

Formulation of Emulsion with A Combination of Microalgae *Chlorella sp.* with Gourami Fish Oil (*Osphronemus gouramy L.*)

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ABSTRACT

Introduction: *Chlorella sp.* is a type of microalgae that has high nutritional value and is often cultivated for various purposes such as medicines or supplements. One of the efforts that can be made to optimize the benefits of *Chlorella sp.* microalgae with a combination of gourami fish oil formulated in an emulsion preparation. **Aims:** determine the best formula and physical stability of *Chlorella sp.* microalgae emulsion with gourami fish oil. **Method:** making emulsion with varying concentrations of *Chlorella sp.* microalgae with gourami fish oil (F1= 1:1, F2= 2:1, and F3=3:1). The physical preparation stability test will be carried out such as organoleptic test, homogeneity, pH, viscosity, emulsion type and stability. **Results:** all three formulas met the requirements for physical evaluation of emulsion preparations, only in the stability test there was separation at F3 (3:1). The type of emulsion produced is oil in water (O/W). **Conclusion:** the best formula according to the test results is F1 & F2.

KEYWORDS: Microalgae, *Chlorella sp.*, gourami fish oil, formulation, emulsion preparation.

INTRODUCTION

Microalgae is known as a raw material for the pharmaceutical industry because it contains chemical compounds such as protein, fatty acids, pigments and vitamins (Kurnia, 2018). *Chlorella sp.* is a phytoplankton that is often found in public waters, both fresh waters and marine waters (Wigajatri et al., 2003 in Aprilliyanti et al., 2016). *Chlorella sp.*

microalgae have high nutritional value, making them often cultivated for various purposes such as medicines and food supplements. Advantages of *Chlorella sp.* apart from having high nutritional content, easy care and fast growth, it also has great potential to be developed in cultivation businesses. The cosmopolitan nature of *Chlorella sp.* able to live anywhere except in

places that are very important for life (Aprilliyanti et al., 2016). The ability of photoautotrophic microalgae to produce high-value compounds such as pigments, polyunsaturated fatty acids and vitamins (Solomon et al., 2023). Gourami fish is a type of freshwater fish that is widely kept and cultivated because it has high protein value and low fat so it is in great demand (Patmawati et al., 2022). In making medicinal preparations that have therapeutic value, *Chlorella sp.* microalgae can be combined with gourami fish oil which is rich in benefits, one example is as an antioxidant. When combined with fish oil, this potential bioactivity can be enhanced. Based on research from Husni et al., 2019, fish oil easily undergoes oxidation and is terrible, so to overcome this, it is formulated from an emulsion dosage form (Aprilliyanti et al., 2016). Apart from that, *Chlorella sp.* has a strong taste and smell and is not always liked by many people so emulsion preparations can help mask this unpleasant taste and smell which makes it easier to consume. However, these two materials have different properties where *Chlorella sp.* enters the air phase and the oil produced by the gourami fish enters the oil phase. To overcome this, one way is to formulate gourami fish oil into an emulsion combined with powder obtained from the microalgae *Chlorella sp.*

Basically, emulsions are unstable systems due to the contact between the oil and water phases. To form an emulsion, mechanical forces are required to disperse one phase into

the other. However, emulsions formed without the addition of surface active ingredients will not be stable, and the emulsion phase will begin to separate into different layers based on density differences (Akbari & Nour, 2018). Therefore, in making emulsion preparations, a suitable emulsifier is needed to mix the oil phase and the water phase so that it is expected to increase the stability of the emulsion which has a longer shelf life because it can prevent merging (Aprilliyanti et al., 2016). Tween 80 and span 80 are emulsifiers that are often used and can make emulsions more stable than using a single emulsifier because they can reduce face tension, are able to increase viscosity so that they can form the desired emulsion preparation and can increase stability (Pratiwi et al., 2023)

This research is important to improve knowledge and skills and contribute ideas, information and input so that *Chlorella sp.* microalgae emulsion with gourami fish oil can be used as a medicine or food supplement. Evaluation of the physical properties or stability of the emulsion was carried out from several variations of the formula that had been made using organoleptic parameters, pH, homogeneity, emulsion type test, stability test and viscosity in order to produce good physical properties because it affects the resulting therapeutic.

