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Diversification of Cocoa Drinks with *Chlorella vulgaris* Hydrolysate as a Functional Innovation for Health Benefits

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ABSTRACT

Background: Cocoa has long been known as the main ingredient in making chocolate, which is applied in food such as very popular chocolate drinks. Thus, cocoa is believed to have a promising approach in terms of diversification and innovation of health products. One of them is the fortification of *Chlorella* protein hydrolysate which is considered a sustainable protein source for the future in increasing the protein value of health drinks. The purpose of this study was to evaluate the sensory and physicochemical properties of cocoa drinks fortified with *Chlorella* hydrolysate.

Methods: The experimental design used was a non-factorial completely randomized design (CRD) with 4 treatment levels, in the fortification of *Chlorella* protein hydrolysate into cocoa drinks 0% (HPC_0), 10% (HPC_1), 15% (HPC_2), 20% (HPC_3).

Result: *Chlorella* protein hydrolysate had a protein content of 90.47%. Overall, the sensory value of cocoa drink was highly favored by panelists ($p<0.001$) and the addition 20% of *Chlorella* hydrolysate (HPC_3) obtained the highest taste value. Moreover, the chemical composition for all treatments increased and HPC_3 had the highest protein (11.15%) with 72.97% of solubility and 2.27 ppm of phenol ($p<0.001$). Cocoa drinks can be widely applied worldwide for diversification and innovation of valuable products.

Key words: Cocoa, Microalga, Nutritional content, Protein hydrolysate, Sensory.

INTRODUCTION

Indonesia is one of the world's third largest cocoa producing countries after Gading Coast and Ghana, with a production of 13% (Priyono, 2021; FDA, 2024). Cocoa contains polyphenols and flavonoids that have the potential to act as antioxidants. Around 10% of the dry weight of cocoa beans also contains bioactive compounds such as catechins, anthocyanins and proanthocyanidins (Zugravu and Otelea, 2019). However, cocoa beans also contain theobromine, polyphenols and flavonoids which can cause a bitter taste after being processed into chocolate so that sugar is usually added to beverage products to provide a sweet taste (Goya *et al.,* 2022; Cempaka *et al.,* 2021; Nidhilangelo and Antony, 2024). Along with the development of health and sustainability trends, the diversification and innovation of cocoa-based products are growing. Cocoa can be developed and applied as a health drink.

One interesting breakthrough is the combination of cocoa with nutrient fortification such as *Chlorella* protein hydrolysate. *Chlorella* has attracted the interest of scientists globally because it has essential bioactive compounds such as pigments, proteins, carbohydrates, vitamins and polyunsaturated fatty acids (PUFA) (Lorenzo *et al.,* 2023; Levasseur *et al.,* 2020; Je and Yamaoka, 2022; Balasubramaniam *et al.,* 2021). *Chlorella* has been exploited commercially and the most widely used genera are *Chlorella* for functional foods (Beheshtipour *et al.,* 2012). The potential of *Chlorella* sp. can be used as a nutrient for a food product because it has a high protein nutritional content of 51-58% and antioxidant content such as phenolics (Iriani *et al.,* 2011; Iriani *et al.,* 2017; Iriani *et al.,* 2023., Iriani *et al.,* 2025).

Various studies have revealed that the quality of products such as cheddar cheese, bread and cosmetics after the addition of *Chlorella* has no negative impact on the physicochemical characteristics (Gouveia *et al.,* 2007; Iriani *et al.,* 2023; Diprat *et al.,* 2020; Batista *et al.,* 2017;

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Bhatnagar *et al.,* 2024). For example, chocolate showed low oxidative stability and peroxide value and maintained its quality after fortification with lyophilized and encapsulated *Scenedesmus obliquus* (Hlaing *et al.,* 2020). Yogurt fortified with *Chlorella* (0.25%) improved water retention and maintained whey quality for 28 days of storage (Barkallah *et al.,* 2017). Chocolate milk fortified with *Spirulina*-LEB-18 improved solubility and decreased hygroscopicity (<10%) (Oliveira *et al.,* 2021). Nutritional properties evaluated by the addition of Spirulina (10%) in Saudi Arabian date drinks resulted in good quality for the body's immunity (Aljobair *et al.,* 2021).

