Journal of Current Science and Technology, September - December 2024 Vol. 14 No. 3, Article 51

ISSN 2630-0656 (Online)

Cite this article: Somdee, T., Yangyeun, S., Srikongpan, P., Wongprachum, K., Khodseewong, S., Nonsa-ard, R., Chumroenphat, T., & Somdee, T. (2024). Assessment of nutritional, physiochemical, antioxidant and sensory properties of seasoning powder containing *Asplenium unilaterale* lam. *Journal of Current Science and Technology*, *14*(3), Article 51. https://doi.org/10.59796/jcst.V14N3.2024.51

Journal of Current Science and Technology

Journal homepage: https://jcst.rsu.ac.th

Assessment of Nutritional, Physiochemical, Antioxidant and Sensory Properties of Seasoning Powder Containing *Asplenium unilaterale* Lam

Thidarat Somdee[1,*], Suneerat Yangyeun[1], Piyaporn Srikongpan[1], Kasama Wongprachum[1], Sirapat Khodseewong[1], Rujira Nonsa-ard[1], Theeraphan Chumroenphat[2], and Theerasak Somdee[3]

[1]Faculty of Public Health, Mahasarakham University, Maha Sarakham 44150, Thailand
[2]Aesthetic Sciences and Health Program, Faculty of Thai Traditional and Alternative Medicine, Ubon Ratchathani Rajabhat University, Ubon Ratchathani 34000, Thailand
[3]Faculty of Science, Khon Kaen University, Khon Kaen 40000, Thailand

[*]Corresponding author; E-mail: thidarat@msu.ac.th

Received 25 February 2024; Revised 14 March 2024; Accepted 22 March 2024
Published online 1 September 2024

Abstract

The current study used *Asplenium unilaterale* Lam. with a natural alternative to monosodium glutamate (MSG) , along with plant mixtures to develop a seasoning powder. Four formulations (2-8 w/w of A. *unilaterale* Lam.) were characterized. Furthermore, the nutritional, physiochemical, amino acid profiles, antioxidant, and sensory properties were determined. The ash and carbohydrate content differed significantly among the four seasoning powders containing different concentrations of A. *unilaterale* Lam. The protein content significantly increased in seasoning powders as the A. *unilaterale* Lam. content increased.. The developed products had a water activity range of 0.33–0.40, and the color value ranges, L*, a*, and b*, were 60.42–78.38, 1.14–4.53, and 8.49–13.81, respectively. High concentrations of the non-essential amino acids aspartic acid and glutamic acid were observed, causing an umami taste that increased with A. *unilaterale* Lam. content. The highest antioxidant properties were measured in the sample with added A. *unilaterale* Lam. content. The sensory scores in taste for Sample D (8 w/w of A. *unilaterale* Lam.) received the highest scores, but the overall acceptability of the samples did not significantly vary. Therefore, this seasoning powder potentially has flavor and nutritional properties that provide health benefits and could be used as an alternative plant-derived seasoning product.

Keywords**:** *Asplenium unilaterale Lam.; seasoning powder; nutritional value; antioxidant properties; sensory properties*

1. Introduction

Asplenium unilaterale Lam. is a wild edible fern traditionally consumed in northern Thailand; its common name is Hauor Ti La. Wild edible ferns often serve as an alternative to staple crops (Thakur et al., 2017). Edible ferns are often used as food and spices in Asian countries. In particular, different ethnicities in northern Thailand frequently use many species (Liu et al., 2012; Giri, & Uniyal, 2022; Panyadee et al., 2023). The medicinal properties of *A. unilaterale* Lam. have been studied in many countries, including India and Nepal (Sathiyaraj et al., 2015; Ojha, & Devkota, 2021), but studys has been limited in Thailand. A previous study has shown that differences in genetics and culturing conditions affect the composition of this wild edible fern, especially the nutritionally valuable compounds, such as proteins, polysaccharides, polyphenols, and fatty acids (Nekrasov et al., 2019; Zhu et al., 2019). In addition, *A. unilaterale* Lam. had high protein content (21.13% *dry weight)* and fat content (24.73% *dry weight*), along with phenolic acids and flavonoids associated with antioxidant activity

(Petkov et al., 2022). The Sgaw Karen people use Hauor Ti La to add sweetness to local food, similar to monosodium glutamate (MSG). In the Karen language, Hauor Ti La can be translated as “Hauor” (leaf), “Ti” (water), and “La” (sweet taste) and is referred to as “Doi MSG” (Panyawuthakrai, 2023). Moreover, Hauor Ti La has high glutamic acid content (10.63 g/kg fresh weight) compared to shitake mushroom (2.23 g/kg fresh weight) (Zhao et al., 2021).

Plant-derived seasoning products are a new trend in reducing the sodium content of foods, which has been recommended to prevent high blood pressure and heart disease. Alternative plant-based seasonings can have high levels of phytochemicals and a natural umami taste, which is regarded as MSG-like. Additionally, herb blends are the most studied and successful proven strategy for reducing salt intake, especially when continuous consumption is considered; synergistic effects among herbs often enhance flavor (Taladrid et al., 2020). MSG is a famous seasoning used as a flavor enhancer; its consumption has been reported as a risk factor for non-communicable diseases, especially hypertension (Celestino et al., 2021; Insawang et al., 2012; Shi et al., 2011). Therefore, decreasing MSG intake by replacing it with a safe and healthy plant-based seasoning product could reduce the risk factor for hypertension.

