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Improving the extraction efficiency and functional properties of wheat germ protein by ultrasound-assisted extraction

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Abstract: This study optimised the conditions for ultrasound-assisted extraction (UAE) of defatted wheat germ protein (WGP) and evaluated its effect on the functional properties. Single-factor and orthogonal experiment designs were combined to optimise the UAE extraction condition. The extraction of WGP reached the highest level, at 88.66%, with a solid : liquid ratio of 1 : 25 g·mL^{-1}, pH value of 9.0, ultrasonic time of 10 min, and ultrasonic power at 400 W. Under these conditions, albumin, globulin, prolamin, and glutenin accounted for 32.26, 28.52, 5.42, and 22.40% of total protein, respectively. In addition, this study compared the functional properties of WGP extracted by UAE with the results based on a commercially available soy protein (SP) isolate (SPI). The UAE of WGP had better oil absorption, foaming, and emulsifying properties. Therefore, UAE is a promising technique for food protein extraction because it can change the protein efficiencies and properties of the extract.

Keywords: wheat grain; orthogonal experiment design; single-factor experiment design; protein; cavitation and mechanical effects

Wheat germ (WG), which constitutes about 2–3% of the wheat grain, is an important byproduct of flour processing owing to its valuable nutritional content (Barros et al. 2021). WG is praised as 'the natural nutrient treasure-house and life source of mankind' (Wang et al. 2017) for its high nutritive value and palatability, often used as industrial food sources. The annual global production of WG is estimated to be nearly 25 million tons, which makes it an important milling byproduct for valorisation. However, WG is currently

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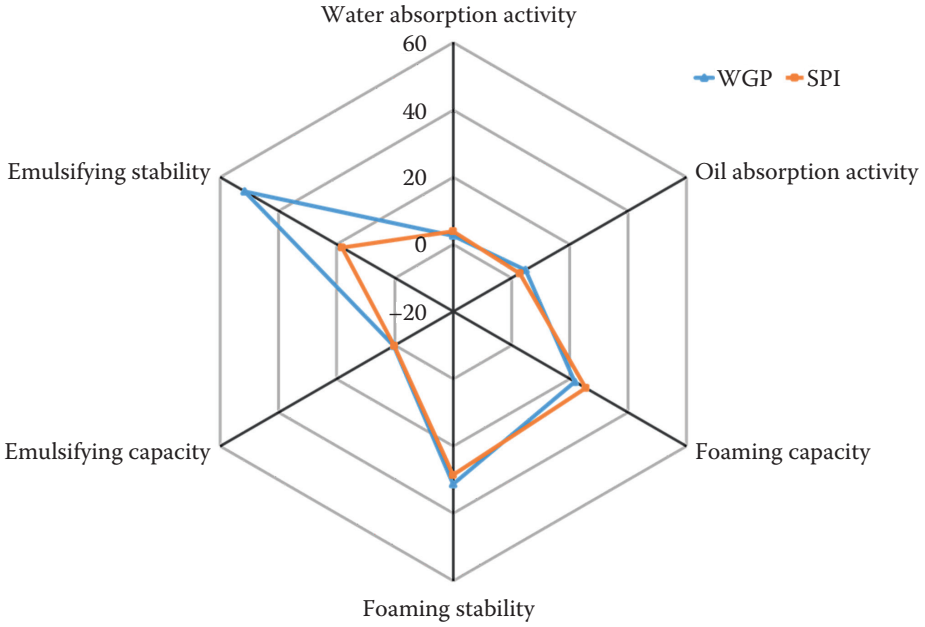

Figure 1. Radar chart of functional characteristics of WGP and SPI

WGP extracted by ultrasound, commercially available SPI; WGP – wheat germ protein; SPI – soy protein isolate

used mainly as animal feed formulations; human consumption of this product remains limited and underexplored (Boukid et al. 2018).

