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Study the Optimal Conditions for the Production of Protein Isolates from Chia Seeds

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Abstract
The current study aimed to produce a protein concentrate and isolate from chia seeds, and studying the chemical composition of them, in addition to determining the optimum conditions (pH of extraction, extraction temperature, extraction time, and mixing ratio) for the production of protein isolates from chia seeds. The alkaline extraction method was used to obtain the protein isolate and then sedimentation at the isoelectric point (pH = 4.5). The results of the chemical composition of the whole, defatted, concentrated, and protein isolate of the chia seeds indicated that the protein isolate of chia seeds was significantly superior in terms of protein content, where the percentage of protein reached 83.7%, while the percentage of moisture, fat, fiber, ash and carbohydrates were significantly decreased compared to the whole and defatted seeds and the protein concentrate of chia seeds. The results of the research also indicated that the best pH for protein extraction was 12, as it gave an extraction percentage of 78.77%, while the best extraction temperature was 60 ° C, with an extraction percentage of 73.8%.In addition, the extraction time of 60 minutes gave the highest protein extraction percentage (78.60%), and the mixing ratio of 30: 1 (w / v) was significantly superior to give the best extraction percentage for chia seed protein isolate (79.56) %.
Keywords: Chia seeds, the protein isolate of chia seeds, the protein concentrate of chia seed, optimal conditions for the protein isolate of chia.

INTRODUCTION
Chia is an annual herbaceous plant that belongs to the Lamiaceae family, where it is spread in southern Mexico and northern Guatemala, and in recent years, chia has been cultivated for commercial purposes in many countries such as Argentina, Colombia, Ecuador, Peru, Australia and some other countries (Busilacchi, al et, 2013). There are about 88 species of these plants spread in the Turkish Anatolian plateau, and this region is the main source of the Asian species for these plants (Ixtaina et al., 2008). Chia plants are planted twice a year and are classified under the mint family (Labiatae) (Cahill et al., 2002), (Bresson., 2009). Chia seeds are considered a pseudocereal due to their high oil content. They are also oilseeds widespread in some countries, including Central America, and are considered the largest genetic diversity in the Pacific slope from central Mexico to northern Guatemala (Cahill, 2004). Chia seeds are small, about a few millimeters long and have an oval shape, and few are oblate in shape with a width to thickness of about (1 mm) (Diaz & Aguilera., 2012). The color of the seed is either black, gray, or black dotted to white, or there is a slight difference in shape where the white seeds are larger, thicker, and wider compared to the black seeds but

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they are equal in nutritional value, as the protein content in black chia seeds is about 16.9%, while the white seeds are 16.5. As for its fiber content, it is about 32.6% and 32.4%, respectively (Knez Hrnčič et al., 2018). Chia seeds are a traditional food in Central and South America and are currently consumed on a large scale due to their benefits and the oil extracted from them. They have recently been described as an important food substance that promotes human health due to the biologically active ingredients they contain, especially in maintaining a healthy level of fats, where the presence of phenolic acid contributes to this effect, and Omega-3 and Omega-6 oils, found in chia seeds (Vuksan et al., 2007); (Vuksan et al., 2010).

