International Food Research Journal 32(4): 1053 - 1069 (August 2025)
Journal homepage: *http://www.ifrj.upm.edu.my*

Effects of different drying techniques on physico-chemical, techno-functional, and structural characteristics of kenaf (*Hibiscus cannabinus* L.) seed protein isolates

[1,2,3]Hamim, N. A., [4]Saari, N., [4]Wan Ibadullah, W. Z., [2,5]Dos Mohamed, A. M., [4,6,7]Anwar, F. and [1]*Karim, R.

[1]*Department of Food Technology, Faculty of Food Science and Technology, Universiti Putra Malaysia, Serdang, Selangor, Malaysia*
[2]*Section of Food Engineering Technology, Universiti Kuala Lumpur, Malaysian Institute of Chemical and Bioengineering Technology, Alor Gajah, Malacca, Malaysia*
[3]*Inkubator Teknologi Makanan MARA, Kepong Industrial Park, Kuala Lumpur, Malaysia*
[4]*Department of Food Science, Faculty of Food Science and Technology, Universiti Putra Malaysia, Serdang, Selangor, Malaysia*
[5]*Venus Biotech Sdn. Bhd., Villaraya Industrial Park, Semenyih, Selangor, Malaysia*
[6]*Institute of Chemistry, University of Sargodha, Sargodha, Pakistan*
[7]*Faculty of Health Sciences, Shinawatra University, Bang Toey, Pathum Thani, Thailand*

Article history

Received:
8 February 2025
Received in revised form:
5 August 2025
Accepted:
29 September 2025

Keywords

kenaf seed protein powder, freeze-drying, spray-drying, oven-drying, techno-functional properties

Abstract

Kenaf (*Hibiscus cannabinus* L.) is a promising source of raw material for the production of protein isolate due to its high levels of protein and essential amino acids. During production, the drying stage plays a crucial role in shaping the physico-chemical, functional, and structural properties of the derived protein isolates. The present work investigated and compared the physico-chemical, techno-functional, thermal, and structural properties of freeze-dried (FD), spray-dried (SD), and oven-dried (OD) kenaf seed protein isolate (KSPI) powder. Morphological observation revealed that SD isolate had a collapsed and wrinkled surface, whereas those of FD and OD isolates had non-collapsed, non-porous, and crystalline structures. SD isolate demonstrated exceptional protein solubility, emulsification activity, foaming ability, and stability. Among the three isolates, OD exhibited a significantly higher water absorption capacity, while FD showed a significantly higher oil absorption capacity. All the tested isolate powders exhibited endothermic peak with high T_d (84.25 - 96.00°C), indicating good thermal stability of the isolates. In comparison to OD and FD isolates, SD isolate demonstrated a much greater ΔH, indicating a lower degree of protein denaturation. Different drying methods did not result in the dissociation of protein subunits in KSPI based on SDS-PAGE data. Additionally, the FD isolate contained a higher amount of β-sheet structures and a lower proportion of random coil structures compared to SD and OD isolates. Overall, the present work indicated that freeze-, spray-, and oven-drying produced different colours, particle size distribution, crystal and surface morphology, and protein solubility, with several desirable functional and structural properties of KSPIs. These findings offered valuable insights into selecting a suitable drying procedure that yields KSPIs with the desired functionality, thus aiding in optimising their applications in the food industry.

DOI
https://doi.org/10.47836/ifrj.32.4.12

Introduction

Considering the potential for widespread protein deficiencies in the future, one of the main concerns is the search for sustainable and alternative protein sources. Presently, there is a growing interest in investigating novel protein sources derived from plants, microorganisms, and insects (Paivarinta *et al*., 2020; Kurek *et al*., 2022; Liceaga *et al*., 2022; Ma *et al*., 2024; Zhang *et al*., 2024). Plant-based proteins are one of the increasingly popular alternative protein sources, and crucial to a wholesome and sustainable food production system. Increasing consumer concerns about the taste, sustainability, types, and

*Corresponding author.
Email: rosaz@upm.edu.my

health effects have led to a rising demand for plant protein (Schreuders *et al*., 2019; Paivarinta *et al*., 2020; Kurek *et al*., 2022). In this regard, animal proteins have gradually been replaced in recent years by plant proteins derived from fava beans (Multari *et al*., 2015), peanuts (Boukid, 2022), soy (Qin *et al*., 2022), peas (Wang *et al*., 2023), and lentils (Galvão *et al*., 2024). With a few exceptions, such soy and potato proteins, animal proteins often have higher Digestible Indispensable Amino Acid Score (DIAAS) than plant-based proteins. The world's plant protein supply quantity shows a rising trend between 2010 - 2019; the protein supply quantity was increased up to 0.55, 0.39, 2.33, 0.67, 2.00, and 4.33% for beans, peas, pulses, nuts, soybeans, and groundnuts, respectively (FAO, 2022).

Legumes, which include peas, soybeans, lentils, lupins, chickpeas, broad beans, and mung beans, are among the primary plant protein sources in the human diet. Despite their restricted availability, legume protein stand out from other plants due to their high levels of functional vegetable protein (Brishti *et al*., 2017; Schreuders *et al*., 2019; Kurek *et al*., 2022). Meanwhile, oilseed may provide a substantial contribution to a consistent supply of protein (Ancuţa and Sonia, 2020; Kotecka-Majchrzak *et al*., 2020; Kumar *et al*., 2024). Many oilseeds and/or oil seed meals, including hemp, chia, flax, evening primrose, rapeseed, glandless cottonseed, milk thistle, nigella, pumpkin, sesame, safflower, and sunflower seeds have been utilised as sources of protein in the food industry recently, providing a well-balanced profile of amino acids (Kotecka-Majchrzak *et al*., 2020; Kurek *et al*., 2022; Kumar *et al*., 2024).

According to the Sustainable Development Goals (SDGs) framework, researchers are increasingly focusing on utilising underexplored agro-industrial by-products and waste materials to produce food products. Due to the emphasis on sustainable plant protein sources, several new and underused seed sources are being investigated (Ancuţa and Sonia, 2020). For instance, kiwi fruit seeds have been studied for their potential as a high-protein source (Deng *et al*., 2014), and sour cherry kernels have similarly shown promise as a protein source (Kasapoğlu *et al*., 2021). Other examples of underutilised materials include the defatted seeds of watermelon, muskmelon (Qin *et al*., 2022), and roselle (*Hibiscus sabdariffa* L.) seeds (Tounkara *et al*., 2013), all of which are rich in high-quality functional proteins.

Additionally, kenaf (*Hibiscus cannabinus* L.) seeds, which are often regarded as agricultural waste, have also been explored for their potential as a high-protein source, with studies evaluating their protein yield and functional properties (Ibrahim *et al*., 2021). Kenaf is a short-term fibrous plant primarily grown in tropical and temperate climates, thriving in areas with abundant solar radiation and high rainfall patterns (Ayadi *et al*., 2017). Known for its high-quality industrial pulp, kenaf is widely recognised for its versatility in fibre production, which is used in both technical and textile applications, including carpets, canvases, ropes, cordages, and sacks (Alexopoulou *et al*., 2013). Additionally, extracts from kenaf leaves have potential uses in herbal cosmetics, particularly for skin whitening and anti-aging purposes (Sim and Nyam, 2021). A large quantity of kenaf seeds is produced during the harvesting or processing of kenaf. Despite their high nutritional value, these seeds are frequently wasted as agricultural waste, rather than being valorised into useful products (Giwa Ibrahim *et al*., 2019). Kenaf seeds consist of high levels of dietary fibre, vegetable oil, and protein, as well as phenolic antioxidants. They could be employed as a value-added natural ingredient in the development of functional foods and nutraceuticals that may benefit human health and wellness (Chan *et al*., 2013; Giwa Ibrahim *et al*., 2019).

Kenaf seeds are a good dietary source of high-quality, functional protein; the seed extracts, in particular, can be used as a plant-based milk substitute (Karim *et al*., 2020). Several studies have also demonstrated that kenaf seeds can be used to produce kenaf-based drinks and tofu (Ibrahim *et al*., 2020; Karim *et al*., 2020). Similarly, kenaf seeds can be processed into kenaf seed protein isolate (KSPI), which, due to its high protein content, has potential applications in a variety of functional foods. Hamim *et al*. (2025) optimised various parameters for efficient extraction of KSPI, and found that it holds significant potential for the development of sustainable food products, while Ibrahim *et al*. (2021) employed alkaline extraction to produce kenaf seed meal protein concentrate (KSPC) and lyophilised kenaf seed milky extract protein concentrate (KSMEPC). Based on a study of the proximate composition, amino acid profile, and techno-functional properties of the protein concentrates,

KSPC demonstrated notable food product development potential.

