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Application of basil leaf extracts to decrease *Spirulina platensis* off-odour in increasing food consumption

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Abstract

Spirulina platensis is a cyanobacterium that has high nutritional content and bioactive compounds. However, the amount of *spirulina* added into foods can cause a decrease in sensory characteristics, especially through the generation of odour. The present work thus aimed to evaluate the effect of soaking *spirulina* in basil leaf extract with varying *spirulina* to basil ratios on its off-odour compound (geosmin and 2-methylisoborneol), sensory characteristics and nutrition contents. *Spirulina* was soaked in basil leaf extract for 30 min with ratios of 1:3, 1:4, and 1:5 (w/v), and then dried at 40°C for 17 h. The results showed that when compared to non-soaked *spirulina* control sample, at a ratio of 1:3 (w/v), the geosmin compound which caused off-odour in *spirulina* was not reduced; while at a ratio of 1:4 (w/v) it was reduced to 52.17%, and at ratio of 1:5 (w/v) it was reduced by 100%. Hedonic scale and phycocyanin content of *spirulina* also increased with increasing amount of basil leaf extract used. Soaking *spirulina* in basil leaf extract increased the levels of aspartic acid, glutamic acid, serine, glycine, histidine, arginine, threonine, alanine, valine, isoleucine, leucine, phenylalanine and tyrosine by 3.57% to 57.39%, and decreased the levels of proline and lysine by 8.67%. Qualitatively, soaking *spirulina* in basil leaf extract caused the presence of methyl undecanoate fatty acids, linolelaidic acid methyl ester, gamma-linolenic acid methyl ester, and cis-4,7,10,13,16,19-docosahexaenoate which were not present in the control sample.

Introduction

Spirulina platensis is one of the largest biomass of cyanobacteria (blue-green microalgae) found in water. It is well known as a source of protein (60 - 70 g/100 g) of high biological value, gamma-linolenic acid, vitamin B12, pro-vitamin and minerals (especially iron). *Spirulina* also has 61% digestibility that is 19% higher than *Chlorella* (Muys *et al*., 2019). Extensive literature is available on the health benefit of *spirulina* as a hypolipidemic agent (Narmadha *et al*., 2012) and its ability to fight against diabetes and obesity (Anitha and Chandralekh, 2010). Therefore, *spirulina* can be utilised in the formulation of novel functional food products (Park *et al*., 2018).

Fradique *et al*. (2010) demonstrated the incorporation of *Chlorella vulgaris* and *Spirulina maxima* in fresh pasta which increased the chemical compositions of the pasta without affecting its culinary quality. Some works have also been published on innovative and functional food products integrated with microalgae such as pasta, biscuits and gelled desserts (Batista *et al*., 2012; Fradique *et al*., 2013; El-Baky *et al*., 2015; Singh *et al*., 2015).

Previous study conducted by Agustini *et al*. (2016) found that increased usage of *spirulina* in ice cream could decrease its hedonic value especially odour and taste. The maximum concentration of *spirulina* that could be used in ice cream is 1.2%. This is due to the odorous substances found in *S. platensis*. Several studies have examined earthy and musty odour compounds formed by cyanobacteria. Geosmin and 2-methylisoborneol (MIB) are cyanobacteria secondary metabolites that are responsible for off-odours (Burr *et al*., 2012; Deng *et al*., 2012; Zhang *et al*., 2013). In some cases, odorants can cause an off-flavour when added into food product, resulting in significantly negative impact on consumers' acceptance. Therefore, pre-treatment is needed to reduce or remove the odorants in *S. platensis*.

Basil (*Ocimum basilicum*) is an aromatic plant native to Southeast Asia, and is commonly used in

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various cuisines. The plant contains essential oils such as oxygenated monoterpenes and phenylpropane derivatives. Applications of essential oils from basil vary from usage in flavour liqueurs, confectionery, bakery and meat products. According to literature review on Basil aroma profile, terpenes are present at overwhelmingly high levels, followed by alcohols and aldehydes. Of the terpenes, methyl chavicol is the main compound. Additionally, the aroma-active compounds of basils have been investigated by using aroma extract dilution analysis (AEDA). The application of AEDA revealed 18 aroma-active compounds. Among them, linalool and methyl chavicol had the greatest flavour dilution (FD) factors giving floral-lemony and floral aroma, respectively (Sonmezdag *et al*., 2018). In addition, basil has hepatoprotective, antifungal, antibacterial and antioxidant activities due to its flavonoid and phenolic compounds (Carro *et al*., 2013; Bernhardt *et al*., 2015).

