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Analysis of Bioactive Components of Pakcoy Microgreens (*Brassica rapa* L.) on Variations of Planting Media

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ABSTRACT

Microgreens are new foods with bioactive compounds that are beneficial for human health. Microgreens *Brassica rapa* L. are small-scale, naturally occurring vegetable crops that possess a high antioxidant value and natural nutrient density.The profile of bioactive compounds is influenced by variations in growing media on microgreens *Brassica rapa* L. including rice husk charcoal, cocopeat, and top soil.The purpose of this study was to analyze the increase in flavonoid, chlorophyll and carotenoid compounds in several variations of growing media: This research was conducted using a Non-Factorial Randomized Group Design (RAK) with three treatment levels, namely top soil, top soil + rice husk charcoal, top soil + cocopeat with five replications. Parameters measured were flavonoid compounds, chlorophyll a b, and carotenoids. Results: the analysis showed that the best planting media were rice husk charcoal, cocopeat, and top soil, respectively had a significant effect on the increase in Chlorophyll (42.12 mg L-1) and Carotenoids (10.63 µmol/L) Flavonoids (19.19 µmol g-1). Conclusion: The best recommended planting medium is rice husk charcoal which shows a significant effect on increasing Chlorophyll (42.12 mg L-1) Carotenoids (10.63 µmol/L) and cocopeat which shows a significant effect on increasing flavonoid content (19.19 µmol g-1).

Keywords: *Bioactive, Growing Media, Microgreen, Planting Media, Treatment Levels*

1. INTRODUCTION

Microgreens are sprout-like plants that grow longer than sprouts. According to Luang-In et al., (2021) microgreens are plants from the vegetable or herbal group that can be harvested 7-14 days after planting when the cotyledons have appeared so that the nutritional content of microgreens is high. when the cotyledons have appeared so the nutritional content of the microgreens is high. Consuming microgreens is very beneficial for the body because it has 4 - 40 times the amount of nutrients and vitamins of mature plants with mineral and antioxidant content (Valupi et al., 2021). Therefore microgreens can be an alternative vegetable that will help reduce global food insecurity, and the resulting complications such as malnutrition (Abaajeh et al., 2023). *Brassica rapa* L. contains flavonoid, chlorophyll and carotenoid compounds. Chlorophyll and carotenoids function as antidotes to degenerative diseases and cancer (Dewi et al., 2023). Flavonoids function as antioxidants that protect the body from free radicals. Flavonoids are widely used as anticancer, antimicrobial, antiviral, antioxidant, neuroprotective and antiproliferative agents (Ullah et al., 2020). The nutritional content per 100 grams of *Brassica rapa* L includes 2.30 grams of protein, 0.030 grams of fat, 4 grams of carbohydrates, 220.50 mg calcium, 38.40 mg phosphorus, 2.90 mg iron, 6.4 vitamin A, 0.009 vitamin B, 102 mg vitamin C (Sanif et al., 2017). Utilizing small areas of land such as house yards is one of the breakthroughs in community activities to improve body nutrition and income. Using the right planting medium can provide optimal growth for mustard greens. The composition of the planting medium can also influence growth and increase the bioactive content of the green vegetable *Brassica rapa* L. Microgreens can be planted with various planting media mixtures such as husk charcoal, cocopeat and egg shells. Husk charcoal has good organic material for maintaining soil moisture because when added to the soil it can bind water and then release it into the micro pores to be absorbed by plants. According to Marjenah et al., (2016) husk charcoal has a large surface area that can absorb nutrients and encourage the growth of good microorganisms so that it can significantly increase plant growth. According to Nehru et al., (2021) rice husk charcoal is a mixture of planting media that can bind water and nutrients so that it is used to fertilize plants because of its fragile nature and structure and contains 0.32% N, 15% P2O, 31% K2O, 0.95% Ca, and 180 ppm Fe, 80 ppm Mn, 14.1 ppm Zn and pH 6.8. Cocopeat is good for plants because cocopeat is able to bind and store water strongly and contains nutrients such as phosphorus. According to Ramadhan (2017) and Gbollie et al., (2021) and Gbollie et al. (2022), cocopeat is a planting medium obtained from the process of crushing coconut fiber which produces fiber. The advantage of cocopeat as a planting medium is that it can bind and store water strongly, and contains nutrients such as calcium (Ca), magnesium (Mg), potassium (K), sodium (Na) and phosphorus (P) as well as electrical conductivity (DHL).Flavonoids are included in the group of secondary plant metabolites which have a polyphenylic structure and are found in many fruits and vegetables. According to Arbiyani et al., (2023) and Noer et al., (2018) flavonoids are secondary metabolite compounds of the polyphenol group which come from green plant extracts and have active effects such as anti-viral, anti-inflammatory, cardioprotective, anti-diabetic, anti-cancer and antioxidant.Chlorophyll is a pigment found in plants with many functions for plant life processes by converting light energy into chemical energy. In chloroplasts there is not only chlorophyll which is the substance that causes the green color of leaves, but there are also other color pigments, namely

