EFFECTS OF ENZYME-DIGESTED PROTEIN HYDROLYSATES DERIVED FROM *Clarias batrachus* (CLARIAS CATFISH) VISCERA ON THE GROWTH OF *Brassica rapa subsp. chinensis* (BOK CHOY)

By

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ABSTRACT

Effects of Enzyme-digested Protein Hydrolysates Derived from *Clarias batrachus* (Clarias Catfish) Viscera on The Growth of *Brassica rapa* subsp. *chinensis* (Bok Choy)

KOH CHARL TSEN

Protein hydrolysate (PH) is a nitrogen-rich material composed of amino acids that serves as an effective liquid fertiliser to promote vegetative growth in crops. Although numerous studies have reported the beneficial effects of PH on plant development, limited information is available regarding the influence of specific PH sources and concentrations on optimal plant growth and biochemical properties. In this study, *Brassica rapa* subsp. *chinensis* (Bok Choy) was treated with varying concentrations (0, 2000, 4000, 6000, and 8000 mg/L) of *Clarias batrachus* (Clarias Catfish) protein hydrolysate under a randomised complete block design over 35 days. Growth parameters, including plant weight, height, total chlorophyll content (TCC), total phenolic content (TPC), total flavonoid content (TFC), and antioxidant potential, were assessed. Heavy metal contents, lead and cadmium were also measured to ensure compliance with food safety standards. The results demonstrated that treatment with 6000 mg/L PH yielded the highest growth and biochemical performance, with significant increases in shoot and root biomass, TCC, TPC, TFC, and antioxidant activity. While all PH-treated groups showed improved biomass compared to the control, a decline was observed at 8000 mg/L. In conclusion, Clarias catfish protein hydrolysate markedly enhanced both the growth and antioxidant capacity of Bok Choy, with 6000 mg/L identified as the optimal concentration. These findings highlight the potential of fish-derived protein hydrolysate as a sustainable and efficient biofertilizer for improving crop productivity and nutritional quality.

Keywords: protein hydrolysate; *Brassica rapa* subsp. *chinensis*; *Clarias batrachus*; biochemical content; biofertilizer

Subject Area: QD415 – 436 Biochemistry

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FACULTY OF SCIENCE

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Date: 30/4/2025

PERMISSION SHEET

It is hereby certified that **KOH CHARL TSEN** has completed this project entitled "**Effects of Enzyme-digested Protein Hydrolysates Derived from *Clarias batrachus* (Clarias Catfish) Viscera on The Growth of *Brassica rapa* subsp. *chinensis* (Bok Choy)"** under the supervision of Dr Wong Fai Chu (Supervisor) and Dr Chai Tsun Thai (Co-supervisor) from the Department of Chemical Science, Faculty of Science.

I understand that the University will upload a softcopy of my final year project in PDF format into the UTAR Institutional Repository, which may be made accessible to the UTAR community and the public.

Yours truly

Koh Charl Tsen

(KOH CHARL TSEN)

(30/4/2025)

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PBS	Phosphate Buffer Saline
DNA	Deoxyribonucleic acid
TPC	Total Phenolic Content
TFC	Total Flavonoid Content
HNO_3	Nitric Acid
Chl_{α}	Chlorophyll α
Chl_{β}	Chlorophyll β
C_{x+c}	Carotenoids
Cd	Cadmium
Pb	Lead
PH	Protein Hydrolysate

CHAPTER 1
INTRODUCTION

1.1 Introduction

Agriculture is the cornerstone of every country and civil society. Agriculture practices are more convenient to support the growth of a society than hunting. A successful agricultural practice not only benefits our own country but also helps our country to export goods to other countries and earn benefits. However, this scenario can only be achieved in big countries such as the United States and China, where their food self-sufficiency rate are 86.5% and 65.8% respectively. On the other hand, Malaysia's food self-sufficiency rate is only 44.7% for vegetables (The Malaysian Reserve, 2023). Malaysia has a diverse agricultural sector that plays a significant role in its economy, contributing approximately 12% to the nation's GDP and employing about 16% of the population (Rulia Akhtar, 2023). However, the dominant plantations are oil palm, rubber, cocoa and paddy rice. Hence, Malaysia is required to import fresh goods (fruits and vegetables) from other countries to fulfil society's needs, such as the USA, China and Egypt (Rulia Akhtar, 2023). Besides that, sustainability and efficiency have become the most important parameters to focus on nowadays. For example, fossil fuels require a million years to be formed and extracted. In contradiction, these fuels in our vehicles just burn and become energy in the blink of an eye. The world keeps developing and requires more energy to sustain itself. However, the supply of resources is far more deficient to meet the global demand. In the 1970s, the global was facing a big crisis in fossil fuels, and the reason is simple: the world economy was under rapid development and required a huge demand for energy to develop new infrastructure.

However, the process of harvesting fossil fuels is far behind the rapid development of the economy. But luckily, this global issue has been overcome by a solution. The solution was not because the world invented new machinery to harvest fossil fuels or found a huge amount of it. However, the world has noticed the importance of sustainability and efficiency. Most of the scientist had revised their methodology on using the fuels, and they found that most of the machinery was consuming much but worked less than expected. Therefore, they keep modifying the technology to make it work more efficiently with less fuel consumption. For example, the Ford Taurus executive car in 1989 required 1 gallon of fuel to run 18 miles. Compared to now, a Chevrolet Suburban SUV in 2021 only required 1 gallon of fuel to run 18.1 miles. Hence, the methodology to achieve sustainability and efficiency becomes crucial to maintaining the Earth's ecosystem as well as all races.

Before the year 2022, the farmers used chemical fertilisers to boost their crop yield had been their agricultural practice for a long time. But after the day of Russo-Ukraine War started again on 24 February 2022, most things had changed. The American and European Union had cut down the economic relationship with Russia as a punishment for invading Ukraine (CFR.org Editors, 2022). On the other hand, Russia also shut off the Nord Stream. This series of actions had a major impact on the chemical plants of chemical fertiliser in Germany, such as EuroChem Group, Grupa Azoty, and ICL Group LTD (CFR.org Editors, 2022). One of the raw materials to make chemical fertilisers is methane gas, which is combined further with nitrogen gas to produce urea and ammonia as chemical fertilisers. Without the supply from Nord Stream, the entire production line and supply chain of the chemical fertiliser had broken up, which further caused the global food crisis. The Russo-Ukraine War has brought up the price of chemical fertiliser. The outcome of the event raised the price of goods and other consumables, and the consumers had to bear the consequences. Hence, every country ought to have contingency plans to replace the usage of chemical fertilisers.

Based on the news reported in Malaysia, our country keeps importing vegetables and fruits worth RM51.3 billion in 2017 and exceeded RM52 billion in 2018, and these figures are still increasing. This is because our land in Malaysia is not enough to produce crops to meet the local demand. Not to mention, the war event had worsened the situation for using chemical fertilisers and pesticides as agricultural practices. In recent years, there have been several methods, such as using protein hydrolysate to replace chemical fertilisers. Several studies have supported that using compost and protein hydrolysate can replace chemical fertilisers to enhance the crop's yield and overall health. In addition, the protein hydrolysate can be mixed with water as a foliar spray on the crops to induce similar pesticide effects. Apart from that, Osman, et al. also discovered that using protein hydrolysates digested by pepsin and papain promotes the productivity of *Pisum sativum*, dry seed weight, and lectin production against pest invasion. However, the protein hydrolysates as foliar applications do not work as strongly as chemical fertilisers and pesticides. This is because the effect of the protein hydrolysates takes a longer time for the plant to absorb and undergo a complex biochemical action to carry out the enhancing effects. On the other hand, chemical fertilisers and pesticides are simple molecules such as nitrogen, phosphorus and potassium which can be directly used by the plant and have out immediate effect. However, the use of chemical fertilisers and pesticides has violated the Sustainable Development Goals. The use of chemical fertilisers and pesticides will flow into the underground water and further to the river stream due to their high solubility. These chemicals will become a feed to the microorganisms and algae and cause an increase in biochemical oxygen demand (BOD) and algae bloom. On the other hand, organic fertilisers such as protein hydrolysates are larger molecules, which slowly degrade into smaller molecules for the plant to absorb. By comparing both organic and chemical fertilisers, the former can meet the requirements of the sustainable development plan.

In the current global trend, smart food waste management has also become an important milestone in achieving sustainable development plans. This management aims to reduce the environmental impact and maximise resource efficiency. The enhancement of the reuse and recycling of materials will cut down the intake of natural resources to achieve sustainability. Fish have been a good source of food for humans from ancient times until now. It has been described as a perfect diet not only for its taste as well as its ease to be digestion. The biological structure of the fish protein was simpler than red meat and poultry; the fish proteins have shorter muscle fibres compared to red meat and poultry. In addition, fish have less fat content than red meat and poultry. The study also shows that fish contain higher proportions of essential amino acids, vitamins and essential fatty acids, which are beneficial for the human diet. For these reasons, fish is considered healthier to be consumed compared to other red meats and poultry. Fish consumption increased proportionally over these years due to the growing population and health benefits. The most consumable fish in the current trend is catfish. Hence, tons and tons of fish by-products, such as viscera, were generated and left untreated. In Malaysia, the Clarias Catfish (*Clarias batrachus*) is the most consumed. However, consumers only consume the fillets. The other parts of Clarias Catfish, such as viscera and bones, are treated as waste and dumped into the waste field. The degradation of these materials will produce a huge amount of greenhouse gases, such as methane gas, which harm the environment and the ozone layer. These materials that were once part of the fish constitute up to 70% of the fish biomass, and the viscera constitute around 20% of its that rich in proteins and polyunsaturated lipids. With the aim of food waste management in valuing the reuse of materials, the proteinaceous Clarias Catfish waste can be treated to become protein hydrolysates.

Protein hydrolysates are produced by exposing plant or animal proteins to hydrolysis treatments, such as the enzymatic digestion approach. During the hydrolysis process, the peptide bonds in the proteins are broken, releasing their building blocks, such as peptides and amino acids. With their smaller sizes, peptides and amino acids in protein hydrolysates have higher permeability, and better absorbability and may confer unique biological activities when applied to vegetables. Enzymatic hydrolysis leads to an interesting peptide and amino acid-rich product with high nutritional value and bioactive properties. In this study, the Bok Choy (*Brassica rapa* subsp. *chinensis*) will be used as a model vegetable to test the effect of Clarias Catfish protein hydrolysate on the growth of 35 days. After the harvest, the Bok Choy from each treatment will be tested for their length, weight, total chlorophyll, total phenolic, total flavonoid content, antioxidant potential and heavy metal contents.

1.2 Research Rationale

Several studies have reported the beneficial effects of fish-derived protein hydrolysates in enhancing the growth of plants such as broccoli (*Brassica oleracea* var. *italica*) and other leafy vegetables. These studies indicate that fish protein hydrolysate (FPH) is a nutrient-rich material that can act as a biostimulant to promote plant growth (Pathak, et al., 2017; Ricci, et al., 2019). However, the specific type of fish and the anatomical part used for hydrolysate preparation are rarely detailed. Consequently, the effects of FPH derived specifically from fish viscera on plant physiological and biochemical traits remain unclear. Furthermore, there is limited information on the use of protein hydrolysate obtained from *Clarias batrachus* (Clarias Catfish) the most consumed freshwater species in Malaysia (Ahmad, et al., 2016) for plant cultivation. Therefore, this study aims to produce protein hydrolysate from *Clarias batrachus* viscera and evaluate its effects on the growth of *Brassica rapa* subsp. *chinensis* (Bok Choy).

1.3 Research Objectives

1. To investigate the effects of protein hydrolysate (PH) application on the growth of Bok Choy (*Brassica rapa* subsp. *chinenesis*) on its physical characteristics and chlorophyll contents.

2. To evaluate the influence of PH application on the antioxidant composition and antioxidative potential of Bok Choy, including total phenolic and flavonoid

contents as well as radical scavenging activities.

3. To determine the levels of heavy metals (Cd and Pb) in the PH and PH-treated Bok Choy using atomic absorption spectrometry (AAS).

CHAPTER 2

LITERATURE REVIEW

2.1 *Brassica rapa* subsp. *chinensis* (Bok Choy)

Brassica rapa subsp. *chinensis* is the scientific name of Bok Choy, which is commonly found in Southeast Asia (Soetan, et al., 2010). The Brassicaceae family consist of 372 genera and 4060 species (Soetan, et al., 2010). This family's most widely recognised species are *Brassica oleracea*, *Brassica rapa*, *Brassica napus*, *Raphanus sativus* and others. Within the species, there are also diverse into a few other subspecies. Take Brassica rapa as an example, the subspecies of *Brassica rapa* are turnip (*B. rapa* subsp *rapa*), Chinese cabbage (*B. rapa* subsp. *pekinensis*), Japanese mustard greens (*B. rapa* subsp. *narinosa*), rapeseed (*B. rapa* subsp. *oleifera*), Bok choy (*B. rapa* subsp. *chinensis*) and others (Baslam, et al., 2013).

Bok Choy originated in China and was introduced to Asian countries, including Malaysia, by Chinese immigrants. Bok Choy is one of the fast-growing vegetables, such as lettuce, choy sum and others that will grow within 3 to 8 weeks. Bok Choy was usually planted from seeds and required 6 to 7 weeks to mature and be harvested. The Bok Choy is a highly tolerant vegetable which can survive under full sun and slight frost, but it cannot tolerate cold temperatures below 10 $^{\circ}C$. The agricultural practices for planting Bok Choy will be in a double row with rows spaced 10 inches (25 cm) apart, and plant the seeds 2 inches (5 cm) apart and barely cover them with soil. The pH of the soil was maintained around 6.0 – 7.5 to promote the optimum growth of the Bok Choy, as it aids the plant in nutrient availability and uptake. The Bok Choy also requires an adequate amount of water to stay hydrated. However, the watering procedure was dependent on the weather conditions; the Bok Choy was a heavy feeder, which required more water under full sun or summer to stay hydrated. The agricultural practices of watering the Bok Choy were usually carried out in the morning and evening to keep the Bok Choy hydrated. The mature Bok Choy usually had 20 - 25 centimetres of height, 10 - 12 leaves, and 140 – 150 mm^2 total leaf surface area. The mature Bok Choy had a smooth, slightly curved, bright white, firm stem connected to its dark green leaves with noticeable white veins across the surface. When the Bok Choy was raw, it was described as crunchy with a green flavour; when it was cooked, it had a mild mustard-like and earthy sensation. The Bok Choy was not a seasonal crop which could be grown and harvested throughout the year. Hence, Bok Choy is one of the most marketable and demanding vegetables in the world, especially nowadays. Recently, people have raised their awareness of their health and started to eat more greens to get healthier. Studies have shown that a cup (170 grams) of raw Bok Choy consists of 1 g of fibre, 9 kcal of calories, 1 g of protein, 1.5 g of carbohydrates, 0.2 g of fats, 0.95 mg of vitamin A, a trace amount of vitamin C and other beneficial vitamins (Baslam, et al., 2013). According to a report by The Country in 2021, the demand for greens, including Bok Choy, had spiked up to 400% over the last 2 – 3 years.

Bok Choy was one of the vegetables described as a heavy feeder since it had fast-growing abilities. The Bok Choy requires a huge amount of nitrogen, phosphorus and potassium to support their rapid growth. Nitrogen was the source of the Bok Choy to synthesise amino acids to further develop into proteins, chlorophyll and other essential molecules (Verdoliva, et al., 2021). In an adequate source of nitrogen, the Bok Choy will show lush and green foliage on its leaves. For example, a pyrrole ring is a molecule that is built from one nitrogen atom and four carbon atoms. Four pyrrole rings assemble continuously to form chlorophyll, which is a pigmentation molecule from biochemical biosynthesis for the use of photosynthesis (Verdoliva, et al., 2021). Lack of nitrogen sources in plants will lead to consequences such as yellowing of leaf veins, leaf chlorosis, growth redundancy and other plant diseases (Othman, 2013). The plants have a higher affinity towards nitrate and ammonia for their nitrogen sources, as they are water-soluble and found most abundant in the soil (Leal, et al., 2020).

In addition, phosphorus plays a crucial role in flora development, such as root development, flowering, fruiting and energy transfer (Vimala and Sukra, 2010). Phosphorus was present in most metabolic pathways, as well as nucleotide synthesis. For example, adenosine triphosphate (ATP) transfers dihydrogen phosphate ions to the energy-requiring substances in the plant to facilitate the chemical and biological reactions in cells (Baron, 2019). Besides that, the DNA inside the cell requires phosphate as the backbone for further sequencing as well as nucleic acid biosynthesis. Therefore, a lack of phosphorus will lead to several plant diseases, such as stunted growth and low reproduction rates (Stone, 2017).

The phosphorus deficiency in the plant will show necrotic, dark spots and purple colouration on the leaves (Stone, 2017).

Lastly, the potassium helps the Bok Choy to maintain its vigour as well as other physiological processes such as water uptaking, development of strong cell walls, disease resistance, etc. (Ferrarezi, et al., 2015). The potassium remains an ion in the cell, which frequently flows within the plant, especially in the rooting system (Ferrarezi, et al., 2015). Therefore, insufficient potassium in plants will lead to a weak rooting system and poor water uptake (Martínez, 2010). As a result, the plant tends to wilt due to insufficient water (Martínez, 2010). The symptoms of a lack of potassium in plants are chlorosis and necrosis at the lower edges of the leaves (Rouphael and Colla, 2005). The Bok Choy also requires a minimum amount of other minerals such as magnesium, sulfur, iron, zinc and others to coordinate the function of its enzymatic activities. In short, the Bok Choy requires a large amount of nutrients to promote its growth.

Based on previous research, the light intensity and nitrogen availability were the major factors to influenced the quality and growth of Bok Choy; the former will affect the Bok Choy quality compared to the latter. In Xia Hao, et al. (2020) study, she pointed out that high light intensity (350 μmol m^{-2} s^{-1}) aids the Bok Choy to obtain higher anthocyanin contents in the leaves, but when the light intensity is higher than 350 μmol m^{-2} s^{-1}, the anthocyanin contents were significantly dropped. In Hughes, et al. (2005) study, they used neutral density cloth to shade the leaves evergreen herb Galax to get different light intensity environments from 0 to 10000 μmol m^{-2} s^{-1}. The result showed that the anthocyanin content increases linearly with light intensity. The anthocyanin content of the leaves from 0% shade treatment was 23 times lower than that from the 80% shade treatment. On the other hand, the nitrogen availability will affect the fresh shoot and root weight. Based on Xia Hao, et al. (2020) study, the Bok Choy fresh shoot weight increases linearly with the nitrogen availability, but the trend decreases when the nitrogen content is more than 10.5 mmol/L. On the other hand, the fresh root weight had decreased linearly with the nitrogen content, where the fresh root weight of Bok Choy was highest at 2.5 mmol/L but lowest at 18.5 mmol/L. Several studies have demonstrated that nitrogen deficiency induces the production of root biomass but inhibits the growth of aerial or shoot organs such as leaves. (Albert, et al., 2009; Luo, et al., 2013; York, et al., 2015).

2.2 Protein Hydrolysate and Its Potential

Protein hydrolysates (PH) contain many peptide fragments of different sizes and amino acids. The PH was produced by treating the proteinaceous plant or animal with hydrolysis treatments such as acidic, alkaline or enzymatic approaches. The proteins will be broken down into smaller size of peptide fragments and further degraded into the smallest free amino acids by breaking the peptide bonds. Before the proteins were hydrolysed, the oligopeptide with the protein blocks was considered inactive as they were stacked within it. After the treatment, these oligopeptides became active, which is known as bioactive peptides. It had greater function with their specific sequence composition and promoted various potential health benefits compared to native protein molecules, such as antioxidative potential, antihypertensive, antimicrobial and others (Chai, et al., 2021). Studies found that protein hydrolysates contain small sizes of peptides and amino acids, which have high permeability and absorbability and promote unique biological activities in plant growth (Budseekoad, et al., 2018; Jain and Anal, 2016). The character of protein hydrolysate was determined by its molecular size, content of amino acids, and sequence of bioactive peptides. Each protein source, such as fish, soybean, chickpeas, milk and others, will produce different amounts of amino acid content after the treatment. Apart from that, the methods of the treatment, such as chemical, enzymatic and microbial treatment, will contribute different amino acid contents from the protein sources. In most cases, enzymatic and microbial approaches were preferable in producing the protein hydrolysates compared to other methods, as they were more biologically friendly, easier to scale up and had shorter reaction times. The method of protein hydrolysis was dependent on the source of the protein. For example, acidic, alkaline and bacterial keratinases were recommended as approaches to digest harder keratinised protein sources such as feathers, wools, beaks and others. In the other cases, enzymatic and microbial approaches were recommended to hydrolyse the proteins as they were faster and environmentally friendly.

