



Effect of acid-hydrolyzed soybean waste as a biostimulant on cos lettuce growing using two types of hydroponic systems

 **Aryanis Mutia Zahra**¹

¹ School of Bioresources and Technology, King Mongkut's University of Technology Thonburi, Bangkok 10150, Thailand

 **Apiradee Uthairatanakij**^{1,*}

² Pilot Plant Development and Training Institute, King Mongkut's University of Technology Thonburi, Bangkok 10150, Thailand

 **Natta Laohakunjit**¹

³ Institute of Agricultural Technology, Suranaree University of Technology, Nakhon Ratchasima 30000, Thailand

 **Pongphen Jitareerat**¹

 *Corresponding author: apiradee.uth@kmutt.ac.th

 **Nattapon Kaisangsri**²

 **Arak Tira-umphon**³

Article History

Submission: 19 September 2024

Revised: 14 July 2025

Accepted: 29 July 2025

Keywords

Plant growth

Phytochemical

Protein hydrolysate

Sustainable agriculture

Abstract

Background and Objective: Plant biostimulants produced from agricultural waste have been reported to promote plant growth, stimulate phytochemical content, and promote the valorization of agricultural by-products. This study investigated the impact of soybean-derived protein hydrolysate (SPH) on lettuce production in hydroponic systems using a reduced chemical fertilizer solution concentration (FSC).

Methodology: Cos lettuce (*Lactuca sativa* L.) was cultivated using both deep-water culture (DWC) and nutrient film technique (NFT) hydroponic systems. The test used a factorial design arranged in a randomized complete block design (RCBD) with two factors: SPH concentration (0 mL/L [SPH0] and 1.0 mL/L [SPH1]) and FSC (half- and full-strength Hoagland nutrient solutions). Each treatment combination was replicated twice with 30 plants per unit, where growth, biomass, and phytochemical parameters were analyzed using two-way ANOVA and Duncan's multiple range test.

Main Results: SPH1 significantly enhanced plant growth and phytochemical content in both hydroponic systems ($P < 0.05$). In half-strength nutrient solutions with SPH1, lettuce head diameter and biomass increased by 1.16- and 1.17-fold in deep-water culture, and by 1.16 and 1.61-fold in

nutrient film technique, respectively, with greener and more intense color than the control. Additionally, the total phenolic contents, total flavonoid contents, DPPH• scavenging activity, ascorbic acid content, soluble protein content, and nitrate content increased by 1.47, 1.47, 1.11, 1.02, 1.33, and 1.23-fold, respectively, in the DWC system, and by 1.40, 2.40, 2.98, 1.15, 1.69, and 1.71-fold, respectively, in the NFT system.

Conclusions: The findings of this study indicated that adding SPH1 in both systems enhanced lettuce growth, yield, and quality and proved to be an effective method for improving nutritional contents and antioxidant activities while enabling reduced chemical fertilizer use. Although nitrate content increased, levels remained within acceptable daily intakes.

How to cite : Mutia Zahra, A., A. Uthairatanakij, N. Laohakunjit, P. Jitareerat, N. Kaisangsri and A. Tira-umphon. 2025. Effect of acid-hydrolyzed soybean waste as a biostimulant on cos lettuce growing using two types of hydroponic systems. Thai J. Agric. Sci. 58(3): 236–251.

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INTRODUCTION

Sustainable agricultural practices have gained momentum, optimizing resource use and reducing environmental impacts to address global food security and climate change. Overuse of chemical inputs threatens ecosystems, prompting a shift toward environmentally friendly and resilient agriculture. Plant biostimulants (PBs) from agricultural by-products have emerged as a promising approach to enhance plant growth, crop yield, quality, and resilience (Zhang *et al.*, 2024). PBs support crop production and yield stability under various stresses, such as high salinity (El-Nakhel *et al.*, 2022), nutrient deficiencies (Ottaiano *et al.*, 2021), suboptimal temperature (Popko *et al.*, 2018; Casadesús *et al.*, 2020), and improve lettuce crop performance and metabolic traits (Choi *et al.*, 2022). Supplementation with pig blood-derived hydrolysate increases bioactive compounds and yield (Zhou *et al.*, 2022), legume-derived hydrolysates enhance nitrogen uptake efficiency and photosynthesis (Di Mola *et al.*, 2020), and seaweed extract delays leaf senescence and prolongs shelf life (Miceli *et al.*,

2021). PBs also regulate hormone-like compounds, metabolic enzymes, biochemical and morphological alterations, supporting crop output by modulating carbon and nitrogen metabolisms (Schiavon *et al.*, 2008; Casadesús *et al.*, 2020), and can reduce chemical fertilizer and pesticide use, thereby minimizing environmental impact (Zhang *et al.*, 2024).

Soybean meals (SM), a by-product of the soybean processing industry, are abundant and underutilized. The soybean oil processing industry produces 1,000 g of SM per 1,210 g of processed soybeans (Cai *et al.*, 2022). In 2024, China produced 78.41 million metric tons of SM, 30% of the global total, while Thailand produced 5.07 million metric tons, primarily used as animal feed from cooking oil extraction (Cai *et al.*, 2022; USDA, 2024). Utilizing various hydrolysis techniques, including chemical and enzymatic hydrolysis, may transform SM into soybean protein hydrolysate (SPH), a biostimulant rich in organic compounds and nutrients (Matsumiya and Kubo, 2011; Mohana Dass *et al.*, 2021). Matsumiya and Kubo (2011) reported an effective hydrolysis of

SPH using an alkaline protease loaded with root hair-promoting peptide, stimulating the length and number of root hairs in tomatoes, mustard greens, and lettuce. Another study by Mohana Dass *et al.* (2021) found that enzymatically hydrolyzed SPH increased phytochemical, photosynthetic pigment, and harvested yield in hydroponic lettuce grown at varying PH concentrations. While some studies have shown promising results using SPH in various crops, its effects on hydroponically grown lettuce, particularly in different hydroponic systems and at varying fertilizer concentrations, have not been thoroughly investigated.

Integrating biostimulants into hydroponic systems combines two sustainable agricultural strategies. Hydroponic efficiently use water and space, enabling year-round production with reduced pesticides and fertilizer inputs (O’Sullivan *et al.*, 2019). Nutrient film technique (NFT) and deep-water culture (DWC) hydroponic systems are widely adopted for their simplicity, low cost, and effectiveness. NFT exposes roots to a thin nutrient film for improved oxygenation, while DWC submerges roots in nutrient solutions for continuous nutrient availability (Bracino *et al.*, 2020). However, the potential synergy between PBs and hydroponic systems remains underexplored. Despite its potential as a plant biostimulant, limited research addresses the effects of acid-hydrolyzed soybean-derived waste in hydroponic lettuce cultivation. This study evaluates the efficiency and cost-effectiveness of acid hydrolysis in comparison to enzymatic approaches, offering it superior for large-scale applications. Acid hydrolysis effectively degrades proteins into smaller peptides and free amino acids, leading to an increased degree of hydrolysis, enhanced solubility, and substantial yield of amino acid recovery (Nolsøe and Undeland, 2009). Protein hydrolysates containing more free amino acids are better absorbed by plants (Di Mola *et al.*, 2020; Ottaiano *et al.*, 2021). Moreover, comparative research assessing biostimulant efficacy across hydroponic systems and fertilizer concentrations

is limited. Therefore, this study aims to evaluate the effectiveness of acid-hydrolyzed soybean waste as a biostimulant for improving lettuce growth, yield, and quality in both NFT and DWC hydroponic systems with reduced fertilizer inputs.