The demand for protein consumption continues to rise in relation to the increases in the world population. The quest for new alternatives and inexpensive, high-quality protein sources has become an important research trend in recent years. Wheat germ protein (WGP) is a highly nutritive material extracted from defatted WG (DWG) that contains > 30% protein. This material has been classified as an exceptionally effective animal protein owing to its enrichment in 17 amino acids, particularly threonine, lysine, and methionine, which are essential amino acids deficient in many cereal grains (Koç and Özçıra 2019). Therefore, it is important to improve the protein extraction efficiency and the functional properties of WGP.

In recent years, research results have shown that functionality of WGP is less than that of soy protein (SP) (Hettiarachchy et al. 1996). Functional properties are directly affected by physical and chemical agents that are often used in protein extraction and can thus be used to evaluate the extracted protein functionality (Pojić et al. 2018). The traditional approach used to extract WGP, such as alkaline extraction and isoelectric precipitation, has low protein extraction efficiency and causes severe environmental pollution due to the high amounts of alkaline or acerbic wastewater produced. Ultrasound-assisted extraction (UAE) has been recognised as a promising new clean approach in food technology for its low environmental impact (Ojha et al. 2020). UAE can increase the extraction yield by ~ 20% over that obtained using conventional methods and can improve the functional properties mainly through cavitation and mechanical effects widely used in processes of plant and animal protein extraction (Ojha et al. 2020; Bernardi et al. 2021). However, information for improving the protein extraction efficiency of UAE and the functional properties of WGP remains limited.

The present study combines single-factor and orthogonal experiment designs. The present study combines single-factor and orthogonal experiment designs to optimise the UAE extraction condition of WGP from DWG to improve extraction efficiency and functional properties. Moreover, the functional properties of WGP are further studied by comparing the results with those obtained using soy protein (SP) (Figure 1). In summary, this research systematically studies the effect of ultrasonic conditions on WGP, lays a foundation for the extraction of WGP and its application in food processing, and promotes agricultural development and environmental improvement.

MATERIAL AND METHODS

Material. The defatted wheat germ (DWG) used in this study was obtained from Langxue Flour Food Co., Ltd. (Langfang, China). The material was dried to a con-

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stant weight and was pulverised to flour consistency using a medicinal herb grinder (HC-700, Zhejiang, China).

All other chemicals and reagents were obtained from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). Deionised water was used throughout the experiment.

Extraction yield of wheat germ protein (WGP). The protein content was determined by applying the Coomassie Brilliant Blue G-250 method (Cheng et al. 2019), and the semitrace Kjeldahl method (Bo et al. 2015) was used to determine the total WGP content. The WGP yield was obtained as the ratio percentage of the total supernatant protein content to the total WGP content.

Single-factor experiment design. The effects of the solid : liquid ratio (1 : 10, 1 : 15, 1 : 20, 1 : 25, and 1 : 30), extraction time (10, 20, 30, 40, and 50 min), ultrasonic power (0, 200, 300, 400, and 500 W), extraction temperature (20, 30, 40, 50, and 60 °C), and water solvent pH (8, 9, 10, 11, and 12) on the extraction yield of the WGP were investigated first using a single-factor experiment design.

Orthogonal experiment design. Based on the single-factor experiment design, an orthogonal experiment design L_{16} (4^5) (five factors and four levels) was adapted to further screen the experimental factors (at extraction temperature 50 °C). As shown in Tables S1 and S2 in the Electronic Supplementary Material (ESM), the extraction was conducted with four factors and four levels.

Determination of WGP composition. The Osborne method (OSB) (Caligiani et al. 2018) was used to sequentially extract WGP from DWG based on its solubility in water, 5% NaCl, 0.1 M NaOH, and 70% ethanol, respectively. The globulin, glutenin and prolamin fractions were obtained following Wang et al. (2017); the Coomassie Brilliant Blue method was applied to measure the protein content in the supernatant.

Determination of WGP isoelectric point To analyse the WGP isoelectric point, 10 equal portions of WGP extract were measured. The pH ranges were adjusted using HCl and NaOH solutions, and after standing for 40 min, the samples were centrifuged at 4 000 rpm for 15 min. The Coomassie Brilliant Blue method was again applied to determine the protein content in the supernatant.

