The Optimal Condition for Carrot Seeds Protein Isolate Preparation and Characterization

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Abstract

This study was conducted to prepare carrot seed protein isolate (CSI) from defatted carrot seed protein concentrate (CSP) using alkaline extraction and isoelectric precipitation. The optimum conditions for preparing the CSI were as follows: extraction pH 10, precipitation pH 4, temperature 50C˚, a 1:30 w/v (concentrate: water) mixing ratio, and an extraction time of 240 min. The protein content and yield of the CSI under these conditions were 87.29% and 15.93g/100g of concentrate, respectively. Albumin and globulin were the predominant storage proteins in carrot seeds. HPLC analysis found that the major amino acids in the CSI were valine, lysine, methionine, isoleucine, and phenylalanine, while the proportion of essential amino acids was significantly higher than that reported for ideal food proteins. While the functional properties results demonstrated superiority of the protein isolate in terms of water and fat absorption, solubility, emulsification, and foaming ability and stability, it was also observed that pH affected the foaming and emulsification properties and stability of the samples under study. The overall results indicate that carrot seed protein isolate could be a promising protein source for food products.

Keywords: Protein concentrate, Carrot seed, Protein isolate yield, Storage proteins.

Introduction

Due to growing consumer concerns about the safety of animal products and their derivatives, interest has recently increased in the search for new plant-based protein sources as alternatives to animal products [1]. The World Health Organization has recommended consuming plant-based protein instead of animal protein, which contains high amounts of saturated fat and cholesterol [2]. It can also be a better alternative to more expensive animal products. For this reason, plant proteins have become a major source of dietary protein in developing countries, with increasing protein consumption and the problem of protein deficiency leading to the emergence of malnutrition diseases [3]. Therefore, it has become necessary to search for non-traditional sources of protein isolate. Over the past 30 years, interest in plant seeds and their use as protein concentrates has grown significantly due to their functional and nutritional properties [4, 5].

Carrot (*Daucus carota* L.) is an herbaceous plant of the Apiaceae family and is the most important member of that family [6]. Carrot is a biennial plant with a life cycle of 12–24 months that stores large amounts of carbohydrates. The carrot plant grows to a height of 30-100 cm or more and is native to temperate (subtropical) climates [7,8]. It was first used as a medicinal plant in Central Asia and later became an important global crop grown worldwide [9]. The crop is cultivated on an area of 1.131 million hectares worldwide, and its global production exceeds 40 million tons annually

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[10]. Its seeds are small, brown, oval-shaped, and can be eaten raw or cooked. They are a good source of vitamins (K, A, and C) [11], and also contain protein, fiber, ash, and fat. The seeds also contain many plant components such as phenolics, flavonoids, carotenoids, and a series of volatile compounds [12,13]. The seeds also have antioxidant, antidiabetic, anticancer, antihypertensive, antifungal, antibacterial, and anti-inflammatory properties [14].

Plant protein concentrates or isolates are typically prepared by alkaline extraction, and the extracted protein is then precipitated either by lowering the pH to its isoelectric point or by heating [15,16]. The high protein content of protein isolates extracted from seeds through precipitation constitutes a potential protein source for food industry applications, and this potential benefit is based on their functional properties [17,18]. Functional properties are chemical and physical properties that influence the behavior of proteins in the diet during processing, storage, and consumption, such as solubility, foaming ability, gelation, and emulsifying properties [19]. Plant proteins are commonly classified into four classes: albumin, globulin, prolamin, and glutelin, based on their solubility in different solvents, such as water, salt, alcohol, and alkali solutions. The proportions of these four classes vary depending on the plant [20]. Plant protein isolates are the most refined form of proteins and often have improved taste and appearance compared to the original meal, making them more suitable for use as nutritional and functional ingredients in many food products (e.g., baked goods, etc.) [21,22]. However, only limited information is available on the relative physicochemical and functional properties of carrot seed protein isolate. In this regard, there is a need to study the physicochemical properties of the isolated protein (e.g., amino acid composition, protein structure, and potential biological activities) to provide a better understanding of its properties and potential applications in the food industry. To date, there has been no relevant study on the preparation and characterization of carrot seed protein isolate. Therefore, the present study aimed to identify the optimal conditions for preparing carrot seed protein isolate and to study the amino acid composition, protein classification, and functional properties of the protein concentrate and isolate.

