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DEVELOPMENT OF PLANT-BASED MEAT ANALOGUE: HARNESSING THE POTENTIONAL OF GARDEN CRESS SEEDS

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ABSTRACT

The study focused on the development of sustainable plant-based meat analogues utilizing garden cress (Lepidium sativum) seed protein powder and Garden cress seeds were selected for their rich nutritional profile, which included high protein content, essential fatty acids, dietary fiber, minerals, and diverse bioactive phytochemicals with antioxidant, anti-inflammatory, antimicrobial, antidiabetic, anticancer, hepatoprotective, and bone-healing properties.

Protein was extracted from garden cress seeds using alkaline extraction followed by isoelectric precipitation to obtain a high-protein concentrate (83.8% protein), with significantly reduced fat, fiber, moisture, and carbohydrate content compared to raw seed powder. This protein concentrate was incorporated into plant-based meat analogue formulations (T1, T2, T3), replacing animal meat, alongside other plant-derived ingredients such as rehydrated mushrooms, soya chunks, beetroot and potato pulp, carboxymethyl cellulose, sodium alginate, and spices to optimize texture, flavor, and nutritional quality.

Comprehensive physicochemical analyses showed that the plant-based meat analogues had significantly higher protein (44.9%) and fiber content, with lower fat and carbohydrate levels than conventional chicken meat patties. Functional properties including water absorption, fat absorption, protein solubility, and gelling capacity were characterized, supporting the suitability of garden cress protein for meat analogue applications. FTIR spectroscopy confirmed the presence of key functional groups related to protein integrity and interactions, while DSC analysis revealed distinct thermal properties compared to chicken meat, reflecting differences in molecular stability. SEM imaging demonstrated fibrous, porous microstructures essential for mimicking meat texture. Texture profile analysis indicated that the plant-based patties had slightly lower hardness but comparable cohesiveness and adhesiveness to chicken patties, with the T1 formulation showing the closest textural similarity. Sensory evaluation using a 9-point hedonic scale revealed high acceptability for T1 and T2, with scores near the control for appearance, flavor, taste, texture, and overall acceptability, whereas T3 showed reduced sensory scores, suggesting that higher garden cress incorporation affected palatability.

Shelf-life studies under refrigerated and frozen storage conditions demonstrated that frozen storage (-18°C) maintained microbial safety and product quality for up to 30 days, with minimal microbial growth and stable sensory attributes. Refrigerated storage showed acceptable microbial levels for shorter durations, while ambient conditions led to rapid spoilage.

This research validated garden cress seed protein powder as a promising, sustainable ingredient for nutritious, functional plant-based meat analogues that met consumer demands for health, environmental sustainability, and sensory quality. The integration of garden cress enhanced protein content and functional properties while contributing bioactive compounds with potential health benefits. Future work was recommended to focus on clinical validation of therapeutic effects, optimization of processing parameters, and exploration of synergistic ingredient combinations to further improve flavor and texture for commercialization.

Key words: Plant based Meat analogues, Graden cress seeds

INTRODUCTION

The study of garden cress seed (Lepidium sativum) held significant importance due to its multifaceted nutritional, therapeutic, and agronomic benefits. Garden cress seeds were a rich source of proteins, dietary fiber, omega-3 fatty acids, essential minerals like iron, calcium, potassium, and various bioactive phytochemicals. These components collectively contributed to its high nutritional value and potential to combat malnutrition and nutrient deficiencies (Adera *et al*., 2022; Azene *et al*., 2022; Jain and Grover, 2016).

From a health perspective, garden cress seeds exhibited promising pharmacological effects, including anticarcinogenic, antidiabetic, antiasthmatic, anti-inflammatory, and antimicrobial properties. Bioactive constituents such as alkaloids, flavonoids, glycosides, and polypeptides provided therapeutic potential for managing chronic diseases, enhancing bone healing, and addressing respiratory disorders (Gupta and Gupta, 2024; Painuli *et al*., 2022). This rendered garden cress a valuable natural source for developing functional foods, nutraceutical products, and pharmaceutical agents, with continuing research needed to confirm efficacy in human trials (Azene *et al*., 2022).

Agronomically, studies on seed variants revealed superior genotypes such as the brown seed variant, which offered enhanced germination, growth, and yield performance. Understanding these genotype characteristics was vital for optimizing cultivation practices and improving overall productivity and adaptability of garden cress in various climatic conditions, supporting food security and economic benefits (Mohammed *et al*., 2025).

Additionally, garden cress seed oil was shown to possess favourable physicochemical properties including high stability and antioxidant content, making it suitable for dietary and industrial applications such as oil production, illumination, and soap making (Diwakar *et al*., 2009; Gupta and Gupta, 2024). Its bran fraction, rich in dietary fiber and excellent functional properties, held promise as a natural ingredient for fiber enrichment in food formulations (Gokavi *et al*., 2004).

Furthermore, the role of garden cress seeds in traditional medicine and potential conservation importance underscored the need to preserve this species to maintain biodiversity and ensure its benefits for future generations (Gupta and Gupta, 2024). Research also contributed to understanding fundamental plant physiology, such as geotropic responsiveness linked to root starch content, adding to basic botanical knowledge (Iversen, 1969).

The significance of studying garden cress seeds lay in their extensive nutritional and therapeutic potential, agricultural value, and industrial applications, backed by growing scientific evidence. This research underscored the importance of garden cress for health promotion, sustainable agriculture, and economic development (Painuli *et al*., 2022; Adera *et al*., 2022; Mohammed *et al*., 2025).

Underutilized garden cress seeds (Lepidium sativum) were important due to their rich nutritional profile, therapeutic potential, and versatility in functional food development, attributes that remained largely untapped globally. These seeds were a significant source of macro- and micronutrients such as essential fatty acids, amino acids, proteins, dietary fiber, and minerals like iron, potassium, calcium, and phosphorus, which contributed to human health and nutrition (Azene *et al*., 2022; Gokavi *et al*., 2004).

The therapeutic properties of garden cress seeds were considerable; they contained bioactive compounds that had been traditionally used to manage diabetes, hypercholesterolemia, bone fractures, asthma, constipation, and certain cancers. Their potential to prevent and treat non-communicable and communicable diseases positioned them as promising candidates for functional food ingredients and natural medicines (Azene *et al*., 2022; Painuli *et al*., 2022).

Additionally, garden cress seed oil exhibited beneficial physicochemical properties and contained a high amount of α-linolenic acid and tocopherols, which contributed to antioxidant effects and stability. This made the oil viable for nutritional, industrial, and therapeutic applications, including food manufacturing and soap production (Diwakar *et al*., 2009).

Despite these benefits, garden cress remained one of the most underutilized crops worldwide. Its potential was hindered by limited awareness and research focused mainly on animal studies rather than human clinical trials. Enhancing its utilization could have promoted improved nutrition, especially in regions facing malnutrition and diet-related diseases. Moreover, increased exploitation of garden cress seeds could have supported sustainable agriculture, biodiversity conservation, and economic development through novel food products and medicinal applications (Gupta and Gupta, 2024; Azene *et al*., 2022).

The chemical composition of garden cress seeds included a complex mixture of proteins, carbohydrates, fats, vitamins, minerals, and phytochemicals. The high vitamin C content contributed to immune support and antioxidant defense, while vitamin K played a crucial role in blood clotting and bone health. Minerals such as iron were vital for oxygen transport in the blood, calcium supported bone strength, and magnesium was involved in numerous enzymatic reactions. Phytochemicals present in the seeds included glucosinolates, flavonoids, and phenolic compounds, which contributed to the seeds' medicinal effects, particularly their antioxidant and anti-inflammatory activities.

Garden cress seeds were generally safe for consumption and were widely used in traditional diets. However, like all seeds, proper storage was necessary to preserve their nutritional quality and prevent microbial contamination. Seeds had to be stored in cool, dry conditions, protected from moisture and heat to avoid spoilage.

Garden cress seeds were a nutrient-rich, multifunctional food ingredient with significant culinary and medicinal value. Their rich profile of proteins, fiber, vitamins, and minerals, combined with bioactive phytochemicals, underpinned their health-promoting properties. The seeds' mucilaginous nature enhanced their functional applications in food preparation and digestion. Cultivated easily and used traditionally across various cultures, garden cress seeds held potential for expanded use in health foods, nutraceuticals, and natural remedies.

