



Techno-Functional and Physicochemical Properties of Caper Seed Protein: A Comparative Study of Extraction Methods

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Abstract

This study investigates the valorization of *Capparis ovata* seeds as a sustainable source of high-quality plant protein. Protein powders were produced via four extraction methods: standard alkaline (CAE), enzyme-assisted (EAE), ohmic heating-assisted (ODE), and a combined ohmic heating-enzyme-assisted process (CODE-EE). Structural characterization using FTIR and SEM revealed preserved protein conformations and distinct morphological features across methods. SDS-PAGE analysis indicated molecular weights of 19–47 kDa. Amino acid profiling demonstrated high levels of essential amino acids, including isoleucine, leucine, lysine, tryptophan, glutamic acid, and arginine, with ohmic heating and enzyme treatments enhancing their abundance. The extraction method significantly influenced techno-functional properties: CODE exhibited the highest water-holding and oil-holding capacities (WHC: 3.18 g/g; OBC: 3.64 g/g), CODE and CODE-EE achieved maximal foaming capacity ($110.0\% \pm 2.0$ and $109.0\% \pm 1.0$), and CODE-EE showed superior emulsifying activity and stability, whereas CAE displayed the lowest performance. The exciting potential of caper seeds as a valuable plant-based protein source is demonstrated by this study, which also reveals how the CODE-EE process efficiently yields protein powder with a high protein content and superior functional qualities.

Keywords *Capparis ovata* L. · Amino acid profile · Plant protein · Green extraction methods · SDS-PAGE · Techno-functional properties

Introduction

By 2050, global population projections estimate a surpass of 9 billion individuals, which will lead to a nearly 70% increase in global demand for food and fiber (FAO, 2018; United Nations, 2022). Historically, animal proteins have been the primary dietary protein source; however, several factors, including rising costs, limited nutritional resources, vulnerability to climate change, health risks, and the depletion of freshwater resources, are driving the shift toward plant-based proteins (Cao et al., 2024; Sun-Waterhouse et al., 2014). Plants are regarded as bioproduction systems for high-value compounds, primarily producing metabolites such as proteins, carbohydrates, nucleic acids, and lipids, all of which are essential for life. According to FAO (2010), plant-based proteins account for 57% of the total global protein supply, while meat contributes 18%, dairy 10%, fish and shellfish 6%, and other animal products 9%. Proteins, which are complex and diverse molecules, are essential for maintaining the structure and function of living cells. Plant-based

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proteins, categorized as vegan foods, are rich in amino acids that are easily absorbed by the body, thereby offering benefits for treating various diseases. In addition, they are high in fiber, polyunsaturated fatty acids, carbohydrates, and oligosaccharides. Beyond their role in providing amino acids, vegetable-based proteins are vital in food formulations due to their techno-functional properties, including water retention, oil absorption, emulsification, and foaming abilities (Cao & Mezzenga, 2019).

Caper seed (*Capparis ovata* L.) is notable for its exceptional adaptability to extreme environmental conditions, making it a promising source of nutrition, particularly considering projected droughts intensified by climate change. The caper's extensive root system (Olmez et al., 2006) enables it to sustain photosynthesis under extreme radiation and temperature conditions without sustaining damage (Levizou et al., 2004). It is primarily cultivated in countries bordering the Mediterranean region, with an estimated average annual production of 10,000 tons (Tlili et al., 2011). Caper seeds contain a protein content of approximately 22–23% (Duman & Özcan, 2014), in addition to their high oil content, which is lower than that of conventional protein sources such as soybean (36–40%) but comparable to pea seeds (20–25%) (Banaszkiewicz, 2011; Shen et al., 2022). Upon oil removal, the remaining pulp serves as a rich and concentrated source of protein, highlighting its potential value for industrial applications. Although the amino acid composition of caper seed protein has not been documented in the literature, chia seeds, which share similarities with caper seeds, are rich in essential amino acids such as isoleucine, leucine, and valine, as well as non-essential amino acids like glutamic acid, aspartic acid, and arginine, which are known to enhance immune function (Timilsena et al., 2016).

The existing literature underscores the importance of plants as a key protein source, highlighting the growing interest in research within this domain. Numerous studies have explored various plant sources, alternative extraction methods, and the characterization of protein-based extracts. Among these methods, alternatives such as microwave-assisted protein extraction from bran (e.g., sesame, wheat, rice), ultrasound-assisted protein extraction from byproducts of the milling industry, oilseeds (e.g., soybean, rapeseed), and enzyme-assisted extraction from pulp/biomass have been investigated (Kumar et al., 2021). Recent research has also focused on the extraction of bioactive compounds from diverse plant materials using the ohmic heating-assisted extraction technique. However, there is a lack of research on the extraction of proteins from plant sources using ohmic heating-assisted methods (Pereira et al., 2016).

Considering the unexploited potential of *Capparis ovata* L. seeds as a resilient plant protein source and the limited literature on electro-thermal extraction applications for proteins, this study aims to evaluate the efficacy of novel

extraction protocols on protein recovery from defatted caper seeds.

A comparative assessment was conducted utilizing four distinct methodologies: conventional alkaline extraction (CAE), conventional enzyme-assisted extraction (CEE), ohmic heating-assisted extraction (CODE), and a hybrid ohmic heating-enzyme-assisted extraction (CODE-EE). Beyond yield optimization, the primary objective is to elucidate the impact of these extraction mechanisms, particularly the thermal and non-thermal effects of ohmic heating—on the physicochemical profile and techno-functional behavior (solubility, emulsification, foaming properties) of the resulting isolates. Furthermore, comprehensive structural characterizations using SDS-PAGE, FTIR spectroscopy, and SEM imaging were performed to establish the structure–function relationships of the extracted proteins.

Material and Methods

Material

Caper seeds (*Capparis ovata* L.) were purchased from a local supplier (Aşçı Murat®, Burdur, Turkey). The seeds were finely ground, and the resulting particles were sieved through a 500-mesh sieve to standardize the particle size.

All chemicals and solvents were of analytical grade and used. Sodium hydroxide (NaOH), hydrochloric acid (HCl), sodium dodecyl sulfate (SDS), ethanol (C₂H₅OH), β-mercaptoethanol, glycine, acrylamide, N, N'-methylenebisacrylamide, tetramethylethylenediamine (TEMED), ammonium persulfate (APS), and Coomassie Brilliant Blue were purchased from Sigma-Aldrich Chemical Co. (St. Louis, MO, USA). Petroleum ether (analytical grade) was obtained from Merck KGaA (Darmstadt, Germany). Alcalase® 2.4 L and Viscozyme® L were purchased from Novozymes (Bagsværd, Denmark). The Pierce™ Bradford Protein Assay Kit (950 mL), bovine serum albumin (BSA), and PageRuler™ Prestained Protein Ladder were obtained from Thermo Scientific™ (Thermo Fisher Scientific, Waltham, MA, USA).

Defatting Process

To optimize the protein extraction process and prevent the formation of oil globule barriers, which can hinder the extraction efficiency, the oil phase was removed from the ground seeds using petroleum ether. Following defatting, the seed particles were subjected to drying in an oven (Mikrotest, MSD2.50D, Turkey) at 38 °C until the residual organic solvent was completely evaporated, and the moisture content was reduced to less than 10%. The dried material was then homogenized and stored at –40 °C under vacuum in polyethylene packaging material with

low moisture and oxygen permeability for subsequent processing.

The total oil content in the sample was determined using the Soxhlet extraction method, as outlined by the AOAC guidelines (AOAC, 1990), with the process repeated in triplicate. The total oil content was calculated, and the results were presented as the mean $\pm$ standard error.

Protein Extraction

A total of four extraction procedures were employed to obtain cured protein powder from defatted caper seeds. Prior to conducting the extraction methods, preliminary experiments were carried out to determine key parameters including the solid-to-liquid ratio, medium pH, temperature, enzyme concentration, and potential enzyme combinations. The solid-to-liquid ratio (w/v), pH, and temperature were systematically varied within the ranges of 1:10 to 1:30, 2 to 11, and 40 to 60 °C, respectively. For enzyme-assisted extraction, two types of enzymes (Viscozyme® L (Novozymes, Bagsværd, Denmark), Alcalase® L (Novozymes, Bagsværd, Denmark)) were investigated. Experiments were conducted separately for each enzyme as well as for combinations of the enzymes. The optimal enzyme concentrations were determined based on the water-soluble protein content of the resulting extracts. Additionally, the pH value used for protein precipitation was tested, with the most efficient phase separation occurring at a pH of 4.5. Throughout the preliminary trials, the extraction time was fixed at 70 min, and the shaker speed was maintained at 80 rpm to ensure consistency across experiments.

Alkaline Extraction (CAE)

In this study, the alkaline extraction procedure described by Ivanova et al. (2017) was adapted with some modifications. Initially, defatted caper seeds were combined with distilled water at a solid-to-liquid ratio of 1:10 (w/v). Based on preliminary optimization, the suspension pH for alkaline extraction was adjusted to 11.0 using NaOH, and the extraction temperature was maintained at 60 °C. The extraction was carried out for 70 min, during which the suspension was continuously mixed using a magnetic stirrer at 80 rpm. Upon completion of the extraction, the liquid–solid mixture was centrifuged at 3000 $\times$ g for 20 min, and the supernatant was subsequently collected for further analysis.

