Sustainable Food Technology

ROYAL SOCIETY OF CHEMISTRY

PAPER

View Article Online
View Journal

Cite this: DOI: 10.1039/d5fb00437c

Studies on the physiochemical, thermal, structural and functional attributes of vetch starch (*Vicia villosa*) and protein from Kashmir valley

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Vicia villosa (vetch), a leguminous plant and a significant source of starch and protein, is primarily used for animal nutrition, but it also has potential for human consumption. This study aimed to isolate starch and protein from vetch seeds and evaluate them based on various physicochemical, functional, structural, and thermal characteristics. The extracted protein and starch exhibited high purity with a C-type crystalline structure. The chemical composition of starch revealed moisture (7.7 ± 0.20), protein (0.09 ± 0.01), fat (0.02 ± 0.00) and ash (0.03 ± 0.01) contents, while that of protein showed moisture, fat, and ash in the range of 9.2 ± 0.3, 2.3 ± 0.26 and 2.05 ± 0.05 g/100 g. SEM revealed an oval or kidney-shaped starch structure, while a heterogenous lamellar structure with some particle aggregation in the case of vetch starch. The DSC analysis demonstrated early gelatinization with increased enthalpy (6.94 J g^{-1}) in vetch starch, suggesting a stable crystalline region and adequate heat resistance whereas vetch protein displayed two separate denaturation peaks (86.19 °C and 227.86 °C), affirming the existence of vicilin (7S) and legumin (11S) fractions, which provide structural stability and functionality during processing. SDS-PAGE displayed polypeptide sub-units ranging between 25 kDa and 75 kDa in molecular weight with the majority of bands characteristic of vicilin and legumin. The pasting behavior of vetch starch showed an effective swelling tendency with strong gel forming capacity, as evident from the high peak, setback and final viscosities – attributes favorable for thickening, texturizing, and film production. Also, vetch protein demonstrated promising functional properties (emulsifying, gelling, and foaming), highlighting its potential applications in food, packaging and various industrial sectors.

Received 29th July 2025
Accepted 16th October 2025

DOI: 10.1039/d5fb00437c

rsc.li/susfoodtech

Sustainability spotlight

The present study provides a pathway for technological valorization of the underutilized pulse variety *Vicia villosa*. Utilizing *Vicia villosa*, a natural and underutilized legume, promotes biodiversity, decreases reliance on conventional crops, and strengthens local agricultural markets. Vetch is well-known for its abundant protein content and nutritional value, making it a sustainable plant protein source. Vetch protein isolates have superior emulsifying and foaming capabilities, indicating possible applications in food formulations. Also, vetch starch has remarkable properties like a high gelatinization temperature, making it a viable source for both the food and non-food sectors. Thus, the physiochemical and functional investigation of vetch starch and protein suggests the potential for biodegradable film synthesis, providing environmentally acceptable alternatives to synthetic packaging. Overall, the research aims to promote zero-waste valorization and circular bioeconomy models by extracting starch and protein from locally available biomass, improving efficient use of resources and facilitating the transition towards green protein, collectively aimed at building a sustainable and resilient food ecosystem.

1 Introduction

Global population was calculated to be 8.2 billion in 2024 (ref. 1) which is likely to continue growing for the next 50 to 60 years, reaching approximately 9.8 billion in 2050 and 10.3 billion by the mid-2080s.[2] With this drastic increase in global population, the need for food is expected to increase by 50%, necessitating a boost in agricultural productivity.[3] This spike in population, along with altering dietary preferences toward a protein-rich diet and increasing environmental concerns about conventional livestock farming, has put enormous pressure on agricultural systems to find novel sources of protein. Additionally, the increased need for sustainable, cheaper and new sources of plant-based carbohydrates led to studies on different legume varieties. Although legumes like soybeans, chickpeas, and lentils are widely employed, lesser-known and underutilized legumes provide unexplored potential for the development of nutritional and functional ingredients. *Vicia villosa*, which is also known as hairy vetch, is one of approximately 150 species that comprise the genus *Vicia* and the family Fabaceae (legume). It is a cool-season, creeping-growth legume, cultivated for green manure, pasture, silage, and grain for cattle feed under the

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native name "kalamatar". The smooth, spherical, black or brown vetch seeds have a diameter between 3.5 and 5.0 mm and are categorized as an underutilized legume. Currently found throughout the world, vetch is essentially considered a multi-purpose crop because it is utilized as pasture or silage for livestock and as green manure or a cover crop in agriculture.[4] However, these plants are native to tropical Africa, North and South America, East and Central Asia, and Europe. *Vicia villosa* seeds are considered a good source of carbohydrates, protein, fiber, and minerals like magnesium, iron, potassium, *etc.* and are valuable for human well-being. They also contain various phenolic compounds and flavonoids, including daidzein, quercetin, formononetin, diosmetin, kaempferol, luteolin, myricetin, petunidin, malvidin, *P*-hydroxy benzoic acid and gallic acids.[5] It is mostly used as a source of protein for poultry and cattle, but it can also be consumed by humans. This leguminous plant produces seeds that have significantly high starch content ranging from 30–47% (DW basis).[6] The seeds of *V. villosa* have significant amounts of crude protein, ranging from 17–25% and in some studies up to 28%.[7–9] Thus, vetch is not only an important source of starch and protein for farm animals, but it also offers a potential diet for humans. *Vicia villosa*, as a non-conventional source of starch, has the ability to broaden the range of necessary functional qualities required to generate high-value food products and promote the use of sustainable agricultural resources.

Even though legumes like soybean, chickpea, *Vicia sativa* and lentil have been investigated, *Vicia villosa* (especially from the Kashmir valley) has received less attention despite having similar or higher protein and starch content. Existing studies on vetch species have mostly concentrated on their proximate composition and value as animal feed, paying little attention to their techno-functional, structural, or thermal properties. Furthermore, integrated investigations utilizing multi-technique analyses, including differential scanning calorimetry (DSC), X-ray diffraction (XRD), rapid visco analysis (RVA), and scanning electron microscopy (SEM), are still lacking for *V. villosa*. Therefore, by offering a thorough assessment of the protein and starch extracted from *Vicia villosa*, this study advances current knowledge by systematically characterizing its physicochemical, thermal, structural, and functional properties, emphasizing the plant's potential as an underutilized, sustainable source of functional ingredients. By investigating these properties, it is possible to assess their potential applications in various food systems to highlight the importance of vetch seeds as a novel source for broader utilization beyond agricultural uses.

2 Materials and methods

2.1 Materials and chemical reagents

Vetch (*Vicia villosa*) seeds were procured from Srinagar local markets. Seeds were cleaned of dust and other contaminants and stored at 20 °C for future use. The study employed only analytical-grade substances.

2.1.1 Starch extraction. Isolation of starch from vetch seeds was performed in accordance with the technique described by Wani *et al.*[10] with slight modifications. 1 kg of sample was soaked in 4 L of distilled water for 12 hours at 4 °C. Seed coats were removed manually. Cotyledons were pulverized with water in a blender for 2–3 minutes. The slurry was diluted ten times (v/v) with distilled water and adjusted to pH 10 with 0.1 M NaOH. The slurry was constantly stirred with a magnetic stirrer for 1 hour before filtering through a 75 μm mesh sieve to separate the fiber. The filtered slurry was centrifuged at 3000×*g* for 30 minutes at 10 °C (REMI CPR-24, REMI Laboratory Instruments, Mumbai, India). After centrifugation, the aqueous fraction was collected to extract proteins, while the sediment was scraped and the bottom white part was recovered as starch. The collected starch was dried at 40 °C in a hot air oven and was ground and stored in airtight containers for further analysis.

