



## Physicochemical Characteristics and ACE Inhibitory Activity of Duck Egg White Powder Peptide Fermented with *Candida Metapsilosis* Ta.22

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### ABSTRACT

The potential of bioactive peptides to function as antihypertensive agents is one of the functional characteristics of duck eggs that can be promoted. These peptides can be derived from egg white through fermentation using yeast microbes. The peptides produced by fermentation vary in type depending on the microbial strain and fermentation conditions. This study aims to evaluate the ACE-inhibitory activity and the functional properties of duck egg white powder produced through fermentation using *Candida metapsilosis* isolate Ta.22. The highest ACE-inhibitory activity was obtained from egg white peptides fermented for 24 hours at 0.2% concentration is 79.5%. The resulting product exhibited the following chemical characteristics: moisture content of 7.53%, ash content of 5.7%, protein content of 73.62%, carbohydrate content of 1.96%, reducing sugar content of 2.86%, fat content of 0.03%, and pH value of 9.11. Physical properties included a yield of 12.38%, foaming capacity of 113.8%, foam stability of 84.5%, color intensity values of L\* 90.65%, a\* 1.32%, and b\* 9.23%. These findings indicate that fermented duck egg white peptides possess promising functional and bioactive properties, supporting their potential use as natural antihypertensive agents.

**Key words:** *C. metapsilosis* Ta.22, Duck egg white peptide, ACE inhibitor, Physicochemical properties

### INTRODUCTION

Duck eggs possess several functional properties, one of which is the potential of their bioactive peptides to act as antihypertensive agents. These peptides can be obtained through the hydrolysis of ovalbumin (Benedé and Molina 2020) or ovotransferrin (Rathnapala et al. 2021). Although hydrolysis is commonly performed on whole egg white (Li et al. 2024). Bioactive peptides derived from ovalbumin hydrolysis have been shown to exhibit antihypertensive activity (Maggonagé al. 2024). Hydrolysis of proteins using the enzyme Alcalase has been reported to show high ACE-inhibitory activity of 74% (Vásquez et al. 2024). Alcalase is an alkaline protease frequently employed to produce protein hydrolysates rich in bioactive peptides capable of inhibiting angiotensin-converting enzyme (ACE) (Tacias-Pascasio et al. 2020).

Bioactive peptides can also be isolated using techniques such as ultrafiltration or chromatography

(Marcet et al. 2022). While crude bioactive peptides are often used as functional components in food products, purified peptides are typically utilized in the pharmaceutical industry (Jiang et al. 2024). Furthermore, hydrolysis with bioactive potential can indicate the suitability of animal- or plant-based proteases (Mazorra et al. 2018; Bueno Gavilá et al. 2019), or a combination thereof, for efficient peptide production (Zhang et al. 2019). Selecting appropriate proteases for hydrolysis can enhance the functional properties of food products and their derivatives (Ceylan et al. 2023).

Based on recent research, enzymes derived from microbial sources exhibit significant advantages over gastrointestinal enzymes in producing more potent bioactive peptides (Cruz Casas et al. 2021). Trypsin, a digestive enzyme, typically produces fewer peptides and demonstrates lower hydrolytic efficiency when compared to microbial-derived enzymes such as alcalase (Abeyrathne et al. 2016). Different hydrolytic enzymes operate optimally

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at varying temperatures. For instance, alcalase exhibits optimal activity at 50°C (Jahandideh et al. 2018). Incubation temperature is a critical factor influencing enzyme activity, and excessively high temperatures may damage the protein structure in egg components. Notably, specific egg proteins begin to denature at temperatures as low as 45°C.