Among published works on the application of basil, none has reported the application of basil leaf extract to decrease the off-odour of *S. platensis*. Therefore, the present work aimed to evaluate the effect of different ratios of *S. platensis* to basil leaf extract on its off-odour compounds (geosmin and 2-methylisoborneol), sensory characteristics, and nutritional values.

Materials and methods

Materials

Freshwater *S. platensis* powder was obtained from PT Neoalgae (Central Java, Indonesia) in 2017. Basil leaves were purchased from the local market in Semarang (Central Java, Indonesia), blended using aquadest (10:1, w/v), and filtered using a nylon screen. Next, 10 g of *S. platensis* powder was soaked for 30 min in basil leaf extract. The ratios between *spirulina* and basil leaf extract used were 1:3, 1:4, and 1:5 (w/v) as previously described by Agustini *et al*. (2016). Drying was later carried out by spreading mixed paste of *spirulina* and basil leaf extract on glass plates measuring 10 × 10 cm, and oven-dried at 40°C for 17 h. *S. platensis* that had not been soaked in basil leaf extract was used as a control.

Analysis of off-odour

Off-odour concentrations within *S. platensis* were analysed using the solid-phase microextraction (SPME) method to first isolate the volatile compounds which were later identified using GC-MS (Hurlburt *et al*., 2009). Briefly, *S. platensis* powder (1 g) in 22 mL SPME vial was heated to 80°C, and the SPME fibre (Sigma-Aldrich) was inserted into the instrument for a 30 min adsorption period. Standard solutions of geosmin and 2-methylisoborneol (100 ppm in methanol) were purchased from Sigma-Aldrich (St. Louis, MO, USA). The solutions were diluted to 200 ppb in hexane to produce stock solutions.

The samples were run on a GC Agilent 7890A and MS Agilent 5975C with Triple Axis Detector (Agilent Technologies, Mississauga, Canada). The original injection was performed with a programmable pressure temperature vaporiser which enabled for large-volume injection, and reduced volume inlet liner was also used in the pressurised temperature vaporiser inlet. A 30 m × 250 µm i.d. capillary column HP-5MS (J&W Scientific, Folsom, USA) with 0.25 µm stationary phase was used. Hydrogen was used as the carrier gas at flow rate of 1.8 mL/min. The oven was initially held for 1 min at 50°C, then gradually increased by 10°C/min to 200°C, retained for 1 min, and then 20°C/min to 250°C which was retained for 5 min. Selected ions of the base peaks and molecular ion for geosmin (m/z 112 and 182) and MIB (m/z 95 and 168) were monitored at dwell times of 100 µs. Quantitation was determined by integrating the base peak area.

Sensory evaluation of S. platensis

Sensory evaluation for all samples were performed by 30 untrained panellists consisted of 13 men and 17 women aged between 20 and 22. The panellists evaluated the samples in terms of appearance, texture, and odour with hedonic scale (9-point scale where the smallest number indicated the most unpleasant).

Analysis of amino acid profile

The amino acids profile in the samples was determined by high-performance liquid chromatography (HPLC) (Sharoba, 2014). Acid (HCl) hydrolysis method was used for aspartic acid, threonine, serine, glutamic acid, proline, glycine, alanine, valine, isoleucine, leucine, tyrosine, phenylalanine, histidine, lysine and arginine. A Licrosper 100 RP 18 column (4 mm × 125 mL) was used to separate the compound. The mobile phase consisted of two solvents: methanol and mercaptoethanol. Shimadzu RF-138 fluorescence detector was used at excitation wavelength of 360 nm and emission wavelength of 460 nm. After hydrolysis, amino acid analyser (Sykam Gmbh, Germany) with an integrator (Axxiom Chromatography Inc.) was used for the quantification of the amino acids.