2.1.2 Protein extraction. The vetch protein was extracted using the alkaline extraction and acid precipitation technique, explained by Wani *et al.*[11] The aqueous fraction obtained during centrifugation from starch extraction, was adjusted to pH 4.5 (isoelectric pH) with 0.1 N HCl to precipitate the proteins. The protein was then recovered by centrifugation at 8000×*g* for 10 min at 5 °C. The supernatant was removed and the protein curd was collected and washed with distilled water. The protein curd was re-dispersed in distilled water, adjusted to pH 7 with 0.1 N NaOH, and then freeze-dried as protein isolate (BK-FD10P, BIOBASE, BIODUSTRY, Shandong, China). The freeze dried protein isolate was stored in airtight glass containers at 4 °C for further use.

2.2 Physico-chemical properties

2.2.1 Proximate composition. Determination of moisture, protein, fat and ash contents was performed according to AOAC methods[12] and is reported in grams per 100 grams. The Kjeldahl method was used to determine the protein content of the vetch protein isolate in accordance with accepted AOAC protocols.[12] The nitrogen content was converted to protein content using a conversion factor of 6.25. All measurements were carried out in triplicate.

2.2.2 Yield. Starch yield (%) was estimated by calculating the grams of pure starch isolated from 1000 grams of vetch seeds and multiplying by 100.[13] However, protein isolate recovery and yield were assessed using the method proposed by Sofi *et al.*[14]

Protein isolate recovery was measured by weighing the extracted isolates per 100 g of vetch seed, while the protein yield of isolates was calculated using the following equation.

$$\text{Protein yield} = \text{protein recovery} \times \frac{\text{isolate protein(g/100 g)}}{\text{vetch protein(g/100 g)}}$$

2.2.3 Apparent amylose content. The apparent amylose content (AAC) was calculated by using the iodine-blue method as described by Wani *et al.*[15] with minor modifications. 20 mg of starch sample was added to 10 mL of 0.5 M KOH and properly mixed to prepare a suspension. The suspension was poured into a 100 mL volumetric flask and was diluted with distilled water up to the mark. A 10 mL aliquot of the test starch mixture was

pipetted out into a 50 mL volumetric flask and then 5 mL of 0.1 M HCl and 0.5 mL of iodine reagent were added. The mixture was then diluted to 50 ml with distilled water and left to stand for 5 min. Absorbance of the sample was recorded at 625 nm and the amylose content was calculated using a standard calibration curve developed from standard blends of amylose and amylopectin.

2.2.4 Color. The color of the starch and protein was assessed *via* a Color Flex spectrocolorimeter (Hunter Lab Color flex EZ Model No. 45/0, Serial No. CFEZ2243) after standardization employing Hunter Lab color standards. The color parameters L^* (darkness to lightness), a^* (green to red) and b^* (blue to yellow) values were analyzed.

2.2.5 Swelling and solubility index. The starch samples were analyzed for the swelling and solubility index following the protocol of Wani *et al.*[16] A starch sample (0.2 g dry basis) was placed in a pre-weighed centrifuge tube and filled with 10 mL distilled water. The starch slurry was then heated in a water bath at 50, 60, 70, 80, and 90 °C for 30 min with vortexing every 5 min. The samples were allowed to cool at room temperature, followed by centrifugation at 5000×*g* for 15 min. Pre-weighed moisture dishes were used to decant each supernatant. Then the centrifuge tubes were weighed and the gain in weight was expressed as the swelling index. Moisture dishes were dried at 110 °C for 12 hours before cooling in a desiccator at room temperature. The increase in weight of the moisture dishes was determined as the solubility index.

2.2.6 Syneresis. Syneresis of vetch seed starch was measured using the method of Sofi *et al.*[17] with minor modifications. 3 g of starch was suspended in 50 mL of distilled water and heated in a water bath (REMI-RSB-12, REMI Elektrotechnik Limited, India) at 90 °C in separate centrifuge tubes for 30 min. The starch suspensions were stored for 1, 2, 3, 4, and 5 days in separate centrifuge tubes for each day. The syneresis was determined as the percentage of water released following centrifugation at 3000×*g* for 10 min.

2.2.7 Light transmittance. Starch gels were prepared to calculate (%) light transmittance by the method described by Wani *et al.*[16] An aqueous starch suspension (1% DB) was prepared by heating the slurry for 30 min at 90 °C in a water bath (REMI-RSB-12, REMI Elektrotechnik Limited, India) with constant stirring at 70 rpm. The suspension was cooled at room temperature for 1 hour. Then the samples were stored under refrigeration for 5 days and transmittance was calculated by measuring the absorbance after every 24 hours at 640 nm against water as a blank using a UV spectrophotometer (SP-UV 300, SPECTRUM, PerkinElmer Company, New York).

2.2.8 Freeze–thaw stability. Gelatinized starch pastes were examined for freeze–thaw stability according to the methodology of Hoover & Ratnayake[18] with some modifications. An aqueous starch suspension (3 g w/v) was made in 50 mL distilled water in centrifuge tubes, followed by heating in a water bath at 90 °C for 30 min. The gelatinized gels were cooled at 4 °C for 16 hours, followed by freezing at −16 °C for 24 hours. To determine the freeze–thaw stability, the gels frozen at −16 °C for 24 hours were thawed at room temperature for 6 hours and then refrozen again at −16 °C. Five such freeze–thaw cycles were performed, and the tubes were centrifuged at 1000×*g* for 20 min at 20 °C. The released water was used as a measure of the freeze–thaw stability of starch pastes.

2.3 Pasting properties

Pasting parameters of vetch seed starch were computed by means of a Rapid-visco Analyzer (Tech Master, Pertain Instruments, Warriewood, Australia) using the procedure described by Wani *et al.*[16] An aqueous starch suspension containing 3 g of starch (10.7%, w/w) on a 14% moisture basis was prepared in an aluminum canister (28 g capacity). Initially, the sample was held at 50 °C for 1 min, then heated to 95 °C at a rate of 12.2 °C and maintained at this temperature for 2.5 min, followed by cooling to 50 °C at a rate of 11.8 °C min^{-1} and again held for 2 min. During the test, the paddle rotated continuously at a speed of 160 rpm, except for the initial 10 seconds when it rotated faster at 960 rpm for proper dispersion of the sample.

2.4 Differential scanning calorimetry (DSC)

Vetch starch and protein samples were subjected to differential scanning calorimetry in order to determine their onset (T_{onset}), peak (T_{peak}), and end (T_{end}) temperatures as well as their enthalpy (ΔH). A Differential Scanning Calorimeter (Perkin Elmer, Model: DSC 6000) was used to monitor the DSC thermograms of the protein and starch samples. Samples of 4.1 mg (starch) and 4.9 mg (protein) were hermetically sealed and then heated in aluminum pans between 20 and 150 °C for starch and 20 to 250 °C for protein at a scanning rate of 10 °C min^{-1}. As a reference, an empty pan was used.

2.5 SDS-PAGE

The molecular weight of unmodified vetch protein isolate was determined by SDS-PAGE utilizing a BIO-RAD Mini-PROTEAN system, as described by Strauch & Lila[19] with minor changes. A powdered protein sample of 0.2 mg was solubilized in 500 μL Laemmle buffer (5×) and denatured for 20 min at 85 °C. The sample was then subjected to centrifugation at rpm of 15 000 for 5 min. Afterwards the gels were run at a steady voltage of 90 V for 2 to 2.5 hours at room temperature in a premixed 12% resolving gel (Acryl-bis, Tris, SDS, APS and TEMED). Then the gels were stained using Hi-Media Coomassie Brilliant Blue Stain (G-250, MB092-25G) for 1.5 hours and finally de-stained with double distilled water overnight at a shaking speed of 12. The gels were again rinsed with double-distilled water until protein bands started appearing on the gel and imaged with a Chem-iDoc MP Imaging System (Invitrogen by Thermo Fischer Scientific, Model: CL1500, Singapore). The molecular weights were estimated using a standard from 11 to 75 kDa.