The enzymatic and microbial approaches share a common characteristic in that where use enzymes to perform the hydrolysis treatment. The only difference between them was the presence of free-moving enzymes during the treatment. In microbial treatment, microorganisms such as bacteria and yeast retain the enzymes within themselves to digest the proteinaceous sources into smaller bioactive peptides and amino acids. Whereas the enzymatic approaches used the free-moving isolated enzyme from the microorganisms to perform the hydrolysis.

Therefore, the enzymatic approaches cost more than microbial approaches. However, the turnover rate was higher in enzymatic approaches as it reacted directly to the proteins, while the microorganisms required a series of actions to break down the protein, such as phagocytosis. Meanwhile, microbial proteases gave different degrees of efficiency and specificity in protein hydrolysis, which induces higher complexity in comprehension. According to Hou, et al. (2017), microbial digestion faces difficulties in maintenance and inconsistency in the amino acid productions as variable including inconsistency of fermentation conditions, which becomes a major flaw of this method.

On the other hand, enzymatic approaches were a complex process since the enzyme is highly specific to the accessibility of peptide bonds. Besides that, enzymatic reactions on protein hydrolysis were sensitive to temperature and pH. The enzymes only work optimally in their desired temperature and pH, and they are susceptible to denaturing when there is a fluctuation of temperature and pH (Clemente, 2000). For example, the alcalase works optimally within pH 6.5 to 8.5 and a temperature of 30 to 65 ^{o}C. If the pH is not within the range and the temperature exceeds 65 ^{o}C, the alcalase will be denatured (Clemente, 2000). As a result, the hydrolysis process will become unsuccessful. The enzymatic hydrolysis in protein hydrolysate preparation only requires a mild temperature and pH, which reduces the risk of side reactions and loss of amino acids. These reasons have become the advantages of using enzymatic hydrolysis as an approach to prepare protein hydrolysate. Therefore, enzymatic approaches are considered environmentally friendly as they comply with the sustainability development plan. However, the presence of natural inhibitors from the sample had become a drawback of enzymatic approaches (Andriamihaja, et al., 2013). Alcalase (protease) was produced from the *Bacillus lincheniformis* and is commonly used in PH production (Muzaifa, et al., 2012).

According to Dai, et al. (2014) study, acid and alkaline hydrolysis were not considered a sustainable method in the long run, as the chemicals used during the process were hazardous and not environmentally friendly. Although the chemical hydrolysis provided a shorter hydrolysis duration and a lower cost. These methods will lead to the loss of certain amino acids. For example, acid hydrolysis will convert asparagine into aspartame, glutamine into glutamate and destroy tryptophan (Pasupuleti, 2010). In alkaline hydrolysis, there will be a significant recovery of tryptophan compared to acid hydrolysis, but there will also be a total loss of amino acids as most of it becomes foams (Pasupuleti, 2010). Lastly, treatment of acid and alkaline often results in poor quality of the PH as the chemicals from the treatment have destroyed most of the useful amino acids (Kristinsson & Rasco, 2000).

An optimal enzyme-substrate ratio of 1 to 10 for PH production using alcalase was shown in the previous study (Chai, et al., 2021). Based on Doucet, et al. (2003) study, the alcalase shows high specificity in breaking the peptide bonds in many amino acids, such as tyrosine and glutamine. The Bradford and ninhydrin methods were used to determine the degree of completion of the PH. The former was used to evaluate the protein concentration in the PH by binding the Coomassie blue dye with protein and read at 595 nm. The former was used to evaluate the amino acid concentration in a sample where it binds the amino group of amino acids to form a colour complex and is read at 570 nm (Friedman, 2004).

2.3 Protein Hydrolysate vs Compost

Compost is a nutritious soil-like material degraded naturally by microbes such as bacteria and fungi from biological waste materials. On the other hand, the protein hydrolysates were treated through acidic, alkaline, or enzymatic treatments by humans manually controlled. Hence, the timeframe for both products to be completed was different. The protein hydrolysates were able to complete faster than the compost. For example, the protein hydrolysate requires around 6 to 12 hours to degrade the protein into smaller peptide chains and amino acid fragments; the compost, however, requires a minimum of 3 months to finish its process.

By comparing their durations, protein hydrolysates had outperformed the compost. Yet, the compost has the advantage of containing a variety of nutrients. The materials to make compost were biodegradable and often found as food waste, manure, sewage sludge and others (Weil and Brady, 2017). These big molecules of materials were later degraded by the environmental microbes into smaller molecules such as simple sugars, peptides, amino acids, fatty acids, mineral ions, and especially humic acids. Humic acids are the most common product found in the compost. It is a ubiquitous redox-active compound with a high molecular weight containing blocks of aromatic rings and aliphatic chains (Hwang, et al., 2019). The elements inside the humic acid are carbon, hydrogen, oxygen, and nitrogen, with a small amount of sulphur and phosphorus. The composition of the humic acids can vary based on the sources of plant and animal matter (Gumisiriza, et al., 2020). The humic acid itself is a large and complex molecule which can act as a nutrient reservoir in the soil (Gumisiriza, et al., 2020). Besides that, the hydrogen bonds from the humic acids can hold the water molecules, which improves the soil water infiltration and water retention.

On the other hand, the contents in protein hydrolysates were much simpler than in compost. The protein hydrolysates only contain peptide fragments, amino acids, and a small number of mineral ions. On the contrary, the amino acid content from the protein hydrolysates was much higher than the compost. This is because the protein hydrolysates were free from microbes. Hence, most of the amino acids and their nutrients were preserved. Unlike the compost, microbes such as bacteria and fungi will utilise an amount of nutrients from the compost for their survival and growth. As a result, the nutrients in the compost will fluctuate in different batches, while the nutrients in the protein hydrolysates will be standardised.

Besides that, the procedure for producing the compost was complicated as there were many factors to influenced it. The underlying logic of compost is the degradation of materials using microbes, and these microbes only perform optimally in the right humidity and temperature. Besides that, there are other factors, such as the type of materials and duration will affect the high quality of compost. For example, the standard quality of compost using fruit peels and coconut husks requires a temperature range from 45 ºC to 65 ºC to favour the aerobic microbes to ferment the biodegradable materials (Ricci, et al., 2019). At the sametime, the humidity in the environment must be set between 40% to 60% to provide the necessary hydration for microbial metabolic activities such as proliferations, growth and enzymatic activities (Ricci, et al., 2019). Apart from that, the compost piles must be regularly folded to release the heat from the decomposition and improve the ventilation. On the other hand, the protein hydrolysates have an easier procedure. The fixed amount of protein will be treated with either acid, alkaline or enzyme for a certain duration to obtain the protein hydrolysates. Among the approaches, the enzymatic treatments are more specific as they will be affected by temperature, pH and the type of enzyme used. For example, a 1:10 ratio of soy protein isolate: alcalase was treated at 50 ºC for 6 hours to obtain the soy protein hydrolysates (Shaila, 2022). Hence, it can be said that the procedure of protein hydrolysates was less sophisticated than composting.

In conclusion, protein hydrolysates contain more advantages to compost as it has shorter procedures, shorter durations, easier to monitor and standardise.

2.4 Effects of Protein Hydrolysate on Crops

The protein hydrolysates made from animal or plant waste were able to enhance overall plant growth and development. Improvement of root growth was detected in PH-treated plants based on a previous study by Rouphael, et al. (2017). The PH had triggered the enzymes for the nitrogen and carbon metabolism, thus stimulating the secondary metabolites. In the further report from Ertani, et al. (2016), the PH aids the plant to increase nutrient uptake and leads to rapid growth of the plant. In agronomic practices, the protein hydrolysates can be applied to the plants by foliar applications or drenched into the soil. In foliar applications, the protein hydrolysates were mixed with small amounts of surfactant to increase the absorption into the cells, such as the cuticle and stomata, before they reached the mesophyll cells. In soil drenching applications, the PH was absorbed by the root cells and later distributed to other parts of the plant (Subbarao, et al., 2015). By comparing the foliar applications and soil drenching, the former had a higher rate of protein hydrolysate absorption compared to the latter, as there was less microbial competition on the leaves than on the roots. The foliar application was unable to penetrate the plant's waxy cuticle on its own due to different polar substituents, and it required a surfactant to aid it. However, the presence of the surfactant may wipe off the waxy cuticle of the leaves, which will reduce the water retention of the leaves and further increase the susceptibility to dehydration (Kenny, et al., 2019).

Based on the report of Kenny, et al. (2019), the foliar applications were applied to achieve short-term outcomes, while the soil drenching was used to observe a long-term effect. Recent studies showed that PH had regulated P-solubilising and N_2-fixing bacteria in the soil, which were beneficial to the plant (Moe, 2013). Besides that, Calvo, et al. (2019) observed that PH had increased the nitrogen absorption of the plant and the plant growth. Another study reported by Wilson, et al. (2018) discovered that PH served as a bio-stimulant on the maize plant (*Zea mays* L.) by enhancing its growth and development. The author concluded that the presence of the PH had increased the activities of nitrate reductase and malate dehydrogenase (Wilson, et al., 2018). The author also mentioned the PH had stimulated the enzymes in glycolysis and the Krebs cycle for carbon metabolism (Wilson, et al., 2018). Apone, et al. (2010) also reported that PH induced the plant's secondary metabolism, which further led to better toleration against stress such as drought and oxidative conditions.

However, the exact mechanisms had yet to remain unknown although the PH promote a variety of metabolic processes in plants. The study from Ertani, et al. (2013) showed that Alfalfa-derived PH (*Medicago sativa* L.) boosted the enzyme activities in plants, further influencing and helping in the upregulation of gene expression in the secondary metabolism, such as phenolic compound biosynthesis. The PH showed hormone-like action when tested on maize (*Zea mays* L.); the PH-treated maize showed an increase in shoot growing rate compared to the control. Furthermore, tomato plants (*Solanum lycopersicum* L.) treated with plant-based PH showed greater physical properties (plant weight and length) compared to the non-treated plants (Colla, et al., 2015). In the Ertani, et al. (2008) study, the dwarf pea plants (*Pisum sativum* L.) were treated with plant-based PH and an increase in shoot length was observed, which demonstrated that PH possessed a gibberellin-like activity.

2.5 *Clarias batrachus* Composition and Nutritional Values

Clarias batrachus, also known as the walking catfish, has several common names in different countries. For example, *Clarias batrachus* is known as Clarias Catfish in Malaysia, and Thai hito or Thailand catfish in Thailand. Clarias Catfish is a species of catfish found in freshwater. Its strong adaptation also allows it to live in brackish water and terrestrial areas. This was because of the existence of its unique gills, which can keep its gills in moisture for a long period to travel on land if necessary. It is commonly found in an environment such as irrigation channels, lakes, wetlands and paddy fields. Clarias Catfish existed mainly in Asia countries such as Malaysia, Singapore, Thailand, Vietnam, the Philippines and others. Therefore, it has become a common dish for most people in different countries. Different countries had their unique way and cultures to process the fish. For instance, Clarias Catfish was usually deep fried, grilled or cooked with sambal in Malaysia; in Thailand usually cooked with hot and sour Tomyam soup. In recent years, Clarias Catfish has also been found in the wild of Western countries such as the USA. Clarias Catfish was classified as detritivores, which have a broad choice of diet; it can be fed on organic waste or any other dead organic matter. Its behaviour was highly predatory, which would consume almost anything offered from the environment or human activities. Therefore, it had been labelled as an aggressive invasive species due to its raging behaviour, high tolerance and indiscriminate feeding habits of the local food chains.

Clarias Catfish had an elongated body with a narrow head, alongside irregular bumps on its spine. On average, the Clarias Catfish ranged between 8 to 47 cm long and weighed 1.2 kg. The Clarias Catfish had a flattened and broad head, small eyes and a wide mouth with a typical catfish appearance. The most significant appearance of Clarias Catfish is having four pairs of whiskers and a long dorsal fin that can be extended two-thirds of its body length.

The Clarias Catfish can be divided into four body parts, which are meat, head, bones, skin, gills and viscera. In general, the meat of the Clarias Catfish, head with bones, skin and gills with viscera each contribute 44.66 %, 37.66 %, 5.98 % and 11.70 % respectively to the fish's total weight (Haryati, et al., 2020). One hundred grams of Clarias Catfish per serving constitutes 18 g of proteins, 2.9 g of fats, 0.5 g of carbohydrates, 237 mg of omega-3 fatty acids and 337 g of omega-6 fatty acids (Cecilia, 2019). In Haryati, et al. (2020) study, the edible part of fish fillet had huge amounts of proteins (18.24 ± 0.09 %) compared to other parameters such as fats (4.16 ± 0.01 %) and carbohydrates (0.42 ± 0.01 %). Therefore, it's a good source of protein for a diet.

Component	**White flesh (%wb)**
Moisture content	75.94±0.02
Ash content	1.25±0.05
Protein content	18.24±0.09
Fat content	4.16±0.01
Carbohydrates	0.42±0.01

Table 2.4: Major contents of Clarias Catfish.

Pipecolate is a non-proteinaceous product from lysine catabolism. It serves as a regulator of immunity to fight against invasive pathogens such as bacteria and fungi. The lysine from the protein hydrolysate was used for the synthesis of pipecolate. In the study conducted by Maurizio, et al. (2021), the exogenous sources of amino acids such as glutamate, aspartate and alanine were shown to produce synergistic and positive effects on tomato growth. These amino acids from the protein hydrolysate act as precursors for various primary and secondary metabolites, which enhance the plant's resistance to environmental stress (Maurizio, et al., 2021).

2.6 Photosynthetic Pigments

Chlorophylls and carotenoids play an important role in photosynthesis. The level of these photosynthetic compounds can easily affect the photosynthetic activity (Knecht, et al., 2015). Besides that, the level of these photosynthetic compounds can be altered by external factors such as nutrient availability, environmental stress, and quality of light. For example, chlorophylls are green in colour due to the presence of magnesium ions. Thus, increasing the nutrition availability with amino acids as the precursor or magnesium ions will increase the level of chlorophylls. On the other hand, the magnesium ions in the chlorophylls also play another important role. The magnesium ions inside the chlorophyll will assist it by absorbing the red and blue light. The red and blue light have a higher quantum yield of carbon dioxide assimilation (Knecht, et al., 2015). Therefore, the red and blue light fulfil the plant's requirements to utilise the carbon dioxide for energy production (Nicolle, et al., 2004). The captured light will trigger the photosynthetic pathway to produce carbohydrates and energy from carbon dioxide (Nicolle, et al., 2004). Thus, the leaf of the plant appears to be green as the chlorophyll absorbs the red and blue light, and reflects all the green light to the environment. The chlorophylls can be further differentiated into chlorophyll α and chlorophyll β. The higher plants have higher chlorophyll α than chlorophyll β (Maiani, et al., 2009). In higher plants, monitoring of the chlorophyll α and β ratio demonstrated the plant's physiological status, as the ratio is affected by internal and environmental stress (Maiani, et al., 2009).

Besides that, the carotenoids, which are known as carotene, are found as coloured pigments in plants. These coloured pigments were discovered to benefit human health as they can serve as antioxidants, immune system enhancers, and anticancers (Dai & Mumper, 2010). Consuming chlorophyll can increase the amount of magnesium ions in the human body and further stimulate the red blood cells to transport more oxygen to all parts of the body cells. Besides that, it can assist in the assimilation of calcium and other heavy metals within the human body and reduce reactive oxygen species (ROS) to minimise damage to the cells (Dai & Mumper, 2010). The study found that α-carotene, β- β-carotene, and lutein were beneficial to human health. For example, lutein shows a positive effect on human vision (Seeram, et al., 2006). Studies showed that taking lutein in our daily meals helps to reduce the incidence of age-related eye disease (Seeram, et al., 2006). In addition, the α-carotene and β-carotene possess anticancer properties. Studies have shown that these carotenoid compounds could induce cell cycle arrest and stop metastasis (Shukitt-Hale, et al., 2009).

In general, chlorophyll metabolism can be categorised into 3 main phases. The amino acid, glutamate, was utilised and became chlorophylls during the first phase. The chlorophylls will further differentiate into chlorophyll α and chlorophyll β, and this process is known as the chlorophyll cycle. Finally, the third phase involves the degradation of chlorophyll α (Rüdiger, 2002). Carotenoids are another type of photosynthetic molecule which show yellow-red pigments in most fruits and vegetables (Ruiz, et al., 2012). Carotenoids cannot be made and are only available by dietary uptake (McGraw, et al., 2006).

2.7 Oxidative Stress

Oxidative stresses were associated with the free radicals. Free radicals are molecules which contain one or more unpaired electrons, which readily accept an electron. Therefore, they were highly unstable and reactive. It was prone to oxidising the other stable molecules by taking their electrons away from them. After the free radicals have reacted with the stable molecules at a high energy output, they will produce a new free radical from the stable molecules and cause a cascade effect. A study conducted by Sen (2001) showed that free radicals are prone to react with oxygen and produce reactive oxygen species (ROS). The phenomena were inevitable, but organisms have an amount of antioxidants in their body to resolve these problems. These antioxidants can be synthesised by our own body or through food consumption. For example, living organisms synthesise endogenous antioxidants such as lipoid acid and L-arginine, whereby vitamins C and E can be consumed as exogenous antioxidants. However, in the current days, there is more existence of ROS in the environment as well as in our bodies. Therefore, it causes oxidative stress within our body and damages our healthy cells (Cooper, et al., 2002). Undesirable amounts of ROS will cause apoptosis and inflammation in the healthy cells, and it also further disrupts the normal cellular function and causes cell ageing, Parkinson's, Alzheimer's disease and cancer (Golden, et al., 2002).

The presence of antioxidants becomes more essential these days as the ROS increases. The antioxidants showed higher affinity toward the ROS and combined with it to reduce the level of free radicals or even stop the cascade effects. Hence, it reduces oxidative stress and damage to important molecules in the body, such as proteins and DNA (Powers & Lennon, 1999). Besides that, the liver in living organisms also produces some enzymatic antioxidants, such as catalase and superoxide dismutase, to carry out the antioxidant effects. Through study, phenolic compounds and flavonoids also show high antioxidant effects against ROS compared to vitamins C and E (Finaud, et al., 2006). These antioxidative enzymes were produced by healthy cells and highly regulated by the DNA to inhibit oxidative stress, such as lipid peroxidation (Gill & Tuteja, 2010). SOD is a type of metalloenzyme found abundantly in liver cells that readily reacts with ROS, such as the dismutation of oxygen into hydrogen peroxide, and leaves it for the CAT or GPX to eliminate it. The CAT was found abundantly in peroxisomes, and it helps in eliminating the products from the SOD.. It breaks down the hydrogen peroxide into smaller molecules during the fatty acid oxidation (Chelikani, et al., 2004). Apart from that, GPX is found abundantly in mitochondria to eliminate the hydrogen peroxide in water and lipid peroxides, but its activity is highly dependent on its selenium catalyst (Góth & Páy, 2004).

CHAPTER 3

MATERIALS AND METHODS

3.1 Overview of Process

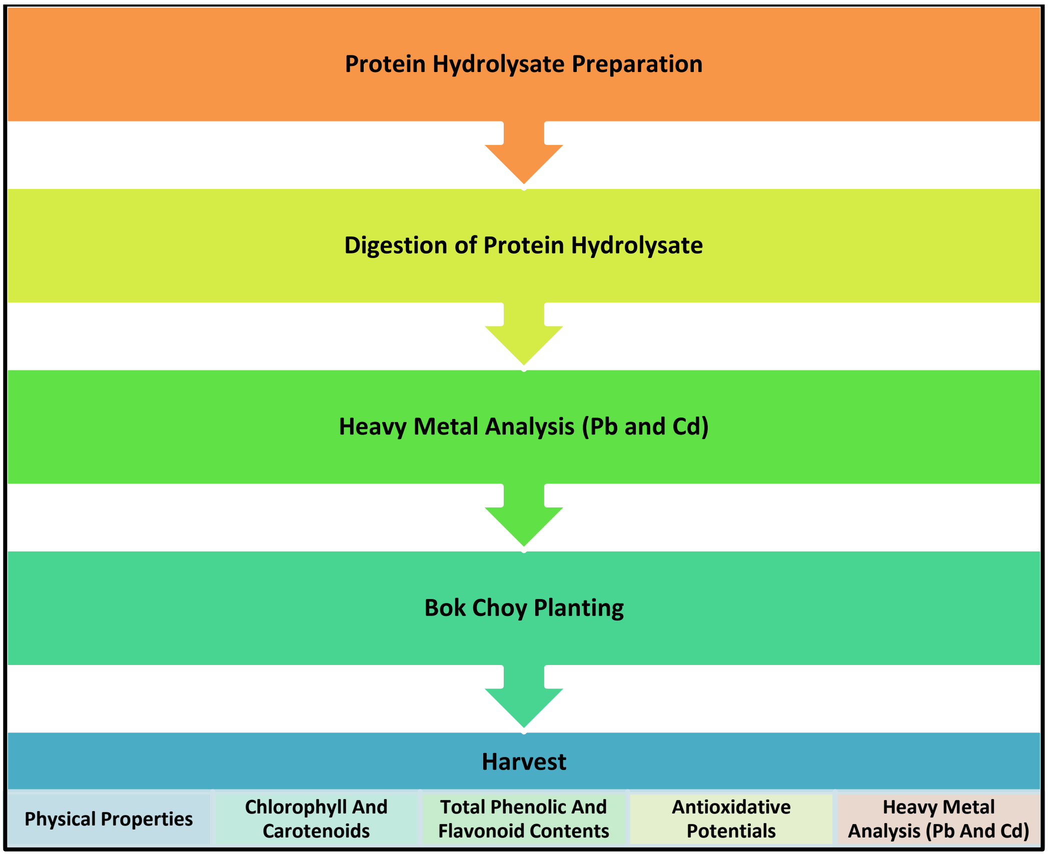

Figure 3.1: Overview of the process in this study.

