

EFFECT OF EGG WHITE PRE-TREATMENTS ON ANTIOXIDANT ACTIVITY OF ITS HYDROLYSATE

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Received: November 30, 2020; Revised: June 28, 2021; Accepted: June 29, 2021

Abstract

Egg white is considered as one of the best food proteins and hydrolysis can be applied to increase its nutritional value and reveal antioxidant activity. Low molecular weight of egg white hydrolysate was also beneficial for the chronic kidney disease (CKD) sufferers. However, hydrolysis of egg white protein typically results in relatively low yield. Thus, pre-treatment methods to increase yield of hydrolysate should be sought. The objective of this research was to compare the use of ultrasound, microwave, and steam sterilization (121°C) prior to hydrolysis of egg white by 10% (w/w protein) Alcalase on degree of hydrolysis (DH) and antioxidant activity of the hydrolysate. Ultrasound at intensity of 41.5 W/cm² applied to boiled egg white was found to produce hydrolysate with the highest DH of 45.4%. Antioxidant activities of egg white hydrolysate were examined by 2, 2'-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid (ABTS) radical scavenging activity, ferric reducing antioxidant power (FRAP), and ferrous ion chelating capacity (FICC) assays. Raw egg white subjected to sterilization for 5 min resulted in the hydrolysate exhibiting the strongest ABTS radical scavenging activity with approximately 64% inhibition. In addition, 30-min steam sterilization the most powerful pre-treatment yielding peptides with FRAP of 22 µM Trolox equivalence. Boiled egg white subjected to ultrasound at intensity of 41.5 W/cm² obtained hydrolysate with FICC of 1800 µM EDTA equivalence. Since there were several measured parameters, and pre-treatment methods affected these parameters differently.

Keywords: Egg white hydrolysate, Ultrasound, Microwave, Steam sterilization, Antioxidan

Introduction

Chicken egg white is assumed as the best source of protein, because many proteins are developed within the egg white, such as ovalbumin, ovotransferrin, lysozyme, ovomucin, and ovomucoid (Abeyrathne *et al.*, 2018). Ovalbumin contains nearly 54% of total protein of egg white, considering as the major protein presence in the egg white. High amount of protein has been found to be

beneficial as diet therapy for several diseases, including chronic kidney disease (CKD). Albumin is one the most prominent protein presence in human body to maintain osmotic balance endogenously as its main function. Albumin metabolism is synthesized by the liver and only certain amount is filtered by glomerular membrane of kidney. Studies revealed that healthy people loss

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merely 10-20 mg/day of albumin through urinary process. The intensive losses of albumin often cause hypoalbuminemia, bearing a meaning for a state of albumin lower than 3.0 g/dL (Mazzaferro and Edwards, 2020). The main cause of hypoalbuminemia has been established over the years, either caused by the lack of hepatic albumin synthesis or abnormality of kidney glomerular filtration. The perpetual condition of hypoalbuminemia excessively leads to CKD and dialysis treatment or kidney transplantation is necessary in the nearly future to suppress mortality rate. Therefore, exogenous albumin intake from egg white can be helpful to overcome the lack of albumin for the sufferers (Nicholson *et al.*, 2008).

Hydrolysis of egg white into protein hydrolysate is expected to have high nutritional value with various bioactivities, including antioxidant activity (Chen *et al.*, 2012). Aside from that, low molecular weight of protein hydrolysate also has a vital contribution in albumin absorption due to degradation of liver capability of the sufferers to synthesize albumin (Mazzaferro and Edwards, 2020). Nevertheless, conventional enzymatic hydrolysis process sometimes comes up with relatively low yield and time consuming. Nowadays, there is a huge attraction in technology to be developed and become the most suitable approach in hydrolysate production which is often applied prior to hydrolysis, including maximizing hydrolysis process, shortening hydrolysis time, enhancing degree of hydrolysis (DH), and promoting antioxidant activity (Ketnawa and Liceaga, 2016).

