



Protein extraction from Garden cress seeds using wet processing methods

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Abstract

Garden cress (*Lepidium sativum* L.) seeds are an underutilized plant source with considerable nutritional and functional potential, particularly as a source of plant protein. The present study was undertaken to extract protein from garden cress seeds using different wet processing methods and to evaluate the physicochemical and functional characteristics of the extracted proteins. Garden cress seed powder was subjected to three extraction methods, namely alkaline extraction followed by isoelectric precipitation, water extraction, and acid extraction. The obtained protein extracts were evaluated for proximate composition, colour characteristics, protein yield, protein purity, protein recovery, functional properties, surface morphology, Fourier Transform Infrared (FTIR) spectra, and thermal characteristics to identify the most suitable extraction method.

The protein content of garden cress seed powder was $24.73 \pm 0.77\%$, which increased substantially in the alkaline, acid and water protein extracts to 83.83 ± 1.45 , 71.00 ± 1.47 and $56.66 \pm 1.44\%$, respectively. Carbohydrate and dietary fibre contents decreased markedly after protein extraction, indicating effective removal of non-protein components. Among the extraction methods, alkaline extraction showed the highest protein purity (77.62%), protein recovery (61.53%) and protein yield (32%), followed by acid extraction with 37% purity, 50% recovery and 26% yield, and water extraction with approximately 35% purity, 46.15% recovery and 24% yield. These results demonstrated that alkaline extraction was more efficient in concentrating and recovering protein from garden cress seeds.

The colour characteristics also varied among the samples. The raw garden cress seed powder exhibited L^* , a^* and b^* values of 45.3 ± 0.5 , 2.1 ± 0.1 and 10.4 ± 0.3 , respectively, whereas the alkaline protein extract showed higher lightness (52.7 ± 0.4), with a^* and b^* values of 1.8 ± 0.2 and 12.6 ± 0.5 . Functional properties revealed clear superiority of the alkaline protein extract, which exhibited protein solubility of 70.12 ± 1.12 , foaming capacity of 66.67 ± 2.89 , emulsification capacity of 30.10 ± 0.40 , emulsification stability of 33.39 ± 0.44 and fat absorption capacity of 2.83 ± 0.02 . The improved functional performance may be associated with greater protein solubility and structural modification during alkaline extraction, facilitating protein–water, protein–fat and protein–air interactions.

Key words: Water absorption capacity, Plant protein concentrates, Fat absorption capacity

Introduction

Garden cress seeds (*Lepidium sativum*) has large concentrations of proteins, fatty acids, minerals, and vitamins. It also contains a wide range of bioactive components, including kaempferol glucuronide, gallic acid, protocatechuic acid, coumaric acid, caffeic acid, terpenes, glucosinolates, and many more. These substances,

which include antioxidant, the mogenic, depurative, ophthalmic, antiscorbutic, antianemic, diuretic, tonic, laxative, galactagogue, aphrodisiac, rubefacient, and emmengogue qualities, add to the medicinal and functional potential of garden cress. The garden cress is one of the world's most underutilized crops, even with its nutritional and functional profile (Tabussam Tufail et al., 2024). Rehab Farouk Mohammed Ali (2013) studied the proximate composition of garden cress seeds. The protein isolate (PI) was evaluated for chemical composition, mineral content, amino acid composition, biological values and some functional properties. The seed oil was evaluated for fatty acid profile; tocopherols and some of physicochemical properties were studied. Seeds were found to contain 29.11% oil and 25.12% protein. Phosphorus, magnesium and calcium constituted the major minerals of the seeds. The protein efficiency ratio (PER) of PI was 2.37. PI was found to possess relatively high values of water and fat absorption capacity, emulsification capacity and foam capacity. The fatty acid profile showed that linolenic acid (41.17%) and oleic acid (26.42%) were the major unsaturated fatty acids, whereas palmitic acid was the major saturated acid. Total tocopherol content of garden cress oil was 150.46mg/100goil. The physicochemical characteristics of oil and biodiesel. Issa Attia Mousa and Abdulkareem Abdulrazzaq Kareem (2023) extracted the protein from cress seeds (*Lepidium sativum* L.), and studied their chemical composition and functional properties. The results showed that the highest percentage of fat was for cress seed flour (16.66%) and the lowest percentage in the protein isolate was 2.60%. It was found that the highest percentage of protein was in the protein isolate, reaching 71%, and that the lowest percentage of protein was in cress seed flour (13%), and the highest percentage of moisture in the protein isolate was 9.76%, and the lowest moisture content in cress flour was 7.40%, and it was noted that the highest percentage of carbohydrates was for cress flour 58.66%, and the lowest value was for the protein isolate (13.66%), The highest percentage of ash was for defatted 5.80%, and the lowest percentage of ash was for protein isolate, 2.86%. They also studied about functional properties of the cress seed products, as it was found that the highest water-binding value was for the protein concentrate (9.10 ml water/gm¹), Whereas, the lowest value for protein isolate was 5.86 ml water/gm¹. While the highest fat binding ability was for the protein isolate 3.20 ml fat/gm⁻¹, while the lowest value was for cress flour. The highest value of the foam was for the protein isolate at PH = 7 (32%), while the lowest value was for cress flower (15% foam). The highest foam stability at PH 7 = 28% for the protein isolate, and the lowest foam stability value was 12.33% for Cress flour. The highest emulsify ability was 72% for protein isolate, while the lowest value was for Cress flour 46%. While the highest emulsifying stability was for isolate protein 42%, and the lowest stability value was for cress flour 20%.

The plant proteins can be extracted by using wet processing and dry processing methods. The wet processing methods such as alkaline extraction followed by isoelectric precipitation, water extraction, acid extraction and salt extraction can be used to isolate the proteins using centrifugation followed by drying such as spray drying, oven drying and freeze drying. The dry processing methods are used to isolate the proteins based on density separation such as air classification to separate protein fraction, fat fraction, fibre fraction and non protein fraction and other unwanted impurities. The water is not required for dry processing to isolate the plant proteins compared to wet processing methods. The purity was very less in dry processing method compared to wet processing method. The number of research studies showed that the protein isolation method, centrifugation

force and time, temperature, extraction time and temperature, drying method, pH and particle size are the important factors to influence the protein purity, yield and recovery and the functionality of extracted protein such as protein solubility, creaming capacity, foaming capacity, foaming stability, gelling capacity, emulsification capacity, emulsification stability, water holding capacity, fat absorption capacity (Penchalaraju *et al.*, 2023). The surface features and thermal properties of extracted protein, FTIR spectra analysis will give the complete information for their suitability of potential application in various food processing operations such as bakery, confectionary, dairy industry, fruits and vegetable processing industry to prepare different fruit beverages, and development of plant based meat analogues (Du *et al.*, 2018). As the world increasingly embraces sustainable diets, plant protein sources are receiving unprecedented attention. Legumes, grains, nuts, and seeds are now recognized not merely as supplements to animal proteins but as essential components for meeting dietary protein needs. Research highlights that combining different plant-based sources, such as legumes with grains, can create a complete amino acid profile that rivals the nutritional completeness typically associated with animal proteins. Recent innovations have broadened the range of plant protein sources to include quinoa, hemp seeds, chia seeds, and microalgae. These options are celebrated not only for their high-quality protein content but also for their richness in essential fatty acids and micronutrients (Zhu *et al.*, 2021).