However, the application of algae biomass in large quantities can cause green color and fishy taste which are usually unacceptable in the product (Iriani *et al.,* 2017). In addition, nutritional quality depends on the type of microalgae and processing conditions which may have low digestibility and lower nutrient availability (Qazi *et al.,* 2021; Muys *et al.,* 2019). One way to overcome this may be to improve the functional properties of *Chlorella* in the form of hydrolysate. *Chlorella* protein hydrolysate shows interesting properties such as antioxidant, antihypertensive, anti-inflammatory, anticancer and antimicrobial (Cunha *et al.,* 2022). Through the hydrolysis process, the rigid cell wall of *Chlorella* becomes weak, making it easier for protease reactions to access intracellular proteins (Cunha *et al.,* 2022). In addition, hydrolyzed microalgae peptides also have physical properties such as solubility, emulsifying and foaming which can be useful for industrial applications that have an impact on improving the nutritional value of food products. Therefore, fortification of *Chlorella* protein hydrolysate in cocoa beverage products is one of the best ways to improve overall healthy food intake with low side effects compared to intact protein and free amino acids from *Chlorella* biomass (Iriani *et al.,* 2017; Morgan *et al.,* 2021; Ornella *et al.,* 2024). This reason is also supported by Pushpa *et al.* (2018) that beverages formulated through the protein hydrolysis process can enhance nutritional quality, making them a health drink recommended by the WHO.

Thus, cocoa processed and consumed in the form of delicious beverages is believed to have a promising approach for *Chlorella* hydrolysate fortification. The utilization of algae proteins to improve texture and nutritional value has been carried out for more than 20 years (Ferreira *et al.,* 2021; Bhatnagar *et al.,* 2024). Previous studies have revealed that the beneficial properties of *Chlorella* can be further enhanced in the form of protein hydrolysates. This is also because *Chlorella* biomass has a green color and a fishy taste that interferes with consumer preferences and product sensory. Therefore, instead of adding algae biomass as a whole, isolating proteins derived from algae and adding them to food products can be a promising solution to overcome this problem.

To the author's knowledge, there has been no comprehensive study on the application and fortification of *Chlorella* proteins in cocoa. Considering that the global algae protein market has been growing and continues to increase to expand. The purpose of this study was to provide a comprehensive interpretation of cocoa formulations fortified with *Chlorella* protein hydrolysate as functional drink. This study also summarized the effects of *Chlorella* hydrolysate fortification on the sensory, nutritional, solubility and phenolic properties of cocoa. This latest study related to cocoa product development helps in-depth insight into the current trends related to algae protein extraction which is very important for the successful commercialization of algae protein in the cocoa industry.

MATERIALS AND METHODS

Time and place

This research was conducted from August to December 2024. Samples were processed and analyzed at the Fishery Products Processing Laboratory, Fishery Products Biotechnology Laboratory, Fishery Products Chemistry Laboratory, Faculty of Fisheries and Marine Sciences, Universitas Riau.

Materials and equipment

The main materials used in this study consisted of cocoa powder and *Chlorella vulgaris* was cultivated in the Fishery Products Biotechnology Laboratory, Faculty of Fisheries and Marine Science Universitas Riau, Indonesia. Other ingredients were distilled water, H_2SO_4 (Merck, Germany), ethanol 96% (Merck, Germany), ethanol 70% (Merck, Germany), ethyl acetate (Merck, Germany), hexane (Merck, Germany), PP indicator (phenolpthalein) (Merck, Germany), kjeldhal tab catalyst (Merck, Germany), 45% NaOH, 20%, boric acid (H_3BO_3) (Merck, Germany), methyl blue and methyl red indicators (Merck, Germany), HNO_3 (Merck, Germany). The equipment used in this study were digital scales (Kova), incubator (binder), measuring cylinder (Pyrex), beaker (Pyrex), spatula, Erlenmeyer flask (Pyrex), hot plate (Thermo Scientific), porcelain cup (Oem), filter paper (Hellma), desiccator (Duran) and UV-Vis spectrophotometer (Optima).

