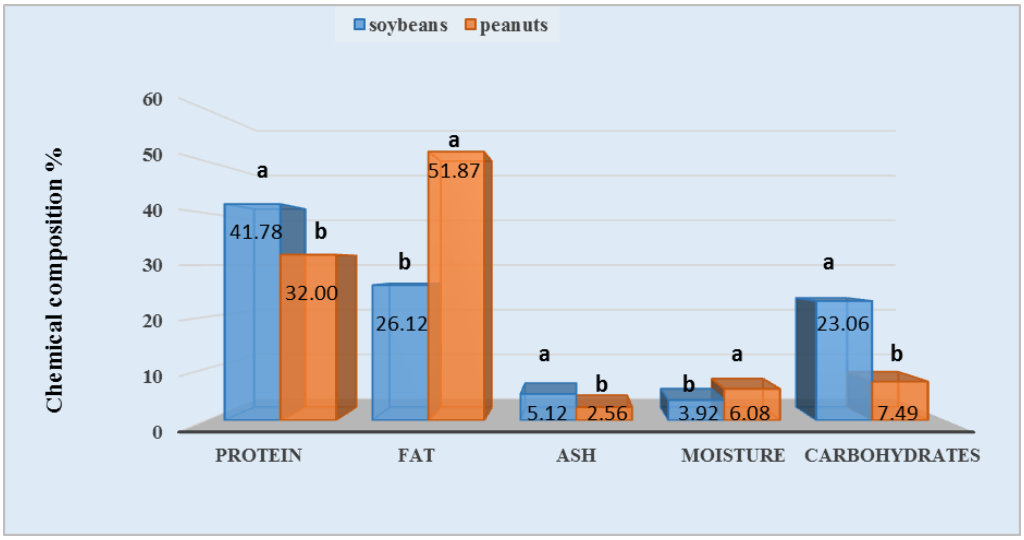

Figure (1) chemical composition of soybeans and peanuts.

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Gel electrophoresis (SDS-PAGE)

Figure (2) shows the results of the electrophoresis of peanut and soybean proteins, soybeans protein has seven bundles, with the partial weights ranged between 18.6-86.2 (kDa) while eleven bundles appeared in peanut proteins with the partial weights ranged between 14.6-68.1 ((kDa), their molecular weights were calculated by the standard curve in fig. (3).