The most commonly used technologies in research area are high hydrostatic pressure, microwave, and pulsed electric field (Marciniak *et al.*, 2018). Nevertheless, the research of using technology as pre-treatment method prior to hydrolysis is still limited. Therefore, ultrasound (US), microwave (MW), and steam sterilization (ST) are proposed in this research. Ultrasound is proven to generate smaller particle sizes of protein and exposure protein cleavage sites, which facilitates better enzyme accessibility during hydrolysis. This action is attributed to high shear force and cavitation generated during ultrasound (Stefanovic *et al.*, 2014). Microwave irradiation is absorbed by dipole compounds, which cause molecular rotation within the electric field. Consequently, the temperature rises up rapidly and could disrupt protein aggregation, leading to greater enzyme accessibility (Vanier, 2013). Steam sterilization produces high pressure and temperature to extensively denature the protein. Protein denaturation enhances enzyme accessibility (Sezdi and Yoleri, 2013).

By improving enzymatic cleavage during hydrolysis through pre-treatment technologies could increase DH that may give impact on antioxidant activity (Mason, 1996). Thus, in this study several chemical-based antioxidant assays are carried out, including 2, 2'-azinobis-3-ethylbenzothiazoline-6-sulfonic acid (ABTS) assay, ferric reducing antioxidant power (FRAP) assay, and ferrous ion chelating capacity (FICC) assay. Therefore, the aim of this research was to compare DH and antioxidant activity of egg white hydrolysate prepared from 3 various pre-treatments aforementioned.

Material and Methods

Chicken eggs were purchased from Suranaree University of Technology Farm. The egg white was separated from the yolk and used as raw egg white sample. In addition, cooked egg white sample was prepared by boiling at 95°C for 30 min and used as a control. Raw and cooked egg white samples were added deionized water to ratio of 1: 20 (w/v protein). For ultrasound pre-treatment, samples were sonicated using ultrasonic probe with a 12-mm titanium tip (Qsonica Ultrasonic Processor, LLC, Newtown, CT 06470, USA) at frequency of 20 kHz with an intensity of 4.6, 22.1, 41.5 W/cm² for 10 min (pulse duration of on-time 5 s and off-time 5 s). For microwave irradiation, samples were subjected to microwave digestion unit (Ethos One, Milestone Srl, Sorisole (BG), Italy) at different temperatures (121°C for 2 min, 110°C for 30 s, 85 and 95°C for 1 min). Temperature was controlled manually by the microwave system. Subsequently, a raised temperature could be detected by subjecting a probe directly to the sample. Microwave quickly increased to the desired temperature prior to significant decrease to its initial temperature. The time required to reach the desired temperature was recorded by the system, further written as aforementioned. For steam sterilization (Tomy SX-700, Tomy Digital Biology Co., Ltd., Tagara, Nerima-ku, Tokyo, Japan), samples were steamed at 121°C for 5, 15, and 30 min.

Ultrasound, microwave, and steam sterilization treated samples and controls were hydrolyzed by 10% (w/w protein) Alcalase at 60°C pH 8.5-9 for 4 h. Subsequently, the reaction was inactivated at 95°C for 10 min and sample was subjected to centrifugation process at 10,000 x g for 10 min at 4°C. The supernatant was then adjusted to pH 7 and analyzed for the α -amino acid content using TNBS assay to determine DH according to the method of Adler-Nissen (1979).

ABTS stock solution was made from 7.4 mM 2,2'-Azinobis (3-ethylbenzothiazoline-6-sulfonic

acid) diammonium salt mixed with 2.6 mM $K_2S_2O_8$ dissolved in 10 mM phosphate buffer pH 7.4. ABTS working solution was prepared from 1 ml ABTS stock solution and 40 ml of 10 mM phosphate buffer pH 7.4 ($A_{734\text{ nm}}=0.730\pm0.05$). ABTS assay was carried out according to the method of Arnao (2000), in which 1.98 ml of ABTS working solution was added to 20 μ l of diluted hydrolysate sample. The mixture was then kept in dark room for 5 min and the absorbance was then measured at 734 nm against blank. Trolox was used as a standard for ABTS and the result was expressed as percent scavenger activity at the same peptide concentration of 0.01 mg Leucine/ml.

