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FULL PAPER

Evaluation of the volatile compound basil leaf (*Ocimum basilicum*) intervention on *Spirulina platensis*

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Abstract

Microalgae *Spirulina platensis* contains high protein which can be used as functional food, but it has several undesirable volatile compounds. *Ocimum basilicum* contains various essential oils from the monoterpene class that can be used to reduce volatile compounds causing an off-odour in *Spirulina platensis*. This study was conducted to evaluate the intervention *of O. basilicum* on nature and microencapsulated *S. platensis* powder after *O. basilicum* treatment against the volatile compounds. The research consisted of two intervention models. *S. platensis* powder soaked with basil extract (1:4, w/v) and then dried by freeze-drying method without microencapsulation (DSB). The *S. platensis* powder was soaked in basil extract (1:4, w/v) then dried by freeze-drying method and microencapsulated (MSB) after. *Spirulina platensis* powder was used as a control (SP). A solid-phase microextraction was used with the combination of GC-MS to identify volatile compounds. The results showed that the main volatile compounds in *Spirulina* are medium-chain alkanes. Other compounds that contributed to this off-odour are geosmin (0.02%), 2-Methylisoborneol (0.28%), β-ionone (4.94%). The intervention of *O. basilicum* in *S. platensis* resulted in a decrease in these volatile compounds. Geosmin was not detected by intervention with *O. basilicum* either by microencapsulation (MSB) or without microencapsulation (DSB). Therefore, the intervention of *O. basilicum* can reduce off-odour in *S. platensis* and can be applied as a functional food.

1. Introduction

Spirulina platensis is a biomass of cyanobacteria as a single cell protein producer which is rich in protein, essential vitamin and minerals, making it very appropriate to be used as a food source (Jung *et al.,* 2019; Kameshwari *et al.,* 2020). The amino acids composition *Spirulina* protein is among the best, exceeding the amino acids in soybeans, useful as a protein supplement in the treatment of malnutrition in children (Saranjaj and Sivasakthi, 2014). *Spirulina platensis* contains bioactive compounds such as phenols, phycocyanin and polysaccharide which have bioactive as antioxidants, anti-inflammation and immunostimulants. The use of *Spirulina* as a functional food provides various human health benefits (Finamor *et al.,* 2017). However, *Spirulina* added to food can reduce sensory characteristics in the presence of an off-odour (Agustini *et al.*, 2019).

The most common volatile compounds produced by cyanobacteria are geosmin and 2-methylisoborneol which causes a muddy-earthy-musty type flavour. Apart from these compounds, medium-chain alkane, β-ionone, β-cyclocitral are the main off-odour compounds in *Spirulina* (Suurnakki *et al.,* 2015; Milovanović *et al.,* 2015). Geosmin and 2-Methylisoborneol have very low human sensory limits, they are disliked by consumers even at low concentrations (Lindholm-Lehto *et al.,* 2019).

Ocimum basilicum is an aromatic plant that produces essential oils with a distinctive mint aroma. Essential oil is used in providing flavour and aroma to food to increase consumer acceptance of a product (Shaaban *et al.*, 2012; Trivedi *et al.*, 2014). Aromatic plants contain essential oils that play a specific role in aromas, such as chavicol, linalool and eugenol. The compounds are used in the food industry to reduce off-odours and act as an antioxidant (Kiferle *et al.*, 2011; Barros *et al.*, 2013;

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Koroch *et al*., 2017). The instability of essential oil in *O. basilicum* characterised by its susceptibility to oxygen, temperature and light whereby it requires microencapsulation to maintain its aroma and bioactivity (Majeed *et al*., 2015).

Microencapsulation can be used to protect active compounds during the processing and storage stages. This technique has been widely used to mask taste and aroma to enhance the organoleptic properties of a product. Microencapsulation is also used in a significant reduction in aromatic odours considering it as a more user-friendly product when dealing with an unpleasant odour (Gondal *et al.,* 2017). Limited information on the intervention of a substance against volatile compounds to increase *Spirulina* consumption is available. Several studies on *Spirulina* to date were used as nutraceuticals and pharmaceuticals (Sowjanya and Manjula, 2016). However, the aroma and taste of *S. platensis* have not been studied further. The objective of this study was to evaluate the intervention of *O. basilicum* extract on *Spirulina* powder and microencapsulation treatment against volatile compounds.

