Study of the chemical composition, functional, and nutritional properties of millet seed protein isolate

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Abstract:

The current study aimed to produce a protein isolate from millet seeds and to study its chemical composition and functional and nutritional properties. The study also examined the chemical composition of millet seed powder, defatted millet seed powder, and millet seed protein isolate. The highest protein percentage was observed in the protein isolate group at 71.25%, while the millet powder group had the lowest at 6.83. studied the mineral content of the above products, and observed that the element with the highest percentage in these products was magnesium, reaching 1069.00 mg/kg. studied the essential amino acid content of millet seed productsand found it to be (41.97, 39.76, and 40.14)% for millet seed powder, defatted millet seed powder, and protein isolate, respectively. found the highest value for the essential amino acid index (EAAI) in millet seed powder (82.70). found the highest biological value (BV) in millet seed powder (78.44%). The highest nutritional index (NI) value for millet protein isolate was 56.55. Studying the functional properties of the different products, we noted that the highest water absorption value was for the protein isolate group, reaching 3g/g. The highest fat absorption was observed with the protein isolate, at 3.6 g/g. found that the highest emulsifying Capacity was for the protein isolate, reaching (47.82)%. The highest foaming value was also for the protein isolate, reaching (20)%.

Keywords : Protein Isolate ; Chemical composition ; minerals ; Amino acids ; Functional and nutritional properties

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1. Introduction

A large portion of the protein in the human diet is consumed from animal foods such as meat and dairy products. The demand for protein-rich foods is increasing significantly globally as consumers become increasingly aware of the vital role of protein in human health. With growing awareness of environmental pollution and the adverse health effects of animal products, consumers are turning to plant-based alternatives rich in protein. Plant-based options are also rich in biologically active compounds, including polyphenols, antioxidants, vitamins, and dietary fibre. The protein content inplantbased sources such as grains (6-15%), legumes (20-38%), nuts (18-38%), and seeds (9-30%) is relatively higher than its content in milk (3-5%), while it is close to its content in meat (23%) (1). Cereals are among the most important food sources of energy, carbohydrates, and plant proteins globally. However, only about 41% of total cereal production is directed towards human consumption, while approximately 35% is allocated to animal feed. Despite their high nutritional value, cereals remain underutilized as a sustainable and healthy source of plant protein (2). Millet (*Pennisetum glaucum*) is a versatile and nutritious grain belonging to the grass family (Poaceae). Millet has been cultivated for over 10,000 years and thrives in dry and semi-dry conditions (3). Millet is considered an important traditional food known for its nutritional diversity and plays a pivotal role in ensuring human health and well-being because it is rich in nutrients, including dietary fibre, protein, vitamins, and minerals. Because it is gluten-free, millet can be used as an alternative for patients who are allergic to wheat. Millet contains bioactive compounds, such as flavonoids, lignin, and phenolic acids, that reduce inflammation and act as antioxidants. Millet has positive health benefits for blood sugar, cardiovascular disease, and obesity, as well as for the human digestive system (4). Proteins and isolated proteins are used in the food industry for their functional properties such as their ability to bind fats, emulsify, hydrate, enhance flavor retention, improve mouthfeel, foam formation and Stability, gelling, adhesion, rheological properties (viscosity, elasticity), edible coatings and films, and improve texture in products such as beverages, ice cream, cheese, yogurt, and other food products. Proteins improve the functional and nutritional properties of food products (5). Several studies have been conducted to produce protein isolates from various sources, including okra seed protein isolate (6). Soy protein isolate (7). Chickpea protein isolate (8). Cress seed protein isolate (9). Red lentil protein isolate (10). The current study aimed to produce a protein isolate from millet seeds and to study its chemical composition, mineral and

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amino acid content, and its functional and nutritional properties.

2. **Materials and Method**

2.1. Sample Collection

The study used millet seed samples obtained from local markets in Baghdad Governorate, which were cleaned of clinging dirt and impurities by hand and by sieve to obtain clean seeds ready for grinding.