FRAP assay was carried out according to the method reported by Benzie and Strain (1996). FRAP reagent was prepared from 25 ml of 300 mM acetate buffer solution pH 3.6 ml, 2.5 ml of 10 mM TPTZ solution, and 2.5 ml of 20 mM $FeCl_3 \cdot 6H_2O$ solution. FRAP assay was determined by adding 1 ml FRAP reagent into 100 μ l of diluted sample and incubated in water bath at 37°C for 15 min. The absorbance was read at 593 nm and result was expressed as μ M Trolox equivalent at the same peptide concentration of 0.091 mg Leucine/ml.

FICC assay was performed based on method reported by Carter (1971) by adding 1400 μ l of DI water and 100 μ l of 2 mM $FeCl_2$ into 100 μ l of diluted samples. The mixed solution was then incubated in dark room temperature for 3 min. Subsequently, 400 μ l of 5 mM ferrozine was added to the mixed solution and incubated at dark room temperature for 10 min. The absorbance was read at 562 nm and results were expressed as μ M EDTA equivalent at the same peptide concentration of 0.05 mg Leucine/ml.

All experiments were tested in triplicate and analyzed using one-way ANOVA. The difference among means was performed using Duncan's multiple range test (DMRT) at 95% confidence level using SPSS software (SPSS Inc., Version 21.0, Chicago, IL, USA).

Results and Discussions

Intensity was important feature involving optimum US treatment. Our preliminary study demonstrated that 10-min US pre-treatment on egg white showed the highest DH and antioxidant activity as compared to those of either lower or higher sonication times (data not shown). One of variable to calculate intensity was obtained from amplitude. The setting condition was programmed based on previously reported by Stefanovic *et al.* (2014) modified by various range of intensities. Intensity was mostly associated with the wave energy produced by US, as intensity rose up, the cavitation occurred in more violent fashion, generating smaller size of boiled egg white. This resulted in larger surface area of boiled egg white particles, allowing greater extent of enzyme accessibility (Wang *et al.*, 2018).

Ultrasound at intensity of 41.5 W/cm² on boiled egg white increased DH to approximately 45.4% ($p<0.05$; Table 1), indicating that peptide bonds were cleaved to a greater extent in ultrasound-treated sample. In contrast, control of raw egg white, raw egg white at intensity of 4.6 and 22.1 W/cm² turned into a gel-like material after 4 h hydrolysis. Therefore, DH of these samples could not be determined because of the lack of the supernatant. Intensity of 4.6 and 22.1 W/cm² generated low shear force and cavitation which might be insufficient to break particles of raw egg white and induce gel network formation.

There was a variety of DHs regarding the effect of intensity. The DH value of hydrolysate increased when boiled sample was treated with US at 4.6 W/cm² as compared to the control after 1 h hydrolysis. However, the increase of intensity to 22.1 W/cm² resulted in lower DH value ($p<0.05$, Table 1). The collapse bubble due to shear force and cavitation of US at intensity of 22.1 W/cm² was accumulated as larger aggregate speculating faster cross-linking reaction might occur, reducing

Table 1. Degree of hydrolysis of egg white hydrolysates prepared from ultrasound pre-treatment

Hydrolysis time (h)	Hydrolysates				
	Control	Boiled EW at 4.6 W/cm ²	Boiled EW at 22.1 W/cm ²	Boiled EW at 41.5 W/cm ²	Raw EW at 41.6 W/cm ²
1	26.58 $\pm$ 0.62 ^{dB}	31.49 $\pm$ 0.11 ^{CB}	29.31 $\pm$ 0.07 ^{DC}	34.05 $\pm$ 0.03 ^{DA}	19.32 $\pm$ 0.02 ^{CE}
2	32.21 $\pm$ 1.09 ^{CC}	31.36 $\pm$ 0.03 ^{DC}	33.72 $\pm$ 0.04 ^{CB}	35.63 $\pm$ 0.55 ^{CA}	19.32 $\pm$ 0.02 ^{CD}
3	37.35 $\pm$ 0.01 ^{BB}	36.98 $\pm$ 0.01 ^{BC}	35.19 $\pm$ 0.01 ^{BD}	38.29 $\pm$ 0.01 ^{BA}	19.58 $\pm$ 0.16 ^{BE}
4	38.55 $\pm$ 0.01 ^{AC}	39.05 $\pm$ 0.01 ^{AB}	35.58 $\pm$ 0.07 ^{AD}	45.37 $\pm$ 0.40 ^{AA}	20.05 $\pm$ 0.01 ^{AE}