Enzyme-Assisted Extraction (CEE)

Enzyme-assisted extraction was performed using Viscozyme® L (Novozymes, Bagsværd, Denmark) and Alcalase®

2.4 L (Novozymes, Bagsværd, Denmark). Based on preliminary experiments, the optimal parameters for enzyme concentration, pH, temperature, and extraction time were established. It was observed that Alcalase® 2.4 L (Novozymes, Bagsværd, Denmark) did not significantly enhance protein extraction efficiency, and its combination with Viscozyme® L (Novozymes, Bagsværd, Denmark) did not offer notable improvements compared to using Viscozyme® L (Novozymes, Bagsværd, Denmark) alone. Consequently, the enzyme-assisted extraction process continued solely with Viscozyme® L (Novozymes, Bagsværd, Denmark). For the Viscozyme-assisted extraction, the suspension's pH was adjusted to 2.5 using 1 M HCl. Defatted caper seeds were then mixed with distilled water at a solid-to-liquid ratio of 1:10 (w/v), and an enzyme solution was added to achieve a final enzyme concentration of 37.5 FBG units per 100 g. The mixture was incubated at 60 °C for 70 min. Following incubation, the supernatant was collected by centrifugation at 3000 $\times$ g for 20 min.

Ohmic Heating-Assisted Extraction (CODE)

The ohmic heating-assisted extraction process was another method investigated in this study. The system used for this process consisted of an AC power generator (output range 0–1000 V, 20 A AC at 50–60 Hz) (Eraktek Innovation, Konya, Turkey) and a modular Teflon (polytetrafluoroethylene) extraction cell (dimensions: 10 cm $\times$ 10 cm $\times$ 15 cm, L $\times$ W $\times$ H), equipped with titanium square electrodes (10 cm $\times$ 10 cm). The ohmic heating power generator facilitated the monitoring of real-time output voltage (V), current (A), as well as both instantaneous and set temperature (°C) data. The extraction temperature was controlled via an on/off switch, with an additional thermocouple integrated into the power supply for monitoring. Heating of the extraction medium was provided by following the ramp-up method including three temperature levels (first step: 50 °C, second step: 55 °C, and final step: 60 °C). After the set level of 60 °C was attained, the system continuously monitored the medium temperature fluctuation in the range of $\pm$ 1 °C by on/off control method for the remaining extraction time.

Preliminary experiments were conducted to determine the optimal extraction parameters, including potential difference (V), pH, temperature, and extraction time. Based on these trials, the solid-to-liquid ratio, pH, and extraction temperature were set to 1:10 (w/v), 11, and 60 °C, respectively. The extraction was performed at a constant potential difference of 500 V. The extraction process was carried out using an ohmic-heating power supply capable of delivering a constant 60-Hz sinusoidal alternating current. The electric field strength was adjustable, and in this study, an electric field intensity of 50 V/cm was applied, with a fixed extraction

time of 70 min. Ohmic *Heating-Assisted Enzyme Extraction* (CODE-EE).

The same system designed for the ohmic heating-assisted extraction, including the extraction cell and power generator, was also employed for a combined process involving both ohmic heating and enzyme-assisted extraction. The extraction was conducted at a constant potential difference of 500 V, corresponding to a voltage gradient of 50 V/cm, for a duration of 70 min. For the enzyme-assisted component, Viscozyme® L (Novozymes, Bagsværd, Denmark) was selected as the enzyme, with a concentration of 37.5 FBG units per 100 g of caper seeds. Considering the thermal stability of the enzyme, the extraction temperature was set at 60 °C to ensure optimal enzyme activity during the process.

Protein Powder Production

To obtain crude protein powder (CPP), a modified version of the method described by Timilsena et al. (2016) was employed. In this procedure, the extracts obtained from defatted caper seeds via various extraction techniques were first subjected to protein precipitation, with the supernatants being discarded. The pH of the extract was adjusted to 4.5 using 1 N NaOH or 1 M HCl, and the mixture was kept at 4 °C overnight to allow protein precipitation. Following the cold storage period, solid–liquid phase separation occurred, and the mixture was centrifuged at 3000×g to remove the supernatant. The resulting moist precipitate was then freeze-dried using a laboratory-scale freeze dryer (operating at a pressure of 0.8 mbar and condenser temperature of −50 °C) (Heto, Drywinner DW 3, Denmark). The resulting crude protein powder was ground, vacuum-packed in bags with low moisture and oxygen permeability, and stored at −40 °C until further analysis was performed.

$$\text{Protein Recovery(\%)} = \frac{\text{Amount of protein recovered}}{\text{Amount of protein in the raw material}} \times 100 \quad (1)$$

Characterization of Protein Powders

Amino Acid Profile

Amino acid composition of the defatted caper seed and protein powder samples was determined by the method described by Dimova (2003). The analysis was performed using an HPLC system equipped with a UV detector (Shimadzu, Series 20, Ultra-Fast Liquid Chromatography). First, the samples were subjected to acid hydrolysis with 6 N HCl at 110 °C for 24 h in sealed tubes. Following hydrolysis, the acid was removed via freeze-drying, and the resulting residues were dissolved in a coupling solvent. Pre-column derivatization was performed using phenylisothiocyanate (PITC). The derivatization reagent was

Moisture Content and Water Activity

The moisture content (MC) of the protein powder sample was determined using a moisture analyzer (DBS 60–3, Kern-Sohn GmbH, Germany) at a temperature of 105 °C. The water activity (a_w) of the sample was measured at 25 ± 2 °C using a water activity meter (Thermoconstanter TH 200, Novasina, Axair Ltd., Switzerland).

Proximate Analysis

Approximately 1–2 g of the protein powder was weighed into a porcelain crucible and subjected to combustion at 550 °C in an ash oven (Nüve MF 120, Turkey) until the resulting ash turned white. The crucible was then cooled, reweighed, and the ash content was calculated, expressed as grams of ash per 100 g on a dry basis. For the determination of total protein content, the nitrogen content of the samples was first measured using elemental analysis (Mazı, 2024). This value was then multiplied by a factor of 6.25, which had been previously determined through elemental analysis using a bovine serum albumin standard, to calculate the total protein content (AOAC, 2000). The total carbohydrate content was estimated using the subtraction method.

Protein Recovery

Protein recovery is a critical parameter for assessing the efficiency of the extraction method employed. It is defined as the ratio of the total protein content in the extracted crude protein powder to the total protein content in the raw material. The protein recovery is expressed as a percentage (%), calculated according to Eq. 1:

freshly prepared as a mixture of PITC, water, ethanol, and triethylamine (1:1:7:1, v/v/v/v). The reaction was carried out at room temperature for 15 min using 0.4 mmol of the sample, after which the solvent was removed by freeze-drying. Thin-layer chromatography (TLC) was used to monitor the derivatization efficiency using silica gel 60 plates with ethanol–water (3:1, v/v) as the mobile phase. Visualization was performed using iodine vapors and a 0.2% ninhydrin solution in acetone.

Separation of phenylthiocarbamyl (PTC) amino acid derivatives was achieved on a reversed-phase C18 column (LiChrosolv RP-C18, 250×4.5 mm, 5 µm) with a flow rate of 1 mL/min. Detection was determined at 254 nm. The mobile phase consisted of methanol (Solvent A) and 12 mM sodium phosphate buffer at pH 5.5 (Solvent B). A gradient solvent

system was applied from 10 to 50% Solvent A over 30 min at a flow rate of 1 mL/min. The column temperature was 40 °C.

Amino acids were identified by comparing their retention times with those of standard PTC-amino acids. The retention times of the PTC-amino acid derivatives were determined under the chromatographic conditions described above. The retention times (t_R , min) were as follows: glutamic acid (2.77), aspartic acid (5.00), serine (5.77), methionine (6.97), valine (7.83), threonine (9.00), proline (10.00), alanine (10.93), histidine (11.23), arginine (13.83), tyrosine (16.83), glycine (18.40), leucine (20.03), isoleucine (25.53), cysteine (27.77), tryptophan (29.79), phenylalanine (31.70), and lysine (33.27). The retention time of phenylisothiocyanate (PITC) was 34.27 min.

Molecular Size Determination of Protein Fractions

The molecular size distribution of proteins in the crude protein powder was characterized using Tricine-SDS-PAGE (Sodium Dodecyl Sulfate–Polyacrylamide Gel Electrophoresis), following the method described by Laemmli (1970). Protein separation was based on molecular weight, with smaller proteins migrating more rapidly through the polyacrylamide matrix. Electrophoresis was performed using the Bio-Rad® system with a 12% separating gel and a 4% stacking gel. The gels were cast between glass plates (10×8 cm and 10×7.5 cm), and polymerization was conducted at room temperature. Following polymerization of the separating gel, distilled water was layered on top to ensure a uniform interface and subsequently removed prior to pouring the stacking gel. After full gel polymerization, protein samples (25 µL per well) were loaded alongside a PageRuler™ Plus Prestained Protein Ladder (#26,620 Thermo Scientific) to facilitate molecular weight estimation of the resolved bands. Electrophoresis was initiated at 90 V for 1 h and was then increased to 110 V for an additional 2 h. Upon completion, the gel was stained overnight using Coomassie Brilliant Blue and destained for 45 min to visualize protein bands.

FTIR Spectroscopy

The crude protein powder and defatted caper seed samples were analyzed with an IR Affinity-1 Spectrometer (Shimadzu Corporation, Kyoto, Japan) with an attenuated total reflection (ATR) attachment. The sample was analyzed in the 4000–500 cm^{-1} region with 32 scans at 4 cm^{-1} resolution. The obtained spectrum was analyzed by comparison with each other and with the literature.