2.6 Fourier transforms infrared (FTIR) spectroscopy

The FTIR spectra of starch and protein samples were captured on a FTIR spectrophotometer (PerkinElmer) coupled to an ATR accessory at room temperature. Finely ground starch and protein powders were measured in the mid-infrared region with

a resolution of 4 cm^{-1} and over a spectral range of 500–4000 cm^{-1}.

2.7 Scanning electron microscopy (SEM)

To interpret SEM micrographs of vetch starch and protein granules, a field emission scanning electron microscope (JSM-6480 LV, JEOL, Japan) was used following the protocol of Jafari *et al.*[20] The dried samples were coated with gold particles using a sputter coater before examination. The pictures were taken at an acceleration voltage of 15 kV.

2.8 X-ray diffraction (XRD)

The crystalline structure of vetch starch and protein samples was analyzed with an X-ray diffractometer (Model: D8 Advance DAVINCI, Bruker AXS Inc. Madison, WI, USA) according the methodology of de Oliveira Filho *et al.*[21] The samples were scanned over a 2θ range of 5 °C to 40 °C at an average temperature of 3.2° per minute with a step value of 0.0131°.

2.9 Functional properties

2.9.1 Water and oil absorption capacity. Water and oil absorption capacities were assessed following the procedure of Stone *et al.*[22] with certain alterations. Starch/protein (2.5 g on db) was mixed with 30 mL of distilled water/oil in a pre-weighed centrifuge tube (50 mL). The samples were vortexed (Digital Vortex Mixer, Labtronics, Model: LT82) every 20 seconds for 30 min and then centrifuged (REMI CPR-24, REMI Laboratory Instruments, Mumbai, India) at 3000×*g* for 20 min. The supernatant was carefully decanted without disturbing the pellet. The centrifuge tube containing the pellet was weighed. The gain in weight divided by the original weight of the sample was reported as the percentage of water/oil absorption capacity.

2.9.2 Foaming capacity (FC) and foaming stability (FS). The foaming capacity and foaming stability of vetch protein isolate were determined according to the procedure of Wani *et al.*[23] with some modifications. A 2% (w/v db) aqueous protein dispersion at pH 4, 7, and 10 was homogenized at 10 000 rpm for 1 min in a 250 mL measuring cylinder with a high-speed homogenizer (IKA, Ultra Turrax, Model: T 18 digital, Germany). Foaming capacity was calculated as the percent increase in the volume of foam of protein dispersion upon homogenization. The foam stability was analyzed by measuring the volume of foam over time.

2.9.3 Emulsion capacity (EC) and emulsion stability (ES). Emulsifying properties like EC and ES were calculated by the turbidimetric method developed by Pearce & Kinsella.[24] An emulsion was prepared by homogenizing 2 mL refined (soyabean) oil and 6 mL of 0.2% protein solution adjusted at pH 3, 5, and 7 using a mechanical homogenizer at 10 000 rpm for 1 min. Immediately after homogenization, 50 μL of emulsion was pipetted out from the bottom of the centrifuge tube into 5 mL of 0.1% (w/v) SDS solution at 0 and 10 min. The absorbance of the resulting emulsion was recorded as initial absorbance (A_0) and the absorbance after 10 min (A_{10}) at 500 nm using a UV spectrophotometer (SP-UV 300, SPECTRUM, PerkinElmer Company, New York). The absorbances measured at (A_0) and (A_{10}) after emulsion formation were used to calculate the emulsifying activity index (EAI) and the emulsion stability index (ESI).

$$\mathrm{EAI}\left(\mathrm{m^2\ g^{-1}}\right) = \frac{2 \times 2.303 \times A_0 \times \mathrm{DF}}{c \times \phi \times \theta \times 10\,000}$$

$$\mathrm{ESI(min)} = \frac{A_0}{(A_0 - A_{10}) \times 10}$$

where DF is the dilution factor (100), c is the initial concentration of protein (g mL^{-1}), φ is the optical path (0.01), θ is the oil volume fraction of the emulsion (0.25) and A_0 and A_{10} are the absorbances of the emulsion at 0 and 10 min, respectively.

2.10 Statistical analysis

All studies were carried out in triplicate and the data were presented as the average of three readings. An analysis of variance (ANOVA) with a significance level of 5% was done and Duncan's test was performed for assessing differences between means using commercial data analysis software (SPSS Inc., Chicago, IL, USA). Statistical analysis was performed using OriginPro 2018 software (OriginLab Corporation, Northampton, MA, USA).

3 Results and discussion

3.1 Physico-chemical properties

3.1.1 Proximate composition. The chemical composition of native vetch starch and protein included moisture, ash, protein, and fat (Table 1). Vetch starch and protein showed moisture content ranging from 7.7 to 9.2 g/100 g, fat content ranging from 0.02 to 2.3 g/100 g and ash content between 0.03 and 2.05 g/100 g. The protein content of vetch starch was found to be 0.09 g/100 g, while that of vetch protein was observed to be 85.66 g/100 g.[25]

The carbohydrate content of vetch starch was estimated to be 91.16 (g/100 g), respectively. Also, the apparent amylose content of vetch seed starch was 31.25 g/100 g. These values are consistent with the findings of Tarahi *et al.*[26] for bitter vetch starch, Wani *et al.*[27] for Bengal gram starch, and Wani *et al.*[11] for kidney bean protein isolates.

3.1.2 Yield. The isolated starch yield was 285.6 g per kilogram of vetch seeds, which falls within the reported range of 220–450 g kg^{-1} for the majority of legumes.[17]

Protein isolate recovery of vetch protein was calculated to be 13 g/100 g, whereas protein yield was estimated to be 46.4 g/

Table 1 Proximate composition of vetch starch ($n = 3$)[a]

Parameter	Vetch starch	Vetch protein
Moisture (g/100 g)	7.70 ± 0.20	9.20 ± 0.30
Protein (g/100 g)	0.09 ± 0.01	85.66 ± 0.57
Fat (g/100 g)	0.02 ± 0.00	2.30 ± 0.26
Ash (g/100 g)	0.03 ± 0.01	2.05 ± 0.05
Carbohydrate (g/100 g)	92.16 ± 0.00	ND

[a] ND: not-detected. Values expressed are mean ± standard deviation.

Table 2 Color, yield and amylose content of vetch starch[a]

Parameter	Vetch starch	Vetch protein
Amylose (g/100 g)	31.25 ± 0.50	ND
Yield (g/100 g)	28.56 ± 0.23	
Protein isolate recovery (g/100 g)	ND	13 ± 1.50
Protein yield (g/100 g)	ND	50.61 ± 3.90
Color		
L^*	93.93 ± 0.15	83.51 ± 0.46
a^*	−0.14 ± 0.01	3.80 ± 0.25
b^*	6.48 ± 0.09	18.20 ± 0.26

[a] ND = not detected. Values expressed are mean ± standard deviation.

100 g (Table 2). The observed data regarding the recovery of vetch protein isolates are consistent with the results reported by Stone *et al.*[22] and Sofi *et al.*[14] for pea protein and chickpea protein isolates.

3.1.3 Color. The color characteristics of protein powder were significantly different from those of starch. The lightness (L) value of starch was 93.93 and that of vetch protein was 83.51 (Table 2). Similar lightness values were also observed by Mukhtar *et al.*[28] and Schumacher *et al.*[29] for oat grain starch and pea protein isolates. The 'a' and 'b' color values of vetch starch were reported to be −0.14 ± 0.01 and 6.48 ± 0.09 while those of protein were 3.8 and 18.2, respectively. The obtained results are consistent with those obtained by Shubeena *et al.*[30] and Shevkani *et al.*[25] The higher L^* value of starch indicates its greater whiteness and purity, which is generally desirable for applications requiring transparent appearance (like edible films or coatings). In contrast, the higher a^* (redness) and b^* (yellowness) values of protein suggest a more pigmented appearance, likely arising from residual pigments or Maillard reaction products formed during protein extraction. Even though color characteristics are influenced by extraction technique, particle size, and processing procedure, they can be highlighted in terms of their possible applications. For example, lightness in starches and protein powders is frequently thought to be associated with purity, transparency, and quality; color is a crucial factor in terms of consumer acceptability and perception, particularly for biodegradable films intended for food use. The stability of the product can be indirectly indicated by changes in L^*, a^*, or b^* values, which would also show oxidative or degradative changes during storage which may affect the functional stability of the film as well as its aesthetic appeal. Therefore, the color values (L^*, a^*, or b^*) of vetch starch and protein isolates are essential markers of film transparency, appearance, and potential light-barrier functioning, which assist in determining their applicability for packaging applications.

