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CHARACTERIZATION OF COCOA FUNCTIONAL DRINK PREPARATION FORTIFIED *Chlorella vulgaris*

Atiqah Nurul Vattaah[1], Dian Iriani[1*], N. Ira Sari[1]
[1]Department of Fish Product Technology, Faculty of Fisheries and Marine,
Universitas Riau, Pekanbaru, 28293 Indonesia
*dian.iriani@lecturer.unri.ac.id

ABSTRACT

The cocoa drink is a beverage derived from processed cocoa beans. The antioxidant content of cocoa can be used as a basis for cocoa beverage development. One form of diversification of processed cocoa products is made drinks with *Chlorella vulgaris* fortification, which is rich in nutrients. This study aims to determine the effect of *Chlorella vulgaris* powder fortification on the quality characteristics of cocoa preparations and determine the best concentration for making cocoa drinks. The research method was experimental, using a factorial Completely Randomized Design (CRD) consisting of 4 different levels of formula concentration treatment, namely cocoa drink processing without *Chlorella vulgaris* powder fortification (KC0), 10% *Chlorella vulgaris* powder fortification (KC1), 15% *Chlorella vulgaris* powder fortification (KC2) and 20% *Chlorella vulgaris* powder fortification (KC3). The research parameters observed were characteristics of appearance, odor, flavor, and texture in hedonic testing, proximate composition (moisture, fat, protein, ash, carbohydrate content), solubility, antioxidant activity, and total phenolic testing. Based on the results showed that cocoa drink with 15% *Chlorella vulgaris* powder fortification (KC2) is the best concentration with organoleptic characteristics such as blackish brown cocoa drink, slightly fragrant chocolate odor typical of *Chlorella vulgaris* and cocoa, a slightly bitter taste, typical of *Chlorella vulgaris* and a slightly thick texture. The proximate composition is 3.18% moisture content, 11.10% fat, 7.95% protein, 3.86% ash and 73.92% carbohydrate. Meanwhile, the solubility is 67.69%, antioxidant activity is 177.92 mg/L, and phenolic content is 1.14 µg/mL. Therefore, this cocoa drink can be used as a functional drink.

Keywords: Fortification, *Chlorella vulgaris*, Cocoa, Drink

1. INTRODUCTION

Nowadays, people are increasingly aware of the importance of taking care of their health. When consuming beverages, they not only look at taste and nutritional fulfillment but also consider the functional aspects of food for health. Indonesia is one of the largest cocoa-producing countries in the world, ranking third after Côte d'Ivoire and Ghana, with production amounting to 13% of the world's total cocoa production[1]. The main antioxidant phytonutrients present in cocoa beans are polyphenols and flavonoids, and 10% of the dry weight of cocoa beans is composed of polyphenols. The most abundant flavonoids in cocoa powder are catechins, anthocyanins, and proanthocyanidins[2].

The theobromine, polyphenols, and flavonoids in cocoa beans give chocolate its bitter taste[3]. Polyphenolic compounds contained in cocoa beans and products that have antioxidant and health properties have the potential to be used as the basis for the development of healthy cocoa drinks. One form of diversification of processed cocoa products is by processing them into drinks fortified with *Chlorella vulgaris* powder. *Chlorella vulgaris* has 17 amino acid profiles with a total of 301.52 mg/g, 7 fatty

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acids 84.32 mg/g, and secondary metabolites, namely alkaloids 109.471 mg/L, flavonoids 82.111 mg/L, saponins 1342.222 mg/L, tannins 411.591 mg/L, and phenolics 151.889 mg/L, protein (more than 53%), vitamin A (beta-carotene), and CGF (*Chlorella Growth Factor*) and antioxidant content of 32.74%[4-6]. *Chlorella vulgaris* is not only useful as a food ingredient but also has various potentials in the health and cosmetic sectors[7] and even for detoxifying heavy metals in wastewater[8].

