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# Analysis of Sulforafan Content in Hybrid Broccoli Supplies in MS Media Delivered NAA and BAP and Methionine in Vitro

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**Abstract:** ***Broccoli is a vegetable plant that contains sulforaphane as an antioxidant. These compounds are useful for curing cancer. Therefore, research has been carried out on the analysis of sulforaphane content in hybrid broccoli sprouts. The purpose of this research : 1. to determine the sulforaphane content of hybrid Broccoli sprouts growing on MS media 2. To determine the difference in sulforaphane content of hybrid broccoli sprouts grown on media that was given a combination of NAA; BAP and methionine The design used is factorial in RAL with 3 factors, namely A. NAA 0; 0.5 ; 1 ppm B. BAP 0 ; 1 ; 2 ppm and C. methionine 0 g; 25 g and 50 g. Each treatment was repeated 3 times. Variables observed were sprout height, sprout weight and sulforaphane content. The data were analyzed by analysis of variance and continued with the 5% BNT test. The results of the analysis of variance showed that there was an interaction between the treatment of NAA, BAP and Methionine and had a significant effect on the height of hybrid broccoli sprouts. Furthermore, the results showed that the highest sprouts were found in the N0B0M0 treatment or without NAA, BAP and Methionine treatment, which was 10.60 cm. While the lowest in the combination treatment N0.5B0M50 and N0.5B2M50 is 0.80 cm. The higher the concentration of NAA, BAP and Methionine can suppress the increase in shoot height of the hybrid broccoli sprouts. Furthermore, the results of the analysis of variance showed that there was no interaction between NAA, BAP and Methionine on the weight of sprouts but there was an interaction between BAP and methionine in a significant effect on the weight of sprouts. The results of the analysis of variance showed that there was an interaction between NAA, BAP and methionine on sulforaphane biosynthesis. The NoB1M25 combination had the highest sulforaphane content compared to the other treatments and was significantly different from the other treatments.***

**Keywords:** Hybrid Broccoli Sprouts, NAA, BAP, Methionine, Sulforaphane Biosynthesis

## 1. Introduction

Brassicacea is a vegetable plant that belongs to the cabbage family.This type of cabbage is quite popular as a food ingredient.Broccoli is most similar to cauliflower, except that broccoli is green, while cauliflower is white.Broccoli is a plant that lives in cold weather habitats (Anonymous. 2009).

Furthermore, cabbage or cabbage and Chinese cabbage are only consumed for the leaves.These plants are endemic plants which are only found in certain places and can adapt in several places in Indonesia.In addition to containing nutrients, broccoli, cauliflower, cabbage and Chinese cabbage contain a compound that is very beneficial for human health.

Cabbage-type vegetables are said to be the richest in antioxidants, both in terms of quantity and type.An important antioxidant compound stored in this type of cabbage is sulforaphane.In addition, this vegetable contains beta-carotene, quercetin, glutathione.Broccoli, cauliflower or white cauliflower, cabbage, and Chinese cabbage are particularly important for women, as they remove harmful, potentially cancer-promoting excess estrogen.For people with diabetes mellitus (diabetes mellitus), these vegetables help reduce soaring blood sugar levels, because these vegetables are very rich in chromium microminerals.For this reason, it is recommended that people with diabetes eat more often the vegetables of the cabbage group, especially broccoli (Apriadji, 2008).

Various groups call cabbage as a superfood.Many studies have shown how this natural food is rich in substances that are beneficial to health.A recent study in England indicated that broccoli and other types of cabbage also have important substances that can repair and restore the function of blood vessels damaged by diabetes.Substance called sulforaphane in this plant, has a major role in restoring blood vessels. Sulforaphan is able to stimulate the production of enzymes that can protect blood vessels and reduce molecules that cause significant cell damage.Brassicacea-type vegetables such as broccoli were previously associated with a lower risk of heart attack and stroke (Keck and Finley, 2004).

This Brassicaceae vegetable contains fat, protein, carbohydrates, fiber, water, iron, calcium, minerals, and various vitamins (A, C, E, ribolamin, nicotinamide).These vegetables contain sulforaphane, which is a cancer-fighting compound.So that broccoli and other cabbage vegetables are grouped as medicinal plants (Jeffery and Araya, (2008). Broccoli and cabbage types have the ability to accelerate disease healing and prevent and inhibit the development of cancer cells in the body, especially cancers related to

hormones, such as cancerbreast cancer in women and prostate cancer that threatens men. This has been proven through research conducted by an epidemiological team from Harvard University. This plant is very good for people with diabetes. The content of chromium and fiber can regulate blood sugar levels. Broccoli and other types of cabbage strengthen cellsIf consumed from a young age, it prevents bone loss (osteoporosis) in old age. Brassicaceae also has the ability to prevent skin diseases such as abscesses or boils (Dalimartha, 2005) and (Jeffery and Araya, 2008).