Material and Methods

Prepare samples:

In this study, carrot seeds of the Italian-originated and locally used Nantes Advanced cultivar were used, which were obtained from the local seed markets of Najaf, Iraq. Chemicals and solvents were purchased from BDH (England), Sigma-Aldrich (Germany), and Chem Cruz (USA) The seeds were first finely ground using a laboratory grinder several times, then sieved to obtain a fine powder, and then frozen in plastic bags at -18C˚ until use.

Preparation of defatted and concentrated carrot seed powder:

Defatted carrot seed powder was prepared according to the method described by [23], then defatted carrot seed protein concentrate was prepared by mixing defatted carrot seed powder with ethanol (70%) for 2 hours, grinding the product and passing it through a 120 mesh, and storing it at 4C˚ for further analysis.

Preparation of carrot seed protein isolate:

Carrot seed protein isolate was prepared according to the method described by [24], by mixing carrot seed protein concentrate with distilled water in different ratios (1:10-1:40 w/v). The protein was extracted at various temperatures (30–60C˚) for (15–240 min). The pH was then adjusted to (2, 4, 6, 8, 9, 10, 11, 12) to determine the best pH for

protein solubility. The mixture was centrifuged at 10000_xg for 20 min. The supernatant was then collected, and the pH was adjusted to (3, 3.5, 4, 4.5, 5, 5.5) to precipitate the protein. The supernatant was then centrifuged again at 10000_xg for 20 min. The precipitate was collected and washed with distilled water several times. A small amount of water was then added. Distilled to precipitate, pH adjusted to 7, freeze-dried, and stored at 4C˚ for further analysis.

Estimation of the yield of protein isolate:

The protein isolate yield was estimated according to [25] using the following equation:

[Yield (%) = (Weight of protein isolate (g)) / (Weight of protein concentrate (g)) x100]

Estimation of protein content:

The total nitrogen content of defatted, concentrated, and isolated carrot seed powder was determined using a Kjeldahl apparatus based on the method described in [26].

Determination of carrot seed proteins:

The types of proteins (albumin, globulin, prolamin, glutelin) present in defatted, concentrated, and isolated carrot seed powder were determined by relying on the difference in their solubility in different solutions (water, salt, alcohol, alkaline solutions) according to the method described by [27].

Determination of Amino acid content on carrot seed protein isolate:

The amino acid content of carrot seed protein isolate was estimated using high-performance liquid chromatography (HPLC) technology, specifically the Solvent Delivery System 2100, equipped by the German company, with UV/vis detection, according to the injection program and the method described by [28].

Estimation of functional properties:

Water absorption capacity (WAC):

The water absorption capacity of the concentrated and isolated protein of carrot seed powder was measured according to the method described by [29], by weighing (1g) of samples and then placing them in test tubes with a capacity of (15ml) and gradually adding (10ml) of distilled water with stirring using a magnetic stirrer and leaving it for (30 min) at room temperature (2±25C˚). Then, the centrifugation process was carried out at a speed of (2000_xg) for (20min), and then the supernatant was obtained. After that, the tube with the sample was weighed, and the amount of bound water was measured according to the following equation:

$$\mathbf{WAC} = \frac{(\mathbf{W_2})-(\mathbf{W_1})}{(\mathbf{W_0})}$$

Where:

W_0 = (Weight of dry sample).

W_1 = (Weight of tube+ dry sample before adding water).

W_2 = (Weight of tube+ weight of precipitate after adding water).

Fat absorption capacity (FAC):

The fat absorption capacity of the concentrated and isolated protein of carrot seed powder was measured according to the method described by [29], by weighing (1g) of samples in a pre-weighed 15ml centrifuge tube. Then, the samples were mixed with (10ml) of sunflower fat, and the mixture was left at room temperature (2±25C˚) for 30min. Then, the centrifugation process was carried out at a speed of (5000_xg) for 30min, and then the supernatant was carefully removed. After that, the tube was weighed with the sample, and the fat absorption capacity was measured according to the following equation:

$$\mathbf{FAC} = \frac{(\mathbf{F_2}) - (\mathbf{F_1})}{(\mathbf{F_0})}$$

Where:

F_0 = (Weight of dry sample).