SEM Imaging

Micro-surface imaging was performed before and after protein extraction of defatted caper seed, and the structural

change caused by the applied extraction process at the microscopic level was investigated. At the same time, micro-surface imaging was also performed on the crude protein powder sample (FEI QUANTA FEG-250, Thermo-Fischer Scientific, USA).

Determination of Techno-Functional Properties

Water Holding (WHC) and Oil Binding Capacity (OBC)

To determine WHC (g water/g protein) and OBC (g oil/g protein), 0.1 g of crude protein powder was mixed with 1 mL of distilled water and then stirred using a vortex for 2 min. The mixture was then centrifuged at 13,000×g for 30 min. After the supernatant was properly discarded, the weight of the solid pellet was measured. To determine the WHC, the following equation (Eq. 2) was used:

$$WHC(\%) = \left(\frac{A1}{M} \right) \times 100 \quad (2)$$

A1, Weight of aqueous pellet after centrifugation (g); M, weight of the sample (g).

To determine OBC, 1 mL of corn oil was mixed with 0.1 g of crude protein powder, and the mixture was stirred for 1 min. The mixture was centrifuged at 13,000 g for 30 min. After the supernatant was properly discarded, the weight of the solid pellet was measured. Finally, OBC was calculated using Eq. 3:

$$OBC(\%) = \left(\frac{A2}{M} \right) \times 100 \quad (3)$$

A2, weight of oily pellet after centrifugation (g); M, weight of the sample (g).

Emulsion Activity (EA) and Emulsification Stability (ES)

EA and ES values of the crude protein powder sample were determined by a modified version of the method described by Hadidi et al. (2021). For EA, the weighed powder was mixed with 10% (w/v) phosphate buffer (0.01 M, pH 7.4) and incubated on a shaker for 24 h for complete hydration. Following, the sample was centrifuged at 2700×g for 15 min. For analysis, 0.9 ml of the sample supernatant was mixed with 0.3 ml of corn oil and homogenized (IKA T18, Germany) at 6000 rpm for a min. A 7-ml SDS was added to 0.1 ml of the sample-oil mixture, and the absorbance value was recorded at 340 nm. For evaluating ES, the absorbance was re-measured after 10 min at a wavelength of 340 nm. EA and ES values were calculated using Eqs. 4 and 5 given below:

$$EA\left(\frac{m^2}{g}\right) = \frac{2 \times 2.303 \times A_0 \times N}{c \times \varphi \times 10000} \quad (4)$$

$$ES, dak = (A_0/\Delta A) \times t \quad (5)$$

A_0 represents the absorbance of the diluted emulsion right after homogenization, N is the dilution factor ($\times 70$), φ is the oil volume fraction of the emulsion, c is the concentration (g/mL), ΔA is the absorbance change between 0 and 10 min (A_0 – A_{10}), and t is the time interval of 10 min.

Foaming Capacity (FC) and Foam stability (FS)

The method proposed by Hadidi et al. (2021) was modified to determine the FC and FS of a protein powder sample. For this purpose, 5 mL of distilled water was mixed with 0.1 g of crude protein powder. The resulting mixture was homogenized by a homogenizer (IKA T18, Germany) at 6000 rpm for 2 min. The total volume was then measured at time zero to determine the FC of the sample (V^*). The total volume was measured again 10 min following homogenization (V^{**}) to determine the FS. Equations 3 and 4 were used to calculate the FC (%) and FS (%) values as seen below:

$$FC(\%) = \left[\frac{(V^* - V)}{V} \right] \times 100 \quad (6)$$

$$FS(\%) = \left(\frac{V^{**}}{V^*} \right) \times 100 \quad (7)$$

V , total volume measured before homogenisation (mL), V^* , total volume measured at 0 min after homogenisation (mL), V^{**} , total volume measured at 10 min after homogenisation (mL).

Protein Solubility (PS)

To determine the PS of the crude protein powder, a protein suspension was prepared in potassium phosphate buffer (0.01 M) using crude protein powder at 1% (w/v), and the mixture's pH value was adjusted to the range of 2–10 using 1 M NaOH and/or 1 M HCl. The suspension was shaken on an orbital-shaker incubator (Four E'S Scientific, MI0103002, China) for one night at room temperature. After this, the solution was centrifuged for 20 min at $13,000 \times g$. As a control, a 1% (w/v) protein solution in 0.1 M NaOH was prepared and centrifuged at $13,000 \times g$ for 20 min. The Bradford method was used to determine protein solubility. PS was calculated according to Eq. 8 below:

$$Solubility, \% = \left(\frac{P1}{P2} \right) \times 100 \quad (8)$$

$P1$, protein content of sample (%), $P2$, protein content of control (%).

Statistical Analysis

Statistical analysis was performed using SPSS version 29.0. Differences among treatments were assessed using analysis of variance (ANOVA), followed by Duncan's multiple-comparison test to determine specific group differences. The number of replicates was three unless otherwise stated. Results are presented as means $\pm$ standard errors (SE).

Results and Discussion

Extraction and Proximate Analysis of Caper Seed Protein Powders

Initially, caper oil (38.35 g per 100 g of untreated caper seeds) was extracted using the Soxhlet method, serving as a by-product prior to crude protein isolation. Following oil removal, the defatted caper seed powder (DCS) exhibited a moisture content of 5.4 ± 0.02 g/100 g dry weight (dw), a water activity (a_w) value of 0.49 ± 0.00 , and an ash content of 1.88 ± 0.04 g/100 g dw, as presented in Table 1. The low a_w value, remaining below 0.6, indicates that the resulting protein powders are microbiologically stable, with enzyme activity significantly limited under such conditions.

Proximate analysis further revealed that the defatted caper seeds contained a substantial protein content (41.67 ± 1.27 g/100 g dw), emphasizing their potential as a valuable plant-based protein source. Defatted caper seeds contain approximately 42% protein, indicating their potential as a concentrated plant protein source. Moreover, capers are drought-tolerant and can grow in arid and marginal soils with minimal irrigation, although their limited cultivation currently restricts large-scale industrial protein production. These promising findings underscore the importance of identifying and optimizing efficient protein extraction methodologies to maximize yield and quality.

In pursuit of this objective, three environmentally friendly extraction techniques including ohmic heating-assisted extraction (CODE), enzyme-assisted extraction (CEE), and combined ohmic heating-assisted enzyme extraction (CODE-EE) were employed for the recovery of crude protein powders. Additionally, conventional alkaline extraction (CAE) was applied as a reference method. Notably, this study reports for the first time the application of ohmic heating-assisted extraction for protein recovery from defatted caper seeds.

Table 1 Proximate composition and protein recovery yields of crude protein powders extracted from defatted caper seed powder by different techniques

	DCS	CAE	CEE	CODE	CODE-EE
Protein	41.67 ± 1.27	66.48 ± 0.66 ^c	90.20 ± 0.54 ^b	55.28 ± 0.38 ^d	99.10 ± 0.09 ^a
Ash	1.88 ± 0.04	0.53 ± 0.09 ^b	0.51 ± 0.02 ^b	0.98 ± 0.03 ^a	0.50 ± 0.09 ^b
Carbohydrate	56.45 ± 1.35	32.99 ± 0.65 ^b	9.29 ± 0.53 ^c	43.74 ± 0.37 ^a	0.40 ± 0.08 ^d
Moisture content	5.40 ± 0.02	8.96 ± 0.09 ^a	4.53 ± 0.54 ^b	3.99 ± 0.22 ^{b,c}	2.92 ± 0.35 ^c
Water activity	0.49 ± 0.00	0.36 ± 0.00 ^a	0.15 ± 0.00 ^b	0.11 ± 0.00 ^c	0.12 ± 0.00 ^c
Protein recovery (%)		78.72 ± 1.59 ^b	86.87 ± 0.20 ^a	84.93 ± 0.73 ^a	86.91 ± 0.14 ^a

DCS defatted caper seed powder, CAE caper protein powder produced by alkaline extraction, CEE caper protein powder produced by enzyme extraction, CODE caper protein powder produced by ohmic heating-assisted extraction, CODE-EE caper protein powder produced by ohmic heating-assisted enzyme extraction. Protein, ash, and carbohydrate content were calculated on a dry basis. Different letters in the same line are significantly various ($p < 0.05$)

The proximate composition of the resulting protein powders is summarized in Table 1. Overall, the compositional characteristics of the powders obtained via green extraction methods were comparable. All samples were subjected to drying until the moisture content fell below 10%, in accordance with the experimental protocol. For the CAE-derived sample, drying was similarly completed upon reaching this threshold. Water activity values were consistent with the corresponding moisture contents. In terms of ash content, all samples exhibited levels below 1%. Nevertheless, the powder obtained through CODE demonstrated a notably higher ash content, approximately twice that of the other samples, suggesting potential differences in matrix interactions or extractability during the extraction process.