Different uppercase letters in each row indicate significant difference in mean $\pm$ standard deviation ($n = 3$) values of hydrolysates as an effect of various ultrasound intensities. Different lowercase letters in each column indicate significant difference in mean $\pm$ standard deviation ($n = 3$) values of hydrolysates as an effect of hydrolysis time.

hydrolysis rate. Ma *et al.* (2016) reported there was a decrease on hydrolysis process along with the increase of US intensity due to intensive destruction on pectinase structure. The subsequent increment of DH was clearly observed at maximum intensity of 45.1 W/cm². It indicated complete degradation of protein structure, leading to optimum hydrolytic reaction. Similar behavior was also monitored after 3 h and 4 h hydrolysis. The results demonstrated that US at intensity of 22.1 W/cm² was insufficient pre-treatment as DH was found unstable. Ultrasound intensity could be one of prominent factor determining DH of hydrolysates, however, another important factor could be attributed to hydrolysis time.

The highest DH values for all hydrolysate samples were found after 4 h hydrolysis (Table 1), indicating that DH was dependent on hydrolysis time. The control sample (boiled egg white) hydrolysate showed about 38.55% DH after 4 h hydrolysis. But, hydrolysates prepared from boiled egg white subjected to US showed higher DH than that of control ($p < 0.05$, Table 1). Longer hydrolysis time of 4 h allowed enzyme on optimum peptide bond cleavage more efficiently than those of shorter times. The result was similar to those reported for raw egg white (Stefanovic *et al.*, 2018) and poultry meal (Nchienzia *et al.*, 2010). Using similar enzyme/substrate concentration and exposure US time, the discrepancy of DH was caused by hydrolysis time. Therefore, 4 h was selected as a suitable hydrolysis time for other pre-treated egg white.

Apparently, DH values were also affected by the boiling process as all pre-treatment methods for boiled egg white hydrolysate showed higher DH than raw egg white counterparts. Heating process causes protein unfolding, leading to exposure of protein active site and thus clearly increased enzyme accessibility of boiled egg white (Stefanovic *et al.*, 2014).

Ovoglobulin G2 and G3 as protease inhibitors were reported to denature at 92.5°C (Guha *et al.*, 2018). Thus, microwave at several range of temperatures were assessed to study effect of the presence and inactivation of protease inhibitors (at 85°C and 95°C), combination effect of inactivated protease inhibitors and particle explosion (at 110°C), and comparison temperature to that of steam sterilization (at 121°C). Among hydrolysates of MW-treated raw egg white, 85°C showed slightly higher DH ($p < 0.05$, Figure 1a), indicating that the presence of protease inhibitors was likely inactivated by MW. In contrast, the combination effect aforementioned at 95°C and 121°C succeeded to produce boiled egg white hydrolysate with relatively high DH (Figure 1a).

Microwave (Figure 1b) seemed to lower DH values of both raw and boiled egg white when compared to the control ($p < 0.05$). High power of microwave could possibly increase temperature within a short time, resulting in exposure of protein cleavage site. The main cause of low DH of hydrolysates prepared from MW-treated egg white was high microwave power accelerated protein aggregation, hampering enzymatic hydrolysis (Huang *et al.*, 2017). Nevertheless raw egg subjected to MW resulted in hydrolysates with lower DH than those of boiled egg white. To our knowledge, microwave heated from within part of sample which could directly absorbed by polar compound (Ketnawa and Liceaga, 2016), generating huge explosion of protein particles. The effect of MW seemed difficult to perform on raw egg white particle due to the increase of collision between unfolded molecules after being exploded. It led to form more compact structure (Gladovic *et al.*, 2020). Enzyme accessibility was found limited on this form, thus, low DH was a significant effect.

