

# Impact of steaming and roasting heat-treatment on physico-chemical, functional and digestibility of walnut kernel

Xiaoyan Zhao<sup>1</sup>, Xiangrui Ren<sup>2</sup>, Hongkai Liu<sup>2</sup>, Meng Wang<sup>1</sup>, Xiaowei Zhang<sup>2</sup>, and Haifang Hu<sup>3</sup>

<sup>1</sup>University of Jinan

<sup>2</sup>Affiliation not available

<sup>3</sup>Xinjiang Academy of Forestry Sciences

June 1, 2023

## Abstract

In order to develop the application of walnut kernel, the effect of steaming and roasting treatment on the physicochemical and functional properties of walnut kernel at 95 °C for different time (15, 20 and 30 min) was investigated, and compared to those of untreated sample. Scanning electron microscopy suggested that heating treatment had a notable effect on the microstructure of walnut kernel, especially the steam heating. Both treatments significantly increased the enthalpy, vitro protein digestibility, viscosity, G' and G'' (P < 0.05), the order from high to low was steaming > roasting > untreated. All samples contained the amounts of essential amino acids, the amino acid score (AAS) of samples by steaming was the highest compared to that of the untreated and roasting, and the only limiting amino acid of walnut kernel before or after heat treatment was lysine. In addition, the protein of walnut kernel after heating treatment with the extension of time contained more  $\alpha$ -helix and random coil structures compared to the untreated sample, while  $\beta$ -sheet and  $\beta$ -turns structures decreased. Moreover, the thermal treatment could cause the changes of the water/oil holding capacity, foaming and emulsifying properties of walnut kernel flour. When there were differences between the results of steaming and roasting samples, it was concluded that the water played an important role in steaming. These results suggested that the thermal treatment as an effective approach could improve the physico-chemical, structural and functional properties of walnut kernel and be potentially applied in the food processing.

## Introduction

Walnut (*Juglans regia* L.) belongs to a tree nut due to consisting of a hard nutshell protecting the kernel. It is widely concerned and popular for its high nutritional value. Walnut is distinguished by high oil content (43.3-78.48%) of unsaturated fatty acids (about 70% total fatty acids) [1]. Besides, walnut also an excellent source of protein, carbohydrate and polyphenolic compounds [2,3]. Especially, the defatted walnut meal is discarded as a by-product of oil extraction. It consists of the abundant protein (about 55 d.w%) and carbohydrate (about 28 d.w%), which can act as the high nutritional components in food products [4]. Food and Drug Administration and European Food Safety Authority declares that the walnut kernel can decrease the risk of heart disease, cardiovascular disease, cancer, hypertension and all-cause [5]. In short, the walnut kernel has the characteristics of improving human health [5]. The references reported that thermal treatment had significant influence on polyphenols content, vitamin E content and fat acid profile [6], and also improved the utilization and availability of protein and reduced the anti-nutritional factors [7]. In addition, the studies proved that the quality of walnut meal flour was not damaged under mild thermal treatment procedures [6], and applied as a functional ingredient in food industry [3].

However, the utilization of walnut in the food system is often limited partially because of the digestibility and allergy of walnut protein [8]. The thermal processing can conquer these shortcomings, which was proved

that it could be used to process beans and cereals, such as soybean, common bean [9,10]. In general, the thermal processing method is a common processing, which includes wet-heat and dry-heat treatment. The wet-heat method transfers heat to food through steam or water, such as boiling, steaming and cooking in simmering liquid; while the dry-heat transfers to food by hot air, such as roasting and air frying [9].

Roasting method is a kind of typical food hot processing, which can improve the digestibility, eating qualities and bioavailability of food products by modifying the physicochemical properties and structure of food components [11]. The reason is due to biochemical changes in process of roasting, such as hydrolysis, oxidation, reduction, and resulting reactions from pyrolysis [11]. These changes also will be helpful to enhance the color, flavor, taste active compounds, and antioxidants and decrease the microbiological pollution, natural poison and enzyme inhibitors in food [11]. The steaming method is considered as one of the most common mechanical heating methods with the features of low cost and convenience in food industry, which can affect the texture, color, digestibility, structure and antioxidant compounds in food material [12-14]. Moreover, it can also avoid the leaching of nutritional compositions [12].

Meanwhile, there is absent from the systematic report on the effects of roasting and steaming methods on the physicochemical and functional properties of walnut meal flour [6]. Such information is needed to strengthen the study for this species from walnut meal as a major source of protein and other nutritional requirements for healthy growth. Therefore, the purpose of this study was to compare the effects of roasting and steaming methods under different treatment time at the same heating temperature on the *vitro* digestibility, physicochemical and functional properties of walnut meal flour.

## Procedures

### Material

Dried walnut (Xinxin No. 2) was supported by Xinjiang Academy of Forestry Sciences (Urumqi, China) and harvested in September of 2022. The shelled walnut kernel formed from a proximate composition of  $20.08 \pm 1.38\%$  protein,  $5.11 \pm 0.18\%$  moisture content,  $63.22 \pm 5.12\%$  oil and  $1.71 \pm 0.06\%$  ash [15]. The norleucine was bought from Sigma Chemical Co. (St Louis, MO, USA). The pepsin ( $3000 \text{ U mg}^{-1}$ ) and pancreatin from porcine pancreas ( $4000 \text{ U mg}^{-1}$ ) were purchased from the Novozymes (Bagsværd, Denmark). All other reagents were analytical grade, and bought from Sinopharm Chemical Reagent Co., Ltd (Beijing, China).

### Sample Preparation

The obtained shelled walnut kernel was thermally processed according to a previously described method [9]. The moist-heat processing method was as following: five hundred grams of shelled walnut kernel was steamed in boiled water for 15, 20 and 30 min (S15, S20 and S30) at a temperature of  $95 \pm 1.5^\circ\text{C}$ , which were cooled to room temperature, frozen for 24 h at  $-18^\circ\text{C}$ , and lyophilized for 48 h at  $-55^\circ\text{C}$ . The dry heated processing method was as following: five hundred grams of shelled walnut kernel was roasted in an air fryer (HD9252, Philips, Qingdao, Shandong, China) at  $95 \pm 1.5^\circ\text{C}$  for 15, 20 and 30 min (R15, R20 and R30). The raw was as the untreated sample. Total samples were 7 (1 untreated + three moisture heated + three dry heated). The defatted walnut kernel flour (100 mesh screen) by ground processing contained about protein  $44.85\% \pm 1.21\%$ , oil  $15.65\% \pm 1.08\%$ , moisture content  $5.32 \pm 0.53\%$  and ash  $3.09 \pm 0.51\%$ .

### Scanning Electron Microscopy (SEM) Analysis

The surface morphological characteristics of samples with different thermal treatment were observed using a SU8100 scanning electron microscope (Hitachi High- Technologies Corporation, Tokyo, Japan). The sample powder was stuck on a specimen holder using a double-sided conductive adhesive and then coated with a 10 nm gold film by ion sputtering. An accelerating voltage of 15 kV was used during observation.<sup>16</sup> The sample images were obtained at a magnification of 3000 times.

### Amino Acid Composition

The determination of total amino acids of the walnut kernel flours (WKF) was performed by high performance liquid chromatography (HPLC, Agilent 1260, Agilent Technologies Co., Ltd., Palo Alto, CA, USA, USA)

according to the described previously method of Trigui et al [17]. The HPLC system was equipped with two columns (Styragel HMW7 and HMW6; Styragel, Waters, Milford, MA, USA). Two hundred milligrams of samples were hydrolyzed by 15 mL 6 M HCl for 24 h at 120 °C. Then, the hydrolyzed samples were mixed with 40 mL of 0.2 M citrate buffer (pH 2.2). Norleucine was added as the standard solution with the amount of 1mL. The mixture solution was diluted to 100 mL by citrate buffer. After the samples were filtered by a 0.2 µm nylon filter, which was detected by HPLC. The amino acid contents were expressed as g 100g<sup>-1</sup> protein. Amino Acid Score (AAS) of amino acid composition in samples was expressed as following the equation [18].

$$\text{AAS (\%)} = \frac{\text{mg of amino acid in 1 g of test protein}}{\text{mg of amino acid in requirement pattern}} \times 100(1)$$

### Fourier Transform Infrared (FTIR) Spectroscopy

FTIR spectra of samples were scanned at room temperature by a Bruker Vertex 70 FTIR spectrometer (Bruker Daltonics, Bremen, Germany) as reported previously [9]. Different testing samples were acquired in KBr tablets. The spectra were collected with a scanning range of 4000-500 cm<sup>-1</sup> at a resolution 4 cm<sup>-1</sup> and 32 scans. The secondary structure of protein in untreated, moist-heated, and dry-heated walnut kernel was estimated by a second derivative in the amide I region (1700–1600 cm<sup>-1</sup>). Deconvolution of the second derivative of FTIR was obtained by Savitsky–Golay algorithm from Origin Pro 2021 software (Hearne Scientific Software).

### Thermal Properties

The thermal properties of walnut kernel flour were measured by a differential scanning calorimeter (DSC, TA Q20, New Castle, USA). Around 3.0 mg of sample was blended with 12 µL distilled water and locked in an aluminum pan. The pans were kept at room temperature overnight before determining. The samples were heated from 20 to 120 °C. Heating rate was 10 °C min<sup>-1</sup>. The reference was the empty pan.<sup>19</sup> The onset temperature(T<sub>0</sub>), peak temperature(T<sub>p</sub>), conclusion temperature (T<sub>c</sub>) and enthalpy change (*H*) were used to reflect the thermal properties of samples [19].

### Rheological Properties

Rheological properties of samples were investigated according to a previous study [20]. Samples were prepared in 10 mg mL<sup>-1</sup> deionized water at 95 oC for 20 min, and kept mixing for 60 min to ensure complete solubilization, then equilibrated 30 min at ambient temperature. After processing, the sample was laid in an aluminum parallel plate and analyzed by a Discovery HR-2 rheometer (TA Instruments Ltd., New Castle, DE). The experimental conditions were as following: 40 mm diameter, 1 mm gap and 25 oC temperature. The strain sweeping from 0.1 to 10% was performed at 1 Hz frequency. The frequency sweeps with a fixed strain of 1% were measured by the range from 100 to 0.01 rad s<sup>-1</sup> at room temperature within the linear viscoelastic range. The storage modulus (G') and loss modulus (G'') were automatically recorded. Data were collected according to the logarithmic mode and performed in triplicate.

### Functional Properties

Changes of water holding capacity (WHC) and oil holding capacity (OHC) were determined according to the methods described by Choe et al. with minor modifications [9]. The sample (1.0 g) was placed in pre-weighed 25 mL centrifuge tubes and dispersed in 10 mL of distilled water/soybean oil under magnetic stirring with occasional vortex agitations for 10 min at room temperature. Afterwards, the samples were centrifuged at 4000 x g for 15 min at ambient temperature. Then, the centrifuge tube containing sediment was weighed. The WHC or OHC was appraised as g water or oil per g dry sample, respectively.

