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The *Chroococcus Turgidus* Supplements in Jelly Candy Formulation as Antioxidant Agent

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Abstract: The high demand for natural product ingredients with health benefits has led to the development and innovation in the confectionery industry. Jelly candy supplemented with microalgae extract could be an attractive solution. *Chroococcus turgidus* contains high protein and other metabolites that have potential as antioxidants. This study was conducted to evaluate and analyze the addition of biomass powder of the *C. turgidus* extract to the jelly candy formulation. Proximate analysis, total phenol content analysis, antioxidant analysis, physicochemical properties, and organoleptic analysis of jelly candy products were conducted. The result showed that the water-soluble extract of *C. turgidus* had the highest total phenol content and FRAP values, as much as 10.882 mg GAE/g and 22.5158 M eq Fe^{2+}, respectively. Microalgae *C. turgidus* has carbohydrates 3.6 mg/L, protein 32.8 mg/L, and crude fat 3.1 mg/g. The supplementation of microalgae *C. turgidus* can increase the proximate content of jelly candy. The combination of adding 1.5% biomass powder and 1% water-soluble extract with heat treatment at 80°C is the best jelly candy formulation. These results provide an overview of the potential of *C. turgidus* extract supplementation as an alternative to functional candy with antioxidant and nutritional improvement properties that have not been previously reported.

Keywords: *Chroococcus turgidus*, jelly candy, formulation, antioxidant.

果冻糖果配方中添加膨胀色球藻作为抗氧化剂

摘要:

对具有健康益处的天然产品成分的高需求导致了糖果行业的发展和创新。添加微藻提取物的果冻糖果可能是一个有吸引力的解决方案。膨胀色球藻含有高蛋白质和其他具有抗氧化剂潜力的代谢物。本研究旨在评估生物质粉末的添加情况，并分析果冻糖果配方中的膨胀色球藻提取物。本研究旨在评估向果冻糖果配方中添加生物质粉末和膨胀色球藻提取物的情况。研究了果冻糖果产品的近似分析、总酚含量、抗氧化剂分析、理化性质和感官分析。结果表明，膨胀色球藻水溶性提取物的总酚和FRAP(铁还原抗氧化能力测定)值最高，分别高达10.882毫克通用电气工程师.g^{-1}和 22.5158米当量铁2+。微藻膨胀色球藻具有碳水化合物 3.6毫克升$^{-1}$、蛋白质32.8毫克升$^{-1}$和粗脂肪3.1毫克g^{-1}。微藻膨胀色球藻的添加可以增加果冻糖果的近似含量。添加1.5%生物质粉和1%水溶性提取物并在80° C下热处理的组合是最好的果冻糖果配方。这些结果可以概述补充膨胀色球藻提取物作为功能性糖果替代品的潜力。这些结果概述了膨胀色球藻提取物补充剂作为具有抗氧化和营养改善特性的功能性糖果的替代品的潜力，这些特性以前从未报道过。

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关键词: 膨胀色球藻, 果冻糖果, 配方， 抗氧化剂.

1. Introduction

Jelly candy is a kind of confectionery product whose main ingredient is sugar. This candy is loved by consumers, especially children, because of its soft and sticky texture [1]. On the other hand, high consumption of this confectionery product may cause negative effects on human health due to its sugar content and other additives [2-4]. In children, it has been linked with health problems such as obesity, stress-driven anxiety, impulsiveness, and addictive behavior [4]. Sucrose, often used in candy formulation, has a high glycemic index and contributes to high blood sugar [1]. This sugar can be substituted with other low-calorie sweeteners, such as stevia [5].

Nowadays, the consumer demand for more natural food products providing health benefits has increased, including confectionery industries. A current trend is to innovate confectionery products based on natural ingredients with antioxidant properties [3]. Microalgae have the potential for nutritional application because of their high protein content and other valuable metabolites [6]. Microalgae produce higher protein than terrestrial crops with a yield of 4-15 tons/ha/year, higher than soybean, which is 0.6-1.2 tons/ha/year [7]. They have a well-balanced chemical composition and are rich in polyunsaturated fatty acids, pigments, vitamins, polysaccharides, sterols, and other bioactive compounds. Applying microalgal biomass or metabolites is a good innovation for developing healthier food products [8].