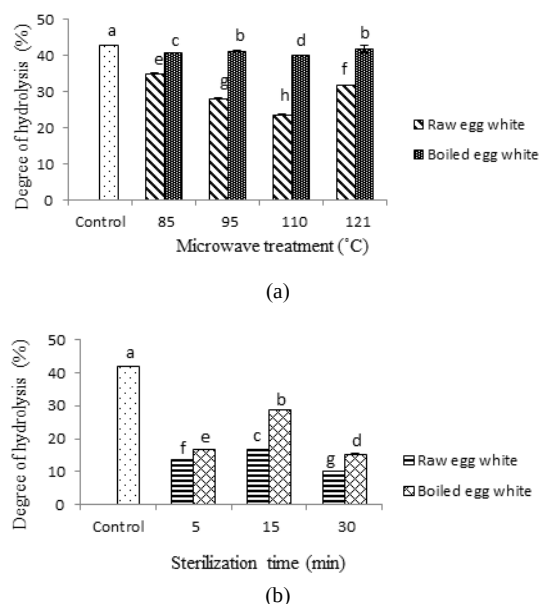


Figure 1. Degree of hydrolysis of egg white hydrolysates. (a) effect of microwave pre-treatment at 1400 W hydrolyzed for 4 h; (b) effect of steam sterilization pre-treatment at 121°C hydrolyzed for 4 h. Control was boiled egg white without any pre-treatments. Mean $\pm$ standard deviation ($n=3$) values were presented. Different letters indicate significant differences ($p < 0.05$).

Steam sterilization at 121°C for 15 min was commonly used for sterilization process. Since steam sterilization was working under combination of steam and pressure (Zhang *et al.*, 2018), minimizing the dramatic changes of protein properties was carried out by adjusting sterilization time to 5 min. In contrast, 30-min sterilization pre-treatment was used to study the effect of excessive structural changes at prolonging time. All of those condition were expected to involve in antioxidant activity. Sterilization pre-treatment produced hydrolysates with much lower than that of control ($p < 0.05$, Figure 1b). It was presumably due to protein denaturation which induced protein aggregation, decreasing enzyme accessibility (Sezdi and Yoleri, 2013).

At the same temperature of 121°C, DH values of ST-treated sample were lower than that of MW-treated. Note that energy of ST, which was transferred from outside into the inside part of protein (Ketnawa and Liceaga, 2016), likely promoted aggregation, further impeding enzyme mobility. As mentioned, hydrolysates of ST-treated boiled egg white showed slightly higher as compared to those of raw egg white. Steam sterilization mostly affected in protein solubility (Zhang *et al.*, 2018). In coagulated form of boiled egg white it enabled the steam to breakdown protein aggregates into more soluble components. Meanwhile the extreme condition of sterilization likely led to insoluble aggregate formation. Therefore, our study revealed that among three pre-treatment technologies, ultrasound became the most potential treatment to be applied in boiled egg white prior to hydrolysis because it yielded hydrolysate with the highest DH value.

ABTS assay is the most common method of antioxidant activity evaluation via electron or hydrogen donation, which was applicable on wide range sample characteristics due to high solubility both in water and organic solvent (Mahalgaes *et al.*, 2008). ABTS scavenging activity of ultrasound-treated hydrolysate was illustrated in Figure 2a, in which boiled egg white with ultrasound treatment at intensity of 41.5 W/cm² and hydrolyzed for 4 h was the highest with about 50% scavenging activity. As mentioned previously, intensity induced cavitation to produce small peptides upon enzymatic hydrolysis, which could contribute in ABTS scavenging activity. Therefore, the higher ultrasound intensity, the stronger inhibition of ABTS radicals would be generated. This result was in agreement with Guerra-Almonacid *et al.* (2019) who reported that *Erythrina edulis* hydrolysates treated by ultrasound exhibited stronger ABTS radical scavenging activity than control. It presumably contains tyrosine at C-terminal of

peptides which acts as electron donor to stabilize ABTS radical.

