Antioxidant and Anti-tyrosinase Activities of *Halymenia durvillei* Water Extract Containing R-Phycoerythrin Before and After Microencapsulation

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Abstract

Halymenia durvillei water extract (HDWE) contains a dark-pink colored pigment of R-phycoerythrin (R-PE), which its stability is influenced by temperature and pH and possesses biological activities. Microencapsulation could be the solution to preserving the nature of R-PE. This study characterized the HDWE emulsion and analyzed the R-PE content, antioxidants, and anti-tyrosinase activities before and after microencapsulation using sodium caseinate and maltodextrin (M-HDWE). *Halymenia durvillei* was extracted in distilled water (ratio 1:2, w/v) for 24 hours at 4 °C. Sodium caseinate to maltodextrin coating ratios were 1:2; 1:4; 1:6 and 1:12. Emulsion (viscosity, color, and R-PE content) and powder characteristics (surface morphology) were determined using Brookfield DV2T, ColorFlex EZ Hunterlab, UV-VIS spectrophotometry, and Jeol-SEM, respectively. The antioxidant and anti-tyrosinase activities were determined using the Ferric Reducing Antioxidant Power (FRAP) and mushroom tyrosinase assays. The Commision Internationale d'Éclairage (CIE) L* a* b* colorimetry result showed that the more maltodextrin and the lower viscosity, the more intense the emulsion color. Despite HDWE having roughly six-fold more R-PE content, all M-HDWE treatment samples exhibited equal R-PE contents (p>0.05). The M-HDWE 1:2; 1:4; 1:6; and 1:12 microcapsules had more wrinkled spheres and fewer cracks than M-HDWE 0. The M-HDWE 1:2 had 33.10% lower antioxidant activity than the HDWE and was the highest compared to other M-HDWE compositions (17.15±1.27 µM/µg extract, p<0.05). Meanwhile, all M-HDWE had low anti-tyrosinase activity. It can be concluded that microencapsulation could be a solution for preserving HDWE's antioxidant activity but not its anti-tyrosinase activity.

Keywords: *Halymenia durvillei*, microencapsulation, characteristics, bioactivity

Introduction

Phycoerythrin (PE) is one of the pigmented proteins that have a bright red fluorescence color from the phycobiliprotein group. Phycobiliproteins are differentiated into four types: phycoerythrin (PE)/ pinkish-purple color; phycoerythrocyanin (PC)/orange color; phycocyanin (PC)/blue color; and allophycocyanin (AP)/bluish-green (Filaire et al., 2019). Based on the source, PE is generally accumulated in cyanobacteria (C-PE), a particular family of red algae called Bangiales microalgae (B-PE), and red macroalgae (R-PE). PE is a water-soluble protein and is a natural pigment, so it is considered to be safer for human consumption than synthetic pigments. PE color pigment from red seaweed *Grateloupia turuturu* and red microalgae *Porphyridium cruentum* showed the pigment's stability was influenced by light exposure and pH (Munier et al., 2014).

The color of B-PE from *Rhodomonas salina* was stable from light exposure until eight hours (Marraskuranto et al., 2019). In addition, B-PE from *P. cruentum* showed purple color at pH below 3, pink color between pH 3-12, and colorless at pH above 12 (Gonzalez-Ramirez et al., 2014). According to a patent (IDS000005148) entitled 'Natural red pigment extracting from *Halymenia durvillei* (Munifah et al., 2022), its R-PE content and antioxidant activity were stable within pH of 4 to 10. In addition, R-PE content was stable at 30-50 °C after 10 minutes of incubation and drastically decreased after 10 minutes at 60 °C, whereas its antioxidant activity remained constant. PE is the most dominant pigment in red algae (R-PE) which is used for photosynthesis and gave the algae blackish-

purple color (Munifah et al., 2022). R-PE could be extracted from *Colaconema farmosanum* (Lee et al., 2021), *Euchema sp., Gelidium sp., and Halymenia sp.* (Khatulistiani et al., 2020).

Halymenia durvillei is a very common species in the Indian and West Pacific Oceans and can be found in Indonesian waters like Java, Borneo, Komodo Island, Sumba, and Sulawesi. Based on its morphology, *H. durvillei* has cartilaginous with a slight surface in the color dark pink to dark red (Arguelles, 2022; De Smedt et al., 2001). The biochemical compositions of *H. durvillei* are 13.02 % protein, 1.29 % fat, 53.65% carbohydrates, 39.54 % ash, and 28.41% carrageenan yield (Kho et al., 2016). In addition, alkaloid, flavonoid, phenolic, tannin, and saponin compounds were also detected both in its R-PE extract and thallus (Mantiri et al., 2021).