The ability to effectively use plant proteins in food processing is greatly influenced by their techno-functional properties, which is highly affected by protein extraction and drying methods. Different extraction and drying techniques can yield proteins with varying functional characteristics, even when derived from the same source. A suitable drying method is essential when producing protein isolate powders. Although drying might increase storage stability, it causes protein to partially denature, which changes its functional properties, and results in irreversible, insoluble aggregates. Freeze-, spray-, and oven-drying are the most often utilised protein drying methods. Freeze-drying is frequently used to assess protein functionality, whereas spray-drying is employed for large-scale commercial protein manufacturing. Oven-drying is less costly, and the drying temperature can be set lower than the protein denaturation temperature (Feyzi *et al*., 2017), but the residence time is substantially longer. It has previously been shown that drying affects the structural and functional properties of mung bean, fenugreek, chickpea, peanut, quinoa, chia, and rice dreg protein isolates (Zhao *et al*., 2013; Ghribi *et al*., 2015; Gong *et al*., 2016; Timilsena *et al*., 2016; Feyzi *et al*., 2017; Brishti *et al*., 2020; Shen *et al*., 2021). To date, no comparative study has investigated the effects of different drying methods on the properties of KSPIs. Therefore, the present work aimed to assess the impact of three drying methods—freeze-, spray-, and oven-drying—on the physico-chemical, functional, thermal, and structural properties of KSPI.

Materials and methods

Materials

Kenaf seeds of variety V36 were free gift from Lembaga Kenaf dan Tembakau Negara plantation in Perlis, Malaysia. Kenaf seeds were thoroughly cleaned to remove contaminants, foreign particles, dirt, and any broken, discoloured, or other foreign seeds. The seeds were then rinsed under running tap water, and pre-dried in an oven (Constance FCH-9146A, Germany) for 1 h at 40°C. After cleaning, the kenaf seeds were ground using an IKA M20 Universal Mill (Germany), and the resulting particles were sieved through a 50-mesh screen to obtain the kenaf seed powder.

Preparation of protein isolate

Prior to protein extraction, kenaf seed flour was defatted with petroleum ether using FOSS Soxtec 2050 (Denmark) at a 1:4 (w/v) ratio for 1 h, and this process was repeated three times. The defatted kenaf flour was placed in a fume hood for at least 24 h to evaporate the petroleum ether. Protein isolate was extracted following the alkaline solubilisation and acid precipitation methods described by Mariod *et al*. (2010) with some modifications. Briefly, 100 g defatted kenaf flour was dispersed in 5,000 mL distilled water, and the suspension was stirred for 1 h at room temperature with pH maintained at 9.0 using 1 M NaOH at 50°C, followed by centrifugation at 4,500 *g* for 15 min at 4°C using Hitachi high-speed refrigerated centrifuge model CR22N (NuAire, Inc., Plymouth, USA). The supernatant was collected and adjusted to pH 4.5 with 1.0 M HCl to precipitate protein, followed by centrifugation. The pellet was rinsed and collected, and subjected to three different independent drying techniques.

Production of protein isolate by different drying methods

The freeze-dried isolate was produced by initially freezing the kenaf seed protein curd at -20°C for 48 h, followed by freeze-drying at -30°C under a pressure of 120 Pa for another 48 h using a VirTis Benchmark Freeze Dryer (SP Scientific, Pennsylvania, USA). The spray-dried isolate was obtained by diluting the protein curd at a 1:4 (w/v) ratio with distilled water along with 15% maltodextrin as wall material, homogenising, and spray-drying it using a pilot-scale spray drier (Model Niro 2000 type A; GEA Niro A/S, Soeborg, Denmark) with a rotary atomiser at an inlet temperature of 160°C, an outlet temperature of 90°C, and a flow rate of 6 mL/min. The oven-dried powder was prepared by drying the protein curd in a convection oven at 50°C for 24 h (Memmert GmbH + Co. KG, Schwabach, Germany).

Physico-chemical properties

Colour properties and browning index

The colour of KSPIs was measured using a digital colorimeter (Model CR-410, Konica Minolta, Tokyo, Japan). Three colour components were monitored, including L* (- black to + white), a* (- green to + red), and b* (-blue to + yellow) values. The instrument was calibrated using a white standard with

L* = 88.05, a* = 1.44, and b* = -4.27 prior to colour determination. The browning index was calculated using Eq. 1 according to Gonzales *et al*. (1999):

$$Browning\ index = \frac{x-0.31}{0.172} \times 100 \quad \text{(Eq. 1)}$$

where, x = (a* + 1.75L*) / (5.645L* + a* – 0.3012b*).

Particle size analysis

The particle size distribution of KSPIs was evaluated using a laser diffraction particle size analyser (Model Mastersizer 2000, Malvern Instruments Ltd., Worcestershire, UK). The refractive indices of the dispersion (water) and the particles were 1.33 and 1.44, respectively. Four size distribution features were determined at D_{10}, D_{50}, D_{90}, and $D_{[4,3]}$. The instrument was pre-programmed to analyse each sample in triplicate.

Morphological analysis

The surface morphology of KSPI powder was observed using a scanning electron microscope (Model JSM-5200. JEOL Ltd., Tokyo, Japan). An accelerating potential of 15 kV was employed during the test. Approximately, 0.01 g sample were deposited on aluminium stubs using double-sided adhesive carbon conductive tape, and coated with a thin gold layer with the help of gold sputter. Images were observed at 500× magnification levels.

Molecular weight analysis

The SDS-PAGE electrophoresis was performed according to Laemmli (1970) in a Mini-PROTEAN Tetra electrophoresis gel (Bio-Rad, Hercules, CA, USA).

Amino acids profile

The amino acids profile of KSPIs was determined following the method of Waters Corporation (1993). In a 15-mL test tube, 0.5 g of KSPI was added, followed by 5 mL of 6.0 mol/L HCl. The mixture was hydrolysed for 24 h at 110°C in an oven. Then, the hydrolysed sample was mixed with 4 mL of 2.5 mmol/L α-aminobutyric acid and 20 mL of deionised water. The aliquot was degassed with nitrogen gas purging for 10 min before being filled to 100 mL with deionised water. A 0.22 µm membrane filter was used to filter the aliquot into a sample vial. A calibration curve with an internal standard– 400 µL of 2.5 mmol/L Waters Amino Acid Hydrolysate Standard mixture (external standard stock solution), was prepared by mixing with 40 µL of 2.5 mmol/L AABA (internal standard stock solution) and 560 µL of deionised water in a sample vial. Prior to standard and sample derivatisation, AccQ.Tag Flour Reagent Powder (2A) was reconstituted by transferring 1.0 mL of Waters AccQ Flour Reagent diluent (2B) into 2A powder. The mixture was vortexed for 10 sec before being placed in a 55°C oven for 10 min to dissolve the powder. For derivatisation, 10 µL of calibration standard or hydrolysed sample were transferred into the Eppendorf tubes, and 70 µL of Waters AccQ Flour Borate Buffer (BF) and 20 µL of reconstituted AccQ reagent were added to each tube. The tubes were vortexed for 10 sec prior to incubation at 55°C for 10 min. The aliquot from each tube was transferred into an HPLC autosampler vial (Merck, Darmstadt, Germany), and injected into an HPLC (Agilent Technologies, German) equipped with a fluorescent detector (Agilent Technologies, Germany) at excitation wavelengths of 250 nm, and emission wavelengths of 395 nm. The amino acids were separated using the AccQ.Tag column (Nova-Pak TM C18, 4 µm) at a flow rate of 1 mL/min. Using the calibration curve, the quantity of each amino acid was determined based on the standard equation for each amino acid.

Techno-functional properties

Protein solubility

The solubility of KSPIs was determined as a function of pH according to Feyzi *et al*. (2015). Protein solutions of 1.5% concentration were prepared in deionised water. The dispersions were treated with 1.0 M HCl or NaOH to achieve a certain pH (2 - 11), and stirred at room temperature for 30 min. The slurry was centrifuged at 4,000 *g* for 15 min to separate the supernatants. Protein content was determined by the Bradford method (Bradford, 1976).

Water and oil absorption capacity

The water and oil absorption capacity were determined according to Ibrahim *et al*. (2021) with slight modification. A centrifuge tube was pre-weighed, and 0.5 g (W_0) of KSPI were added (W_1) and dispersed in 5 mL of distilled water or vegetable oil for water or oil absorption capacity determination. The centrifuge tube was vortexed for 2 min, and allowed to stand for 1 h at ambient temperature. The dispersion was then centrifuged at 3,000 *g* for 10 min. The supernatant was carefully discarded, and the contents were allowed to drain at 45° angle for 1 h.

The weight of the centrifuge tube (W_2) was calculated using Eq. 2:

$$\text{WAC / OAC } \left(\frac{g}{g}\right) = \frac{W_2 - W_1}{W_0} \times 100 \quad \text{(Eq. 2)}$$

where, W_0 = weight of protein sample; W_1 = weight of centrifuge tube and protein sample; and W_2 = weight of centrifuge tube and protein after water/oil was absorbed.