Extraction and hydrolysis of *Chlorella* protein

The method introduced by Winarni *et al.,* (2022) and Gao *et al.* (2020) with modified. *Chlorella* was cultured for 12 days under room temperature. *Chlorella* was harvested by centrifugation for 4 minutes, at 4000 rpm to obtain a paste. Furthermore, it was lyophilized with a freeze dryer at a temperature of -63.5°C for 3 days to obtain algae powder. Furthermore, the algae powder was extracted with 0.3 M NaCl (1:10 w/v) at pH 10 and incubated for 3 hours at 5°C. Then, it was centrifuged at 4000 rpm for 26 minutes. The supernatant was precipitated for 10 minutes using 0.5 M HCl until pH 4.2. It was centrifuged again and the precipitate was mixed with distilled water (2:1 v/v) at pH 7.2 using 1 N NaOH and it was lyophilized using a freeze dryer. Furthermore, the algae protein was dissolved with 5%

phosphate buffer and 2% pancreatin enzyme was added in the substrate (Gharehbeglou *et al.,* 2024). Then, it was incubated at 50°C for 3 hours and inactivated at 90°C for 15 minutes. The solution was centrifuged at 4000 rpm for 20 minutes and the supernatant was lyophilized using a freeze dryer at -63.5°C for 6 hours.

Procedure for making cocoa drinks with *Chlorella* hydrolysate

The cocoa drink formulation refers to Cempaka *et al.,* (2021) which was modified as shown in Table 1. The concentrations of *Chlorella* hydrolysate were 0% (HPC_0), 10% (HPC_1), 15% (HPC_2) and 20% (HPC_3), this percentage was obtained from the total amount of cocoa powder used.

Sensory evaluation

Organoleptic analysis refers to SNI (2006). *Chlorella* protein hydrolysate was dissolved using 200 mL of hot water for 2 minutes and added 2 spoons of cocoa. As many as 80 untrained panellists for this evaluation. This analysis provides a preference value for the quality of product characteristics according to the numerical scale that had been given. The scores given range from 1 to 9 (1 = very much dislike; 6 = like; 9 = very much like). The higher of value, the more preferred of the product.

Proximate analysis

Chlorella protein hydrolysate was measured for protein, fat, carbohydrate, water, ash and fiber analysis according to AOAC (2005).

Solubility test

Solubility testing based according to Mosii *et al.* (2023) was carried out to measure the solubility level of the cocoa-*Chlorella* powder produced. A total of 1 g of powder (a) was dissolved in 20 mL of distilled water then filtered it with Whatman No. 42 filter paper. Before use, the filter paper was dried in an oven at 105°C for 30 minutes and weighed (b). After filtering, the filter paper was dried again in an oven for 1 hour at a temperature of 105°C. After that, the filter paper was cooled in a desiccator and then weighed until a constant weight was achieved (c).

$$\text{Solubility in water (\%)} = 1\text{-} \left(\frac{c - b}{a}\right) \times 100\%$$

Analysis of phenol content

Total phenol analysis refers to Hazra *et al.,* (2008). A total of 5 mg was dissolved in 5 mL of demineralized water. The sample dilution results were taken as much as 1 mL, 1 mL of 96% ethanol, 5 mL of distilled water and 0.5 mL of 50% Folin Ciocalteau reagent were added. The mixture was left for 5 minutes and 1 mL of 5% Na_2C_3 was added. The mixture was homogenized and then incubated in the dark for one hour. The standard used was gallic acid with concentrations of 20, 40, 60, 80 and 100 ppm. The standard solution and sample were measured using a UV-Vis spectrophotometer at a wavelength of 725 nm. The concentration values of the standard solution and the absorbance of the blank were plotted on the x and y axes respectively in the linear regression equation:

$$y = ax + b$$

The equation formed is used to obtain the total compound value (mg GAE/G). The calculation of total phenol uses the following formula:

$$F = C \times FP/a$$

Description:
F = Phytochemical compound (mg GAE/g extract).
C = Standard concentration (mg/L).
a = Linear regression.
FP = Dilution factor.