A wide variety of microalgae-based nutritional supplements can be found on the market. Moreover, they can also be incorporated into food products such as biscuits, cookies, pasta, bread, yogurt, and candies [8-9]. In addition to health benefits, this variety of microalgae-based functional foods increases consumer attractiveness [9]. Major microalgae species widely commercialized and used as human supplements and food products are *Spirulina*, *Chlorella vulgaris*, *Haematococcus pluvialis*, and *Dunaliella salina* [8-9]. Other promising microalgae in this application are *Chroococcus turgidus*, a species belonging to cyanobacteria (blue-green algae). According to [10], *C. turgidus* has a protein content of 0.31 mg/L, almost similar to *Spirulina* sp. 0.35 mg/L. Other research also reported its potential as an antioxidant agent [6]. As explained in [11], *C. turgidus* is a cyanobacteria species that exhibited high free radical scavenging activity.

Based on the potential of bioactive compounds and the limitations of using microalgae *C. turgidus* in food application, this study focused on adding *C. turgidus* into jelly candy products. The addition of microalgae *C. turgidus* is intended as a source of protein and antioxidants. It is expected to be an alternative functional candy that is beneficial for consumers, especially children.

2. Materials and Methods

2.1. *C. Turgidus* Cultivation

The unicellular microalgae C. turgidus was the collection of the Research Center for Biotechnology. Modified Johnson medium pH 7 was used as the cultivation medium, as mentioned in [12]. The algae were cultivated at a 2 L culture bottle with constant light and aeration for five days. The cultures were harvested by centrifugation at 6000 rpm for 5 min at 4°C. The biomass was then dried at 60oC until thoroughly dried.

2.2. Preparation of *C. Turgidus* Extract

C. turgidus dried biomass (40 g) was macerated using food-grade ethanol and water with a ratio of 1:10 (w/v) for 3 h and repeated the cycle until the biomass turned pale. The extract was then collected with centrifugation at 6000 rpm for 10 min. Finally, the ethanol extract was evaporated using a rotary evaporator (Janke & Kunkel RV 05-ST, Germany) at 40°C, while the water-soluble extract was freeze dry at -40°C.

2.3. Biomass Analysis

Total carbohydrate of C. turgidus biomass was measured using the phenol sulfuric acid method [13]. Next, total protein was analyzed using the Kjeldahl method following Mihajev et al. [14]. Finally, total lipid was calculated gravimetrically following the method of Bligh and Dyer [15].

2.4. Total Phenolic Content

The total phenolic content of ethanol extract and water-soluble extract of C. turgidus was measured using a modified colorimetric Folin-Ciocalteu method [16]. The samples were prepared at 1000 μg/mL. The gallic acid was used as a standard and prepared in a series concentration (0, 10, 20, 30, 40, and 50 ppm). The Folin-Ciocalteu reagent, a mixture of phosphotungstic and phosphomolybdic acids, is reduced to tungsten and molybdenum blue oxides. The absorbance was read at 765 nm spectrophotometrically (Hitachi V-3900 H, Japan). The total phenol contents were expressed as mg gallic acid equivalent (GAE) per gram dry weight biomass (dwb).

2.5. Antioxidant Analysis Using FRAP

Antioxidant activity was measured using FRAP

(Ferric Reducing Antioxidant Power) following Benzie and Strain method [17]. The FRAP assay has measured the reduction of ferric iron and 2,3,5-triphenyl-1,3,4-triaza-azoniacyclopenta-1,4-diene chloride to the blue ferrous complex by antioxidant under the acidic condition [18]. The absorbance of the reaction mixture was monitored at 593 nm spectrophotometrically (Hitachi V-3900 H, Japan). The FRAP unit is the reduction of one mole of Fe^{3+} to Fe^{+2}.