Analysis of fatty acid profile

The fatty acid profile was analysed using a gas-chromatography instrument (Hewlett Packard 6890 series Palo Alto, California, USA) with novel technology (Sharoba, 2014). The oils extracted from all samples were converted to their corresponding methyl ester using boron trifluoride methanol complex (14% w/v). The mixture was maintained at 105°C for 1 h, then was discontinued with the addition 0.5 mL of distilled water. The extracted fatty acid methyl esters (FAMEs) were dissolved in heptane for GC analysis. The column was set up with an HP-FFAP capillary column (25 m × 0.32 mm i.d., 0.5 µm film thickness); initial oven temperature of 130°C (1 min), increased at 2.5°C/min to a final temperature of 230°C (10 min); injector temperature 230°C, detector temperature 250°C; helium carrier gas with a spilt ratio of 20:1, and flow rate of 1 mL/min. FAMEs were identified by comparison of their retention time with respect to pure standard FAMEs purchased from Sigma-Aldrich (St. Louis, MO, USA). The FAMEs were quantified according to their percentage area, obtained by integration of the peaks. The results were expressed as a content of individual fatty acids in the lipid fraction.

Phycocyanin content

Samples (40 mg) were placed in a 10 mL centrifuge tube containing 100 mM phosphate buffer (pH 7.0) and stored at 4°C overnight before centrifugation to separate the blue supernatant. C-phycocyanin calculation was determined using the absorbance ratio (Setyoningrum and Azimatun, 2015). Phycocyanin content was determined using Eq. 1:

$$\text{Phycocyanin content}\,(\%) = (\text{Abs} \times \text{v}) / (3.39 \times \text{w} \times \text{w}_{\text{dry}}) \times 100\,\% \quad \text{(Eq. 1)}$$

where Abs = absorbance at 620 nm, v = volume of solvent (mL), 3.39 = coefficient of C-Phycocyanin at 620 nm, w = weight of sample (mg), w_{dry} = % dry weight of sample.

Statistical analysis

Phycocyanin content data obtained was expressed as mean of triplicates (n = 3) ± standard deviation. The data was subjected to the analysis of variance followed by multiple comparisons using Tukey HSD. Data of sensory analysis was processed using Kruskal Wallis followed by Man Whitney test. The parametric and non-parametric data were analysed using SPSS version 17.0 (International Business Machines Corporation, USA).

Results and discussion

Off-odour assessment

Reduction of geosmin content in *spirulina* was found at the ratio of *spirulina* to basil leaf extract ratios of 1:4 and 1:5 (w/v), resulting in respective reduction values of 52.17% and 100%. However, at 1:3, basil leaf extract was not at all able to reduce the geosmin content in *spirulina*. The decrease in the geosmin content could be due to the decrease in the amount of unsaturated fatty acid in *spirulina*. According to a study conducted by Bou-Maroun and Cayot (2011), lipids can act as solvents for volatile compounds. Thus, delipidation removes not only lipids, but also aroma compounds.

On the contrary, the analysis of 2-methylisoborneol (MIB) content revealed an increase in concentration with increasing amount of basil leaf extract used. Substantially, 1:5 increased MIB content by 114.28%, while 1:4 increased MIB content by 80.95%. The increase in MIB content could be due to the basil leaf extract which also contained MIB. As stated by Bernhardt *et al*. (2015), dried basil leaves contain isoborneol compounds as much as 0.10% to 0.27%.

The odour compounds identified in the present work, geosmin and MIB, were respectively described as earthy and musty (Mahmoud and Buettner, 2017). Though *spirulina* has been soaked in basil leaf extract, the concentrations of geosmin and MIB in *spirulina* found were still higher than what were found in *Mycrocystis aeruginosa*, where geosmin ranged from 308.00 ng/L to 795.00 ng/L, and MIB ranged from 22.16 ng/L to 54.00 ng/L (Zhang *et al*., 2013). If put into context, humans are able to detect earthy or musty taste and odour at very low concentrations of 10 and 30 ng/L, respectively (Olsen *et al*., 2016).

