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Exploring the potential of kenaf seed proteins produced via freeze, spray and vacuum oven drying: A comparison with commercial soy and whey proteins

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ABSTRACT

The effect of freeze drying (FD), spray drying (SD) and vacuum oven drying (VOD) on the structural, thermal, physicochemical and techno-functional properties of kenaf seed proteins was studied. Their functional performance was also compared with commercial soy protein (SP) and whey protein (WP). Microstructural observation revealed distinct morphologies, with FD exhibiting a plate-like structure, SD producing spherical particles and VOD resulting in a block structure. FD and SD exhibited similar molecular weight distributions, greater structural flexibility and lower surface hydrophobicity compared to VOD. All drying methods exhibited high and comparable thermal denaturation temperature. SD produced the lightest-coloured protein powder, closely resembling the colour of commercial SP and WP. The essential amino acids content of FD, SD and VOD was comparable to that of SP and WP. Based on functional performance, FD and SD showed the highest solubility, oil holding capacity (OHC) and foaming properties; FD exhibited excellent emulsion properties; VOD demonstrated superior water holding capacity (WHC); and SD exhibited the best gelling properties. Notably, FD, SD and VOD of kenaf seed protein outperformed commercial SP and WP in most functional properties, except for solubility and gelling (WP) and WHC (SP), highlighting their promising potential as sustainable plant-based protein alternative.

1. Introduction

The demand for affordable, nutritious and sustainable alternative protein ingredients has tremendously surged due to the growing global population (Özdemir et al., 2022; Hadidi et al., 2024). Currently, animal- and plant-derived proteins, such as whey, egg, soybean, pea and wheat, are commercially and widely used in the food industry. Nevertheless, several drawbacks such as allergenicity and poor functional performance associated with some of these proteins, address the need for alternative sustainable protein sources (Ge et al., 2021; Sá et al., 2020).

Kenaf (*Hibiscus cannabinus* L.), a biennial fibre crop from the Malvaceae family, is emerging as a promising source of sustainable protein. Globally, kenaf cultivation has expanded in many countries such as the USA, China, India, Thailand and Malaysia. Kenaf seeds, which are often discarded as industrial by-products, contain a high protein content (20 % to 36 %) and are rich in essential amino acids (Sim & Nyam, 2021; Arulrajah et al., 2023; Ibrahim et al., 2021). Despite growing interest in kenaf seeds, research on their proteins remains limited, with most studies primarily focusing on conventional alkaline extraction methods (Hamim et al., 2025; Ibrahim et al., 2021; Mariod et al., 2010). Notably, the effect of different drying methods on the physicochemical,

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structural, thermal and techno-functional properties of kenaf seed protein have not been previously investigated.

In the food industry, freeze drying (FD), spray drying (SD) and vacuum oven drying (VOD) are widely used techniques for processing food proteins (Lin et al., 2021). Among these drying techniques, SD is extensively used due to several advantages, such as less-time consuming, cost-effective and capable of producing uniform good-quality protein powder. However, SD can also cause more shrinkage and result in denser particles. In contrast, protein powders produced by FD tend to retain their native structure more effectively due to reduced thermal stress, although the process is time-consuming and expensive (Dong et al., 2024; Kanwate, Ballari & Kudre, 2019). VOD, while a simple dehydration technique, involves high heat, which can lead to significant degradation of thermal sensitive products and can be expensive in industrial scale production (Shen, Tang & Li, 2021). Previous studies have reported that operational differences in drying methods, particularly variations in duration and temperature, can significantly influence the physicochemical and structural properties of proteins, thereby affecting their functional performance (Brishti et al., 2020; Kapoor et al., 2024; Shen et al., 2021; Siddiqui et al., 2024). For example, peanut protein concentrate obtained with spray and freeze drying produced proteins with distinct structures and functionalities, in which freeze drying process yielded the best-performing protein (Liu et al., 2019). Mune and Sogi (2015) reported that freeze-dried cowpea and bambara bean protein concentrates exhibited superior oil absorption capacity but exhibited the lowest solubility profiles compared to samples produced by cabinet and vacuum drying methods. In another study conducted by Shen, Tang and Li (2021), quinoa protein isolates produced by freeze drying, spray drying and vacuum drying displayed significant structural differences. The freeze-dried protein isolate exhibited good oil holding capacity as well as emulsion and gelling properties, while the spray-dried protein isolates showed better solubility and water absorption capacity. On the other hand, thermal processing can cause protein oxidation, driven by several chemical modification such as thiol oxidation, aromatic hydroxylation and formation of carbonyl group, thereby negatively affecting quality (e.g., colour & flavour) of the final products (Bordignon et al., 2023; Chen et al., 2022; Estrada et al., 2018). Protein oxidation occurred in insect proteins processed with different drying treatments, in which hot-air drying caused the most severe effect followed by vacuum drying and freeze drying (Wang et al., 2022).

Thus, investigating the impact of different drying methods on proteins can optimise and enhance their application in the food industry. Therefore, for the very first time, this work comprehensively investigated the effects of FD, SD and VOD on the structural, thermal, physicochemical and techno-functional properties of kenaf seed proteins. Additionally, we compared the functional performance of kenaf seed proteins obtained using these drying methods with that of commercial proteins from soy (SP) and whey (WP). This research provides a scientific basis for choosing the most compatible drying method to produce alternative high-quality plant-based kenaf seed protein for food applications.

2. Materials and methods

2.1. Materials and reagent

Kenaf seeds (variety V36) was procured from National Kenaf and Tobacco Board, Kelantan, Malaysia. Commercial soy protein (protein content=91 %) was purchased from Pharmanutri Enterprise, Selangor, Malaysia, while whey protein (protein content=80 %) was procured from Purefit Nutrition, Selangor, Malaysia. All chemicals used were of analytical or HPLC grade.

2.2. Kenaf seed protein extraction by alkaline solubilisation-acid precipitation

Prior to protein extraction, kenaf seeds powder was defatted by mixing 100 g (seed powder) with 300 mL petroleum ether. The defatting process was performed at room temperature for 30 min and repeated twice for efficient oil removal. Then, 50 g of the defatted seed meal was suspended in 1000 mL distilled water and mixed for 30 min. Next, the pH of the mixture was adjusted to 10 using 1 M NaOH and incubated at 45 °C and 200 rpm for 2 h. The mixture was then centrifuged at 5000 g, 15 min (Himac CR22N, Hitachi-Koki, Ibaraki, Japan) to obtain supernatant containing the solubilised proteins. Following that, 1 M HCl was added gradually to reach a pH value of 4.5 and kept in chiller overnight (4 °C) for protein precipitation. Finally, the protein was obtained by centrifugation at 10,000 *g* for 10 min at 4 °C (Ibrahim et al., 2021). Prior to drying, the obtained protein was rinsed, neutralised (pH 7) and diluted (1:4 w/v). For freeze drying (FD), the protein solution was kept at −40 °C and dried using ScanVac Coolsafe freeze-dryer at −100 °C, and a pressure of 1.19 mbar (LaboGene, Lynge, Denmark). For spray drying, the protein solution was homogenised and dried using a pilot scale spray dryer (GEA Mobile Minor, GEA Process Engineering Niro A/S, Soeborg, Denmark) operated at 160 °C and 60 °C for inlet and outlet temperature, respectively, and a flow rate of 22 mL/min. Vacuum oven drying (VOD) was carried out using vacuum oven at 600 mbar and 45 °C (WTC Binder, Tuttlingen, Germany). All dried protein samples were stored at 4 °C for further analysis. The protein content of the obtained protein powders was 85.83 %, 87.33 % and 81.04 % for FD, SD and VOD, respectively, determined using Kjeldahl (conversion factor 6.25) following the AOAC methods (AOAC, 2005).

