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Application of enzyme-digested soy protein hydrolysate on hydroponic-planted lettuce: Effects on phytochemical contents, biochemical profiles and physical properties

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A B S T R A C T

Plant-derived protein hydrolysates (PH) offer many promising benefits and applications. In this paper, PH was prepared from soy-based processing waste via enzymatic-digestion method and supplemented into hydroponic grow medium solution. The hydroponic-planted lettuces were then harvested and assessed for their selected phytochemical contents, biochemical parameters, antioxidative enzymes and mineral contents. Additionally, the lettuce's physical properties were assessed. Based on our results, increases in three phytochemical contents were observed, in a PH concentration-dependent manner (0–0.01 mg/mL). Similar trends were also observed for chlorophyll and carotenoid contents. Harvested lettuce length and fresh weight peaked at 0.01 mg/mL PH treatment group, but not in a PH concentration-dependent manner. Whereas, for other physical properties (lettuce leaf surface area, root length, root weight), no significant difference was detected. Through this study, we are hoping to contribute toward the potential PH application as agricultural nutrient supplement for hydroponic plants, with accompanied improvements in harvest yields and nutritional contents.

Introduction

Plant-derived biochemical components represent a vast library of candidates with promising benefits and applications. Among them, the potential rewards of plant-derived protein hydrolysates (PH) are particularly promising. In general, PH is derived from the parental proteins via enzymatic hydrolytic digestion, microbial proteolytic actions or fermentation (Chai et al., 2021). Following these hydrolytic processes, plant protein macromolecules are converted into smaller-sized protein hydrolysates and peptides, accustomed with their unique physical and biological properties. Previous studies had reported on the various applications and bioactivities of PH, ranged from health-promoting nutrient supplements, bioactive potentials, to various therapeutic applications, including anti-microbial, anti-diabetic and anti-cancer (Chai et al., 2020). In addition, several recent studies reported on the applications of pH as agricultural nutrient supplement in soil-based planting (Consentino et al., 2020; Nurdiawati et al., 2019).

On the other hand, hydroponic plating system is an increasingly popular method for horticultural and plant food production. In hydroponic system, the planted crops are grown in a soilless system, by suspending their roots in grow medium solution, with reduced interferences from environmental factors such as soil quality, irrigation and climate. In addition, hydroponic system also enables better utilization of land space, as well as close proximity to the targeted consumer markets (Sharma et al., 2019; Treftz & Omaye, 2016). Popular plant food produced using hydroponic system including but not limited to lettuces, herbs, tomatoes, strawberries and other horticulture plants. However, unlike the vast variety of commercial fertilizers available for traditional soil-based planting, relatively limited commercial nutrient supplements are currently available for application in hydroponic planting, especially those using PH derived from enzymatic-digested processing-wastes.

In this paper, we aim to determine the effects of protein hydrolysate (PH) on hydroponic planted lettuce. We would like to test if PH could

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help to improve the hydroponic crop's yield, as well as the phytochemical and biochemical contents. Here, PH was firstly prepared from soy-based processing waste via enzymatic digestion method and then mixed into the hydroponic grow solution at different concentrations. Following the PH-treatments, the harvested lettuce samples were assessed for their selected phytochemical contents (total phenolics, total flavonoids, and total hydroxycinnamic acids). Additionally, the harvested lettuces samples were also tested for their selected biochemical parameters (chlorophyl, carotenoid, ascorbic acid, and antioxidant enzymes). Lastly, the harvested lettuce weight and other physical properties that may affect consumer purchasing desires (lettuce length and leave surface area) were also assessed, along with factors that may affect the plant's nutrient uptakes (root length and weight). Through this study, we are hoping to contribute toward the potential PH application as nutrient supplement for hydroponic plants, with accompanied improvements in harvest yields and nutritional contents.

Materials and methods

Protein hydrolysate preparation

Protein hydrolysate (PH) was prepared using collected soy waste from food-processing sector. Firstly, soy protein isolate was obtained by suspending 100 g of soy waste in 500 mL deionized water at a ratio of 1:5, stirred for 1 h at room temperature, followed by heating for 20 min at 90 °C. Following centrifugation, the collected supernatant was adjusted to 80% ammonium sulfate saturation to precipitate the soy protein from the mixture. Then, the isolated soy proteins were dialyzed overnight using dialysis tubing (molecular weight cut-off: 6–8 KDa). Next, PH was prepared by incubating a mixture containing 0.5 g isolated soy protein and 0.05 g of protease (Alcalase), in 100 mL of 50 mM Phosphate Buffer Saline (PBS) for 6 h at 50 °C. Following heat-inactivation (20 min at 100 °C), the prepared PH was cooled on ice and stored at −20 °C for further use (Chai et al., 2021; Quah et al., 2018).

