ผลของการเตรียมเบื้องต้นด้วยสารละลายแมกนีเซียมซัลเฟตต่อฤทธิ์ต้านอนุมูลอิสระและปริมาณคลอโรฟิลล์ของผำอบแห้ง

Effect of Magnesium Sulfate Solution Pretreatment on Antioxidant Properties and Chlorophyll Content of Dried Wolffia (*Wolffia globosa*)

ปภากร ศรีสอน[1*] พีรรัตน์ ดวงติ๊บ[1] และ กานต์รวี ฮั่นสกุล[1]

Paphakorn Srisorn[1*], Peerarat Doungtip[1] and Kanrawee Hunsakul[1]

[1]คณะวิทยาศาสตร์และเทคโนโลยี มหาวิทยาลัยเทคโนโลยีราชมงคลตะวันออก จังหวัดชลบุรี

[1]Faculty of Science and Technology, Rajamangala University of Technology Tawan-ok, Chonburi Province

*Corresponding Author: paphakorn_sr@rmutto.ac.th

Received: September 28, 2025 **Revised:** August 01 2026 **Accepted:** August 19, 2026

บทคัดย่อ

ผำ (*Wolffia globosa*) ซึ่งเป็นพืชน้ำที่อุดมไปด้วยสารอาหารและมีถิ่นกำเนิดในภูมิภาคเอเชียตะวันออกเฉียงใต้กำลังได้รับความสนใจในฐานะแหล่งโปรตีนและสารออกฤทธิ์ทางชีวภาพที่ยั่งยืน อย่างไรก็ตามกระบวนการแปรรูปหลังการเก็บเกี่ยวมักส่งผลกระทบต่อลักษณะปรากฏและสมบัติเชิงหน้าที่ งานวิจัยนี้จึงมุ่งศึกษาผลของการเตรียมการเบื้องต้น (pretreatment) ด้วยสารละลายแมกนีเซียมซัลเฟต ($MgSO_4$) ที่มีต่อการรักษาสีและสมบัติการต้านอนุมูลอิสระของผำอบแห้ง วิธีการศึกษาทำโดยนำผำสดมาลวกในสารละลาย $MgSO_4$ ที่ระดับความเข้มข้นร้อยละ 0.1, 0.3, 0.5 และ 0.7 (น้ำหนักต่อปริมาตร) ก่อนนำไปอบแห้งภายใต้สภาวะควบคุม จากนั้นทำการประเมินค่าพารามิเตอร์สี ได้แก่ ค่าความสว่าง (L*) ค่าความเป็นสีแดง/เขียว (a*) และค่าความเป็นสีเหลือง/น้ำเงิน (b*) ด้วยเครื่องสเปกโตรโฟโตมิเตอร์ พร้อมทั้งประเมินฤทธิ์การต้านอนุมูลอิสระด้วยวิธี DPPH radical scavenging assay และ FRAP assay ผลการศึกษาพบว่าสิ่งทดลองที่ใช้ความเข้มข้นของสารละลาย $MgSO_4$ ร้อยละ 0.7 (น้ำหนักต่อปริมาตร) จะให้ปริมาณคลอโรฟิลล์รวมสูงสุด (6,147.31 มิลลิกรัมต่อกิโลกรัม) แต่การคงอยู่ของคลอโรฟิลล์นี้ไม่ได้ส่งผลต่อการรักษาสภาพสี การเตรียมการเบื้องต้นด้วย $MgSO_4$ ทำให้ค่าความสว่าง (L*) และความเป็นสีเขียวลดลงอย่างมีนัยสำคัญ (โดยมีค่า a* ติดลบน้อยลงเมื่อเทียบกับสิ่งทดลองควบคุม) ($p < 0.05$) นอกจากนี้กระบวนการดังกล่าวยังทำให้ปริมาณสารประกอบฟีนอลิกทั้งหมดและความสามารถในการต้านอนุมูลอิสระลดลงอย่างมีนัยสำคัญเมื่อเปรียบเทียบกับผำอบแห้งที่ไม่ผ่านการลวก ($p < 0.05$) การค้นพบนี้ชี้ให้เห็นว่าแม้การลวกด้วยสารละลาย $MgSO_4$ จะช่วยปกป้องคลอโรฟิลล์ในระดับเคมี แต่กลับส่งผลเชิงลบต่อลักษณะปรากฏด้านความเป็นสีเขียว ความสว่าง และคุณสมบัติการต้านอนุมูลอิสระ ดังนั้นการนำกระบวนการเตรียมการเบื้องต้นนี้ไปใช้จึงควรประเมินถึงความคุ้มค่าระหว่างการรักษาเสถียรภาพของคลอโรฟิลล์กับการสูญเสียคุณภาพทางด้านสีและสารประกอบเชิงหน้าที่

คำสำคัญ: ผำ, สารละลายแมกนีเซียมซัลเฟต, การรักษาสี, สมบัติการต้านอนุมูลอิสระ, ปริมาณคลอโรฟิลล์