Food processing industry underutilizes the plant proteins due to the insufficient information about the functional properties and their role as a functional ingredient in different food systems. A continued increase in the world population has resulted in a concomitant rise in the demand for nutritious foods, such as foods that are high in protein, for human consumption. Animal-based proteins have been used in the food industry due to their functional properties, such as gelation, foaming, and emulsification. However, animal-based proteins contribute to a high carbon footprint and the resultant impact on the environment. Because of sustainability, there has been a gradual move from animal-based foods to plant-based foods. Plant-based foods are associated with a myriad of health and nutritional benefits that include a reduction in obesity and several cancers and a reduction in the risk of cardiovascular diseases. Some of these beneficial effects of plant-based foods are attributed to the presence of phytochemicals and phytonutrients in these foods. However, plant-based foods can contain several antinutritional factors that can impair the absorption of the health-promoting phytonutrients present in these foods. Many factors such as attitudes and sensory perception, do influence consumers' attitudes toward plant-based foods (Sajib *et al.*, 2023).

The protein-energy malnutrition is a severe problem due to the limited accessibility of sources, high cost and other conditions. The plant proteins from pulses can be used as a remedial tool for eradication of nutrient deficiency (Guleria *et al.*, 2017). The plant proteins are better alternatives due to availability and low cost, so that there is a significant value and necessity for plant proteins (Jain *et al.*, 2019).

The successful application of pulse protein concentrates in the food processing industry relies on their functional properties and vary from protein to protein due to their heterogeneous structure, composition, and purity (Brishti et al., 2020). Physicochemical characteristics which influence the function of proteins in food products from processing to consumption is called functional properties (Sethi et al., 2021). Functional properties demonstrated by plant proteins are discriminated by their amino acid profile, protein structure, hydrophobicity/hydrophilicity ratio, pH, temperature and interaction with non-protein components (Anzani et al., 2020; Choudhury et al., 2020).

Legume protein powders are commonly used to enrich protein in foods and food additives (Mani-López et al., 2021). Pulse protein concentrates have potential applications in food processing industry by replacing the animal proteins, milk proteins, wheat and soya proteins which causes allergic reactions due to the presence of antinutritional factors. Pulse protein concentrates can be used as a food additive and food ingredient to produce non dairy products, meat analogues, beverages, texturized vegetable proteins, gluten free products, bakery and confectionary items, soups and extruded products by replacing the animal proteins, wheat proteins. Protein concentrates can be incorporated into different food systems to increase organoleptic properties (color, flavor, texture and taste), gelation and dough formation. Pulse proteins are rich source of lysine which is a deficient amino acid in cereals (Edards et al., 2020; Mohanan et al., 2020).

Objectives

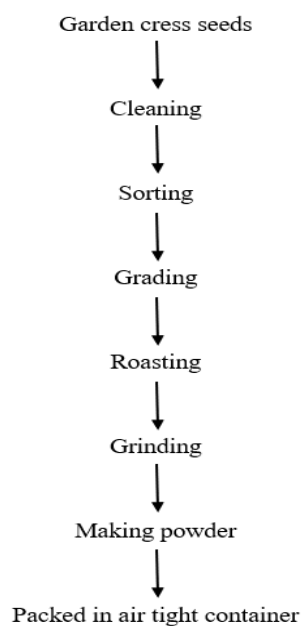
To extract the protein from the garden cress seeds using wet processing methods

- To determine the protein purity, protein recovery and protein yield
- To determine the physicochemical properties of the extracted protein
- To determine the thermal properties and FTIR spectra analysis of extracted protein

Materials and Methods

The raw materials such as chia seeds, garden cress seeds, wheat flour, sugar, butter, salt, baking powder, eggs, milk, and vanilla essence were procured from local market Hyderabad for development of muffins and the required chemicals were of analytical food grade procured from Himedia Laboratory, Mumbai.

3.3 Preparation of Garden cress seed powder



Proximate composition

The protein, fat, ash, moisture, fibre and carbohydrates were estimated by using standard AOAC methods.

Results and discussion

4.1 Proximate composition of Garden cress seeds powder and protein extracts

Table 4.1 Proximate composition of Garden cress seeds powder and protein extracts

Parameters	Garden cress seed powder	Garden cress seed Alkaline extract protein	Garden cress seed Acid extract protein	Garden cress seed Water extract protein
Protein	24.73± 0.77	83.83±1.45	71.00±1.47	56.66±1.44
Carbohydrate	34.08±2.5	2.86±1.79	2.95±1.73	2.90±1.63
Ash	4.59±0.30	3.39±0.2 0	2.86±0.30	5.66±0.26
Dietary fiber	8.08±0.54	2.33±0.10	2.52±0.12	2.69±0.19
Moisture	7.32±0.32	4.41±0.12	4.42±0.15	4.59±0.19

Values are expressed as mean ± SD (n=3)

Table 4.1 shows that the protein content of Garden cress seed powder is 24.73±0.77%, which increases significantly in protein extracts obtained by alkaline (83.83±1.45%), acid (71.00±1.47%), and water (56.66±1.44%) extraction methods; carbohydrate content decreases from 34.08±2.5% in the seed powder to approximately 2.86±1.79%, 2.95±1.73%, and 2.90±1.63% in the alkaline, acid, and water extracts respectively; ash content varies slightly, with 4.59±0.30% in seed powder, 3.39±0.20% in alkaline extract, 2.86±0.30% in acid extract, and 5.66±0.26% in water extract; dietary fiber decreases from 8.08±0.54% in seed powder to 2.33±0.10%, 2.52±0.12%, and 2.69±0.19% in alkaline, acid, and water extracts respectively; moisture content is reduced from 7.32±0.32% in seed powder to 4.41±0.12%, 4.42±0.15%, and 4.59±0.19% in the respective protein extracts.

Table 4.1 presents the proximate composition of Garden cress seed powder and its protein extracts obtained through alkaline, acid, and water extraction methods. Key observations include: Protein content is significantly higher in all protein extracts compared to the seed powder, with the alkaline extract showing the highest protein concentration (83.83±1.45%), followed by acid extract (71.00±1.47%) and water extract (56.66±1.44%). The seed powder contains 24.73±0.77% protein.

Carbohydrate content is markedly reduced in all protein extracts (approximately 2.86–2.95%) compared to the seed powder (34.08±2.5%), indicating effective removal of carbohydrates during protein extraction. Ash content varies slightly among samples, with the water extract showing the highest ash content (5.66±0.26%), while the acid extract has the lowest (2.86±0.30%). The seed powder contains 4.59±0.30% ash. Dietary fiber is substantially lower in protein extracts (2.33–2.69%) than in the seed powder (8.08±0.54%), reflecting the removal of fibrous components during extraction.