2.2. Preparing millet seed powder

prepared millet seed powder by grinding clean, impurity-free seeds in a household grinder. After grinding, the millet powder was packed into sealed polyethene bags and stored at 4°C until use.

2.3. Preparing defatted millet seed powder

Defatted millet seed powder was prepared according to the method described by (11) with some modifications. The millet seed powder was treated with hexane at a 10:1 (w/v) ratio with continuous stirring using a magnetic mixer for 3 hours. Afterwards, filtration was carried out using filter paper No. 1, and the resulting product was collected, dried at room temperature for 24 hours, then ground and sieved using a 300-micrometre sieve. It was then stored in the freezer until use.

2.4. Preparation of Protein Isolate

The protein isolate was prepared according to the method described by (12) with some modifications. This was done by dissolving defatted millet seed powder in water at a 30:1 (w/v) ratio, then adjusting the pH to 10 with 1 M sodium hydroxide. The mixture was stirred with a magnetic stirrer for 1 hour, then centrifuged at 10,000 x g for 30 minutes. The filtrate was then separated from the precipitate. The filtrate was collected, and the pH was adjusted to 4 with 1 M hydrochloric acid. Then, centrifugation was performed at 10,000 × g for 30 minutes, and the precipitate was separated from the filtrate. The pH was then adjusted to 7, and the precipitate was dried, crushed, and stored in a freezer.

2.5. Chemical composition determination

Chemical estimations were carried out according to the standard method described in (13). Moisture, fat, ash, protein, and minerals were estimated using methods No. (925.10, 920.39, 923.03, 984.13, 942.05) respectively.

2.5.1. Determination of carbohydrate

estimated the carbohydrate content in millet seed powder, defatted millet seed powder, and millet seed protein isolateaccording to the equation shown, as stated by (14).

Carbohydrates = 100 – (% Moisture + % Fat + % Protein + % Ash).%

2.6. Amino acid estimation

The amino acid content in millet seed powder, defatted millet seed powder, and millet seed protein isolate of the samples under study was estimated using a high-performance liquid chromatography (HPLC) device (Sykam, Germany), according to the method described by (15).

2.7. Functional properties Determination:

2.7.1. Water Absorption Capacity (WAC

The water absorption capacity of millet seed powder, defatted millet seed powder, and millet seed protein isolate was estimated according to the method described by(11). Weighed 1 gram of the sample into a pre-weighed 15 mL test tube, then added 10 mL of distilled water while stirring with a glass rod. The mixture was left to stand at room temperature for 30 minutes, then centrifuged at 2000 x g for 20 minutes. The amount of water bound was calculated as follows:

WAC = (W2 – W1) / WO

WO = Weight of dry sample.

W1 = Weight of tube + dry sample before water addition.

W2 = Weight of tube + Weight of precipitate after water addition.

FAC (2.7.2. Fat Absorption Capacity

calculated the fat absorption capacity of millet seed powder, defatted millet seed powder, and millet seed protein isolate according to (11). calculated the fat absorption capacity of millet seed powder, defatted millet seed powder, and millet seed protein isolateinto a pre-weighed 15 mL test tube and mixed with 5 mL of corn oil. incubated the mixture at room temperature for 30 minutes, then centrifuged at 5000 x g for 20 minutes. The filtrate was carefully removed, along with the tube and the sample. The fat absorption capacity was measured in grams per gram of sample using the following equation:

FAC = (F2 – F1) / FO

FO = Weight of dry sample.

F1 = Weight of tube + Weight of dry sample before oil addition.

F2 = Weight of tube + Weight of precipitate after oil addition.