Those compounds indicate that R-PE from *H. durvillei* has a good potency as cosmetics additive because its active ingredient could prevent skin redness by reducing and maintaining the skin microbiome *Corynebacterium kroppenstedtii* (Filaire et al., 2019). Besides that, *H. durvillei* had the highest R-PE content amongst red macroalgae species found in Banten, Indonesia. Additionally, it was reported that it has the most beautiful color and has the most potent antioxidant and anti-tyrosinase activities (Khatulistiani et al., 2020). So, in this study, we would like to explore their antioxidant and anti-tyrosinase activities if we microencapsulate the pigment. Several studies showed that microencapsulation is one way for preserving R-PE quality and bioactivities (Hsieh-Lo et al., 2019).

Microencapsulation is the process to modify a solid or liquid into a microcapsule form (0.2-5,000 µm). The coating material of carbohydrate-based such as maltodextrin can be used to protect the substance from any harm caused by environmental influences (Jyothi et al., 2010). Maltodextrin also can prevent the Maillard reaction of some food components like fats, oils, vitamins, minerals, and colorants. Besides the carbohydrate-based coating materials, there are also protein-based coating materials such as sodium caseinate. Sodium caseinate has superior emulsifying properties and resistance to heat denaturation. Both coating materials were used to encapsulate phycocyanin and showed great performance to protect the quality of the pigment (Ilter et al., 2021). Our earlier research discovered that microencapsulating *H. durvillei* water extract (HDWE) with a single coating ingredient (maltodextrin) produced poor microcapsule quality (Munifah et al., 2019). Therefore, a combination of maltodextrin and sodium caseinate was utilized to encapsulate the HDWE in this study. The microencapsulation technique was conducted using spray dry methods.

Spray-dry is one of the methods used in the microencapsulation technique. The benefits of using spray drying are the constant of powder specification, applying an automatic machine, and can be applied for dehydration of heat-sensitive materials, which are controlled by airflow and temperature to produce a powder with a low percent water content (Ilter et al., 2021; Vega & Roos, 2006). There was limited information about the antioxidant and anti-tyrosinase performance of microencapsulated *H. durvillei* water extract (M-HDWE). Therefore, this study proposed to characterize the HDWE emulsion formulated before drying and to analyze the R-PE content, antioxidants, and anti-tyrosinase activities before and after microencapsulation with sodium caseinate and maltodextrin.

Material and Methods

Plant Materials

Halymenia durvillei were collected from Binuangeun Coastal Zone, Banten, Indonesia at 6^0 50’41" S-105^0 52’49' E, at ± 4 m depth in March 2019. Samples were identified by their morphological characteristics based on literature (Arguelles, 2022; De Smedt et al., 2001). First, all collected samples were washed and rinsed using seawater, packed into dark plastic bags, and stored in the styrofoam bag by implementing a cold-chain system. Then, samples were sent to the Research Center for Marine and Fisheries Product Processing and Biotechnology, Ministry of Marine Affairs and Fisheries, Jakarta, Indonesia in less than 24 hours. Samples were then immediately stored in cold storage at -20 ^{o}C until the extraction process.

Extraction and Formulation

The ratio of sample biomass to ultra-filtered water (Adrona Crystal Water Purifier) was 1:2 (w/v), where 100 g of *H. durvillei* were macerated in 200 mL distilled water and incubated for 24 hours. The resulting extract was separated using a centrifuge at 2,800 *xg* (Centrifuge Scientific), at 25 ^{o}C for 20 minutes. In this study, the formulation of the coating material was based on our previous study in which the HDWE was coated with varying dilution ratios (1:3, 1:5, and 1:7 v/v) and maltodextrin percentages (10, 20, and 30%). Despite this, the M-HDWE single-coating material (maltodextrin) investigation did not produce optimal results (Munifah et al., 2019). Therefore, we continued to combine maltodextrin and sodium caseinate to encapsulate HDWE. The HDWE was diluted in distilled water (1:7 v/v) and added with 10% coating materials. The formula of maltodextrin and sodium caseinate is shown in Table 1.