Emulsifying properties

The emulsion capacity (EC) and emulsion stability (ES) was determined according to Sethi *et al*. (2021). Briefly, 2.0 g of KSPIs was mixed with 20 mL each of distilled water and corn oil in centrifuge tube. Subsequently, the mixture was centrifuged at 3,000 *g* for 10 min. The emulsion layer's height was measured and used to calculate the emulsifying capacity using Eq. 3:

$$\text{EC } \left(\frac{mL}{mL}\right) = \frac{V_2}{V_1} \times 100 \quad \text{(Eq. 3)}$$

where, V_1 = volume of the emulsion layer; and V_2 = volume of the whole layer.

The emulsion stability (ES) was determined by heating the centrifuged sample at 80°C for 30 min, and then cooling it at room temperature. The mixture was then centrifuged for 10 min at 3,000 *g*. The emulsion layer's height was measured and used to calculate emulsification stability using Eq. 4:

$$\text{EC } \left(\frac{mL}{mL}\right) = \frac{V_2}{V_1} \times 100 \quad \text{(Eq. 4)}$$

where, V_1 = volume of the emulsion layer after heating; and V_2 = volume of the whole layer.

Foaming properties

The foaming capacity (FC) and foaming stability (FS) was determined according to Brishti *et al*. (2017). Briefly, 2.0 g of KSPI was mixed with 100 mL of distilled water, blended for 1 min with a Waring blender (WARINGLAboratory Science, Model 7010BU), and the mixture was immediately transferred to a 250-mL graduated measuring cylinder. The foam value was measured before and after whipping (Lawhon and Cater, 1971), and the foaming capacity was calculated using Eq. 5:

$$\text{FC } \left(\frac{mL}{mL}\right) = \frac{V_2}{V_1} \times 100 \quad \text{(Eq. 5)}$$

where, V_1 = volume of protein solution before whipping; and V_2 = volume of protein solution after whipping.

The foaming stability was measured by monitoring the change in foam volume after 15 min at room temperature, and calculated using Eq. 6:

$$\text{FC } \left(\frac{mL}{mL}\right) = \frac{V_t}{V_0} \times 100 \quad \text{(Eq. 6)}$$

where, V_t = volume of foam at time, t; and V_0 = initial foam volume.

Thermal properties

The thermal properties were measured using a differential scanning calorimeter (DSC) (Mettler Toledo 822e, GmbH, Switzerland) according to Brishti *et al*. (2017). An aluminium 40 µL DSC crucible was used to hold a 5 - 10 mg of KSPI. A temperature ramp rate of 10°C/min in the range of 0 - 200°C was used to heat the sample. The STARe software was used to analyse the thermograms specifically the denaturation temperature (T_d) and enthalpies (ΔH) of the protein isolate.

Structural properties

Secondary structure characterisation

Fourier Transform Infrared Spectroscopy (FTIR) was used to determine the secondary structure of KSPI. An FTIR spectrometer (Thermofisher Scientific Nicolet 6700) fitted with an Attenuated Total Reflectance (ATR) cell was used to record the infrared spectra of KSPI. The isolates were placed on the ATR crystal, and pressed down to ensure good contact. The spectrometer was subjected to constant dry air purging. The spectra were observed in the range of 4000 - 600 cm^{-1} (average of 120 spectra at 4 cm^{-1} resolution), and referenced against empty cell. Origin Pro 9.0 (OriginLab MA, USA) was used to detect overlapping peaks in the amide-I (1700 - 1600 cm^{-1}) region using self-deconvolution and curve fitting procedures.

Statistical analysis

The statistical analysis was performed using the Minitab statistics package version 18.1 (2017). The data from the storage analyses were subjected to One-way analysis of variance (ANOVA), followed by the Tukey multiple comparison test to compare the difference between mean values. Two-way ANOVA was also used to assess the significance of interaction

effects. Means and standard deviations were provided, with significance defined as $p < 0.05$.

Results and discussion

Physico-chemical properties

Colour

Colour properties of KSPI powder were significantly different ($p < 0.05$) when treated at different drying techniques (Table 1). All samples showed minimal reddish colour, but had stronger yellowish colour, as shown by the small a* and large b* values. Freeze-dried (FD) and oven-dried (OD) KSPI powder were brownish in colour, while spray-dried (SD) KSPI were creamy white. Compared to FD and OD, SD had the highest L* value, indicating its higher degree of lightness, as shown by its creamy white colour. This agreed with Brishti *et al*. (2020), who reported that spray-dried mung bean protein isolate had the highest L*, but the lowest a* and b* values. However, OD had the darkest colour as indicated by the lowest L* value. This was consistent with the lowest lysine content observed in the OD sample (Table 2), and further supported by the high browning index shown in Table 1. According to Feyzi *et al*. (2017), a lower lysine content indicates the occurrence of the Maillard reaction, as lysine is a key indicator of this process.

Particle size distribution

Table 3 shows the particle size distribution of KSPI from different drying techniques. The lowest D_{10}, D_{50}, D_{90}, and $D_{[4,3]}$ values were found in SD, followed by FD and OD, implying that SD produced the smallest particle size. This agreed with the highest L* values because small particle size enabled a bigger surface area to reflect more light compared to FD and OD (Hu *et al*., 2010). Brishti *et al*. (2020) reported similar results for freeze-, spray-, and vacuum-dried isolated mung bean proteins. SD powder exhibited a narrower and more uniform distribution, whereas FD and OD proteins had comparable patterns (Table 3). Similar findings were reported by Shen *et al*. (2021) on the particle size distribution pattern of quinoa protein isolates.

Crystal morphology

Solid food materials can be crystalline, semicrystalline, or amorphous. In order to confirm the amorphous structure of KSPIs, XRD testing was carried out. Figure 1 demonstrates that the main amorphous haloes, a broad band with a maximum at $2\theta = 20$, can be seen in the XRD patterns of all KSPI samples. Amorphous samples exhibit a broad peak, while crystalline samples display sharp signals (Ma *et al*., 2018). The peaks between 10° and 20° correspond to α-helix and β-sheet structures (Zhao *et al*., 2015; 2019), respectively. A higher content is indicated by a greater area of the peaks (Wang *et al*., 2024). All the proteins in KSPI exhibited peak intensities at $2\theta = 20°$ that were higher than those at 10° (Figure 1). Huang *et al*. (2017) also reported a dominant amorphous halo at $2\theta = 20°$ for various proteins, including soy protein isolate, peanut protein, chickpea protein concentrate, and fish gelatine. This suggested that the β-sheet content of KSPI was higher than its α-helix content, which was consistent with the proportions of secondary structures observed (Table 4).

A low intensity of typical diffraction peaks at 8.5° indicates the presence of α-helix, which was particularly noticeable in SD. This agreed with the secondary structure proportion findings (Table 4), which demonstrated that SD had higher α-helix content than the other two samples. SD exhibited the highest peak intensity at 20°, implying that SD had the most β-sheet content in the secondary structure. This was also consistent with the results from secondary structure proportion where SD possessed the highest β-sheet structure than the other two samples.

The diffraction patterns of KSPIs showed both amorphous and crystalline regions, with a predominant amorphous structure and poor crystallinity. It was significantly easier for SD to absorb moisture since the components comprised of amorphous powder that are more hygroscopic than those of crystalline material. Therefore, it is crucial to store them in air-tight containers. Several sharp peaks observed in FD samples indicated that they were in crystalline form. Crystal formation in FD samples was caused by the creation of ice crystals during the freezing process, which sublimed during freeze-drying (Zhao *et al*., 2013). It is interesting to note that the intensity level of OD KSPI powder, as demonstrated in the diffractograms, was much lower than for the other two powder products. As seen in Table 4, this result was triggered by the conversion of the α-helix and β-sheet structures into other secondary structures such the random coil and β-turn.

Table 1. Colour parameters of freeze-dried (FD), spray-dried (SD), and oven-dried (OD) kenaf seed protein isolates.

Colour	FD	SD	OD
L*	62.29 ± 0.64[b]	78.15 ± 0.61[a]	46.58 ± 0.98[c]
a*	7.16 ± 0.07[a]	3.14 ± 0.13[b]	6.91 ± 0.16[a]
b*	26.72 ± 0.31[a]	11.11 ± 0.19[c]	16.54 ± 0.42[b]
Browning index	12.33 ± 0.05[b]	5.37 ± 0.15[c]	13.87 ± 0.35[a]

Means ± S.D. in similar row followed by different lowercase superscripts are significantly difference at $p < 0.05$.

Table 2. Amino acid profile of freeze-dried (FD), spray-dried (SD), and oven-dried (OD) kenaf seed protein isolates.

