



# Sesame seed protein: Amino acid, functional, and physicochemical profiles

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## Abstract:

Sesame (*Sesamum indicum* L.) is an erect herbaceous annual plant with flat seeds. It is one of the oldest cultivated oilseed plants in the world, especially popular in Africa and Asia.

The present research objective was to describe a sesame protein isolate, i.e., its amino acid profile, functional and physicochemical properties, zeta potential, and hydrodynamic diameter. The surface charge and hydrodynamic diameter in aqueous solutions were obtained for standard sesame seeds, defatted sesame seeds, and the sesame protein isolate.

Defatted sesame seeds yielded the following optimal parameters: salt concentration – 0.6 M, pH – 7, iso-electric point (pI) – 4. The sesame protein isolate was rich in methionine content, which is rare in other plant proteins, but its lysine content was lower than in other isolates. The sesame protein isolate displayed almost identical zeta potential profiles with its pH. The decreasing pH increased the zeta values gradually from the lowest negative value to the highest positive value. The zeta potentials of standard and defatted sesame seeds at pH 7 were –23.53 and –17.30, respectively. The hydrodynamic diameter of the sesame protein isolate (0.33 µm) was smaller than that of sesame seeds (2.64 µm) and defatted sesame seeds (3.02 µm). The sesame protein isolate had a water holding capacity of 1.26 g/g and an oil holding capacity of 3.40 g/g. Its emulsifying properties looked as follows: emulsion capacity – 51.32%, emulsion stability – 49.50%, emulsion activity index – 12.86 m<sup>2</sup>/g, and emulsion stability index – 44.96 min, respectively. These values are suitable for the sesame protein isolate and are consistent with the literature.

The sesame protein isolate was a good source of protein (88.98%). Using sesame proteins as functional components can be an important basis for better knowledge of the relationship between electrical charge interactions in food matrices and the structure, stability, shelf life, texture, structural and functional properties of food. Research prospects include the effects of sesame protein isolates on various food systems.

**Keywords.** Sesame protein isolate, amino acid profile, zeta potential, functional properties, solubility

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## INTRODUCTION

Sesame (*Sesamum indicum* L.) is an erect herbaceous annual plant. Its seeds are considered one of the oldest oilseed plants in human history. For instance, peoples of Africa and Asia have cultivated them for many centuries. Nowadays, sesame seeds and oil are used in food production and different industries. Global sesame production was approximately 2.5 million tons in 1997 and 6.5 million tons in 2019 [1].

Sesame seeds are remarkably rich in oil and protein. They contain 45.46–59.28% oil, 21.43–25.77% protein,

2.70–5.10% moisture, 2.89–5.44% ash, 3.20–7.31% fiber, and 4.33–19.33% carbohydrates [2, 3]. The chemical composition of sesame seeds varies according to many factors, e.g., genetics, climate, environment, growth, maturation stage, harvest time, analytical methods, etc. [4].

Sesame proteins have a high potential to be used as an ingredient in the food industry because they increase the nutritional value of foods. One of the main properties of sesame proteins is that they contain such essential amino acids as tryptophan and methionine.

Sesame flour and protein isolates are rich in sulfur-containing amino acids (3.8–5.5%) with methionine prevailing (2.5–4.0%) [5–7]. Most plant proteins, e.g., soy protein, wheat flour, barley grain, ground rice, cornmeal, etc., contain low sulfur amino acids. Therefore, sesame proteins are unique because they contain high amounts of sulfur amino acids [4]. As a result, sesame proteins can serve as a complementary food to legumes and cereals. The polymeric structure of sesame proteins also improves the textural and rheological properties of the food they complement by increasing the acceptability of the final product [8].

Emulsifying capacity and emulsion stability are essential parameters in selecting a protein for particular industrial processes. Proteins prevent coalescence and reduce tension of the water-oil interface. The stabilizing effect of a protein in an emulsion comes from the membrane matrix that surrounds the oil droplet and prevents it from coalescing [5]. Proteins also have a good oil and water holding capacity, foaming stability, and solubility. The importance of these characteristics depends on the type of food product where the protein isolate is used. For example, high foaming and emulsifying protein isolates are preferred in salad dressings and soups.

In contrast, protein isolates with high water and fat holding capacity are preferred in products that contain meat fat [4]. Onsaard *et al.* reported that the emulsifying properties and solubility of sesame protein isolates can be used to replace soy protein isolates [9]. Achouri *et al.* found that sesame protein isolates had lower water retention and oil holding capacities than soy protein isolates [3]. They also proved that the foaming and emulsifying properties of sesame protein isolates are pH-dependent.

Sesame protein isolates or concentrates are easy to obtain by the isoelectric precipitation method. Due to its different functional properties and easy extraction procedures, sesame protein has become a popular commercial protein with a wide range of applications. Sesame protein isolates have a good heat stability and high molecular weight, which makes them suitable for film-forming utilization [10]. However, very few publications feature the zeta potential of sesame protein isolates and the average particle size (hydrodynamic diameter) in an aqueous solution. Most of them focus on the zeta potential and hydrodynamic size measurement of emulsions produced from sesame protein isolates [11, 12].

The industrial role of sesame seeds as a potential source of protein is growing. As a result, composition, physicochemical profile, and functional properties of sesame-isolated proteins are attracting more and more scientific attention. For proper industrial use, isolated proteins need to have a proper effect on specific processed foods. Therefore, food science needs more data on utilization of a specific protein [6]. For instance, very little information is available about how extraction

conditions, e.g., salt concentration and pH, affect the extractability of proteins from sesame seeds.

Therefore, the main objectives of this study were as follows: (I) to investigate the extractability of proteins from sesame seeds in water and at different NaCl concentrations (0, 0.3, 0.6, 0.9, and 1.2 M) as a function of pH; (II) to describe the sesame protein isolate and to examine some of its functional properties; (III) to investigate the zeta potential and particle size of the sesame protein isolate in aqueous solution, (IV) to examine the amino acid profile of the sesame protein isolate.