Seeing the benefits of these vegetable plants, it is necessary to examine which growth phase and which type of cabbage has the highest sulforaphane content in vivo.In a previous study, several types of cabbage were analyzed in different phases and the result was a high sulforaphane content in the sprout phase of broccoli compared to other cabbages (Tilaar, PoliiMandang and Pinaria, 2018).Furthermore, the next research in 2019 was conducted on hybrid broccoli seeds and the Lucky Taichung type which were grown on MS media treated with NAA, BAP, where hybrid sprouts obtained high yields by administering 1 ppm NAA without BAP.Because of that, the plan is to carry out research on the analysis of sulforaphane content.The research was in vitro culture on hybrid broccoli seeds grown on MS media which was given a combination of NAA, BAP , Methionine to get the sprouts and continued with the analysis of the sulforaphane content in the sprouts.

## 2. Materials and Methods

This research was conducted in the Unsrat Faculty of Agriculture's Biotechnology Lab and the Chemical Engineering Department's laboratory, State Polytechnic of Malang.This research was conducted for 1 year.

**Tools and Materials**
The tools used are sonicleaner, hot plate, centrifuge, mortal and other glassware, Liquid Chromatography Mass spectroscopy Mass Spectroscopy device, XR-ODSIL shim-Pack column (50 mm x 2.0 mm I.D.), mobile phase A : 5 mmol/l ammonium formate-water, mobile phase B : acetonitrile, flow rate 0,3 ml/minute, injection volume 5 ul, column temperature 40 0 C, gas flow 1.5 l/minute, dry gas pressure 10 l/minute.

The materials used are 2 weeks old sprouts of green cauliflower (broccoli), chemical compounds for extraction.These materials include methanol p.a, acetonitrile, ammonium formate and distilled water, and standard sulforaphane.

**Methods**
The design used is factorial in RAL with 3 factors namely A. NAA 0;0.5 ;1 ppm B. BAP 0 ;1 ;2 ppm and C. 0 ;25 ;50 g methionine .Each treatment was repeated 3 times.Variables observed: germination time, sprout weight and sulforaphane content.Data were analyzed by analysis of variance and continued with the 5% BNT test. Procedure Broccoli sprout material extraction and sulforaphane analysis.

**Extraction and isolation of sulforaphane**
Sulforaphan extraction begins with weighing the sprouts, green cauliflower (broccoli), and cauliflower seeds.These ingredients are weighed on a digital scale.Then put in mortal and added with 1 -2 ml of methylchloride and crushed until crushed.The crushed broccoli shoots were transferred to a flash tube and added with 25 – 50 ml of methylchloride. Sonification was then carried out for 30 minutes to remove sulforaphane from the hybrid broccoli sprout tissue.Then the resulting sulforaphane extract is filtered using waltmen paper and transferred to a tube and placed on a heating box or hot plate with a temperature of 70 to 80 oC to produce a supernatant.To this dry residue, 5 ml of NaSO4 was added and heated again on the hot plate at 70 to 80 o C until the supernatant was dry.To this dry residue, 10 ml of acetonitrile was added, then filtered with waltmen paper and the solution was centrifuged for 15 minutes at 4000 rpm.Finally, the solution containing the residue was transferred to the microtube and put into the LC tandem MS to determine the sulforaphane content of the shoots.

**Qualitative and quantity analysis of sulforaphane**
Qualitative and quantitative analyzes for sulforaphane were carried out with the MSMS Plus LC tool.Mobile phase A : 5 mmol/l ammonium formate-water, mobile phase B : acetonitrile, flow rate 0.3 ml/min, injection volume 5 ul, column temperature 40 0 C, gas flow 1.5 l/min, gas pressuredry 10 l/min.The UV wavelength used to detect sulforaphane is 254 nm.Before the analysis was carried out, the mobile phase and sample solution were first filtered with a 0.4 um cellulose acetate PTFE membrane filter.Standard sulforaphane obtained from the Biopharma Science Center was used as the comparator compound.Qualitative analysis was carried out by comparing the retention time between standard sulforaphane and the sample retention time.If there is a compound in the sample that has the same retention time as standard sulforaphane, then that compound is sulforaphane.Coinjection of standards and samples was carried out to ensure the presence of sulforaphane in the samples.