Amino acid (mg/g sample)	FD	SD	OD
Aspartic acid	35.52 ± 4.32	36.63 ± 1.96	30.50 ± 2.09
Glutamic acid	87.95 ± 7.98	87.75 ± 7.91	77.88± 7.18
Serine	31.52 ± 1.88	28.64 ± 0.40	32.00 ± 2.06
Glycine	16.13 ± 1.63	16.03 ± 1.80	17.44 ± 0.94
Histidine	14.25 ± 1.26	14.59 ± 0.27	15.36 ± 1.23
Arginine	98.50 ± 2.67	99.12 ± 2.45	109.26 ± 3.30
Threonine	14.01 ± 1.69	13.95 ± 1.43	16.18 ± 2.74
Alanine	24.85 ± 1.40	24.32 ± 1.36	23.71 ± 1.30
Proline	20.10 ± 2.26	19.14 ± 3.46	19.45 ± 2.41
Tyrosine	17.76 ± 1.93	18.92 ± 2.02	19.19 ± 1.42
Valine	32.71 ± 1.06	30.80 ± 0.06	32.72 ± 1.96
Methionine	7.73 ± 1.25	4.13 ± 1.51	7.94 ± 1.83
Isoleucine	33.82 ± 2.32	21.82 ± 0.77	34.04 ± 0.71
Leucine	73.57 ± 3.55	64.66 ± 2.84	73.93 ± 4.41
Phenylalanine	44.51 ± 1.70	42.19 ± 1.76	47.96 ± 1.34
Lysine	25.55 ± 1.05	40.88 ± 1.69	23.60 ± 2.69
Total Amino Acid	578.48	563.57	581.16
Acidic	123.47	124.38	108.38
Basic	138.30	154.59	148.22
Polar-without-charge	186.76	185.89	175.75
Hydrophilic	208.80	222.44	195.52
Hydrophobic	253.42	223.09	257.19
Essential	246.15	233.02	251.73
Non-essential	332.33	330.55	329.43

Table 3. Particle size distribution of freeze-dried (FD), spray-dried (SD), and oven-dried (OD) kenaf seed protein isolates.

Particle size distribution (µm)	Drying technique		
	FD	SD	OD
D_{10}	149.89 ± 5.42[b]	4.87 ± 0.33[a]	77.87 ± 7.78[c]
D_{50}	354.03 ± 9.85[a]	13.98 ± 1.46[b]	483.11 ± 10.70[a]
D_{90}	695.15 ± 11.24[a]	30.80 ± 6.77[c]	823.12 ± 11.66[b]
$D_{[4,3]}$	389.06 ± 9.01[b]	18.38 ± 1.09[c]	465.33 ± 8.79[a]

D_{10}, D_{50}, and D_{90} represent the corresponding particle size, which is smaller than 10, 50, and 90% of the sample particles, respectively. $D_{[4,3]}$ represents the particle diameter of volume. Means ± S.D. in similar row followed by different lowercase superscripts are significantly difference at $p < 0.05$.

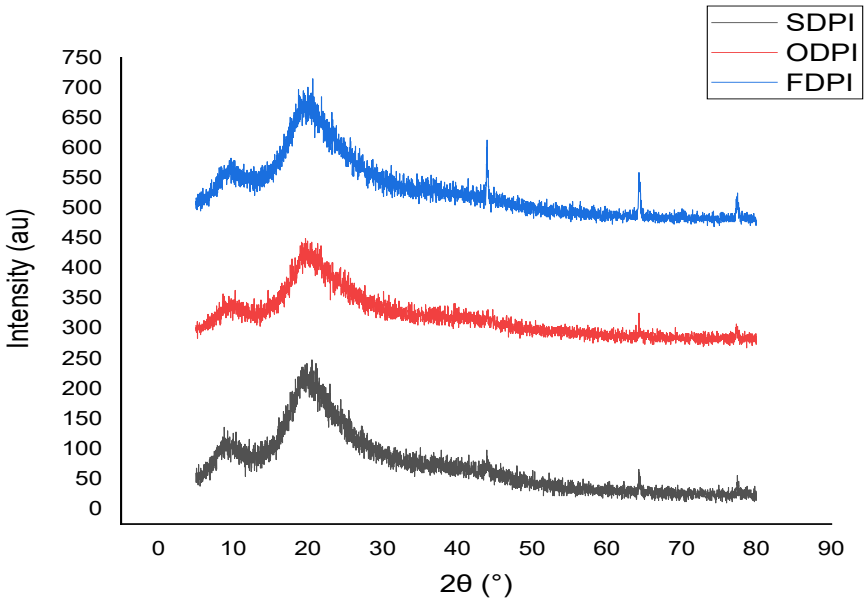

Figure 1. XRD spectrum for freeze-dried (FD), spray-dried (SD), and oven-dried (OD) kenaf seed protein isolates.

Table 4. Percentage of secondary structure of freeze-dried (FD), spray-dried (SD), and oven-dried (OD) kenaf seed protein isolates.

	FD	SD	OD
α-helix	22.37 ±1.58^{a}	22.27 ± 1.23^{a}	18.02 ± 1.14^{b}
β-sheet	38.44 ± 1.92^{a}	34.84 ± 1.33^{b}	24.41 ± 1.46^{c}
β-turn	22.29 ± 1.52^{b}	13.64 ± 1.44^{c}	26.94 ± 1.59^{a}
Random coils	16.30 ± 1.31^{c}	22.19 ± 1.95^{b}	28.21 ± 2.51^{a}

Means ± S.D. in similar row followed by different lowercase superscripts are significantly difference at $p < 0.05$.

Morphological characterisation

Scanning electron micrographic studies of KSPI powders (30 - 500 µm) obtained by different drying processes are shown in Figure 2. The microstructure of FD was non-collapsed, non-porous, and compact crystalline structure (Figure 2a). During freeze-drying, the formation of ice crystal in KSPIs prevented shrinkage and collapse of the structure and shape, resulting in a small change in volume (Ratti, 2001). On the other hand, the microstructure of OD powder (Figure 2c) appeared more compact, with irregular particles, sharp edges, and substantial indentations. Brishti *et al*. (2020) observed a similar structure in oven-dried mung bean protein isolate powder, attributing this compactness to prolonged drying at high temperatures (24 h, 50°C). This process likely caused water molecules to migrate from the interior of the sample, and evaporate rapidly from the surface. Spray-drying KSPI powder (Figures 2e and 2f) exhibited spherical or oval shape and smooth surface particles due to the impact of the spray-drying condition, which was maintained at an inlet temperature of 160 ± 2°C during drying. The collapsed and wrinkled shape of SD might have been due to the uneven shrinkage of protein and water-containing particles (Gong *et al*., 2016).

Protein profiling

SDS-PAGE was carried out to assess the changes in protein pattern of KSPI. The protein molecular weight distribution pattern of FD, SD, and OD are shown in Figure 3. Different drying methods did not result in the dissociation of protein subunits in KSPI because all KSPIs exhibited the same band pattern. This agreed with Zhao *et al*. (2013), who reported that the subunit composition of rice protein isolates were not affected by spray- and freeze-drying. Based on Figure 3, OD exhibits weaker bands compared to FD and SD. This can be attributed to the longer drying duration at high temperatures (24 h, 50°C), which likely caused protein denaturation, aggregation, and cross-linking, resulting in reduced protein solubility, and subsequently weaker bands (Feyzi *et al*., 2017).

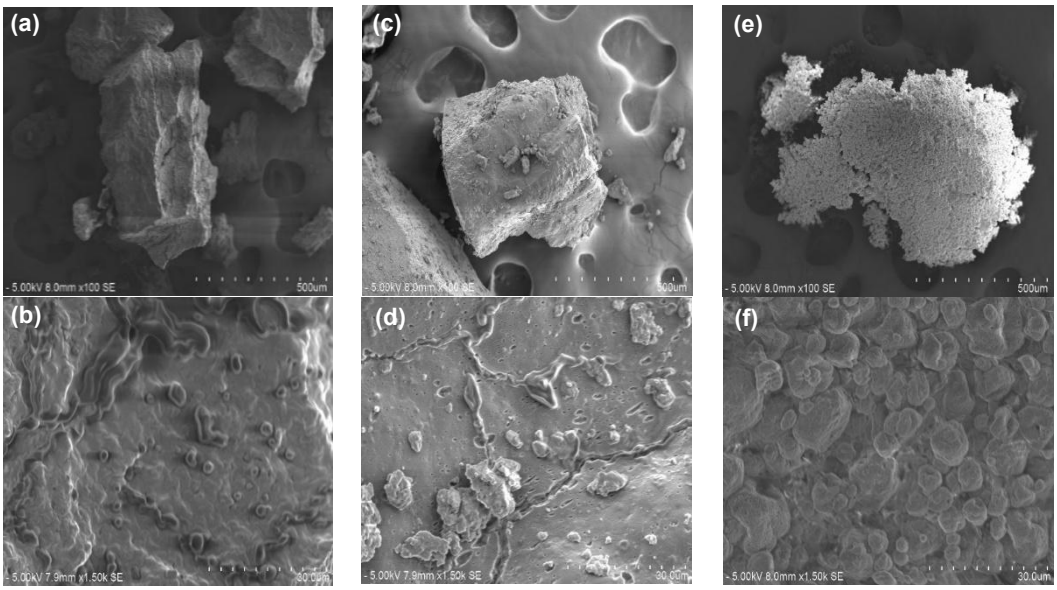

Figure 2. Scanning electron micrographs (SEM) of kenaf seed protein isolates by freeze-drying [**(a)** and **(b)**], oven-drying [**(c)** and **(d)**], and spray-drying [**(e)** and **(f)**]. Magnification of 100× [**(a)**, **(c)**, and **(e)**] and 300× [**(b)**, **(d)**, and **(f)**], 50 kV.