## STUDY OBJECTS AND METHODS

**Materials and chemicals.** The sesame (*Sesamum indicum* L.) seeds were supplied by Pro Gıda, Olam Group Co (Samsun, Turkey). The corn oil was purchased from a local market (Samsun, Turkey). The diethyl ether and sodium hydroxide (NaOH) were obtained from Isolab (Wertheim, Germany). All other chemicals and standards were obtained from Merck (Darmstadt, Germany) and Sigma-Aldrich (USA). A Milli-Q water purification system (Millipore, Massachusetts, USA) was used to obtain deionized water.

**Optimal pH, NaCl concentration, and iso-electric point for protein extractability.** Sesame seeds were milled in a kitchen blender (AR1133, Arzum, Turkey) and defatted by constant stirring with diethyl ether (1:4 w/v, sesame/solvent) at room temperature for 3 h. The resulting mix was defatted four more times using the same sesame/solvent proportion. After that, the defatted sesame was filtered using filter paper and dried at 40°C overnight [9]. After drying, the defatted sesame seeds were used for protein extraction.

In order to find the optimal conditions for protein isolation, we determined the optimal salt concentration and pH by modifying the method described by Achouri *et al.* in [3]. Dispersions of defatted sesame seeds (10% w/v) were prepared using different concentrations of sodium chloride (0, 0.3, 0.6, 0.9, and 1.2 M) and shaken at 300 rpm at 35°C for 2 h. The dispersions were divided into test tubes by approximately 10 mL. There, their pH was adjusted from 2 to 11 (in 1 unit increments) using 1N HCl or 1N NaOH. The dispersions were continuously stirred at room temperature for 2 h, and their pH was kept at the desired level (2–11). The samples were sonicated for 30 min and centrifuged at 2060×g for 30 min (NF 1200R, Nuve, Ankara, Turkey).

Then, 0.1 mL was taken from the supernatant and added to 3 mL of Bradford's reagent. After 5 min, the absorbance of the mix was registered using a T80+ spectrophotometer (PG Instruments Limited, Leicestershire, UK) at 595 nm wavelength. The pH and salt concentration of the samples with the highest protein content were chosen as optimal conditions to maximize protein isolation.

The iso-electric point (pI) precipitation method was used for protein isolation. The most appropriate pI was determined by the method described by Yuzer *et al.* in [13]. First, a 10% sample dispersion was prepared with the optimal concentration of NaCl solution and adjusted to the optimal pH value for extraction. The dispersion was shaken at 300 rpm for 2 h. After shaking, 10 mL of the liquid part from each sample was poured into six centrifuge tubes. Their pH values were adjusted between 3.5–6.0 with a 0.5-unit change and vortexed for 1 min in a WN-2800 Vortex Mixer (Weightlab Instruments, Istanbul, Turkey). The samples were centrifuged at 3000×g 4°C for 20 min. The protein amount in the supernatants was determined using the Bradford method. The pH value with the lowest protein amount was determined as the iso-electric point of the sample.

**Preparation of the sesame protein isolate.** The optimal salt concentration and pH values were determined as 0.6 and 7 M, respectively (Fig. 1), to produce a 1:10 (w/v) sample suspension. The suspension was stirred at 35°C at 300 rpm for 2 h. The supernatant portion containing the soluble proteins was obtained by filtration. In order to increase the protein amount and yield, we mixed the remaining solid using the same salt solution and pH and removed the supernatant by filtration.

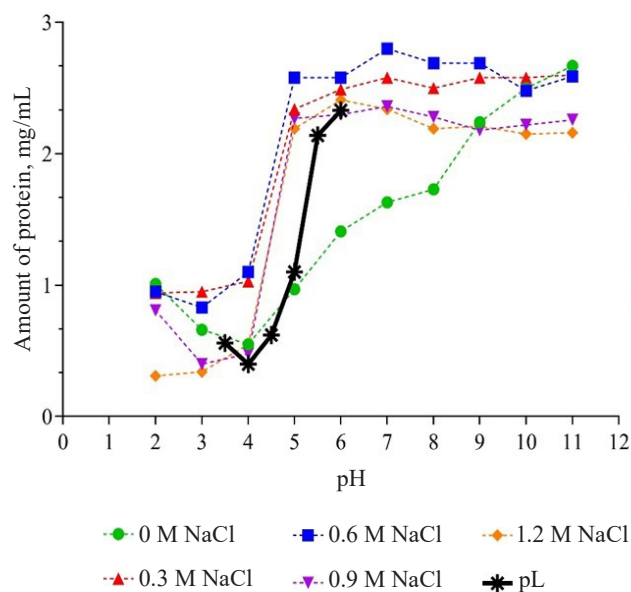
Afterward, the pH values of the samples were adjusted to the isoelectric point (pH 4, Fig. 1). The proteins were allowed to precipitate and kept at +4°C for 18 h. At the end of the period, the supernatant was removed by centrifugation at 3000×g at 4°C for 30 min to recover the precipitated proteins. The resulting precipitates were washed with distilled water. The pH was then neutralized to 7. The protein isolates were dried with a lyophilizer at –105°C and 100 mbar (ScanVac Coolsafe 110-4 Pro, Labogene, Lyngø, Denmark). The resulting sesame protein isolate was stored at –20°C. The protein yield, %, was calculated as follows [3]:

$$\text{Protein yield} = \frac{w_{\text{SPI}} \times \text{PA}_{\text{SPI}}}{w_{\text{DSS}} \times \text{PA}_{\text{DSS}}} \times 100 \quad (1)$$

where SPI is the sesame protein isolate; DSS is the defatted sesame seeds; *w* is the weight, g; PA is the protein amount, %.

**Proximate composition and pH analysis.** Standard sesame seeds, defatted sesame seeds, and sesame protein isolates were tested for moisture, crude protein (N×6.25), ash, and crude fat according to the AOAC methods [14]. The pH values were determined by a Starter 2100 pH-meter (OHAUS, USA) as follows: 10% (w/v) dispersions of each sample were tested in triplicates.

**Zeta potential and hydrodynamic size.** The hydrodynamic size (μm) of the samples was determined using a Malvern Zetasizer Nano Zetasizer (Malvern Instruments, Malvern, UK). The device employed the dynamic light scattering principle with refractive indices 1.330 and 1.476 for water and sesame,



**Figure 1** Optimal isoelectric point, salt concentrations, and pH values in sesame protein isolation

respectively. The zeta potential (mV) was measured with the same equipment based on the electrophoretic action of protein solutions. We prepared 0.05% (w/v) protein solutions of the sesame protein isolate with distilled water. Their pH was adjusted from 2 to 11 with 1N HCl or 1N NaOH. For standard sesame seeds and defatted sesame seeds, the sample dispersions contained 0.1% (w/v) deionized water. Their pH was adjusted to 7 with 1N HCl or 1N NaOH. Each sample was homogenized and measured in triplicates.