Sensory characteristics of S. platensis

Table 2 shows that varying ratios of *spirulina* to basil leaf extract had no significant effects ($p > 0.05$) on the appearance of *spirulina* but had significant impact ($p < 0.05$) on the texture and odour of *spirulina*. The increase in the amount of basil leaf extract used could increase the hedonic scale of *spirulina*, especially odour. Sensory evaluation of all samples showed correlations with its geosmin content. Mahmoud and Buettner (2017) stated that odorants detected as potent contributors in the aroma of fish meat correlates with sensory evaluation. In addition, basil contains natural compounds that are capable of removing off-odours. The two most highlighted compounds being referred here are geraniol (34.89%) and citral (25.31%) (Saha *et al*., 2012).

Table 2. Sensory characteristics of Spirulina platensis.

Ratio of spirulina to basil leaf extract	Appearance	Texture	Odour
Control	7.40 ± 1.45[a]	6.60 ± 1.45[a]	4.06 ± 1.45[a]
1:3	7.46 ± 1.45[a]	6.67 ± 1.39[a]	4.47 ± 1.57[a]
1:4	7.40 ± 1.22[a]	6.80 ± 1.42[ab]	6.07 ± 1.14[b]
1:5	7.67 ± 1.09[a]	7.47 ± 1.25[b]	6.07 ± 1.14[b]

Different superscripts on the same column indicate significant difference.

Table 3. Amino acids profile in Spirulina platensis.

Type of amino acid	Amino acid content (ppm)			
	Ratio of spirulina to basil leaf extract			
	Control	1:3	1:4	1:5
Aspartic acid	46,053.43	47,699.34	47,821.11	44,940.08
Glutamic acid	68,361.74	77,706.53	77,578.06	73,714.20
Serine	22,219.62	28,576.96	29,809.61	30,277.47
Glycine	24,154.04	33,460.65	33,410.53	31,728.92
Histidine	81,65.22	12,413.02	12,051.74	11,531.09
Arginine	36,744.15	44,376.08	41,208.99	37,298.43
Threonine	26,459.57	32,896.97	31,698.10	30,964.71
Alanine	37,147.35	42,395.25	41,926.30	40,946.82
Proline	20,527.52	20,694.33	19,950.32	19,911.57
Valine	33,705.82	35,607.12	35,315.69	34,861.24
Isoleucine	30,815.05	32,330.41	32,190.50	31,579.26
Leucine	48,222.84	51,093.39	50,383.49	49,213.80
Phenylalanine	26,498.71	39,387.40	38,268.50	35,418.73
Lysine	29,368.39	27,519.01	27,397.47	26,823.09
Tyrosine	20,747.95	32,654.72	30,689.23	28,821.62

Amino acids profile

With the exception of serine, there was a decrease in the amino acid content of *spirulina* with increasing volume of basil leaf extract used (Table 3). However, when compared to the control sample, *spirulina* soaked in basil leaf extract showed an increase in amino acids ranging from 3.57% to 57.39%, excluding aspartic acid, proline and lysine. The highest amino acid content in *spirulina* found was glutamate which is associated with the taste of umami and also causes fishy odour on *spirulina*. Glutamic acid content in the treatment ratio of 1:3 amounted to 77,706.53 ppm and decreased by 5.13% at 1:5 treatment (73,714.20 ppm). This could be due to the basil leaf extract which is able to dissolve organic components of *spirulina*. Basil leaves contain essential oils that can function as antioxidant, antibacterial, fungistatic and insecticidal agents. Monoterpenes and phenylpropanoids are the prevalent groups present in these essential oils (Nakamura *et al*., 2009; Carović-Stanko *et al*., 2010; Varga *et al*., 2017).

Leucine is the second highest amino acid contained in *spirulina*. Similar result was found by Dewi *et al*. (2016), where the amino acid concentration of leucine was second to glutamate. *Spirulina* proteins can undergo denaturation in a treatment or process with a temperature of > 60°C. In the present work, the *spirulina* paste was oven-dried at 40°C. Agustini *et al*. (2015) states that *spirulina* processing incorporating high temperature ranging from 40 - 50°C does not give any significant effect on its nutritional quality. Hence, the process conducted in the present work was not likely to decrease the amino acid content.