2.3. Structural properties

2.3.1. Scanning electron microscopy (SEM)

The microstructure of all the proteins (FD, SD & VOD) was observed using SEM (JSM-5200, JEOL, Tokyo, Japan) at 10 kV. The proteins samples were fixed on an aluminium plate and coated with gold layer prior to viewing.

2.3.2. Fourier transform infrared (FTIR) spectroscopy

The effect of different drying methods (FD, SD & VOD) on the secondary structure components of kenaf proteins was investigated using FTIR spectrometer (PerkinElmer, Connecticut, USA). The spectrums were obtained in the wavelength region between 450 and 4000 cm^{-1} with a resolution of 10 cm^{-1} and 32 scans. The secondary structure components were estimated by Fourier self-deconvolution (FSD) of the Amide I region (1600–1700 cm^{-1}) (OriginPro, Massachusetts, USA)

2.3.3. Surface hydrophobicity (H_0)

All the protein samples were dissolved in phosphate buffer (10 mM, pH 7) and serially diluted to obtain a series of concentration. Then, a total of 4 mL was withdrawn from each concentration and mixed with 20 μL of 8 mM ANS (8-anilino-1-naphthalenesulfonic acid) probe in a black microplate. The relative fluorescent intensities of protein plus ANS and blank (protein without ANS) were recorded at 390/470 nm excitation/emission using a spectrofluorophotometer (SpectraMax Gemini XPS, Molecular Devices LLC., California, USA). H_0 was determined from the slope by plotting relative fluorescent intensity against protein concentration as linear regression (Shi et al., 2024).

2.3.4. Protein profile by SDS-PAGE

Protein profile of kenaf protein (FD, SD & VOD) was obtained using a Mini-PROTEAN Tetra electrophoresis system (Bio-Rad, CA, USA) with 14 %/4 % separating gel/stacking gel under reducing conditions, following the procedure reported by Qoms et al. (2022).

2.4. Thermal properties

About 10 mg of each protein samples dried with different methods were sealed in hermetic aluminum TA pan and heated in the range 30 to 180 °C at heating rate of 10 °C/min using differential scanning calorimeter (DSC) (823E, Mettler Toledo, Greifensee, Switzerland). The results were processed using the Star^e software (Mettler Toledo, Greifensee, Switzerland).

2.5. Physicochemical properties

2.5.1. Colour parameters

The colour parameters of kenaf proteins (FD, SD & VOD) and commercial proteins (SPI & WPI) were analysed using a digital colorimeter (Model CR-410, Konica Minolta, Tokyo, Japan) based on Hunter scale of L*, a* and b* values. Browning index (BI) was calculated as previously described (Dong et al., 2024).

2.5.2. Amino acid profile

The Pico-Tag pre-column phenyl isothiocyanate (PITC) derivatisation method developed by White, Hart and Fry (1986) was used to analyse and quantify the amino acids of kenaf protein samples and commercial soy and whey, with some modifications. Complete hydrolysis of all samples were performed in 15 mL 6 M HCl under anaerobic condition (flushed with nitrogen gas for 15 min) at 110 °C for 24 h. The chromatographic analysis was performed on high-performance liquid chromatography (HPLC) (LC 20AT, Shimadzu, Kyoto, Japan) with ZORBAX Eclipse Plus C18 column (4.6 mm × 150 mm, 3.5 μm) (Agilent, California, USA) at an absorbance of 254 nm. Samples (10 μL) were injected and eluted at 1 mL/min with a gradient of mobile phase A (100–0 %) and mobile phase B (0–100 %) at a column temperature of 43 °C. The mobile phase A prepared with 0.1 M ammonium acetate and adjusted to pH 6.5 using acetic acid, while the mobile phase B consisted of 0.1 M ammonium acetate, acetonitrile and methanol at a ratio of 44:46:10 (v/v/v). The amino acids compositions were calculated by comparing the area of each amino acid in the protein samples with the area in the standard amino acids.

2.6. Techno-functional properties

The protein solubility of kenaf proteins (FD, SD & VOD) and commercial proteins (SPI & WPI) were determined at pH ranging from 2 to 12 using Bradford assay based on BSA standard curve (Bradford, 1976). The capacity of all the protein samples to hold water (WHC) and oil (OHC) at pH 7 was investigated as reported by Ibrahim et al. (2021). Gelling properties of all the protein samples were investigated at protein concentrations ranging between 2 and 20 % (pH 7) to determine the least gelation concentration (LGC) (Ge et al., 2021). Emulsion properties, emulsion activity index (EAI) and emulsion stability index after 15 min (ESI), were investigated for all the protein samples at a concentration of 1 % (w/v) and different pH values (3, 5, 6, 7 & 8) as described by Qoms et al. (2023). Foaming properties, foaming capacity (FC) and stability after 60 min (FS), were investigated for all the protein samples at a concentration of 1 % (w/v) and varying pH values (3, 5, 6, 7 & 8) (Karimi et al. (2024).

2.7. Statistical analysis

Data are reported as mean ± standard deviation of triplicate measurements. One-way ANOVA with post hoc Tukey test was used to determine the significant differences at $p < 0.05$ using Minitab 18 (Minitab Inc., PA, USA).

3. Results and discussion

3.1. Structural properties of kenaf seed proteins

3.1.1. Microstructure and surface morphology

The microscopic morphology of kenaf seed proteins dried via different methods was markedly distinct (Fig. 1). The FD protein sample exhibited an irregular, plate-like structure with a smooth surface and shallow cavities (Fig. 1A–C). Ice crystals formed during the freezing process can interconnect with protein molecules, and during sublimation, the insufficient force to break these ice crystals into smaller droplets may result in the plate-like structure. Additionally, when protein molecules surround the ice crystals, the sublimation of these crystals may lead to the formation of cavities (Mutlu & Korkmaz, 2024). In contrast, the spray-dried kenaf seed protein featured a smooth, spherical structure with concave indentations (Fig. 1D–F). During the spray drying process, a high temperature is applied for a short residence time. When the protein solution enters the drying chamber, it disperses into small droplets and quickly comes into contact with hot dry air, rapidly losing moisture and forming a spherical structure. Furthermore, the rapid dehydration combined with increased surface tension result in the formation of concave features on the protein surface (Nie et al., 2023; Wang et al., 2022). While a thick, rough and dense block structure was observed for VOD (Fig. 1G–I), likely due to the layering of solute (protein) and solvent (water) during the extended heating and drying period. Under vacuum drying conditions, proteins gradually settle at the bottom of the tray while water transfers through the sample and rapidly evaporates, resulting in the observed block structure (Ye et al., 2024). The observed rough structure in VOD could be attributed to protein aggregation as a result of partial unfolding of protein molecules that lead to the exposure of hydrophobic patches that are typically buried within the protein interior (Cao et al., 2023).