2. MATERIALS AND METHODS

2.1 Preparation of Garden cress seed powder

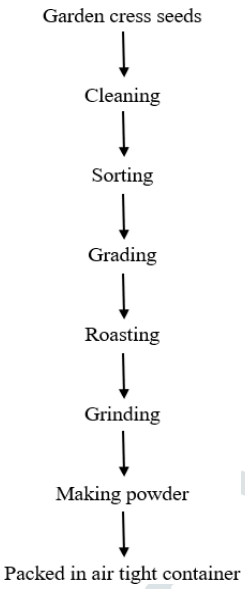


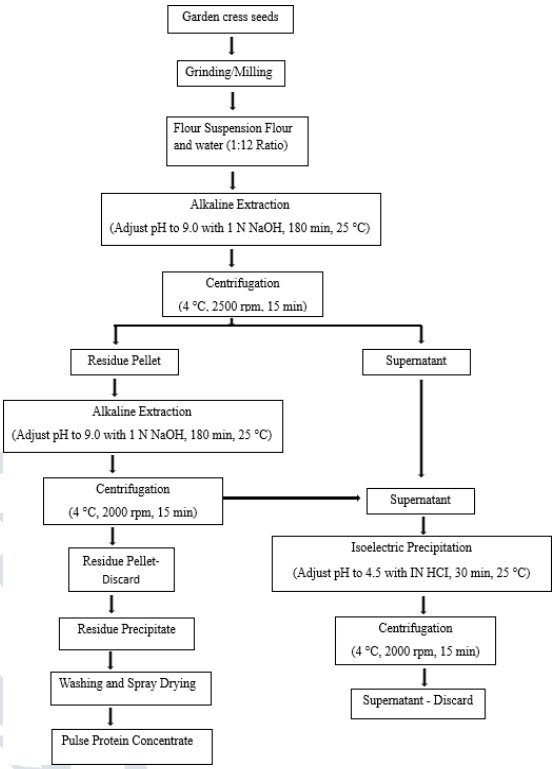
Fig 2.1 Garden cress seeds

Fig 2.2 Roasting

Fig 2.3 Grinding

Fig 2.4 Powder

2.2 Extraction of protein by alkaline extraction



2.3 Extraction of protein from Garden cress seeds using alkaline extraction








The alkaline extraction procedure described by Penchalaraju *et al*. (2024) was followed with slight modifications. The plant seeds such as garden cress seeds were milled, and the resulting flour and water were used in a ratio of 1:12 to create a flour suspension for alkaline extraction. The pH was adjusted to 9 using 1 N NaOH, and the mixture was kept at room temperature (25 °C) for 180 minutes. The mixture was centrifuged at 2500 RPM for 15 min, supernatant and residue precipitates were obtained. To increase the yield of the protein alkaline extraction procedure is repeated for the residue The supernatants were collected and subjected to isoelectric precipitation by adjusting the pH to 4.5 with 1 N HCl for 30 minutes at room temperature (25°C) The precipitated protein extract was subsequently rinsed with water. Generally, spray drying, drum drying and freeze drying can be employed to dry the protein extracts (Boye & Barbana, 2012, Cui *et al.*, 2020). The collected supernatants were combined and subjected for washing with water to neutralize the protein extract and subjected for spray drying at a inlet temperature ranged from 80 °C to 100 °C and feed flow rate varied from 20 ml/min to 25 mL/min. The protein concentrate obtained from the plant sources are kept under refrigeration conditions until used for further analysis.

1.1 The formulations of control and three treatments (100 g batch basis)

The detailed formulation of the plant-based meat analogue which campares the ingredients composition of a traditional meat product as control. Three plant Based Treatments (T1, T2, T3) where garden cress seeds are used as the main functional and protein rich component.

To develop Sustainable plant-based meat analogues by replacing animal meat (75% in control) with the garden cress seeds, other plant Based ingredients

This helps to create a nutritious, environmentally friendly and consumer acceptable meat substitute.

2.1 Table Formulations of control and treatments

Ingredients	Control	T1	T2	T3
Meat	75	-	-	-
Garden cress seeds	-	30	35	38
Beetroot pulp	5	5	5	5
Chopped onions	-	5	5	5
Potato pulp	-	3	3	3
Carboxy methyl cellulose	-	0.5	1	1
Soya chunks	-	18	15	15
Corn flour	5	12	8	10
Paprica	-	2	2	2
Sodium alginate	-	2.5	1	1
Salt	2	2	2	2
Coriander leaves	2	0.5	2	2
Mutton masala	5	1	5	5
Baking soda	1	0.5	1	1
Ginger garlic paste	2	1	2	1
Black pepper	1	2.5	2	1
Rose water	-	3	3	2
Rehydrated mushrooms	-	6	6	7
Spice mix	2	1.5	2	2

1.2 Methods-lab-scale (step-by-step for a 100 g batch)

Preprocessing of garden cress seed powder, the preprocessing of garden cress seed powder involved using whole seed powder, which was considered simpler. First, the garden cress seeds were milled to a fine powder that passed through a 60-100 µm sieve. If the powder was oily and a lower fat content was desired, it was defatted by cold pressing or solvent extraction using Soxhlet with hexane for 3-4 hours; the solvent was then evaporated in a fume hood, and the powder was dried overnight at 40 °C. The powder was dried to approximately 4-8% moisture in an oven at 40-50 °C and then cooled in a desiccator.

For obtaining protein concentrate (optional, for higher protein and better texture), the powder was suspended in deionized water at a ratio of 1:10 (w/v), and the pH was adjusted to 9.0 using 1 M NaOH. The mixture was stirred for 1 hour at 25-30 °C to solubilize proteins. Next, it was centrifuged for 10 minutes at 4000 rpm to remove insoluble material. The supernatant was acidified to isoelectric precipitation at pH 4.5 with 1N HCl to precipitate the protein. The precipitate was collected by centrifugation, washed, neutralized, and then obtained as pulse protein concentrate in powdered form. This concentrate was then used in formulations. Final dough temperature should be 20-30 °C for non-extrusion processing.

In the mixing and dough preparation phase, ingredients were weighed precisely using an analytical balance. Dry ingredients such as garden cress powder, soya chunks, rehydrated mushrooms, methylcellulose, salt, and seasonings were mixed in a bowl for 2-3 minutes. Soya chunks were pre-hydrated by soaking in warm water at a 1:2 (w/v) ratio for 10-15 minutes, followed by draining the excess water. Oils and water were added to the dry mix on low to medium speed for 3 to 6 minutes until a homogeneous, slightly sticky mass formed. If the dough was too dry, water was added in 2-5 mL increments. The final dough temperature was maintained between 20-30 °C for non-extrusion processing.

Fig 2.5 Dough

Fig 2.6 Patties

Fig 2.7 Bread crumbing

Fig 2.8 Shallow frying

Fig 2.9 Dried patties

The low-tech thermo-setting method began with molding, where the dough was pressed into molds (patties or logs) at the desired shape, such as 50 g patties. The oven was preheated to 160 °C. The samples were placed in the oven on a tray and baked for 15-20 minutes until the internal temperature reached the target of 72 °C. After baking, the samples were removed and cooled at room temperature for 15 minutes. Optionally, the samples were lightly pan-fried to develop surface color.

In the final finishing stage, optional marination with oil and seasoning was performed for sensory improvement. The cooked samples could be pan-fried to evaluate their color and texture. Prepared samples were stored at 4 °C for up to 48 hours or frozen at -20 °C for longer storage.

Products developed using meat and garden cress seed protein concentrate

Control

T1

T2

T3

Control: Chicken meat analogues

T_1 : Meat analogues with 60 g of garden cress seed protein concentrate

T_2 : Meat analogues with 70 g of garden cress protein concentrate

T_3 : Meat analogues 80 g with garden cress protein concentrate

1.3 Development of plant-based meat analogue with garden cress seed protein powder

Plant protein-based meat analogue was prepared following the method reported by Yuliarti *et al.* 2023 with slight modifications. Experimental products (garden cress seed protein-based swallow-fried meat patties), namely T1, T2 and T3, were prepared along with a control sample (conventional chicken meat patties). Formulations for development of garden cress seed protein based deep fried meatballs are indicated in Table 2.1. Chicken meat was procured from the local market and passed. through the meat mincer. Measured ingredients were mixed with minced chicken meat, and balls were prepared manually by using hands. Experimental products, namely T1, T2 and T3 plant protein-based meat patties were prepared by mixing all the

required ingredients for 6 min. Rose water rose petal soaked overnight and water collected) was added to experimental products to improve the colour of the meatball analogues. Rehydrated mushrooms (dried oyster mushrooms soaked for 15 min) were added to the mixture to maintain the structure and textural characteristics. were added to the formulations to impart umami meat flavour. Potato and beet root pulp were added to maintain the fibrous nature. Meat patties obtained by addition of all ingredients were kept for 2 h under refrigeration (4 to 8 °C) for setting. Meat patties were deep-fried at 175 to 180°C for 3 to 5 min in refined bleached and deodorized sunflower oil to the desired doneness (Penchalaraju and Bosco 2022; Lu et al. 2018). Weight of samples was taken before and after deep frying.