**Amino acid analysis.** The amino acid analysis of the sesame protein isolate relied on a liquid chromatography tandem mass spectrometry (LC-MS/MS). The Agilent Infinity 1260 HPLC system consisted of a binary pump, a degasser, and an autosampler coupled with an Agilent 6460 Triple Quad Mass Spectrometer (Agilent Technologies, CA, USA) using the method described by Bilgin *et al.* in [15]. We used a Jasem LC-MS/MS amino acid analysis kit (Sem Laboratories Devices Pazarlama San. ve Tic. Inc., Istanbul, Turkey) with a modified sample preparation procedure to measure amino acid concentrations.

The samples were hydrolyzed as follows. We placed 0.1 g of sample in a screw-cap glass tube, added 4 mL of acidic hydrolysis reagent, and hydrolyzed it at 110°C for 24 h. When the hydrolysate was cooled to room temperature, it was centrifuged at 4000 rpm for 5 min. Then, 100 μL of supernatant was poured into a vial with 900 μL of distilled water. This dilution process was repeated once more to obtain a 4000-fold diluted hydrolysate of the sample. Following the hydrolysis process, the kit sample preparation was applied as follows. We poured 50 μL of diluted hydrolysate into a vial and added 50 μL of the stable isotope-labeled internal standard mix and 700 μL of reagent 1. The mix was vortexed for 5 s. After all the samples underwent

the same procedure, they were injected into the LC-MS/MS system. The calibration curve required for quantifying amino acids was obtained by preparing the five-point calibration set in accordance with the kit sample preparation without hydrolysis and reading in the LC-MS/MS system.

We injected 3  $\mu$ L of the prepared sample into the Jasem amino acid analytical column set at 30°C in HPLC. The chromatographic separation analysis took 7.5 min with mobile phase A and B gradient programmed for 0.7 mL/min. The tandem electrospray ionization mass spectrometer performed mass spectrometric detection in the positive ionization mode. The mass detector parameters were as follows: gas temperature – 150°C, gas flow – 10 L/min, capillary voltage – +2000 V, and nebulizer pressure – 40 psi.

**Scanning electron microscopy (SEM).** The surface morphology of the sesame protein was determined by a scanning electron microscope (SEM, JSM-700 LF JEOL, Japan) at a voltage acceleration of 10 kV. In order to increase the electrical conductivity of the samples and obtain a clearer image, the surface of the samples was coated with a 10-nm gold-palladium alloy (Quorum SC7620, UK) before measurement. The scanning electron microscopy images had three different magnifications: 5000 $\times$ , 10 000 $\times$ , and 30 000 $\times$ .

**X-ray diffraction (XRD).** The X-ray diffraction analysis (Rigaku Smartlab, Germany) was performed to investigate the crystallinity of the samples. The analysis conditions were as follows: room temperature,  $2\theta$  from 5 to 40°, scanning rate – 1°/min, step size – 0.01°, power – 40 kV, Cu K $\alpha$  radiation – 30 mA [11].

**Fourier transform infrared spectroscopy (FTIR).** Fourier transformed infrared spectroscopy determined the functional groups of the samples and their interactions. The secondary structure of sesame protein isolates was determined using a Platinum ATR equipped with a FTIR Tensor 27 spectrophotometer (Bruker Optics Inc., USA). The measurements were made at the wavelength range between 400 and 4000  $\text{cm}^{-1}$ .

**Differential scanning calorimetry (DSC).** The thermal properties of the samples were studied by differential scanning calorimetry (DSCQ2000, TA Instruments, USA). Approximately 10 mg of each sample was hermetically sealed in aluminum containers. The samples were then analyzed under the following conditions: nitrogen atmosphere, flow rate – 30 mL/min, heating rate – 10°C/min between 25 and 400°C [16]. An empty aluminum pan served as a reference. The initial temperature ( $T_0$ ), peak temperature ( $T_p$ ), and reaction enthalpy ( $\Delta H$ ) of the samples were determined using Universal Analysis 2000, version 4.5A software (TA Instruments, USA).

**Thermogravimetric analysis (TGA).** The thermal stability of the samples was evaluated using thermogravimetry (Q600 SDT, TA Instruments, USA). Approximately 10 mg of each sample in platinum cups was studied under the following conditions: nitrogen atmosphere, heating rate – 10°C/min between 30 and

600°C, flow rate – 50 mL/min. An empty platinum capsule served as a reference [17]. The results were evaluated using Universal Analysis 2000 software 4.5A (TA Instruments, USA).

**Solubility.** Solubility percentage was determined as a function of pH (2–11). We prepared 0.5% (w/v) protein dispersions of the sesame protein isolate with deionized water. The pH of the dispersions was adjusted 2 to 11 using 1N HCl or 1N NaOH. The suspensions were stirred in a magnetic stirrer for 1 h at ambient temperature. Then, the suspensions were centrifuged at 3000 $\times g$  at room temperature for 30 min [18]. After centrifugation, we performed the protein analysis in the supernatant using the Bradford method. The bovine serum albumin standard curve was used to calculate the amount of protein. The solubility, %, was found according to Eq. (2):

$$\text{Solubility} = \frac{\text{Protein content in supernatant}}{\text{Total protein content in sample}} \times 100 \quad (2)$$

**Water and oil holding capacities.** Approximately 50 mg of the protein isolate was dispersed in a 2-mL centrifuge tube by adding 1.5 mL of distilled water or commercial corn oil and mixed with a vortex at room temperature for 20 s. After mixing, the tubes were capped and held at room temperature for 30 min. The tubes were centrifuged at 14 000 rpm for 20 min. After the supernatant was carefully decanted at a 45° angle, we weighed the centrifuge tube with the sediment. The water or oil retained content was determined by weighing the tubes with the sediment. The water (WHC) and oil (OHC) holding capacities were expressed as 1 g of water or oil absorbed per 1 g of the sesame protein isolate, respectively. They were found according to Eq. (3):

$$\text{WHC or OHC} = \frac{M_2 - M_1}{M_0} \quad (3)$$

where  $M_0$  is the weight of the sample;  $M_1$  is the weight of the centrifuge tube;  $M_2$  is the weight of the centrifuge tube after the oil or water has been removed [18].