3.1.2. Secondary structure

Fig. 2A shows the FTIR spectra of kenaf proteins produced using different drying methods (FD, SD & VOD) in the range 4000 to 500 cm^{-1}. The amide I band (1600–1700 cm^{-1}) was used to estimate the secondary structure composition (Fig. 2B and C). The secondary structure components were identified as follows; β-sheet (1600–1640 cm^{-1}), random coil (1640–1650 cm^{-1}), α-helix (1650–1660 cm^{-1}) and β-turn (1660–1700 cm^{-1}) (Ai et al., 2019). It was noted that drying methods influenced the distribution pattern of secondary structural components of kenaf seed proteins. The vacuum oven-dried protein possessed the highest β-sheet content of 48.82 % compared to the FD (36.33 %) and SD (33.44 %), which could be attributed to partial unfolding and refolding of protein during the prolonged heat treatment, favouring protein aggregation and thus higher content of β-sheet (Brishti et al., 2020; Ma et al., 2022). On the other hand, the random coil segment was found the lowest in VOD at 12.50 %, while higher in FD and SD at 31.46 % and 35.53 %, respectively. The proportion of both β-sheet and α-helix structures contributes to protein compactness, while random coil and β-turn structures reflect flexibility and loose structure (Liu et al., 2022; Qoms et al., 2023). Our results showed that VOD produced proteins with a higher degree of compactness, as reflected by the sum of β-sheet and α-helix structures (60.11 %), compared to FD (41.39 %) and SD (41.76 %). Conversely, both FD and SD led to the production of proteins with more flexible structures, as indicated by the sum of random coil and β-turn proportions (FD=58.61 % & SD=58.24 %) compared to VOD (39.89 %). These differences in secondary structural composition could influence the functional performance of the proteins.

3.1.3. Surface hydrophobicity

The surface hydrophobicity reflects the degree of hydrophobic amino acids exposed on a protein surface and is influenced by protein unfolding and denaturation during processing methods such as freezing, heating and/or drying. This property can also affect intramolecular protein-

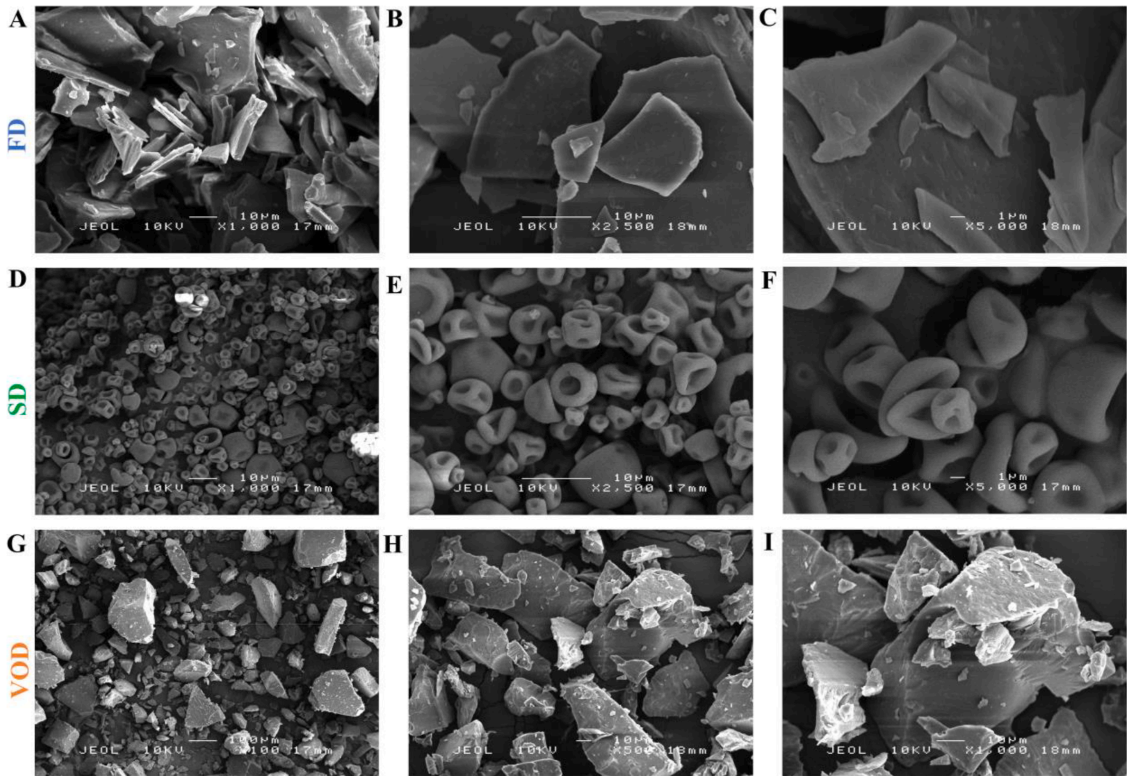

Fig. 1. Microstructure and surface morphology of kenaf seed proteins obtained by freeze drying (FD) (A, B & C) spray drying (SD) (D, E & F) and vacuum oven drying (VOD) (G, H & I).

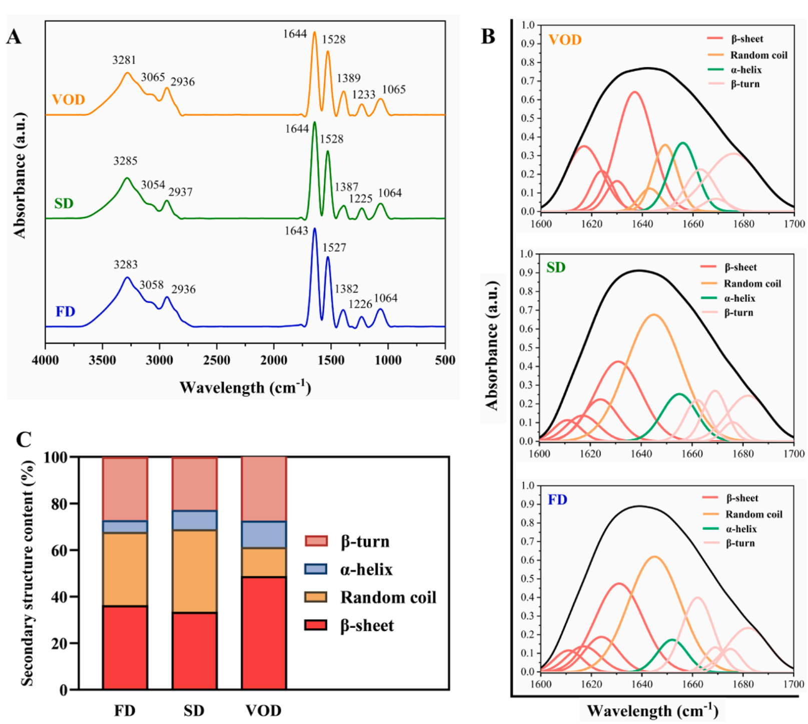

Fig. 2. FTIR spectra in the range 4000 to 500 cm^{-1} (A), peak fitting of amide I region (B) and secondary structure composition (C) of kenaf seed proteins obtained using freeze drying (FD), spray drying (SD) and vacuum oven drying (VOD).

protein and protein-lipid interactions and is crucial for techno-functional properties such as solubility, emulsification and foaming (Lin et al., 2020; Shen et al., 2021). Based on Fig. 3A, the surface hydrophobicity of all kenaf seed proteins was apparently varied, which may be attributed to protein unfolding and denaturation occurring during different drying processes. The VOD protein sample exhibited the highest ($p < 0.05$) surface hydrophobicity of 1784.43, followed by SD (1308.97) and FD (1171.87). This can be explained by the structural