2.7.3. emulsifiability and emulsification stability

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The emulsifying Capacity of millet seed powder, defatted millet seed powder, and millet seed protein isolate was estimated according to the method of (16), with some modifications. 5 ml of 2% of each sample was homogenized with 5 ml of corn oil, and the mixture was centrifuged at 3500 rpm for 5 minutes. The height of the emulsion layer relative to the total content in the test tube, and the emulsifying Capacity was estimated using the following equation:

% Emulsifying Capacity = (Height of Emulsion Layer / Height of Total Content) × 100

estimated emulsion stability according to (17) by heating the mixture in a water bath at 90°C for 30 minutes, then immediately cooling it with tap water. The Stability was calculated using the following equation:

Emulsion Stability % = (Volume after heating / Volume before heating) × 100

2.7.4. Foam capacity and foam stability

The foaming Capacity of millet seed powder, defatted millet seed powder, and millet seed protein isolate was estimated according to the method described by (18). This was done by whisking 0.5 g of each sample with 50 mL of distilled water using a laboratory mixer for 3 minutes until foam formed. monitored the resulting foam volume to record the results. Foaming capacity was expressed as the percentage increase in Volume due to mixing. estimated foam stability by measuring the foam volume recorded after 30 minutes of storage. Foaming capacity and Stability were calculated using the following equation:

%Foaming Capacity = (Volume after mixing – Volume before mixing) / Volume before mixing) × 100

%Foam Stability = (Volume after storage – Volume before mixing) / Volume before mixing) × 100

2.8. Determination value Nutritional

2.8.1. amino acid score determination (AAS)

The amino acid score of millet seed powder, defatted millet seed powder, and millet seed protein isolate in the studied samples was estimated based on the method described by (19), according to the following equation:

Amino acid score = (Percentage of essential amino acids in the sample%) /(Percentage of essential amino acids recommended by the FAO%) × 100

)2.8.2. Essential amino acid index (EAAI

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The amino acid index of millet seed powder, defatted millet seed powder, and millet seed protein isolate from the samples was estimated according to (20), as follows:

$$EAAI = \sqrt[6]{\frac{(His \times Thr \times Val \times Leu \times Isoleu \times Lys)a}{(His \times Thr \times Val \times Leu \times Isoleu \times Lys)b}}$$

Where:

a = Total essential amino acids in the sample.

b = Total essential amino acids in the standard protein (egg or casein).

2.8.3. The Biological Value (BV)

The biological value of millet seed powder, defatted millet seed powder, and millet seed protein isolate from the studied samples was estimated according to the method proposed by (21), as follows:

$$Bv = 1.09 \times EAAI - 11.7$$

Where:

EAAI = Essential Amino Acid Index.

Bv = Biological Value.

2.8.4. Nutritional Index (NI)

The nutritional index value was estimated according to (22). using the following equation:

$$NI = (EAAI \times Protein\ \%) / 100$$

Where:

NI = Nutritional Index.

EAAI = Essential Amino Acid Index.

3. Results and Discussions

3.1. Chemical composition

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Table (3-1) shows the chemical composition of millet seed powder, defatted millet seed powder, and isolated millet seed protein. The results showed that the moisture content of each of (millet seed powder, defatted millet seed powder, and millet seed protein isolate) was (7.08, 8.16, 8.01)%, respectively, and that the highest value was for the defatted millet seed powder group. The difference was significant at $p \leq 0.05$ compared to the other two samples, and the lowest percentage was observed in the millet seed powder group. The difference was not significant at the $p \leq 0.05$ level compared to the other sample. These differences are attributed to the different manufacturing processes applied to these samples during the study. The oil content of millet seed powder, defatted millet seed powder, and millet seed protein isolate was 6.94%, 0.42%, and 0.30%, respectively. The highest oil content was observed in the millet seed powder group, with a significant difference ($p \leq 0.05$) compared to the other two samples. The lowest oil content was observed in the protein isolate group, with no significant difference ($p \leq 0.05$) compared to the other samples. This variation in oil content is attributed to different manufacturing processes, including isolation and milling, which remove a significant percentage of the oil. reported that millet seeds contain approximately 7% oil, and the oil content varies with variety, location, soil fertility, milling method, seed freshness, oil extraction method, and solvent type (23). A study conducted by (9) on arugula seeds showed that the highest percentage of oil is found in arugula seed powder (16.66%), and that the percentage of oil in arugula seed protein isolates (2.60%) is lower. The ash content of millet seed powder, defatted millet seed powder, and millet seed protein isolates was 2.16%, 2.16%, and 0.50%, respectively. The highest ash content was observed in millet seed powder and defatted millet seed powder, at 2.16% and 2.16%, respectively.] found the lowest ash contentin the millet seed protein isolate. The difference was significant at $p \leq 0.05$ compared to the other two samples. The variation in ash content is attributed to the milling and isolation processes, as well as the manufacturing processes employed during the study. The results showed that the protein percentage for each of (millet seed powder, defatted millet seed powder, millet seed protein isolates) was (6.83, 14.37, 71.25)%, respectively, and that the highest protein percentage was in the protein isolate group. The difference was significant at a probability level ($p \leq 0.05$) compared to the other two samples, and the lowest percentage was in the millet seed powder group. The difference was significant at a probability level ($p \leq 0.05$) compared to the other two samples. The increase in protein concentration is due to various manufacturing processes that remove most non-protein substances, such as fat, carbohydrates, and