Abstract

Wolffia globosa, a nutrient-dense aquatic plant native to Southeast Asia, has gained attention as a sustainable source of protein and bioactive compounds. However, post-harvest processing often compromises its visual appeal and functional properties. This study investigates the effect of magnesium sulfate ($MgSO_4$) solution pretreatment on color retention and antioxidant properties of dried Wolffia. Fresh Wolffia were blanched in varying concentrations of $MgSO_4$ at 0.1%, 0.3%, 0.5%, and 0.7% w/v and dried under controlled conditions. Color parameters including lightness (L*), redness/greenness (a*), and yellowness/blueness (b*) were measured using a spectrophotometer, while antioxidant activity was assessed via DPPH radical scavenging assay and FRAP assay. Results indicated that while the 0.7% w/v $MgSO_4$ treatment yielded the highest total chlorophyll content (6,147.31 mg/kg), this chemical retention did not

translate to color preservation. $MgSO_4$ pretreatments significantly decreased lightness (L*) and greenness (resulting in less negative a* values compared to the unblanched control) ($p < 0.05$). Furthermore, the pretreatments caused a significant reduction in total phenolic content and antioxidant capacity compared to the control dried Wolffia ($p < 0.05$). These findings suggest that while $MgSO_4$ blanching effectively protects chlorophyll chemically, it negatively impacts the greenness, lightness, and antioxidant properties of dried Wolffia. Therefore, the trade-off between chlorophyll stabilization and the loss of color quality and functional compounds must be carefully evaluated for product applications.

Keywords: Wolffia, Magnesium sulfate solution, Color retention, Antioxidant properties, Chlorophyll content

1. Introduction

Wolffia globosa, commonly known as watermeal, is the smallest flowering plant on Earth and a promising source of plant-based protein, dietary fiber, and bioactive compounds. Indigenous to Southeast Asia, Wolffia has long been consumed in traditional diets and is now gaining global attention as a sustainable ingredient for functional foods and nutraceuticals due to its nutritional composition and potential for human food applications (Appenroth et al., 2018). Its rapid growth rate, minimal land requirement, and high nutritional density position it as a strategic crop for addressing food security and environmental sustainability (Appenroth et al., 2018; Masavang et al., 2022).

Despite its nutritional potential, the commercialization of Wolffia-based products faces challenges related to post-harvest stability, particularly in maintaining visual appeal and bioactivity during drying and storage. Color degradation and loss of antioxidant capacity are common issues that compromise consumer acceptance and functional efficacy (Oliveira et al., 2016). These changes are often driven by enzymatic browning, pigment oxidation, chlorophyll degradation, and structural damage during thermal processing or inappropriate drying conditions, which may reduce nutritional value and bioactive compounds in dried duckweed products (Kaur et al., 2018; Prosridee et al., 2023). Therefore, pretreatment strategies that can improve color retention while minimizing the loss of bioactive compounds are important for the development of dried Wolffia products (Minh et al., 2019).

Magnesium sulfate ($MgSO_4$), a food-safe inorganic salt, has been investigated for its potential role in pigment stabilization, particularly in relation to chlorophyll-containing materials. Mg^{2+} ions are associated with the structural stability of chlorophyll, in which magnesium is located at the center of the porphyrin ring; displacement of this central Mg^{2+} during processing can promote the conversion of chlorophyll to pheophytin, resulting in dull green or brownish discoloration (Bohn et al., 2004). Previous studies have suggested that $MgSO_4$ or salt-assisted blanching can help retain color and chlorophyll content in dried Wolffia, although its effect on antioxidant-related properties may vary depending on processing conditions and the leaching of water-soluble phenolic compounds during blanching (Workneh et al., 2014; Khan et al., 2022; Doungtip et al., 2025).

Although $MgSO_4$ has been reported to contribute to pigment retention, its application as a pretreatment for aquatic plants such as *Wolffia globosa* remains insufficiently clarified, particularly when visual quality and functional properties are evaluated together. In addition, limited information is available on the trade-off between chlorophyll retention and the possible reduction of phenolic content and antioxidant activity caused by blanching. Therefore, this study aimed to evaluate the effects of $MgSO_4$ solution pretreatment at different concentrations on color characteristics, water activity, proximate composition, total phenolic content, antioxidant properties, and chlorophyll content of dried *Wolffia*

globosa. The findings are expected to provide useful information for optimizing pretreatment conditions that balance visual quality and functional properties in dried Wolffia products.

2. Materials and methods

2.1 Preparation of Dried Wolffia

Fresh *Wolffia globosa* samples were collected from Tonkla Bon Din Community Enterprise, Ban Bueng, Chonburi and thoroughly rinsed with distilled water to remove surface impurities. The samples were drained to remove excess surface water before treatment. The study was designed to evaluate one control and four $MgSO_4$ pretreatment conditions. The control consisted of fresh Wolffia that was rinsed and drained without $MgSO_4$ pretreatment, while the treated samples were blanched in $MgSO_4$ solutions at concentrations of 0.1%, 0.3%, 0.5%, and 0.7% (w/v). A fixed sample-to-solution ratio of 500 g of powder / 1 L of solution was used for all treatments. Each treatment involved immersing the samples in the respective $MgSO_4$ solution at 80 °C for 3 min. After blanching, the samples were immediately cooled in cold water (4–8 °C) for 10 min and drained for 30 min to ensure uniform moisture removal before drying. All samples were dried in a hot-air oven at 50 °C for 8 h or until the samples reached a moisture content of 10%. The dried samples were then ground using a laboratory grinder and passed through a 100-mesh sieve. The ground samples were stored in aluminum foil pouches protected from light at 4-8 °C until analysis.