3.2 Chemicals and Consumables

Table 3.2: List of chemicals and consumables with their origin.

Chemicals and Consumables	Origin
Ammonium sulfate	Bendosen
Dialysis Tube	Fisher Scientific
Phosphate Buffer Saline (PBS)	OXOID
Alcalase	Sigma-Aldrich
Bok Choy Seeds	TRIO
Sand	Sin Seng Huat Seeds Sdn. Bhd.
Cocopeat	Sin Seng Huat Seeds Sdn. Bhd.
Potassium dihydrogen phosphate	SYSTERM
Dipotassium hydrogen phosphate	SYSTERM
Quercetin	ACROS ORGANICS
Ethanol	QReC
Acetone	Emsure
Folin-Ciocalteu Reagent	Chemiz
Sodium carbonate	QRec
Gallic acid	R&M Chemical
Sodium nitrite	QReC
Aluminium chloride hexahydrate	QReC
Sodium hydroxide	Merck
Cadmium	Merck
Lead	Merck

3.3 Apparatus and Machines

Table 3.3: List of apparatus and machines with their model.

Apparatus and Machines	Model/Brand
Centrifuge Machine	Velocity 14R
Water bath	SASTEC
Microplate Reader	FLUOstar OMEGA, BMG Labtech
Drying Oven	Memmert
Electronic Balance	Sartorius
Refrigerator	LG
Flame Atomic Absorption Spectrometer (FAAS)	Agilent FAAS 280FSAA
Vortex	Genie Scientific Industries
UV-Visible Spectrophotometer	THERMO Scientific (GENESYS 10)

3.4 Preparation Of Protein Hydrolysates

Protein hydrolysates from the viscera of Clarias Catfish were prepared using the enzymatic digestion approach using the protease alcalase. The Clarias Catfish viscera was first taken out from fresh Clarias Catfish brought in Econsave (Kampar). The fish viscera were blended until no chunks remained. The samples were then suspended in deionised water at the mass (g): volume (mL) ratio of 1:3 and stirred for 30 min at 25 °C. The mixture was defatted by using n-hexane at the mass (g): volume (mL) ratio of 1:2 and stirred for 60 min at 25 °C. The mixture was left for 30 minutes to decant the oil from the viscera with the n-hexane in a separation funnel. The mixture was separated from the viscera oil and n-hexane. The mixture was heated at 90 °C for 30 min to eliminate any preexisting visceral enzymatic activities. The samples were centrifuged at 8000 rpm for 40 min to remove any water-insoluble substances. The supernatants were brought up to 80% saturation with ammonium sulfate and stirred at 4 °C for 1 hour. Precipitated proteins were pelleted after centrifugation at 20000 rpm at 4 °C for 40 min. The proteins isolated were subjected to separation and purification using dialysis tubing and centrifugal filtration (Quah, et al., 2018).

To prepare protein hydrolysate, 0.5 g of Clarias Catfish protein was mixed with 100 mL of 50 mM PBS buffer. An amount of 0.05 g of alcalase was added to the mixture. The optimal pH for alcalase was between 6.5 – 8.5; the pH in the experiment was controlled at pH 7.0 as an intermediate. The mixture was digested by the enzymes in a 50 ºC water bath for 7 hours. A volume of 1 mL of the protein hydrolysate samples was taken from the mixture from the 1^{st} to the 7^{th} hours. Each tube of hydrolysate samples was heated to inactivate the protease at 100 ºC for 10 minutes, then stored at -20 ºC. The samples were freeze-dried before carrying out the biochemical test to verify the complete digestion of the protein into its constituent amino acids (Wong, et al., 2019).

3.5 Testing Of Biochemical Profiles of The Prepared Protein Hydrolysates

This step was performed to verify the complete digestion of the protein into amino acids. Alcalase primarily cleaves at glutamic acid residues, with secondary cleavage sites including methionine, leucine, tyrosine, lysine, glutamine, phenylalanine, tryptophan, alanine, and serine. Bradford and Ninhydrin methods were applied to determine the protein and amino acid contents, respectively (Chai, et al., 2015; Lee, et al., 2018). For the Bradford method, 60 µL of the sample will be added to 940 µL of Bradford reagent, the mixtures were sat for 15 minutes and read at 595 nm, and the readings will be compared to the protein standard curve using Bovine serum albumin. For the ninhydrin method, 1 mL of ninhydrin reagent will be added to 2 mL of the sample, and the mixture will be placed into a 90 ºC water bath for 10 minutes. After cooling to room temperature, 5 mL of 95% ethanol will be added to the mixture. Absorbance were recorded at 570 nm and compared to the amino acids standard curve using L-methionine (Ninhydrin Reagent Protocol, Sigma, 2018).

Based on preliminary data, the alcalase used 6 hours to complete the digestion at 50 ºC water bath with pH 7.0.

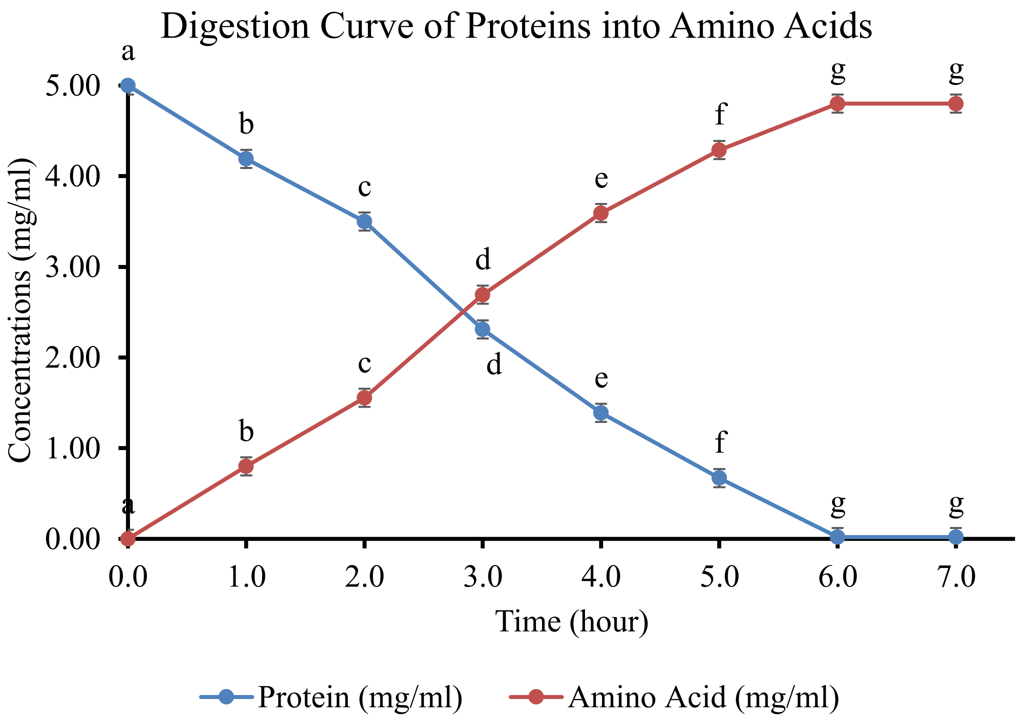

Figure 3.5: Digestion curve of Clarias Catfish protein into protein

hydrolysate. The protein content from Clarias Catfish showed a decreasing trend, while the amino acid content showed an increasing trend from 0.0 to 7.0 hours. Data are reported as mean ± SE values (n=3). Superscripts (a-g) indicate statistically significant differences ($p < 0.05$).

3.6 Testing Of Heavy Metal Content of Protein Hydrolysates

Testing of the heavy metal content of cadmium (Cd) and lead (Pb) proceeded with slight adjustment from Uddin, et al. (2016). One millilitre of PH was added to 10 mL of 65% nitric acid (HNO_3). The mixture was boiled for 15 minutes. The mixture was cooled to room temperature and filtered. The filtrate was added with deionised water, 50 mL, as the final amount. Quantification of Cd and Pb was performed using the FAAS machine.

Table 3.6: Experimental conditions for Cd and Pb determination using FAAS.

Element	Standard solution (mg/L)	Wavelength (nm)	Slit (nm)	Lamp intensity	Type of gas	Gas Flow (L/min)	
						Oxidant	Acetylene
Cd	0.3-1.2	228.8	0.5	40	Air/C_2H_2	13.5	2
Pb	2.5-10.0	217	1	20	Air/C_2H_2	13.5	2

The PH made from Clarias catfish viscera were tested for lead (Pb) and cadmium (Cd) due to their high toxicity and strong bioaccumulation in the organ, especially in the liver. The liver accumulates heavy metals because it functions as the primary detoxification organ. The heavy metals will bind to cysteine-rich metallothioneins that form stable metal-thiolate clusters (Mahmoud, et al., 2020). Other amino acids, such as histidine, aspartic acid, and glutamic acid, also provide functional groups (imidazole and carboxyl) for metal binding (Mahmoud, et al., 2020). Pb and Cd are prioritised over other heavy metals because of their low tolerable intake and well-documented chronic effects: Pb affects the nervous, hematopoietic, and cardiovascular systems, while Cd targets the kidneys and bones and is classified as a human carcinogen (Barst, et al., 2018). Monitoring these metals ensures that the PH is safe for crop application and compliant with regulatory standards.

3.7 Planting Site and Experimental Design

The experiment was conducted at an open area between Block D and E (4° 20' 17.9232" N, 101° 8' 37.1904" E) in UTAR Kampar. The coconut coir and sand were sterilised with an autoclave machine and put in an oven at 50 ºC for 24 hours to ensure complete dryness. The Bok Choy seeds were germinated in mesh pots containing coconut coir as support. 50 mL of distilled water was added to each seed for 7 days. The successfully germinated plant samples were transferred into the polybags (5’ x 5' dimensions) with full sand and arranged with a randomised complete block design (RCBD). The plants were 10 cm away from each other.

Sand was used as an inert medium with low nutrient content, allowing for clearer observation of the effects of the protein hydrolysate (PH) itself. Similarly, Islam, et al. (2016) employed sterilised sand as a growth medium to evaluate the impact of chromium-tolerant plant growth-promoting bacteria on maize under controlled conditions, ensuring that nutrient variability was minimised.

The plant samples were divided into non-treatment and treatment groups. In the treatment groups, a gradient of PH (2000, 4000, 6000 and 8000 mg/L) will be added to the soil every 3 days. The plant samples were harvested after the optimal growth period of 35 days. The weight and length of the harvested plant (shoot and root) were measured and recorded.

3.8 Measurement of Physical Parameters

3.8.1 Surface Area of Leaf (cm^2)

Leaf surface area was measured after the harvest of plant samples. The three largest green leaves were selected for the measurement by multiplying the width and length of the giant leaves.

3.8.2 Wet Plant Length (cm)

The length of the shoot and root of the plant sample were measured and recorded after the harvest by using a tape measure.

3.8.3 Wet Plant Weight (g)

After the harvesting was completed, the plant samples were washed to remove any soil from the roots. Next, the plants were separated into two individual shoots and root systems. An electronic balance (Sartorius) was used to measure and record the individual weight of the shoot and root system from each plant sample.

3.8.4 Measurement of Total Chlorophyll and Carotenoid Contents

Lichtenthaler & Buschmann (2001) was used as a reference to determine the concentration of plant pigments. Firstly, a piece of green healthy leaf was chosen and cut into smaller pieces from the harvested crops from each category. The leaves were immersed in 10 millilitres of 98% acetone at 4 ºC for 24 hours. Three different absorbances were used and inserted into the following formula to measure the concentrations of plant pigments in units of (µg/mL):

$$C_{\alpha}\ (\mu g/mL) = 11.24\ [OD\ (661.6)] - 2.04\ [OD(664.8)]$$

$$C_{\beta}\ (\mu g/mL) = 20.13\ [OD(644.8)] - 4.19\ [OD(661.6)]$$

$$C_{x+c}\ (\mu g/mL) = [1000\ [OD(470)] - 1.90\ (C_{\alpha}) - 63.14\ (C_{\beta})]/214$$

Equation 3.8: Equations for calculating the plant pigments by using the value of absorbance at 470 nm, 661.6 nm, and 664.8 nm.

3.9 Preparation of Dried Plant Extract

The leaves from the plant samples were separated from the plant body and dried in an oven for 6 hours at 60 ºC. The dried leaves were ground into fine powder. A gram of ground leaves was weighed and mixed with 19 mL of deionised water. The mixture was then heated at 90 ºC for an hour. The mixture was then filtered with a clean cheesecloth to remove the leaves.The extracts were centrifuged at 10,000 rpm for 15 minutes. The extracts were stored at -20 ºC for further analysis.

3.10 Determination of Antioxidative Contents

3.10.1 Determination of Phytochemicals

Phytochemical contents (total phenolic content and total flavonoid content) were evaluated using previously published conditions from the harvested plant samples. Folin-Ciocalteu reagent was used to evaluate the total phenolic content (TPC) and reported as mg gallic acid equivalents /g dry matter (mg GAE /g DM). Aluminium chloride reagent was used to evaluate the total flavonoid content (TFC) and reported as mg quercetin equivalents /g dry matter (mg QE /g DM). In the Folin-Ciocalteu colourimetric technique, the procedure started by mixing the reaction mixture with 1.0 mL of plant extract with 0.2 mL of 10% (v/v) FC reagent. The mixture was rested at room temperature for 3 minutes. Next, the addition of 0.8 millilitres of seven hundred millimolar sodium carbonate into the mixture was performed. The mixture was left for 2 hours at 25 ºC. The mixture was read at 765 nm. Gallic acid was used as a standard, and the unit for TPC is (mg GAE/ g DM) (Ainsworth & Gillespie, 2007).

The TFC in the Bok Choy was determined by using the Zou, et al. (2004) study as a reference. Firstly, 0.2 millilitres of plant extract was added with 0.15 millilitres of 5% (w/v) $NaNO_3$ and left for 6 minutes at 25 ºC. Next, the reaction mixture was added with 0.15 millilitresof 10% (w/v) $AlCl_3 \cdot 6H_2O$ and left for 6 minutes at 25 ºC. The reaction mixture was followed by adding 0.8 millilitres of 10% (w/v) NaOH and left for 15 minutes at 25 ºC. Lastly, it was read at 510 nm. Quercetin was used as a standard, and the unit for TFC was (mg QE/ g DM).

3.10.2 Determination of Antioxidative Potential

Antioxidative scavenging assays (DPPH radical scavenging assay and ABTS radical cation scavenging ability) will be applied to Bok Choy to evaluate its antioxidative potential.

For the DPPH radical scavenging assay, the method was assessed as described previously with modifications (Thaipong, et al., 2006; Lim and Quah, 2007). DPPH stock solution was prepared by dissolving 24 mg of DPPH into 100 mL of ethanol and stored at -20 °C until needed. The DPPH working solution was prepared by mixing 10 mL of stock solution with 45 mL of ethanol. To 1 mL of DPPH working solution, 50 µl of extract was added. The mixture was left in the dark for 30 min before its absorbance was read at 517 nm. A blank was prepared for each sample in which the DPPH solution was replaced with ethanol. DPPH radical scavenging activity was calculated using the following formula:

$$\text{DPPH radical scavenging activity (\%)} = (A_{control} - A_{sample})/A_{control} \times 100$$

Equation 3.10.1: Equations for calculating the DPPH radical scavenging activity (%).

A_{sample} is the absorbance in the presence of a plant extract. Ascorbic acid will be used as the positive control. Results will also be presented as EC_{50} values, which represent concentrations of extracts required to scavenge 50% of the DPPH radicals.

ABTS radical cation ($ABTS^+$) scavenging activity of the extracts will be determined as described in Re, et al. (1999) with modifications. To prepare the $ABTS^+$ stock solution, an equal volume of ABTS solution (8 mg/mL) will first be mixed with potassium persulfate (1.32 mg/mL). The mixture will be kept in the dark for 12 hours at room temperature. Then, an $ABTS^+$ working solution will be prepared by diluting the $ABTS^+$ stock solution with potassium phosphate buffer (100 mM, pH 7.4) to obtain an absorbance of 0.700 ± 0.005 at 734 nm. For measurements, 0.1 mL of extract will be added to 1 mL of $ABTS^+$ working solution. The mixture will be kept in the dark for 10 min before its absorbance is read at 734 nm. Ascorbic acid will be used as the positive control. $ABTS^+$ radical scavenging ability (%) will be calculated as shown below:

$$ABTS^+ \text{ radical scavenging ability (\%)} = (A_{control} - A_{sample}) / A_{control} \times 100$$

Equation 3.10.2: Equations for calculating the DPPH radical scavenging activity (%).

Where $A_{control}$ is the absorbance of the control reaction (without plant extract) and A_{sample} is the absorbance in the presence of a plant extract.

3.11 Measurement of Heavy Metal Contents In Plant Samples

Testing of the heavy metal content of cadmium (Cd) and lead (Pb) proceeded with slight adjustment from Uddin, et al. (2016). The Bok Choy leaves were dried and ground into fine powder. Then, 0.1 grams of the powder was added to 10 millilitres of 65% HNO_3. The mixture was boiled for 15 minutes. The mixture was cooled to room temperature and filtered. The filtrate was diluted with deionised water to 50 mL for a final amount. Quantification of Cd and Pb was performed using the FAAS machine.

Table 3.11: Experimental conditions for Cd and Pb determination using FAAS.

Element	Standard solution (mg/L)	Wavelength (nm)	Slit (nm)	Lamp intensity	Type of gas	Gas Flow (L/min)	
						Oxidant	Acetylene
Cd	0.3-1.2	228.8	0.5	40	Air/C_2H_2	13.5	2
Pb	2.5-10.0	217	1	20	Air/C_2H_2	13.5	2

3.12 Data Analysis

Data are presented as mean ± standard errors (n=5). SAS (Version 9.4) was used to perform data analysis. All data were analysed with the ANOVA test, and the means of significant differences were assessed by using Fisher's LSD test at a 0.05 level of probability.

CHAPTER 4

RESULTS

4.1 Digestion Curve of Protein Hydrolysate

The optimal pH for alcalase was between 6.5 – 8.5, while the temperature was between 45 – 65 ºC. Hence, the pH and the temperature in the experiment were controlled at pH 7.0 and 50 ºC as intermediate. Alcalase primarily cleaves at glutamic acid residues, with secondary cleavage sites including methionine, leucine, tyrosine, lysine, glutamine, phenylalanine, tryptophan, alanine, and serine. In this experiment, the specific activity of alcalase from Sigma-Aldrich was 2.4 U/mg. Therefore, the activity concentration of alcalase in this experiment was 12 U/mL.

The Clarias Catfish protein was digested with alcalase for 7 hours to turn the protein into amino acids. In general, the protein content from Clarias Catfish showed a decreasing trend from 100^{a} % to 0^{g} %, while the amino acid content showed an increasing trend from 0^{a} % to 100^{g} % 0.0 to 7.0 hours. However, the digestion trend stopped at 6.0 hours, and the protein and amino acid content remained with relatively small changes at subsequent hours. Therefore, it can be concluded that the digestion of Clarias Catfish protein in such conditions mentioned in the methodology was optimum at 6.0 hours.