Proteins from plant sources are available, cheap and safer than animal sources, which cause concern among many consumers. Therefore, it has become necessary to pay attention to plant proteins, especially in many countries that suffer from economic and nutritional problems, especially the problem of protein deficiency and increased consumption in those countries, so the need has become urgent to extract and develop of protein isolates from some grains by unconventional methods (Rangel et al., 2004) (Laskar & Mariod., 2010);). In the past few years, interest in searching for plants with high protein sources has increased and due to consumer concerns about the health safety of animal products and their derivatives, in addition to that, they can be considered a better and cheaper alternative to expensive animal products , as plant proteins have become a major source of dietary protein in many developing countries, and with the increased interest in protein consumption and the problem of protein deficiency, it has become necessary to search for non-traditional sources for protein isolation (Mariod et al., 2010). Horax et al.(, 2011) explained that there are ways to isolate and concentrate protein, including the chemical method of alkaline extraction, as it is considered one of the common and simple methods to obtain relatively pure protein, and this extraction is usually carried out under alkaline conditions and at a pH between 8 to 10, then protein precipitation at the isoelectric point of a protein by lowering the pH, followed by a centrifugation process to increase protein isolation, or the use of the enzymatic method, where the process of preparing protein isolates is carried out by adding proteolytic enzymes or by self-degradation by means of self-enzymes present in tissues (Adler and Nissen, 1982). Since chia seeds have high protein content, the present study aimed to produce protein isolates from chia and studying the optimal conditions for its production.

MATERIALS AND METHODS

Samples preparation

Chia seeds were obtained from the local markets of Baghdad, and after cleaning the samples from all impurities, dirt and other remnants of the seeds, they were ground using an electric grinder to obtain the grain powder, then packed in sealed polyethylene bags and then preserved until use.

Preparing defatted chia seed powder

The defatted chia seed powder was prepared according to Karim and Shakir, (2016) as the chia seeds were ground using a laboratory mill and the oil was removed using a cold method by mixing the ground chia seeds with hexane in a ratio of 20: 1 (seed: solvent) and the process was repeated again to ensure that the largest possible amount of fat was removed,

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then the drying process of the defatted seeds was carried out at a temperature of 50 ° C for a period of 24 hours, then grinded, sifted, and then kept at a temperature of -18 ° C until use.

Preparation of Chia seed protein concentrate

According to Karim and Shakir, (2016) chia seed protein concentrate was prepared from defatted chia seed powder, as the chia seed powder was mixed with ethanol prepared at a concentration of 70% with a mixing ratio of 10: 1 (seeds: ethanol) with stirring for two hours at room temperature (30 ± 2) ° C, then collecting the sediment and dissolving with a quantity of distilled water, then centrifuging at a speed of 10000 xg, after which grinding and keeping at a temperature of -18 ^{0}C until use.

Determining the optimum conditions for the production of protein isolate for chia seeds

Determine the Best Extraction Solution

Dissolve 4 g of chia seed protein concentrate in 40 ml of distilled water and adjust the pH to (8, 10, and 12) using NaOH solution (1M) with stirring for 45 minutes at room temperature (30 ± 2) C ^{0}. The centrifugation process was carried out at a speed of 10000 x g for 20 minutes, the filtrate was collected, and its pH was adjusted to 4.5 using a (1M) HCl solution. The centrifugation process was performed again at a speed of 10000 x g for 20 minutes, then the filtrate was collected, dried, weighed and the percentage of nitrogen in it was estimated using the micro kjeldahl method and 5.70 was used as a protein factor.

Determine the Best Extraction Time

The best time to extract the chia seed protein was determined by weighting 3 g of chia seed protein concentrate and mixed with distilled water in a ratio of 1: 30 (w / v) with stirring, then the pH was adjusted to 12 using NaOH (0.1 M) solution with Stirring for a period of (15, 30, 60) minutes, then the centrifugation process was carried out at a speed of 10000 xg for a period of 20 minutes, and the rest of the steps were completed according to what was mentioned in the method of protein extraction referred to in paragraph (2-2-1).

Determine the Best Temperature for Extraction

Determine the best temperature for chia seed protein extraction by mixing(3 g) of chia seed protein concentrate with distilled water in a ratio of 1: 30 (w / v) with stirring, then adjust the pH to 12 using NaOH (0.1 M) solution with stirring for 60 minutes at different temperatures (30,45,60 ° C), then a centrifugation process was conducted at a speed of 10000 xg for a period of 20 minutes, and the rest of the steps of the protein extraction method referred to in paragraph (2-2-1) were completed.