2.6. Formulation and Production of Jelly Candy

Control jelly candy was made according to Purwaningtyas et al. [19] formula with a modification. The formulation of jelly candies with *C. turgidus* biomass and the water-soluble extract is shown in Table 1. The sugar used in this study is stevia sugars with a constant concentration for all formulations, both treatment, and control. The resulting gels were molded and kept at room temperature, and the gelled candies were then waxed with corn starch.

Table 1 Formulation of jelly candy

Ingredients (g)	Treatments				
	F1	F2	F3	F4	Control
Gelatin	20.00	20.00	20.00	20.00	20.00
Stevia sugar	5.00	5.00	5.00	5.00	5.00
Citric acid	1.00	1.00	1.00	1.00	1.00
Dried biomass of *C. turgidus*	1.00	1.00	1.50	1.50	0
Water-soluble extract of *C. turgidus*	0.10	0.10	0.10	0.10	0
Water	72.90	72.90	72.40	72.40	74.00
Temperature (°C)	60	80	60	80	80

2.7. Physicochemical Properties of Jelly Candy

Jelly candies samples were homogenized with water until completely dissolved. Moisture and ash content were determined gravimetrically using an oven at 525°C and 100°C, respectively (Indonesian National Standard (SNI) 01-2891-1992 point 5.1 and point 6.1) [20]. Total fat was determined using Soxhlet, followed by hydrolysis. The total protein was analyzed by Kjeldahl (Indonesian National Standard (SNI) 01-2891-1992 point 7.1) [20]. While, the total carbohydrates were determined by differences of total solid reduced with a sum of total fat, protein, moisture, and ash.

2.8. Organoleptic Analysis of Jelly Candy

The organoleptic analysis was carried out with untrained panelists of 30 consumers of various ages. This test used the jelly candy formulation with the addition of *C. turgidus* biomass and extract (F1-F4). The test consisted of a five-point hedonic scale to express how the appearance, color, aroma, texture, and taste were to those preferred by the panelists, i.e., 1 = too weak, 2 = a little weak, 3 = just as I like it, 4 = a bit strong, 5 = too strong.

3. Results

3.1. Biomass Analysis of *C. Turgidus*

C. turgidus is a cyanobacterium, which has a bright blue-green color. They sometimes have a rough granular, oval, or rod-shaped colony [21]. The cultivation of *C. turgidus* was carried out for ten days to accumulate the biomass for further analysis.

The biomass contents of *C. turgidus* are shown in Table 2. A previous study [10] obtained the carbohydrate and protein contents of *C. turgidus* being 1.5 mg/L and 31 mg/L, respectively. While in this study, the carbohydrate content was two times higher (3.6 mg/L), and the protein content was almost similar. On the other hand, the lipid content is 3.1 mg/g, which is less than in [22] (10 mg/g).

Table 2 Biomass contents of *C. turgidus*

Biomass content	Yield
Carbohydrate	3.6 mg/L
Protein	32.1 mg/L
Lipids	3.1 mg/g dried biomass

The differences in yield of microalgae biomass content could happen as the origin of the strain, nutrition, and environmental factors [23-24]. The *C. turgidus* used in this study was from the Central Java seashore. Protein content in *C. turgidus* has amino acid essential that human body needs *i.e*. methionine (4.34 mg/0.5 g), phenylalanine (2.98 mg/g), leucine (2.43 mg/0.5 g), lysine (0.1323 mg/0.5g), while the non-amino acids essential ingredients consist of cysteine (3.455 mg/0.5g), proline and tyrosine (1.989 mg/0.5 g), ascorbic acid (1.56 mg/0.5 g), and alanine (0.0054 mg/0.5 g) [25]. Therefore, supplemented of *C. turgidus* in jelly candy proposed to add the value of jelly candy with amino acid essential.

3.2. Total Phenolic Compound of *C. Turgidus*

The goal of this study was to supplement jelly candy with biomass and extract of *C. turgidus.* Therefore, the biomass and the extracts were evaluated for the total phenolic compound.

The total phenolic compound of *C. turgidus* biomass and extract are given in Table 3. The highest total phenolic compound was found in the water-soluble extract (10.89 mg GAE/g dbw).