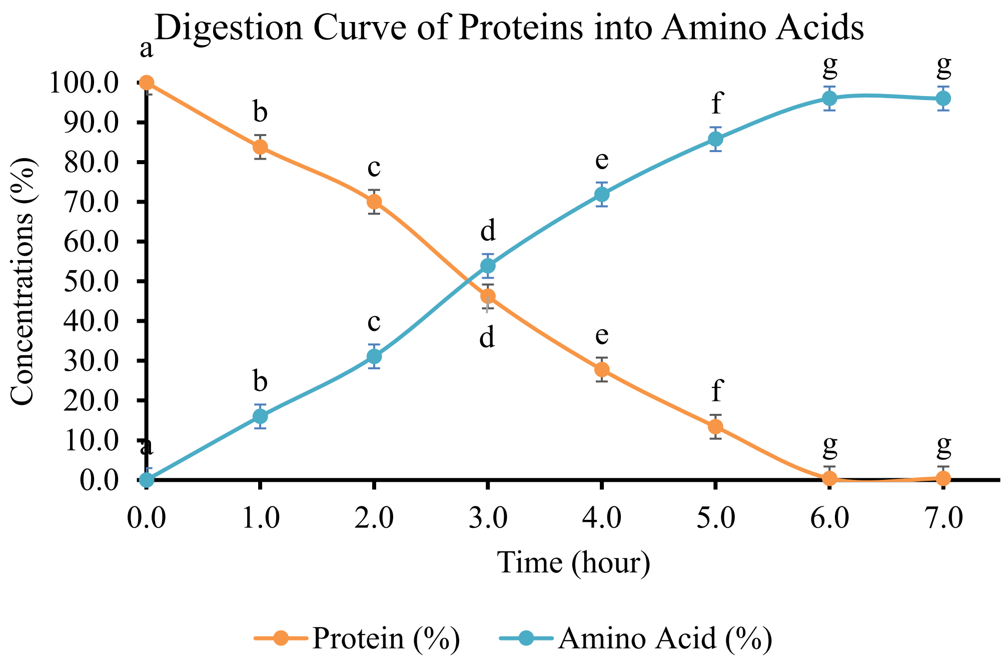

Figure 4.1: Digestion curve of Clarias Catfish protein into protein hydrolysate. The protein content from Clarias Catfish showed a decreasing trend, while the amino acid content showed an increasing trend from 0.0 to 7.0 hours. Data are reported as mean ± SE values (n=3). Superscripts (a-g) indicate statistically significant differences ($p < 0.05$).

4.2 Physical Parameter (Harvested)

All plant samples were planted for 28 days and treated with different concentration ranges of PH from 0 mg/L to 8000 mg/L. A group of plant samples was grown without treatment (0 mg/L) to serve as the control group. The effects of the different concentrations of PH were evaluated by the physical parameters of the harvested plant. In this experiment, the number of leaves counted only included fully expanded and healthy green leaves to assess vegetative growth. Senescent or damaged leaves were excluded from the count. This was to ensure the data reflect active plant growth and photosynthetic performance.

In general, the physical parameters of the plant samples were directly proportional to the increasing concentration of PH, but the trend started to decline from 6000 mg/L to 8000 mg/L. The result showed the Bok Choy treated with a 6000 mg/L PH had the highest score in shoot length (16.05 ± 0.12 cm). By comparing the treatment of 6000 mg/L with non-treatment, the shoot length had increased 2.43-fold from non-treatment. Other physical parameters, such as Bok Choy dried shoot and root weight, number of leaves, and leaf surface area, also showed the significance of the increasing trend with increasing PH concentration.

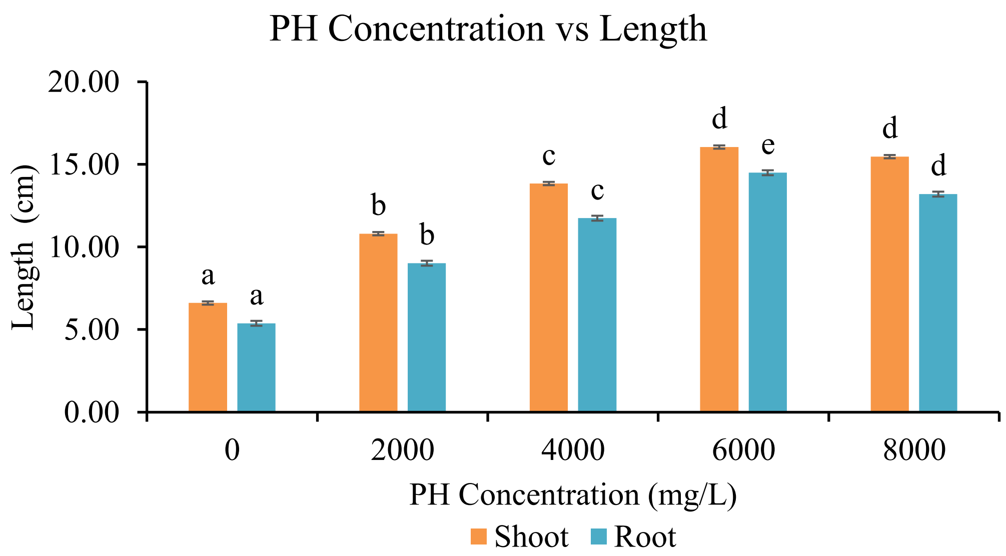

Figure 4.2.1: Effect of increasing PH concentration on plant shoot and root length. The shoot and root length of the plant (cm) showed an increasing trend with increasing PH concentration. Data are reported as mean ± SE values (n=5). Superscripts (a-e) indicate statistically significant differences ($p < 0.05$).

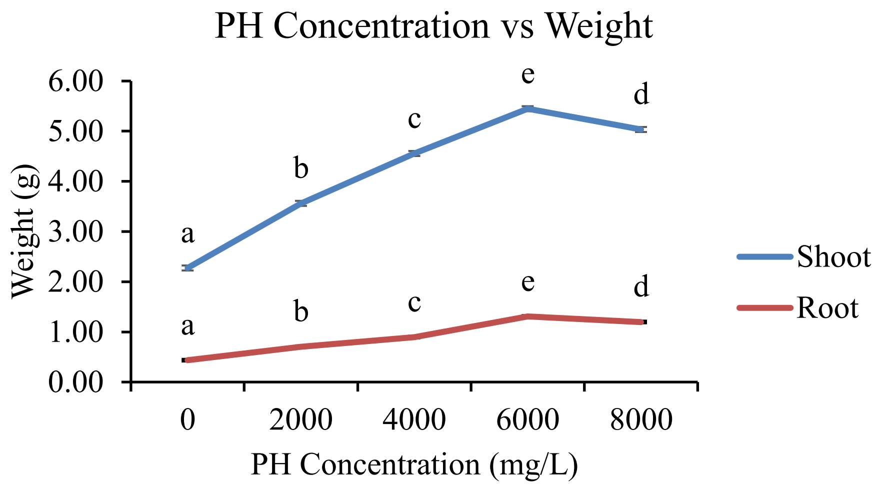

Figure 4.2.2: Effect of increasing PH concentration on plant dried shoot and root weight. The dried shoot and root weight of the plant (g) after harvesting from the treatment and non-treatment showed an increasing trend. Data are reported as mean ± SE values (n=5). Superscripts (a-e) indicate statistically significant differences ($p < 0.05$).

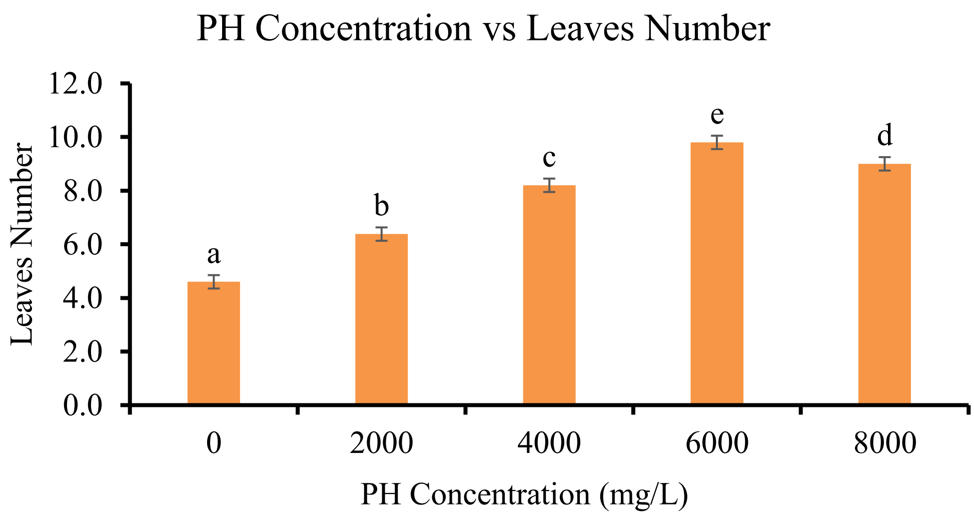

Figure 4.2.3: Effect of increasing PH concentration on the number of leaves from the plant. The number of leaves of the plant showed an increasing trend with increasing PH concentration. Data are reported as mean ± SE values (n=5). Superscripts (a-e) indicate statistically significant differences ($p < 0.05$).

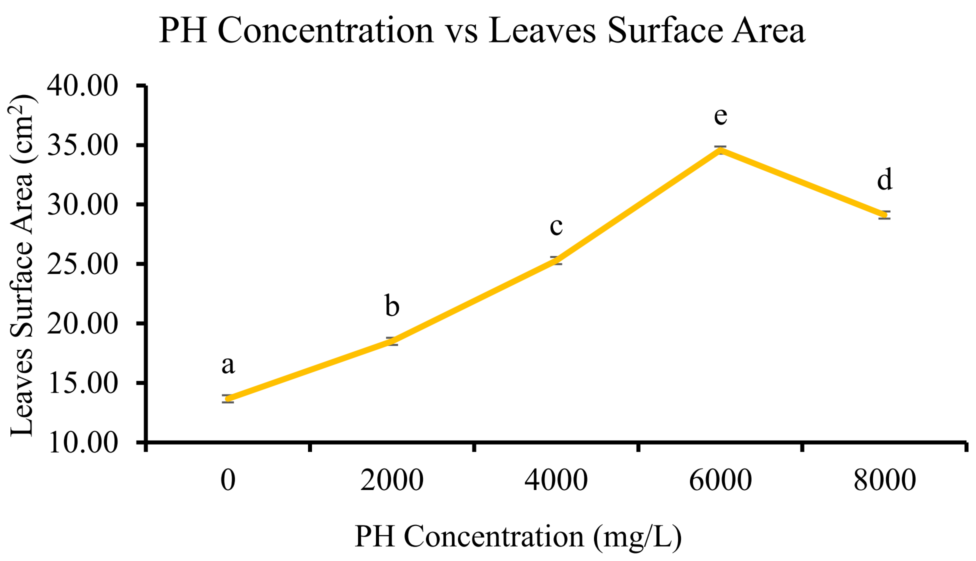

Figure 4.2.4: Effect of increasing PH concentration on the plant leaves surface area. The surface area of the leaves from the plant (cm^2) showed an increasing trend with increasing PH concentration. Data are reported as mean ± SE values(n=5). Superscripts (a-e) indicate statistically significant differences ($p < 0.05$).

4.3 Total Chlorophyll and Carotenoid Content

The chlorophyll and carotenoid contents of the freshly harvested Bok Choy were immersed in acetone for 24 hours and measured at the OD(661.6), OD(644.8), and OD(470). The data were inserted into formulas to get the concentration of chlorophyll and carotenoid contents. In general, the chlorophyll and carotenoid contents increased proportionally to the increasing PH concentrations. However, the trend started to decline from 6000 mg/L to 8000 mg/L with PH treatment. For example, the plant samples from the 6000 mg/L treatment had a higher chlorophyll α content than 8000 mg/L (5.086 ± 0.050 μg/mL and 4.601 ± 0.050 μg/mL, respectively). Besides that, the plant samples with PH treatment of 6000 mg/L had the highest content of chlorophyll α, chlorophyll β, and carotenoids (5.086 ± 0.050 μg/mL, 6.801 ± 0.101 μg/mL and 0.6109 ± 0.0409 μg/mL, respectively) among the treatments. The plant samples with 6000 mg/L PH treatment had increased by 394%, 416%, and 149% on chlorophyll α, chlorophyll β, and carotenoids compared to the control group.

Table 4.3.1: **Effect of increasing PH concentration on plant chlorophyll and carotenoid content.** The leaf pigments of the plants (μg/mL) showed an increasing trend with increasing PH concentration. Data are reported as mean ± SE values (n=5). Superscripts (a-e) indicate statistically significant differences ($p < 0.05$).

PH (mg/L)	Contents (μg/mL)		
	Chlorophyll α	Chlorophyll β	Carotenoids
0	1.288 ± 0.030^{a}	1.635 ± 0.047^{a}	0.4108 ± 0.0209^{a}
2000	3.215 ± 0.030^{b}	3.621 ± 0.049^{b}	$0.4976 \pm 0.0125^{a,b}$
4000	4.174 ± 0.049^{c}	5.405 ± 0.103^{c}	0.5270 ± 0.0380^{b}

6000	5.086 ± 0.050^{e}	6.801 ± 0.101^{e}	0.6109 ± 0.0409^{b}
8000	4.601 ± 0.050^{d}	5.696 ± 0.057^{d}	0.6077 ± 0.0249^{b}

4.4 Phytochemical Contents

The total phenolic contents (TPC) and total flavonoid contents (TFC) of the harvested Bok Choy were determined to examine the effect of increasing the PH concentration. In general, the values of TPC and TFC from the harvested Bok Choy increased proportionally with the increasing treatment. The highest score of TPC and TFC (1.096 ± 0.003 mg/mL and 0.312 ± 0.001 mg/mL, respectively) from harvested Bok Choy was at the most significant percentage of compost to soil mix ratio (40%). Compared to untreatedBok Choy (0%), the overall rise in TPC and TFC was 198% and 65% respectively.

Table 4.4: Effect of increasing PH concentration on plant phytochemical contents. The total phenolic content (mg GAE/mL) and total flavonoid contents of plants (mg QE/mL) showed an increasing trend with increasing PH concentration. Data are reported asmean ± SE values (n=5). Superscripts (a-e) indicate statistically significant differences ($p < 0.05$).

PH (mg/L)	TPC (mg GAE/mL)	TFC (mg QE/mL)
0	0.390 ± 0.003^{a}	0.624 ± 0.017^{a}
2000	0.719 ± 0.002^{b}	0.825 ± 0.007^{b}
4000	0.830 ± 0.002^{c}	1.059 ± 0.007^{c}
6000	0.961 ± 0.004^{e}	1.215 ± 0.011^{e}
8000	0.907 ± 0.002^{d}	1.142 ± 0.014^{d}

4.5 Heavy Metal Contents In PH And PH Treated Bok Choy

The heavy metal contents in the PH and plant samples were investigated using the FAAS. Two heavy metals, cadmium and lead, were evaluated, and they were found in the PH sample.

The PH made from Clarias catfish viscera were tested for lead (Pb) and cadmium (Cd) due to their high toxicity and strong bioaccumulation in the organ, especially in the liver. The liver accumulates heavy metals because it functions as the primary detoxification organ. The heavy metals will bind to cysteine-rich metallothioneins that form stable metal-thiolate clusters (Mahmoud, et al., 2020). Other amino acids, such as histidine, aspartic acid, and glutamic acid, also provide functional groups (imidazole and carboxyl) for metal binding (Mahmoud, et al., 2020). Pb and Cd are prioritised over other heavy metals because of their low tolerable intake and well-documented chronic effects: Pb affects the nervous, hematopoietic, and cardiovascular systems, while Cd targets the kidneys and bones and is classified as a human carcinogen (Barst, et al., 2018). Monitoring these metals ensures that the PH is safe for crop application and compliant with regulatory standards.

Table 4.5: Heavy metal contents in Clarias Catfish PH and increasing PH treated plant samples. Heavy metal contents in Clarias Catfish PH and PH-treated plants. Data are reported as mean ± SE values (n=3). Superscripts (a-b) indicate statistically significant differences ($p < 0.05$).

Sample	[Pb]/ppm	[Cd]/ppm
PH	0.2765 ± 0.0116^{a}	0.1654 ± 0.0011^{a}
0 mg/L	0.0196 ± 0.0076^{b}	0.0311 ± 0.0004^{b}
2000 mg/L	0.0131 ± 0.0015^{b}	0.0299 ± 0.0003^{b}
4000 mg/L	0.0197 ± 0.0000^{b}	0.0325 ± 0.0016^{b}
6000 mg/L	0.0164 ± 0.0041^{b}	0.0309 ± 0.0006^{b}
8000 mg/L	0.0084 ± 0.0015^{b}	0.0347 ± 0.0010^{b}

4.6 Antioxidative Potential In PH-Treated Bok Choy

The antioxidative potential in plant samples was investigated by using a DPPH radical scavenging assay and ABTS radical cation scavenging ability.

Table 4.6: Effect of increasing PH concentration on plant antioxidative potential. The plant's antioxidative potential showed an increasing trend with increasing PH concentration. Data are reported as mean ± SE values (n=5). Superscripts (a-d) indicate statistically significant differences ($p < 0.05$).

PH (mg/L)	Antioxidative Potential (%)	
	DPPH	$ABTS^+$
0	20.14 ± 0.63^a	19.60 ± 0.16^a
2000	22.89 ± 0.60^b	31.00 ± 0.21^b
4000	27.21 ± 0.80^c	33.22 ± 0.25^c
6000	28.06 ± 0.44^d	35.90 ± 0.08^c
8000	27.93 ± 0.47^d	35.41 ± 0.17^c

CHAPTER 5

DISCUSSION

5.1 Physical Parameter (Harvested)

Farming is the easiest way to harvest the produce among the agricultural activities. Fresh products require a relatively short amount of time to mature and mass-produce. However, several variables affected the sufficiency of crops, such as the availability of fertiliser. The commercial fertilisers came in big packages and required heavy transport to reach from the seller to the farmer. Therefore, the supply chains need some time to complete the series of actions. On the other hand, protein hydrolysates were more convenient compared to commercial fertilisers as it is much smaller and more concentrated. It required a small amount to provide positive effects to the crops. Besides that, studies have reported that PH has improved crop productivity by enhancing its growth, phytochemicals and others (Calvo, et al., 2014; Colla, et al., 2015). In this study, the Bok Choy was grown in sand treated with increasing Clarias Catfish PH (0, 2000, 4000, 6000, 8000 mg/L) for 28 days to test its effectiveness on plant biochemical and physical parameters. The physical parameters, which also refer to plant size, may influence the consumer's purchase decisions.

In general, the physical parameters of the Bok Choy showed increasing trends with increasing PH treatments from 0 mg/L to 8000 mg/L. As shown in Figure 4.2.1 until Figure 4.2.4, the Bok Choy with 6000 mg/L PH treatment scored the highest, followed by 8000 mg/L, 4000 mg/L, 2000 mg/L and the control group.

By taking Figure 4.2.1 as an example, the length of the shoot of the Bok Choy from 6000 mg/L PH treatment had increased 2.43-fold (16.05 ± 0.12^{e} cm) compared to the control group (6.61 ± 0.21^{a} cm). However, the parameters showed a decreasing trend when the Bok Choy was treated with 8000 mg/L PH. Other physical properties of the plant also showed significant changes with increasing PH treatments.

Colla, et al. (2014) applied a plant-derived ‘PH Trainer’ to cultivate corn (*Zea mays* L.) in their previous study. The results of the treated corn showed by the author had a higher coleoptile elongation rate than the untreated corn. She stated that the presence of nitrogenous compounds such as ammonia, bioactive peptides, and tryptophan was the major factor for the positive effect of PH. The author highlighted that tryptophan plays a major role in stimulating the physical growth of the corn, as tryptophan was the precursor of indole-3-acetic acid biosynthesis (Colla, et al., 2015). Therefore, the plant cells were stimulated and underwent elongation and proliferation. In general, the plant will uptake the tryptophan consistently and stimulate the auxin biosynthesis pathway. As a result, the plant cell will perform cell proliferation at a higher rate. Besides that, the spinach treated with protein hydrolysate made from Indian sardine waste had an increased 40% increase in the yield associated with better root development compared to the control (Dewang, et al., 2020). Moreover, the lettuce treated with protein hydrolysate made from rainbow trout waste had increased by around 25% in leaf number, stem diameter and weight based on the study by Xu, et al. (2017).

Despite the positive effect of PH on the growth of crops, there were some factors which would lead to inhibition. Excess use of fish waste PH causes the inhibition of growth on lettuce as it leads to soft rot damage (Xu, et al.,2017). Besides that, excess PH reduces the dissolved oxygen and inhibits the growth of the plant (Xu, et al., 2017). Dewang, et al. (2021) shared similar results on treating the spinach with feather-derived PH. The PH had a positive effect on the plant by upscaling the PH concentration. However, the spinach growth staggered when the concentration applied was beyond the optimum.

One of the limitations was the PH did not compared with the effects of commercial fertiliser treatments, which limits the ability to evaluate their relative effectiveness, cost efficiency, and practicality under real agricultural conditions. Furthermore, environmental parameters such as the pH, electrical conductivity, and dissolved oxygen content of the growth medium were not assessed, even though these factors could influence plant growth by altering nutrient availability and microbial activity following PH application. In addition, despite the overall positive trend in plant growth with increasing PH concentration, a decline was observed at the highest concentration (8000 mg/L). The underlying cause of this inhibition was not determined. Therefore, further investigation is required to determine the environmental and physiological mechanisms responsible for these inhibitory effects and to establish the optimum PH concentration for sustainable crop growth.