Determine the Best Mixing Ratio

The best mixing ratio was determined by weighting (3 g) of chia seed protein concentrate and mixing it with distilled water and using mixing ratios that ranged between (10: 1), (20: 1) and (30: 1) v / w, followed by the process of adjusting the pH to 12 using a solution of 0.1M NaOH, the centrifugation process was carried out at a speed of 10,000 xg for a period of 20 minutes, and the rest of the steps were completed to extract the protein referred to in paragraph (2-2-1).

Prepare a Chia Protein Isolate

The chia seeds were collected after cleaning and grinding by a laboratory mill, and the fat was removed by treating the seeds with hexane after a 24-hour soaking at a ratio of 1: 10 w / v, and the protein concentrate was prepared for the defatted seeds by treatment with ethanol

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1: 10 w / v with stirring for two hours, then the chia seed concentrate was mixed with water in a ratio of 1: 30 w / v, then adjust the pH to 12 using a solution of NaOH (0.1M), stirring at a temperature of 60 ° C for 60 minutes, then centrifuging at a speed of 10,000 xg for a period of 20 minutes and at a temperature of 10 ° C. The filtrate was collected and the pH was adjusted to 4.5 using a solution of HCl (1M) for the purpose of sedimentation, after which it was centrifuged at a speed of 10,000 xg for 20 minutes at a temperature of 10 ° C, and the sediment was adjusted and the pH was adjusted to 7 and dried and then stored until use (López & Galante., 2018).

Chemical estimates of whole, defatted, concentrated, and protein isolates of chia seeds:

Determination of moisture content:

The moisture content of whole seeds, defatted, concentrated, and isolate proteins were estimated for the studied samples according to (A.O.A.C., 2008).

Protein Percentage Estimation:

The protein percentage of whole and defatted, concentrated and protein isolate seeds was estimated for the studied samples using the micro kjeldahl method, as mentioned in (A.O.A.C, 2008).

Ash Percentage Estimation:

Ash percentage was estimated for whole seeds, defatted, concentrated, and protein isolate seeds for the studied samples as stated in (A.O.A.C., 2008).

Fat Percentage Estimation:

Fat percentage of whole and defatted, concentrated and protein isolate seeds was estimated using the soxhlet method, as mentioned in (A.O.A.C., 2008).

Determination of the Percentage of Fibers:

The percentage of fiber was estimated for whole and defatted, concentrated and protein isolates seeds according to (A.O.A.C., 2008).

Estimating the Total Carbohydrate Percentage:

The percentage of carbohydrates was estimated for whole, defatted, concentrated, and protein isolates for the studied samples, according to the difference, according to (A.O.A.C., 2008).

2-5: Statistical Analysis

A statistical analysis of the obtained results was performed using Complete Random Design (CRD) (SAS., 2004) . Analysis of variance (ANOVA) with Duncan's multiple range tests at a significance level of $P < 0.05$ was used to compare treatment means.

RESULTS AND DISCUSSION

Chemical Composition of Whole, Defatted, Concentrated and Protein Isolates of Chia Seed

Table (3-1) shows the results of the chemical composition of whole, defatted, concentrated, and protein isolate of chia seeds, where it is noticed from the table that the protein content in whole chia seeds reached 22.2%, and this percentage is close to what Rafaela et al. (2014) and Maira, et al., (2014), where they indicated that the percentage of protein in white and black chia seed was 25.32% and 23.11%, respectively, while Noshi,(2018) was found in white and black chia seeds, the protein content was 16.57% and 18.37%, respectively. The results of the table also indicated that a significant increase in the percentage of crude protein

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in the defatted, concentrated and protein isolate seeds which reached (38.7, 54.8 and 83.9) %, respectively, after it was 22.2% in the whole seeds, because the removal of fat by treatment with hexane for a period of time (5 hours) and treating the concentrate with ethanol, which also caused the removal of part of the fats, complex materials and phenols (Karim & Shakir, 2016). , Saka et al. (2012) found that the percentage of protein in the defatted, concentrated, and protein isolate for the powder of confort seed (36.4% , 45.6% 80.50)%, respectively, the results of the statistical analysis indicated that there were significant differences in the protein ratios between the whole seeds, the defatted, the concentrate and the protein isolate of chia seeds.