MATERIAL AND METHODS

Materials

The materials used in this research include

samples of microalgae *Chlorella sp.*, Gourami fish, NaOH (Emsure), microalgae feed, Methanol p.a (Supelco), Tween 80 (Sigma Aldrich), Span 80 (Aloin), Butylated Hydroxytoluene (BHT, by ZAG Kimya), Methylparaben (by Golden Era), *flavoring agent* (melon flavoring code B046), and Methylene blue (ROFA Laboratorium Centre).

Sample Preparation for Microalgae *Chlorella sp.*

The process of sampling the microalgae *Chlorella sp.* carried out at the Lombok Mariculture Center, West Sekotong Village, Sekotong District, West Lombok Regency, West Nusa Tenggara. Next the *Chlorella sp.* sample was cultured to obtain *Chlorella sp.* which is denser. Then a filtering process was carried out to obtain *Chlorella sp.* gel before *Chlorella sp.* dried. *Chlorella sp.* gel which is formed is then dried by placing it on a flat container and drying with the help of sunlight until *Chlorella sp.* powder is formed.

Sampling Gourami Fish

A total of 9.4 kg was taken at the Freshwater Fish Farm in Gerung Village, Lingsar District. After cleaning and removing some parts of the fish such as the head, tail, and other parts, a net yield of about 478 grams was obtained. Extraction of gourami fish was carried out to obtain a crude extract from gourami fish that had previously been cleaned and then centrifuged to obtain gourami fish oil. The filtered gourami extract is then centrifuged to separate oil and water.

Emulsion Formulation

The preparation was formulated using three different formulas with several variations in the concentration of *Chlorella sp.* microalgae powder in each formula. In formula 1 (F1), formula 2 (F2), and formula 3 (F3), concentrations of *Chlorella sp.* microalgae powder and gourami fish oil were used respectively as 2%: 2%, 4%: 2%, and 6%: 2 %.

Physical Evaluation of Emulsion Preparations

Evaluation of the preparations carried out on the three formulas includes organoleptic tests, homogeneity tests, pH tests of the preparations, emulsion type tests, viscosity tests, and emulsion stability tests. Evaluation was carried out to find out the best formula from the three formulas created.

Procedure

Before proceeding to the drying stage, harvesting of the microalgae was carried out using the flocculation technique using NaOH which was then dried until the *Chlorella sp.* biomass was obtained. Dry with a water content of less than 10% (Kumalasari et al., 2014 in Kurnia, 2018). A total of 5 L of *Chlorella sp.* microalgae was added with 5 grams of NaOH, waited for 15 minutes until the *Chlorella sp.* microalgae settled, then filtered with a satin cloth. *Chlorella sp.* microalgae gel was dried under sunlight with a cover for two days.

The gourami fish is heated at 100°C without adding water until the oil is released.

Table 1. Emulsion preparation formula

Material	Function	F1	F2	F3
<i>Chlorella sp.</i> powder	Active substance (water phase)	2%	4%	6%
Gourami fish oil	Active substance (oil phase)	2%	2%	2%
BHT	Antioxidant	0.02%	0.02%	0.02%
Tween 80	Emulgator	2.5%	2.5%	2.5%
Span 80	Emulgator	2.5%	2,5%	2.5%
Sorbitol 70%	Sweetener	35%	35%	35%
Metylparaben	Preservative	0.2%	0.2%	0.2%
Melon flavoring	<i>Flavoring agent</i>	q.s	q.s	q.s
Aquades	Solvent	ad 100%	ad 100%	ad 100%

The results obtained are then filtered using filter paper to separate impurities that are still mixed with the raw fish oil extract. The extraction results were then centrifuged at a speed of 7000 rpm for 20 minutes (Iwo, 2019)

Emulsion Formulation

The formula that will be used in the formulation is as follows (Table 1). *Chlorella sp.* powder is dissolved using hot water at 80°C with a ratio of 1:10 on a hot plate. Methyl paraben is added when the *Chlorella sp.* powder has dissolved. Next, a 70% sorbitol solution was made by mixing 70 g of sucrose with 100 mL of hot water at 80°C. Ingredients that are in the oil phase such as fish oil, Span 80, and BHT are homogenized in a glass beaker. Ingredients which are in the water phase such as a solution of *Chlorella sp.* powder and methylparaben, Tween 80, Sorbitol 70%, and *Flavoring agent* (melon flavoring) are homogenized in different beakers. The oil phase was added little by little into the water phase and the chili sauce was homogenized using an ultraturax at a speed of 5600 rpm (Puertas & Vazquez, 2021)

