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Comparative Study of Chlorophyll Content in *Wolffia globosa* (Khai-phum, a Thai aquatic plant) with *Chlorella* sp. and *Spirulina* sp. for the Evaluation of its Potential as a Superfood

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Abstract

The global demand for sustainable superfoods has increased interest in *Wolffia globosa* (Khai-phum), a rich bioactive profile aquatic plant. However, its chlorophyll profile, which is a key indicator of bioactivity, still lacks standardized comparative data. This study quantified the chlorophyll content of *Wolffia globosa* and compared it with commercial *Chlorella* sp. and *Spirulina* sp. to scientifically evaluate its superfood potential.

Pigments were extracted using food-grade 95% ethanol under controlled parameters. Chlorophyll-a and chlorophyll-b were quantified by UV-Vis spectrophotometry. Physical color was evaluated using the CIELAB system, followed by Pearson correlation analysis between color coordinates and pigment concentrations.

Results demonstrated that dehydrated *W. globosa* yielded 1.51 ± 0.02 mg/g DW of chlorophyll-a, 0.45 ± 0.02 mg/g DW of chlorophyll-b, and 1.96 ± 0.01 mg/g DW of total chlorophyll, with significant variance among species ($p < 0.05$). When compared to benchmarks, the chlorophyll profile of *Wolffia globosa* presented lower overall extractable yields but significantly higher chlorophyll-b than *Spirulina* sp. Furthermore, a significant positive correlation emerged between the $a*$ value and chlorophyll-a content ($r = 0.766$, $p < 0.05$), and an exceptionally strong correlation between the $b*$ value and chlorophyll-b content ($r = 0.900$, $p < 0.01$). This statistical finding highlights an optical paradox where the intense visual greenness of *Wolffia globosa* did not

directly translate to high extractable pigment yields, which may be attributed to its intact multicellular matrix limiting solvent diffusion.

In conclusion, this study provides a standardized benchmark for the chlorophyll yields of *Wolffia globosa*, scientifically supporting its bioactive potential. These findings support its potential as a superfood candidate, establishing a foundation to enhance food security and drive the Thai bioeconomy.

Keywords: *Wolffia globosa*, Chlorophyll Quantification, Superfood, *Chlorella* sp., *Spirulina* sp., Spectrophotometry

Introduction

Over the last few decades, functional and high-nutrient food trends have been rising significantly due to a recognition of how diets impact humans' health. As a result, it changes overall mindset and people start to be looking for more sustainable ways to consume food (Fanzo et al., 2022). Consequently, the term 'superfood' has gained more attention. Even though there is still no formal regulatory definition for it but it is generally a term to refer to foods significantly high in nutrition and bioactive compounds with a strong connection to environmental sustainability and plant-based solutions (Tacer-Caba, 2019; Fernández-Ríos et al., 2022; Diaz et al., 2023; Torres-Tiji et al., 2020).

Among these superfoods, chlorophyll is often overlooked as a mere pigment, but it is a potent bioactive compound with profound implications for anti-aging and regenerative medicine. Chlorophyll and its derivatives are remarkable scavengers of reactive oxygen species (ROS), which helps mitigate oxidative stress, a primary driver of cellular aging and chronic diseases (Pérez-Gálvez et al., 2020; Martins et al., 2023). Chlorophyll-a captures light energy and converts it into chemical energy (Björn et al., 2009), while chlorophyll-b absorbs more light wavelengths, facilitating more efficient photosynthesis (Masuda & Fujita, 2008). Studies indicate that chlorophyll has anti-cancer, antimutagenic, and chemopreventive properties, while also impacting lipid digestion and gastrointestinal absorption (Hayes & Ferruzzi, 2020; Wang et al., 2022; Kocaoğlu Cenkci & Kaya, 2022). Therefore, foods with high chlorophyll content have strong potential as functional anti-aging superfood candidates.