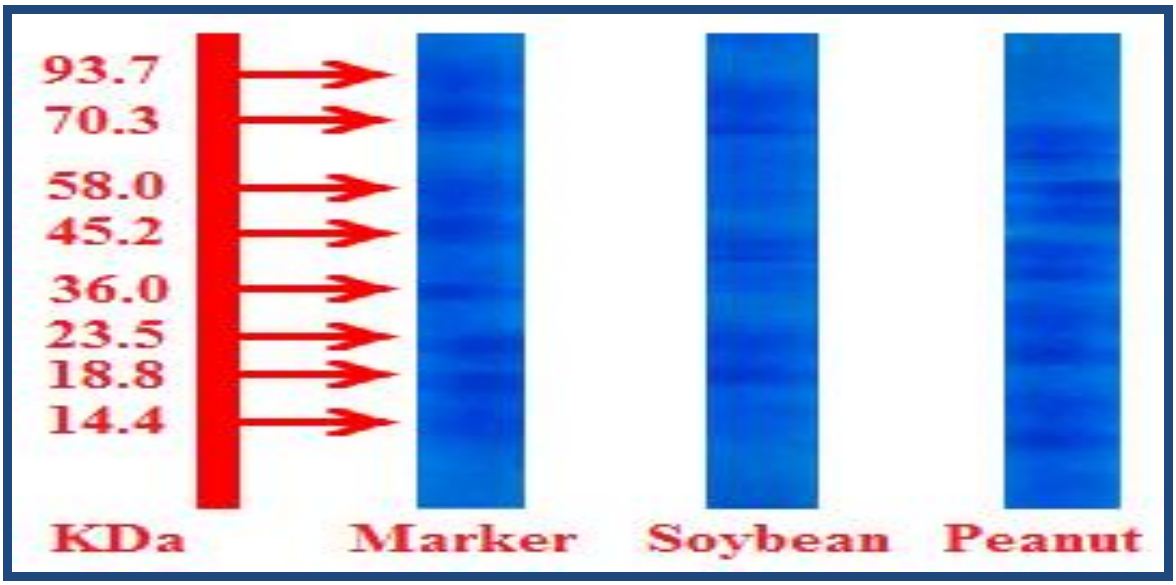

Figure 2: Electrophoresis of proteins

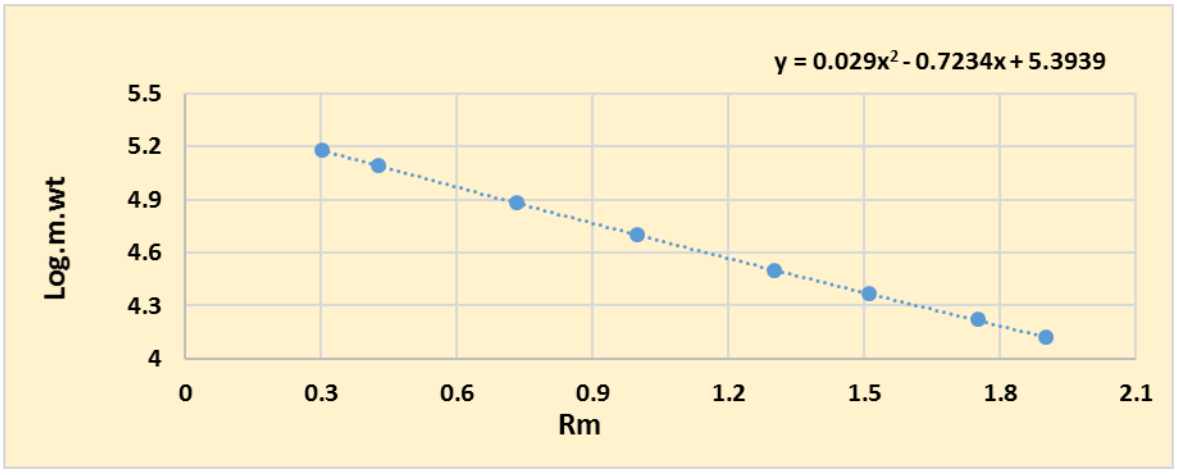

Figure (3) standard curve of marker

These results were in accordance with (Astuti *et al*, 2018) who found 7 bundles in soybeans and 12 bundles in peanuts, whose molecular weights ranged between 20-83 kDa.

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Table (1) molecular weights (kDa) of the separated soybean and peanut protein bundles

Bundles	Peanut proteins	Soybean proteins
1	**68.1**	**86.2**
2	**60.5**	**69.4**
3	**43.8**	**66.5**
4	**35.7**	**52.9**
5	**27.4**	**41.7**
6	**21.9**	**38.3**
7	**19.8**	**18.6**
8	**17.9**	
9	**16.02**	
10	**15.3**	
11	**14.6**	

Solubility

Figure (4) shows the solubility of peanut and soybean proteins. Three solvents were used for these properties water, calcium chloride and sodium phosphate buffer solution). Statistical analysis of the results showed a statistically significant variations ($p<0.05$) between the three different solvents with solubility of peanut and soybean proteins. The highest value of peanut and soybean proteins solubility was 73% and 52% using calcium chloride solution, while the less solubility was 28% and 23% at buffer solution for peanut and soybean proteins respectively. As protein solubility is one of more substantial properties due to its ability to influence on another properties, It was observed that the minimum solubility of protein was at buffer solution and the upper limit of solubility was at calcium chloride solution, with observed of changing in protein color, and there was a similarity between the solubility pattern of soybean proteins and peanut proteins.