Moreover, hydrolysate prepared from US-treated raw egg white possessed the lowest DH (Figure 1a), showing relatively high scavenging activity (Figure 2a), regardless of hydrolysis time. The result was an evident that peptide size was not a major factor affecting scavenging activity, high proportion of hydrophobic amino acids (e.g. His, Trp, Phe, Pro, Gly, Lys, Ile, and Val) might contribute to such property. Zou *et al.* (2016) reported that hydrophobic amino acids showed ability to form hydrophobic environment which enables peptides to convert ABTS radical into stable product.

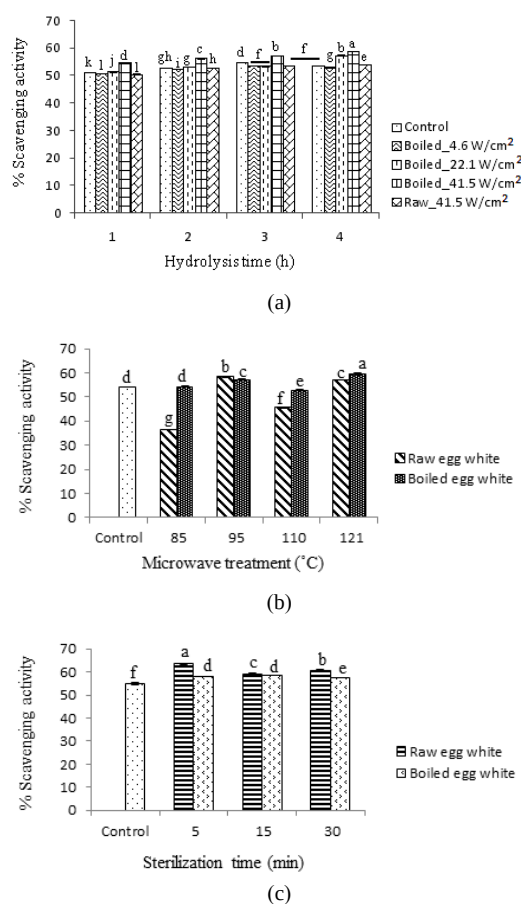


Figure 2. ABTS radicals scavenging activity of egg white hydrolysates. (a) effect of ultrasound pre-treatment; (b) effect of microwave pre-treatment; (c) effect of steam sterilization pre-treatment; percent inhibition was at 10 µg Leucine/ml peptide concentration in reaction and used Trolox as a standard. Mean±standard deviation (n=3) values were presented. Different letters indicate significant differences ($p < 0.05$).

Microwave pre-treatment on boiled egg white at 121°C resulted in the hydrolysate exhibiting the highest ABTS radicals scavenging activity about 60% ($p < 0.05$; Figure 2b). However, 5-min ST pre-treatment yielded raw egg white hydrolysate exhibiting ABTS radicals scavenging activity higher than those treated by microwave at the same temperature ($p < 0.05$; Figure 2c). Similar to ABTS scavenging activity, peptide chain length was not an important indicator on reducing power ability. Dryakova *et al.* (2010) also reported that there was no direct correlation between DH of whey protein hydrolysates and antioxidant activity. Hydrolysates with high scavenging ability might contain abundant amount of peptides with His residues. Zou *et al.* (2016)

reported that imidazole ring in His showed strong contribution as proton donor to scavenge radical in oxidation reaction.

This result revealed that steam sterilization could be used to improve ABTS radicals scavenging activity of egg white hydrolysate. Low molecular weight peptides and certain amino acid sequence were responsible for antioxidant activity (Lin *et al.*, 2013; Chalamaiah *et al.*, 2016). Therefore, the forthcoming research in either amino acid analysis or molecular weight determination of all hydrolysates was needed to explain some critical factors governing antioxidant activity of egg white hydrolysate.

FRAP assay is typically used to establish the possible correlation between reducing power and antioxidant activity of hydrolysates by donating electron (Lassoued *et al.*, 2015). Although DH was the lowest (Table 1), US pre-treatment on raw egg white at intensity of 41.5 W/cm² resulted in hydrolysate with the highest reducing power ability of 9 μ M Trolox equivalence after 1 h hydrolysis (Figure 3a). Similar to that of US pre-treatment, majority of hydrolysates prepared from MW-treated egg white with higher DH (Figure 1a) showed much lower reducing power ability (Figure 3b). Apparently, amino acid composition was the major factor involving reducing power ability. Zou *et al.* (2016) reported that hydrolysates with Asp and Gln residues were potential as electron donor which contributed in reducing power ability. In the case of reducing power, thus, peptide sizes could be ruled out. Finally, microwave was the least effective pre-treatment to obtain hydrolysate with reducing power ability.