Recently *Chlorella* sp. and *Spirulina* sp. have become well-known benchmarks in superfood discussions due to their high chlorophyll, protein and vitamin content (Abreu et al., 2023; Ladjal-Ettoumi et al., 2024; Davani et al., 2022; Kratzer & Murkovic, 2021). However, the focus on them has created a 'blind spot' for other high-potential species like *Wolffia globosa*. Commonly known as the world's smallest flowering plant, *Wolffia globosa* is rich in proteins, minerals and bioactive compounds (Appenroth et al., 2018; Xu et al., 2021; Monthakantirat et al., 2022). Research points out its various beneficial properties including anticancer protein fractions, prebiotic potential, maintaining iron homeostasis and strengthening postprandial glucose response (Siriwat et al., 2023; Dhamaratana et al., 2025; Yaskolka Meir et al., 2019; Zelicha et al., 2019).

Aside from its benefits, developing *Wolffia globosa* into a functional food ingredient requires effective post-harvest stabilization, as thermal processing may adversely affect heat-sensitive compounds like chlorophyll. Currently there is still no clear standardized data specifying the chlorophyll content of *Wolffia globosa* especially in comparison to established standards such as *Chlorella* sp. and *Spirulina* sp. This lack of information creates a critical knowledge gap. Therefore, the specific objectives of this study are:

1. To quantify the chlorophyll-a, chlorophyll-b, and total chlorophyll content in *Wolffia globosa* by using a spectrophotometric methodology under controlled laboratory conditions.
2. To compare the chlorophyll profile of *Wolffia globosa* with commercially established microalgae benchmarks, *Chlorella* sp. and *Spirulina* sp.
3. To evaluate the potential of *Wolffia globosa* as a chlorophyll-rich raw material for sustainable food ingredient development.
4. To assess the efficacy of whole-flake extraction as a cost-effective processing method to preserve phytochemical stability and maximize value retention.

Scope of Research

This study focused on *Wolffia globosa* harvested from a semi-open cultivation pond in Korat Thailand. This source was selected to control environmental variance better than natural wild sources. The extraction process was strictly limited to using food-grade 95% ethanol as the solvent. This solvent was chosen to ensure safety and align with food industry standards for superfood evaluation.

Research Methodology

This study was a laboratory-based experimental investigation comparing the chlorophyll profile and colorimetric attributes of *Wolffia globosa* with commercial *Chlorella* sp. and *Spirulina* sp. powders. To ensure experimental reliability and accuracy, standard operational parameters and calibrated instruments were monitored under uniform environments. The formal procedures were systematically conducted through the following sequential stages:

1. Colorimetric Analysis: Biomass samples were spread homogeneously to maintain standard spatial uniformity. Chromatic coordinate attributes were recorded using a calibrated Chroma Meter under the standard CIELAB color space dimensions to determine surface lightness (L^*), greenness (a^*), and yellowness (b^*) profiles. The CIELAB system is widely recognized as a highly accurate standard method for evaluating the colorimetric quality of aquatic plants and microalgae. For instance, a recent study by Ladjal-Ettoumi et al. (2024) successfully utilized the CIELAB system to precisely quantify the physical color of *Chlorella* sp. and *Spirulina* sp. biomasses, linking these values directly to their respective pigment concentrations. By adopting this established method, the colorimetric evaluation in this study aligns with international accuracy standards, allowing for an objective and precise physical comparison.

2. Pigment Extraction and Processing: 1 gram of dried biomass from each species was used. *Wolffia globosa* was harvested from a local pond in Korat Thailand. Commercial organic powders of *Chlorella* sp. and *Spirulina* sp. were also used in this study. Each sample was mixed with 50 mL of food-grade 95% ethanol to make a 1:50 (w/v) ratio. The mixture was homogenized at a low-speed of 2800 rpm for 5 minutes at 25 °C. This condition was chosen to mix the solvent well without breaking the cell wall of the *W. globosa* whole flake. The reason to keep the intact whole-flake structure instead of mechanical grinding is to prevent frictional heat and avoid exposing the sensitive chlorophyll to natural enzymes. This approach preserves cellular integrity and chemical stability. It helps inhibit chlorophyll degradation into pheophytin. Therefore,

this method provides a realistic evaluation of pigment recovery under a minimal processing condition. After that the mixture was centrifuged at 5000 rpm for 5 minutes at 4 °C to separate the solid waste. The clear liquid was then collected and diluted at a 1:10 ratio with 95% ethanol for the next analysis.

