The Effect of Basil (Ocimum basilicum i.) Leaf Extract in Immersion Stage Against Profile of Volatile Compound on Spirulina Platensis Powder

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Abstract. Spirulina platensis produces secondary metabolites that can affect the aroma and reduce the level of direct consumption. This study aims to determine the change of chemical parameters (protein, water, fat and thiobarbituric acid) and volatile compounds contained in S. platensis after immersion with basil leaf extract. The method was soaking of S. platensis powder in basil leaf extract with ratio of 1: 0 (Control), 1: 3 (A), 1: 4 (B), 1: 5 (C) with three replications. Data of chemical parameters from S. platensis in this research processed by analysis of variance continued with Tukey analysis, and data of volatile compound were analyzed descriptively. The results showed an increase ratio of S platensis powder and basil leaf extract results in increase of water and protein content, on the other hand the fat content and the number of TBA decrease i.e. 58.79-68.87% of protein content, 9.45-15.29% of water contents, 1.70-7.43% of fat contents and 0.55-2.03 mg/kg of TBA values. The result of this study wasthere were 14 compounds of S. platensis powder in control treatment, in A and B treatments there were 8 compounds of S. platensis powder and also 3 and 4 compounds addition from basil leaf extract, respectively. The C treatment there were 6 compounds of S. platensis powder and 8 compounds addition from extract. The dominate of volatile compoundsshowed in all treatments were hexadecane, octadecyne, eicosyne, tetradecanoid acid, octadecadienoic acid, and octadecanoic acid.

1. Introduction
One of the commercially known microalgae is S. platensis. S. platensis is the microorganism cell that grows well in fresh water or sea water [1]. Algae can be used as ingredient in some food, dietary supplement, food additives, functional food, and algae can produce hundred tons product per year. Microalgae can be considered as innovative food ingredients which are rich in nutrients like protein, polyunsaturated fatty acid, carotenoids, vitamins, minerals, and fenolat as bioactive molecules [2].

There are two types of Spirulina, fresh water and seawater. The striking difference of the two types Spirulina is in odors produced. Spirulina in fresh water has less unpleasant odors than Spirulina in seawater. Fresh water Spirulina has less mineral content than Spirulina of seawater, the odors are influenced by marine environment or fresh water Spirulina can be conditioned in such a way by farmers. Environmental factors not only affect photosynthesis and biomass productivity of the cell but can affect the metabolic activity, which has an impact on the dynamics of the cell’s composition [3].

In addition, algae contain different secondary metabolites (e.g., vitamins, saccharide, volatile compounds, and phenol) which can be used as antioxidant substances, and antibiotics. Food products that are made from algae can involve not only positive but also negative effects in the mammalian organism. For example, the content of toxic elements that are higher (e.g. cadmium or fucotoxins) from undesirable algae food products [4]. The Spirulina has many nutrients that are good for food to people of all ages and lifestyles. Spirulina contains with Gamma–linolenic Acid (GLA), B vitamins,
minerals, enzymes, and chlorophyll. The other valuable nutrients of Spirulina are carotenoids, sulfolipid, ficosianin, superoxide dismutase, RNA and DNA [5].

Gas Chromatography-Flame Ionization Detector (GC-FID) and Gas Chromatography-Mass Spectrometry (GCMS) detected 77 volatile compounds identified and calculated as 85 compounds on Ocimum basilicum L. Seven compounds with the highest concentrations are the main compounds. This compound is oxygenated monoterpenes (1.8-linalool, cineole, linalool acetate) derivative, fenylpropane (methyl chavicol, eugenol, methyl trans-cinnamate) and sesquiterpene hydrocarbon (trans-bergamotene) [6].

Aromatic herbs, Basil (O. basilicum) can be consumed direct or added to the processing industry. Essential oils are rated in the international market and widely used in food industry, cosmetics and medicines. Basil oil has high economic value due to the presence of specific substances such as lineol, estragol, linalool, eugenol, methyl cinamato, limonene and geraniol [7].