Natural flavorings have been developed from soybeans, algae, mushrooms, tomatoes, and peanuts, all containing substances with an umami flavor (Zhao et al., 2019). The molecules most strongly associated with umami are amino acids, such as aspartic acid and glutamic acid; both are abundant in mushrooms (Somdee et al., 2021). The edible ferns used in this study are sold in local markets and used to add a flavor comparable to MSG (Panyawuthakrai, 2023). Edible ferns, such as Hauor Ti La, are rich in nutritional and bioactive compounds, including glutamic acid and phenolic compounds (Panyawuthakrai, 2023).

Using plant seasoning powder with additives that can add an umami taste similar to MSG has been suggested to reduce sodium intake and the risk factor for hypertension. Hauor Ti La is already used to replace MSG by the Sgaw Karen in Maehongson Province (Panyawuthakrai, 2023). *A. unilaterale* Lam., or Hauor Ti La, is often associated with an umami taste due to its high glutamic acid content (Liu et al., 2012). Therefore, its potential use as a natural replacement for MSG has been studied to determine whether these plant-based ingredients can be made into a reduced sodium seasoning powder.

2. Objectives

The evaluates the nutritional value, physical properties, amino acid composition, antioxidant compounds, antioxidant properties, and sensory evaluation of different amounts of *A. unilaterale* Lam.–based seasoning powder products.

3. Materials and methods

3.1 Sample collection and chemical reagents

Fresh *A. unilaterale* Lam. was obtained from the local market in Maehongson Province, northern Thailand, and identified by its botanical morphology by researchers in the Biology Department of Mahasarakham University (Thailand). Other ingredients, including spices, local herbs, and sugar, were purchased from a local market in Mahasarakham Province. The *A. unilaterale* Lam., spices, and herbs were pretreated in a hot air oven at 50°C until they reached dryness. The seasoning powder products were prepared for this experiment; their composition is shown in Table 1. *A. unilaterale* Lam. was added in concentrations of 0, 2, 4, 6, and 8 g (w/w). All chemicals used for the proximate characteristics, amino acid, and antioxidant property determinations were of analytical reagent grade.

Table 1 Ingredients of seasoning powder products

Ingredients	Amount (w/w)				
	Control	A	B	C	D
A. unilaterale Lam. (g)	0	2	4	6	8
Onion (g)	4.20	4.20	4.20	4.20	4.20
Radish (g)	4.9	4.9	4.9	4.9	4.9
Garlic (g)	4.2	4.2	4.2	4.2	4.2
Coriander root (g)	8.0	8.0	8.0	8.0	8.0
Black pepper (g)	4.0	4.0	4.0	4.0	4.0
Sugar (g)	6.1	6.1	6.1	6.1	6.1

3.2 Nutritional value analysis

The nutritional values (moisture, ash, fat, protein, and total carbohydrates) was determined according to the Association of Official Analytical Chemists process (AOAC, 2016). The moisture content was determined using an electric oven (Model OV-440, Gallenkamp, United Kingdom) and drying the samples to constant weights. The samples were analyzed in triplicate. Results are expressed in percentage (%). The ash content was examined using a muffle furnace (L3/11/B170, Nabertherm GmbH, Bremen, Germany): 5.0 g portion of the sample was weighed and heated at 600°C for 12 h. The fat content was examined using the Soxhlet method, in which 5 g of the sample was placed in a fat-free extraction thimble and capped lightly with cotton wool. Then, the sample was extracted with petroleum ether, and the resulting crude lipids were estimated as g/100 g dry weight of sample. The protein content was measured using the Kjeldahl method, which consists of three-step acid digestion of 5 g of sample with concentrated H_2SO_4, alkalization and steam distillation of the acid digest with 5 mL of 40% (w/v) NaOH, and quantification of trapped NH_3 by titration with 0.01 M HCl solution. The amount of crude protein was then calculated by multiplying the nitrogen percentage of the digest by 6.25. The total carbohydrates were computed by subtracting the sum of the moisture, protein, lipid, and ash contents (%) from 100.

3.3 Physiochemical analysis

3.3.1 Water activity evaluation

The water activity (a_w) was determined using a water activity meter (*LANDTEK WA-60A*, Guangzhou, China). The analysis was performed in triplicate.

3.3.2 Color value measurement (*L*, a*, b**)

The color values are comprised of three axes: L*, which represents darkness to lightness with a value from 0 to 100; a*, which represents greenness to redness with a value from −128 to +127; and b*, which represents blueness to yellowness with a value from −128 to +127. They were measured using an NH300 portable colorimeter (3NH, Shenzhen, China).

3.4 Amino acid determination

The amino acid profile was measured using liquid chromatography with tandem mass spectrometry (LC-MS/MS) (Chumroenphat et al., 2019). The LC-MS/MS analysis was *performed* on an LC-MS-8030 triple-quadrupole mass spectrometer (Shimadzu, Kyoto, Japan) operated in ESI mode and an LC-20AC series high-performance liquid chromatography (HPLC) system (Shimadzu). A 2-µL test sample was introduced into the system. The HPLC system analyzed the amino acid content under the following conditions: a mobile phase of solvent (A), 0.1% formic acid in water, and (B) the solution was diluted with methanol (1:1). The autosampler needle was purged with methanol before and after the aspiration of the sample. The flow rate of the mobile phase was set at 0.2 mL/min, and the column temperature was set at 38°C. Amino acid identifications were performed using MS/MS mode: multiple reaction monitoring (MRM) mode was used, the capillary voltage was 4.5 kV (positive mode, ESI (+)), the cone voltage was 1.72 kV, and the ion source temperature was 400°C. The amino acids were identified by their m/z values and by comparison with the retention time of standards. All other settings were analyte-specific and were auto-optimized by the flow injection of 2 µL of a solution in methanol containing 1 ppm of one analyte. To prepare a 100 g sample for analysis, each sample was extracted with 0.5 mL of 0.05 M aqueous HCl–ethanol (1:1, v/v), mixed for 5 min with a vortex mixer, and immediately centrifuged at 12,000 g at 4°C for 15 min (Thiele et al., 2012).

