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Off-odour Identification from Volatile Organic Compounds (VOCs) of *Spirulina*

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Abstract. *Spirulina platensis* is a common cyanobacteria microalga with high nutrition and bioactive compound sources. The addition of spirulina in foods and beverages improves nutrition and bioactive compound content. However, certain species of cyanobacteria are known to produce various compounds causing off-odour. This study investigates the chemical profile and volatile organic compounds (VOCs) in spirulina biomass and determine off-odour potency. The spirulina extract was analysed phytochemical qualitatively and GC-MS (Gas Chromatography-Mass Spectrometry). The Spectra mass was compared to the mass spectral database and profile of chemical compound libraries. The result shows, phytochemical analysis positively contains of alkaloids, flavonoids, tannins, saponins, and terpenoids. A total of 155 volatile compounds consisting of classes acid, alcohol, aldehyde, alkene, benzene, ether, ester, ketone, sulphur-contain, and terpene were identified. The off-odour VOC content such as phytol; cyclopropanebutanoic acid, 2- [[2 - [[2 - [(2- pentylcyclopropyl) methyl) cyclopropyl) methyl) cyclopropyl) methyl]-, methyl ester; 3.7.11.15-tetramethyl-2-hexadecen-1-ol; Imidazole, 2-fluoro-5-(2-carboxyvinyl)-; β-ionone; and N,N-Dimethyl-O-(1-methyl-butyl)-hydroxylamine, were detected in spirulina. The odour descriptions of off-odour VOCs are floral, balsamic, powdery, waxy, rancid, sweaty, woody, alkali, and fish-like. The off-odour content of VOCs might influence food's sensory odour, with spirulina added in excessive quantities.

1 Introduction

Spirulina platensis is a common cyanobacteria microalga with high nutrition and bioactive compound sources. Spirulina is substantial for commercially produced high-value molecules, including carotenoids, long-chain polyunsaturated fatty acids (PUFA), proteins, and pigment [1]. The addition of spirulina has been studied and applied in a variety of food products,

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including pasta, snack bars, dark cholate, cookies and beverages [1,2,3,4,5]. The additions spirulina improve the nutrient content, carotenoid [1,2,3], and total phenol [4,5].

Besides the beneficial properties, spirulina also produces numerous volatile compounds, which can cause an unpleasant odour (off-odour) [6]. When microalgal biomass is added to food products, its odour may influence the sensory properties of the final products. However, increasing the addition of spirulina in food products can improve the contain of bioactive compounds and antioxidant capacity but decrease the panellist acceptance [4,5].

The consumer acceptance and purchase intention of a food product will be primarily affected by odour and flavour. The fortified dark chocolate with spirulina has consumer preferences with lowest value (55.8%) [7]. The purchase intention consumers of the cookies fortified by spirulina have lower values than formulation without additioning spirulina [4]. The fishy odour of these volatile components reduces consumer acceptance and inhibits the development of edible algae [1,8].

Volatile organic compounds (VOCs) are chemical substances in nature and plant cultivation. Spirulina, a species of blue-green algae, is one of the most VOC-rich substances. There are 50 different types of spirulina VOCs components that have been discovered, and some of them are responsible for the authentic aroma such as β-ionone, heptadecane and dihydrvactinodiolide [9]. The environmental and taxonomical factors determine the composition of chemical content and VOC in microalgae biomass [10]. The environmental factor of cultivation such as, temperature, salinity, illumination, pH, nutrient [10] and cultivation methods [11].

Composition of VOC in substances detection by Gas Chromatography (GC) separation techniques [9,10]. Gas Chromatography (GC) is a technique for separating chemicals found in a sample. The retention time (RT) is the time it takes for a certain compound to transit the length of the column and is the defining property [12]. A literature search showed a few information about composition of volatile compound in spirulina and their profiles and potency of off-odour.

The antioxidant capacity of spirulina in functional foods increases with the amount added, nevertheless it has low sensory acceptability scales [4,5]. Deodorization is a way to increase spirulina capacity without reducing sensory acceptability of odour. Deodorization spirulina with masking technique can removes odour, but also protects colour and maintains the function of spirulina [8]. The characterization of volatile component and determine their chemical odour profiles of spirulina was the main objective of this study. Information about the profile and odour of VOC components and it is necessary to consider actions such as deodorization or masking when using increased amounts of spirulina in food.