3. Spectrophotometric Measurement: Absorbance profiles of the diluted target extracts were quantified at 664 nm and 649 nm using standard quartz cuvettes using a UV-Vis Spectrophotometer with pure 95% ethanol as the blank calibration. The recorded values were then calculated using simultaneous equations and converted to a dry weight basis (mg/g DW) according to the formulas derived by Lichtenthaler (1987).

Statistical Analysis

All verified analytical datasets were processed using IBM SPSS Statistics and expressed as Mean ± SD with the statistical significance level set at $\alpha = 0.05$. The evaluation was conducted through the following criteria:

1. Shapiro–Wilk and Levene's Tests: Performed to assess data normality and homogeneity of variance prior to the primary analysis.

2. Coefficient of Variation (CV): Calculated to monitor data precision across replicates, using an acceptance threshold of CV ≤ 5%.

3. One-way ANOVA and Tukey's HSD: Applied for parametric mean comparisons and pairwise variations of pigment yields and color coordinates among species.

4. Kruskal–Wallis H-test: Utilized as a non-parametric alternative when data violated normality or variance assumptions.

5. Pearson Correlation Coefficients (*r*): Applied to examine the mathematical relationships between physical CIELAB color parameters and biochemical pigment concentrations.

Results

1. Physical Color Analysis

Table 1 Physical color analysis of various microalgae and *Wolffia globosa* (Fresh vs. Dried)

Sample	**Lightness (*L*∗)**		**Greenness (*a*∗)**		**Yellowness (*b*∗)**	
	Mean	SD.	Mean	SD.	Mean	SD.
Chlorella sp.	25.95^{a}	0.5	−8.01^{b}	0.08	20.66^{a}	0.25
Spirulina sp.	18.26^{b}	0.75	−6.06^{c}	0.09	4.16^{d}	0.01
W. globosa (Fresh)	26.04^{a}	0.46	−9.71^{a}	0.19	11.22^{c}	0.34
W. globosa (Dried)	25.83^{a}	1.22	−9.39^{a}	0.25	14.39^{b}	0.19
***p*-value**	<0.001 ∗		<0.001 ∗		<0.001 ∗	

Note Values are presented as x̄ ± SD of independent triplicates (n = 3). Different superscript letters (a, b, c, d) within the same column (in the vertical direction) indicate statistically significant differences among different microalgae and *Wolffia* samples according to One-way ANOVA followed by Tukey's HSD post-hoc test ($p < 0.05$).

The physical color coordinates ($L*a*b*$) of the biomass samples were evaluated using the CIELAB system. For the greenness coordinate ($a*$), both fresh and dehydrated *Wolffia globosa* exhibited the highest degree of greenness, with values of −9.71 ± 0.19 and −9.39 ± 0.25, respectively. These values were statistically comparable to each other but significantly greener ($p < 0.05$) than both commercial microalgae benchmarks, *Chlorella* sp. (−8.01 ± 0.08) and *Spirulina* sp. (−6.06 ± 0.09). Regarding lightness ($L*$), *Chlorella* sp. (25.95 ± 0.50) and dried *Wolffia globosa* (25.83 ± 1.22) were statistically comparable, whereas *Spirulina* sp. was found to be significantly darker (18.26 ± 0.75, p < 0.05). For the yellowness coordinate (b ∗), *Chlorella* sp. yielded the highest value (20.66 ± 0.25), followed by dried *Wolffia globosa* (14.39 ± 0.19) and fresh *Wolffia globosa* (11.23 ± 0.34). Among all tested materials, *Spirulina* sp. displayed the lowest yellowness at 4.16 ± 0.01.