3.5 Sample extraction

The extraction method was performed on 0.5 g of seasoning powder, which was soaked in 10 mL of ethanol overnight at room temperature. The suspension was filtered through a Whatman No. 42 filter paper, and the residue was washed with ethanol. The insoluble residue was discarded. The filtrate was evaporated in a water bath at 50°C to a final volume of 1 mL for the evaluation of the antioxidant properties (Somdee et al., 2016).

3.6 Antioxidant compounds

3.6.1 Total phenolic content (*TPC*)

The TPC of all seasoning powders was determined according to the Folin–Ciocalteu procedure (Yawadio et al., 2008). In this procedure, 0.2 mL of Folin–Ciocalteu reagent and 2 mL of 7.5% sodium carbonate were combined with the sample and incubated for 30 min at room temperature. The absorbance measurement was performed at 725 nm. Gallic acid was used as the standard, and the TPC is reported as mg of gallic acid equivalents.

3.6.2 Total flavonoid content (TFC)

The Total flavonoid content (TFC) of the seasoning powders was determined by colorimetric analysis using the method reported by Abu-Bakar et al., (2009). Five hundred microliters of the sample were added to 2.25 mL of distilled water, followed by 0.15 mL of 5% $NaNO_2$ solution. After 6 min, 0.3 mL of 10% $AlCl_3$ solution was added. After 5 min, 1.0 mL of 1 M NaOH was added. The TFC is expressed as mg of catechin equivalents (CE).

3.7 Antioxidant properties

3.7.1 2,2-Diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity

The 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity was determined using a modification of the method of Brand-Williams et al., (1995). Briefly, 2 mL of 0.20 mM DPPH ethanol solution was mixed with 0.2 mL of the samples and incubated at room temperature in the dark for 30 min. After that, the mixture was measured at the absorbance of 517 nm using a spectrophotometer.

The percentage of DPPH scavenging activity was determined from the equation:

$$\% \text{ scavenging activity} = 100 \times [1 - (A_E/A_D)],$$

where A_E is the absorbance of the DPPH solution with the extract added, and A_D is the absorbance of the DPPH solution without the extract (Amarowicz et al., 2004).

3.7.2 Ferric reducing antioxidant potential (FRAP) assay

The Ferric reducing antioxidant potential (FRAP) assay was done following the method of Benzie, & Strain (1996). The antioxidant potential of the sample was determined from a $FeSO_4 \cdot 7H_2O$ standard curve using linear regression to calculate the FRAP values of the sample. The FRAP solution mixture with 1 mL of $FeCl_3 \cdot 6H_2O$ acetate buffer (pH 3.6), 1 mL of 10 mM 2,4,6-tripyridyl-s-triazine solution in 40 mM HCl, and 1 mL $FeCl_3 \cdot 6H_2O$ solution was mixed with 0.15 mL of the samples for 30 min in the dark. The spectrophotometry readings of the products were then taken at 593 nm.

3.8 Sensory evaluation

To analyze and quantify consumer acceptance of the seasoning powder products, including appearance, flavor, and texture, a 9-point hedonic scale was used (1= dislike extremely, 5 = neither like nor dislike, 9= like extremely). Untrained consumers (n=30) were selected from the staff and students of the Faculty of Public Health, Mahasarakhan University, Thailand, to sensory test the seasoning powder products in the form of hot soup by using 25 g of the sample cooked with 1.0 L of freshwater. The sample was served within 10 minutes of cooking to control the serving temperature. The test was performed in an individual testing box under the daylight-fluorescent lights of the Faculty of Public Health, Mahasarakhan University.

3.9 Statistical analysis

The data were collected in triplicate for statistical analysis. The data was recorded as mean ± standard deviation and analyzed using SPSS (version 11.5 for Windows 98, SPSS Inc.). The results were examined by one-way analysis of variance (ANOVA) with a post hoc (Duncan's) test, and statistical differences of $p < 0.05$ were considered statistically significant.

4. Results and Discussion

The development of seasoning powder products from *A. unilaterale* Lam. and the assessment of the nutritional potential, physicochemical properties, amino acid profiles, antioxidant properties, and sensory evaluation are worthwhile, and similar analysis can be applied to seasoning powders from innovative plant-based seasoning products with an MSG-like taste using AOAC methods, LC-MS/MS, colorimetric, and a nine-point hedonic scale.

4.1 Nutritional value of seasoning powders

The proximate characteristics of the seasoning powders produced from *A. unilaterale* Lam. were determined and are shown in Table 2. The moisture content of the seasoning powder products (5.07%–6.96%) increased with increasing *A. unilaterale* Lam. content. The moisture levels of all four seasoning powder products were low because the raw materials repel moisture.