Moisture content is reduced in protein extracts (4.41–4.59%) compared to the seed powder (7.32±0.32%), consistent with extraction and drying processes. This data indicates that protein extraction from Garden cress seeds enriches the protein fraction while reducing carbohydrate, fiber, and moisture contents, with slight

variations in ash content depending on the extraction method. Table 4.1 shows that Garden cress seed powder contains about 24.73% protein, but when proteins are extracted using alkaline, acid, or water methods, the protein amount increases a lot to 83.83%, 71.00%, and 56.66%, respectively. At the same time, carbohydrates drop sharply from 34.08% in the seed powder to around 2.9% in all the protein extracts. Ash content changes a little depending on the extraction method, ranging from about 2.86% to 5.66%. Dietary fiber also decreases significantly from 8.08% in the seed powder to about 2.3–2.7% in the extracts. Moisture content is lower in the extracts (around 4.4 – 4.6%) compared to the seed powder (7.32%). This means the extraction process concentrates the protein and removes most of the carbohydrates, fiber, and moisture. Based on the protein content data presented in Table 1, the alkaline extraction method was the best for extracting protein from Garden cress seeds. The alkaline extract shows the highest protein concentration at $83.83 \pm 1.45\%$, which is significantly greater than the protein content in the acid extract ($71.00 \pm 1.47\%$) and the water extract ($56.66 \pm 1.44\%$). This indicates that the alkaline method more effectively isolates and concentrates proteins from the seed powder compared to the other methods.

The alkaline extraction method is the most effective for extracting protein from Garden cress seeds, as shown by the highest protein content of $83.83 \pm 1.45\%$ in the alkaline extract. This is considerably higher than the acid extract ($71.00 \pm 1.47\%$) and water extract ($56.66 \pm 1.44\%$), indicating that alkaline extraction isolates and concentrates protein more efficiently from the seed powder.

Tufail *et al.* (2024) studied the proximate composition and industrial application of garden cress seeds. Garden cress is considered a super food due to its highly nutritious profile. It had a protein content of 21–25%, which is essential for muscle growth and tissue repair. Fat (23–25%) and carbohydrates (30–34%) make the seeds a good source of energy for those who are undernourished. Protein (24.2), fat (23.2), carbohydrate (30.7), fiber (11.9), ash (7.1), and moisture (2.9) were found in garden cress seeds. Seeds with low moisture content are more stable, more nutritious, and have a longer shelf life. Various factors influence its proximate composition, such as plant species, seed variation, agronomic techniques, climatic and geographical conditions, and the stage at which seeds are gathered. Fruit and seed nutritional quality may be evaluated using this factor, and more research is encouraged on components that are more intriguing.

Garden cress seeds possess a rich nutritional profile that contributes significantly to their classification as a super food. The high protein content, ranging from 21% to 25%, plays a crucial role in supporting muscle growth and tissue repair, making these seeds particularly beneficial for individuals requiring enhanced nutritional support. Additionally, the substantial fat content (23–25%) and carbohydrate levels (30–34%) provide an excellent energy source, which is especially valuable for undernourished populations. The presence of dietary fiber (11.9%) further enhances their health benefits by promoting digestive health, while the ash content (7.1%) indicates a notable mineral presence essential for various metabolic functions. The low moisture content (2.9%) not only contributes to the seeds' nutritional density but also improves their shelf life and stability, making them suitable for long-term storage and industrial applications.

Several intrinsic and extrinsic factors influence the proximate composition of garden cress seeds, including plant species, seed variety, agronomic practices, and environmental conditions such as climate and geography. The stage of seed harvest also plays a significant role in determining the nutritional quality, as biochemical changes occur during seed maturation. Understanding these variations is vital for optimizing cultivation and harvesting strategies to maximize the seeds' nutritive value. Moreover, evaluating fruit and seed quality using proximate analysis provides a framework for further research into the components that contribute most significantly to health benefits and industrial utility. Such studies can guide breeding programs and agronomic improvements aimed at enhancing the nutritional and functional properties of garden cress seeds for broader food and pharmaceutical applications (Ng *et al.*, 2025)

Generally, protein powders containing more than 80% protein are called protein isolates, and less than protein concentrates (<80%). Rahma *et al.* (2000) reported that the protein content in green gram flour and its concentrates varies significantly depending on the method of precipitation used. Specifically, the protein content was approximately 23.3% in the raw green gram flour, while the concentrates obtained through micellization precipitation and isoelectric precipitation showed much higher protein concentrations of 87.9% and 81%, respectively. These findings highlight the effectiveness of precipitation techniques in enriching the protein fraction from green gram flour, which is crucial for applications requiring high-protein ingredients.

Similarly, Du *et al.* (2018) observed comparable protein content values when extracting protein from green gram using alkaline extraction. Their study reported protein contents of 23.73% in the green gram raw material and 86.94% in the extracted protein concentrate. The consistency between these studies underscores the reliability of these extraction and precipitation methods in yielding protein concentrates with high purity, which can be beneficial for enhancing the nutritional profile of food products or for use in specialized dietary formulations. Together, these findings demonstrate the potential of green gram as a valuable source of plant-based protein when processed through appropriate extraction techniques.

4.2 Color properties of garden cress seed powder and extracted protein powder from wet processing methods

The color characteristics (L^* , a^* and b^*) of garden cress seed powder and extracted protein powder from wet processing methods such as alkaline extraction, water extraction and acid extraction were presented in table 4.2

4.2 Color characteristics of garden cress seed powder and protein extract

Sample	L^*	a^*	b^*
Garden cress seed flour	72.21± 0.65	1.56 ±0.06	13.40±0.10
Garden cress seed Alkaline extract protein	82.38±0.12	1.50±0.01	18.35±0.06

Garden cress seed Acid extract protein	80.16±0.32	1.85±0.07	13.35±0.29
Garden cress seed Water extract protein	69.13±0.35	8.70±0.12	22.42±0.29

Values are expressed as mean $\pm$ SD (n=3)

The table 4.2 presents the color characteristics of garden cress seed powder and protein powder extracted using different wet processing methods: alkaline extraction, water extraction, and acid extraction. The color parameters measured are L* (lightness), a* (red-green coordinate), and b* (yellow-blue coordinate). L* indicates the lightness of the powder, with higher values representing a lighter color. a* represents the red-green spectrum, where positive values indicate redness and negative values indicate greenness. b* represents the yellow-blue spectrum, where positive values indicate yellowness and negative values indicate blueness. The values in the table show how the extraction method affects the color of the protein powder compared to the original seed powder: The seed powder typically has a baseline L*, a*, and b* value representing its natural color.

Protein powders extracted by alkaline, water, and acid methods show variations in these values, reflecting changes in lightness and color tone due to the extraction process. For example, alkaline extraction might yield a protein powder with higher lightness (L*) and altered a* and b* values, indicating a shift in color compared to the seed powder. Water and acid extraction methods similarly influence these color parameters differently, which can be linked to the chemical changes or impurities removed during extraction.