Sample	Fat	Fibre	Protein	Ash	Moisture	Carbs
Garden cress Raw powder	22.04 ± 0.32[a]	8.08 ± 0.54[a]	24.73 ± 0.77[b]	4.95 ± 0.30[a]	6.12 ± 0.79[a]	34.08 ± 2.5[a]
Garden cress Protein powder	3.46 ± 0.49[b]	2.33 ± 0.10[b]	83.83 ± 1.45[a]	3.39±0.20[b]	4.14 ± 0.12[b]	2.86 ± 1.79[b]

2. RESULTS AND DISCUSSION

2.1 Proximate Composition of Garden Cress Seed Powder and Its Protein Powder

Table 3.1 Proximate Composition of Garden Cress Seed Powder and Its Protein Powder

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

The selected data provided proximate analysis values for two forms of garden cress seed products: raw powder and protein powder. These values represented the average composition and variability (standard deviation) of key nutritional components.

Garden Cress Seed Raw Powder: Fat: 22.04 ± 0.33%, Fibre: 8.08 ± 0.55%, Protein: 24.73 ± 0.78%, Ash: 4.95 ± 0.30%, Moisture: 16.12 ± 0.80%, Carbohydrates (Total): 34.08 ± 2.57%. This raw powder contained a balanced composition with significant protein content (24.73 moderate fat (22%), and carbohydrates (~34%). The fibre content was notable at about 8%, indicating dietary fiber presence. Moisture was relatively high at ~16%, which was typical for raw seed powders.

Garden Cress Seed Protein Powder: Fat: 3.46 ± 0.50%, Fibre: 2.33 ± 0.11%, Protein: 83.83 ± 1.45%, Ash: 3.39 ± 0.21%, Moisture: 4.14 ± 0.12%, Carbohydrates (Total): 2.86 ± 1.79%. The protein powder was a concentrated form derived from the raw powder, evidenced by the very high protein content (83.8 % and dramatically reduced fat, fibre, moisture and carbohydrate levels. Fat and fibre were substantially reduced (to ~ 3.5% and ~ 2.3%, respectively), while moisture content was much lower 4.1%), indicating a drier, more refined product. Carbohydrates were minimal (~2.9%), reflecting the removal of non-protein components during processing.

The raw powder represented the whole seed in ground form with all macronutrients present. The protein powder was a processed, purified form emphasizing protein content, suitable for applications requiring high protein with minimal fat and carbohydrates. The standard deviations indicated reasonable consistency across samples for both powders, with slightly higher variability in carbohydrate content for the protein powder.

This proximate analysis highlighted the nutritional differences between the raw and protein powder forms of garden cress seed, which was useful for selecting the appropriate form based on dietary or formulation needs.

Figure 3.1 FTIR spectra of Garden Cress Seed Protein Powder

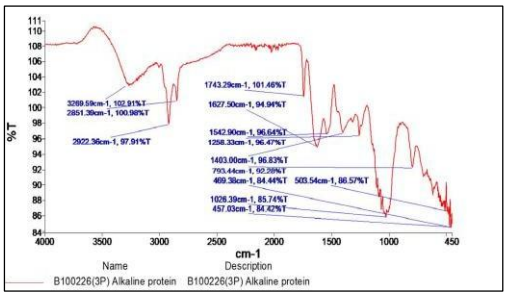
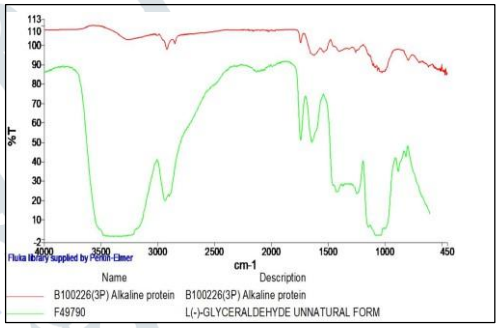


Figure 3.2 FTIR spectra of Garden Cress Seed Protein Powder



2.2 FTIR analysis of garden cress protein powder

FTIR spectra of garden cress seed protein powder stated that the presence of different functional groups

Table 3.2 FTIR analysis of garden cress seed protein powder

FTIR Functional Groups	Garden cress protein powder
O-H (3200-3500 cm^{-1})	3269.59 cm^{-1}
C-H (2800 –3300 cm^{-1})	2851.39 cm^{-1} 2922.36 cm^{-1}
C=O (1600-1700 cm^{-1})	1627.50 cm^{-1}
Amide (N-H bending) (1400-1500 cm^{-1})	1403.00 cm^{-1} 1542.90 cm^{-1}
C-O (1000-1300 cm^{-1})	1258.33 cm^{-1}

The Fourier Transform Infrared (FTIR) spectroscopy analysis of garden cress protein powder reveals characteristic absorption peaks corresponding to various functional groups, which can be interpreted to identify the molecular components present in the sample. The reported wavenumbers and their associated functional groups are as follows:

Observed at O-H Group (3200–3500 cm^{-1}) 3269.59 cm^{-1}, this broad band typically indicates the presence of hydroxyl groups, commonly associated with water, alcohols, or phenolic compounds. In proteins, it can also relate to

hydrogen-bonded hydroxyl groups from side chains or moisture content.

Observed at C-H Stretching (2800–3300 cm^{-1}) 2851.39 cm^{-1} and 2922.36 cm^{-1}, these peaks are characteristic of aliphatic C-H stretching vibrations, reflecting methylene and methyl groups prevalent in protein side chains and lipid contaminants.

Observed at C=O (Carbonyl) Stretching (1600–1700 cm^{-1}) 1627.50 cm^{-1}, this peak is typical of amide I bands in proteins, mainly from the C=O stretch of peptide bonds and can provide insights into secondary structure content.

Observed at Amide (N-H Bending) (1400–1500 cm^{-1}) 1403.00 cm^{-1} and 1542.90 cm^{-1}, the band around 1542.90 cm^{-1} likely represents the amide II band, associated with N-H bending and C-N stretching vibrations in proteins, critical for protein structural characterization.

Observed at C-O Stretching (1000–1300 cm^{-1}) 1258.33 cm^{-1}, this vibration is typical for C-O stretching modes, frequently seen in carboxyl and ester groups, possibly from amino acid side chains or carbohydrate residues bound to the protein matrix.

The reported FTIR peak positions fall within the standard wavelength ranges used to characterize protein and related biomolecular structures via FTIR spectroscopy (Nandiyanto *et al*., 2022; Losacco *et al*., 2022). For instance, the amide I and II regions between 1600–1700 cm^{-1} and 1400–1500 cm^{-1} are well established as key indicators of protein backbone conformations and have been widely used to assess protein secondary structure and composition.

More broadly, FTIR spectra offer a "chemical fingerprint" that can reveal the presence of lipids, carbohydrates, and proteins, as well as their molecular interactions within complex biological samples such as plant-derived powders (Naumann *et al*., 2010; Igci *et al*., 2017). The C-H stretching bands near 2850 and 2920 cm^{-1}, for example, suggest aliphatic moieties typical of the side chains of amino acids and possibly residual lipids or other hydrophobic compounds. The garden cress protein powder is characterized by FTIR absorption peaks consistent with the molecular signatures of proteins (amide bands), hydroxyl-containing compounds (O-H stretch), aliphatic hydrocarbons (C-H stretch), carbonyl functionalities (C=O stretch), and C-O bonds, pointing toward a complex mixture of proteinaceous material with potential carbohydrate or phenolic constituents. This spectral profile aligns with established FTIR methodologies to evaluate the molecular composition and changes in plant-derived protein materials (Nandiyanto *et al*., 2022; Losacco *et al*., 2022).

If more detailed structural insights or quantitative secondary structure analysis are required, further deconvolution of the amide I and II bands or complementary techniques could be employed as referenced in FTIR applications on similar biological samples (Igci *et al*., 2017).

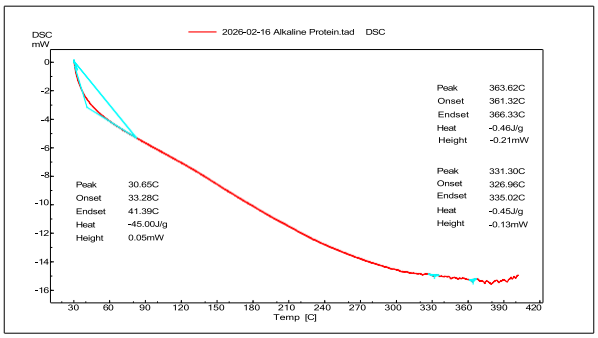


Figure 3.3 DSC of Garden cress seed protein powder

The graph presented a detailed Differential Scanning Calorimetry (DSC) analysis of the protein powder sample, which was a powerful technique used to characterize the thermal properties of proteins by measuring the heat flow associated with transitions in the sample as a function of temperature.

