

# Preparation of Face Mask from Microalga *Chlorella* sp. and Its Potential as Antiaging

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## ABSTRACT

The mask with natural extracts is more acceptable by consumers at present. The *Chlorella* sp. is one of the microalgae which contain the antioxidant compound. The research aimed to obtain the best concentration of *Chlorella* sp. in the manufacturing of masks and to evaluate the sensory, physical properties, chlorophyll a b, carotenoid and its antioxidant compound. Non-factorial Completely Randomized Design with 4 level treatments was used in this study with different concentrations of *Chlorella* sp. powder whereas MW<sub>0</sub> (without chlorella powder), MW<sub>1</sub> (0.4% chlorella powder), MW<sub>2</sub> (0.5% chlorella powder), MW<sub>3</sub> (0.6% chlorella powder), triplicated. The parameter assay was observed for sensory (color, texture, and odor), physical properties (homogeneity, pH, spreadability, drying time), chlorophyll a, b, carotenoid and antioxidant activity. Based on the result of this study shows that the MW<sub>3</sub> with 0.6% of chlorella powder was the best treatment with sensory value (8.28, 7.08, 7.37); physical properties (homogenous, 5.3, 7.5 cm, 24-30 minutes), respectively. Meanwhile, chlorophyll a 0.67 mg/L, chlorophyll b 0.89 mg/L, total chlorophyll 1.63 mg/L, carotenoid 0.84 mg/L, the antioxidant activity of *Chlorella* sp. mask was 246.70 mg/L. Therefore, the present study suggested that the *Chlorella* sp. mask could be used in cosmeceutical products, and potentially as an antiaging.

**Key words:** Antiaging, Antioxidant activity, *Chlorella* sp, Face mask.

## INTRODUCTION

There are two types of algae, macro and microalga, and *Chlorella* sp. is one of the green microalga containing functional sources, which has very useful for health. *Chlorella* in its utilization can be used for non-food products as well as for functional food products.

*Chlorella* has fast growth; it can be harvested within 12 days at a laboratory scale with continuous lighting.<sup>1</sup> *Chlorella* contains phenolic compounds, carotenoid and chlorophyll a b.<sup>2,3</sup> Moreover, it contains 51-58% protein, 28-32% oil, 4-5% nucleic acids,<sup>4</sup> and 32.74% antioxidants.<sup>5</sup> Therefore, it can be applied as a health food in green ones,<sup>6</sup> and cosmeceutical products.<sup>7</sup> Cosmeceuticals are cosmetic products that have functional ingredients that can improve skin texture and against free radicals. Cosmetics can improve light contour, skin tone, texture, and reduce wrinkles by providing the nutrients needed by the skin.<sup>8</sup> One of the cosmetics with a lot of use in the community is a face mask.

A face mask is an example of a product that is often used by the public, which is applied to the face after cleansing, containing protein, minerals, and vitamins. Face masks can be removed by cleaning directly with water. It also can clean by wiping with a damp cloth until it's clean.<sup>9</sup> Practical masks that can be removed immediately without rinsing are known as peel-offs. The application of peel-off masks on the face is expected to keep the skin clean and fresh, by removing dead skin cells and dirt, so that it can restore and improve the freshness and softness of the skin, and reduce fine wrinkles on the face with regular use.<sup>10</sup> Peel-off masks can hydrate the skin, prevent the skin from wrinkles, premature

aging, acne, shrink pores, relax facial muscles, cleanse and moisturize the skin, as well a skin refresher.<sup>11</sup>

One of the factors causing wrinkles on the face is free radicals, so the skin needs compounds that can be used as an inhibitor or against free radicals that cause wrinkles on the skin, namely antioxidants. Antioxidants are molecules with the ability to stabilize, ward off, and inactivate free radicals before they attack cells, and have the ability to inhibit or delay oxidation, so that Anti-aging is a process of preventing and slowing down the effects of aging, to become fresher, more beautiful and more youthful.<sup>9</sup> Antioxidants play a role in reducing the rate of change due to aging.<sup>12</sup>

To avoid masks made with synthetic ingredients that harm the skin, hence, *Chlorella* sp. is used as a source of natural antioxidants. Therefore, this research aimed to evaluate the sensory, physical properties, chlorophyll, carotenoid, and antioxidant activity of *Chlorella* face mask.

