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Effect of Soaking of NaCl Solution on Reduction of Calcium Oxalate and Size of Amylum on Purple Yam (*Dioscorea alata* L.)

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A B S T R A C T

Purple yam (*Dioscorea alata* L.) is a traditional food as a source of alternative carbohydrates and potentially as food functional ingredients. Purple yam contains high carbohydrate and some antioxidant compounds but contains high enough Ca oxalate. This study aims to reduce the content of Ca oxalate by soaking in NaCl solution and analyze its effect on amylum size in purple yam. The research used Completely Randomized Design (CRD) with the single factor of NaCl concentration. Treatment of soaking with different concentrations of 5%, 10%, 15% and with four replications for each treatment. The parameters observed were number and size of crystal Ca oxalate (µm), Ca oxalate content (ppm) and decrease percentage, and amylum size (µm). The data were analyzed using Analysis of Variance (ANOVA) at 95% confidence level and continued by multiple-range test *Duncan's Multiple Range Test* (DMRT). The results showed that soaking in NaCl solution had an effect on Ca oxalate content of each treatment but did not affect on the number and size of crystals Ca-oxalate and amylum size. The best treatment was found in the treatment of P2 (10% NaCl) which can reduce Ca oxalate to 22.89% with oxalate content of 78.92 ppm. The higher concentration of NaCl in the solution increasingly affect the reduction of oxalate content on purple yam.
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Keywords: Amylum, Ca oxalate, *Dioscorea alata* L., Purple yam.

I. INTRODUCTION

The fulfillment of staple food needs, especially rice in Indonesia, face a severe problem due to disturbance such as drought, flood or pest disease. In Indonesia, diversification foodstuff of carbohydrate from rice is not lead to corn, sorghum, tubers, or sago, unless to imported wheat. Indonesia is noted by the Food and Agriculture Organization (FAO) as the fifth largest wheat importer country in the world (Yalindua, 2014).

Local food security in Indonesia can be maintained by utilizing the potential of non-rice food, one of them is *Dioscorea* or yam. *Dioscorea* has long been known and utilized in the life of Indonesian society. Two prominent forms of *Dioscorea* utilization are food and medicine. One of the species found in Indonesia is *Dioscorea alata* L. (purple yam) (Abdillah, 2015). The purple color of the yam is due to a purplish-colored pigment which is an anthocyanin that usually spreads irregularly on the tubers (Yalindua, 2014). Purple yam has an average carbohydrate content of 81.54%; protein 4.53%; 0.39% fat; glucomannan 35.70% and calcium oxalate 0.35% (Wibawa *et al*., 2011).

Lack of information about uwi tuber makes it lack the use of both as food and as raw materials of food industry. Purple uwi tubers can be used as flour, and their starch can be taken, although special handling is needed to overcome the mucus of tubers (Nadia and Hartari, 2012). Additionally, the oxalate crystals contained within the tubers cause itching if not appropriately treated (Wibawa *et al*., 2011). Ca oxalate crystal purple yam was found in the form of rafida in large numbers. In every 3,707,058.61 μm^2 Ca oxalate crystals were found as many as 62 pieces with an average length of each piece were 158.479 µm (Kumalawati, 2017).

Mayasari (2010) used various concentrations of salt solution (NaCl) to reduce the oxalate content of Bogor taro. The soaking solution of NaCl 10% for 60 minutes was able to reduce oxalate with the highest average of 96.83%.

The reduction of Banten taro oxalate using NaCl solution in Marliana's research (2011) obtained the largest percentage of oxalate reduction (90.29%) in the concentration of 10% NaCl with soaking time of 150 minutes.

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According to Chotimah and Fajarini (2013) who performed oxalate reduction research on sente or taro by boiling with NaCl solution, the highest decrease of calcium oxalate content at boiling of 6% NaCl solution, temperature 80° C for 30 minutes were able to reduce oxalate content by 60%.

Reduction of oxalate levels can be made by soaking in a solution of acid, alkaline and salt (to reduce oxalate levels of insoluble crystalline solid) and soaking in warm water (to reduce levels of soluble oxalate compounds in the form of oxalic acid). A good solution for the reduction of calcium oxalate compounds includes citric acid, chloride, sodium chloride and calcium hydroxide/whiting (Lukitaningsih, 2012; Chotimah and Fajarini, 2013; Mayasari, 2010). Soaking in the solutions intended to eliminate the itching due to the oxalate content present in the tubers. With the reduction of oxalate content on tubers, it will produce food sources that can be used as an alternative food of Indonesia (Marliana, 2011). This research was conducted to determine the effect of soaking of purple yam in NaCl solution to reduce calcium oxalate.

II. Material And Methods

The research was conducted at Biology Laboratory in Department of Biology, Faculty of Science and Mathematics, Diponegoro University in October 2017 until January 2018. Purple yam sampling was performed using purposive sampling method in Mranggen Demak area. Samples were harvested at the age of 12 months.