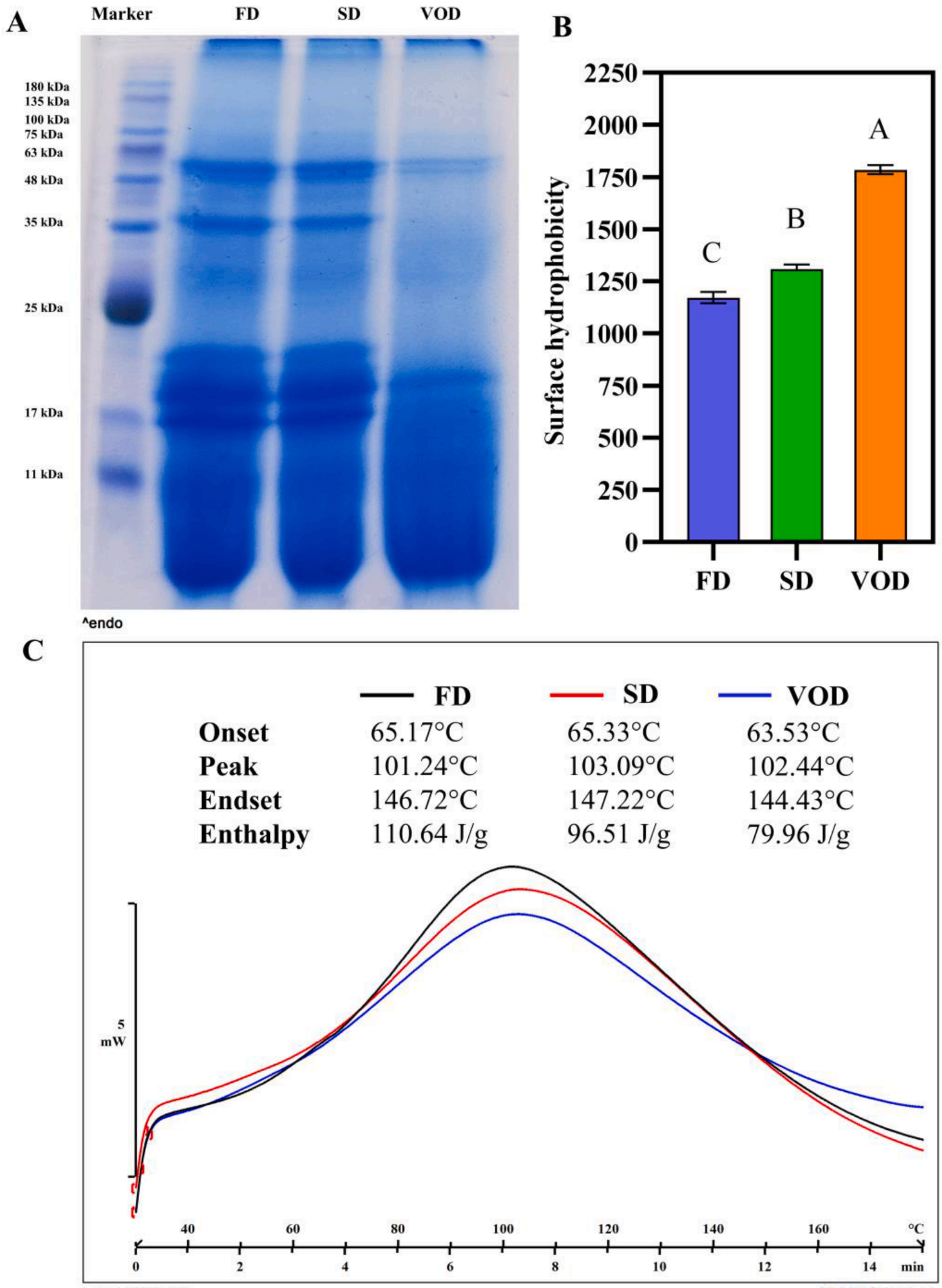

Fig. 3. Protein profile (A), surface hydrophobicity (B), and DSC thermogram (C) of kenaf seed proteins obtained using freeze drying (FD), spray drying (SD) and vacuum oven drying (VOD).

unfolding and partial denaturation occurred due to the slow dehydration/delayed drying process during the vacuum oven drying, which likely exposes hydrophobic groups buried within the protein core to the surface. Similarly, the high temperature applied during SD for a short duration could also cause a certain degree of protein unfolding and denaturation, resulting in moderately high surface hydrophobicity. In contrast, the lowest surface hydrophobicity of FD could be attributed to the fact that this process involves lower temperature, which minimises the extent of protein structural unfolding and denaturation (Lin et al., 2021; Nie et al., 2023). This observation is in line with findings by Li et al. (2022) and Feyzi et al. (2018b), who reported that VOD produced proteins with higher surface hydrophobicity, compared SD and FD.

3.1.4. Protein molecular weight profile

Fig. 3B shows the effect of different drying methods (FD, SD & OD) on the kenaf protein profile. It is noted that drying techniques affected the protein banding patterns and their intensities. Among the drying methods, FD and SD proteins had similar protein profile (pattern & intensities), while VOD protein sample showed noticeable differences compared to FD and SD. The molecular weight distribution of all kenaf protein samples ranged from 10 to 69 kDa. Six major band at 54, 53, 36, 21, 18 and 16 kDa were clearly observed in both FD and SD proteins. However, the major bands were less intense in VOD and most of them disappeared, except for the bands at 54, 53 and 18 kDa. These differences could be due to that protein dried using vacuum oven drying experienced some structural modifications and certain degree of denaturation as the protein was exposed to high temperature for a longer duration compared to FD and SD (Ye et al., 2024). This observation further supports the findings of surface morphology and surface hydrophobicity analyses, where the VOD-obtained protein sample demonstrated partial structural modifications. Similarly, Ye et al. (2024) investigated the effect of SD and VOD on the protein profile of camphor seed kernel protein and found that VOD process induced significant protein aggregation and denaturation. Also, Zhang et al. (2022) noted that heat treatment via microwave drying caused severe aggregation and denaturation of insect protein, while FD treatment did not alter the protein banding pattern. In another study, FD and SD processed rice bran proteins showed similar protein profiles, consistent with our findings (Abd Rahim et al., 2023).

3.2. Thermal properties

The DSC thermogram and thermal properties (denaturation peak & enthalpy) of kenaf seed proteins dried with FD, SD and VOD are presented in Fig. 3C. All the kenaf protein samples possessed high thermal stability with single endothermic peak having a denaturation temperature (T_d) of 101.24 °C (FD), 103.09 °C (SD) and 102.44 (VOD), indicating that different drying method did not affect their thermal stability. This observation is linked to the high content of β-sheet structure, wherein the proportion of β-sheet structure was the highest in FD and VOD, and the second highest in SD (as discussed in Section 3.1.2.). The β-sheet structure contributes to a more compact and highly ordered protein structure, making the protein thermally stable (Qoms et al., 2023). Kenaf seed proteins dried with different methods possessed higher thermal stability (101.24–103.09 °C) compared to other proteins obtained with different drying techniques such as Antarctic krill (62.70–80–44 °C) (Lin et al., 2020), fenugreek (61.80–92.30 °C) (Feyzi et al., 2018b) and rice bran (74.23–92.49 °C) (Abd Rahim et al., 2023).

The thermal denaturation enthalpy (ΔH) of kenaf seed proteins followed the order FD>SD>VOD, with values of 110.64, 96.51 and 79.96 J/g, respectively. This trend correlates with the proportion of thermally susceptible α-helix structure presents in kenaf seed protein samples (as discussed in Section 3.1.2). The VOD sample had the highest α-helix content, followed by SD and FD, which explains the lower energy required for denaturation in VOD compared to SD and FD (Feyzi et al., 2018b; Nie et al., 2023). Another contributing factor is the surface hydrophobicity of the proteins, as lower surface hydrophobicity typically indicates a lower degree of denaturation and consequently higher ΔH (Brishti et al., 2020). Our findings showed that FD protein sample had the lowest surface hydrophobicity with the highest ΔH. Conversely, VOD had the highest surface hydrophobicity which exhibited the lowest ΔH. Based on the thermal behaviour study, kenaf seed proteins dried using FD, SD and VOD can be incorporated in food system requiring high heat treatment.