3.1.4 Swelling and solubility index. Swelling power is an indicator of the water-retaining ability of starch molecules during gelatinization and is commonly used to distinguish between different forms of starches. When starch is heated in water, the hydrogen bonds that exist between water and the hydroxyl groups of amylose and amylopectin are disrupted, causing swelling and disassociation of the starch granules. As a result, the granules release some of their amylose content. Thus, the swelling capacity is the ratio of the deposited gel to the dry weight of starch, which depends on multiple factors including the amylose/amylopectin ratio, branch length of amylose and amylopectin, and the distribution of molecular weight.[31] Solubility refers to the percentage of starch released into the supernatant during the determination of swelling capacity. Solubility is a function of temperature, with higher values at increased temperatures and lower values at lower temperatures.

The swelling index (SI) ranged between 2.75 and 13.71, and 2.90 to 11.09 g g^{-1} for vetch starch and protein respectively. The SI of both vetch starch and protein showed an increase with the increase in temperature from 50 °C to 90 °C (Table 3). The increase in swelling power could be attributed to the breakdown of intermolecular interactions, such as hydrogen bonding, as temperature increases. The solubility index also increased with increasing temperature from 50–90 °C. The water solubility index of vetch starch ranged from 0.002 to 0.19 and from 0.03 to 0.14 for vetch protein. These results are comparable with those reported for wheat, yellow pea starch, and cowpea cultivars[32–34] but little higher than that for wheat which may be due to the difference in amylose content. The swelling power is highly influenced by the amylose content; materials with a lower amylose concentration often have higher swelling power. The moderate swelling capacity of vetch starch provides significant water-binding ability and suggest its possible application in food systems that require viscosity development, thickening, or moisture retention. Additionally, because of its neutral solubility, it is advantageous for films used for packaging materials. Vetch protein, on the other hand, has lower swelling and solubility, which suggests that it has more structural stability and regulated water uptake. These qualities are beneficial for high-protein food products or composite edible films where integrity and decreased moisture sensitivity are crucial.

Table 3 Swelling and solubility of vetch starch (n = 3)[a]

Parameter	Vetch starch	Vetch protein
Swelling index (g g^{-1})		
50 °C	2.75 ± 0.04^{a}	2.90 ± 0.04^{a}
60 °C	3.81 ± 0.14^{a}	4.10 ± 0.17^{a}
70 °C	7.17 ± 0.32^{b}	6.96 ± 0.11^{b}
80 °C	11.14 ± 0.17^{c}	10.66 ± 0.32^{c}
90 °C	13.71 ± 0.21^{d}	11.09 ± 0.21^{c}
Solubility index (g g^{-1})		
50 °C	0.002 ± 0.00^{p}	0.03 ± 0.025^{p}
60 °C	0.03 ± 0.01^{p}	0.20 ± 0.03^{p}
70 °C	0.11 ± 0.02^{q}	0.29 ± 0.02^{q}
80 °C	0.16 ± 0.01^{r}	0.13 ± 0.02^{r}
90 °C	0.19 ± 0.02 ^{s}	0.14 ± 0.03 ^{s}

[a] Values expressed are mean ± standard deviation. Means in the row with different superscripts are significantly different at $p \leq 0.05$.

Table 4 Syneresis, light transmittance and freeze–thaw of vetch starch ($n = 3$)[a]

Parameter	Vetch starch
Syneresis (%)	
0 h	00[a]
24 h	36.51 ± 0.51[b]
48 h	40.57 ± 0.52[c]
72 h	43.32 ± 0.43[d]
96 h	45.52 ± 0.47[d]
120 h	48.25 ± 0.23[e]
Light transmittance (%)	
0 h	5.15 ± 0.34[c]
24 h	3.68 ± 0.02[b]
48 h	1.72 ± 0.01[a]
72 h	1.27 ± 0.03[a]
96 h	0.73 ± 0.05[a]
120 h	0.49 ± 0.01[a]
Freeze–thaw (%)	
0 h	00[a]
24 h	3.30 ± 0.17[a]
48 h	11.56 ± 0.25[b]
72 h	17.70 ± 0.20[c]
96 h	27.33 ± 0.35[d]
120 h	30.43 ± 0.40[d]

[a] Values expressed are mean ± standard deviation. Means in the row with different superscripts are significantly different at $p \leq 0.05$.

3.1.5 Syneresis. Syneresis is the process that occurs when a starch gel releases water following cooling or repeated freeze–thaw cycles, primarily due to starch retrogradation, caused by re-association of amylose to produce a more organized, crystalline structure. The extent of syneresis depends on amylose content, amylose–lipid complexes, and granule size, where high amylose and low molecular weight granules favor retrogradation, while larger granules and B-type granules, with less amylose, shorter amylopectin chains, retrograde less than A-type granules.[35,36] Syneresis of vetch starch is presented in Table 4.

An increase in the syneresis of starch gels was observed with an increase in storage duration at 4 °C. Vetch starch displayed the highest (48.25 ± 0.23) syneresis on the 5th day of storage, while the lowest was reported on day zero of storage. One possible explanation for this rise is the interaction between dispersed amylose and amylopectin during storage, which creates junction zones and causes water to be expelled, leading to phase separation. Also, grains with high amylose content exhibit greater syneresis because amylose molecules tend to retrograde more quickly during cooling or storage. These findings were in accordance with the findings of Sofi *etal.*[17] for *Vicia faba* (broad bean) starch.

3.1.6 Light transmittance. The transmittance of starch gels was significantly reduced after 120 hours of refrigerated storage (Table 4). The light transmittance of starch gels dropped from 5.15 to 0.49% after 120th hour of refrigerated storage. The reduction in light transmittance of vetch starch gels with increasing storage duration may be attributed to granule swelling and the leaching of amylopectin and amylose, which results in the formation of turbidity and lower transmittance in vetch starch gels upon refrigerated storage. Jafari *et al.*[20] also reported an increase in light transmittance for Bengal gram starch with an increase in storage period. The decrease in light transmittance of vetch starch gels after refrigerated storage suggests increasing retrogradation and opacity. This property improves gel rigidity and stability, making it ideal for puddings, bakery fillings, and desserts. Greater molecular reassociation and gel strength may increase stiffness and barrier characteristics in edible films and coatings, thus increasing their structural integrity and decreasing permeability.

3.1.7 Freeze–thaw stability. The tendency of a substance to preserve its chemical, physical and functional characteristics over successive cycles of both freezing and thawing is known as freeze–thaw stability. This is a crucial feature of food items and other materials since it influences their shelf life and quality. Freeze–thaw stability also increased with an extended storage period (Table 4). The lowest freeze–thaw stability of starch pastes was observed on day 0, while the highest freeze–thaw stability (30.43 ± 0.40) was observed on day 5 after five freeze–thaw cycles. Wani *et al.*[16] also reported an increase in freeze–thaw stability with longer storage. The increase in freeze–thaw values of starch is primarily caused by the retrogradation of starch, where the starch molecules gradually re-align and crystallize, leading to increased water release and a firmer texture. An increase in freeze–thaw stability of vetch starch after storage shows increased resistance to structural disintegration upon freezing and thawing. Functionally, this implies increased textural rigidity and water retention in frozen or refrigerated foods, making it appropriate for foods such as frozen desserts, sauces, and pastries. In edible films and coatings, better freeze–thaw stability improves flexibility, reduces fractures, and retains barrier properties under changing temperature conditions, thus extending product shelf life.