Alcalase obtained from *Bacillus licheniformis* has been shown to produce higher ACE-inhibitory activity, reaching 273.83µg protein/mL, compared to pepsin (171.87µg protein/mL) and thermolysin (81.07µg protein/mL) (Liu et al. 2018). Furthermore, hydrolysis of egg yolk proteins using alcalase from *Bacillus subtilis*, in combination with protease N, achieved a hydrolysis degree of 25.3% and a solubility of 98%, outperforming hydrolysis using trypsin (Ting et al. 2011). Another study reported an even higher degree of hydrolysis (43.2%) using alcalase from *Bacillus* species, which was 50% greater than the hydrolysis achieved using papaya-derived collopulin (Noh and Suh 2015). These findings indicate that a higher degree of hydrolysis leads to a more effective breakdown of egg white proteins.

These results suggest that alcalase derived from microbial sources is a promising agent for bioactive peptide production. However, the use of alcalase derived from yeast in protein hydrolysis remains underexplored. Moreover, bioactive peptides produced through alcalase hydrolysis are not always fully compatible with enzymes produced by the human body (Cruz Casas et al. 2021). Therefore, this study aims to determine the ACE inhibitor activity physicochemical properties, and functional properties of duck egg white peptides fermented using yeast isolate *C. metapsilosis* Ta.22.

## MATERIALS AND METHODS

### Collection of eggs

The duck eggs used in this study were obtained from Cihateup ducks in West Java, Indonesia. The *C. metapsilosis* isolate was sourced from the fermentation of salted duck eggs stored at the Laboratory of Animal Product Processing Technology, Faculty of Animal Husbandry, Universitas Padjadjaran.

### Preparation of starter culture and duck egg white powder

Two streaks of *C. metapsilosis* were inoculated into 5mL of NaCl broth and incubated at 25°C for 24 hours. Subsequently, 0.1mL (0.1%) of the resulting culture was transferred into 100mL NaCl medium and incubated at 25°C for 48 hours. The resulting *C. metapsilosis* culture was used for the fermentation process in egg white powder preparation, as modified from the method described by Nusa et al. (2017). Fresh duck eggs were manually separated to obtain the egg whites, which were then acidified with 5% citric acid until reaching a pH of 6.5. The egg whites were pasteurized in a water bath (Julabo TW20) at 62°C for 3 minutes. After pasteurization, the egg whites were fermented using the prepared *C. metapsilosis* culture. The fermentation process was carried out at 25°C for 24 and 48 hours in an incubator (Memmert IF55).

### Chemical properties analysis

Moisture and ash content were determined using the

gravimetric method as outlined by AOAC (2005). The mass difference before and after drying was used to calculate the moisture content. Following the moisture analysis, the same sample was used to determine ash content. Ash content was calculated by comparing the weight of the resulting ash to the initial sample weight and expressed as a percentage. Fat content was measured according to (AOAC, 2005) procedures using a Soxhlet extraction method. The fat content was calculated by comparing the weight of extracted fat to the initial sample weight and expressed as a percentage. Carbohydrate levels were determined using the Luff-Schoorl method (Handayani and Hidayati 2024). Protein content was analyzed using the Lowry method (Shen, 2019). Protein concentration was determined by measuring the absorbance and comparing it to a standard curve. The final protein content was calculated as follows

$$\text{Final protein content} = \text{protein weight/sample weight} \times 100\%.$$

Where protein weight = sample volume x protein concentration.

Reducing sugar levels were assessed using the dinitrosalicylic Acid (DNS) method with UV/VIS spectrophotometry. Absorbance was measured at 550nm using a UV/VIS spectrophotometer. A linear regression equation from the glucose standard curve calculated the reducing sugar content (Puspita et al. 2020). To measure pH, 10g of the sample was dissolved in 100mL of distilled water and homogenized. The pH of the homogenized solution was measured using a digital pH meter (Hanna HI2210-02).