3.3. Physicochemical properties of kenaf seed proteins in comparison to commercial SP and WP

3.3.1. Colour properties

The colour of food ingredients is one of the most crucial considerations in the food industry, as it greatly influences consumer acceptability of the final products. The effect of drying processes on the visual appearance and colour properties of kenaf seed proteins, compared with soy and whey proteins, is displayed in Fig. 4. The SD protein powder exhibited a creamy white colour, similar to commercial SP and WP, while the FD protein powder appeared golden-yellow, and the VOD protein powder displayed a darker brownish hue. This observation aligns with the L* (lightness) values, wherein the SD (64.74), SP (65.81) and WP (69.18) had significantly ($p < 0.05$) higher lightness values compared to FD (50.23) and VOD (40.2), indicating greater lightness. Similarly, SD (6.73), SP (5.77) and WP (7.70) had significantly ($p < 0.05$) lower degrees of BI than FD (13.89) and VOD (20.09). The slightly high BI of FD may result from the cold burn due to the exposure of protein to low temperature for an extended period (Özdemir et al., 2022). The highest BI of VOD could be due to the Maillard reaction as a result of the interaction between amino groups of amino acids (particularly lysine) and aldehydes (from carbohydrates) during heating in the vacuum oven drying which led to the formation of darker colour (Lin et al., 2021; Özdemir et al., 2022), in accordance with the lowest content of lysine in VOD as presented in Table 1. These findings are in line with previous work investigating the effect of drying methods on the colour properties of plant-based proteins from different sources such as camphor seed kernel protein (Ye et al., 2024), yam soluble protein (Nie et al., 2023), hempseed protein (Dong et al., 2024; Lin et al., 2021) and grass pea protein (Feyzi et al., 2018a).

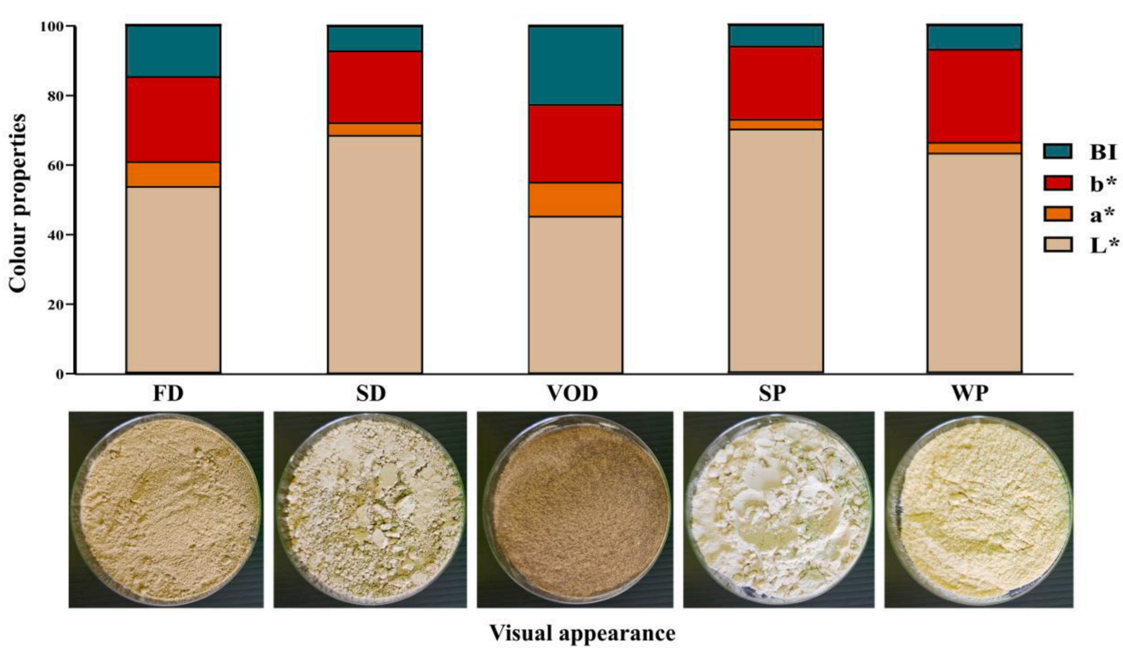

Fig. 4. Colour properties and visual appearance of kenaf seed proteins obtained using freeze drying (FD), spray drying (SD) and vacuum oven drying (VOD) compared to commercial soy protein (SP) and whey protein (WP).

Table 1
Amino acid compositions and amino acid scores of kenaf seed proteins obtained using freeze drying (FD), spray drying (SD) and vacuum oven drying (VOD) compared to commercial soy protein (SP) and whey protein (WP).

Amino acid (AA) (mg/g)	FD	SD	VOD	SP	WP	FAO/WHO
Essential AA						
Threonine	51.70±3.83 (2.07)	54.72±2.27 (2.19)	48.10±2.36 (1.92)	50.36±0.80 (2.01)	50.54± 4.03 (2.02)	25
Methionine	12.85±1.03	12.46±0.76	12.30±0.14	10.67±0.01	15.73±0.17	
Phenylalanine	46.63±3.23	48.61±0.58	47.00±2.98	51.86±0.36	31.02±1.98	
Lysine	36.48±0.23 (0.76)	38.44±0.18 (0.80)	34.43±0.34 (0.72)	56.58±1.08 (1.18)	64.97±0.38 (1.35)	48
Valine	45.31±1.89 (1.13)	48.01±0.05 (1.20)	46.37±0.72 (1.16)	43.57±0.47 (1.09)	44.86±2.38 (1.12)	40
Isoleucine	67.85±0.15 (2.26)	74.74±0.33 (2.49)	69.71±1.81 (2.32)	67.78±0.65 (2.26)	74.48±0.79 (2.48)	30
Leucine	74.31±3.57 (1.22)	84.41± 1.93 (1.38)	72.81±1.57 (1.19)	74.00±3.33 (1.22)	68.47±7.30 (1.12)	61
Histidine	15.73±0.90 (0.98)	17.40±0.19 (1.09)	15.59±0.83 (0.97)	19.42±0.11 (1.21)	10.40±0.71 (0.65)	16
Total essential AA	350.85±12.78	378.87±0.54	346.31±3.36	374.24±4.02	360.47±2.23	
Non-essential AA						
Tyrosine	26.77±3.66	29.04±0.31	26.70±1.70	33.64±0.56	22.16±0.19	
Cysteine	18.48±2.80	22.24±0.45	21.42±1.34	23.69±0.37	28.41±0.66	
Serine	41.98±0.56	45.78±0.95	38.18±1.78	44.85±0.85	36.65±0.15	
Glycine	36.92±1.48	39.75±1.14	35.14±2.45	36.45±0.69	13.41±0.45	
Glutamic acid	178.86±1.16	178.28±1.88	143.37±2.44	176.32±4.04	133.64±2.95	
Proline	34.02±1.29	37.53±0.64	33.36±2.66	45.35±0.66	44.98±1.52	
Alanine	48.60±1.94	52.21±0.51	42.26±3.40	34.47±0.42	9.73±0.50	
Arginine	42.01±2.55	43.91±0.26	37.08±1.02	50.43±0.85	76.68±2.78	
Aspartic acid	72.14±1.06	58.35±0.60	49.97±0.78	100.06±2.46	75.22±2.44	
Total non-essential AA	499.78±8.78	507.08±4.71	427.48±11.12	545.28±10.17	440.89±10.97	
Sulphur AA	31.32±3.83 (1.36)	34.70±0.31 (1.51)	33.72±1.20 (1.47)	34.36±0.36 (1.49)	44.15±0.83 (1.92)	23
Aromatic AA	73.40±6.88 (1.79)	77.65±0.89 (1.89)	73.70±1.28 (1.80)	85.50±0.20 (2.09)	53.18±2.17 (1.30)	41
Hydrophilic AA	438.90±8.17	436.88±0.90	366.72±1.62	498.03±8.04	448.10±11.72	
Hydrophobic AA	356.34±14.71	387.09±2.57	350.50±5.59	361.35±2.21	311.43±4.10	
Essential AA/total AA (%)	41.24±0.46	42.77±0.26	44.76±0.88	40.70±0.71	44.99±0.77	
Total AA	850.64±21.56^{B}	885.96±4.17^{AB}	773.78±7.75^{C}	919.52±6.15^{A}	801.35±8.74^{C}	

AA indicates amino acid.
Hydrophilic amino acids: aspartic, threonine, serine, glutamic acid, lysine, histidine and arginine. Hydrophobic amino acids: proline, alanine, valine, methionine, isoleucine, leucine, tyrosine and phenylalanine. Sulphur amino acids: methionine and cysteine. Aromatic amino acids: phenylalanine and tyrosine.
Values in parentheses indicate the amino acid score compared to the FAO/WHO (2013) for adult requirements.
Different letters indicate a statistically significant difference ($p < 0.05$) between different samples.