MATERIALS AND METHODS

Protein Hydrolysate Preparation and Testing on Hydroponic Lettuce

Soybean protein hydrolysate (SPH) was produced by dissolving 100 g of soy waste in 500 mL of 6 N hydrochloric acid (HCl, ANaPure 37%) at a 1:5 w/v ratio, stirring it for 6 hours at 95 °C, and adjusting the pH to 6.5 using calcium hydroxide (Ca(OH)₂). Following centrifugation, the supernatant was filtered, stored at 4 °C, and the resulting hydrolysate solution (approximately 450 mL per 100 g soy waste) was used directly in further trials without additional concentration or evaporation. SPH is abundant in total and free amino acids, with the ten highest compositions shown in Table 1.

Green Cos lettuce (*Lactuca sativa* L.) was germinated for 48 hours in a petri dish with moistened tissue paper, 0.5 cm sprouts were then placed in 3.5 × 3.5 cm sponge blocks and maintained in a growth chamber at 25–27 °C and 70% relative humidity for 2 weeks. A white luminous lamp provided 12 hours of photoperiod illumination with a PPFD of 100 μmol/m²/s. After this initial nursery period, seedlings were then transferred to either a Styrofoam box for deep-water culture (DWC) or a hydroponic PVC set for the nutrient film technique (NFT). Plants were placed in net pots spaced 12 cm apart and installed in a greenhouse equipped with a 60–85% sunshade net and a 50 L water reservoir for each treatment. Within the greenhouse, environmental conditions were monitored but not actively controlled during the cultivation period (September 1–30, 2023) at the School of Bioresources and Technology facility at the Bangkhuntien campus, King Mongkut’s University of Technology Thonburi

(KMUTT), Bangkok, Thailand (13°34'35.5"N, 100°26'33.0"E; 6 m above sea level). The recorded environmental condition values included temperature (24, 53, and 31 °C for min, max, and average), relative humidity (31, 97, and 71%), and light intensities (0, 10,000, and 1,762 lux), which were measured with a KIMO sensor and shown in Figure 1.

Table 1 Total and amino acid compositions present in soybean protein hydrolysate

Amino acids	Total amino acids (% total)	Free amino acids (% total)	Function*
Aspartic acid	16.58	17.59	Germination stimulation
Serine	8.19	8.73	Chlorophyll synthesis stimulation Precursor of auxin
Glutamic acid	27.69	27.76	Germination stimulation Growth stimulator and regulator Precursor of other amino acids
Glycine	6.75	7.84	Precursor of chlorophyll
Threonine	5.49	4.51	Germination stimulation
Alanine	6.14	6.83	Hormone and chlorophyll metabolism stimulation
Proline	8.37	8.74	Anti-stress agent Regulation of water balance
Valine	7.10	3.48	Precursor of auxin
Lysine	6.96	8.54	Germination stimulation Chlorophyll synthesis stimulation
Phenylalanine	6.73	5.98	Germination stimulation
Total EAA (% total)	26.28	22.52	
Total BCAA (% total)	7.10	3.48	
Total HAA (% total)	35.09	32.87	

Note: Total EAA = total essential amino acid calculated from the sum of threonine, valine, lysine, and phenylalanine; Total BCAA = total branched-chain amino acid calculated from the sum of valine; Total HAA = total hydrophobic amino acid calculated from the sum of alanine, phenylalanine, valine, proline, and glycine; *Function was listed based on Popko *et al.* (2018).

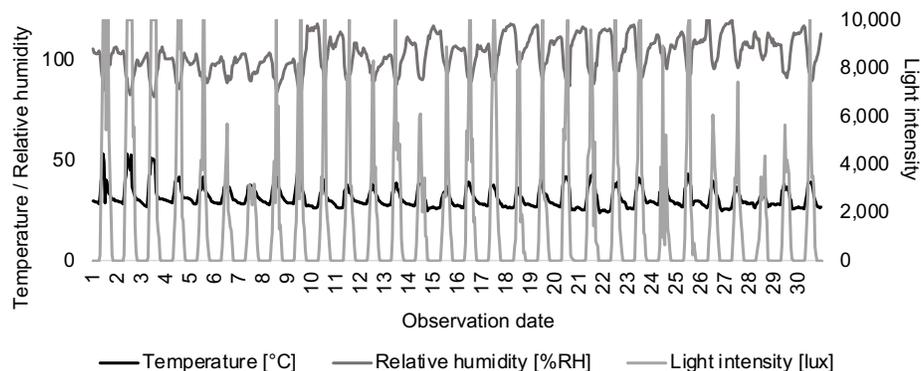


Figure 1 Environmental conditions inside the greenhouse during the cultivation period

Plants were continually immersed in Hoagland nutrient solutions prepared as fertilizer A (101 g KNO_3 , 236.15 g $\text{Ca}(\text{NO}_3)_2$, and 7 g Fe-EDTA per liter) and fertilizer B (57.515 g $\text{NH}_4\text{H}_2\text{PO}_4$, 246.48 g $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 2.86 g H_3BO_3 , 1.26 g MnCl_2 , 0.28 g ZnSO_4 , 0.08 g CuSO_4 , 0.02 g Na_2MoO_4 per liter), with pH 6.5–7.0 (Wang *et al.*, 2022a). Four combinations of Hoagland chemical fertilizer solution concentration (FSC) and soy protein hydrolysate (SPH) doses (0- and 1.0-mL/L) were used for both hydroponic systems: control half strength without SPH (HS), control full strength without SPH (FS), half strength treated with SPH 1.0 mL/L (HS+SPH1), and full strength treated with SPH 1.0 mL/L (FS+SPH1). The electrical conductivity (EC, dS/m) was adjusted to 1.40, 2.20, 1.80, and 2.40 for HS, FS, HS+SPH1, and FS+SPH1. SPH was applied directly to the nutrient solution four times on days 3, 8, 13, and 18 after transplanting, corresponding with the day of adjusting EC. Lettuces were randomly harvested on day 25 after transplanting (DAT).