The ash content of the seasoning powder products (3.41%–4.00%) increased with increasing *A. unilaterale* Lam. content. Ash indicates the presence of various minerals in food and can indicate whether the food is adulterated (Wen et al., 2010). In particular, the sodium contents of all the seasoning powder products were between 5.50 and 21.00 mg/100 grams (data not shown). Less than 2 g of sodium per day is recommended by the World Health Organization for the prevention of cardiovascular diseases (World Health Organization, 2012). Our seasoning powders from *A. unilaterale* Lam. are a mixture of plant products, and these results are consistent with Mitchell et al., (2013), who showed that replacing salt with mixtures of herbs (oregano, garlic, and rosemary) could reduce the use of salt by 40%–56%.

The fat content of the seasoning powder products was between 2.00% and 2.92%, which is low. A previous study has shown that edible ferns have very low fat content (Chettri et al., 2018). The protein content was lower in the control than in the seasoning powder samples; the range was 8.39%–11.82%. Significantly higher amounts of protein were found in the samples containing *A. unilaterale* Lam. powder. Tan et al., (2016) demonstrated that ferns contain more protein than many common plant-based foods, such as lettuce (2.38%), turmeric (1.16%), and peas (7.2%) (Shin et al., 2019). The carbohydrate content in the seasoning powder products was high, reaching 73.59%–80.13%. Thus, based on our results, seasoning powders containing *A. unilaterale* Lam. may be used as a nutritional product due to the high amount of protein.

4.2 Physiochemical analysis of seasoning powder

The physical properties of the seasoning powders containing *A. unilaterale* Lam. are shown in Table 3. Sample D had the highest a_w, followed by A, B, C, and the control. The value of a_w increased with the *A. unilaterale* Lam. content. Nevertheless, the a_w of all samples were safe for consumption according to the quality control guidelines in Thai regulations, which state that a_w should be below 0.65 (Thai Community Product Standards, 2004). Similar results were reported by Mahendradatta et al., (2011), who found that at a_w less than 0.51 that microorganisms cannot grow. The color of the ingredients used to prepare the powders was similar, but the lightness (L*) was significantly different. The highest L*value was found in the control, followed by A, B, C, and D, where D was dark with a high *A. unilaterale* Lam. content. The seasoning powder products developed in this study have red (1.14–6.98) and yellow (8.49–13.81) shades, and the shades of red and yellow decreased with high *A. unilaterale* Lam. content.

4.3 Amino acid determination

The amino acid profiles of the seasoning powders containing *A. unilaterale* Lam. are presented in Table 4. The highest essential amino acid content in the seasoning powders was threonine, with a range of 13.77–446.04 µg/g. The most abundant non-essential amino acid was glutamic acid, which was between 4.04 and 90.98 µg/g. Our study shows that seasoning powder containing *A. unilaterale* Lam. has high threonine and glutamic acid contents, which form the basis of the sweet and umami qualities, respectively. These findings are similar to those of a previous study in which a wild edible fern (*Diplazium maximum*) had high levels of glutamic acid (Sareen et al., 2021; Arai et al., 1973). Brown seaweed (konbu) is an umami plant containing up to 2240 mg of free glutamic acid per 100 g of dry weight (Ninomiya et al., 2002). Additionally, *A. unilaterale* Lam. should be used in seasoning powders because its high level of glutamic acid and the associated umami taste can replace MSG, consumption of which is a risk factor for hypertension. The results from the present study suggest that seasoning powder products containing *A. unilaterale* Lam. may be a good option for introducing the umami flavor.

In China, 64 species of edible ferns are used for soup (Yun et al., 2009; Cao et al., 2007). Additionally, Taladrid et al., (2020) have shown that many plant-derived seasonings, such as turmeric, garlic, and coriander, have good consumer acceptability when used as sodium replacements. Importantly, the amino acid profile in these products suggests an umami taste and some biological activity, especially antioxidant activity (Rhyu et al., 2020).

Table 2 Nutritional value of seasoning powders products

Nutrition values (per dry weight 100 g)	Seasoning powder products				
	Control	**A**	**B**	**C**	**D**
Moisture	5.07 ± 1.71^d	5.31 ± 0.06^c	6.51 ± 0.14^b	6.87 ± 0.09^a	6.96 ± 0.02^a
Ash	3.41 ± 0.02^e	3.51 ± 0.05^d	3.80 ± 0.01^c	3.93 ± 0.05^b	4.00 ± 0.01^a
Fat	2.00 ± 0.02^d	2.09 ± 0.09^d	2.51 ± 0.03^c	2.92 ± 0.07^b	3.63 ± 0.08^a
Protein	8.39 ± 0.08^e	9.69 ± 0.04^d	10.11 ± 0.01^c	11.08 ± 0.01^b	11.82 ± 0.05^a
Carbohydrate	81.13 ± 0.84^a	79.40 ± 0.06^b	77.07 ± 0.06^c	75.20 ± 0.48^d	73.59 ± 0.04^e

Values are expressed as mean ± SD of triplicate measurements (n = 3).
The means with different letters in each row are significantly different ($p < 0.05$).

Table 3 The physical properties of seasoning powder products

Physical properties	Seasoning powder products				
	Control	**A**	**B**	**C**	**D**
a_w	0.32±0.01^b	0.33±0.01^b	0.31±0.17^b	0.37±0.17^c	0.40±0.01^a
Color					
L*	837.3±0.76^a	7.838±0.16^b	7.187±0.94^c	67.32±0.62^d	60.42±0.44^e
a*	6.98±0.50^a	4.53±0.24^b	2.07±0.21^c	1.57±0.10^c	1.14±0.07^c
b*	14.08±0.11^a	1.381±0.14^a	1.175±0.29^b	10.96±0.40^c	8.49 ±0.10^d

Values are expressed as mean ± SD of triplicate measurements (n = 3).
The means with different letters in each row are significantly different ($p < 0.05$).