Among the drying methods, SD produced the lightest-coloured kenaf seed protein powder, closely resembling the colour profile of commercial soy and whey protein powders. Although FD produced a slightly yellowish protein powder, it remains visually appealing due to its golden hue. In contrast, VOD resulted in protein powder with the darkest colour, indicating the poorest colour quality.

3.3.2. Amino acid profile

The effect of different drying methods (FD, SD & OD) on the amino acid profile of kenaf seed protein, in comparison to commercially available soy and whey proteins, are presented in Table 1 with the FAO/WHO (2013) requirements for adults. Among the drying methods, FD (850.64 mg/g) and SD (885.96 mg/g) protein samples contained significantly ($p < 0.05$) higher total amino acids compared to VOD (769.78 mg/g). Interestingly, the total amino acids content of SD was comparable ($p > 0.05$) to commercial SP (919.52 mg/g), while both SD and FD had higher ($p < 0.05$) total amino acids content than commercial WP (801.35 mg/g). Among all the protein samples, VOD exhibited the lowest total amino acids content but showed no significant ($p > 0.05$) difference when compared with WP. The amino acids glutamic acid, aspartic acid, leucine and isoleucine were the most abundant in all the protein samples, like most of storage proteins (Ge et al., 2021; Shen et al., 2021; Zhu et al., 2019). A similar trend was observed in the distribution of hydrophilic and hydrophobic amino acids, with hydrophilic amino acids being predominant in all the protein samples.

The essential amino acids cannot be synthesised by the human body and must therefore be obtained externally from amino acid-rich diet. The essential amino acids content of kenaf seed proteins obtained with different drying methods was high and ranged between 346.31 mg/g to 378.87 mg/g, constituting 41.24 % to 44.76 % of the total amino acids, comparable to the commercial SP (374.24 mg/g; 40.70 %) and WP (360.47 mg/g; 44.99 %). Among the essential amino acids, leucine and isoleucine were found to be the most predominant in all the protein samples, with isoleucine known to positively affect muscle development (Akyüz & Ersus, 2021). The application of plant-based proteins in the food industry is often limited by their incomplete essential amino acids profile, thus we compared the essential amino acids of FD, SD, VOD, SP and WP with FAO/WHO (2013) for adult requirements. The results showed that all the essential amino acids in all the protein samples met the recommended amounts with amino acid scores >1, except for lysine in FD (0.76), SD (0.80) and VOD (0.72), and histidine in FD (0.98), VOD (0.97) and WP (0.65). Based on these amino acid scores, kenaf seed proteins obtained using FD, SD and VOD contained a considerable amount of sulphur-containing amino acids and leucine, unlike typical plant-based proteins. However, they were deficient in histidine (FD & VOD) and lysine (FD, SD & VOD), thus it is recommended to be blended with other proteins to produce a high-quality protein powder blend suitable as a supplementary ingredient for food application.

3.4. Techno-functional properties of kenaf seed proteins in comparison to commercial SP and WP

3.4.1. Protein solubility

Solubility is one of the most crucial properties of proteins determining their functional performance, as it influences other techno-functional properties such as foaming and emulsification. The solubility profiles of FD, SD, VOD, SP and WP in the pH range of 2 to 12 are displayed in Fig. 5A. The solubility curves of all the kenaf seed protein samples (FD, SD & VOD) exhibited a pronounced U-shape distribution. In contrast, the U-shape was less prominent for SP and WP. Nevertheless, all protein samples had a minimal solubility around pH 4 and 5, approaching pH levels near the isoelectric point. At the isoelectric point, proteins exhibit no net surface charge because of the balance between its cationic and anionic residues thus, decreasing the intermolecular repulsive force between protein molecules, causing to the formation of large bulky aggregate via strong hydrophobic interactions, which weakens protein-water interactions and consequently solubility. Conversely, protein solubility increased at pH values away from the isoelectric point (below/above). In these conditions, protein acquire cationic charge (below isoelectric point) or anionic charge (above isoelectric point) on the surface, facilitating electrostatic repulsion between protein molecules, thus promoting protein dispersion in aqueous phase and enhances solubility (Deng et al., 2019; Li et al., 2022).

Among the kenaf seed proteins obtained by different drying methods, SD and FD exhibited almost similar solubility at all the pH, except near the isoelectric point around pH 4 and 5, where SD showed higher solubility. VOD, however, displayed the lowest solubility at all the pH values. Protein solubility is often influenced by several factors such as the balance between hydrophilic and hydrophobic amino acids, protein structural conformation as well as the extent of protein denaturation (Zhang et al., 2022). Based on our findings, the hydrophobic/hydrophilic ratios were almost similar for all the kenaf seed protein samples, suggesting this factor likely did not significantly affect solubility differences. Instead, the observed differences in solubility were primarily attributed to differences in protein structural conformation and microstructure. As reflected by the secondary structure analysis, SD and FD with a more flexible structures (higher content of random coil & β-turn) tend to have fewer intramolecular hydrogen bonds and more solvent-accessible surface area, thus contributing to efficient protein-water interactions (Ajayi et al., 2024; Yeasmen et al., 2024). Additionally, as described in the microstructure analysis, the shallow cavities and concave indentations observed in the FD and SD protein samples likely created localised pockets for water accumulation, maintaining a hydrated structure (Lili et al., 2015). Overall, all kenaf seed protein samples obtained by FD, SD and VOD exhibited higher solubility across all the pH values compared to the commercial plant-based SP but lower solubility than the commercial animal-based WP. These findings suggest that kenaf seed proteins can serve as a new alternative plant-based protein in the food industry.

