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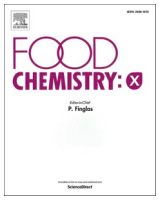

Effect of microwave treatment and water-bath heating treatment on the performance of glutenin from Tiger nut seed meal: Insights into changes in structural characteristics, functional properties, and in vitro gastrointestinal digestibility

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ABSTRACT

In this study, the structural characteristics, functional properties, and in vitro gastrointestinal digestibility of glutenin from Tiger nut seed meal (TNSMG) treated by microwave (140–700 W, 20–60 s) and water-bath heating (40–100 °C, 10–30 min) were investigated. Analysis of the surface hydrophobicity, intrinsic fluorescence spectroscopy and Fourier transform infrared spectroscopy indicated that both microwave and water-bath heating treatments caused structure changes of TNSMG. The results showed an increase in the exposure of sulfhydryl groups and the content of β-sheet, coupled with a decrease in the content of α-helix and β-turn. These structural changes contributed to the improved solubility, foamability, emulsification properties, and digestibility of TNSMG under proper thermal treatment conditions. TNSMG exhibited the best solubility (68.48%) and foamability (85.56%) after water-bath heating treatment for 20 min at 80 °C. Furthermore, TNSMG showed the best emulsification property (9.61 m^2/g) and digestibility (78.58%) when treated by microwave treatment at 560 W for 40 s.

1. Introduction

The extensive utilization of proteins in the food industry can be attributed to their high nutritional value and diverse range of functional properties (Wolfe et al., 2018). Proteins not only provide energy and essential amino acids for humans but also exhibit excellent foamability, emulsification property and gel property (Xue et al., 2022). These properties make proteins valuable as quality improvers in the food industry. However, protein's spatial structure, functional properties and digestibility can be influenced by external factors such as temperature, pressure, pH value, salt concentration, and ethanol.

Heat treatment has a wide range of applications in food processing, including water-bath heating, baking, microwave, and high pressure. Among them, microwave treatment and water-bath heating treatment are extensively employed due to their convenience, safety, and environmental friendliness. The two methods can not only improve functional properties of protein but also enriches its taste profile (Huang et al., 2022; Peng et al., 2016). The popularity of microwave treatment technology is increasing due to its principle of generating heat through molecular rearrangement-induced internal friction, offering advantages such as improved product quality, enhanced thermal efficiency, and reduced processing time (Aguilar-Reynosa et al., 2017). Similarly, water-bath heating treatment is also extensively employed within the food industry due to its capability to provide uniform heat distribution and enabling precise control over reaction temperatures. The utilization of water-bath heating treatment techniques is indispensable in various food processing procedures, such as pasteurization and sterilization (Peng et al., 2016). Research by Ma et al. (2022) demonstrated a noteworthy enhancement in the emulsification of *Cocos nucifera* L. globulins when heated at 90 °C. Additionally, trout frame protein, when pretreated with microwave for 5 min at 90 °C, exhibited a reduced level of anti-nutritional factors, thereby showing the higher digestibility

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(Ketnawa et al., 2018). However, proteins are sensitive to thermal processing, and their nutritional and functional properties may suffer under inappropriate processing conditions. For instance, Bassogog et al. (2022) found that *moringa oleifera* seed proteins isolate exhibited excellent emulsification properties at room temperature. Nevertheless, when exposed to a higher temperature (80 °C), its emulsification properties deteriorated. Similarly, previous research carried out by Chen et al. (2023) demonstrated that when the heating temperature exceeded 85 °C, the digestibility of myofibrillar protein decreased. This is likely due to the changes in secondary structure, leading to a more compact structure within protein aggregates. Thereby, the accessibility of gastrointestinal digestive enzymes was influenced, ultimately reducing protein digestibility. Therefore, appropriate thermal treatments conditions are crucial for protein processing to maximize the effectiveness of proteins.

Glutenin from Tiger nut seed meal (TNSMG) is a kind of high-quality protein, containing 18 distinct amino acids, with essential amino acids accounting for 46.03%, surpassing the WHO/FAO model's specified value of 36% for adults (Cui et al., 2021). In addition, the high content of lysine in tiger nut protein can provide nutritional complementarity with other cereal proteins (Yu et al., 2022). However, the solubility and emulsifying properties of natural TNSMG are relatively poor (Li et al., 2024). It is meaningful to improve the functional properties and applications of TNSMG through heating treatment. Given that microwave treatment and water-bath heating treatment are common methods in food processing, it is crucial to explore the alterations in the structure, functional properties, and digestibility of TNSMG under the two treatments. Thus, the functional properties and digestibility of TNSMG under different microwave and water-bath heating treatment conditions were detected. Structural alterations of TNSMG before and after thermal treatment were also investigated through analysis of surface hydrophobicity (H_0), fluorescence spectroscopy and Fourier transform infrared (FTIR) spectroscopy. Our work seeks to identify optimal treatment conditions to enhance the performance of TNSMG, and provides theoretical underpinning for its effective utilization in thermal treatments.

2. Materials and methods

2.1. Materials

Cold-pressed tiger nut meal was procured from Tangshan Kunyuan Sanhe Agricultural Technology Co., Ltd. (Hebei, China). β-Mercaptoethanol and Ammonium 8-aniline-1-naphthalene sulfonate (ANS) were obtained from Yuanye Bio-Technology Co., Ltd. (Shanghai, China). Potassium bromide and sodium dodecyl sulfate were acquired from Aladdin Biochemical Technology Co., Ltd. (Shanghai, China). *O*-phenyldiformaldehyde and Sodium tetraborate were procured from Solarbio Technology Co., Ltd. (Beijing, China). Pepsin, trypsin, and bovine serum albumin (BSA) were procured from Sigma-Aldrich (Israel). Hydrochloric acid, sodium hydroxide and hexane were obtained from Beijing Chemical Reagent (Beijing, China).