Previous studies have shown that cocoa drinks fortified with *Arthrospira platensis* microalgae can improve consumer characteristics, preferences, and purchase intention[9]. In addition, Fransisca[10] also fortified cocoa drinks by adding *Spirulina* powder (1.5 g) and 60% stevia sugar substitution, which is the best formula based on hedonic test and antioxidant content testing. Cocoa drinks fortified with microalgae *Chlorella vulgaris* have not been widely studied so far. *Chlorella vulgaris* is a source of high protein and antioxidants, and cocoa, with its polyphenol content, will provide additional nutrients that are good for the body.

Based on the explanation above, the author is interested in conducting research on the fortification of *Chlorella vulgaris* powder to cocoa drinks by testing organoleptic, proximate content, solubility, antioxidant activity, and total phenol so that it can become a functional beverage product that has high economic value.

2. RESEARCH METHOD

Time and Place

This research was conducted from June to July 2024 at the Fishery Product Technology Laboratory and Fishery Product Chemistry Laboratory, Faculty of Fisheries and Marine Sciences, Universitas Riau and Agriculture Laboratory, Faculty of Agriculture, Universitas Islam Riau.

Method

The research method used is experimental, namely processing cocoa drinks with *Chlorella vulgaris* powder fortification. The research design used is a non-factorial complete randomized design (CRD) with four treatment levels, namely, KC_0 (without *Chlorella vulgaris* powder), KC_1 (10% *Chlorella vulgaris* powder), KC_2 (15% *Chlorella vulgaris* powder), KC_3 (20% *Chlorella vulgaris* powder) with three replications so that the number of experimental units is 12 units. The amount of *Chlorella vulgaris* powder was obtained from the percentage of cocoa powder used.

Procedures

Making *C.vulgaris* Powder

Chlorella vulgaris samples are the result of cultivation in the Microbiology and Biotechnology Laboratory of Fishery Products, Faculty of Fisheries and Marine Sciences, Universitas Riau, Pekanbaru. Furthermore, *Chlorella vulgaris*, which is ready to harvest, is separated between the media and sediment using a centrifuge for 5 minutes at 3000 rpm. Then, the sediment obtained was dried in the sun (temperature ± 30°C) for 1-2 days. After that, the dried *Chlorella vulgaris* was pulverized using a mortar and then sieved using a 100-mesh sieve until Chlorella vulgaris powder was obtained, which was ready to be used for making cocoa drink preparations[11].

Making Cocoa-*Chlorella* Drink

The procedure for making cocoa drink, according to Fransisca[10,] was modified by adding 6.5 g of cocoa powder with additional ingredients (stevia sugar, granulated sugar, vegetable creamer, CMC, and salt), then mixing *Chlorella vulgaris* powder according to the concentration (10, 15, and 20%) and stirring until homogeneous. The dough that has been homogenized is then packed in airtight plastic according to the dosage. Then, produce cocoa-*chlorella* powder. All ingredients were dissolved in water at 80^0C, and as much as 200 mL of water was stirred until all ingredients were dissolved in water.

Organoleptic tests

Cocoa-*Chlorella* drink samples were evaluated by semi-trained panelists (n=25). The acceptability was evaluated by using 9-point hedonic scale ranging from (1) dislike extremely, (2) dislike very much, (3) dislike moderately, (4) dislike slightly, (5) neither like nor dislike, (6) like slightly, (7) like moderately, (8) like very much, and (9) like extremely. These nine scales are used to evaluate the acceptance of cocoa-*Chlorella* drink samples for four attributes: appearance, odor, texture, and flavor[12].

Proximate Analysis

The proximate analysis procedure on cocoa drink preparation fortified with *Chlorella vulgaris* aims to determine the content of the main components in the material, such as moisture, ash, protein, fat, and carbohydrate content, carried out based on AOAC[13] procedures.

Solubility Tests

Solubility measurement was conducted to determine the solubility of cocoa beverage preparation with *Chlorella vulgaris* powder fortification. The test begins with weighing 1 g of powder sample. (a) which is then dissolved in 20 mL of distilled water. The solution was filtered with Whatman No. 42 filter paper. Before use, the filter paper was dried in an oven at 105^0C for 30 minutes and then weighed (b). After the filtration process, the filter paper used was dried again in an oven at 105^0C for 1 hour. The filter paper was then cooled in a desiccator and weighed again until it reached a fixed weight (c)[13].