Determination of nitrogen solubility indices. The method of Rajarathnam et al. (2016) was used to determine the nitrogen solubility indices (NSI) of the WGP.

Water and oil-absorption activity. The water and oil absorptions with WGP were evaluated following Wang et al. (2017). SP was used as the control, and each experiment was repeated triplicate times.

Foaming capacity and foaming stability. The foaming properties, such as capacity and stability, were determined as described by Bandyopadhyay et al. (2008). SP was used as the control, and each experiment was repeated three times.

Emulsifying activity and emulsion stability. The emulsifying properties, such as emulsifying activity and emulsion stability, were determined as Wang et al. (2017) described. SP was used as a control, and each experiment was repeated three times.

Gel electrophoresis. Sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) was conducted on a discontinuous buffer system using 15% separation gel and 5% concentration gel. The protein samples were dissolved in 1.5 mol·L^{-1} Tris-HCl (pH 8.8) and 1 M Tris-HCl (pH 6.8) buffer solutions containing 10% (*w*/*v*) SDS, 30% gel mother liquor, 10% persulfate (*w*/*v*), and tetramethylethylenediamine (TEMED) and were then heated in boiling water for 5 min before electrophoresis. For each sample, 10 μL was applied to each lane. After running at a constant current of 20 mA for 3 h, the separated gel was stained with 0.25% Coomassie Brilliant Blue (R-250) in 50% trichloroacetic acid.

Statistical analysis. All extractions and studies were conducted in triplicate, and the means with standard deviation were reported. The data collected were subjected to analysis of variance (ANOVA), where $P < 0.05$ denotes the presence of a statistically significant difference.

RESULTS AND DISCUSSION

Effects of single factors on the extraction efficiency of WGP. As shown in Figure 2A, the WGP extraction rate positively correlated with the solid : liquid ratio and increased within a certain range. When the solid : liquid ratios were 1 : 20 g·mL^{-1} and 1 : 25 g·mL^{-1}, the WGP extraction rates reached 67.10% ± 0.25% and 67.30% ± 0.16%, respectively. Then, the extraction rate gradually decreased with a continuous increase in the solid : liquid ratio. An excessively high solid : liquid ratio is unsuitable for mass production. Therefore, the optimal choice was a solid : liquid ratio of 1 : 20 g·mL^{-1}.

The WGP extraction rate reached a maximum of 82.31% ± 1.62% at pH 9.0 (Figure 2B). Then, as the pH continued to increase, the protein extraction rate gradually decreased. This occurred likely because strong alkali conditions will destroy the WGP structure, which causes some of the nutrients to be lost (Liu et al. 2013). The WGP extraction rate increased with an increase in the extraction time from 10 to 30 min, ultimately reaching 66.29% ± 0.22% at 30 min (Figure 2C). As the