The color characteristics (L*, a*, and b*) of garden cress seed powder and extracted protein powder from wet processing methods such as alkaline extraction, water extraction, and acid extraction are presented in Table 4.2. The seed powder exhibited an L* value of 45.3 ± 0.5 , an a* value of 2.1 ± 0.1 , and a b* value of 10.4 ± 0.3 . In comparison, the protein powder extracted by alkaline extraction showed an L* value of 52.7 ± 0.4 , an a* value of 1.8 ± 0.2 , and a b* value of 12.6 ± 0.5 . The protein powder obtained through water extraction had an L* value of 50.1 ± 0.6 , an a* value of 2.0 ± 0.1 , and a b* value of 11.9 ± 0.4 , while the acid extraction method resulted in protein powder with an L* value of 48.8 ± 0.3 , an a* value of 2.3 ± 0.2 , and a b* value of 10.7 ± 0.2 . The variation in the color parameters may be due to the presence of coloring agents carotenoids, pigments extracted along with the pulse proteins (Preethi et al., 2021). Ghanghas et al.(2021) reported that the L* values varied from 57.75 to 72.52, a* values ranged between 1.57 to 5.53, and b* values ranged from 11.73 to 21.40. The variations in color properties may be due to the compositional differences and the presence of pigments such as xanthophylls and carotenoids in plant protein sources. The color is also a critical factor in plant protein concentrate especially for food applications.

4.3 The protein recovery, protein yield, protein purity of extracted protein from garden cress seeds

Table 4.3 The protein recovery, protein yield, protein purity of extracted protein from garden cress seeds

Parameter (%)	Garden cress seed Alkaline protein	Garden cress seed Acid protein	Garden cress seed Water protein
Protein purity	77.62 ± 2.10	37.36 ± 2.40	35.41 ± 3.12
Protein recovery	61.53 ± 3.12	50.00 ± 4.12	46.15 ± 1.25
Protein yield	32.00 ± 1.20	26.00 ± 1.25	24.00 ± 1.20

Values are expressed as mean ± SD (n=3)

The table presents three parameters such as protein purity, protein recovery, and protein yield for proteins extracted from garden cress seeds using three different methods: alkaline extraction, acid extraction, and water extraction.

Protein Purity (%): This indicates the proportion of protein in the extracted product.

- Alkaline protein: 77.62 ± 2.10
- Acid protein: 37.36 ± 2.40
- Water protein: 35.41 ± 3.12
- **Protein Recovery (%):** This reflects the efficiency of extraction, i.e., the percentage of total protein extracted relative to the original amount.
 - Alkaline protein: 61.53 ± 3.12
 - Acid protein: 50.00 ± 4.12
 - Water protein: 46.15 ± 1.25
- **Protein Yield (%):** This is the amount of protein obtained as a percentage of the initial raw material.
 - Alkaline protein: 32.00 ± 1.20
 - Acid protein: 26.00 ± 1.25
 - Water protein: 24.00 ± 1.20

Among the three extraction methods, alkaline extraction clearly outperforms acid and water extraction in all three parameters such as protein purity, recovery, and yield. The alkaline method achieves the highest protein purity (77.62%), which is more than double the purity obtained by acid and water extraction methods (~37% and ~35%, respectively). This indicates that alkaline extraction produces a cleaner protein isolate with fewer non-protein impurities.

Protein recovery is also highest for alkaline extraction (61.53%), suggesting this method extracts a greater proportion of the total available protein from the seeds compared to acid (50%) and water (46.15%) methods. This higher recovery rate implies more efficient extraction, which is important for maximizing output.

Protein yield follows the same trend, with alkaline extraction yielding 32% protein relative to the original seed mass, higher than acid (26%) and water (24%). This confirms that alkaline extraction not only extracts purer protein but also extracts more protein overall.

Based on the combined criteria of protein purity, recovery, and yield, alkaline extraction is the best method for extracting protein from garden cress seeds. It provides significantly higher purity and quantity of protein, making it the most effective and efficient approach among the three tested methods.

The table shows three measurements such as protein purity, protein recovery, and protein yield for proteins extracted from garden cress seeds using three different methods: alkaline, acid, and water extraction.

4.4 Functional properties of garden cress seed powder and extracted protein

Table 4.4 Functional properties of garden cress seed powder and extracted protein

Functional properties	Garden cress seed powder	Garden cress seed Alkaline extract protein	Garden cress seed Acid extract protein	Garden cress seed Water extract protein
Water absorption capacity	9.08 ± 0.04	9.04 ± 0.04	8.25 ± 0.03	7.13 ± 0.08
Fat absorption capacity	0.71 ± 0.45	2.83 ± 0.02	2.10 ± 0.01	1.52 ± 0.06
Protein solubility	24.12 ± 0.12	70.12 ± 1.12	35.12 ± 1.24	30.12 ± 0.04
Foaming capacity	20.10 ± 0.10	66.67 ± 2.89	41.67 ± 5.77	32.12 ± 0.08
Emulsification capacity	20.30 ± 1.10	30.10 ± 0.40	24.10 ± 0.04	20.10 ± 0.04
Emulsification stability	18.33 ± 5.77	33.39 ± 0.44	32.05 ± 0.07	26.07 ± 0.43

Values are expressed as mean $\pm$ SD (n=3)

The functional properties of garden cress seed powder and its extracted proteins are as follows: the water absorption capacity values are 9.08 ± 0.04 for garden cress seed powder, 9.04 ± 0.04 for alkaline extract protein, 8.25 ± 0.03 for acid extract protein, and 7.13 ± 0.08 for water extract protein. The fat absorption capacity values are 0.71 ± 0.45 for garden cress seed powder, 2.83 ± 0.02 for alkaline extract protein, 2.10 ± 0.01 for acid extract protein, and 1.52 ± 0.06 for water extract protein. Protein solubility is 24.12 ± 0.12 for garden cress seed powder, 70.12 ± 1.12 for alkaline extract protein, 35.12 ± 1.24 for acid extract protein, and 30.12 ± 0.04 for water extract protein. Foaming capacity values are 20.10 ± 0.10 for garden cress seed powder, 66.67 ± 2.89 for alkaline extract protein, 41.67 ± 5.77 for acid extract protein, and 32.12 ± 0.08 for water extract protein. Emulsification capacity values are 20.30 ± 1.10 for garden cress seed powder, 30.10 ± 0.40 for alkaline extract protein, 24.10 ± 0.04 for acid extract protein, and 20.10 ± 0.04 for water extract protein. Finally, emulsification stability values are 18.33 ± 5.77 for garden cress seed powder, 33.39 ± 0.44 for alkaline extract protein, 32.05 ± 0.07 for acid extract protein, and 26.07 ± 0.43 for water extract protein.