The emulsifying capacity (EC) and emulsifying stability (ES) were evaluated according to the described method of Di et al. with small modifications [21]. Briefly, 5 mL of soybean oil and 5 mL of sample solution (80 mg mL<sup>-1</sup>) was mixed. Then the mixture was homogenized by a high-speed shearing blender (PT-10-35GT, BUCHI Labortechnik AG, Switzerland) at 10, 000 rpm for 2 min at room temperature and centrifuged

at 3000 xg for 5 min at room temperature. The emulsion formed was admitted to keep for some minutes until the emulsion layer was stable. EC was calculated as follows Di et al:<sup>21</sup>

$$EC (\%) = \frac{H_2}{H_1} \times 100 (2)$$

Where  $H_1$  was the total height of liquid, cm;  $H_2$  was the height of the emulsified layer, cm.

The above emulsion was centrifuged again after heating at 80 degC for 30 min and then cooled at room temperature. ES was expressed by the following equation Di et al [21]:

$$ES (\%) = \frac{H_4}{H_3} \times 100 (3)$$

Where  $H_3$  was the total height of the liquid after heating; cm;  $H_4$  was the height of emulsion layer after heating, cm.

The foaming capacity (FC) and foaming stability (FS) were evaluated according to the described method of Di et al. with small modifications [21]. Sample solutions were prepared at 10 mg mL<sup>-1</sup> and pH of the phosphate buffer was adjusted to 7.0. The solutions were whipped in graduated plastic tubes using a homogenizer (PT-10-35GT, BUCHI Labortechnik AG, Switzerland) at 10,000 rpm for 3 min at room temperature. The volume was recorded before and after whipping. FC and FS were reported by the formula (4).

$$FC (\%) = \frac{V_1 - V_0}{V_0} \times 100 (4)$$

Where  $V_0$  represented the volume prior to homogenization, mL;  $V_1$  represented the volume after homogenization, mL.

Following, the determination of FS was performed by a similar procedure and the residual foam volume was measured after sample keeping for 30 min at ambient temperature. The formula (5) was applied to express the FS value.

$$FS (\%) = \frac{V_3 - V_0}{V_0} \times 100 (5)$$

Where  $V_3$  represented the residual volume after standing, mL.

### In Vitro Digestibility (IVPD)

The vitro digestibility of samples by thermal treatment conditions was determined using the method of Hao et al [22] and Di et al. [21]. The vitro gastric and intestinal digestion were simulated by the pepsin and trypsin, respectively. The digestive base fluid (simulated intestinal fluid (SIF) and simulated gastric fluid (SGF)) was prepared according to Hao et al. before the experiment [22]. The experimental steps were as follows: the sample solution (1g 100mL<sup>-1</sup>) and an equal amount of SGF solution and pepsin (2000 U mL<sup>-1</sup>) were mixed, then the pH of mixture solution was adjusted to 2.0 with 1 M HCl. Thereafter, the mixture was incubated in Shaking Water Bath with 150 rpm at 37 °C. The digestive solution was collected at different time including 0, 1, 5, 10, 30, 60 and 120 min. The gastric digestion was terminated by boiling water bath immediately for 10 min. The digestion solution was centrifugated at 4700 x g for 15 min at 8 degC. Then, the bicinchoninic acid (BCA) method was used to determine the protein content in supernatant [21]. The vitro digestibility rate of walnut meal flour was expressed according to the following equation.

$$IVPD (\%) = \frac{PC_0 - PC_t}{PC_0} \times 100 (6)$$

Where  $PC_0$  was the initial protein content;  $PC_t$  was the protein content after simulated in vitro digestion.

SIF solution and sample solution digested by pepsin was mixed in a ratio of 1:1 (v/v) and the trypsin solution with the activity of 1000 U mL<sup>-1</sup> was added at the same time. The pH of mixture solution was adjusted to 7.0 with 1 M NaOH. Then, the mixture was incubated and oscillated in a water bath at 37 °C for 0, 1, 5, 10, 30, 60 and 120 min. Finally, the intestinal digestion was ended in ice bath for 30 min. After centrifugation, the protein content of supernatant was measured as above described. All data was done in triplicate.

### Statistical Analysis

The analysis and experiments of samples were expressed in triplicate. The data were shown as mean + standard deviation and analyzed by the statistical software SPSS 19.0 (IBM, New York, USA). The differences between means were performed by a one-way analysis of variance (ANOVA). The significant differences of means were confirmed by the Tukey's test with a significance value  $P < 0.05$ .

## Results and Discussion

### Morphological Characterization of Heat-treated Walnut Kernel

The samples had shown a significant variation in microstructure and they varied in the sizes and shapes (Fig. 1). SEM micrographs indicated that the thermal processing could modify the microstructure of samples, which varied in the sizes and shapes compared to the untreated sample (Fig. 1). The morphology of untreated walnut kernel flour presented a complex spherical to oblong/elliptical granular structures together with the smaller particles and irregularly shaped cellular fragments. The granular structures might be starch granules, while the protein bodies were displayed by the shape of smaller spheres particles [24]. Generally, the protein, starch, cellulose molecules etc. were partly or completely embedded together, for example the protein body substance was surrounded by the smaller particles with spherical or irregular in shape [24]. Here, the roasting and steaming at 100 °C for 15, 20 and 30 min had marked influence on the surface of granules compared to the untreated sample. The clumping phenomenon in samples of thermal treatment began to appear, and the change extent varied with heating time (Fig. 1). The part granule integrity in WKF was kept after thermal treatment for 15 min, whereas it was observed to significantly change at 20 and 30 min ( $P < 0.05$ ). Both flours treated by steaming and roasting showed aggregated microstructure with irregular surfaces. However, the aggregate of sample processed by steaming was obviously larger than that of roasting ( $P < 0.05$ ). The results indicated that the steaming had maximally influence on walnut kernel compared to roasting treatment, which was likely attributed that the gelatinization was easy to occur due to the presence of water in steaming [25].

### Amino Acid Composition Analysis

Amino acid (AA) composition is an important chemical property of protein. The reason is that it can determine the nutritional value of protein [26]. AA composition of the untreated and heat-processed samples of walnut kernel is exhibited in Table 1. The data of experiments revealed that the protein in different WKF samples included 16 amino acids, including 7 essential amino acids (EAA) and 9 nonessential amino acids (NEAA) (Table 1). No significant difference was found in the total content of AA between untreated, roasting and steaming samples for 15 and 20 min ( $P > 0.05$ ). The AA content of the sample was significantly higher than that of other samples when it was steamed for 30 min ( $P < 0.05$ ). From the Table 1, it was observed that the amount of acidic AA in samples was higher than alkaline AA, which suggested that the WKF had the acidic characteristic [27]. These findings were in agreement with those previously reported for walnut meal flour [27]. Glutamic (Glu), aspartic (Asp), and Arginine (Arg) in all WKF were relatively abundant (Table 1), Asp contents in untreated, S15-S30 and R15-30 were 7.13, 5.77-7.24 and 6.04-5.10 g 100g<sup>-1</sup>, respectively; Glu contents were 19.15, 15.83-15.50 and 18.60-17.66 g 100g<sup>-1</sup>, respectively; Arg contents were 13.26, 13.48-15.18 and 13.90-13.31 g 100g<sup>-1</sup>, respectively.

In addition, EAA content could reflect the nutritional value of protein [28]. All samples contained EAA contained (except Tryptophan). The total EAA contents in untreated, S15, S20, S30, R15, R20 and R30 were 26.72, 26.93, 27.89, 30.19, 27.07, 26.11 and 25.74 g 100g<sup>-1</sup>, respectively, which occupied 30.42, 33.33, 32.12, 33.23, 31.08, 31.12 and 31.2 % of the total AA content, respectively. Therefore, the walnut kernel obtained by steam heating could be regarded as high quality of natural proteins due to being composed of the most of the EAA (Table 1). After different heat treatments, the content of EAA in protein of walnut kernel changed to a certain extent compared to the untreated sample. The steam treated samples were significantly higher than the untreated and roasted samples ( $P < 0.05$ ). After steaming, the total amount of total EAA in protein increased significantly ( $P < 0.05$ ) with the increase of heating time. Roasting treatment had no significant effect on the total EAA content of walnut protein ( $P > 0.05$ ). The results could be explained by their different processing methods. The thermal stability of protein was comprehended by the composition

of AA.<sup>17</sup> The higher the content of hydrophobic AA in the protein, the more stable the protein was, which suggested that the more stable protein contained the higher total hydrophobic AA content. The hydrophobic AA content of the steaming treated walnut kernel gradually increased compared with the untreated, while the roasting treated walnut kernel gradually decreased, which was due to a thermodynamically less stable for the protein of walnut kernel. However, there was the small difference for total hydrophilic AA among samples.

It is generally believed that the closer the Amino Acid Score (AAS) is to 100, the closer the AA pattern in samples is to the recommended value,<sup>18</sup> which suggests that the nutritional value of sample is higher. AAS of untreated and heated walnut kernels is observed in Table 1. For walnut kernel samples before and after heat treatment, the AAS of rest AA except lysine was closest to 100 or more. It was illustrated that these amino acids could basically meet the recommended requirements [18]. Lysine was the first limiting amino acid in walnut kernel samples according to the AA score (Table 1) [18]. It was worth noting that the lysine AAS by steaming treatment increased from 39.78 to 47.56%, while roasting treatment decreased from 39.78 to 37.11%. This showed that the steaming treatment could improve the nutritional value of protein in kernel. These results proved that the AAS of walnut kernels by steaming processing significantly increased compared to the untreated and roasting processing ( $P < 0.05$ ). Although the AAS of histidine, valine, leucine, isoleucine, phenylalanine and threonine had decreased after roasting treatment, it was still close to the recommended value of WHO/FAO/UNU Expert Consultation [18], and AAS of methionine by roasting treatment for 15 min increased from 71.76 to 77.65%. These data indicated that the two kinds of heat treatment could improve the nutritional value of protein in walnut kernel. The amino acid content of each component of protein in walnut kernel changed after different heat treatment, but most EAA could still meet the recommended requirements. Therefore, the walnut protein by different thermal processing could be used as a high-quality protein ingredient in food [29].

In addition, the different heat treatments also changed the amount of AA composition of the protein in walnut kernel. The results showed that the content of other AA contents except for glutamic gradually increased with the extension of steaming treatment time. It indicated that the steaming processing led to a significant increase in major AA contents, which might be contributed to the release of bound amino acids during steaming with water [30]. The increase in the amino acid levels after the steaming of walnut kernel could inspire utilization in food formulations. It was worth noting that the content of AA components changed little when the roasting time was 15 min; when the roasting time was extended to 20 and 30 min, other AA contents except for arginine reduced. This phenomenon could interpret the higher concentration of hydrophobic AA in steamed walnut kernel. Compared with the untreated and steaming processing samples, the major AA content and total AA of samples in roasting processing from 15 to 30 min gradually reduced. This might be due to little water in roasting processing, which would lead to the partial degradation or loss of protein or the result of Maillard reaction between amino acids and sugars [31].