carotenoids, phycocyanin, phycoerythrin and fucoxanthin. According to Dharmadewi (2020); Mehdipoor et al., 2021 and Martin et al., 2023, the role of chlorophyll in the body is as an antioxidant. Therefore, chlorophyll is currently widely extracted and consumed as a food supplement. Carotenoids are natural dyes that come from plants. Carotenoids give foods yellow, orange and red colors. There are several types of carotenoids, namely astasantin, fucoxanthin, beta carotene, lycopene, lutein, and others. According to Maleta et al., (2018); Ko, EY, Lee et al., 2023 and Suttisansanee et al., 2023, carotenoids function as antioxidants that can protect the body from free radicals. Carotenoids are found in many fruits and vegetables and have potential anti-cancer activity.

2. MATERIAL AND METHODS

Brassica rapa L seeds, Nauli F1 variety, top soil, cocopeat, husk charcoal, egg shell, bamboo, plastic rope, water, 96% ethanol, PA methanol, Sodium Acetate, AlCl3, Quarsetin, Aquadest, aluminum foil, filter paper, and materials other supporters.

The tools used are a hoe to clean the yard that will be used for research, a seed pot measuring 30 x 21 x 4 cm is used as a planting container, a ruler is used to measure planting distances, a protective net is used to cover the plant area so that pests do not damage the plants, gembor is used to water the plants, plastic clips are used to contain the plants after harvest, analytical scales are used to weigh the samples, writing instruments are used to take notes, blenders are used to grind the samples, 20 cc and 100 cc urine pots are used to hold the extracted samples, beaker glass used to store sample solutions, measuring cups, test tubes, 10 ml and 5 ml measuring flasks, dropper pipettes, micropipettes used to take solutions, vortexes used to homogenize solutions, watch glasses used to weigh sample extracts, evaporator cups, water baths, ovens , spatula, UV-Vis spectrophotometer to measure absorbance values, and other supporting materials.

This research used a non-factorial randomized block design (RAK), namely: top soil, top soil + charcoal husk (1:1), top soil + cocopeat (1:1) with five replications. Research Implementation: 15 seed trays were prepared which were filled with each planting medium. Then 300 seeds are provided and soaked for 8 hours per tray. Plant maintenance is carried out by watering twice a day which is adjusted to environmental conditions. Harvesting is carried out after the plants are 10 HST and then the flavonoid, chlorophyll and carotenoid content will be tested in the laboratory. Flavonoid Compound Content: Total flavonoid content analysis is a measurement of the total flavonoid content contained in the sample. The method used is colorimetry and UV-Vis spectrophotometry and the reagent used is AlCl3. Preparation of Quercetin Standard Stock Solution: Weigh 10 mg of quercetin, dissolve it with methanol to obtain a volume of 100 mL to obtain a quercetin solution with a concentration of 100 ppm. Determination of the Maximum Wavelength of Quercetin: Pipette 2 mL of 100 ppm quercetin standard stock solution, add 0.1 mL of AlCl3 and 0.1 mL of CH3COONa and 3 mL of distilled water, then incubate for 25 minutes. The maximum wavelength was measured using a UV-Vis spectrophotometer in the range 400 nm - 800 nm. Determination of the Maximum Wavelength of Quercetin. Pipette 2 mL of 100 ppm quercetin standard stock solution, add 0.1 mL AlCl3 and 0.1 mL CH3COONa and 3 mL distilled water, then incubate for 25 minutes. The maximum wavelength was measured using a UV-Vis spectrophotometer in the range 400 nm - 800 nm. Determination of Operation Time: 2 ml of quercetin solution with a concentration of 100 µg/ml is pipetted, put into a 5 ml volumetric flask. Added 0.1 ml of 10% aluminum chloride solution,