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minerals. confirmed that several factors, including pH, temperature, flour solubility, and solvent-to-flour ratio, influence protein extraction (24). The study agreed with a study on cress seed protein isolate, which found that the highest protein content was in the protein isolate (71%) and the lowest in cress seed powder (13%) (9). It was observed that the carbohydrate content of each of (millet seed powder, defatted millet seed powder, and millet seed protein isolates) was (76.99, 74.89, and 19.94)%, respectively, with the highest value being for the millet seed powder group. The difference was significant at a probability level of ($p \leq 0.05$) compared to the other sample. The lowest value was observed in the protein isolate group, and the difference was significant ($p \leq 0.05$) compared with the other two samples.

Table (3-1): Chemical compositions of millet seed powder, defatted millet seed powder, and millet seed protein isolates.

Carbohydrates%	Protein %	Ashes %	Oil %	Moisture %	**Transactions** / **Product**
76.99 a	6.83 c	2.16 a	6.94 a	7.08 b	Millet Seed Powder
74.89 a	14.37 b	2.16 a	0.42 b	8.16 a	Defatted Millet Seed Powder
19.94 b	71.25 a	0.50 b	0.30 b	8.01 a	Millet Seed Protein Isolate

3.2. Mineral content

Table (3-2) shows the concentrations of mineral elements in millet seed powder, defatted millet seed powder, and millet seed protein isolates. The results showed that the iron concentration for each of (millet seed powder, defatted millet seed powder, and millet seed protein isolates) was (15.80, 18.70, and 270) mg/kg, respectively. The Table shows that the highest value was for the protein isolate group. The difference was significant at a probability level of ($p \leq 0.05$) compared to the other two