5.2 Chlorophyll and Carotenoids

The chlorophyll and carotenoid contents were evaluated to evaluate the effect of increasing PH concentration on the Bok Choy on chlorophyll α, chlorophyll β, and carotenoid contents. From the result shown, the plant samples in the 6000 mg/L treatment group had the highest chlorophyll α and chlorophyll β, and carotenoid contents (5.086 ± 0.050^e µg/mL and 6.801 ± 0.101^e µg/mL, respectively) compared to other treatment and non-treatment groups. However, the carotenoid contents showed no significant changes between the control and PH-treated Bok Choy, which required more research to evaluate the implications of Clarias Catfish PH.

Chlorophyll and carotenoids are essential for plants. They are pigments to enable the plants to perform photosynthesis. Chlorophyll shows green in colour, while the carotenoids provide orange-coloured pigments in fruits and vegetables such as carrots and tomatoes. Carotenoids are one of the antioxidants that benefit our body by boosting the immune system and providing protection against free radicals (Rinawati, et al., 2020). The main function of chlorophyll and carotenoids is to harvest solar energy and trigger photosynthesis. It further engages the carbon fixation process by converting the carbon dioxide into carbohydrates, which help in synthesising energy for growth and development (Rinawati, et al., 2020).

Several factors affect the extraction of pigments, such as extraction techniques, types of solvent used, duration of the extractions, leaf anatomy, and tissue structure. Studies have shown that several solvents can be used to extract chlorophyll and carotenoids, such as methanol, ethanol, and acetone (Minocha, et al., 2009). In comparison, acetone was the most effective extraction solvent, as it can extract the non-polar carotenoids and provide a sharp chlorophyll peak during the analysis (Vimala & Poonghuzhali, 2015). Besides that, Su, et al. (2010) highlighted that using acetone for chlorophyll extraction was fast, reliable, and low degradation rate. A study was carried out by Xu, et al. (2017) using fish PH made from fish waste to grow the lettuce. The author stated that lettuce with the treatment has higher total chlorophyll content than the control. Besides that, Ertani, et al. (2013) showed tomato plants treated withplant-derived PH had higher chlorophyll content. In the Luziatelli, et al. (2016) study, the authors used plant-derived PH to grow Bok Choy. The result showed a similar result as the Bok Choy with treatment has higher chlorophyll content than the control. The author found that a high level of the amino acid glycine from the PH is one of the reasons for the increased chlorophyll concentration. The author also highlighted that glycine acts as a precursor to produce a porphyrin ring as the core component of chlorophyll. Therefore, the abundance of glycines was taken up by the plant to produce more porphyrin rings, which were later used to synthesise more chlorophyll.

Although Bok Choy treated with 6000 mg/L of Clarias Catfish PH showed the highest chlorophyll α, chlorophyll β, and carotenoid contents, this study had several limitations. Firstly, the biochemical mechanisms behind the increase in pigment levels were not explored, and photosynthetic performance was not evaluated to confirm functional improvement. Additionally, variations in extraction efficiency, solvent polarity, and possible pigment degradation during handling may have influenced the measured pigment concentrations. The use of a single extraction solvent also limited the assessment of optimal pigment recovery. Lastly, the short cultivation period under controlled conditions may not fully reflect pigment responses under field conditions or during longer growth cycles.

5.3 Phytochemicals

Phytochemicals are natural biological compounds that are found in most dietary plants. These phytochemicals play a crucial defence mechanism by protecting the plant from pathogenic infections. Phenolic compounds play crucial roles in scavenging reactive oxygen species (ROS), maintaining chloroplast integrity, and regulating auxin-mediated cell expansion, thereby promoting photosynthetic efficiency and vegetative development (Grace, 2005; Peer, et al., 2013). On the other hand, flavonoid compounds act as both antioxidants and signalling molecules that modulate auxin transport, enhance root branching, and protect chlorophyll from oxidative degradation (Buer, et al., 2010; Agati, et al., 2012).

Phytochemicals were also found to be a potential drug candidate to treat and prevent human diseases. By comparing the phytochemicals to other synthetic drugs, they are found to be safer with fewer side effects, as they are natural products. Besides that, Sasidharan, et al. (2011) stated that phytochemicals have the properties of anticancer, antibacterial, antioxidant, antifungal, and wound-healing properties. In general, the bioactivities of the plant contribute to the type of phytochemical produced. Polyphenolic compounds are the most popular phytochemicals to study, containing phenolic acids and flavonoids (Garcia, et al., 2011). These polyphenolic compounds are secondary metabolites that are found in a variety of green plants. These compounds serve different functions such as signalling, detoxifying, UV filter, and pollinator attractants. In the Samanta, et al. (2011) study, the authors stated that phenolic acids and flavonoids are essential in an individual's diet to stay healthy.

The phytochemical content evaluated in this study is total phenolic content (TPC) and total flavonoid content (TFC) from the Bok Choy treated with increasing Clarias catfish pH concentration. In general, the TPC and TFC increased proportionally to the increasing PH concentration. The Bok Choy with the 6000 mg/L Clarias Catfish PH treatment has the highest TPC and TFC (0.961 ± 0.004^{e} mg GAE /mL and 1.215 ± 0.011^{e} mg QE/mL, respectively). However, the trend from 6000 mg/L PH treatment to 8000 mg/L had dropped (0.907 ± 0.002^{d} mg GAE /mL and 1.142 ± 0.014^{d} mg QE/mL, respectively).

Most studies concluded that PH applications were beneficial and increased the plant TPC and TFC. In the Shetty, et al. (2003) study, the authors used PH made from fish waste to evaluate the effect of growth on fava beans (*Vicia faba*). The author shows that fava beans treated with the fish PH had higher total phenolic contents than the control. In the following studies, the author found that fava beans with fish PH treatment had enhanced their key enzymes in the pentose phosphate pathway (Shetty, et al. 2003). The author also claimed that the sugar substrate from the pentose phosphate pathway acts as a precursor and stimulates the phenolic biosynthesis of the fava bean and its growth (Shetty, et al. 2003). Alfalfa plant (*Medicago sativa* L.) PH had increased TFC in corn by enhancing the flavonoid biosynthesis pathway. The study also showed that the presence of nitrogen had stimulated the activity of key enzymes in the phenylpropanoid biosynthesis, which further stimulated the flavonoid biosynthesis pathway (Ertani, et al., 2013; Shetty & McCue, 2003). This biochemical enhancement likely contributed to the higher chlorophyll content, biomass accumulation, and antioxidant potential observed in Bok Choy, indicating that PH application not only promotes nutrient supply but also activates the plant’s endogenous defence and growth regulation systems (Sharma, et al., 2019).

Although the application of the PH had increased the total phenolic content (TPC) and total flavonoid content (TFC) of Bok Choy, several limitations should be noted. This study did not investigate the specific phenolic or flavonoid compounds present, which limits understanding of which individual metabolites contributed most to the observed increase. Additionally, the biochemical pathways responsible for enhanced phytochemical synthesis such as enzyme activity in the phenylpropanoid or pentose phosphate pathways were not examined. The analysis also relied on colorimetric assays (Folin–Ciocalteu and aluminium chloride methods), which may be influenced by other reducing substances and thus provide only approximate estimations of TPC and TFC.

5.4 Determination of Heavy Metals

The PH made from Clarias catfish viscera were tested for lead (Pb) and cadmium (Cd) due to their high toxicity and strong bioaccumulation in the organ, especially in the liver. The liver accumulates heavy metals because it functions as the primary detoxification organ. The heavy metals will bind to cysteine-rich metallothioneins that form stable metal-thiolate clusters (Mahmoud, et al., 2020). Other amino acids, such as histidine, aspartic acid, and glutamic acid, also provide functional groups (imidazole and carboxyl) for metal binding (Mahmoud, et al., 2020). Pb and Cd are prioritised over other heavy metals because of their low tolerable intake and well-documented chronic effects: Pb affects the nervous, hematopoietic, and cardiovascular systems, while Cd targets the kidneys and bones and is classified as a human carcinogen (Barst, et al., 2018). Monitoring these metals ensures that the PH is safe for crop application and compliant with regulatory standards.

The FAAS machine was used to estimate the level of heavy metals (Pb and Cd) in the Clarias Catfish PH and treated plant samples to study the heavy metal absorption and bioaccumulation. Lead (Pb) and cadmium (Cd) were found in the samples, including Clarias Catfish PH, treated and non-treated plant samples.

In contrast, the heavy metal contents in Clarias Catfish PH were higher than in treated and non-treated plant samples. For example, the Pb and Cd each scored 16.9 and 5.4 fold in Clarias Catfish PH compared to 6000 mg/L treated plant samples. However, no significant changes were observed between the control and treatment groups.

Heavy metals such as Pb and Cd were known to bioaccumulate in various organs of fish, especially in the liver and gills. The liver of the fish, as well as other organisms, was a primary site for various metabolic and detoxification processes. Hence, the liver itself stored high amounts of heavy metals such as Pb and Cd compared to other tissues such as muscles (Farhan, et al., 2023). Besides that, the gills were susceptible to accumulating heavy metals as they had a large surface area exposed to the water. Hence, it also showed a significant concentration of heavy metals (Pb and Cd) due to direct exposure to the environment (Norhazirah, et al., 2023).

According to the European Union Regulation on leafy vegetables, the maximum allowable level of both Pb and Cd was 0.1 mg/L (European Union Regulation, 2021). Based on the results, the heavy metal contents in treated and non-treated plant samples were acceptable and safe for consumption. However, most studies have shown that the *Brassica* family, particularly *Brassica juncea* and *Brassica rapa* in bioaccumulating heavy metals such as Pb and Cd (Alicja, et al., 2017; Xia, et al., 2024). The study showed *Brassica* species had specific biochemical mechanisms to handle the heavy metals. Arleta, et al. (2021) observed that *Brassica juncea* had activated an antioxidative and detoxification pathway to handle the oxidative damage caused by the heavy metals. Apart from that, the study from Miguel, et al. (2015) also indicated the presence of metal transporters and chelation mechanisms in the *Brassica* genus, such as phytochelatins, to sequester the heavy metals (Pb and Cd) within the plant tissues and reduce the toxicity. On the contrary, the results obtained from experiments showed a low quantity of heavy metals from the treated and non-treated Bok Choy, despite there being higher Pb and Cd found from the Clarias Catfish PH.

There were several reasons to explain this phenomenon. Firstly, there was lower metal availability in the environment, and the concentration of heavy metals (Pb and Cd) was relatively low for the plant to absorb. The plant samples were grown in polybags with sand medium. Therefore, the heavy metals (Pb and Cd) may wash away with the daily irrigation and cause a relatively lower concentration.

Besides that, soil pH and beneficial minerals such as iron, magnesium and calcium may compete with the heavy metals, which further reduce the accessibility to Bok Choy roots. Based on the study of Jelena, et al. (2021), the presence of manganese, zinc and copper can compete with lead (Pb) from the plant root binding sites. In addition, Sun, et al. (2021) showed that metal cations had lower solubilities at higher pH. Hence, the Pb and Cd may form insoluble compounds with hydroxide or carbonate ions, thus reducing the uptake by *Brassica rapa* (Sun, et al., 2021).

Although the study confirmed that PH contained detectable levels of lead (Pb) and cadmium (Cd), but the treated Bok Choy samples still remained within the safe limits established by the European Union. However, several limitations should be considered. Firstly, only two heavy metals (Pb and Cd) were analysed, while other potentially hazardous elements such as mercury (Hg), arsenic (As), and chromium (Cr) were not evaluated which limits the assessment of PH safety. Secondly, the experiment did not quantify the total or bioavailable metal fractions in the growth medium, making it difficult to determine the extent of metal mobility or leaching during irrigation. The exclusive use of sand as the growing medium may have underestimated metal retention and plant uptake compared to natural soils with higher organic matter and cation exchange capacity. In addition, only a single crop cycle and PH concentration series were tested, providing limited insight into long-term accumulation risks or potential metal buildup in successive planting cycles. Finally, the study did not assess the distribution of heavy metals among plant tissues (roots, stems, and leaves), which would have offered a more detailed understanding of metal translocation, bioaccumulation patterns, and food safety implications.

5.5 Determination of Antioxidative Potential

Antioxidants are a group of molecules that readily react with free radicals, which are also referred to as reducing agents (Lobo, et al., 2010). As a result, it neutralises the free radicals in the body by donating one of its electrons to them (Lobo, et al., 2010). Hence, the free radicals were finally stabilised and reduced the negative outcomes to the body. Besides that, the antioxidants can terminate the chain reactions initiated by the free radicals at the beginning (Masaki, 2010). Thus, it can early reduce the impact of free radicals such as inflammation, lipid peroxidation, protein alteration and others (Masaki, 2010). Based on the study, the antioxidative compounds found in most plants were phenolic and flavonoid compounds such as quercetin, catechins, hydroxybenzoic acids and others (Xu, et al., 2017).

DPPH radical scavenging assay and ABTS radical cation scavenging ability were used to evaluate the antioxidative potential in treated and non-treated Bok Choy. The former method was more sensitive to flavonoid compounds, while the latter was more sensitive to phenolic compounds (Natividad, et al., 2020). Both methods provided more insightful details on the phytochemical compounds from the treated and non-treated Bok Choy. The results showed higher $ABTS^+$ radical scavenging activities than DPPH radical scavenging activities. However, there were no significant changes among the 6000 mg/L and 8000 mg/L PH-treated Bok Choy on $ABTS^+$ radical scavenging activities; and 4000, 6000 and 8000 mg/L PH-treated Bok Choy on DPPH radical scavenging activities.

The antioxidation capacities of plants are often linked to their level of phenolic and flavonoid contents. Consistently, plant samples with high contents of total phenolic were also found to possess high levels of total flavonoid. The plant samples treated with increasing Clarias catfish pH also possess an increasing trend in radical scavenging activities. The *Brassica* family possesses a strong variety of phytochemical compounds in combating oxidative stress and inflammation, thereby contributing to health benefits when consumed. Studies have shown that the *Brassica* family contains abundant phenolic acids such as gallic acids, chlorogenic acids, cinnamic acid derivatives and ferulic acids (María, et al., 2010). Besides that, a previous study also showed the *Brassica* family is rich in flavonoids such as quercetin, kaempferol, and isorhamnetin (Sohail, et al., 2018). Therefore, it remains to be determined whether the aforementioned phenolic and flavonoid compounds were responsible for the radical scavenging activities observed in plant samples treated with Clarias Catfish PH.

In the previous study, an increase in the radical scavenging activities was observed when the plants were treated with fish PH, as it also increased the phenolic and flavonoid compounds in the plant (Shetty, et al., 2003; Vasconcelos, et al., 2009). Besides that, Sun, et al. (2024) showed that the application of fish PH can increase the phenolic compounds in the plant, which further aids the plant to handle abiotic stress and defend itself from pathogens. In addition, Mehdi, et al. (2023) stated that PH made from the viscera of commercial fish species that were rich in amino acids will activate the key enzymes in phenylpropanoid biosynthesis and pentose-phosphate pathway in the plant, which further produces more phenolic and flavonoid compounds in the plant. Apart from that, Horii, et al. (2007) also reported similar results on the positive effect of fish PH by increasing the free radical scavenging activities in soybeans.

Although the DPPH and ABTS assays revealed that Bok Choy treated with PH had exhibited higher antioxidant activity, several limitations should be considered. First and foremost, there were only two in vitro assays were used to assess the antioxidant potential, which primarily reflect chemical reactivity rather than the physiological antioxidant capacity of the plant. The results may therefore not fully represent the in vivo antioxidant performance or enzymatic defence responses, such as the activities of superoxide dismutase (SOD), catalase (CAT), or peroxidase (POD). Lastly, the study did not identify or quantify specific phenolic or flavonoid compounds responsible for the observed radical scavenging effects, limiting mechanistic interpretation.

CHAPTER 6

CONCLUSION

6.1 Conclusion and Limitations

In conclusion, PH made from Clarias Catfish viscera brought positive effects to the Bok Choy in its physical growth and phytochemical compounds. The growth of Bok Choy showed a positive correlation to the dosage of Clarias Catfish PH. The dose-dependent relationship stopped at 6000 mg/L. Treatment of 8000 mg/L shows overnutrition and starts to pose a negative impact on Bok Choy growth. Bok Choy treated with 6000 mg/L Clarias Catfish PH exhibited the highest yield in physical growth parameters, phytochemical content, and chlorophyll content. While improvements were also observed in carotenoid content and radical scavenging activity, the differences were less pronounced. Furthermore, the levels of heavy metals lead (Pb) and cadmium (Cd), in the PH, were within the permissible limits established by the European Union, confirming its safety for agricultural application. Overall, 6000 mg/L enzyme-digested Clarias Catfish viscera PH demonstrated its potential as an effective organic fertilizer.

6.2 Future studies

In future studies, other vegetative crops or herbs could be explored as plant models to further evaluate the potential of Clarias catfish viscera protein hydrolysate (PH). Comparative assessments between this PH and commercial fertilizers should also be conducted to determine their relative efficiency, nutrient bioavailability, and cost-effectiveness. In addition, amino acid profiling and peptide mass fingerprinting using LC-MS/MS or MALDI-MS could be employed after enzymatic digestion to better characterise the peptide composition and identify bioactive sequences responsible for plant growth promotion. Quantification of plant hormones such as auxins and cytokinins should also be performed to understand the biochemical mechanisms for the growth enhancement. Moreover, analyzing the concentration of aromatic amino acids such as tryptophan, phenylalanine, and tyrosine may provide insights into their role as precursors in major plant hormone biosynthetic pathways and secondary metabolite production. Further investigation into the distribution of metal ions in the PH and their accumulation in plants is also recommended to ensure both agronomic efficiency and environmental safety. Finally, comprehensive food safety evaluations of PH-treated crops should be conducted especially in other heavy metals such as mercury, arsenic, and chromium before consumer application to confirm compliance with international food safety standards.

REFERECES

Abdelmawgoud, S. M. S., Aziz, H. H. A., Shibi, A. A. A., & Qabeel, M. A.-S., 2021. A Comparative Economic Study of Bok Choy by Hydroponics and Conventional Agriculture (With Soil) in Greenhouses: A Case Study in the Nubaria Region. *Asian Journal of Agricultural Extension, Economics & Sociology*, 39(2), 126-140.

Ainsworth, E. A. & Gillespie, K. M., 2007. Estimation of total phenolic content and other oxidation substrates in plant tissues using Folin-Ciocalteu reagent. *Nature Protocols*, 2(4), pp. 875-877. doi: 10.1038/nprot.2007.102.

Ali, M. F., Ali, U., Jamil, M. A., Awais, M., Khan, M. J., Waqas, M. & Adnan, M. (2021) 'Hydroponic Garlic Production: An Overview', Agrinula: *Jurnal Agroteknologi dan Perkebunan*, 4(1), pp. 73–93. doi: 10.36490/agri.v4i1.138

Anwar, A., Yu, X. & Li, Y., 2020. Seed priming as a promising technique to improve growth, chlorophyll, photosynthesis and nutrient contents in cucumber seedlings. *Notulae Botanicae Horti Agrobotanici Cluj-Napoca*, 48(1), 116-127.

Apone F., Tito A., Carola A., Arciello S., Tortora A., Filippini L., Monoli I., Cucchiara M., Gibertoni S., Chrispeels M.J., Colucci G. (2010). A mixture of peptides and sugars derived from plant cell walls increases plant defense responses to stress and attenuates ageing-associated molecular changes in cultured skin cells. *Journal of Biotechnology*, 145(4): 367–376. DOI:10.1016/j.jbiotec.2009.11.021.

Shetty, K. & Wahlqvist, M. (2004) 'Phenolic antioxidant biosynthesis in plants for functional food application: integration of systems biology and biotechnological approaches', *Food Biotechnology*, 17(2), pp. 67-97. doi: 10.1080/08905430410001724846.British Broadcasting Corporation (BBC) (2022) *Food Safety News* [online]. Available at: https://www.bbc.com/ (Accessed: 8 November 2024).

Bulgari, R., Cocetta, G., Trivellini, A. & Vernieri, P., 2015. Biostimulants and crop responses: A review. *Biological Agriculture and Horticulture*, 31(1), 1-17.

Cerdán, M., et al., 2013. Effect of commercial amino acids on iron nutrition of tomato plants grown under lime-induced iron deficiency. *Journal of Plant Nutrition and Soil Science*, 176(6), 859-866.

Chew, B.P. & Park, J.S. (2004) 'Carotenoid action on the immune response', *The Journal of Nutrition*, 134(1), pp. 257S-261S. doi: 10.1093/jn/134.1.257S.