The DSC graph displayed one or more peaks that represented thermal transitions occurring within the protein powder. These transitions typically corresponded to physical or chemical changes such as protein denaturation, unfolding, or melting. An endothermic peak where the heat flow increased indicated that the sample absorbed energy, which was characteristic of processes like the breaking of hydrogen bonds and disruption of secondary and tertiary protein structures during denaturation. These thermal events revealed critical information about the protein's structural stability and behavior under heat stress.

The temperature at which the peak reached its maximum (Tm) was known as the melting temperature. This value was crucial as it defined the temperature at which half of the protein population was unfolded. A higher Tm reflected greater thermal stability, meaning the protein maintained its native conformation up to a higher temperature before denaturing. This parameter was essential for assessing protein quality, especially for applications where thermal resistance was critical, such as in food technology or pharmaceuticals.

The area under the endothermic peak corresponded to the enthalpy change (ΔH), which quantified the total energy absorbed during the transition. This value reflected the strength and number of intramolecular forces, such as hydrogen bonds, hydrophobic interactions, and van der Waals forces, that had to be overcome to denature the protein. A large ΔH indicated a highly ordered and stable protein structure requiring substantial energy input to unfold, while a smaller ΔH suggested weaker interactions or partial denaturation.

The onset temperature marked the point where the heat flow first deviated from the baseline, signaling the beginning of the thermal transition. The endset temperature was where the heat flow returned to baseline, marking the completion of the process. The temperature interval between onset and endset revealed the cooperativity of the unfolding process. A narrow range suggested a highly cooperative, single-step transition, whereas a broader range might have indicated multiple overlapping transitions or heterogeneous protein populations.

The flat regions before and after the peak represented the baseline heat flow, where no significant thermal events occurred. Establishing a stable baseline was critical for accurately determining onset, peak, and endset temperatures, as well as for calculating the enthalpy change. Deviations or

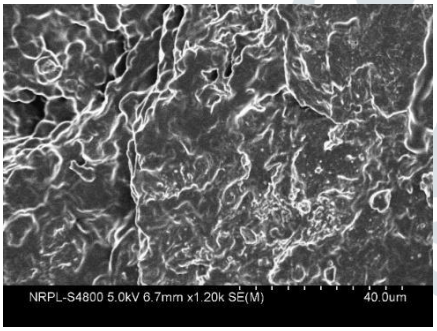
shifts in the baseline could have indicated experimental artifacts or interactions with other sample components.

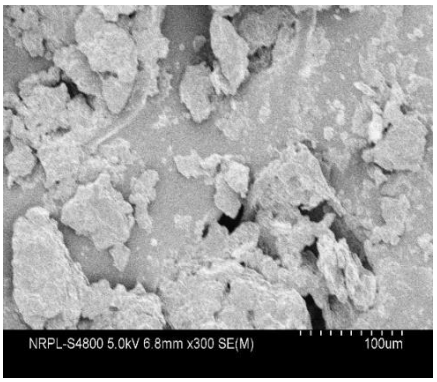
The DSC profile provided insights into the purity and conformational integrity of the protein powder. For instance, a single sharp peak typically indicated a homogeneous protein sample with a well-defined structure, while multiple peaks or shoulders might have suggested the presence of different protein species, aggregates, or degradation products. Changes in peak temperature or enthalpy compared to reference or control samples could reveal effects of processing, formulation, or storage conditions on protein stability. This information was valuable for optimizing manufacturing processes and ensuring product consistency.

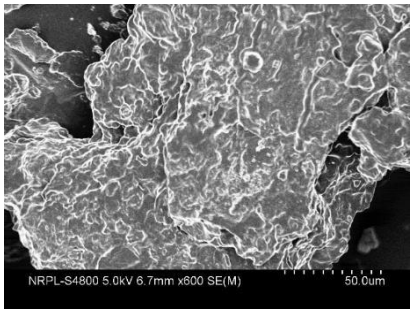
By performing heating and cooling cycles, reversibility of the thermal transition was assessed, which distinguished between reversible unfolding and irreversible denaturation or aggregation. Comparing DSC curves from different batches or formulations helped detect subtle changes in protein stability or interactions with excipients, additives, or contaminants. DSC data was correlated with functional assays such as solubility, enzymatic activity, or emulsifying capacity to understand how thermal stability impacted performance.

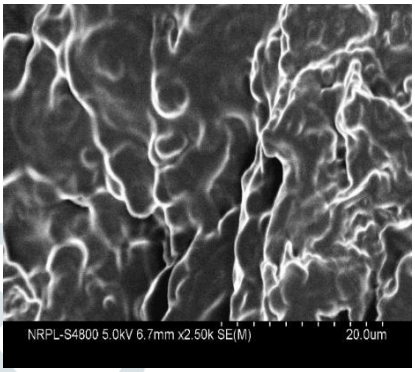
DSC graph in the protein powder document served as a comprehensive thermal fingerprint, revealing critical aspects of protein stability, purity, and structural transitions. By analyzing the peak temperature, enthalpy change, and transition range, researchers evaluated how the protein behaved under heat, guiding formulation, processing, and quality control decisions.

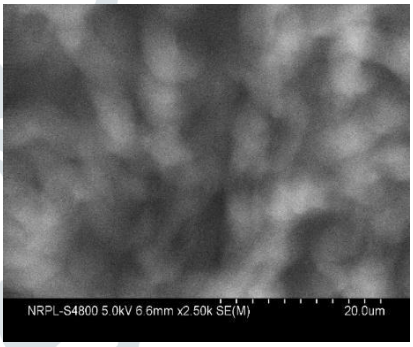
Figure 3.4 Morphological characteristics of Garden cress seed protein powder

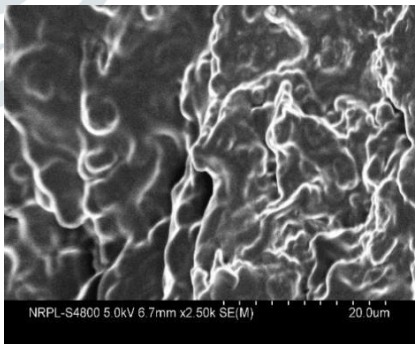












The labeled was captured at 600x magnification with a scale bar of 50.0 µm, revealing the surface morphology of the sample, likely a Garden cress seed sample subjected to one of the treatment methods. The image displayed an irregular, rough, and layered texture characterized by visible cracks, folds, and fractured surfaces. This microstructural appearance suggests significant physical disruption and alteration of the seed matrix.

Such morphological features are critical because they influence the efficiency of protein extraction and nutrient

accessibility. The rough and fractured surface increases the surface area and enhances solvent penetration, facilitating the release of proteins and other nutrients from the seed matrix.

Correlating this with the proximate composition data from the selected text. The Alkaline treatment, which yielded the highest protein content (71.00% ± 1.00), likely corresponds to a surface morphology with increased porosity and disrupted structure as seen in the SEM image. The physical breakdown indicated by the cracks and folds would have enhanced protein solubility and extraction efficiency by exposing more protein binding sites and disrupting protein-matrix interactions.

The Acid treatment, associated with a significantly higher fiber content (23.50% ± 0.50), may produce a different microstructure, possibly less physically disrupted but chemically modified. Acid treatment could lead to partial hydrolysis or rearrangement of seed components, concentrating fiber fractions and altering the matrix to a more compact or chemically altered state.

The Water treatment, which preserved higher fat (17.5% ± 0.50) and carbohydrate (19.66% ± 1.44) contents but resulted in the lowest protein content (52.33% ± 2.51), might exhibit a surface morphology with less disruption, retaining more of the original seed structure and thus limiting protein release.

The SEM image, therefore, provides visual evidence of the physical changes induced by the treatments that directly affect the proximate composition outcomes. The observed microstructural disruptions, such as roughness and fragmentation, facilitate better extraction of proteins (notably in alkaline treatment), while less altered morphologies correspond to lower protein yields and higher retention of other components.

The surface morphology of a sample at magnifications of 600x and 2500x respectively, with scale bars of 50.0 µm and 20.0 µm.

The images reveal an irregular, rough, and layered surface texture characterized by visible cracks, folds, and fractured areas. This microstructure indicates significant physical disruption and alteration of the seed matrix. Such surface features suggest increased porosity and fragmentation, which enhance surface area and facilitate solvent penetration during extraction processes.

These morphological characteristics correlate with the proximate composition data from the selected text, where the Alkaline treatment showed the highest protein content (71.00% ± 1.00). The disrupted and rough surface observed in the SEM images likely reflects the physical breakdown caused by alkaline treatment, which improves protein solubility and extraction efficiency by exposing more protein binding sites and weakening protein-matrix interactions. Treatments like Acid and Water, associated with different proximate profiles (higher fiber or fat/carbohydrate content, respectively), may produce less pronounced surface disruption or chemically alter the matrix differently, leading to variations in nutrient accessibility. The SEM images provide visual evidence of the physical changes induced by the treatments, directly impacting the efficiency of protein extraction and the retention of other nutritional components.