### Physical properties measurement

Yield percentage was determined by using the AOAC (2005) method. 1g sample was dissolved in 100mL of distilled water and filtered using filter paper. The yield was then calculated as the percentage of the retained residue relative to the initial sample mass. Foaming capacity and foam stability were evaluated following the method by Ulug et al. (2021). Foam volume generated from the egg white was compared to the initial volume of the egg white solution. Foam stability was assessed by observing the volume of foam retained over time. Emulsion stability was tested using mayonnaise formulated with egg white powder. A 1mL mayonnaise sample was centrifuged at 4000rpm for 15 minutes. Emulsion stability was calculated using the following:  $100 - (\text{volume of separated phase/volume of mayonnaise} \times 100\%)$ . Color analysis was performed using a chromameter to obtain L\*, a\*, and b\* values. These values were then processed using the Nix sensor application for standardized color interpretation (Yulkifli et al. 2019).

### ACE inhibitory activity measurement

The antihypertensive activity of the samples was assessed following the method described by Eckert et al. (2013). A 40µL sample (comprising hydrolysate, peptide fractions, or peptides) was mixed with 160µL of Hippuryl-His-Leu (HHL) substrate solution (5mmol/L in 100mmol/L potassium phosphate buffer containing 300mmol/L NaCl, pH 8.3) and incubated at 37°C for 5 minutes. To initiate the ACE enzymatic reaction, 20µL (2mU) of ACE solution was added, followed by further incubation at 37°C for

30min. The enzymatic reaction was terminated by adding 150µL of 1M HCl. Hippuric acid produced from the reaction was extracted with 1mL of ethyl acetate, then vortexed. A 750µL aliquot of the upper organic layer was transferred to a test tube and evaporated under vacuum. The resulting residue was reconstituted in 800µL of distilled water, and absorbance was measured at 228nm using a UV spectrophotometer. Each sample was analyzed in triplicate. The ACE inhibitory activity was calculated by using the following formula:

$$\text{ACE Inhibition(\%)} = \left( \frac{\text{Blank absorbance} - \text{Sample absorbance}}{\text{Blank absorbance}} \right) \times 100$$

### Statistical Analysis

This study was conducted as a laboratory experiment using a Nested Completely Randomized Design (CRD). The experimental design included two factors: concentration, with three levels (0.2, 0.4, and 0.6%), and fermentation duration, nested within concentration, with two levels (24 hours and 48 hours). Each treatment was replicated four times. When a significant difference was found, Duncan's Multiple Range Test was applied for post hoc comparison (Gaspersz 1995).

## RESULTS

### ACE inhibitory activity of duck egg white powder peptide

The results showed that prolonged fermentation time of duck egg white using *C. metapsilosis* significantly increased ACE (angiotensin-converting enzyme) inhibitory activity. This finding suggests that extended fermentation enhances protein breakdown, leading to the production of peptide sequences that are likely responsible for ACE inhibitory (antihypertensive) effects. Egg white is a nutritionally rich food source known to contain bioactive compounds with various biological activities, including antihypertensive properties.

### Effect of duck egg white powder peptide on the chemical properties

Based on the research results, the length of fermentation on making egg flour showed significant results ( $P < 0.05$ ) in terms of ash content, protein content, carbohydrate content, and reducing sugar. On the other hand, water content, fat content, and pH value showed the same effect ( $P > 0.05$ ). There was a difference between the fermentation times of 24 and 48 hours.

### Effect of duck egg white powder peptide on the physicochemical characteristics

*C. metapsilosis* produces protease enzymes. Therefore, the functional properties of egg white powder show similar results between treatments. Yield, foaming capacity, foaming stability, and emulsion stability values show non-significant results ( $P > 0.05$ ). Meanwhile, color intensity shows significant results ( $P < 0.05$ ) at the  $a^*$  and  $b^*$  values.

## DISCUSSION

### ACE inhibitory activity of duck egg white powder peptide

The ACE inhibitory activity obtained in this study

(Table 1) was higher compared to the findings of Nahariah et al. (2015), who reported an activity range of 50.57% to 75.06% for egg white. This increase may be attributed to the proteolytic nature of *C. metapsilosis*, which enables the production of more specific peptides that contribute to greater ACE inhibitory activity. According to Ramos et al. (2015), *C. metapsilosis* exhibits a proteolytic capability of up to 93.8%, supporting its effectiveness in producing bioactive peptides from egg white proteins.

