



Drying Of Microalgae *Chlorella Sorokiniana* on Antioxidant Activity and Formulation Application on Peel Off Gel Masker

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ABSTRACT

Unhealthy environmental conditions due to air pollution can cause the emergence of free radicals. Peel off gel mask is very practical and easy to apply mask by applying evenly on the face and after draying, the mask can be removed immediately without the need to rinse. An alternative that can be chosen and is safe in overcoming skin problems is to use natural active ingredients, such as microalgae. *Chlorella sorokiniana* has nutritional content, such as water content (6.54%), carbohydrates (18.08%), protein (46,80%), fat (19.93%), and minerals (7.3%). In addition, bioactive compound such as alkaloids, flavonoids, phenolics, saponins, and terpenoids. The purpose of this study was to dry microalgae *C. sorokiniana* at the best drying time conditions, testing proximate and bioactive compounds, such as total phenolics, flavonoids, and antioxidant activity, as well as formulating and evaluating *C. sorokiniana* microalgae powder mask preparations. The independent variables in this study include the drying time of microalgae biomass *C. sorokiniana* 30, 60, 90, 120, and 150 minutes, as well as the concentration of microalgae powder *C. sorokiniana* 5, 10, 15, 20, 25% for the application of peel off gel mask preparations, while the dependent variables in this study include proximate content, total phenolic, total flavonoid, antioxidant activity, and evaluation of mask preparations. The procedures in this study include: cultivation, harvesting, and collection of microalgae biomass, drying process with time variations, proximate testing and bioactive compounds, as well as formulation and evaluation of *C. sorokiniana* microalgae powder mask preparations. The results showed that the best drying time for *C. sorokiniana* microalgae was 120 minutes with a constant drying rate. In proximate testing, including water content (9.27%), ash (4.28), carbohydrates (10.67%), fat (7.56%), protein (31.39%), and crude fiber (0.25%) which shows that the nutritional content of microalgae *C. sorokiniana* is quite high which can be used to nourish the skin. In addition, the total phenolic content (24.30 mgGAE/g), total flavonoids (19.70 mgQE/g), and antioxidant activity (543.79 mg/L) which shows the antioxidant content is very weak. In the evaluation of masks, organoleptic, pH, homogeneity, spreadability, dry time, and hedonic tests were carried out with 5 different formulations. Based on the evaluation results, the 4th formula has a formulation that meets the requirements and a high level of liking.

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Introduction

Unhealthy environmental conditions due to air pollution such as cigarette smoke, incomplete combustion from motorized vehicles, pollutants, and sun exposure can cause the emergence of free radicals. Skin is one of the body's protective tissues that will undergo an aging process as a person ages or is caused by other external factors, such as

genetic factors, gene mutations, lifestyle, environment and the influence of free radicals [1]. Currently, many cosmetic products made from natural ingredients are safe and beneficial for skin health. Cosmetics with natural ingredients are believed not to cause side effects [2]. One of the cosmetic products that can be made with natural ingredients is a face mask.

Facial masks are skincare products used to treat and manage facial skin problems, such as dull, oily skin and acne. One of the masks that can be used is a peel

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off gel mask. The peel off gel mask is a mask that is very practical and easy to apply by applying it evenly on the face and after drying, the mask can be removed immediately without the need to rinse [3]. Another advantage of peel off gel masks is that they have occlusive properties that can retain moisture in the facial skin so that the active ingredients are more easily absorbed into the facial skin, easily lifted like an elastic membrane, so they are easy to apply and do not cause pain when removed and can be a solution to various skin problems such as acne and large pores, wrinkles and premature aging [4]. An alternative that can be chosen and is safe in overcoming skin problems is to use natural active ingredients, such as microalgae.

Microalgae are microorganisms that can be utilized in the food, pharmaceutical, and cosmetic industries because microalgae biomass contains several bioactive components, such as lipids, amino acids, antioxidants (astaxanthin, β -carotene, dimethyl sulfoniopropionate, mycosporine and several other carotenoids) [5], polysaccharides, pigments (chlorophyll, carotenoids, and pico biliproteins), antibacterial, vitamins such as A, B-1, B-2, B-6, B-12, and C [5], and polyphenols. In this study, microalgae *Chlorella sorokiniana* was used as a source of antioxidants. *C. sorokiniana* has nutritional content, such as water content (6.54%), carbohydrates (18.08%), protein (46.80%), fat (19.93%) and minerals (7.3%) [6]. In addition, bioactive compounds such as alkaloids, flavonoids, phenolics, saponins and terpenoids have potential as antioxidants.