**Emulsifying properties.** We diluted 0.25 g of the protein isolate with 5 mL of distilled water. The pH value was adjusted to 7.0 using 1N HCl or 1N NaOH. After that, we added 5 mL of corn oil to the protein solution and mixed using a vortex for 1 min. Afterward, the emulsion was centrifuged at 1100 $\times g$  for 5 min, and the emulsion capacity (EC, %) was determined as in Eq. 4. To define the emulsion stability, the samples were heated in a water bath at 80°C for 30 min and cooled rapidly under running water and ice. The samples were again centrifuged at 2291 $\times g$  for 5 min, and emulsion stability (ES, %) was calculated as in Eq. (5) [19]:

$$\text{EC} = \frac{\text{Height of emulsified layer in the tube}}{\text{Height of the total contents in the tube}} \times 100 \quad (4)$$



**Table 1** Proximate composition, pH, zeta potential, and particle size of standard sesame seeds, defatted sesame seeds, protein isolates, and protein extraction yield (n = 3)

Sample	Moisture, %	Protein, %	Crude fat, %	Ash, %	pH	Zeta potential, mV	Particle size, µm	Protein extraction yield, %
Sesame seeds	2.68 ± 0.01	25.55 ± 0.14	56.83 ± 0.45	2.96 ± 0.02	6.41 ± 0.03	-23.53 ± 0.45	2.64 ± 0.49	–
Defatted sesame seeds	2.14 ± 0.22	57.37 ± 0.23	6.64 ± 0.18	5.94 ± 0.25	6.23 ± 0.01	-17.30 ± 1.28	3.02 ± 0.98	–
Protein isolate	1.26 ± 0.06	88.98 ± 0.13	0.82 ± 0.25	–	6.98 ± 0.19	-14.20 ± 1.01	0.33 ± 0.05	43.76

Data represent as mean ± SD. Means with different letters in the same column are significantly different ( $p < 0.05$ )

$$ES = \frac{\text{Height of emulsified layer after heating}}{\text{Height of the emulsified layer before heating}} \times 100 \quad (5)$$

The emulsion activity index and emulsion stability index were determined according to the method developed by You *et al.* [20]. We mixed 300 mg of the protein isolate sample with 30 mL of purified water (1% protein dispersion) and then added 10 mL of corn oil. The pH of the mix was adjusted to 7 with 1N HCl or 1N NaOH. The mix went through a homogenizer (Ultra-Turrax T25 digital, IKA, Staufen, Germany) at 20 000 rpm for 1 min. Immediately after the emulsion was formed, 50 µL of emulsion sample from its lower part (liquid phase) was put into a tube and diluted by adding 5 mL of a 0.1% (w/v) sodium dodecyl sulfate (SDS) solution. The absorbance of the resulting mix at 500 nm was measured in the spectrophotometer. The emulsion activity index (EAI, m<sup>2</sup>/g) was calculated as in Eq. (6):

$$EAI = \frac{2 \times 2.303 \times A_0 \times N}{c \times \phi \times 10000} \quad (6)$$

where,  $A_0$  is the absorbance at 0 min;  $N$  is the dilution factor (100);  $c$  is the concentration of the protein dispersion (0.01 g/mL);  $\phi$  is the volumetric fraction of the oil (10/40 = 0.25).

After 10 min, 50 µL of the emulsion was taken from its lower part (liquid phase) and diluted with 5 mL of a 0.1% (w/v) sodium dodecyl sulfate solution. The absorbance registered at 500 nm was used in Eq. (7) to calculate the emulsion stability index (ESI, min):

$$ESI = \frac{A_0 \times t}{A_0 - A_{10}} \quad (7)$$

where,  $A_{10}$  is the absorbance at 10 min after the homogenization process;  $t$  is the holding time of the emulsion (10 min).

**Foaming properties.** We diluted 3% (w/v) dispersions of the sample with distilled water and adjusted to pH 7 using 1 N HCl or 1 N NaOH. Then the mix was homogenized at 11 000 rpm for 2 min using an Ultra-Turrax homogenizer. The mix was immediately transferred to a 100-mL graduated cylinder. We recorded the total volumes, liquid volumes, and volumes of the remaining foams after 10 and 30 min of storage at

room temperature. The foaming capacity (FC, %) and foaming stability (FS, %) were calculated using Eqs. (8) and (9) [18]:

$$FC = \frac{V_1 - V_0}{V_0} \times 100 \quad (8)$$

$$FS = \frac{V_2}{V_1} \times 100 \quad (9)$$

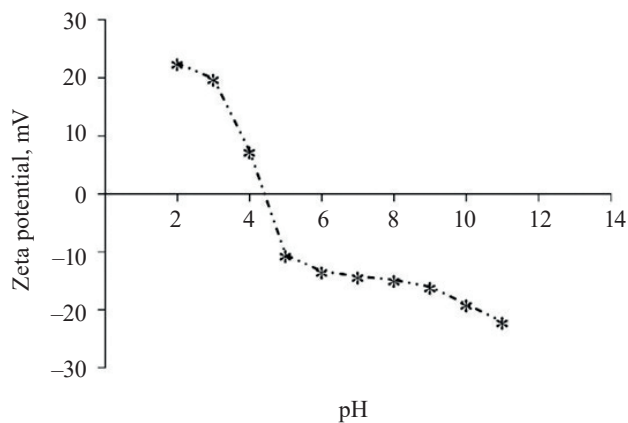
where  $V_1$  is the volume of foam instantly after homogenization;  $V_0$  is the volume before homogenization;  $V_2$  is the volume of foam remaining after 10 and 30 min at room temperature.

**Statistical analysis.** We used SPSS Statistics 26.0 (IBM, New York, USA) to evaluate the data and Duncan’s multiple comparison test to evaluate significant ( $p < 0.05$ ) differences.

