Preparation of protein concentrate from quinoa seeds and study of its chemical composition and functional properties

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Abstract

The study included preparing a protein concentrate from quinoa seeds, studying its chemical composition and comparing it with whole and defatted seeds. Chemically, there were significant differences between each of the whole seed powder, the seeds removed from the fat, and the protein concentrate in terms of the percentage of protein, and the protein center recorded the highest protein percentage, amounting to 38%. While the defatted seeds contained the highest percentage of ash, amounting to 2.6% compared to the whole seeds and the protein concentrate. The results indicated that the highest percentage of moisture, fat and carbohydrates were in the whole seeds compared to the seeds removed from the fat and the protein concentrate, while the seeds removed from the fat recorded the highest percentage of fiber 5.2% compared to the seeds removed from the fat and the protein concentrate which recorded the lowest percentage of fiber 2.0%. The results showed the functional properties, which included the ability to hold water and bind the fat for each of the whole and defatted seed powder and the protein concentrate at different pH numbers, including pH (12, 7, 4). The protein concentrator gave the highest ability to hold water at pH 7, followed by the defatted seeds, while whole seeds recorded the least ability to hold water. As for the ability to bind fat, the protein center also recorded the highest ability to bind fat at pH 4, followed by the defatted seeds, while the powder was recorded. Whole seeds are less amenable to attachment. The results of the ability to form foam indicated the superiority of the protein concentrate, which gave the highest ability to form at pH 12 followed by the defatted seeds, while the whole seed powder recorded the least ability to form foam. As for the stability of the foam after 15, 30, 60 minutes, the results indicated the superiority of the seeds. Defatted at pH 12 by giving it the best foam stability after 60 minutes, followed by protein concentrate and then whole seed powder at the same pH. As for the results of the capacity and stability of the emulsion, it indicated the superiority of the protein concentrate by giving it the best emulsification ability and at pH 12 and the best stability of the emulsion, followed by defatted seeds, while the whole seed powder recorded less emulsifiability and less stability of the emulsion.

Keywords: quinoa seeds, protein concentrate, functional properties, chemical composition

INTRODUCTION

Quinoa scientific name *Chenopodium quinoa Willd* is a dicotyledonous plant that belongs to the family Chenopodi aceae, native to the Andes region of South America, it has gained increasing interest worldwide due to its nutritional and functional properties in addition to the possibility of its cultivation in extreme climatic conditions, in terms of soil, precipitation Rainfall, temperature, salinity and aridity therefore these characteristics make it important in areas prone to food insecurity and also it can grow at a level up to 4500 meters above sea level [8].

Quinoa contains all the necessary amino acids, and therefore it is considered a complete protein and is much easier to digest than animal proteins, and it contains a lower

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percentage of fat. significantly during the eighties[26]. Many agricultural scientists were interested in introducing this crop to their countries because of its high nutritional value, and its high protein content ranging between 16-18%, while the protein rate does not exceed 13% in wheat crops, and the amino acid lysine is twice as high compared to wheat. And due to the high nutritional value of this grain, the NASA space agency used it as a balanced and complete food for astronauts, as the seeds represent the main part of the quinoa plant used in human nutrition, as it can be consumed whole, like rice, and ground to make flour and to prepare baked or fermented products, and the leaves are also used in feeding Humans are like other leafy vegetables such as spinach[12]. The functional characteristics of the protein is the change in the food that expresses the composition of the amino acids and their interactions, as the increase in the polar amino acids leads to an increase in solubility, emulsification and foaming[24].

The functional properties of proteins are affected by the different physicochemical properties of food depending on the conditions of preparation, processing, storage and consumption, as they directly affect the quality in addition to the sensory characteristics of the food, including size, shape, amino acid sequence, charge distribution, net charges, hydrophilic and hydrophobic groups, different protein structures, water percentage, flexibility or hardness of a molecule Protein and its ability to interact with other compounds[13]. Protein efficiency is one of the most important functional characteristics in food systems, which includes emulsification capacity and emulsion stability, and its importance appears in the manufacture of some types of foods such as sauce, bread and mayonnaise[24].