Table 4 Amino acid profile of seasoning powder products

Amino acid	Amino acid contents (μg/g)				
	Control	**A**	**B**	**C**	**D**
Essential					
arginine	2.1±0.07^e	5.23±0.05^c	5.18±0.15^d	5.41±0.11^b	6.77±0.34^a
histidine	5.58±0.03^c	8.54±0.20^b	8.78±0.28^b	8.63±0.19^b	9.39±0.44^a
isoleucine	4.59±0.11^c	33.00±1.17^b	35.46±2.09^b	34.03±2.36^b	55.12±1.55^a
leucine	4.87±0.37^d	29.56±1.78^c	30.68±0.28^c	32.11±0.60^b	47.73±2.88^a
lysine	42.47±0.31^d	118.76±0.77^c	127.64±1.79^b	120.19±0.69^b	159.62±6.26^a
methionine	0.19±0.01^d	2.09±0.06^c	2.62±0.13^b	2.60±0.20^b	3.88±0.31^a
phenylalanine	2.31±0.07^d	23.00±0.64^c	23.93±2.01^c	26.52±0.35^b	32.62±0.74^a
threonine	13.77±0.24^e	295.5±2.80^d	308.81±4.87^c	333.66±2.05^b	446.04±9.96^a
tryptophan	1.84±0.41^c	3.63±0.22^b	3.76±0.28^b	3.48±0.38^b	4.49±0.16^a
valine	2.13±0.08^d	9.96±0.30^c	11.41±0.92^b	9.97±0.78^c	13.1±0.49^a
Non-Essential					
alanine	2.75±0.20^d	10.71±0.46^c	11.89±0.12^b	12.31±1.11^b	15.32±1.40^a
asparagine	4.72±0.15^c	11.43±0.64^b	11.79±0.84^b	10.84±0.14^b	13.65±0.21^a
aspartic acid	15.82±0.19^d	51.57±0.28^c	55.45±0.65^b	53.35±0.51^b	76.68±0.43^a
cysteine	0.56±0.03^c	0.61±0.03^a	0.57±0.01^b	0.57±0.03^b	0.56±0.02^c
glutamine	14.16±1.1^d	19.67±0.22^b	19.19±0.57^b	17.95±0.64^c	35.34±0.59^a
glutamic acid	4.04±0.38^d	65.91±0.66^c	75.82±2.31^b	77.85±1.51^b	90.98±2.34^a
glycine	0.97±0.03^c	6.22±0.13^b	6.90±0.19^a	0.82±0.07^d	0.60±0.15^e
proline	2.56±0.16^d	8.37±0.27^b	7.83±0.16^c	8.17±0.52^b	10.52±0.13^a
serine	2.19±0.02^d	3.08±0.23^c	3.35±0.11^b	3.21±0.16^b	3.84±0.07^a
tyrosine	3.01±0.41^d	26.15±0.64^c	27.04±0.22^c	28.89±0.40^b	34.52±2.09^a
Total amino acids	**153.62±1.75^e**	**742.90±7.44^d**	**781.74±4.03^c**	**790.58±1.22^b**	**1035.70±15.67^a**

Values are expressed as mean ± SD of triplicate measurements (n = 3).
The means with different letters in each row are significantly different ($p < 0.05$).

Table 5 Antioxidant activity and compounds of seasoning powder products

Seasoning powder products	Antioxidant activity		Antioxidant compounds	
	DPPH (% scavenging activity)	**FRAP (mmol $FeSO_4$/100 g dry weight)**	**TPC (mg/100 g dry weight)**	**TFC (mg/100 g dry weight)**
Control	26.89 ± 1.21^e	129.56 ± 2.74^d	20.44 ± 0.92^e	20.89 ± 0.64^e
A	32.89 ± 2.04^d	127.65 ± 1.23^d	27.83 ± 1.32^d	23.24 ± 1.11^d
B	37.39 ± 1.44^c	135.65 ± 2.23^c	33.39 ± 2.04^c	27.65 ± 1.23^c
C	40.89 ± 0.45^b	156.12 ± 0.96^b	38.17 ± 1.88^b	30.89 ± 1.11^b
D	48.01 ± 1.31^a	160.81 ± 1.19^a	43.07 ± 1.89^a	33.12 ± 1.43^a

The mean ± SD of triplicate measurement, the means with different letters in each column are significantly different ($p < 0.05$).

Table 6 The sensory evaluation of seasoning powder products

Sensory test	Seasoning powder products				
	Control	A	B	C	D
Appearance [ns]	5.20 ± 0.80	4.60 ± 1.83	4.80 ± 1.80	4.80 ± 1.86	5.90 ± 1.61
Color	6.26 ± 0.09[a]	5.20 ± 1.92[b]	4.70 ± 1.29[b]	4.05 ± 1.30[b]	4.05 ± 1.13[b]
Flavor	6.80 ± 1.35[a]	6.10 ± 0.96[b]	5.60 ± 0.93[c]	4.50 ± 0.68[d]	3.80 ± 1.00[e]
Taste	4.87 ± 1.01[a]	4.60 ± 1.48[a]	5.80 ± 1.22[b]	4.60 ± 1.50[a]	4.87 ± 1.70[a]
Overall acceptability [ns]	5.40 ± 1.52	5.30 ± 1.22	5.50 ± 1.40	5.30 ± 1.67	5.30 ± 1.70

The means with different letters in each row are significantly different ($p < 0.05$).