## RESULTS AND DISCUSSION

**Proximate composition and pH analysis.** Table 1 illustrates the proximate composition of standard sesame (*Sesamum indicum* L.) seeds, defatted sesame seeds, sesame protein isolate, and protein extraction yield. Defatted sesame seeds had a greater content of ash and protein. The protein content rose from 25.55% in the standard sesame seeds to 57.37% in the defatted sesame seeds. Probably, the ash content increased as a result of the proportional decrease in the amount of crude fat. The crude fat content of the sesame seeds decreased because it was affected, first, by the solvent during the defatting process and, second, by the further separation of oil during centrifugation. As a result, the crude fat content of the standard sesame seeds, defatted sesame seeds, and sesame protein isolate were 56.83, 6.64, and 0.82%, respectively. The moisture content of the samples varied between 1.26 and 2.68%. These results confirmed those reported in [2, 3, 21]. The protein content of the sesame protein isolate was 88.98%. It was slightly lower than 90.50% achieved by Sharma *et al.*, but higher than 86.33% obtained by Fathi *et al.* and 81.66% obtained by Saatchi *et al.* [6, 10, 12]. The protein extraction yield was determined as 43.76% according to the extraction conditions used in this study.

The pH values of the standard sesame seeds, defatted sesame seeds, and sesame protein isolates were 6.41, 6.23, and 6.98, respectively (Table 1). An insignificant change in pH occurred between standard and defatted



**Figure 2** Effect of pH on the zeta potential of the sesame protein isolate

sesame seeds ( $p > 0.05$ ), followed by an increase in the sesame protein isolates as a result of the neutralization in the last stages of isolate production. Unfortunately, very few publications are available on the pH of sesame and its products.

**Zeta potential and hydrodynamic size.** Food systems include electrically charged particles that interact with the environment and with each other. Electric charge interactions significantly affect food structure, stability, rheological behavior, precipitation, texture, color, shelf life, and flavor. The zeta potential ( $\zeta$ ) is one of the most practical characteristics in investigating electrical relations in food matrices. Functional characteristics of proteins are affected by their foaming and emulsifying capacity, solubility, gelation, surface activity, conformational stability, and interaction with polysaccharides. The zeta potential is important for determining the aggregation and interactions of compounds [22].

Figure 2 shows the zeta potential of the sesame protein isolates at pH 2–11. The aqueous solution of the sesame protein isolate had a neutral charge at pH 4–5. This range included the iso-electric point pH 4 determined for precipitation during the isolation process. The sesame protein isolate displayed almost identical zeta potential profiles with its pH. As the pH increased from 2 to 11, the zeta values gradually changed from the highest (positive) value to the lowest (negative) value. This result was consistent with the fact that the surface charge can change gradually from negative to positive due to the gradual protonation of carboxyl groups and deprotonation of amino groups of proteins [23]. The zeta potential of standard and defatted sesame seeds at pH 7 were  $-23.53$  and  $-17.30$  mV, respectively (Table 1). We found no previous publications on the surface charge in aqueous solutions of standard and defatted sesame seeds, which makes this research relevant. The obtained data revealed how electrical charge interactions in sesame seeds and sesame-containing food components may affect the structure, stability, shelf life, and texture of the finished product.

**Table 2** Amino acid composition of the sesame protein isolates

Amino acid	Amount, g/100 g
Essential amino acids	
Phenylalanine	$4.47 \pm 0.00$
Isoleucine	$2.32 \pm 0.01$
Leucine	$5.68 \pm 0.00$
Lysine	$2.82 \pm 0.01$
Methionine	$2.78 \pm 0.00$
Threonine	$3.45 \pm 0.00$
Valine	$3.52 \pm 0.00$
Histidine	$2.51 \pm 0.01$
Non-essential amino acids	
Arginine	$11.86 \pm 0.01$
Alanine	$4.33 \pm 0.00$
Aspartic acid	$6.92 \pm 0.00$
Cysteine	$1.57 \pm 0.00$
Glutamic acid	$17.78 \pm 0.02$
Glycine	$2.26 \pm 0.00$
Proline	$3.55 \pm 0.00$
Serine	$4.21 \pm 0.00$
Tyrosine	$2.90 \pm 0.00$
Total non-essential amino acids	55.38
Total essential amino acids	27.55
Total acidic amino acids <sup>a</sup>	24.70
Total basic amino acids <sup>b</sup>	17.19
Total sulfur amino acids <sup>c</sup>	4.35

Data represent a mean  $\pm$  SD (n = 2)

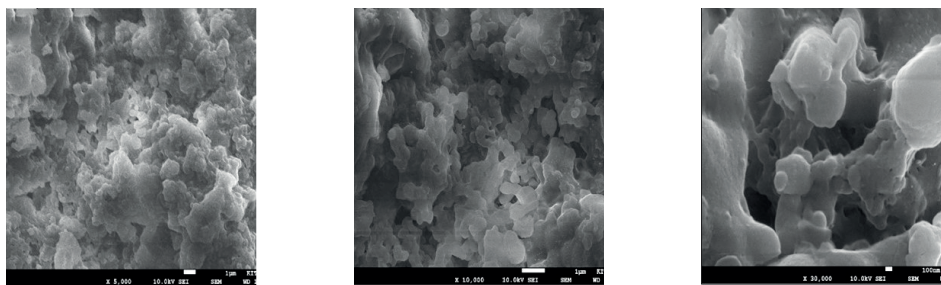
<sup>a</sup> Acidic amino acids: aspartic acid and glutamic acid

<sup>b</sup> Basic amino acids: histidine, lysine, and arginine

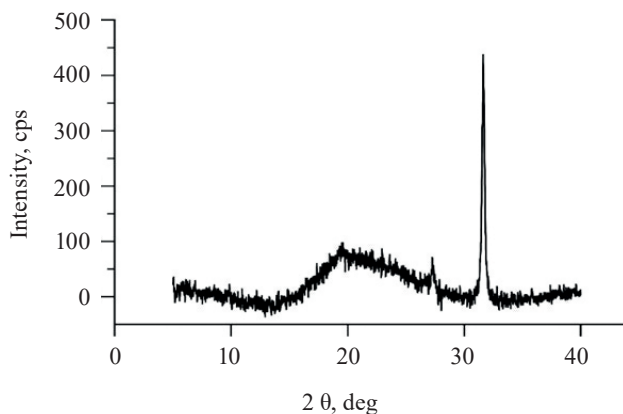
<sup>c</sup> Sulfur amino acids: methionine and cysteine