Materials and methods

1- Sample Collection

After collecting the quinoa seeds from the local markets in the holy city of Najaf, they were cleaned, impurities were removed from them, and washed well to remove the soapy substance, then they were dried at 40 °C for 24 hours, ground using a laboratory mill, and sifted to obtain a fine powder. They were placed in polyethylene bags and kept in freezing at a temperature -18 C°[18].

2- Preparation of Defatted Quinoa Seeds

The defatted quinoa seed powder was prepared according to quinoa seeds were ground using a laboratory mill, and the oil was removed using the cold method, by mixing the ground quinoa seeds with hexane in a ratio of 20:1 (model: solvent) and the process was repeated again. To ensure the removal of the largest possible amount of fat, then the dried seeds were dried at a temperature of 50 C^0 for 24 hours, then ground and sifted using a sieve, then kept at a temperature of -18 C° until use[16].

3- Preparation of Quinoa Protein Concentrator

The protein concentrator of quinoa seeds was prepared from a powder of defatted quinoa seeds according to[16], where the defatted quinoa seed powder was mixed with ethanol prepared at a concentration of 70% in a mixing ratio of 10:1 (seeds: ethanol) with stirring for a period of time. Two hours at a temperature (30 ± 2) °C, then centrifugation was carried out at a speed of 5000 revolutions / min for 10 minutes, then the precipitate was collected, ground and kept at a temperature of -18°C until use.

4- Chemical Composition of Quinoa Seeds, Defatted Seeds and protein Concentrator

The chemical composition of whole quinoa seed powder, defatted seed powder and protein concentrate was estimated, which included the following:

A- Determination of Moisture Content

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$$\text{Moisture (\%)} = \frac{\text{Weigh the eyelid with the sample before drying} - \text{Weigh the eyelid with sample before drying}}{\text{weight of original sample}} \times 100$$

B- Determination of fat Content

The fat percentage of quinoa seed samples was estimated using the succiolit apparatus and according to the method described in [1], and the lipid percentage was estimated using the following equation:

Fat percentage = (empty weight of the flask + fatty matter) – (empty weight of the flask) / weight of the sample) x 100

C- Determination of Protein Content

The percentage of total protein was estimated in the studied seed samples based on the amount of total nitrogen as mentioned in[1], and using the Micro kjeldahl method. The percentage of total protein was calculated by multiplying the value of nitrogen by the general protein coefficient of foods.

$$\text{Nitrogen (\%)} = \frac{\text{Consumer Volume of HCl} \times \text{Molarity of Acid} \times \text{Nitrogen Equivalent(0.014)}}{\text{weight of sample}} \times 100$$

As for the percentage of protein, it was estimated using the following equation:

Crude protein in the sample(%) = Nitrogen in the sample(%) × 5.7

D- Determination of Fiber Content

The percentage of fibers was estimated according to the method described in [1], and the percentage of fibers was calculated as follows:

$$\text{Fiber (\%)} = \frac{\text{Weigh the eyelid with the deposit after drying} - \text{Weigh the eyelid with ash after burning}}{\text{weight of sample}} \times 100$$

E- Determination of Ash Content

The ash percentage was estimated according to A.O.A.C., (2008). The ash percentage was calculated according to the following equation:

$$\text{Ash (\%)} \frac{\text{Weigh the eyelid with ash - Weigh the empty eyelid(gm)}}{\text{weight of sample(gm)}} \times 100$$

F- Total carbohydrates

Carbohydrates were estimated using the difference (100- the sum of the other components (moisture, fat, ash, protein, and fiber)).