As for the moisture content, it reached 19.90% in whole seeds. This result was higher than what Ixtaina et al., (2008) and Munoz et al., (2012) who found that the moisture content of white seeds was 7.2%, while the moisture content in black seeds was 6.6. %.As for the moisture content of the defatted seeds, the concentrate and the protein isolate, it reached (13.61, 8.12, 2.73)%, respectively, and Naji (2016), Chatterjee et al (2016), Sharma et al., (2015) found that the percentage of moisture in the protein isolate that they obtained from sesame seeds amounted to (7.50,7.20,6.86)%, respectively. The results of the statistical analysis indicated that there were significant differences in the moisture contents between the whole seeds and the defatted, concentrated, and protein isolate of the chia seeds.

The results of the table indicate that the percentage of fat in whole chia seeds was 33.1%, and this percentage came close to what was mentioned by Rafaela, et al.,(2014), where they indicated that the percentage of fat in chia seeds is 30.22%, It is similar to what was mentioned by FSAI., (2016), as it reached 30.1%, and the result also converged with what was found by lxtaina, et al.(, 2011) when they indicated that the percentage of fat in white chia seeds reached 30%, while (Noshi., 2018) indicated that the percentage of The fat in white chia seeds was 35.6%, while in black seeds it was 34.6%. The results of the table also indicated that there was a significant decrease in the percentage of fat in the defatted, concentrate and protein isolate, which recorded (8.2, 4.7, 1.6) %, respectively. The results of the statistical analysis also indicated that there were significant differences in the fat percentages between the whole seeds, the defatted, the concentrate and the protein isolate of the chia seeds, and it was found by Abdelatief et al ., (2010) that the percentage of fat in the concentrate of cowpea was 0.01%.

As for the percentage of fibers shown in Table (4-1), it was in whole, defatted, concentrated and protein isolate chia seeds (18.6, 19.89, 10.59, 4.4) %, respectively, and this result is less than what was found by FSAI,(2016), when it was 28.8. It is also less than what was mentioned by Maira, et al.,(2014), as it was 34.46% in whole white and black chia seeds. The reason for this difference in the percentage may be due to the difference in the type of grains depending on their variety, degree of maturity, harvest timing, agricultural environment and storage conditions (Dhingra et al. ., 2012). Onsaardet al.,(2010) indicated that the percentage of fiber in the concentrate of sesame seed reached (46.3)%, while Sharma et al. (2016) indicated that the percentage of fiber in the sesame seed isolate was (0.03%). While Chatterjee et al., (2015) indicated that the percentage of fiber was (2.5) % in the sesame seed protein isolate. . The results of the statistical analysis indicated that there were significant differences in the percentage of fiber between whole seeds, defatted, concentrated,

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and protein isolate of chia seeds.

The ash content in Table (4-1) indicates that the percentage of ash in whole chia seeds reached 4.7%, and this result is consistent with the range mentioned by Maira, et al., (2014) as it reached 4-5%, while it was less than what he found by Noshi (2018) on white and black chia seeds, where the ash content was 1.68% and 1.45%, respectively, as for the ash percentage in the defatted, concentrated, and protein isolate seeds, it reached (5.8, 4.8, and 3.5)% respectively, and it is noticed that there are differences between the percentages obtained by the researchers, and this may be due to the difference in the varieties used and the method and conditions of extraction. The results of the statistical analysis indicated that there was no significant difference in ash percentages between the whole seeds and the protein concentrate, while there were significant differences between the whole seeds with the defatted and the protein isolate of the chia seeds.