Table 1. Formulation of coating materials for 250 mL *H. durvillei* water extract (HDWE)

No	Sample code	Sodium caseinate (g)	Maltodextrin (g)	Sodium caseinate: maltodextrin
1	M-HDWE 0 (control)	0	25	-
2	M-HDWE 1:2	8.33	16.67	1:2
3	M-HDWE 1:4	5.00	20.00	1:4
4	M-HDWE 1:6	3.57	21.43	1:6
5	M-HDWE 1:12	2.03	22.97	1:12

Notes: M-HDWE (Microencapsulated *H. durvillei* water extract)

The range of sodium caseinate and maltodextrin ratio in this study was modified from previous studies (Harimurti et al., 2019; Syafi'i et al., 2016). The resulting emulsions were homogenized using a homogenizer (Ultra Turax) at 10,000 rpm for 30 minutes. The viscosity and color of the M-HDWE formulation were checked before the drying process. During the procedure, the spray dryer (Buchii) had the following settings: inlet temperature 160 °C; outlet temperature 140 °C, aspirator temperature 90 °C; pump mode 5-7; and nozzle mode 2. After the process was finished, the powder extracts were stored at 4 °C and used as a sample for antioxidant and anti-tyrosinase assay.

Viscosity and Color Analysis of the Emulsion Before the Spray Drying Process

The viscosity test was started by adding a 15 mL sample emulsion into a cylinder of viscometer (Brookfield DV2T) and measured. The color test was started by adding a 100 mL sample emulsion into the chamber of a colorimeter (Colorflex EZ Hunterlab). Identifying the color of the sample was conducted using L* a* b* interpretation. L* is for lightness or clarity, -a* is for green, +a* is for red, –b* is for blue, and +b* is for yellow (Rathore et al., 2012). Color visualization was determined from the website www.nixsensor.com. The color difference (ΔE) can be equation below (Zhang et al., 2020):

$$\Delta E: \sqrt{(L^* \text{ sample} - L^* \text{ control})^2 + (a^* \text{ sample} - a^* \text{ control})^2 + (b^* \text{ sample} - b^* \text{ control})^2}$$

Scanning Electron Microscope (SEM)

Following Ganesan and Shanmugam (2020), the particle size and external morphology of M-HDWE were observed using Scanning Electron Microscope (SEM). The M-HDWE powder was hooked up into the stub and coated with gold, then the external particle size and morphology of M-HDWE powder were examined using SEM (Jeol-JCM6000). The magnification was accommodated depending on the size of M-HDWE.

R-Phycoerythrin (R-PE) Analysis

R-PE content was determined using a microplate reader spectrophotometer (Multiskan GO, Thermo Scientific) to measure their light absorption in the aqueous extract with various wavelength PE absorption spectra (455, 565, and 592 nm). The R-PE content was estimated by the following equation below (Beer and Eshel, 1985):

$$[\text{R-PE}] \text{ (mg per mL)} = [(A565 - A592) - (A455 - A592) \times 0.20] \times 0.12$$

Ferric Reducing Antioxidant Power (FRAP) Assay

Acetate buffer 300 mM (pH 3.6) was prepared by diluting 3.1 g sodium acetate trihydrate to 16 mL glacial acetic acid per liter distilled water; hydrochloric acid 40 mM was prepared by diluting 3.4 mL hydrochloric acid per liter distilled water; 2,4,6-Tri(2-pyridyl)-s-triazine (TPTZ) (Sigma) 10 mM was prepared by diluting 3.12 g of TPTZ per liter 40 mM hydrochloric acid; and iron (III) chloride hexahydrate 20 mM was prepared by diluting 5.406 g per liter distilled water. Mixed the TPTZ solution, iron (III) chloride hexahydrate solution, and buffer acetate solution to make FRAP Reagent (Benzie & Strain, 1996). Iron (II) sulfate heptahydrate (Merck) was used as a standard curve (0, 5, 10, 20, 40, 80, and 100 µM) (Nursid et al., 2019).

Sample solutions (10 mg/mL) with the amount of 10 µL were added into the well and then added with 150 µL FRAP reagent. Samples were incubated for 30 minutes and then were read the absorbance using a microplate reader spectrophotometer (Multiskan Go, Thermo Scientific) at λ= 594 nm. FRAP value was expressed in µM/µg extract by interpolating data from the Iron (II) sulfate heptahydrate standard curve.