3.4.2. Water holding (WHC) and oil holding (OHC) capacities

WHC and OHC of proteins are crucial properties that affect the texture and flavour-binding potential of food products. As shown in Fig. 5B, VOD-obtained protein possessed significantly ($p < 0.05$) higher WHC (3.56 g water/g protein) compared to SD (2.21 g water/g protein) and FD (1.84 g water/g protein). The lower WHC values observed for FD and SD could be attributed to the higher protein solubility compared to

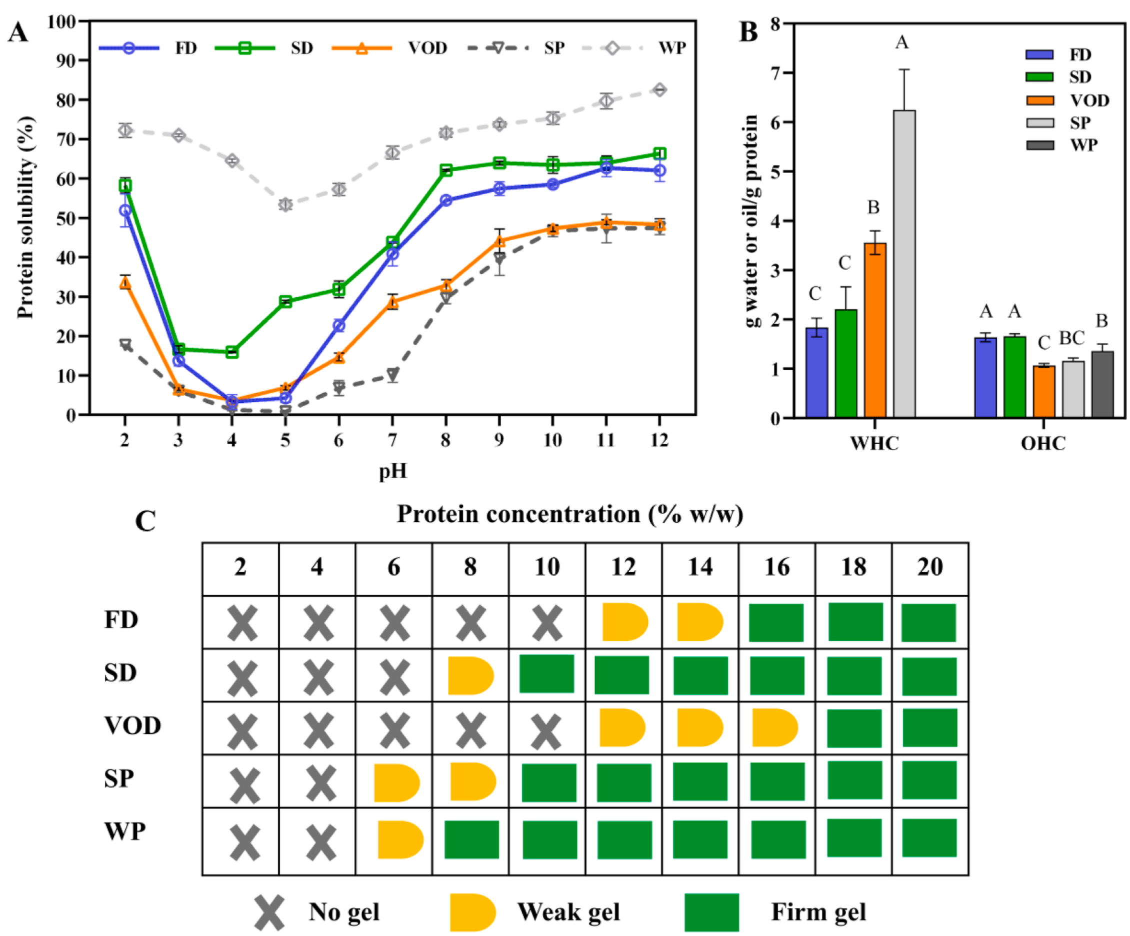

Fig. 5. Solubility profile (A), WHC and OHC (B) and LGC (C) of kenaf seed proteins obtained using freeze drying (FD), spray drying (SD) and vacuum oven drying (VOD) compared to commercial soy protein (SP) and whey protein (WP).

VOD. Soluble proteins tend to disperse easily in water, consequently lowering their ability to retain water molecules within their structure (Qoms et al., 2023; Zhang et al., 2022). When comparing the WHC of kenaf seed proteins (FD, SD & VOD) with the commercial SP and WP, SP displayed significantly ($p < 0.05$) higher WHC (6.25 g water/g protein), while WP showed no WHC as it was completely dissolved. For OHC, FD (1.64 g oil/g protein) and SD (1.66 g oil/g protein) exhibited significantly ($p < 0.05$) higher capacities than VOD (1.07 g oil/g protein). This could be due to the shallow cavities and concave indentations morphology observed in the FD and SD protein samples, which create pockets on the protein surface that trap and hold oil molecules (Özdemir et al., 2022). These results agree with hemp seed meal protein, where FD-protein displayed higher OHC compared to VOD and oven-dried proteins (Lin et al., 2021). Also, Mutlu and Korkmaz (2024) reported that camelina seed proteins dried using FD and SD showed better OHC compared to VOD. In another study, Lin et al. (2020) observed higher OHC for antarctic krill protein dried using SD compared to hot air drying. Nonetheless, they observed an opposite trend for WHC, with FD and/or SD showing better WHC than oven drying (OD). Interestingly, FD- and SD-kenaf protein demonstrated significantly ($p < 0.05$) higher OHC compared to commercial SP (1.16 g oil/g protein) and WP (1.36 g oil/g protein). These findings suggest that kenaf seed proteins dried using different drying methods have relatively high WHC and OHC, thus have great potential to be incorporated into food system that requires water and oil binding simultaneously, such as meat analogue, sausage and burger patty, in order to enhance juiciness and flavour retention in the final product.

3.4.3. Least gelation concentration (LGC)

The ability of proteins to form thermally induced gels/three-dimensional network for holding water, lipids, flavours, sugars and other food ingredients, is essential in food manufacturing. As shown in Fig. 5C, SD exhibited the lowest LGC at 10 %, followed by FD at 16 % and VOD at 18 %. Interestingly, the SD-kenaf protein showed an LGC comparable to SP (10 %) and slightly higher than WP (8 %). Proteins with a high content of random coil structure can form strong gels at low concentrations, while a high content of α-helix structure is associated with weaker gelling properties. This is in line with our findings, where the SD was dominated by random coil and had the lowest α-helix content compared to FD and VOD. The high content of random coil in SD likely enhanced its ability to form strong bonds with neighbouring protein molecules (Kapoor et al., 2024). Another possible reason is the high protein solubility observed in SD allowed more protein molecules to participate in gel formation, reducing the concentration needed to form a firm gel compared to FD and VOD (Tang et al., 2024). These results indicate that SD-obtained kenaf seed protein has excellent gel-forming ability at concentrations comparable to commercial SP, making it beneficial in food systems that require good gelling properties such as meat analogues and meat products. The finding of this study is consistent with Brishti et al. (2020), who reported that SD and FD-dried mung bean proteins required lower LGCs (12 %) compared to oven-dried protein (18 %). Similarly, Locali-Pereira and Nicoletti (2025) found that pigeon pea protein exhibited a lower LGC of 10 % when produced by FD and SD, whereas oven-dried protein required 12 % to form a stable gel.

3.4.4. Foaming properties

The foaming capacity (FC) and foam stability (FS) of kenaf seed proteins obtained by different drying methods, in comparison to commercial proteins (SP & WP), as influenced by pH values are illustrated in Fig. 6A and B. The kenaf seed proteins dried using different methods showed higher foaming properties ranging from 38.33 % to 129.33 % and 60.67 % to 92.40 % for FC and FS, respectively, compared to SP (FC=22.67 %−96.67 %; FS=24.80 %−70.66 %) and WP (FC=23.33 %−62.00 %; FS=5.51 %−49.43 %). Among the drying methods, FD and SD showed the highest FC and FS at all pH values, compared to VOD. These findings are consistent with those reported by Locali-Pereira and Nicoletti (2025), who studied the effects of different drying techniques, including SD, FD and OD, on the functional properties of pigeon pea protein concentrate. Similar to the present study, they found that SD and FD produced protein concentrate had superior foaming capacities compared to those obtained via OD. Also, Lin et al. (2021) reported that FD produced hemp seed meal protein with better foaming properties compared to VOD and OD. The excellent foaming behaviour displayed for FD and SD potentially due to their high solubility and structural flexibility, allowing efficient dispersion and adsorption at the air-water interface, leading to greater air entrapment with faster interfacial film formation (Kapoor et al., 2024). Additionally, their relatively high surface hydrophobicity promotes effective adsorption and interaction at the air-water interface, which enhances both foaming ability and stability