Evaluation of The Physical Stability

Organoleptic test

The emulsion organoleptic test is a qualitative test carried out by observing the color and aroma of the emulsion preparation (Fatta et al., 2023)

Homogeneity test

The homogeneity test is carried out by dripping three drops of the preparation onto the slide and then observing whether there are solid particles, lumps, sediment, or inhomogeneous oil globules (Fatta et al., 2023)

pH test

pH testing is carried out using litmus paper and compared with a pH indicator. Oral emulsion preparations have a pH range of around 5.5-7.5 (Husni et al., 2019)

Viscosity test

Testing is carried out to see the viscosity level of the preparation. Tests were carried out using a Brookfield viscometer (Fatta et al., 2023). Emulsions with higher viscosity show better emulsion stability. A good oral emulsion viscosity value is in the range of 50-5000 cP (Kumar et al., 2014)

Emulsion type test

The emulsion type test was carried out using methylene blue which can give a blue color to O/W type emulsions. *Oil in water* (O/W) is the oil phase dispersed as droplets in the entire outer phase of water (Attama et al., 2016 in Wafa & Betha, 2023). A positive result indicates the color will dissolve in the emulsion.

Stability Test

The emulsion stability test was carried out by centrifuging the preparation for 10 minutes at a speed of 3600 rpm. An emulsion can be said to be stable if there is no separation (Wafa & Betha, 2023)

RESULT AND DISCUSSION

Microalgae Sample Culture Process *Chlorella sp.*

The culture process was carried out for one week and the results obtained were samples of 15 L of *Chlorella sp.* microalgae. The darker color of the microalgae sample indicated that the number of microalgae colonies was increasing.

Filtering and Drying Sample of Microalgae *Chlorella sp.*

A 15 L sample of *Chlorella sp.* microalgae was precipitated using NaOH in a ratio of (1:1). The addition of NaOH aims to kill microalgae. *Chlorella sp.* is a microalgae whose life is spread floating in sea water, therefore NaOH which has an alkaline pH will cause extreme environmental conditions so

that the microalgae can die and settle (Kurnia, 2018). The settled *Chlorella sp.* biomass is filtered using a chamois cloth. Kanebo cloth was chosen as a filter because it has a very small pore diameter (60 μ). The filtered residue is rinsed again with distilled water to remove salts that are still present in the biomass (Kurnia, 2018). The results of the filtration will produce wet microalgae biomass in gel form. The results of *Chlorella sp.* microalgae gel were 194 grams. The *Chlorella sp.* microalgae gel was then dried in the air for one week and the dry *Chlorella sp.* microalgae yield was 21.3 grams.

Yield is the comparison of the dry weight of the product produced with the weight of the raw materials (Sari et al., 2021). The yield is calculated based on the comparison of the final weight (the weight of the extract produced) with the initial weight (the weight of the cell biomass used) multiplied by 100% (Sari et al., 2021). Determining the yield serves to determine the levels of secondary metabolites carried by the solvent, but cannot determine the type of compound (Sari et al., 2021)

The yield result of *Chlorella sp.* powder, namely 10.9 %, can be said to be good or meets the requirements, where the greater the yield value indicates the greater the value of the

Tabel 2. Yield result

Result	<i>Chlorella sp.</i> (g)	Gourami fish oil (g)
Dry sample	194	478
Oil yield	21.3	55.69
Percent	10.9%	11.6%

extract produced. The requirement for the yield of thick extract is that the value is not less than 10% (Kemenkes RI, 2017).

Fish Oil Extraction

A crude extract of gourami fish oil was obtained and then centrifuged to separate the water and oil in the sample. The fish oil yield after centrifugation was obtained as much as 55.69 grams. The yield of gourami fish oil meets the requirements, namely not less than 10% with the following calculation.

Yield Results

The following table shows the results of the yield of chlorella sp fish oil and gourami fish (Table 2)