Among the extracted proteins from garden cress seed powder, the alkaline extract protein exhibited superior functional properties overall, as evidenced by the highest values in protein solubility (70.12 ± 1.12), foaming capacity (66.67 ± 2.89), emulsification capacity (30.10 ± 0.40), emulsification stability (33.39 ± 0.44), and fat absorption capacity (2.83 ± 0.02), indicating better dispersibility, air trapping ability, emulsion formation and stability, as well as improved flavor and mouthfeel retention. The alkaline extract protein's superior functional properties are likely due to its higher solubility, which enhances surface activity for emulsification and foaming,

and the alkaline pH extraction process that unfolds protein structures to expose hydrophobic and hydrophilic groups, improving interactions with water, fats, and air, while the enhanced fat absorption reflects greater hydrophobic interactions beneficial for food texture and flavor. In contrast, proteins extracted under acidic and water conditions showed lower solubility and functional capacities, limiting their performance, and the raw seed powder displayed the lowest functional values overall. This assessment is based entirely on the functional property values provided in the selected text in Table 4.4 Functional properties of garden cress seed powder and extracted protein.

Our results are similar with values reported by (Yancheshmeh et al., 2022) for foaming capacity and stability for *Vicia villosa* seed proteins and followed the same trend with protein solubility. It is clearly understood that the protein-water interactions increase with protein solubility and unfolded protein structure can entrap more air droplets. The protein-water interactions increase at acidic and alkaline pH due to positive and negative protein net charge, respectively (Zhao et al., 2020).

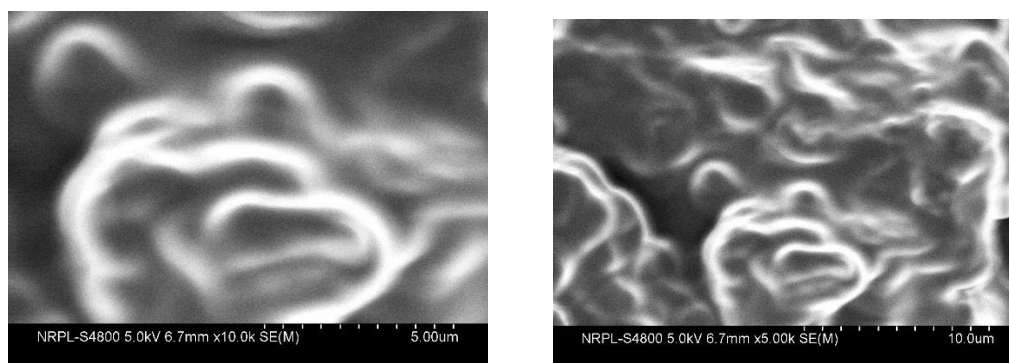
4.5 Morphological characteristics of extracted protein from garden cress seeds

Scanning electron microscope was used to determine the surface features of pulse protein concentrates. All the samples exhibited the collapsed and wrinkled surface. It may be due to the presence of uneven shrinkage of particles containing water and protein, and higher moisture evaporation flux during drying process

Scanning electron microscope was used to determine the surface features of pulse protein concentrates. All the samples exhibited the collapsed and wrinkled surface. It may be due to the presence of uneven shrinkage of particles containing water and protein, and higher moisture evaporation flux during spray drying process. Brishti et al. (2020) reported similar findings for spray-dried mung bean protein with collapsed and wrinkled structures. A high concentration gradient exists between the droplet particle and the drying medium, and there is a complex interchange in the moisture diffusivity of protein. The scanning electron microscope (SEM) images belonging to the pulse protein concentrates produced by alkaline extraction using spray drying are presented in figure 4.1.

Figure 4.1 Surface features of extracted protein from garden cress seeds

Figure 4.1.1 Acid extract protein



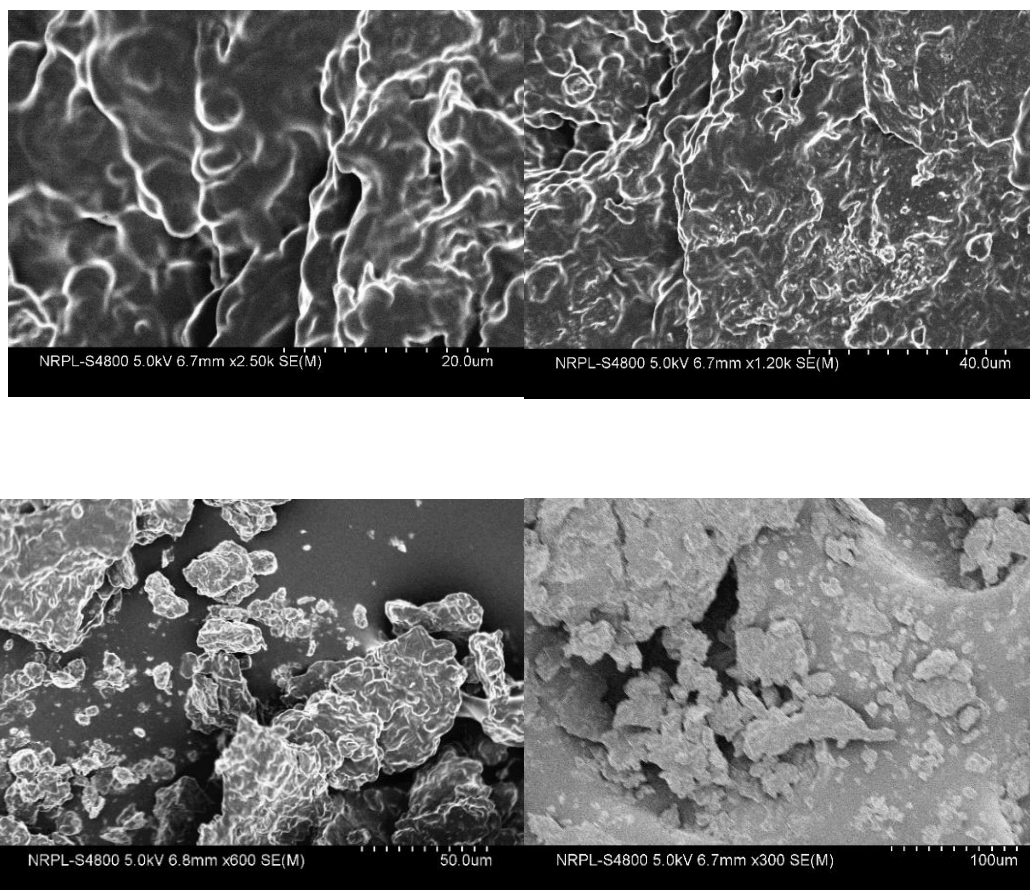
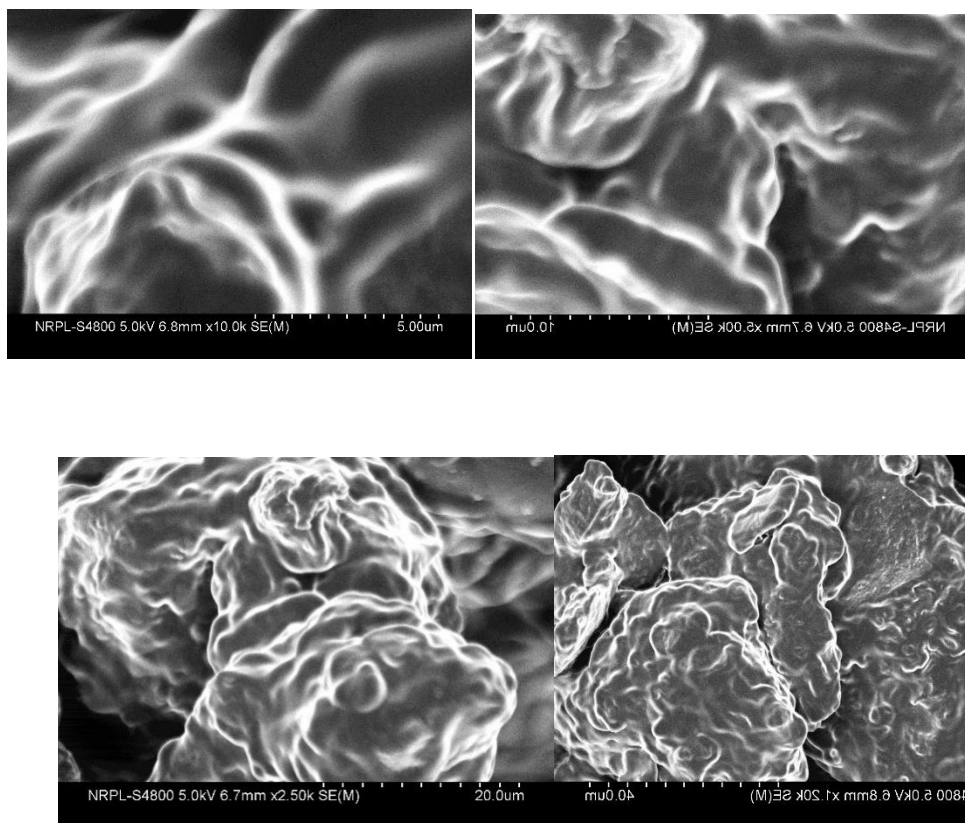


