

# Trypsin Inhibition Activity and Functionality of Whiteleg Shrimp (*Litopenaeus Vannamei*) Head Protein Hydrolysate

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Trypsin inhibition activity (TIA) of whiteleg shrimp (*Litopenaeus vannamei*) head (WLSH) protein hydrolysate was investigated. Alcalase hydrolysis was performed under a determined condition, including a 1:7 (w/v) WLSH powder to water ratio, an enzyme to substrate (E:S) ratio of 30 U/g protein, and a 3 h of hydrolysis, to produce a hydrolysate demonstrating the TIA of  $1190.0 \pm 25.8$  trypsin inhibition units (TIU)/mg protein. Beyond 50% of this activity was retained after the hydrolysate was treated at pH range of 3 to 8 or heated for 90 min at 100°C. Regarding functional features, within the pH range from 3 to 8, the WLSH hydrolysate expressed notable solubility, heat stability, foaming and emulsifying properties. Moreover, average capacities of holding water and oil of the hydrolysate were ascertained. Besides, the hydrolysate could be served as an amino acid (AA) supplement which provided at least 8 essential AAs with the proportion of 44.59% of the hydrolysate's total AAs. Furthermore, membrane ultrafiltration further enhanced the TIA of the hydrolysate, with its 1-3 kDa peptide fraction exhibiting the TIA of  $1614.40 \pm 32.20$  TIU/mg protein. These findings would benefit the development of natural trypsin inhibition products from the WLSH, which could be employed in different areas.

## 1. Introduction

Trypsin inhibitors have presented their potential applications in different fields including food technology, pharmaceuticals, and agronomy (Klomklao et al., 2016). In food products, they impeded endogenous and microbial proteases in protein-based food products, mitigating the degradation of myofibrillar proteins, thereby maintaining their texture (Singh and Benjakul, 2017). Regarding medicinal applications, trypsin inhibitors interpret their role in managing obesity via obstructing the digestion of proteins, avoiding the excessive absorption of free AAs (Awosika and Aluko, 2019). In addition, the inhibition of pancreatic trypsin leads to the accumulation of undigested proteins in the intestine, elevating the release of cholecystokinin, a hormone generating satiety (Awosika and Aluko, 2019). Besides, the prevention of unbalanced intake of AAs decreases ammonia and urea levels in blood, protecting the kidney from overwork (Awosika and Aluko, 2019). Moreover, as reported by Klomklao et al. (2016), protease inhibitors can be used to address cancer along with immune-related, neurodegenerative, and heart diseases. Considering the field of agriculture, trypsin inhibiting agents were thought to be a new generation of insecticides, which disrupted AA balance and induced the depletion of essential AAs, resulting in pest mortality (Hemmati et al., 2021). Previous publications on TIA of a hydrolysate derived from pea proteins (Awosika and Aluko, 2019), and protein extracts from squid ovary (Singh and Benjakul, 2017), tamarind seeds (Gomes et al., 2024), yellowfin tuna roe (Klomklao et al., 2016), and cowpea (El-latif, 2015), highlighted the potency of protein-based trypsin inhibitors.

According to the prediction of the Vietnam's Ministry of Industry and Trade, the quantity of WLSHs is 450.000 tons in 2025. This study aimed to convert the WLSHs into a trypsin inhibition hydrolysate, employing the alcalase hydrolysis. Investigations on effects of WLSH powder to water ratio, E:S ratio and hydrolysis time on TIA of the WLSH hydrolysate were performed to select a suitable level for each factor. The hydrolysate gained under the selected hydrolysis condition was further subjected to analyses, encompassing hydrolysis degree (DH), AA profile, techno-functional features (solubility, heat stability, interfacial properties, water- holding capacity (WHC) and oil-holding capacity (OHC)), and bioactivity stability against pH and heat treatments. Furthermore,

membrane fractionation was applied to separate the hydrolysate into 5 various peptide fractions (>30, 10-30, 3-10, 1-3 and <1 kDa) and tested for their TIA. The result would benefit for deeper studies, regarding hydrolysate purification, peptide sequencing, and their applications. This is the very first study investigating TIA of WLSH protein hydrolysate and its peptide fractions, which could be further tested for use as a natural trypsin inhibitor.