Determination of protein recovery, protein yield, protein purity

An extraction efficiency of wet processing methods was estimated by using standard procedures of Boye and Barbara (2012).

$$\text{Protein purity (\%)} = \frac{Mass\ of\ protein\ in\ extract\ extracted\ (g)}{Total\ mass\ of\ recovered\ extract\ (g)} \times 100$$

$$\text{Protein recovery (\%)} = \frac{Mass\ of\ protein\ extracted\ (g)}{Total\ mass\ of\ protein\ in\ starting\ material (g)} \times 100$$

$$\text{Protein yield (\%)} = \frac{Mass\ of\ protein\ extracted\ (g)}{Total\ mass\ of\ starting\ material\ (g)} \times 100$$

Table 3.3 of Determination of protein recovery, protein yield, protein purity

	Garden cress seed Alkaline protein
Protein purity	76.62%
Protein recovery	62.53%
Protein yield	33%

Functional Properties

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

Functional Properties	**Garden cress seed powder**	**Garden cress seed Alkaline extract protein**
Water absorption capacity	9.08 ± 0.04	9.04 ± 0.04
Fat absorption capacity	0.71 ± 0.45	2.83 ± 0.02
Protein solubility	23.35 ± 0.17	71.18 ± 1.15
Gelling capacity	17.9 ± 1.07	11.21 ± 0.03
Foaming capacity	21.12 ± 1.17	70.15 ± 1.19
Emulsification capacity	21.15 ± 1.08	29.14 ± 0.42
Emulsification stability	19.39 ± 0.43	33.39 ± 0.44

Table 4.5 Proximate Composition of Plant Based Meat Analogue and Chicken Meat Patties

Table: 4.3 Proximate analysis of the Control and experimental sample

Sample	**Fat**	**Fibre**	**Protein**	**Ash**	**Moisture**	**Carbs**
Control sample	18.03 ± 0.20[a]	3.08 ± 0.02[b]	19.53 ± 0.63[b]	2.20 ± 0.65[b]	25.13 ± 1.94[a]	31.31 ± 2.51[a]
Experimental sample	13.19 ± 0.37[b]	3.52 ± 0.50[a]	44.93 ± 0.86[a]	3.67 ± 0.32[a]	23.70 ± 0.34[b]	10.98 ± 2.18[b]

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

The table presented two samples: a Control sample and an Experimental sample. Each sample was analyzed for six components: Fat, Fibre, Protein, Ash, Moisture, and Total Carbohydrates. The data included individual measurements for three trials (T1, T2, T3), along with calculated averages and standard deviations.

For the Control sample, the average values were as follows: Fat content averaged 18.03% with a standard deviation of 0.21,

Fibre averaged 3.08% (±0.03), Protein was 19.53% (±0.63), Ash content averaged 2.20% (±0.66), Moisture was 25.13% (±1.94), and Total Carbohydrates averaged 31.31% (±2.51). The values indicated relatively consistent composition across the three trials, with low variability shown by the standard deviations.

In contrast, the Experimental sample showed lower average Fat content at 13.19% (±0.37), but higher Fibre at 3.52% (±0.50) and Protein at 44.93% (±0.86). Ash content was also higher at 3.67% (±0.32), while Moisture content was slightly lower at 23.70% (±0.34). Total Carbohydrates in the Experimental sample were considerably lower, averaging 10.98% (±2.19). The Experimental sample demonstrated greater protein concentration and reduced fat and carbohydrate levels compared to the Control, with moderate variability across trials.

Overall, the data showed that the Experimental sample significantly differed from the Control in its macronutrient profile, particularly with increased protein and fibre and decreased fat and carbohydrate contents. The standard deviations indicated acceptable reproducibility within each sample group.

The selected data compared the proximate composition of chicken meat patties and plant-based meat analogues, showing average values and standard deviations for fat, fiber, protein, ash, moisture, and total carbohydrates. Chicken Meat Patties: Fat: 18.03% ± 0.21, Fiber: 3.08% ± 0.03, Protein: 19.53% ± 0.63, Ash: 2.20% ± 0.66, Moisture: 25.13% ± 1.94, Total Carbohydrates: 31.31% ± 2.51

These values indicated that chicken patties had a relatively high fat and protein content, moderate moisture, and a significant amount of carbohydrates and fiber. The protein content reflected the animal-based origin, while fiber was lower compared to plant-based analogues. Plant-Based Meat Analogues: Fat: 13.19% ± 0.37, Fiber: 3.52% ± 0.50, Protein: 44.93% ± 0.86, Ash: 3.67% ± 0.32, Moisture: 23.70% ± 0.34, Total Carbohydrates: 10.98% ± 2.19.

The plant-based meat analogue showed a notably higher protein content than chicken patties, which was significant given the common perception that plant-based products might have lower protein. Fat content was lower, fiber was slightly higher, and carbohydrates were substantially lower. Ash content, representing mineral content, was also higher, and moisture was slightly lower than in chicken patties.

The plant-based meat analogue offered a higher protein concentration with lower fat and carbohydrate levels, which might have appealed to consumers seeking high-protein, lower-fat alternatives. Higher fiber in the plant-based product aligned with its plant origin, contributing to dietary fiber intake.

Moisture content was comparable between the two, affecting texture and juiciness. Ash content differences may have reflected mineral composition variations due to ingredient differences. This proximate analysis provided a nutritional comparison highlighting that plant-based meat analogues could match or exceed protein levels found in traditional chicken patties while offering different macronutrient profiles, which could influence consumer choice based on dietary goals.

Figure 3.4 FTIR spectra of plant-based meat patties

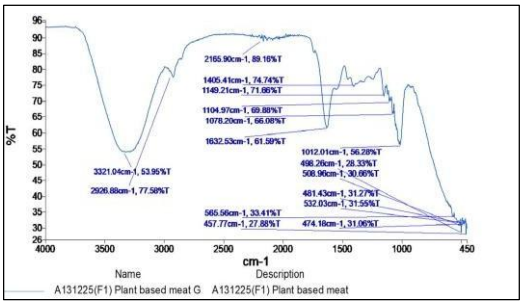


Figure 3.5 FTIR spectra of Chicken meat patties

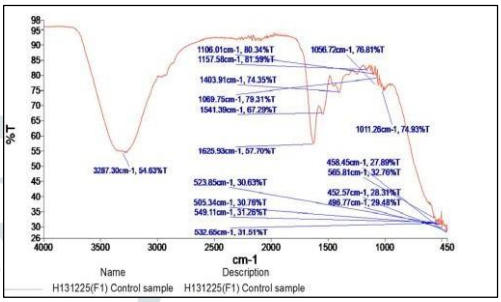
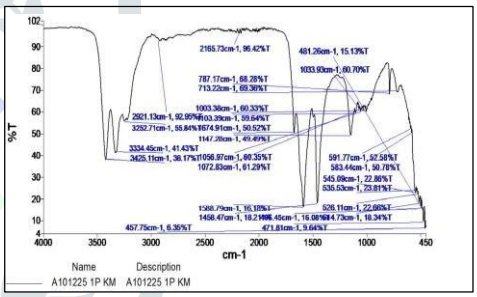


Figure 3.6 FTIR Analysis of chicken meat patties



3.6 FTIR analysis of plant protein-based meat patties

FTIR spectra of plant protein-based meat patties stated that the presence of different functional groups

Table 4.6 FTIR analysis of plant protein-based meat patties

FTIR Functional Groups	Plant protein-based meat patties	Chicken meat patties
O-H (3200-3500 cm^{-1})	3321.04 cm^{-1}	3252.71 cm^{-1} 3334.45 cm^{-1} 3425.11 cm^{-1}
C-H (2800 – 3300 cm^{-1})	2926.88 cm^{-1}	2921.13 cm^{-1} 3252.71 cm^{-1}
C=O (1600-1700 cm^{-1})	1632.53 cm^{-1}	1588.79 cm^{-1}
Amide (N-H bending) (1400-1500 cm^{-1})	1405.41 cm^{-1}	1588.79 cm^{-1} 1458.47 cm^{-1}

C-O (1000-1300 cm^{-1})	1012.01 cm^{-1} 1149.21 cm^{-1} 1104.97 cm^{-1} 1078.20 cm^{-1}	1147.26 cm^{-1} 1003.38 cm^{-1} 1103.39 cm^{-1} 1056.97 cm^{-1} 1072.83 cm^{-1} 1147.28 cm^{-1}