Analysis of Physical, Pigment, Antioxidant, and Phytochemical Content of Harvested Lettuce

Random lettuce samples were taken from ten replications of each treatment. Plant height, head diameter, and root length were measured in centimeters, and total plant biomass was recorded in grams of fresh weight (FW). The color was determined by measuring lightness (L) and hue angles (Ho) at three random points on mature leaves' dorsal surfaces. Photosynthetic pigment and phytochemical content were determined using the ultraviolet spectrophotometry method, modifying previously published conditions. Total chlorophyll and carotenoid contents were measured by incubating fresh lettuce in N, N-dimethylformamide and recording absorbances at 663, 645, and 470 nm (Wellburn, 1994), reported as mg/g FW conditions. Total phenolic content (TPC) was measured using the Folin-Ciocalteu reagent at

760 nm (Attard, 2013), reported as mg gallic acid equivalents per gram FW (mg GAE/g FW). Total flavonoid content (TFC) was measured using an aluminum chloride reagent at 510 nm (Zhishen *et al.*, 1999), reported as mg quercetin equivalents per gram FW (mg QE/g FW). Ascorbic acid content was determined using the 2,4-dinitrophenylhydrazine (DNPH), indophenol, and thiourea mixtures (Matthews and Hall, 1978), determined absorbance at 540 nm and reported as mg ascorbic acid equivalents per 100 g FW (mg AsA/100 g FW). Soluble protein content was measured using the Coomassie brilliant blue G-250 dye method at 595 nm (Bradford, 1976), reported as mg/100 g FW with bovine serum albumin standard. Nitrate content was measured using salicylic acid, sulfuric acid, and NaOH mixtures at 410 nm (Cataldo *et al.*, 1975), and it was reported as mg/g FW with KNO_3 as standard. Lastly, the DPPH• radical scavenging rate was determined using 100 $\mu\text{mol/L}$ DPPH solution at 517 nm and expressed as DPPH• inhibition (Tadolini *et al.*, 2000).

Statistical Analysis

Each treatment combination was repeated two times for each hydroponic system, with 30 plants per experimental unit, applying a factorial randomized complete block design (RCBD). Physical parameters were analyzed with 10 plants, while photosynthetic pigment and phytochemical content were analyzed with 5 mixed biological replicates from six lettuce plants. A two-way analysis of variance (ANOVA) was performed to compare chemical fertilizer solution concentration and hydroponic systems using the SPSS 19 software program. Duncan's multiple range test (DMRT; $P < 0.05$) was used to compare the means of independent measured variables. Principal component analysis (PCA) was utilized to visually represent the overall correlation between treatment and parameters (Rouphael *et al.*, 2021; Wang *et al.*, 2022a). Pearson's correlation

coefficients were employed to quantify the strength of the associations among parameters in Cos lettuce (Zhou *et al.*, 2022). The data and figures were analyzed and created using the OriginPro 2024 software application.

RESULTS AND DISCUSSION

Plant Growth and Biomass of Lettuce

The interaction between chemical fertilizer soluble concentration and soybean protein hydrolysate (FSC × SPH) was significant ($P < 0.05$) for growth and biomass parameters in both hydroponic systems (Figure 2). However, for plant height, only the main effect of the hydroponic system (DWC vs. NFT) was significant. Application of SPH enhanced lettuce growth and biomass compared to the untreated group. Root length and total plant biomass were dependent on FSC, with full-strength nutrient solutions yielding higher values than half-strength. The NFT system exhibited significantly greater head diameter, root length, and total plant biomass than DWC, while lettuce grown in DWC had higher plant height than NFT. The FSC × SPH interaction suggests potential for optimizing nutrient management, reducing chemical fertilizer use, and supporting sustainability. The results show that SPH improves lettuce growth, increasing crop yield and quality, and highlight synergy between biostimulant and hydroponic systems.

Other research indicated that NFT and DWC hydroponic systems yielded comparable growth outcomes at the full bloom of lettuce cultivation in a controlled environment (Griffith *et al.*, 2023). However, Yang *et al.* (2024) observed seasonal variations in lettuce yield and quality in climate-controlled greenhouses; NFT systems produced higher yields but more tip burn in summer, whereas DWC resulted in better yield and nutritional content in fall. The observed disparities between NFT and DWC systems are primarily due to differences in oxygen availability for root development and overall plant growth. In NFT, the thin stream of

nutrient solution partially exposes plant roots to air, improving oxygen delivery and supporting growth, while in DWC, poor aeration can deplete oxygen levels in fully submerged roots (Bracino *et al.*, 2020). Furthermore, waterlogging can develop when the roots become excessively dense, impeding the passage of oxygen and decreasing the average yield (Ide *et al.*, 2022). The application of soybean-derived protein hydrolysates has been shown to significantly enhance plant growth and biomass accumulation, as these biostimulants supply free amino acids and peptides that improve nutrient uptake, utilization, and physiological functions (Di Mola *et al.*, 2020). These benefits are consistent with previous reports highlighting that vegetal protein hydrolysates improve morpho-physiological traits and yield in sweet basil, tomato, and lettuce (Rouphael *et al.*, 2021; Choi *et al.*, 2022), supporting the potential of integrating protein hydrolysates into hydroponic systems to optimize crop performance under varying environmental and system-specific conditions.

Colorimetric Parameters and Photosynthetic Pigment of Lettuce

The interaction between chemical fertilizer soluble concentration and soybean protein hydrolysate (FSC × SPH) was significant ($P < 0.05$) for color parameters in both hydroponic systems (Figure 3). SPH application increased lettuce hue angles and reduced lightness, indicating enhanced green intensity and deeper color compared to the untreated group. The improvement in colorimetric parameters closely aligns with the observed increases in photosynthetic pigment content, as higher FSC and SPH1 application further elevated these pigment levels. A significant interaction ($P < 0.05$) between FSC × SPH was also observed for total chlorophyll and carotenoid content, with lettuce grown in DWC exhibiting higher pigment concentrations than those in NFT. These findings highlight SPH1's crucial role in promoting chlorophyll

and carotenoid synthesis, which directly contributes to the observed improvements in color parameters. Elevated pigment levels not only enhance the nutritional quality and visual appeal of lettuce but also increase its market value and health benefits (El-Nakhel *et al.*, 2022; Aires *et al.*, 2023; Yang *et al.*, 2024).

The results of this study demonstrate that the application of SPH1 significantly improves lettuce color quality by increasing chlorophyll and carotenoid content, which not only intensifies the green coloration of leaves but also enhances photosynthetic efficiency and overall plant health, leading to greater visual appeal and marketability of harvested lettuce

(El-Nakhel *et al.*, 2022). A presumed mechanism is closely linked to improved nitrogen use efficiency (Mohana Dass *et al.*, 2021; Choi *et al.*, 2022). SPH1 may stimulate enzyme activity and support a vigorous root system, resulting in elevated leaf nitrogen concentration that boosts photosynthetic efficiency and facilitates sugar transport to growth sinks, contributing to overall plant development (Rouphael *et al.*, 2021). DWC systems provide a consistently nutrient-rich solution to plant roots and generally maintain better water quality than NFT (Hooks *et al.*, 2022; Yang *et al.*, 2024). This steady nutrient availability supports higher chlorophyll and pigment synthesis in leaves (Hooks