2.2 Percent Yield of Dried Wolffia

The percent yield of dried Wolffia was calculated to determine the mass recovery after pretreatment and drying. The initial fresh weight of each sample was recorded before pretreatment, and the dried sample weight was recorded after hot-air drying. The percent yield was calculated using the following equation:

$$\text{Yield (\%)} = \frac{\text{Dried sample weight}}{\text{Initial fresh weight}} \times 100 \quad (1)$$

2.3 Color Analysis Using the CIE L*a*b* System

Color parameters—Lightness (L*), redness/greenness (a*), and yellowness/blueness (b*), were measured using a spectrophotometer (CM-3500d, Konica Minolta, Japan). Color values were expressed according to the CIE L*a*b* system, where L* represents lightness, a* indicates redness (+a*)/greenness (–a*), and b* indicates yellowness (+b*)/blueness (–b*). Dried Wolffia powder that had passed through a 100-mesh sieve was placed in a flat, opaque sample holder to minimize external light interference and ensure uniform sample presentation. The surface was leveled to minimize shadows or uneven reflection, allowing for consistent instrumental color measurement of the powder samples. Color measurements were performed at 3 different points in triplicate for each treatment, and the results were expressed as mean ± standard deviation.

2.4 Water Activity Analysis

Water activity (a_w) of dried Wolffia samples was measured using a water activity meter (Series 3 TE, Aqua Lab, USA) at temperature, at 25 ± 2 °C. Dried Wolffia powder that had passed through a 100-mesh sieve was placed in the sample cup to an appropriate depth according to the instrument requirements, and measurements were performed in triplicate for each treatment. Values are expressed on a scale from 0 to 1, where 1.0 represents pure water. The results were reported as mean ± standard deviation.

2.5 Chemical Composition Analysis

Moisture, ash, crude protein, crude fat, and crude fiber contents were determined according to the Official Methods of Analysis of AOAC INTERNATIONAL (2023) using AOAC Official Methods 925.10, 923.03, 981.10, 920.39, and 962.09, respectively. Protein content was determined using a nitrogen-to-protein conversion factor of 6.25. Carbohydrate content was calculated by difference as follows:

$$\text{Carbohydrate (\%)} = 100 - [\text{moisture (\%)} + \text{protein (\%)} + \text{fat (\%)} + \text{ash (\%)} + \text{crude fiber (\%)}] \quad (2)$$

All analyses were performed in triplicate, and the results were expressed as mean ± standard deviation.

2.6 Antioxidant Properties

Dried Wolffia powder extract was prepared using a modified method described by Yadav et al. (2024). Briefly, 0.2 g of dried Wolffia powder was extracted with 10 mL of distilled water. The mixture was sonicated at 4 ± 2 °C, frequency of about 120 kHz for 25 min, followed by centrifugation at 9,000 rpm for 15 min at 4 ± 2 °C. The supernatant was collected and used for the analysis of antioxidant properties and total phenolic content. All analyses were performed in triplicate, and the results were expressed as mean ± standard deviation.

2.6.1 DPPH Radical Scavenging Assay

DPPH radical scavenging activity was determined according to Maduwanthi & Marapana (2021), with slight modifications. The extract (0.1 mL) was mixed with 0.02 mM DPPH solution (10%) in ethanol, (1 mL), vortexed, and incubated in the dark for 30 min at room temperature. Absorbance was measured at 517 nm using a spectrophotometer (UVmini-1240, Shimadzu, Japan). Trolox 10 – 100 µg/ml was used to construct the standard curve, and the results were expressed as mg Trolox equivalent/g sample.

2.6.2 Ferric Reducing-Antioxidant Power (FRAP) Assay

FRAP assay was performed according to Kubola & Siriamornpun (2008), with slight modifications. The FRAP reagent was prepared by mixing 300 mM acetate buffer, 20 mM $FeCl_3 \cdot 6H_2O$, and 10 mM TPTZ in 40 mM HCl at a ratio of (ratio, e.g., 10:1:1). The extract (20 µL) was mixed with FRAP reagent (150 µL) and 30 µL of acetate buffer and incubated at 37 °C for 4 min. Absorbance was measured at 593 nm using a spectrophotometer. Ferrous sulfate (0.1–1.0 mM) was used to construct the standard curve, and the results were expressed as µmol Fe(II)/g sample.