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samples. The lowest value was observed in the millet seed powder group, and the difference was significant ($p \leq 0.05$) compared to the other two samples. The results showed that the zinc concentration for each of (millet seed powder, defatted millet seed powder, and millet seed protein isolate) was (4.30, 6.62, 0) mg/kg, respectively, and the highest value was for the defatted millet seed powder group. The difference was significant at a probability level of ($p \leq 0.05$) compared to the other two samples. It was noted that the zinc element did not appear in the millet seed protein isolate. The calcium concentration for (millet seed powder, defatted millet seed powder, and millet seed protein isolate) was (262.0, 283.0, and 26.2) mg/kg, respectively. The Table shows that the highest value was observed in the defatted millet seed powder group, and the difference was significant ($p \leq 0.05$) compared to the other two samples. The lowest value was observed in the protein isolate group, and the difference was also significant ($p \leq 0.05$) compared to the other two samples. The phosphorus concentrations of millet seed powder, defatted millet seed powder, and millet seed protein isolate were found to be 615.0, 672.0, and 94.0 mg/kg, respectively. The highest concentration was observed in the defatted millet seed powder group, with a significant difference ($p \leq 0.05$) compared to the other two samples. The lowest concentration was observed in the protein isolate group, with a significant difference ($p \leq 0.05$) compared to the other two samples. It was observed that the magnesium concentration of (millet seed powder, defatted millet seed powder, and millet seed protein isolate) was (986.0, 1069.0, and 185.0) mg/kg, respectively. The highest value was observed in the defatted millet seed powder group, and the difference was significant ($p \leq 0.05$) compared with the other two samples. The lowest value was observed in the protein isolate group, and the difference was significant ($p \leq 0.05$) compared to the other two samples. The results showed that the manganese concentration in millet powder, defatted millet powder, and millet protein isolate was 28.1, 27.2, and 1.8 mg/kg, respectively. The highest concentration was observed in the millet powder group, with a significant difference ($p \leq 0.05$) compared to the other two samples. The lowest concentration was observed in the protein isolate group, with a significant difference ($p \leq 0.05$) compared to the other two samples. The variation in the percentage of certain minerals, whether higher, lower, or absent, is attributed to the manufacturing processes involved, such as milling and isolation, in producing the millet protein isolate. These processes resulted in varying levels of certain minerals. noted that environmental conditions such as high salinity, water scarcity, and high temperatures affect the mineral content of foods(25). confirmed that the hulling process

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reduces antinutrients that inhibit mineral bioavailability by forming complexes, thereby improving mineral bioavailability (26). reported that millet contains significantly higher levels of minerals, such as magnesium, manganese, and phosphorus, than other grains (27).

Table (3-2) Concentration of mineral elements of millet seed powder, defatted millet seed powder, and protein isolate of millet seeds.

Mn (mg/kg)	Mg (mg/kg)	P (mg/kg)	Ca (mg/kg)	Zn (mg/kg)	Fe (mg/kg)	Minerals / Product
28.1 a	986.0b	615.0 b	262.0 b	4.30 b	15.80 b	Millet Seed Powder
27.2 a	1069.0 a	672.0 a	283.0 a	6.62 a	18.70 a	Defatted Millet Seed Powder
1.8 b	185.0 c	94.0 c	26.2 c	0.00 c	270 c	Millet Seed Protein Isolate

3.3. Amino acids

Table (3-3) shows the amino acid ratios of millet seed powder, defatted millet seed powder, and millet seed protein isolate, and the amino acid ratios recommended for adults by the Food and Agriculture Organisation and the World Health Organisation (28). The results showed that the percentage of essential amino acids in millet seed powder was (2.39, 3.85, 5.77, 3.98, 8.66, 4.82, 9.34, 2.98)% for each of (histidine, threonine, valine, methionine, phenylalanine, isoleucine, leucine, and lysine), respectively. The highest percentage was for the essential amino acid (leucine), and the lowest was for the essential amino acid (histidine). The percentage of non-essential amino acids in millet seed powder was (11.42, 20.55, 5.71, 5.0, 6.86, 8.55)% for each of (aspartic acid, glutamic acid, serine, glycine, alanine, and tyrosine). The sequence showed the highest percentage of the non-essential amino acid (glutamic acid), and the lowest percentage of the non-essential amino acid (glycine). found the percentage of essential amino acids in defatted millet seed powder to be (2.35, 3.61, 5.45, 3.89, 8.26, 4.56, 8.84, 2.80)% for histidine, threonine,