Anti-tyrosinase Analysis

The anti-tyrosinase analysis was conducted following the previous research (Khatulistiani et al., 2020). Sample stocks were prepared by dissolving 10 mg of M-HDWE powder into 1 mL (10,000 ppm) buffer phosphate solution (pH 7) and then diluting it to 1,000 ppm. An extract of 70 µL was added into the microplate well, then 30 µL of 333 unit per mL tyrosinase enzyme (Sigma) was added into the well and incubated for 5 minutes at 25 °C. After the

incubating processes were done, a-110 µL of L-DOPA 12 mM as a substrate was added into the well, then incubated again for 30 minutes at 25 °C. The absorbances were read using Multiskan Go (Thermo Scientific) at λ= 475 nm. Kojic acid (Sigma Aldrich) was used as a standard (62.5, 125, 250, and 500 ppm). The inhibition percentage was calculated by the following equation:

$$\text{Inhibition (\%):} \frac{\text{Abs control - (Abs sample - Abs control sample)}}{\text{Abs control}} \text{ x } 100\%$$

Data Analysis

Spray drying was performed in triplicates on each M-HDWE formulation. Analyses of antioxidant, anti-tyrosinase activity, CIE L* a* b* color measurement, the color difference (ΔE), viscosity, and yield percentage were performed in triplicates. SPSS 26 software was utilized for the One-Way ANOVA statistical analysis. The M-HDWE's particle size and its morphology were described descriptively.

Results and Discussion

Yield and Viscosity

The viscosity is vital when creating an emulsion of microencapsulated formula for the spray drying process. If the emulsion was very viscous, it will block the circulation of internal droplets on the spray dryer (Babu & Amamcharla, 2022). M-HDWE 1:2 emulsion showed to be the highest viscosity. It was 32.57% higher than M-HDWE 0 (Table 2). The more sodium caseinate that is added to the formula, the higher its viscosity. Although, the viscosity of the emulsion did not correlate with the M-HDWE yield percentage (p = 0.706) in this study. The maximum yield was from M-HDWE 1:6 (34.34%), it was 70.76% higher than M-HDWE 0 which has the least viscosity. This could happen because much of the microcapsule was attached to the spray dry cylinder during the process, causing the M-HDWE yield amount in each treatment to fluctuate. In addition, there were also much of droplets stuck and clogged the spray dryer column and trapped in the filter and the cyclone, which made the microencapsulated yield less than 100% (Tarigan et al., 2018).

Table 2. The characteristics and R-PE content of Microencapsulated *H. durvillei* water extract (M-HDWE) emulsion

Sample	Viscosity (Cp)	Yield (%)	R-PE content (mg/mL)
M-HDWE 1:2	4.62±0.04^{a}	24.93±0.07^{a}	0.014±0.001^{a}
M-HDWE 1:4	3.92±0.04^{b}	19.84±0.00^{a}	0.015±0.001^{a}
M-HDWE 1:6	3.63±0.02^{c}	34.34±0.04^{b}	0.014±0.001^{a}
M-HDWE 1:12	3.51±0.05^{d}	18.00±0.01^{a}	0.016±0.002^{a}
M-HDWE 0	3.50±0.04^{d}	20.11±0.09^{a}	0.016±0.001^{a}
**FD-HDWE			0.106

Notes : ^{a,b,c,d} showed a significant difference between the formula (p < 0.05)** Freeze-dried *H. durvillei* water extract (FD-HDWE) (Khatulistiani et al., 2020)

In contrast, the viscosity has a strong correlation with M-HDWE color. Viscosity has a strong positive correlation with L* and a* (R = 0.823; p = <0.001 and R= 0.836; p = <0.001, respectively), whereas it has a strong negative correlation with b* (R = -0.752; p = 0.001). The more sodium caseinate in the formula made the emulsion more viscous, thus, it caused the lighter color of the M-HDWE (Table 3).

R-Phycoerythrin Content

The R-PE spectrum of M-HDWE in this study was slightly shifted, which could be caused by the particle of the coating materials. Practically, to measure the R-PE purification value, the sample must be centrifuged to clean up the dirt in the sample. The dirt may interfere with the crude extract absorbance measurement in the process. Dirt or debris made a linear baseline slope change, but as long as the range between the baseline and pigment absorbance peaks were similar, the R-PE value could be calculated (Beer & Eshel, 1985). Besides, Sampath-Wiley and Neefus (2007) reported that the absorbance of *Porphyra purpurea* also did not begin at zero, a baseline value of zero was reached only at wavelengths 730 nm and more. Based on Table 2, the R-PE value for all treatments (M-HDWE 0, 1:2, 1:4, 1:6, and 1:12) was not significantly different (p > 0.05). It can be concluded that the different ratios of coating materials in this study did not affect the R-PE content.