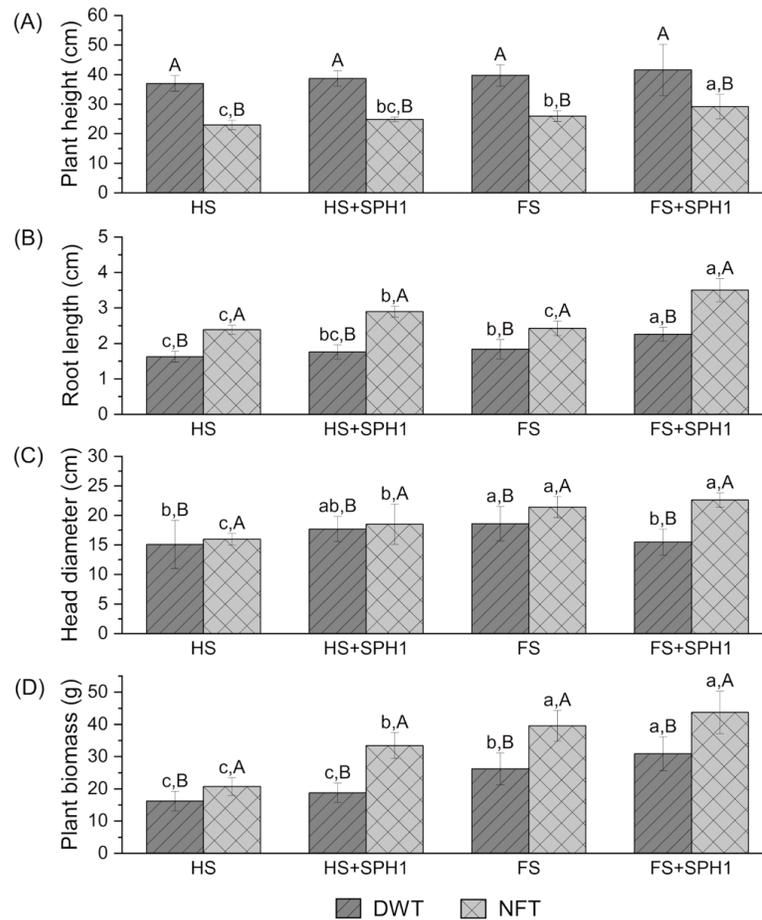


Figure 2 Plant height (A), root length (B), head diameter (C), and total plant biomass (D) of Cos lettuce grown under different hydroponic systems (DWT = deep-water; NFT = nutrient film) and treatments (HS = half-strength; FS = full-strength; SPH1 = soybean protein hydrolysate). Lower-case letters indicate significant nutrient solution changes within each system, while upper-case letters show differences between hydroponic systems for the same solution ($P < 0.05$).

et al., 2022; Aires *et al.*, 2023; Yang *et al.*, 2024). Additionally, mild stress conditions in DWC, such as nutrient fluctuations, may trigger increased pigment production as a protective response (Aires *et al.*, 2023; Yang *et al.*, 2024). These findings align with prior research reporting increased chlorophyll and carotenoid contents in hydroponically grown lettuce and mint treated with various protein hydrolysates, including those derived from soybean, pig blood, and legumes (Mohana Dass *et al.*, 2021; Choi *et al.*, 2022; Zhou *et al.*, 2022). This mechanistic understanding supports the role of biostimulants in regulating

metabolic pathways, enabling more targeted and effective use of biostimulants for optimizing crop quality, resilience, and resource efficiency in hydroponic systems (Schiavon *et al.*, 2008; Zhang *et al.*, 2024).

Phytochemical Content, Ascorbic Acid Levels, and Antioxidant Capacity of Lettuce

The interaction of FSC × SPH treatments significantly influenced ($P < 0.05$) the total phenolic content (TPC), total flavonoid content (TFC), DPPH• radical scavenging activity, and ascorbic acid content,

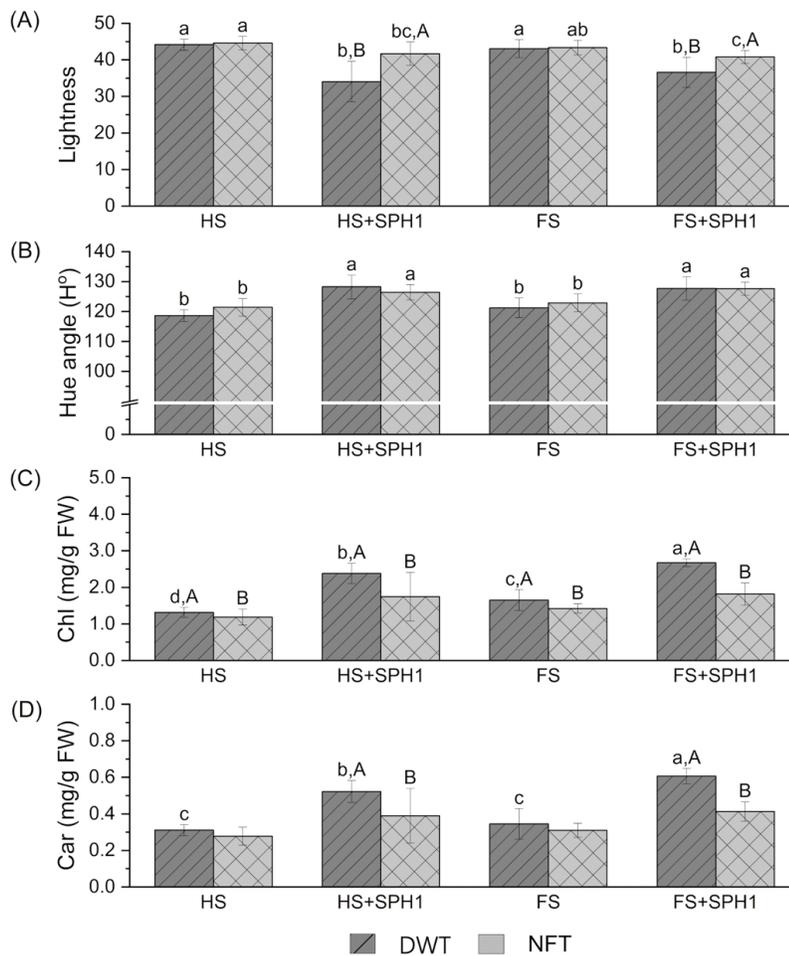


Figure 3 Lightness (A), hue angle (B), total chlorophyll (Chl; C), and total carotenoids (Car; D) of Cos lettuce grown under different hydroponic systems (DWT = deep-water; NFT = nutrient film) and treatments (HS = half-strength; FS = full-strength; SPH1 = soybean protein hydrolysate). Lower-case letters indicate significant nutrient solution changes within each system, while upper-case letters show differences between hydroponic systems for the same solution ($P < 0.05$).

but only in NFT system (Figure 4). In this study, no additional oxygenation techniques were used, pumps in NFT systems served solely for water recirculation. López-Pozos *et al.* (2011) reported that a thin nutrient solution stream in NFT systems can enhance oxygen supply in warm climates by partially exposing plant roots to air, which can be further improved by optimizing tray slope and channel gaps. However, the absence of supplemental oxygenation in both NFT and DWC systems represents a clear limitation of this study, potentially restricting phytochemical

production, particularly in DWC, where roots are fully submerged and oxygen availability is lower. Yang *et al.* (2024) demonstrated that additional oxygenation in DWC mitigates low oxygen conditions, improving plant performance. Similarly, higher dissolved oxygen concentrations in hydroponic systems correlate to increased yield, antioxidant compounds, and ascorbic acid content in Butterhead lettuce (Yang *et al.*, 2024). Future studies should incorporate enhanced oxygenation strategies to better assess their impact on phytochemical accumulation and crop quality.