0.1 ml of 1 M sodium acetate and 3 ml of distilled water. Measure the absorbance of the solution at a wavelength of 440 nm every 1 minute and observe the time the solution begins to produce a stable absorbance, which will be used as the operating time. Pipette 0.5 mL of quercetin standard solution each; 0.975 mL; 1.45 mL; 1.925 and 2.4 mL and put into each 5 mL measuring flask then add methanol to obtain a solution with a concentration of 10 ppm; 19.5ppm; 29ppm; 38.5ppm; and 48 ppm. Pipet 2 mL of each concentration and add 0.1 mL of AlCl3 and 0.1 mL of CH3COONa as well as 3 mL of distilled water, then incubate for 25 minutes. The absorbance of each concentration was measured by UV-Vis spectrophotometry at a maximum wavelength of 440 nm. The quercetin calibration curve and linear regression equation y = ax + b were obtained.

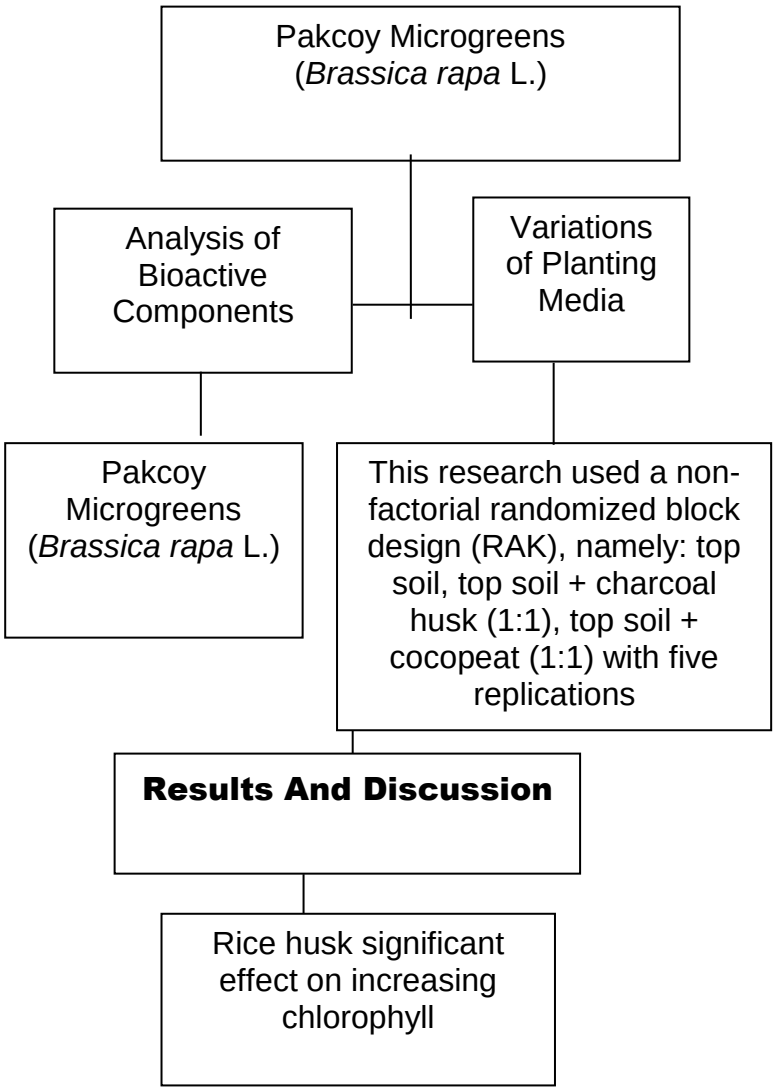

Figure 1. Stages of research

Determination of Total Extract Flavonoid Content: Weighed 10 mg of thick extract, dissolved in 10 mL of methanol solvent to obtain a concentration of 1000 ppm. 2 mL of the solution was pipetted, added 0.1 mL AlCl3 and 0.1 mL CH_3COONa and 3 mL distilled water, then incubated for 25 minutes.Absorbance was measured by UV-Vis spectrophotometry at a maximum wavelength of 427 nm. Determination of Total Extract Flavonoid Content: Weighed

10 mg of thick extract, dissolved in 10 mL of methanol solvent to obtain a concentration of 1000 ppm. 2 mL of the solution was pipetted, added 0.1 mL AlCl3 and 0.1 mL CH3COONa and 3 mL distilled water, then incubated for 25 minutes. Absorbance was measured by UV-Vis spectrophotometry at a maximum wavelength of 427 nm. Determination of Total Extract Flavonoid Content: Weighed 10 mg of thick extract, dissolved in 10 mL of methanol solvent to obtain a concentration of 1000 ppm. 2 mL of the solution was pipetted, added 0.1 mL AlCl3 and 0.1 mL CH_3COONa and 3 mL distilled water, then incubated for 25 minutes. Absorbance was measured by UV-Vis spectrophotometry at a maximum wavelength of 427 nm. Chlorophyll and Carotenoid Content: Analysis of chlorophyll content was carried out using the Wintermans and De Monts (1965) method at the age of 11 