The R-PE content of freeze-dried *H. durvillei* water extract (FD-HDWE) was already determined in the previous investigation, in which the R-PE content was 0.106 mg/mL (Khatulistiani et al., 2020). It is around six-fold higher than the M-HDWE in this study. It can happen because the FD-HDWE was more concentrated than M-HDWE. The spectrum absorbance of each red algae species might be a little bit different; it is because of the variation of phycoerythrobilin to phycourobilin molar ratios. Rennis and Ford (1992) showed a slight difference spectrum of R-PE from 48 red algae species. Likewise, Saluri et al. (2020) showed that the absorbance spectrum of R-PE from five red algae from the Baltic Sea and four red algae from Japan Coast were also kind of different.

Table 3. CIE L* a* b*of Emulsion of *H. durvillei* water extract (HDWE) Containing R-Phycoerythrin Formulated with Sodium Caseinate and Maltodextrin Before Drying

Sample	Color			ΔE	Color Visualization[1]
	L*	a*	b*		
M-HDWE 0	26.89±0.02 [e]	23.52±0.04 [e]	32.15±0.03 [e]	-	
M-HDWE 1:2	35.51±0.05 [a]	29.23±0.11 [a]	16.15±0.04 [a]	19.05±0.02 [a]	
M-HDWE 1:4	33.39±0.18 [b]	28.05±0.39 [b]	19.31±0.12 [b]	15.09±0.11 [b]	
M-HDWE 1:6	32.06±0.18 [c]	26.73±0.42 [c]	20.83±0.17 [c]	12.86±0.53 [c]	
M-HDWE 1:12	30.55±0.04 [d]	25.60±0.07 [d]	24.16±0.01 [d]	9.03±0.03 [d]	

Note: [a,b,c,d,e] showed significant difference between the formula ($p < 0.05$), [1]color visualization was illustrated by online website www.nixsensor.com (nix™ Color Sensor, 2023)

Color

Describing color using a colorimeter is completely necessary because the perception of color using human senses is very subjective. The instrument measured the color in a numeric value and could differentiate one color from another color. Identifying the color of the sample in this study was using L* a* b* interpretation. L* is for lightness or clarity, -a* is for green, +a* is for red, –b* is for blue, and +b* is for yellow (Rathore et al., 2012).

As can be seen in Table 3, M-HDWE 0 had the lowest L* values, whereas M-HDWE 1:2 had the greatest. Since there was no sodium caseinate in the M-HDWE 0 formula, the sample presented the clearest visually. The sodium caseinate gave the emulsion a cloudy appearance. This research determined that the dark pink emulsion color of M-HDWE 0 was produced by mixing the colors red (+a*) and yellow (+b*). The M-HDWE 0 treatment, which has the darkest pink visual appearance, exhibited the lowest +a* value and the highest +b* value, whereas the M-HDWE 1:2 treatment, which has the lightest pink color, showed the highest +a* value and the lowest +b* value.

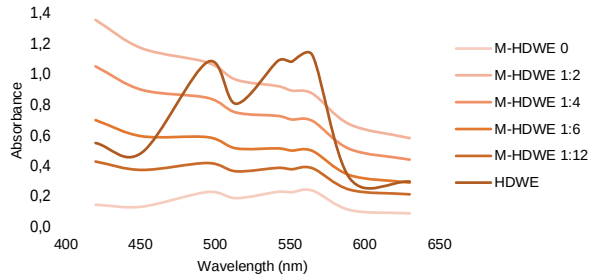

Figure 1. R-PE absorbance spectrum of *H. durvillei* water extract (HDWE) and Microencapsulated *H. durvillei* water extract (M-HDWE) formula.

Ganesan and Shanmugam (2020) utilized the R-PE pigment from *Kappaphycus alvarezi* as natural colorant for ice cream product. The pigment gave a beautiful pink color to the ice cream until 90 days of storage. The color stability of carbonated drinks was also determined by Sudhakar et al. (2014), the color of those drinks that were preserved with 0.5% NaCl declined to less than 50% until 100 days of storage. Besides, R-PE was also can be utilized as a natural colorant and active compound for cosmetics (Lee et al., 2021b).

SEM

The particle size of powder extract was between 11-50 µm for all treatments in this study. Wrinkled sphere microcapsule was dominating in M-HDWE 1:2, 1:4, 1:6, and 1:12, meanwhile the percentage of the irregular sphere and cracked microcapsule were far lower than M-HDWE 0. In addition, irregular sphere microcapsule was dominating in M-HDWE 0, with cracked microcapsule (Figure 3).