F_1 = (Weight of tube+ weight of dry sample before adding fat).

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F_2 = (Weight of tube+ weight of precipitate after adding fat).

Emulsifying capacity estimation:

The emulsifying capacity and emulsion stability were estimated according to the method described by [30]. 5ml of the concentrated and isolated protein of carrot seed powder, prepared at a concentration of 0.25%, was mixed with 5ml of sunflower fat at different pH levels (4, 7, 10). The mixture was then homogenized using a homogenizer at a speed of 10,000 rpm for 1 min. After that, the centrifugation process was carried out at a speed of (3500_xg) for (5min). Then, the volume of the emulsion layer was measured using a graduated cylinder, and the percentage (%) of emulsifying capacity was calculated using the following equation:

Emulsification capacity (%) = (emulsion layer volume) / (total volume) ×100

Emulsion stability:

The stability of the emulsion was estimated by placing the prepared emulsion in a water bath for (30min) at a temperature of (85C˚), after which the centrifugation process was carried out at a speed of (3500_xg) for (5min), then the size of the emulsion layer was measured using a graduated cylinder, and the percentage (%) of emulsion stability was calculated using the following equation:

Emulsion stability (%) = (emulsion layer volume after heating) / (total volume before heating) ×100

Estimation of foam capacity and stability:

The foaming capacity and stability of the concentrated and isolated protein of carrot seed powder were estimated according to the method described by [31]. 50mL of sample suspensions were prepared at a concentration of 1% and different pH levels (4, 7, 10). They were then placed in 150ml glass beakers and mixed using an electric mixer at maximum speed for 1min. After that, they were transferred to a graduated cylinder with a capacity of 100ml. The foam volume was measured before and after mixing, and the percentage of foaming capacity was calculated using the following equation:

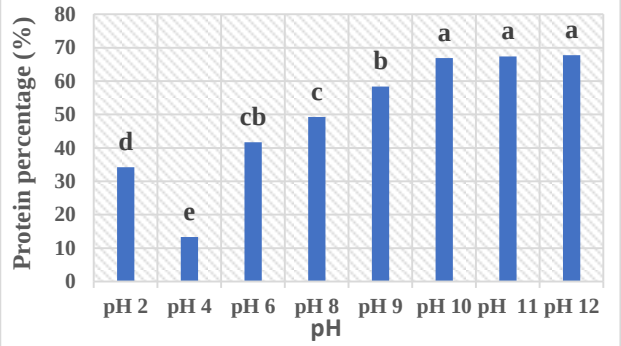
Foaming ability (%) = [(total volume after mixing - total volume before mixing) / total volume before mixing] ×100

The foam stability was estimated by measuring the volume of foam formed after 15, 30, 45, and 60 min. and the percentage (%) of foam stability was calculated using the following equation:

Foam stability (%) = (Foam volume at given time) / (Foam volume at zero time) ×100

Estimation of solubility:

The solubility of the concentrated and isolated protein of carrot seed powder was



estimated according to the method described by [32], by dissolving (50mg) of the samples in (20ml) of distilled water, then adjusting the pH to (2,4,6,8,10), then the solution was placed on a magnetic stirrer for (1hour) while ensuring that the pH was constant, then the centrifugation process was carried out at a speed of (10000_xg) for (15min), after which the supernatant was collected and the protein content was measured using the Microkjeldahl method, and the percentage (%) of protein solubility was calculated using the following equation:

Protein solubility (%) = (protein percentage in supernatant) / (protein percentage in sample) ×100

Statistical analysis:

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The Statistical Analysis System (SAS) [33] program was used to analyze the data to study the effect of different factors on the studied characteristics, according to the completely randomized design (CRD), and the significant differences between the means were compared using the least significant difference (LSD) test at a significant level (P≤0.05).