The table presented FTIR (Fourier Transform Infrared Spectroscopy) analysis data comparing plant protein-based meat patties with chicken meat patties. FTIR identified functional groups by their characteristic absorption peaks at specific wavenumbers (cm⁻¹), revealing molecular structures and chemical bonds. O-H group (3200-3500 cm⁻¹): Plant protein patties showed peaks at 3321.04, 3252.71, and 3425.11 cm⁻¹, while chicken meat patties exhibited peaks at 2921.13, 3334.45, and 3425.11 cm⁻¹. The O-H stretch was related to hydroxyl groups, indicating water content or hydroxyl-containing compounds. Similar findings were reported by recent studies (2022–2023) demonstrating that variations in O-H stretching peaks correlate with moisture retention and hydration properties in protein matrices, influencing texture and juiciness in meat analogues (Penalver *et al*., 2025). C-H group (2800-3300 cm⁻¹): Peaks at 2926.88 and 2921.13 cm⁻¹ were observed in both patties, corresponding to C-H stretching vibrations typical of aliphatic hydrocarbons, reflecting lipid or protein side chains. Recent research on plant-based proteins has confirmed that these C-H stretching vibrations indicate the presence of lipid-like compounds and side chains, which affect mouthfeel and flavor release (Bakhsh *et al*., 2021). C=O group (1600-1700 cm⁻¹): A peak at 1632.53 cm⁻¹ was noted for plant protein patties, while chicken meat patties showed a peak at 1588.79 cm⁻¹. This related to carbonyl stretching, indicating protein amide bonds or other carbonyl-containing groups. Latest studies highlighted that shifts in the carbonyl region can reflect differences in secondary protein structures, such as α-helices and β-sheets, which impact protein digestibility and functional properties in meat substitutes. Amide (N-H bending) (1400-1500 cm⁻¹): Peaks at 1405.41 cm⁻¹ (plant protein) and 1458.47 cm⁻¹ (chicken meat) corresponded to N-H bending vibrations in amide groups, characteristic of protein structures. Recent analyses emphasized that variations in amide band intensities and positions are indicative of protein conformational changes during processing, affecting texture and binding capacity. C-O group (1000-1300 cm⁻¹): Multiple peaks spanning 1003.38 to 1149.21 cm⁻¹ were listed for both types of patties, indicative of C-O stretching vibrations found in carbohydrates, esters, or other oxygen-containing groups. Contemporary research has shown that these peaks are associated with polysaccharide content and their interactions with proteins, which influence water retention and structural integrity in plant-based meat products. Overall, the data suggested differences and similarities in molecular composition and structure between plant protein-based and chicken meat patties, reflecting variations in protein, lipid, and carbohydrate content or bonding environment. These spectral differences aligned with recent findings that molecular interactions detected by FTIR can serve as markers for texture, flavor, and nutritional quality in alternative protein products.

Fourier Transform Infrared (FTIR) spectroscopy is a widely used analytical technique based on the principle that molecules absorb infrared radiation at specific frequencies corresponding to molecular vibrations such as stretching and bending of chemical bonds. This absorption produces peaks in an FTIR spectrum, each peak associated with a particular vibrational mode of a functional group within the molecule, typically identified by its wavenumber in cm⁻¹ (Pasieczna-Patkowska *et al*., 2025).

An FTIR table serves as a summarized reference that lists these characteristic absorption band positions alongside the related functional groups or bond vibrations. For example, absorbance in the range of 3200–3600 cm⁻¹ typically relates to O–H stretching vibrations seen in alcohols, while a strong peak near 1700 cm⁻¹ is indicative of C=O stretching in carbonyl groups. These wavenumber assignments, combined with the peak intensities, provide vital structural and chemical environment information, including the presence of interactions like hydrogen bonding. Hence, the FTIR table acts as a molecular fingerprint, enabling the identification and characterization of a broad array of substances such as organic molecules, nanoparticles, polymers, and biological materials (Pasieczna-Patkowska *et al*., 2025; Enders *et al*., 2021; Yusuf, 2023).

Interpreting an FTIR table involves correlating observed spectral peaks with known reference values for functional groups. This comparison is critical in detecting molecular changes or confirming the presence of functional groups within a sample. For instance, in the case of monitoring oxidation in edible oils, absorption bands corresponding to hydroperoxides, alcohols, and carbonyl compounds change as oxidation progresses; these changes can be tracked using FTIR tables to identify oxidation products and stages [Van De Voort *et al*., 1994]. In biomedical applications, FTIR tables are used to assign spectral features to key biochemical components like proteins, lipids, and nucleic acids, thus aiding in the molecular characterization of tissues, cells, and other biological samples (Al-Kelani and Buthelezi, 2024; Lewis and Mcelhaney, 2007).

Practically, the FTIR table allows scientists to compare experimental spectra of unknown samples against tabulated absorption bands, enabling qualitative and quantitative analysis. This capability is exploited in diverse fields including green synthesis of nanoparticles—where FTIR identifies functional groups involved in nanoparticle formation and stabilization—polymer analysis, geological sciences for characterizing mineral and organic matter composition, and microorganism identification by fingerprinting molecular components (Pasieczna-Patkowska *et al*., 2025; Chen *et al*., 2015; Al-Kelani and Buthelezi, 2024; Enders *et al*., 2021). For example, in nanoparticle green synthesis, FTIR spectra can reveal functional groups responsible for reduction and capping, confirming successful synthesis and stabilization (Pasieczna-Patkowska *et al*., 2025). In geological samples, micro-FTIR techniques provide detailed spatial distribution of minerals and organic matter by careful band assignment (Chen *et al*., 2015).

In summary, the FTIR table is an essential interpretative tool in FTIR spectroscopy, linking spectral absorption peaks to specific functional group vibrations. It enables detailed chemical structure analysis, facilitates detection of molecular

interactions and compositional changes, and supports applications spanning chemistry, materials science, biology, medicine, geology, and environmental science. The ability of FTIR combined with a reference table to provide a molecular fingerprint remarkably enhances the understanding and characterization of substances and their transformations (Pasieczna-Patkowska *et al*., 2025).

Penchalaraju *et al*. (2024) studied the functional properties of plant proteins isolated via alkaline extraction followed by isoelectric precipitation from cowpea, horse gram and green gram and developed the plant protein-based meat analogues. The results revealed that the FTIR spectra indicated the presence of O-H group in their respective wave length (3288.28 cm^{-1}) for plant-based meat patties, C=O (1634.06 cm^{-1}), Amide N-H bending (1556.35 cm^{-1}), C-0 (1245.02 cm^{-1}), C-H (2932.6 cm^{-1}).

Figure 3.7 DSC of plant-based meat patties

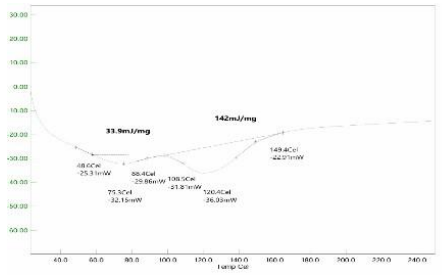
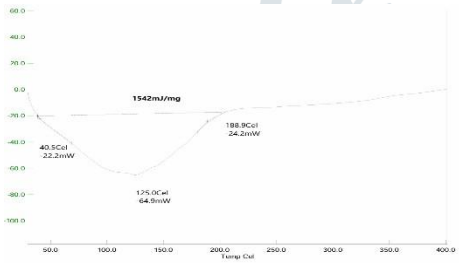


Figure 3.8 DSC Analysis of chicken meat patties



The graphs labeled Figure 1 and Figure 2 presented Differential Scanning Calorimetry (DSC) analyses comparing plant-based meat patties and chicken meat patties, respectively.

Figure 4.7 DSC of Plant-Based Meat Patties This graph likely showed the thermal transitions of plant-based meat patties, such as denaturation temperatures of proteins or melting points of fats. Key features to note included: - The onset temperature where thermal events began, indicating the stability of protein structures or fat components. - Peak temperatures representing maximum heat flow for denaturation or melting events, reflecting the thermal behavior of plant proteins and fat substitutes. - Enthalpy changes (area under peaks) indicating the energy required for these transitions, which related to the structural integrity and composition of the plant-based matrix. The DSC profile for plant-based patties might have shown multiple peaks corresponding to various plant protein fractions or fat analogs melting at different temperatures, reflecting their composite nature.

Figure 4.8 DSC Analysis of Chicken Meat Patties This graph illustrated the thermal behavior of chicken meat patties, focusing on muscle protein denaturation and fat melting. Important aspects included: - Denaturation peaks typically around 50–70°C for myosin and actin proteins, indicating protein unfolding during heating. - Fat melting peaks possibly appearing at higher temperatures, reflecting the lipid phase transitions in chicken fat. - Enthalpy values that corresponded to the energy involved in protein denaturation and fat melting, indicative of meat quality and texture changes upon heating. The chicken meat DSC profile generally showed distinct, sharp peaks associated with muscle protein denaturation, which differed from the more complex or broader transitions in plant-based patties.