4.4 Antioxidant compounds

The antioxidant compounds, including TPC and TFC, in the four seasoning powder products and the control are shown in Table 5. The TPC (27.83–43.07 mg gallic acid equivalents/100 g dry weight) of the seasoning powders were higher than that of the control (20.44 mg gallic acid equivalents/100 g dry weight), with sample D having the highest value. There were significant differences between the seasonings containing different concentrations of *A. unilaterale* Lam. powder. The TFC was evaluated as a proxy for phytochemical compounds in the seasoning powder products. The highest TFC of the seasoning powder products corresponded with the highest TPC and was also found in sample D.

Previous studies have hypothesized that plant-based condiments are a good source of phytochemical compounds (Novais et al., 2021). Corresponding with our results, the TPC and TFC of the seasoning powder products containing *A. unilaterale* Lam. and another local herb mixture were high in the samples with high *A. unilaterale* Lam. contents. Additionally, phenolic and flavonoid compounds, the major secondary metabolites in plants, are related to phytochemical properties, such as anti-inflammatory, cytotoxic, and antioxidant activity (Hu et al., 2022). Thus, the high amounts of phenolic and flavonoid compounds in the seasoning powder products greatly strengthen their antioxidant activity.

4.5 Antioxidant activity

Antioxidant activity can be due to several reaction mechanisms (Santos-Sánchez et al., 2019). In this study, two methods (DPPH and FRAP) were used to identify the antioxidant activity of the seasoning powder products (Table 5). The DPPH radical scavenging results are presented as the inhibition percentage and show a significant increase (*$p < 0.05$*) in the inhibition percentage with increasing *A. unilaterale* Lam. powder content. Meanwhile, the antioxidant activities of the FRAP assay are expressed as the concentration of antioxidants with Fe^{3+} reduction capacity equivalent to that of 1 mM $FeSO_4$. The FRAP assay showed the same results as the DPPH assay.

Previous research has shown different groups of compounds in mixed plant products, many with phytochemical properties, such as phenolic and flavonoid compounds (Pereira et al., 2015). Importantly, these plant compounds can enhance biological activities through synergistic or additive effects (Cunha et al., 2012). To the best of our knowledge, the mixtures of plants used to develop seasoning powder products are an excellent source of bioactive compounds and can benefit human health (Novais et al., 2021). Seasoning powder products containing *A. unilaterale* Lam. with plant mixtures contain phenolic compounds and flavonoids that contribute greatly to their antioxidant activity.

5. Conclusion

A. *unilaterale* Lam. seasoning powder products are a source of high-quality plant-based protein with many functional properties, especially suitable for enhancing flavor and adding bioactive phytochemicals. Additionally, this product could be an alternative to MSG due to its high glutamic acid content. In conclusion, the study shows the benefits of A. *unilaterale* Lam. and promotes its use as a food and application as a seasoning powder.

6. Acknowledgements

The research was financially supported by Faculty Public Health, Mahasarakham University, Thailand (Grant year 2023).

7. References

Abu-Bakar, M. F., Mohamed, M., Rahmat, A., & Fry, J. (2009). Phytochemicals and antioxidant activity of different parts of bambangan (Mangifera pajang) and tarap (Artocarpus odoratissimus). *Food Chemistry*, *113*(2), 479–483. https://doi:10.1016/j.foodchem.2008.07.081

Amarowicz, R., Pegg, R. B., Rahimi-Moghaddam, P., Barl, B., & Weil, J. A. (2004). Free-radical scavenging capacity and antioxidant activity of selected plant species from the Canadian prairies. *Food Chemistry*, *84*(4), 551-562. https://doi.org/ Sathiyaraj, G., Muthukumar10.1016/S0308-8146(03)00278-4

AOAC. (2016). Offcial Methods of Analysis of AOAC International. Retrieved from https://www.aoac.org/wp-content/uploads/2019/08/app_f.pdf

Arai, S., Yamashita, M., & Noguchi, M. (1973). Taste of L-Glutamyl oligopeptides in relation to their chromatographic properties. *Agricultural and Biological Chemistry*, *37*(1), 151–156. https://doi.org/10.1080/00021369.1973.10860638

Benzie, I. F., & Strain, J. J. (1996). The ferric reducing ability of plasma (FRAP) as a measure of “antioxidant power”: the FRAP assay. *Analytical Biochemistry*, *239*(1), 70-76. https://doi.org/10.1016/S0076-6879(99)99005-5

Brand-Williams, W., Cuvelier, M. E., & Berset, C. L. W. T. (1995). Use of a free radical method to evaluate antioxidant activity. *LWT-Food Science and Technology*, *28*(1), 25-30. https://doi.org/10.1016/S0023-6438(95)80008-5

Cao, Q. M., Zhong, H. Y., Li, Z. H., & Sun, C. B. (2007). Exploitation and utilization study of fern starch in China. *Food Research and Development*, *28*(12), 168-171.