Results and Discussion

Optimal conditions for preparing carrot seed protein isolate

Effect of pH on protein solubility:

Figure 1 shows the protein solubility at different pH values (2, 4, 6, 8, 9, 10, 11, 12) when carrot seed protein concentrate was mixed with distilled water at a ratio of 1:10 (w/v) and incubated at 30C˚ for 45 min. The highest protein solubility was obtained at pH 10, 11, and 12. Statistical analysis results indicated that the differences were insignificant between them, so pH 10 was chosen, while there was a significant difference (p≤0.05) between all pH values. Found [34] that protein solubility increases with increasing pH after the isoelectric point, reaching its highest value at pH 10. Reported [35] that pH 10 is optimal for the preparation of sesame seed protein isolate. Reported [36] that the best pH for the solubility of peanut powder protein is 10 and that using a pH higher than that is not preferable due to undesirable changes, such as protein denaturation, color change, which may affect the functional properties and sensory qualities of the resulting protein isolate.

Figure 1. Effect of different pH values of solubility on the extraction efficiency of protein isolate (L.S.D. 0.05 = 7.589).

Effect of mixing ratio of water:

Figure 2 shows the effect of adding different levels of water to the concentrated carrot seed powder on the efficiency of protein extraction. Different mixing ratios (1:10 – 1:40) (w/v) were applied at pH 10 and an incubation time of 30C˚ for 45 min. The lowest protein extraction rate was achieved at a mixing ratio (1:10 w/v) of 29.30%, while the highest protein extraction rate was achieved at a mixing ratio (1:40 w/v) of 71.83%. The results of the statistical analysis indicated that the differences were not significant between the ratios (1:30, 1:40). Therefore, the mixing ratio (1:30) was chosen, which achieved a protein extraction rate of 71.42%. In contrast, the differences were significant between the remaining ratios. Reported [37]that the optimum mixing ratio for producing protein isolate from defatted sesame seed powder was 1:30 w/v when using different mixing ratios (1:10 – 1:40). Noted [38] that the mixing ratio 1:10 w/v was not effective for protein extraction from sesame seeds, which could be due to the high viscosity of the extraction mixture and insufficient solvent, both of which hinder protein solubility.

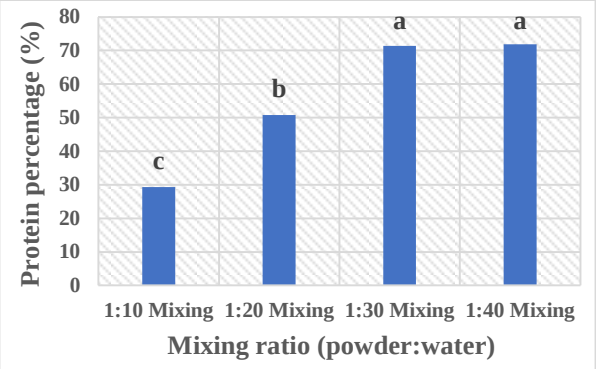

Figure 2. Effect of mixing ratio on protein extraction from carrot seed powder (L.S.D. 0.05 = 8.074).

Effect of temperature on extraction:

Figure 3 shows the effect of different extraction temperatures (30-60C˚) at pH 10 and a mixing ratio of 1:30 w/v for 45 min on protein extraction of concentrated carrot seed powder. The highest protein extraction was observed at 60C˚, reaching 64.45%, but 50C˚ was chosen at 57.11% due to the appearance of protein denaturation odor at 60C˚. The lowest protein extraction was at 30C˚, reaching 51.32%. The statistical analysis

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results indicated significant differences between different temperatures. These results differed from those of [35] and agreed with [37], who indicated that protein extraction increases with increasing extraction temperature, due to improved solubility of solids and reduced viscosity of the extraction solution.