## FTIR Analysis of Walnut Kernel During Thermal Treatment

The untreated and heated walnut kernel samples were analyzed by FTIR spectroscopy to study the change in amide I frequency ((1600–1700  $\text{cm}^{-1}$ ), which chiefly implies the C = O stretch vibration of the amide group [28]. The corresponding secondary structure of protein includes  $\alpha$ -helix (1648 and 1664  $\text{cm}^{-1}$ ),  $\beta$ -sheet (1615–1637  $\text{cm}^{-1}$  and 1682–1700  $\text{cm}^{-1}$ ),  $\beta$ -turn (1664–1681  $\text{cm}^{-1}$ ) and random coil (1637 to 1648  $\text{cm}^{-1}$ ) [32]. Table 2 shows the percentage content of the secondary structure of protein in walnut kernel treated with different time of steaming and roasting. With the processing time continued to increase, the change in the  $\beta$ -sheet and  $\beta$ -turns structures gradually slowed down, while the  $\alpha$ -helix and random coil gradually rose up. The percentage of  $\beta$ -sheet structure (47.16%) was the highest in untreated sample, and the random coil occupied the lowest percentage (12.86%). It was interesting that the protein secondary structure in walnut kernel significantly changed after the steaming and roasting treatment. As shown Table 2, the relative contents of  $\beta$ -sheet (39.91% and 35.67%) and  $\beta$ -turns (19.98% and 19.19%) of the samples by steaming and roasting treatment for 30 min decreased, respectively, which decreased by 18.17% and 36.79%, 28.61% and 41.79% compared with the native, respectively. Furthermore, the relative contents of  $\alpha$ -helix (24.44% and 25.92%)

and random coil (14.47% and 16.85%) of samples by steaming and roasting treatment for 30 min increased, respectively, which increased by 50.91% and 54.44%, 11.13% and 23.68% compared with the untreated, respectively. The results exhibited that the  $\beta$ -sheet and  $\beta$ -turns structure of protein in heat-treated walnut kernel were transformed to  $\alpha$ -helix and random coil structures. It was supported by the investigation of Vanga et al. [33], which found that the  $\alpha$ -helix and random coil structures of protein in peanut with an increase in the heat treatment time increased. The reason for this phenomenon was that the original rigid structure of the protein was destroyed to a certain extent. Therefore, the flexibility of protein increased and the conformation transformed from order to disorder [28,34]. On the other hand, the hydrogen bonds of protein might be destroyed by heat, which led to a greater degree of random distribution of molecular free energy [28,35]. As a consequence, it was concluded that a set of reactions in walnut kernels after steaming and roasting treatment occurred a set of reactions, such as protein denaturation and aggregation, which would cause the interconversion between  $\alpha$ -helix,  $\beta$ -sheet,  $\beta$ -turn and random coil structures [28].

At the same time, it was also found that the different secondary structures of protein was presented in steaming and roasting walnut kernels due to two distinct thermal processing (moisture and dry heating method) (Table 2). It was obvious that the content of  $\beta$ -sheets was higher in walnut kernel by roasting treatment for 30 min compared to the steaming treatment (36.67%), which accounted for 39.91% of the whole secondary structure (Table 2). On the contrary, a low content of the  $\alpha$ -helix and random coil was determined for walnut kernel by roasting treatment for 30 min, which was 24.44% and 14.47%, respectively, while that of walnut kernel by steaming treatment was higher (25.92% and 16.85%, respectively). It could be inferred that the steaming and roasting heating would cause the structural changes of protein in walnut kernel, which was attributed to the denaturation, cross-linking and aggregation of protein through heating treatment.<sup>36</sup>

### Differential Scanning Calorimetry (DSC) Analysis

The thermal properties of the protein in both heating treated (steaming and roasting) and untreated walnut kernel samples are carried out by DSC. The denaturation peak temperature ( $T_p$ ), onset temperature ( $T_o$ ), endset temperature ( $T_e$ ) and denaturation enthalpy ( $\Delta H$ ) of protein in walnut kernel before and after heating treatment are summarized in Table 3.  $T_p$ ,  $T_o$ ,  $T_e$  and  $\Delta H$  play the important roles in the thermal properties of proteins, which is attributed to being closely connected with the protein conformation.  $T_p$  is the temperature at which protein denatures,  $T_o$  represents the initial temperature of the protein denaturation,  $T_e$  is the temperature of at which protein denaturation ends, while  $\Delta H$  represents the energy required during protein denaturation [21,37]. As shown Table 3, there was a significant difference for the  $T_e$  and  $T_p$  of protein in walnut kernel before and after heating treatment ( $P < 0.05$ ). The results showed that the heating processing increased the  $T_e$  and  $T_p$  values of protein, the highest  $T_p$  values were observed after 15 min of roasting and 30 min of steaming, which was 102.83 °C and 102.22 °C, respectively, the highest  $T_e$  values were found after 15 min of roasting and 20 min of steaming, which was 116.04 °C and 114.50 °C, respectively. While  $T_{onset}$  in all samples slightly changed. With increasing steaming and roasting time, there is no significant change in  $T_p$  ( $P > 0.05$ ), and  $T_o$  and  $T_e$  of other samples except roasting for 15 min also had not the significant difference ( $P > 0.05$ ). It was also found that the denaturation enthalpy ( $\Delta H$ ) of protein in walnut kernel increased after steaming and roasting treatment (Table 3). The increase of random coil and  $\alpha$ -helix proportions in protein can reflect the changes of  $\Delta H$  [38]. As can be shown in Table 3, there are an increase in the  $\Delta H$  values of roasted and steamed walnut kernel compared to that of untreated sample, which suggested that the heating treatment could increase the unfolding molecular structure of protein and change the stable structure of protein [39]. The value of  $\Delta H$  was the highest in roasting and steaming for 20 min, which was 7.07 and 8.69 J/g, respectively. The result indicated that heating (roasting and steaming) treatment at 95 °C improved the denaturation temperature and  $\Delta H$  values of protein in walnut kernel. However, it was obvious that  $\Delta H$  was lower in dry-heated (roasting) samples compared to the moist-heat (steaming) samples as a result of both different thermal processing methods.

### Rheological Properties

The rheological properties of walnut kernel flours by roasting and steaming treatment with different time

were accessed as shown in Fig. 2. The storage and loss modulus were  $G'$  and  $G''$ , respectively, which could reflect the elasticity and viscosity of samples [40]. Fig. 2 A and B exhibit the  $G'$  and  $G''$  changes of untreated and heating processing samples. It could be observed that the  $G'$  of all samples was greater than the  $G''$ , which reflected the dominant viscoelastic solid-like behavior in all samples [41]. As shown in Fig.2 (A and B), the  $G'$  and  $G''$  values of other samples except for the untreated and R15 increased slightly with increasing angular frequency, suggesting if the samples formed a gel, it could be stable and strong [42]. However, the  $G'$  and  $G''$  of untreated and R15 samples gradually with increasing frequency increased, which suggested that the gel network structure of two samples was more fragile [43]. Compared with untreated walnut kernel, heating treatment resulted in the increase of  $G'$  and  $G''$  values, which was explained that the protein molecular interaction was improved due to the denaturation and exposure of hydrophobic groups during heat processing [44]. It was worthy of remark that  $G'$  and  $G''$  values of steaming treatment were higher than that of roasting processing, which was attributed that the steam contained water during heating. de Vries et al. reported that the addition of a small amount of water in protein could promoted the hydrated protein aggregates during heating [45], which would cause the increase of  $G'$  and  $G''$  values. These results indicated that the rheological properties of walnut kernel were significantly altered by different heat treatments [43].

Fig. 2 C describes the flow behavior of the walnut kernel flours with different heating methods and time. As can be seen in Fig. 2 C, with the increase of shear rate, all sample viscosity gradually reduced, which showed the shear thinning behavior (pseudoplastic) [21]. It was explained that the partial destruction of the protein network structure in walnut kernel was caused by the increase of shear rate, which would decrease the fluidity of the protein and the viscosity [21]. Compared to the untreated walnut kernel, the initial viscosity of R15-R30 and S15-S30 was increased, the order of viscosity values from high to low was steaming > roasting > untreated sample. In addition, with the extension of steaming and roasting time, the viscosity of the samples increased, this might be related with the changes in the protein structure after heating. It was supposed that the heating treatment for 15-30 min could cause the crosslinking of internal molecules [43], namely, the formation of three-dimensional network structure occurred due to the changes of protein conformation in samples. on the other hand, the heat treatment could increase the number of active molecules per unit volume, which enhanced the effective collisions and interactions between molecules [43]. It was concluded that the appropriate heat treatment could lead to the unfolding of protein molecules and enhance the interaction of molecules [43]. These findings further proved that the heat processing could be helpful to result in the more interaction behavior and improve the elastic properties of samples [43].

### In Vitro Protein Digestibility (IVPD)

The vitro protein digestion rate of untreated, steaming and roasting walnut kernel was evaluated. The results of in vitro protein digestibility of walnut kernel before and after heating are shown in Fig. 3. For all samples, the rate of digestion in the intestine were higher than those in the stomach because of the higher number of catalytic sites on trypsin, which was supported by described finding of Fang et al.<sup>40</sup> Compared with untreated walnut kernel, the IVPD of protein increased after heating, which indicated that the heat-processing could enhance IVPD to some extent, which was agreement with the results of Chinma et al. [46]. The reason might be that heating treatment could improve the hydrolysis activity of intrinsic protease and the solubility of protein in walnut kernel, and remove the protease inhibitors, tannins, and phytic acid [46,47]. This also indicated that the heat-processing could potentially improve the digestibility of protein in food systems [48]. The digestion of protein is also related to its secondary structure [49]. The reference reported that the  $\alpha$ -helix content of proteins with heating treatment increased and the  $\beta$ -sheet content decreased, which would lead to an increase of the food digestibility [50]. The results in this study were agreement with the finding of Carbonaro et al. [50]. R30 and S30 presented the highest percentage of protein digestibility (gastric digestion: 15.42% and 17.83%, respectively; intestinal digestion: 22.37% and 27.31%, respectively), the lowest IVPD was identified in untreated sample (gastric digestion: 4.18%; intestinal digestion: 7.58%). It was attributed to the unfolding of protein structure during heating treatment, that was that the more hydrophobic groups were exposed, which would result in proteinase to produce the additional binding sites. Then, the protein skeleton was decomposed and the IVPD increased at last [21]. It was interesting that the digestibility of samples by steaming treatment was significantly higher than that of roasting treatment ( $P <$



0.05). One of the main contributing factors to this phenomenon might be the water in steaming [30]. Protein application value can be reflected by IVPD, which also influences the quality of protein absorbed by the body. In general, the high-quality proteins have high IVPD [21]. As more amino acids from the protein backbone are released by proteolysis, which can be better digested and absorbed by the body [21]. Consequently, these results proved that the proper heat treatment could influence the structural characteristic (tertiary and secondary structure) of protein, which would be helpful to enhancing the occupying coefficient of protein in walnut kernel and expanding the applications in food products.