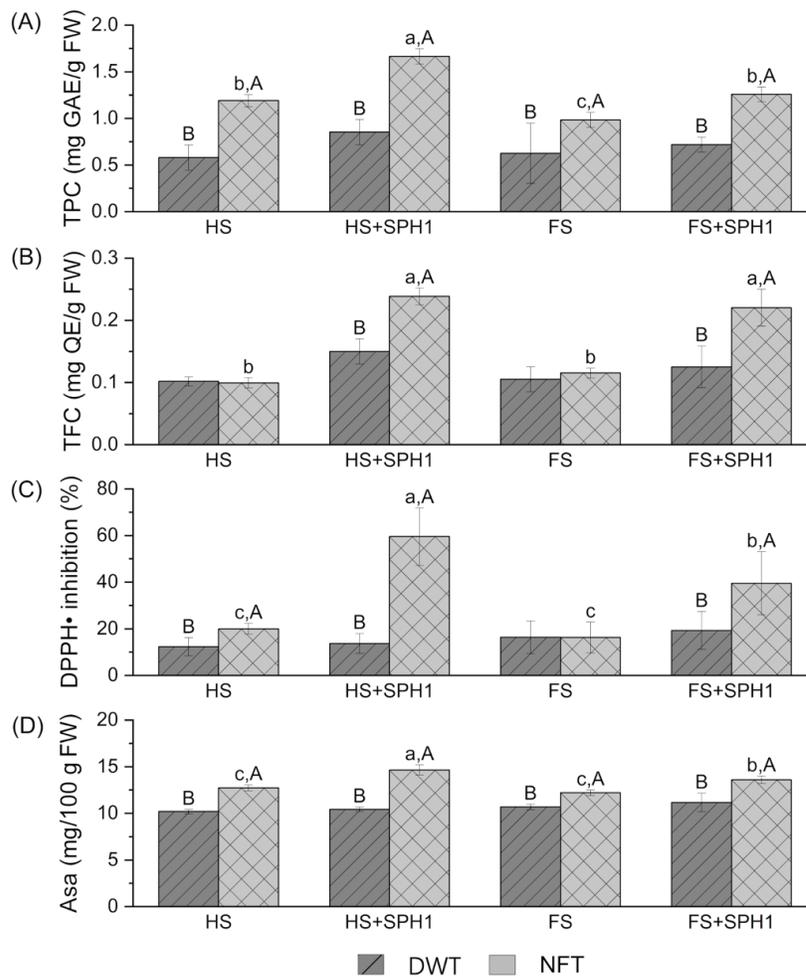


Figure 4 Total phenolic contents (TPC; A), total flavonoid contents (TFC; B), DPPH• scavenging activity (C), and ascorbic acid content (Asa; D) of Cos lettuce grown under different hydroponic systems (DWT = deep-water; NFT = nutrient film) and treatments (HS = half-strength; FS = full-strength; SPH1 = soybean protein hydrolysate). Lower-case letters indicate significant nutrient solution changes within each system, while upper-case letters show differences between hydroponic systems for the same solution ($P < 0.05$).

Moreover, the enhancement of TPC, TFC, DPPH• scavenging activity, and ascorbic acid levels with SPH1 treatment highlight the potential of soybean-derived protein hydrolysates as effective biostimulants in hydroponic systems. Enhanced enzyme gene expression, particularly phenylalanine ammonia-lyase (PAL) biosynthesis, helps explain how SPH1 promotes phenolic and flavonoid content accumulation (Mohana Dass *et al.*, 2021; Zhou *et al.*, 2022). The rise in TPC and TFC, alongside ascorbic acid and antioxidant activity, indicates that phenolics and flavonoids are the primary contributors to antioxidant enhancement following protein hydrolysate treatment (Zhou *et al.*, 2022). Among treatments, HS+SPH1 yielded the highest phytochemical levels, suggesting a promising nutrient management strategy. These findings suggest protein hydrolysate can alleviate nutrient limitations while regulating phenylalanine levels (Casadesús *et al.*, 2020; Zhou *et al.*, 2022). Additionally, H₂O₂ may promote phenolic accumulation under low nitrogen supply in a half-strength nutrient application, indicating biostimulants act through multiple mechanisms to mitigate oxidative stress and enhance crop resilience under stress (Wang *et al.*, 2022b). Furthermore, lettuce treated with half-strength nutrients may exhibit decreased citrate cycle activity and increased glucose and sucrose levels, reallocating nitrogen and carbon towards phenolic via phenylalanine biosynthesis (Casadesús *et al.*, 2020; Zhou *et al.*, 2022). These findings emphasize the necessity for focused studies on biostimulants use across hydroponic systems to optimize benefits and improve crop quality and sustainability in hydroponic lettuce cultivation.

Soluble Protein and Nitrate Levels in Lettuce

The interaction between fertilizer soluble concentration and soybean protein hydrolysate treatments (FSC × SPH) significantly influenced soluble protein content in both deep-water culture

and nutrient film technique (Figure 5A), demonstrating SPH's effectiveness as a biostimulant across hydroponic systems. The observed increase in soluble protein content likely results from accelerated growth in SPH1-treated plants, which demands enhanced root nutrient uptake for protein biosynthesis. This finding aligns with previous research showing that protein hydrolysate applications, such as those derived from alfalfa and algae, can upregulate nitrogen metabolism enzymes at the transcriptional level and boost total soluble protein in crops like maize and spinach (Schiavon *et al.*, 2008; Ottaiano *et al.*, 2021). Similarly, Cos lettuce treated with SPH1 exhibited increased nitrate content in both hydroponic systems, under half-strength and full-strength nutrient solutions (Figure 5B). In contrast, alfalfa protein hydrolysate application in maize increased total protein but decreased nitrate content in roots and leaves (Schiavon *et al.*, 2008). Conversely, seaweed extract and microalgae treatments increased both total protein and nitrate content in lettuce (Miceli *et al.*, 2021). This disparity emphasizes the complexity of plant responses to biostimulants and the need for crop-specific research.