Protein content (g/100 g dw) and protein recovery yields of the crude powders are presented in Table 1. The final protein purity of the isolates obtained by each method corresponds to the total protein content of the recovered crude protein isolates (Table 1). No additional purification steps were performed. Notably, the CODE-EE method maintained protein purity at levels comparable to the other extraction methods, indicating that the more extensive cell wall disruption did not lead to significant co-extraction of non-protein components. Among the extraction methods, CODE-EE yielded the highest protein content (99.10 ± 0.09 g/100 g dw), followed by CEE (90.20 ± 0.54 g/100 g dw), CAE (66.48 ± 0.66 g/100 g dw), and CODE (55.28 ± 0.38 g/100 g dw). Statistically significant differences ($p < 0.05$) were observed among the extraction techniques, with CODE-EE demonstrating superior protein enrichment capacity. Similarly, the protein recovery yields were significantly affected by the extraction method ($p \leq 0.05$), with the highest recovery efficiencies (approximately 84–86%) achieved through green extraction approaches, particularly CODE-EE, CODE, and CEE, whereas CAE resulted in comparatively lower yields. These findings are consistent with previous reports, such as those by Mazi et al. (2021), who observed enhanced protein recovery from sesame seeds using enzyme-assisted extraction. The improved efficiency observed in the present study

is attributed to the action of carbohydrase enzymes, which hydrolyze high-molecular-weight carbohydrates within the seed cell wall matrix, thereby reducing carbohydrate-protein interactions and facilitating greater protein release Turker and Isleroglu (2024). Taken together, these results highlight the effectiveness of green extraction techniques, particularly ohmic heating-assisted enzyme extraction, for the sustainable and efficient recovery of plant-based proteins from defatted caper seeds.

Characterization of Protein Powders

Amino Acid Compositions

Amino acid composition is a crucial indicator for the evaluation of protein quality of any source, including plant-based ones. Thus, the caper seed was examined to this extent. Extracted crude protein powder and defatted caper seed were both investigated, and their amino acid compositions were determined. It was seen that the samples include essential amino acids, leucine, isoleucine, and phenylalanine (Table 2). Leucine and isoleucine are crucial for protein synthesis, muscle repair, and regulation of blood glucose levels. Leucine also stimulates wound healing and growth hormone production, while isoleucine supports muscle metabolism, immune function, and energy regulation (Rehman et al., 2023). Phenylalanine serves as a precursor for neurotransmitters critical for cognitive functions and mood regulation. It also contributes to protein structure and enzyme function (Gasmi et al., 2022). This literature information reveals the importance of those amino acids; thus, the current samples are considered significant in the food industry and human diet in case of utilization as a protein supplement.

The results show that caper seed is also a good source of non-essential amino acids. The dominant amino acids detected in both caper seeds and caper protein powders obtained by different extraction methods are glutamic acid and arginine among the non-essential amino acids. According to a published study,

Table 2 Amino acid composition of defatted caper seed and caper seed protein powders obtained using different extraction methods

Amino acids	DCS	CAE	CEE	CODE	CODE-EE
Essential amino acids (mg/100 g)					
Thr (Threonine)	0.56 ± 0.00	0.84 ± 0.00	0.36 ± 0.02	0.74 ± 0.01	0.46 ± 0.01
Val (Valine)	1.16 ± 0.00	1.84 ± 0.01	6.36 ± 0.04	2.60 ± 0.02	6.43 ± 0.04
Met (Methionine)	0.15 ± 0.00	0.44 ± 0.00	0.67 ± 0.01	0.78 ± 0.00	0.91 ± 0.00
Ile (Isoleucine)	0.60 ± 0.00	1.68 ± 0.00	4.33 ± 0.03	2.61 ± 0.02	4.50 ± 0.03
Leu (Leucine)	1.78 ± 0.00	2.54 ± 0.04	6.69 ± 0.02	3.81 ± 0.02	7.10 ± 0.03
Phe (Phenylalanine)	1.30 ± 0.00	2.25 ± 0.00	5.89 ± 0.03	3.36 ± 0.02	6.81 ± 0.05
Lys (Lysine)	1.03 ± 0.00	2.61 ± 0.00	1.37 ± 0.00	3.45 ± 0.04	2.31 ± 0.02
Non-essential amino acids (mg/100 g)					
Asp (Aspartic acid)	1.15 ± 0.00	4.42 ± 0.02	6.77 ± 0.02	3.13 ± 0.02	4.23 ± 0.07
Ser (Serine)	0.65 ± 0.00	1.08 ± 0.01	0.03 ± 0.00	0.89 ± 0.02	0.16 ± 0.00
Glu (Glutamic acid)	4.24 ± 0.00	6.60 ± 0.01	27.60 ± 0.14	10.91 ± 0.07	21.89 ± 0.12
Gly (Glycine)	1.01 ± 0.00	1.43 ± 0.01	2.82 ± 0.01	2.53 ± 0.04	2.88 ± 0.00
Ala (Alanine)	1.53 ± 0.00	1.81 ± 0.05	5.82 ± 0.03	2.96 ± 0.01	6.32 ± 0.04
Tyr (Tyrosine)	0.51 ± 0.00	1.43 ± 0.00	2.95 ± 0.02	2.18 ± 0.02	3.07 ± 0.03
His (Histidine)	ND	1.08 ± 0.00	1.20 ± 0.00	0.65 ± 0.01	1.51 ± 0.00
Arg (Arginine)	4.05 ± 0.00	3.25 ± 0.00	18.68 ± 0.12	5.65 ± 0.04	15.81 ± 0.04
Pro (Proline)	1.53 ± 0.00	1.83 ± 0.00	4.68 ± 0.05	2.07 ± 0.01	4.80 ± 0.01

DCS defatted caper seed powder, CAE caper protein powder produced by alkaline extraction, CEE caper protein powder produced by enzyme extraction, CODE caper protein powder produced by ohmic heating-assisted extraction, CODE-EE caper protein powder produced by ohmic heating-assisted enzyme extraction

glutamic acid-rich protein fractions can boost immunity and central nervous system function, and glutamate also has effective antioxidant properties (Sandoval-Oliveros & Paredes-López, 2013). Also, glutamic acid acts as a major excitatory neurotransmitter, which is crucial for brain function, energy production, and detoxification processes (Kumar et al., 2023; Pope et al., 2024). Another abundant amino acid, arginine, plays a vital role as a precursor in the synthesis of nitric oxide, proline, and other important molecules, which are essential for optimal growth, blood pressure regulation, wound healing, and immune function (Wu et al., 2021). To our knowledge, there has not been any study in the literature examining the amino acid profile of caper seeds. Thus, the current study contributes to this lack of knowledge in the literature.

Molecular Weight Profiling of Caper Seed Protein Powders

The differences in protein profiles in crude protein powders obtained using different extraction methods and in defatted caper seed powder were examined in terms of band color intensity and thickness. The results are presented in Fig. 1.

Data analysis of protein fraction bands of the bands in the protein profile was performed using Gel Analyzer (23.1.1) software. In lane 2, bovine serum albumin (BSA) standard protein was used as a control and that was located at 74 kDa, as expected. In the present study, the electrophoretic band of caper seed in lane 3 was distributed predominantly at 47, 32,

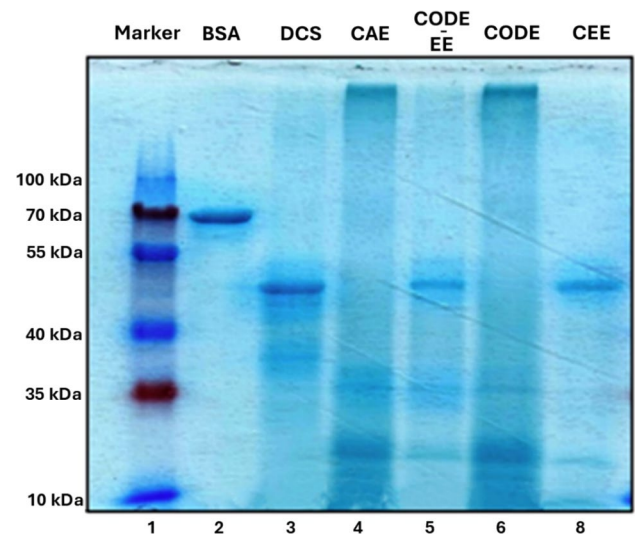


Fig. 1 SDS-PAGE Analysis of defatted caper seed proteins (lane 3) and protein powders extracted by CAE, CODE-EE, CODE, and CEE methods (lanes 4–7); Lane 1: Marker, Lane 2: BSA Standard

and 24 kDa. Protein extraction from caper seeds across various regions revealed the highest glutelin and lowest albumin content. SDS-PAGE analysis showed that the proteins had relatively small molecular weights (20–66 kDa) and varied protein band patterns among different sources (Wang et al., 2023), supporting the protein fraction distribution observed in the present study. In lane 4, the protein fractions in the protein powder produced by the CAE method were found at 27 and 17 kDa, while a similar

pattern was observed at 27, 19, and 17 kDa in the protein powder produced by the CODE method in lane 6. In lane 5, the protein fractions in the protein powder produced by the CEE method were found at 49, 27, and 19 kDa, while a similar pattern was observed at 48 and 19 kDa in the protein powder produced by the CODE-EE method in lane 7. The common bands between the protein powders were found at 19 and 27 kDa.

FTIR Spectroscopy

The peak analysis of the specific FTIR spectrum ranges enables detailed chemical composition evaluation of the samples; thus, it has become popular in scientific studies including food science applications. The FTIR spectrum examination has been reported as reliable, accurate, and sensitive data analysis. In the field of food science, FTIR spectroscopy is used to analyze a variety of compounds, including proteins, lipids, carbohydrates, and nucleic acids. Distinct wavelength values have been associated with the different functional groups of chemical structure (Table 3).

Recent advances in FTIR spectroscopy have revealed detailed insights into protein structure. The amide I, II, and III bands ($1700\text{--}1500\text{ cm}^{-1}$) reflect protein backbone conformations, enabling the determination of secondary structures. The amide I band ($1700\text{--}1600\text{ cm}^{-1}$) correlates strongly with α -helix, β -sheet, and unordered structures in proteins. Peptide bonds mainly contribute to protein absorbance in the $1700\text{--}1500\text{ cm}^{-1}$ region, while amino acid side chains (e.g., glutamic acid, aspartic acid, glutamine, asparagine, lysine, arginine, and tyrosine) influence the amide II region (Table 3). Carboxylate groups of aspartic and glutamic acids absorb between 1580 and 1520 cm^{-1} , and the intensity of these bands indicates protein content (Subramanian & Rodriguez-Saona, 2009).