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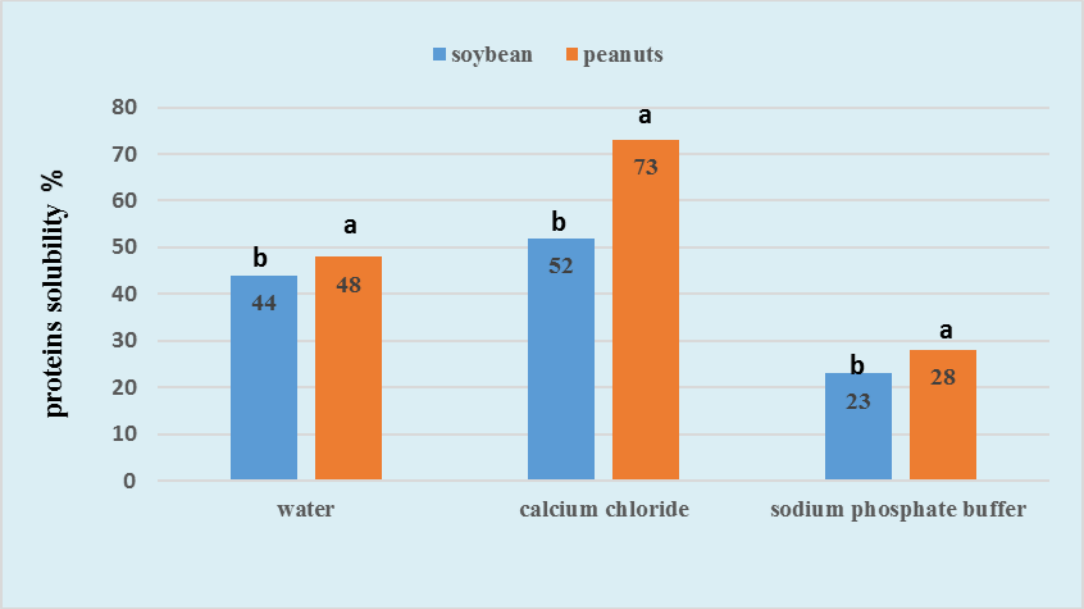

Figure (4) solubility of soybean and peanut proteins

These results were similar to Shafiqur *et al*, (2018) who found that the least solubility of soybeans and peanuts was at pH 3.5 – 4, and the upper limit of solubility was at pH 10. In the lower pH the proton the amino and carboxyl groups in the amino acid chains in forms of NH3 and COOH so the total charges positive in most protein molecules, when pH was increases, some of the groups of carboxyl are separated into COO^- and H^+ and the positive charges decreases associated with proteins up to neutral points (Cherry and McWatters, 1975). Proteins cannot be hydrated due to changes in the third and quaternary composition of proteins, So, the solubility reaches the minimum value, But when pH increasing, the amino groups dismantle into NH2 and H^+ and the total protein charges becomes negative outcome the COO^- groups presence, So can be dissolved and hydrated. the pH increase is improves electrostatic repulsion, and this increase of the proteins solubility (Shafiqur *et al*, 2018).

Gelation

The results in Table (2) and Figure (5) show the gel strength of soybean and peanut proteins by using different concentrations of 6%,8%, 10% proteins and two concentrations of calcium chloride 20 and 30 mM with different temperatures of 80 and 100 °C.