## 2. Materials and methods

### 2.1 Materials

WLSHs, obtained from the Vietnam Food company, were washed, and dried at 90°C for 20 min, followed by a further drying process at 60°C until achieving unchanged weight. The resulting WLSH were ground, filled in sealed polyacrylamide packages, which were placed in the dark at room temperature. Moisture and protein contents of the WLSH powder were determined to be  $5.36 \pm 0.12\%$  and  $54.5 \pm 0.5\%$ , using the Association of Official Analytical Collaboration (AOAC) 950.46B and AOAC 990.03 methods (AOAC, 2023), respectively. The Alcalase® 2.5L preparation was obtained from Novozymes. All other chemicals employed in this study were classified as analytical grade, provided by Sigma-Aldrich, Merck, Biobasic, and Thermo Scientific Chemicals.

### 2.2 Methods

The WLSH powder was blended in distilled water with a desired ratio, then heated for 10 min at 90°C for endogenous enzyme inactivation. pH of the blend was then brought to 7.5 using 1 M HCl or 1 M NaOH solution before adding the Alcalase at a determined E:S ratio. The hydrolysis process was performed at 55°C for a required time. A 10 min period of heating at 90°C was done again to inactivate the alcalase. After that, the obtained mixture was centrifuged to gain the WLSH hydrolysate (the supernatant), which was subsequently lyophilized and preserved at -20°C until used (Vo et al., 2025b). Effect of WLSH powder to water ratios varying from 1:5 to 1:9 (w/v) on TIA of the hydrolysates was first investigated, in which a 50 U/g protein E:S ratio and a 4 h of hydrolysis were set. The obtained hydrolysates were examined for their TIA, accordingly, a WLSH powder to water ratio at which the activity peaked was chosen. Identical approach was implicated to establish suitable levels for E:S ratio and hydrolysis time in the tested ranges from 20 to 70 U/g protein, and between 2 h and 6 h, respectively. After these investigations, the obtained WLSH hydrolysate was fractionated using centrifugal devices, attached with decreasing molecular-weight-cutoff membranes of 30, 10, 3, and 1 kDa (Macroprep, Pall Laboratory, USA). The resulting five peptide fractions of <1, 1-3, 3-10, 10-30, and >30 kDa were tested for their TIA. DH, AA composition, functional characteristics (solubility, heat stability, foaming property, emulsifying property, WHC and OHC), and bioactivity stability against pH and heat treatments of the WLSH hydrolysate with the highest TIA were evaluated using our previously published methods (Vo et al., 2025b).

TIA of WLSH hydrolysates was assessed according to the method of El-latif (2015) with a minor adjustment. A mixture of 0.5 mL of the hydrolysate and 0.5 mL of trypsin solution (60 µg/mL, prepared in Tris-HCl buffer pH 8 (0.01M), containing CaCl<sub>2</sub> (0.02 M)) was kept at 37°C for 15 min. Then, 2.5 mL of 1 mM N $\alpha$ -Benzoyl-DL-arginine p-nitroanilide solution (dissolved in distilled water) was added and the mixture was maintained at 37°C for further 15 min. Trypsin was inactivated using 0.5 mL of acetic acid solution (30% v/v) and the mixture's absorbance at wavelength of 410 nm was recorded. A control in which the hydrolysate was substituted by distilled water was prepared in parallel. One TIU was defined as a 0.01 decrease in the absorbance of the inhibitor-containing mixture compared to that of the control. TIA of the hydrolysate was calculated as follows:

$$\text{TIA (TIU/mg protein)} = \frac{A_c - A_s}{0.01 \times m_{\text{protein}}} \quad (1)$$

where  $A_c$  and  $A_s$  denotes the absorbance at 410 nm of the control and the sample, in order;  $m_{\text{protein}}$  is soluble protein weight in the hydrolysate, which was determined using the Lowry method (Vo et al., 2025b). The chemical compound 4-(2-aminoethyl)benzenesulfonyl fluoride hydrochloride (AEBSF) served as standard.