The results of Table (4-1) showed the percentages of carbohydrates in whole, defatted, concentrated and protein isolate of chia seeds, where the percentage of carbohydrates in whole seeds was (10.5) %. The results of the table also indicated that there were no significant differences between the whole and defatted seeds and the protein concentrate, reaching (13.80 and 16.99)% for each of the defatted seeds and the protein concentrate, respectively, while it was noticed that there was a significant decrease in the percentage of carbohydrates to the protein isolate, where the percentage decreased to 4.07%, and this decrease is due to the increase in protein concentration as a result of the precipitation process. Noshi (2017) indicated that the percentage of carbohydrates in whole white and black chia seeds was 35.8% and 33.38%, respectively, and FSAI (2016) stated that the percentage of carbohydrates in whole chia seeds was 31.4%, the differences in the results obtained compared to what others have found can be attributed to the difference in the variety of seeds, where they are grown, and their environmental conditions. Sharma et al. (2016) found that the percentage of carbohydrates in the protein isolate of sesame seeds was 0.08%. The results of the statistical analysis indicated that there were significant differences in the ratios of carbohydrates between the whole, defatted, concentrated and protein isolate of the chia seeds.

Table (3-1) the Chemical Composition of Whole, Defatted, Concentrated, and Protein Isolate of Chia Seeds

Samples	**Protein %**	**Moisture %**	**Fat %**	**Fiber %**	**Ash %**	**Carbohydrates %**
Whole Seed	22.2a	19.90d	24.1d	18.6c	4.7b	**10.5b**
Defatted	38.7b	13.61C	8.2c	19.89d	5.8c	**13.8b**
Concentrate	54.8c	8.12b	4.7b	10.59b	4.8b	**16.99b**
Isolate	83.7d	2.73a	1.6a	4.4a	3.5a	**4.07a**

- The different letters in one column indicate the presence of significant differences

Determine the Optimum Conditions for Preparing a Protein Isolate

The best pH of the Extraction Solution

The results of Fig (3-1) showed the efficiency of protein isolate extraction of chia seeds at pH

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numbers (8, 10.12), whereas the extraction efficiency of the isolate protein for chia seeds (67.11, 72.60, 78.77) % respectively. It was noticed that the best pH for preparing chia seeds protein isolate was 12, as it gave an extraction rate of 78.77% at a mixing ratio of (1:20) (w/ v) and then extraction at a pH of 4.5. The reason is that the resultant charge on the protein surface at the isoelectric point is minimal, which leads to protein aggregation and deposition through hydrophobic bonds, and the charge net increase in the acid and base media, thus increasing the repulsion between protein molecules, which leads to its solubility, and the reason is that the net charge on the surface of the protein, at pH 4.5 it is the lowest possible, so the protein tends to clump and settle at the bottom of the solution Noshi (2018). As the results of the statistical analysis indicated that there were significant differences in the efficiency ratios of extraction with respect to the pH numbers used in preparing the protein isolate for chia seeds. Naji (2016) found that pH 10 was the best pH in the preparation of sesame seed protein isolate, as its protein extraction efficiency reached 67.23%. Onsaard et al. (2010) reported that the best pH of protein isolate preparation from sesame was 11, and the protein extraction efficiency was 35.5% when mixed with water at a ratio of 1: 10 (w / v). Also, Gerde et al, (2013) concluded that pH 11 is the best in preparing a defatted and non-defatted Nannochloropsis microalgae protein isolate with an extraction efficiency of (56.9, 40.50) %, respectively.