Protein hydrolysates, composed of amino acids and peptides, are readily absorbed by plants, enhancing nitrogen uptake and metabolism (Di Mola *et al.*, 2020; Ottaiano *et al.*, 2021). Studies indicate that protein hydrolysates may boost nitrate reductase activity and glutamine synthetase, enzymes critical for nitrogen assimilation and incorporation into organic molecules (Schiavon *et al.*, 2008). Additionally, protein hydrolysates promote root growth and development, increasing root surface area and nutrient absorption capacity, including nitrates and inorganic nitrogen (Matsumiya and Kubo, 2011; Casadesús *et al.*, 2020). In this study found nitrate content in Cos lettuce following SPH1 treatment ranged from 0.19 to 0.24 mg/g FW, well within acceptable daily intakes (ADIs) limits set by

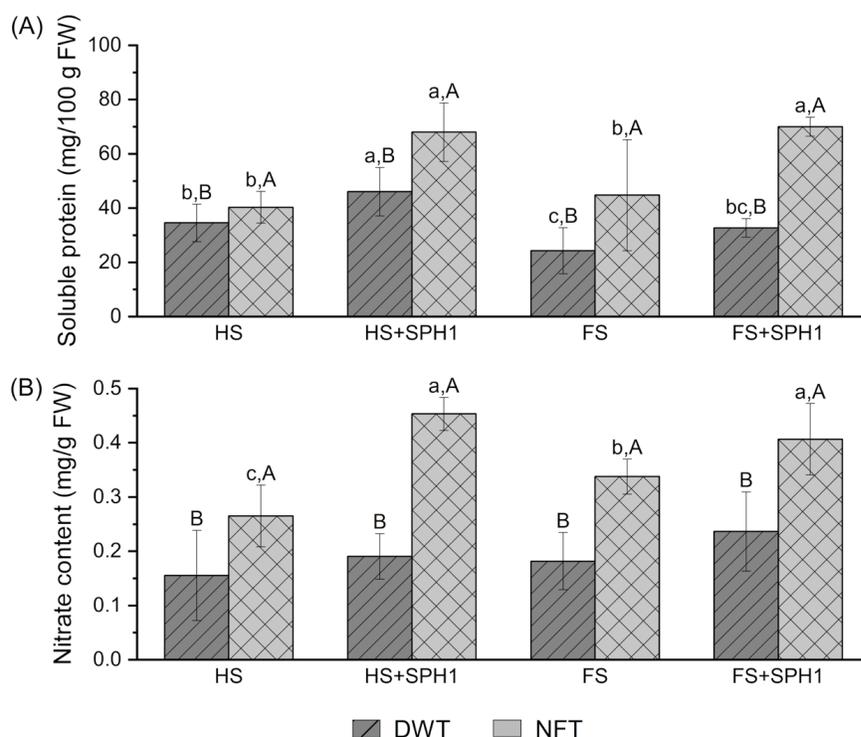


Figure 5 Soluble protein content (A) and nitrate content (B) of Cos lettuce grown under different hydroponic systems (DWT = deep-water; NFT = nutrient film) and treatments (HS = half-strength; FS = full-strength; SPH1 = soybean protein hydrolysate). Lower-case letters indicate significant nutrient solution changes within each system, while upper-case letters show differences between hydroponic systems for the same solution ($P < 0.05$).

the Joint FAO/WHO Expert Committee on Food Additives (JECFA), which range from 3.6 to 7.3 mg/kg body weight, corresponding to 220 to 440 mg daily for a 60 kg person (European Food Safety Authority, 2008).

Soybean Protein Hydrolysate Improves Lettuce Yield and Quality

Pearson correlation analysis (Figure 6A) revealed strong positive correlations between total biomass and root length (0.68), TPC, TFC, DPPH• scavenging activity, nitrate content (0.44–0.49), soluble protein content (0.57), and ascorbic acid content (0.69). Color and pigment parameters also showed notable correlations, with hue angle positively related to chlorophyll (0.55) and lightness negatively

related to both chlorophyll and carotenoid (−0.62). Optimal growth conditions, such as adequate light, water, and nutrients, can promote plant development and biomass accumulation, often through enhanced photosynthesis and nutrient uptake, which in turn increase phytochemical levels (Yang *et al.*, 2024). Strong correlations between DPPH• scavenging activity and TPC (0.78), TFC (0.82), and ascorbic acid (0.78) suggest that these components play a significant role in antioxidant activity, supporting the plant's defense against oxidative stress (Wang *et al.*, 2022b).

Principal component analysis (PCA) showed that the first two principal components (PC1 and PC2) explained 67.34% of the variance, with PC1 accounting for 44.74% and PC2 for 22.60%

(Figure 6B). The PCA biplot revealed distinct clusters separating control and SPH1-treated plants, indicating that SPH1 significantly alters lettuce's physiological and biochemical profile. Some overlap in data points suggests similar growth trends and biochemical responses within groups. The segregation of FSC × SPH treatments in Cos lettuce was evident along the PC1 and PC2 axes, with HS (DWC), FS (DWC), HS+SPH1 (NFT), and FS+SPH1 (NFT) grouping along PC1, and HS+SPH1 (DWC), FS+SPH1

(DWC), HS (NFT), and FS (NFT) along PC2. Notably, HS+SPH1 (NFT) and FS+SPH1 (NFT) clustered in the center-right, correlating with most parameters, including biomass, root length, TPC, TFC, DPPH• scavenging activity, ascorbic acid, nitrate, and soluble protein. In contrast, HS+SPH1 (DWC) and FS+SPH1 (DWC) grouped in the upper-left quadrant, positively correlated with plant height, chlorophyll, and carotenoids.

