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RESEARCH ARTICLE

The impact of fermentation duration on pH, antioxidant properties and consumer preference of *Spirulina* sp. beverage

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Abstract

Fermentation enhances the nutritional value of antioxidants in food. Fermentation breaks down the cell wall structure of the ingredients and activates enzymes that release bound compounds, such as antioxidant phenolics. This study aimed to assess the effect of fermentation time on *Spirulina* sp. regarding antioxidant activity, pH, consumer preference and low molecular weight bioactive compounds. The results showed that the best fermentation time was the first day with the highest antioxidant activity of 86.82 % a pH value of 4.76; phytochemical screening analysis indicated the presence of positive compounds from the flavonoid, alkaloid, steroid, triterpenoid, catechol and phenolic groups; total phenolic content of 147.548 µg/mL; presence of 9 essential amino acids and seven non-essential amino acids; consumer preference level of $7.22 < \mu < 7.50$; and the primary low molecular weight bioactive compound is hexadecenoic acid with an area of 55.77 %. The findings indicated that beverages fermented with *Spirulina* sp. possess the potential to serve as promising health-functional beverages, given their abundance of active compounds that are advantageous to health.

Keywords: antioxidant; consumer awareness; fermentation; phytochemical; *Spirulina* sp.

Introduction

Microalgae are one of the most promising sources of bioactive compounds and ingredients for new food products. Nutrients derived from the sea and other bioactive marine sources have excellent potential as functional food ingredients. The advantages of microalgae depend on their anticancer, antioxidant and anti-inflammatory activities (1). The compositional balance of microalgae can be utilized to enhance the nutritional value of food, making them highly valuable as functional food ingredients. Microalgae possess various lipids, carbohydrates, antioxidants and other components (2). *Spirulina* sp. is a prokaryotic microalgae that is capable of photosynthesis due to its blue pigment, phycocyanin, which serves as the primary pigment for the photosynthesis process, along with the green pigment chlorophyll A (3). This gives *Spirulina* sp. cells a blue-green colour. *Spirulina* sp. belongs to the non-heterocystous, non-branching, multicellular filamentous microalgae group and can be identified by a characteristic open left-hand helix along its filaments. The nutritional content of *Spirulina* sp. consists of approximately 60-70 % proteins, 15-25 % carbohydrates, 6-8 % fats, 7-13 % minerals, 8-10 % fibers and approximately 3% water. *Spirulina* sp. is also a natural source of provitamin A in the form of beta-carotene, with a content of approximately 23,000 IU per 10 g of biomass (4).

The antioxidant compounds in *Spirulina* sp. play a crucial role in protecting the body from the negative effects of free radicals, which can lead to various diseases. Free radicals are molecules with one or two unpaired electrons. This group can be expanded by adding other compounds that are not radicals but possess oxidative properties (5). Antioxidants and enzymes such as superoxide dismutase (SOD), glutathione peroxidase, or catalase can neutralize radicals by transforming reactive species into stable compounds (6). The composition and health benefits of consuming *Spirulina* sp. (or its derivatives, including proteins, lipids, carbohydrates, vitamins, minerals and pigments) make it a potential essential food in the future. They can be used as an ingredient in the development of functional foods. However, its main challenge lies in the fact that most microalgae have a fishy aroma and taste and a less attractive color (7).

Fermentation methods can impart unique aroma, taste and texture characteristics to a product. Fermentation is a metabolic process in which carbohydrates are oxidized to produce energy without the need for an external electron acceptor (8). This process is one of the oldest and most cost-

effective food preservation and processing techniques. During fermentation, numerous biochemical changes occur that can affect the nutritional compounds and consequently influence the properties of the end product (9). Fermentation is a natural method that can enhance the levels of vitamins and essential amino acids, reduce the content of anti-nutrients, increase protein content and improve the appearance, taste and aroma of food (10). Not only does fermentation increase the number of free phenolics, but it also enhances the level of bound phenolics, which can be detected through increased extraction power, with fermentation exhibiting a stronger effect. The fermentation process facilitates the release of phenolic compounds, particularly gallic acid, chlorogenic acid, p-hydroxybenzoic acid and p-coumaric acid while increasing the antioxidant content (11). Fermentation also increases the content of flavonoids and saponins, which are bioactive compounds. This entire process helps improve sensory quality and food safety. The antioxidant content and levels of bioactive compounds increase during the fermentation process.

The primary challenge is addressing the strong and fishy taste of Spirulina, which is difficult to mask in food formulations, thereby influencing consumer preference. Recent studies demonstrated that buttermilk fortified by *S. platensis* could improve nutraceutical and sensory characteristics (12). Another report suggested that *S. platensis* powder fermented with *Pediococcus acidilactici* increased the gamma-aminobutyric acid and L-glutamic acid (13). These results suggested that Spirulina has versatility in various fermented beverage applications. During fermentation, cyanobacterial peptides and other bioactive compounds trapped within the cell wall of *Spirulina* sp. are released, as evidenced by increased antioxidant capacity and protein fragmentation in the fermented samples (14). Microorganism-mediated biotransformation processes alter the chemical components of the substrate, providing a preservative effect and enhancing nutrient viability. However, as the fermentation time lengthens, the antioxidant content in fermented *Spirulina* sp. tends to decrease. Prolonged fermentation also results in a decrease in pH, making the beverage more acidic (15). Therefore, there is very little supporting data that determines the optimal fermentation time for *Spirulina* sp., which might affect its characteristics and properties in fermented beverages. This study aims to investigate the effects of fermentation times on the antioxidant content, pH, panellists' acceptance and potential bioactive compound components with antioxidant potential in fermented *Spirulina* sp. using the best fermentation times treatment.