3.2 Pasting properties

The pasting behavior of starch encompasses a series of intricate processes that occur post-gelatinization, involving irreversible swelling, solubilization, crystallite melting, the release of amylose, loss of order, birefringence and gel formation. The extent of these changes is determined by starch type, concentration, temperature, solutes, and shear, resulting in viscosity development, making starch useful for both culinary and non-food applications, particularly in imparting texture. Granular swelling is thought to be the primary cause of crystallite disintegration during gelatinization process.[37]

The pasting properties of vetch starch are given in Table 7. Peak viscosity (PV) is the tendency of starch to expand in response to a temperature increase prior to physical breakdown. Peak viscosity is expected to be affected by the degree of amylose leaching, the formation of amylose–lipid complexes, friction among swollen granules, and competition for free water between leached amylose and ungelatinized granules. A greater peak viscosity may be beneficial for their utilization as

a thickening agent in food systems.[10] Peak viscosity of native vetch starch was found to be 2378.66 cP.

Trough viscosity (TV) is the viscosity drop, following peak viscosity as a result of disruption of swollen granules due to increased temperature and shearing force.[17] The TV of starch paste was 1698 cP, showing a decrease from peak viscosity.

Breakdown viscosity (BDV) represents the differences in peak viscosity and trough viscosity and measures the degree of disintegration in the swollen granules of starch. Vetch starch showed a BDV of 680 cP.[10]

Final viscosity indicates an increase in gel viscosity due to decreased movement of water molecules as the temperature drops. The final and setback viscosities are largely due to re-ordering or polymerization of leached out amylose & long linear amylopectin. FV of vetch starch was found to be 3497.66 cP.[6]

Setback viscosity (SBV) represents the reorganization capacity of linear and branched starch chains; thus, it measures the retrogradation potential of starch. It is the restoration of viscosity that occurs during the cooling of a hot starch slurry. The SBV of vetch starch was observed to be 1799.33 cP.[35]

Pasting temperature is the lowest temperature required for cooking. A lower pasting temperature suggests weaker resistance to swelling and disintegration. The pasting temperature of vetch starch was 76.80 °C. Similar results for all pasting properties have been observed by Wani *et al.*[10] and Fu *et al.*[6] for kidney bean and vetch starches.

3.3 Differential scanning calorimetry (DSC)

DSC is a simple and fast technique that requires no special sample preparation. It is also recognized as an environmentally friendly approach because it does not require any harmful chemicals or solvents. It has contributed significantly to the study of the thermal characteristics of polymers like carbohydrates and proteins. DSC is a thermo-analytical technology that determines the amount of heat required to raise a sample's temperature compared to a reference material at varying temperatures. The difference in heat flow is then measured as a peak. The area below the curve, which shows whether the thermal process is exothermic (creating energy) or endothermic (consuming energy), is exactly proportional to the enthalpy change.[38] The DSC curve of vetch starch and protein exhibits an endothermic peak revealing the gelatinization behavior of these molecules. The DSC analysis of vetch starch exhibits a major endothermic peak at the beginning which can be attributed to the presence of higher initial moisture content. The onset (T_o) temperature was 54.58 °C which indicates possible pre-damage and early gelatinization of amylopectin. This also highlights the loss of birefringence and aligns with lower pasting viscosity. Owing to a greater gelatinization temperature, the peak temperature (T_p) is found to be at 68.22 °C. An early onset and a slight delayed peak temperature indicate the possible amylose complexation and the reduction in native water mobility. It may also reflect a greater concentration of amorphous regions and variations in granule size. This effect also continued, resulting in a higher gelatinization enthalpy of 6.94 J g^{-1}. The enthalpy values are slightly lower compared to previously reported results which may be due to a greater proportion of amylose double helices relative to the overall crystallinity. The concluding temperature (T_c) was 71.06 °C which further validates the presence of double helices. The vetch protein isolate exhibits two endothermic peaks. The first denaturation (T_d) or peak temperature of protein is at 86.19 °C indicating the denaturation of vicilin type protein (7S) with an enthalpy of 140.378 J g^{-1}. This enthalpy reflects the area change upon denaturation (*i.e.* heat required for inducing denaturation). The second denaturation (T_d) at 227.86 °C indicates relatively higher thermal stability for the legumin type sub-protein fraction (11S).[39] The increased stability is also related to the purity and origin of the protein isolate. This also indicates the possible transition of a rubbery state and attainment of glass transition temperature (T_g). In this case of vetch protein isolate, it can be observed that the linearity in the confrontational mobility and its alignment in an orderly fashion would have increased crystallinity, thereby leading to higher T_d and T_g. Time, coupled with temperature, alters the protein structure, starting with unfolding and denaturing, leading to a reversible glassy state. The attainment of vitrification (glassy state) in gelled systems and dehydrated products can be positively influenced by these protein isolates. For polymer processing, it is required to increase the moisture content of the protein isolate so that the melting or transition is better prevented. The enthalpy also indicates the orderly change of protein from its native to denatured condition. Here, the enthalpy was found to be 35.050 J g^{-1} which reflects its resistance to breaking hydrophobic bonds and causing minimal damage to its orderly structure. The exact understanding and reason can be assessed by supporting data on viscosity and XRD.

3.4 SDS-PAGE

SDS-PAGE is the most preferred gel electrophoretic method for protein analysis. It works on the principle of separation of proteins based on their size and thus can be used to determine the molecular mass of proteins. SDS is an anion-based detergent that unfolds and intensely binds with proteins to produce linear polypeptide chains. The results of SDS-PAGE analysis are shown in Fig. 7. Vetch protein expresses a less complex protein profile in which polypeptide sub-units range between 25 kDa and 75 kDa in molecular weight. Overall, most of the bands and the peptides that have been identified are associated with storage proteins like vicilin and legumin. However, several other metabolic proteins were also detected as small constituents. Visual examination of the SDS-PAGE profile shows that the majority of strong bands emerge in three regions; 23, 35 and 70 kDa. The sub-units corresponding to 70 kDa belong to convicilin and the polypeptide units around 35 kDa correspond to acidic polypeptide of legumin subunits the polypeptide subunits at 23 kDa may be assigned to the basic polypeptide of 11S legumin. The unique bands around 97 kDa may be related to aggregates resulting from the splitting and joining of disulfide bonds among acidic and basic polypeptide units. These polypeptide patterns were comparable to the observations of

Chen *et al.*[40] and Ladjal-Ettoumi *et al.*[41] However, a clear band between 11 and 18 kDa was found which may correspond to the water-soluble metabolic protein albumins. These findings are consistent with the findings of Schumacher *et al.*[29] for pea protein isolates.

3.5 Fourier transforms infrared (FTIR) spectroscopy

FTIR spectroscopy is a potent analytical instrument for identifying polymeric materials. It is used to quantify particular functional groups where the strength of absorbance peaks correlates with the quantity of functional groups present in the sample. The FTIR spectrum of vetch starch is illustrated in Fig. 1. The spectra represent peaks at different wavenumbers. The broad and dominant peak was found at 3302.6 cm^{-1} which represents –OH stretching, indicating the abundance of hydroxyl groups in starch granules. The broadness of the band represents substantial inter- and intra-molecular hydrogen bonding which plays a vital role in defining starch crystal structure, gelatinization, and retrogradation tendency.[42] The second absorption band at 2932.74 cm^{-1} was ascribed to the stretching of the C–H bond of CH_2 groups in glucose units of starch, which indicates the aliphatic nature of starch molecules. The band at 1642.08 cm^{-1} is associated with bending oscillations of H–O–H groups showing hydrogen bonding among the molecules, which in turn indicates bound water molecules within the amorphous regions of starch. The peak at 1337.31 cm^{-1} is ascribed to the twisting of $–CH_2$ and the band at 1155.4 cm^{-1} depicts C–O, C–C and O–H bond stretching and folding or bending of C–O–H. A sharp band at 1077.91 cm^{-1} was related to the vibration of C–O–H deformation and C–C and C–O stretching. Another sharp band at 998 cm^{-1} represents the existence of α-(1,4)-glycosidic linkages and C–O–C stretching vibrations, validating the structural identity of amylose and amylopectin chains. The results of FTIR spectra were in agreement with the data of Tahari *et al.*[26] for bitter vetch starch.