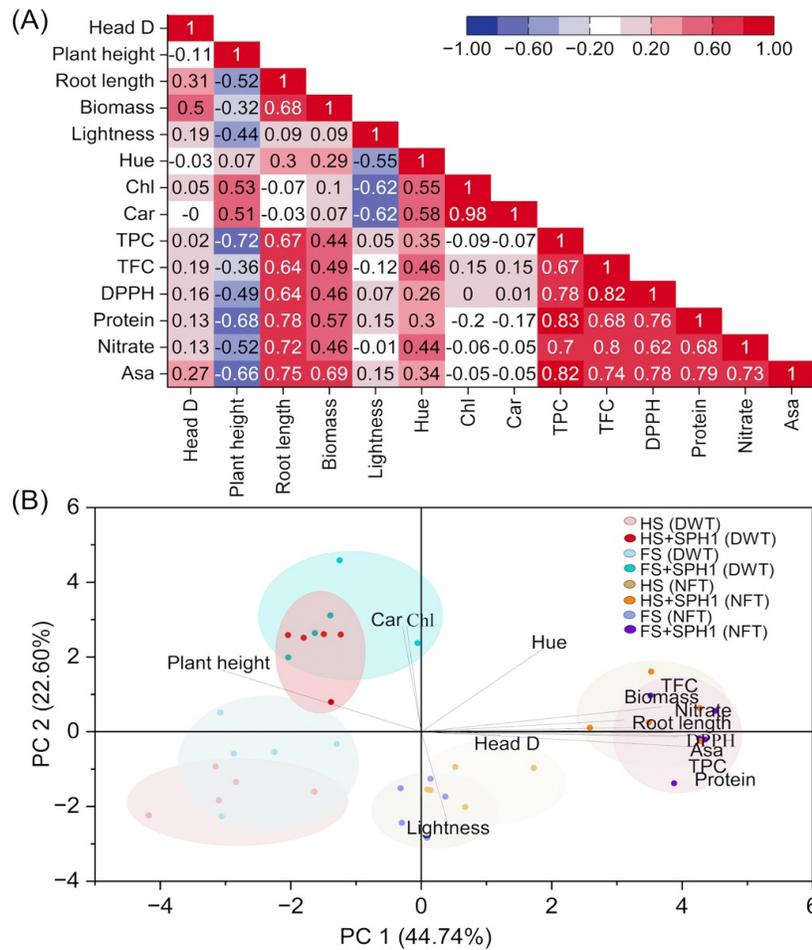


Figure 6 Pearson's correlation coefficients (A) and PCA biplot (B) showing the relationship between examined parameters in Cos lettuces grown under different hydroponic systems (DWT = deep-water; NFT = nutrient film) and FSC x SPH treatments (HS, HS+SPH1, FS, and FS+SPH1; HS = half-strength; FS = full-strength; SPH1 = soybean protein hydrolysate). Head D = head diameter; Chl = total chlorophyll; Car = total carotenoids; TPC = Total phenolic contents; TFC = total flavonoid contents; DPPH = DPPH• scavenging activity; Protein = soluble protein content; Nitrate = nitrate content; Asa = ascorbic acid content.

CONCLUSIONS

This study highlights the considerable potential of soybean-derived protein hydrolysate (SPH1) as a plant biostimulant in hydroponic lettuce cultivation, promoting plant growth, yield, and phytochemical content while maintaining acceptable nitrate levels in both deep-water culture and nutrient film technique hydroponic systems. Pearson correlation analysis indicated robust positive correlations between total biomass and characteristics, including soluble protein, ascorbic acid, and antioxidant activity, implying that enhanced growth is intricately associated with elevated phytochemical accumulation. Principal component analysis (PCA) corroborated these findings, demonstrating distinct separation between control and SPH1-treated plants, with the treated groups clustering at elevated values for critical growth and quality parameters. SPH1 also contributes to sustainable farming by diminishing the reliance on chemical fertilizers and mitigating their environmental impact. Additional studies are required to clarify its fundamental mechanisms and enhance application

approaches, as well as to assess economic feasibility and resolve regulatory concerns for successful commercial implementation in hydroponic agriculture.

CONFLICT OF INTEREST

The authors declare that they have no conflicting financial interests or personal relationships that may have potentially influenced the investigation presented in this publication. All authors granted their consent for publication.

ACKNOWLEDGEMENTS

This research received financial support and sponsorship from the Petchra Pra Jom Klao Doctoral Scholarship provided by King Mongkut's University of Technology Thonburi (KMUTT) under agreement number 56/2565 to Ms. Aryanis Mutia Zahra. We appreciate the technical assistance provided by Dr. Chalida Cholmaitri and Mr. Apichai Jenjob, who are both members of the Postharvest Plant Physiology Laboratory, SBT KMUTT.

REFERENCES

- Aires, L.M.I., K. Ispolnov, T.R. Luz, H. Pala and J.S. Vieira. 2023. Optimization of an indoor DWC hydroponic lettuce production system to generate a low N and P content wastewater. *Processes*. 11(2): 365. <https://doi.org/10.3390/pr11020365>.
- Attard, E. 2013. A rapid microtitre plate Folin-Ciocalteu method for the assessment of polyphenols. *Cent. Eur. J. Biol.* 8(1): 48–53. <https://doi.org/10.2478/s11535-012-0107-3>.
- Bracino, A.A., R.S. Concepcion, E.P. Dadios and R.R.P. Vicerra. 2020. Biofiltration for recirculating aquaponic systems: A review, pp. 1–6. *In: The Proceedings of 2020 IEEE 12th International Conference on Humanoid, Nanotechnology, Information Technology, Communication and Control, Environment, and Management (HNICEM)*. Manila, Philippines. <https://doi.org/10.1109/HNICEM51456.2020.9400136>.
- Bradford, M.M. 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal. Biochem.* 72: 248–254. [https://doi.org/10.1016/0003-2697\(76\)90527-3](https://doi.org/10.1016/0003-2697(76)90527-3).
- Cai, L., X. Gong, H. Ding, S. Li, D. Hao, K. Yu, Q. Ma, X. Sun and M.A. Muneer. 2022. Vermicomposting with food processing waste mixtures of soybean meal and sugarcane bagasse. *Environ. Technol. Innov.* 28: 102699. <https://doi.org/10.1016/j.eti.2022.102699>.

- Casadesús, A., M. Pérez-Llorca, S. Munné-Bosch and J. Polo. 2020. An enzymatically hydrolyzed animal protein-based biostimulant (Pepton) increases salicylic acid and promotes growth of tomato roots under temperature and nutrient stress. *Front. Plant Sci.* 11: 953. <https://doi.org/10.3389/fpls.2020.00953>.
- Cataldo, D.A., M. Maroon, L.E. Schrader and V.L. Youngs. 1975. Rapid colorimetric determination of nitrate in plant tissue by nitration of salicylic acid. *Commun. Soil Sci. Plant Anal.* 6(1): 71–80. <https://doi.org/10.1080/00103627509366547>.
- Choi, S., G. Colla, M. Cardarelli and H.J. Kim. 2022. Effects of plant-derived protein hydrolysates on yield, quality, and nitrogen use efficiency of greenhouse grown lettuce and tomato. *Agronomy.* 12(5): 1018. <https://doi.org/10.3390/agronomy12051018>.
- Di Mola, I., E. Cozzolino, L. Ottaiano, S. Nocerino, Y. Roupael, G. Colla, C. El-Nakhel and M. Mori. 2020. Nitrogen use and uptake efficiency and crop performance of baby spinach (*Spinacia oleracea* L.) and Lamb's lettuce (*Valerianella locusta* L.) grown under variable sub-optimal N regimes combined with plant-based biostimulant application. *Agronomy.* 10(2): 278. <https://doi.org/10.3390/agronomy10020278>.
- El-Nakhel, C., E. Cozzolino, L. Ottaiano, S.A. Petropoulos, S. Nocerino, M.E. Pelosi, Y. Roupael, M. Mori, and I. Di Mola. 2022. Effect of biostimulant application on plant growth, chlorophylls and hydrophilic antioxidant activity of spinach (*Spinacia oleracea* L.) grown under saline stress. *Horticulturae.* 8(10): 971. <https://doi.org/10.3390/horticulturae8100971>.
- European Food Safety Authority. 2008. Nitrate in vegetables-scientific opinion of the panel on contaminants in the food chain. *EFSA Journal.* 689: 1–79. <https://doi.org/10.2903%2Fj.efsa.2008.689>.
- Griffith, M.A.C., G.P. Buss, P.A. Carroll, X. Yang, J.L. Griffis Jr., G. Papkov, S. Bauer, K. Jackson and A.K. Singh. 2023. The comparative performance of nutrient-film technique and deep-water culture method of hydroponics for GREENBOX technology. *Agric. Sci.* 14: 1108–1120. <https://doi.org/10.4236/as.2023.148074>.
- Hooks, T., L. Sun, Y. Kong, J. Masabni and G. Niu. 2022. Effect of nutrient solution cooling in summer and heating in winter on the performance of baby leafy vegetables in deep-water hydroponic systems. *Horticulturae.* 8(8): 749. <https://doi.org/10.3390/horticulturae8080749>.
- Ide, R., A. Ichiki, T. Suzuki and Y. Jitsuyama. 2022. Analysis of yield reduction factors in processing tomatoes under waterlogging conditions. *Sci. Hortic.* 295: 110840. <https://doi.org/10.1016/j.scienta.2021.110840>.
- López-Pozos, R., G.A. Martínez-Gutiérrez, R. Pérez-Pacheco and M. Urrestarazu. 2011. The effects of slope and channel nutrient solution gap number on the yield of tomato crops by a nutrient film technique system under a warm climate. *HortScience.* 46(5): 727–729. <https://doi.org/10.21273/hortsci.46.5.727>.
- Matsumiya, Y. and M. Kubo. 2011. Soybean peptide: Novel plant growth promoting peptide from soybean, pp. 215–230. *In: H. El-Shemy, (Ed.), Soybean and Nutrition.* Intech Open, London, UK.
- Matthews, R.F. and J.W. Hall. 1978. Ascorbic acid, dehydroascorbic acid and diketogulonic acid in frozen green peppers. *J. Food Sci.* 43(2): 532–534. <https://doi.org/10.1111/j.1365-2621.1978.tb02347.x>.
- Miceli, A., F. Vetrano and A. Moncada. 2021. Influence of *Ecklonia maxima* extracts on growth, yield, and postharvest quality of hydroponic leaf lettuce. *Horticulturae.* 7(11): 440. <https://doi.org/10.3390/horticulturae7110440>.