2.6.3 Total Phenolic Content

Total phenolic content was determined using a modified Folin–Ciocalteu colorimetric method described by Wolfe et al. (2003). The extract (20 µL) was mixed with 10% of Folin–Ciocalteu reagent (110 µL) and allowed to react for 6 min. Then, 10% of sodium carbonate solution (70 µL) was added, and the mixture was incubated for 30 min at room temperature in the dark. Absorbance was measured at 765 nm using a spectrophotometer. Gallic acid (15–270 µg/ml) was used to construct the standard curve, and the results were expressed as mg gallic acid equivalent/g sample.

2.7 Total Chlorophyll Content

Chlorophyll a, chlorophyll b, and total chlorophyll contents were determined according to Rajput & Patil (2017), with slight modifications. Briefly, 1.0 g of dried Wolffia powder was extracted with 80% acetone under light-protected conditions and centrifuged at 5,000 rpm for 5 min. The supernatant was collected and adjusted to a final volume of 100 mL with 80% acetone. The absorbance of the extract was measured at 645 and 663 nm using a spectrophotometer. All analyses were performed in triplicate. To align

with the reported results, chlorophyll contents were calculated and expressed as milligrams per kilogram (mg/kg) of sample using the following equations:

$$\text{Chlorophyll a (mg/kg)} = [(12.7 \times A663) - (2.69 \times A645)] \times \frac{V}{W} \quad (3)$$

$$\text{Chlorophyll b (mg/kg)} = [(22.9 \times A645) - (4.68 \times A663)] \times \frac{V}{W} \quad (4)$$

$$\text{Total chlorophyll (mg/kg)} = [(20.2 \times A645) + (8.02 \times A663)] \times \frac{V}{W} \quad (5)$$

where

A645 and A663 are the absorbance values measured at 645 and 663 nm, respectively

V is the final volume of the extract (mL)

W is the sample weight (g).

2.8 Statistical Analysis

The experiment was designed using a completely Randomized Design (CRD). Data were expressed as mean ± standard deviation (SD) from triplicate analyses (n = 3), unless otherwise stated. Statistical differences among treatments were analyzed using one-way analysis of variance (ANOVA) at a significance level of $p < 0.05$. Differences among treatment means were further evaluated using Duncan's multiple range test. Statistical analyses were performed using IBM SPSS Statistics version 27.0 (IBM Corp., Armonk, NY, USA).

3. Results

3.1 Percent yield and water activity of dried Wolffia

After drying, the percent yield of dried Wolffia decreased significantly with increasing $MgSO_4$ concentration (Table 1). The untreated dried Wolffia (control) sample showed the highest yield (%) of 3.74 ± 0.05. As the $MgSO_4$ concentration increased from 0.1% to 0.7%, the yield continuously declined from 3.63 ± 0.09 to the lowest value of 3.22 ± 0.02. In contrast, the water activity (a_w) values of all $MgSO_4$-treated samples were significantly higher than that of the untreated sample (0.49 ± 0.01). However, the a_w values did not increase proportionally with the $MgSO_4$ concentration. The highest a_w value was observed in the 0.1% $MgSO_4$ treatment (0.58 ± 0.01). When the $MgSO_4$ concentration increased to 0.3%, 0.5%, and 0.7%, the a_w values decreased slightly to 0.53 ± 0.01, 0.53 ± 0.01, and 0.52 ± 0.01, respectively, but remained higher than the control.

Table 1. Percent yield and water activity (a_w) of dried Wolffia under different $MgSO_4$ blanching concentrations

Treatment	Yield (%)	a_w
Dried Wolffia	3.74 ± 0.05^{a}	0.49 ± 0.01^{c}
$MgSO_4$ 0.1 %	3.63 ± 0.09^{b}	0.58 ± 0.01^{a}
$MgSO_4$ 0.3 %	3.53 ± 0.04^{c}	0.53 ± 0.01^{b}
$MgSO_4$ 0.5 %	3.34 ± 0.02^{d}	0.53 ± 0.01^{b}
$MgSO_4$ 0.7 %	3.22 ± 0.02^{e}	0.52 ± 0.01^{b}

Note: Data are expressed as mean ± standard deviation (n=3). Different superscript letters (a–e) within the same column indicate significant differences ($p < 0.05$).

3.2 Color analysis using CIE L*, a*, b* system of dried Wolffia

After drying, $MgSO_4$ blanching significantly affected the color values of dried Wolffia (Table 2). Compared with the untreated dried Wolffia, all $MgSO_4$-treated samples exhibited significantly lower L* values (ranging from 15.41 ± 0.29 to 16.89 ± 0.33, compared to 21.88 ± 0.18 for the control), indicating reduced lightness and a darker appearance. The b* values also decreased after $MgSO_4$ treatment (from 20.66 ± 0.25 to a range of 14.35–15.14), suggesting a reduction in yellowness**.** For the a* value, all samples remained in the negative range, indicating the presence of green pigment. However, $MgSO_4$-treated samples showed significantly less negative a* values (-3.18 to -4.16) than the untreated control (-5.40). Since all measured a* values were well below zero, this shift must be interpreted strictly as reduced greenness rather than an increase in redness. The visual appearance of the samples shown in Figure 1 is highly consistent with these instrumental color results. As depicted, the untreated dried Wolffia (T1) displays a vibrant, lighter green color, which directly corresponds to its higher L* (lightness), higher b* (yellowness), and more negative a* (stronger greenness) values. In contrast, the $MgSO_4$-treated samples (T2–T5) generally appear visually darker, duller, and less intensely green, accurately reflecting the objective spectrophotometric measurements of reduced lightness, reduced greenness, and reduced yellowness.