Solubility (%) = $(1 - (\frac{c-b}{a})$ x100%)

Antioxidant activity tests

An antioxidant activity test was conducted using the DPPH method with the help of a spectrophotometer. This test involved four samples of cocoa drink preparation with different dosage variations of *Chlorella vulgaris* powder. The procedure began with the preparation of a sample mother solution of 100 ppm, namely by dissolving 10 mg of the sample with 100 mL of PA methanol. The 50 ppm DPPH stock solution was prepared by dissolving 5 mg of DPPH compound in 100 mL of PA methanol. Furthermore, the control solution was prepared by mixing 2 mL of PA methanol and 1 mL of 50 ppm DPPH solution. For the sample test, each solution consisted of 2 mL of sample solution and 2 mL of DPPH solution. This mixture was incubated for 30 minutes at 27^0C until there was a color change caused by DPPH activity. Each sample was tested three times (triplo). After incubation, the absorbance value of the sample was measured using a Uv-vis spectrophotometer at a wavelength of 517 nm. Antioxidant activity was calculated based on the percentage of DPPH inhibition by each sample[14].

Inhibition activity (%) = $\frac{blank\ absorbance - Sample\ Absorbance}{Blank\ absorbance}$ x 100%

Total phenol tests

The total phenol content in cocoa drink preparation with *Chlorella vulgaris* powder fortification was tested using the *Follin-Ciocalteau* method. Samples with a certain volume were put into a test tube, and then distilled water was added until the total volume reached 5 mL. Next, 0,5 mL of *Follin-Ciocalteau* reagent was added to the tube, then the mixture was vortexed and allowed to stand for 5 minutes.

After that, 1 mL of Na_2CO_3 solution (2%) was added, and the mixture was again vortexed and allowed to stand for 60 minutes in the dark. The absorbance value of the sample was measured using a spectrophotometer at a wavelength of 765 nm. The total polyphenol content in cocoa drink preparation with *Chlorella vulgaris* powder fortification was expressed as phenol equivalents in µg/mL extract[15].

Statistical analysis

The experiment was carried out in 3 repetitions, and the results were expressed as the mean±std.deviation. The experimental results were analyzed using ANOVA, and differences between treatments ($p<0.05$)

were carried out using the Duncan test software SPSS version 26

3. RESULT AND DISCUSSION

Organoleptic Test

The results of organoleptic testing on cocoa drinks with Chlorella vulgaris powder fortification can be seen in Table 1.

Based on the results showed that a cocoa drink with 15% *Chlorella vulgaris* powder fortification (KC_2) is the best concentration with organoleptic characteristics such as blackish brown cocoa drink, slightly fragrant chocolate aroma typical of *Chlorella vulgaris,* and cocoa, a slightly bitter taste typical of *Chlorella vulgaris* and a slightly thick texture.

Table 1. Organoleptic value of cocoa drink with *Chlorella vulgaris* fortification

The level of preference	KC0	KC1 (10%)	KC2 (15%)	KC3 (20%)
Appearance	7.71 ± 0.02^{c}	7.67 ± 0.02^{bc}	7.61 ± 0.02^{b}	6.87 ± 0.06^{a}
Odor	7.40 ± 0.04^{c}	7.07 ± 0.06^{b}	6.97 ± 0.06^{b}	6.49 ± 0.05^{a}
Flavor	7.51 ± 0.02^{b}	7.56 ± 0.04^{b}	8.13 ± 0.06^{c}	5.24 ± 0.04^{a}
Texture	$7.75\pm0,05^{b}$	$7.71\pm0,05^{b}$	$6.67\pm0,05^{b}$	$6.83\pm0,05^{a}$

Notes: Numbers followed by different letter notations mean the effect of treatment is significantly different.

Chemical and physical tests

The results of chemical and physical testing on cocoa drink preparations with *Chlorella vulgaris* powder fortification can be seen in Table 2.