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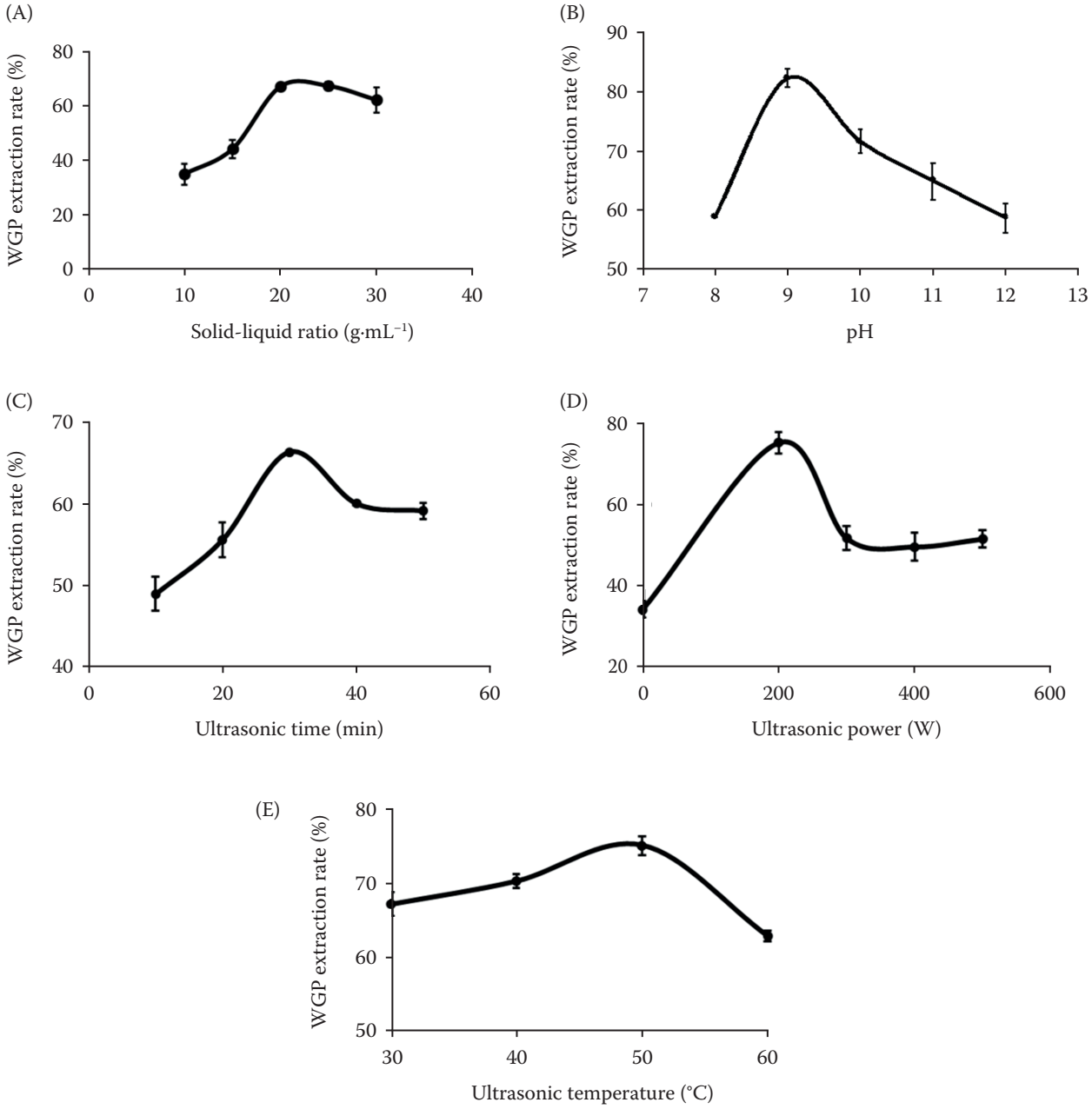

Figure 2. Effects of various single factors on the extraction efficiency of WGP: effects of differences in the (A) solid : liquid ratio, (B) water solvent pH, (C) ultrasonic time, (D) ultrasonic power, and (E) ultrasonic temperature on the WGP extraction rate

WGP – wheat germ protein

ultrasonic treatment time was prolonged, the extraction rate gradually weakened, likely because the hydrophobic groups exposed by ultrasound recombined or aggregated over time to form more stable structures (Lv et al. 2019). At low ultrasonic power (< 200 W), the WGP extraction rate changed rapidly. The WGP extraction peaked at 75.14% ± 2.55% at 200 W (Figure 2D), suggesting that the unstable WGP clusters were aggressively divided into small soluble aggregates. Under higher ultrasonic power (> 200 W), the extraction rate dropped sharply. Then it tended to stabilise, which can be attributed to noncovalent interactions during aggregation and repolymerization (Jiang et al. 2014). When the temperature was 50 °C, the extraction rate of WGP was the highest, at 75.17% ± 1.27% (Figure 2E), which is similar to the results reported by Li et al. (Li et al. 2018).