The highest Amide A, Amide I, Amide II, and Amide III peaks are observed in the protein powder produced through CODE-EE, CEE, CAE, and CODE, respectively (Fig. 2). In this context, when the FTIR results are correlated with the protein content findings, the state of the Amide A, Amide I, Amide II, and Amide III bands shows a high degree of similarity. Our study shows a strong correlation between FTIR spectra and amino acid contents, notably for arginine, lysine, glutamic acid, aspartic acid, and tyrosine. As a result of the careful analysis, the spectra of protein powders obtained using the CODE-EE extraction method showed the highest levels in both the amide 1 and amide 2 regions ($1450\text{--}1750\text{ cm}^{-1}$). This distinction is particularly pronounced at 1560 cm^{-1} , where the separation of protein powders obtained by the CODE-EE extraction method from other methods is evident, indicating a highly dispersed region for N–H and C–H bonds.

SEM Imaging

The surface structure of protein powders obtained through four different extraction methods (CAE, CEE, CODE, CODE-EE) and the powder samples being their lyophilized supernatant phases stored after the protein precipitation step of the extraction process were investigated using SEM (FEI, QUANTA FEG 250, Thermo-Fischer, USA). The results are presented in Figs. 3 and 4. SEM images were examined at magnifications ranging from 1000 to 5000 $\times$.

Figure 3a and b present the images of defatted caper seeds prior to protein extraction, revealing cellular structures and globular protein formations. In Fig. 3c, d, e, and f, which depict the supernatant phases remaining after protein extraction using different methods, it can be observed that the cells were deformed,

Table 3 Functional groups and wavenumber (Subramanian & Rodriguez-Saona, 2009)

Mode	Frequency (cm^{-1})	Potential Energy Distribution (%)	Amino Acid	Bond	Frequency (cm^{-1})
Amide-I	~ 1655	Bending C–C–N: 11% Stretching C–N: 15% Stretching C–O: 83%	Asp	–COO [–]	1584
				–COOH	1713
			Glu	–COO [–]	1567
				–COOH	1706
Amide-II	~ 1560	Stretching N–C: 9% Stretching C–N: 33% Wagging N–H: 49% Wagging C–O: 12% Stretching C–C: 10%	Asn	–C=O	1648
				–C ₃ N ₃ H ₅	1608
			Arg		1586
				–OH	1615
				–OH	1515
				–O [–]	1603
Amide-III	~ 1300	Wagging N–H: 52% Stretching C–N: 14% Stretching C–C: 18% Wagging C–O: 11%	Tyr	–O [–]	1500
				–OH	1586
Amide-A	~ 3300	Stretching N–H: 95%		–O [–]	

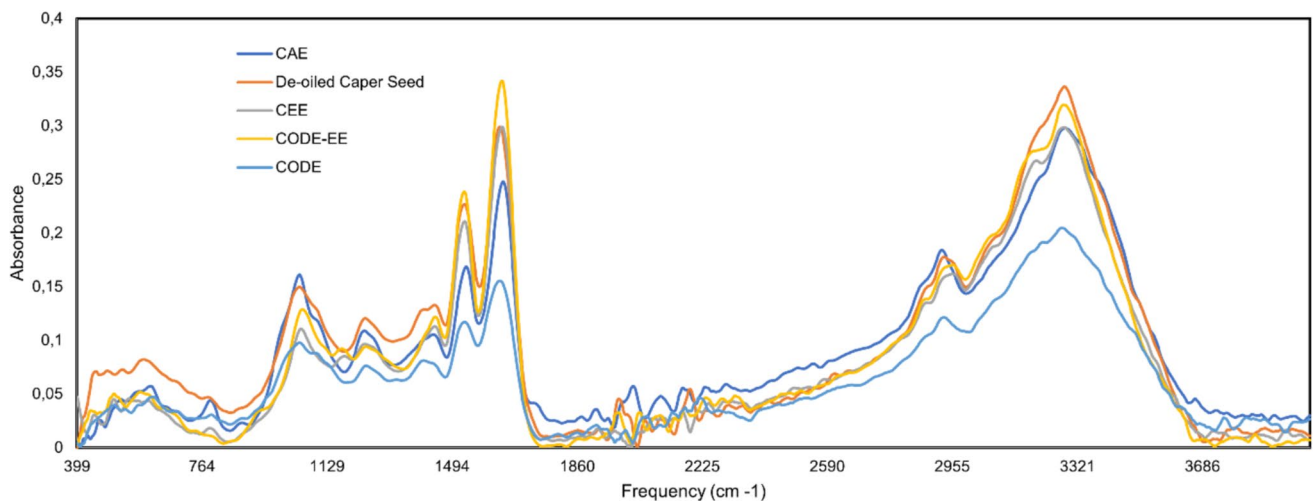


Fig. 2 FTIR diagram of defatted caper seed and caper protein powders. *CAE, caper protein powder produced by alkaline extraction; CEE, caper protein powder produced by enzyme extraction; CODE,

caper protein powder produced by ohmic heating-assisted extraction; CODE-EE, caper protein powder produced by ohmic heating-assisted enzyme extraction

their cytoplasm had shrunk, and globular protein structures—presumed to be removed—were no longer present. Figure 3c corresponds to the supernatant phase remaining after alkaline extraction. The image reveals that while the cytoplasm has drained, the integrity of the cell walls is maintained. In contrast, enzyme-assisted extraction, ohmic-assisted extraction, and ohmic-assisted enzyme extraction exhibit significant structural damage like broken cell walls and degradation in it (Fig. 3d, e, and f). This suggests that the protein molecules within the cell matrix are also removed during those extraction processes.

The determined total protein content of the samples was in agreement with those observations. In other words, extraction methods dependent on higher extraction yield were emphasized in SEM analysis, as well. Compared to the alkaline extraction method, other investigated ones revealed higher potential in terms of crude protein extraction from caper seeds. Previous studies on protein extraction using various techniques have reported that the disordered, irregular, and vacuolated structures observed in SEM images post-extraction are due to cellular damage caused by the extraction process. Moreover, a greater extent of damage and irregularity in the structures correlates with higher extraction efficiency (Görgüç et al., 2019; Ortega et al., 2024). In this context, Fig. 3f shows that the supernatant phase remaining after ohmic-assisted enzyme extraction exhibits the presence of more damaged and irregular structures. Additionally, the hollow, honeycomb-like voids observed post-extraction provide insight into the extent of extraction efficiency. When correlated with the total protein content and protein yield results presented in the results section, the data supports the notion that ohmic-assisted enzyme extraction is the most efficient method for protein extraction from defatted caper seeds. Evaluating the images of the supernatant phases after enzyme extraction and ohmic-assisted extraction indicates that these methods affect the

material structurally in distinct ways. Enzyme extraction creates more irregular and larger structures, while ohmic-assisted extraction results in more regular and smaller structures. Therefore, combining these two methods, which exhibit different effects on the material, is considered to enhance the efficiency of protein extraction from caper seeds.

SEM images of protein powders obtained from defatted caper seeds using different extraction methods are presented in Fig. 4a, b, c, and d. As shown in Fig. 4, the presence of globular protein structures is particularly pronounced in the powders obtained through enzyme-assisted extraction and ohmic-assisted enzyme extraction, indicating that these structures were transferred to the extraction medium and finally into protein powders (Fig. 4b and d). The globular structures observed in the protein powders obtained through ohmic-assisted extraction were less pronounced compared to the other two methods, as they almost disappeared in the alkaline extraction method (Fig. 4a and c). In this context, correlating the images with the content of the produced protein powders suggests that the higher amino acid content and nearly 99% total protein levels in the powders obtained by CODE-EE and CEE methods support the notion that the globular structures observed in the images could be proteins.

Techno-Functional Properties

Water Holding (WHC) and Oil Binding Capacity (OBC)

The expression of WHC and OBC is based on the amount of water or oil, respectively. Protein conformation, molecular weight, surface polarity/hydrophobicity, processing technique, and amino acid content all affect the WHC and