- Mohana Dass, S., T.T. Chai, H. Cao, A.L. Ooi and F.C. Wong. 2021. Application of enzyme-digested soy protein hydrolysate on hydroponic-planted lettuce: Effects on phytochemical contents, biochemical profiles and physical properties. *Food Chem. X.* 12: 100132. <https://doi.org/10.1016/j.fochx.2021.100132>.
- Nolsøe, H. and I. Undeland. 2009. The acid and alkaline solubilization process for the isolation of muscle proteins: State of the art. *Food Bioproc. Tech.* 2: 1–27. <https://doi.org/10.1007/s11947-008-0088-4>.
- O’Sullivan, C.A., G.D. Bonnett, C.L. McIntyre, Z. Hochman and A.P. Wasson. 2019. Strategies to improve the productivity, product diversity and profitability of urban agriculture. *Agric. Syst.* 174: 133–144. <https://doi.org/10.1016/j.agsy.2019.05.007>.
- Ottaiano, L., I. Di Mola, E. Cozzolino, C. El-Nakhel, Y. Rouphael and M. Mori. 2021. Biostimulant application under different nitrogen fertilization levels: Assessment of yield, leaf quality, and nitrogen metabolism of tunnel-grown lettuce. *Agronomy.* 11(8): 1613. <https://doi.org/10.3390/agronomy11081613>.
- Popko, M., I. Michalak, R. Wilk, M. Gramza, K. Chojnacka and H. Górecki. 2018. Effect of the new plant growth biostimulants based on amino acids on yield and grain quality of winter wheat. *Molecules.* 23(2): 470. <https://doi.org/10.3390/molecules23020470>.
- Rouphael, Y., P. Carillo, F. Cristofano, M. Cardarelli and G. Colla. 2021. Effects of vegetal-versus animal-derived protein hydrolysate on sweet basil morpho-physiological and metabolic traits. *Sci. Hortic.* 284: 110123. <https://doi.org/10.1016/j.scienta.2021.110123>.
- Schiavon, M., A. Ertani and S. Nardi. 2008. Effects of an alfalfa protein hydrolysate on the gene expression and activity of enzymes of the tricarboxylic acid (TCA) cycle and nitrogen metabolism in *Zea mays* L. *J. Agric. Food Chem.* 56(24): 11800–11808. <https://doi.org/10.1021/jf802362g>.
- Tadolini, B., C. Juliano, L. Piu, F. Franconi and L. Cabrini. 2000. Resveratrol inhibition of lipid peroxidation. *Free Radic. Res.* 33(1): 105–114. <https://doi.org/10.1080/10715760000300661>.
- USDA (United States Department of Agriculture). 2024. Thailand: Oilseeds and Products Annual. Available Source: <https://fas.usda.gov/data/thailand-oilseeds-and-products-annual-8>, July 21, 2024.
- Wang, W., C. Zhang, M. Shang, H. Lv, B. Liang, J. Li and W. Zhou. 2022b. Hydrogen peroxide regulates the biosynthesis of phenolic compounds and antioxidant quality enhancement in lettuce under low nitrogen condition. *Food Chem. X.* 16: 100481. <https://doi.org/10.1016/j.fochx.2022.100481>.
- Wang, Z., R. Yang, Y. Liang, S. Zhang, Z. Zhang, C. Sun, J. Li, Z. Qi and Q. Yang. 2022a. Comparing efficacy of different biostimulants for hydroponically grown lettuce (*Lactuca sativa* L.). *Agronomy.* 12(4): 786. <https://doi.org/10.3390/agronomy12040786>.
- Wellburn, A.R. 1994. The spectral determination of chlorophylls a and b, as well as total carotenoids, using various solvents with spectrophotometers of different resolution. *J. Plant Physiol.* 144(3): 307–313. [https://doi.org/10.1016/S0176-1617\(11\)81192-2](https://doi.org/10.1016/S0176-1617(11)81192-2).
- Yang, T., U. Samarakoon and J. Altland. 2024. Growth, phytochemical concentration, nutrient uptake, and water consumption of butterhead lettuce in response to hydroponic system design and growing season. *Sci. Hortic.* 332: 113201. <https://doi.org/10.1016/j.scienta.2024.113201>.
- Zhang, X., J. Yin, Y. Ma, Y. Peng, O. Fenton, W. Wang, W. Zhang and Q. Chen. 2024. Unlocking the potential of biostimulants derived from organic waste and by-product sources: Improving plant growth and tolerance to abiotic stresses in agriculture. *Environ. Technol. Innov.* 34: 103571. <https://doi.org/10.1016/j.eti.2024.103571>.

- Zhishen, J., T. Mengcheng and W. Jianming. 1999. The determination of flavonoid contents in mulberry and their scavenging effects on superoxide radicals. *Food Chem.* 64(4): 555–559. [https://doi.org/10.1016/S0308-8146\(98\)00102-2](https://doi.org/10.1016/S0308-8146(98)00102-2).
- Zhou, W., W. Zheng, H. Lv, Q. Wang, B. Liang and J. Li. 2022. Foliar application of pig blood-derived protein hydrolysates improves antioxidant activities in lettuce by regulating phenolic biosynthesis without compromising yield production. *Sci. Hortic.* 291: 110602. <https://doi.org/10.1016/j.scienta.2021.110602>.