Chiew, Y. L., Spångberg, J., Baky, A., Hansson, P. A. & Jönsson, H. (2015) 'Environmental impact of recycling digested food waste as a fertilizer in agriculture — A case study', *Resources, Conservation & Recycling*, 95, pp. 1-14. doi: 10.1016/j.resconrec.2014.11.015.

Choi, I.S., Cho, E.J., Moon, J.-H. & Bae, H.-J. (2015) 'Onion skin waste as a valorization resource for the by-products quercetin and bio-sugar', *Food Chemistry*, 188, pp. 537-542. doi: 10.1016/j.foodchem.2015.04.024.

Colla, G. (2014) 'Biostimulant action of a plant-derived protein hydrolysate produced through enzymatic hydrolysis', *Frontiers in Plant Science*, 5(448), pp. 1-6. doi: 10.3389/fpls.2014.00448.

Colla, G., Cardarelli, M., Bonini, P. & Rouphael, Y. (2017) 'Foliar applications of seaweed-based compost increase yield but differentially modulate fruit quality of greenhouse tomato', *HortScience*, 52(9), pp. 1214-1220. doi: 10.21273/HORTSCI11923-17.

Colla, G., Hoagland, L., Ruzzi, M., Cardarelli, M., Bonini, P., Canaguier, R. & Rouphael, Y. (2017) 'Biostimulant action of protein hydrolysates: unraveling their effects on plant physiology and microbiome', *Frontiers in Plant Science*, 8, Article 2202. doi: 10.3389/fpls.2017.02202.

Dai, Z., Wu, Z., Jia, S. & Wu, G. (2014) 'Analysis of amino acid composition in proteins of animal tissues and foods as pre-column o-phthaldialdehyde derivatives by HPLC with fluorescence detection', *Journal of Chromatography B*, 964, 116-127.

Despommier, D. (2011) 'The vertical farm: controlled environment agriculture carried out in tall buildings would create greater food safety and security for large urban populations', *Journal of Consumer Protection and Food Safety*, 6, pp. 233-236. doi: 10.1007/s00003-011-0675-2.

Development of an Automatic Microcontroller System for Deep Water Culture (DWC). Shah Alam, IEEE 9th International Colloquium on Signal Processing and its Applications.

Dinesh, B., Yadav, B., Reddy, R. D., Padma, A. S. & Sukumaran, M. K. (2015) 'Determination of ascorbic acid content in some Indian spices', *International Journal of Current Microbiology and Applied Sciences*, 4(8), pp. 864-868.

Domingues, D. S., Takahashi, H. W., Camara, C. A. P. & Nixdorf, S. L. (2012) 'Automated system developed to control pH and concentration of nutrient solution evaluated in hydroponic lettuce production', *Computers and Electronics in Agriculture*, 84, pp. 53-61. doi: 10.1016/j.compag.2012.02.006.

Doucet, D., Otter, D.E., Gauthier, S.F. & Foegeding, E.A. (2003) 'Enzyme-induced gelation of extensively hydrolysed whey proteins by Alcalase:

peptide identification and determination of enzyme specificity', *Journal of Agricultural and Food Chemistry*, 51(21), pp. 6300-6308. doi: 10.1021/jf026242v.

Eckhardt, U., Grimm, B. & Hörtensteiner, S. (2004) 'Recent advances in chlorophyll biosynthesis and breakdown in higher plants', *Plant Molecular Biology*, 56(1), pp. 1-14. doi: 10.1007/s11103-004-2331-3.

Enhances Bok Choy Tolerance to Alkalinity and Salinity. Frontiers in Plant Science, 8(131), 1-12.

Ertani, A., Cavani, L., Pizzeghello, D., Brandellero, E., Altissimo, A., Ciavatta, C. & Nardi, S. (2009) 'Biostimulant activity of two protein hydrolysates in the growth and nitrogen metabolism of maize seedlings', *Journal of Plant Nutrition and Soil Science*, 172(2), pp. 237-244. doi: 10.1002/jpln.200800174.

Ertani, A., Pizzeghello, D., Francioso, O., Tinti, A. & Nardi, S. (2016) 'Biological activity of vegetal extracts containing phenols on plant metabolism', *Molecules*, 21(2), Article 205, pp. 1-14. doi: 10.3390/molecules21020205.

Ertani, A., Schiavon, M., Muscolo, A. & Nardi, S. (2013) 'Alfalfa plant-derived biostimulant stimulate short-term growth of salt stressed Zea mays L. plants', *Plant and Soil*, 364(1), pp. 145-158. doi:10.1007/s11104-012-1335-z.

Fehrenbach, E. & Northoff, H. (2001) 'Free radicals, exercise, apoptosis, and heat shock proteins', *Exercise Immunology Review*, 7, pp. 66-89. doi: 10.1080/026404101750165481.

Ferrarezi, R.S., Weaver, G.M., van Iersel, M.W. & Testezlaf, R. (2015) 'Subirrigation: historical overview, challenges, and future prospects', *HortTechnology*, 25(3), pp. 262-276. doi: 10.21273/HORTTECH.25.3.262.

Finaud, J., Lac, G. & Filaire, E. (2006) 'Oxidative stress: relationship with exercise and training', *Sports Medicine*, 36(4), pp. 327-358. doi: 10.2165/00007256-200636040-00004.

Firdaus, R. B. R., Tan, M. L., Rahmat, S. R. & Gunaratne, M. S. (2020) 'Paddy, rice and food security in Malaysia: A review of climate change impacts', *Cogent Social Sciences*, 6(1), Article 1818373. doi: 10.1080/23311886.2020.1818373.

Friedman, M. (2004) 'Applications of the Ninhydrin Reaction for Analysis of Amino Acids, Peptides, and Proteins to Agricultural and Biomedical Sciences', *Journal of Agricultural and Food Chemistry*, 52(3), pp. 385-406. doi: 10.1021/jf030490p.

Gallie, D.R. (2013) 'L-Ascorbic Acid: A Multifunctional Molecule Supporting Plant Growth and Development', *Scientifica*, 2013, Article 795964, pp. 1-24. doi: 10.1155/2013/795964.

Gao, J., Si, C. & He, Y. (2015) ‘Application of soybean residue (okara) as a low-cost adsorbent for reactive dye removal from aqueous solution’, *Desalination and Water Treatment*, 53(8), pp. 2266-2277. doi: 10.1080/19443994.2013.865568.

Gao, J.-F., Zhang, Q., Wang, J.-H., Wu, X.-L., Wang, S.-Y. & Peng, Y.-Z. (2011) ‘Contributions of functional groups and extracellular polymeric substances on the biosorption of dyes by aerobic granules’, *Bioresource Technology*, 102(2), pp. 805-813. doi: 10.1016/j.biortech.2010.08.119.

Garcia, A.L., Madrid, R., Gimeno, V., Rodriguez-Ortega, W.M., Nicolas, N. & Garcia-Sanchez, F. (2011) ‘The effects of amino acids fertilization incorporated to the nutrient solution on mineral composition and growth in tomato seedlings’, *Spanish Journal of Agricultural Research*, 9(3), pp. 852-861. doi: 10.5424/sjar/20110903-399-10.

Genç, E. & Atici, Ö. (2019) ‘Chicken feather protein hydrolysate as a biostimulant improves the growth of wheat seedlings by affecting biochemical and physiological parameters’, *Turkish Journal of Botany*, 43(1), pp. 67–79. doi: 10.3906/bot-1804-53.

Gill, S. S. & Tuteja, N. (2010) ‘Reactive oxygen species and antioxidant machinery in abiotic stress tolerance in crop plants’, *Plant Physiology and Biochemistry*, 48(12), pp. 909-930. doi: 10.1016/j.plaphy.2010.08.016.

Golden, T.R., Hinerfeld, D.A. & Melov, S. (2002) ‘Oxidative stress and aging: beyond correlation’, *Aging Cell*, 1(2), pp. 117-123. doi: 10.1046/j.1474-9728.2002.00015.x.

Gruda, N. (2009) ‘Do soilless culture systems have an influence on product quality of vegetables?’, *Journal of Applied Botany and Food Quality*, 82(2), pp. 141-147. doi: 10.18452/9433.

Gumisiriza, M. S., Ndakidemi, P. A. & Mbega, E. R. (2020) ‘Memoir and farming structures under soil-less culture (hydroponic farming) and the applicability for Africa: a review’, *Agricultural Reviews*, 41(2), pp. 139-145. doi: 10.18805/ag.R-137.

Gupta, U. C. & Gupta, S. C. (2014) ‘Sources and Deficiency Diseases of Mineral Nutrients in Human Health and Nutrition: A Review’, *Pedosphere*, 24(1), pp. 13-38. doi: 10.1016/S1002-0160(13)60077-6.

Gurav, R. G. & Jadhav, J. P. (2013) ‘A novel source of biofertilizer from feather biomass for banana cultivation’, *Environmental Science and Pollution Research*, 20(7), pp. 4532-4539. doi: 10.1007/s11356-012-1405-z.

Gurfinkel, D. M. & Rao, A. V. (2003) ‘Soyasaponins: The relationship between chemical structure and colon anticarcinogenic activity’, *Nutrition and Cancer*, 47(1), pp. 24-33. doi: 10.1207/s15327914nc4701_3.

Hancock, R.D. & Viola, R. (2005) ‘Improving the nutritional value of crops through enhancement of L-ascorbic acid (vitamin C) content: rationale and biotechnological opportunities’, *Journal of Agricultural and Food Chemistry*, 53(13), pp. 5248-5257. doi: 10.1021/jf0503863.

Hansen, L., Skeie, G., Landberg, R., Lund, E., Palmqvist, R., Johansson, I., Dragsted, L.O., Egeberg, R., Johnsen, N.F., Christensen, J., Overvad, K., Tjønneland, A. & Olsen, A. (2012) ‘Intake of dietary fiber, especially from cereal foods, is associated with lower incidence of colon cancer in the HELGA cohort’, *International Journal of Cancer*, 131(2), pp. 469-478. doi: 10.1002/ijc.26381.

Heck, J. X., Hertz, P. F. & Ayub, M. A.–Z. (2002) ‘Cellulase and xylanase production by isolated Amazon Bacillus strains using soybean industrial residue based solid-state cultivation’, *Brazilian Journal of Microbiology*, 33(3), pp. 213-218. doi: 10.1590/S1517-83822002000300005.

Hoque, M.A., Akhter Banu, M.N., Okuma, E., Amako, K., Nakamura, Y., Shimoishi, Y. & Murata, Y. (2007) ‘Exogenous proline and glycinebetaine increase NaCl-induced ascorbate-glutathione cycle enzyme activities, and proline improves salt tolerance more than glycinebetaine in tobacco Bright Yellow-2 suspension-cultured cells’, *Journal of Plant Physiology*, 164(11), pp. 1457-1468. doi: 10.1016/j.jplph.2006.10.004.

Horii, A., McCue, P. & Shetty, K. (2007) ‘Seed vigour studies in corn, soybean and tomato in response to fish protein hydrolysates and consequences on phenolic-linked responses’, *Bioresource Technology*, 98(11), pp. 2170-2177. doi: 10.1016/j.biortech.2006.08.030.

Hughes, N.M., Neufeld, H.S. & Burkey, K.O. (2005) ‘Functional role of anthocyanins in high-light winter leaves of the evergreen herb Galax urceolata’, *New Phytologist*, 168(3), pp. 575-587. doi: 10.1111/j.1469-8137.2005.01546.x.

McCarthy, A. L., O’Callaghan, Y. C. & O’Brien, N. M. (2013) ‘Protein Hydrolysates from Agricultural Crops—Bioactivity and Potential for Functional Food Development’, *Agriculture*, 3(1), pp. 112-130. doi: 10.3390/agriculture3010112.

Ilahi, F., Fazilah, W., Desa, A. & Mohammud Che, H. (2017) ‘Effects of root-zone cooling on Bok Choy grown in tropical conditions in a coir-perlite mixture’, *Horticulture, Environment and Biotechnology*, 58(1), pp. 1-4. doi: 10.1007/s13580-017-0123-3.

Jackson, C.J.C., Dini, J.P., Lavandier, C., Rupasinghe, H.P.V., Faulkner, H., Poysa, V. & Buzzell, D. (2002) ‘Effects of processing on the content and composition of isoflavones during manufacturing of soy beverage and tofu’, *Process Biochemistry*, 37(10), pp. 1117-1123. doi: 10.1016/S0032-9592(01)00323-5.

Maksimović, J., Pivić, R., Stanojković-Sebić, A., Jovković, M., Jaramaz, D. & Dinić, Z. (2021) 'Influence of soil type on the reliability of the prediction model for bioavailability of Mn, Zn, Pb, Ni and Cu in the soils of the Republic of Serbia', *Agronomy*, 11(1), p. 141. doi: 10.3390/agronomy11010141.

Jovicich, E., Cantliffe, D.J. & Stoffella, P.J. (2003) '"Spanish" pepper trellis system and high plant density can increase fruit yield, fruit quality, and reduce labor in a hydroponic, passive-ventilated greenhouse', *Acta Horticulturae*, 614, pp. 255-262. doi: 10.17660/ActaHortic.2003.614.37.

Kang, S.W., Seo, S.G. & Pak, C.H. (2009) 'Capillary wick width and water level in channel affects water absorption properties of growing media and growth of Chrysanthemum and Poinsettia cultured in C-channel subirrigation system', *Korean Journal of Horticultural Science and Technology*, 27(1), pp. 86-92.

Kano, K., Kitazawa, H., Suzuki, K., Widiastuti, A., Odani, H., Zhou, S., Chinta, Y. D., Eguchi, Y., Shinohara, M. & Sato, T. (2021) 'Effects of organic fertilizer on bok choy growth and quality in hydroponic cultures', *Agronomy*, 11(3), p. 491. doi: 10.3390/agronomy11030491.

Karakaya, S. (2004) 'Bioavailability of phenolic compounds', *Critical Reviews in Food Science and Nutrition*, 44(6), pp. 453-464. doi: 10.1080/10408690490886683.

Kasai, N., Murata, A., Inui, H., Sakamoto, T. & Kahn, R.I. (2004) 'Enzymatic high digestion of soybean milk residue (okara)', *Journal of Agricultural and Food Chemistry*, 52(18), pp. 5709-5716. doi: 10.1021/jf035067v.

Kaur, G. & Asthir, B. (2015) 'Proline: a key player in plant abiotic stress tolerance', *Biologia Plantarum*, 59(4), pp. 609-619. doi: 10.1007/s10535-015-0549-3.

Kaur, N., Chugh, V. & Gupta, A.K. (2014) 'Essential fatty acids as functional components of foods – a review', *Journal of Food Science and Technology*, 51(10), pp. 2289–2303. doi: 10.1007/s13197-012-0677-0.

Quah, Y., Mohd Ismail, N., Ooi, J.L.S., Affendi, Y.A., Abd Manan, F. & Wong, F.-C. (2018) 'Identification of novel cytotoxic peptide KENPVLSLVNGMF from marine sponge Xestospongia testudinaria, with characterization of stability in human serum', *International Journal of Peptide Research and Therapeutics*, 24(1), pp. 189-199. doi: 10.1007/s10989-017-9604-6.

Khan, F. A., Kurklu, A., Ghafoor, A., Ali, Q., Umair, M. & Shahzaib (2018) 'A review on hydroponic greenhouse cultivation for sustainable agriculture', *International Journal of Agriculture, Environment and Food Sciences*, 2(2), pp. 59-66. doi: 10.31015/jaefs.18010.

Khare, S.K., Jha, K. & Gandhi, A.P. (1995) 'Citric acid production from okara (soy-residue) by solid-state fermentation', *Bioresource Technology*, 54(3), pp. 323-325. doi: 10.1016/0960-8524(95)00155-7.

Kielland, K., McFarland, J. & Olson, K. (2006) ‘Amino acid uptake in deciduous and coniferous taiga ecosystems’, *Plant & Soil*, 288, pp. 297-307. doi: 10.1007/s11104-006-9117-0.

Kim, H. J., Cho, Y. S., Kwon, O.-K., Cho, M. W., Hwang, J. B., Bae, S.-D. & Jeon, W.-T. (2005) ‘Effect of pH and EC of hydroponic solution on the growth of greenhouse rose’, *Asian Journal of Plant Sciences*, 4(4), pp. 348-355. doi: 10.3923/ajps.2005.348.355.

Kim, J.A., Jung, W.S., Chun, S.C. & Yu, C.-Y. (2006) ‘A correlation between the level of phenolic compounds and the antioxidant capacity in cooked-with-rice and vegetable soybean (Glycine max L.) varieties’, *European Food Research & Technology*, 224(2), pp. 259-270. doi: 10.1007/s00217-006-0377-y.

Kim, M.J., Moon, Y., Tou, J.C., Mou, B. & Waterland, N.L. (2016) ‘Nutritional value, bioactive compounds and health benefits of lettuce (Lactuca sativa L.)’, *Journal of Food Composition and Analysis*, 49, pp. 19-34. doi: 10.1016/j.jfca.2016.03.004.

Kizhedath, A. & Suneetha, V. (2011) ‘Estimation of chlorophyll content in common household medicinal leaves and their utilization to avail health benefits of chlorophyll’, *Journal of Pharmacy Research*, 4(5), pp. 1412-1413.

Kloubert, V. & Rink, L. (2015) ‘Zinc as a micronutrient and its preventive role of oxidative damage in cells’, *Food & Function*, 6(10), pp. 3195-3204. doi: 10.1039/C5FO00630A.

Kratky, B.A. (1993) ‘A capillary, non-circulating hydroponic method for leaf and semi-head lettuce’, *HortTechnology*, 3(2), pp. 206-207. doi: 10.21273/HORTTECH.3.2.206.

Lakhiar, I.A., Gao, J., Syed, T.N., Chandio, F.A. & Buttar, N.A. (2018) ‘Modern plant cultivation technologies in agriculture under controlled environment: A review on aeroponics’, *Journal of Plant Interactions*, 13(1), pp. 338-352. doi: 10.1080/17429145.2018.1472308.

Lakkireddy, K., Kondapalli, K. & Rao, K.S. (2012) ‘Role of hydroponics and aeroponics in soilless culture in commercial food production’, *Research & Reviews: Journal of Agricultural Science & Technology*, 1(1), pp. 26-35. doi: 10.37591/rrjoast.v1i3.800.

Landrum, J.T. & Bone, R.A. (2001) ‘L-Ascorbic Acid: a multifunctional molecule supporting plant growth and development’, *Scientifica*, 2013, pp. 1-24. doi: 10.1155/2013/795964.

Leal, L. Y. C., Souza, E. R., Júnior, J. A. S. & Santos, M. A. (2020) ‘Comparison of soil and hydroponic cultivation systems for spinach irrigated with brackish water’, *Scientia Horticulturae*, 274, Article 109616, pp. 1–11. doi: 10.1016/j.scienta.2020.109616.

Lee, S. & Lee, J. (2015) ‘Beneficial bacteria and fungi in hydroponic systems: types and characteristics of hydroponic food production methods’, *Scientia Horticulturae*, 195, pp. 206-215. doi: 10.1016/j.scienta.2015.09.011.

Lee, Y.-B., Lee, H.J. & Sohn, H.S. (2005) ‘Soy isoflavones and cognitive function’, *The Journal of Nutritional Biochemistry*, 16(11), pp. 641–649. doi: 10.1016/j.jnutbio.2005.06.010.

Li, B., Qiao, M. & Lu, F. (2012) ‘Composition, nutrition, and utilization of okara (soybean residue)’, *Food Reviews International*, 28(3), pp. 231-252. doi: 10.1080/87559129.2011.595023.

Li, S., Zhu, D., Li, K., Yang, Y., Lei, Z. & Zhang, Z. (2013) ‘Soybean curd residue: composition, utilization, and related limiting factors’, *ISRN Industrial Engineering*, 2013, Article 423590, pp. 1-8. doi: 10.1155/2013/423590.

Li, S., Liu, Z., Li, J., Liu, Z., Gu, X. & Shi, L. (2022) ‘Cow manure compost promotes maize growth and ameliorates soil quality in saline-alkali soil: Role of fertilizer addition rate and application depth’, *Sustainability*, 14(16), Article 10088, pp. 1-18. doi: 10.3390/su141610088.

Lichtenthaler, H.K. & Buschmann, C. (2001) ‘Chlorophylls and carotenoids: measurement and characterization by UV-VIS spectroscopy’, *Current Protocols in Food Analytical Chemistry*, 1(1), pp. F4.3.1–F4.3.8. doi: 10.1002/0471142913.faf0403s01.

Llorach, R., Martínez-Sánchez, A., Tomás-Barberán, F.A., Gil, M.I. & Ferreres, F. (2008) ‘Characterisation of polyphenols and antioxidant properties of five lettuce varieties and escarole’, *Food Chemistry*, 108(3), pp. 1028-1038. doi: 10.1016/j.foodchem.2007.11.032.