## MATERIALS AND METHODS

The *Chlorella* sp. powder as a raw material in the manufacturing of face masks was obtained from the Microbiology and Biotechnology Laboratory Faculty of Fisheries and Marine Science Universitas Riau, Indonesia. The face mask was prepared in 4 concentrations: 0% (MW<sub>0</sub>/control) without *Chlorella* sp. powder, 0.4% *Chlorella* sp. powder (MW<sub>1</sub>), 0.5% *Chlorella* sp. powder (MW<sub>2</sub>), and 0.6% *Chlorella* sp. (MW<sub>3</sub>) powder, all treatment was three replicated with Non-factorial completely randomized. The parameter assay was observed for for sensory (color, texture, and odor), physical properties (homogeneity, pH, spreadability, drying time), chlorophyll a, b, carotenoid and antioxidant activity.

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## Materials and equipment

The material used in the manufacturing of face mask: *Chlorella* sp. powder, ethanol 70%, ethanol 96%, aquadest, PVA (Polyvinyl alcohol), HPMC (Hydroxypropyl methylcellulose), Glycerin, Methylparaben, propylparaben), fosfat 0,2 M,  $K_3Fe(CN)_6$  1%, TCA,  $FeCl_3$  0,1%, oxalate solution, ascorbat solution, and consumables are tissue, aluminum foil, and label paper. Equipment was used autoclave model HVE-50 by Japan, chemical glass (Pyrex), measuring cup, Erlenmeyer (Pyrex), drop pipette (Pyrex), analytical balance (Radwag-Polandia, AS 220.R2 Plus), stopwatch, centrifuge model K241R by United Kingdom, pH meter, sieve 100 mesh, and freeze dryer (Lyovapor, L-200), LaMotte *smart Spectro spectrophotometer* model 2000-01 Brand Smart Spectro by USA.

## RESEARCH PROCEDURE

### Preparation of *chlorella* sp. powder

*Chlorella* sp. was cultivated for 12 days with continuous lighting, after that *chlorella* was harvested by centrifugation for 10 minutes at 2500 rpm, after obtaining *chlorella* paste, then dried in a freeze dryer for 3 days at a temperature of  $-50^{\circ}C$ , the *chlorella* powder obtained was filtered through a 100 mesh sieve.

### Preparation of face mask

The *Chlorella* sp. face mask was prepared with a formulation in Table 1. The concentration of *Chlorella* sp. powder is obtained from the total ingredients used. So the weight of the *Chlorella* sp. powder used for the  $MW_0$  treatment was 0 gr,  $MW_1$  0.63 gr,  $MW_2$  0.78 gr,  $MW_3$  0.93 gr.

### Procedure in manufacturing of *chlorella* sp. face mask<sup>13</sup>

The initial stage was carried out by adding 250 mL of hot aquadest ( $70-80^{\circ}C$ ) at 23.55 grams of PVA until it swelled for a facial base, followed by adding 50 mL of cold aquadest at 14.71 grams of HPMC and stirring constantly until it swelled. In the next step, 5.88 grams of methylparaben was dissolved in 44.15 mL of glycerin and 0.29 grams of propylparaben was dissolved in 44.15 mL of 70% ethanol. All ingredients are mixed and added with *Chlorella* sp. powder, which has been dissolved in 12 mL of distilled water little by little until homogeneous.

For more details, the research procedure can be seen in Figure 1.

## RESEARCH ANALYSIS

### Sensory evaluation

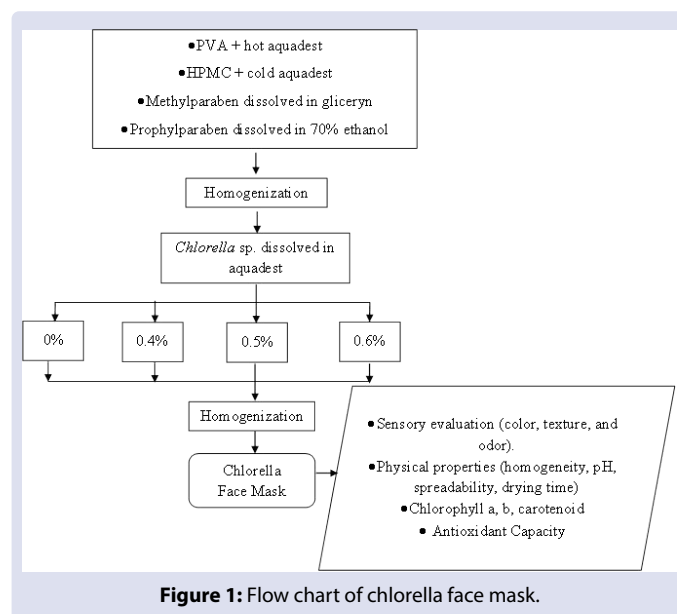
Sensory evaluation aims to determine the sensory value of the preparation which includes color, texture, and odor. This observation used a quality test with 25 semi-trained panelists consisting of students from the Department of Fisheries Product Technology, Faculty of Fisheries and Marine Science, Universitas Riau.