## Functional Properties

### Water and Oil Holding Capacity

Because the water holding capacity (WHC) and oil holding capacity (OHC) affect the application of samples in food, it is necessary to study the functionality [51]. In this study, the effect of different heat treatments on WHC and OHC of walnut meal flours were assessed. WHC of untreated and treated WKF is depicted in Table 4. Compare to the untreated and roasting samples, WHC values of steaming samples increased significantly ( $P < 0.05$ ). The results were supported by finding of Choe et al. [9], they found that moist-heat treatment could significantly increase WHC of whole seeds. The first reason possibly was the structural changes of components in walnut kernel during heating, which would lead to absorbing more water and improving the hydration property [9]. Another reason was that the phenomenon of starch swelling or gelatinization or damaging in walnut kernel occurred during steaming treatment, which would cause the increase of WHC due to more exposed hydroxyl regions with interacting with water [9]. Finally, it also probably was due to the higher content of hydrophilic amino acids in walnut kernel during steam processing [9]. On the contrary, the roasting walnut kernel showed the higher OHC than that of untreated or steaming treatment (Table 4), which was attributed to the protein denaturation as well as the exposure of more hydrophobic regions [52].

However, WHC of R30 was significantly lower than untreated, while there was non-significantly ( $P > 0.05$ ) between untreated and R15. Additionally, with the extending of heating time, WHC gradually reduced. Untreated sample had WHC of  $2.84 \text{ g g}^{-1}$  while as steaming and roasting treated flours had WHC ranging from  $4.43$  to  $3.45 \text{ g g}^{-1}$  and  $3.32$  to  $1.85 \text{ g g}^{-1}$ , respectively. It was interesting that the walnut kernel by roasting treatment showed the highest OHC, which was  $5.67$ - $5.73 \text{ g g}^{-1}$ , the untreated sample exhibited the lowest OHC of  $1.21 \text{ g g}^{-1}$ . However, no significant differences in OHC were observed between roasting samples for 15, 20 and 30 min. In steaming samples for 15, 20 and 30 min, the decrease in OHC might be related with the low concentration of hydrophobic amino acids (Table 1) [46], which had a high attraction for oil. Therefore, OHC of S15 significantly was higher than that of S20 and S30. Because WHC and OHC altered with heat processing, which should be thought over when the food formulas were developed.

### Foaming Capacity and Stability

The foaming capacity (FC) and stability (FS) of the samples are reported in Table 4. The results clearly showed that the FC and FS of the samples by roasting and steaming treatment were higher than that of the untreated sample (Table 4). Untreated sample had a FC (62.13%) and FS (43.30%) significantly lower than heating samples ( $P < 0.05$ ). The steaming and roasting samples both had good foaming properties with FC (65.44-68.60% and 69.17-71.11%, respectively) and FS (80.07- 67.48% and 80.11-48.18% respectively). With the changes of heating methods, the FC and FS of the samples changed. The results showed that the FC and FS of samples by steaming treatment were slightly different than the roasting. When the roasting time was 30 min, the FC of sample reached the maximum value, while FS with the extending of time gradually reduced. However, FC during the steaming was not significantly different between S15, S20 and S30, but the FS gradually decreased with time from 15 to 30 min. The functional properties of protein can be altered due to the changes of the secondary and tertiary structure [22]. The conformation changes of protein also would cause the changes of foaming properties [53]. some researchers have reported that the partial unfolding of proteins can improve the FC and FS of protein [22]. In the present study, the infrared data had shown that the number of unfolded structures in protein increased. Therefore, the application of walnut kernel flour depended on the processing conditions, ingredient formulation during heating treatment.

## Emulsifying Capacity and Stability

The above study showed that different heating methods and time affected the conformation of protein in samples. It was trusted that the structure changes could influence the efficacy of evolving oil droplets due to the different of morphology and surface groups, thus, the functional properties of protein might be affected [54]. The emulsion capacity (EC) reflected the adsorption capacity of protein at the water-oil interface, and the emulsion stability (ES) indicated the retention ability of protein at the oil-water interface after emulsion [54]. Table 4 shows the emulsion capacity (EC) and emulsion stability (ES) of untreated and treated walnut kernel. Untreated sample had the EC of 44.32%. EC of roasting and steaming treated walnut kernel samples ranged from 55.42 to 56.82% and from 52.37 to 56.38%, respectively. EC of walnut kernel heated for 15, 20 and 30 min increased significantly ( $P < 0.05$ ). However, EC of untreated walnut kernel exhibited a significant ( $P < 0.05$ ) difference compared to the heated samples. It was attributed that the heating treatment could cause the changes of native structure or surface properties of proteins in walnut kernel, which promoted more dissolution and the adsorption at the interfacial area [22]. Furthermore, no differences were found between the R15, R20, R30, S15 and S30. In contrast, there was a significant but minor decrease in EA (52.37%) for S30 compared to other samples. In general, the emulsifying properties of samples are due to depending on the strength of the formed interfacial films of protein at the oil-water interface [55]. The increase of protein adsorption and decrease of interfacial energy would hinder the separation of two phases, which resulted in the high EC [56].

Table 4 also shows the variation of the ES at different treatment methods and time. The ES of samples with heating treatment was significantly ( $P < 0.05$ ) higher compared to untreated sample. It was further proved that the samples by heating treatment had better ES due to the better surface characteristics that could be beneficial to hinder the aggregation of droplet [57]. Untreated sample represented ES of 74.21% (Table 4). The ES of R15-R30 and S15-S30 increased by 92.46 - 96.39% compared to untreated samples, which indicated a greater capability of the protein polymer in heat-treated samples to adsorb on the surface of the oil droplet. It was concluded that the heating treatment had the ability to enhance the ES of protein in walnut kernel. It was farther confirmed that the unfolding of proteins would be helpful to improving the surface assimilation and maintenance of processing proteins at the oil-water interface, which would lead to the better diffusion and ES [57]. On the whole, the protein from heated walnut kernel might more reliably accumulate liquids on the surface, which lead to a relatively stable emulsion. Therefore, the samples obtained by heat treatment of walnut kernel would improve the stability in applications of food products [57].

## Conclusion

The thermal treatment significantly influenced the physicochemical, structural, functional and digestibility properties of protein in walnut kernel flour in a temperature-dependent action. SEM micrographs reveled the walnut kernel flour by roasting and steaming treatment more sensitive than the untreated, while the steam treatment had more influence on walnut kernel flour than the roasting treatment. The secondary structures of protein in walnut kernel flours were affected by heating as measured by FTIR, the protein structure changes of samples for steamed processing happened to a greater extent than that of roasted ones. It was found that the heat treatment could result in unfolding of protein, which had significantly affected the functionality of samples. The results showed that the roasting and steaming treatment affected the amino acid composition and amino acid score (AAS), such as the roast processing decreased the AAS for essential amino acids compared to the untreated sample, while the steam processing increased the AAS. But the lysine was the only limiting amino acid for all samples.

It was noted that the digestibility, water holding capacity and oil holding capacity, foaming and emulsion capacities and stabilities of walnut kernel flour during heat processing increased compared to untreated, however, with the extension of heating time, these functional properties would produce the different changes. At the same time, the walnut kernel flours prepared by heating treatment were characterized by high enthalpy ( $H$ ), *storage modulus*( $G'$ ), *loss modulus*( $G''$ ) and *viscosity* compared to that of untreated sample, the order of these values from high to low was:  $H > G' > G'' > \eta$ .

**Author Contribution Xiaoyan Zhao** : Project administration, Resources, Supervision, Funding acquisition, Writing – review & editing. **Haifang Hu** : Project administration, Resources, Supervision, Funding acquisition. **Xiangrui Ren** : Investigation, Writing – original draft. **Xiaowei Zhang** : Data curation, Methodology, Writing - original draft. **Hongkai Liu** : Conceptualization, Software. **Meng Wang** : Validation, Formal analysis.

**Acknowledgments** Financial support of this work by National Natural Science Foundation of China (Grant No. 21406133) and Xinjiang Key Scientific and Technological Project (Grant No. 2021A02002-2).

**Data availability** Xiaoyan Zhao can be contacted for the data

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

## Reference

1. Grilo FS, Wang SC. (2021). Walnut (*Juglans regia* L. ) volatile compounds indicate kernel and oil oxidation. *Foods* 10:329
2. Liu Q, Zhang DJ, Huang Q. (2021). Engineering miscellaneous particles from media-milled defatted walnut flour as novel food-grade Pickering stabilizers. *Food Res Int* 147:110554
3. Ruiz-Caro P, Espada-Bellido E, García-Guzmán JJ, Bellido- Milla D, Vázquez-González M, Cubillana-Aguilera L, Palacios-Santander JM. (2022). An electrochemical alternative for evaluating the antioxidant capacity in walnut kernel extracts. *Food Chem* 393:133417
4. Chen YM, Pei HM, Dai QY, Zhang CM, Kong XZ, Hua YF. (2022). Raw walnut kernel: A natural source for dietary proteases and bioactive proteins. *Food Chem* 369:130961
5. Alasalvar C, Salvadó JS, Ros E. (2020). Bioactives and health benefits of nuts and dried fruits. *Food Chem* 314:26192
6. Santos J, Alvarez-Ortí M, Sena-Moreno E, Rabadán A, Pardob JE, Oliveira MP. (2018). Effect of roasting conditions on the composition and antioxidant properties of defatted walnut flour. *J Sci Food and Agric* 98:1813–1820
7. Samadi Theodoridou K, Yu PQ. (2013). Detect the sensitivity and response of protein molecular structure of whole canola seed (yellow and brown) to different heat processing methods and relation to protein utilization and availability using ATR-FT/IR molecular spectroscopy with chemometrics. *Spectrochim.Acta A Mol Biomol Spectrosc* 105:304–313
8. Borres MP, Sato S, Ebisawa M. (2022). Recent advances in diagnosing and managing nut allergies with focus on hazelnuts, walnuts, and cashew nuts. *World Allergy Organization Journal* 15:100641
9. Choe U, Osorno JM, Ohm J, Chen B, Rao J. (2022). Modification of physicochemical, functional properties, and digestibility of macronutrients in common bean (*Phaseolus vulgaris* L. ) flours by different thermally treated whole seeds. *Food Chem* 382:132570
10. Zheng H, Yan G, Lee Y, Alcaraz C, Marquez S, de Mejia EG. (2020). Effect of the extrusion process on allergen reduction and the texture change of soybean protein isolate-corn and soybean flour-corn mixtures. *Inn Food Sci Emerg Technol* 64: 102421
11. Sruthi,NU, Premjit Y, Pandiselvam R, Kothakota A, Ramesh SV. (2021). An overview of conventional and emerging techniques of roasting: Effect on food bioactive signatures. *Food Chem* 348:129088
12. Ceccanti C, Pellegrinib E, Guidi L. (2021). Effect of superheated steam and conventional steam roasting on nutraceutical quality of several vegetables. *LWT* 149:112014