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Table 1. One-way ANOVA table

Factor	SD	d*f*	Mean[2]	*F*	Statistical significance
Solid : liquid ratio	870.04	20	43.50	1.06	***
Ultrasonic time	463.60	20	23.18	0.30	**
Ultrasonic power	185.99	20	9.30	0.05	*
Water solvent pH	877.27	20	43.86	1.08	****
Ultrasonic temperature	79.05	15	5.27	0.02	\

\, *, **, ***, **** $P > 0.05$, $P < 0.05$, $P < 0.01$, $P < 0.001$, and $P < 0.0001$, respectively; SD – standard deviation; d*f* – degree of freedom

At higher temperatures (> 50 °C), the WGP extraction rate began to decrease, likely because the high temperature caused the protein to denature, resulting in a decrease in the dissolution rate. Further, excessively high temperatures can weaken the cavitation effect of ultrasonic waves, which creates more cavitation bubbles in the solvent. For this reason, the water solvent pH, ultrasonic extraction time, ultrasonic power and extraction temperature with 9.0, 30 min, 200 W, and 50 °C were selected.

In addition, one-way ANOVA was applied to the single-factor results. As shown in Table 1, the extraction temperature's statistical significance was insignificant ($P > 0.05$), indicating the slightest effect on the protein extraction rate. Some studies have reported that temperature does not affect protein extraction (Jambrak

Table 2. Orthogonal experiment results

Test number	Factor				WGP extraction rate* (%)
	solid : liquid ratio (A)	ultrasonic time (B)	ultrasonic power (C)	solvent pH (D)	
1	1	1	1	1	43.73 ± 4.11^{e}
2	1	2	2	2	61.07 ± 3.92^{c}
3	1	3	3	3	46.20 ± 1.02^{e}
4	1	4	4	4	55.53 ± 1.98^{c}
5	2	1	2	3	55.56 ± 3.27^{c}
6	2	2	1	4	34.08 ± 4.01^{f}
7	2	3	4	1	52.43 ± 2.22^{d}
8	2	4	3	2	52.27 ± 1.97^{d}
9	3	1	3	4	49.33 ± 1.19^{d}
10	3	2	4	3	58.89 ± 3.33^{c}
11	3	3	1	2	55.23 ± 4.44^{c}
12	3	4	2	1	36.47 ± 2.11^{f}
13	4	1	4	2	88.66 ± 3.21^{a}
14	4	2	3	1	74.35 ± 2.54^{b}
15	4	3	2	4	76.42 ± 1.78^{b}
16	4	4	1	3	76.02 ± 2.22^{b}
k1	51.632	59.320	52.265	51.745	–
k2	48.585	57.098	57.380	64.308	–
k3	49.980	57.570	55.538	59.168	–
k4	78.862	55.072	63.878	53.840	–
Range (R)	30.278	4.248	11.612	12.562	–
Primary and secondary order	A > D > C > B				–
Optimal combination	A_4 (1 : 25)	B_1 (10 min)	C_4 (400 W)	D_2 (pH 9.0)	–

WGP – wheat germ protein; *results are a mean ± standard deviation (SD); $^{a–f}$values within rows with different letters are significantly different ($P < 0.05$)

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Table 3. Protein distribution of DWG protein (mean ± SD)

Protein	Proportion in DWG (%)	Proportion in total protein (%)
Albumin	10.72 ± 0.34	32.26 ± 0.22
Globulin	9.50 ± 0.18	28.52 ± 0.13
Prolamin	1.80 ± 0.09	5.42 ± 0.06
Glutenin	7.46 ± 0.32	22.40 ± 0.41
Total	29.48 ± 0.29	88.54 ± 0.54

SD – standard deviation; DWG – defatted wheat germ

et al. 2014; Preece et al. 2017). Accordingly, excluding the temperature control parameters can reduce the cost. Thus, we conducted an orthogonal analysis on other factors, including the solid : liquid ratio, ultrasonic time, ultrasonic power, and water solvent pH.