5- Functional properties of quinoa seed powder, defatted seed powder and protein Concentrate

A- Water Holding Capacity (WHC)

The water-holding ability of whole and defatted seed powder and protein concentrate of quinoa seeds was measured according to[21], which included mixing 1g of the sample separately with 20 ml of distilled water in a centrifuge tube and mixing using a Vortex mixer for 5 minutes and adjust the pH to (12,7,4) and leave for 15 minutes making sure that the pH is stable. Then centrifugation was carried out at 10000 cycles/min for 10 minutes, and the water holding capacity was expressed as the weight of water absorbed by 1gm of the sample, according to the equation below:

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$$WHC(Water\ g / Sample\ g) = \frac{W2 - W1}{W1}$$

W0 = weight of sample
W1= Tube weight + sample weight before adding water
W2= Tube weight + sediment weight after removing water

B- fat Holding Capacity (OHC)

The fat-holding ability of whole and defatted seed powder and the protein concentrate of quinoa seeds were measured according to [21]. About the ability to bind the fat by the number of grams of oil absorbed per gram of the sample and according to the equation below:

$$OHC\ fat\ g / Sample\ g = \frac{F2-F1}{F0}$$

F0 = wiegth of sample
F1= Tube weight + sample weight before adding oil
F2= Tube weight + sediment weight after removing oil

C- Emulsion Properties

The emulsifiability and stability of the emulsion were measured according to [24], by mixing 5 gm of whole and defatted seed powder and protein concentrate of quinoa seeds prepared separately at a concentration of 0.25% with 5 ml of sunflower oil at different pH numbers. (12, 7, 4) Then the mixture was homogenized at a speed of 10000 rpm for 1 minute, followed by centrifugation at 3500 rpm for 5 minutes. The size of the emulsion layer was measured using the graduated cylinder, and the percentage of emulsifiability was calculated using the equation:

$$Emulsification\ Ability = \frac{Emulsion\ layer\ volume}{Total\ volume}\ 100$$

D- Emulsion Stability

The stability of the emulsion was measured by placing the prepared emulsion in a water bath at a temperature of 95 C^0 for 30 minutes, then the centrifugation process was carried out at a speed of 3500 rpm for 5 minutes, and the size of the emulsion layer was measured using the graduated cylinder[7].The stability of the emulsion was calculated using the following equation:

$$Emulsification\ Ability = \frac{Emulsion\ volume\ after\ heating}{Total\ volume\ before\ heating}\ 100$$

E- Foaming Properties

The foaming ability of each of the whole and defatted seed powder and the protein concentrator of quinoa seeds was studied separately according to[9], where 50 ml of the sample suspension was prepared separately at a concentration of 1% and at pH numbers (4, 12, 7) and placed in glass flasks with a capacity of 150 ml and mixed using the electromixer at the maximum speed for 1 minute, then transferred to a graduated cylinder with a capacity of 100 ml and the volume was measured before and after mixing with the electromixer and the ability to form foam was measured using the following equation:

$$Foaming\ Ability = \frac{Total\ volume\ after\ whisking}{Total\ volume\ before\ whisking}\ 100$$

F- Foam Stability

The foam stability was measured by measuring the volume of foam formed after (15, 30, 60) minutes. The foam stability was calculated using the following equation:

$$Foam\ Stability = \frac{Foam\ volume\ at\ a\ given\ time}{Foam\ volume\ at\ zero\ time}\ 100$$

Results and Discussion

1- The chemical composition of quinoa seeds

Table (1) shows the chemical composition of each of the whole and defatted seeds and the protein center of the quinoa seeds. It is noted from the table that the moisture content of the whole seeds amounted to 9.86%, and this result was less than what was reported by the US Department of Agriculture for the year 2018 that the moisture content of quinoa seeds is 13.3 %. As for the percentage of moisture in the seeds, the fat removed and the protein concentrate, it was (7.38, 5.47)%, respectively[5], found that the percentage of moisture in the defatted chia seeds and the protein concentrate were (13.61, 8.12)% respectively.

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The results of the statistical analysis indicated that there were significant differences in the moisture percentages between whole and defatted seeds and the protein content of quinoa seeds.