DAP. Chlorophyll is extracted by grinding the leaves using 96% ethanol. Then filtered using filter paper to get 25 ml of leaf extract. Measured with a UV/Vis spectrophotometer at wavelengths of 649 nm (chlorophyll b) and 665 nm (chlorophyll a) and 96% ethanol (blank) as a neutralizer, then calculated using the formula Chlorophyll a = (13.36 x A665) - (5 .19 x A649); Chlorophyll b = (27.43 x A649) - (8.12 x A665); Total chlorophyll = (5.24 x A665) + (22.24 x A649); Carotenoids (1000*A470) - (2.13*Chl a) - (97.63*Chl b) / 209 in µmol/L units.12 x A665); Total chlorophyll = (5.24 x A665) + (22.24 x A649); Carotenoids (1000*A470) - (2.13*Chl a) - (97.63*Chl b) / 209 in µmol/L units.12 x A665); Total chlorophyll = (5.24 x A665) + (22.24 x A649); Carotenoids (1000*A470) - (2.13*Chl a) - (97.63*Chl b) / 209 in µmol/L units.

3. RESULT AND DISCUSSION

The highest average flavonoid content was found in the top soil + cocopeat treatment, namely 19.19 mgQE/g. Flavonoids in plants are synthesized to protect plants from bacterial infections. The increase in flavonoids in microgreens is assisted by the use of good planting media such as cocopeat which has the ability to store water well so that the availability of water can be a transport for nutrients to be dissolved and easily absorbed by plants. However, it is suspected that cocopeat planting media contains tannin compounds which can inhibit plant growth but can increase phenolic compounds such as flavonoids as a response to plant stress. Therefore, tannins are beneficial for plants because they increase antioxidants, one of which is flavonoids. This is supported by Arbiyani et al., (2023); and Esati et al., (2021), flavonoids are secondary metabolite compounds of the polyphenol group which come from green plant extracts and have active effects such as anti-viral, anti-inflammatory, cardioprotective, anti-diabetic, anti-cancer and antioxidant. Tungmunnithum et al., 2018; Kumar et al., 2023 and Nakabayashi et al., (2014), also stated that flavonoids are phenolic compounds derived from secondary metabolite compounds which play an active role in the physiological mechanisms of plants to defend themselves from stress conditions.