Orthogonal analysis of WGP extraction efficiency. To further screen the experimental factors, an orthogonal experiment L_{16} (4^5) was conducted to investigate the effects of other parameters on the extraction rate. As shown in Table 2, the extraction was performed under the following conditions: a solid : liquid ratio of 1:25 g·mL^{-1}, pH of 9.0, ultrasonic power of 400 W, and extraction time of 10 min. The importance order of four experimental factors in terms of R was A > D > C > B, or solid : liquid ratio > water solvent pH > ultrasonic power > extraction time, and the WGP extraction rate was 88.66% ± 3.21%. This result also indicates that $A_4B_1C_4D_2$ was the optimal process combination for extracting WGP.

Composition of WGP. The optimal extraction conditions for UAE of WGP were obtained through single-factor and orthogonal experiment optimisations discussed in sections 3.1 and 3.2. In particular, the rate of total protein of the DWG extracted by UAE was 36.65% ± 0.56%, and the protein content of WGP was 90.87% ± 1.66%, similar to those of commercially available SP, at 91.39% ± 1.19%. The information on the molecular weight and distribution pattern of the WGP components was obtained by SDS-PAGE treatment. The bands were between 11 and 116 kDa (Figure S1, ESM).

The components and content of soluble protein in the WGP samples were determined by applying the OSB method for classifying the protein extracted. As shown in Table 3, albumin was the predominant protein, amounting to 10.72%± 0.34% in the DWG and 32.26% ± 0.22% in total protein. In addition, globulin, prolamin, and glutenin accounted for 28.52% ± 0.13%, 5.42% ± 0.06%, and 22.40% ± 0.41% of the total protein, respectively. These results agree with those reported by Wang et al. (2017).

WGP characteristics. As shown in Figure 3A, the WGP solution was processed using various pH values, and the ions in the solution changed to precipitate protein. The protein content in the supernatant was significantly different, and the WGP content was lowest when the pH was 4.0. This indicates that the isoelectric point of WGP is pH 4.0. The protein solubility increased at pH values below and above the isoelectric point because, under such conditions, the protein has negative or positive net charges, which enables more water to interact with the protein molecules.

The dissolution of WGP can be expressed by the NSI, which increases with an increase in pH value; that is, the dissolution of protein gradually increases under this condition. The dissolution of WGP was lowest near the isoelectric point (pH = 4.0), as shown in (Figure 3B). No net charge was present on the WGP at the

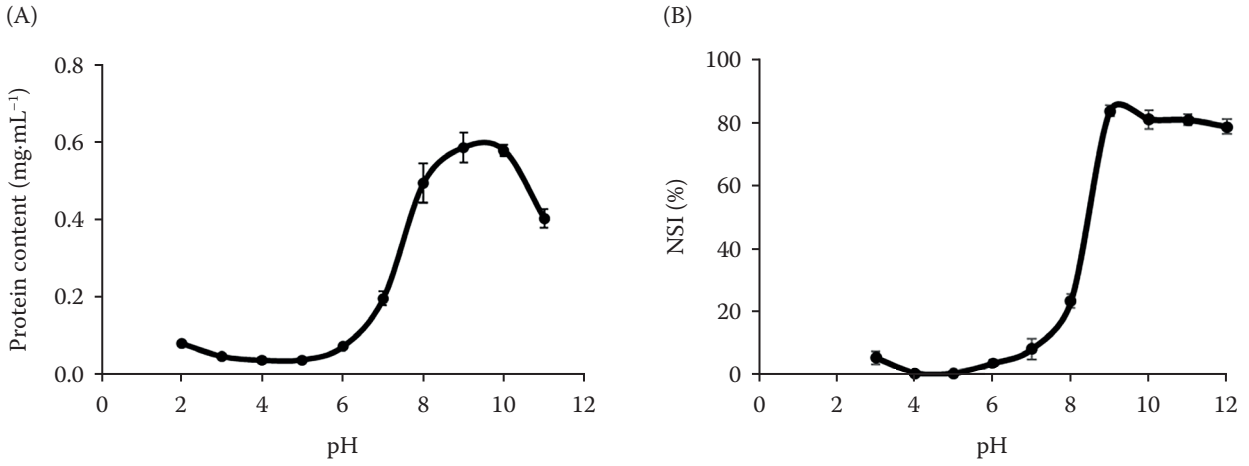

Figure 3. Characteristics of WGP: (A) isoelectric point and (B) nitrogen solubility index (NSI)