2. Materials and methods

2.1 Materials

Spirulina powder was obtained from Brackishwater Aquaculture Fisheries (BBPBAP) Jepara (Central Java, Indonesia) in 2019. *O. basilicum* was purchased from a traditional market in Semarang, Central Java. This study covers treatments of *Spirulina platensis* powder that was not soaked in *O. basilicum* as a control sample (SP), *S.platensis* powder soaked with basil extract (1:4, w/v) then dried by freeze-drying method without microencapsulation (DSB) and *S. platensis* powder soaked with basil extract (1:4, w/v) then dried by freeze-drying method and then microencapsulated (MSB). *Spirulina* powder was soaked with *O. basilicum* leaf extract (1:4, w/v) and microencapsulation was conducted using maltodextrin (CV. Multi Kimia Raya, Semarang, Indonesia) combined with gelatin (Xian, Biof Bio-Technology, Cina) with the concentration of 9:1 (w/w). The drying process used was the freeze-drying method (Heto Powerdry LL 1500, Germany) with a temperature of -100°C for 48 hours. Sample preparation for the intervention of *O. basilicum* in *Spirulina* was conducted as previously described by Handiani *et al*. (2019).

2.2 Solid-phase micro extraction analysis

Solid-phase micro extraction (SPME) analysis was carried out with 30 mins exposure to a 2 cm DVB/ CAR/ PDMS fibre followed by analyte desorption at 220°C for 3 mins. Briefly, 3.5 g of *S. platensis* powder in 22 mL SPME bottles are heated to 80°C. 10 ppm of methanol (Sigma-Aldrich, Saint Louis, MO, USA) was added as an internal standard. Sample preparation stages such as extraction, purification, and concentration are combined into one step and one device that was directly connected to the Gas Chromatography Mass Spectrophotometer (Hulburt *et al.,* 2009).

2.3 GC-MS analysis

The samples were run on a GC Agilent 7890A and MS 5975C with a Triple Axis detector. A 30 m x 250 µm i.d. capillary column HP-5MS (J and W Scientific, folsom, USA) with 0.25 µm stationary phase was used. Helium was used as a carrier gas at a flow rate of 1.8 mL/min. The oven was initially held for 1 min at 50°C, then gradually increased by 3°C/min to 120°C for 1 min. Then, at a temperature of 5°C/min to 220°C which was retained for 0 min. The ions of volatile compounds in *S. platensis* were observed over a period of 100 µs. The quantity of each volatile compound from *Spirulina* and intervention *O. basilicum* in powder and *Spirulina* microencapsulation were evaluated.

2.4 Identification and quantification of volatile compounds

Identification of all volatile compounds obtained SPME analysis on the comparison of experimentally obtained compounds mass spectra with mass spectra available in NIST14 database. The quantification analysis was performed using Advanced Chemistry Development through the integration of the peak area of the chromatograms.

3. Results and discussion

The chromatographic profile was investigated to identify peaks of major and minor volatile compounds in each sample as shown in Figures 1 - 3. The result obtained from the determination of volatile compounds from the intervention of *O. basilicum* in *Spirulina* powder and microencapsulation was shown as % chromatographic peaks of the total ion chromatogram (Table 1). The result showed that medium-chain alkanes were the main component of the volatile compounds in *Spirulina*. It has been proven that cyanobacteria have the capacity to produce natural hydrocarbon from fatty acids (Coates *et al.,* 2014). The enzyme responsible for alkane production in cyanobacteria are acyl-acyl carrier protein (Acyl-ACP) reductase (AAR) and aldehyde-deformulating oxygenase (ADO). These two enzymes work in series and in a complex manner which efficiently converts long-chain fatty acyl ACP or fatty acyl CoA into hydrocarbons (Gao *et al.,* 2020). Pentadecane, hexadecane and heptadecane are the main hydrocarbon compounds in cyanobacteria (Milovanović

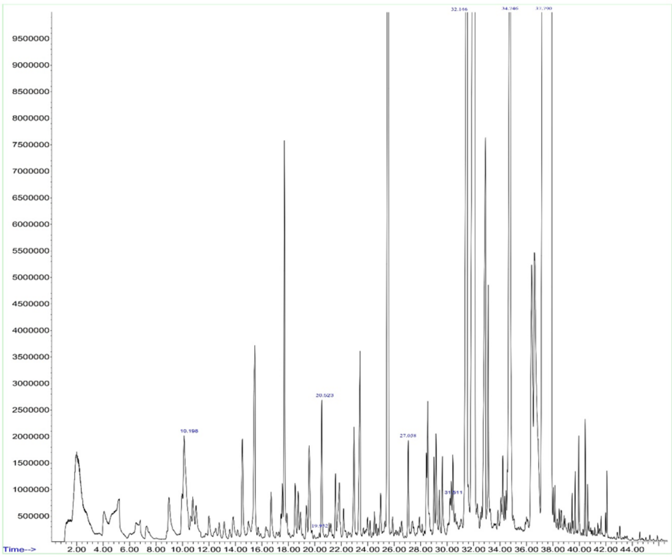

Figure 1. Chromatogram of the volatile compounds of *Spirulina platensis* (SP)