Materials and methods

Sample collection

The main ingredient used in this research is fresh *Spirulina* sp., obtained from *Perseroan Terbatas* (PT) Alga Bioteknologi Indonesia, Gunungpati, Semarang, Indonesia. *Spirulina* sp. is packaged in plastic to maintain its freshness and stored using cube ice to ensure its cold chain. The yeast used in this study is *Saccharomyces cerevisiae* in dry yeast form. *S. cerevisiae* was purchased from a grocery store. The powdered *Spirulina* sp. is shown in Fig. 1.

Fig. 1. Powdered *Spirulina* sp.

Preparation of Fermented Spirulina sp.

The ingredients were prepared by weighing all necessary components, such as *Spirulina* sp. (4 %) and sugar (10 %). *Spirulina* sp. was pasteurized by boiling in water at 75˚C for 30 minutes. *S. cerevisiae* was introduced at a concentration of 0.05 % following the temperature decrease. Subsequently, the solution was gently agitated until it became homogeneous. The fermentation procedure was carried out in a sealed and clean container that was maintained at room temperature for 0, 1, 3 and 5 days consecutively (modified from the method of Okechukwu et al. (16). The ingredients of fermented *Spirulina* sp. can be seen in Table 1.

Table 1. Fermented *Spirulina* sp. formulation.

Ingredients	Quantity (%)			
	0 day	1 day	3 day	5 day
Spirulina sp.	4 %	4 %	4 %	4 %
Sugar	10 %	10 %	10 %	10 %
Yeast	0.05 %	0.05 %	0.05 %	0.05 %

Phytochemical analysis

Phytochemical analysis was conducted qualitatively by observing the sample's color changes after adding reagents. The colour differences between phytochemical components in the sample were identified based on previous research (modified from the method of Roghini and Vijayalakshmi (17). The summarised phytochemical methods are summarised in Table 2.

Table 2. Phytochemical testing.

Compound Identification	Testing Method
Flavonoids	A 2 mL extract and 1 mL of 2N sodium hydroxide was added. The presence of yellow indicates
Alkaloids	2 mL HCl was added to 2 mL of extract and a few drops of Mayer's reagent. Green or white
Steroids	To 1 mL of extract, an equal volume of chloroform is added and a few drops of concentrated sulfuric acid are added. The appearance of a brown ring
Triterpenoids	0.5 mL extract was treated with 2 mL of chloroform and sulfuric acid. The formation of a red-brown color at the interface indicates the presence of
Catechol (Condensed Tannins)	2 mL of 5 % ferric chloride was added to 1 mL of extract. The formation of dark blue or greenish black indicates the presence of catechol.
Phenolic	2 mL of distilled water, followed by a few drops of 10 % ferric chloride, was added to 1 mL of extract. The formation of blue or green colour indicates the presence of phenols.

Antioxidant activity

The fermented Spirulina was centrifuged at a speed of 2000 rpm for 5 minutes and then the supernatant was collected. The supernatant was then mixed with 50 µM DPPH at ratio 1:1 (v/v) and incubated for 1 hour. A color change from purple to yellow indicates the efficiency of free radical scavenging. The absorbance of the sample was measured using a UV-Vis spectrophotometer at a wave length of 517 nm. Antioxidant activity was calculated using equation (1) (5)):

$$\% \text{ Inhibition} = \frac{\text{Blank Absorbance - Sample Absorbance}}{\text{Blank Absorbance}} \times 100 \quad \text{(Eqn. 1)}$$

Blank absorbance is DPPH solution without samples and sample absorbance is DPPH solution reacted with fermented *Spirulina* sp. on varying fermentation times.

Determination of Total phenolic content

The total phenolic analysis method uses Folin-Ciocalteu reagent (0.5 mL), 20 g of Na_2CO_3 and distilled water. The concentration of standard solution series is prepared using gallic acid, including 0, 5, 15 and 20 ppm. Next, a Na_2CO_3 solution was prepared by weighing 20 g of Na2CO3 and dissolving it in distilled water to a total volume of 100 mL. Sample and standard solutions (0.1 mL) were placed in vials, then 7.9 mL of distilled water and 0.5 mL of Folin Ciocalteu reagent were added. Incubated for 8 minutes while shaking. Then, 1.5 mL of 20 % Na_2CO3 solution was added, followed by a 2-hour incubation period. The absorbance was measured at a wavelength of 765 nm. Total phenolic was calculated using equation (2) (modified from the previous method (18,19)):

$$\text{Total Phenol} = \frac{C_1 \times V}{m} \quad \text{(Eqn. 2)}$$

Where, C1 : Gallic acid concentration (mg/L), m : Weight of the extract (g) and V: Extract volume (L)

pH (Potential of Hydrogen)

pH analysis is conducted based on the National Standardization Agency of Indonesia, using a pH meter to determine the acidity level of a solution. Firstly, the pH meter is calibrated using a pH 4 buffer solution and dried with soft tissue. Then, the sample is poured into a beaker glass for measurement. Next, the pH electrode of the pH meter is immersed into 10 mL of the sample until a stable reading is obtained and the pH value is recorded. After the measurement is complete, the electrode is cleaned with mineral-free water.