Table 2. Color values (L*, a*, and b*) of dried Wolffia under different $MgSO_4$ blanching concentrations

Treatment	Color values		
	L*	a*	b*
Dried Wolffia	21.88±0.18^{a}	-5.40±0.05^{d}	20.66±0.25^{a}
$MgSO_4$ 0.1 %	15.41±0.29^{c}	-3.18±0.04^{a}	15.14±0.23^{b}
$MgSO_4$ 0.3 %	16.89±0.33^{b}	-3.74±0.04^{b}	14.83±0.11^{b}
$MgSO_4$ 0.5 %	16.24±0.09^{b}	-4.16±0.02^{c}	14.35±0.02^{c}
$MgSO_4$ 0.7 %	16.22±0.62^{b}	-4.13±0.08^{c}	14.89±0.29^{b}

Note: Data are expressed as mean ± standard deviation (n=3). Different superscript letters (a–d) within the same column indicate significant differences ($p < 0.05$).



Dried Wolffia | $MgSO_4$ 0.1 % | $MgSO_4$ 0.3 % | $MgSO_4$ 0.5 % | $MgSO_4$ 0.7 %

Figure 1. Appearance of dried Wolffia under different $MgSO_4$ blanching concentrations

3.3 Chemical composition of dried Wolffia

After drying, the proximate composition of dried Wolffia was significantly affected by the $MgSO_4$ blanching treatments (Table 3). It was observed that blanching with higher concentrations of $MgSO_4$ solution resulted in a significant increase in carbohydrate content. The carbohydrate content rose continuously from 50.42 ± 0.01 in the untreated dried Wolffia to the highest value of 55.07 ± 0.03 in the

0.7% $MgSO_4$ treatment. Conversely, increasing the $MgSO_4$ concentration led to a significant decrease in protein content, which declined from 18.49 ± 0.02% in the control to 16.06 ± 0.01 in the 0.7% treatment. Additionally, the ash and crude fiber contents generally decreased at higher $MgSO_4$ concentrations (0.3%–0.5%) compared to the 0.1% treatment, although the crude fiber content exhibited a notable recovery at the 0.7% concentration (11.25 ± 0.02).

Table 3. Proximate composition of dried Wolffia samples under different $MgSO_4$ blanching concentrations.

Treatment	Moisture (%)	Protein (%)	Fat (%)	Ash (%)	Fiber (%)	Carbohydrate (%)
Dried Wolffia	10.45 ± 0.05^{b}	18.49 ± 0.02^{a}	3.92 ± 0.01^{c}	4.91 ± 0.01^{a}	12.16 ± 0.01^{a}	50.07 ± 0.01^{e}
$MgSO_4$ 0.1 %	10.80 ± 0.10^{a}	17.55 ± 0.06^{b}	4.89 ± 0.01^{ab}	4.56 ± 0.01^{b}	11.25 ± 0.02^{b}	61.75 ± 0.05^{d}
$MgSO_4$ 0.3 %	10.35 ± 0.05^{bc}	16.92 ± 0.10^{c}	4.90 ± 0.01^{a}	4.34 ± 0.01^{c}	10.73 ± 0.01^{c}	64.23 ± 0.07^{c}
$MgSO_4$ 0.5 %	10.38 ± 0.02^{bc}	16.50 ± 0.10^{d}	4.81 ± 0.01^{b}	4.30 ± 0.01^{c}	9.61 ± 0.01^{d}	64.96 ± 0.19^{b}
$MgSO_4$ 0.7 %	10.32 ± 0.02^{c}	16.06 ± 0.01^{e}	3.00 ± 0.10^{d}	4.30 ± 0.02^{c}	9.43 ± 0.01^{e}	65.39 ± 0.02^{a}

Note: Data are expressed as mean ± standard deviation (n=3). Different superscript letters (a–e) within the same column indicate significant differences ($p < 0.05$).

3.4 Total phenolic content and antioxidant properties of dried Wolffia

The total phenolic content and antioxidant properties of dried Wolffia samples are presented in Table 4. The untreated dried Wolffia showed the highest total phenolic content (4.25 ± 0.05 mg GAE/g sample) and DPPH radical scavenging activity (4.50 ± 0.18 mg Trolox eq./g sample). $MgSO_4$ blanching generally reduced total phenolic content and DPPH activity compared with the untreated sample. Among the $MgSO_4$-treated samples, the lowest total phenolic content and DPPH activity were observed in the 0.5% $MgSO_4$ treatment, whereas the 0.7% $MgSO_4$ treatment showed slightly higher values than the 0.5% treatment.