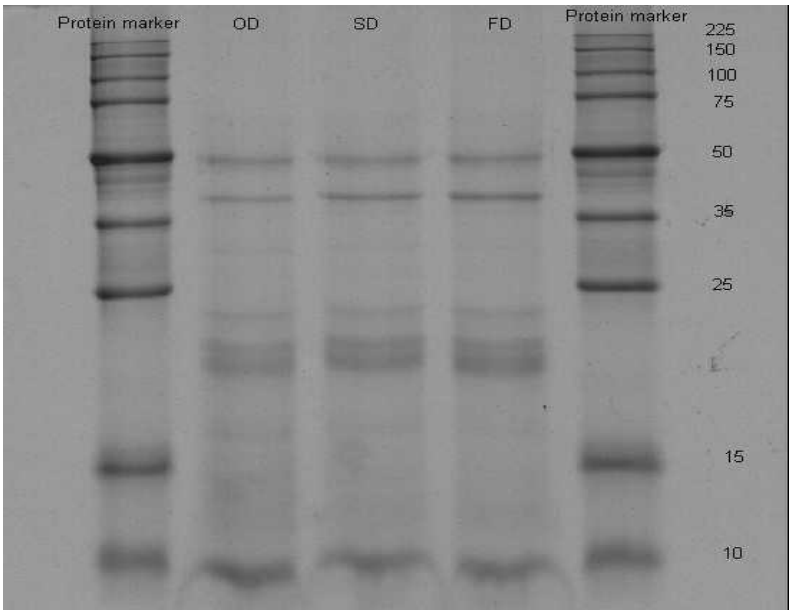

Figure 3. Electrophoresis pattern of oven-dried (OD), spray-dried (SD), and freeze-dried (FD) kenaf seed protein isolates. Lane 1: Marker; Lane 2: Oven-dried (OD); Lane 3: Spray-dried (SD); and Lane 4: Freeze-dried (FD).

Amino acid composition

Amino acid composition (hydrophobic, acidic, basic, and charged residues) of a protein sample has a significant impact on its functionality. The net surface charge of a protein molecule can be obtained by comparing the ratios of basic and acidic amino acids. Amino acid composition of FD, SD, and OD of KSPI are shown in Table 2. SD had lower total amino acid content (563.57 mg/g protein) than FD (578.48 mg/g protein) and OD (583.16 mg/g protein). The vast reduction in SD might have been due to high temperature (160°C inlet temperature and 90°C outlet temperature) compared to FD (-30°C) and OD (50°C). Similar results have been reported by Shen *et al*. (2021).

Plant-based proteins are commonly composed of higher amounts of amino acids, even though they may lack one or two essential amino acids such as aspartic acid, glutamic acid, and arginine. These amino acids are critical in human metabolic functions (Qin *et al*., 2022). Interestingly, the tested KSPIs were found to be rich in aspartic acid, glutamic acid,

and arginine, with total amounts of 221.97 mg/g for FD, 223.5 mg/g for SD, and 217.64 mg/g for OD proteins. This agreed with previous research by Ibrahim *et al*. (2021), although the levels were significantly higher than those reported for kenaf seed protein concentrate. The increased concentration of these amino acids in the present work compared to earlier studies could have been due to the focus on protein isolate in the present work rather than protein concentrate. Aside from that, different varieties of kenaf seeds may contribute to varying levels of these amino acids.

Iwe *et al*. (2001) discovered that the amino acids that are most strongly affected by reactions occurring during heating are tryptophan, cysteine, methionine, histidine, lysine, and arginine. These amino acids, particularly lysine, are employed as indicators for the Maillard reaction, which reflects the degradation of protein quality. SD sample revealed the highest lysine concentration (40.88 mg/g protein), while OD sample exhibited the lowest lysine concentration (23.60 mg/g protein). This agreed with the lowest L* value in OD (Figure 4) which was confirmed by a reduced lysine concentration.

(a) **(b)** **(c)**

Figure 4. Freeze-dried **(a)**, spray-dried **(b)**, and oven-dried **(c)** kenaf seed protein isolate.

Techno-functional properties of KSPIs

Protein solubility

Low soluble proteins have limited functional properties and applications in the food industry (Kinsella, 1979). Protein isolate with high solubility is desirable for high-quality food products such as milk and tofu. Figure 5 shows the protein solubility for FD, SD, and OD KSPI at pH 2 - 12. Similar U-shaped curves were apparent in all of these samples. The highest solubility of KSPI was observed under extreme acidic (pH 2) and alkaline (pH 12) conditions, while the lowest solubility was recorded at pH 4.5, which is the isoelectric point of KSPI. Notably, the solubility of SD protein was comparatively higher than that of FD and OD proteins in both extremely acidic (pH 2) and alkaline (pH 8 - 12) environments. This agreed with the results of the SEM morphology analysis (Figure 2), which revealed that the SD powder exhibited porous particle morphology, whereas FD and OD powders were more compact. The porous structure of SD may facilitate protein solubility by enhancing protein hydration. Additionally, the XRD spectrum (Figure 1) supported this finding, showing that SD was more amorphous than FD and OD, with a pronounced diffraction peak at 8.5° for SD. Similar results were reported by Timilsena *et al*. (2016) and Zhao *et al*. (2013), who found that spray-dried samples exhibited the highest protein solubility.

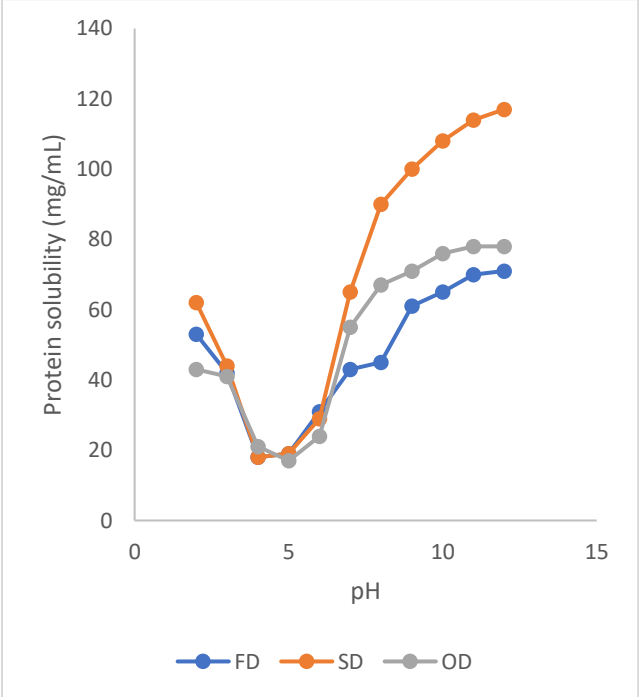

Figure 5. Protein solubility profile of freeze-dried (FD), spray-dried (SD), and oven-dried (OD) kenaf seed protein isolates at pH 2 - 12.

Water and oil absorption capacity

The terms WAC and OAC relate to the quantity of water and oil that can be absorbed by one gram of sample, respectively. Both these parameters have crucial implications in food applications, such as the ability to prevent fluid leakage from a food product during storage or processing (Jarpa-Parra, 2018), as well as the ability to retain oil in doughnuts, baked goods, and ground meat formulations (Toews and Wang, 2013). As shown in Table 5, the WAC of SD (1.37 g/g) is significantly lower than FD and OD. This might have been due to the formation of a moisture-resistant film during the spray-drying process (Ghribi *et al*., 2015). A similar pattern was reported by Brishti *et al*. (2020), who found that spray-dried mung bean protein isolate exhibited significantly lower WAC compared to freeze- and oven-dried mung bean protein isolates. Furthermore, the WAC of kenaf seed protein concentrate reported by Mariod *et al*. (2010) was significantly higher, at 3.39 g/g, than the findings of the present work. The difference could be explained by various protein structures, conformational changes, and the availability of polar amino acids on the protein surface due to different drying techniques (Mune Mune and Sogi, 2015). The present work suggested that FD and OD could be added to food product formulations that require significant water retention.