2 Materials and methods

2.1 Extraction and phytochemical analysis

Spirulina platensis powder purchase from PT. Algaepark Indonesia Mandiri, Klaten Indonesia. Spirulina powder was macerated with 1:10 (w/v) 70% ethanol solvent and incubated for 24 hours using a shaking incubator at 125 rpm. Maceration is repeated until filtrate produces a colour close to clear. Maceration is filtered and evaporated using a rotary evaporator at 50°C until a dry paste is formed. Phytochemical screening for the presence of the following; alkaloids, flavonoid, saponin, triterpenoid, steroid and tannins were carried out following methods previously described [11].

2.2GC-MS analysis and identification of compound

Ethanolic extract of spirulina filtered using a 0.45 µm PTFE filter syringe into a 2 mL tube. The filtrate was put into a 2 mL amber GC vial, and split injected into the GCMS/MS (Agilent 7890B). The Agilent HP-5MS column used was 30 min length, 0.25mm in diameter, 0.25 µm thick and helium carrier gas. The column oven temperature 100°C, temperature injection was 230°C and read in MS (Agilent 7000). The identification of the compounds was based on comparison of their retention times (RT), and mass spectra with database NIST/EPA/NIH libraries spectra and literature.

2.3Determine odour of VOCs

The highest peak area and retention time of VOCs is used to identify chemical profiles and odour based on literature, and libraries databases. The database of chemical profiles and odour descriptor are accessed at the online platform www.thegoodscentscompany.com and www.smolecule.com [12].

3 Result

Table 1. Phytochemical analysis of the ethanolic extract *Spirulina platensis*

Bioactive compound	Inference
Alkaloid	+
Flavonoid	+
Tannin	+
Saponin	+
Triterpenoid	+

Ethanolic extract of *Spirulina platensis* positively contains of alkaloid, flavonoid, tannin, triterpenoid, and saponin as bioactive compound (Table 1.). Bioreactor cultivation of *spirulina platensis* has phytochemical compound was flavonoids, phenols, steroids and saponins [11]. Different cultivation method and environmental factor has a more varied of chemical component [10]. The GC-MS analysis layout (chromatogram) of spirulina extract can be seen in Figure 1.