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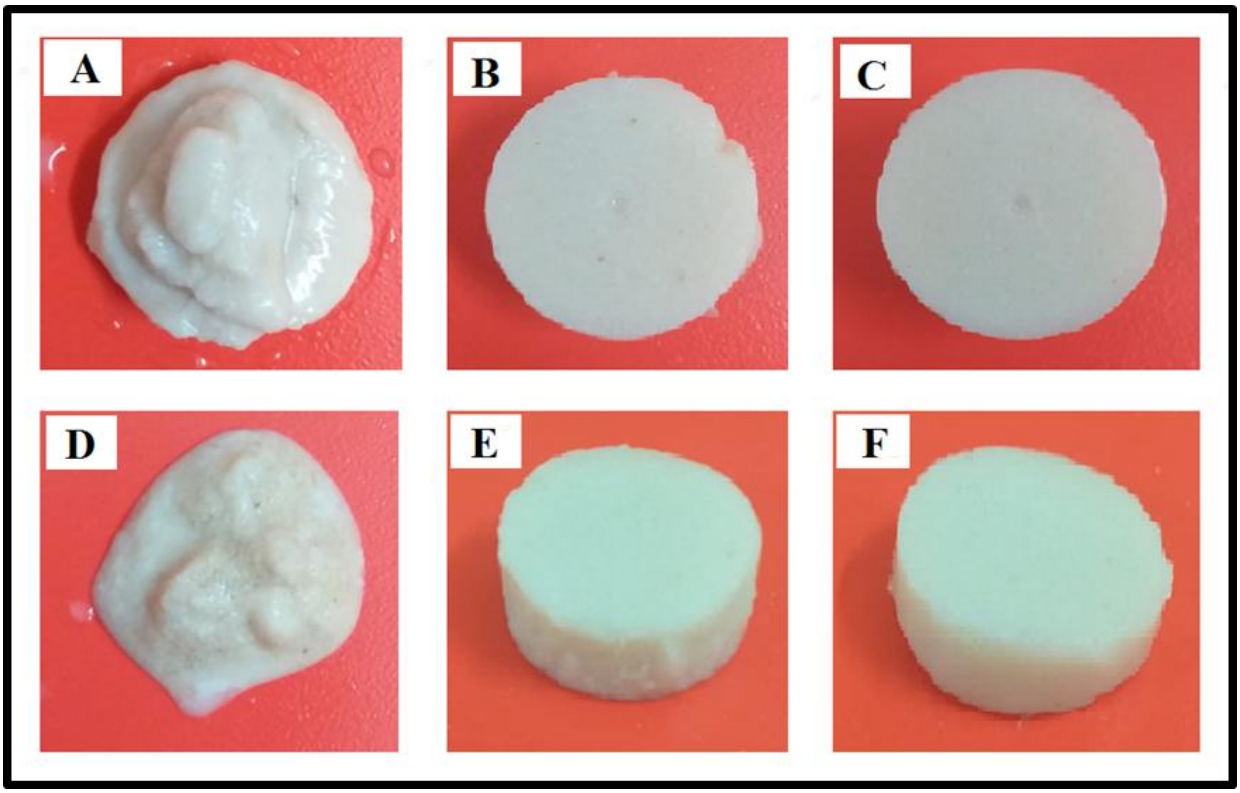

Figure (5) gel images: (A) peanut proteins 6%; (B) peanut proteins 8%; (C) peanut proteins 10%; (D) soybean proteins 6%; (E) soybean proteins 8%; (F) soybean proteins 10% with calcium chloride 30 mM at 100 °C.

The results showed the presence of gel in all the concentrations used, as the strength of the gel increased with increasing concentration. Also, the gel became stronger by increasing the concentration of calcium chloride, as the gel was more powerful when using calcium chloride 30 mM than the concentration of 20 mM, with an effect on the color and texture. There is also a noticeable effect of temperature on gel strength, the gel becomes greater strong by using 100°C compared with 80 °C**.**

Table (2) gelation of soybean and peanut proteins

Concentration of proteins			**NaCl concentration**	**Temperature °C**	**Samples**
%10	%8	%6			
+ + +	+	+	20	80	**Soybean proteins**
+ + +	+	+	30	80	
+ + +	+	+	20	100	

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+ + +	++	+	30	100	
+ + +	+	+	20	80	**Peanut proteins**
+ + +	+	+	30	80	
+ + +	+	+	20	100	
+ + +	++	+	30	100	

+ Weak gel ;++ Good gel ; + + + Very good gel

These results were similar to (Xin *et al*, 2010) who found that there was a significant effect of temperature (80-120 °C) and calcium chloride (20 - 40 m M) on gel strength, it is assumed that this effect mostly arises due to increased exposure to the functional gropes present in protein synthesis, leading to improved interactions between protein molecules, and the addition of calcium. This effect is likely due to the formation of increased calcium through hydrophobic reactions, or increased salt bridge formation (Xingfeietal,2019).

CONCLUSION

The peanut and soybean proteins were isolated by electrophoresis. Both proteins contain a different bundles groups. Using calcium chloride solution gave highest value solubility of peanut and soybean proteins, while buffer solution gave the less solubility.The gelation test gave the highest which was increased by increasing protein , sodium chloride concentration and temperature.

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