Lu, T., Yu, H., Wang, T., Zhang, T., Shi, C. & Jiang, W. (2022) ‘Influence of the electrical conductivity of the nutrient solution in different phenological stages on the growth and yield of cherry tomato’, *Horticulturae*, 8(5), p. 378. doi: 10.3390/horticulturae8050378.

Luna, M.C., Martínez-Sánchez, A., Selma, M.V., Tudela, J.A., Baixauli, C. & Gil, M.I. (2013) ‘Influence of nutrient solutions in an open-field soilless system on the quality characteristics and shelf life of fresh-cut red and green lettuces (Lactuca sativa L.) in different seasons’, *Journal of the Science of Food and Agriculture*, 93(2), pp. 415–421. doi: 10.1002/jsfa.5777.

Luziatelli, F., Ficca, A.G., Colla, G., Svecova, E. & Ruzzi, M. (2016) ‘Effects of a protein hydrolysate-based biostimulant and two micronutrient-based fertilizers on plant growth and epiphytic bacterial population of lettuce’, *Acta Horticulturae*, 1148, pp. 43-48. doi: 10.17660/ActaHortic.2016.1148.5.

Maiani, G., Periago Castón, M.J., Catasta, G. & Toti, E. (2009) ‘Carotenoids: Actual knowledge on food sources, intakes, stability and bioavailability and their protective role in humans', *Molecular Nutrition & Food Research*, 53(Suppl. 2), pp. S194-S218. doi: 10.1002/mnfr.200800053.

Malaguti, M., Dinelli, G., Leoncini, E., Bregola, V., Bosi, S., Cicero, A.F.G. & Hrelia, S. (2014) 'Bioactive peptides in cereals and legumes: agronomical, biochemical and clinical aspects', *International Journal of Molecular Sciences*, 15(11), pp. 21120–21135. doi: 10.3390/ijms151121120.

Malar, S., Sahi, S.V., Favas, P.J.C. & Venkatachalam, P. (2015) 'Mercury heavy-metal-induced physiochemical changes and genotoxic alterations in water hyacinths [*Eichhornia crassipes* (Mart.)]', *Environmental Science and Pollution Research*, 22(6), pp. 4597-4608. doi: 10.1007/s11356-014-3576-2.

Martínez, F., Castillo, S., Carmona, E. & Avilés, M. (2010) 'Dissemination of Phytophthora cactorum, cause of crown rot in strawberry, in open and closed soilless growing systems and the potential for control using slow sand filtration', *Scientia Horticulturae*, 125(4), pp. 756-760. doi: 10.1016/j.scienta.2010.05.010.

Martos, I. E. & Rupérez, P. (2009) 'Indigestible fraction of okara from soybean: composition, physicochemical properties and in vitro fermentability by pure cultures of *Lactobacillus acidophilus* and *Bifidobacterium bifidum*', *European Food Research & Technology*, 228(5), pp. 685-693. doi: 10.1007/s00217-008-0979-7.

Mateos-Aparicio, I., Mateos-Peinado, C., Jiménez-Escrig, A. & Rupérez, P. (2010) 'Multifunctional antioxidant activity of polysaccharide fractions from the soybean by-product okara', *Carbohydrate Polymers*, 82(2), pp. 245-250. doi: 10.1016/j.carbpol.2010.04.020.

Matkowski, A., Zielińska, S., Oszmiański, J. & Lamer-Zarawska, E. (2008) 'Antioxidant activity of extracts from leaves and roots of *Salvia miltiorrhiza* Bunge, *S. przewalskii* Maxim., and *S. verticillata* L.', *Bioresource Technology*, 99(16), pp. 7892-7896. doi: 10.1016/j.biortech.2008.02.013.

Maucieri, C., Nicoletto, C., Junge, R., Schmautz, Z., Sambo, P. & Borin, M. (2018) 'Hydroponic systems and water management in aquaponics: A review', *Italian Journal of Agronomy*, 13(1), pp. 1–11. doi: 10.4081/ija.2017.1012.

McGraw, K.J., Crino, O.L., Medina-Jerez, W. & Nolan, P.M. (2006) 'Effect of dietary carotenoid supplementation on food intake and immune function in a songbird with no carotenoid coloration', *Ethology*, 112(12), pp. 1209-1216. doi: 10.1111/j.1439-0310.2006.01280.x.

McMillan, H. & Small, J. (2016) *Impact of soil acidity on crop yield and management in central western NSW*. [online] Grains Research and Development Corporation. Available at: https://grdc.com.au/resources-and-publications/grdc-update-papers/tab-content/grdc-update-papers/2016/07/soilacidity-crop-yield-impacts-and-management-in-central-western-nsw [Accessed 13 Nov. 2022].

Nikoo, M., Regenstein, J.M. & Yasemi, M. (2023) 'Protein hydrolysates from fishery processing by-products: Production, characteristics, food applications, and challenges', *Foods*, 12(24), p. 4470. doi: 10.3390/foods12244470. [online] Available at: https://doi.org/10.3390/foods12244470

Michalak, A. (2006) 'Phenolic compounds and their antioxidant activity in plants growing under heavy metal stress', *Polish Journal of Environmental Studies*, 15(4), pp. 523-530.

Minocha, R., Martinez, G., Lyons, B. & Long, S. (2009) 'Development of a standardized methodology for quantifying total chlorophyll and carotenoids from foliage of hardwood and conifer tree species', *Canadian Journal of Forest Research*, 39, pp. 849-861.

Moe, L.A. (2013) 'Amino acids in the rhizosphere: from plants to microbes', *American Journal of Botany*, 100(9), pp. 1692-1705.

Mohammed, S.B. & Sookoo, R. (2016) 'Nutrient Film Technique for commercial production', *Agricultural Science Research Journal*, 6(11), pp. 269-274.

Mou, B. (2005) 'Genetic variation of beta-carotene and lutein contents in lettuce', *Journal of the American Society for Horticultural Science*, 130(6), pp. 870-876.

Mukherjee, S. (2018) 'Novel perspectives on the molecular crosstalk mechanisms of serotonin and melatonin in plants', *Plant Physiology and Biochemistry*, 132, pp. 33-45.

Naharul, M.A. (2021) 'Aim for food security, not self-sufficiency', *The Malaysian Reserve*, [online]. Available at: https://themalaysianreserve.com/2021/01/21/aim-for-food-security-not-self-sufficiency/ [Accessed 8 Nov. 2024].

Naik, S., Barman, D., Rampal & Medhi, R. (2013) 'Evaluation of electrical conductivity of the fertilizer solution on growth and flowering of a cymbidium hybrid', *South African Journal of Plant and Soil*, 30(1), pp. 33-39.

Nardi, S., Pizzeghello, D., Schiavon, M. & Ertani, A. (2016) 'Plant biostimulants: physiological responses induced by protein hydrolysate-based products and humic substances in plant metabolism', *Scientia Agricola*, 73(1), pp. 18-23.

Nicolle, C., Rock, E., Rémésy, C., Doyle, M.E. & Demigné, C. (2004) 'Characterisation and variation of antioxidant micronutrients in Bok Choy', *Journal of the Science of Food and Agriculture*, 84(15), pp. 2061-2069.

Noble, J.E. & Bailey, M.J.A. (2009) 'Chapter 8 Quantitation of protein', *Methods in Enzymology*, 463, pp. 73-95.

Nurdiawati, A., Rachman, F., Hakim, A.L., Haryanto, A. & Wicaksono, B. (2019) 'Liquid feather compost as a potential fertilizer to increase growth and yield of patchouli (*Pogostemon cablin* Benth) and mung bean (*Vigna radiata*)', *International Journal of Recycling of Organic Waste in Agriculture*, 8, pp. 221-232.

Ofosu-Anim, J., Blay, E.T. & Frempong, M.E. (2006) 'Effects of organic manure on okra (*Abelmoschus esculentus* (L.) Moench) production', *Journal of Applied Horticulture*, 8(2), pp. 155-158.

Osvald, J., Petrovic, N. & Demsar, J. (2001) 'Sugar and organic acid content of tomato fruits (*Lycopersicon lycopersicum* Mill.) grown on aeroponics at different plant density', *Acta Alimentaria*, 30(1), pp. 53-61.

Othman, A.B. (2013) *Investigation on the potential of orange peel waste in the production of useful homemade solution*. [pdf] Pahang: Universiti Malaysia.

Palande, V., Zaheer, A. & George, K. (2018) 'Fully automated hydroponic system for indoor plant growth', *Procedia Computer Science*, 129, pp. 482-488.

almer, J., Thorburn, P.J., Biggs, J.S., Dominati, E.J., Probert, M.E., Meier, E.A., Huth, N.I., Dodd, M., Snow, V., Larsen, J.R. & Parton, W.J. (2017) 'Nitrogen cycling from increased soil organic carbon contributes both positively and negatively to ecosystem services in wheat agroecosystems', *Frontiers in Plant Science*, 8(713), pp. 1-13.

Paran, E., Novack, V., Engelha, Y.N. & Hazan-Halevy, I. (2009) 'The effects of natural antioxidants from tomato extract in treated but uncontrolled hypertensive patients', *Cardiovascular Drugs and Therapy*, 32(2), pp. 145-151.

Parida, A.K., Das, A.B. & Mittra, B. (2004) 'Effects of salt on growth, ion accumulation, photosynthesis and leaf anatomy of the mangrove, *Bruguiera parviflora*', *Trees*, 18, pp. 167-174.

Pfaltzgraff, L.A., De Bruyn, M., Cooper, E.C., Budarin, V.L., Clark, J.H. & Shuttleworth, P.S. (2013) 'Food waste biomass: a resource for high-value chemicals', *Green Chemistry*, 15(2), pp. 307-314.

Phantong, P., Machikowa, T., Saensouk, P. & Muangsan, N. (2018) 'Comparing growth and physiological responses of *Globba schomburgkii* Hook. f. and *Globba marantina* L. under hydroponic and soil conditions', *Emirates Journal of Food and Agriculture*, 30(2), pp. 157-164.

Pinto, M.C.D. & Gara, L.D. (2004) 'Changes in the ascorbate metabolism of apoplastic and symplastic spaces are associated with cell differentiation', *Journal of Experimental Botany*, 55(408), pp. 2559-2569.

Powers, S.K. & Lennon, S.L. (1999) 'Analysis of cellular responses to free radicals: focus on exercise and skeletal muscle', *Proceedings of the Nutrition Society*, 58(4), pp. 1025-1033.

Puupponen-Pimiä, R., Nohynek, L., Alakomi, H.-L. & Oksman-Caldentey, K.M. (2005) 'Bioactive berry compounds—novel tools against human pathogens', *Applied Microbiology and Biotechnology*, 67(1), pp. 1-18.

Quah, Y., Mohd Ismail, N. I., Ooi, J. L. S., Affendi, Y. A., Abd Manan, F., Wong, F.-C. & Chai, T.-T. (2017) 'Identification of novel cytotoxic peptide KENPVLSLVNGMF from marine sponge *Xestospongia testudinaria*, with characterization of stability in human serum', *International Journal of Peptide Research and Therapeutics*, 24(1), pp. 189-199. doi: 10.1007/s10989-017-9604-6.

Quitain, A.T., Oro, K., Katoh, S. & Moriyoshi, T. (2006) 'Recovery of oil components of okara by ethanol-modified supercritical carbon dioxide extraction', *Bioresource Technology*, 97(13), pp. 1509-1514.

Randhir, R. & Shetty, K. (2003) 'Light-mediated fava bean (*Vicia faba*) response to phytochemical and protein elicitors and consequences on nutraceutical enhancement and seed vigour', *Process Biochemistry*, 38(6), pp. 945-952.

Rasit, N., Lim, H.F. & Ghani, W.A.W.A.K. (2019) 'Production and characterization of eco enzyme produced from tomato and orange wastes and its influence on the aquaculture sludge', *International Journal of Civil Engineering and Technology*, 10(3), pp. 967-980.

Redondo-Cuenca, A., Villanueva-Suárez, M.J. & Mateos-Aparicio, I. (2008) 'Soybean seeds and its by-product okara as sources of dietary fibre: measurement by AOAC and Englyst methods', *Food Chemistry*, 108(3), pp. 1099-1105.

Riemersma, R.A., Rice-Evans, C.A., Tyrrell, R.F., Clifford, M.N. & Lean, M.E.J. (2001) 'Tea flavonoids and cardiovascular health', *QJM: An International Journal of Medicine*, 94(5), pp. 277-282.

Rinaldi, V.E.A., Ng, P.K.W. & Bennink, M.R. (2000) 'Effects of extrusion on dietary fiber and isoflavone contents of wheat extrudates enriched with wet okara', *Cereal Chemistry*, 77(2), pp. 237-240.

Rinawati, M., Sari, L.A. & Pursetyo, K.T. (2020) 'Chlorophyll and carotenoids analysis spectrophotometer', *IOP Conference Series: Earth and Environmental Science*, 441(1), pp. 1-21.

Ritter, E., Riga, P., Angulo, B. & Herrán, C. (2001) 'Comparison of hydroponic and aeroponic cultivation system for the production of potato minitubers', *Potato Research*, 44(2), pp. 127-135.

Rodríguez-Concepción, M. (2010) 'Supply of precursors for carotenoid biosynthesis in plants', *Archives of Biochemistry and Biophysics*, 504(1), pp. 118-122.

Global Food Security: Challenges and Policies by Mark W. Rosegrant & Sarah A. Cline (2003) *Science*, 302(5652), pp. 1917-1919.

Rouphael, Y. & Colla, G. (2005) ‘Growth, yield, fruit quality and nutrient uptake of hydroponically cultivated zucchini squash as affected by irrigation systems and growing seasons’, *Scientia Horticulturae*, 105(2), pp. 177-195.

Rüdiger, W. (2002) ‘Biosynthesis of chlorophyll b and the chlorophyll cycle’, *Photosynthesis Research*, 74(2), pp. 187-193.

Ruiz-Sola, M.Á. & Rodríguez-Concepción, M. (2012) ‘Carotenoid biosynthesis in *Arabidopsis*: a colorful pathway’, *The Arabidopsis Book*, 10, pp. 1-29.

Ruth, A.A., Babatunde, A.W., Oyekunle, O.J. & Rapheal, K.F. (2017) ‘Growth and yield attributes of soybean (*Glycine max* L) in response to cassava peel compost and inorganic fertilizer’, *Research on Crops*, 18(4), p. 618.

Salvia miltiorrhiza Bunge, S. przewalskii Maxim. & S. verticillata L. (2008) ‘Antioxidant activity of extracts from leaves and roots of *Salvia miltiorrhiza* Bunge, *S. przewalskii* Maxim., and *S. verticillata* L.’, *Bioresource Technology*, 99(16), pp. 7892-7896. doi:10.1016/j.biortech.2008.02.013

Calvo, P., Nelson, L. & Kloepper, J.W. (2014) ‘Agricultural uses of plant biostimulants’, *Plant and Soil*, 383, pp. 3-41..

Samanta, A., Das, G. & Das, S.K. (2011) ‘Roles of flavonoids in plants’, *International Journal of Pharmaceutical Science and Technology*, 6(1), pp. 12-35.

Sambo, P., Nicoletto, C., Giro, A., Pii, Y., Valentinuzzi, F., Mimmo, T., Lugli, P., Orzes, G., Mazzetto, F., Astolfi, S., Terzano, R. & Cesco, S. (2019) ‘Hydroponic solutions for soilless production systems: issues and opportunities in a smart agriculture perspective’, *Frontiers in Plant Science*, 10(923), pp. 1-17.

Sankhalkar, S., Komarpant, R., Dessai, T.R., Simoes, J. & Sharma, S. (2019) ‘Effects of soil and soil-less culture on morphology, physiology and biochemical studies of vegetable plants’, *Current Agriculture Research Journal*, 7(2), pp. 181-188. doi: 10.12944/CARJ.7.2.06.

Sardare, M.D. & Admane, S.V. (2013) ‘A review on plant without soil – hydroponics’, *International Journal of Research in Engineering and Technology*, 2(3), pp. 299-304.

Sasidharan, S., Chen, Y., Saravanan, D., Sundram, K. M. & Yoga Latha, L. (2011) ‘Extraction, isolation and characterization of bioactive compounds from plants’ extracts’, *African Journal of Traditional, Complementary and Alternative Medicines*, 8(1), pp. 1-10. doi: 10.4314/ajtcam.v8i1.60483.

Savvas, D. (2003) ‘Hydroponics: a modern technology supporting the application of integrated crop management in greenhouses’, *Journal of Food, Agriculture and Environment*, 1(1), pp. 80-86.

Scartezzini, P. & Speroni, E. (2000) ‘Review on some plants of Indian traditional medicine with antioxidant activity’, *Journal of Ethnopharmacology*, 71(1-2), pp. 23-43.

Scott, J., Rébeillé, F. & Fletcher, J. (2000) ‘Folic acid and folates: the feasibility for nutritional enhancement in plant foods’, *Journal of Science of Food and Agriculture*, 80(7), pp. 795-824.

Seeram, N.P., Lee, R., Hardy, M. L. & Heber, D. (2006) ‘Blackberry, black raspberry, blueberry, cranberry, red raspberry, and strawberry extracts inhibit growth and stimulate apoptosis of human cancer cells in vitro’, *Journal of Agricultural and Food Chemistry*, 54(25), pp. 9329-9339.

Semananda, N.P.K., Ward, J.D. & Myers, B.R. (2018) ‘A semi-systematic review of capillary irrigation: The benefits, limitations, and opportunities’, *Horticulturae*, 4(23), pp. 1-15.

Sen, C.K. (2001) ‘Antioxidant and redox regulation of cellular signaling: introduction’, *Medicine and Science in Sports and Exercise*, 33(3), pp. 368-370.

Sgherri, C., Quartacci, M.F., D’Abrosca, B., Ranieri, A., Navari-Izzo, F. & Izzo, R. (2010) ‘Levels of antioxidants and nutraceuticals in basil grown in hydroponics and soil’, *Food Chemistry*, 123(2), pp. 416-422.

Sharma, N., Kumar, V., Singh, S., Meena, R. & Verma, A. (2018) ‘Hydroponics as an advanced technique for vegetable production: An overview’, *Journal of Soil and Water Conservation*, 17(4), pp. 364-371.

Shehrawat, P., Sindhu, N. & Singh, B. (2015) ‘Agricultural waste utilization for healthy environment and sustainable lifestyle’, *Annals of Agri Bio Research*, 20(1), pp. 110-114.

Sheikh, B. A. (2006) ‘Hydroponics: Key to sustain agriculture in water stressed and urban environment’, *Pakistan Journal of Agriculture, Agricultural Engineering and Veterinary Sciences*, 22(2), pp. 53-57.

Shetty, K. & McCue, P. (2003) ‘Phenolic antioxidant biosynthesis in plants for functional food application: integration of systems biology and biotechnological approaches’, *Food Biotechnology*, 17(2), pp. 67-97.

Shetty, K., 2004. Role of proline-linked pentose phosphate pathway in biosynthesis of plant phenolics for functional food and environmental applications: a review. *Process Biochemistry*, 39(7), pp.789-804.

Shohag, M. J. I., Wei, Y. & Yang, X., 2012. Changes of folate and other potential health-promoting phytochemicals in legume seeds as affected by germination. *Journal of Agricultural and Food Chemistry*, 60(36), pp.9137-9143.

Shoily, S. J., 2021. Soil Reaction: Soil pH, sources of H^+ & OH^- ions. [online] Available at: https://plantlet.org/soil-reaction-soil-ph-sources-of-h-oh-ions/ [Accessed 14 November 2024].

Shukitt-Hale, B., Cheng, V. & Joseph, J. A., 2009. Effects of blackberries on motor and cognitive function in aged rats. Nutritional Neuroscience, 12(3), pp. 135-140.

Silberbush, M. & Ben-Asher, J., 2001. Simulation study of nutrient uptake by plants from soilless cultures as affected by salinity build-up and transpiration. Plant and Soil, 233(1), pp. 59-69.

Silva, L., Valdés-Lozanoa, D., Escalante, E. & Gasca-Leyva, E., 2018. Dynamic root floating technique: An option to reduce electric power consumption in aquaponic systems. Journal of Cleaner Production, 183, pp. 132-142.

Silva, R. R. d., 2017. Bacterial and fungal proteolytic enzymes: production, catalysis and potential applications. Applied Biochemistry and Biotechnology, 183, pp. 1-19.

Smid, E. J. & Lacroix, C., 2013. Microbe-microbe interactions in mixed culture food fermentations. Current Opinion in Biotechnology, 24(2), pp. 148-154.

Smirnoff, N. & Wheeler, G. L., 2000. Ascorbic acid in plants: biosynthesis and function. Critical Reviews in Biochemistry and Molecular Biology, 35(4), pp. 291-314.