2. Research methods

2.1. Research materials

The main materials used are Spirulina platensis powder from CV. NeoalgaeSukoharjo, basil leaf (Ocimum basilicum L.) from traditional market.

2.2. Basil leaf Extracts preparation with modifications [8]

1500 g of basil leaves was added with 300 ml aquadest and grinded. The addition of aquadest makes the Basil leaves easily ground. The slurry was then filtered by using Then filter with filter fabric and the extract resulted was approximately 1500 ml.

2.3. Treatment of S.platensis with basil leaf extract

10 g of Spirulina powder was blended with water extracts of Basil leaf with different ratio i.e: 1 : 3; 1 : 4; 1 : 5 of S.platensis and basil leaf extract. The mixture was spread at the plate glass that has been layered with a plastic, plastic on the glass make it easier in process, the plastic will easily absorb heat, and then dried at 40°C for 17 hours, and the mixture was then stored in a tight container.

2.4. Analysis of volatile compounds with modifications [9]

Volatiles that are absorbed by fiber were adsorbed by thermal heat port injection gas chromatography QP2010S SHIMADZU for 5 minutes at 300°C (splitless mode). Identification of compounds based on the mass spectrum is compared to a database of Spectra and checked with the standards.

2.5. Analysis of nutrition contents [10]

Determination of water content by Gravimetric method, protein content by Micro Kjeldahl method, fat by Soxhlet method and thiobarbituric acid (TBA) by [11].

3. Result and discussion

3.1. Volatile compounds of Spirulina platensis with basil leaf extract

There were 14 volatile compounds have been detected by Gas Chromatography Mass Spectrometry (GCMS) on control treatment and decrease from 14 became 11 compounds in A (1:3) treatment because some compounds were not detected, the compounds are 2(4H) - Benzofurane, 5,6,7,7a-Tetrahydro – 4.4 7a Trimethyl, Pentadecanioic Acid, Hexadecatrienoic Acid, Hexadecadienoic Acid dan Oxacyclotetradecane. B (1:4) treatment has the same number of compounds with A treatment because some compounds appear from the Basil leaf extract, and there were 15 compounds in C treatment. Volatile compounds from basil leaf extract (Ocimum basilicum L.) contribute to increase the volatile compounds of Spirulina. The detected compounds by Gas Chromatography-Mass Spectrometry (GCMS) shown in Table 1.

Volatile compound is a compound that can evaporate, so can affect the aroma and flavor of the product. Flavor also includes the sensing of volatile compounds by the nose. The sense of trigeminal
nerve perceptions to detect chemical irritations [12]. The volatile organic compounds are soluble in the water and in the air, they can make some effects temporal and spatial. In the case generated biologically Volatile Organic Compound (VOC) is a secondary metabolite microalgae [13]. Treating the algae with different treatment basil leaf extract, resulted in different amount of volatile compounds. Basil leaf extract has volatile compounds in which can give some new volatile compounds and will be combining the origin volatile compounds. Many aromatic compounds can be found in all species of algae and most of these compounds are derivatives of benzene dicarboxylate acid, esters of benzoic acid, tetraphthalic, dimethylphthalate, methylvinyl esters and derivatives carboxylic acid esters of fenantran [14]. This research was in line with previous study [15], stated that pentadecane and heptadecane are a compound derived from palmitic and stearic acid decarboxylation of green blue microalgae.