Table 3 Total phenolic compound of *C. turgidus* in different types of solvents

Extract	Total Phenolic Content	
	µg GAE/ml	mg GAE/g dwb
Water-soluble extract	10888.16	10.89
Ethanol extract	9835.54	9.84
Biomass	7638.89	7.64

According to [26], the extraction process separated one or several compounds from biomass. Therefore, the total phenolic compounds in extracts are higher than in the biomass. The total phenolic compound in the water-soluble extract is slightly higher than in ethanol extract (9.84 mg GAE/g dwb). The difference value of the total phenolic compound in water-soluble and ethanol extracts is influenced by the polarity of the solvent [27]. Another previous study [28] mentioned that the total phenolic compound extracted with polar solvent obtained a higher value. The phenolic compound interacted with hydrogen in a polar solvent.

3.3. Antioxidant Activity of *C. Turgidus*

The antioxidant activity of *C. turgidus* extracts was determined by FRAP assay. In general, the FRAP method was used to monitor the antioxidant activity in plant and food products [29].

The antioxidant activity of *C. turgidus* was given in Table 4. In this experiment, the antioxidant activity of the water-soluble extract was higher than ethanol extract. The higher antioxidant activity of the water-soluble extract is linear with its total phenolic compound. According to Noreen et al. [30], antioxidant activity is strongly affected by the phenolic compound. *C. turgidis* is one of the *Cyanobacteria* species that exhibited high free radical scavenging activity [11].

Table 4 Antioxidant activity of *C. turgidus* extract

Extract	FRAP (μM eq Fe^{2+})
Water-soluble Extract	22.52
Ethanol Extract	19.10

3.4. Physicochemical Analysis of Jelly Candy

The jelly candies were formulated using gelatin, stevia sugar, citric acid, flavor, and water as main ingredients. The formulation of each candy jelly variation is mentioned in Table 1. Based on the total phenolic contents and antioxidant activity, the extract used for the jelly formulation is water-soluble. The results of the physical-chemical analysis are shown in Table 5.

The moisture of all treatment formulas was not normally distributed and was significantly different from the control. The control has the highest moisture content among the others (19.48 ± 0.36). The jelly candy with additional 1.5% *C. turgidus* biomass and processing temperature at 80ºC has the lowest moisture content (17.00 ± 0.08). Water in confection industries is necessary for processing the raw material into final products, and water will affect the texture of candy and is the main factor of shelf life [31]. The four different formulations and control have a high moisture content. However, it is still in the range of moisture content in gummy or jelly candy, about 8-22% [31].

Total ash representing the mineral content was found to be 0.41 ± 0.01% in control which increased in *C. turgidus* supplemented jelly candies as 1.40 ± 0.43 percent in 1.5 g *C. turgidus* biomass. The data showed the addition of *C. turgidus* biomass and processing temperature affecting the total ash of jelly candy. As found in [32], the total ash of bovine gelatin is in the range of 0.39 ± 0.16%. Total ash in jelly candy was influenced by the type of gelatin used and how it was processed. The gelatin used for food production should have total maximum ash of 2% [33].

The total lipid of control jelly candy and other formulations is not significantly different (Table 5). According to [32], the total lipid of bovine skin gelatin was 0.47 ± 0.03%. While in this research, approximately 0.42% to 0.44%. Moreover, the total protein of jelly candy was slightly different in the control formulation (64.75 ± 0.14%), while the other formulation reached the highest protein content of F4 (65.68 ± 0.01%). This data showed that protein was a major component, and the addition of biomass and extract of *C. turgidus* enhanced the protein content. The total carbohydrate content in the jelly candy was calculated based on the difference by subtracting 100% of the nutrient content of the sample with water, ash, protein, and fat content. The average carbohydrate in jelly candy ranges from 15% (Table 5).