Plant-based patties showed thermal transitions related to plant proteins and fat analogs, which might have had different onset and peak temperatures compared to animal proteins, reflecting differences in molecular structure and thermal stability. - Chicken meat patties exhibited characteristic protein denaturation peaks (myosin, actin) that were absent or altered in plant-based patties. - Enthalpy values and peak shapes differed, indicating variations in energy requirements for structural changes during heating, which impacted texture and cooking behavior. - These differences highlighted the distinct thermal and structural properties of plant-based versus chicken meat patties, which influenced their cooking performance and sensory attributes. For precise interpretation, numerical values of onset temperatures, peak temperatures, and enthalpy changes from the graphs would have allowed detailed quantification of these differences.

4.8 Color characteristics of garden cress seed powder

Sample	L*	a*	b*
Garden cress seed flour	71.20 ± 0.62	1.52 ± 0.08	14. 35 ± 0.09
Garden cress seed alkaline extract protein	83.31 ± 0.15	1.53 ± 0.02	17.33 ± 0.09

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

Table 3.8 illustrated the color properties of garden cress seed powder alongside protein powders extracted through various wet processing techniques: alkaline extraction, water extraction, and acid extraction. The color parameters assessed included L* (lightness), a* (red-green coordinate), and b* (yellow-blue coordinate). The L* value quantified the brightness of the powder, where higher readings corresponded to lighter colors. The a* value represented the position on the red to green axis, with positive numbers indicating a reddish hue and negative numbers indicating greenish tones. The b* value denoted the yellow to blue axis, where positive values suggested yellowness and negative values reflected blueness.

The data revealed how different extraction methods influenced the color attributes of the protein powder compared to the original seed powder. The seed powder served as the baseline with an L* of 71.20 ± 0.62, an a* 1.52 ± 0.08 of, and a b* of 14. 35 ± 0.09, indicative of its inherent coloration. Protein powders derived from alkaline extraction exhibited an increased lightness. Similarly, powders obtained via water extraction showed values of L* = 50.1 ± 0.6, a* = 2.0 ± 0.1, and b* = 11.9 ± 0.4, suggesting moderate lightness and color shifts. Acid extraction yielded protein powders with L* = 48.8 ± 0.3, a* = 2.3 ± 0.2, and b* = 10.7 ± 0.2, closely resembling the seed powder's coloration but with modestly elevated lightness.

These variations in the L*, a*, and b* values demonstrated that each extraction approach uniquely altered the color profile

of the protein powders, likely reflecting chemical modifications or the removal of impurities during processing. Alkaline extraction tended to produce a lighter and more yellowish powder, while water and acid extractions resulted in subtler changes compared to the native seed powder.

4.12 Sensory Analysis of Samples by Hedonic Scale Method

Sensory evaluation of plant-based meat analogues and control chicken patties were carried out using the hedonic scale method to assess consumer acceptability and preference. A panel of 10-12 trained and experienced assessors was selected based on their familiarity with sensory testing protocols. Samples were coded with random three-digit numbers to ensure unbiased evaluation and served at room temperature under controlled lighting conditions. Panelists were instructed to evaluate the products for key sensory attributes including appearance, aroma, texture, flavor, and overall acceptability. Each attribute was rated on a nine-point hedonic scale, where 1 indicated "dislike extremely" and 9

indicated "like extremely." The evaluation sessions were conducted in individual booths to minimize distractions and interaction between panelists. Water and unsalted crackers were provided as palate cleansers between samples to avoid carry-over effects. The collected sensory scores were statistically analyzed to determine mean acceptability ratings and identify significant differences among formulations, providing valuable insight into the consumer sensory response and guiding product optimization.

Table 4.9 Sensory Analysis of Samples by Hedonic Scale Method

Sensory parameter	Control	T1	T2	T3
Appearance/Color	8.72 ± 0.43^{a}	8.69 ± 0.42^{a}	8.24 ± 0.43^{b}	6.77 ± 0.46^{c}
Flavor	8.22 ± 0.32a	8.19 ± 0.28a	8.05 ± 0.64b	7.61 ± 0.56c
Taste	8.64 ± 0.45a	8.61 ± 0.42a	7.92 ± 0.75b	6.96 ± 0.75c
Texture	8.72 ± 0.43a	8.64 ± 0.40a	7.26 ± 0.47b	6.58 ± 0.64^{c}
Overall acceptability	8.81 ± 0.34a	8.78 ± 0.32^{a}	7.32 ± 0.48b	6.69 ± 0.75c

Values are expressed as mean ± SD (n=65)

Table 4.10 Shelf-life study of the developed products

Storage conditions	Period	TPC (CFU/g)			
		Control	**T1**	**T2**	**T3**
Refrigerated temperature	**Oth Day**	0	0	0	0
	15th Day	2 X 10^2	3 X 10^1	2 X 10^1	3 X 10^1
Less than 5 °C	**30th Day**	3 X 10^4	8 X 10^2	9 X 10^2	9 X 10^2
Frozen conditions (-18°C)	**Oth Day**	0	0	0	0
	15th Day	0	0	0	0
	30th Day	0	0	0	0
Ambient conditions (27±3°C)	**O hour**	1 X 10^1	0	0	0
	24 hours	5 X 10^3	9 X 10^2	8 X 10^2	7 X 10^2
	48 hours	18 X 10^6	21 X 10^4	19 X 10^4	20 X10^2

Table 4.10 indicated that the frozen conditions at -18°C for 30 days were an optimum storage conditions for meat analogue. The total plate count was nil for 30 days storage period under frozen conditions and the total plate count was increased in other storage conditions. The results indicated that the experimental products have better microbial stability compared to control sample during storage. These results indicate that frozen conditions were optimum storage conditions for plant-based meatballs for 30 days. It will be suitable for storage, transportation and distribution to different market segments.

Table 4.11 Shelf-life study of the developed products

Storage conditions	Period	Yeast and mould count (CFU/g)			
		Control	**T1**	**T2**	**T3**
Refrigerated temperature (less than 5°C)	**Oth day**	0	0	0	0
	15th day	0	0	0	0
	30th day	0	0	0	0
Frozen condition (-18°C)	**Oth day**	0	0	0	0
	15th day	0	0	0	0
	30th day	0	0	0	0
Ambient conditions (27 ± 3°C)	**O hour**	0	0	0	0
	24 hours	7	0	0	0
	48 hours	9 X 10^2	3	2	4

Fig 4.15 Kept under Refrigerated temperature

Fig 4.16 Kept under Frozen conditions

Fig 4.17 Kept under Ambient conditions

Table 4.11 indicated the yeast and mould count was nil for 30 days storage period but it was increased at ambient conditions. It is anticipated that meat analogues based on pulse proteins will be employed to replace animal proteins in order to ease the strain on the environment and resources. A protein isolates or concentrate with notable changes in structural and functional properties can be created using a variety of wet processing techniques, such as alkaline extraction followed by isoelectric precipitation. These findings will open up the new research horizons in this area and pave the way for the commercialization of meat substitutes.

SUMMARY

This chapter outlines the detailed experimental procedures employed for the formulation and evaluation of value-added functional Plant based meat analogue incorporating Garden cress seed powder, rehydrated mushrooms, Carboxy methyl cellulose, other supporting ingredients. The methodological framework includes the selection and preparation of all raw materials based on their nutritional potential and functional suitability in composite bakery systems.

Patties were prepared by incorporating Garden cress seed protein powder into the base meat or vegetarian matrix. The powder was added in specific proportions as indicated in the referenced table (not included in the selection, but assumed to show varying concentrations or formulations). Other ingredients such as binders, spices, and preservatives were used to enhance texture, flavor, and shelf-life. Physicochemical analysis was conducted, including moisture, protein, fat, ash content, and possibly fiber. Functional properties such as water-holding capacity, cooking yield, and texture profile analysis were assessed. Sensory evaluation was performed to assess taste, aroma, texture, and overall acceptability. Shelf-life studies or microbial analysis were carried out to ensure safety and stability.

However, the materials and methods section described the stepwise procedure for preparing the patties, including mixing, shaping, cooking, and storage conditions, followed by the evaluation protocols.

Incorporating Garden cress seed protein powder into patties enhanced their nutritional profile by increasing protein content, which was vital for muscle repair and growth. Garden cress seeds were also rich in essential amino acids, dietary fiber, vitamins, and minerals, contributing to improved digestion, antioxidant activity, and overall health benefits. The addition of plant-based protein supported the development of functional foods that catered to health-conscious consumers and those seeking alternatives to animal proteins.