Extracts of Basil leaf (Ocimumbasilicum L.) have a role as the aromatic plant for some food. The result analysis using Gas Chromatography-Mass Spectometry (GCMS) detected Basil consists of 54 components with 9 major components with content above 2% [16]. The 9 components are linalool (2.03%), Z-sitral (7.02%), methyl eugenol (4.88 %), 3-metilsiklopent-2 enona acid (3.78%), methyl heksadekanoat (2.48%), acid ethyl heksadekanoat (17.72%), ethyl acid 9oktadekenoat (10.62%) and ethyl oktadekanoat acid (14.83%). Compounds contained in Basil are dependent on environmental conditions. The results showed that A treatment (1:3) detected several compounds of alkanes, esters, compounds and hydrocarbons. As many as six compounds, i.e. 2 (4 h)-benzofurane orange 4.7a trimethyl, pentadecanoic acid, hexadecenoic acid, hexadecatrienoic acid, oxacyclotetradecane was not detected in this treatment, no detection of six such compounds because the granting of basil leaf extract were added. However, this study found that there was need less basil leaf extract to add into S. platensis for removing off odour of S. platensis. B treatment (1: 4) was the best treatment compared with other treatments because there were only found 11 volatile compounds. This means that basil leaf extract may affect some volatile compounds on S. platensis as compared with controls decreased, even some volatile compounds of S. platensis was not detected, other volatile compounds from basil leaf extract still dominate and make some new compounds. C treatment (1: 5) had higher variations of volatile compounds than other treatments. This treatment detected one volatile compound, named Azulene. Azulene was one of aromatic compound that can be found in many fruits or flowers derived from basil leaf extract. Azulene is one of the unique aromatic and type of non-benzene ingredients, and has different characters from other benzenoid aromatics such as naphthalene [17]. In addition, azulene is a blue aromatic hydrocarbon, has a strong dipole moment, and other unique characters.

3.2. Nutrition contents of Spirulina with basil leaf extract

The nutrition contents of water, protein, and fats contribute to the quality and affect on the volatile compounds of S. platensis. The nutrition contents are presented in Table 2. The addition of basil leaf extract into S.platensis could increase 58 to 69% of protein content. Protein increasing occurred because basil leaf extract also had a protein content that contributed protein levels in each treatment. Protein levels of basil ranged from 3 to 5 g per 100 g samples so that affect to the Spirulina protein, Spirulina has high enough protein levels. High protein content from microalgae (60 - 65% in Spirulina biomass), and advantage of protein from microalgae is high digestibility and balanced essential amino acid content [19]. Protein levels are very useful for body's metabolism, and design the immune system. Protein and carbohydrates from algae in the small intestine give benefit to humans by stimulate the immune response indirectly through the response of microbes [20].
| No. | Basil Leaf Extract | Spirulina platensis (control) | Ratio of Spirulina with Basil Leaf Extract |
|-----|--------------------|--------------------------------|--------------------------------------------|
|     |                    |                                | 1:3 | 1:4 | 1:5 |
| 1   | 1,8-Cineole        | 2-4H - Benzofuran, 5,6,7,7a   | -   | -   | -   |
| 2   | Terpinolene        | Tetrahydro - 4,4,7 aTrimethyl  | -   | -   | -   |
| 3   | β-(E)-Ocimene      | Hexadecane                     | Hexadecane | Hexadecane | Hexadecane |
| 4   | Linalool           | Octadecyne                     | Octadecyne | Octadecyne | Octadecyne |
| 5   | Borneol            | Eicosyn                         | Eicosyn | Eicosyn | Eicosyn |
| 6   | Methyl eugenol     | Pentadecanoic Acid             | -   | -   | -   |
| 7   | Eugenol            | Hexadecanoic Acid              | -   | -   | -   |
| 8   | γ-Gurjunene        | Tetradecanoic Acid             | Tetradecanoic Acid | Tetradecanoic Acid | Tetradecanoic Acid |
| 9   | α-Epi-cadinol      | Octadecadienoic Acid           | Octadecadienoic Acid | Octadecadienoic Acid | Octadecadienoic Acid |
| 10  | α-Cadinol          | Octadecanoic Acid              | Octadecanoic Acid | Octadecanoic Acid | Octadecanoic Acid |
| 11  | β-(Z)-Elemenone    | Hexadecatrienoic Acid          | -   | -   | -   |
| 12  | β-(Z)-Santalol     | Octadecanoate                  | -   | -   | -   |
| 13  | 1,8-Cineole        | Oxacyclotetradecane            | -   | -   | -   |
| 14  | Terpinolene        | Hexadecanoic Acid              | Hexadecanoic Acid | -   | -   |
| 15  | β-(E)-Ocimene      | Ethyl                           | Ethyl | Ethyl | Ethyl |
| 16  |                    | Octadecanoate                  | Octadecanoate | Octadecanoate | Octadecanoate |
| 17  |                    | Benzenedicarboxylic Acid       | Benzenedicarboxylic Acid | Benzenedicarboxylic Acid |
| 18  |                    | Undecane                       | Undecane | Undecane | Undecane |
| 19  |                    | Dodecane                       | Dodecane | Dodecane | Dodecane |
| 20  |                    | Decanedienoic Acid             | -   | -   | -   |
| 21  |                    | Ethyl cis-4-decenoate          | -   | -   | -   |
| 22  |                    | Azulene                        | -   | -   | -   |
| 23  |                    | Ethyl Tridecanoate             | -   | -   | -   |
| 24  |                    | 2-Decanone, 5,9-dimethyl       | -   | -   | -   |
| 25  |                    | Heptadecane                    | -   | -   | -   |
| 26  |                    | Tetracosanoic acid             | -   | -   | -   |
| 27  |                    | Hexadecatrienoic acid          | -   | -   | -   |