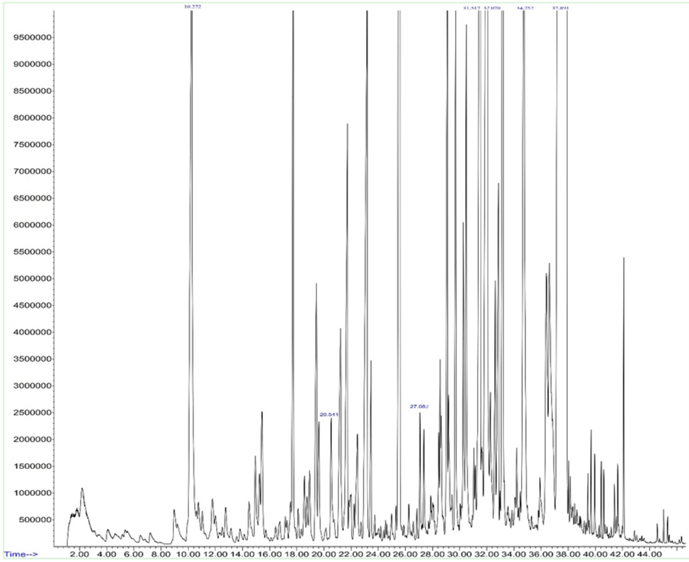

Figure 2. Chromatogram of the volatile compounds of DSB sample

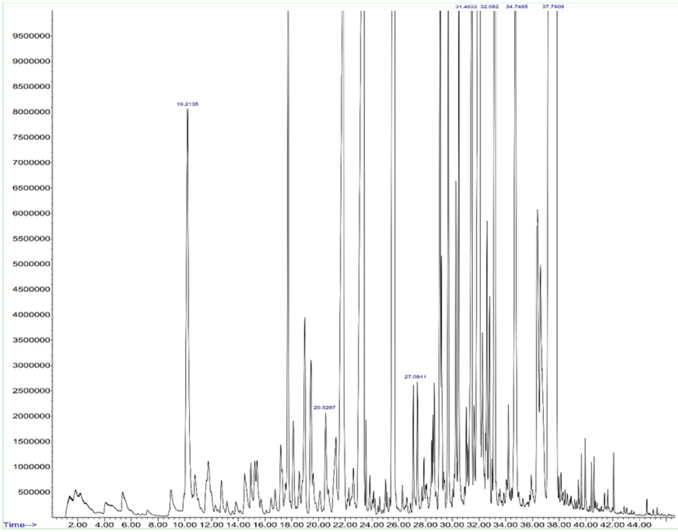

Figure 3. Chromatogram of the volatile compounds of MSB sample

Table 1. Volatile compounds detected in the samples (SP, DSB and MSB)

Volatile compounds	SP		DSB		MSB	
	RT	% area	RT	% area	RT	% area
2-Pentylfuran	10.198	0.31	10.2728	2.07	10.2135	1.54
Geosmin	19.9529	0.02		-		-
β-cyclocitral	20.5237	0.41	20.5415	0.43	20.5297	0.43
2-Methylisoborneol	27.0583	0.28	27.0821	0.29	27.0941	0.28
β-ionone	31.5119	4.94	31.5177	4.27	31.4822	3.01
Pentadecane	32.1065	5.92	32.0707	4.3	32.0827	4.45
Hexadecane	34.7465	4.57	34.7523	3.64	34.7465	3.41
Heptadecane	37.7909	61.15	37.8918	49.92	37.7909	47.28

et al., 2015).