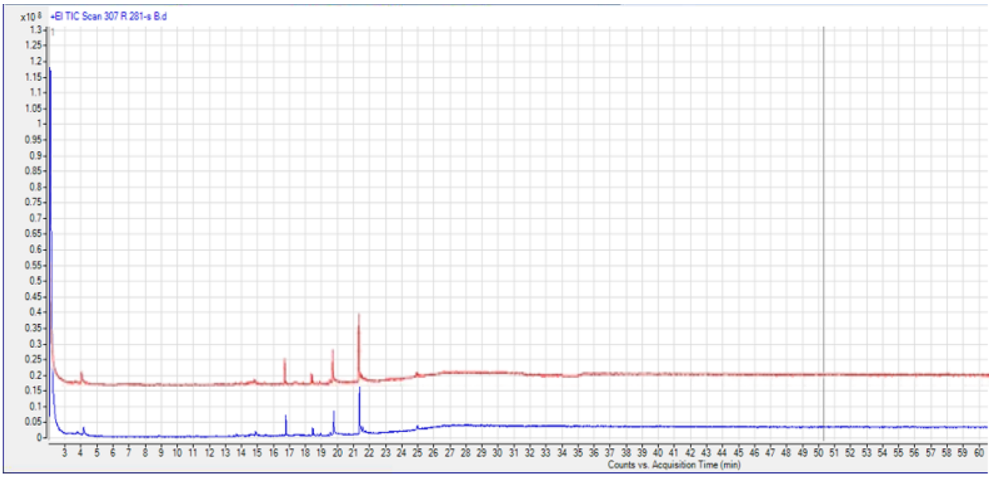

Fig. 1. The chromatogram layout of *Spirulina platensis* extract

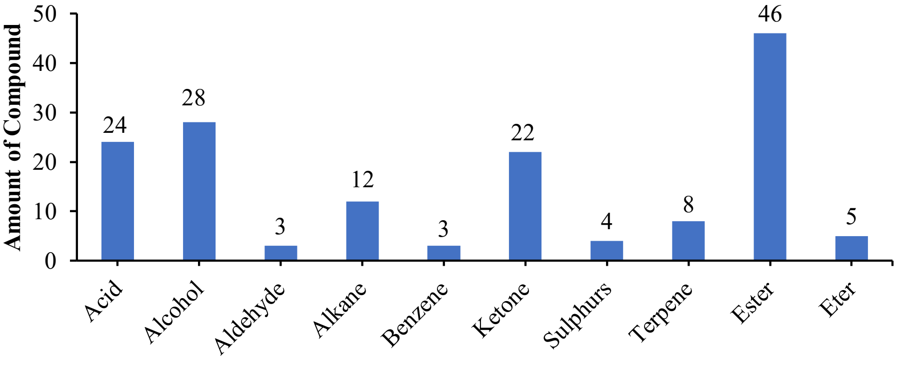

Fig. 2. The volatile organic compounds (VOCs) of *Spirulina platensis*

A total of 155 VOC of spirulina has been identified using GC-MS analysis in acid (24 compounds), aldehyde (3), alcohol (28), alkene (12), benzene (3), ether (5), ester (46), ketone (22), sulphur-contain (4), and terpene classes (8) (Figure 2). Ester is a significant class with 46 of the total amounts of VOC. The VOCs from ester class is cyclopropanebutanoic acid, 2-[[2-[[2-[(2-pentylcyclopropyl)methyl)cyclopropyl)methyl)cyclopropyl)methyl]-, methyl ester (Table 2). Peak area of ester was small in individual compound of each VOCs. The recent study reported esters are one of the major functional groups present in the structures of prodrugs and bioactive compound of plant [13]. Spirulina is microalga that rich of fatty acids, proteins and another beneficial component. Alcohol in microalgae is formed by secondary decomposition of hydroperoxides fatty acid [10]. The main VOCs from alcohol class are 1,2,3-propanetriol, 3.7.11.15-Tetramethyl-2-hexadecen-1-ol, 3,7,11-Trimethyl-1-dodecanol, Ethanol, 2-(9,12-octadecadienyloxy), (ZZ)-, and phytol (Table 2).

The main volatile organic compounds (VOCs) present as indicated by the highest peaks (%) shown in the chromatogram layout (Figure 1) which identifies compounds of spirulina using GC-MS instruments. The main VOCs are phytol; palmitic acid; Glycerin; 1-Monolinoleoylglycerol trimethylsilyl ether, and 3.7.11.15-tetramethyl-2-hexadecen-1-ol.