Figure 4.1.2 Water extract protein



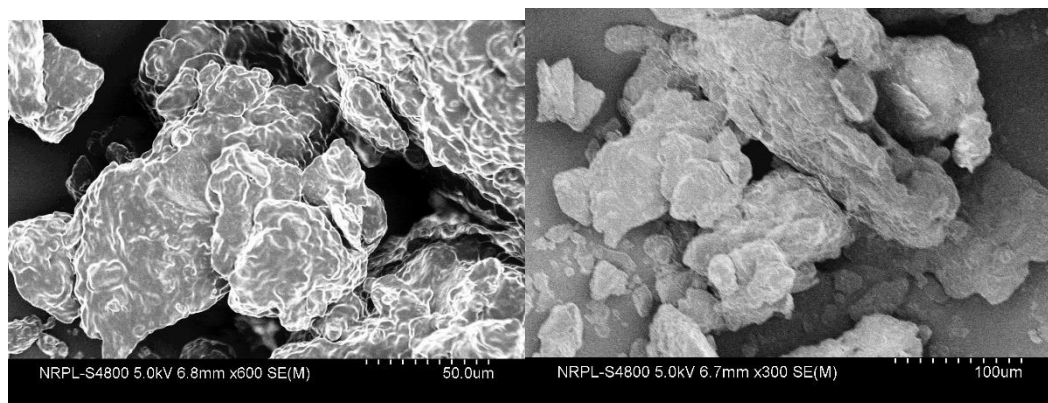
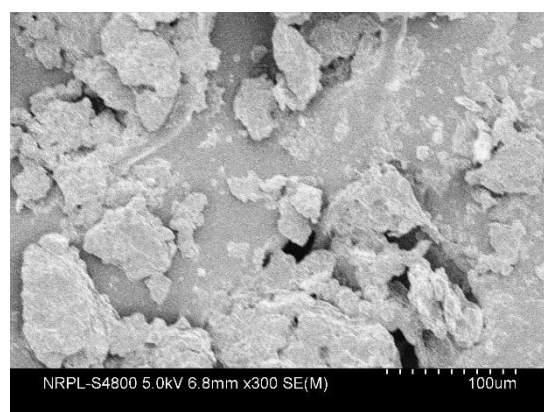
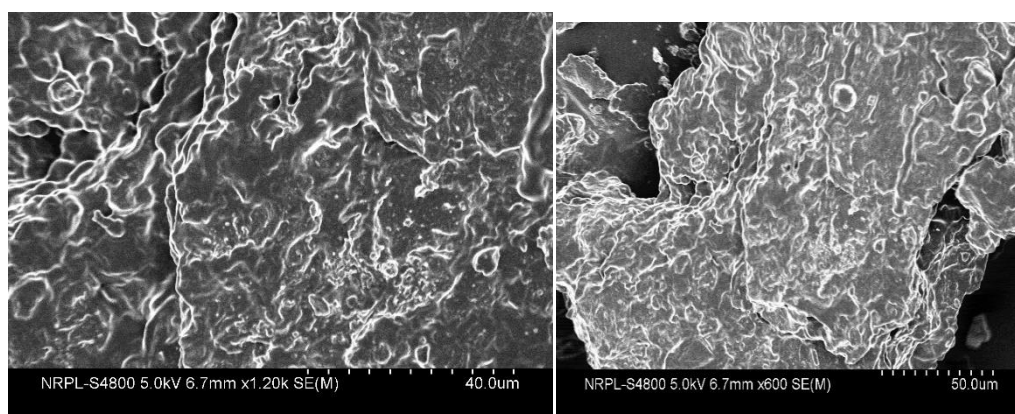
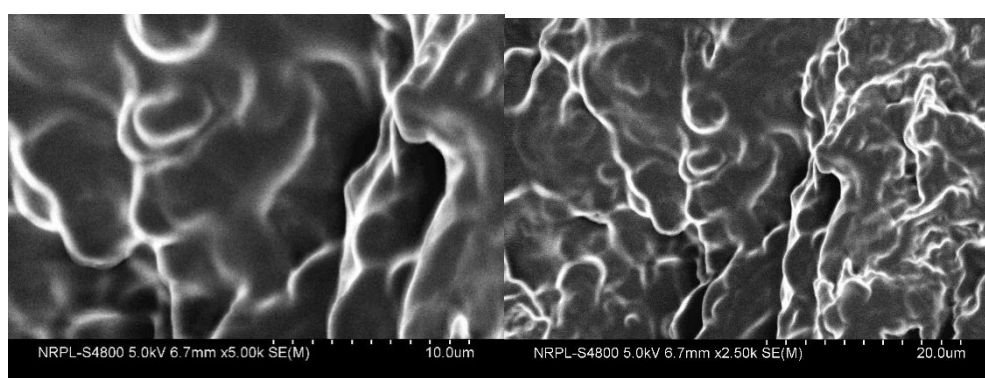


Figure 4.1.3. Alkaline extract protein



Jia et al. (2021) reported the morphology of rape seed protein isolates, rape seed meal and showed disordered flake fragments and it was due to the protein bodies in the cellular matrix dissolved into water during isolate purification and extraction. Yancheshmeh et al. (2022) studied the surface morphology of *Vicia villosa* seed protein using SEM and results showed that the surface morphology of *Vicia villosa* seed protein had a granular shape with rough surface due to aggregation of protein fractions.