Oil absorption capacity (OAC) is the ability to retain oil in foods such as doughnuts, baked goods, and ground meat formulations (Toews and Wang, 2013). The OAC of FD, SD, and OD samples of KSPI are given in Table 5. The data clearly indicate that the OAC of FD (1.33 g/g) is significantly higher than that of SD (1.13 g/g) and OD (1.24 g/g). This increased OAC may be attributed to enhanced protein-fat interactions and the superior fat-binding ability of non-polar amino acid side chains (Brishti *et al*., 2020). Various studies have reported different OAC for kenaf seed protein, ranging between 8.24 - 8.71 g/g (Mariod *et al*., 2010), and 1.38 g/g (Ibrahim *et al*., 2021) for kenaf seed protein concentrate. In food formulations, proteins must be able to bind with fat because fat entrapment enhances taste absorption, and creates a pleasant mouthfeel. But SD with its low fat-binding ability is not suitable as meat substitute or extender to enhance flavour retention and improve mouthfeel in meat analogues.

Table 5. Techno-functional properties of freeze-dried (FD), spray-dried (SD), and oven-dried (OD) kenaf seed protein isolates.

	FD	SD	OD
Water absorption capacity (WAC)	1.44 ± 0.02	1.37 ± 0.02	1.46 ± 0.02
Oil absorption capacity (OAC)	1.33 ± 0.02	1.13 ± 0.02	1.24 ± 0.03
Emulsifying capacity (EC)	77.05 ± 2.20	83.12 ± 2.45	75.83 ± 2.29
Emulsifying stability (ES)	67.19 ± 2.20	75.63 ± 2.31	65.43 ± 2.40
Foaming capacity (FC)	55.03 ± 2.15	106.03 ± 4.55	52.93 ± 2.70
Foaming stability (FS)	2.67 ± 0.12	18.03 ± 1.20	2.17 ± 0.03

Emulsifying properties

Proteins can act as emulsifiers by forming a film or skin around oil droplets dispersed in an aqueous medium to prevent phase separations and structural changes to the emulsion system as a result of processes like coalescence, creaming, flocculation, or sedimentation. This is possible because proteins have an amphiphilic molecular structure (Boye *et al*., 2010). The emulsifying properties of proteins are commonly evaluated by measuring the Emulsifying Capacity (EC) and Emulsifying Stability (ES). EC is defined as the maximum quantity of oil that can be emulsified by a standard amount of protein under given conditions (Pearce and Kinsella, 1978), while ES measures the emulsion's resistance over a predetermined period of time.

Higher emulsifying activities are generally beneficial for applications in the food industry. The EC for SD samples was notably higher at 83.12 mL/mL, compared to FD at 77.05 mL/mL, and OD at 75.83 mL/mL (Table 5). Additionally, the ES values for SD were 75.63 mL/mL, significantly surpassing FD at 67.19 mL/mL, and OD at 65.43 mL/mL. This agreed with Brishti *et al*. (2020), who observed that spray-dried mung bean protein isolate exhibited greater emulsifying activity Index (EAI) and ES values compared to freeze- and oven-dried counterparts. Feyzi *et al*. (2017) similarly reported that the EAI and ES values for spray-dried fenugreek protein isolate were higher than those for freeze- and vacuum-dried samples. The increased EAI and ES values of the spray-dried samples can be attributed to

the heating treatment during the spray-drying process, where an output temperature of 90°C induces partial unfolding of globular proteins. This unfolding exposes hydrophobic amino acid residues, enhancing adsorption at the oil-water interface (Brishti *et al*., 2020).

Foaming properties

Foaming properties (FC and FS) are desirable in various situations, and used in food systems for aeration and whipping (Timilsena *et al*., 2016). Foams are created when proteins unfold to form an interfacial skin at the air-water interface, which reduces surface tension, and prevents the collapse of air bubbles by reducing surface tension (Boye *et al*., 2010). Foaming properties of proteins are generally expressed as FC which indicates the amount of foam formed per volume of protein solution due to air incorporation, and FS, which measures the ability of the protein system to retain air in the form of bubbles throughout ageing (Jarpa-Parra, 2018). The foaming capacity of SD samples stood out significantly at 106.03 mL/mL compared to FD at 55.03 mL/mL, and OD at 52.93 mL/mL (Table 5). This agreed with the solubility results depicted in Figure 5, where higher protein solubility indicates the samples' ability to migrate towards the water-air interface, and encapsulate air bubbles (Feyzi *et al*., 2017). Moreover, the FS values for SD were notably higher at 18.03% compared to FD at 2.67%, and OD at 2.17% (Table 5). Thus, it can be inferred that SD, with its exceptional foaming properties, is suitable for usage as a foaming ingredient in various food products such as cakes, breads, whipped creams, ice creams, and some baked goods.

Thermal properties

DSC was used to analyse the magnitude of the changes in thermal properties caused by the disruption of protein structures. The denaturation temperature (T_d) of a protein reflects its thermal stability, with higher T_d indicating greater stability. ΔH indicates the amount of energy required to denature the protein, which is the net energy value required to break the hydrogen bonds (endothermic) and hydrophobic interactions (exothermic) (Ma and Harwalkar, 1991). Based on Table 6, all samples exhibited endothermic peak with high T_d (84.25 - 96.00°C), indicating good thermal stability of KSPI, making it a potential ingredient for food products that require a high processing temperature. The denaturation temperature was similar with the denaturation temperature reported by Mariod *et al*. (2010) for V36 kenaf seed protein concentrate (81.8°C).

Table 6. Thermal properties of freeze-dried (FD), spray-dried (SD), and oven-dried (OD) kenaf seed protein isolates.

Thermal properties	FD	SD	OD
T_d (°C)	84.25 ± 0.25^b	96.00 ± 0.33^a	95.93 ± 0.16^a
T_o (°C)	44.48 ± 0.25^c	49.57 ± 0.44^b	51.25 ± 0.19^a
T_e (°C)	143.72 ± 0.28^c	148.91 ± 0.21^b	164.23 ± 0.25^a
ΔH (J g^{-1})	156.86 ± 3.59^c	198.30 ± 4.83^a	164.23 ± 2.21^b

Means ± S.D. in similar row followed by different lowercase superscripts are significantly difference at $p < 0.05$.

The T_d of SD sample (96.00°C) was significantly ($p > 0.05$) higher than that of OD (95.93°C), and FD (84.25°C), as well as ΔH suggesting that a lower degree of protein denaturation in SD compared to OD and FD samples. This could have been due to the fact that spray-drying is a quick procedure, and the drying residence time of KSPI inside the drying chamber is short (30 sec), resulting in lower protein denaturation. On the other hand, prolonged oven drying (24 h, 50°C) denatures protein molecules to a higher extent, resulting in reduced ΔH. According to Sanfelice and Temussi (2016), protein denaturation in FD may result *via* intermolecular reactions involving S-S bonds during concentrations by freezing.

Structural properties

Changes in secondary structure during drying

The amide-I region (1700 - 1600 cm^{-1}) in protein IR spectra is the most sensitive to secondary structure due to the correlation to a slightly different C=O stretching frequency in the spectrum due to its

unique molecular geometry and hydrogen bond (Krimm and Bandekar, 1986). Consequently, the amide-I region of KSPI was evaluated to determine the ratio of different secondary structures. Based on previous study, secondary structures in amide I, ranging from 1620 - 1640 cm^{-1}, represents β-sheet or extended structure; 1640 - 1644 cm^{-1} represents random coil; 1650 - 1656 cm^{-1} represents α-helix; and 1660 - 1700 cm^{-1} represents β-turn (Farrell *et al*., 2002).

The percentage of the β-sheet, a-helix, random coil, and β-turn determined through the curve fitting is given in Table 4. There were notable variations in the secondary structures of the kenaf seed proteins produced using various dry techniques. KSPI, like the majority of other plant protein isolates with predominant globulins, exhibited a bigger proportion of β-helix than α-helix. The results also suggested that the alteration in the secondary structural features was highest in OD KSPI, where β-sheets were mostly altered to β-turns and random coils due to denaturation. The intensity of bands between 1660 and 1640 cm^{-1} increased significantly as the random coil and β-turn contents increased while the β-sheet content decreased. The antiparallel β-sheet caused the bands to move from 1635 cm^{-1} to a lower wavenumber. This shift implied that the strength of hydrogen bonds in the β-sheet structure increased as the protein structure unfolded (Susi and Byler, 1985; Tang and Ma, 2009). The higher intensity of the band at 1660 cm^{-1} could be attributed to C=O stretching of the glutamine side chain. The broadening can be attributed to the distortion of the native secondary structure caused by the establishment of intra- and intermolecular hydrogen bonds between glutamine side chains and backbone peptide groups (Tang and Ma, 2009). All of these observations demonstrated that OD KSPI had higher variation in structural conformation than SD and FD KSPIs. Timilsena *et al*. (2016) also found that SD protein isolate was the least denatured due to the minimum secondary structural alterations in chia seed proteins.