WGP – wheat germ protein

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isoelectric point, and the resulting lack of repulsive forces enabled the interaction between the protein molecules to increase. The dissolution increased progressively with an increase in pH, with the maximum value of 83.63% ± 1.01% observed at pH 9.0. This increase in dissolution can be attributed to the protein unfolding or denaturation, in which ultrasound destroys the interaction of hydrophobic molecules inside the protein and increases the surface hydrophobicity and molecular movement (Jiang et al. 2014).

Comparison of functional characteristics between WGP and SP. As shown in Figure 4A, the WGP had

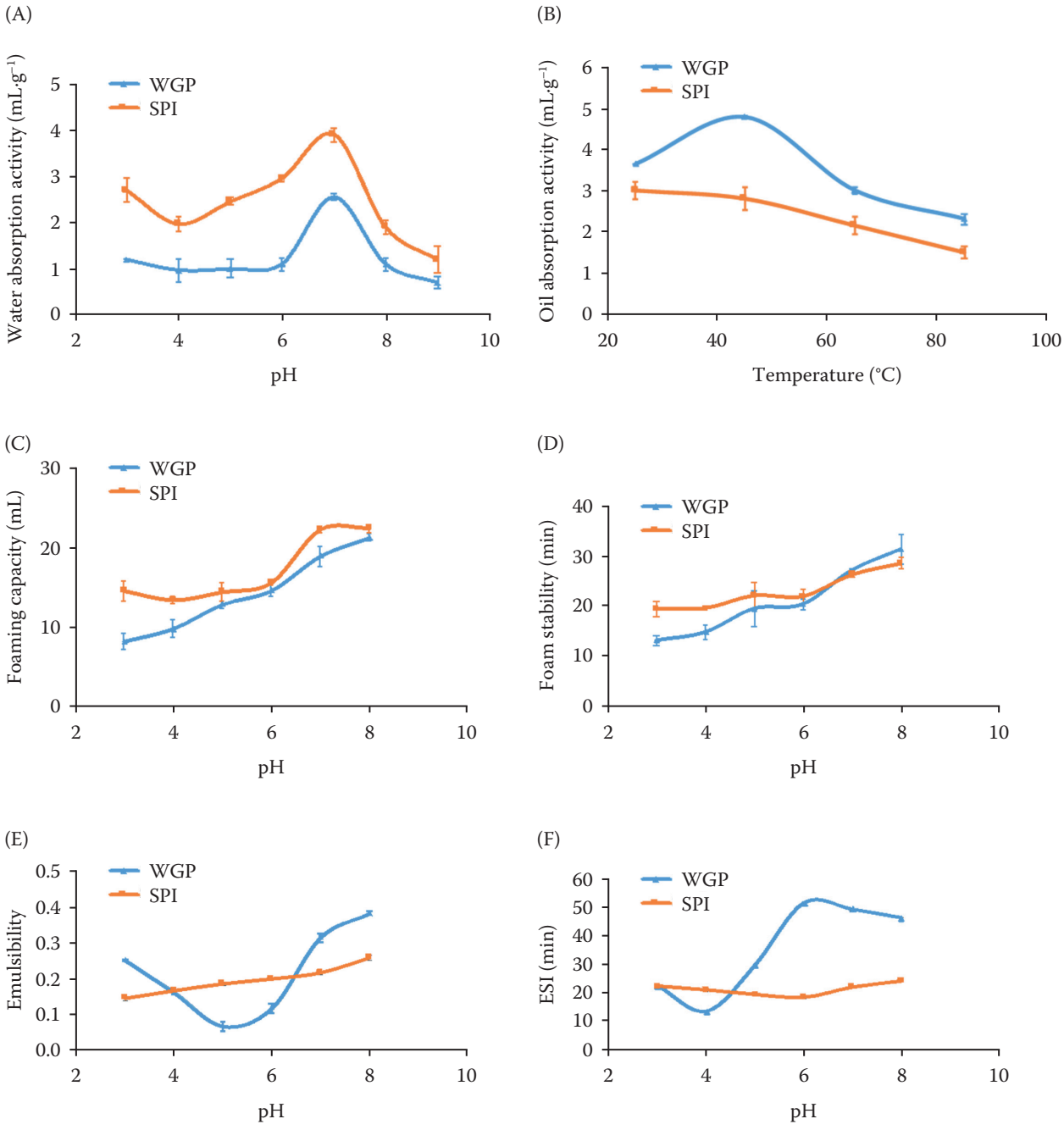

Figure 4. Comparison of functional characteristics of WGP extracted by ultrasound and commercially SPI: (A) effect of pH on water absorption activity of WGP and SPI, (B) effect of temperature on the oil-absorption activity of WGP and SPI, (C) effect of pH on foaming capacity of WGP and SPI, (D) effect of pH on foam stability of WGP and SPI, (E) effect of pH on emulsibility of WGP and SPI, (F) effect of pH on emulsification stability of WGP and SPI

WGP – wheat germ protein; SPI – soy protein isolate; ESI – emulsifying stability

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less effective water absorption compared with the SP isolate (SPI), although the effect of pH on the two proteins was similar. The highest water absorption, 2.55 ± 0.071 g·mL^{-1} for WGP and 3.90 ± 0.071 g·mL^{-1} for SPI, was obtained at pH 7.0. When the pH continued to increase, the water-holding capacity of the proteins decreased significantly. This occurred likely because the protein's solubility also increases as the solution's alkalinity increases. This leads to the destruction of the protein's spatial structure, which decreases the water absorption capability of the protein.

The oil absorption of WGP extracted by ultrasound was better than that of the commercially available SPI. As shown in Figure 4B, the oil absorption of the WGP reached 4.86 ± 0.12 g·mL^{-1} at a temperature of 45 °C, whereas that of the SPI was only 2.80 ± 0.38 g·mL^{-1}. The increase in oil absorption of WGP can be attributed to partially unfolded proteins. Ultrasound and temperature cause more functional groups in the molecule to be exposed to the outside, thereby enhancing the interaction between the protein and oil (Wang et al. 2017).