Table 1 illustrates the hydrodynamic dimension measurements for the standard sesame seeds, defatted sesame seeds, and sesame protein isolates. The functionality and emulsifying properties of proteins depend on their molecular size [24]. The hydrodynamic diameter of the sesame protein isolates ( $0.33 \mu\text{m}$ ) was smaller than that of standard sesame seeds ( $2.64 \mu\text{m}$ ) and defatted sesame seeds ( $3.02 \mu\text{m}$ ). Ordinary and defatted sesame seeds may have formed larger aggregates through strong hydrophobic interactions in water. These values were similar to those reported in previous scientific publications. For instance, Rahmati *et al.* determined the hydrodynamic dimensions between  $100$ – $200$  nm at pH 7 in bean protein isolates [24]. Mozafarpour *et al.* reported the hydrodynamic radius of soy protein isolates as  $0.32$ – $0.68 \mu\text{m}$  [25].

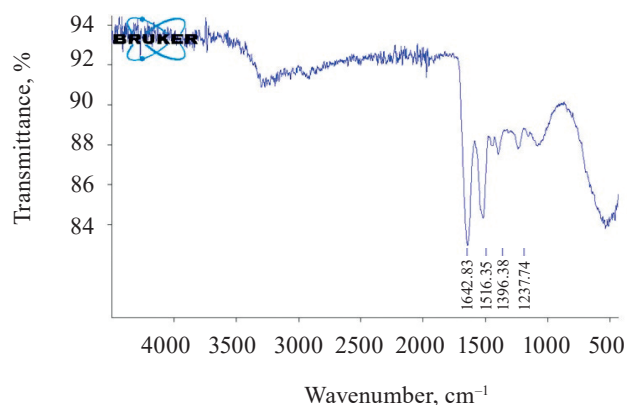
**Amino acid composition.** Table 2 shows the amino acid composition of the sesame protein isolates. Glutamic acid, arginine, and aspartic acid were  $17.78$ ,  $11.86$ , and  $6.92$  g/100 g, respectively. They proved to be the predominant amino acids in the isolate, which confirmed other sesame protein studies [11, 26–29]. Leucine ( $5.68$  g/100 g) appeared to be the most abundant essential amino acid, which approximated the results reported by Yang *et al.* and Lawal *et al.* [27, 28]. The



**Figure 3** Scanning electron microscopy of the protein isolate: (a) 5000× magnification; (b) 10 000× magnification; (c) 30 000× magnification



**Figure 4** X-ray diffraction pattern of the sesame protein isolate



**Figure 5** Fourier transform infrared spectroscopy of the sesame protein isolate

sesame protein isolates also contained sulfurous amino acids, primarily methionine, which are rarely found in other plant proteins. However, methionine was more abundant than cysteine, which was also observed by Saatchi *et al.* and Fasuan *et al.* [11, 26].

The sesame protein isolates owed their acidic quality to their high acidic/basic amino acid ratio. The fact that the iso-electric point of the protein is in the acidic pH region can be attributed to the high amount of acidic amino acids, e.g., glutamic and aspartic acids, in its content (Figs. 2 and 5).

The sesame protein isolate was richer in such essential amino acids as phenylalanine, methionine, threonine, valine, and histidine than hemp, soy, and pea proteins reported by Gorissen *et al.* [30]. They also had more leucine and isoleucine than hemp and soy proteins but less than pea protein. However, the sesame protein isolates were insufficient in lysine compared to soy and pea proteins.

**Scanning electron microscopy.** The structural morphology of the sesame protein isolate was examined with the aid of scanning electron microscopy at three different magnifications (Fig. 3). The particles of the sesame protein isolate powder were irregular in shape and not uniform. The surface was wrinkled, and the particles were agglomerated. Saini *et al.* also reported that the alkali extraction and iso-electric precipitation technique applied to sesame protein isolates transformed

the microstructure of the protein into a compact structure with a wrinkled surface [7].

**X-ray diffraction (XRD).** Figure 4 shows the crystal structure of the sesame protein isolate obtained by X-ray diffraction. The image revealed a broad and diffuse background at 19.5°, which means that the major structure of the isolate was amorphous. Other sharp peaks ( $2\theta = 27.3^\circ$  and  $31.6^\circ$ ) could be attributed to minerals or other compounds remaining after protein isolation. These results were consistent with those reported in previous publications [7, 11, 31, 32].

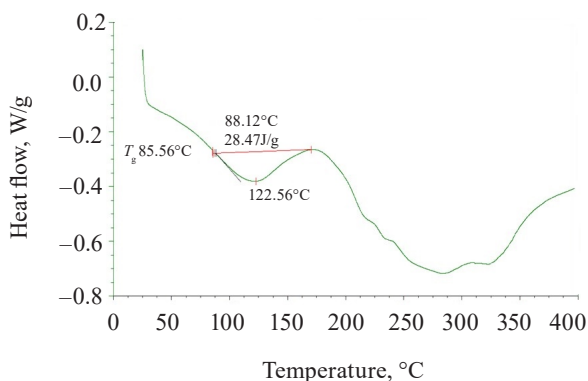
**Fourier transform infrared spectroscopy (FTIR).** Figure 5 shows the spectra demonstrated by the Fourier transform infrared spectroscopy. The obtained spectra made it possible to determine the functional groups. The sesame protein isolate showed some typical features of the protein spectrum, namely Amide I ( $1642\text{ cm}^{-1}$ ), Amide II ( $1516\text{ cm}^{-1}$ ), and Amide III ( $1238$  and  $1396\text{ cm}^{-1}$ ) that result from the stretching and bending vibrations, typical of the protein backbone [12, 27, 33]. The characteristic peaks in Amide I and Amide II were predominantly due to N-H stretching vibrations. In Amide III, they were attributed to C-N stretching and N-H bending vibrations [12, 27]. In particular, the density of the Amide I group was stronger than that of Amide II and Amide III. Saatchi *et al.* observed the Amide I, Amide II, and Amide III bands of sesame protein concentrate at  $1635$ ,  $1518$ , and  $1234\text{ cm}^{-1}$ ,