Fig. 2 presents the FTIR spectra of vetch protein. A prominent absorption band at 3276.31 cm^{-1} describes the symmetrical and asymmetrical stretching vibrations of peaks of O–H in proteins, and its intensity reflects the extent of intermolecular interactions and hydration capacity of proteins. A band at 2927 cm^{-1} represents the stretching vibration absorbance peak of aliphatic C–H groups in proteins.[43] A sharp band at 1633.52 cm^{-1} indicates the absorption peak produced through the stretching vibration of the amide I band (C═O), a highly susceptible region for examining protein secondary structures such α-helix, β-sheet, and random coil conformations, while another sharp band at 1527.82 cm^{-1} was associated with the bending vibration of the amide II band (N–H) in proteins. The absorption peak near 1453.96 cm^{-1} displayed the bending vibration from the absorption peak of $–CH_2–$, both for proteins as well as polysaccharides, suggesting that the protein isolate may include trace amounts of carbohydrates. Furthermore, the absorption peak at 1233.32 cm^{-1} corresponds to N–H bending, and the band at 920.29 cm^{-1} usually corresponds to C–H vibrations in insoluble fibers or side chain groups. Similar results were also observed in FTIR spectra of pea protein.[44] The presence of amide I, II, and III bands, together with supplementary side-chain absorptions, confirms that vetch protein predominantly displays a globular shape characterized by substantial β-sheet conformations and some related carbohydrate residues.

The FTIR study elucidates the structure–function relationship of vetch biopolymers. Significant intermolecular linkages that improve the starch and protein isolates' ability to bind water and expand are provided by the strong O–H stretching and broader –OH bands. Prominent amide I and II bands of vetch protein indicate organized β-sheet structures, suggesting enhanced heat stability and significant gel-forming capacity. The solubility, water retention, and overall functionality of the protein and starch extracted from vetch are all directly impacted by these molecular interactions.

3.5.1 Scanning electron microscopy (SEM). SEM is a high-resolution imaging tool for micro and nanoscale particles. SEM images display a unique three-dimensional appearance and help in interpreting the surface morphology of several substances by detecting the secondary electrons produced by the metallic coating on the specimen which is used to generate the image. In the context of starch and protein, SEM is used to examine the granule shape, structure, and size of different types

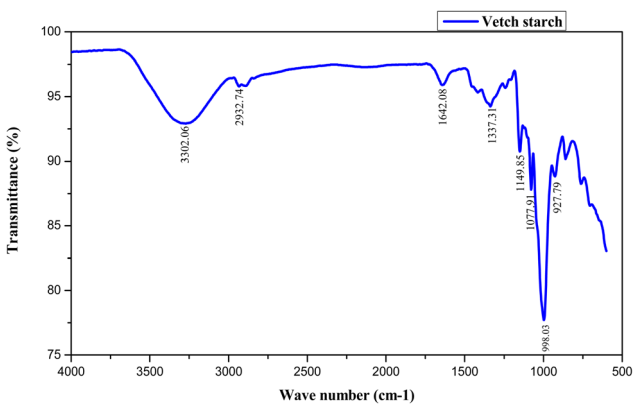

Fig. 1 FTIR spectra of vetch starch.

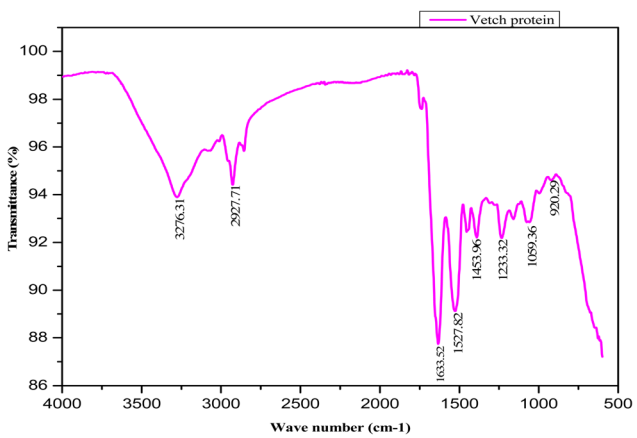

Fig. 2 FTIR spectra of vetch protein.

of starch and protein obtained from various sources. The morphology of vetch starch granules is shown in Fig. 3. As evident from the figure, starch granules of vetch were oval or ellipsoid to kidney-shaped with more or less rough surface and having variable sizes. Smaller and larger granules coexisted within the heterogeneous granule size range, which was roughly 4 to 20 μm. The existence of fractures on the surface of some larger starch granules could be helpful in facilitating rapid penetration of water during processing. The shape and size of hairy vetch (*Vicia villosa*) starch are almost similar to those of bitter vetch, mung bean and common vetch starches. However, *Vicia villosa* starch granules are larger than soybean and oat starch granules[6,28] but smaller than kidney bean, pea and faba bean starch granules.[10,17,45] Granule size arrangement is an essential element of starch functionality; smaller granules favor lower gelatinization temperatures, whereas larger granules lead to increased viscosity and gel strength. The combination of both types in vetch starch may provide an appropriate balance between thickening capacity and processing ability.[6] Several studies on bitter vetch (*Vicia ervilia*), common vetch (*Vicia sativa*), pea and mung bean have shown similar shapes.[6,26,45,46] Moreover, granule size and morphology influence both the viscosity and gelatinization properties of starch. For instance, surface roughness and fractures may enhance molecular interactions with proteins, polyphenols, or other additives, thereby improving compatibility in starch–protein composite films and potentially enhancing barrier and mechanical properties.

SEM image analysis of vetch protein revealed a heterogeneous lamellar structure with some particle aggregation giving it a near-spherical morphology with particle size ranging from 1 to 8 μm, Fig. 4. As is evident from the figure the surface roughness varies suggesting an irregular or non-uniform protein matrix. There is a dense and well-connected network with minimal fractures which depicts its good mechanical stability. This information is in agreement with that mentioned by Zhang *et al.*[47] and Barreras-Urbina *et al.*[48] for common vetch protein and wheat protein.[47,48] The protein is appropriate for emulsification applications because of its rough and heterogeneous surface morphology and pores, which are likely to improve water absorption, solubility, and interfacial activity. Strong molecular associations are suggested by the dense, interconnected matrix with few fractures, which may result in better mechanical stability and film-forming capabilities.[49]

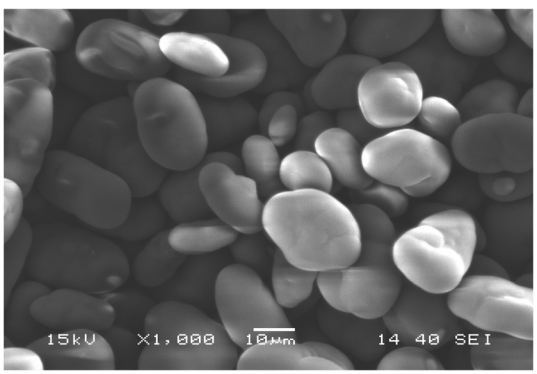

Fig. 3 SEM image of vetch starch at different magnifications.