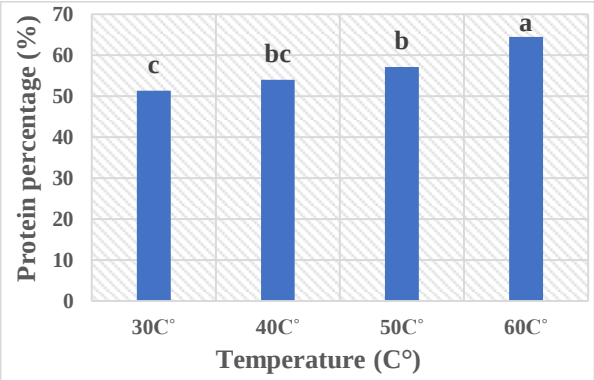

Figure 3. Effect of temperature on the extraction efficiency of protein isolate (L.S.D. 0.05 = 3.194).

Effect of extraction time:

Figure 4 shows protein extraction over different periods (15-240 min) at pH 10, a mixing ratio of 1:30 w/v, and incubation at 50C°. The highest protein extraction rate was recorded after 240 min (69.26%), and the lowest extraction rate was recorded at 15 min (38.10%). The differences were significant between all times, so 240 min was chosen. Found [39] that the extraction period (2-4 h) for tomato seed powder achieved the highest extraction rate for the production of protein isolate. Concluded [40] that the best extraction time for milk thistle seed protein isolate was 180 min when using different extraction times (60-180 min).

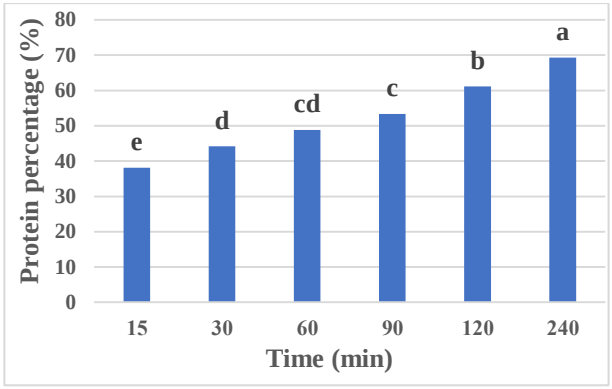

Figure 4. Effect of time on the extraction efficiency of protein isolate (L.S.D. 0.05 = 5.263).

Effect of pH on protein precipitation:

Figure 5 shows the protein precipitation ability at different pH values (3, 3.5, 4, 4.5, 5, 5.5), after protein solubility at pH 10 and mixing ratio (1:30 w/v), and incubating at 50C° for 240 min. The highest protein precipitation rate was obtained at pH 4, which was used in the production of protein isolate. Found [23] that pH 4 was the best for okra seed protein precipitation when using different pH values (2-6). Also showed [37] that the best pH for the production of defatted sesame seed protein isolate was pH 4, which achieved the highest protein content.

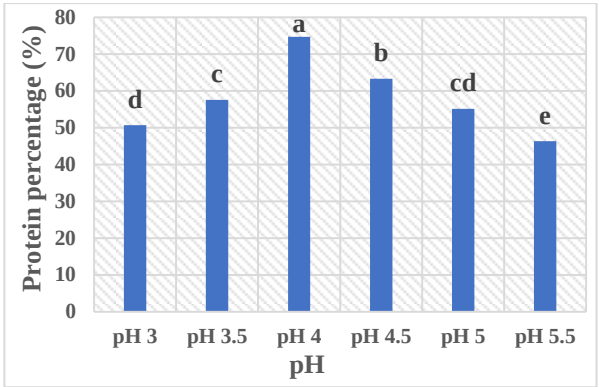

Figure 5. Effect of different precipitation pH on the extraction efficiency of protein isolate (L.S.D. 0.05 = 4.083).

Carrot seed powder protein isolate:

Table 1 shows the percentage of protein and types of storage proteins in the defatted, concentrated, and isolated carrot seed protein powder, along with the calculation of the