**Table 1:** ACE inhibitor of duck egg white powder peptide

Treatment	ACE inhibitor
	.....%.....
T(0.2%;24 jam)	75.75 <sup>a</sup>
T(0.2%;48 jam)	79.50 <sup>b</sup>
T(0.4%;24 jam)	76.00 <sup>a</sup>
T(0.4%;48 jam)	77.00 <sup>a</sup>
T(0.6%;24 jam)	77.25 <sup>a</sup>
T(0.6%;48 jam)	78.75 <sup>b</sup>

Values followed by different letters in a column differ significantly ( $P < 0.05$ ).

In the second phase of this study, the enzymes produced by *C. metapsilosis* demonstrated significant activity toward peptides derived from ovalbumin protein, achieving hydrolysis efficiency exceeding 95%. It is likely that the observed ACE (angiotensin-converting enzyme) inhibitory activity during fermentation originated from these ovalbumin-derived peptides. Ovalbumin is known to be the most abundant protein in egg white, with a molecular weight of approximately 45kDa, comprising 386 amino acids, and possessing protease inhibitory properties (Ding et al. 2016). When hydrolyzed by proteolytic enzymes, ovalbumin can yield peptides exhibiting ACE inhibitory, antimicrobial, and antioxidant activities (Liu et al. 2018).

Interestingly, generating ACE inhibitory peptides does not necessarily require a specific protease or protein substrate. However, small peptides, typically consisting of fewer than 10 amino acids, tend to demonstrate higher ACE inhibitory activity (Aluko, 2015). Both the type of protease used and the nature of the protein substrate play crucial roles in the release of bioactive peptides. Therefore, selecting an appropriate protease is essential to obtain hydrolysates with the desired functional peptide profile (Tavano, 2013). Peptides such as RADHPFL and YAEERYPIL, derived from egg white, have been identified as having high ACE inhibitory activity (Miguel et al. 2004). Conversely, other studies have reported peptides with low ACE inhibitory potential. For instance, peptides FFGRCVSP and ERKIKVYL, extracted from ovalbumin (OVA), showed limited ACE inhibitory effects (Fujita et al. 2000). This reduced efficacy may be attributed to the degradation or modification of egg-derived peptides during passage through the gastrointestinal tract, bloodstream, or liver (Hartmann and Meisel 2007).

### Effect of duck egg white powder peptide on the chemical properties

Protein structural changes during fermentation increase the number of exposed active groups, thereby enhancing the water-binding capacity of the material. Consequently, the moisture content in duck egg white powder increases with longer fermentation durations (Pujimulyani et al. 2001). During fermentation, *Candida*

species metabolize glucose and galactose through glycolysis under aerobic conditions, generating pyruvic acid. This intermediate is then converted into Acetyl-CoA and enters the Krebs cycle, producing carbon dioxide (CO<sub>2</sub>), water (H<sub>2</sub>O), and energy in the form of ATP (Paige and Lydia 2023). Incomplete drying processes can also contribute to elevated moisture content, as residual water in the material may not fully evaporate (Clavaud et al. 2020). Pan drying, also referred to as layered drying using an oven, is employed to reduce processing time and cost. However, careful management of drying layer thickness is critical to ensuring optimal air circulation within the oven for efficient drying performance.

Potassium is a macro element essential for yeast metabolism, functioning as a cofactor for various enzymes involved in oxidative phosphorylation, protein biosynthesis, and carbohydrate metabolism. In abnormal yeast strains, the role of potassium can be substituted by magnesium or sodium; however, such substitution leads to a reduction in fermentation efficiency (Ribeiro-Filho et al. 2022). In the present study, it was observed that increasing the concentration of *C. metapsilosis* corresponded with higher ash content in the resulting flour. This increase may indicate that fermentation was not proceeding efficiently, possibly due to impaired potassium utilization as a metabolic cofactor. Higher ash content generally signifies an increased mineral concentration, which, while contributing positively to nutritional value, is often undesirable in flour products as it can impart a darker coloration. Across all fermented substrates, ash content increased post-fermentation. This rise is attributed to the high glucose content in the medium, which is converted into organic acids and hydrolyzed into other compounds, including minerals, ultimately elevating the ash levels. Previous research by Pratama et al. (2023) supports these findings, indicating that fermentation using *Kluyveromyces* not only increases ash content but also enhances protein levels in chicken egg white flour.