2.2. Preparation of the TNSMG

Dried tiger nut seed meal (TNSM) ground using a blender and mixed with n-hexane at the ratio of 1:5 (*w/v*) for 3 h. The precipitate was harvested through centrifugation and repeated twice. The solvent was evaporated for 12 h and the defatted TNSM powder (DTNSMP) was kept in a freezer at −20 °C until used.

The Osborne method was used to extract the TNSMG with slight modifications (Kizzie-Hayford et al., 2015). Briefly, the DTNSMP (100 g) introduced into 1 L distilled water with magnetic stirring for 1 h, then centrifuged (10,000 rpm, 20 min at 4 °C). The precipitate was added to 1 L of NaCl (0.5 M) for 1 h and centrifuged at 10000 rpm for 20 min at 4 °C. Globular proteins were present in the supernatant, which was then discarded. The precipitation was sequentially combined with NaOH (0.1 M), then centrifuged at 10000 rpm for 20 min at 4 °C to obtain the supernatant as TNSMG. The steps were performed twice to recover most of TNSMG. The supernatant obtained from twice extractions was mixed, and adjusted the pH to the isoelectric point of the protein using HCl (1 M) to precipitate the TNSMG.

The solutions were incubated at 4 °C, subsequently centrifuged (8000 rpm, 15 min at 4 °C) to get precipitates. The precipitates were redissolved and the pH was adjusted to 7. Then, the protein samples underwent dialyzed with 3.5 kDa dialysis bag and freeze-dried in vacuo, the protein content of TNSMG was 80.16%.

2.3. Microwave treatment and water-bath heating treatment on TNSMG

For microwave treatment, TNSMG solution (3 mg/mL) was treated at 0, 140, 280, 420, 560, and 700 W power levels for 20, 40, and 60 s, respectively.

For water-bath heating treatment, TNSMG solution (3 mg/mL) underwent varying temperatures of 0, 40, 60, 80, and 100 °C for 10, 20, and 30 min, respectively. After completing the thermal treatment process, a portion of the sample was rapidly cooled (−20 °C, 5 min) and stored at 4 °C (usable within one week), while the other part was freeze-dried for subsequent experiments.

2.4. H_0

H_0 was determined using the method of Yang et al. (2021). ANS (8 mmol/L, 20 μL) was used as a fluorescent probe and mixed with TNSMG samples (0.05–0.5 mg/mL, 4 mL), then incubated in darkness for a duration of 15 min. Subsequently, the fluorescence intensity was detected using an Rf-5301pc fluorescence spectrophotometer (Hitachi, Tokyo, Japan), the excitation wavelength was configured at 390 nm, the emission wavelength was configured at 470 nm.

2.5. Intrinsic fluorescence spectroscopy

The fluorescence spectroscopy of TNSMG (0.2 mg/mL) was analyzed with a fluorescence spectrophotometer, with an excitation wavelength fixed at 290 nm. The scanning range was set from 300 to 450 nm, and the voltage was maintained at 700 V.

2.6. FTIR

Briefly, a mixture of 2 mg TNSMG and 198 mg KBr was compressed into sheets. The spectra were scanned 32 times in the range of 400 to 4000 cm^{-1} using a Fourier infrared spectrometer (Vertex70, Bruker, Germany).

2.7. Solubility

The TNSMG was dissolved in deionized water following various thermal treatments and centrifuged at 8000 rpm at 4 °C for 15 min. Further, the TNSMG content in the supernatant under various treatment conditions was quantified using BSA as a reference standard (Mohan & Mellem, 2020). The TNSMG content was determined by BCA Protein Quantification Kit (P0010, Shanghai Beyotime Biotechnology Co., Ltd). Solubility was estimated using the formula:

$$\text{Solubility}\ (\%) = \frac{\text{TNSMG content of supernatant}}{\text{TNSMG content of protein sample}} \times 100\% \quad (1)$$

2.8. Foaming ability (FA) and foam stability (FS)

The FA and FS of TNSMG were determined with modification based on the previous study (Arteaga et al., 2020). A high-speed homogenizer was employed to homogenize TNSMG solution (3 mg/mL) at 12000 rpm

for 1 min. Subsequently, the total volume of the solution was assessed. FA and FS were estimated using the formula with V (volume before centrifuge), V_0 (volume after stirring) and V_{30} (volume after standing for 30 min):

$$FA(\%) = \frac{V_0 - V}{V} \times 100\% \quad (2)$$

$$FS(\%) = \frac{V_{30} - V}{V_0 - V} \times 100\% \quad (3)$$

2.9. Emulsifying activity (EAI) and emulsifying stability (ESI)

EAI and ESI of TNSMG were assessed by a previous method with minor modifications (de Souza et al., 2020). The TNSMG solution (2 mg/mL) was homogenized (10,000 rpm, 1 min) with soybean oil. Then, 50 μL of solution from the bottom of the container was combined with 5 mL of SDS solution (0.1%) immediately, and the absorbance of the resulting solution was determined at 500 nm. The EAI and ESI were calculated using the formula with A_0 and A_{10} (the absorbance value of solution at 0 min and 10 min):

$$EAI\ (m^2/g) = 2T\frac{A_0 \times N}{\varphi \times L \times C \times 10000} \quad (4)$$

$$ESI\ (min) = \frac{A_0 \times 10}{A_0 - A_{10}} \quad (5)$$

N: dilution factor; $T = 2.303$; L: optical diameter; φ: volume fraction of oil (0.25); C: initial concentration of protein solution (2 mg/mL).

2.10. Digestibility

The in vitro gastrointestinal digestion of TNSMG was conducted following the method of Liu et al. (2022). To simulate gastric digestion, 10 mL of simulated gastric fluid (SGF) containing pig pepsin at a concentration of 2000 U/mL was preheated in a water-bath heating treatment at 37 °C. Then, the TNSMG (10 mL , 5 mg/mL) was added to the SGF solution, and made the final pH at 3.0 using HCI solution. Further, the samples were digested by SGF at 37 °C, and each sample was collected at various time points of the digestion (0, 5, 10, 20, 30, 45, 60, 90, and 120 min). NaOH solution was used as a termination reaction solution.