Quantification of Amino Acids

The implementation method based on AOAC (2005) states that the composition of amino acids can be determined using HPLC (High-Performance Liquid Chromatography). Before analysis, HPLC should be flushed with the eluent for 2-3 hours. The same applies to cleaning the injection with pure water until it is thoroughly clean. The analysis of amino acids using HPLC consists of four steps: protein hydrolysis, drying, derivatization and injection, as well as amino acid analysis. The separation was performed on a reversed-phase C18 column (250 × 4.6 mm, 5 µm particle size) with 254 nm wavelength of the UV–vis diode array detector. The column was held at 50 ± 1 °C with a thermostatically controlled column heater. Solvent A consisted of sodium acetate pH 6.6 with glacial acetic acid, which was mixed with acetonitrile (40:60). Solvent B consisted of solvent A and acetonitrile (40:60). Amino acids were identified by retention times and quantified by external standard technique using the amino acid standard. Amino acid levels were expressed in g/100 g of sample. (20).

Hedonic test

A hedonic evaluation was conducted on fermented *Spirulina* sp., involving 30 untrained panelists. While lacking formal training, these panelists possess the capacity to discern and articulate their reactions to sensory assessments of the product. Analysis parameters include color, aroma and taste. Panelists are provided with a form containing hedonic scores for fermented *Spirulina* sp. Information from the hedonic scale is as follows: a score of 9 = like extremely, 8 = like very much, 7 = like moderately, 6 = like slightly, 5 = neutral, 4 = dislike slightly, 3 = dislike moderately, 2 = dislike very much and 1 = disliked extremely (21).

GC-MS (Gas Chromatography-Mass Spectrometry)

GC-MS analysis using Shimadzu QP2010S GC-MS with the following conditions: a 30mm Rtx5MS column with an internal diameter of 0.22mm. Helium was used as the carrier gas with the following injector parameters: a temperature of 320°C, a pressure of 13.7 kPa, a total flow rate of 40 mL/minute, a column flow rate of 0.50 mL/minute, a linear velocity of 25.90 cm/second, a purge flow of 3 mL/minute and a split ratio of 73.0. The column temperature was programmed to start at 70° C (maintained for 5 minutes) and continue up to 300°C (maintained for 52 minutes) with a temperature increase rate of 10°C/minute. The ion source temperature was set at 250°C and the interface temperature was set at 320°C. In the chromatogram, the number of compounds in the extract is indicated by peaks, while the names/types of compounds are interpreted based on the spectral data for each peak using a library approach in the GC-MS database.

Data analysis

Data analysis for this study was conducted using statistical tests with the SPSS 16 application, including tests for normality, homogeneity and ANOVA (Analysis of Variance). A completely randomized design was used for homogeneous treatments. The obtained data showed a normal and homogeneous data distribution, allowing for further analysis using ANOVA. The analysis of sensory evaluation data for fermented *Spirulina* sp. used the non-parametric Kruskal-Wallis's test, followed by the Mann-Whitney test.

Results and Discussions

Antioxidant activity

Fermentation time treatment on *Spirulina* sp. significantly influenced ($p<0.05$) the antioxidant activity according to the analysis of variance (ANOVA) results. Post-hoc tests using LSD (Least Significance Different) revealed significant differences among all fermentation time treatments of *Spirulina* sp. The highest value was obtained from fermented *Spirulina* sp. with a

fermentation time of the first day, which showed an inhibition percentage value of 86.82 % (Fig. 2). The inhibition percentage is calculated by measuring the difference in absorbance between the extract and DPPH. Fermented *Spirulina* sp. exhibits antioxidant activity through the presence of antioxidant compounds that react with free radicals via a proton donor mechanism at the hydroxyl group. These compounds may contain carbonyl, hydroxyl and polyhydroxyl groups, which confer antioxidant activity. In addition to the hydroxyl group, the sulphate groups in sulphated polysaccharides also act as antioxidants. Moreover, flavonoid and phenolic compounds can also contribute to the antioxidant properties (22). The Spirulina fermentation results in a significant increase in chlorophyll and carotenoid content, which contributes to its enhanced antioxidant properties (12). Additionally, the fermentation process leads to the upregulation of stress response-related proteins, further boosting the antioxidant capacity of the beverage. This is consistent with findings from other fermented foods, such as wheat, where natural fermentation has been shown to increase total phenolic and flavonoid contents, resulting in more effective antioxidant activity compared to unfermented samples (23).

Antioxidant activity analysis showed an increase in % inhibition on the first day of fermentation treatment, from 62.96 % to 86.82 %. Antioxidant activity on the third day of treatment decreased from 86.82 % to 80.66 %. A further decrease in antioxidant activity from 80.66 % to 70.56 % was observed on the fifth day of fermentation treatment. Antioxidant capacity is related to the chemical structure of phenolic compounds. The total phenolic and flavonoid content increases in fermented samples, which enhances their antioxidant potential compared to non-fermented ones (24). The antioxidant activity of phytochemical compounds is influenced by various factors such as temperature, pH and solvent. These factors need to be well controlled to obtain consistent results regarding antioxidant capacity. During the fermentation process, an increase in ethanol concentration and temperature can enhance the solubility of phenolic compounds while reducing their content in the product (25).