A study by Jiang et al. (2020) reported that prolonged fermentation leads to a decrease in optical density. This phenomenon is due to the microbial production of proteolytic enzymes capable of hydrolyzing proteins (Bu et al. 2010). Specifically, the percentage of ovalbumin protein (40kDa) decreased from 72.54% to 70.77% after 9 hours of fermentation, while the peptide fraction (5kDa) increased from 2.02% to 2.76%. These findings indicate that longer fermentation times result in a reduction of crude protein content in the duck egg white flour (Jach et al. 2022). Further explained that during fermentation, protease enzymes produced by yeast degrade complex proteins in egg white into smaller amino acids and peptides. This enzymatic activity contributes to the overall reduction in total protein content in the final fermented egg product.

Table 2 illustrates that as the fermentation time increases (24 and 48 hours), there is a corresponding decrease in the carbohydrate content (total sugars) of the duck egg white flour. In this study, *C. metapsilosis* not only demonstrated the ability to hydrolyze proteins into peptides but also exhibited the capability to degrade sugars present in the duck egg white. Yeast is known to metabolize monosaccharides, disaccharides, and certain trisaccharides through enzymatic carbohydrate synthesis (Yoon et al. 2003). The degradation of sugars may indirectly enhance peptide release due to increased protease activity, as reflected by the higher levels of protein hydrolysis observed (Asaithambi et al. 2022) emphasized that sugar breakdown through yeast fermentation yields higher efficiency compared to conventional hydrolysate methods. The results of this study further indicate a consistent decrease in reducing sugar content with extended fermentation time. This suggests that *C. metapsilosis* effectively reduces glucose levels in duck egg white through the enzymatic action of zymase, which facilitates the release of hydrogen ions from glucose to produce alcohol and carbon dioxide. Under aerobic conditions, glucose undergoes oxidation, where carbon atoms bind with oxygen (Ramos et al. 2015).

The fat content in this study complies with the Indonesian National Standard (SNI 01-4323) for egg white flour (1996), which specifies a maximum fat level of 1%. Fat content in egg white flour is directly linked to rancidity; excess fat can accelerate lipid oxidation, which not only deteriorates flavor and aroma but can also alter protein structure through amino acid degradation. Therefore, maintaining a low fat content is crucial, as it extends shelf life and preserves the sensory and nutritional qualities of the product. The results support the significance of minimizing lipid levels to ensure product stability during storage.

The research analysis revealed that variations in the concentration of *C. metapsilosis* and the nested fermentation duration did not significantly affect the pH value of the duck egg white flour. In all treatments, the pH remained consistently high, above 9. This elevated pH can be attributed to the yeast strain used, *C. metapsilosis*, which was isolated from salted egg products known for their high pH environments. Notably, the ideal pH for optimal protease activity in this strain is above 8.5, supporting its performance under alkaline conditions.

### Effect of duck egg white powder peptide on the physicochemical characteristics

Based on Table 3, show the results of the effect of fermentation treatment on physicochemical characteristics. The average yield of duck egg white flour in this study was lower than that reported by Fakhruzy et al. (2023), who