Data presented as average of three independent trials  $\pm$  standard deviation were analyzed using Excel software, while the Statgraphics Centurion 18 software was used to ascertain statistically significant differences.

## 3. Results and discussion

### 3.1 Influence of hydrolysis parameters on TIA of WLSH hydrolysate

This study found that with the increasing WLSH powder to water ratio from 1:5 to 1:9 (w/v), TIA of the WLSH hydrolysate rose and peaked at the ratio of 1:7 (w/v), then declined (Figure 1a). It could be explained that decreasing the WLSH powder to water ratio from 1:9 (w/v) to 1:7 (w/v) increased the substrate concentration, ameliorating the hydrolysis reaction rate. As a result, a high quantity of bioactive peptides was released into the obtained hydrolysate, boosting its TIA (Eberhardt et al., 2021). However, low WLSH powder to water ratios (1:5

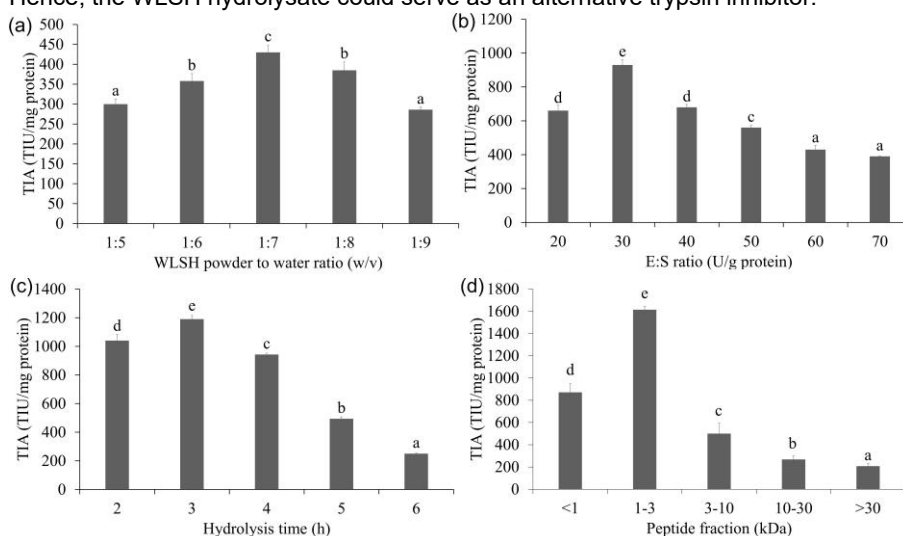
and 1:6 (w/v)) may trigger the solution to be too viscous, mitigating the molecular diffusion rates, thus reducing collision probability of enzymes and substrates (Vo et al., 2025b). Consequently, decreased TIAs of the WLSH hydrolysates were seen in these low ratios.

Within the tested E:S ratio range of 20–70 U/g protein, TIA of the WLSH hydrolysate peaked at 30 U/g protein (Figure 1b). This could be attributed to the sufficient amount of enzyme at the E:S ratio of 30 U/g protein, facilitating the liberation of active sequences from the WLSH native proteins, ultimately enhancing the TIA of the resulting hydrolysate (Vo et al., 2025b). Alternatively, reduced activities of the WLSH hydrolysates at higher E:S ratios (40–70 U/g protein) might be due to the appearance of competitive inhibition or enzyme aggregation when using excessive amount of enzymes, impeding their binding to substrates and the generation of bioactive peptides (Vo et al., 2025a). Unnecessary enzymes may also degrade trypsin inhibition peptides into inactive fragments, thus lowering the inhibition activity against trypsin of the obtained hydrolysates (Vo et al., 2025b).