Phytochemical analysis

Based on the antioxidant activity analysis results in fermented *Spirulina* sp., the highest percentage inhibition of antioxidant compounds was obtained at 86.82 % on the first day of fermentation treatment. Subsequently, the screening of phytochemicals was conducted using the 0-day (control) fermentation treatment as a comparison to determine the content of phytochemical compounds in the fermented *Spirulina* sp. treatments at 0-day and 1st-day fermentation. The results of the qualitative screening for phytochemicals in fermented *Spirulina* sp. with different fermentation times are presented in Table 3.

The results obtained from the qualitative screening of phytochemicals in the 0-day (Control) and 1st-day fermentation treatments of *Spirulina* sp. showed the presence of compounds belonging to the groups of flavonoids, alkaloids, steroids, triterpenoids, catechol and phenolics. A previous study exhibited that *Spirulina* fermented with *Saccharomyces cerevisiae* upregulated the most differentially expressed proteins compared to non-fermented Spirulina water extract, which predict to enhance its antioxidant ability (26). On the other hand, Spirulina

Table 3. Results of qualitative screening for phytochemicals in fermented *Spirulina* sp.

Compound identification	Results	Treatment	Results
Flavonoids	Orange, Brick Red, Pink, Dark Red	0 Day	+
		1 Day	+
Alkaloids	Orange Yellow Precipitate, Brick Red Precipitate	0 Day	+
		1 Day	+
Steroids	Blue Green Colour	0 Day	+
		1 Day	+
Triterpenoids	Red, Purple, Brown	0 Day	+
		1 Day	+
Catechol (Condensed Tannins)	Red precipitate	0 Day	+
		1 Day	+
Phenolic	Dark Green Color, Black Blue Color	0 Day	+
		1 Day	+

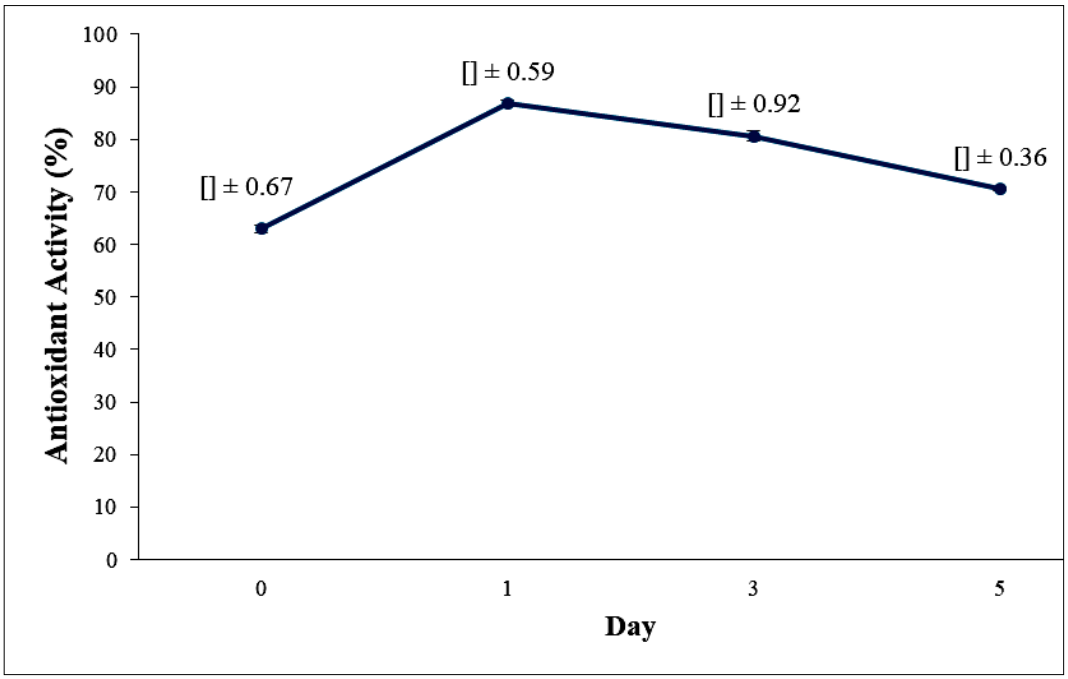


Fig. 2. Antioxidant activity in each treatment.

fermented with *Lactiplantibacillus plantarum* 122 resulted in increased levels of L-glutamic acid and gamma-aminobutyric acid (GABA). The highest contents of L-glutamic and gamma-aminobutyric acids (4062 and 228.6 mg/kg, respectively) were found in 24 and 48 h of fermentation. Additionally, fermentation increased the content of saturated fatty acids and omega-3 in Spirulina, while monounsaturated fatty acids and omega-6 were reduced (13).

Determination of total phenolic content

The quantitative assay of total phenolics based on the optimum results of the best antioxidant activity on the first day and the 0 days as a comparison is presented in Table 4.

Table 4. Results of quantitative analysis of total phenolics in fermented *Spirulina* sp.

