Nutrients and Anti-nutrients Content Analysis of Bogor Taro Mutant Clone (*Colocasia esculenta*)

F Nurilmala and D Mardiana

1 Nusa Bangsa University, Jl. KH Sholeh Iskandar Km 4 Cimanggu, Bogor 16166, Indonesia

E-mail: febi@unb.ac.id

**Abstract.** Bogor Taro is an important local staple food, a potential substitute to rice as carbohydrate source in Indonesia. However, its production in the last decade tends to decline due to unsuccessful breeding program. Taro contains both nutrients (carbohydrate) and anti-nutrients, such as calcium oxalate that cause itching sensations when consumed. At present, through somaclonal variation technology by gamma radiation, six mutant-clones have been produced. We investigated technology to improve carbohydrate content and to depress calcium oxalate on the six mutant-clones available. We used iodometric titration to examine carbohydrate levels, permanganometric titration to measure calcium oxalate levels, argentometric titration to determine cyanide acid levels, and extraction method-gravimetric for saponins. We found, there were three mutant clones which have higher carbohydrate and lesser calcium oxalate content compared to the parent, namely B521 (5 Gy gamma radiation), B1023 (10 Gy gamma radiation) and B1511 (15 Gy gamma radiation) clones which will be proliferate to be a superior seeds.

1. **Introduction**

The primary source of carbohydrate for the majority of Indonesians is rice, even though there are alternatives besides rice as the primary source of carbohydrate such as taro. Of the many tubers, taro is a good rice substitute as a food source due to has a high starch content reaching 70-80% [1]. There exist other nutrients inside taro such as carbohydrate as well as anti-nutrients that could impair the nutrients. Anti-nutrients are chemical matters that have evolved on plants to defend biological functions between plants. Anti-nutrients diminish the utilization of nutrients (proteins, vitamin and minerals in particular) thus effectively preventing optimal utilization of nutrients and decreasing the nutritional value [2]. Cultivation is expected to disable anti-nutrients factors and increase the availability of bioactive compound. However, the health risk for consumers in a high amount of anti-nutrients residue must not set aside.

One of the anti-nutrients found in taro is an oxalate compounds. This compound could cause itches. Food consumption with a high amount of oxalate can be detrimental to health due to the formation of oxalate stones also known as kidney stones, resulting in renal disorder [3]. Besides oxalate compounds, there is also cyanide acid found in taro. Cyanide is a poison to every living thing, could obstruct breathing due to the hindering of ferricytochrome oxidase enzyme process of oxygen intake for breathing, and cause imperfect cell growth [4]. Side effects caused by HCN poisoning also include headaches, vomiting, and dizziness. The human body could tolerate HCN when consumed within the reasonable amount. A standard was assigned by FAO that specifies tubers with the HCN amount of 50 mg/kg below is still safe to be consumed. Another compound which included as anti-nutrients on
taro is saponin. Generally tastes bitter and foams when shaken with water. It is also poisonous to some cold-blooded animals [2].

The development of genetic variation on Bogor Taro aims to a higher carbohydrate and lower calcium oxalate compared to the parent has been done through the somaclonal variation induction by gamma radiation treatment at a dose of 5, 10, and 15 Gray to the culture of Bogor Taro apical bud and has acclimatized 5 mutant clones, namely B012, B521, B1022, B1023, and B1511 [5]. The mutant clones were then analyzed to find out the content of nutrition (carbohydrate) and anti-nutrition (calcium oxalate, cyanide, and saponin).

2. Materials and Methods

Plant materials obtained from 4-6 months old acclimated mutant clones and the parent from Nusa Bangsa University’s collection. Sample preparation began with washing the taro's corms, peeled, and washed again in running water, then cut into match-sized parts and soaked in water for one night. The sample is blanched for 5 minutes and dried in the oven at 70 °C for 24 hours. Dried sample are then grinding to 100 meshes.

Determining carbohydrate level according to iodometric titration method that 1 gram sample put into 500 ml Erlenmeyer flask, added 25 ml of 1.25% H₂SO₄, and then boiled for 3 hours using upright cooler. Chill and neutralize with 3.25% NaOH using red litmus and move to the 100 ml volumetric flask and squeeze until line marks with distillation water and then strain. Using 25 ml pipette, move the distillate into 500 ml Erlenmeyer flask, add 25 ml of Luff solution, add some boiling stones, and 10 ml of distilled water. The mixture is then heated again in upright cooler, and the mixture should be boiled within 3 minutes, boil for 10 minutes straight, then immediately chill inside an ice-filled tub. After chilled, add ten ml of 30% KI solution and 25 ml of 25% H₂SO₄, then slowly titrate with 0.1 N TiO solution with 0.5% starch indicator.

Calcium oxalate determined using permanganometric titration method. Two grams of the sample are macerated at room temperature with 20 ml of 0.15% citric acid for 6 hours. Strain the solution, and the filtrate is then acidized and precipitated with 5 ml 10% tungstophosphoric acid and then centrifuged for 5 minutes at 1500 rpm. Supernatant and precipitation dissolved with 2 M H₂SO₄, and then heated until 70-80°C and then titrated with 0.01 M KMNO₄.