13. Feng CS, Tian L, Jiao YD, Tan YQ, Liu C, Luo YK, Hong H. (2022). The effect of steam cooking on the proteolysis of pacific oyster (*Crassostrea gigas*) proteins: Digestibility, allergenicity, and bioactivity. Food Chem 379:132160
14. Wu ZX, Li DY, Shen M, Wang ZY, Wang ZW, Liu YX, Bai YH, Zhou DY. (2022). Effect of different *sous-vide* cooking conditions on textural properties, protein physiochemical properties and microstructure of scallop (*Argopecten irradians*) adductor muscle. Food Chem 394:133470
15. AACC. (2022). American association of cereal chemists approved methods (10th ed.). St. Paul, MN: AACC
16. Lu XX, Chang RR, Lu H, Ma RR, Qiu LZ, Tian YQ. (2021). Effect of amino acids composing rice protein on rice starch digestibility. LWT 146:111417
17. Trigui, I. Yaich H, Zouari A, Cheikh-Rouhou S, Blecker C, Attia H, Ayadi MA. (2021). Structure-function relationship of black cumin seeds protein isolates: Amino-acid profiling, surface characteristics, and thermal properties. Food Struc 29:100203
18. WHO/FAO/UNU. (2007). Expert Consultation, Protein and Amino Acid Requirements in Human Nutrition. World Health Organization Technical Report Series, pp. 1–265 <https://doi.org/10.1181/9789241209356>
19. Miao WB, Ma SY, Peng XG, Qin Z, Liu HM, Cai XS, Wang XD. (2021). Effects of various roasting temperatures on the structural and functional properties of starches isolated from tigernut tuber. LWT 151:112149
20. Lei YQ, Gao SH, Xiang XL, Li XT, Yu XW, Li SG. (2021). Physicochemical, structural and adhesion properties of walnut protein isolate-xanthan gum composite adhesives using walnut protein modified by ethanol. Int J Biolog Macromol 192:644–653
21. Di Y, Li X, Chang XW, Gu RJ., Duan X, Liu FG, Liu XB, Wang YT. (2022). Impact of germination on structural, functional properties and in vitro protein digestibility of sesame (*Sesamum indicum* L.) protein. LWT 154:112651
22. Hao LL, Sun JW, Pei MQ, Zhang GF, Li C, Li CM, Ma XK, He SX, Liu LB. (2022). Impact of non-covalent bound polyphenols on conformational, functional properties and in vitro digestibility of pea protein. Food Chem 383:132623
23. Hill HD, Straka JG. (1988). Protein determination using bicinchoninic acid in the presence of sulfhydryl reagents. Anal Biochem 170(1):203–208
24. Shevkani K, Kaur R, Singh N, Hlanze DP. (2022). Colour, composition, digestibility, functionality and pasting properties of diverse kidney beans (*Phaseolus vulgaris*) flours. Curr Res Food Sci 5:619–628
25. Correia I, Nunes A, Barros AS, Delgadillo I. (2010). Comparison of the effects induced by different processing methods on sorghum proteins. J Cereal Sci 51:146–151
26. Denardi-Souza T, Massarolo KC, Tralamazza SM, Badiale-Furlong E. (2018). Monitoring of fungal biomass changed by *Rhizopus oryzae* in relation to amino acid and essential fatty acids profile in soybean meal, wheat and rice. Cyta Journal of Food 16(1):156–164
27. Sze-Tao KWC, Sathe SK. (2000). Walnuts (*Juglans regia* L.): proximate composition, protein, solubility, protein amino acid composition and protein in vitro digestibility. J Sci Food Agric 80(9):1393–1401
28. Liao X, Wang S, Li Y, Olajide TM, Zhai X, Qian J, Miao S, Huang JY. (2022). Effects of “nine steaming nine sun-drying” on proximate composition, protein structure and volatile compounds of black soybeans. Food Res Int 155:111070
29. Zhu YY, An SS, Wang L, Dong JL, Shen RL. (2022). Effects of Thermal Treatments on the Nutritional Quality of Quinoa Protein Isolates. Journal of the Chinese Cereals and Oils Association (Chinese) 37(6):75–82

30. Maetens E, Hettiarachchy N, Dewettinck K, Horax R, Moens K, Moseley DO. (2017). Physicochemical and nutritional properties of a healthy snack chip developed from germinated soybeans. *LWT* 84:505–510
31. Paucar-Menacho LM, Dueñas M, Peñas E, Frias J, Martínez-Villaluenga C. (2018). Effect of Dry Heat Puffing on Nutritional Composition, Fatty Acid, Amino Acid and Phenolic Profiles of Pseudocereals Grains. *Polish J Food Nutr Sci* 68(4):289–297
32. Zhao XY, Chen FS, Xue WT, Lee L. (2008). FTIR spectra studies on the secondary structures of 7S and 11S globulins from soybean proteins using AOT reverse micellar extraction. *Food Hydrocoll* 22(4):568–575
33. Vanga SK, Singh A, Kalkan F, Garipey Y, Orsat V, Raghavan V. (2016). Effect of Thermal and High Electric Fields on Secondary Structure of Peanut Protein. *Int J Food Propert* 19(6):1259–1271
34. Luo X, Wang Q, Wu YY, Duan WS, Zhang YF, Geng F, Song HB, Huang,Q, An FP. (2022). Mechanism of effect of heating temperature on functional characteristics of thick egg white. *LWT* 154:112807
35. Tornberg E, Ediriweera N. (2005). Coalescence stability of protein-stabilized emulsions. *Food emulsions and foams* 52–63
36. Wu Z, Gao RP, Li H, Liao X, Tang X, Wang XG, Su ZM. (2022). How steaming and drying processes affect the active compounds and antioxidant types of *Gastrodia elata* Bl. *f. glauca* S. chow . *Food Res Int* 157:111277
37. Kaushik P, Dowling K, McKnight S, Barrow CJ, Wang B, Adhikari B. (2016). Preparation, characterization and functional properties of flax seed protein isolate. *Food Chem* 197:212–220
38. Liu X, Wu JH, Xu JH, Mao DZ, Yang YJ, Wang ZW. (2016). The impact of heat-moisture treatment on the molecular structure and physicochemical properties of Coix seed starches. *Starch Staerke* 68(7–8):662–674
39. Lavoisier A, Vilgis TA, Aguilera JM. (2019). Effect of cysteine addition and heat treatment on the properties and microstructure of a calcium-induced whey protein cold-set gel. *Curr Res in Food Sci* 1:31–42
40. Fang YZ, Huang QF, Lin YZY, Ge H, Huang GR, Jiang H. (2022). Physicochemical, digestive and rheological properties of protein from tuna by subcritical dimethyl ether: Focus on process-related indexes. *Food Chem* 372:131337
41. Huang ZG, Wang XY, Zhang JY, Liu Y, Zhou T, Chi SY, Gao F, Li J, Tian B, Shi WT, Bi CH. (2022). Effect of heat treatment on the nonlinear rheological properties of acid-induced soy protein isolate gels modified by high-pressure homogenization. *LWT* 157:113094
42. Jiao B, Shi A, Liu H, Sheng X, Liu L, Hu H, Adhikari B, Wang Q. (2017). Effect of electrostatically charged and neutral polysaccharides on the rheological characteristics of peanut protein isolate after high-pressure homogenization. *Food Hydrocoll* 77:329–335
43. Yan XH, Zhao JX, Zeng ZL, Ma MM, Xia JH, Tian WR, Zhang GH, Gong XF, Gong D, Yu P. (2022). Effects of preheat treatment and polyphenol grafting on the structural, emulsifying and rheological properties of protein isolate from *Cinnamomum camphora* seed kernel. *Food Chem* 377:132044
44. Malik MA, Saini CS. (2019). Heat treatment of sunflower protein isolates near isoelectric point: Effect on rheological and structural properties. *Food Chem* 276: 554–561
45. de Vries A, Jansen D, van der Linden E, Scholten E. (2018). Tuning the rheological properties of protein-based oleogels by water addition and heat treatment. *Food Hydrocoll* 79:100–109
46. Chinma CE, Abu JO, Adedeji OE, Aburime LC, Joseph DG, Agunloye GF, Adebo JA, Oyeyinka SA, Njobeh PB, Adebo OA. (2022). Nutritional composition, bioactivity, starch characteristics, thermal and microstructural properties of germinated pigeon pea flour. *Food Biosci* 49:101900

47. Ayala-Rodríguez VA, López-Hernández AA, Lomelí ML, González-Martínez BE, Vázquez-Rodríguez JA. (2022). Nutritional quality of protein flours of fava bean (*Vicia faba* L.) and in vitro digestibility and bioaccessibility. *Food Chemistry: X* 14:100303
48. Cui RB, Yoo MJY, Zhu F. (2022). Comparison of microwave and conventional heating on physicochemical properties and phenolic profiles of purple sweetpotato and wheat flours. *Food Biosci* 46:101602
49. Yang Y, Wang Z, Wang R, Sui X, Qi B, Han F, Li Y, Jiang LZ. (2016). Secondary structure and subunit composition of soy protein in vitro digested by pepsin and its relation with digestibility. *BioMed Res Int* 2006:1–11
50. Carbonaro M, Maselli P, Nucara A. (2012). Relationship between digestibility and secondary structure of raw and thermally treated legume proteins: A fourier transform infrared (FT-IR) spectroscopic study. *Amino Acids* 43(2):911–921
51. Joshi AU, Liu C, Sathe SK. (2015). Functional properties of select seed flours. *LWT* 60(1):325–331
52. Stone AK, Parolia S, House JD, Wang N, Nickerson MT. (2021). Effect of roasting pulse seeds at different tempering moisture on the flour functional properties and nutritional quality. *Food Res Int* 147:110489
53. Sui X, Sun H, Qi B, Zhang M, Li Y, Jiang L (2018). Functional and conformational changes to soy proteins accompanying anthocyanins: Focus on covalent and non-covalent interactions. *Food Chem* 245:871–878
54. Dong WY, Zhang XY, Ding LX, Liu C, Ai MH, Jin YG, Isobe K, Handa A, Cai ZX. (2023). Enhancement of emulsification properties by modulation of egg white protein fibril structure with different heating times. *Food Hydrocoll* 135:108203
55. Damodaran S. (2017). Amino acids, peptides and proteins. In: Damodaran, S., Parkin, K. L. (Eds.), *Fennema's Food Chemistry*, fifth ed. CRC Press, Boca Raton, pp. 235–356
56. Wani IA, Sultan N, Qadir N, Ahmad T. (2021). Physico-chemical, functional and antioxidant properties of wild barnyard grass (*Echinochloa crusgalli* L.) seed flour as affected by gamma-irradiation. *Rad Phys Chem* 183:109409
57. Tang CH. (2020). Globular proteins as soft particles for stabilizing emulsions: Concepts and strategies. *Food Hydrocoll* 103:105664