Culture media is important in utilizing *C. sorokiniana* as an antioxidant. Utilization of waste in microalgae culture can reduce production costs and become one of the solutions to handling waste. Previous studies have shown that *C. sorokiniana* can grow well in wastewater such as domestic waste, livestock waste [7], dairy waste, and tofu liquid waste [8]. In Indonesia, many tofu industries contribute liquid waste of around 20 million cubic meters per year. The selection of tofu industry liquid waste is motivated by the large number of tofu industries in Indonesia ranging from small and medium enterprises to large-scale industries and opens up potential opportunities for the utilization of tofu industry liquid waste which is one of the environmental pollution issues [9]. Therefore, so that this waste does not cause pollution, it should be utilized for microalgae culture media.

One way to obtain a good microalgae biomass is to dry the *C. sorokiniana* microalgae by choosing the

appropriate method depending on the nature of the material and the compounds to be isolated [10]. Drying is a preservation method carried out to reduce the water content of a product so as to reduce enzyme and microbial activity and increase product shelf life. Antioxidant compounds are highly changeable, sensitive, unstable and susceptible to degradation. One of the factors that cause antioxidant compounds to degrade is the drying process. In this study using an oven, so it is expected that the drying time is faster than drying using sunlight which takes longer [11]. The research was designed to determine the optimum drying process conditions in the manufacture of dried microalgae by the effect of drying time.

Methods

Microalgae biomass *C. sorokiniana* that has been cultivated in tofu liquid waste, then harvested on the 7th day. Next, drying was carried out with a time variation of 30, 60, 90, 120, and 150 minutes at 50°C, then calculated the % moisture content of the drying results and analyzed the proximate content, including moisture, ash, fat, protein and carbohydrate content, phytochemical screening, total phenolic and flavonoid, and antioxidant activity. The dried biomass of microalgae was formulated to make peel off gel mask preparations with six different formulations and evaluated the characteristics of peel off gel mask preparations, including organoleptic, pH, homogeneity, spreadability, drying time, and hedonic tests.

Results and Discussions

Microalgae *Chlorella sorokiniana* Biomass Drying

The drying process of *C. sorokiniana* microalgae biomass aims to reduce the water content to have an appropriate water content, thus increasing storage and avoiding the growth of unwanted fungi and microorganisms [12]. Based on the drying test of *C. sorokiniana* microalgae biomass with a variation of drying time of 30, 60, 90, 120, 150 minutes at 50°C, the following results were obtained:

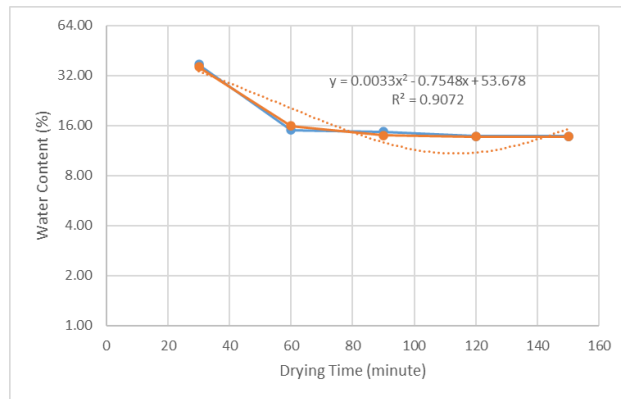


Figure 1. Relationship between Moisture Content and Drying Time

Based on the research results in **Figure 1**, it shows that the best drying time is at 120 minutes with a moisture content of 13.81% (U_1) and 13, 75% (U_2). When the drying time increases, the percentage of evaporated water content will *decrease*. High temperatures will be very effective and can cause a rapid and constant decrease in moisture content [13]. This is shown based on the amount of free water content in microalgae biomass that moves to the surface and undergoes evaporation is the cause of a large decrease in water content that occurs at the beginning of drying so that a constant water content is obtained.

Proximate Testing of *Chlorella sorokiniana* Microalgae Powder

Proximate analysis is a method used to identify the nutritional content of food or feed. Microalgae *C. sorokiniana* contains carbohydrates, vitamins, proteins, and lipids. The results of proximate analysis of *C. sorokiniana* microalgae powder can be seen in **Table 1**.