Emulsion

In this research, the formulation used several excipients such as Tween 80 and Span 80 as emulsifiers, Sorbitol 70% as sweetener, Butylated Hydroxytoluene (BHT) as antioxidant, Methyl, paraben as preservative, Aquades, and Melon flavoring as flavoring agent. The choice of Tween 80 and Span 80 as emulsifiers is because this type of emulsifier is often used and can make a more stable

emulsion compared to using a single emulsifier because it is able to reduce surface tension, can increase viscosity so that it can form the desired emulsion preparation and can increase stability (Pratiwi et al., 2023). In making liquid preparations, stabilizers are also needed, one of which is antioxidants (Fickri, 2019). The content of free fatty acids in gourami fish oil can increase, one of which is due to the influence of temperature and heating time which occurs in the process of making emulsion preparations so that oxidation and hydrolysis reactions occur in the oil. This reaction will reduce the desired quality of the oil. To prevent this oxidation, synthetic antioxidants or natural antioxidants can be added. One of the synthetic antioxidants most widely used in food products is BHT (Butylated Hydroxytoluene) (Gultom & Ginting, 2018). The use of BHT as an antioxidant aims to reduce the rancidity of emulsion preparations caused by gourami fish oil. The preservative such as methylparaben in the preparation aims to make the preparation long-lasting and can be used repeatedly (Fickri, 2019) also considering that the emulsion preparation is a liquid that is easy for

Table 3. Result of evaluation of emulsion preparations

Evaluation	F1	F2	F3	Standard
Organoleptic	Light green and smells like melon	Light green and smells like melon	Dark green and smells like melon	Green and smells like melon
Homogeneity	Homogeneous	Homogeneous	Homogeneous	Homogeneous
pH	6.27±0.12	6.20±0.01	6.20±0.02	5.5-7.5
Viscosity (cP)	420.2±0,34	395.7±0.25	286.3±0.43	50-5000
Emulsion type	O/W	O/W	O/W	O/W or W/O
Stability	No separation	No separation	separation	No separation

microbes to grow, so a preservative is needed in the preparation. As an added value, melon flavoring and a sweetener combination of 70% sorbitol are used so that the preparation can provide comfort for users when consumed.

Results of Physical Evaluation of Emulsion Preparations

Based on Table 3, the organoleptic test shows almost the same results between F1, F2, and F3, but there is a slight difference in F3 which shows a darker green color. *Chlorella sp.* as an active substance is broken down into particles that are dispersed in the water phase which increases the concentration of the water phase (Tania et al., 2020). In F3, the concentration of microalgae *Chlorella sp.* used was greater than fish oil with a percentage of 3:1, but these results were still within the normal range. The homogeneity test found that the three preparations can be said to be homogeneous, with the requirement in the homogeneity test is that the oil phase and water phase are not homogeneously separated. In testing the emulsion type, methylene blue reagent is used. If methylene blue is dispersed in the preparation, the type of emulsion formed is oil in water (O/W) (Sopianti et al., 2021)

Based on the viscosity test, the emulsion preparations for the three formulas still meet the normal requirements for viscosity tests, namely the range of 50-5000 cP. However, in the results of statistical testing using One Way Anova, the significant results of <0.05 indicate

that there are differences in viscosity results in each formula.

The result shows that the highest viscosity is in F1 with a combination of *Chlorella sp.*: fish oil, namely 1: 1. This is because F1 is a formula that has the smallest concentration of water phase which can increase the value of the viscosity of the preparation. So, the lower the concentration of the water phase, the higher the viscosity level of preparation.

This study measured pH using a pH meter with accurate and fast measurement results, but this measurement method is relatively more expensive because it uses tools (Wibowo & Ali, 2019). The pH measurement results of the three formulas with the three replications are by the pH target for emulsion preparations (5.5-7.5) (Husni et al., 2019). After statistical testing, the *P-value* for pH was 0.740 (> 0.05) which showed no significant difference in the pH of all formulas.

F1 and F2 have good stability, but F3 has poor stability or separation occurs. This can be caused because the dissolving temperature of *Chlorella sp.* with water on a hot plate is not consistent or the temperature is low. According to Baskara et al. 2020, a low mixing temperature will cause separation in the preparation because the temperature in the manufacturing process has just reached the boiling point of the emulsion and there is not enough time during stirring. This causes the particles to be unable to stabilize the emulsion, resulting in the cream preparation experiencing separation (Baskara et al., 2020).

In addition, emulsion stability can be influenced by the concentration ratio between the water phase and the oil phase where the concentration of the water phase is too high can result in separation between the water phase and the oil phase (Mu'Awanah et al., 2014). This is what happened in F3.

CONCLUSION

Based on the three formulas above, they have met the requirements for the physical stability test of emulsion preparations except that in the F3 stability test where separation occurs due to the high concentration of the water phase. In the organoleptic test, the higher the concentration of *Chlorella sp.*, the darker the color of the preparation (F3). In the viscosity test, the lower the concentration of *Chlorella sp.*, the higher the viscosity value (F1). So the best formula is F1 and F2.

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