**Table 3** Differential scanning calorimetry and thermogravimetric analysis results of the sesame protein isolates

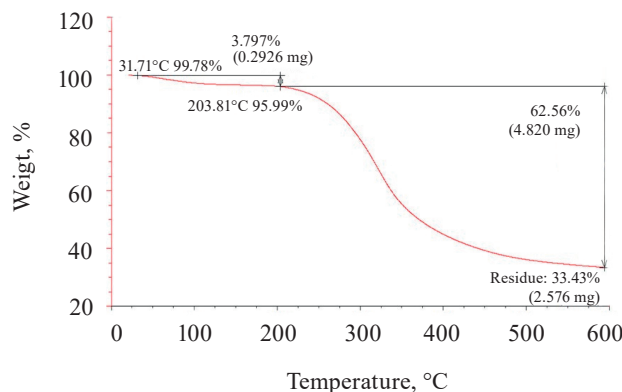
	Glass transition temperature ( $T_g$ ), °C	Denaturation onset temperature ( $T_0$ ), °C	Peak or denaturation temperature ( $T_p$ ), °C	Enthalpy of denaturation ( $\Delta H$ ), J/g	Thermal decomposition initial temperature ( $T_{d1}$ )*, °C	Weight loss ( $\Delta_{w1}$ )*	Thermal decomposition initial temperature ( $T_{d2}$ )**, °C	Weight loss ( $\Delta_{w2}$ )**	Residue, %
Protein isolate	85.56	88.12	122.56	28.47	31.71	3.80	203.81	62.56	33.43

Data represent as mean  $\pm$  SD (n = 4)

\*first stage of degradation, \*\*second stage of degradation



**Figure 6** Differential scanning calorimetry of the sesame protein isolate



**Figure 7** Thermogravimetric analysis of the sesame protein isolate

respectively [12]. These results are very similar to the Fourier transform infrared spectroscopy results obtained for the sesame protein isolate in the current study. Our results were also compatible with other studies [7, 27].

**Differential scanning calorimetry (DSC).** Differential scanning calorimetry is a convenient method to evaluate protein thermal stability and conformational changes. Figure 6 shows differential scanning calorimetry results for the sesame protein isolate. In our study, the thermograms exhibited endothermic peaks, which are usually attributed to protein denaturation [18].

For the sesame protein isolate,  $T_0$  started at 88.12°C, and peak temperature  $T_p$  was at 122.56°C, while the  $\Delta H$  value was 28.47 J/g. The amorphous fractions of the protein formed a glass transition ( $T_g$ ), and the  $T_g$  value was 85.56°C. Available publications on the thermal stability of sesame proteins give a wide range of results. Sharma and Singh determined the  $T_g$  value of the sesame protein isolate as 37°C,  $T_0$  as 98.25°C, and  $T_p$  as 207°C [32]. Saini *et al.* reported  $T_0$  as 176.07°C,  $T_p$  as 210.78°C, and  $\Delta H$  as 63.72 J/g [7]. They attributed the high denaturation temperature to higher non-polar residues in the proteins. However, Saatchi *et al.* reported the  $T_g$  temperature of the sesame protein concentrates as 100.54°C and the  $\Delta H$  value as 51.10 J/g [11]. Yang *et al.* determined  $T_0$  as 55.55–61.26°C,  $T_p$  as 112.39–113.69°C, and  $\Delta H$  as 203.4–220.8 J/g [27].

**Thermogravimetric analysis (TGA).** Thermogravimetry presents the change in mass with progression in temperature and, therefore, can determine the physical

and chemical structural changes during the thermal conversion of biomass to food products [27, 34]. Table 3 describes the thermogravimetric analysis while Fig. 7 illustrates the thermogram of the sesame protein isolate. The samples exhibited weight loss in two stages. A slight weight loss was observed in the samples at temperatures below 100°C, probably, due to water removal. The second and significant primary decrease in the mass of the samples was observed in the range of 250–450°C.

For the sesame protein isolate, the first degradation started at 31.71°C with a weight loss of 3.80%, while the second degradation started at 203.81°C with a weight loss of 62.56%. The weight loss below 100°C could be attributed to the water loss and the decomposition of the quaternary structure of proteins. Temperatures above 100°C are known to denature protein subunits and promote protein aggregate formation through electrostatic, hydrophobic, and disulfide exchange binding mechanisms [35]. At 203°C, the weight loss resulted mainly from the cleavage of the covalent bond between the peptide bonds of amino acids. Further heating caused dissociation of  $\alpha$ -globulins (2S),  $\beta$ -globulins (11S), and the secondary structure opening in the subunit.

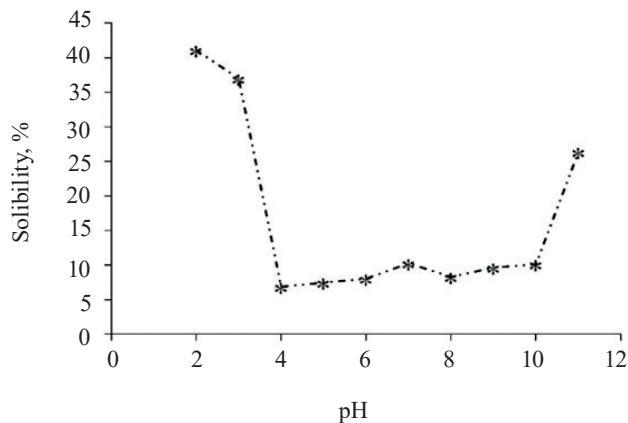
The sesame protein isolate was rich in sulfur-containing amino acids methionine and cysteine, which decomposed between 220 and 250°C. The weight loss continued at around 300°C, probably, due to the splitting of S–S, O–N, and O–O junctions [7]. Our thermogravimetry results were consistent with those



**Table 4** Water and oil holding capacity, as well as foaming and emulsifying properties of the sesame protein isolates

Protein isolate	Water holding capacity, g/g	Oil holding capacity, g/g	Emulsion capacity, %	Emulsion stability, %	Emulsion activity index, m <sup>2</sup> /g	Emulsion stability index, min	Foaming capacity, %	Foaming stability after 10 min, %	Foaming stability after 30 min, %
Protein isolate	1.26 ± 0.04	3.40 ± 0.26	51.32 ± 1.86	49.50 ± 0.71	12.86 ± 0.89	44.96 ± 0.01	36.21 ± 2.44	22.79 ± 0.41	20.26 ± 0.36

Data represent as mean ± SD (n = 4)

**Figure 8** Effect of pH on solubility of the sesame protein isolate

obtained by Saini *et al.*, Yang *et al.*, and Sharma and Singh [7, 27, 32]. Yang *et al.* reported a two-stage weight loss [27]. They attributed the first weight loss to water evaporation at 59.02°C, while the second weight loss, which occurred at 182.58°C, was probably caused by protein weight loss.