As seen in Figure 1c, TIA of the WLSH protein hydrolysate increased along with the prolongation of hydrolysis period from 2 to 3 h, then significantly declined. A 3-h of hydrolysis may offer a sufficient time for the effective interactions between enzyme and substrate molecules, resulting in abundance of bioactive peptides in the gained hydrolysate (Vo et al., 2025b). However, prolonged periods of hydrolysate may cleave active sequences formed in the early stage into small ones with decreased TIA (Vo et al., 2025a). Additionally, the overexposure of hydrophobic regions on these small peptide's surfaces could lead to peptide agglomeration, lowering the activity of the hydrolysate (Souza et al., 2025).

The appropriate levels of these hydrolysis parameters in the alcalase hydrolysis process of WLSHs were found, including WLSH powder to water ratio of 1:7 (w/v), E:S ratio of 30 U/g protein, and a 3 h duration of hydrolysis.

The resulting hydrolysate displayed a DH of  $17.6 \pm 0.2\%$  and a TIA of  $1190.0 \pm 25.8$  TIU/mg protein, surpassing that of the intact WLSH proteins by 2.7 times. As compared to AEBSF, a specific irreversible inhibitor of trypsin, the hydrolysate exhibited a 13.5 fold lower in TIA. However, the TIA of the WLSH hydrolysate was significantly greater than that of protein extract derived from tamarind seeds (381.65 TIU/mg protein) (Gomes et al., 2024), and cowpea (120.20 TIU/mg protein) (El-latif, 2015). Kunitz-type proteins were the most common trypsin inhibitor in plants, but their long-term uptake may trigger pancreatic carcinogenesis (Biswas and Purohit, 2025). Hence, the WLSH hydrolysate could serve as an alternative trypsin inhibitor.



**Figure 1:** Influence of (a) WLSH powder to water ratio, (b) E:S ratio and (c) hydrolysis time on TIA of WLSH hydrolysate. (d) TIA of peptide fractions from the WLSH protein hydrolysate. Bars marked with different letters indicate statistically significant differences ( $p < 0.05$ ).

### 3.2 TIA of peptide fractions separated from the WLSH hydrolysate

Among the five peptide fractions gained from the WLSH hydrolysate, the 1-3 kDa fraction exhibited the highest TIA of  $1614.40 \pm 32.20$  TIU/mg protein (Figure 1d). This activity was 1.35 times higher than that of the whole hydrolysate, which may result from the ultrafiltration-induced diminution of antagonistic effects in the peptide fraction (Awosika and Aluko, 2019). Moreover, small peptides in the 1-3 kDa fraction might take advantage in tightly binding to trypsin's active site (Meriño-Cabrera et al., 2020). However, the low TIA of the <1 kDa peptide fraction may be attributable to its high content of very small peptides or free AAs that did not effectively attach to trypsin. This hypothesis was supported by the findings of Meriño-Cabrera et al. (2022) that dipeptides expressed a lower affinity to trypsin compared to tetra- or hexa peptides did. Additionally, Awosika and Aluko (2019) reported that TIA of the <1 kDa fraction of the pea protein hydrolysate was lower than that of its higher

molecular weight fractions. Hence, the 1-3 kDa fraction of the WLSH hydrolysate could be acted as a potential trypsin inhibiting substance or subjected to further investigations on identification of bioactive AA sequences.

### 3.3 Amino acid composition of the WLSH hydrolysate

Arg took the highest proportion in the WLSH hydrolysate, reaching 13.51% of its total AA content (Table 1). This AA contributed to the TIA of the WLSH hydrolysate via forming hydrogen bonds, salt bridges, or ionic links with trypsin, which was confirmed by molecular docking simulation results in the studies of Meriño-Cabrera et al. (2022) and Kvetkina et al. (2022). Besides, charged AAs (Glu, Asp, and Lys), comprising 17.58% AA composition of the WLSH hydrolysate, were recognised to involve in interactions with trypsin (Kvetkina et al., 2022). In addition, Hemmati et al. (2021) had recorded the role of AAs with hydroxyl side chains (Ser and Tyr) in TIA of the containing peptides. Moreover, hydrophobic AAs, particularly Pro, Val and Ile, were determined to create non-polar contacts or van der Waals forces with trypsin (Hemmati et al., 2021). In this study, these hydroxyl-containing and hydrophobic AAs constituted 10.14% and 14.86% overall AAs concentration of the WLSH hydrolysate, respectively. Relating to nutritional aspects, the WLSH hydrolysate in this study could supply at least 8 out of 9 essential AAs, with their ratio to total AAs of the hydrolysate reaching 44.59% (Table 1).