Sample (Day)	Results (µg GAE/mL)
0	118.943
1	147.548

The fermentation treatment of 0 days showed a result of 118.943 µg/ml, while the fermentation treatment of the first day showed a result of 147.548 µg/ml. The fermentation time affects the phenolic content in fermented *Spirulina* sp. *Saccharomyces cerevisiae* yeast influences the phenolic compound content in fermented *Spirulina* sp., leading to an increase due to the yeast's ability to form phenolic compounds (27). This increase in phenolic content occurs during alcohol fermentation, possibly due to yeast activity. It has been reported that microbial enzymes such as tannase, glucosidase and cellulase produced during fermentation can facilitate the release of phenolic compounds by disrupting the plant cell structure (28), indicating an increase in phenolic content during fermentation. Similar with our results, the addition of 0.25 % Spirulina were increased in total phenolic content, reaching 4.21 mg/g GAE (12).

Fermentation also results in the release of microbial enzymes, producing more freely available plant chemical compounds such as flavonoids, tannins, alkaloids and phenylpropanoids (24, 29). The antioxidant capacity is closely related to the chemical structure of phenolic compounds (15). A compound can be classified as a natural antioxidant because it possesses reducing power, free radical scavenging, chelating agents and singlet oxygen quenchers. Phenolic compounds play various roles in plants, including self-protection against UV radiation for survival and environmental adaptation (30). Several studies have shown that phenolic compounds with high content function as potent antioxidants (5, 31).

pH (potential of hydrogen)

The results of the pH analysis for fermented *Spirulina* sp. with different fermentation times are presented in Fig. 3.

Fermentation time treatment on *Spirulina* sp. significantly influenced ($p<0.05$) the pH according to the analysis of variance (ANOVA) results. Post-hoc tests using LSD (Least Significant Difference) revealed significant differences among all fermentation time treatments of *Spirulina* sp. The pH decreases during the fermentation process (Fig. 3). After adding yeast, the pH decreases from 5.46 ± 0.141 for the control treatment to approximately 3.28 ± 0.145 on the final day of fermentation. The results indicate that longer fermentation decreases pH, which is caused by an increase in the content of organic acids (32). As the fermentation times increase, the pH of the beverage decreases due to the production of acidic compounds that adjust the optimal pH for yeast growth. During fermentation, *Saccharomyces cerevisiae* not only produces alcohol but also generates various byproducts, such as organic acids. Microorganisms break down fermentable carbohydrates into end products such as organic acids, carbon dioxide and alcohol. In line with our results, Spirulina addition (fresh or dry) to yoghurt generally leads to a faster decrease in pH value during the fermentation process. The rapid acidification is likely due to the rich nutrient content of Spirulina, which provides an excellent substrate for lactic acid bacteria and other fermentative microorganisms (33). However, another study reported that the addition of spirulina and wheat germ powder to functional fruit juices had little effect on pH and acidity. This suggests that the impact of Spirulina on pH may depend on various factors, including the base beverage composition and the specific fermentation conditions.

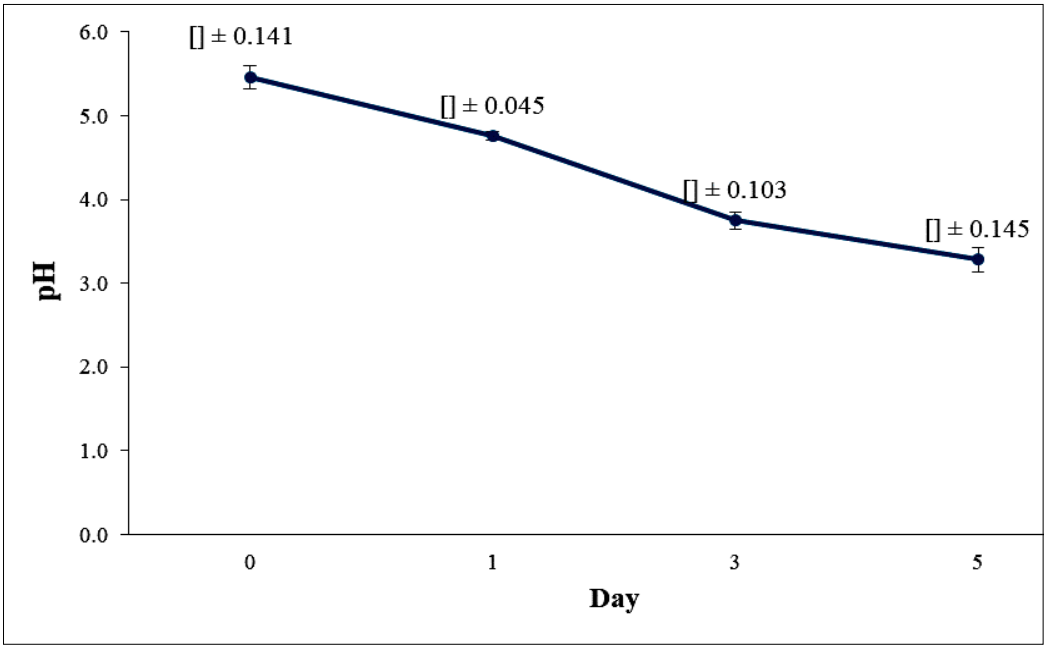


Fig. 3. pH (Potential of Hydrogen).