According to Choudhury et al. (2021), the average particle size of the spray-dried powder is approximately 5-5,000 µm, which was observed for all M-HDWE treatments in this study. Microcapsules with compact and smaller particle sizes prevent active substances from releasing freely. It also indicates the improved quality of the microcapsule. Not only the particle size but also morphology reveals the quality of microencapsulation products. There should be a few cracks on the coating's surface. Microcapsules with cracks would make it simple for the core substance to escape and increase the active ingredient's resistance to degradation (Dewi et al., 2016). In comparison to M-HDWE 0, the microcapsule morphology of all M-HDWE treatments combined with maltodextrin and sodium caseinate exhibits fewer cracks. Moreover, Li et al. (2017) found that the microcapsule's fluidity decreased as its morphology became more wrinkled.

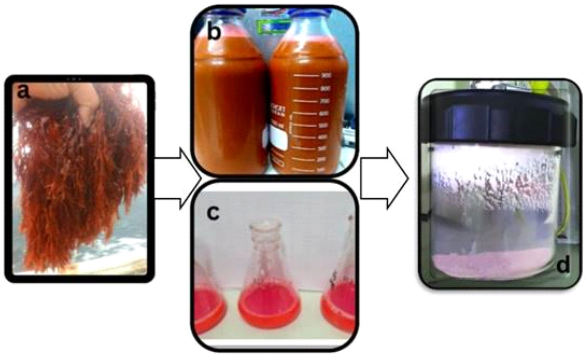

Figure 2. (a) Fresh *Halymenia durvillei* collected from Binuangen Coast; (b) Concentrated *H. durvillei* Water Extract (HDWE); (c) Diluted HDWE; (d) Microencapsulated- *H. durvillei* Water Extract (M-HDWE) powder.

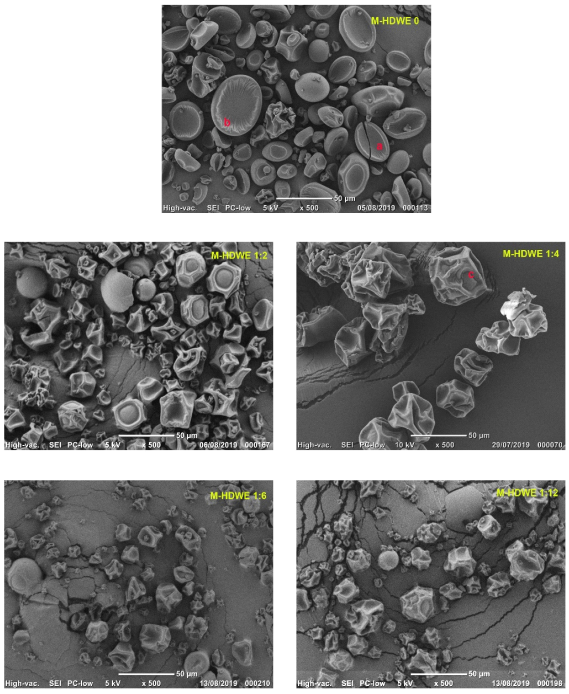

Figure 3. The surface morphology of Microencapsulated *H. durvillei* extract (M-HDWE) powder; a=cracked microcapsule; b= irregular sphere microcapsule; c= wrinkled sphere microcapsule.

Antioxidant Activity

The samples were water-soluble protein pigments (Freitas et al., 2022) and contain carbohydrates from maltodextrin. Carbohydrates are not soluble in any kind of alcohol solvent but are highly soluble in water, therefore, FRAP assay was chosen to determine its antioxidant activity (Moon & Shibamoto, 2009). Besides, compared with another antioxidant assays, phycoerythrin has a greater activity in FRAP. It is because phycoerythrin promotes activity by scavenging the reactive oxygen species via redox reaction (Sonani et al., 2014). FRAP measured the effectiveness of some antioxidants to reduce Fe from Fe^{3+}-TPTZ complex into Fe^{2+} -TPTZ by transferring its electron (Benzie & Devaki, 2018).