To simulate intestinal digestion, bile salts (5 mg/mL) and porcine trypsin (250 U/mL) were added to 10 mL of simulated intestinal fluid (SIF) to simulate intestinal digestion. Further, 10 mL of gastric digest was mixed with SIF, and then the final pH of the mixture was adjusted to 7.0 using NaOH solution. Following, the samples were continuously shaken at 37 °C for a duration of 2 h. Afterwards, the digestive fluid mixtures were taken out at intestinal digestion time point of 5, 10, 20, 30, 45, 60, 90 and 120 min and boiled for 10 min to stop the process of digestion. The samples were centrifuged (8000 rpm, 15 min at 4 °C) and the concentration of TNSMG in supernatant were measured by BCA protein assay.

2.11. Statistical analyses

The experiments were repeated three times, and the dates were presented as mean value ± standard deviation. Statistical analyses ($p < 0.05$) were conducted using SPSS software version 24.0 (IBM Corporation, Chicago, IL, USA).

3. Results and discussion

3.1. H_0 analysis

H_0 is commonly employed as a reliable indicator of protein conformation. Moreover, it has been documented that H_0 significantly influences the functional properties of proteins, including foaming, emulsification, and gelation (Sahil et al., 2022). As illustrated in Fig. 1A, an increase in microwave treatment power over a short duration of 20 s didn't result in a significant alteration in H_0 of TNSMG. The H_0 of TNSMG initially increased, followed by a subsequent decrease in response to variations in microwave power, reaching its peak value of 2784.33 at 560 W for 40 s. One possible reason was that, when the TNSMG exposed to microwave heating, the hydrogen bonding and intermolecular interactions of TNSMG weaken, resulting in the unfolding of its molecular structure. Subsequently, hydrophobic groups, once concealed within the proteins, gradually became exposed on the surface with increasing microwave treatment power and duration (Han et al., 2021). However, with a microwave treatment power of 700 W, an increase in the duration of heating resulted in a reduction in H_0. The possible reason for this phenomenon was that protein molecules aggregated, and the surface hydrophobic residues in TNSMG combined to each other and cannot be exposed, thereby reducing the H_0, this finding was consistent with previous research of Han et al. (2019).

Compared with microwave treatment, the impact of water-bath heating treatment on H_0 of TNSMG was less significant. As shown in Fig. 1B, the value of H_0 exhibited a positive correlation with treatment time at the temperatures below 80 °C, and reached its maximum of 2672.67 at 80 °C for 20 min. This was due to with the extension of heating time, protein molecules undergo spatial recombination, exposing the hydrophobic region of their core, which led to an increase in H_0 (Bu et al., 2022). On the contrary, H_0 exhibited an inverse relationship with the duration of heating when the temperature exceeded 80 °C. The reason for this phenomenon was that when the heating temperature exceeded the denaturation temperature, the TNSMG structure unfolded and became disordered, resulting in the exposure of internal hydrophobic groups (Ren et al., 2018). However, with the rising temperature, protein aggregation occurred, causing a partial burial of the hydrophobic binding site and consequently resulting in a reduction of H_0 (Cao et al., 2019).

3.2. Intrinsic fluorescence spectroscopy analysis

The utilization of intrinsic fluorescence spectroscopy can characterize the alterations of protein structure. In Fig. 2, it can be observed that both microwave treatment and water-bath heating treatment effectively increased fluorescence intensity and caused a red-shift in the maximum emission wavelength compared to untreated protein. As depicted in Fig. 2A and D, the fluorescence intensity exhibited an upward trend with increasing microwave treatment power and water-bath heating treatment temperature during short heating durations (20 s for microwave treatment and 10 min for water-bath heating treatment). Concurrently, the emission peak wavelength red-shifted from 335.0 nm to 338.8 nm under microwave treatment and to 338.2 nm under water-bath heating treatment, respectively. The reason for these changes was that the molecular configuration of TNSMG became unfolded, causing amino acid residues to relocate from the interior to polar surface of TNSMG (Ling et al., 2019). As illustrated in Fig. 2C and F, when subjected to microwave treatment for 60 s and water-bath heating treatment for 30 min, the fluorescence intensity of TNSMG exhibited a decrease, accompanied by a blue-shift in the emission peak with increasing microwave treatment power and water-bath heating treatment temperature. One possible explanation was that the exposed groups on the surface were more likely to interact with each other as the treatment intensity increased, resulting in fluorescence quenching. Another potential reason was the formation of larger aggregates of the protein by the increased temperature, which encased the fluorescent chromophore (Ling et al., 2019). These observations align with the results of Zheng et al. (2023), the fluorescence intensity of cod protein decreased with the prolonged heating. This was due to the exposure of fluorophores in the protein as the heating time was extended from 30

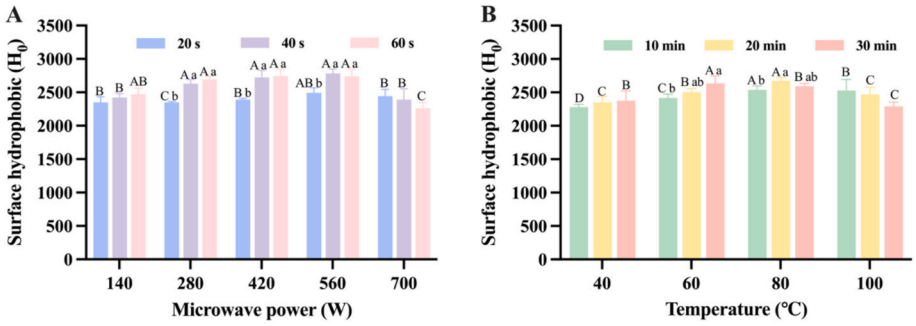

Fig. 1. Effects of different treatment conditions on surface hydrophobic of glutenin from tiger nut seed meal (TNSMG). (A) Microwave treatment; (B) Water-bath heating treatment. The letters (A-D) mean significant differences between groups ($p < 0.05$); The letters (a-c) mean significant differences within groups ($p < 0.05$), and unlabeled letters mean no significant difference.