### Determination of homogeneity of *chlorella* face mask

Put the mask preparation on a slide and then observe the presence or absence of coarse particles contained in the mask preparation.<sup>14</sup>

**Table 1: The formulation of face mask.<sup>13</sup>**

Ingredients	Weight (gr)			
	$MW_0$	$MW_1$	$MW_2$	$MW_3$
<i>Chlorella</i> sp. powder	0	0.63	0.78	0.93
PVA	23.55	23.55	23.55	23.55
HPMC	14.71	14.71	14.71	14.71
Glycerin (mL)	44.15	44.15	44.15	44.15
Methylparaben	5.88	5.88	5.88	5.88
Propylparaben	0.29	0.29	0.29	0.29
Ethanol 70% (mL)	44.15	44.15	44.15	44.15
Aquadest (mL)	312	312	312	312



**Figure 1: Flow chart of chlorella face mask.**

### Determination of pH of *chlorella* face mask

The pH measurement uses a calibrated pH tool. A total of 1 gram of face mask preparation is dissolved in 100 mL of distilled water. The pH meter is immersed in the solution until the pH shows a number, which is the pH of the face mask preparation.<sup>10</sup>

### Determination of spreadability of *chlorella* face mask

The test was carried out with as much as 1 gram of the mask preparation being given a load of 100 grams and left for 1 minute, then the spreadability of the mask preparation was measured.<sup>15</sup>

### Determination of drying time of *chlorella* face mask

This test is done by applying the preparation as much as 1 gram on the back skin evenly, waiting for it to dry and can be peeled off. Then the drying time is calculated.<sup>16</sup>

### Determination of chlorophyll a, b, carotenoid content of *chlorella* face mask<sup>17</sup>

Testing for chlorophyll a, b and carotenoid content was carried out where the chlorophyll a, b and carotenoid content was tested using a spectrophotometer with a wavelength (Abs) of 652, 665 and 470 nm. As much as 5 mg of *Chlorella* sp extract was dissolved using methanol solvent with a concentration of 5 mg extract/5 mL, then measured the wavelengths of 480, 630, 645 and 665 nm. Calculate the pigment concentration with the formula below:

$$\text{Klorofil } a \text{ } (\mu\text{g/mL}) = 16,72 \text{ (Abs 665)} - 9,16 \text{ (Abs 652)}$$

$$\text{Klorofil } b \text{ } (\mu\text{g/mL}) = 34,09 \text{ (Abs 652)} - 15,28 \text{ (Abs 665)}$$

$$\text{Karotenoid } (\mu\text{g/mL}) = 1000 \text{ (Abs 470)} - 1,63 \text{ (Ka)} - 104,96 \text{ (Kb)} / 221$$

### Determination of antioxidant capacity of *chlorella* face mask<sup>18</sup>

5 mg of the extract was dissolved in 5 mL of 96% ethanol, then 1 mL pipetted, added 1 mL of 0.2 M phosphate buffer (pH 6.6) and 1 mL of 1%  $K_3Fe(CN)_6$  after that, incubated for 20 minutes at  $50^{\circ}C$ . After incubation, 1 mL of TCA was added and centrifuged at 3000 rpm for 10 minutes. After centrifuging, 1 mL of the top layer was pipetted into a test tube, and 2 mL of distilled water and 1 mL of 0.1%  $FeCl_3$  were added. The solution was allowed to stand for 10 minutes and the absorbance was measured at 720 nm. As a blank used a mixture of oxalate solution.

Calibration curves were made using ascorbic acid solutions of various concentrations. FRAP values are expressed in mg equivalent of ascorbic acid/g extract. The % inhibition value is calculated using the following formula:

$$\% \text{ Resistance (Inhibition)} = \frac{(A \text{ control} - A \text{ sample})}{A \text{ control}} \times 100\%$$

Information:

A Control = Absorbance does not contain sample

A Sample = Absorbance of sample

The sample concentration values and inhibition percentages were plotted respectively on the x and y axes in the linear regression equation, the equation formed was  $y=a+bx$ , used to find the IC50 value (50% inhibitor concentration) of each sample tested by stating the y value is 50 and the x value will be obtained as IC50. This value represents the concentration of the sample solution required when reducing FRAP free radicals by 50%.