**Table 2:** Physicochemical properties of duck egg white powder peptide

Treatment	Water content	Ash content	Protein content	Carbohydrate content	Reducing sugar	Fat content	pH
				%			
T(0.2%;24h)	7.53 <sup>a</sup>	5.70 <sup>a</sup>	73.62 <sup>a</sup>	2.96 <sup>a</sup>	2.86 <sup>a</sup>	0.03 <sup>a</sup>	9.11 <sup>a</sup>
T(0.2%;48h)	8.66 <sup>a</sup>	5.64 <sup>a</sup>	67.52 <sup>b</sup>	1.31 <sup>b</sup>	1.09 <sup>b</sup>	0.02 <sup>a</sup>	9.12 <sup>a</sup>
T(0.4%;24h)	7.63 <sup>a</sup>	5.37 <sup>a</sup>	68.73 <sup>a</sup>	3.33 <sup>a</sup>	3.02 <sup>a</sup>	0.03 <sup>a</sup>	9.35 <sup>a</sup>
T(0.4%;48h)	8.86 <sup>a</sup>	5.68 <sup>a</sup>	63.66 <sup>b</sup>	2.12 <sup>b</sup>	2.41 <sup>a</sup>	0.03 <sup>a</sup>	9.16 <sup>a</sup>
T(0.6%;24h)	8.37 <sup>a</sup>	5.35 <sup>a</sup>	66.50 <sup>a</sup>	1.60 <sup>a</sup>	1.98 <sup>a</sup>	0.02 <sup>a</sup>	9.13 <sup>a</sup>
T(0.6%;48h)	8.94 <sup>a</sup>	5.31 <sup>b</sup>	67.81 <sup>a</sup>	0.94 <sup>b</sup>	0.76 <sup>a</sup>	0.03 <sup>a</sup>	9.06 <sup>a</sup>

Values followed by different letters in a column differ significantly (P<0.05).

**Table 3:** Physicochemical characteristics of duck egg white powder

Treatments	Yield	Foaming capacity	Foaming stability	Emulsion stability	Color intensity		
			(%)		L*	a*	b*
P(0.2%;24h)	12.38 <sup>a</sup>	113.8 <sup>a</sup>	69.8 <sup>a</sup>	84.5 <sup>a</sup>	90.65 <sup>a</sup>	1.32 <sup>a</sup>	9.23 <sup>a</sup>
P(0.2%;48h)	12.97 <sup>a</sup>	135.0 <sup>a</sup>	40.3 <sup>a</sup>	57.3 <sup>a</sup>	90.36 <sup>a</sup>	2.36 <sup>b</sup>	11.12 <sup>a</sup>
P(0.4%;24h)	12.68 <sup>a</sup>	120.0 <sup>a</sup>	76.0 <sup>a</sup>	81.0 <sup>a</sup>	91.89 <sup>a</sup>	1.61 <sup>a</sup>	8.99 <sup>a</sup>
P(0.4%;48h)	13.18 <sup>a</sup>	137.5 <sup>a</sup>	42.0 <sup>a</sup>	63.0 <sup>a</sup>	91.20 <sup>a</sup>	2.25 <sup>b</sup>	9.24 <sup>a</sup>
P(0.6%;24h)	13.01 <sup>a</sup>	117.5 <sup>a</sup>	68.0 <sup>a</sup>	66.8 <sup>a</sup>	92.54 <sup>a</sup>	1.48 <sup>a</sup>	7.48 <sup>a</sup>
P(0.6%;48h)	12.60 <sup>a</sup>	124.5 <sup>a</sup>	52.3 <sup>a</sup>	54.0 <sup>a</sup>	92.65 <sup>a</sup>	1.71 <sup>a</sup>	7.03 <sup>a</sup>

Values followed by different letters in a column differ significantly (P<0.05).

obtained yields ranging from 13.13 to 14.58% after 6 to 24 hours of fermentation. According to Fadhila et al. (2023), the reduction in yield can be attributed to the action of yeast during fermentation, which consumes glucose in the egg white, thereby decreasing the final mass of the flour product. Additionally, yield loss may result from carbon dioxide evaporation during the drying process, which contributes to further mass reduction.