Table 1. Flavonoid Content in Green Vegetables *Brassica rapa* L.

Growing media	Mean (mgQE/g)/SD
Topsoil	13.60bc ± 2.49bc
Top Soil + Charcoal Husk (1:1)	18.24ab ± 3.65ab
Top Soil + Cocopeat (1:1)	19.19a ± 5.40a

Notes: Different numbers and letters in the columns indicate differences significant according to the Duncan Test at the 5% test level.

Figure 2. *Brassica rapa* L microgreens 10 days after planting

Chlorophyll Content

The highest average chlorophyll content was found in the top soil + rice husk charcoal treatment, namely 42.12 µmol/L. This is thought to be because rice husk charcoal has a high carbon content and can improve soil structure so that the roots can easily absorb water well and can fix the available N nutrient content which is useful for plants for the formation of chlorophyll in the leaves. This is in accordance with Carsidi et al., (2021) and Andaresta et al., (2022) who stated that the absorption of nitrogen nutrients in plant leaf tissue will be used as raw material for the formation of chlorophyll. In general, the chlorophyll in *Brassica rapa* L mustard green plants is green and varied, which means that green mustard plants do not experience water stress. This is in accordance with the statement by Song & Banyo (2011) in the journal Carsidi et al., (2021) that if plants lack water, it will affect all aspects of plant growth such as physiological, biochemical, anatomical and morphological processes. In line with these findings, Libutti et al., 2020 and Chrysargyris et al., (2019) observed that biochar material has a high K content and an alkaline pH, thereby increasing the pH of the planting medium and increasing the concentration of N, K, and P in the leaves.

Table 2. Chlorophyll content in green vegetables *Brassica rapa* L.

Growing media	Average (mol/L)/ SD
Topsoil	38.97c ± 1.30
Top Soil + Charcoal Husk (1:1)	42.12a ± 3.68
Top Soil + Cocopeat (1:1)	41.39ab ± 4.18

Notes: Different numbers and letters in the columns indicate differences significant according to the Duncan Test at the 5% test level.

Carotenoid content

The highest average carotenoid content was found in the top soil + charcoal husk treatment, namely 10.63278 µmol/L. This is because the mixture of top soil and husk charcoal has a pH that is closest to normal, namely 5.0 compared to other planting media, where the pH can help in the absorption of nutrients in plants. This is supported by Tsai et al., 2020 and Jaaf et al., (2022), namely that if the pH conditions are normal, the absorption of nutrients by plants will not experience obstacles, so the growth rate of the plants will increase. Another factor that increases the carotenoid content is that the carotenoid content increases at the age of 1 week to 3 weeks or in the lag phase. This is in accordance with Hendriyani et al., (2018) and Kirigia et al., (2018) who stated that the carotenoid content increases at the age of 1 week to 3 weeks or in the lag phase. This increase is thought to be because during that week the plants experienced an initial growth phase

where there would be an increase in pigment production for the photosynthesis process.

Table 3. Carotenoid content in green vegetables *Brassica rapa* L.

Growing media	Average(µmol/L)/ SD
Topsoil	3.81d ± 0.12
Top Soil + Charcoal Husk (1:1)	10.63a ±0.35
Top Soil + Cocopeat (1:1)	7.01c ± 0.78

Notes: Different numbers and letters in the columns indicate differences significant according to the Duncan Test at the 5% test level.

CONCLUSION

The best recommended planting medium is rice husk charcoal which shows a significant effect on increasing chlorophyll (42.12 mg/L), carotenoids (10.63 µmol/L) and cocopeat which shows a significant effect on increasing flavonoid content (19.19). mgQE/g).

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