Table 1. Proximate Analysis of Microalgae *C. sorokiniana*

| Parameter | Satuan | Results |
|--------------|--------|---------|
| Moisture | % | 9.27 |
| Ash | % | 4.89 |
| Fat | % | 7.56 |
| Protein | % | 31.39 |
| Carbohydrate | % | 10.67 |
| Crude Fiber | % | 0.25 |

Table 1 shows that the moisture content of *C. sorokiniana* microalgae powder is 9.27%. In dry food samples, moisture content is often associated with the stability index especially when stored. The higher the moisture content of a food ingredient, the faster the growth of microbial organisms, such as mold, bacteria, so to avoid microbial growth in food ingredients, it is necessary to reduce the moisture

content in accordance with the quality requirements of food ingredients in order to extend shelf life [14]. According to BPOM RI, (2019) concerning Safety and Quality Requirements for Traditional Medicines, the required water content of a food ingredient is less than 10% so that the resulting water content meets the quality standards. The nutritional profile shows the results of the ash content in *C. sorokiniana* microalgae of 4.89%. The results of this ash content meet the requirements based on SNI 01-7085/2005 ash content in simplisia which is max 8%. Minerals contained in microalgae have an important role in their utilization in the health sector so that their application in masks can nourish the skin well.

Microalgae *C. sorokiniana* has a fat content of 7.56%, based on these results the fat content meets the standard requirements of $\leq 38\%$ although the resulting levels are still low. Bioactive lipid compounds derived from microalgae are increasingly recognized as a potential alternative to conventional synthesis in the field of cosmetics and skin care. Microalgal lipids play an important role in cosmetics as moisturizers, emollients, surfactants, emulsifiers, and bioactive ingredients [15]. Then *C. sorokiniana* microalgae has a protein content of 31.39%. The protein content of microalgae depends on the use of media and the content of nutrients contained in the growth media. Mirkoalga species *Spirulina platensis* which has a high protein content of 60-70% can be used as a bioactive compound for the skin and prevents wrinkles. In its application in mask preparations, high protein and fat content has an important role in moisture and smoothness in facial skin.

The carbohydrate content in microalgae *C. sorokiniana* is 10.67%. The carbohydrate content can be formulated in cosmetic preparations as moisturizers or thickeners. In addition, polysaccharides from microalgae can be used as bioactive biomaterials that benefit the skin, including antioxidant, anti-melanogenic, and anti-aging properties. Therefore, it can be utilized in the field of skin health for cosmetic applications [16]. The crude fiber content of microalgae *C. sorokiniana* is 0.25%. Crude fiber is fiber that cannot be digested, and has no nutritional value, but is very important in the body. Fiber has a role for the body, one of which is to facilitate digestion to be smooth.

Phytochemical Screening of Microalgae *Chlorella sorokiniana* Powder

Phytochemical screening of *C. sorokiniana* microalgae powder was carried out to determine the class of secondary metabolite compounds contained in *C. sorokiniana* microalgae powder. Based on the research results in **Table 2**, *C. sorokiniana* microalgae powder contains alkaloids, flavonoids, steroids, phenolics, and saponins.

Table 2. Phytochemical Screening Analysis of Microalgae *C. sorokiniana* Powder

| Testing | Results |
|-----------------|---------|
| Alkaloids: | |
| a. Wagner | +++ |
| b. Dragendorff | ++ |
| c. Mayer | +++ |
| Flavonoids | + |
| Steroids | +++ |
| Phenolic/Tanine | +++ |
| Saponin | + |

The results of phytochemical screening tests on *C. sorokiniana* microalgae powder have never been carried out. However, previous research by [10] showed that the ethanol extract of *C. sorokiniana* had positive results for alkaloids, flavonoids, phenolics, saponins, and steroids/terpenoids. Based on the results of phytochemical screening of *C. sorokiniana* microalgae powder, compounds that are thought to have antioxidant activity are flavonoids and phenolics/tannins. Flavonoid and phenolic compounds have an effect as antioxidants and are able to capture free radicals [17] so that they can be used to treat skin damage due to free radicals.

Total Phenolic and Flavonoids, and Antioxidant Activity of Microalgae *Chlorella sorokiniana* Powder

Determination of phenolic content in *C. sorokiniana* microalgae powder by Folin-Cioacalciu method and expressed by gallic acid equivalent mass. Based on the results of the study (**Table 3**), the total phenolic content of *C. sorokiniana* microalgae powder was 24.30 mgGAE/g. Antioxidant activity is due to the presence of phenolic compounds that have the ability to donate hydrogen atoms or electrons to form stable radical intermediates. According to Nakiboglu et al., (2007) the ability to capture DPPH free radicals is strongly influenced by OH groups contained in phenolic compounds. Bioactive compounds in microalgae that act as potent absorbers of free radicals are phenolic constituents and flavonoids. *C. vulgaris* extract has been tested and found to be a strong antioxidant in vitro [18]. Determination of total flavonoid content in *C. sorokiniana* microalgae powder using UV-VIS

spectrophotometric method. Based on the research results (**Table 3**), the total flavonoid content in *C. sorokiniana* microalgae powder was 19.70 mgQE/g. The total flavonoid content can be influenced by the drying process, the higher the drying temperature and time, the lower the total flavonoid content will be due to heat exposure that can degrade flavonoid components in microalgae. Cosmetic applications of flavonoids, such as UV protection, anti-aging, antioxidant and anti-inflammatory effects.