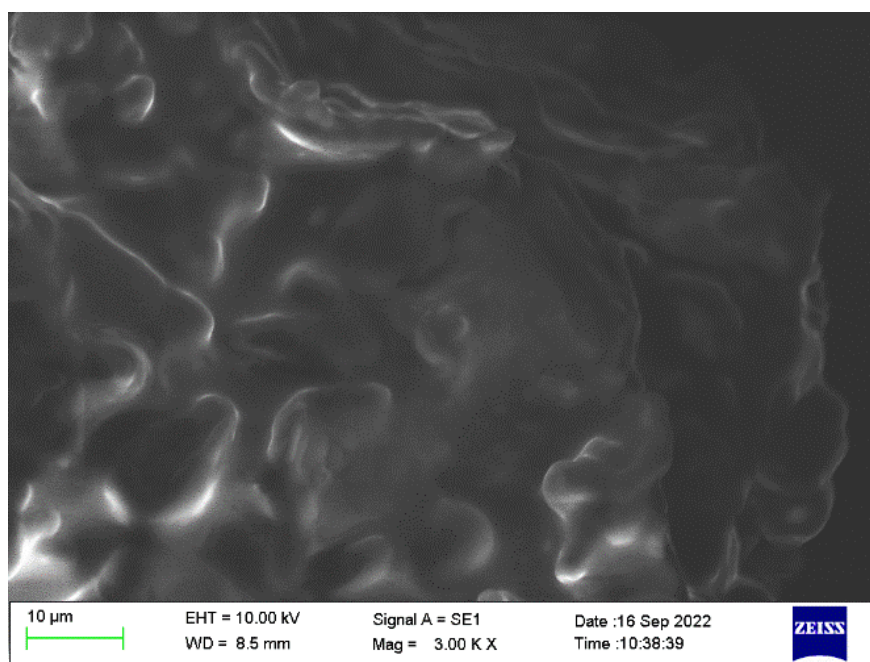
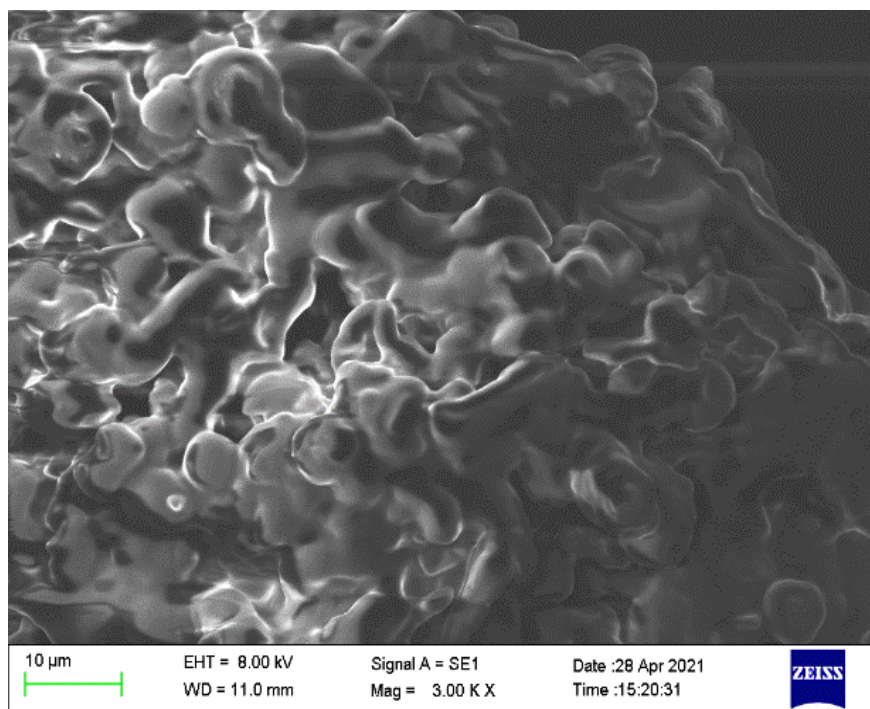
Figure captions:

Fig. 1. Scanning electron microscopy (SEM) images of the flours from walnut kernel with roasting and steaming treatment. A: untreated walnut kernel flour; B-D: walnut kernel treated by steaming for 15, 20 and 30 min, respectively; E-G: walnut kernel treated by roasting for 15, 20 and 30 min, respectively.

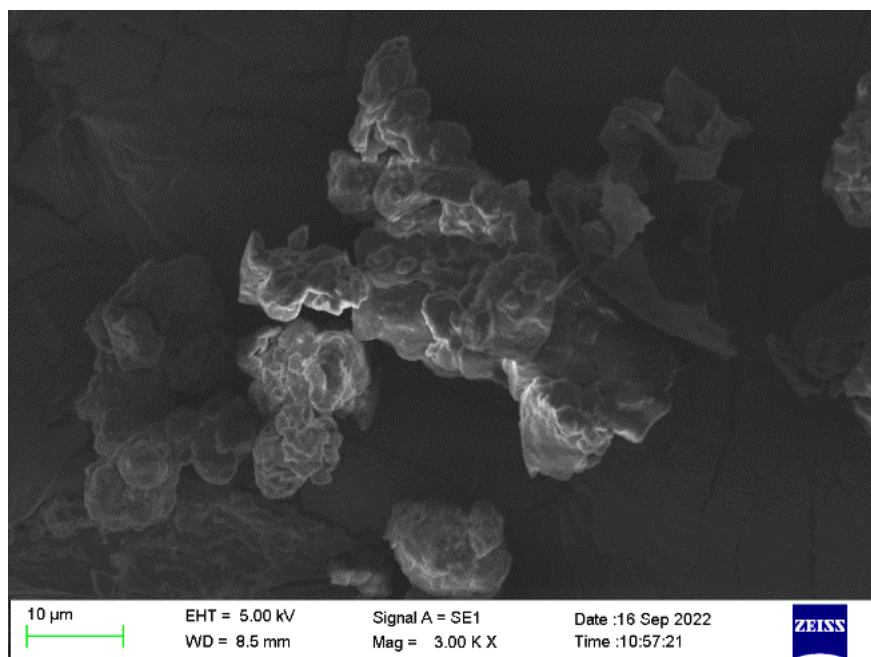
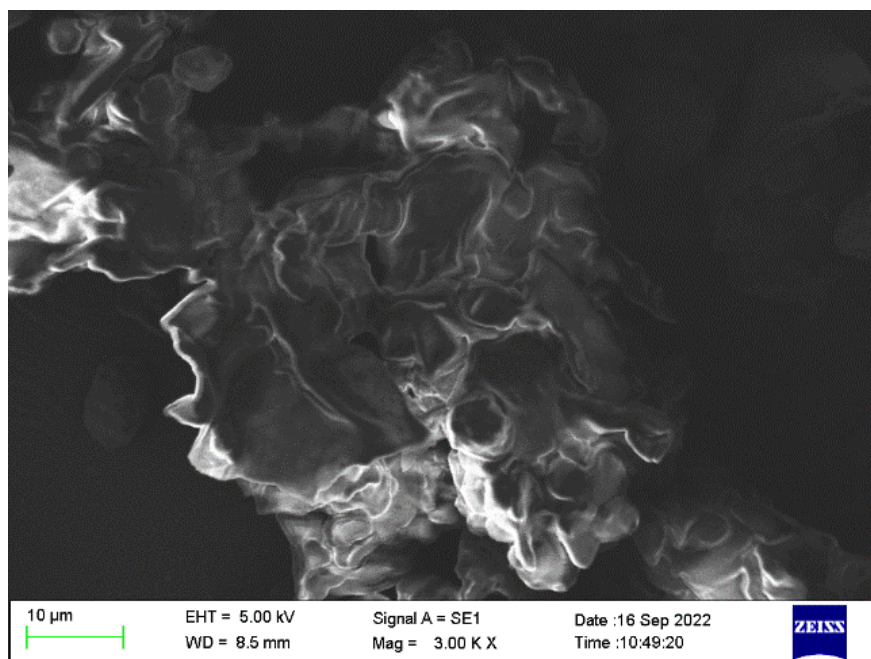
Fig. 2. The rheology properties of the walnut kernel flours with roasting and steaming treatment. A: Plots of storage modulus  $G'$ ; B: Plots of loss modulus  $G''$ ; C: viscosity.

Fig. 3. The determined digestibility (%) curves of walnut kernel flours with roasting and steaming treatment during (A) gastric digestion and (B) intestinal digestion.

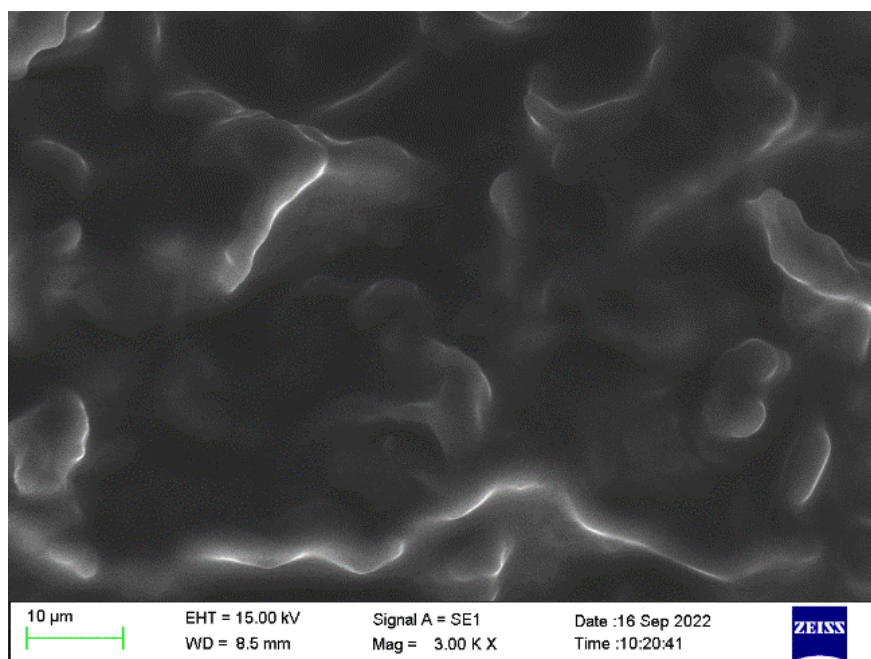
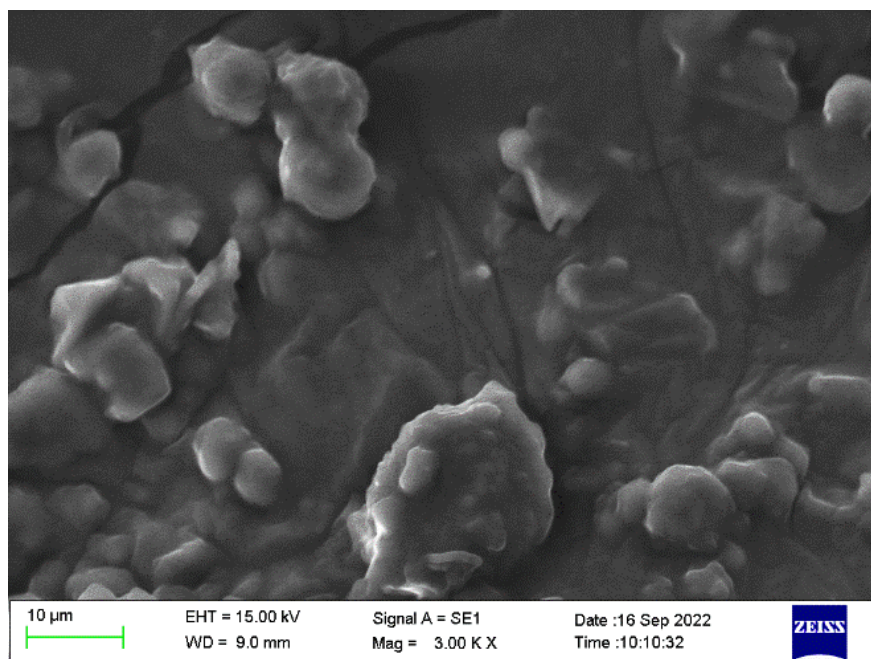
Fig. 1.