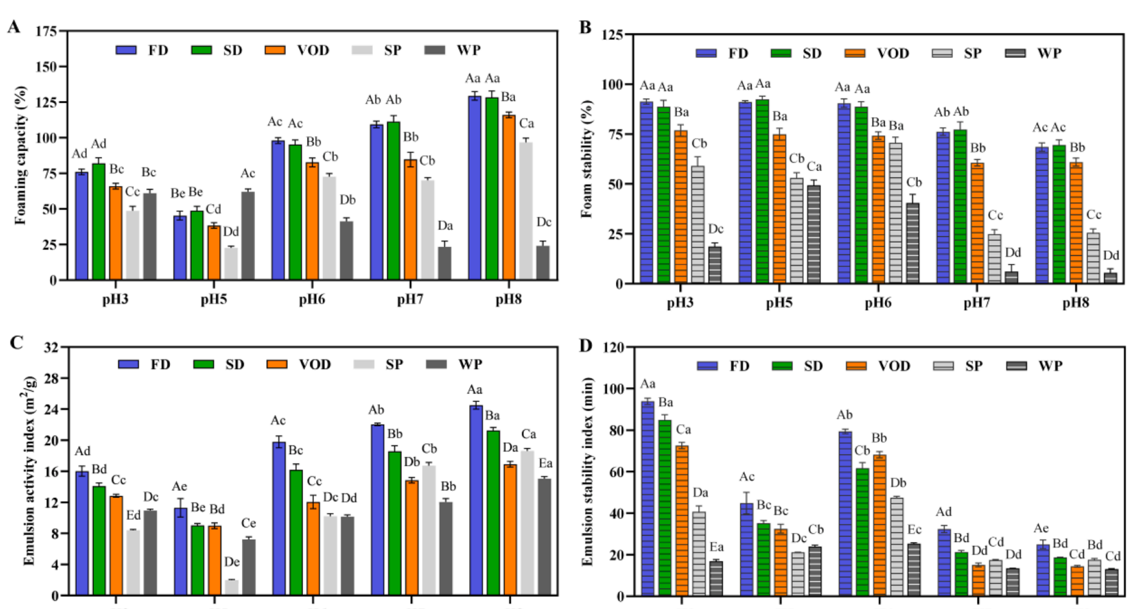

Fig. 6. Foaming properties (A&B) and emulsion properties (C&D) of kenaf seed proteins obtained using freeze drying (FD), spray drying (SD) and vacuum oven drying (VOD) compared to commercial soy protein (SP) and whey protein (WP).

(Yeasmen et al., 2024; Zhao et al., 2022).

The effect of pH on the foaming properties of FD, SD, VOD and SP showed a similar pattern. FC increased as the pH moved away from the isoelectric point (pH 3, 6, 7 & 8). Conversely, FS showed an opposite trend, with significantly ($p < 0.05$) higher values observed under acidic conditions (pH 3, 5 & 6). The increased in FC at pH above or below the isoelectric point can be attributed to an increased net charge on the protein surface, which improves solubility and structural flexibility, thereby accelerating the diffusion of proteins at the air-water interface to rapidly encapsulate air and foam formation. However, an excessively high net charge in alkaline conditions may reduce protein-protein interactions, hindering elastic film formation, consequently lowering FS (Ge et al., 2021). We also observed that the animal-based WP exhibited distinct behaviour compared to plant-based proteins (kenaf & soy). WP showed the highest FC near the isoelectric point (pH 3 & 5), with a significant reduction as the pH increased (pH 6, 7 & 8), this trend was also observed by Marinova et al. (2009). Based on these findings, kenaf seed proteins obtained through FD, SD and VOD exhibited superior foaming properties under different pH environments compared to commercial SP and WP. Thus, the kenaf seed proteins could serve as promising ingredients in food products that rely on foam formation such as bakery products, mousses, whipped toppings or aerated confectioneries.

3.4.5. Emulsification properties

Fig. 6C and D displays the effect of pH on the emulsion activity index (EAI) and emulsion stability index (ESI) of kenaf seed protein dried using FD, SD and VOD, compared to commercial proteins SP and WP. The results showed that FD, SD and VOD had superior EAI (8.98 m^2/g–24.50 m^2/g) and ESI (14.39 min–93.83 min) compared to SP (EAI=1.98 m^2/g–18.60 m^2/g; ESI=17.51 min–47.44 min) and WP (EAI=7.23 m^2/g–15.06 m^2/g; ESI=13.09 min–25.34 min). However, SP demonstrated a better EAI than VOD at pH 7 and 8. Among the drying methods, FD exhibited slightly but significantly ($p < 0.05$) higher emulsification properties than SD and VOD, which can be attributed to the synergistic effect of high surface hydrophobicity, structural flexibility, solubility and oil-binding capacity. On the other hand, the effect of pH on EAI showed that all proteins (FD, SD, VOD, SP & WP) displayed a close trend with their solubility profiles. The highest EAI values were observed at pH values far from the isoelectric point (pH 3, 6, 7 & 8), whereas the highest ESI values were found under acidic conditions (pH 3, 5 & 6), at pH levels far from the isoelectric point (below/above), electrostatic repulsion between protein molecules is maximised, enabling more soluble proteins to adsorb effectively at the oil-water interface (Shen et al., 2021). Conversely, excessive repulsions between negatively charged groups in an alkaline environment may lead to the formation of thinner and less cohesive films/viscoelastic networks that can prevent effective protein adsorption at the oil-water interface, leading to reduced emulsion stability (Fan et al., 2022). Overall, kenaf seed protein obtained through FD and SD displayed superior emulsification properties over a wide range of pH compared to commercial SP and WP, therefore they could be ideal ingredients for food products requiring emulsification such as meat analogues, sauces, spreads or salad dressings. Similarly, Kanwate, Ballari and Kudre (2019) reported that swim bladder gelatin produced by SD and FD exhibited superior emulsification properties compared to that produced by VOD. Likewise, Brishti et al. (2020) observed that mung bean protein obtained through SD and FD had better emulsification properties than oven-dried protein.

3.5. Conclusion

The current study comprehensively investigated the effect of drying technologies, freeze drying (FD), spray drying (SD) and vacuum oven drying (VOD), on the structural, physicochemical and techno-functional properties of kenaf seed proteins. Furthermore, the functional performance of the obtained proteins was investigated against two commercial protein isolates (soy & whey). Our findings revealed that drying methods significantly influenced the properties of kenaf seed proteins, with FD and SD being the best-performing protein samples compared to VOD. Therefore, kenaf seed protein functionalities can be fine-tuned depending on the desired properties of the final products. Interestingly, the functional performance of kenaf seed proteins obtained with different drying methods were superior to the commercial proteins, except for solubility and gelling (whey) and WHC (soy). The current work highlights the potential of kenaf seed protein as a promising alternative to commercially available plant and animal-based proteins, particularly when processed using freeze or spray drying methods.

Ethical statement

The research presented does not involve any animal or human study.

CRediT authorship contribution statement

Brisha Arulrajah: Writing – review & editing, Writing – original draft, Validation, Software, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Mohammed S. Qoms:** Writing – review & editing, Writing – original draft, Validation, Software, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Ines Barkia:** Resources, Writing – review & editing. **Wan Zunairah Wan Ibadullah:** Resources. **Muta Harah Zakaria:** Resources. **Nazamid Saari:** Writing – review & editing, Supervision, Resources, Project administration, Funding acquisition.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Data availability

Data will be made available on request.

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