2. Quantification of chlorophyll-a, chlorophyll-b, and total chlorophyll content

Table 2 Chlorophyll-a, chlorophyll-b, and total chlorophyll content (mg/g DW) of *Chlorella* sp., *Spirulina* sp., and *Wolffia globosa*

Sample	**Chlorophyll-a (mg/g DW)**		**Chlorophyll-b (mg/g DW)**		**Total Chlorophyll (mg/g DW)**	
	Mean	SD.	Mean	SD.	Mean	SD.
Chlorella sp.	5.55^{a}	0.02	1.55^{a}	0.06	7.10^{a}	0.05
Spirulina sp.	5.22^{b}	0.01	0.14^{c}	0.03	5.36^{b}	0.02
Wolffia globosa (Dried)	1.51^{c}	0.02	0.45^{b}	0.02	1.96^{c}	0.01
***p*-value**	<0.001 *		<0.001 *		<0.001 *	

Note DW indicates dry weight. Values are presented as $\bar{x} \pm$ SD of independent triplicates (n = 3). Different superscript letters (a, b, c) within the same column (in the vertical direction) indicate statistically significant differences among different microalgae and *Wolffia* samples according to One-way ANOVA followed by Tukey's HSD post-hoc test ($p < 0.05$).

The chlorophyll content of *W. globosa*, *Chlorella* sp., and *Spirulina* sp. are presented in Table 2. The spectrophotometric analysis revealed significant variations in the pigment profile across the three species ($p < 0.05$). The concentration of chlorophyll-a was highest in *Chlorella* sp. (5.55 ± 0.02 mg/g DW), followed by *Spirulina* sp. (5.22 ± 0.01 mg/g DW), and *W. globosa* (1.51 ± 0.02 mg/g DW). For chlorophyll-b, *Chlorella* sp. contained the highest amount (1.55 ± 0.06 mg/g DW). Notably, the chlorophyll-b content in *W. globosa* (0.45 ± 0.02 mg/g DW) was significantly higher than that of *Spirulina* sp. (0.14 ± 0.03 mg/g DW) ($p < 0.05$). Consequently, the total chlorophyll content showed a significant difference among the samples ($p < 0.05$), with *Chlorella* sp. yielding the highest total concentration (7.10 ± 0.05 mg/g DW), followed by *Spirulina* sp. (5.36 ± 0.02 mg/g DW), while *W. globosa* yielded the lowest extractable amount (1.96 ± 0.01 mg/g DW).

3. Correlation Analysis

Pearson correlation coefficients (r) were calculated to examine the statistical relationships between the physical color coordinates (L^* a^* b^*) and the extracted chlorophyll concentrations. The analysis revealed a strong positive correlation between total chlorophyll and chlorophyll-a content (r = 0.965, $p < 0.01$). Furthermore, a significant positive correlation was observed between the a^* value and chlorophyll-a content (r = 0.766, $p < 0.05$). Additionally, a strong positive correlation was observed between the b^* coordinate and chlorophyll-b content (r = 0.900, $p < 0.01$). Among the physical color parameters, a strong negative correlation was found between lightness (L^*) and greenness (a^*) (r = −0.885, $p < 0.01$).