### Data analysis

The data physical properties obtained were presented in the form of tables and figures and then analyzed descriptively. Data sensory, chlorophyll, carotenoid, and antioxidant were analyzed with ANOVA using Software SPSS 19.

## RESULT AND DISCUSSION

### Sensory evaluation

The different concentration of chlorella powder has effect to color, texture, and odor of face. The value of sensory evaluation can be seen in figure 2 and table 2.

Based on figure 2 and table 2 shown that, the more chlorella powder given, the quality color is better which is a bright green color, fragrant and specific to chlorella as well as the odor, but not with texture. *Chlorella* sp. powder obtained has a dry and smooth texture and is dark green in color, this is due to the nutrient content in the growth medium which produces more chlorophyll in *Chlorella* sp., where the nutrient content is needed by *Chlorella* sp.<sup>6</sup>

### Physical properties

**Homogeneity of chlorella face mask:** The *Chlorella* sp. face mask from all treatments has similar homogeneity, specifically no lumps and no coarse grains. The homogeneity assay can be seen in Figure 4.

Based on Figure 4, all the treatments obtain the best homogeneity. The homogeneity of the distribution shows that the bioactive compounds contained in it are also evenly distributed. This is following the BPOM 1979<sup>19</sup> statement, that the uniform distribution of bioactive indicates that the preparation has a homogeneous composition.

**pH of chlorella face mask:** pH all the treatments of different concentrations of chlorella powder in the face mask manufacturing have a range of 5.3-5.5.

Based on Table 3, can be seen that the range of pH all of the treatments fulfills the pH skin. This is following the statement of Troy and

**Table 2: The sensory evaluation of chlorella face mask.**

Treatments	Parameters		
	Color	Texture	Odor
MW <sub>0</sub>	2,23 <sup>a</sup> ±0,05	7,83 <sup>c</sup> ± 0,33	4,23 <sup>a</sup> ± 0,68
MW <sub>1</sub>	6,23 <sup>b</sup> ±0,17	7,08 <sup>a</sup> ± 0,08	6,60 <sup>b</sup> ± 0,60
MW <sub>2</sub>	7,99 <sup>c</sup> ± 0,39	7,40 <sup>b</sup> ± 0,29	7,00 <sup>b</sup> ± 0,35
MW <sub>3</sub>	8,28 <sup>c</sup> ± 0,21	7,08 <sup>a</sup> ± 0,14	7,37 <sup>b</sup> ± 0,36



**Figure 2:** Face mask with different concentration of chlorella powder.



**Figure 3:** Chlorella with 0.6% concentration of chlorella powder.



**Figure 4:** The homogeneity assay of chlorella face mask.

**Table 3: pH of chlorella face mask.**

Treatments	pH
MW <sub>0</sub>	5.5
MW <sub>1</sub>	5.4
MW <sub>2</sub>	5.3
MW <sub>3</sub>	5.3

**Table 4: Spreadability of chlorella face mask.**

Treatments	Spreadability
MW <sub>0</sub>	7.0
MW <sub>1</sub>	7.0
MW <sub>2</sub>	7.5
MW <sub>3</sub>	7.5

**Table 5: Drying time of chlorella face mask.**

Treatments	Drying Time
MW <sub>0</sub>	25-35
MW <sub>1</sub>	25-34
MW <sub>2</sub>	25-32
MW <sub>3</sub>	24-30

**Table 6: Chlorophyll and carotenoid content of chlorella face mask.**

Treatment	Chlorophyll-a	Chlorophyll-b	Total Chlorophyll	Carotenoid
MW <sub>0</sub>	0,31 ± 0,005 <sup>a</sup>	0,32 ± 0,010 <sup>a</sup>	0,65 ± 0,015 <sup>a</sup>	0,27 ± 0,010 <sup>a</sup>
MW <sub>1</sub>	0,62 ± 0,010 <sup>b</sup>	0,37 ± 0,005 <sup>a</sup>	1,02 ± 0,015 <sup>b</sup>	0,59 ± 0,020 <sup>b</sup>
MW <sub>2</sub>	0,61 ± 0,015 <sup>b</sup>	0,55 ± 0,025 <sup>b</sup>	1,22 ± 0,010 <sup>c</sup>	0,66 ± 0,015 <sup>b</sup>
MW <sub>3</sub>	0,67 ± 0,040 <sup>b</sup>	0,89 ± 0,045 <sup>c</sup>	1,63 ± 0,015 <sup>d</sup>	0,84 ± 0,445 <sup>c</sup>