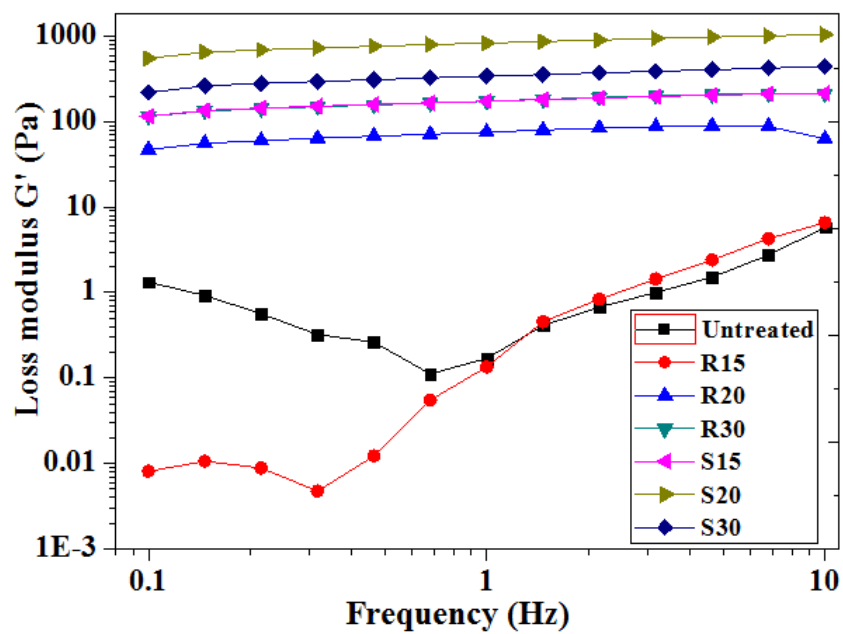
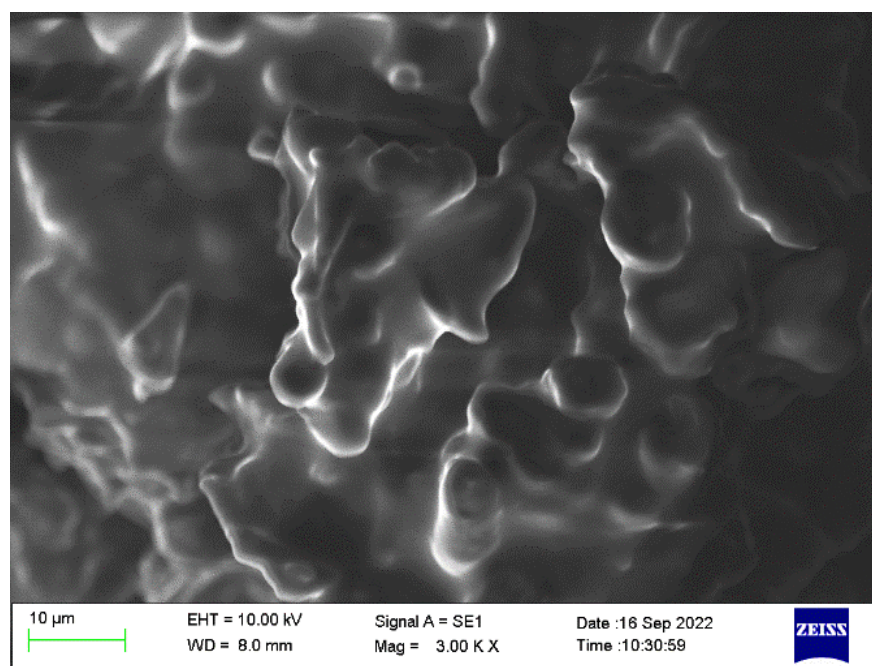


Fig. 2

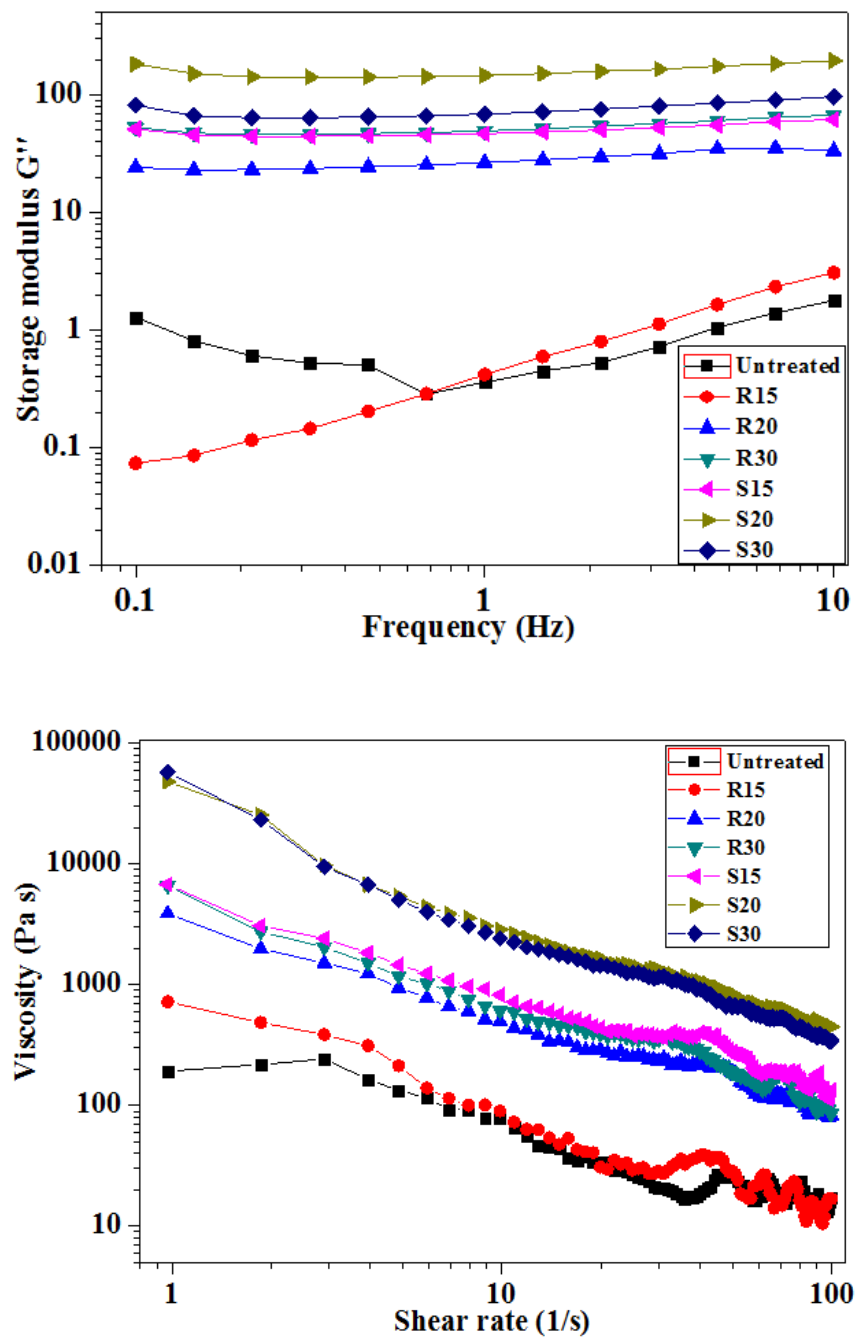


Fig. 3

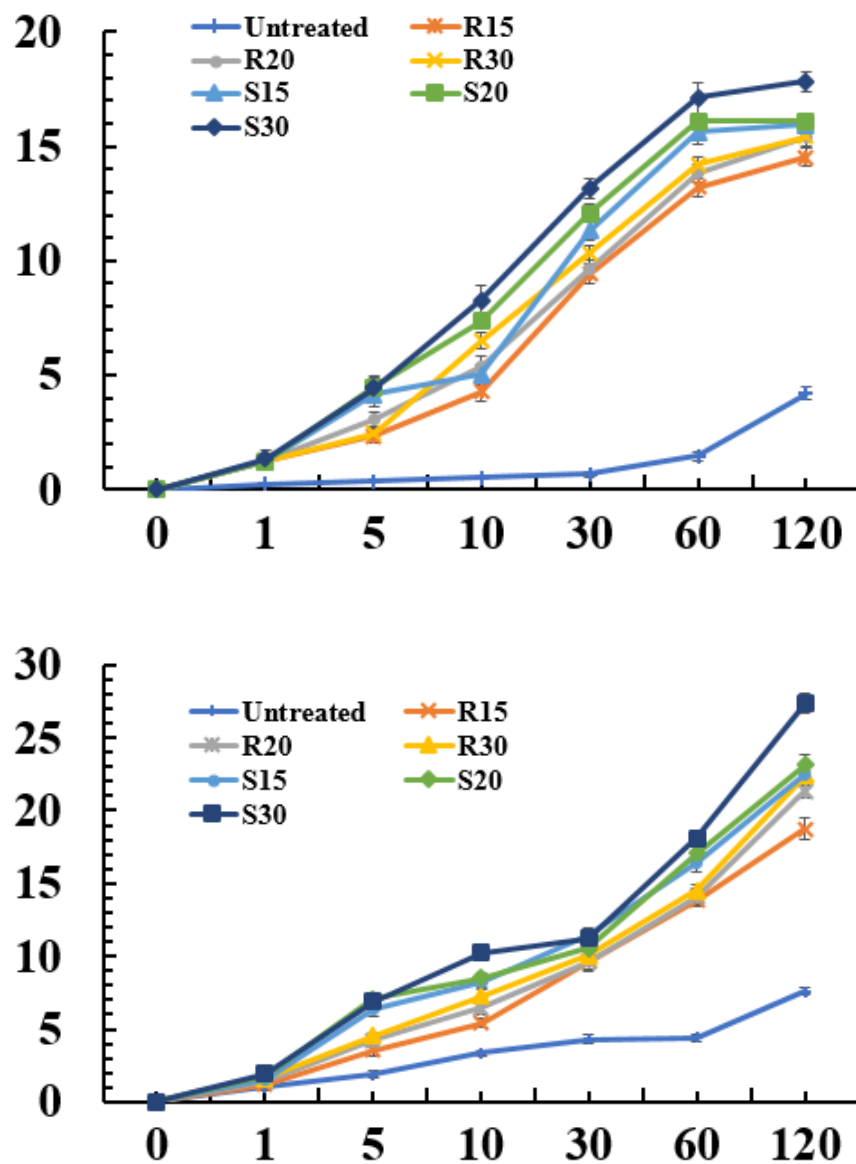


Table 1

The amino acid composition (g 100g<sup>-1</sup> protein) and amino Acid Score for adults (%) of the untreated and heat-processed walnut kernel with high content of protein.