Table 1: AA profile of the WLSH hydrolysate

AAs	Content (mg/100g)	Ratio to TAA (%)	AAs	Content (mg/100g)	Ratio to TAA (%)
His	20	1.35	Asp	20	1.35
Ile	50	3.38	Glu	70	4.73
Leu	140	9.46	Gly	110	7.43
Lys	170	11.49	Pro	90	6.08
Met	30	2.03	Ser	70	4.73
Phe	130	8.78	Tyr	80	5.41
Thr	40	2.70	TEAA*	660	44.59
Val	80	5.41	THAA*	810	54.73
Ala	180	12.16	TPAA*	550	37.16
Arg	200	13.51	TAA*	1480	100

\* TEAA: Total essential AAs (His, Ile, Leu, Lys, Met, Phe, Thr, Val), THAA: Total hydrophobic AAs (Ile, Leu, Met, Phe, Val, Ala, Gly, Pro), TPAA: Total polar AAs (Asp, Glu, Ser, His, Thr, Arg, Lys), TAA: Total AAs.

### 3.4 pH and thermal stability of TIA of the WLSH hydrolysate

Figure 2a indicated that TIA of the WLSH hydrolysate was stable in pH ranging from 3 to 8, with the relative activity exceeding 63%, while it was unstable at extreme acidic or alkaline pH values. Strong intermolecular Coulomb forces, mediated by charged side chains at intensely acidic or alkaline conditions, cause the unfolding of peptides, which may negatively impact on the peptide's bioactivity (Vo et al., 2025a). Moreover, deamination and racemization reactions at alkaline pH may further decrease TIA of peptides (Vo et al., 2025b).

The WLSH hydrolysate totally retained its original TIA after being heated at 100°C for 15 min (Figure 2b). After that, the relative activity gradually dropped to 22.22±0.16% when increasing heating time up to 180 min. The preservation of TIA of the WLSH hydrolysate after a 15 min of heating might be attributed to its high content of hydrophobic AAs with bulky side chains or aromatic rings (Table 1). These AAs create compact cores for the containing peptides, promoting their overall robustness under thermal stress (Vo et al., 2025b).

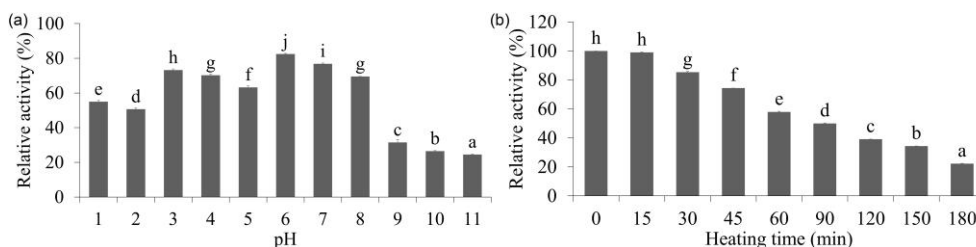


Figure 2: Bioactivity stability of the WLSH hydrolysate against (a) pH and (b) thermal treatment. Bars marked with different letters indicate statistically significant differences ( $p < 0.05$ ).