For FRAP activity, the untreated dried Wolffia exhibited the highest value (44.73 ± 0.43 μmol Fe(II)/g sample). The 0.1% $MgSO_4$ treatment showed a comparable FRAP value (43.76 ± 0.68 μmol Fe(II)/g sample) and was not significantly different from the untreated sample. However, FRAP values decreased markedly in the 0.3–0.7% $MgSO_4$ treatments, with the lowest value observed in the 0.5% $MgSO_4$ treatment. Overall, $MgSO_4$ blanching, particularly at concentrations of 0.3% and above, was associated with reduced phenolic content and antioxidant activity in dried Wolffia.

Table 4. Total phenolic content and antioxidant properties of dried Wolffia.

Treatment	Total phenolic content (mg GAE/g sample)	Antioxidant activity	
		DPPH (mg Trolox eq./g sample)	FRAP (μmol Fe(II)/g sample)
Dried Wolffia	$4.25\pm.0.05^{a}$	4.50 ± 0.18^{a}	44.73 ± 0.43^{a}
$MgSO_4$ 0.1 %	4.20 ± 0.02^{b}	4.10 ± 0.02^{b}	43.76 ± 0.68^{a}
$MgSO_4$ 0.3 %	2.20 ± 0.02^{c}	1.87 ± 0.10^{c}	19.48 ± 0.74^{b}
$MgSO_4$ 0.5 %	1.40 ± 0.01^{e}	1.02 ± 0.03^{e}	11.91 ± 0.10^{d}
$MgSO_4$ 0.7 %	1.89 ± 0.02^{d}	1.56 ± 0.02^{d}	15.87 ± 0.62^{c}

Note: Data are expressed as mean ± standard deviation (n=3). Different superscript letters (a–e) within the same column indicate significant differences ($p < 0.05$).

3.5 Total chlorophyll content of dried Wolffia

The chlorophyll a, chlorophyll b, and total chlorophyll contents of dried Wolffia samples are shown in Table 5. Overall, $MgSO_4$ blanching treatments significantly enhanced the retention of both chlorophyll fractions compared to the untreated control ($p < 0.05$). In the untreated dried Wolffia, chlorophyll a and chlorophyll b contents were 2,862.05 ± 0.01 mg/kg and 1,172.17 ± 0.02 mg/kg, respectively, resulting in a total chlorophyll content of 4,034.21 ± 0.11 mg/kg. For the $MgSO_4$-treated samples, both chlorophyll a and chlorophyll b levels were consistently higher than those of the control. Across all treatments, chlorophyll a remained the dominant pigment fraction compared to chlorophyll b. The 0.7% $MgSO_4$ treatment demonstrated the most effective pigment preservation, yielding the highest concentrations of chlorophyll a (3,906.66 ± 0.01 mg/kg), chlorophyll b (2,240.65 ± 0.02 mg/kg), and total chlorophyll (6,147.31 ± 0.01 mg/kg). Interestingly, the pigment retention did not follow a simple linear concentration-dependent trend; while the 0.3% $MgSO_4$ treatment showed highly effective retention (chlorophyll a: 3,796.73 ± 0.01 mg/kg; chlorophyll b: 2,109.86 ± 0.02 mg/kg), a significant drop occurred at 0.5% $MgSO_4$ before reaching the maximum peak at 0.7%. These results indicate that $MgSO_4$ blanching, particularly at 0.7%, successfully protects both chlorophyll a and b molecules from degradation during the drying process.

Table 5. Total chlorophyll content of dried Wolffia

Treatment	Chlorophyll a (mg/kg)	Chlorophyll b (mg/kg)	Total chlorophyll (mg/kg)
Dried Wolffia	2862.05±0.01^{e}	1172.17±0.02^{d}	4034.21±0.11^{e}
$MgSO_4$ 0.1 %	3280.77±0.17^{d}	1069.09±0.57^{e}	4349.85±0.05^{d}
$MgSO_4$ 0.3 %	3796.73±0.01^{b}	2109.86±0.02^{b}	5906.59±0.04^{b}
$MgSO_4$ 0.5 %	3315.41±0.01^{c}	1530.41±0.01^{c}	4845.82±0.02^{c}
$MgSO_4$ 0.7 %	3906.66±0.01^{a}	2240.65±0.02^{a}	6147.31±0.01^{a}

Note: Data are expressed as mean ± standard deviation (n=3). Different superscript letters (a–e) within the same column indicate significant differences ($p < 0.05$).

4. Discussion

The application of magnesium sulfate ($MgSO_4$) blanching pretreatments significantly influenced the physicochemical properties, proximate composition, and visual characteristics of dried Wolffia. The observed decrease in percentage yield at higher $MgSO_4$ concentrations could be attributed to the potential loss of dry mass and the leaching of water-soluble compounds into the blanching medium. This leaching mechanism might also explain the alterations in proximate composition; as $MgSO_4$ concentration increased, protein content significantly decreased from 18.49% to 16.06%, while the relative carbohydrate proportion increased continuously from 50.42% to 55.07%. Regarding water activity (aw), all $MgSO_4$-treated samples exhibited higher aw values compared to the unblanched control. A possible explanation for this anomaly is that the combined thermal treatment and ionic stress might have altered the plant tissue structure, potentially facilitating the release of previously bound water into the free water pool. However, within the treated groups, increasing the $MgSO_4$ concentration from 0.1% to 0.7% resulted in a slight progressive decrease in aw, which could reflect a concentration-dependent interaction between the sulfate salt and water molecules (Fomenko et al., 2025).