The foaming properties of SPI and WGP gradually increased with an increase in pH, with the SPI showing slightly better performance (Figures 4C and 4D). This difference in foaming performance can be attributed to increased protein solubility and rapid expansion into a bonding layer around the gas/air droplets (Bernardi et al. 2021). However, the foaming stability of the WGP was better than that of SPI when the pH was greater than 7.0. These results indicate that the foaming property of WGP produced by UAE might be more suitable than SPI in various food products.

As shown in Figures 4E and 4F, the emulsifying properties of the WGP showed a tendency to decrease first and then with an increase in pH, which was the lowest performance observed near pH 4.0. A likely explanation is that WGP exhibits its lowest solubility near the protein isoelectric point, resulting in less protein available for emulsification. The protein molecules charged as the pH level increased, and the oil dispersed in the water. This promoted forming a thin film between the oil and water, thereby preventing droplet flocculation and coalescence and enhancing the emulsification. Compared with those of the SPI, the emulsifying properties of WGP were higher at pH > 7.0.

CONCLUSION

Ultrasound-assisted treatment was used to promote WGP extraction and improve the protein's functional properties. Optimising the ultrasound wave extraction conditions increased optimising the extraction rate of the WGP was increased to 88.66%. Moreover, functional characteristics such as water absorption, oil absorption, foaming properties, and emulsifying properties were better than those obtained when SPI was employed. The use of ultrasound to increase the solubility of plant protein offers new opportunities in the food industry because solubility tends to have a positive impact on other functional properties.

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