In contrast, 30-min ST pre-treatment on raw egg white yielded hydrolysate with lower DH ($p < 0.05$; Figure 1c), but the highest reducing power ability of 22 μ M Trolox equivalence was obvious ($p < 0.05$; Figure 3c). Moreover, at the same temperature of 121°C, lower reducing power of hydrolysates prepared from MW pre-treatment was clearly observed as compared to those of ST. The result was similar to that reported by Sonklin *et al.* (2018) revealing that mungbean meal protein hydrolysate with the highest molecular weight of >10 kDa exerted high FRAP ability. However, Lin *et al.* (2013) reported that egg white protein hydrolysate prepared from Alcalase with molecular weight <1 kDa exhibited excellent FRAP ability. Therefore, peptide size was not the only factor controlling reducing power of hydrolysate. Amino acid sequence could also play a vital role in reducing power ability of the egg white protein hydrolysates. Among 3 pre-treatment technologies, steam sterilization was the most promising method,

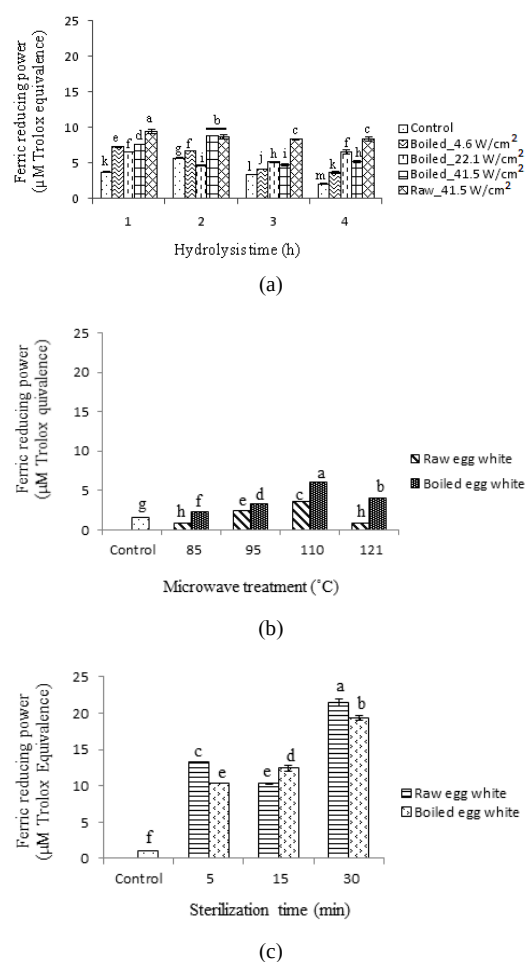


Figure 3. FRAP of egg white hydrolysates. (a) effect of ultrasound pre-treatment; (b) effect of microwave pre-treatment; (c) effect of steam sterilization pre-treatment. Peptide concentration was 91 μ g Leucine/ml in reaction. Mean $\pm$ standard deviation ($n=3$) values were presented. Different letters indicate significant differences ($p < 0.05$).

yielding hydrolysate with the highest reducing power ability.