It is widely known that the secondary structure of most globular proteins changes due to the loss of an α -helix, as shown by a significant decrease in the intensity of the amide I band around 1651 cm^{-1} (Kong and Yu, 2007; Tang and Ma, 2009). In general, the β-sheet structure is more stable than the α-helix. Proteins with a high proportion of β-sheet structure typically exhibit high denaturation temperatures (Damodaran and Parkin, 2017).

Conclusion

The present work evaluated the comparative effects of three drying techniques on the physico-chemical, techno-functional, thermal, and structural properties of powdered kenaf (*Hibiscus cannabinus* L.) seed protein isolate (KSPI). The findings indicated that freeze-dried (FD), spray-dried (SD), and oven-dried (OD) KSPIs exhibited comparable colour, particle size distribution, crystal and surface morphology, protein solubility, with several desirable functional and structural properties. SD KSPI had the highest L* values due to its smaller particle size, which provided a larger surface area to reflect more light compared to FD and OD KSPI. Morphological analysis revealed that the SD sample exhibited a collapsed and wrinkled surface, while the FD and OD samples maintained non-collapsed, non-porous, and crystalline structures. The SD KSPI demonstrated excellent protein solubility, emulsification activity, and stability, as well as foaming activity and stability. In contrast, the OD sample showed superior water absorption capacity, whereas the FD sample displayed significantly higher oil absorption capacity. All samples presented an endothermic peak with a high denaturation temperature (T_d) ranging from 84.25 to 96.00°C, indicating good thermal stability of KSPI. Compared to the OD and FD samples, the SD sample had a higher enthalpy change (ΔH), suggesting less protein denaturation. The SDS-PAGE results indicated that different drying processes did not cause separation of protein subunits in KSPI. However, FD samples exhibited more β-sheet structures and lower random coils compared to SD and OD counterparts. In terms of food applications, FD KSPI appeared to be best suited for the production of low-fat bread products and meat extenders. Meanwhile, SD KSPI showed potential for use in meat emulsions (such as meatloaf, bologna, and sausage), foam-type products (such as whipped toppings and ice cream), and beverages. OD KSPI, on the other hand, appeared to be well-suited for meat extenders. These findings offered valuable insights into selecting a drying procedure that yields KSPI powders with the desired functionality, thus aiding in optimising their applications in the food industry.

Acknowledgement

The authors extend heartfelt thanks to the National Kenaf and Tobacco Board (LKTN),

Ministry of Plantation and Commodities, Malaysia, for generously funding the present work (vote no.: 6300910). Gratitude is also expressed to Universiti Putra Malaysia (UPM) for providing state-of-the-art laboratory facilities that significantly contributed to the successful completion of the present work. Furthermore, special appreciation goes to the Malaysian Institute of Chemical and Bioengineering Technology, University of Kuala Lumpur (UNIKL MICET), for granting the scholarship and study leave to the first author, and to the Asia Food Validation Centre in Kepong, Kuala Lumpur, for providing essential laboratory facilities. Their steadfast support played a pivotal role in advancing this research endeavour.

References

Alexopoulou, E., Papatheohari, Y., Christou, M. and Monti, A. 2013. Origin, description, importance, and cultivation area of kenaf. In Monti, A. and Alexopoulou, E. (eds). Kenaf: A Multi-Purpose Crop for Several Industrial Applications, p. 1-15. London: Springer.

Ancuţa, P. and Sonia, A. 2020. Oil press-cakes and meals valorization through circular economy approaches: A review. Applied Sciences 10(21): 1-31.

Ayadi, R., Hanana, M., Mzid, R., Hamrouni, L., Khouja, M., Hanachi, A. S., … and Hanachi, A. S. 2017. *Hibiscus cannabinus* L . - Kenaf: A review paper. Journal of Natural Fibers 14(4): 1-19.

Boukid, F. 2022. Peanut protein - An underutilised by-product with great potential: a review. International Journal of Food Science and Technology 57(9): 5585-5591.

Boye, J., Zare, F. and Pletch, A. 2010. Pulse proteins: Processing, characterization, functional properties and applications in food and feed. Food Research International 43(2): 414-431.

Bradford, M. M. 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Analytical Biochemistry 72(1-2): 248-254.

Brishti, F. H., Chay, S. Y., Muhammad, K., Ismail-Fitry, M. R., Zarei, M., Karthikeyan, S. and Saari, N. 2020. Effects of drying techniques on the physicochemical, functional, thermal, structural and rheological properties of mung bean (*Vigna radiata*) protein isolate powder. Food Research International 138: 109783.

Brishti, F. H., Zarei, M., Muhammad, S. K., Ismail-Fitry, M. R., Shukri, R. and Saari, N. 2017. Evaluation of the functional properties of mung bean protein isolate for development of textured vegetable protein. International Food Research Journal 24(4): 1595-1605.

Chan, K. W., Khong, N. M. H., Iqbal, S., Mansor, S. M. and Ismail, M. 2013. Defatted kenaf seed meal (DKSM): Prospective edible flour from agricultural waste with high antioxidant activity. LWT - Food Science and Technology 53(1): 308-313.

Damodaran, S. and Parkin, K. L. 2017. Fennema's food chemistry. 5th ed. United States: CRC Press.

Deng, J., Sun, T., Cao, W., Fan, D., Cheng, N., Wang, B., … and Yang, H. 2014. Extraction optimization and functional properties of proteins from kiwi fruit (*Actinidia chinensis* Planch.) Seeds. International Journal of Food Properties 17(7): 1612-1625.

Farrell, H. M., Qi, P. X., Wickham, E. D. and Unruh, J. J. 2002. Secondary structural studies of bovine caseins: Structure and temperature dependence of β-casein phosphopeptide (1-2) as analyzed by circular dichroism, FTIR spectroscopy, and analytical ultracentrifugation. Journal of Protein Chemistry 21(5): 307-321.

Feyzi, S., Varidi, M. and Javad, M. 2017. Effect of drying methods on the structure, thermo and functional properties of fenugreek (*Trigonella foenum graecum*) protein isolate. Journal of the Science of Food and Agriculture 98(5): 1880-1888.

Feyzi, S., Varidi, M., Zare, F. and Varidi, M. J. 2015. Fenugreek (*Trigonella foenum graecum*) seed protein isolate: Extraction optimization, amino acid composition, thermo and functional properties. Journal of the Science of Food and Agriculture 95(15): 3165-3176.

Food and Agriculture Organization (FAO). 2022. FAOSTAT yearbook 2020. United States: FAO.

Galvão, A. M. M. T., Costa, G. F., Santos, M., Pollonio, M. A. R. and Hubinger, M. D. 2024. Replacing the animal fat in Bologna sausages

using high internal phase emulsion stabilized with lentil protein isolate (*Lens culinaris*). Meat Science 216: 109589.

Ghribi, A. M., Gafsi, I. M., Blecker, C., Danthine, S., Attia, H. and Besbes, S. 2015. Effect of drying methods on physico-chemical and functional properties of chickpea protein concentrates. Journal of Food Engineering 165: 179-188.

Giwa Ibrahim, S., Karim, R., Saari, N., Wan Abdullah, W. Z., Zawawi, N., Ab Razak, A. F., … and Umar, R. A. 2019. Kenaf (*Hibiscus cannabinus* L.) seed and its potential food applications: A review. Journal of Food Science 84(8): 2015-2023.

Gong, K. J., Shi, A. M., Liu, H. Z., Liu, L., Hu, H., Adhikari, B. and Wang, Q. 2016. Emulsifying properties and structure changes of spray and freeze-dried peanut protein isolate. Journal of Food Engineering 170: 33-40.

Gonzales, A. P., Burin, L. and Mari. 1999. Color changes during storage of honeys in relation to their composition and initial color. Food Research International 32: 185-191.

Hamim, N. A., Saari, N., Wan, Z., Manan, A., Mohamed, D., Anwar, F., … and Karim, R. 2025. Optimization of extraction for efficient recovery of kenaf seed protein isolates: Evaluation of physicochemical and techno-functional characteristics. Journal of the Science of Food and Agriculture 105(2): 2032-2047.

Hu, X. Z., Cheng, Y. Q., Fan, J. F., Lu, Z. H., Yamaki, K. and Li, L. T. 2010. Effects of drying method on physicochemical and functional properties of soy protein isolates. Journal of Food Processing and Preservation 34(3): 520-540.

Huang, L., Ding, X., Dai, C. and Ma, H. 2017. Changes in the structure and dissociation of soybean protein isolate induced by ultrasound-assisted acid pretreatment. Food Chemistry 232: 727-732.

Ibrahim, G., Noh, N. A. M., Saari, N. and Karim, R. 2020. Water soaking temperature of kenaf (*Hibiscus cannabinus* L.) seed, coagulant types, and their concentrations affected the production of kenaf-based tofu. Journal of Food Processing and Preservation 44(5): e14549.