Testing of prepared soy PH on hydroponic lettuce

The hydroponic plant that had been chosen as testing model was Green Coral Lettuce (*Lactuca sativa* L.). Firstly, germinated lettuce samples in mesh pots were placed into covered hydroponic containers following the Kratky method, with exposure to natural photo-period and ambient daily temperatures (Kratky, 2005). The lettuce roots were submerged into a commercial hydroponic grow medium solution (Well Grow Seeds Co.), whcih was prepared by diluting 5 mL of Hydroponic Solution A (Ca, NO_3, NH_4, Fe, K) and Solution B (H_2PO_4, SO_4, K, Mg, B, Cu, Mo) with 2 L of distilled water, and the lettuce roots of the hydroponic plants were suspended into this prepared hydroponic medium solution. These young lettuce plant samples were then divided into treatment groups (with soy PH) and a control group (without soy PH). In the three treatment groups, the preparared soy PH was added into the hydroponic grow media, at concentrations of 0.001, 0.01, and 0.1 mg/mL, respectively. These hydroponic-planted lettuce samples were then allowed to grow for the next nine weeks, with pH maintained at 6.0 and fresh grow media changed at three weeks interval. After harvested, the fresh lettuce weights and other physical properties that may affect consumer's purchasing desires (lettuce length and leave surface areas) were measured and recorded, along with factors that may affect the plant's nutrient uptakes (root length and weight).

Determination of phytochemical contents

Phytochemical contents in the harvested lettuce samples were determined using previously published conditions (Wong, Chai, & Hoo, 2012). Briefly, Total phenolic content (TPC) was determined using Folin-Ciocalteu reagent and reported as mg gallic acid equivalents /g dry matter (mg GAE /g DM). Total flavonoid content (TPC) was determined using aluminium chloride reagent and reported as mg quercetin equivalents /g dry matter (mg QE /g DM). Total hydroxycinnamic acid content (THC) was determined using Arnow's reagent and reported as mg caffeic acid equivalents /g dry matter (mg CAE /g DM). The absorbances were monitored at 765, 510 and 490 nm, respectively (Chai et al., 2015; Wong, Chai, & Hoo, 2012). Lastly, ascorbic acid content in the harvested lettuce was determined using 2, 6-dichlorophenol indophenol (DCPIP) method, with ascorbic acid as standard and reported as mg/g.

Biochemical profiles and mineral contents of harvested lettuces

After harvested, 1 g of hydroponic-grown lettuce samples were homogenized in an iced mortar and pestle using 10 mL of extraction buffer containing 50 mM phosphate buffer (pH 7.4), 0.5 mM ascorbate and 1 mM EDTA. The mixture was then centrifuged at 10,000 rpm for 15 min, and the supernatant was collected and used for further analysis.

The superoxide dismutase (SOD) activity was determined as previously reported, by measuring the ability of lettuce extract to inhibit the photochemical reduction of nitroblue tetrazolium (NBT), using a 3 mL reaction mixture containing 100 mM potassium phosphate buffer (pH 7.8), 0.1 mM EDTA, 13 mM methionine, 2.25 mM NBT (Sigma-Aldrich), 60 μM riboflavin (Sigma-Aldrich), and leaf extract sample (25–100 μL of 50 mg/mL). After 15 min of fluorescent light exposure, the 560 nm absorbance was determined (Beauchamp & Fridovich, 1971; Malar, Sahi, Favas, & Venkatachalam, 2015). At the same time, the catalase (CAT) activity of lettuce extract was determined using a 3 mL reaction mixture containing 50 mM potassium phosphate buffer (pH 7.0), 0.25 mL leaf extract sample and 60 mM hydrogen peroxide (H_2O_2). Next, the decrease in absorbance at 240 nm was monitored to calculate the H_2O_2 decomposition (extinction coefficient of 43.6 M^{-1} cm^{-1}) (Dhindsa, Plumb-Dhindsa, & Thorpe, 1981; Malar, Sahi, Favas, & Venkatachalam, 2015).