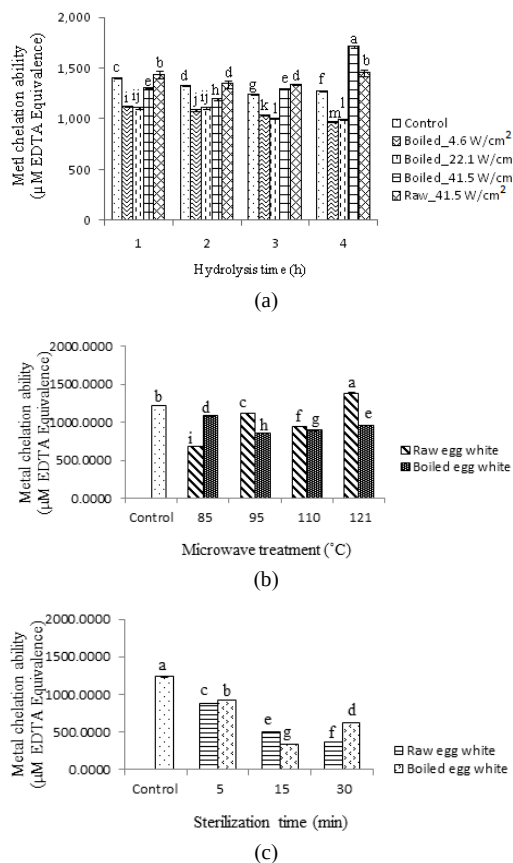


Figure 4. FICC of egg white hydrolysates. (a) effect of ultrasound pre-treatment; (b) effect of microwave pre-treatment; (c) effect of steam sterilization pre-treatment at peptide concentration of 0.05 mg Leucine/ml in reaction. Mean±standard deviation (n=3) values were presented. Different letters indicate significant differences ($p < 0.05$).

The presence of ovotransferrin within egg white which was considered to have antioxidant activity by binding or chelate ferrous ion and transport to the body could be proven using metal chelation assay (Abeyrathne *et al.*, 2018). Ultrasound-treated boiled egg white at intensity of 41.5 W/cm² showed the potent metal chelation activity after 4 h hydrolysis (Figure 4a), indicating that the highest metal chelation was accompanied by the highest DH (Table 1). Shorter peptides presumably contained concentrated carboxyl groups of acidic amino acids which was essential as metal ion chelator (Hou *et al.*, 2019).

Hydrolysates prepared from MW pre-treatment at 121°C, with higher DH ($p < 0.05$; Figure

1a), also showed higher metal chelation activity as compared to those of ST ($p < 0.05$; Figure 4b and Figure 4c). It was likely a shorter peptide might contain strong metal-binding ability. A study by Korczek *et al.* (2019) showed that an increase in DH was correlated to metal chelating ability. In addition, lower DH of all hydrolysates prepared from ST pre-treatment was concomitant with the lower metal ion chelation activity. The extreme condition of ST pre-treatment enhanced the cross-linking reaction of denatured protein being more resistant to enzyme during hydrolysis (Salazar-Villaneda *et al.*, 2016). It likely impeded the exposure of amino acids containing peptides as ion chelator.

Nevertheless, microwave and steam sterilization pre-treatments on egg white did not improve chelating activity of the hydrolysates (Figure 4b and Figure 4c) as compared to the control without pre-treatment. The difference of chelation ability might be dependent on peptide structure and chain length (Saiga *et al.*, 2003). This result was in same agreement with Carrasco-Castilla *et al.* (2012) in which purified phaseolin hydrolysate exhibited strong copper chelating ability at peptide size of <1 kDa. Short peptide of palm kernel kernel expeller glutelin-1 (PKEG-1) also showed remarkable chelating capacity due to interaction between the charged groups and metal ion (Zheng *et al.*, 2017). Alcalase hydrolysis of Nile tilapia resulted in hydrolysate with DH of 40%, exhibiting the most potent ferrous ion chelator capable to lower metal ion oxidation (Yarnpakdee *et al.*, 2014).

Conclusions

Ultrasound at intensity of 41.5 W/cm² of boiled egg generated hydrolysate with the highest DH of 45.4% and FICC about 1800 μM EDTA equivalence. In addition, steam sterilization for 5 min yielded hydrolysate with the highest ABTS radical scavenging activity of 60% inhibition and steam sterilization for 30 min yielded hydrolysate with the highest FRAP about 22 μM Trolox equivalent. Microwave did not yield any effects on both DH and antioxidant activities. Therefore, DH and antioxidant activities of egg white hydrolysate were varied with pre-treatment method applied on it.

Acknowledgements

This work was financially supported by the Food Innovation for Safety and Value Creation of Nakhonchaiburin, Suranaree University of Technology (SUT3-305-61-12-06). One research one grant (OROG) scholarship was also highly appreciated.

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