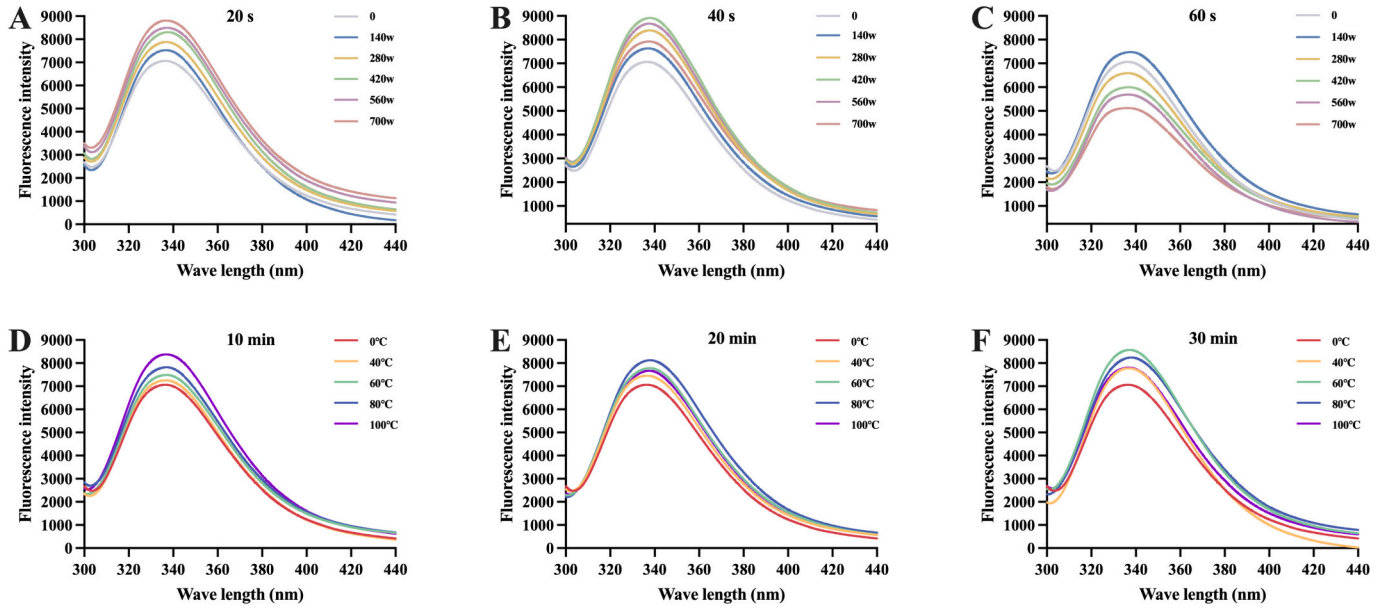

Fig. 2. The intrinsic fluorescence spectroscopy of TNSMG. (A) Microwave treatment for 20 s at different power levels; (B) Microwave treatment for 40 s at different power levels; (C) Microwave treatment for 60 s at different power levels; (D) Water-bath heating treatment for 10 min at different temperature; (E) Water-bath heating treatment for 20 min at different temperature; (F) Water-bath heating treatment for 30 min at different temperature.

min to 120 min, interacting with the polar solvent and led to fluorescence quenching, which in turn reduced the fluorescence intensity.

Overall, the intrinsic fluorescence results were in alignment with those obtained from H_0. When the microwave power was <420 w and within 40 s, and the water-bath heating temperature is lower than 80 °C and within 20 min, the emission peak red-shifted with increased time, consistent with the increase in H_0. However, when the heat treatment exceeded the strength, the emission peak blue-shifted, and H_0 decreased. These results indicating that microwave treatment and water-bath heating treatment induced alterations in the tertiary structure of TNSMG.

3.3. FTIR analysis

The secondary structure of TNSMG were assessed by FTIR. The content of secondary structures of TNSMG under various conditions of microwave and water-bath heating treatment were illustrated in Table 1. Over a brief period of 20 s, the content of β-sheet and random coil conformations in TNSMG witnessed a slight increase, whereas the α-helix decreased in response to the rising microwave power. This finding suggested that microwave treatment under appropriate conditions could promote collisions between protein molecules, part of the α-helix structure was transformed into random coil conformation which made protein become disordered (Malik & Saini, 2019). Under 40 s, with the increased of microwave power, the content of β-sheet and random coil conformation slightly decreased first and then increased, and the α-helix increased first and then decreased. When the protein was treated for a long time (60 s), the β-sheet and random coil conformation in the protein basically showed a decrease, and the α-helix showed an overall increase trend. The content of the β-turn remained essentially unchanged during the treatment. These results might be attributed to alterations in hydrogen bond between TNSMG and the formation of protein aggregated under excessive power and time (Dong et al., 2021).

However, in contrast to the results obtained with microwave treatment, β-turn and random coil conformation of TNSMG did not change significantly after water-bath heating treatment. As the temperature increased, the α-helix of the TNSMG gradually decreased and the β-sheet increased within 20 min of treatment periods. The observation indicated that water-bath heating treatment disrupted the secondary structure of TNSMG, leading to alterations in hydrophobic interactions and hydrogen bond, promoting the exposure of hydrophobic residues and facilitating protein folding. Previous studies of Chang et al. (2020) demonstrated a positive correlation between the β-sheet content and the hydrophobicity in protein molecules, exhibiting a similar variation

Table 1
The secondary structural percentage of glutenin from tiger nut seed meal after microwave and water-bath heating treatment. Different superscripts within a raw indicate a significant distinction ($p < 0.05$).