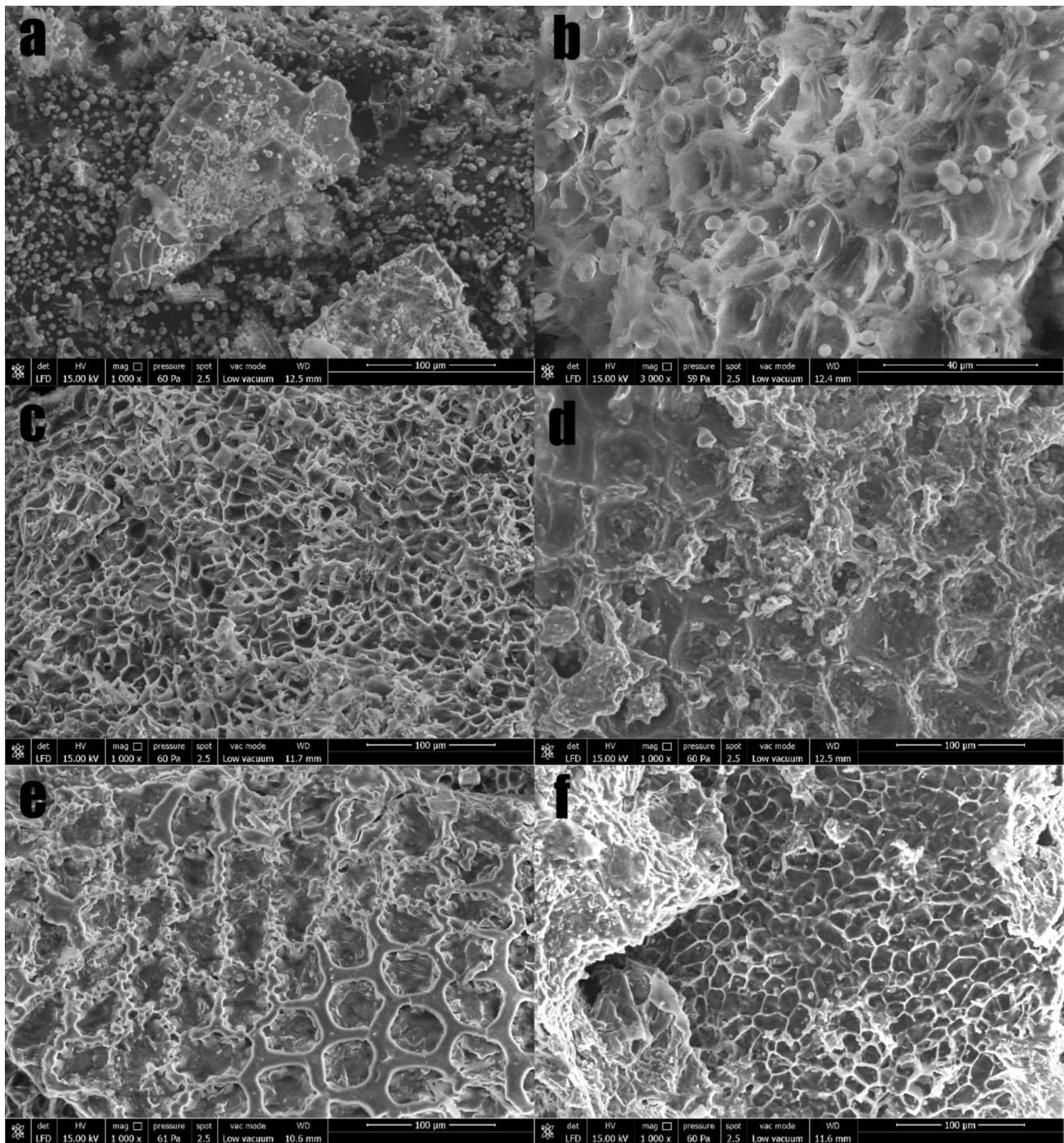


Fig. 3 The SEM images of defatted caper seed and the supernatant phases remaining after protein extraction from defatted caper seed. **a** ground defatted caper seed powder (1000 $\times$), **b** ground defatted caper seed powder (3000 $\times$), **c** supernatant phase remaining after alkaline extraction (pH: 11) (1000 $\times$), **d** supernatant phase remain-

ing after enzyme-assisted (Viscozyme® L (Novozymes, Bagsværd, Denmark)) extraction (1000 $\times$), **e** supernatant phase remaining after ohmic-assisted extraction (1000 $\times$), **f** supernatant phase remaining after ohmic-assisted enzyme extraction (1000 $\times$)

OBC values of protein powders (Hadidi et al., 2021). Proteins with low WHC may be more susceptible to moisture exposure during storage, whereas protein powders with high WHC can dehydrate other dietary ingredients. Therefore,

choosing proteins that have a high WHC is essential, particularly when a rapid rehydration rate is crucial (Haque et al., 2015). Another techno-functional property, the OBC values of protein powders, is important in the food formulation

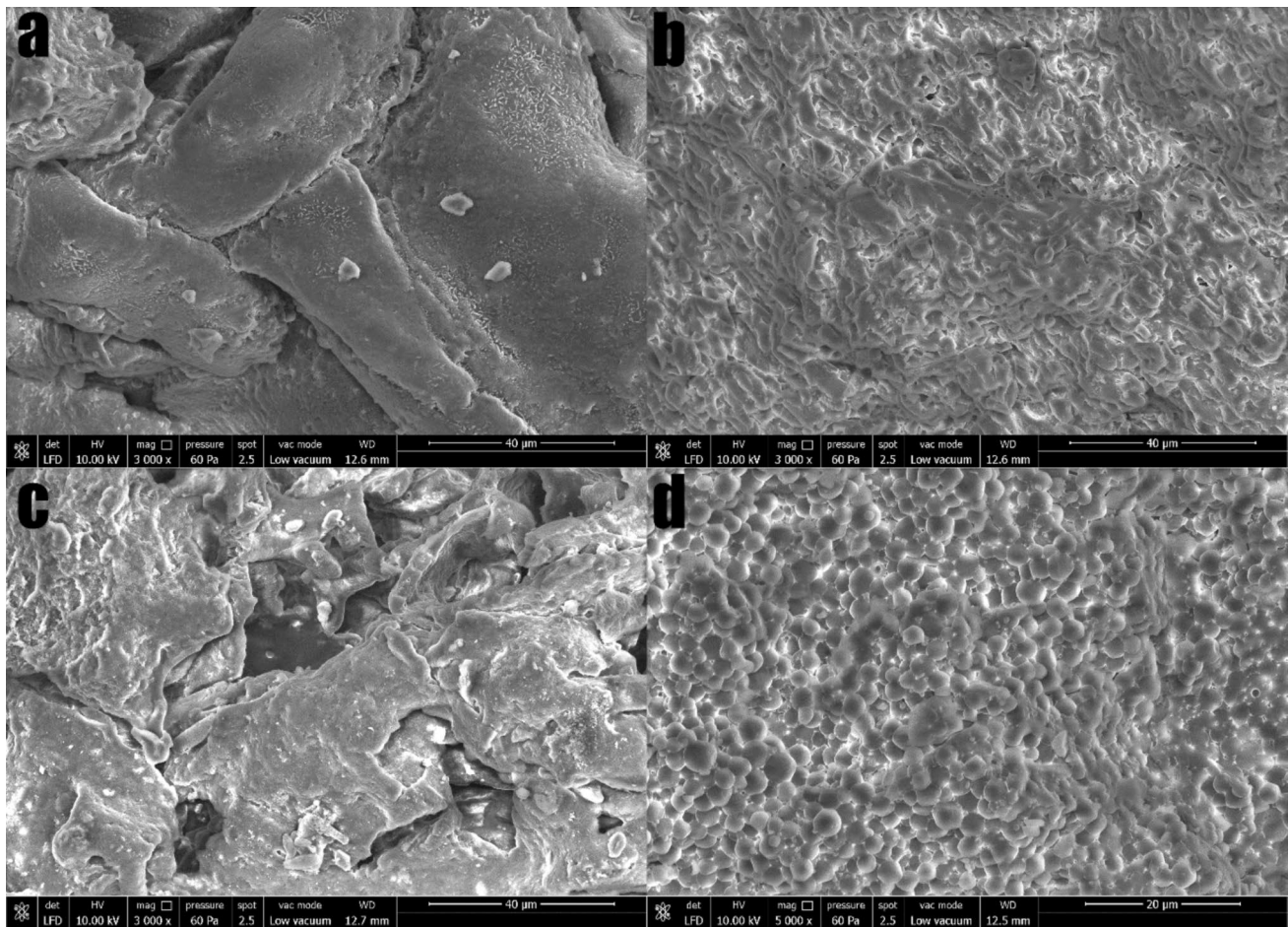


Fig. 4 SEM images of protein powders produced from defatted caper seeds. **a** protein powder produced by alkaline extraction (pH: 11) (3000×), **b** protein powder produced by enzyme-assisted (Viscozyme® L (Novozymes, Bagsværd, Denmark)) extraction (3000×),

c protein powder produced by ohmic-assisted extraction (3000×), **d** protein powder produced by ohmic-assisted enzyme extraction (5000×)

efforts like preparing sausages, cake batter, mayonnaise, and salad dressings, since these products need high-fat absorption capacity (Chen & Yang, 2023).