Table 2. The profiles of volatile organic compounds (VOCs) *Spirulina platensis*

Volatile Organic Compounds	Classes	Formula	Retention time (min)	Peak %	Odor descriptor
1,2,3-propanetriol (Glycerin)	Alcohol	$C_3H_8O_3$	4.171	56.5	Mild, Odorless[a]
N,N-Dimethyl-O-(1-methyl-butyl)-hydroxylamine	Ether	$C_7H_{17}NO$	4.171	15.7	Fish-like [14]
Imidazole, 2-fluoro-5-(2-carboxyvinyl)-	Acid	$C_6H_5FN_2O_2$	8.863	22.9	Alkali [15]
3-Buten-2-one, 4-(2,6,6-trimethyl-1-cyclohexen-1-yl)- (β-ionone)	Ketone	$C_{15}H_{24}$	14.092	17	Woody and Floral [16]
2(4H)-Benzofuranone, 5,6,7,7a-tetrahydro-4,4,7a-trimethyl-, (R)-	Ketone	$C_{11}H_{16}O_2$	14.906	19.9	Sweet, fruity, and woody[a]
Heptadecane	Alkane	$C_{17}H_{36}$	16.780	14.4	Odorless[a]
3.7.11.15-Tetramethyl-2-hexadecen-1-ol	Alcohol	$C_{20}H_{40}O$	18.446	31.9	Floral balsamic powdery waxy [b]
3,7,11-Trimethyl-1-dodecanol	Alcohol	$C_{15}H_{32}O$	18.546	24.1	Faint odor[a]
13-Heptadecyn-1-ol	Alkane	$C_{17}H_{32}O$	18.730	14.8	Fatty, mushrom, roasted, grassy[b] [14]
Ethanol, 2-(9,12-octadecadienyloxy), (ZZ)-	Alcohol	$C_{20}H_{38}O_2$	18.945	8.84	alcoholic, medic [19]
Cyclopropanebutanoic acid, 2-[[2-[[2-[(2-pentylcyclopropyl) methyl)cyclopropyl)methyl) cyclopropyl)methyl]-, methyl ester	Ester	$C_{25}H_{42}O_2$	19.398	29.3	Rancid, sweaty [20]
Palmitoleic acid	Acid	$C_{16}H_{30}O_2$	19.567	12.3	Greasy and grassy [21]
n-Hexadecanoic acid/Palmitic acid	Acid	$C_{16}H_{32}O_2$	19.767	58.6	Odorless[a]
Phytol	Alcohol	$C_{20}H_{40}O$	21.372	65.5	Floral balsamic powdery waxy[b]
17-Octadecynoic acid	Acid	$C_{18}H_{32}O_4$	21.571	3.87	Sweaty/waxy [22]
1-Monolinoleoylglycerol trimethylsilyl ether	Ether	$C_{27}H_{54}O_4Si_2$	24.989	33.3	Fermented acid [23]

[a]Odour descriptor from: https://www.smolecule.com/html.
[b]Odour descriptor from: http://www.thegoodscentscompany.com/data/rw1040391.html

The volatile organic compounds (VOCs) produced by algae vary significantly depending on several factors, such as algae growth stage, species, light, salinity, carbon and nitrogen sources [25]. The production and emission of VOCs has corelation with defensive algal cells resisting ROS under abiotic stresses in environment, allelopathic agent to inhibit another competitor, and defensive against predator [26]. Nitrogen source in growth medium was shown to negatively affect the production of 2-methylisoborneol (MIB) and geosmin [6]. The (MIB) and geosmin was not detected or less peak area is that mean the probability to separate this compound in the extract is lowest.

The odourants present in microalgae belong essentially to alcohols (1,5-octadien-3-ol and trans-2-octen-1-ol), aldehydes (2,4-decadienal, hexanal, octanal, 2-octenal, trans-2,cis-6-nonadienal, and nonanal), terpenes (α-ionone and β-ionone), and ketone (3,5-octadien-2-one) groups [27]. However, not all odorant in VOCs has a negative impact (off-odour) in sensory assessment. The off-odour of VOCs were phytol; 3.7.11.15-tetramethyl-2-hexadecen-1-ol; cyclopropanebutanoic acid, 2- [[2- [[2- [(2- pentylcyclopropyl) methyl) cyclopropyl) methyl) cyclopropyl) methyl]-, methyl ester; Imidazole, 2-fluoro-5-(2-carboxyvinyl)-; and 3-Buten-2-one, 4-(2,6,6-trimethyl-1-cyclohexen-1-yl)- (β ionone). The odour descriptor of each off-odour VOCs is floral, balsamic, powdery, waxy [11]; rancid, sweaty [13]; and woody [22] alkali [15], and fish-like [14] (Tabel 2). The β-ionone is one of off-odour compound of spirulina that has responsible in aroma acceptance [9].

4 Discussion

In this study phytochemical contain positively contains of triterpenoids or terpene, alkaloids, flavonoids, saponins, and tannins (Table 1). In the other hand study reported *Spirulina platensis* from bioreactor cultivation has phytochemical bioactive compound was; flavonoids, phenols, steroids and saponins [11]. The presence of bioactive terpenes in the volatile components of plants, therefore, validated its therapeutic importance in the treatment of skin-related diseases [17,24]. A total 155volatile compounds spirulina identified as acid, alcohol, aldehyde, alkene, benzene, ether, ester, ketone, sulfur-contain, and terpene classes (Fig. 2). *Haematoccocus pluvialis* powder identified a total of 401 VOCs as esters, ketone, and aldehydes were the major VOCs contributing to the odour [12]. Different species, culturing, and posh-harvest processing has also different odour component and concentration (VOCs) of microalgae [8].