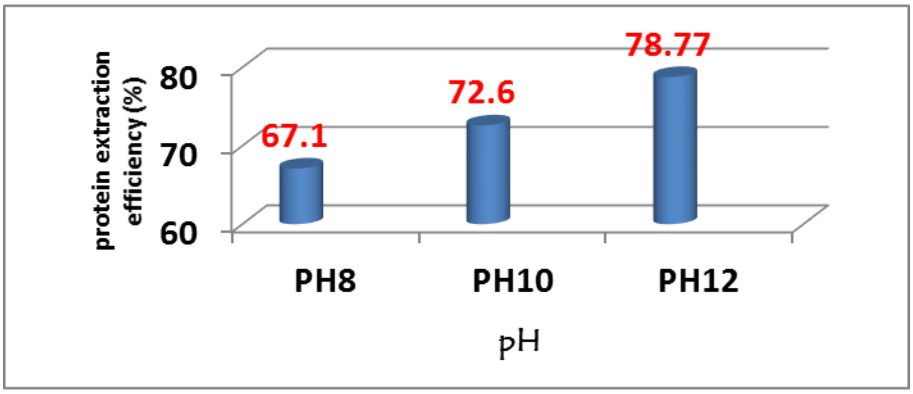

Figure (3-1) the effect of pH on the extraction efficiency of chia seed protein isolate

The Best Time to Extract the Protein Isolate

Figure (3-2) shows the effect of the extraction time on the extraction efficiency of the protein isolate for chia seeds, as three extraction periods were tested (15,30,60) minutes, where the percentage of extraction efficiency of the protein isolate reached 48.06, 50.23, 78.60%, respectively. Where the study showed that the best time for extraction was 60 minutes as it gave the best protein extraction rate (78.6%) and that the reason for this may be due to the fact that 60 minutes is sufficient to break down cells and dissolve the largest possible amount of protein. The results of the statistical analysis also indicated that a time of 60 minutes gave the best extraction rate and it differs significantly from 15 and 30 minutes. In a study conducted on sesame seeds, it was found that the best time to extract the protein isolate for sesame seeds is 60 minutes, as the extraction efficiency reached (78.6%), as indicated by Naji.,(2016) and Essa et al., (2015) that Increasing the extraction time beyond 60 minutes did not lead to an increase in the efficiency of the extraction of sesame seed protein isolate.

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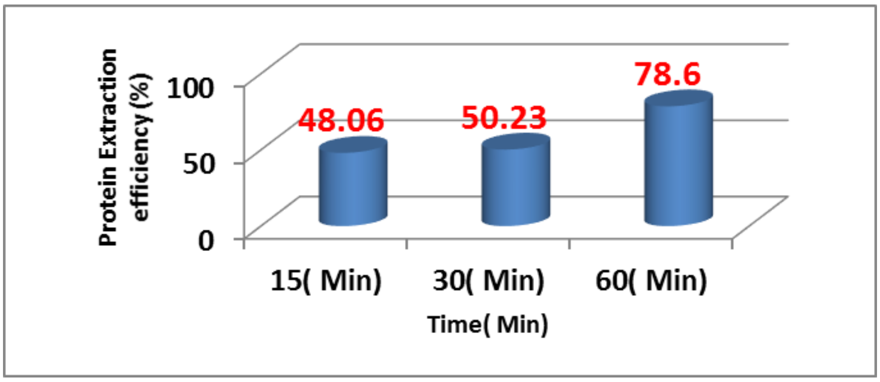

Figure (3-2) the effect of extraction time on the extraction efficiency of chia seed protein isolate

The Best Temperature for Protein Isolates Extraction

Figure (3-3) shows the effect of different temperatures (30, 45, 60) ° C on the efficiency of extracting the protein isolate from chia seeds, as the efficiency of extracting the protein isolate from chia seeds reached (62.13, 65.43 ,73.80)%, respectively, where the temperature of 60 ° C was significantly higher, by giving the best extraction rate (73.8%) and it was significantly different from 45 ° C and 30 ° C. The reason for the high efficiency of protein extraction may be due to the decrease in the viscosity of the solution and the increase in the kinetic energy of the particles with the increase in the temperature, which leads to the chances of the mass transfer between the solution and the sample, which in turn leads to an increase in the solubility of the protein (Naji 2016). Essa et al (2015) found that the best extraction temperature for sesame seed protein was 50 ° C, where an extraction efficiency was given to the protein isolate of 94.64% while the best extraction temperature was (45 ° C) for the same seeds and with an extraction efficiency of the protein reached 90.87%. (Naji 2016).