Sample

	AA	Untreated AAS*	Untreated AA	S15 AA
Essential amino acids	Essential amino acids	Essential amino acids	Essential amino acids	Essential amino acids
Histidine	2.74±0.03 <sup>b</sup>	182.67±0.53 <sup>C</sup>	2.87±0.07 <sup>c</sup>	2.87±0.07 <sup>c</sup>
Lysine	1.79±0.01 <sup>bc</sup>	39.78±0.60 <sup>BC</sup>	1.79±0.02 <sup>bc</sup>	1.79±0.02 <sup>bc</sup>
Valine	3.93±0.04 <sup>c</sup>	100.77±0.35 <sup>B</sup>	3.97±0.02 <sup>c</sup>	3.97±0.02 <sup>c</sup>
Leucine	6.95±0.04 <sup>b</sup>	117.80±0.58 <sup>A</sup>	7.01±0.06 <sup>b</sup>	7.01±0.06 <sup>b</sup>

Isoleucine	3.00±0.02 <sup>c</sup>	100.00±0.51 <sup>C</sup>	3.03±0.04 <sup>c</sup>	3.03±0.04 <sup>c</sup>
Phenylalanine	4.19±0.03 <sup>c</sup>	220.53±0.66 <sup>C</sup>	4.11±0.01 <sup>b</sup>	4.11±0.01 <sup>b</sup>
Threonine	2.90±0.02 <sup>c</sup>	126.09±0.47 <sup>B</sup>	2.94±0.06 <sup>cd</sup>	2.94±0.06 <sup>cd</sup>
Methionine	1.22±0.06 <sup>b</sup>	71.76±0.23 <sup>B</sup>	1.21±0.08 <sup>b</sup>	1.21±0.08 <sup>b</sup>
Non- essential amino acids	Non- essential amino acids	Non- essential amino acids	Non- essential amino acids	Non- essential amino acids
Aspartic	Aspartic	6.13±0.16 <sup>e</sup>	6.13±0.16 <sup>e</sup>	5.77±0.16 <sup>e</sup>
Glutamic	Glutamic	18.15±0.34 <sup>c</sup>	18.15±0.34 <sup>c</sup>	15.83±0.34 <sup>c</sup>
Arginine	Arginine	13.26±0.22 <sup>a</sup>	13.26±0.22 <sup>a</sup>	13.48±0.22 <sup>a</sup>
Serine	Serine	5.55±0.03 <sup>b</sup>	5.55±0.03 <sup>b</sup>	5.62±0.03 <sup>b</sup>
Tyrosine	Tyrosine	2.23±0.06 <sup>c</sup>	2.23±0.06 <sup>c</sup>	2.39±0.06 <sup>c</sup>
Cysteine	Cysteine	0.35±0.02 <sup>c</sup>	0.35±0.02 <sup>c</sup>	0.27±0.02 <sup>c</sup>
Alanine	Alanine	4.14±0.02 <sup>b</sup>	4.14±0.02 <sup>b</sup>	4.14±0.02 <sup>b</sup>
Proline	Proline	4.04±0.02 <sup>ab</sup>	4.04±0.02 <sup>ab</sup>	4.03±0.02 <sup>ab</sup>
Glycine	Glycine	5.28±0.13 <sup>b</sup>	5.28±0.13 <sup>b</sup>	5.32±0.13 <sup>b</sup>
Total EAA	Total EAA	26.72±0.12 <sup>a</sup>	26.72±0.12 <sup>a</sup>	26.93±0.12 <sup>a</sup>
TAA	TAA	85.85±2.32 <sup>a</sup>	85.85±2.32 <sup>a</sup>	83.78±2.32 <sup>a</sup>
EAA/TAA(%)	EAA/TAA(%)	30.42±0.01 <sup>a</sup>	30.42±0.01 <sup>a</sup>	33.33±0.01 <sup>a</sup>
Hydrophobic AA	Hydrophobic AA	32.75±0.17 <sup>b</sup>	32.75±0.17 <sup>b</sup>	32.82±0.17 <sup>b</sup>
Hydrophilic AA	Hydrophilic AA	11.03±0.11 <sup>b</sup>	11.03±0.11 <sup>b</sup>	11.22±0.11 <sup>b</sup>
Alkaline AA	Alkaline AA	17.79±0.12 <sup>a</sup>	17.79±0.12 <sup>a</sup>	18.14±0.12 <sup>a</sup>
Acidic AA	Acidic AA	24.28±0.06 <sup>a</sup>	24.28±0.06 <sup>a</sup>	21.60±0.06 <sup>a</sup>

The values were presented as the mean ± standard deviation in triplicate. AA: amino acid; AAS: amino acid score (mg of amino acid in 1 g of test protein/mg of amino acid in requirement pattern) × 100; EAA: essential amino acid; TAA: total amino acid; R: roasting; S: steaming.

\*WHO/FAO/UNU (2007) Expert Consultation Report for adults.

<sup>a-g</sup> Means within a row with different letters were significant difference of the AA composition between samples for each amino acid ( $P \leq 0.05$ ).

<sup>A-F</sup> Means within a row with different letters were a significant difference of the AAS between samples for each essential amino acid ( $P \leq 0.05$ )

Table2

Effect of steaming and roasting treatment on secondary structure of protein in walnut kernel.

Sample	Secondary structure content (%)	Secondary structure content (%)	Secondary structure content (%)	Secondary structure content (%)
	a-helix	β-sheets	β-turns	Random coil
Untreated	11.81±0.28 <sup>a</sup>	47.16±0.72 <sup>c</sup>	27.21±0.41 <sup>d</sup>	12.86±0.41 <sup>d</sup>
R15	14.48±0.32 <sup>b</sup>	47.57±0.29 <sup>c</sup>	15.40±0.31 <sup>a</sup>	12.52±0.31 <sup>a</sup>
R20	19.75±0.34 <sup>d</sup>	48.56±0.48 <sup>c</sup>	20.42±0.35 <sup>b</sup>	11.26±0.35 <sup>b</sup>
R30	24.44±0.41 <sup>e</sup>	39.91±0.32 <sup>b</sup>	19.98±0.25 <sup>b</sup>	14.47±0.25 <sup>b</sup>
S15	15.38±0.43 <sup>b</sup>	47.21±0.37 <sup>c</sup>	22.48±0.24 <sup>c</sup>	12.72±0.24 <sup>c</sup>
S20	18.87±0.36 <sup>c</sup>	46.21±0.63 <sup>c</sup>	20.69±0.27 <sup>b</sup>	13.15±0.27 <sup>b</sup>
S30	25.92±0.32 <sup>f</sup>	36.67±0.33 <sup>a</sup>	19.19±0.19 <sup>b</sup>	16.85±0.19 <sup>b</sup>

The values were presented as the mean ± standard deviation in triplicate; R: roasting; S: steaming.

The different superscript letter of values in same column suggested significantly different ( $P < 0.05$ ) according

to Tukey's test.

Table 3

Thermal behaviour of untreated, steaming and roasting walnut kernel flour

Sample	$H (Jg^{-1})$	$T_{onset} (^{\circ}C)$	$T_{peak} (^{\circ}C)$	$T_{end} (^{\circ}C)$	$T (C)$
Untreated	4.53±0.02 <sup>a</sup>	92.76±1.03 <sup>a</sup>	98.83±0.27 <sup>a</sup>	109.08±0.2 <sup>a</sup>	16.32±0.09 <sup>a</sup>
R15	6.69±0.12 <sup>b</sup>	94.34±0.41 <sup>b</sup>	102.83±0.2 <sup>b</sup>	116.04±0.26 <sup>c</sup>	21.70±0.11 <sup>c</sup>
R20	7.07±0.11 <sup>c</sup>	91.76±0.06 <sup>a</sup>	100.37±0.86 <sup>b</sup>	114.35±0.11 <sup>b</sup>	22.59±0.13 <sup>e</sup>
R30	6.24±0.12 <sup>b</sup>	92.24±1.02 <sup>a</sup>	101.76±0.31 <sup>b</sup>	115.12±0.25 <sup>b</sup>	22.88±0.11 <sup>e</sup>
S15	7.73±0.12 <sup>d</sup>	91.52±0.43 <sup>a</sup>	100.70±0.56 <sup>b</sup>	113.51±0.02 <sup>b</sup>	22.01±0.08 <sup>d</sup>
S20	8.69±0.13 <sup>e</sup>	93.45±1.12 <sup>a</sup>	101.97±0.63 <sup>b</sup>	114.50±0.13 <sup>b</sup>	21.05±0.16 <sup>c</sup>
S30	7.11±0.11 <sup>c</sup>	93.39±0.92 <sup>a</sup>	102.22±0.15 <sup>b</sup>	114.18±0.08 <sup>b</sup>	20.79±0.05 <sup>b</sup>

The values were presented as the mean ± standard deviation in triplicate. R: roasting; S: steaming.

The different superscript letter of values in same column suggested significantly different ( $P < 0.05$ ) according to Tukey's test.

$T_{onset}$ : Onset temperature;  $T_{peak}$ : peak temperature;  $T_{end}$ : conclusion temperature;  $\Delta T$ :  $T_{end} - T_{onset}$ ;  $\Delta H$ : enthalpy; R: roasting; S: steaming.

Table 4

Functional properties of untreated and heated walnut kernel flours ( $n = 3$ ).

Sample	WHC (g g <sup>-1</sup> )	OHC (g g <sup>-1</sup> )	EC (%)	ES (%)	FC (%)	FS (%)
Untreated	2.84±0.03 <sup>b</sup>	1.21±0.01 <sup>a</sup>	44.32±1.26 <sup>a</sup>	74.21±1.15 <sup>a</sup>	62.13±1.13 <sup>a</sup>	43.30±1.23 <sup>a</sup>
R15	3.32±0.16 <sup>c</sup>	5.67±0.12 <sup>c</sup>	55.42±2.21 <sup>c</sup>	96.24±2.41 <sup>b</sup>	65.44±1.15 <sup>b</sup>	80.07±2.06 <sup>e</sup>
R20	2.81±0.01 <sup>b</sup>	5.73±0.09 <sup>c</sup>	56.82±1.77 <sup>c</sup>	96.39±2.08 <sup>b</sup>	64.36±1.11 <sup>b</sup>	80.13±1.97 <sup>e</sup>
R30	1.85±0.04 <sup>a</sup>	5.68±0.03 <sup>c</sup>	56.51±1.42 <sup>c</sup>	95.64±3.68 <sup>b</sup>	68.60±0.53 <sup>c</sup>	77.48±1.82 <sup>c</sup>
S15	4.43±0.08 <sup>e</sup>	5.34±0.11 <sup>c</sup>	56.34±1.31 <sup>c</sup>	95.62±2.75 <sup>b</sup>	69.17±0.36 <sup>d</sup>	80.11±1.52 <sup>e</sup>
S20	3.86±0.03 <sup>d</sup>	4.55±0.13 <sup>b</sup>	56.38±1.27 <sup>c</sup>	92.46±3.13 <sup>b</sup>	71.11±1.12 <sup>d</sup>	78.39±1.37 <sup>d</sup>
S30	3.45±0.01 <sup>c</sup>	4.62±0.11 <sup>b</sup>	52.37±1.08 <sup>b</sup>	96.35±2.95 <sup>b</sup>	69.32±0.58 <sup>d</sup>	75.18±2.07 <sup>b</sup>

Values expressed were mean ± standard deviation in triplicate. WHC and OHC: water holding capacity and oil holding capacity, respectively; FC and FS: foaming capacity and foaming stability, respectively; EC and ES: emulsifying capacity and emulsifying stability, respectively; R: roasting; S: steaming.

Means in the column with different superscript were significantly different at  $P < 0.05$  according to Tukey's test.