Fatty acid profile

As can be seen in Table 4, methyl palmitate yielded the highest concentration as compared to other fatty acid compounds in *S. platensis*. This indicates that *S. platensis* has a high saturation level and thus can easily experience oxidation. However, soaking *spirulina* in basil leaf extract at higher concentrations could reduce the levels of methyl palmitate and methyl palmitoleate which are saturated fatty acids. At a ratio of 1:4, basil leaf extract reduced methyl palmitate by 9.49% and methyl palmitoleate by 13.35%; while at a ratio of 1:5, basil leaf extract reduced methyl palmitate by 33.70% and methyl palmitoleate by 42.65%. The reduction observed could be the consequence of the presence of essential oils in basil leaf extract that dissolved saturated fatty acids in *spirulina*.

The results also showed that soaking *spirulina* in basil leaf extract generated the presence of methyl undecanoate, linolelaidic acid methyl ester, gamma-linolenic acid methyl ester, and all cis-4,7,10,13,16,19-docosahexaenoate which were not present in the control. In addition, soaking *spirulina* in basil leaf extract with a higher concentration increased the content of methyl linolenat from 90.13 ppm to 6,491.08 ppm and methyl cis-5,8,11,14,17-eicosapentanoate from 160.24 ppm to 7,392.69 ppm. Basil has high antioxidant activities due to its flavonoid and phenolic compounds (Carro *et al*., 2013). The oxidative deterioration of muscle lipid is responsible for the deterioration of nutritional and sensory properties of meat and meat products (Faustman *et al*., 2010).

Table 4. Fatty acids profile in Spirulina platensis.

Type of fatty acid	Fatty acid content (ppm)			
	Ratio of spirulina to basil leaf extract			
	Control	1:3	1:4	1:5
Methyl undecanoate	n.d.	34,255.59	27,350.61	2,2151.74
Methyl palmitate	606,510.11	676,331.39	548,951.99	402,085.82
Methyl palmitoleate	32,140.02	36,268.34	27,847.06	18,431.59
Linolelaidic acid methyl ester	n.d.	7,585.17	98,796.50	230,557.34
Gamma-linolenic acid methyl ester	n.d.	225,791.96	225,791.96	93,879.44
Methyl linoleate	16,570.22	16,367.26	15,387.69	3,047.97
Methyl linolenat	90.13	2,355.54	4,573.96	6,491.08
Methyl cis-5,8,11,14,17-Eicosapentanoate	160.24	2,766.49	5,723.82	7,392.69
All cis-4,7,10,13,16,19-Docosahexaenoate	n.d.	n.d.	n.d.	5,637.75

n.d. = not detected.

Phycocyanin content

The phycocyanin content among all treatments showed a significant difference ($p < 0.05$), where the higher basil leaf extract added, the higher the phycocyanin content of *spirulina* (Table 1). The phycocyanin content amounted to 0.047%. This implies that the increase in the level of phycocyanin in correlation with the amount of basil leaf extract used could be due to the presence of phycocyanin in the extract. The highest phycocyanin content was found in *spirulina* with soaking treatment in basil leaf extract at a ratio of 1:5 (4.07%). The essential oils in basil leaf extract can also protect the organic compounds in *spirulina* during the heating process. The results obtained were comparatively lower than the results found by Setyoningrum and Azimatun (2015), where the content of phycocyanin in *S. platensis* ranged from 6.92% to 17.2%.

Table 1. Geosmin, MIB, and phycocyanin contents in Spirulina platensis.

Spirulina to basil leaf extract ratio	Geosmin content (%)	MIB content (%)	Phycocyanin content (%)
Control	0.23	0.21	1.74 ± 0.56^{a}
1:3	0.23	0.19	3.83 ± 0.16^{b}
1:4	0.11	0.38	3.91 ± 0.00^{b}
1:5	ND	0.45	4.07 ± 0.03^{b}

The phycocyanin content is expressed as the average of triplicate ($n = 3$) ± standard deviation. MIB: 2-methylisoborneol. Different superscripts on the same column indicate significant difference ($p < 0.05$).

Conclusion

Based on the results of the present work, it can be concluded that, in general, an increase in the amount of basil leaf extract used demonstrated a positive effect, namely a decrease in the content of geosmin and saturated fatty acids and an increase in hedonic scale, unsaturated fatty acids, and phycocyanin content in *spirulina*. But this is not the case with some amino acids which actually decreased with increasing amounts of basil leaf extract.

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