Chlorophyll and carotenoids contents were determined according to published conditions, by incubating 0.5 g of stripped fresh lettuce into 10 mL of 98% acetone. After overnight incubation, the absorbances at 661.6, 644.8 and 470.0 nm were recorded to calculate the concentrations of chlorophyll *a* (c_a), chlorophyll *b* (c_b) and the sum of leaf carotenoid (c_{x+c}) (Lichtenthaler & Buschmann, 2001). Mineral content analysis using atomic absorption spectroscopy (AAS) was performed using published conditions with some modifications (Uddin et al., 2016). Briefly, 0.1 g of the powdered lettuce sample was added into 10 mL 65% nitric acid (HNO_3) and boiled for 15 min. After cooling at room temperatue and filtered, the filtrate was then topped up to the final volume of 50 mL with distilled water. The following eight selected minerals: aluminium (Al), cadmium (Cd), calcium (Ca), copper (Cu), Iron (Fe), magnesium (Mg), lead (Pb) and zinc (Zn) were then analyzed using a flame atomic absorption spectrometer (FAAS) (Agilent Flame Atomic Absorption Spectrometer, Model 280FSAA), by following the absorbances at 309.3, 422.7, 228.8, 324.8, 248.3, 285.2, 217.0, 213.9 nm, respectively.

Physical characteristics of harvested lettuces

After harvested, the hydroponic-grown lettuces were assessed for their selected physical parameters. Lettuce length and weight were measured using the aerial parts of the harvests, and reported as centimeter (cm) and gram (g), respectively. For the determination of lettuce leaf surface areas, twenty six healthy lettuce leaves were selected from each treatment group, and a digital camera was used to capture the outlined tracing of each leaf sample. The captured images were then analyzed by ImageJ software (NIH) to determine the leave surface areas and reported as square centimeter (cm^2). Lastly, the lettuce root length and weight were measured and reported as cm and g, respectively.

Table 1
Lettuce extracts tested for their total phenolic content (TPC), total flavonoid content (TFC), total hydroxycinnamic acid content (THC), and ascorbic acid.

PH Conc. (mg/ml)	TPC (mg GAE/ g DW)	TFC (mg QE/ g DW)	THC (mg CAE/ g DW)	Ascorbic acid (mg/g)
0	0.27 ± 0.00^{c}	3.18 ± 0.03^{c}	1.53 ± 0.02^{c}	0.39 ± 0.02^{b}
0.001	0.29 ± 0.00^{b}	3.44 ± 0.03^{b}	1.59 ± 0.02^{b}	0.37 ± 0.01^{b}
0.01	0.30 ± 0.00^{a}	3.58 ± 0.04^{a}	1.69 ± 0.00^{a}	0.59 ± 0.01^{a}
0.1	0.26 ± 0.00^{d}	3.53 ± 0.04^{a,b}	1.67 ± 0.02^{a}	0.29 ± 0.00^{c}

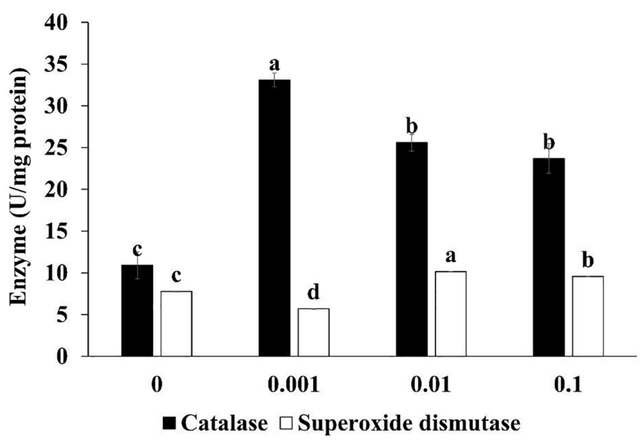

Fig. 1. Antioxidant enzymes contents in harvested lettuces, reported as Unit (U) per milligram of protein.

Data analysis

Data are presented as mean ± standard errors (n = 4). The statistical analysis was performed using SAS (Version 9.4). Data were analyzed by the ANOVA test, and means of significant differences were separated using Fisher's Least Significant Difference (LSD) test at the 0.05 level of probability.