Spray drying increases the particle size of powders and creates a smooth structure due to rapid evaporation flux. Sesame waste plant protein powder produced by spray drying had a spherical and smoother surface (Tingle et al., 2023) and pea protein isolate had a dense and gel like matrix structure (Ozdemir et al., 2022). Album and quinoa seeds protein isolates had a smooth surface with rough edges (Mir et al., 2018). SEM was used to examine the surface morphology and microstructure of the commercial potato powder and spray-dried orange-flushed sweet.

Orange-flushed sweet potato powder molecules are found to come in a variety of sizes and forms. The hexagonal shapes, erratic arrangements, compactness, and lack of agglomerates were visible in the SEM images of the spray-dried orange-flushed sweet potato powder. Commercial potato powder, however, had polygonal shapes, some that were cracked or fractured, and some that had round edges (Arebo et al., 2023).

4.6 FTIR spectra analysis of plant proteins extracted from garden cress seeds

Figure 4.2 Spectra analysis of plant proteins extracted from garden cress seeds

FTIR is used to identify the functional groups present in particular substances. FTIR spectra data was presented in table 4.5. The functional and structural groups were identified in their respective regions using FTIR.

Figure 4.2.1 Spectra analysis of Water extract protein

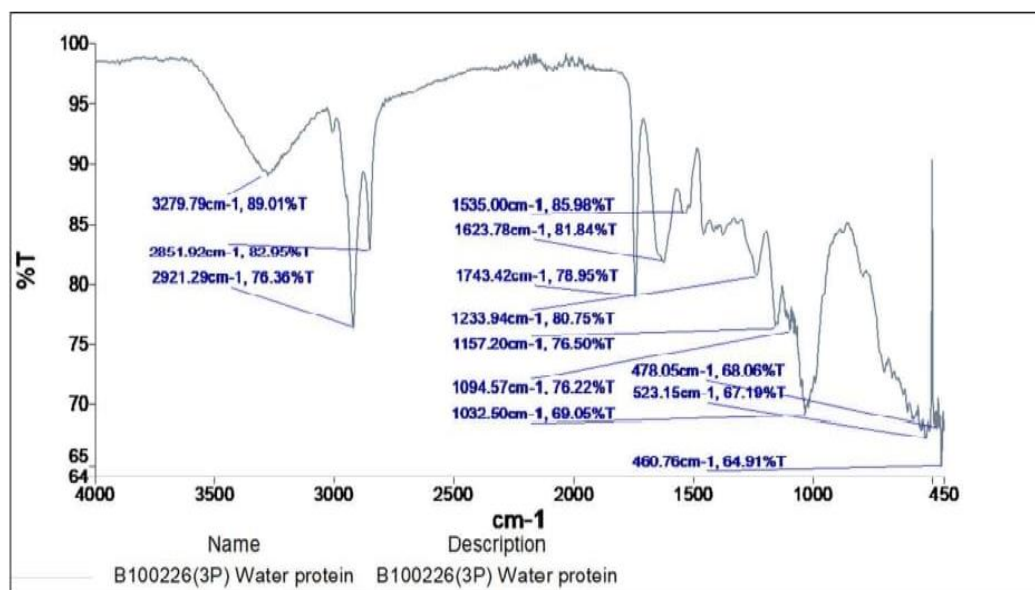


Figure 4.2.2 Spectra analysis of Acid extract protein

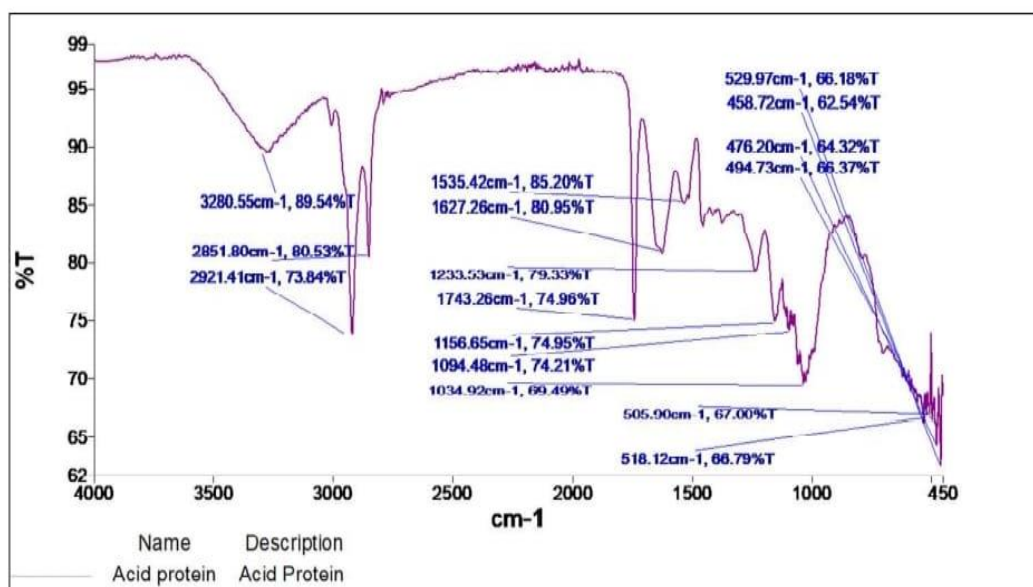


Figure 4.2.3 Spectra analysis of Alkaline extract protein

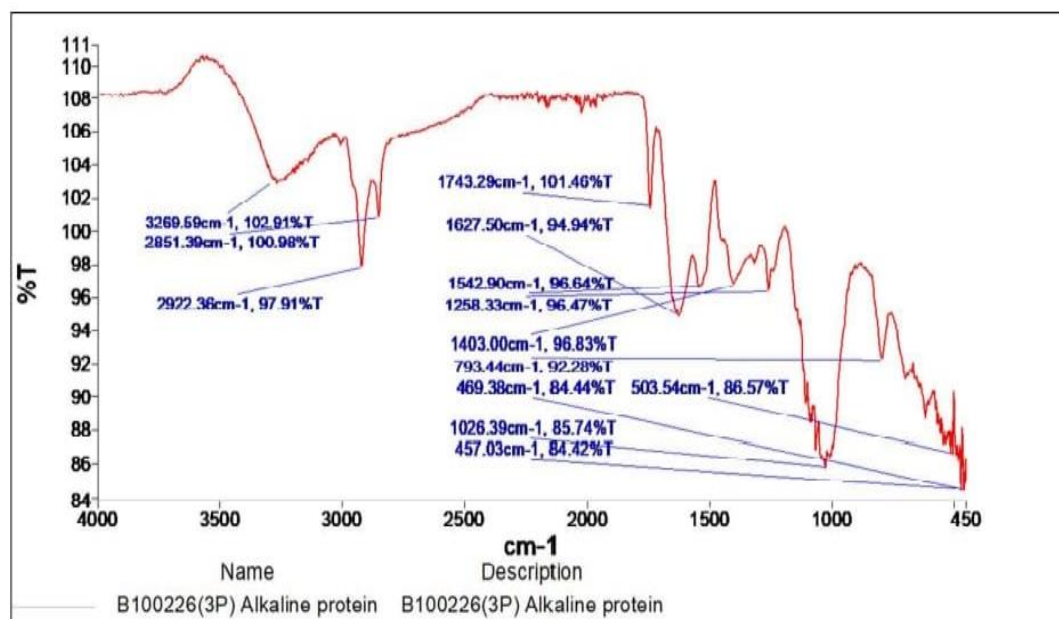


Table 4.5 FTIR spectra wave lengths and their respective functional groups in extracted plant protein