FRAP value (antioxidant activity) was interpolated according to Iron (II) sulfate heptahydrate calibration curves ($y = 0.0015x + 0.1089$, $r^2 = 0.9985$). Positive control in this test was ascorbic acid (0.1 mg/mL). Besides, the concentration of the sample was 10 mg/mL. The ascorbic acid antioxidant activity was two times higher than the antioxidant activity of HDWE (25.61±1.55 µM/µg extract). Meanwhile, the M-HDWE 1:2 antioxidant activity was 33.10% lower than HDWE. It might have happened because the actual R-PE content in 10 mg/mL HDWE is almost six times higher than the R-PE content of 10 mg/mL M-HDWE 1:2.

Based on Table 4, antioxidant activity gradually decreased linearly with maltodextrin concentration on M-HDWE formulations. The higher maltodextrin concentration means the less sodium caseinate in the formula. M-HDWE 1:2 has the highest antioxidant activity (17.15±1.27 µM /µg extract). It is because the amount of sodium caseinate in the formula was also the highest. Sodium caseinate was resistant to heat denaturation during the spray drying process, so it could protect the bioactive compound inside. The least antioxidant activity is M-HDWE 1:12 (10.81±0.08 µM/µg extract) which has the lowest sodium caseinate in the formula. This phenomenon was also in accordance with a study conducted by Dewi et al., (2016). Their study showed that the antioxidant activity of microencapsulated PC (a phycobiliprotein pigment) increased significantly by the increase in carrageenan concentration; the stability of bioactive during the spray drying process was strongly influenced by the combination of coating materials. The mixture of maltodextrin and carrageenan was more effective in the protection against lipid oxidation than only maltodextrin as a coating material, even in the high temperature of the spray drying process. There was just some antioxidant activity of red algae water extract that was already determined.

The M-HDWE 1:2 in this study has a higher FRAP value compared with red seaweeds extract from Binuangeun, Banten, include *Splatogosum solieri*, *Gigartina chauvanii*, *Gracillaria edulis*, and *Hypnea* sp (Nursid et al., 2016). The numerous antioxidant activity assay and the various standard calibration curves also made the comparison of FRAP value from another research intricate. Furthermore, the antioxidant activity of microencapsulated HDWE containing R-PE

Table 4. Antioxidant and Anti-tyrosinase Activity of *H. durvillei* water extract Powder Before (HDWE) and After Microencapsulation (M-HDWE) with Sodium Caseinate and Maltodextrin

Sample (10 mg/mL)	Antioxidant Activity (μM/μg extract)	Tyrosinase inhibitor (%)
HDWE	25.61±1.55 ^{e}	48.75±11.4 ^{b}
M-HDWE 1:2	17.15±1.27 ^{d}	7.30±1.41 ^{a}
M-HDWE 1:4	15.33±0.53 ^{c}	1.66±2.15 ^{a}
M-HDWE 1:6	13.67±0.24 ^{b}	3.11±2.44 ^{a}
M-HDWE 1:12	12.50±0.18 ^{a}	4.04±0.13 ^{a}
M-HDWE 0	10.81±0.08 ^{a}	4.45±1.26 ^{a}
Ascorbic Acid (0.1 mg/mL)	52.63±0.23 ^{f}	-
Kojic Acid (0.1 mg/mL)	-	51.38±7.14 ^{b}

Note: :- ^{a,b,c,d,e,f} means a significant difference ($p < 0.05$) between the sample

has never been reported. However, the investigation regarding the antioxidant activity of purified R-PE from *Bangia atropurpurea* has already been determined. It was reported that the FRAP value of purified R-PE from the red algae *B. atropurpurea* had 54.81±0.31 mg gallic acid equivalents (GAE)/g dry weight (DW), while its positive control had a value of 65.77±0.12 mg GAE/g DW (Punampalam et al., 2018). In comparison to these results, the FRAP value of R-PE from HDWE was almost 50% lower than its positive control, indicating that R-PE from HDWE must be purified before microencapsulation to increase its antioxidant activity. R-PE which weights lower than 3 kDa was found to have high antioxidant activity that can be used as essential pharmaceutical and biological importance (Brabakaran et al., 2020).

Free radical oxidation caused abnormalities in the human body, including cell aging. The antioxidant activity of some protein compounds was active in the side chain of amino acids. Charged amino acid residues and hydrophobic amino acids also have an important role in antioxidant activity. Its antioxidant material depressed the age-associated function to slow cell aging (Patel et al., 2018). It was in line with PE from *Lyngbia* sp. (marine cyanobacteria) were proved that it can prolong *C. elegans'* life. Phycoerythrin from *Phormidium* sp. could decline the Reactive Oxygen Species (ROS) activity in the mouse fibroblast and human fibroblast (Sonani et al., 2014, 2017). Therefore, R-PE can also be used as pharmaceutical material.