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valine, methionine, phenylalanine, isoleucine, leucine, and lysine, respectively. Leucine had the highest percentage of essential amino acids, while histidine had the lowest. The percentage of non-essential amino acids in defatted millet seed powder was (10.79, 20.38, 5.42, 5.03, 6.47, 8.12, 3.94)% for aspartic acid, glutamic acid, and serine. Glycine, alanine, tyrosine, and cysteine) respectively, with the highest percentage for the non-essential amino acid (glutamic acid), and the lowest percentage for the non-essential amino acid (cysteine). observed that the percentage of essential amino acids in the millet seed protein isolate was (2.29, 3.77, 4.47, 3.85, 8.33, 4.61, 8.98, 2.84)% for histidine, threonine, valine, methionine, phenylalanine, isoleucine, leucine, and lysine, respectively. Leucine had the highest percentage of the essential amino acids, while histidine had the lowest. The percentage of non-essential amino acids in the millet seed protein isolate was (11.0, 19.76, 5.47, 4.99, 6.58, 8.21, 3.75)% for aspartic acid, glutamic acid, serine, and glycine. Alanine, tyrosine, and cysteine) respectively, with the highest percentage for the non-essential amino acid (glutamic acid), and the lowest percentage for the non-essential amino acid (cysteine). In a study of the Brusso millet variety to determine its protein content and essential amino acid composition, the researchers found that its protein content was similar to that of wheat. However, Brusso millet protein was significantly richer in the essential amino acids leucine, isoleucine, and methionine (29). The proportions of the amino acids lysine and methionine are generally lower in plant proteins compared to animal proteins (30),(31),(32). stated that millet is richer in the amino acid lysine than most grains and can be used as a supplementary protein source (33) indicated that non-essential amino acids (aspartic, glutamic, and alanine) are typically found in high concentrations in plant protein extracts (34). confirmed that these non-essential amino acids are beneficial to the human body because they can react with free radicals, thus preventing oxidative damage (35). found, in a study of amino acids in finger millet, kudu, and sorghum, a high content of essential amino acids, particularly sulfur-containing amino acids (cysteine and methionine) (36).

noted that millet flour contains higher levels of methionine, leucine, isoleucine, phenylalanine, and valine compared to wheat and rice (37).

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Table (3-3) Amino acid ratio of millet seed powder, defatted millet seed powder and millet seed protein isolate.

Recommendations FAO/* WHO %	Amino acids in milk and eggs %	Millet Seed Protein Isolate %	Defatted millet seed powder %	Millet Seed Powder %	Product / Amino Acid
-	-	11.0	10.79	11.42	Aspartic acid
-	-	19.76	20.38	20.55	Glutamic acid
-	-	5.47	5.42	5.71	Serine
1.9	2.2	2.29	2.35	2.39	Histidine
-	-	4.99	5.03	5.0	Glycine
4.0	4.7	3.77	3.61	3.85	Threonine
-	-	6.58	6.47	6.86	Alanine
-	-	8.21	8.12	8.55	Tyrosine
-	-	3.75	3.94	0	Cystine
3.9	6.6	5.47	5.45	5.77	Valine
-	-	3.85	3.89	3.98	Methionine
-	-	8.33	8.26	8.66	Phenylalanine
3.3	8.6	4.61	4.56	4.82	Isoleucine
5.9	5.4	8.98	8.84	9.34	Leucine
5.8	7	2.84	2.80	2.98	Lysine
2.5	5.7	7.6	7.83	3.98	Cys + Met
6.3	9.3	16.54	16.38	17.21	Phe + Tyr
-	-	-	-	-	Tryptophane
24.8	34.5	40.14	39.76	41.79	EAA
-	-	59.58	60.15	57.09	NEAA

FAO/WHO (2007) EAA: Essential amino acids. NEAA: Non-essential amino acids.

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3.4. Nutritional properties

Table (3-4) Table (3-5) shows the nutritional characteristics of millet seed powder, defatted millet seed powder, and millet seed protein isolate. The results showed that the Essential Amino Acid Index (EAAI) values for millet seed powder, defatted millet seed powder, and millet seed protein isolate were (82.70, 78.27, 79.37), respectively. As shown in the Table below, the highest value was for millet seed powder, and the lowest value was for defatted millet seed powder. studied the nutritional index (NI) value of millet seed powder, defatted millet seed powder, and millet seed protein isolate and reached (5.64, 11.24, 56.55), respectively. The Table shows that the highest value was for millet seed protein isolate, and the lowest was for millet seed powder. determined the biological value (BV) of millet seed powder, defatted millet seed powder, and millet protein isolateto be 78.44, 73.61, and 74.81, respectively. This value indicates the amount of protein the body can utilize. The same Table shows that the highest value was for defatted millet seed powder, and the lowest value was for defatted millet seed powder. found that adding fish protein isolate to meatballs increased the EAAI (Energy-Assisted Amino Acid Index) from 94.37% in the meat sample to 96.78% in the (25% fish protein isolate + meat) sample. He also noted an increase in the biological value (BV) from 91.4% to 93.68% for both samples (38).