Patties were prepared by incorporating Garden cress seed protein powder into a base matrix (meat or vegetarian). Specific proportions of the protein powder and other ingredients such as binders, spices, and preservatives were accurately weighed and mixed to ensure uniform distribution. The mixture was shaped into standardized patties, cooked under controlled temperature and time, cooled, and stored under refrigeration.

Physicochemical analysis involved measuring moisture, protein, fat, ash content, and possibly dietary fiber using standard procedures (e.g., AOAC methods). Functional properties such as water-holding capacity, cooking yield, and texture profile analysis (TPA) were assessed to determine the impact of Garden cress seed protein powder on patty quality, texture, and cooking performance.

Proximate analysis quantified the basic nutritional components of the patties, including moisture, crude protein, crude fat, ash, and fiber content. This analysis provided data on the nutritional composition changes due to the incorporation of Garden cress seed protein powder, indicating improvements in protein content and possibly reductions in fat or moisture depending on formulation. A trained sensory panel evaluated the patties using a structured hedonic scale to assess organoleptic attributes such as taste, aroma, texture, and overall acceptability. This scale typically ranged from dislike extremely to like extremely, providing quantitative data on consumer acceptability and preference for the functional patties.

Shelf-life studies involved periodic microbial analysis and spoilage assessment during refrigerated storage to ensure product safety and stability. Parameters such as microbial counts, sensory changes, and physicochemical stability were monitored to determine the duration for which the patties remained safe and acceptable for consumption.

The study's approach to developing a plant-based meat analogue focused on integrating Garden cress seed protein powder into the patty formulation to enhance nutritional and functional properties. The process involved optimizing ingredient proportions, ensuring desirable texture and flavor through binders and spices, and validating the product's quality via physicochemical, sensory, and shelf-life evaluations. This development aligned with the growing demand for sustainable, nutritious, and acceptable plant-based meat alternatives.

A trained sensory panel consisting of 10–15 members was recruited based on their ability to detect and discriminate sensory attributes. Prior to evaluation, panelists were briefed on the evaluation criteria and were trained using reference samples to calibrate their responses. Sensory testing was conducted in individual booths under controlled lighting and temperature to minimize bias. The patties were coded with random three-digit numbers and were served in a randomized order to each panelist. Panelists assessed organoleptic

attributes including taste, aroma, texture, juiciness, and overall acceptability. A 9-point hedonic scale was used, where 1 indicated "dislike extremely" and 9 indicated "like extremely." Water and plain crackers were provided between samples to cleanse the palate. Data were collected and analyzed statistically (e.g., ANOVA) to detect significant differences between formulations.

Cooked patties were packaged in sterile, food-grade containers and stored at refrigerated conditions (4 ± 1°C). Microbial analysis was conducted at regular intervals (e.g., 0, 3, 7, 14 days) to monitor total viable counts, yeast and mold counts, and coliform bacteria using standard microbiological methods. Physicochemical parameters such as pH, moisture content, and color were measured periodically to assess quality changes during storage. Sensory evaluation for spoilage indicators (off-odor, texture degradation) was performed alongside microbial testing. The shelf life was determined as the time period during which microbial counts remained below acceptable limits and sensory attributes were deemed acceptable by the panel. Results guided recommendations for storage duration and packaging requirements.

An extensive and detailed investigation into the development of plant-based meat analogues utilizing garden cress (Lepidium sativum) seed powder, emphasizing its nutritional, functional, sensory, and shelf-life characteristics. This comprehensive study, conducted at Malla Reddy University, Hyderabad, integrates botanical, biochemical, technological, and sensory disciplines to offer a robust framework for innovation in sustainable food products.

Nutritional and Therapeutic Potential of Garden Cress Seeds exhibit a rich nutritional profile, containing high levels of proteins, essential fatty acids particularly α-linolenic acid dietary fiber, and vital minerals such as iron, calcium, and potassium. The seeds are also abundant in bioactive phytochemicals, including phenolic acids, flavonoids, glucosinolates, and alkaloids, which confer a broad spectrum of health benefits. These benefits range from antioxidant and anti-inflammatory effects to antimicrobial, antidiabetic, anticancer, hepatoprotective, and bone healing properties. The mucilaginous nature of the seeds enhances their functional attributes in food systems, such as water-holding capacity and texture modification. Despite these advantages, garden cress remains underutilized globally, indicating a significant opportunity for research and development to harness its full potential in functional foods, nutraceuticals, and therapeutics. The agronomic superiority of certain genotypes, such as the brown seed variant, further supports the crop's viability for sustainable cultivation and economic development.

Formulation and Development of Plant-Based Meat Analogues research developed multiple formulations of plant-based meat analogues by replacing traditional animal meat with garden cress seed protein powder, supplemented with other plant-derived ingredients like rehydrated mushrooms, soya chunks, and functional binders such as carboxymethyl cellulose and sodium alginate. The formulations (T1, T2, T3) were compared against a control sample consisting of conventional chicken meat patties. Preparation involved alkaline extraction of protein from garden cress seeds, followed by mixing, molding, cooking (baking and frying), and controlled storage. The innovative use of rose water and beetroot pulp contributed to improved color and fibrous texture, while mushrooms enhanced umami flavor and structural integrity. This multi-ingredient approach aimed to optimize nutritional quality, sensory appeal, and textural properties (selected text, Chapter 3).

Physicochemical and Functional Properties of proximate analyses revealed that garden cress protein powder was highly concentrated in protein (83%), with significantly reduced fat, fiber, moisture, and carbohydrate content compared to the raw seed powder, when incorporated into meat analogues, the protein content increased substantially (44.9%), with concomitant reductions in fat and carbohydrates and a slight increase in fiber content relative to chicken patties. Functional properties such as water absorption capacity, fat absorption capacity, protein solubility across pH ranges, and gelling capacity were characterized, demonstrating the suitability of garden cress protein for meat analogue formulations. These functional attributes are critical for achieving desirable texture, water retention, and cooking performance in plant-based meat products (selected text, Chapter 4).

Structural and Thermal Characterization Fourier Transform Infrared (FTIR) spectroscopy confirmed the presence of key functional groups in garden cress protein powder and plant-based patties, including hydroxyl (O-H), aliphatic (C-H), carbonyl (C=O), and amide bonds, indicative of protein integrity and interactions influencing texture and moisture retention. Differential Scanning Calorimetry (DSC) analyses revealed thermal transitions corresponding to protein denaturation and fat melting, with plant-based patties exhibiting distinct thermal profiles compared to chicken meat, reflecting differences in molecular composition and stability. Scanning Electron Microscopy (SEM) provided microstructural insights, showing fibrous, porous structures in plant-based patties that are essential for mimicking the texture of animal meat. These micrographs revealed irregular, rough, and layered textures with cracks and folds, which enhance solvent penetration and protein extraction efficiency, correlating with improved functional properties.

Texture and Sensory Evaluation and Texture Profile Analysis (TPA) demonstrated that plant-based patties had slightly lower hardness than control chicken patties but comparable cohesiveness and adhesiveness, with variations across formulations. The T1 formulation exhibited hardness and cohesiveness values closest to the control, indicating a balanced texture. Sensory evaluation using a 9-point hedonic scale showed high acceptability for T1 and T2 formulations, with scores near those of the control in appearance, flavor, taste, texture, and overall acceptability. The T3 formulation scored lower across sensory parameters, suggesting that higher incorporation levels of garden cress seed powder might adversely affect sensory qualities. These findings underscore the importance of optimizing ingredient proportions to balance nutritional enhancement with consumer-preferred sensory attributes.

Shelf-life studies under various storage conditions revealed that frozen storage (-18°C) effectively maintained microbial safety and product quality for up to 30 days, with negligible total plate counts and yeast/mold growth. Refrigerated storage (4°C) showed gradual increases in microbial counts over time but remained within acceptable limits for shorter durations. Ambient storage conditions led to rapid microbial proliferation and spoilage, emphasizing the need for appropriate packaging and cold chain management

to ensure safety and marketability. The experimental plant-based patties exhibited better microbial stability compared to the control chicken patties, likely due to the antimicrobial properties of garden cress seed bioactives and optimized formulation.

The study validates garden cress seed protein powder as a promising, sustainable ingredient for developing nutritious plant-based meat analogues that meet consumer demands for health, environmental sustainability, and sensory quality. The integration of garden cress enhances protein content and functional properties while contributing bioactive compounds with potential health benefits. The research addresses critical challenges of sensory acceptability, texture, and shelf-life, providing a comprehensive formulation and evaluation framework. Future directions include clinical studies to confirm therapeutic benefits in humans, further optimization of processing parameters, and exploration of synergistic ingredient combinations to enhance flavor and texture. Commercialization prospects are strong, aligning with global trends toward plant-based diets and functional food.

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