Quantitative analysis to obtain sulforaphane concentration is obtained by converting the sample area to the standard area whose concentration is known in the standard caliber curve.The standard calibration curve was obtained from the data on the area of various concentrations of standard sulforaphane and then a relationship was made between the area and the sulforaphane content.

The observed variables are sprout height,sprout weight, sulforaphane qualitatively and quantitatively from hybrid broccoli sprouts

**Data analysis**
Data was collected, tabulated and statistically analyzed for conclusions to be drawn.Research data will be presented in table form, according to the type of data obtained in this study.Statistical tests to determine differences in the mean sprout height, sprout weight and sulforaphane content in broccoli sprouts were carried out by measuring the height of the sprouts, weighing the weight of the hybrid broccoli sprouts from each treatment and analyzing the sulforaphane content according to the 2-week-old sprouts of the broccoli.

## 3. Results and Discussion

**Sprout Height**

The results of the analysis of variance showed that there was an interaction between the NAA, BAP and Methionine treatments on the height of hybrid broccoli sprouts with significantly different effects on caterpillars.Because of this, a 5% BNT test was carried out, treatments that affected the height of hybrid broccoli sprouts originating from Tomohon Market stalls/stalls.The highest sprouts were found in the N0B0M0 treatment or without NAA, BAP and Methionine treatment, namely 10.60 cm.While the lowest was in the combination treatment N0.5B0M50 and N0.5B2M50 which was 0.80 cm.The results of the 5% BNT test showed that the treatment without NAA, BAP and Methionine was the highest from hybrid broccoli sprouts.The higher the concentration of NAA, BAP and Methionine can suppress the height growth of the hybrid broccoli sprouts.Providing NAA as auxin should increase shoot height but the opposite occurs.This is possible at a concentration of 0.5 ppm and 1 ppm there is too much concentration so that it can inhibit growth.In the treatment without the administration of NAA, BAP and methionine it was very high reaching 10.60 cm so it was different from all the treatments given NAA, BAP and methionine either alone or in combination of the three treatments.Furthermore, the 1 ppm BAP treatment without NAA and methionine was quite high which was not different from the N0B2M50, N0.5B0M0, N0.5 B1 treatment without methionine and N0.5B1M50 and N1B2M25 but different from the other treatments.In the NoBoM25 treatment, the height of the hybrid broccoli sprouts was different from the NoBoMo and NoB1Mo treatments, but the height of the hybrid broccoli sprouts was not different from the other treatments.Furthermore, it can be seen in table 1.

**Table 1:** Effect of NAA, BAP and Methionine Interaction on Sprout Height

| No | Treatment | Height (cm) |
|---|---|---|
| 1 | NoBoMo | 10,60e |
| 2 | NoBoM25 | 1,47 abc |
| 3 | NoBoM50 | 1,47 abc |
| 4 | NoB1Mo | 2,60 d |
| 5 | NoB1M25 | 1,23 abc |
| 6 | NoB1M50 | 1,50 abc |
| 7 | NoB2Mo | 1,53 abc |
| 8 | NoB2M25 | 1,40 abc |
| 9 | NoB2M50 | 1,77 bcd |
| 10 | N0,5BoMo | 1,77 bcd |
| 11 | N0,5BoM25 | 1,27 abc |
| 12 | N0,5BoM50 | 0,80 a |
| 13 | N0,5B1Mo | 1,75 bcd |
| 14 | N0,5B1M25 | 1,23 abc |
| 15 | N0,5B1M50 | 1,77 bcd |
| 16 | N0,5B2Mo | 1,57 abc |
| 17 | N0,5B2M25 | 1,13 abc |
| 18 | N0,5B2M50 | 0,80 a |
| 19 | N1BoMo | 1,63 abc |
| 20 | N1BoM25 | 1,17 abc |
| 21 | N1BoM50 | 1,03 abc |
| 22 | N1B1Mo | 1,53 abc |
| 23 | N1B1M25 | 2,03 cd |
| 24 | N1B1M50 | 1,10 abc |
| 25 | N1B2Mo | 1,43 abc |
| 26 | N1B2M25 | 1,70 abcd |
| 27 | N1B2M50 | 1,33 abc |
| BNT 5% = 0.91 | | |
| Note: Numbers followed by the same letter are not significantly different based on BNT test at 5% level | | |

**Sprout Weight**

The results of the analysis of variance showed that there was no interaction between NAA, BAP and Methionine but there was an interaction between BAP and methionine in their effect on sprout weight.The highest sprout weight was in the treatment without BAP and Methionine while the lowest was in the combination treatment of 1 ppm BAP and 25 g

Methionine.The treatment without BAP and Methionine was different from the B1M50 and B1M25 treatments but not the other treatment combinations.The higher methionine without BAP inhibits the weight gain of hybrid broccoli sprouts but when combined with BAP, the two support each other in spurring the weight gain of the hybrid broccoli sprouts, even though these two factors slightly inhibit the weight gain of the hybrid broccoli sprouts.