Table 5 Physicochemical analysis of *C. turgidus* candy in different formulation

Properties	Formulation				
	F1	F2	F3	F4	Control
Moisture (g/100 g)	17.02 ± 0.19	17.17 ± 0.13	17.43 ± 0.45	17.00 ± 0.08	19.48 ± 0.36
Total ash (g/100 g)	1.35 ± 0.01	0.95 ± 0.01	1.15 ± 0.01	1.40 ± 0.43	0.41 ± 0.01
Total lipid (g/100g)	0.44 ± 0.01	0.44 ± 0.01	0.43 ± 0.01	0.45 ± 0.02	0.42 ± 0.01
Protein (g/100 g)	65.05 ± 0.01	65.15 ± 0.06	65.50 ± 0.01	65.68 ± 0.01	64.75 ± 0.14
Carbohydrate (g/100g)	15.22 ± 0.13	15.82 ± 0.03	15.95 ± 0.46	15.22 ± 0.05	15.43 ± 0.07

3.5. Organoleptic Analysis of Jelly Candy with C. *Turgidus* Addition

Jelly candies were prepared by the formulation given in Table 1, supplemented by biomass and water-soluble extract of *C. turgidus*. Organoleptic analysis of the four formulations (F1 – F4) was carried out on 30 untrained panelists, including color, aroma, taste, and texture (Figure 1). These four tests are important factors in the behavior of choosing and accepting food consumption [34].

Color is one of the crucial factors of foods quality and acceptability. Color in food can come from the natural color of the food itself or be added during processing [35-36].

The preference color of the panelist was the jelly candy with an F4 formulation having a dark blue-green color.

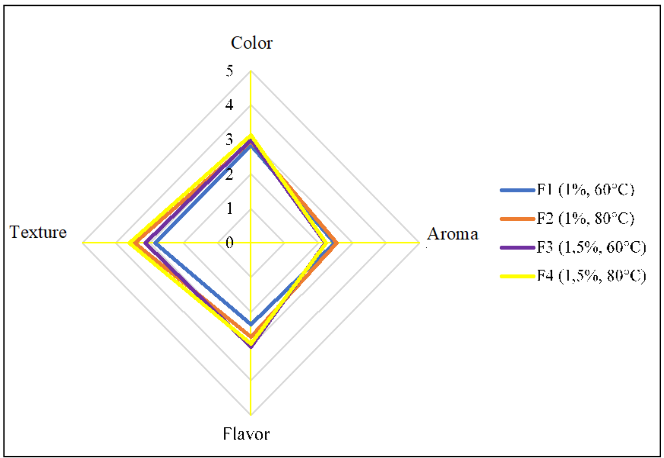

Fig. 1 Organoleptic analysis of jelly candies using a five-point hedonic scale

The jelly candy color was influenced by the addition of biomass powder and water-soluble extract of *C. turgidus*. Cyanobacteria are known to have chlorophyll, carotenoid, and phycobiliprotein functioning as natural colorants [37].

The aroma of jelly candies in this research was affected by additional flavor, gelatin, and microalga. Regarding the aroma of jelly candies, most panelists preferred the candy with F3 formulation. Regarding texture and flavor, the panelist most panelists prefer to F4 formulation. Texture analysis on confectionery products includes hardness, elasticity, and overall characteristics detected by the sense of taste [2, 38]. In the jelly candy, the type of hydrocolloid and sugar have a significant effect on the texture of the resulting candy [39].

4. Conclusions

Dry biomass and water-soluble extract of C. turgidus were successfully added to the jelly candy formulation. The water-soluble extract was chosen over the ethanol extract due to its total phenolic compounds and antioxidant activity. Based on the organoleptic and proximate analysis, the F4 formulation with 1.5% microalgae and heat treatment at 80°C gave the best jelly candy formulation.

It was revealed that the addition of microalgae C. turgidus could increase the proximate value of jelly candy. In other words, it can be assumed that supplementation of jelly candy with C. turgidus could be an alternative functional candy with health benefits for customers.

These results provide new information regarding the application of microalgae C. turgidus in the confectionery field that has not been previously reported. In addition, strategies to improve the appearance and quality of the product need to be considered, especially to anticipate the specific aroma of microalgae.

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