Antioxidant testing in this study was carried out using the DPPH (2,2-diphenyl-1-picrylhydrazyl) method. Based on the research results (**Table 3**), the sample of *C. sorokiniana* microalgae powder obtained an IC₅₀ of 543.79 mg/L. This study proves that *C. sorokiniana* microalgae powder has antioxidant activity characterized by the ability to reduce free radicals in DPPH. The resulting IC₅₀ value has active antioxidant activity with a very weak category when compared to the level of antioxidant strength. Microalgae *C. sorokiniana* is a source of antioxidants that can be used as functional ingredients for food, cosmetic, pharmaceutical, and industrial applications. This microalgae has water content so it needs to be dried to facilitate the use and increase the shelf life of microalgae biomass [19]. However, heating can alter the chemical composition and antioxidant properties of microalgae.

Tabel 3. Analysis of Total Phenolic, Total Flavonoid, and Antioxidant Activity

| Testing | Unit | Result |
|----------------------|------|--------|
| Total Phenolic | mg/L | 24.30 |
| Total Flavonoids | mg/L | 19.70 |
| Antioxidant Activity | mg/L | 543.79 |

Formulation and Evaluation of Gel Peel Off Mask Preparation of Microalgae Powder *Chlorella sorokiniana*

This study uses *C. sorokiniana* microalgae powder as an active ingredient in the preparation of peel off gel masks because it contains primary and secondary metabolite compounds, such as carbohydrates, fats, proteins, phenolics, flavonoids and several other compounds that are useful for health and beauty [15]. Characterization testing of *C. sorokiniana* microalgae powder, including organoleptic test, pH test, homogeneity test, spreadability test, drying time test, and hedonic test.

The results of organoleptic test observations (**Table 4**) that have been carried out before storage F0 is colorless and odorless, F1 is yellow, F2 is greenish

yellow, F3 and F4 are green, and F5 is dark green with a distinctive smell of microalgae and a thick form. After storage, the results obtained in the form, color, and smell consistency of the preparation did not change. Based on organoleptical observations of the resulting mask preparation, it can be concluded that there were no changes during the 14-day storage.

Tabel 4. Organoleptic Test Results of *C. sorokiniana* Peel Off Gel Mask

| Formula | Organoleptic Test | | |
|---------|--------------------------------|-----------------|----------|
| | Before & After 14 Days Storage | | |
| | Shape | Color | Odor |
| F0 | Viscous | Colorless | Odorless |
| F1 | Viscous | Yellow | Typical |
| F2 | Viscous | Greenish Yellow | Typical |
| F3 | Viscous | Green | Typical |
| F4 | Viscous | Green | Typical |
| F5 | Viscous | Dark Green | Typical |

The pH test is carried out to determine the pH value of the preparation whether it is appropriate or not, the peel off gel mask is a preparation that is applied to the facial skin so that the pH value of the preparation must be in accordance with the pH of the facial skin, namely 4.5-6.5 [20]. Because if the pH value of a preparation is too acidic < 4.5 it will cause skin irritation, while if the pH value of a preparation is > 6.5 or too basic it can cause scaly skin. Based on the results of the pH test (Table 5) all formulas were observed to have good pH values because they were still in the range of 4.5-6.5, namely 4.96-6.13.

Tabel 5. pH Test Result of Peel Off Gel Mask of *C. sorokiniana*

| Formula | pH Value | Requirement | Result |
|---------|----------|----------------|-----------|
| F0 | 6.07 | 4.5 – 6.5 [20] | Qualified |
| F1 | 6.13 | | Qualified |
| F2 | 6.06 | | Qualified |
| F3 | 5.97 | | Qualified |
| F4 | 5.75 | | Qualified |
| F5 | 4.95 | | Qualified |

Homogeneity test is a test that plays a role in the process of forming pharmaceutical preparations to determine whether the formulation ingredients are homogeneous or not, homogeneity is indicated by the absence of coarse particles in the preparation when applied to transparent glass. Based on the results of the study (Table 6), all formulas of peel off gel mask preparations show that the blend of formulation ingredients meets the requirements and

is homogeneously dispersed. *C. sorokiniana* microalgae powder has a very weak antioxidant level, so homogeneous distribution must exist in microalgae powder and mask base so that the antioxidant effectiveness can be the same and harmonized.