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protein isolate yield. The table shows that the protein percentage was (30.35, 46.75, and 87.29) %, respectively. There were significant differences between treatments at the (P≤0.05) level. The increase in protein concentration is because the different manufacturing processes led to the removal of most of the non-protein elements, such as carbohydrates, fats, and minerals. As for the types of storage proteins based on the solubility of the protein in different solutions, and according to the Osborne classification, the same table shows that the predominant storage proteins in carrot seeds are albumin and globulin. As for the protein isolate yield, the results showed that every 100g of concentrated carrot seed powder yields 15.93g of protein isolate. Found [41] that the protein content of the defatted powder, concentrate, and isolated Conophor seeds was 36.4, 45.6, and 80.5%, respectively. Noted [42] a higher protein content when preparing the concentrated and isolated protein of cowpea seeds, as it was (22.9%) in the seed powder and (77.6%) in the isolated protein. Studied [43] the storage proteins of the seeds of six important medicinal plants belonging to the Apiaceae family and found that the percentage of albumin and globulin was the predominant percentage in the Apiaceae family. Indicated [44] that the yield of the isolated flaxseed protein extracted at pH 12 was 17.2% as a protein isolate.

Table 1. Percentage of protein and types of stored proteins in defatted, concentrated, and isolated carrot seed protein powder with protein isolate yield.

Product	Protein (%)	(%)Protein type				
		Albumin	Globulin	Prolamin	Glutelin	Insoluble protein
Defatted powder	30.35	10.20	8.43	3.13	5.26	4.28
Protein concentrate	46.75	9.25	14.15	2.91	8.38	11.60
Protein isolate	87.29	39.72	19.01	1.38	12.38	9.32
L.S.D. 0.05	7.138*	5.441*	4.572*	1.088*	3.517*	2.095*
) *P≤0.05(						
Protein isolate yield	15.93g Protein Isolate / 100g Carrot Seed Concentrate					

*Values are from duplicates.

Amino acid estimation of protein isolate:

Table 2 presents the amino acid ratios in the protein isolate of carrot seeds and the recommended amino acid ratios [45]. It was observed that there were 21 amino acids in the protein isolate, including nine essential amino acids, where valine recorded the highest percentage (29.31%), followed by the amino acids lysine, methionine, isoleucine, phenylalanine, cysteine, tyrosine, glutamic acid, serine, leucine, hydroxyproline, proline, glycine, tryptophan, glutamine, histidine, asparagine, alanine, aspartic acid, threonine, and arginine. The results of the table indicate that the protein isolate has high levels of essential amino acids, which amounted to (77.36%) of the total amino acids, except that it contains a low percentage of threonine compared to the recommendations [46]. This decrease can be easily compensated for by other protein sources. It was also noted that the percentage of essential amino acids was higher than the soybean protein isolates for the acids valine, methionine, lysine, and phenylalanine, while the soybean protein isolate was superior for the acids leucine, threonine, and histidine. Studied [47] the

amino acid composition of three varieties of fenugreek seeds (*Trigonella* L.) and indicated that the powder of the fenugreek seed varieties contains a high percentage of the amino acid lysine. Also studied [48] the amino acid composition of *Calliandra surinamensis* seed protein and indicated that the seed protein contained all nine essential amino acids in varying concentrations, with leucine, methionine, lysine, isoleucine, and valine being the most abundant. The amino acid composition of a carrot seed protein isolate was evaluated for the first time in this study.

Table 2. Amino acid content of carrot seed protein isolates and comparison with amino acids of milk, egg, and soy protein isolate.

Amino acids	Protein isolate %	recommendations FAO/WHO %	Amino acids in milk and eggs %	Soy protein isolate %
Alanine	0.64			3.4
Valine	29.31	3.5	6.6	1.1
Leucine	2.28	2.8	5.4	4.1
Isoleucine	6.28	6.6	8.6	6.8
Methionine	6.85			1.1
Phenylalanine	6.10			5.2
Glycine	2.04			3.4
Serine	2.42			4.2
Threonine	0.42	3.4	4.7	3.0
Cysteine	4.25			4.5
Tyrosine	3.50			3.2
Aspartic acid	0.40			9.9
Glutamic acid	2.81			17
Lysine	23.76	5.8	7	5.2
Arginine	0.28			6.6
Histidine	1.10	1.9	2.2	2.3
Asparagine	1.00			
Tryptophan	1.26	1.1	1.7	1.2
Hydroxy proline	2.12			
Proline	2.07			
Glutamine	1.11			
Methionine + Cysteine	11.10	2.5	5.7	
Phenylalanine + Tyrosine	9.60	6.3	9.3	
EAA	77.36	33.9	51.2	
NEAA	22.64			
P	43.17			
NP	56.83			

{EAA} Essential amino acids, {NEAA} Non-essential amino acids, {P} Polar amino acids, {NP} Non-polar amino acids.