Results and discussion

Determination of phytochemical contents

Numerous studies have previously reported on how plant phytochencal contents are directly linked to their bioactivities and pharmacological potentials (Cao et al., 2017; Teng et al., 2019; Wong, Tan, & Chai, 2016). In order to determine the effect of protein hydrolysate (PH) treatments, the harvested hydroponic lettuce samples were firstly tested for the phytochemical contents in three categories, namely total phenolic content (TPC), total flavonoid content (TPC), and total hydroxycinnamic acid content (THC). Here, when treated using PH concentrations ranged from 0 to 0.01 mg/mL, increasing concentrations of TPC, TFC, and THC were detected in the PH-treated lettuce (Table 1). However, no further increase in phytochemical content was detected, in lettuce samples treated with higher PH concentration (0.1 mg/mL). Similar trend was observed for ascorbic acid contents. The overall increase ranged from 1.10 to 1.13 folds, compared to non-treatment group (zero PH) (Table 1). Previously, several studies had also reported on increases in plant's phenolic content and ascorbic acid, following the PH applications in both soil-based and hydroponic-based plantings (Caruso et al., 2019; Consentino et al., 2020; Ertani et al., 2019).

Biochemical profiles and mineral contents of harvested lettuces

After protein hydrolysate (PH) treatments, the harvested hydroponic lettuces were also characterized for their biochemical profiles (chlorophyll, carotenoid and antioxidant enzymes), as well as mineral contents. These selected parameters were tested, as they may affect the harvested lettuce's antioxidant and stress tolerance, photosyntesis and growth rates, as well as nutritional contents (Güneş, Kordali, Turan, & Usanmaz Bozhüyük, 2019; Han, Zhang, & Skibsted, 2012; Mourato, Martins, & Cuypers, 2009). For antioxidant enzymes contents, we focused on studying catalase (CAT) and superoxide dismutase (SOD). Based on our results (Fig. 1), higher CAT and SOD contents were detected in PH-treated lettuces, in the PH concentration ranges of 0.001–0.1 and 0.01–0.1 mg/mL, respectively. In these mentioned PH concentration ranges, higher CAT (2.17–3.04 folds) and SOD (1.23–1.31folds) were detected, compared to the control group (zero PH) (Fig. 1). However, we could not rule out the possbility that the elevated CAT and SOD levels were caused by increased stress levels in PH-treated lettuces.

Table 2
Lettuce extracts tested for their chlorophyll *a* (Chl a), chlorophyll *b* (Chl b) and carotenoid (C_{x+c}) contents.

PH Conc. (mg/ml)	Chl_a (μg/ml)	Chl_b (μg/ml)	C_{x+c} (μg/ml)
0	5.44 ± 0.03^{d}	1.96 ± 0.07^{d}	1.60 ± 0.02^{d}
0.001	6.49 ± 0.03^{c}	2.60 ± 0.02^{c}	1.97 ± 0.01^{c}
0.01	9.89 ± 0.08^{a}	3.69 ± 0.08^{a}	2.73 ± 0.02^{a}
0.1	9.18 ± 0.02^{b}	3.13 ± 0.04^{b}	2.61 ± 0.01^{b}

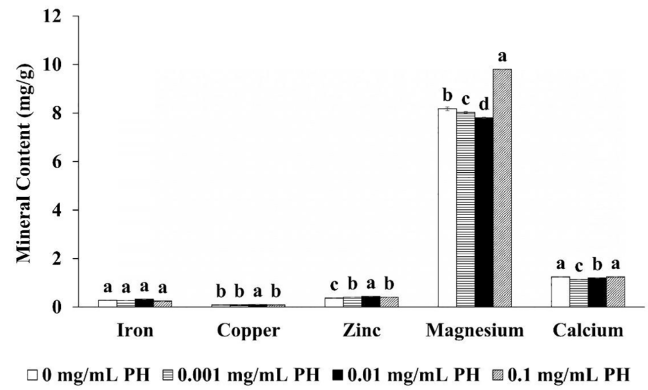

Fig. 2. Harvested lettuce samples tested for their mineral contents. Data are reported as mean ± SE values (n = 4). Different superscripts (a-d) indicate statistically significant differences ($p < 0.05$).