Data analysis

The experimental design used in the study of *Chlorella* hydrolysate fortification in cocoa drink was a completely randomized design (CRD) non-factorial with 4 levels of treatment: 0% (HPC_0), 0.5% (HPC_1), 0.75% (HPC_2), 1% (HPC_3) of cocoa weight and tri replicate. Chemical

Table 1: Formulation of *Chlorella* hydrolysate in cocoa drinks.

Ingredients (g)	Treatment			
	HPC_0	HPC_1	HPC_2	HPC_3
Chlorella powder	0	0.65	0.98	1.30
Cocoa powder	6.5	6.5	6.5	6.5
Ste*via* sugar	2.4	2.4	2.4	2.4
Granulated sugar	7.2	7.2	7.2	7.2
Non-dairy creamer	2	2	2	2
CMC	1	1	1	1
Salt	0.1	0.1	0.1	0.1

Descriptions:
HPC_0 (without *Chlorella* hydrolysate protein).
HPC_1 (10% *Chlorella* hydrolysate protein).
HPC_2 (15% *Chlorella* hydrolysate protein).
HPC_3 (20% *Chlorella* hydrolysate protein).

composition data were analyzed and tabulated in table form. Significant differences in treatment ($p<0.001$) using Duncan multiple range test (DMRT), Statistical for Social Science (IBM SPSS 26).

RESULTS AND DISCUSSION

Sensory evaluation

The sensory values of cocoa drink fortitified with *Chlorella* hydrolysate were shown in Table 2. The aroma value of cocoa fortified with *Chlorella* protein hydrolysate as a functional beverage in HPC_0 and HPC_1 was significantly different from HPC_2 and HPC_3 ($p<0.001$). For the taste value, HPC_0 was significantly different from HPC_1, HPC_2 and HPC_3 ($p<0.001$). For the appearance value, HPC_0 was significantly different from HPC_2 and HPC_3, but not significantly different from HPC_1 and HPC_1 was not significantly different from HPC_2 ($p<0.001$). The appearance value also had the same pattern as the texture value ($p<0.001$).

Overall, panellists preferred HPC_3, due to the taste value increased with the addition of *Chlorella* protein hydrolysate, however, the aroma, appearance and texture values tended to decrease. The decrease in value was still in the category preferred by consumers (>7). In this study, panellists tended to detect a slight fishy aroma from cocoa products but the aroma was not as strong as the addition of microalgae biomass in chocolate reported by Hlaing *et al.* (2020). *Chlorella* hydrolysate and microenca psulated algae have better appeal in terms of taste and other sensory aspects aspects (Fradique *et al.,* 2010). This encourages the potential for the development of new microalgae-based products. Therefore, the development of functional foods enriched with microalgae can be a new era for new food products with increased consumer acceptance.

Cocoa nutritional content

Chlorella protein hydrolysate contained 90.47% protein. In this study, the addition of *Chlorella* protein hydrolysate to cocoa can increase protein levels which are good for nutritional needs. *Chlorella* protein hydrolysate showed higher protein content results, but fat content, carbohydrate content and moisture content tended to decrease compared to HPC_0 ($p<0.001$). The nutritional content of cocoa drink fortified Chlorella protein hydrolysate was shown in Table 3.

In this study, the low water content of cocoa can inhibit microbial growth so that it can extend the shelf life of the product (Quek *et al.,* 2007). This reason is supported by GEA Niro Research Laboratory (2010), that the product standard of less than 10% is considered non-hygroscopic. Thus, all treatments showed low hygroscopicity and the desired characteristics for dehydration. In this study, the formulation of adding protein hydrolysate to cocoa can increase protein levels which are good for nutritional needs. HPC_3 has a high protein content (12.12%) from other treatments, even higher than the addition of *Chlorella* microencapsulation to cocoa drinks reported by (Oliveira *et al.,* 2021) which was 12%. This increase in protein levels also increases physical stability, which can be seen from the characteristics of cocoa. HPC_3 also has higher solubility (72.97%) and phenol content (2.27 ppm) (Table 3). The higher solubility was due to more peptide bonds in the *Chlorella* protein hydrolysate, which were enzymatically hydrolyzed into the product (Sinaga *et al.,* 2025).