Method	Time	Intensity	α-helix (%)	β-sheet (%)	β-turn (%)	Random coil (%)
	Initial		26.51 ± 0.28^{a}	38.30 ± 0.61^{g}	19.40 ± 0.34	15.76 ± 0.32^{def}
		140 W	26.28 ± 0.20^{ab}	38.65 ± 0.28^{fg}	19.22 ± 0.33	15.79 ± 0.55^{def}
		280 W	25.54 ± 0.35^{cd}	39.06 ± 0.82^{fg}	19.07 ± 0.43	17.05 ± 0.82^{ab}
		420 W	24.35 ± 0.54^{gh}	40.15 ± 0.07^{cde}	18.89 ± 0.36	17.01 ± 0.37^{abc}
		560 W	23.84 ± 0.28^{hi}	40.32 ± 0.71^{bcd}	18.64 ± 0.55	17.17 ± 0.68^{ab}
	20 s	700 W	23.17 ± 0.66^{i}	41.05 ± 0.57^{abc}	18.38 ± 0.45	17.37 ± 0.42^{a}
		140 W	25.49 ± 0.42^{cd}	39.55 ± 0.74^{def}	19.06 ± 0.47	16.04 ± 0.34^{de}
		280 W	24.81 ± 0.62^{efg}	40.07 ± 0.56^{cde}	18.90 ± 0.52	16.22 ± 0.50^{cde}
		420 W	23.38 ± 0.55^{i}	41.31 ± 0.45^{ab}	18.88 ± 0.35	16.51 ± 0.21^{bcd}
		560 W	23.67 ± 0.51^{i}	41.65 ± 0.63^{a}	18.47 ± 0.56	16.13 ± 0.33^{de}
		700 W	24.55 ± 0.47^{fg}	40.67 ± 0.46^{abc}	18.74 ± 0.33	16.09 ± 0.69^{de}
	40 s	140 W	25.05 ± 0.65^{def}	40.32 ± 0.22^{bcd}	18.87 ± 0.66	15.81 ± 0.43^{def}
		280 W	24.65 ± 0.27^{fg}	41.15 ± 0.46^{ab}	19.04 ± 0.41	15.20 ± 0.58^{fg}
		420 W	25.37 ± 0.43^{cde}	40.84 ± 0.51^{abc}	19.12 ± 0.43	14.68 ± 0.31^{g}
		560 W	25.82 ± 0.31^{bc}	40.33 ± 0.69^{bcd}	19.23 ± 0.56	14.62 ± 0.53^{g}
Microwave	60 s	700 W	26.03 ± 0.08^{abc}	39.18 ± 0.64^{efg}	19.32 ± 0.52	15.52 ± 0.66^{ef}
		40 °C	26.07 ± 0.29^{ab}	38.96 ± 0.58^{d}	19.23 ± 0.46	16.27 ± 0.81
		60 °C	25.53 ± 0.49^{bcde}	38.47 ± 0.45^{cd}	19.07 ± 0.55	16.45 ± 0.52
		80 °C	25.05 ± 0.66^{defg}	39.44 ± 0.44^{bc}	18.89 ± 0.63	16.62 ± 0.34
	10 min	100 °C	24.29 ± 0.60^{g}	40.08 ± 0.34^{ab}	18.70 ± 0.51	16.89 ± 0.49
		40 °C	25.87 ± 0.54^{abcd}	39.04 ± 0.53^{cd}	19.15 ± 0.49	15.94 ± 0.69
		60 °C	25.15 ± 0.27^{cdefg}	39.54 ± 0.25^{bc}	19.00 ± 0.37	16.25 ± 0.50
		80 °C	24.53 ± 0.36^{fg}	40.45 ± 0.48^{a}	18.63 ± 0.46	16.32 ± 0.61
	20 min	100 °C	24.87 ± 0.57^{efg}	40.13 ± 0.58^{ab}	18.91 ± 0.50	16.13 ± 0.32
Water-bath heating	30 min	40 °C	25.13 ± 0.72^{cdefg}	39.68 ± 0.29^{abc}	18.98 ± 0.21	16.21 ± 0.62
		60 °C	25.36 ± 0.67^{bcdef}	39.44 ± 0.58^{bc}	19.02 ± 0.38	16.15 ± 0.24
		80 °C	25.81 ± 0.52^{abcd}	39.01 ± 0.84^{cd}	19.20 ± 0.55	16.02 ± 0.36
		100 °C	25.97 ± 0.54^{abc}	38.80 ± 0.50^{cd}	19.26 ± 0.55	16.00 ± 0.31

trend to our study. However, a long time (30 min) of heat treatment would change the β-sheet into α-helix, which indicated that the α-helix became the main structure to stabilize the protein thermal aggregates.

The impact of microwave treatment on the secondary structure of TNSMG was more pronounced than that of water-bath heating treatment. When the microwave power <420 and within 40 s, there was a significant elevation in the β-sheet content of TNSMG, accompanied by a more notable decreased in α-helix content. The observed results may be attributed to the synergistic interplay between thermal and non-thermal effects induced by microwave treatment. These effects disrupted the hydrogen bonding crucial for α-helix stability, concurrently promoting a towards to β-sheet structure.

3.4. Solubility analysis

The solubility of proteins is a primary functional and serves as the foundation for other functional properties. As shown in Fig. 3, the solubility of TNSMG showing an initial increase followed by a subsequent decline under various microwave treatment and water-bath heating treatment conditions. As depicted in Fig. 3A, a positive correlation between protein solubility and the duration of microwave treatment was observed when the microwave power <280 W. The maximum solubility of 68.88% was achieved at a microwave treatment power of 420 W for 40 s. This phenomenon can be explained by moderate microwave treatment inducing the rotation of internal molecules in TNSMG, disrupting the structure of TNSMG, reducing particle size, and enhancing solubility by promoting the interaction of water molecules. The alternative explanation was that the electrostatic repulsion between TNSMG was intensified, leading to the dispersion of insoluble molecules aggregates and forming more stable structures resistant to gravitational sedimentation (Momen et al., 2018). However, an increased in processing duration at a microwave power of 420 W resulted in a decreased in protein solubility. This was likely due to a decrease in electrostatic repulsion among proteins because of the excessive microwave treatment power. Consequently, proteins were distributed unevenly and reaggregated into insoluble forms, leading to a decline in solubility (Zheng et al., 2020).