Quantification of amino acids

The results of the quantification of amino acids can be seen in Table 5. Amino acid analysis was conducted on samples with a fermentation treatment on the first day, based on the results of the antioxidant activity analysis, which showed the highest % inhibition of 86.82 %. The amino acid analysis results for the first day of fermentation were compared with the study conducted by Sahin et al., using the same yeast, S. cerevisiae and a fermentation time of the second day (27).

Another study reported that Spirulina fermented with lactic acid bacteria strain increased L-glutamic acid levels (34). Interestingly, the fermentation process not only enhances the production of certain amino acids but also affects the overall protein content and quality. Spirulina-enriched probiotic labneh exhibited significantly higher levels of protein compared to control samples (35). This suggests that fermentation may improve the bioavailability and digestibility of Spirulina proteins. The detection of 9 essential amino acids and eight non-essential amino acids was observed on the second day of fermentation, while nine essential amino acids and seven non-essential amino acids were detected on the first day of fermentation. Essential amino acids are amino acids that cannot be synthesized by the body (36). Essential amino acids, including leucine, isoleucine, valine, lysine, methionine and tryptophan, are vital for various physiological processes such as protein synthesis, neurotransmitter production and regulation of metabolic pathways (37). To meet their requirements, they need to be obtained from food sources that can provide them, while non-essential amino acids are amino acids that can be synthesized by the human body (27). During the fermentation process, the amino acid content increases due to the hydrolysis of proteins by fungi into amino acids (38).

Table 5. Results of amino acid analysis in fermented *Spirulina* sp. with 1st day fermentation treatment compared to others.

Amino Acids	Amino Acid Content (mg/kg)	
	Day 1	Day 2
Essential amino acids		
Valine	288.95	1343.36
Methionine	243.09	460.70
Phenylalanine	346.85	1188.56
Histidine	193.64	199.55
Threonine	295.03	858.49
Arginine	1529.42	1220,38
Isoleucine	468.34	495.15
Leucine	566.12	1869.70
Lysine	1398.87	816.74
Non-Essential amino acids		
Aspartic Acid	1089.64	3382.04
Glutamic Acid	1106.43	3986.67
Alanine	3862.12	3489.66
Serine	433.26	1705.89
Glycine	324.75	3477.88
Tyrosine	213.97	640.28
Proline	220.54	2793.61
Cystine	n.d	52.98

(**n.d**) not detected.

Hedonic test

Hedonic analysis was conducted on fermented *Spirulina* sp. with different fermentation times to determine the panellists' acceptance. The results of the hedonic analysis are presented in Table 6. The confidence intervals for fermented *Spirulina* sp. in the 0-day, 1st day, 3rd day and 5th day treatments are as follows: $4.47 < \mu < 4.75$; $7.22 < \mu < 7.50$; $6.45 < \mu < 6.73$; $6.36 < \mu < 6.66$. The first-day fermentation duration showed a preferred acceptance level by the panellists for colour, aroma and taste. Significant differences ($p<0.05$) were observed in the colour and aroma parameters between the 0-day fermentation time and the 1st-day, 3rd-day and 5th-day fermentation durations. In contrast, no significant differences ($p>0.05$) were found between the first-day, third-day and fifth-day treatments. In terms of taste, there were no significant differences (*p>0.05*) between the 3-day and 5-day fermentation durations, but significant differences ($p<0.05$) were observed compared to the 0-day and 1-day fermentation durations. The fermentation of time influences consumer acceptance. While colour is not directly related to nutritional value, it plays a significant role in consumer decision-making when making purchasing decisions. Colour can even be a determining factor for consumers when choosing a food product. Since the visual aspect plays a crucial role in modern consumers' selection of food products, colour is a primary constituent of food and beverages. The fermentation process in *Spirulina* sp. produces a distinctive acidic aroma and a slight alcoholic odour. Panellists preferred the acidic aroma with a hint of alcohol in the fermented *Spirulina* sp. The characteristic fermented aroma is caused by increased acid content during fermentation. A carbon source (sugar), nitrogen (ammonia and amino acids), water and yeast are required to carry out fermentation. The aromatic byproducts produced during the fermentation process can vary significantly depending on the starting material used (39). The increased acid content contributes to the sour taste of the fermented *Spirulina* sp. However, longer fermentation tends to result in a bitter taste due to alcohol content. During the process of alcohol fermentation, yeast utilizes sugars (glucose and fructose) and other components as substrates for its growth, converting them into

Table 6. Results of hedonic analysis on fermented *Spirulina* sp.

Fermentation times (Day)	Specification			Confidence interval
	Colour	Aroma	Flavor	
0	6.07 ± 0.727^{a}	5.23 ± 0.667^{a}	6.17 ± 0.637^{b}	$4.47 < \mu < 4.75$
1	7.07 ± 0.680^{b}	7.23 ± 0.667^{b}	7.77 ± 0.667^{c}	$7,22 < \mu < 7,50$
3	7.27 ± 0.442^{b}	7.13 ± 0.618^{b}	5.37 ± 0.657^{a}	$6,45 < \mu < 6,73$
5	7.03 ± 0.752^{b}	7.13 ± 0.718^{b}	5.37 ± 0.482^{a}	$6,36 < \mu < 6,66$

Data represents the average results of 3 replicates ± standard deviation. Data with different superscript letters in the same column indicate significant treatment differences ($p < 0.05$). Data with the same superscript letter in the same column showed no significant difference between treatments ($p > 0.05$).

ethanol, carbon dioxide and various other metabolic byproducts that impact the chemical composition and sensory quality of the fermented food (40). Interestingly, the fermentation process decreased the proportions of aldehyde, ester and acid compounds that are responsible for algal flavour in Spirulina. Additionally, methylbutanal-D, (E)-hept-2-enal-D and 2-furan methanol acetate were also reduced in fermented Spirulina, indicating a reduction in the strong odour of Spirulina (41).