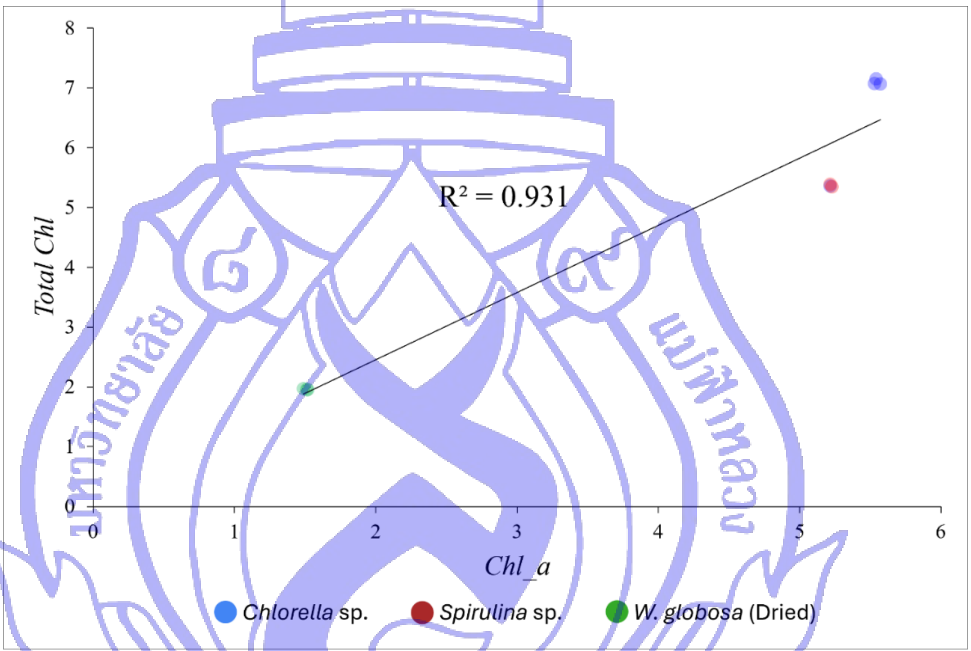

Figure 1 Relationship between chlorophyll-a and total chlorophyll content. This pair exhibits the strongest positive correlation among all measured parameters (r = 0.965, $p < 0.01$)

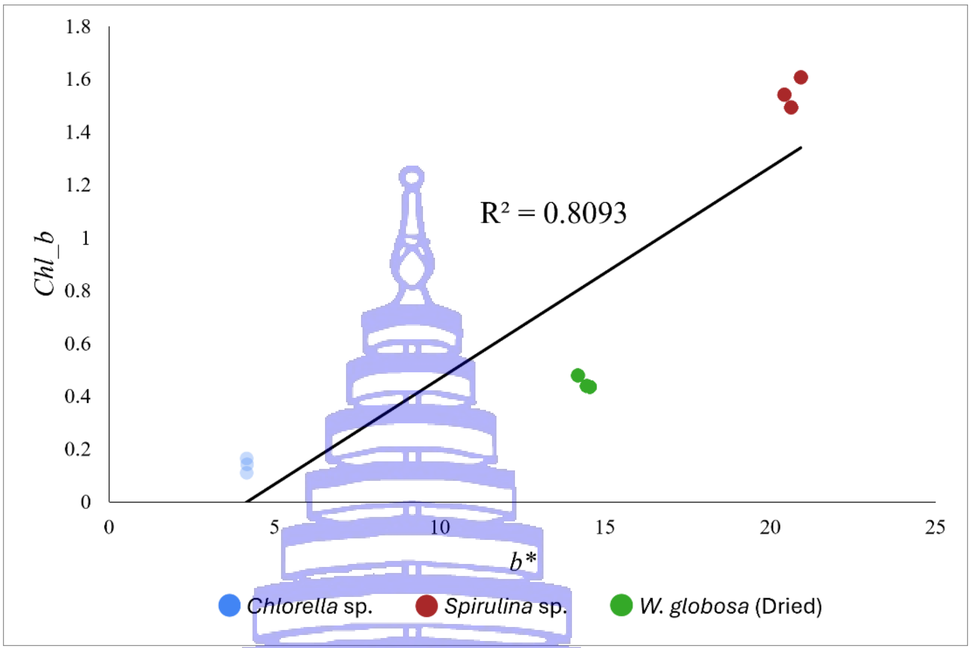

Figure 2 Relationship between b^* value and chlorophyll-b content. This pair exhibits the highest level of statistical significance between a physical color coordinate and pigment concentration ($r = 0.900$, $p < 0.01$).