As depicted in Fig. 3B, the solubility of TNSMG exhibited a direct correlation with both heating time and temperature when the temperature below 80 °C, and reaching the maximum value of 68.48% at 80 °C for 20 min. These findings suggested that appropriate thermal conditions could enhance the protein-water hydrogen bonding structure and promote the association between TNSMG and water molecules. When temperature reached 100 °C, the protein underwent excessive denaturation, leading to molecular reaggregation and a subsequent reduction in solubility.

Based on the above results, microwave treatment and the water-bath heating treatment had almost the same degree of influence on the solubility of TNSMG. To achieve the optimal solubility of TNSMG, microwave treatment conditions of 420 W for 40 s and water-bath heating treatment conditions of 80 °C for 20 min can be used.

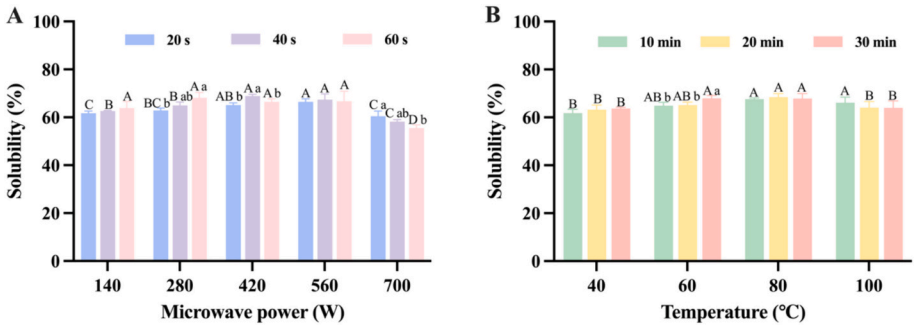

Fig. 3. Effects of different treatment conditions on solubility of TNSMG. (A) Microwave treatment; (B) Water-bath heating treatment. The letters (A-C) mean significant differences between groups ($p < 0.05$); The letters (a, b) mean significant differences within groups ($p < 0.05$), and unlabeled letters mean no significant difference.

3.5. Foaming property

FA and FS of TNSMG under microwave treatment were presented in Fig. 4A and C. A clear proportionality between FA, FS, and the duration of microwave treatment was observed when the microwave treatment power was below 420 W. During this process, the covalent bond of protein molecules was disrupted, leading to a structural expansion which helped the protein to adhere to the air-water interface rapidly, forming a viscous film, promoting foam formation and improving its stability. However, the foaming properties of TNSMG were diminished at high microwave treatment power. It can be ascribed to the excessive microwave power, which may lead to the desorption of TNSMG from the air-liquid interface, causing the interaction with other molecules and forming aggregates (Li et al., 2017).

When TNSMG was subjected to water-bath heating treatment, the FA and FS exhibited a similar trend to that observed under microwave treatment. As depicted in Fig. 4B and D, when the heating temperature was below 80 °C, FA and FS exhibited a positive correlation with the water-bath heating treatment temperature and duration. The possible reasons for these phenomena could be attributed to the moderate heating, which enhanced the aggregation of TNSMG by affecting the electrostatic and hydrophobic interaction among proteins. However, when the heating temperature reached 100 °C, there was a decline in FA and FS. The findings in our investigation aligned with those presented by Ling et al. (2019), who demonstrated a decrease in the foaming properties of protein isolates in rice bran as the temperature increased from 80 °C to 120 °C.

In terms of the effect of the two heat treatments on the foaming properties of TNSMG, under optimal processing conditions, water-bath heating (FA of 85.56%, FS of 75.18%) was more effective compared to microwave treatments (FA of 83.44%, FS of 74.18%).

3.6. Emulsification property

EAI and ESI of TNSMG under microwave treatment and water-bath heating treatment were shown in Fig. 5A - 5D. As can be seen from Fig. 5A, there was a notable increase in EAI (from 6.58 m^2/g to 9.61 m^2/g) when TNSMG was treated by microwave at 560 W for 40 s. Additionally, ESI reached its maximum (39.92 min) at 420 W for 40 s. These findings were attributed to the conformational change induced by