Soetan, K. O., Olaiya, C. O. & Oyewole, O. E., 2010. The importance of mineral elements for humans, domestic animals and plants: a review. African Journal of Food Science, 4(5), pp. 200-222.

Son, J. E., Jeong, B. R., Lee, S. K., Park, K. H., Kim, S. K. & Kim, H. S., 2006. Nutrient-flow wick culture system for potted plant production: System characteristics and plant growth. Scientia Horticulturae, 107(4), pp. 392-398.

Song, W., Zhang, C., Chen, S., Li, X., & Wang, Y., 2004. Tomato Fusarium wilt and its chemical control strategies in a hydroponic system. Crop Protection, 23(3), pp. 243-247.

Spinu, V., Langhans, R. & Albright, L., 1998. Electrochemical pH control in hydroponic systems. Acta Horticulturae, 456(32), pp. 275-282.

Stone, D., 2017. The citrus family tree. Available at: https://www.nationalgeographic.com/magazine/article/explore-food-citrusgenetics [Accessed 27 November 2022].

Su, S., Liu, X., Zhang, Q., Wang, Y., Li, H., 2010. Optimization of the method for chlorophyll extraction in aquatic plants. Journal of Freshwater Ecology, 25(4), 531-538.

Ferrarezi, R.S., Weaver, G.M., van Iersel, M.W. & Testezlaf, R. (2015) ‘Subirrigation: Historical overview, challenges, and future prospects’, *HortTechnology*, 25(3), pp. 262-276. doi: 10.21273/HORTTECH.25.3.262.

Suud, H.M., Syuaib, M.F. & Astika, I.W., 2016. ‘Development of a model for estimating soil nutrient levels by measuring the conductivity of soil electricity’, *Jurnal Keteknikan Pertanian*, 3(2), pp. 35-41.

Tadesse, S. & Assefa, S., 2019. ‘The principal role of organic fertilizer on soil properties and agricultural productivity: a review’, *Agri Res & Tech*, 22(2), pp. 46-50.

Tanaka, A. & Tanaka, R., 2006. ‘Chlorophyll metabolism’, *Current Opinion in Plant Biology*, 9(3), pp. 248-255.

Teixeira, W. F., de Oliveira, R. T., de Souza, R. A., da Silva, F. A., de Oliveira, J. F., & da Silva, J. A. T., 2017. ‘Foliar and seed application of amino acids affects the antioxidant metabolism of the soybean crop’, *Frontiers in Plant Science*, 8(327), pp. 1-14.

Teng, H., Chen, L., Wang, X., Zhang, Y., & Li, J., 2019. ‘Inhibitory effect of the extract from *Sonchus oleraceus* on the formation of carcinogenic heterocyclic aromatic amines during pork cooking’, *Food and Chemical Toxicology*, 129, pp. 138-143.

Umayaparvathi, S., Vinayagam, V., Muthuvel, A. & Saravanan, M., 2014. ‘Antioxidant activity and anticancer effect of bioactive peptide from oyster compost (*Saccostrea cucullata*)’, *Biomedicine and Preventive Nutrition*, 4(3), pp. 343-353.

Vakili, M., Haque, A. A. M. & Gholami, Z., 2012. ‘Effect of manual turning frequency on physico-chemical parameters during the oil palm frond and cow dung composting’, *Caspian Journal of Applied Sciences Research*, 1(2), pp. 49-59.

Vakili, M., Rafatullah, M., Ibrahim, M. H., Salamatinia, B., Gholami, Z. & Zwain, H. M., 2014. ‘A review on composting of oil palm biomass’, *Environment, Development and Sustainability*, 17(4), pp. 691-709.

Valentinuzzi, F., Zuchi, S., Parolini, M., Pii, Y., Vigani, G., Miras-Moreno, B., Lucini, L., Vamerali, T., Pinton, R. & Cesco, S., 2015. ‘Phosphorus and iron deficiencies induce a metabolic reprogramming and affect the exudation traits of the woody plant *Fragaria ananassa*’, *Journal of Experimental Botany*, 66(20), pp. 6483-6495.

Vasconcelos, A. C. F. de, Zhang, X., Ervin, E. H. & Kiehl, J. de C., 2009. ‘Enzymatic antioxidant responses to biostimulants in maize and soybean subjected to drought’, *Scientia Agricola*, 66(3), pp. 395-402.

Verdoliva, S.G., Gwyn-Jones, D., Detheridge, A. & Robson, P. (2021) 'Controlled comparisons between soil and hydroponic systems reveal increased water use efficiency and higher lycopene and β-carotene contents in hydroponically grown tomatoes', *Scientia Horticulturae*, 279, Article 109896. doi:10.1016/j.scienta.2021.109896.

Verma, V. & Sanjay, M.S.S. (2020) 'Hydroponics: A step toward food security', *Science for Agriculture and Allied Sector*, 2(1), pp. 25-31.

Vicaş, S.I., Laslo, V., Pantea, S. & Bandici, G.E. (2010) 'Chlorophyll and carotenoids pigments from Mistletoe (Viscum album) leaves using different solvents', *Analele Universităţii din Oradea – Fascicula Biologie*, 17(2), pp. 213-218.

Cazzonelli, C.I. & Pogson, B.J. (2010) 'Source to sink: regulation of carotenoid biosynthesis in plants', *Trends in Plant Science*, 15(5), pp. 266-274.

Vimala, T. & Poonghuzhali, T.V. (2015) 'Estimation of pigments from seaweeds by using acetone and DMSO', *International Journal of Science and Research (IJSR)*, 4(10), pp. 1850-1854.

Vishwanathan, K.H., Singh, V. & Subramanian, R. (2011) 'Influence of particle size on protein extractability from soybean and okara', *Journal of Food Engineering*, 103(3), pp. 240-246.

Wang, C., Riedl, K.M. & Schwartz, S.J. (2013) 'Fate of folates during vegetable juice processing: deglutamylation and interconversion', *Food Research International*, 53(1), pp. 440-448.

Wang, H.L. & Cavins, J.F. (1989) 'Yield and amino acid composition of fractions obtained during tofu production', *Cereal Chemistry (USA)*, 66(5), pp. 359-361.

Wilson, H.T., Amirkhani, M. & Taylor, A.G. (2018) 'Evaluation of gelatin as a biostimulant seed treatment to improve plant performance', *Frontiers in Plant Science*, 9(1006), pp. 1-11.

Wisniewska, A. & Subczynski, W.K. (2006) 'Accumulation of macular xanthophylls in unsaturated membrane domains', *Free Radical Biology & Medicine*, 40(10), pp. 1820-1826.

Hao, X., Jia, J., Mi, J., Yang, S., Khattak, A.M., Zheng, L., Gao, W. & Wang, M. (2020) 'An optimization model of light intensity and nitrogen concentration coupled with yield and quality', *Plant Growth Regulation*, 92(2), pp. 319-331. doi: 10.1007/s10725-020-00641-0.

Xiao, Z., Lester, G. E., Luo, Y. & Wang, Q. (2012) 'Assessment of vitamin and carotenoid concentrations of emerging food products: edible microgreens', *Journal of Agricultural and Food Chemistry*, 60(31), pp. 7644–7651.

Xu, L. & Geelen, D. (2018) 'Developing biostimulants from agro-food and industrial by-products', *Frontiers in Plant Science*, 9, pp. 1–13.

Yakhin, O. I., Lubyanov, A. A., Yakhin, I. A. & Brown, P. H. (2017) 'Biostimulants in plant science: a global perspective', *Frontiers in Plant Science*, 7, pp. 1–32.

Yao, B., Fang, H., Xu, W., Yan, Y., Xu, H., Liu, Y. & Gong, S. (2014) 'Dietary fiber intake and risk of type 2 diabetes: a dose-response analysis of prospective studies', *European Journal of Epidemiology*, 29(2), pp. 79–88.

Yuan, L., Shi, B., Li, Y., Wu, C., & Wang, C., 2017. 'Fermented soybean meal improves the growth performance, nutrient digestibility, and microbial flora in piglets', *Animal Nutrition*, 3(1), pp. 19–24.

YuanShan, Z., SiJia, G., JinJin, C. & XiaoDong, C., 2019. 'Effects of different nutrient solutions on the acclimatisation of in vitro caladium plantlets using a simplified hydroponic system', *Sains Malaysiana*, 48(8), pp. 1627–1633.

Za, W. L., 2020. *Four Malaysian engineers believe vertical farming offers answer to food sustainability* [Interview], 6 March 2020.

Zhao, X., Qian, S., Jiang, L., Wang, Y. & Li, J., 2007. 'Influences of organic fertilization, high tunnel environment, and postharvest storage on phenolic compounds in lettuce', *HortScience*, 42(1), pp. 71–76.

Zou, Y., Lu, Y. & Wei, D., 2004. 'Antioxidant activity of a flavonoid-rich extract of Hypericum perforatum L. in vitro', *Journal of Agricultural and Food Chemistry*, 52(16), pp. 5032–5039.

Appendices

	Treatment (mg/mL)	0	2000	4000	6000	8000
Shoot	Length (cm)	6.22	11.28	13.77	16.37	15.20
		7.04	10.19	13.98	16.24	15.09
		5.87	11.00	13.34	16.10	15.76
		6.98	10.57	13.45	15.56	15.98
		6.93	10.99	14.66	15.97	15.31
	Mean	6.61	10.81	13.84	16.05	15.47
	Standard Error	0.21	0.17	0.21	0.12	0.15

	Mean ± Standard Error	**6.61 ± 0.21**	**10.81 ± 0.17**	**13.84 ± 0.21**	**16.05 ± 0.12**	**15.47 ± 0.15**
	Weight (g)	2.27	3.73	4.63	5.61	5.03
		2.24	3.46	4.54	5.34	4.95
		2.25	3.71	4.53	5.38	5.08
		2.31	3.57	4.59	5.48	4.99
		2.30	3.34	4.49	5.43	5.12
	Mean	2.27	3.56	4.56	5.45	5.03
	Standard Error	0.01	0.07	0.02	0.04	0.03
	Mean ± Standard Error	**2.27 ± 0.01**	**3.56 ± 0.07**	**4.56 ± 0.02**	**5.45 ± 0.04**	**5.12 ± 0.03**
Root	Length (cm)	5.97	9.39	12.05	14.45	13.36
		5.18	9.16	11.65	14.07	13.24
		5.32	9.31	11.95	13.94	12.80
		5.39	8.23	11.56	15.20	13.13
		5.04	9.00	11.50	14.81	13.47
	Mean	5.38	9.02	11.74	14.49	13.20
	Standard Error	0.14	0.19	0.10	0.21	0.10
	Mean ± Standard Error	**5.38 ± 0.14**	**9.02 ± 0.19**	**11.74 ± 0.10**	**14.49 ± 0.21**	**13.20 ± 0.10**
	Weight (g)	0.46	0.70	0.85	1.28	1.23
		0.39	0.69	0.89	1.31	1.18
		0.44	0.73	0.92	1.32	1.19
		0.43	0.67	0.90	1.29	1.22
		0.46	0.73	0.93	1.34	1.17
	Mean	0.44	0.70	0.90	1.31	1.20
	Standard Error	0.012	0.010	0.012	0.010	0.010
	Mean ± Standard Error	**0.46 ± 0.011**	**0.70 ± 0.010**	**0.90 ± 0.012**	**1.31 ± 0.010**	**1.20 ± 0.010**

Treatment (mg/mL)	0	2000	4000	6000	8000
Leaves Number	4	6	8	9	8
	5	7	9	10	9
	4	6	9	10	9

	5	6	8	10	10
	5	7	7	10	9
Mean	4.6	6.4	8.2	9.8	9.0
Standard Error	0.22	0.23	0.33	0.18	0.28
Mean ± Standard Error	**4.6 ± 0.2**	**6.4 ± 0.2**	**8.2 ± 0.3**	**9.8 ± 0.2**	**9.0 ± 0.3**
Leaves Surface Area	13.46	19.00	24.73	34.28	28.73
	13.34	18.21	26.02	33.70	29.15
	14.04	18.06	24.94	35.45	28.94
	13.57	18.84	25.59	34.87	29.59
	13.92	18.37	25.16	34.57	29.16
Mean	13.67	18.50	25.29	34.57	29.11
Standard Error	0.120	0.162	0.207	0.261	0.128
Mean ± Standard Error	**13.67 ± 0.12**	**18.50 ± 0.16**	**25.29 ± 0.21**	**34.57 ± 0.26**	**29.16 ± 0.13**

Treatment (mg/mL)	0	2000	4000	6000	8000
OD(661.6)	0.128	0.337	0.433	0.521	0.468
	0.134	0.329	0.439	0.533	0.482
	0.139	0.333	0.444	0.540	0.476
	0.137	0.326	0.426	0.535	0.477
	0.134	0.331	0.441	0.541	0.491
Mean	0.134	0.331	0.437	0.534	0.479
Standard Error	0.002	0.002	0.003	0.003	0.003
Mean ± Standard Error	**0.134 ± 0.002**	**0.331 ± 0.002**	**0.437 ± 0.003**	**0.541 ± 0.003**	**0.479 ± 0.003**
OD(644.8)	0.113	0.247	0.350	0.441	0.375
	0.111	0.251	0.352	0.452	0.388
	0.110	0.244	0.367	0.456	0.386

	0.104	0.253	0.369	0.458	0.377
	0.108	0.249	0.359	0.438	0.387
Mean	0.109	0.249	0.359	0.449	0.383
Standard Error	0.001	0.001	0.003	0.004	0.002
Mean ± Standard Error	**0.109 ± 0.001**	**0.249 ± 0.001**	**0.359 ± 0.003**	**0.438 ± 0.004**	**0.387 ± 0.002**
OD(470.0)	0.198	0.341	0.451	0.574	0.494
	0.189	0.342	0.462	0.573	0.499
	0.195	0.339	0.467	0.565	0.502
	0.199	0.341	0.455	0.564	0.506
	0.187	0.343	0.475	0.573	0.491
Mean	0.194	0.341	0.462	0.570	0.498
Standard Error	0.002	0.001	0.004	0.002	0.002
Mean ± Standard Error	**0.194 ± 0.002**	**0.341 ± 0.001**	**0.462 ± 0.002**	**0.570 ± 0.002**	**0.498 ± 0.002**
Chlorophyll A (µg/ml)	1.208	3.284	4.153	4.956	4.495
	1.280	3.186	4.216	5.069	4.626
	1.338	3.245	4.242	5.139	4.563
	1.328	3.148	4.035	5.079	4.592
	1.286	3.212	4.224	5.187	4.729
Mean	1.288	3.215	4.174	5.086	4.601
Standard Error	0.030	0.030	0.049	0.050	0.050
Mean ± Standard Error	**1.288 ± 0.030**	**3.215 ± 0.030**	**4.174 ± 0.049**	**5.086 ± 0.050**	**4.601 ± 0.050**
Chlorophyll B (µg/ml)	1.738	3.560	5.231	6.694	5.588
	1.673	3.674	5.246	6.865	5.791
	1.632	3.516	5.527	6.917	5.776
	1.519	3.727	5.643	6.978	5.590
	1.613	3.625	5.379	6.550	5.733
Mean	1.635	3.621	5.405	6.801	5.696

Standard Error	0.047	0.049	0.103	0.101	0.057
Mean ± Standard Error	**1.635 ± 0.047**	**3.621 ± 0.049**	**5.405 ± 0.103**	**6.801 ± 0.101**	**5.696 ± 0.057**
Carotenoids (µg/ml)	0.4016	0.5139	0.5271	0.6631	0.6198
	0.3782	0.4858	0.5735	0.6069	0.5821
	0.4179	0.5178	0.5138	0.5538	0.6012
	0.4698	0.4659	0.4254	0.5316	0.6743
	0.3866	0.5046	0.5951	0.6989	0.5609
Mean	0.4108	0.4976	0.5270	0.6109	0.6077
Standard Error	0.0209	0.0125	0.0380	0.0409	0.0249
Mean ± Standard Error	**0.4108 ± 0.0209**	**0.4976 ± 0.0125**	**0.5270 ± 0.0380**	**0.6109 ± 0.0409**	**0.6077 ± 0.0249**

Treatment (mg/mL)	0	2000	4000	6000	8000
OD(765)	0.360	0.673	0.791	0.898	0.854
	0.364	0.684	0.785	0.904	0.861
	0.367	0.671	0.781	0.905	0.857
	0.378	0.68	0.775	0.900	0.850
	0.368	0.677	0.779	0.920	0.848
Mean	0.367	0.677	0.782	0.905	0.854
Standard Error	0.003	0.002	0.003	0.004	0.002
Mean ± Standard Error	**0.367 ± 0.003**	**0.677 ± 0.002**	**0.782 ± 0.003**	**0.905 ± 0.004**	**0.848 ± 0.002**
OD(510)	0.277	0.351	0.451	0.512	0.498
	0.252	0.346	0.446	0.527	0.481
	0.268	0.356	0.456	0.513	0.479
	0.278	0.348	0.448	0.521	0.488
	0.279	0.352	0.452	0.51	0.489
Mean	0.271	0.351	0.451	0.517	0.487

Standard Error	0.005	0.002	0.002	0.003	0.003
Mean ± Standard Error	**0.271 ± 0.005**	**0.351 ± 0.002**	**0.451 ± 0.002**	**0.517 ± 0.003**	**0.487 ± 0.003**
TPC	0.382	0.714	0.840	0.953	0.907
	0.386	0.726	0.833	0.960	0.914
	0.390	0.712	0.829	0.961	0.910
	0.401	0.722	0.823	0.955	0.902
	0.391	0.719	0.827	0.977	0.900
Mean	0.390	0.719	0.830	0.961	0.907
Standard Error	0.003	0.002	0.003	0.004	0.002
Mean ± Standard Error	**0.390 ± 0.003**	**0.719 ± 0.002**	**0.830 ± 0.002**	**0.961 ± 0.004**	**0.907 ± 0.002**
TFC	0.651	0.825	1.059	1.203	1.170
	0.592	0.813	1.048	1.238	1.130
	0.630	0.836	1.071	1.205	1.125
	0.653	0.817	1.052	1.224	1.146
	0.655	0.827	1.062	1.198	1.149
Mean	0.624	0.825	1.059	1.215	1.142
Standard Error	0.017	0.007	0.007	0.011	0.014
Mean ± Standard Error	**0.624 ± 0.017**	**0.825 ± 0.007**	**1.059 ± 0.007**	**1.215 ± 0.011**	**1.142 ± 0.014**

Treatment (mg/mL)	0	2000	4000	6000	8000
OD(517)	0.133	0.128	0.123	0.120	0.122
	0.132	0.129	0.124	0.118	0.119
	0.135	0.131	0.122	0.121	0.121
	0.130	0.127	0.118	0.122	0.121
	0.136	0.128	0.120	0.119	0.118
Mean	0.133	0.129	0.121	0.120	0.120

Standard Error	0.001	0.001	0.001	0.001	0.001
Mean ± Standard Error	**0.133 ± 0.001**	**0.127 ± 0.001**	**0.121 ± 0.001**	**0.129 ± 0.001**	**0.132 ± 0.001**
OD(754)	0.563	0.485	0.471	0.448	0.456
	0.565	0.483	0.465	0.451	0.455
	0.567	0.486	0.466	0.452	0.453
	0.566	0.488	0.468	0.450	0.452
	0.561	0.480	0.474	0.449	0.451
Mean	0.564	0.484	0.469	0.450	0.453
Standard Error	0.001	0.001	0.002	0.001	0.001
Mean ± Standard Error	**0.564 ± 0.001**	**0.484 ± 0.001**	**0.469 ± 0.002**	**0.450 ± 0.001**	**0.453 ± 0.001**
Treatment (mg/mL)	0	2000	4000	6000	8000
DPPH	20.83	23.81	26.79	28.57	27.38
	20.00	21.82	24.85	28.48	27.88
	18.67	21.08	26.51	27.11	27.11
	22.16	23.95	29.34	26.95	27.54
	19.05	23.81	28.57	29.17	29.76
Mean	20.14	22.89	27.21	28.06	27.93
Standard Error	0.63	0.60	0.80	0.44	0.47
Mean ± Standard Error	**20.14 ± 0.63**	**22.89 ± 0.60**	**27.21 ± 0.80**	**28.06 ± 0.44**	**27.93 ± 0.47**
ABTS	19.57	30.71	32.71	36.00	34.86
	19.52	31.20	33.76	35.75	35.19
	19.35	30.87	33.71	35.70	35.56
	19.37	30.48	33.33	35.90	35.61
	20.20	31.72	32.57	36.13	35.85
Mean	19.60	31.00	33.22	35.90	35.41
Standard Error	0.16	0.21	0.25	0.08	0.17

Mean ± Standard Error	**19.60 ± 0.16**	**31.00 ± 0.21**	**33.22 ± 0.25**	**35.90 ± 0.08**	**35.41 ± 0.17**

Figure 1: Picture of PH treated Bok Choy.