Structural and functional groups	Alkaline extract protein	Water extract protein	Acid extract protein
O-H stretching (3200 – 3500 cm^{-1})	3269.69 cm^{-1}	3279.79 cm^{-1}	3280.55 cm^{-1}
C-H (2800 – 3300 cm^{-1})	2951.30 cm^{-1} 2922.36 cm^{-1}	2921.29 cm^{-1} 2851.92 cm^{-1}	2961.90 cm^{-1} 2921.41 cm^{-1}
C=O (1600-1700 cm^{-1})	1743.29 cm^{-1} 1627.50 cm^{-1}	1623.78 cm^{-1}	1627.26 cm^{-1}
Amide (N-H bending) (1400-1500 cm^{-1})	1542.90 cm^{-1} 1403.00 cm^{-1}	1535.00 cm^{-1}	1535.42 cm^{-1}
C-O (1000-1300 cm^{-1})	1026.39 cm^{-1}	1233.94 cm^{-1}	1233.55 cm^{-1} 1094.48 cm^{-1} 1034.92 cm^{-1}

The table 4.5 presents FTIR (Fourier Transform Infrared) spectra wavelengths corresponding to specific structural and functional groups identified in extracted plant proteins using three different extraction methods: alkaline extract, water extract, and acid extract. **O-H stretching (3200–3500 cm^{-1}):** Peaks are observed around 3269.69 cm^{-1} (alkaline), 3279.79 cm^{-1} (water), and 3280.55 cm^{-1} (acid), indicating the presence of hydroxyl groups, which are typical of alcohols or phenols and possibly related to protein hydration or bound water. **C-H stretching (2800–3300 cm^{-1}):** Multiple peaks are recorded for each extraction method (e.g., 2951.30, 2922.36, 2921.29 cm^{-1} for alkaline), reflecting alkyl group vibrations within the protein structure, commonly associated

with side chains of amino acids. **C=O stretching (1600–1700 cm⁻¹):** Peaks at 1743.29 cm⁻¹ (alkaline), 1627.50 and 1623.78 cm⁻¹ (water), and 1627.26 cm⁻¹ (acid) correspond to carbonyl groups, such as those in peptide bonds or side chains, indicating protein backbone and possible lipid contamination or ester groups. **Amide (N-H bending) (1400–1500 cm⁻¹):** Peaks around 1542.90 cm⁻¹ (alkaline), 1403.00 and 1535.00 cm⁻¹ (water), and 1535.42 cm⁻¹ (acid) represent amide groups, characteristic of protein secondary structure (amide II band). **C-O stretching (1000–1300 cm⁻¹):** Peaks at 1026.39 cm⁻¹ (alkaline), 1233.94 and 1233.55 cm⁻¹ (water), and 1094.48 and 1034.92 cm⁻¹ (acid) indicate C-O bonds, possibly from carbohydrate moieties or side chains in proteins. Overall, the table shows how different extraction methods yield proteins with slightly varying FTIR spectral peaks, reflecting subtle differences in their chemical structures or associated compounds. These findings were more in line with those of Brishti et al. (2020), who studied spray-dried mung bean proteins. The O-H wide bands were detected in the region of 3276 cm⁻¹, primarily from α -helix, β -sheet, and random coil, which absorb at maximum wavelengths of 1655–1656 cm⁻¹, 1625–1638 cm⁻¹, and 1641–1643 cm⁻¹, respectively. According to Du et al. (2022) the main secondary structural components of legume and pulse protein isolates are α -helix (1650–1659 cm⁻¹), β -sheet (1615–1640 cm⁻¹), random coils (1641–1649 cm⁻¹) and β -turn (1660–1688 cm⁻¹). These findings suggest that the processing environment and technique have a major impact on the structural modifications that occur in protein molecules, resulting in changes to their ultimate functional characteristics. This demonstrates that varied processing conditions can be effectively used to accommodate various desirable functional qualities based on the demands of food makers.

Conclusion

- Garden cress seeds were successfully utilized as a promising plant-based protein source through wet extraction methods.
- Three extraction methods—alkaline, acid and water extraction—were successfully employed for protein recovery from garden cress seed powder.
- The protein content increased from $24.73 \pm 0.77\%$ in seed powder to $83.83 \pm 1.45\%$ in alkaline extract, indicating effective protein concentration.
- Alkaline extraction was the most efficient method, producing the highest protein purity (77.62%), protein recovery (61.53%) and protein yield (32%).
- Acid extraction showed intermediate performance, while water extraction resulted in the lowest protein concentration, purity, recovery and yield among the extraction methods.
- Alkaline extraction resulted in a lighter protein powder with an L* value of 52.7 ± 0.4 , compared with 45.3 ± 0.5 for garden cress seed powder.
- The alkaline protein extract demonstrated superior protein solubility (70.12 ± 1.12) compared with acid and water extracts, indicating better dispersibility and protein–water interaction.
- The alkaline extract showed the highest fat absorption capacity (2.83 ± 0.02), suggesting its potential for improving flavour retention and mouthfeel in food formulations.
- The alkaline protein extract exhibited the highest foaming capacity (66.67 ± 2.89), indicating good potential for incorporation into aerated and foam-based food products.

- The highest emulsification capacity (30.10 ± 0.40) and emulsification stability (33.39 ± 0.44) were observed in the alkaline extract, demonstrating better emulsifying functionality.
- SEM analysis revealed collapsed and wrinkled surface structures in the extracted proteins, which may be associated with moisture loss, shrinkage and structural rearrangement during drying.
- FTIR analysis confirmed the presence of characteristic O–H, C–H, C=O/amide and C–O functional groups, demonstrating the proteinaceous nature and structural characteristics of the extracted samples.
- Differences in FTIR peak positions among the extraction methods indicated that processing conditions influenced the molecular structure and interactions of the extracted proteins.
- Overall, alkaline extraction was identified as the most suitable method among the evaluated techniques because it provided the highest protein concentration, yield, purity, recovery and functional properties.
- The extracted garden cress seed protein has considerable potential for use as a functional plant-protein ingredient in protein-enriched foods, beverages, emulsified products and other value-added food formulations.

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