Table (3-4) Nutritional characteristics of millet seed powder, defatted millet seed powder, and millet seed protein isolate.

Millet Seed Protein Isolate	Defatted Millet Seed Powder	Millet Seed Powder	**Nutritional Properties**
79.37	78.27	82.70	EAAI
74.81	73.61	78.44	BV
56.55	11.24	5.64	NI

(EAAI) Amino Acid Index (NI), Nutritional Index (BV), and Theoretical Biological Value.

The results showed that the degree of essential amino acid (AAS) for millet seed powder was (125.78, 96.25, 147.94, 146.06, 158.30, 51.37)%, for each of (histidine, threonine, valine, isoleucine, leucine, lysine), respectively. It is noted through Table (4-5) that the highest value was for the essential amino acid (leucine), and the lowest value was for the essential

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amino acid (lysine). The results showed that the degree of essential amino acid (AAS) of defatted millet seed powder was (123.68, 90.25, 139.74, 138.18, 149.83, 48.27)%, for each of (histidine, threonine, valine, isoleucine, leucine, lysine), respectively, and the highest value was for the essential amino acid (leucine). The lowest value was for the essential amino acid (lysine). The results showed that the degree of essential amino acid (AAS) of the millet seed protein isolate was (120.52, 94.25, 140.25, 139.69, 152.20, 48.96)% for each of (histidine, threonine, valine, isoleucine, leucine, lysine), respectively, and the highest value was for the essential amino acid (leucine). The lowest value was for the essential amino acid (lysine). Protein quality is determined based on its content of essential amino acids compared to a standard reference pattern; a score of (1.0 or 100%) or more indicates that the protein is complete and provides all of the metabolic needs of humans for essential amino acids and nitrogen (39).

Table (3-5) Essential Amino Acid (AAS) Grade of Millet Seed Powder, Defatted Millet Seed Powder, and Millet Seed Protein Isolate.

Recommendations *FAO/WHO (2007) %	AAS for millet seed protein isolate %	AAS Defatted Millet Seed Powder %	AAS for millet seed powder %	**Amino acids**
1.9	120.52	123.68	125.78	Histidine
4.0	94.25	90.25	96.25	Threonine
3.9	140.25	139.74	147.94	Valine
...	...	...	...	Methionine
...	...	...	...	Phenylalanine
3.3	139.69	138.18	146.06	Isoleucine
5.9	152.20	149.83	158.30	Leucine
5.8	48.96	48.27	51.37	Lysine
2.5	304	313.2	159.2	Methionine + Cystine
6.3	262.53	260	273.17	Phenylalanine + Tyrosine