Celestino, M., Balmaceda Valdez, V., Brun, P., Castagliuolo, I., & Mucignat-Caretta, C. (2021). Differential effects of sodium chloride and monosodium glutamate on kidney of adult and aging mice. *Scientific Reports*, *11*(1), Article 481. https://doi.org/10.1038/s41598-020-80048-z

Chettri, S., Manivannan, S., & Muddarsu, V. R. (2018). Nutrient and elemental composition of wild edible ferns of the Himalaya. *American Fern Journal*, *108*(3), 95-106. https://doi.org/10.1640/0002-8444-108.3.95

Chumroenphat, T., Somboonwatthanakul, I., Saensouk, S., & Siriamornpun, S. (2019). The Diversity of Biologically Active Compounds in the Rhizomes of Recently Discovered Zingiberaceae Plants Native to North Eastern Thailand. *Pharmacognosy Journal*, *11*(5), 1014-1022. https://doi.org/10.5530/pj.2019.11.160

Cunha, A., Nogueira, M., & Roque, O. (2012). *Plantas Aromáticas e Óleos Essenciais Composição e Aplicações*. Fundação Calouste Gulbenkian. Lisboa.

Giri, P., & Uniyal, P. L. (2022). Edible ferns in India and their medicinal uses: A review. *Proceedings of the National Academy of Sciences, India Section B: Biological Sciences*, *92*(1), 17-25. https://doi.org/10.1007/s40011-021-01293-4

Hu, Z., Fang, Y., Yi, Z., Tian, X., Li, J., Jin, Y., ... & Zhao, H. (2022). Determining the nutritional value and antioxidant capacity of duckweed (*Wolffia arrhiza*) under artificial conditions. *LWT-Food Science Technology, 153*, Article 112477. https://doi.org/10.1016/j.lwt.2021.112477

Insawang, T., Selmi, C., Cha’on, U., Pethlert, S., Yongvanit, P., Areejitranusorn, P., ... & Hammock, B. D. (2012). Monosodium glutamate (MSG) intake is associated with the prevalence of metabolic syndrome in a rural Thai population. *Nutrition & Metabolism, 9*, 1-6. https://doi.org/10.1186/1743-7075-9-50

Liu, Y., Wujisguleng, W., & Long, C. (2012). Food uses of ferns in China: a review. *Acta Societatis Botanicorum Poloniae*, *81*(4), 263-270. https://doi.org/10.5586/asbp.2012.046

Mahendradatta, M., Tawali, A. B., Bastian, F., & Tahir, M. (2011). *Optimizing production process of seasoning powder made from fermented fish products*. Retrieve December 10, 2023, from https://www.researchgate.net.

Mitchell, M., Brutnon, N. P., Fitzgerald, R. J., & Wilkinson, M. G. (2013). The use of herbs, spices, and whey proteins as natural flavor enhancers and their effect on the sensory acceptability of reduced-salt chilled ready-meals. *Journal of Culinary Science & Technology*, *11*(3), 222-240. https://doi.org/10.1080/15428052.2013.769869

Nekrasov, E. V., Svetashev, V. I., Khrapko, O. V., & Vyssotski, M. V. (2019). Variability of fatty acid profiles in ferns: Relation to fern taxonomy and seasonal development. *Phytochemistry*, *162*, 47-55. https://doi.org/10.1016/j.phytochem.2019.02.015

Ninomiya, K. (2002). Umami: a universal taste. *Food Reviews International*, *18*(1), 23-38. https://doi.org/10.1081/FRI-120003415

Novais, C., Pereira, C., Molina, A. K., Liberal, Â., Dias, M. I., Añibarro-Ortega, M., ... & Barros, L. (2021). Bioactive and nutritional potential of medicinal and aromatic plant (MAP) seasoning mixtures. *Molecules*, *26*(6), Article 1587. https://doi.org/10.3390/molecules26061587

Ojha, R., & Devkota, H. P. (2021). Edible and medicinal pteridophytes of Nepal: a review. *Ethnobotany Research and Applications*, *22*(August), 1-16. http://dx.doi.org/10.32859/era.22.16.1-16

Panyadee, P., Wangpakapattanawong, P., Inta, A., & Balslev, H. (2023). Very high food plant diversity among ethnic groups in Northern Thailand. *Diversity*, *15*(1), Article 120. https://doi.org/10.3390/d15010120

Panyawuthakrai, R. (2023). Hotila: A Natural Consumption Alternative to MSG. *Rajabhat Chiang Mai Research Journal*, *24*(3), 176-192. https://doi.org/10.57260/rcmrj.2023.262712

Pereira, C., Barros, L., & Ferreira, I. C. F. R. (2015). A Comparison of the Nutritional Contribution of Thirty-nine Aromatic Plants used as Condiments and/or Herbal Infusions. *Plant Foods for Human Nutrition*, *70*, 176–183. https://doi. org/ 10.1007/s11130-015-0476-7

Petkov, V., Slavova, I., Teneva, D., Mladenova, T., Stoyanov, P., & Argirova, M. (2022). Phytochemical study and biological activity of three fern species of the Asplenium genus growing in Bulgaria. *The Natural Products Journal*, *12*(6), 82-90. https://doi.org/10.2174/2210315511666210 512024716

Rhyu, M. R., Kim, Y., & Misaka, T. (2020). Suppression of hTAS2R16 signaling by umami substances. *International Journal of Molecular Sciences*, *21*(19), Article 7045. https://doi.org/10.3390/ijms21197045

Santos-Sánchez, N. F., Salas-Coronado, R., Villanueva-Cañongo, C., & Hernández-Carlos, B. (2019). *Antioxidant Compounds and Their Antioxidant Mechanism. In Antioxidants*; Shalaby, E., (Ed.); London, UK: IntechOpen.