Aging itself could cause various diseases like cancer, diabetes, neurodegenerative disorder, arthritis, etc. Aging could decrease the integrity of biochemical and physiological reactions in the human body (Sonani et al., 2015). Phycoerythrin as Alzheimer's therapeutic potential has already been investigated, PE could inhibit β-site amyloid precursor protein (APP)-cleaving enzyme 1 (BACE1). It was the enzyme that breaks the amyloid-β precursor protein (APP), and accumulates neurotoxic amyloid beta (Aβ) plaques in the human brain. Those biochemical reactions cause Alzheimer's disorder (Chaubey et al., 2019). Moreover, the PE from various red algae (R-PE) was investigated as anti-cancer, antitumor, anti-inflammatory, and immunomodulator (Cian et al., 2012; Pan et al., 2013; Senthilkumar et al., 2013). As mentioned previously, the antioxidant activity of PE could reduce aging. It means that PE also has the potential for cosmetics as an antiaging material.

The majority of skin aging was caused by UV exposure. UV creates oxidative stress in the skin. It raised the matrix metalloproteinases (MMPs) enzyme and made less collagen and elastin production. Those reactions cause the skin to wrinkle, fragile, and lost its elasticity. Skin is the barrier of the human body that is regularly exposed to various environments. The skin's outer layer is called the stratum corneum, which is the target for anti-aging cosmetics. Adding exogenous antioxidants to the skin's outer layer is important to increase its capacity to regenerate, protect, and nourish the deeper layer of the skin (Pouillot et al., 2011). The R-PE in 5.5 to 8.0 pH range was also reported to have a stable antioxidant activity (Wu et al., 2015).

Tyrosinase Inhibitor

The anti-tyrosinase activity of HDWE has 48.75±11.4% inhibition. Meanwhile, the M-HDWE anti-tyrosinase performance declined extremely. Table 4 shows that the more sodium caseinate concentration gave the less tyrosinase inhibition percentage. While M-HDWE 0 (maltodextrin 100%) shows the highest tyrosinase inhibition (7.30±1.41 %), in contrast, the M-HDWE 1:2 shows the least (1.66±2.15%).

There was poor information about the anti-tyrosinase of red algae aqueous extract. Mostly, the study investigated red algae methanolic or ethanolic extracts. Only certain studies that already determined the anti-tyrosinase of red algae aqueous extract, such as *Pyropia yezoensis* water extract. It was reported that it has tremendous anti-tyrosinase activity, which was more than 60% anti-tyrosinase activity at 0.8 mg/mL concentration. Besides, *P. yezoensis* water extracts also enhanced collagen production, thus the extract is potential to be an antiaging and whitening agent (Park

et al., 2021). The protein water-soluble extract of *Palmaria palmata* was also determined, it has 37-56% anti-tyrosinase activity at 10 mg/mL (Harnedy et al., 2014). Those anti-tyrosinase activities were closed to HDWE in this study.

Comparing HDWE and M-HDWE 0 anti-tyrosinase activity, the activity of combination of coating materials formula for HDWE went down to 85.02%. Based on the anti-tyrosinase test result, there is various probability other than the difference of both R-PE content. It could be the sodium caseinate is holding up the compound that is responsible to inhibit tyrosinase activity and makes the active compound is not released during the assay. Other than the antioxidant and anti-tyrosinase activity, there were many R-PE bioactivities that can be determined, such as anti-aging, antiallergic, and so on to support the exploration of marine-based natural colorants for food, cosmetics, and pharmaceutical industries.

Conclusion

Based on L*a*b* color interpretation and visualization by nixsensor™, HDWE has the darkest pink color, while the emulsion of M-HDWE 1:2 has the lightest pink color. R-PE content from all M-HDWE formulas was not significantly different. The coating material combination produced the wrinkled sphere shape of the M-HDWE microcapsule and contained minimum cracks. Based on bioactivity determination, M-HDWE was recommended to be an antioxidant agent for cosmetics, food, and pharmaceutical ingredients. However, microencapsulation using the combination of sodium caseinate and maltodextrin cannot perform the HDWE anti-tyrosinase activity. From this study, it can be concluded that microencapsulation may be a solution for preserving HDWE's antioxidant activity but not its anti-tyrosinase activity. Furthermore, additional research on the M-HDWE's shelf-life is required to assure its characteristic and bioactivity stability.

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Supplementary Materials

Supplementary materials is not available for this article.

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