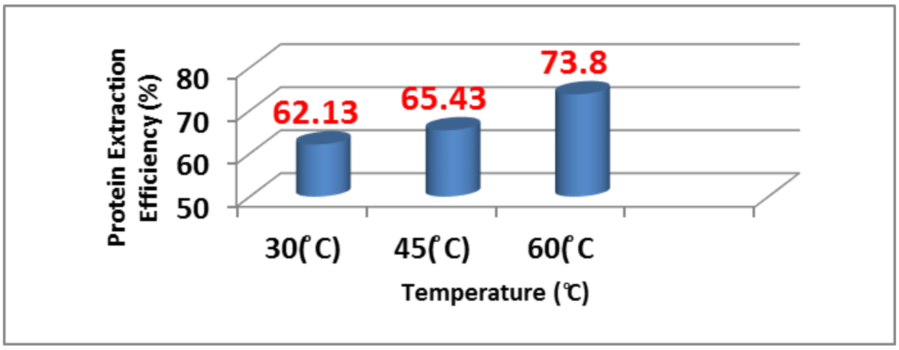

Figure (3-3) the effect of the extraction temperature on the extraction efficiency of the protein isolate of chia seeds

The Best Mixing Ratio for Chia Seed Protein Isolate

Figure (3-4) shows the effect of mixing ratios (1: 10, 1,20: 1,30) w/ v on the efficiency of extracting protein isolates from chia seeds, as the efficiency of extracting chia protein isolates at the above mixing ratios reached (48.10, 57.23, 79.56)% respectively, where it was found that the best mixing ratio was (1: 30) w / v, which gave an extraction efficiency that reached

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79.56 Where the mixing ratio (1: 30) w / v was significantly higher than the remaining mixing ratios. Essa et al., (2015) and Mohamed and Khedr (2004,) indicated that the best mixing ratios were 1: 30 w / v to obtain a high protein isolate from sesame.

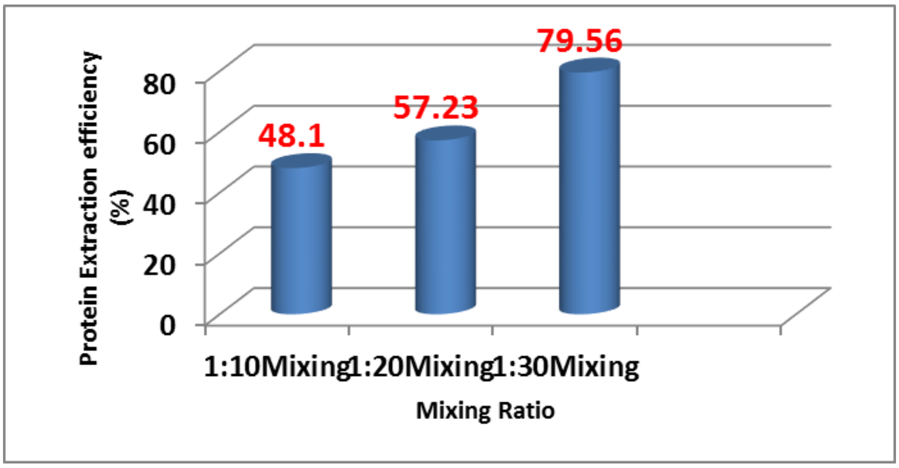

Figure (3-4) the best mixing ratio of chia seed protein isolate

CONCLUSION

We can conclude that it is possible to produce a protein isolate from chia seeds with a protein ratio of more than %80 after determining the optimal conditions for its production, as it can be included in the fortify of different food systems.

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