GC-MS analysis

The results of fermented *Spirulina* sp. with a fermentation time of the first day showed the best results in the antioxidant activity analysis. Subsequently, further testing was conducted for the estimation analysis of compound components using a GC-MS instrument. The GC-MS analysis results for estimating compound components from fermented *Spirulina* sp. are presented in Table 7.

Fig. 4 shows the graph of the analysis. Peaks in the graph, which are relatively close together, indicate different types of chemical components. The height of each peak represents the quantity of the corresponding component. If the percentage of an element in the sample is higher, it will produce a higher peak and conversely, if the rate is lower, it will produce a lower peak. The numbers on the peaks are called retention times, where components with lower boiling points elute first, resulting in shorter retention times. This analysis indicates the percentage of the measured compound area. However, it is essential to note that the percentage area is not an accurate quantitative calculation. Still, it only indicates the concentration of the compounds in the sample based on the measured area. The quality in GC-MS indicates the dominant compounds present in the sample by assessing the percentage similarity (42).

Tetracosamethyl cyclododecasiloxane functions as an antioxidant and antimicrobial agent (43). The compound Dodecamethyl cyclohexasiloxane is an antifungal agent (44). Tetradecamethyl cycloheptasiloxane is an antimicrobial, antiseptic, hair moisturizer and skin moisturizer. Hexadecanoic acid is an antioxidant, 5-alpha-reductase inhibitor, anti-fibrinolytic, hemolytic, antimicrobial, hypocholesterolaemia, nematocide and pesticide and exhibits hemolytic properties (44). Trimethylsilyl ester alpha, 3,4-tris ((trimethylsilyl)oxy) Benzeneacetic acid has roles as an antimicrobial and antibacterial agent (45). The compound 14-Methylpentadecanoic functions as an antioxidant and exhibits antibacterial effects in the leaves of *Polyalthia cinnamomea* (46,47). Decamethyl cyclopentasiloxane is known for its antimicrobial properties (48). Research indicates that ketones, alcohol and furans and olefins produced by LAB and yeast increased after fermentation, thereby enhancing the flavour of Spirulina. Hence, the fermentation of Spirulina by *S. cerevisiae* might be a potential to improve the flavour and functional values, which further diversifies its products (41).

Conclusion

In summary, the fermentation time of *Spirulina* sp. significantly affects the antioxidant activity. The colour and aroma parameters showed significant differences between 0-day fermentation and 1st-day, 3rd-day and 5th-day fermentation ($p<0.05$). However, there were no significant differences ($p>0.05$) among the 1st-day, 3rd-day and 5th-day fermentations. In terms of taste, there were no significant differences ($p>0.05$) between the 3rd-day and 5th-day fermentations, but there were substantial differences compared to 0-day and 1st-day fermentations ($p<0.05$). The bioactive compound content in the 1st-day and 0-day treatments, as the control, included flavonoids, alkaloids, steroids, triterpenoids, catechol and phenolics. The GC-MS analysis of compound components on the 1st day of treatment revealed the presence of Tetracosamethyl cyclododecasiloxane, Hexadecenoic acid and 14-Methylpentadecanoic acid, which have the potential as antioxidants. These findings are based on the optimal fermentation duration, which exhibited the highest antioxidant activity at 86.82 % in the 1st-day treatment. The results suggested that *Spirulina* sp. fermented beverages can be used as a promising healthy-functional drink with many active components that are beneficial to health.