Despite the physical and compositional alterations, chemical analysis indicated that $MgSO_4$ blanching, particularly at 0.7%, successfully retained higher levels of total chlorophyll compared to the untreated control (Negi & Roy, 2000). It is hypothesized that the presence of exogenous Mg^{2+} ions during thermal processing might help stabilize the chlorophyll structure and reduce its conversion to pheophytin, although further mechanistic studies are required to confirm this direct ionic substitution or protection in Wolffia. Interestingly, this enhanced chemical retention of chlorophyll did not translate to improved visual green coloration. Both instrumental color analysis and visual observations demonstrated that $MgSO_4$-treated samples were significantly darker and less green than the control, as evidenced by decreased lightness (L*) and less negative a* values. This discrepancy between chemical pigment content and visual appearance could possibly be driven by non-enzymatic browning reactions during the extended drying process, or by structural changes that altered surface light reflectance.

Furthermore, a significant trade-off was observed between chlorophyll retention and the preservation of bioactive compounds. The $MgSO_4$-treated dried Wolffia exhibited significantly lower total phenolic content (TPC) and antioxidant activities (DPPH and FRAP) compared to the unblanched control. While blanching is theoretically utilized to inactivate oxidative enzymes, the exposure to 80 °C for 3 mins combined with an osmotic gradient from the salt solution might have facilitated the extensive leaching of water-soluble phenolic compounds into the blanching water (Kim et al., 2013; Pichairat & Mahea, 2014). The magnitude of this leaching loss profoundly outweighed any potential benefits of enzymatic inhibition. Ultimately, while 0.7% $MgSO_4$ pretreatment effectively maximizes chemical chlorophyll concentration, it negatively impacts the overall visual lightness, visual greenness, and functional antioxidant capacity of the final dried product.

5. Conclusions and recommendations

Blanching with magnesium sulfate ($MgSO_4$) solution significantly impacts the quality parameters of dried Wolffia. While the treatment, particularly at 0.7% w/v $MgSO_4$, effectively retains the highest levels of chemically extracted total chlorophyll, this chemical retention does not translate to overall visual color preservation. The $MgSO_4$ pretreatment resulted in dried products that were visually darker and less green compared to the unblanched control. Furthermore, the pretreatment caused a reduction in overall yield and significantly decreased the total phenolic content and antioxidant capacity, likely due to the leaching of water-soluble bioactive compounds. Consequently, the application of $MgSO_4$ blanching presents a clear trade-off between maximizing chemical chlorophyll retention and preserving both visual greenness and functional antioxidant properties. Future studies should explore alternative processing conditions to better balance these quality attributes and conduct sensory evaluations for further product applications.

6. References

AOAC International. (2023). Official Methods of Analysis of AOAC international (22nd ed.). Oxford University Press. https://doi.org/10.1093/ 9780197610145.001.0001

Appenroth, K.- J., Sree, K. S., Bog, M., Ecker, J., Seeliger, C., Böhm, V., Lorkowski, S., Sommer, K., Vetter, W., Tolzin-Banasch, K., Kirmse, R., Leiterer, M., Dawczynski, C., Liebisch, G., & Jahreis, G. (2018). Nutritional value of the duckweed species of the genus Wolffia (Lemnaceae) as human food. Frontiers in Chemistry, 6, 483. https://doi.org/ 10.3389/fchem.2018.00483

Bohn, T., Walczyk, T., Leisibach, S., & Hurrell, R. F. (2004). Chlorophyll-bound magnesium in commonly consumed vegetables and fruits: Relevance to magnesium nutrition. Journal of Food Science, 69(9), 347-350. https://doi.org/10.1111/j.1365-2621.2004.tb09947.x

Doungtip, P., Srisorn, P., & Hunsakul, K. (2025). Effect of salt solution combined with blanching on color retention and antioxidant activity in dried Wolffia (Wolffia arrhiza). Journal of Food Technology, Siam University, 20(1), 32-42. https://li01.tci-thaijo.org/index.php/JFTSU/article/view/265825/180193

Fomenko, E. V., Anshits, N. N., Akimochkina, G. V., Ivanenko, T. Y., Morozov, E. V., Yumashev, V. V., Solovyov, L. A., Shestakov, N. P., & Shabanov, V. F. (2025). Experimental study on rapeseed drying characteristics with magnesium sulfate as solid desiccant. Molecules, 30(17), 3604. https://doi.org/10.3390/molecules30173604