Note: (-) means the compound not detected
Table 2. Nutrition contents of Spirulina treated with basil leaf extract

| Treatment | Protein (%)   | Water (%)    | Fat (%)    |
|-----------|--------------|--------------|------------|
| Control   | 58,790 ± 0,190 | 9,450 ± 0,118 | 7,433 ± 0,208 |
| A         | 59,593 ± 0,651 | 10,450 ± 0,269 | 6,100 ± 0,300 |
| B         | 68,300 ± 0,230 | 11,593 ± 0,055 | 3,800 ± 0,200 |
| C         | 68,873 ± 0,651 | 15,296 ± 0,357 | 1,700 ± 0,000 |

Note:
- Data obtained from 4 different measurement points, then averaged 3 reps ± standard deviation
- Data followed by different notation show significant difference (P <0,05)

Water content are closely related to self-life of a food storage. Quality deterioration can be show by unpleasant aroma due to microbial activity that will appear when the storage time is too long or not at the right temperature so it becomes a good medium for the growth of microbes. The high levels of protein and fat in *S. platensis* then it can be a great medium for microbes, drying is the way to minimize the microbial activity on *S. platensis*. Water content of *S. platensis* with basil leaf extracts increased from 10% became 15%, and will increase with increasing of time storage. Thus, the addition of silica gel could be applied for storing to avoid the deterioration of *S. platensis* treated basil leaf extract, because it’s can help to absorb the humidity in storage environment. The water content is 7% to 7.5% as quality standard of Spirulina powder product and it can influenced by habitat.

Volatile compounds in Spirulina are influenced by the fat content. In control treatment, there was 7.43% of fat content. Fat content of Spirulina was decreased when treated with basil leaf extract. The higher concentration of basil leaf extract mixed into Spirulina gave more decreased levels of fat content. Spirulina presented a fat is 5 to 10% of dry weight [22].

Fat levels in a food can be one important factor considering that fat is very easy to react with other factors. Fats oxidation can cause some side effects, such as off odor. Fat metabolism is important in flavor development during storage. The most important enzymes in the formation of volatile compounds are lipoxygenases and lipolytic acyl hydrolases [23].

4. Conclusion
It can be concluded that treatment B (1:4) ratio of *S. platensis* and basil leaf extract has performed the best treatment in reducing the number of volatile compounds (off-odor) from *S. platensis* while keeping good nutritional value of the product.

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