The observation of SPME in the extraction of volatile compounds plays an important role in the formation of the distinctive aroma of *Spirulina* intervention of *O. bacilicum*, it can further be used for flavour analysis. The most important cause of the unpleasant taste and odour in *Spirulina* are geosmin and 2-methylisoborneol which are detected by humans in small concentration levels. The odour threshold for geosmin is 15 ng/L and 2-methylisoborneol is 35 ng/L (Juttner and Wasten, 2007). However, many people detect geosmin and 2-methylisoborneol at lower concentration levels. *Spirulina* in this study naturally produces low concentration of geosmin, 2-methylisoborneol, 2-pentylfuran, β-cyclocitral and β-ionone (Table 1). 2-pentylfuran is a volatile compound found in cyanobacteria (Milovanović *et al.,* 2015). This compound is formed through the auto-oxidation of linoleic acid, the high amount of 2-Pentylfuran is in line

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with the high content of linoleic acid (Krishnamurthy *et al.,* 1967; Xu *et al.,* 2017). The conjugated diene radicals produced from the radical cleavage of 9-hydroxy linoleate can react with oxygen to produce vinyl hydroperoxide, which will then undergo cyclization by alkoxy radicals form 2-pentylfuran (Frankel, 1983).

Ocimum basilicum intervention increased β-cyclocitral content in *Spirulina*, because *O. basilicum* contains citral compounds (da Costa *et al.,* 2014). β-cyclocitral is a volatile compound that displays lytic activity on cyanobacteria. In particular, β-cyclocitral causes an attractive colour change from green to blue during the lysis process. β-cyclocitral provides distinctive properties with maximum absorption, thereby rapidly breaking down chlorophyll a and β-carotene (Harada *et al.,* 2009). The threshold for β-cyclocitral volatile compounds is 750 nM. β-cyclocitral was not detected in live Mycrocystic cells, it was present only at very low concentration of 2.6 amol/cell. Conversely, the rapid cell rupture activates the carotene oxygenase reaction, resulting in a high β-cyclocitral number, around 77±5.5 amol/cell calculated according to the maximum intracellular concentration of 2.2 mM (Juttner *et al.,* 2010). Similar to β-cyclocitral, β-ionone is one of the volatile compounds produced through the degradation of β-caroten cyanobacteria (Garcia-Plazaola *et al.,* 2017).

Ocimum basilicum leaf extract can reduce geosmin compounds in powder and microencapsulation of *Spirulina*. This is because the essential oil content in *O. basilicum* can dissolve geosmin compounds. Essential oil from grape seeds efficiently decreases the concentration of geosmin (Lisanti *et al.,* 2014). Whereas, 2-Methylisoborneol cannot be reduced by the essential oil in *O. bacilicum*. This is due to the isoborneol content found in its essential oils (Joshi, 2013), therefore the 2-methylisoborneol content increases in *S. platensis* with *O. basilicum* intervention. Microencapsulation is carried out to maintain the pleasant aroma created by the intervention of *O. basilicum* in *S. platensis*, therefore increasing its consumption and application in functional food as well as for nutraceutical and pharmaceutical products. da Silva *et al*. (2014) explained that microencapsulation is a technology used in the food industry, one of which is to maintain the aroma of the product. Microencapsulation maintains the stability of aroma or taste during the processing and storage stage to minimize degradation or loss of aroma. In addition, it can improve the quality of the finished product and increase customer satisfaction (Gupta *et al.,* 2016). The aroma encapsulation of essential oils also allows for controlled release (Maes *et al.,* 2019).

The intervention of *O. basilicum* in *Spirulina* powder without microencapsulation (DSB) or microencapsulation (MSB) produced a new volatile compound which results in a new aroma. Vittal *et al.* (2019) describe the extraction of petrichor and essential oils for the production of new fragrance because they produce a pleasant odour from the linalool compound (Elsharif *et al.,* 2015). Stanojevic *et al*. (2018), explained that *O. basilicum* contains 65 active compounds and the highest content are 31.6% linalool and 23.8% methyl chavicol. Toncer *et al*. (2017) explained further that when plants experience flower growth the content of monoterpenes such as linalool increases and decreases along with the completion of the flowering period. The volatile compounds have their respective aroma descriptions. The combination of volatile compounds will cause a distinctive flavour. Essential oils are volatile liquid aroma components that are usually obtained from plants (Hamid *et al.,* 2011).

4. Conclusion

To conclude, the essential oil in *O. basilicum* reduces volatile compounds such as geosmin, β-cyclocitral, β-ionone which causes an off-odour in the powder and *Spirulina* microcapsule. Linalool is a monoterpene compound useful for its purpose in flavour. Therefore, the intervention of *O. basilicum* in *S. platensis* can be applied as a functional food as well as a nutraceutical and pharmaceutical product.

Conflict of interest

The authors declare no conflict of interest.

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