Table 2. Average values obtained on the determination of proximate, physical, and chemical composition

%	KC_0	KC_1 (10%)	KC_2 (15%)	KC_3 (20%)
Composition				
Moisture	3.42 ± 0.03^{c}	3.26 ± 0.03^{b}	3.18 ± 0.02^{b}	2.98 ± 0.10^{a}
Protein	5.50 ± 0.17^{a}	7.49 ± 0.18^{b}	7.95 ± 0.08^{c}	9.52 ± 0.11^{d}
Fat	11.85 ± 0.03^{d}	11.24 ± 0.03^{c}	11.10 ± 0.02^{b}	10.83 ± 0.05^{a}
Ash	2.25 ± 0.01^{a}	3.35 ± 0.04^{b}	3.86 ± 0.04^{c}	5.42 ± 0.05^{d}
Carbohydrate	76.99 ± 0.01^{d}	74.67 ± 0.04^{c}	73.92 ± 0.04^{b}	71.25 ± 0.05^{a}
Physical parameters				
Solubility	59.29 ± 0.09^{a}	65.53 ± 0.03^{b}	67.69 ± 0.03^{c}	70.39 ± 0.04^{d}
Chemical parameters				
Antioxidant activity	436.17 ± 0.03^{d}	191.00 ± 0.08^{c}	177.92 ± 0.05^{b}	145.95 ± 0.12^{a}
Total phenolic	0.65 ± 0.03^{a}	0.77 ± 0.08^{a}	1.14 ± 0.05^{b}	2.16 ± 0.12^{c}

The more *Chlorella vulgaris* powder fortification, the moisture content in the product tends to decrease; this is supported by Gharehbeglou et al.[16], which states that *Chlorella vulgaris* has hygroscopic properties (binds water). Based on the quality requirements of SNI[17], the moisture content in cocoa powder must not exceed 5% (maximum 5%), which means that the moisture content in cocoa drink preparations with *Chlorella vulgaris* powder fortification in this study is still in accordance with applicable standards.

The high ash content obtained in cocoa drink preparations with *Chlorella vulgaris* powder fortification is thought to be caused by the addition of *Chlorella vulgaris,* which causes an increase in mineral content during the manufacturing process. *Chlorella vulgaris* contains iron, which contributes to the formation of hemoglobin so that it can serve as an additional nutrient for people with anemia[18]. Cocoa powder contains calcium, magnesium, potassium, and iron[19], so mixing *Chlorella vulgaris* powder and cocoa powder produces a high ash content.

The addition of *Chlorella vulgaris* powder can increase protein levels. This addition occurs because *Chlorella vulgaris* has a high protein content. This is in line with the statement of Syarif[4], which states that *Chlorella vulgaris* has a protein content above 53%, the highest compared to other living things. Furthermore, based on SNI[17], the fat content of the cocoa drink preparation produced meets the requirements of at least 10%.

The higher the composition of *Chlorella vulgaris* in the cocoa drink preparation, the lower the carbohydrate content. This is due to other nutritional components such as water, ash, protein, and fat when calculated using the difference method. This opinion is in accordance with Roifah et al.[20], which states that if the components of moisture, ash, protein, and fat content increase, the carbohydrate content will decrease and vice versa.

A higher solubility value indicates that less pulp is formed in the product. Better solubility indicates higher product quality, as the product will dissolve faster when mixed with water[21].

The lowest antioxidant activity value is treatment KC0 (436.17 ppm), which is classified as weak antioxidant intensity, and the best IC_{50} antioxidant activity value in treatment KC_3 (145.95 ppm), which is classified as moderate antioxidant intensity. According to Molyneux[22], a compound is considered a very strong antioxidant if its IC_{50} value is less than 50, strong if it is in the range of 50-100, moderate if 100-150, weak if 150-200, and very weak if more than 200. Based on the test results, it is known that the addition of *Chlorella vulgaris* to cocoa can increase the antioxidant content. Antioxidants have many health benefits, including preventing various degenerative diseases such as cancer, heart disease, cataracts, diabetes, liver disease, and premature aging. In addition, antioxidants also play a role in maintaining the quality of food products[23].