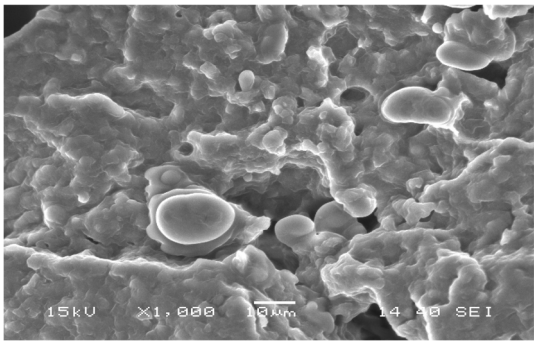

Fig. 4 Scanning electron microscopy (SEM) micrographs of vetch protein.

The protein network's near-spherical aggregates may further strengthen barrier qualities and structural integrity. These characteristics are highly desirable in the context of edible film formation, where the ability to develop a cohesive, crack-free network translates into improved mechanical strength and barrier properties.

3.6 X-ray diffraction (XRD)

XRD is one of the potent non-destructive techniques employed to characterize crystalline materials and is useful to explain crystalline orientation, grain size and crystal defects. Based on XRD patterns, starch granule crystal polymorphs are often classified into three forms: A-type, B-type, and C-type. C-type starches are highly complex mainly due to the varying contents and distributions of A and B-type crystals. Starch extracted from various plant sources exhibits distinct X-ray diffraction spectra. A-Type crystals are commonly found in starches from cereals. B-Type is common in tuber crops and maize. However, a C-type crystalline structure is exhibited by starches from plants like green bananas, tapioca, and peas. As illustrated in Fig. 5, five peaks at 15.3°, 17.29°, 19.8°, 23.23° and 25.9° 2θ values were observed in vetch starch, indicating that this starch is a typical C-type crystal.[16,46,47] The presence of significant and sharp peaks at 17.29° and 23.23° indicates a moderate crystalline structure, suggesting A-type crystallinity, which implies a more compact structure. This packing is linked to decreased water permeability and slower enzymatic digestion. On the other hand, the comparatively reduced intensity of the peak at 15.3° indicates lower crystallinity, which implies a combination of amorphous regions that are mostly caused by irregular amylopectin chains and amorphous amylose matrices.[50] Also, the peak at 19.8° is noteworthy as it indicates the presence of V-type crystallinity, which occurs when amylose forms inclusion networks with lipids or small molecules. Although less significant, this characteristic peak may indicate some level of amylose–lipid interaction in vetch starch, which could affect heating and pasting properties. This semi-crystalline structure is congruent with the structural arrangement of legume starches and is directly related to their

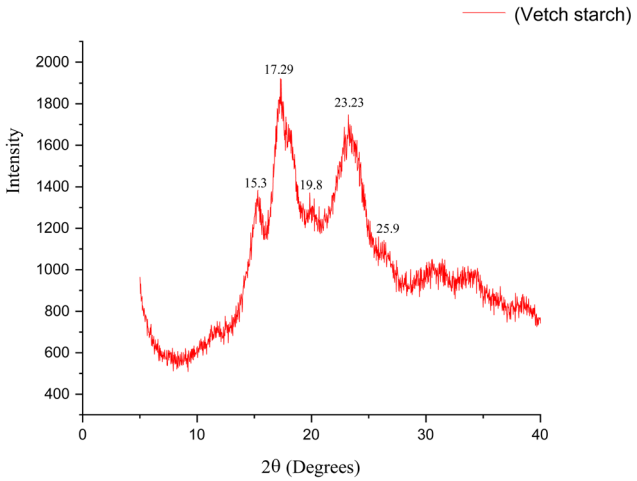

Fig. 5 XRD pattern of vetch starch.

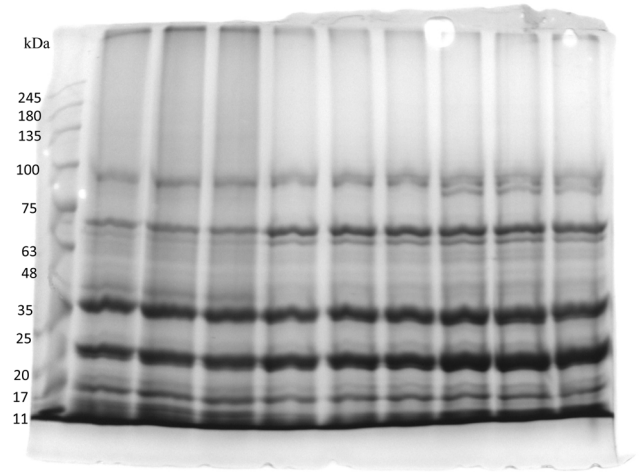

Fig. 7 SDS-PAGE of vetch protein.

gelatinization, retrogradation, and digestible characteristics.[51] Thus, the XRD data validate the C-type crystallinity of vetch starch while also providing information about its structural integrity and functional potential in food and non-food applications. The C-type crystallinity along with moderate A-type and V-type components, signifies a semi-crystalline structure that affects water permeability, gelatinization, retrogradation, and digestibility.

The structural properties of vetch protein, studied by XRD, are shown in Fig. 6. The vetch protein showed two peaks: a high crystalline peak at a 2θ value of 20.0° and a small peak at 9.0°, corresponding to those described by Zhao *et al.*[52] and Jin *et al.*[43] The dominant peak at 20.0° is indicative of β-sheet secondary structures and the small peak around 9.0° shows the presence of α-helix or lamellar structures respectively. The complete diffraction pattern is significantly broad, indicating that the protein has minimal to moderate crystallinity. Thus, it can be suggested that vetch protein exhibits low to moderate crystallinity reflecting the amorphous nature of these proteins and supporting that vetch protein is a globular protein. Also, the protein's amorphous structure implies its versatility in molecular packing, which may affect its solubility, surface activity, and film-forming behavior. Amorphous proteins are more soluble in water and solvents, which improves their hydration and swelling properties. β-Sheets promote strong intermolecular hydrogen bonding and can form ordered aggregates to strengthen the protein network. Vetch protein's increased solubility, hydration, swelling capacity, and film-forming potential are all correlated with low to moderate crystallinity, which includes amorphous patches and significant β-sheet structures. This agrees with SEM observations of dense, linked protein matrices.

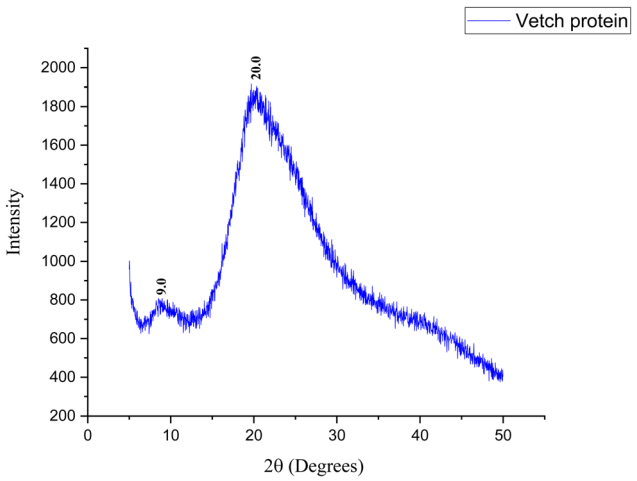

Fig. 6 XRD pattern of vetch protein.

3.7 Functional properties

3.7.1 Water and oil absorption capacity. Water and oil absorption capacity is considered an important functional trait among foods.

The ability of biopolymers like proteins and starches to imbibe water or oil for enhanced consistency in foods is known as their water/oil absorption capacity. The water and oil absorption capacities of native starch and protein are presented in Table 5. WAC is mainly due to the presence of hydrophilic groups on its molecules, causing it to swell and absorb large amounts of water. Vetch starch showed a WAC of 2.43 g g^{-1}. The WAC of a food product is an indicator of the degree of starch gelatinization since it quantifies the starch's capacity to retain water after swelling in excess water, which is equivalent to the

Table 5 WAC and OAC of vetch starch and protein ($n = 3$).[a]

Parameter	Vetch starch	Vetch protein
WAC (g g^{-1})	2.43 ± 0.15	4.62 ± 0.05
OAC (g g^{-1})	1.93 ± 0.05	3.23 ± 0.05

[a] Values expressed are mean ± standard deviation. WAC – water absorption capacity, OAC – oil absorption capacity.