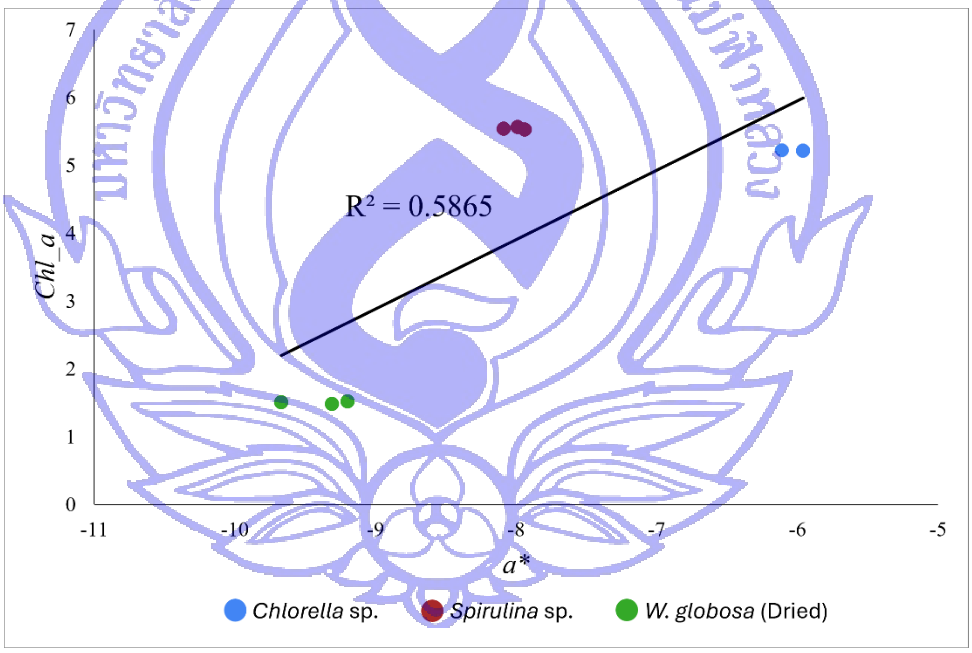

Figure 3 Relationship between a^* value and chlorophyll-a content. This pair demonstrates a significant positive correlation ($r = 0.766$, $p < 0.05$).

Discussion

In this study, the evaluation focuses on the comparative chlorophyll extraction of *Wolffia globosa* (dried), *Chlorella* sp. and *Spirulina* sp. using standardized spectrophotometric analysis. The overall statistical results indicated significant variance ($p < 0.05$) in pigment yields across the species. *Chlorella* sp. exhibited the highest total chlorophyll yield at 7.10 ± 0.05 mg/g DW. This aligns with established industrial benchmarks (Abreu et al., 2023; Yuan et al., 2024). Researchers like Garcia et al. (2019) and Ferreira et al. (2022) point out that advanced industrial micronization effectively disrupts its rigid cell wall to increase the surface area-to-solvent ratio and release pigments trapped within the matrix. Following that, *Spirulina* sp. showed 5.36 ± 0.02 mg/g DW with high chlorophyll-a but only trace chlorophyll-b (0.14 ± 0.03 mg/g DW). Because it is a prokaryotic cyanobacterium, it lacks eukaryotic pathways for chlorophyll-b synthesis. Verma et al. (2024) also notes that its high extractability is attributed to its fragile peptidoglycan membrane which allows rapid solvent penetration.

In contrast, dehydrated *Wolffia globosa* yielded the lowest total chlorophyll at 1.96 ± 0.01 mg/g DW. However, it possessed significantly more chlorophyll-b than *Spirulina* sp. because as a higher plant it utilizes chlorophyll-b for light harvesting (Masuda & Fujita, 2008). Baek et al. (2021) and Dhamaratana et al. (2025) suggest that the lower yield in *Wolffia globosa* is fundamentally attributed to its complex multicellular structure composed of hemicellulose and pectic polysaccharides which may act as a mass transfer barrier. Aside from the plant matrix, Patel et al. (2022) highlights that traditional semi-open pond cultivation in Thailand involves inconsistent nutrient availability, while seasonal fluctuations in sunlight can also lead to lower pigment density compared to highly controlled closed systems (Baek et al., 2021). Furthermore, the decision to maintain the biomass as an intact whole-flake matrix to avoid thermal degradation likely contributed to the limited recovery through passive osmotic diffusion (Barros de Medeiros et al., 2021). Interestingly, a significant 'Optical Paradox' was observed by CIELAB evaluation. Dehydrated *Wolffia globosa* showed the highest physical greenness ($a^* = -9.39 \pm 0.25$) despite having the lowest chemical yield. This suggests that surface color intensity does not always reflect chemical extractability when undisrupted cellular architecture is present. Statistical