Table 3 also shown that the cocoa drink with 20% of Chlorella protein hydrolysate obtained higher value of protein content than cocoa drink fortified Chlorella powder

Table 2: Sensory value of cocoa drink fortified with *Chlorella* hydrolysate.

Treatments	Aroma	Taste	Appearance	Texture
HPC_0	7.68 ± 0.04^{b}	7.67 ± 0.02^{a}	7.52 ± 0.04^{c}	7.37 ± 0.02^{c}
HPC_1	7.57 ± 0.05^{b}	8.13 ± 0.02^{b}	7.45 ± 0.02^{bc}	7.29 ± 0.02^{bc}
HPC_2	7.33 ± 0.02^{a}	8.24 ± 0.04^{b}	7.35 ± 0.02^{ab}	7.23 ± 0.02^{ab}
HPC_3	7.29 ± 0.02^{a}	8.36 ± 0.11^{b}	7.31 ± 0.02^{a}	7.17 ± 0.02^{a}

Data in the same column marked with different manuscripts show significant differences ($p<0.001$).

Table 3: Nutritional content of cocoa fortified with *Chlorella* protein hydrolysate.

Parameters	HPC_0	HPC_1	HPC_2	HPC_3
Moisture (%)	3.46 ± 0.02^{d}	3.26 ± 0.03^{c}	3.09 ± 0.03^{b}	2.91 ± 0.02^{a}
Protein (%)	5.43 ± 0.06^{a}	8.62 ± 0.02^{b}	10.66 ± 0.04^{c}	12.12 ± 0.01^{d}
Fat (%)	11.26 ± 0.00^{c}	11.20 ± 0.00^{b}	11.19 ± 0.01^{b}	11.15 ± 0.03^{a}
Ash (%)	2.27 ± 0.01^{a}	2.29 ± 0.00^{a}	2.35 ± 0.02^{a}	2.78 ± 0.04^{b}
Fiber (%)	3.73 ± 0.07^{a}	3.52 ± 0.06^{a}	3.49 ± 0.05^{a}	3.46 ± 0.06^{a}
Carbohydrate (%)	73.85 ± 0.04^{d}	71.11 ± 0.11^{c}	69.23 ± 0.09^{b}	67.58 ± 0.08^{a}
Characteristics				
Solubility (%)	60.25 ± 0.91^{a}	66.77 ± 0.51^{b}	70.06 ± 0.46^{c}	72.97 ± 0.81^{d}
Phenol (ppm)	0.55 ± 0.04^{a}	0.69 ± 0.08^{a}	1.23 ± 0.06^{b}	2.27 ± 0.14^{c}

Data in the same column marked with different manuscripts show significant differences ($p<0.001$).

(9.52%) (Vattaah *et al.*, 2024a) and it also shown that the cocoa drink had higher protein content than probiotic drink fortified Chlorella (3.77%) (Iriani *et al*., 2024). So, Chlorella protein hydrolysate give the new development in a drink innovation.

The increase in phenolic compound content indicates biological activity in reducing free radicals (Tejano *et al.,* 2019). Secondary metabolites such as phenol can stabilize metabolic disorders (Savitri *et al.,* 2019). This reason is also supported by Lakshmi *et al.* (2024) and Balamurugan *et al.* (2022). Therefore, HPC_3 with addition 20% of *Chlorella* protein hydrolysate in cocoa drink is the best treatment that is promising as a functional drink.

CONCLUSION

In this study, high-quality *Chlorella* protein hydrolysate, with a protein content of 90.47%. Furthermore, fortification of *Chlorella* protein hydrolysate in cocoa had a significant effect on sensory values (taste, aroma, texture, appearance). The chemical composition with the addition of 20% *Chlorella* protein hydrolysate showed an increase in protein content of 12.12%, exhibiting solubility characteristics of 72.97% and phenol content of 2.27 ppm. The results of this study indicate that the 20% of *Chlorella* protein hydrolysate in cocoa drink was the best treatment and demonstrates antioxidant potential as a health drink.

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Disclaimers

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Conflict of interest

The authors declare that there are no conflicts of interest regarding the publication of this article.

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