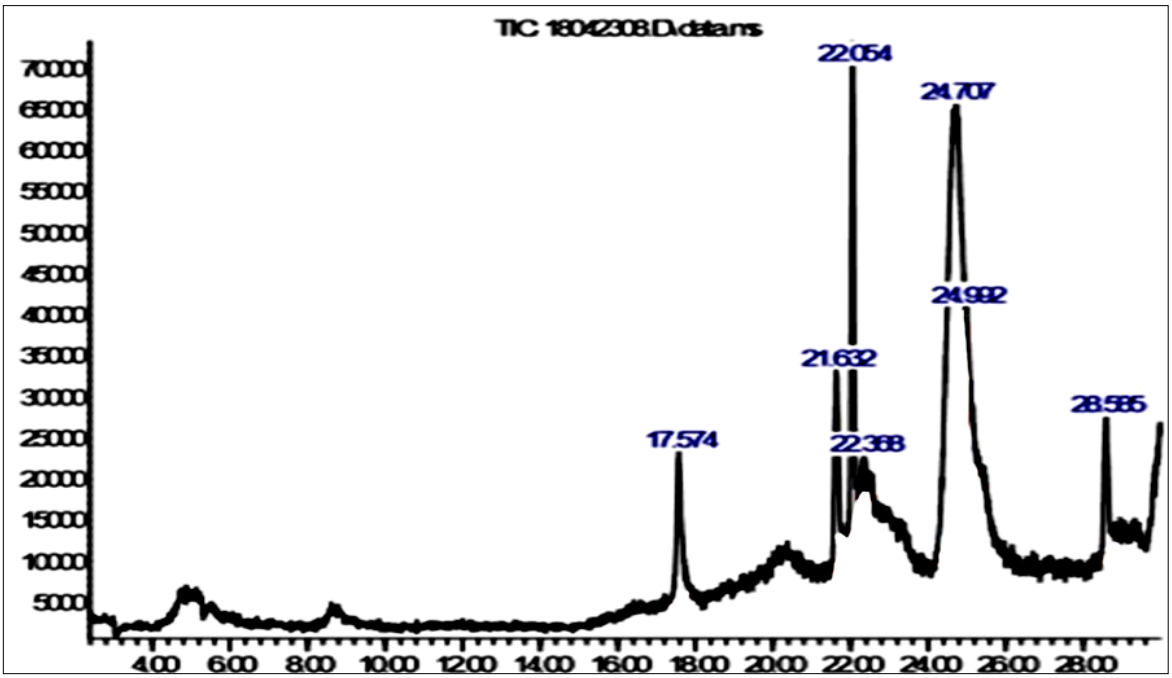
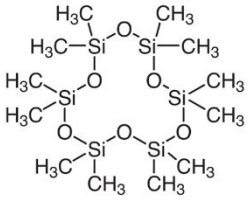
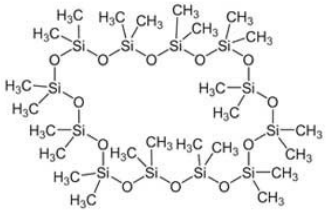
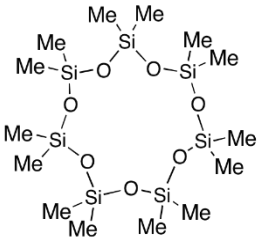
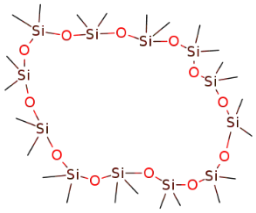
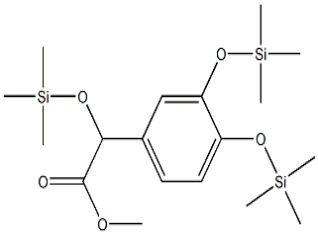
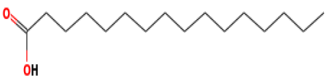
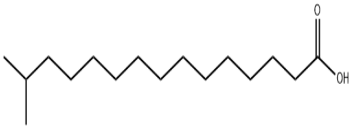
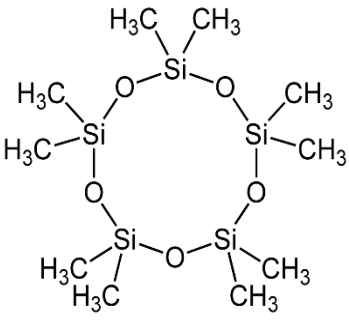


Fig. 4. GC-MS chemical profile of fermented *Spirulina* sp. components. with a long fermentation time of 1st day.

Table 7. Compound results from GC-MS analysis in fermented *Spirulina* sp. with a fermentation times of 1st day.

Retention times (minutes)	Area (%)	Quality (%)	Compound name and formula	2D
17.574	7.34	86	Dodecamethyl cyclohexasiloxane, $C_{12}H_{36}O_6Si_6$	
21.632	7.04	49	Tetracosamethyl cyclododecasiloxane, $C_{24}H_{72}O_{12}Si_{12}$	
22.054	12.98	93	Tetradecamethyl cycloheptasiloxane, $C_{14}H_{42}O_7Si_7$	
22.313	1.58	27	Tetracosamethyl cyclododecasiloxane, $C_{24}H_{72}O_{12}Si_{12}$	
22.368	1.45	35	Trimethylsilyl ester alpha.,3,4- tris ((trimethylsilyl)oxy) Benzeneacetic acid, $C_{20}H_{40}O_5Si_4$	
24.707	55.77	98	Hexadecanoic acid, $C_{16}H_{32}O_2$	
24.992	7.95	98	14-Methylpentadecanoic, $C_{16}H_{32}O_2$	
28.585	5.88	38	Decamethyl cyclopentasiloxane, $C_{10}H_{30}O_5Si_5$	
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Authors' contributions

PHR carried out the design of the study, statistical analysis and drafted the manuscript. TWA helped in statistical analysis, participated in TC measurement and drafted the manuscript. ES statistical analysis, participated in antioxidant test and drafted the manuscript. AA participated in the quantification of amino acids and formal analysis. ADA carried out the phytochemical analysis and drafted the manuscript. EF participated in Spirulina fermentation and drafted the manuscript. ISR participated in chromatography analysis and drafted the manuscript. TAW and MHA participated the hedonic test and statistical analysis. RB conceived of the study and participated in its design, supervision and drafted the manuscript.

Compliance with ethical standards

Conflict of interest: Authors do not have any conflict of interests to declare.

Ethical issues: None

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