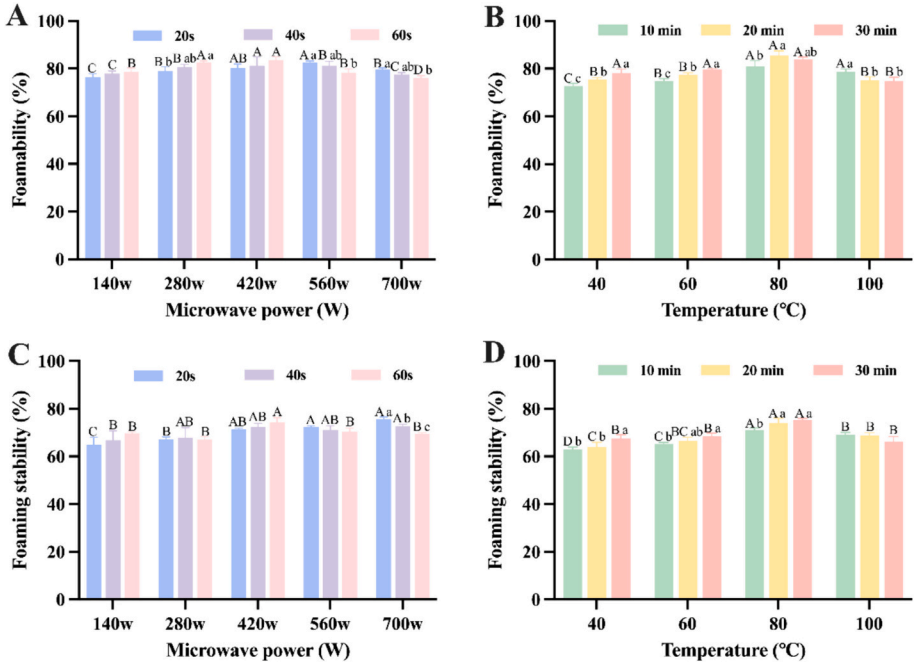

Fig. 4. Effects of different treatment conditions on foaming properties of TNSMG. (A) Foamability after microwave treatment; (B) Foamability after Water-bath heating treatment; (C) Foaming stability after microwave treatment; (D) Foaming stability after Water-bath heating treatment. The letters (A-D) mean significant differences between groups ($p < 0.05$); The letters (a-c) mean significant differences within groups ($p < 0.05$), and unlabeled letters mean no significant difference.

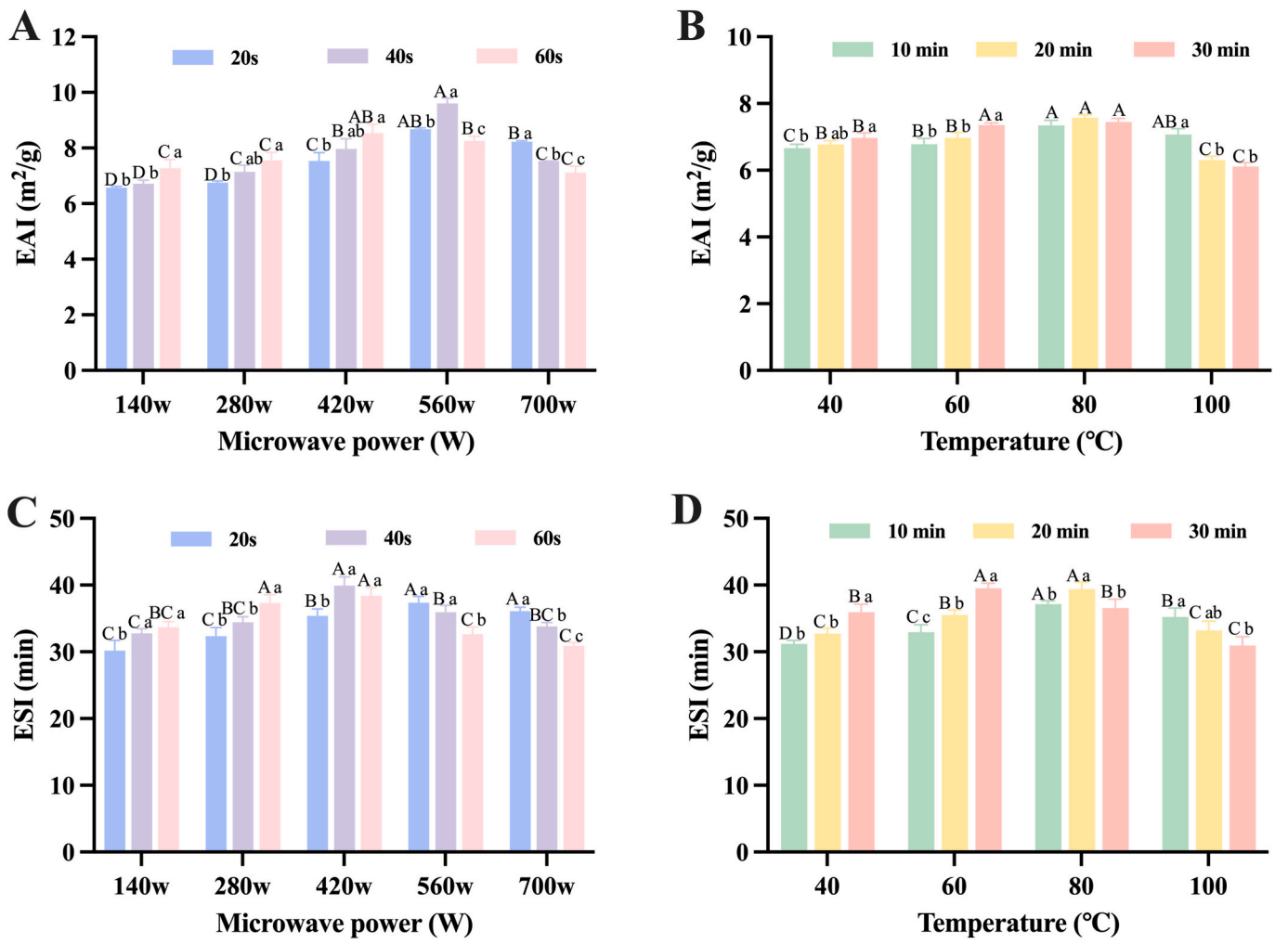

Fig. 5. Effects of different treatment conditions on emulsification properties of TNSMG. (A) Emulsifying activity (EAI) after microwave treatment; (B) EAI after Water-bath heating treatment; (C) Emulsion stability (ESI) after microwave treatment; (D) ESI after Water-bath heating treatment. The letters (A-D) mean significant differences between groups ($p < 0.05$); The letters (a-c) mean significant differences within groups ($p < 0.05$), and unlabeled letters mean no significant difference.

microwave treatment, which exposed the hydrophobic region of the protein. As a result, the heightened hydrophobicity on the protein surface contributed to the improvement of its emulsification property (Zheng et al., 2020). Another possible reason was that the appropriate microwave treatment led to the unfolding of the TNSMG structure, enhancing the molecular flexibility of TNSMG. This increased flexibility allowed the protein to be rapidly absorbed at oil-water interfaces, forming stable monolayer or multilayer structures, which in turn improved its EAI (Wang et al., 2018). The EAI and ESI of TNSMG treated at 700 W for 60 s didn't exhibit significant differences compared to the untreated samples. This result was attributed to the increased microwave power, which caused protein molecules to interact and aggregation occurs. These undesirable changes affect the diffusion and absorption of the protein at the oil-water interface, ultimately impacting its emulsification properties (Yang et al., 2018).