Saponin level decisive according to [6]. Ten grams sample soaked in 100 ml of 20% Ethanol and heated in the water bath at 55°C for 4 hours while being stirred with a stirrer. The mixture then strained, and the residue is extracted again with 100 ml 20% Ethanol. Extraction result is then joined and heated with a water bath at 90 °C until there is 20 ml extraction left. The concentrate then moved into separate funnels (250 ml and ten ml). Diethyl ether is then added and shaken. The liquid layer then moved, and purification process is repeated using 30 ml n-Butanol. The n-Butanol result is washed twice with five ml 5% NaCl. The final mixture then heated with a water bath. After evaporation, the sample is dried with an oven at 105 °C until a constant weight achieved. Saponins level then calculated in percentage.

Determining cyanogenic glycosides as said by [6]. Five grams sample is moved into 800 ml Kjeldahl flask, the sample then macerated with 50 ml water for 2 hours at temperature room. Then added 50 ml water, 10 ml of 0.02 N AgNO₃ and 0.5 ml of liquid HNO₃ homogenized and distilled at the temperature of 95 °C until container volume is 75 ml. Fe Alum (NH₄Fe(SO₄)₂·12H₂O) indicator is then added to the distillate until the color of the mixture turns yellow, then titrated with 0.02 N KCN until brick-red sediments formed.

3. Results and Discussion

We found that the color of the mutant clone flesh corms has changed from its parent. The parent was white, and the mutant clones were yellow and yellowish (Figure 1). The color changed indicated the presence of secondary metabolites such as carotenoid and flavonoid and are essential traits of different cultivars. Compared to the common whitish like the parent of this studies, aspect of the
yellow/yellowish corm flesh are favored more by consumers [7]. This result gives hope to the mutant clones to be well accepted by consumers as a new type of variety.

The content of carbohydrate on various taro mutant clones show that there were four mutant clones have higher carbohydrate compared to the parent (Figure 2). Average carbohydrate spread is around 33.95 g/100 g ingredient. The most substantial rise is on the B1511 clones with an increase of 46.93 g/100 g ingredient or about 211% expansion. We also found the decrease of 50% compared to the parent from B1022 clones. Higher carbohydrate content from its parent can be considered to be an alternative food source so that the society no longer has to be reliant upon rice to fulfill their main food needs. There is no specific amount of carbohydrate needed by the human body per day, although [8] and [9] advises that 40 - 80% total energy consumption be from complex carbohydrate and a maximum amount of 10% from sugar.

![Figure 1.](image) The color of taro mutant clones corm compares to the parent.

The contents of calcium oxalate on various taro mutant clones show that there were four mutant clones which have lower contents of calcium oxalate and only one mutant clone show a higher calcium oxalate content compared to the parent (Figure 2). Calcium oxalate content obtained is in the range of 3.20 – 41.60 mg calcium oxalate/100g ingredient. Average calcium oxalate decrease is 16.64 mg/100 g ingredient and the most substantial calcium oxalate decrease to 90% found on B521 clone. The increase of calcium oxalate content on B1023 and B012 clones was between 20% – 30%. Calcium oxalate caused acridity and itching sensation when consumed. It caused by needle-like oxalate crystals, raphides, that can penetrate the soft skin and cause discomfort in the tissue. Many studies reduce the effect of oxalate by cooking or boiling the taro corms [10-14]. Our study shows that Gamma irradiation can reduce the oxalate content of Bogor taro corms. We expected that some clone like B521 and B1511 could become new varieties with low oxalate content without going through the cooking process.

The contents of saponin on various taro mutant clones show that there were three clones which have lower contents of saponin compared to their parents, four clones which have an equal amount of saponin, and one that has the higher content of saponin to the parent (Figure 2). The range of saponin content is between 17.89 – 20.64 g/100 g ingredient. The most substantial reduction found in B521 clone with a decrease of 7.54%. Saponin is an active compound which foams when shaken in water and at low concentration could cause red blood cell hemolysis [15]. However, saponin can also be
used in the detergents and cosmetics industries as they have excellent foaming properties, moderate detergency and anti-dermatophytic activity [16-18].

The contents of cyanide show that there were four clones have lower cyanide, and only one clone has higher cyanide compared to the parent. The most substantial decrease of cyanide found in B1511 clone, and there is an increase of 34% in cyanide content found in B521 clone. According to [19] the lethal dose of cyanide in a human body is 250mg. So far, all the clones include the parent are lower than the lethal dose of cyanide.

**Figure 2.** Nutrient (Carbohydrate) and Anti-nutrients content of taro mutant clones compares to the parent.

4. Conclusion

There were three mutant clones of Bogor Taro which have a higher carbohydrate content and lower content of calcium oxalate compared to the parent, namely B521, B1023 and B1511 clones which will then be multiplied to meet the needs of superior seeds.

Acknowledgements

Acknowledgements delivered to Ministry of Research, Technology and Higher Education that has funded this research through Applied Product Research Contract No. 1598/K4/KM/2017.

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