**Table 2:** Effect of BAP and Methionine Treatment on Hybrid Broccoli Sprout Weight

| No | Treatment | Sprout Weight (g) |
|---|---|---|
| 1 | BoMo | 0,304 |
| 2 | BoM25 | 0.199 abc |
| 3 | BoM50 | 0.172 a |
| 4 | B1Mo | 0.199 abc |
| 5 | B1M25 | 0.164a |
| 6 | B1M50 | 0.290 bc |
| 7 | B2Mo | 0.200 abc |
| 8 | B2M25 | 0.204 abc |
| 9 | B2M50 | 0.254 abc |
| BNT 5% = 0.11 | | |
| Note: Numbers followed by the same letter are not significantly different based on BNT test at 5% level | | |

**Sulforaphan Content**

The results of the analysis of variance showed that there was an interaction between NAA, BAP and methionine on sulforaphane biosynthesis.The NoB1M25 combination had the highest sulforaphane content compared to the other treatments and was significantly different from the other treatments.General Methionine given alone or given in combination with BAP and NAA can increase the synthesis of sulforaphane at a concentration of 25 g but when combined with a concentration of 50 g it will inhibit the synthesis of sulforaphane.BAP given alone will increase the synthesis of sulforaphane at a concentration of 1 ppm but at a concentration of 2 ppm it can inhibit the synthesis of sulforaphane.When combined with NAA, namely NAA 1 ppm and BAP 1 ppm, it can slightly increase the synthesis of sulforaphane, but the combination of NAA 0 ppm and BAP 1 and 2 ppm, the synthesis of sulforaphane can inhibit it.Likewise for the combination of 0.5 ppm NAA and 1 and 2 ppm BAP.While the combination of NAA and Methionine showed an increase in the synthesis of sulforaphane at 25 g but decreased at a concentration of 50 ppm.

**Table 3:** Effect of NAA, BAP and Methionine Interactions on Sulforaphan Content in Hybrid Broccoli Sprouts

| No | Treatment | Height (cm) |
|---|---|---|
| 1 | NoBoMo | 185,67 c |
| 2 | NoBoM25 | 490,97 p |
| 3 | NoBoM50 | 1,49,85 ab |
| 4 | NoB1Mo | 361,14 kl |
| 5 | NoB1M25 | 729,84 q |
| 6 | NoB1M50 | 389,76 lm |
| 7 | NoB2Mo | 230,91 d |
| 8 | NoB2M25 | 359,10 jk |
| 9 | NoB2M50 | 402,02 mn |
| 10 | N0,5BoMo | 366,70 kl |
| 11 | N0,5BoM25 | 408,24 mn |
| 12 | N0,5BoM50 | 275,86 fg |
| 13 | N0,5B1Mo | 296,41 gh |
| 14 | N0,5B1M25 | 245,28 de |
| 15 | N0,5B1M50 | 178,07 bc |
| 16 | N0,5B2Mo | 279,45 fg |
| 17 | N0,5B2M25 | 415,05 mn |
| 18 | N0,5B2M50 | 146,06 a |
| 19 | N1BoMo | 404,97 mn |
| 20 | N1BoM25 | 448, 85 o |
| 21 | N1BoM50 | 312,90 hi |
| 22 | N1B1Mo | 423,96 no |
| 23 | N1B1M25 | 331,05 ij |
| 24 | N1B1M50 | 266,01 ef |
| 25 | N1B2Mo | 449,53 o |
| 26 | N1B2M25 | 397,42 mn |
| 27 | N1B2M50 | 299,30 gh |
| BNT 5% = 29,80 | | |
| Note: Numbers followed by the same letter are not significantly different based on BNT test at 5% level. | | |

## 4. Conclusion

The height and weight of the sprouts do not determine the synthesis of sulforaphane content but are determined by the growth regulators NAA, BAP and methionine. The highest sulforaphane content was influenced by 1 ppm benzylaminopurine and 25 mg/l methionine

To increase the synthesis of sulforaphane in vitro, hybrid broccoli sprout explants can be used and cultured in MS media with the addition of NAA, BAP and Methionine.The combination of 1 ppm BAP with 25 g of methionine is very good for the synthesis of sulforaphane.

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