Tabel 6. Homogeneity Test Results of *C. sorokiniana* Peel Off Gel Mask

| Formula | Homogeneity | Requirement | Result |
|---------|-------------|---|-----------|
| F0 | Homogeneous | No coarse granules are visible on the preparation | Qualified |
| F1 | Homogeneous | | Qualified |
| F2 | Homogeneous | | Qualified |
| F3 | Homogeneous | | Qualified |
| F4 | Homogeneous | | Qualified |
| F5 | Homogeneous | | Qualified |

The spreadability test aims to determine the area where the gel can spread and spread evenly when used. Based on the results of the spreadability test (Table 7), each formula has varying spreadability, but the spreadability of a good gel mask is 5-7 cm. The measurement results show that the addition of *C. sorokinina* microalgae powder affects the spreadability of the formula, where the higher the concentration of microalgae powder, the lower the spreadability of the preparation. The addition of microalgae powder causes an increase in the viscosity of the preparation so that the resulting spreadability decreases, where the viscosity of the peel off gel mask is inversely proportional to the resulting spreadability.

Tabel 7. Spreadability Test Results of *C. sorokiniana* Peel Off Gel Mask

| Formula | Spreadability | Requirement | Result |
|---------|---------------|--------------|-----------|
| F0 | 5.5 cm | 5 - 7 cm [4] | Qualified |
| F1 | 5.2 cm | | Qualified |
| F2 | 5.2 cm | | Qualified |
| F3 | 5.1 cm | | Qualified |
| F4 | 5.0 cm | | Qualified |
| F5 | 4.8 cm | | Qualified |

The drying time test was carried out with the aim of knowing how long it takes for the preparation to dry completely. Examination of the six formulas showed that each preparation can be peeled off in a time range of 15-30 minutes. Based on the results of the drying time test (Table 8), the dry time results were obtained between 19-30 minutes, this shows that the higher the concentration of *C. sorokiniana* microalgae powder added, the longer it takes for the preparation to dry. This is due to the addition of microalgae making water in the base, so that evaporation takes longer.

Tabel 8. Dry Time Test Results of *C. sorokiniana* Peel Off Gel Mask

| Formula | Dry Time | Requirement | Result |
|---------|------------|-------------|-----------|
| F0 | 21 minutes | | Qualified |
| F1 | 23 minutes | | Qualified |
| F2 | 19 minutes | 15 – 30 | Qualified |
| F3 | 20 minutes | minutes | Qualified |
| F4 | 20 minutes | | Qualified |
| F5 | 30 minutes | | Qualified |

The hedonic test was conducted on 20 untrained panelists to determine the level of liking for the *C. sorokiniana* microalgae peel off gel mask which included texture, color, aroma, homogeneity, and general liking (Figure 2). Based on the results of hedonic testing of *C. sorokiniana* microalgae peel off gel mask preparations, the results of formula F4 were obtained which was the formula with the highest level of preference compared to other formulas on the parameters of texture, color, aroma, homogeneity, and general liking.



Description: (1) really don't like it; (2) don't like it; (3) somewhat like; (4) like; (5) really like it

Figure 2. Hedonic Test Results of *C. sorokiniana* Peel Off Gel Mask

Conclusions

Based on the research results it can be concluded: The best condition of *C. sorokiniana* microalgae drying time is at 120 minutes with constant water content of 13.81% (U1) and 13.75% (U2). Proximate content of microalgae powder *C. sorokiniana* microalgae powder obtained, namely moisture content (9.27%), ash (4.89%), fat (7.56%), protein (31.39%), carbohydrates (10.67%), and crude fiber (0.25%), has bioactive compound content, such as alkaloids, flavonoids, phenolics, steroids, and saponins, as well as total phenolic content (24.30 mg/L), flavonoids (19.70 mg/L), and antioxidant activity (543.79 mg/L) with a very weak category. The results of the evaluation of peel off gel masks with active ingredients of *C. sorokiniana* microalgae powder obtained by formula F4 have a formulation that meets the requirements and a good

level of liking with sensory values of texture (4), color (4), aroma (5), homogeneity (4), and general liking (5).

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