Pearson correlation analysis supported this, showing a strong link between a^* and chlorophyll-a ($r = 0.766, p < 0.05$) and an exceptionally strong link between b^* and chlorophyll-b ($r = 0.900$, $p < 0.01$). This suggests that the b^* value is a highly sensitive and precise indicator of pigment variations. Furthermore, the slight increase in the b^* value after dehydration under a controlled temperature of 50°C (Inta & Lertworasirikul, 2022) likely reflects a minor heat-induced conversion of native chlorophyll into pheophytin, a degradation process described by Hayes and Ferruzzi (2020). In terms of processing, Zhou et al. (2025) highlights a critical trade-off between chemical yield and stability, as mechanical grinding triggers degradation and destroys visual quality. Advanced non-thermal methods like Ultrasound-Assisted Extraction (UAE) could bypass structural barriers without excessive heat, but they involve higher economic costs (Jain et al., 2021; Sun et al., 2024).

However, a notable limitation of this study involves the processing and storage conditions. The fixed dehydration protocol (50°C for 8 hours) was necessary for sample standardization. Nevertheless, prolonged heat and subsequent storage environments can induce partial chlorophyll degradation, which is known as pheophytinization. Because chlorophyll is highly sensitive to external factors, variations in temperature and moisture during storage may negatively affect pigment stability. These factors might have partially reduced the final extractable chlorophyll yields. Therefore, the results reflect the pigment profile specifically under these processing and storage constraints.

Nevertheless, while the 1.96 mg/g DW yield of *Wolffia globosa* is lower than the highly micronized *Chlorella* sp., it still falls within a highly competitive range for natural food-grade supplements. According to previous studies, normal human dietary chlorophyll intake from standard green vegetables is estimated at 26 to 86 mg per day (Hayes & Ferruzzi, 2020; Li et al., 2019). The retained chlorophyll in *Wolffia globosa* suggests that a standard serving size of this minimally processed plant can significantly contribute to this daily requirement. This supports its potential to commercially compete with established microalgae as a natural superfood. Ultimately, these findings support the potential of *Wolffia globosa* as a sustainable superfood with a diverse nutrient profile including 20–48% DW protein, essential amino acids, bioavailable vitamin B12 and iron (Appenroth et al., 2018; Sela et al., 2020). Tagauov et al. (2023) and Yaskolka Meir et al. (2019) demonstrated its synergistic potential to increase hemoglobin and maintain iron homeostasis in humans, which highlights its promise as a high-value functional ingredient. As cultivation and processing standards improve, this rapidly growing plant **has** a strong potential to evolve from a local Thai resource into a standardized international functional food.

Suggestions for Future Research

Based on the current findings, future studies should consider the following:

1. Controlled-environment cultivation may be performed to optimize temperature, pH value, or essential nutrient levels for maximizing chlorophyll synthesis at the source.

2. High-resolution analytical tools, such as HPLC-MS, and the use of varying solvent polarities should be utilized for more precise pigment profiling and complete bioactive results.

3. Total non-thermal processing methods including freeze-drying and cryogenic milling, as well as thermal 'sweet spot' technologies like spray-drying may be explored to prevent pheophytinization and improve the sensory texture of the biomass.

4. To expand the current research toward clinical validation, *in vivo* trials should be performed to measure the bioavailability and actual metabolic uptake of chlorophyll in human subjects. Although *Wolffia globosa* contains a significant amount of chlorophyll, its intact cellular structure may act as a mass transfer barrier. Therefore, it is crucial to verify how effectively the human digestive system can break down this plant matrix and absorb the nutrients.

5. Cost-effective, SME-friendly stabilization frameworks may be developed to elevate *Wolffia globosa* into an international superfood standard within the Thai bioeconomy.

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