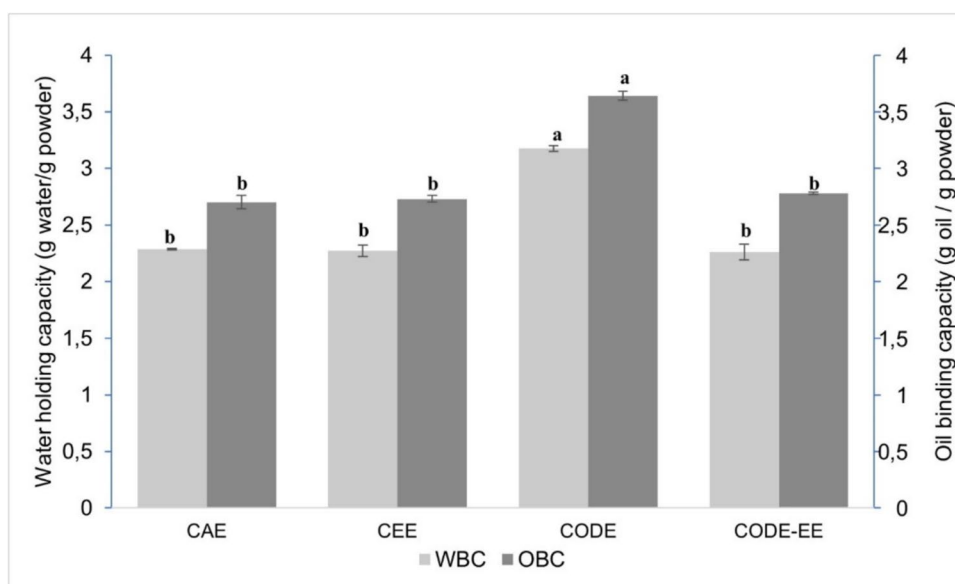
WHC and OBC values of protein powders extracted from defatted caper seed using different extraction techniques are given in Fig. 5. It was determined that the extraction method to extract protein powder from defatted caper seed had a statistically significant effect on WHC and OBC ($p \leq 0.05$). The highest WHC and OBC values were measured for the protein powder obtained by the CODE method, and these values were 3.18 ± 0.03 g water/g powder and $3.64 \text{ g} \pm 0.04$ oil/g powder, respectively, whereas the remaining methods all produced similar values ($p > 0.05$) for the corresponding techno-functional properties. The WHC and OBC results obtained in our study are consistent with trends reported for contemporary plant proteins. Proteins with higher OBC typically exhibit greater exposure of hydrophobic regions, which can enhance interactions with water and oil and support functional performance. Literature reports a positive

correlation between OBC and emulsifying and foaming properties of plant proteins (Kaczmarek et al., 2025). Furthermore, extraction methods significantly influence WHC and OBC by altering protein solubility, hydrophobicity, and molecular flexibility (De Angelis et al., 2024; Korkmaz & Tuncel, 2025). In our study, the CODE and CODE-EE methods exhibited relatively high WHC and OBC, suggesting that extraction-induced structural modifications modulate protein-water and protein-oil interactions.

Foaming Capacity (FC) and Foaming Stability (FS)

The FC and FS values of protein powders extracted from caper seed pulp using different methods are presented in Fig. 6. According to the literature, the foaming capacity and stability of protein powder are both influenced by several factors, including its composition, processing method, temperature, pH, solubility, concentration, mixing time, and the foaming technique (Ma et al., 2022). The results obtained in

Fig. 5 Water-holding and oil-binding capacity of caper protein powders obtained using different extraction methods. *CAE, caper protein powder produced by alkaline extraction; CEE, caper protein powder produced by enzyme extraction; CODE, caper protein powder produced by ohmic heating-assisted extraction; CODE-EE, caper protein powder produced by ohmic heating-assisted enzyme extraction. **Different letters are significantly various ($p \leq 0.05$) (error bars represent standard error).

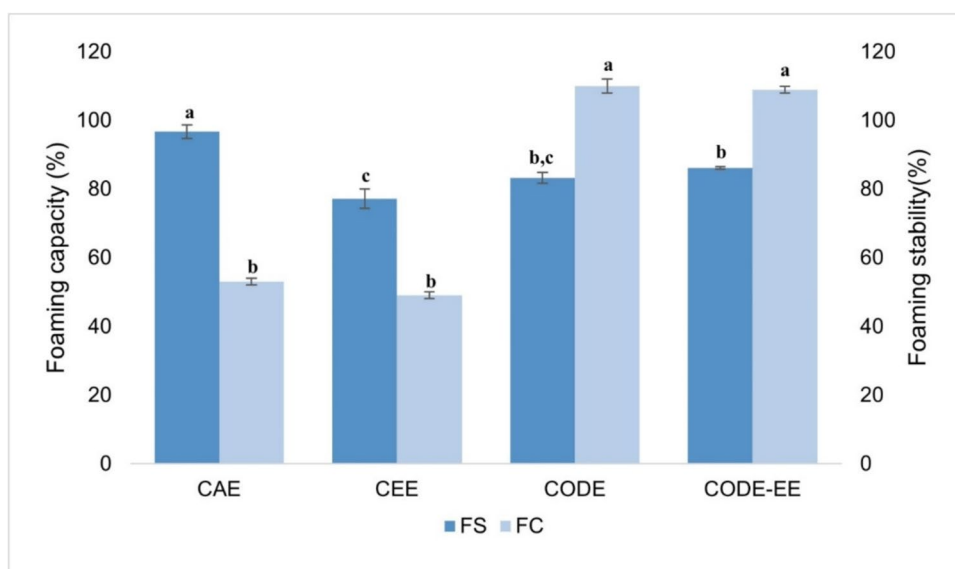


the current study are coincidence with this statement. The extraction method significantly impacted both FC and FS ($p \leq 0.05$). The best FC value was achieved by the extraction methods of CODE ($110.00\% \pm 2.00$) and CODE-EE ($109.00\% \pm 1.00$) for caper seed protein powders. On the other hand, the protein powder sample extracted by the CAE ($96.74\% \pm 1.94$) method showed the highest FS value. In different studies, foaming stability of protein samples is found to be varied to a wide extent. The highest foaming stability was observed for green lentil, pea, and kidney bean proteins, all of which exceeded 90% (Shevkani et al., 2015). Thus, the ohmic heating extraction method positively affected the FC of the protein powders, but this technique did not have the same impact on FS.

Emulsification Activity (EA) and Emulsifying Stability (ES)

Emulsion properties are accepted as key protein characteristics, as they are important for their usage in the food industry. Emulsifying ability and stability, examined to this extent, are two of them for the emulsified-product manufacturing (Görgüç et al., 2019). Figure 7a and b illustrate EA (at 340 nm) and ES (after 10 min) results of caper-seed protein powders. The used crude protein extraction method was seen to be also effective here ($p \leq 0.05$). EA largely relies on peptide diffusion at oil–water interfaces. Protein powders that are smaller and more soluble can enhance this diffusion and improve lipid-protein interactions (Onsaard, 2012). Protein denaturation (accelerated by ohmic-heating extraction) or

Fig. 6 Foaming capacity and foaming stability of caper protein powders obtained using different extraction methods. *CAE, caper protein powder produced by alkaline extraction; CEE, caper protein powder produced by enzyme extraction; CODE, caper protein powder produced by ohmic heating-assisted extraction; CODE-EE, caper protein powder produced by ohmic heating-assisted enzyme extraction. **Different letters are significantly various ($p \leq 0.05$) (error bars represent standard error)



highly soluble, small protein extracts from enzyme extraction are believed to boost emulsion activity by increasing protein binding at interfaces. Similarly, there are literature studies on the potential of protein denaturation to increase emulsion activity (Özdemir et al., 2022).

When the findings of caper seed protein powders were analyzed, it was seen that the protein powder produced by using the CODE-EE method had the highest EA, which was followed by the CODE method, whereas the lowest activity was measured for the sample produced by alkaline extraction (CAE) (Fig. 7). Parallel to EA, the highest value for ES was measured for the sample obtained by CODE-EE, whereas the emulsion stability was found to be weaker for the samples extracted by the methods of all other ones. Interfacial film formation and protein–protein interactions at the oil–water interface are also critical. Research on plant protein emulsifiers such as legume and kernel proteins has shown that while solubility and surface hydrophobicity affect migration to the interface, long-term stability depends on the formation of viscoelastic interfacial films that resist droplet coalescence (Eskandari et al., 2025). Likewise, reviews indicate that controlled unfolding and processing-induced conformational changes can enhance both emulsifying activity and stability by improving amphiphilicity and interfacial adsorption (Lima et al., 2023). Therefore, the superior emulsion stability observed for CODE-EE in our study aligns with the literature, highlighting that holistic functional performance, rather than binding capacity alone, determines emulsion outcomes.

In the literature, soy protein isolate (SPI)—a widely studied plant protein—is reported to have moderate emulsifying activity due to its globulin structure and solubility characteristics, which are often considered lower than those of some animal proteins without further modification (e.g.,

unmodified SPI generally has limited interfacial activity compared with more flexible proteins) (Zhao et al., 2022). In contrast, whey protein isolate (WPI) is well-established as a superior emulsifier because of its high solubility and rapid adsorption at oil–water interfaces, leading to stronger emulsifying capacity and emulsion stability in many food systems (Raj et al., 2026). Based on these established functional benchmarks, our results indicate that caper seed protein isolates exhibit emulsifying properties that are comparable to those of commercial SPI but generally inferior to WPI under similar conditions. This is consistent with literature reports that plant proteins often require structural modification or formulation adjustments to match the interfacial activity of whey proteins (Zhao et al., 2022). Therefore, while caper seed proteins show promising emulsifying behavior relative to SPI, they do not reach the high emulsification performance characteristic of WPI.