**Table 7: Antioxidant capacity of chlorella face mask.**

Treatment	IC <sub>50</sub> (ppm)	Antioxidant Capacity
MW <sub>0</sub>	601,40 <sup>d</sup> ± 0,93	Very weak
MW <sub>1</sub>	518,32 <sup>c</sup> ± 1,07	Very weak
MW <sub>2</sub>	434,77 <sup>b</sup> ± 5,66	Weak
MW <sub>3</sub>	246,70 <sup>a</sup> ± 1,68	Moderate

Beringer<sup>20</sup> that pH 5-7 is acceptable to the skin and pH 4.5-8.0 is a safe limit for topical preparations. pH below the safe limit (alkaline or more acidic) causes the skin to dry quickly, the more difficult it is for the skin to neutralize it, chapped, sensitive, and prone to infection, and even affects skin elasticity.<sup>21</sup>

**Spreadability of *Chlorella* sp. face mask:** Spreadability affects the absorption of substances, the easier it is to apply, the more optimal and maximum absorption of the active substance in the mask preparation on the skin. The spreadability of *Chlorella* sp. face mask is about 7.0-7.5 (Table 4).

Table 4 can be seen that the spreadability of *Chlorella* sp. face mask for control treatments is 7.0 and for CM<sub>2</sub> and CM<sub>3</sub> are 7.5. This is because *Chlorella* sp. powder has fine granules that are homogeneous in size, so it does not affect the spreadability of face mask preparations. The limit of a good dispersion range is between 5-7 cm.<sup>22</sup>

**Drying Time of *Chlorella* sp. Face Mask:** The purpose of this test is to determine the peeling time of the mask on the skin surface. Drying time of *Chlorella* sp. about 24-35 minutes for all treatments.

Based on table 5 can be seen that the *Chlorella* sp. face mask in 0.6% addition of *Chlorella* sp. powder is faster dry than other treatments, this may be due to the chlorella can absorb water. *Chlorella* sp. powder has hygroscopic properties or water-absorbing properties so that it can absorb water and produce the fastest drying time on face masks with the most concentration of *Chlorella* sp. powder.<sup>23</sup>

**Chlorophyll and carotenoid Content:** *Chlorella* sp. contains chlorophyll a and b in the chloroplast. The most important is chlorophyll a.<sup>24</sup> *Chlorella* sp is affected by light intensity because it contains chlorophyll (green pigment) to carry out photosynthesis. Chloroplasts contain two pigments namely chlorophyll and carotenoids. *Chlorella* sp is known to produce several types of carotenoids, such as b-carotene, a-carotene, lutein, zeaxanthin, astaxanthin, and neoxanthin.<sup>25</sup> The chlorophyll and carotenoid content of chlorella face mask shown in table 6.

Based on table 6 can be seen that the highest concentration of chlorella powder given the highest value of bioactive compounds (chlorophyll and carotenoid), and it has significant different with control treatment.

### Antioxidant capacity

The high content of antioxidants in chlorella (32.74%),<sup>5</sup> so that, it can be applied to mask. The effect of different concentration of chlorella powder to mask can be seen in table 7.

In the MW<sub>3</sub> treatment, the best antioxidant results were found, namely moderate, compared to other treatments. This is because the MW<sub>3</sub> treatment was a treatment with the addition of *Chlorella* sp powder, namely 0.6% (0.93 gram), compared to the MW<sub>0</sub> treatment of 0%, MW<sub>1</sub> of 0.4% (0.63 gram), and MW<sub>2</sub> of 0.5% (0.78 gram), so that the chlorophyll content and carotenoids also increased in line with the amount of *Chlorella* sp powder added. Chlorophyll functions as an antioxidant because it can break down excess oxidation in the body and contains enzymes that can neutralize free radical activity.<sup>5</sup> Peel off facial masks can make the face look fresh, can remove dead and dirty skin.<sup>7</sup>

### CONCLUSION

The different concentrations of chlorella powder given affected sensory values, and the treatment with the highest concentration of chlorella powder was the best treatment based on physical properties which had similar homogeneity, specifically no lumps and no coarse grains. The pH of all facial masks fulfills the requirements of the pH range that is acceptable to the skin, with a spreadability of about 7.0-7.5 cm, and a fast drying time of 24-30 minutes. The face mask of chlorella contains bioactive compounds namely chlorophyll, carotenoid, and antioxidants. Hence, the addition of *Chlorella* sp. powder in the manufacturing of face masks could be accepted to and its potential as antiaging consumers.