The average foaming capacity observed in this study was comparatively lower than that reported in previous studies 356.74% (Nusa et al. 2017), 280% (Wibowo and Sudjatinah 2023), and 163.33% (Pratama et al. 2023). This decline in foaming capacity may be attributed to the proteolytic nature of *C. metapsilosis*. In another study, Ramos et al. (2015) documented a proteolytic capability of 60.34% for *Candida* species. Moreover, pH plays a significant role in foaming capacity. Ovalbumin, the primary protein responsible for foam formation, can transform s-ovalbumin during fermentation due to the release of CO<sub>2</sub> and H<sub>2</sub>O, which elevates the pH and alters the protein structure (Wibowo and Sudjatinah 2023).

The results indicate that a 24 hour fermentation period produced higher average foam stability in duck egg white flour compared to a 48 hour fermentation. However, the foam stability values obtained in this study remain lower than those reported by Wibowo and Sudjatinah (2023), who achieved 95.2% foam stability at pH 8.0. This discrepancy may be due to prolonged fermentation, which increases protein degradation from tertiary to secondary and even primary structures, thereby compromising foam stability. Additionally, the pH levels recorded for the duck egg white flour across various treatments averaged 9.2, which may further affect foam performance. The proteolytic activity of *C. metapsilosis* contributes to the reduced ability of proteins to maintain stable foams, as extensive hydrolysis impairs the protein's structural integrity needed for foam retention.

The emulsion stability of emulsions formulated from fermented duck egg white flour ranged between 54.0% and 84.5%, indicating relatively strong stability. These results compare favorably with the findings of Wulandari and Arief (2022), who reported emulsion stability values of 72.4% to 74.0% for mayonnaise made from chicken egg whites. This suggests that the proteins in the duck egg white flour, even after fermentation with *C. metapsilosis*, retain considerable functional integrity in stabilizing emulsions such as mayonnaise.

Protein hydrolysis induced by proteolytic enzymes produced by the yeast plays a critical role in enhancing emulsifying properties. The enzymatic action facilitates the formation of thin interfacial films around oil droplets, which contributes to improved emulsion stability

(Asaithambi et al. 2022). Emulsion stability is achieved when proteins effectively interact with oil phases through coalescence prevention. The emulsifying properties of proteins are influenced by the molecular weight of peptides and their amphiphilic characteristics. Larger protein molecules generally exhibit better emulsifying capabilities (Quan and Benjakul 2019). However, the emulsification efficiency of egg white proteins also strongly depends on external factors such as pH, salt concentration, and polymer ratios. These factors influence ovalbumin's surface hydrophobicity and structural flexibility, enabling more stable emulsion structures (Razi et al. 2023).

The concentration of *C. metapsilosis* had a statistically significant effect on the L\*, a\*, and b\* color values of duck egg white powder. However, the nested fermentation time within the concentration levels showed no significant effect on the L\* and b\* values. A significant effect was observed only in the a\* value, indicating a notable influence of fermentation duration on the redness of the egg white powder. Specifically, the fermentation duration at concentrations P1 (0.2%) and P2 (0.4%) significantly influenced the a\* value between the 24 hour and 48 hour time points (Table 2). In this experimental design, the concentration level was the primary factor affecting the tested parameters. The change in a\* values may be attributed to the progressive fading of pigments in the egg white during extended fermentation, or potentially to the inconsistent granulation during drying, which can lead to uneven color distribution in the final powdered product.

## Conclusion

The duck egg white peptides were achieved through fermentation for 24 hours, and 0.2% exhibited a high ACE inhibitory activity of 79.5%. The chemical composition of the product included 7.53% moisture, 5.7% ash, 73.62% protein, 1.96% carbohydrate, 2.86% reducing sugar, 0.03% fat, and a pH of 9.11. The physical properties comprised a yield of 12.38% of yield, foaming capacity of 113.8%, foam stability of 84.5%, and the color intensity measurements values of L\* 90.65, a\* 1.32, and b\* 9.23. These results demonstrate that fermentation with *C. metapsilosis* not only enhances the functional bioactivity of duck egg white peptides but also maintains desirable physicochemical properties suitable for further application in the food and nutraceutical industries.

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