Functional properties

Water absorption capacity:

Table 3 shows the water absorption capacity of the protein concentrate and isolate of carrot seed powder, which were (2.49, 2.71) g/g, respectively. It was noted that the highest water absorption capacity was for the

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protein isolate, and the difference was insignificant with the protein concentrate at the level ($p \leq 0.05$). These results are due to the increase in the protein percentage, which led to an increase in the water absorption capacity. This is attributed to the protein's ability to bind to water, thanks to the presence of polar amino acids, which constitute 43.17%, as they attract water and form hydrogen bonds with it (Table 2). Stated [49] that the water absorption capacity of the protein isolates of different varieties of pea seeds ranged from (1.88-2.37) g/g. He indicated that these differences are related to the nature of the protein, its type, shape, and amino acid components, in addition to the surface polarity and the number and type of polar groups. While found [50] that the water absorption capacity of both concentrated and isolated okra seed protein was (2.81, 3.36) g/g.

Fat absorption capacity:

Table 3 shows the fat absorption capacity of both carrot seed protein concentrate and isolate, which were (2.03, 2.26) g/g, respectively. The results showed that the highest fat absorption capacity was for the protein isolate, and the difference was significant with the protein concentrate at the level ($p \leq 0.05$). The binding to fat is attributed to the presence of hydrophobic groups in the amino acid composition, which constitute 56.83% (Table 2). These groups contribute to the formation of hydrophobic bonds with lipids, which increases the amount of bound lipids. Reported [49] that the fat absorption capacity of the protein isolates of different varieties of pea seeds ranged from (1.07-1.40) %. Found [50] that the fat absorption capacity of both okra seed protein concentrates and isolate was (2.64, 3.03) g/g.

Table 3. Water and fat absorption capacity of carrot seed protein concentrate and isolate.

Product	symbol	Functional properties	
		Water absorption capacity (g/g)	Fat absorption capacity (g/g)
Protein concentrate	CP	2.49	2.03
Protein isolate	IP	2.71	2.26
T-test		0.287 NS	0.175*
) *P≤0.05(			

*Values are from duplicates.

Emulsifying properties:

Table 4 shows the emulsification ability and emulsion stability of the carrot seed protein concentrate and isolate at different pH levels (4, 7, 10). The emulsification ability of the protein concentrate was (36.12, 45.72, 58.35) %, respectively, while in the protein isolate, it reached (45.37, 71.11, 73.25) %, at the same pH levels. It was noted that the highest emulsification ability was for the protein isolate, and this is due to the high percentage of protein in the protein isolate compared to the protein concentrate, in addition to the presence of impurities such as minerals and fibers that reduce the emulsification ability. Regarding emulsion stability, the highest emulsion stability values were recorded for the protein isolate at different pH levels (4, 7, 10), reaching (47.52, 57.13, 79.44) %, respectively, while the stability values for the protein concentrate reached (17.55, 39.04, 58.29) %, respectively, at the same pH levels. The results of the same table indicated that the emulsifying properties increased with increasing pH, and the differences between the treatments were significant at the ($p \leq 0.05$) level. Reported [51] that, when

studying the technical and functional properties of purslane seeds, the highest level of emulsification ability and stability was for the protein isolate. The results are also consistent with [30] when studying the emulsifying properties of the protein concentrate and isolate of sesame seeds.

Table 4. Emulsification and emulsion stability of carrot seed protein concentrate and isolate.

Product	symbol	Functional properties						L.S.D. 0.05
		Emulsifying capacity(%)			Emulsion stability(%)			
		pH 4	pH 7	pH 10	pH 4	pH 7	pH 10	
Protein concentrate	CP	36.12	45.72	58.35	17.55	39.04	58.29	7.326*
Protein isolate	IP	45.37	71.11	73.25	47.52	57.13	79.44	7.502*
T-test (P≤0.05) *		4.517*	5.743*	5.802*	7.968*	6.021*	6.257*	---

*Values are from duplicates.