As for the percentage of protein in whole quinoa seeds, the same table indicates that the percentage of protein amounted to 17%, and this percentage is lower than what was found by [13], as the percentage of protein in white, black and yellow quinoa seeds reached (21.61, 20, 47, 21.39)%, respectively. The results of the table also indicated that there was a significant increase in the percentage of protein in the fat-removed seeds and the protein concentrate, which reached (24.5, 38)%, respectively, after it was 17% in the whole seeds. Five hours and treatment of the center with ethanol, which also caused the removal of part of the fats, complex substances and phenols, and thus increased protein[16].

The percentage of protein removed and the protein concentrator of Confore powder was (45.6, 36.4)%, respectively. The results of the statistical analysis indicated that there were significant differences in the protein ratios between the whole and defatted seeds and the protein center of the quinoa seeds. Reasons, including the difference in the source of protein and the type of seeds used, as well as the method used for isolation and methods of evaluation, as well as the devices used to estimate the protein[23]. As for the percentage of fat, the results of the table indicate that the percentage of fat in whole quinoa seeds was (5.21%), as this percentage is close to what was mentioned by [16] where it reached 5.53% in quinoa seeds.

The results of the table also indicated that there was a significant decrease in the percentage of fat in the removed seeds and the protein concentrate, as it recorded (1.75, 0.17)%, respectively, and this is expected as a result of the treatment of the seeds with hexane, which removes the fat [27] found that the percentage of fat in the protein center of wheat germ was 0.1%, the results of the statistical analysis indicated that there were significant differences in the percentage of fat between whole and defatted seeds and the protein center of quinoa seeds.

As for the percentage of ash, Table (1) indicates that the percentage of ash in whole quinoa seeds amounted to 2.02%, and this result is consistent with what was stated by the US Department of Agriculture for year 2018, as the percentage of ash reached 2.4%, while the percentage of ash in the seeds removed from the fat and the protein center was 2.4%. (2.6, 1.8)%, respectively. As for [15], I found that the percentage of ash in the protein concentrate of millet and oats amounted to (3.55-0.95)%, respectively. The results of the statistical analysis indicated that there were significant differences in the ash percentages between the whole and defatted seeds and the protein concentrate, as it gave the lowest ash percentage. As for the percentage of carbohydrates in the whole and defatted quinoa seeds and the protein concentrate, the results indicate that the percentage in the whole quinoa seeds amounted to (61.81)%. respectively, as indicated by the US Department of Agriculture for year 2018 that the percentage of carbohydrates in quinoa seeds was 64.2.

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Table (1) The chemical composition of whole and defatted quinoa seeds and protein concentrator

Plant seeds			Components (%)
Quinoa seed protein concentrator	**Quinoa seeds defatted**	**Completely quinoa seeds**	
c 5.47	b 7.38	a 9.86	Moisture
a 38	b 24.5	c 17	Protein
c 0.17	b 1.75	a 5.21	Fat
c 2.0	a 5.2	b 4.1	Fibers
c1.8	a2.6	02 b.2	Ash
c 52.56	b 58.57	a 61.81	Carbohydrate

2- Functional characteristics of whole and defatted seeds and protein concentrator of quinoa seeds

A-Water holding capacity

Table (2) shows the effect of pH numbers (12, 7,4) on the water-holding capacity of whole and defatted seeds and the protein concentrate, where the water-holding capacity of each of the whole and defatted seeds and the protein concentrate was (2.57, 3.74, 1.62) and (2.69 , 3.91, 1.82) and (2.92, 4.13, 1.89) g water/g sample at pH (4, 7, 12) respectively, As the results of the statistical analysis showed that the pH 7 was significantly superior to giving the best ability to hold water, as it reached the highest ability to bind water in the protein center (4.13) g water / g concentrated at pH 7, the ability of the protein to bind water is due to its ability to form hydrogen bonds between water molecules And the polar aggregates of the peptide chains. The reason for the decrease in the ability to bind water at pH 12 may be due to the increase in protein solubility, as the hydrophobic surface activity decreases[3]. Among the factors affecting the ability to bind water are the amino acid composition, pH, and the ratio of hydrophilic to hydrophobic groups on the surface of the protein molecule[7].