Sareen, B., Bhattacharya, A., & Srivatsan, V. (2021). Nutritional characterization and chemical composition of Diplazium maximum (D. Don) C. Chr. *Journal of Food Science and Technology*, *58*(3), 844-854. https://doi.org/10.1007/s13197-020-04598-w

Sathiyaraj, G., Muthukumar, T.,& Ravindran, K. C. (2015). Ethnomedicinal importance of fern and fern allies traditionally used by tribal people of Palani Hills (Kodaikanal), Western Ghats, South India. *Journal of Medicinal Herbs and Ethnomedicine*, *1*(1), 4-9. https://doi.org/10.5455/jmhe.2015-07-08

Shi, Z., Yuan, B., Taylor, A. W., Dai, Y., Pan, X., Gill, T. K., & Wittert, G. A. (2011). Monosodium glutamate is related to a higher increase in blood pressure over 5 years: findings from the Jiangsu Nutrition Study of Chinese adults. *Journal of Hypertension*, *29*(5), 846-853. https://doi.org/10.1097/HJH.0b013e328344da8e

Shin, H. Y., Kim, S. M., Lee, J. H., & Lim, S. T. (2019). Solid-state fermentation of black rice bran with *Aspergillus awamori* and *Aspergillus oryzae*: Effects on phenolic acid composition and antioxidant activity of bran extracts. *Food Chemistry*, *272*, 235-241. https://doi.org/10.1016/j.foodchem.2018.07.174

Somdee, T., Kraiklang, R., Mahaweerawat, C., Yangyuen, S., Mahaweerawat, U., Chumroenphat T., & Somdee, T. (2021). Amino acid, phytochemical compositions and antioxidant activity of inky cap mushroom (Coprinus radiata). *Asian Journal of Chemistry*, *33*(1), 53-56. https://doi.org/10.14233/ajchem.2021.22928

Somdee, T., Mahaweerawat, U., Phadungkit, M., & Yangyuen, S. (2016). Antioxidant Compounds and Activities in Selected Fresh and Blanched Vegetables from Northeastern Thailand. *Chiang Mai Journal of Science*, *43*(4), 834-844.

Taladrid, D., Laguna, L., Bartolome, B., & Moreno-Arribas, V. (2020). Plant-derived seasonings as sodium salt replacers in food. *Trends in Food Science & Technology*, 99, 194-202. https://doi.org/10.1016/j.tifs.2020.03.002

Tan, X. W., Bhave, M., Fong, A. Y., Matsuura, E., Kobayashi, K., Shen, L. H., & Hwang, S. S. (2016). Cytoprotective and Cytotoxic Effects of Rice Bran Extracts in Rat H9c2(2-1) Cardiomyocytes. *Oxidative Medicine and Cellular Longevity*, *2016*(1), Article 6943053. http://dx.doi.org/10.1155/2016/6943053

Thai Community Product Standards. (2004). *Seasoning powder No.494/2547*. ThaiIndustrial Standards Institute, Bangkok, Thailand.

Thakur, D., Sharma, A., & Uniyal, S. K. (2016). Why they eat, what they eat: patterns of wild edible plants consumption in a tribal area of Western Himalaya. *Journal of Ethnobiology and Ethnomedicine*, *13*(70), 1-12. https://doi.org/10.1186/s13002-017-0198-z

Thiele, B., Stein, N., Oldiges, M., & Hofmann, D. (2012). Direct analysis of underivatized amino acids in plant extracts by LC-MS-MS. *Amino Acid Analysis: Methods and Protocols*, *828*, 317-328. https://doi.org/10.1007/978-1-61779-445-2_25

Wen, J., Hu, C., & Fan S. (2010). Chemical composition and nutritional quality of sea cucumbers. *Journal of the Science of Food and Agriculture*, *90*(14), 2469-2474. https://doi.org/10.1002/jsfa.4108

World Health Organization. (2012).Guideline: Sodium Intake for Adults and Children; World Health Organization: Geneva, Switzerland. https://iris.who.int/bitstream/handle/10665/77985/9789241504836_eng.pdf

Yawadio, N., Kikuzaki, H., & Konishi, Y. (2008). Antioxidant activity of various extracts and fractions of Chenopodium quinoa and Amaranthus spp. Seeds. *Food Chemistry*, *106*(2), 760-766. https://doi.org/10.1016/j.foodchem.2007.06.004

Yun, X. L., Zhao, N. W., Pan, L. T., & Zhao, J. H. (2009). Resource distribution and exploitation of edible ferns in Guizhou (II). *Agricultural Science & Technology – Hunan*, *10*(5), 102-106.

Zhao, X., Wang, Y., Zhang, Z., Sun, L., Wei, Y., Bao, X., & Xin, G. (2021). Postharvest short-time partial dehydration affects shiitake mushroom (Lentinus edodes) storage quality and umami taste. *Scientia Horticulturae*, *287*, Article 110274. https://doi.org/10.1016/j.scienta.2021.110274

Zhao, Y., Zhang, M., Devahastin, S., & Liu, Y. (2019). Progresses on processing methods of umami substances: A review. *Trends in Food Science & Technology*, *93*, 125-135. https://doi.org/10.1016/j.tifs.2019.09.012

Zhu, Q. F., & Zhao, Q. S. (2019). Chemical constituents and biological activities of lycophytes and ferns. *Chinese Journal of Natural Medicines*, *17*(12), 887-891. https://doi.org/10.1016/S1875-5364(19)30108-6