**Solubility.** Figure 8 shows the protein solubility profile for the sesame protein isolate. The pH-solubility profile was similar for all proteins, and the solubility in water had a typical U-shaped curve. The sesame protein isolate showed minimal solubility in the isoelectric point region (pH 4–5, Fig. 2). The low protein solubility in the region close to the iso-electric point (pI) values could be attributed to the low net charge in this region. The solubility increased as the pH moved away from the isoelectric point. At pH = 2 and 3, the solubility for the sesame protein isolate was at its highest: 41.11 and 36.99%, respectively. The lowest solubility value of 6.87% was registered at pH 4. These results were consistent with those reported by Achouri *et al.* [3]. The solubility remained low at pH 5–10 because high salt concentration (0.6 M NaCl) during protein isolation might contribute to the adsorption of chloride ions by proteins, thus reducing repulsive interactions between protein molecules [3].

**Water and oil holding capacities.** The water holding capacity of the sesame protein isolate was 1.26 g/g, and the oil holding capacity was 3.40 g/g. The oil holding capacity exceeded the water holding capacity probably because the solubility of the sesame protein isolate was low at neutral pH (10.28%, Fig. 8). Similarly,

Gundogan and Can Karaca reported that the water and oil holding capacities of bean protein isolates were between 1.8–2.1 and 4.0–5.4 g/g, respectively [18]. They also reported that protein isolates with a high oil holding capacity could serve as food components to improve such properties as content, consistency, and viscosity in many products.

**Emulsifying properties.** Table 4 shows such emulsifying properties of sesame protein as emulsion capacity, emulsion stability, emulsion activity index, and emulsion stability index. The emulsion capacity and stability were 51.32 and 49.50%, respectively. Cano-Medina *et al.* reported the highest emulsion capacity values of sesame protein concentrates as 38.0% and the lowest as 19.2% [5]. In the same study, they reported the highest emulsion stability as 50.6% and the lowest as 35.6%. Sharma *et al.* determined the emulsifying activity of sesame protein isolates as 8.70% at pH 9 and 28.96% at pH 12 [6]. Apparently, the differences in the extraction methods and conditions of sesame proteins affect the emulsifying properties.

The emulsion activity index measures the ability of the protein to provide a rapid and adequate coating of the interface area to prevent immediate association and aid in the dispersion of the fat phase [9]. In our study, the emulsion activity index of the sesame protein isolates was 12.86 m<sup>2</sup>/g. Onsaard *et al.* reported a similar emulsion activity index (14.95 m<sup>2</sup>/g) for sesame protein concentrates extracted at pH 9 [9]. However, they had a higher emulsion activity index for the samples extracted at pH 11 and in salt conditions: 96.66 and 49.70 m<sup>2</sup>/g, respectively. Still, our results were consistent with those reported in other studies [3, 26].

Emulsion stability is a time-related property. Interactions of proteins in the oil and aqueous phase affect the stability of the protein film formed at the emulsion interface [9]. Emulsion stability index was 44.96 min, which coincided with the results reported in [9, 26].

However, sesame protein had lower emulsion activity and stability indices compared to other protein isolates, e.g., beans and soy [9, 18]. This result can be explained by the relatively low solubility of sesame protein, compared to that of proteins from other sources.

**Foaming properties.** Table 4 shows the foaming capacity and stability of the sesame protein isolates at pH 7. The foaming capacity and stability after 10 min were 36.21 and 22.79%, respectively. The foaming

stability after 30 min was 20.26%. Good foamability can be associated with flexible protein molecules that reduce surface tension. In contrast, low foaming may be associated with highly regular globular proteins that resist surface denaturation [9]. Sesame flour has the following protein content: 67.3% globulin, 8.6% albumin, 6.9% glutelin, and 1.4% prolamine. The alkaline protein isolate extracted in water at pH 10 and precipitated at pH 4.0 was reported to contain 41.3% albumin, 41.0% glutelin, 14.8% globulin, and 0.8% prolamin [4]. However, an excess of globular proteins can be cited among the reasons for low foaming capacity and stability of the sesame protein isolates. Previous studies also reported relatively low foaming capacity and stability values of sesame protein isolates and concentrates [6, 9, 26].

### CONCLUSION

This study featured sesame (*Sesamum indicum* L.) protein isolates produced from defatted sesame seeds, as well as various properties of standard sesame seeds, defatted sesame seeds, and sesame protein isolates. The extractability of sesame proteins proved sensitive to pH more than to pH 4 and correlated with the increasing NaCl content. The sesame protein isolate appeared to be a good source of protein (88.98%). The sesame protein isolates contained sulfurous amino acids, e.g., methionine (2.78 g/100 g), which is rarely found in plant proteins.

The study is novel in that it reports the zeta potential and hydrodynamic diameter of the sesame protein isolate. The solubility was found to correlate with the

zeta potential. Thus, sesame proteins can serve as prospective functional components. They can cast more light on the relationship between the electrical charge interactions in food matrices and the structure, stability, shelf life, texture, and functions of food.

More data on how these proteins interact with each other and behave in foods can help develop appropriate methods that could detect the effects of sesame protein isolates in foods. In this regard, the behavior of sesame protein isolates in various food systems seems a promising research direction. Our next step will be to study the sesame protein isolate we produced and described in this article in various food systems. We also plan to concentrate on the interactions between sesame paste and sesame isolates, salt concentration, and pH conditions.

### CONTRIBUTION

The authors were equally involved in writing the manuscript and are equally responsible for plagiarism. All authors of this study have read the manuscript and agreed to publish it.

### CONFLICT OF INTEREST

The authors declare no conflict of interests regarding the publication of this article.

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