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Table (2) Water holding ability of whole quinoa seeds, defatted and protein concentrator at different pH

Samples Effect	12	7	4	pH Sample
2.64	1.62	3.74	2.57	Original seeds
2.81	1.82	3.91	2.69	Defatted
2.97	1.89	4.13	2.92	Protein concentrator
LSD=0.0057 (samples effect)	LSD=0.0099 ((interference effect			
LSD=0.0057 **(effect of pH)**	**1.77**	**3.92**	**2.73**	Effect of pH

B- Fat binding ability

Table(3) shows the fat-binding ability of whole and defatted seeds and the protein concentrator of quinoa seeds with different pH numbers, as the results of the table indicated that the fat-binding ability of whole and defatted seeds and the protein status of quinoa seeds amounted to (1.09, 0.93, 0.86) , (1.62, 1.39, 1.28) , (2.71, 2.60, 2.49) g/g sample at pH (4,7,12) respectively. The results indicated that the ability to bind the fat for each of the defatted seeds and the protein concentrator was higher compared to the whole seeds with the superiority of the protein concentrator over the rest of the samples[20]. To reduce the ability to bind the fat, while the superiority of the ability to bind the fat in the protein concentrator may be due to the concentrator containing some non-polar materials such as fibers that are removed when treated with ethanol at a concentration of 70% for two hours [24], the results of the statistical analysis indicated a superiority The protein concentrator at pH 4, 7 and 12 compared to the original and defatted seeds at the same pH.

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Table (3) Fat binding ability of whole and defatted quinoa seeds and protein concentrator at different pH numbers

Samples Effect	12	7	4	pH Sample
0.96	0.86	0.93	1.09	Original seeds
1.43	1.28	1.39	1.62	Defatted
2.60	2.49	2.60	2.71	Protein concentrator
LSD=0.0063 (samples effect)	LSD=0.0109 ((interference effect			
LSD=0.0063 (effect of pH)	**1.54**	**1.64**	**1.81**	Effect of pH

C- Foam capacity and stability

Tables (4) and (5) show the ability to form foam and the stability of foam for whole quinoa seeds and defatted seeds and the protein concentrator at different pH numbers (4, 7, 12), as the ability to form foam for whole seeds was (31.84, 37.79, 41.33)%, and it reached stability (18.33, 20.66, 20) (30.66, 30.33, 35.33) (34.66, 4065, 45.33)% respectively, after (15, 30, 60) minutes, respectively. While the table shows the capacity and stability of the foam for the defatted seeds at the same pH numbers[4], which amounted to (58.53, 60.0, 64.84) respectively, and the stability reached (25.33, 38.33, 45.66) (35, 43, 50) (45.32, 58.82, 60.66)% after passing (60,30,15) minutes, and the foam formation capacity of the protein concentrator at pH (4,7,12) was (72.66, 79.5, 84.15)%, respectively, and the stability was(25, 24.66, 25)(35, 40, 50)(37.66, 40 and 50) percent, respectively, at (15, 30, and 60) minutes[21].

The results of the statistical analysis showed that there are significant differences between the whole seeds and the seeds isolated from the fat and the protein concentrator and there are also significant differences between the pH numbers (4, 7, 12) for the foam capacity and its stability, as the results of the statistical analysis showed a significant superiority of the protein concentrator of the quinoa seeds in relation to the foam capacity at the pH (12), and the pH (12) gave the highest foam capacity and the best stability for it and for each of the whole and defatted seeds and the protein concentrator compared to the rest of the studied pH(22). The results also indicated that the stability of the foam increased with increasing time, as the 60 minute gave the highest foam stability for all studied samples compared to the rest of the periods[10].