Table 6 Functional properties of vetch protein ($n = 3$)[a]

Parameter	pH 3	pH 5	pH 7
Emulsion capacity ($m^2\ g^{-1}$)	48.99 ± 0.67	37.17 ± 0.50	85.11 ± 0.27
Emulsion stability (min)	65.60 ± 0.36	45.96 ± 0.19	97.65 ± 0.60
Parameter	**pH 4**	**pH 7**	**pH 10**
Foaming capacity (%)	86.33 ± 0.57	108 ± 1	129.33 ± 0.57
Foaming stability (min)	50.56 ± 0.55	92.03 ± 0.75	102.96 ± 0.25

[a] Values expressed are mean ± standard deviation.

Table 7 Pasting properties of native vetch starch ($n = 3$)[a]

Pasting property	Vetch starch
PV (cP)	2378.66 ± 20.64
TV (cP)	1698 ± 18.52
BDV (cP)	680 ± 30.51
FV (cP)	3497.66 ± 36.01
SB (cP)	1799.33 ± 15.30
PT (°C)	76.80 ± 0.22
PkT (min)	5.20 ± 0.30

[a] Values expressed are mean ± standard deviation [1 cP = 0.001 Pa s]. PV – peak viscosity, TV – trough viscosity, BDV – breakdown viscosity, FV – final viscosity, SB – set back, PT – pasting temperature, PkT – peak time.

weight of the resulting gel. It depends on the ability of hydrophilic groups to imbibe water molecules as well as the ability of molecules to form gels.[53]

The OAC of starch was found to be 1.93 g g^{-1}. The results align with those observed by Gao *et al.*[54] for pea starch, and Sofi *et al.*[17] for broad bean starch. Oil absorption capacity is primarily due to the physical trapping of oil by the macromolecules, like starch, protein. It serves as an indicator of how quickly proteins attach to fat in food compositions. In dietary systems where optimal oil absorption is desired, the proteins' capacity to interact with oil is significant. It enables flour to have practical applications in various food products. When using flours in food preparation, their ability to absorb oil is crucial for facilitating improvements in mouthfeel and flavor.[53]

The WAC of vetch protein was found to be 4.62 g g^{-1}. The oil absorption capacity of protein is lower as compared to its water absorption capacity and was found to be 3.23 g g^{-1}. The relatively low oil absorption capacity of vetch protein may be ascribed to the extent of denaturation of its protein structure. Comparable results were also observed by Chen *et al.*[40] for common vetch protein and Schumacher *et al.*[29] for different varieties of pea protein isolates.

3.7.2 Foaming capacity (FC) and foaming stability (FS). Foaming is an important property of proteins, in which proteins adsorb at the interface and develop a stabilizing layer around bubbles, which promotes foam formation. Protein in foam reduce surface tension and contribute to the uniform distribution of fine air cells throughout the food structure. The foaming capacity of vetch protein isolates varied at different pH values (4, 7 and 10) ranging from 86.33% to 129.33% and are presented in Table 6. Vetch protein showed the lowest foaming capacity at pH 4 (86.33%) and the greatest at pH 7 (129.33%). At pH 4 and pH 10, protein isolates have the lowest and highest solubility, respectively.

Foam stability is the capacity of foam to preserve its original configuration over time and prevent or retard the coalescence of gas. Foaming stability of protein isolates at different pH values was studied over a 1-hour period (Table 6). The percentage of foam volume that remains after a given time duration is known as foam stability. Vetch protein has the highest foam volume at pH 10 (102.96) and the lowest at pH 4 (50.56). Similar outcomes were also reported by Shen *et al.*[55] and Wani *et al.*[11]

3.7.3 Emulsion capacity (EC) and emulsion stability (ES). Emulsion capacity measures the potential of soluble proteins to shift to the water/oil interface. The EC of proteins may be affected by conditions like pH and ionic conditions which in turn affect their solubility and hence their conformation and functional properties. Emulsion stability refers to the ability of an emulsion to withstand any change in its properties and maintain a uniform composition, preventing the separation of its components during storage. The emulsion capacity and stability of vetch protein were studied at three different pH values (pH 3, pH 5 and pH 7) and are presented in Table 6. Emulsion capacity showed significant variation among different pH values, *viz.*, 48.99 $m^2\ g^{-1}$ (pH 3), 37.17 $m^2\ g^{-1}$ (pH 5) and 85.11 $m^2\ g^{-1}$ (pH 7). At pH 5, emulsion capacity was found to be the lowest, owing to the reduced solubility of proteins around the isoelectric point.

The emulsion solubility index provides a measure of the stability of a diluted emulsion over a specified time duration. Substantial differences in emulsion stability were also observed among different pH values, being highest at pH 7 (97.65 min) and lowest at pH 5 (46.96 min). Greater emulsion stability at neutral pH may be attributed to balanced electrostatic interactions between emulsifier molecules at the oil–water interface, thus preventing droplet aggregation (Table 7). Comparable results were observed by Wani *et al.*[11] for kidney bean protein isolates.

4 Conclusion

This work extensively examined the physicochemical, functional, structural, and thermal properties of starch and protein isolates produced from *Vicia villosa* (vetch) seeds, an underutilized legume with exceptional importance in culinary and industrial applications. The proximate composition revealed a high starch yield and a substantial amount of protein. The apparent amylose content of starch was in the range of other legume starches and the color characteristics of the isolated starch and protein show a negligible amount of any pigment or contaminant. The starch exhibited significant swelling capacity and solubility, accompanied by considerable gel clarity as evidenced by light transmission. Analysis of syneresis and freeze–thaw stability showed that vetch starch produces stable gels

Table 8 DSC gelatinization of native vetch starch

Parameter	Value	
	Vetch starch	Vetch protein
Onset temperature (T_o)	54.58 °C	65 °C
Peak temperature (T_p)	66.22 °C	86.19 °C and 227.86
Conclusion temperature (T_c)	71.06 °C	247 °C
Enthalpy change ΔH	6.94 J g^{-1}	35.050 J g^{-1}

with minimal water segregation during storing and freezing cycles. Thermal analysis by RVA revealed that the vetch starch exhibits an intense peak viscosity (3610.33 cP) and a comparatively low pasting temperature (73.27 °C), providing robust gel forming ability and exceptional resilience to breakdown under shear and heat Table 8. Also, structural analysis by XRD showed C-type crystallinity in starch which is characteristic of legumes, while scanning electron microscopy (SEM) indicated well-organized, elliptical to polygonal starch granules with smooth surfaces. SDS-PAGE examination of the protein isolate revealed a varied molecular weight distribution consistent with legume globulin patterns. In conclusion, vetch protein and starch possess satisfactory functional, structural, and thermal properties, suggesting diverse applications. Specifically, vetch protein shows promise as an emulsifier in plant-based formulations while vetch starch offers potential as a sustainable material for biodegradable packaging. Future research is expected to focus on application-based studies, such as developing and testing vetch starch–protein composite films or edible coatings, to confirm their efficacy in practical applications and improve their commercial use.

Conflicts of interest

There are no conflicts to decalre.

Data availability

All data generated or analysed during this study are included in the manuscript. No new crystallographic data were generated. This study did not involve any human participants.

Acknowledgements

The authors are thankful to the Anusandhan Research Foundation (ANRF), Government of India Erstwhile (SERB scheme) under the SERB-SURE Scheme vide Sanction order SUR/2022/003847, for supporting and funding the research.

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