Cocoa drink preparations fortified with *Chlorella vulgaris* can increase the phenol content in cocoa drinks because of the phenol content in *Chlorella vulgaris*. Research conducted by Iriani et al.[6] reported that *Chlorella vulgaris* has a phenolic content of 151.889 mg/L. Phenolic compounds found in marine algae have various bioactive properties, such as antimicrobial, antiviral, antioxidant, antidiabetic, anticancer, and anti-inflammatory[24].

4. CONCLUSION

Based on the research results of *Chlorella vulgaris* powder fortification of cocoa drink preparations, it can be concluded that it has a significant effect on organoleptic values (appearance, odor, flavor, and texture), proximate values (moisture, ash, protein, fat and carbohydrates), solubility values, antioxidant activity values and also total phenol values. Based on organoleptic tests of cocoa beverages with the best *Chlorella* vulgaris powder fortification with the addition of 15% *Chlorella vulgaris* powder (0.98 g), with characteristics of attractive appearance and blackish brown color, the distinctive odor of *Chlorella vulgaris* and cocoa has a slightly bitter taste typical of Chlorella vulgaris and a slightly thick texture. Proximate values are moisture content (3.18%), ash (3.86%), protein (7.95%), fat (11.10%), and carbohydrates (73.92%), with solubility value (67.69%), antioxidant activity value (177.92 ppm), and total phenols (1.14 µg/mL). Therefore, cocoa drink preparation with *Chlorella vulgaris* powder fortification can be a healthy alternative drink as a functional food that is beneficial for consumption.

REFERENCES

1. Towaha, J. Kandungan Senyawa Polifenol pada Biji kakao dan Kontribusinya terhadap Kesehatan. Balai Penelitian Tanaman Industri dan Penyegar. *SIRINOV*, 2014; 2(1).

2. Zugravu, C., & Otelea, M.R. Dark Chocolate: To eat or not to eat? A review. *Journal of AOAC International*, 2019; 102(5): 1388-1396.
3. Ramlah, S., Yumas, M., & Wahyuni, W. Characteristics of Functional Foods From Cocoa Liquor and Cocoa Powder. *Indonesian Journal of Industrial Research*, 2020; 15(2): 12-26.
4. Syarif, A. *Peningkatan Produksi Biomassa Chlorella vulgaris Buitenzorg dengan Filtrasi Aliran Sirkulasi Medium Kultur pada Pencahayaan Alterasi*. Fakultas Teknik: Universitas Indonesia. Depok, 2008.
5. Iriani, D., Hasan, B., & Sumarto, S. Pengaruh Konsentrasi Ion Fe^{3+} yang Berbeda terhadap Kandungan Klorofil A dan B, Karatenoid dan Antioksidan dari *Chlorella* sp. *Berkala Perikanan Terubuk*, 2017; 45(1): 48-58.
6. Iriani, D., Feliatra, F., Hasan, B., Karnila, R., Chaiyanate, N., & Rozi, R. Effect of Media Formulations on Chlorophyll, Antioxidant Activity of *Chlorella vulgaris* and Its Potential as a Health Supplement. *Jurnal Ilmiah Perikanan dan Kelautan*, 2024; 17(1): 1-15.
7. Iriani, D., Hasan, B., Sari, N.I., & Alfionita, V. Preparation of Face Mask from Microalga *Chlorella* sp. and Its Potential as Antiaging. *Pharmacognosy Journal*, 2023; 15(1).
8. Coronado-Reyes, J.A., Salazar-Torres, J.A., Juárez-Campos, B., & Gonzalez-Hernandez, J.C. *Chlorella vulgaris*, A Microalgae Important to be used in Biotechnology: A Review. *Food Science and Technology*, 2020; 42: e37320.
9. Rafsanjani, M. *Pengaruh Fortifikasi Mikroalga Arthrospira platensis pada Minuman Kakao terhadap Karakteristik, Preferensi dan Minat Beli Konsumen*. Fakultas Pertanian: Universitas Gadjah Mada. Daerah Istimewa Yogyakarta, 2022.
10. Fransisca, C.C. *Fortifikasi Spirulina platensis dan Substitusi Gula Stevia Terhadap Aktivitas Antioksidan dan Penerimaan Konsumen pada Minuman Fungsional Kakao*. Fakultas Pertanian: Universitas Gadjah Mada. Daerah Istimewa Yogyakarta, 2020.
11. Iriani, D., Suriyaphan, O., & Chaiyanate, N. Effect of Iron Concenration on Growth, Protein Content and Total Phenolic Content of *Chlorella vulgaris* Cultured in Basal Medium. *Sains Malaysiana*, 2011; 40(4), 353-358
12. Setyaningsih, D., Anton, A., & Maya, P.S. *Analisis Sensori untuk Industri Pangan dan Agro*. IPB Press, 2010.
13. AOAC. *Method of Analysis*. Washington: Association of Official Analytical Chemistry. USA: AOAC Internasional, 2005.
14. Restuti, A.N.S., Yulianti, A., Oktafa, H., Alfafa, D.S., Yani, F.N., Kurniawati, M., & Wulandari, P. Analisis Aktivitas Antioksidan dan Uji Organoleptik Minuman Cokelat (*Theobroma cacao* L.). *Health Science: Polije Proceedings Series*, 2019; 13-18.
15. Singleton, V.L., & Rossi, J.A. Colorimetry of Total Phenolics with Phosphomolybdic-Phosphotungstic Acid Reagents. *American Journal of Enology and Viticulture*, 1965; 16(3): 144-158.
16. Gharehbeglou, P., Rad, A.H., Akbarbaglu, Z., Sarabandi, K., & Jafari, S.M. Enhancing the Bio-Polymeric Stabilization of Spray-Dried *Chlorella* Phenolic-Rich Extract: Analysis of its Physicochemical, Functional, Structural, and Biological Properties. *Journal of Agriculture and Food Research*, 2023; 14: 100822.
17. SNI 3747:2009. *Standar Nasional Indonesia: Kakao Bubuk*. Badan Standardisasi Nasional. Jakarta, 2009.
18. Chalid, S.Y., Amini, S., & Lestari, S.D. Kultivasi *Chlorella* sp pada Media Tumbuh yang Diperkaya dengan Pupuk Anorganik dan Soil Extract. *Jurnal Kimia Valensi*, 2010; 1(6).
19. Ramlah, S., & Yumas, M. Pengaruh Formulasi dan Asal Biji Kakao Fermentasi terhadap Mutu dan Cita rasa *Dark Chocolate*. *Jurnal Industri Hasil Perkebunan*, 2017; 12(1): 58-75.