The foam capacity and stability of wheat bran were affected by the pH, and the lowest value was observed at an acidic pH. The increase in the foam capacity with an increase in the pH may be attributed to the increase in the total electric charge of the protein and then the increase in the solubility and flexibility of the protein, which results in the diffusion of the protein at the interface (water - air) and surround the air bubbles and thus increase the formation of foam[14]. In a study of the functional properties of the protein concentrator of the wheat embryo and the flour of the defatted fetus, the foaming capacity increased with the increase of the protein concentrator and the increase of the pH. Where the foam capacity and stability were estimated at a concentrator of 1% and 2%, and the highest foaming capacity was at a concentration of 2% and pH of 8[27].

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Table (4) The ability to foam formation of whole quinoa seeds, removed from the fat and the protein concentrate at different pH numbers

Samples Effect	12	7	4	pH Sample
36.99	41.33	37.79	31.84	Original seeds
51.24	54.84	50	48.53	Defatted
76.56	84.15	79.5	72.66	Protein concentrator
LSD=1.906 (samples effect)	LSD=3.301 ((interference effect			
LSD=1.906 (effect of pH)	**63.44**	**57.00**	**54.35**	Effect of pH

Table (5) Foam stability of whole and defatted quinoa seeds and protein concentrator at different pH numbers

pH Effect	Samples Effect	Time			pH	Samples
		60 دقيقة	30 دقيقة	15 دقيقة		
31.88	30.667	34.66	30.66	18.33	4	Original seeds
		40.65	30.33	20.66	7	
		45.33	35.33	20	12	
37.42	44.796	45.32	35.33	25.33	4	Defatted
		58.82	43.66	38.33	7	
		60.66	50.33	45.66	12	
42.41	36.370	37.66	35	25	4	Protein concentrator
		40	40	24.66	7	
		50	50	25	12	
LSD=0.4860 Effect of pH	LSD=0.4860 Effect treatments	LSD=1.4579 interference effect				
LSD=0.4860 (effect of time)		**63.44**	**57.00**	**54.35**		Effect of time

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D- Emulsification capacity and stability

Table (6) shows the effect of pH on the emulsification capacity and stability of whole quinoa seeds, defatted seeds and protein concentrator, as the emulsification capacity of whole seeds was (60, 60, 62.5)% at pH numbers (4, 7, 12) respectively. While the stability Emulsification and at the same pH numbers (50,50,50)%, respectively, and the emulsification capacity of defatted seeds was (55.56, 70.52, 77.78)% at pH numbers (4, 7, 12) respectively[6].

As for the stability of emulsification and at the same pH numbers (50, 61.11, 70.59)%, respectively, the emulsification capacity of the protein center of quinoa seeds was (69.23, 66.66, 86.67)% at pH numbers (4, 7, 12) respectively, while The emulsification stability and the same pH numbers were (61.54, 55.56, 80.00)%, respectively[2]. The results of the statistical analysis showed that the pH number (12) was significantly higher by giving the best emulsification capacity and stability of the emulsion compared to other pH numbers, while there were no significant differences in the stability of the emulsion for whole seeds at pH numbers (4, 7, 12).

The pH (12) gave the best emulsification capacity for the protein isolates of both millet and oats (80.10, 82.85%), respectively[15]. The isolated protein from sunflower gave good stability at neutral pH. The reason is due to the ability of the protein to form an emulsion by absorbing oil and water[19].

Table (6) The capacity and stability of the emulsion for whole and defatted quinoa seeds and the protein concentrator at different pH numbers

Emulsion stability %	emulsification ability %	pH	Samples
50.0	60.0	4	**Completely seeds**
50.0	60.0	7	
50.0	62.5	12	
50.0	55.56	4	**Defatted**
61. 11	70.52	7	
70.59	77.78	12	
61.54	69.23	4	**Protein concentrator**
55.56	66.66	7	
80.0	86.67	12	
LSD=0.8735	**LSD=0.4953**		

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