Protein Solubility

In food processing, solubility is an important parameter of the protein powder applications, as it directly affects the techno-functional properties of protein molecules. The determination of solubility for protein powder extracted from defatted caper seed was conducted over a pH range of 2–11. As illustrated in Fig. 8, the solubility of protein powder is significantly influenced by the ambient pH. Being like other published plant protein studies, the lowest solubility value was observed in the pH range of 4.0–6.0, which is corresponding to the isoelectric point (pI) of the caper proteins, where their net charge is zero and they are aggregated. The medium pH value of this range (pH 4–6) being more acidic or basic caused the solubility increase of caper protein powder (Grossmann & McClements, 2023). The highest

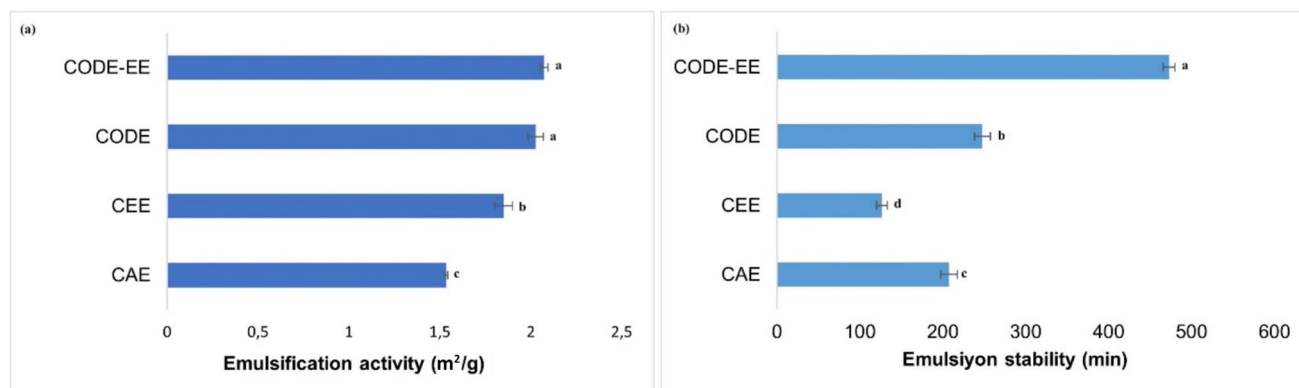


Fig. 7 Emulsification activity and emulsion stability of caper protein powders obtained using different extraction methods. **a** Emulsification activity (m^2/g) and **b** emulsion stability (min). *CAE, caper protein powder produced by alkaline extraction; CEE, caper protein powder produced by enzyme extraction; CODE, caper protein powder

produced by ohmic heating-assisted extraction; CODE-EE, caper protein powder produced by ohmic heating-assisted enzyme extraction. **Different letters are significantly various ($p \leq 0.05$) (error bars represent standard error)

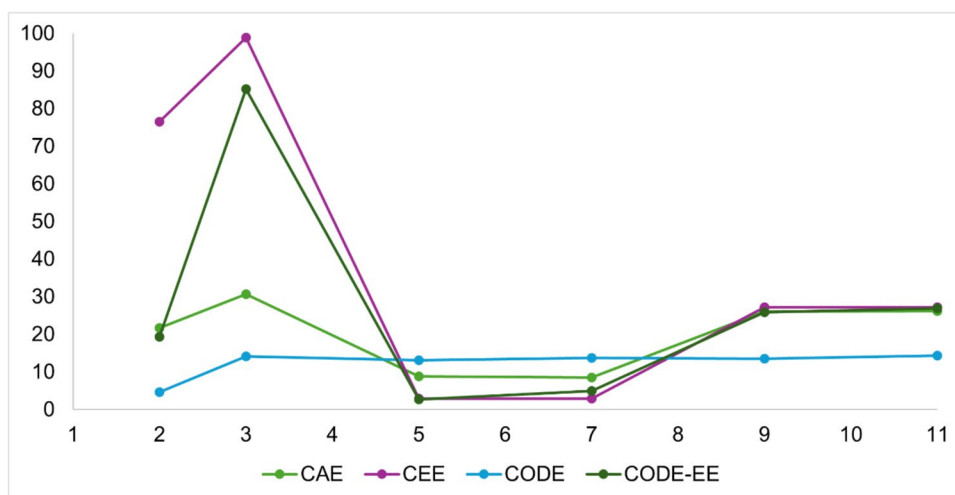
solubility was identified at the pH values of 3 and 11 for acidic and basic medium, respectively, irrespective of the methodology applied for the protein extraction from defatted caper seeds. The solubility curve that was obtained in the current study resembles the curve of proteins extracted from potatoes as reported by Grossmann and McClements (2023).

The extraction-method-dependent solubility change of defatted caper-seed protein powder may be associated with the effect of electric field applied during ohmic heating, since ohmic heating results showed that the solubility decreased for the sample produced by CODE-EE compared to CEE's result and its lowest value was observed for the powder sample extracted by only ohmic-heating application (CODE). In the PEF process, like ohmic heating, the electric current between electrodes may cause structural changes in biomolecules as they reorganize with the electric field. Studies show that external electric fields significantly impact the secondary and tertiary structures of protein molecules, which result in an increase or a decrease in their solubilities (Grossmann & McClements, 2023). In the current study, it was noted that ohmic heating slightly reduced the solubility of the protein powder. Among these studied methods, the protein powders obtained using the CEE method exhibited the highest solubility (98.81%), which was followed by those produced by the CODE-EE, CAE, and CODE methods, which had descending solubility values. The solubility results indicated that the CODE-EE method exhibited significantly lower solubility compared to the other extraction methods ($p < 0.05$). This reduction may be attributed to structural modifications and partial aggregation induced by the combined ohmic and enzymatic treatment, which likely enhanced hydrophobic interactions within the protein structure. In contrast, the CODE-EE process resulted in a significantly higher protein content and protein recovery compared to the CEE method ($p < 0.05$). Although protein recovery between the two approaches was broadly comparable in

practical terms and both methods were performed under the same extraction times, the CODE-EE method was preferred due to its potential advantages in process efficiency. In particular, ohmic heating enables rapid and homogeneous heat generation within the material, which may enhance energy efficiency and improve process control during extraction. Moreover, while the CEE method may be more suitable for formulations requiring high protein solubility, such as beverage systems, CODE-EE may represent a favorable alternative when higher protein yield, energy efficiency, and scalability are prioritized.

The solubility test results indicated that the enzyme-assisted extraction method produced a protein powder with high solubility in the pH range of 2.5–3.0, whereas a high pH value (pH = 11) was required for extracts obtained through non-enzymatic alkaline and ohmic-assisted extraction methods. The solubility characteristics of the extracts can be attributed to their extraction conditions, as extraction itself is a solving phenomenon. Enzyme-assisted extraction was conducted at a pH range of 2.5–6 to optimize enzyme activity, whereas alkaline and ohmic-assisted extractions were carried out at a pH of approximately 11. Consequently, the resulting protein powders exhibited similar pH characteristics conducive to better solubility. Additionally, the solubility of enzyme-assisted extracts was significantly higher than that of the other two methods. This can be attributed to the enzymatic process, which enhances the molecular flexibility of soluble peptides and induces structural changes, improving the solubility of plant proteins (pH 2.0–10.0) (Gao et al., 2023). Furthermore, the smaller particle size of proteins obtained through enzyme-assisted extraction may contribute to their higher solubility. These differences are evident in the SEM images of the obtained protein powders (Fig. 4). Furthermore, when correlating the amount of water-soluble protein with the crude protein powders obtained through CEE and CODE-EE extraction methods, a similar trend is

Fig. 8 The solubility of protein powders obtained by four different extraction methods at different pH values. *CAE, caper protein powder produced by alkaline extraction; CEE, caper protein powder produced by enzyme extraction; CODE, caper protein powder produced by ohmic heating-assisted extraction; CODE-EE, caper protein powder produced by ohmic heating-assisted enzyme extraction



observed, with the highest solubility achieved by protein powders produced using these two methods. Protein solubility is influenced by the amino acids present on the protein's surface, where non-polar amino acids tend to be hydrophobic, while polar amino acids are hydrophilic. Among the hydrophilic amino acids, Asp, Glu, and Tyr play key roles, with Asp and Glu having a particularly strong impact on solubility (Gao et al., 2023). Analysis of the amino acid profile of protein powders obtained via CODE-EE and CEE methods reveals that these solubility-enhancing amino acids are significantly more abundant compared to those obtained through the other two extraction methods.

Conclusion

In this study, proteins were extracted from defatted caper seeds using different protein extraction methods. For the first time, ohmic heating-assisted extraction and the ohmic heating-enzyme combination methods were used for plant protein extraction, proving more successful than traditional alkaline methods, and the study also revealed the amino acid profile of caper seeds. It was found that protein powders obtained by enzyme-assisted extraction and ohmic heating-assisted enzyme extraction had the highest protein content (90.20 ± 0.54 and 99.10 ± 0.09 , respectively). FT-IR, amino acid profile and SEM results confirmed the total protein content results. While soluble protein content was low in protein powders obtained by ohmic-assisted and alkali extraction, soluble protein content was high in protein powders obtained by enzyme-assisted extraction and ohmic heating-assisted enzyme extraction. Furthermore, the study highlighted that the choice of extraction method greatly influences protein functionality, with protein powders produced via ohmic heating-assisted enzyme extraction showing elevated techno-functional properties. Further research is needed on ohmic heating-assisted extraction combined with enzymes, and the results should be thoroughly examined.

Abbreviations CAE: Alkaline extraction; CEE: Enzyme-assisted extraction; CODE: Ohmic heating-assisted extraction; CODE-EE: Ohmic heating-assisted enzyme extraction; FBG: Fungal beta-glucanase; FT-IR,: Fourier transform infrared spectroscopy; SEM: Scanning electron microscope; SDS-PAGE: Sodium dodecyl sulfate–polyacrylamide gel electrophoresis

Authors Contributions Erkan Karacabey: Conceptualization, Methodology, Formal analysis, Investigation, Data Curation, Writing—Original Draft, Visualization, Supervision, Project administration, Funding acquisition. Ayşe Özçelik: Methodology, Investigation, Writing—Review & Editing. Muhammed Mustafa Özçelik: Investigation, Writing—Review & Editing. Bekir Gökçen Mazı: Methodology, Investigation, Writing—Review & Editing. Sebahattin Serhat Turgut: Methodology, Investigation, Writing—Review & Editing.

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Data Availability The data that supports the findings of this study are available on request from the corresponding author.

Declarations

Competing interests The authors declare no competing interests.

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