### ACKNOWLEDGMENT

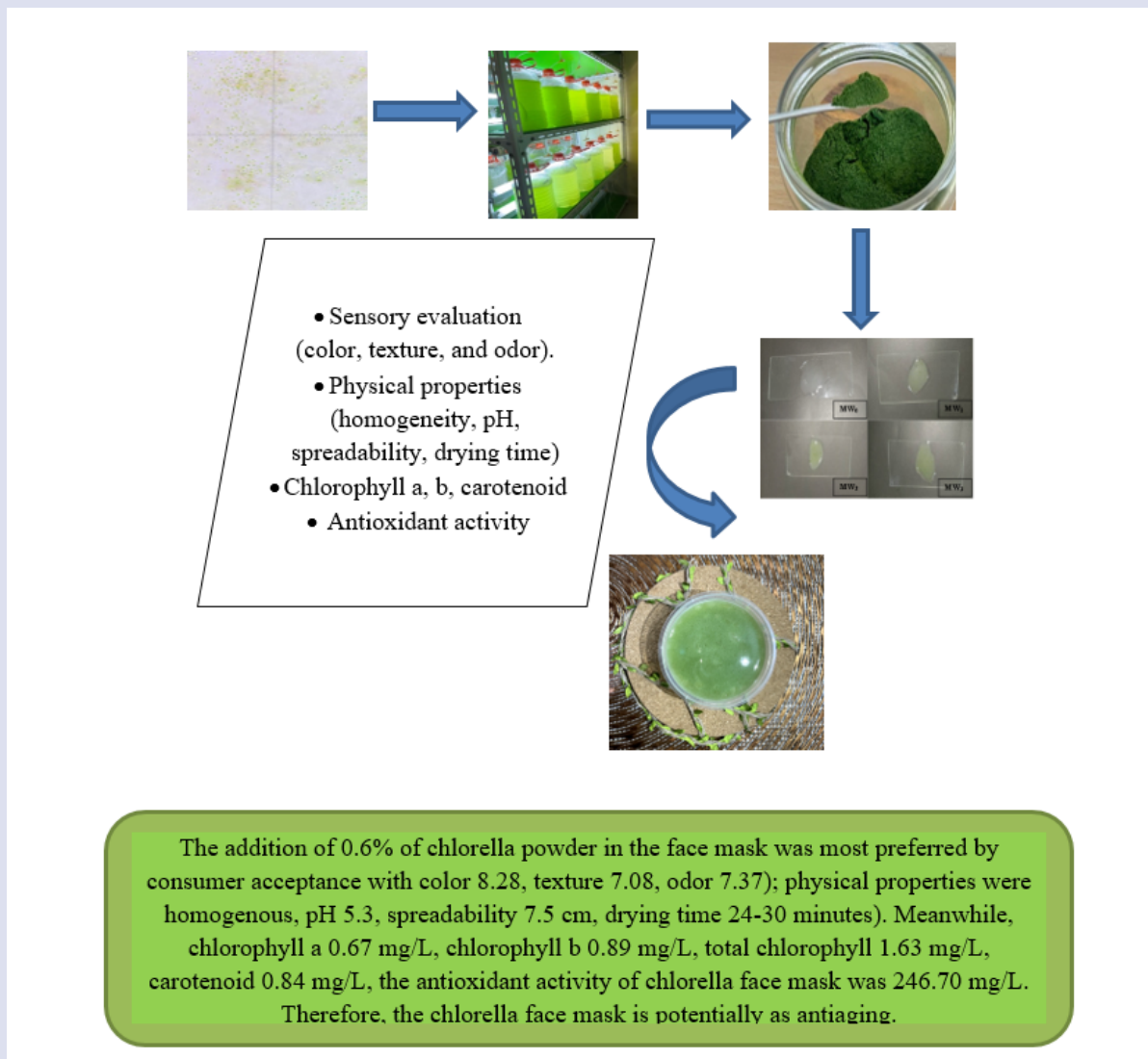
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## GRAPHICAL ABSTRACT



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