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3.5. Functional properties

Table (3-6) shows the functional properties of millet seed powder, defatted millet seed powder, and millet seed protein isolate. The Table shows that the water absorption capacity of millet seed powder, defatted millet seed powder, and millet seed protein isolate was 1.32, 1.34, and 3.2 g/g, respectively. The highest value was observed in the millet seed protein isolate group, with a significant difference ($p \leq 0.05$) compared to the other two samples. The lowest value was observed in the millet seed powder group, with a non-significant difference at $p \leq 0.05$ but a significant difference compared to the other samples. The difference in water absorption capacity between samples is due to the presence of polar and nonpolar groups and their distribution on the protein's outer surface, as well as to pH, which significantly influences water absorption capacity. The results showed that the fat absorption capacity (for millet seed powder, defatted millet seed powder, and millet seed protein isolate) was 1.06, 1.1, and 3.6 g/g, respectively. The Table shows that the highest value was observed in the millet seed protein isolate group, with a significant difference ($p \leq 0.05$) compared to the other two samples. The lowest value was observed in the millet seed powder group, with no significant difference ($p \leq 0.05$) compared to the other samples. The high fat-binding Capacity of the protein is attributed to its hydrophobic groups, which facilitate the formation of hydrophobic bonds, thereby increasing the amount of bound fat. The results showed that the emulsifying power of millet seed powder, defatted millet seed powder, and millet seed protein isolate was 44.77%, 45.58%, and 47.82%, respectively. The highest value was observed in the millet seed protein isolate group, with a significant difference ($p \leq 0.05$) compared to the other two samples. The lowest value was observed in the millet seed powder group, with no significant difference at $p \leq 0.05$ compared with the other samples. The results showed that the emulsification stability for millet seed powder, defatted millet seed powder, and millet seed protein isolate was 96.66%, 96.77%, and 106.06%, respectively. The highest Stability was observed with the millet seed protein isolate, with a significant difference ($p \leq 0.05$) compared to the other two samples. The lowest Stability was observed with millet seed powder, with no significant difference ($p \leq 0.05$) compared to the other two samples. The results showed that the percentage of foam capacity for each of (millet seed powder, defatted millet seed powder, and millet seed protein isolate) was (12, 8, 20)%, respectively. The highest percentage was for the millet seed protein isolate group. The

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difference was significant at the probability level ($p \leq 0.05$) with the other two samples, and the lowest value was for the defatted millet seed powder group. The difference was not significant at the probability level ($p \leq 0.05$), but was significant with the other two samples. The results showed that the foam stability percentage for (millet seed powder, defatted millet seed powder, and millet seed protein isolate) was (8, 8, and 12)%, respectively. The highest percentage was observed in the millet seed protein isolate group, and the difference was significant at a probability level of $p \leq 0.05$ compared to the other two samples. The lowest values were observed in the millet seed powder and defatted millet seed powder groups. found that isolated soy protein exhibited the highest water absorption capacity and the highest oil absorption capacity, at 2.12 ± 0.08 and 3.11 ± 0.12 g/g, respectively (40). stated that proteins extracted from plant sources contribute to the production of the required physical and chemical properties in various types of emulsifiers. The protein's emulsifying ability is attributed to its hydrophobic and hydrophilic properties. The protein reduces the interfacial tension between oil and water, and electrostatic repulsion helps stabilise oil droplets, thereby facilitating emulsion formation (41). indicated that the emulsifying Capacity of proteins depends on the balance between hydrophilic and hydrophobic properties, which is influenced by pH values. They noted that the highest emulsification values were observed at both low and high pH levels (11). stated that the high foaming capacity of protein isolates is due to the formation of surface protein films, which increase the surface area of water and air, thus promoting the encapsulation of air bubbles (42). indicated that the ability to form foam is best demonstrated by proteins with low molecular Weight, high hydrophobicity, high solubility, low net charge at a given pH, and easy and rapid denaturation (43). indicated that foam stability depends on the protein membrane and that low foam stability may be attributed to charge repulsion between protein molecules, which reduces cohesion and viscosity of the protein membranes, thereby weakening foam stability (44).

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Table (3-6) Functional properties of millet seed powder, defatted millet seed powder, and millet seed protein isolate.

Functional properties						Product
Foam stability %	Foam capacity %	Emulsification stability %	Emulsification %	fat absorption capacity (g/g)	Water absorption capacity (g/g)	
8 b	12 b	96.66 b	44.77b	1.06 b	1.32 b	Millet Seed Powder
8 b	8 c	96.77 b	45.58 b	1.10 b	1.34 b	Defatted Millet Seed Powder
12 a	20 a	106.06 a	47.82 a	3.60 a	3.20 a	Millet Seed Protein Isolate

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