The VOCs which have highest peak such as 1,2,3-propanetriol (Glycerin); n-Hexadecanoic acid (Palmitic acid); 2(4H)-Benzofuranone, 5,6,7,7a-tetrahydro-4,4,7a-trimethyl-, (R)- 1-Monolinoleoylglycerol trimethylsilyl ether; and 3,7,11-Trimethyl-1-dodecanol. These VOCs did not include of the off-odour compound which has descriptor: odourless, faint, acid fermented, sweet fruity, and woody [9, 23]. The off-odorous suspect of VOCs such as phytol; 3.7.11.15-tetramethyl-2-hexadecen-1-ol; cyclopropanebutanoic acid, 2- [[2- [[2- [(2- pentylcyclopropyl) methyl) cyclopropyl) methyl) cyclopropyl) methyl]-, methyl ester; imidazole, 2-fluoro-5-(2-carboxyvinyl)-; β-ionone; and N,N-Dimethyl-O-(1-methyl-butyl)-hydroxylamine. The off-odour VOCs has descriptor are floral, balsamic, powdery, waxy, rancid, sweaty, woody, alkali, and fish-like, respectively. Some cyanobacteria reported the production of VOCs, some compounds such as limonene, eucalyptol, β-cyclocitral, α-ionone, β-ionone, and geranylacetone [26]. The β-ionone and other apocarotenoid pigments are also economically important but they exhibit an extremely strong odor threshold, at 0.007 ppb [16]. Increasing of β-ionone compound, strengthens violet-like and floral odour and taste of H. *pluvialis* [12]. Phytol and its derivate, such as 3.7.11.15-Tetramethyl-2-hexadecen-1-ol is an aromatic compound that is detected on spectral FT-IR microencapsulated spirulina, indicating by containing the molecule of hydrocarbon (C-H) [18]. The odour descriptor mentions that phytol is a flavour and fragrance agent with a strong level; its high concentration makes it taste bitter [28].

Spirulina has bioactive compounds and biological activity, such as antioxidant capacity. This makes spirulina a fortified candidate for several functional foods, snacks, and beverages. Some studies reported an increasing addition of spirulina in food related to its antioxidant capacity and its containing bioactive compounds [4,5]. However, this benefit, not linearity with sensorial acceptance of food, was fortified by spirulina. The fortified cookies with the excessive addition of spirulina have increased the antioxidant capacity but not in sensorial scaling and consumers' buying intention [4]. The similar resulted in fortified spirulina in ice cream, panellists reported a distinctive odour and taste from spirulina, even with the addition mint as masking agent [5]. Identifying volatile compounds in spirulina can be considered the initial step toward improving its organoleptic qualities and further studies on fortified spirulina in food [9]. Spirulina preparation using the deodorization or masking method is a solution for high amounts of spirulina without reducing the sensory acceptance of fortified food. Deodorization with masking using oligosaccharide derivate and heating treatment can be applied for removing odour of spirulina. Deodorization with masking using oligosaccharide derivate and heating treatment can be applied to disguise spirulina's odour [8]. The excellent performance is shown by the deodorization of the spirulina heating method by adding 4 g/L of β-cyclodextrin and treating at 30 °C for 40 min [29]. This method not only disguises odour but also protects colour and maintains the function of spirulina protein.

5 Conclusion

The results showed that spirulina powders contain phytochemicals and 155 volatile organic compounds (VOCs). The main VOC spirulina content has a potency of off-odor and detected the unpleasant odor in databases and libraries. Fortified food with excessive spirulina addition might affect the sensory acceptability of odour and taste. Assessing organoleptic attributes is essential when using spirulina for food applications or developing novel and innovative foods. Further research is needed to eliminate the impact of off-odour from VOCs of spirulina. Improving strategies can be done, such as deodorization or masking before fortified spirulina in food.

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