On the other hand, when tested with PH concentrations ranged from 0 to 0.1 mg/mL, both chlorophyll and caratenoid concentrations peaked in lettuce sample treated with 0.01 mg/mL of PH. No further increase in chlorophyll and caratenoid was detected at higher PH concentration (0.1 mg/mL). Compared to the non-treatment groups, the increase in chlorophyll and caratenoid ranged from 1.71 to 1.88 folds (Table 2). Our observation is agreeable with previous studies which reported on increased chlorophyll contents in PH-treated peppermint, maize and patchouli plants, using both soil-based and hydroponic-based plantings (Aktsoglou et al., 2021; Ertani et al., 2019; Nurdiawati et al., 2019). In future studies, it would be interesting to determine the exact mechanism that leads to the increase in chlorophyll contents, following PH-treatment.

In addition, to test how the PH will affect the mineral absorption and bio-accumulation of minerals in hydroponic-grown lettuces, we applied flame atomic absorption spectrometer to detect for the presence of eight selected minerals in our harvested lettuce samples. Here, aluminium, cadmium and lead were not detected in any of our lettuce sample (data not shown). Whereas for the other five mineral elements (iron, copper, zinc, magnesium and calcium), their presences were detected in all lettuce samples (Fig. 2). Previous studies reported on enhanced levels of selected minerals in *Diplotaxis tenuifolia* and maize plants, following PH

Table 3
Physical properties of hydroponic-planted lettuces following nine weeks of pH treatments.

PH Conc. (mg/ml)	Lettuce length (cm)	Lettuce weight (g)	Leave surface area (cm^2)	Root length (cm)	Root weight (g)
0	17.60 ± 2.51^{b,c}	1.46 ± 0.65^{b}	493.76 ± 77.18^{a}	12.60 ± 2.37^{a}	0.05 ± 0.03^{a}
0.001	14.88 ± 2.26^{c}	1.52 ± 0.67^{b}	248.44 ± 40.06^{b}	13.58 ± 2.93^{a}	0.08 ± 0.04^{a}
0.01	25.73 ± 1.76^{a}	3.28 ± 0.53^{a}	531.65 ± 47.65^{a}	18.28 ± 1.84^{a}	0.13 ± 0.02^{a}
0.1	22.08 ± 1.83^{a,b}	2.26 ± 0.33^{a,b}	563.49 ± 65.55^{a}	15.78 ± 0.96^{a}	0.10 ± 0.01^{a}

treatments (Caruso et al., 2019; Ertani et al., 2019). In our study using lettuce, we did not detected any significant difference of mineral contents in our PH-treated lettuces, compared to those in the control group. One interesting exception was the higher magnesium (Mg) content detected in lettuce samples grown at the highest PH concentration (0.1 mg/mL)(Fig. 2). Here, the Mg content is 1.2 fold higher, compared to the control group. The exact mechanism for this observed spike in Mg level remains to be determined.

Physical characteristics of harvested lettuces

To determine the effects of protein hydrolysate (PH) on the hydroponic-grown lettuces, the harvested lettuces were also asesseed for their fresh weights. Additionally, physical properties that may affect consumer purchasing desires (lettuce length and leave surface areas) were also assessed, along with factors that may affect the plant's nutrient uptakes (root length and weight). Compared to the control group (zero PH), harvested lettuce's length and fresh weight peaked at 0.01 mg/mL PH treatment group (Table 3). However, we did not observe any PH concentration-dependent trend. Additionally, no further increase in lettuce length and fresh weight was detected at higher PH concentration. Whereas, for other physical parameters (lettuce's leaf surface area, root length, root weight), no significant difference was detected, compared to the control group (Table 3). On the other hand, in one previous PH study using spinach, increased yield and better root development were reported (Dewang & Devi, 2021). Similarly, another PH study conducted using patchouli, increased leaf area and dry weight were reported (Nurdiawati et al., 2019). For our future study, it would be interesting to study PH prepared from different sources, using different processing parameters.

Conclusion

In conclusion, we had prepared protein hydrolysate (PH) from soy-based processing waste via enzymatic-digestion method, followed by testing on hydroponic-planted lettuces. Increased phytochemicals, chlorophyll and carotenoid contents were observed. Harvested lettuce length and fresh weight peaked at 0.01 mg/mL PH treatment group, but not in a PH concentration-dependent manner. Whereas, for other physical properties (lettuce leaf surface area, root length, root weight), no significant difference was detected. With more similar studies, it is hoped that the application of pH as hydroponic nutrient supplement could be further explored.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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