### 3.5 Solubility and heat stability

Solution pH plays a key role in peptide solubility as it determines their charge density, affecting the strength of their interactions with water molecules (Vo et al., 2025b). The WLSH hydrolysate presented a remarkable solubility, outdoing 76% over pH array from 3 to 8 (Table 2). This improved solubility could be ascribed to its high content of polar AAs (Table 1), facilitating hydrogen bonding with water molecules (Vo et al., 2025a). Heat

treatment causes peptide denaturation, exposing non-polar groups and promoting their hydrophobic interactions. As a consequence, peptide aggregation and precipitation occurs, decreasing solubility of the hydrolysate (Vo et al., 2025a). The WLSH hydrolysate exerted a superior heat stability, with its solubility surpassing 79% in the tested pH range (Table 2). This might be attributed to its abundance of bulky side chain hydrophobic AAs (Ile, Val, Leu, and Phe) (Table 1), which aided to form a compact core inside the peptides, limiting their mobility when subjected to high temperatures (Vo et al., 2025b). These enhanced solubility and heat stability could expand the application range of the WLSH hydrolysate in food formulations.

*Table 2: Functionalities of the WLSH hydrolysate*

Functionalities	pH 3	pH 4	pH 5	pH 6	pH 7	pH 8
Solubility (%)	87.21±0.62 <sup>c</sup>	88.34±0.01 <sup>d</sup>	76.53±0.36 <sup>a</sup>	91.87±0.07 <sup>e</sup>	81.42±0.04 <sup>b</sup>	96.42±0.42 <sup>f</sup>
Heat stability (%)	63°C, 30 min	83.33±0.01 <sup>c</sup>	89.54±0.17 <sup>e</sup>	95.97±0.03 <sup>f</sup>	82.34±0.11 <sup>b</sup>	80.17±0.34 <sup>a</sup>
	93°C, 30 s	85.95±0.49 <sup>c</sup>	90.90±0.06 <sup>e</sup>	96.75±0.40 <sup>f</sup>	83.15±0.08 <sup>b</sup>	79.89±0.33 <sup>a</sup>
FC (%)	Sample*	38.14±0.56 <sup>b</sup>	64.47±0.92 <sup>d</sup>	71.32±1.02 <sup>e</sup>	60.51±0.89 <sup>c</sup>	27.28±0.39 <sup>a</sup>
	Albumin	22.32±0.32 <sup>b</sup>	30.28±1.18 <sup>c</sup>	15.13±0.22 <sup>a</sup>	22.69±0.33 <sup>b</sup>	29.52±0.82 <sup>c</sup>
FS (%)	Sample*	12.71±0.19 <sup>b</sup>	14.88±0.21 <sup>d</sup>	34.43±0.49 <sup>e</sup>	22.69±0.33 <sup>c</sup>	32.24±0.46 <sup>a</sup>
	Albumin	4.96±0.07 <sup>b</sup>	20.19±0.79 <sup>c</sup>	31.09±1.28 <sup>a</sup>	20.17±0.30 <sup>b</sup>	20.48±0.82 <sup>c</sup>
EAI (m <sup>2</sup> /g)	Sample*	21.2±0.41 <sup>d</sup>	7.74±0.23 <sup>a</sup>	14.91±0.3 <sup>c</sup>	13.01±0.2 <sup>b</sup>	21.07±0.32 <sup>d</sup>
	Control**	6.57±0.17 <sup>b</sup>	3.73±0.23 <sup>a</sup>	7.36±0.25 <sup>c</sup>	8.89±0.17 <sup>d</sup>	11.28±0.44 <sup>e</sup>
ESI (min)	Sample*	13.33±0.2 <sup>a</sup>	36.64±1.07 <sup>e</sup>	21.63±0.74 <sup>d</sup>	15.69±0.45 <sup>c</sup>	15.53±0.32 <sup>c</sup>
	Control**	24.39±1.96 <sup>c</sup>	29.68±2.95 <sup>d</sup>	24.6±2.37 <sup>c</sup>	17.13±0.71 <sup>b</sup>	16.25±1.03 <sup>b</sup>

\*Sample: WLSH hydrolysate; \*\*Control: Sodium caseinate. Different letters within the same row indicate statistically significant differences ( $p < 0.05$ ).