As depicted in Fig. 5B and D, the EAI of TNSMG was basically unaffected by the changes in temperature and duration of the water-bath heating treatment compared with the microwave treatment. The ESI of TNSMG exhibited higher values when subjected to 60 °C for 30 min (39.51 min) and 80 °C for 20 min (39.41 min). On the one hand, heating treatment destroyed the structure of TNSMG, exposing its subunits and enhancing the binding ability to oil droplets. On the other hand, this phenomenon might be due to the expansion of hydrophobic residues in TNSMG upon heating, enhancing the surface hydrophobicity of the protein, consequently improving the stability of the emulsion (Peng et al., 2016). However, there was a significant decrease in EAI after subjecting it to water-bath heating treatment at 100 °C for 30 min. Commonly, higher temperature may induce proteins to unfold and aggregate simultaneously, leading to an impact on the hydrophobic group of the protein, thereby, affecting the EAI of TNSMG (Zang et al., 2019).

Comparing the effects of the two heat treatments on the emulsification properties of TNSMG, microwave treatment demonstrated more significant improvements.

3.7. Digestibility of TNSMG

The quantity of free amino groups generated after protein digestion by SGI and SFI reflect the degree of proteolysis, serving as an indicator of the degree of protein digestion (Ketnawa et al., 2018). According to Fig. 6, the digestibility of TNSMG was increased from 60.52% to 78.58% at 560 W for 40 s. This enhancement can be attributed to the accelerated motion of TNSMG with the increase of microwave treatment power and duration, resulting in an enhanced interaction between the enzyme and TNSMG. When the microwave treatment power was 700 W, the prolonged treatment duration led to a decrease in digestibility. This decrease was likely associated with the occurrence of protein aggregation and denaturation, leading to the masking of hydrolysis site on TNSMG under high microwave treatment power (Ketnawa et al., 2018).

For water-bath heating treatment, the maximum digestibility of

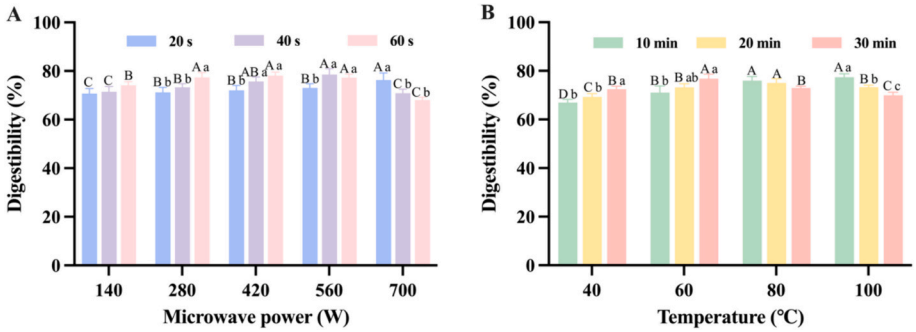

Fig. 6. Effects of different treatment conditions on digestibility of TNSMG. (A) Microwave treatment; (B) Water-bath heating treatment. The letters (A-C) mean significant differences between groups ($p < 0.05$); The letters (a-c) mean significant differences within groups ($p < 0.05$), and unlabeled letters mean no significant difference.

TNSMG reached 77.7% when the treatment condition was 100 °C for 10 min. This was due to appropriate water-bath heating treatment could promote protein unfolding and enhance the exposure of the number of digestive enzyme binding sites, which in turn improved digestibility (Cao et al., 2023). At 80 °C and 100 °C, the digestibility decreased with the increase of treatment time. This undesirable change may be owing to the aggregation of TNSMG induced by prolonged heating time at high temperatures. This aggregation prevented their binding to enzymes, resulting in a decrease in digestibility.

In conclusion, both the two heat treatments could improve the digestibility of TNSMG. Compared with the water-bath heating treatment, the digestibility of TNSMG after microwave treatment was better.

4. Conclusions

In this study, the impacts of microwave treatment and water-bath heating treatment on the structural, functional properties and digestibility of TNSMG were explored. When the microwave power was <420 w and within 40 s, and the water-bath heating temperature is lower than 80 °C and within 20 min, the α-helix was transformed into random coil conformation which made protein become disordered. Moreover, the H_0 and fluorescence intensity of TNSMG increased with extended treatment time, accompanied by a redshift in the fluorescence spectrum peak. This indicated that the structure of TNSMG changed during the heat treatment, more hydrophobic amino acids and hydrophobic groups were exposed. The observed structural alterations provide a foundational understanding for improvements in the functional properties of TNSMG. Among the various thermal treatment conditions, the desirable solubility and foaming property were observed at 80 °C for 20 min, while the highest emulsification property and digestibility were achieved at 560 W for 40 s. In summary, heat treatment effectively changed the structure of TNSMG, and significantly improved its functional properties. These collective findings provide crucial insights for the thermal processing and commercial utilization of TNSMG resources. Furthermore, we suggest that future studies could combine microwave and water-bath heating treatment to further explore the structure-activity relationship and the corresponding changes in functional activity of TNSMG, enhancing its potential as a new food ingredient and nutritional component.

CRediT authorship contribution statement

Yali Yu: Resources, Methodology, Conceptualization. **Xinyu Jiang:** Writing – review & editing, Data curation. **Xiaoyu Lu:** Writing – original draft, Investigation, Data curation. **Rongcan Cai:** Validation, Software. **Yuer Shan:** Visualization, Investigation. **Minglong Tang:** Visualization, Investigation. **Quan Wang:** Visualization, Investigation. **Ye Song:** Validation, Supervision. **Feng Gao:** Supervision, Conceptualization.

Declaration of competing interest

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

Data availability

The data that has been used is confidential.

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