Kaur, N., Aggarwal, P., & Rajput, H. (2018). Effect of different blanching treatments on physicochemical, phytochemical constituents of cabinet dried broccoli. Chemical Science Review and Letters, 7(25), 262-271. https://www.academia.edu/37690771/Effect_of_Different_Blanching_Treatments_on_Physicochemical_Phytochemical_Constituents_of_Cabinet_Dried_Broccoli

Khan, A. A., Syarifah Adilah, M. Y., Mamat, M. H., Yahaya, S. Z., Setumin, S., Ibrahim, M. N., Daud, K., & Abdullah, M. H. (2022). Magnesium sulfate as a potential dye additive for chlorophyll-based organic sensitiser of the dye-sensitised solar cell (DSSC). Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy, 274, 121140. https://doi.org/10.1016/j.saa.2022.121140

Kim, M. H., Kim, J. M., & Yoon, K. Y. (2013). Effects of blanching on antioxidant activity and total phenolic content according to type of medicinal plants. Food Science and Biotechnology, 22(3), 817-823. https://link.springer.com/article/10.1007/s10068-013-0150-5

Kubola, J., & Siriamornpun, S. (2008). Phenolic contents and antioxidant activities of bitter gourd (Momordica charantia L.) leaf, stem and fruit fraction extracts in vitro. Food Chemistry, 110(4), 881-890. 10.1016/j.foodchem.2008.02.076

Maduwanthi, S.D.T., & Marapana, R.A.U.J. (2021). Total phenolics, flavonoids and antioxidant activity following simulated gastro-intestinal digestion and dialysis of banana (Musa acuminata, AAB) as affected by induced ripening agents. Food Chemistry, 339, 127909. https://doi.org/10.1016/j.foodchem.2020.127909

Masavang, S., Winckler, P., Tira-Umphon, A., & Phahom, T. (2022). New insights into moisture sorption characteristics, nutritional composition, and antioxidant and morphological properties of dried duckweed [Wolffia arrhiza (L.) Wimm]. Journal of the Science of Food and Agriculture, 102(5), 2135-2143. https://doi.org/10.1002/jsfa.11555

Minh, N. P., Nhi, T. T. Y., Lam, T. D. B., Nhat, P. M., & Phuong, N. T. T. (2019). Effect of blanching and drying on quality of dried papaya fruit. Journal of Pharmaceutical Sciences and Research, 11(3), 1082-1086. https://www.proquest.com/scholarly-journals/effect-blanching-drying-on-quality-dried-papaya/docview/2206964729/se-2

Negi, P., & Roy, S.K. (2000). Effect of blanching and drying methods on β-carotene, ascorbic acid and chlorophyll retention of leafy vegetables. LWT-Food Science and Technology, 33(4), 295-298. https://doi.org/10.1006/fstl.2000.0659

Oliveira, S. M., Brandão, T. R. S., & Silva, C. L. M. (2016). Influence of drying processes and pretreatments on nutritional and bioactive characteristics of dried vegetables: A review. Food Engineering Reviews, 8, 134-163. https://doi.org/10.1007/s12393-015-9124-0

Pichairat, D., & Mahea, N. (2014). Effect of blanching on the phenolic content and antioxidant capacities of some Southern Thai indigenous vegetables. Rajamangala University of Technology Srivijaya Research Journal, 6(2), 36-46. https://www.repository.rmutsv.ac.th/bitstream/handle/123456789/1223/FullText.pdf?sequence=1&isAllowed=y

Prosridee, K., Oonsivilai, R., Tira-aumphon, A., Singthong, J., Oonmetta-aree, J., & Oonsivilai, A. (2023). Optimum aquaculture and drying conditions for Wolffia arrhiza (L.) Wimn. Heliyon, 9(9), e19730. https://doi.org/10.1016/j.heliyon.2023.e19730

Rajput, R., & Patil, R. (2017). The comparative study on spectrophotometric analysis of chlorophyll and carotenoids pigments from non-leguminous fodder crops. International Journal of Innovative Science, Engineering & Technology, 4(7), 140-148. https://www.researchgate.net/publication/322356853_The_Comparative_Study_on_Spectrophotometric_Analysis_of_Chlorophyll_and_Carotenoids_Pigments_from_Non-Leguminous_Fodder_Crops

Wolfe, K., Wu, X., & Liu, R. H. (2003). Antioxidant activity of apple peels. Journal of Agricultural and Food Chemistry, 51(3), 609-614. https://doi.org/10.1021/jf020782a

Workneh, T., Zinash, A., & Woldetsadik, K. (2014). Blanching, salting and sun drying of different pumpkin fruit slices. Journal of Food Science and Technology, 51(11), 3114-3123. https://doi.org/10.1007/s13197-012-0835-4

Yadav, N. K., Patel, A. B., Debbarma, S., Priyadarshini, M. B., & Priyadarshi, H. (2024). Characterization of bioactive metabolites and antioxidant activities in solid and liquid fractions of fresh duckweed (Wolffia globosa) subjected to different cell wall rupture methods. ACS Omega, 9(18), 19940-19955. https://pubs.acs.org/doi/10.1021/acsomega.3c09674