### 3.6 Foaming property

This study evaluated the foaming property of the WLSH hydrolysate via foaming capacity (FC) and foaming stability (FS), which denoted the ability of peptides to form films for entrapment air and to preserve the air bubbles within the foam, respectively (Vo et al., 2025a). Within the pH ranging from 3 to 8, FCs of the WLSH hydrolysate ranged from 27.28 to 71.32% (Table 2), while it demonstrated FSs between 12.71 and 52.95% (Table 2). The WLSH hydrolysate exhibited significantly higher foaming properties than those of albumin at almost all pH in the tested range, particularly its FC and FS at pH 8 were respectively 2.37 and 1.80 fold greater compared to those of albumin. This enhanced FC of the hydrolysate could be due to its increased solubility and hydrophobic AA content, thus increasing the diffusion and adsorption of its component peptides at the gas-water interface (Wang et al., 2025). Meanwhile, the highest FS of the hydrolysate at pH 8 would be as a result of appropriate peptide-peptide interactions, promoting the formation of strong films capturing air (Vo et al., 2025b). The results indicated that the WLSH hydrolysate can be used as a natural foaming agent in the development of aerated foods, particularly cakes, biscuits, bread, and mousses.

### 3.7 Emulsifying property

This techno-functionality is usually evaluated via emulsifying activity index (EAI) and emulsifying stability index (ESI): the former shows peptide's capacity of forming emulsion, while the latter denotes the emulsion stability over a specific time (Vo et al., 2025b). EAI of the WLSH hydrolysate peaked at pH 8 with the value of  $28.74 \pm 0.37$  m<sup>2</sup>/g (Table 2), while its maximum ESI of  $36.64 \pm 1.07$  min was seen at pH 4 (Table 2). The increased EAI at alkaline pH may be due to the stretch of peptides, which promote the orientation of their hydrophilic and hydrophobic regions at the oil-water interface (Vo et al., 2025a). High solubility of the WLSH hydrolysate at pH 8 (Table 2) suggested that its component peptides could rapidly migrate to and absorb onto the oil-water interface, boosting the hydrolysate's EAI (Souza et al., 2025). Meanwhile, pH 4 may create enough positive charges in the peptide-derived layers enveloping oil droplets, favoring the hydration repulsive forces of these droplets, preventing them from coalescence, finally increasing the ESI (Vo et al., 2025b). Within the tested pH range, the WLSH hydrolysate demonstrated EAI of at least 1.46 times greater than those of sodium caseinate, a protein-originated emulsifier used in food products (Table 2). Alternatively, ESI of the hydrolysate was only higher than that of sodium caseinate at pH 4, surpassing by 1.23 fold (Table 2). The elevated emulsifying property of the hydrolysate may correlate to its remarkable content of lipophilic AAs. These results contribute to extending the WLSH hydrolysate applications in food development as a natural emulsifier.

### 3.8 WHC and OHC

WHC of the WLSH hydrolysate in this study was  $0.89 \pm 0.10$  mL water/g hydrolysate powder, being 2.82 times lower than that of casein. As the hydrolysate demonstrated high solubility, its component peptides were well

dispersed in water, limiting peptide-network formation to entrap water (Vo et al., 2025b). In contrast, the WLSH hydrolysate showed an OHC of  $1.38 \pm 0.16$  mL oil/g hydrolysate powder, equal to that of casein. This could be due to the exposure of buried hydrophobic AAs from the native proteins during alcalase hydrolysis, along with the high amount of hydrophobic AAs in the hydrolysate (Table 1), favoring the its peptides to interact with oil molecules (Vo et al., 2025a). It could be predicted that the fortification of the WLSH hydrolysate into food products could aid in hindering fluid escape during the food processing and storage.

#### 4. Conclusions

This study provided an alternative way to utilize WLSHs, producing trypsin inhibition protein hydrolysate or peptide fractions, possibly used as food enhancer, or an anti-obesity treatment-supporting agent. However, further tests should be done to validate their effectiveness in real-world contexts. The analysis results of the hydrolysate's AA composition, techno-functional characteristics, as well as pH and thermal stability of its bioactivity would be beneficial for future investigations, regarding the fortification of the hydrolysate into food products, or nutraceuticals. Also, the 1-3 peptide fraction from the WLSH hydrolysate could be further studied on sequencing, *in silico* or other analyses for new applications.

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