20. Roifah, M., Razak, M., & Suwita, I.K. Substitusi Tepung Kacang Hijau (*Vigna radiata*) dan Tepung Ikan Tuna (*Thunnus* sp) sebagai Biskuit PMT Ibu Hamil terhadap Kadar Proksimat, Nilai Energi, Kadar Zat Besi, dan Mutu Organoleptik. *Teknologi Pangan: Media Informasi dan Komunikasi Ilmiah Teknologi Pertanian*, 2019; 10(2): 135-146.
21. Mursalin, M., Nizori, A., & Rahmayani, I. Sifat Fisiko-Kimia Kopi Seduh Instan Liberika Tungkal Jambi yang Diproduksi dengan Metode Kokristalisasi. *Jurnal Ilmiah Ilmu Terapan Universitas Jambi*, 2019; *3*(1): 71-77.
22. Molyneux, P. The Use of the Stable Free Radical Diphenylpicrylhydrazyl (DPPH) for Estimating Antioxidant Activity. *Songklanakarin J. Sci. Technol*, 2004; 26(2): 211-219.
23. Fithriani, D. *Potensi Antioksidan Caulerpa racemosa di Perairan Teluk Hurun Lampung*. Sekolah Pascasarjana Institut Pertanian Bogor. Bogor, 2009.
24. Thangaraj, M., Saravana, B.P., Thanasekaran, J., Joen-Rong, S., Manubolu, M., & Pathakoti, K. Phytochemicals of Algae, *Arthospira platensis* (*Spirulina*) *Chlorella vulgaris* (*Chlorella*) and *Azolla pinnata* (Azolla). *GSC Biological and Pharmaceutical Sciences*, 2022; 19(2): 023-043