Ibrahim, S. G., Wan Ibadullah, W. Z., Saari, N. and Karim, R. 2021. Functional properties of protein concentrates of KB6 kenaf (*Hibiscus cannabinus*) seed and its milky extract. LWT - Food Science and Technology 135: 110234.

Iwe, M. O., Van Zuilichem, D. J., Ngoddy, P. O. and Lammers, W. 2001. Amino acid and protein dispersibility index (PDI) of mixtures of extruded soy and sweet potato flours. LWT - Food Science and Technology 34: 71-75.

Jarpa-Parra, M. 2018. Lentil protein: A review of functional properties and food application. An overview of lentil protein functionality. International Journal of Food Science and Technology 53: 892-903.

Karim, R., Noh, N. A. M., Ibrahim, S. G., Wan Ibadullah, W. Z., Zawawi, N. and Saari, N. 2020. Kenaf (*Hibiscus cannabinus* L.) seed extract as a new plant-based milk alternative and its potential food uses. In Noh, N. A. M. (ed). Milk Substitutes, p. 4. United Kingdom: IntechOpen.

Kasapoğlu, K. N., Demircan, E., Eryılmaz, H. S., Karaça, A. C. and Özçelik. B. 2021. Sour cherry kernel as an unexploited processing waste: Optimisation of extraction conditions for protein recovery, functional properties and *in vitro* digestibility. Waste Biomass Valor 12: 6685-6698.

Kinsella, J. 1979. Functional properties of soy proteins. Journal of the American Oil Chemists' Society 56: 242-243.

Kong, J. and Yu, S. 2007. Fourier transform infrared spectroscopic analysis of protein secondary structures. Acta Biochimica et Biophysica Sinica 39: 549-559.

Kotecka-Majchrzak, K., Sumara, A., Fornal, E. and Montowska, M. 2020. Oilseed proteins - Properties and application as a food ingredient. Trends in Food Science and Technology 106: 160-170.

Krimm, S. and Bandekar, J. 1986. Vibrational spectroscopy and conformation of peptides, polypeptides and proteins. Advances in Protein Chemistry 38: 181-364.

Kumar, M., Kumari, N., Prakash, S., Sharma, N., Rajat, Radha, … and Panesar, P. S. 2024. Oilseed meal as a source of protein: Introductory remarks. In Kumar, M., Punia Bangar, S. and Panesar, P. S. (eds). Oilseed Meal as a Sustainable Contributor to Plant-Based Protein. United States: Springer.

Kurek, M. A., Onopiuk, A., Pogorzelska-nowicka, E., Szpicer, A. and Zalewska, M. 2022. Novel

protein sources for applications in meat-alternative products - Insight and challenges. Foods 11: 957.

Laemmli, U. K. 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature 227: 680-685.

Lawhon, J. T. and Cater, C. M. 1971. Effect of processing method and pH of precipitation on the yields and functional properties of protein isolates from glandless cottonseed. Journal of Food Science 36(3): 372-377.

Liceaga, A. M., Aguilar-Toala, J. E., Vallejo-Cordoba, B., Gonzalez-Cordova, A. F. and Hernandez-Mendoza, A. 2022. Insects as an alternative protein source. Annual Review of Food Science and Technology 13: 19-34.

Ma, C. and Harwalkar, V. R. 1991. Thermal analysis of food proteins. Advances in Food and Nutrition Research 35: 317-366.

Ma, M., Ren, Y., Xie, W., Zhou, D., Tang, S., Kuang, M., … and Du, S. K. 2018. Physicochemical and functional properties of protein isolate obtained from cottonseed meal. Food Chemistry 240: 856-862.

Ma, S. L., Sun, S., Li, T. Z., Yan, Y. J. and Wang, Z. K. 2024. Application research and progress of microalgae as a novel protein resource in the future. Critical Reviews in Food Science and Nutrition 65: 5832-5855.

Mariod, A. A., Fathy, S. F. and Ismail, M. 2010. Preparation and characterisation of protein concentrates from defatted kenaf seed. Food Chemistry 123(3): 747-752.

Multari, S., Stewart, D. and Russell, W. R. 2015. Potential of fava bean as future protein supply to partially replace meat intake in the human diet. Comprehensive Reviews in Food Science and Food Safety 14(5): 511-522.

Mune Mune, M. A. and Sogi, D. S. 2015. Functional properties of protein concentrates of cowpea and bambara bean involving different drying techniques. Journal of Food Processing and Preservation 39(6): 2304-2313.

Paivarinta, E., Itkonen, S. T., Pellinen, T., Lehtovirta, M., Erkkola, M. and Pajari, A.-M. 2020. Replacing animal-based proteins with plant-based proteins changes the composition of a whole Nordic diet - A randomised clinical trial in healthy Finnish adults. Nutrients 12: 943.

Pearce, K. N. and Kinsella, J. E. 1978. Emulsifying properties of proteins: Evaluation of a turbidimetric technique. Journal of Agricultural and Food Chemistry 26(3): 716-723.

Qin, P., Wang, T. and Luo, Y. 2022. A review on plant-based proteins from soybean: Health benefits and soy product development. Journal of Agriculture and Food Research 7: 100265.

Ratti, C. 2001. Hot air and freeze-drying of high-value foods: A review. Journal of Food Engineering 49: 311-319.

Sanfelice, D. and Temussi, P. A. 2016. Cold denaturation as a tool to measure protein stability. Biophysical Chemistry 208: 4-8.

Schreuders, F. K. G., Dekkers, B. L., Bodnár, I., Erni, P., Boom, R. M., Jan, A. and Goot, V. D. 2019. Comparing structuring potential of pea and soy protein with gluten for meat analogue preparation. Journal of Food Engineering 261: 32-39.

Sethi, S., Yadav, D. N., Snigdha, S. and Gupta, A. 2021. Optimization of process parameters for extraction of protein isolates from Khesari dhal (*Lathyrus sativus* L). LWT - Food Science and Technology 137: 110368.

Shen, Y., Tang, X. and Li, Y. 2021. Drying methods affect physicochemical and functional properties of quinoa protein isolate. Food Chemistry 339: 127823.

Sim, Y. Y. and Nyam, K. L. 2021. Application of *Hibiscus cannabinus* L. (kenaf) leaves extract as skin whitening and anti-aging agents in natural cosmetic prototype. Industrial Crops and Products 167(1): 113491.

Susi, H. and Byler, D. 1985. Fourier-transform infrared-spectroscopy in protein conformation studies. Journal of the American Oil Chemists' Society 62(4): 631-631.

Tang, C. H. and Ma, C. Y. 2009. Effect of high pressure treatment on aggregation and structural properties of soy protein isolate. LWT - Food Science and Technology 42(2): 606-611.

Timilsena, Y. P., Adhikari, R., Barrow, C. J. and Adhikari, B. 2016. Physicochemical and functional properties of protein isolate produced from Australian chia seeds. Food Chemistry 212: 648-656.

Toews, R. and Wang, N. 2013. Physicochemical and functional properties of protein concentrates from pulses. Food Research International 52(2): 445-451.

Tounkara, F., Amza, T., Lagnika, C., Le, G.-W. and Shi, Y.-H. 2013. Extraction, characterization, nutritional and functional properties of Roselle (*Hibiscus sabdariffa* Linn) seed proteins. Songklanakarin Journal of Science and Technology 35(2): 159-166.

Wang, J., Li, S., Tang, S. and Zhong, Y. 2024. Extraction, structural and functional properties of lipophilic protein isolated from peanut defatted powder. LWT - Food Science and Technology 201: 116235.

Wang, Y., Kim, W., Naik, R. R., Spicer, P. T. and Selomulya, C. 2023. Tuning the pea protein gel network to mimic the heterogenous microstructure of animal protein. Food Hydrocolloids 140: 108611.

Waters Corporation. 1993. Waters AccQ.Tag - Chemistry package instruction manual. United States: Waters Corporation.

Zhang, X., Zhang, T., Zhao, Y., Jiang, L. and Sui, X. 2024. Structural, extraction and safety aspects of novel alternative proteins from different sources. Food Chemistry 436: 137712.

Zhao, Q., Xiong, H., Selomulya, C., Chen, X. D., Huang, S., Ruan, X., … and Sun, W. 2013. Effects of spray drying and freeze drying on the properties of protein isolate effects of spray drying and freeze drying on the properties of protein isolate from rice dreg protein. Food and Bioprocess Technology 6: 1759-1769.

Zhao, X., Liu, H., Zhang, X., Zhu, H. and Ao, Q. 2019. Surface structure and volatile characteristic of peanut proteins obtained through AOT reverse micelles. Colloids and Surfaces B - Biointerfaces 173: 860-868.

Zhao, X., Zhu, H., Zhang, B., Chen, J., Ao, Q. and Wang, X. 2015. XRD, SEM, and XPS analysis of soybean protein powders obtained through extraction involving reverse micelles. Journal of the American Oil Chemists' Society 92(7): 975-983.