Foam properties:

Table 5 shows the foaming ability and stability of the carrot seed protein concentrate and isolate at different pH values (4, 7, 10). The foaming ability of the concentrate and isolate reached (18.23, 30.83, 47.70) % and (25.16, 43.52, 60) %, respectively, at the same pH values. It was noted from the table results that the foaming ability of the isolate was higher than that of the protein concentrate. The reason for the increased foaming ability is due to the formation of protein films on the surface, which increases the surface area (water/air) and reduces the surface tension, which facilitates the encapsulation of air bubbles [50]. The foam stability increases with increasing protein concentration due to the higher viscosity that develops with increasing protein concentration, and also due to facilitating the formation of cohesive protein films on the surface. The results of the same table showed an increase in foaming properties with increasing pH, and the differences between treatments were significant at the (p≤0.05) level. Found [51] that foaming ability and stability increased with increasing protein concentration, indicating that the protein isolate had the highest foaming ability and stability, followed by the protein concentrate. Also reported [29] that the foaming ability and stability of the walnut protein isolate were significantly higher than those of the protein concentrate.

Table 5. Foaming capacity and stability of carrot seed protein concentrate and isolate.

Product	symbol	pH	Functional properties Foam capacity (%)	Foam stability(%) 15min	30min	45min	60min	L.S.D. 0.05
Protein concentrate	CP	pH 4	18.23	69.50	38.72	14.70	0	8.41 *
		pH 7	30.83	78.75	58.15	34.44	12.35	7.55 *
		pH 10	47.70	82.01	66.81	48.59	29.41	8.07 *
Protein isolate	IP	pH 4	25.16	77.82	57.39	36.24	18.08	8.31 *
		pH 7	43.52	83.80	69.55	54.84	36.58	8.94 *
		pH 10	60	86.51	73.06	59.13	43.51	7.63 *
L.S.D. 0.05 (P≤0.05) *			7.601*	6.816*	7.954*	9.027*	7.339*	---

*Values are from duplicates.

Solubility:

Table 6 shows the effect of different pH numbers (2, 4, 6, 8, 10) on the solubility of the protein concentrate and isolate of carrot seeds, as the solubility of the protein concentrate reached (30.76, 12.48, 33.61, 41.83, 50.21) % respectively, while the solubility of the protein isolate reached (36.54, 15.87, 39.73, 58.16, 68.32) % respectively at the same pH numbers. It was noted from the same table that the solubility of the protein isolate was higher than that of the protein concentrate at all pH values. Furthermore, the samples recorded the highest solubility at pH 10. This is due to the electrostatic repulsion forces between the protein charges, which prevent them from aggregating and clumping. The lowest solubility was observed at pH 4, due to the equilibrium of the charges at the isoelectric point. The differences between the treatments were significant at the (p≤0.05) level. Reported [30] that the higher solubility of the protein isolates than that of the protein concentrate may be due to the difference in the quality and type of protein in both the isolate and the protein concentrate. Found [20] that the highest and lowest solubility of the isolate and major fractions of cumin seed protein were at pH 10 and 4.

Table 6. Solubility percentages of concentrate and protein isolate of carrot seeds.

Product	symbol	Functional properties Solubility(%) pH 2	4	6	8	10	L.S.D. 0.05
Protein concentrate	CP	30.76	12.48	33.61	41.83	50.21	6.437*
Protein isolate	IP	36.54	15.87	39.73	58.16	68.32	7.015*
T-test (P≤0.05) *		3.855*	2.959*	4.074*	5.189*	5.361*	---

*Values are from duplicates.

Conclusion

Optimal conditions for the preparation of carrot seed protein isolate, which contains approximately 90% protein, were determined. Furthermore, the functional properties and amino acid composition indicated that the protein isolate could be an interesting alternative in food applications and a potential source for meeting the nutritional requirements of some functional foods. We recommend further studies on the potential uses of carrot seed protein isolate in suitable food products, based on its technical and functional properties.

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