Sample preparation: The outer skin (epidermis) of purple yam was peeled, then sliced with a thickness of 2 mm. Yam sliced soaked in each solution of NaCl (5%, 10%, and 15%) for 60 min. For control (P0), Uwi purple that has been sliced not done soaking, either soaking NaCl and water, P1 (soaking in a solution of NaCl 5%), P2 (soaking in a solution of NaCl 10%) and P3 (soaking in a solution of NaCl 15%). The selected concentration is the result of optimization of the decrease of oxalate content on taro Banten conducted by Mayasari (2010). Based on research Kuswiyanto and Purwaningsih (2016) soaking samples was done with a dose of 100 grams of tubers soaked with 250 ml of solvent.

The number and size of Ca oxalate crystals and the size of the amylum was observed using a photomicrograph at 400x magnification. Ca oxalate crystals were counted and measured is rafida shaped crystals, which belonged to the longest and relatively the same size. Each one of the preparate slides was observed with ten times the calculation, i.e., the preparate shifted to the right and the left patterned zigzag. Each shift was calculated as the number of visible oxalate crystals and measured the length of the oxalate crystals and the length of the amylum. The content of Ca oxalate was measured by permanganometric titration method (Purwaningsih and Kuswiyanto, 2016; Fitriani *et al*., 2016).

This study was conducted using a completely randomized design (CRD) with four treatments and four replications. The data obtained is then analyzed by Analysis of Variance (ANOVA) at 95% confidence level to prove the result, real or not, and then followed by Duncan's Multiple Range Test (DMRT) test at a 95% confidence level. Data were analyzed using SPSS.

III. Results And Discussion

Table 1. Results of the analysis of the average number and length of Ca oxalate crystals, the content and the percentage decrease of Ca oxalate and size of amylum

Treatment	Parameter				
	Ca oxalate crystals		Ca oxalate		Amylum
	Number	Length (µm)	Content (ppm)	Percentage decrease (%)	Length (µm)
P0	117	123,156	102,44^{c}		45,444
P1	102	116,804	88,36^{b}	13,61^{a}	43,539
P2	95	110,903	78,92^{a}	22,89^{b}	43,549
P3	97	112,914	80,77^{a}	20,96^{ab}	43,672

The number followed by the same letter in the same column shows no significant difference based on the Duncan test at the 95% confidence level.

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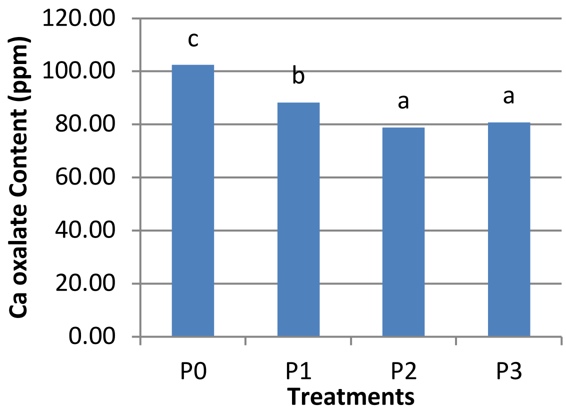

Figure 1. Histogram of the average test result of Ca oxalate content (ppm) in each treatment; P0 (control/without soaking), P1(soaking in 5% NaCl), P2 (soaking in 10% NaCl) and P3 (soaking in NaCl 15%).

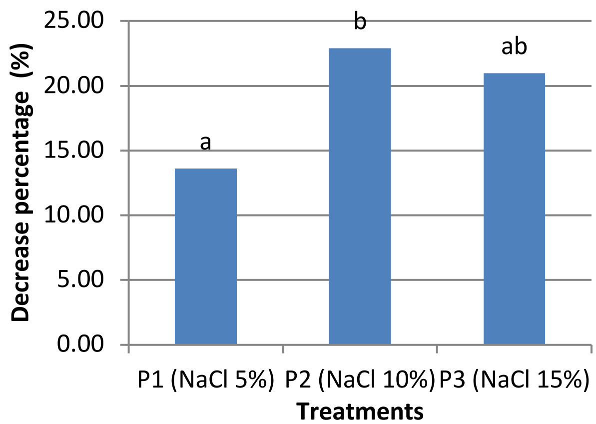

Figure 2. Histogram average percentage decrease of Ca oxalate content in each treatment; P0 (control/without soaking), P1(soaking in 5% NaCl), P2 (soaking in 10% NaCl) and P3 (soaking in NaCl 15%).

The analysis of data showed no significant difference in the number and length of crystals Ca-oxalate and the size of amylum, while the content and percentage of decrease in Ca oxalate showed significantly different results.

3.1 Number and Size of Ca oxalate Crystals

Based on the results of the research, it can be seen that the average yield of oxalate number and length of each treatment although slightly different due to a decrease in number but still in the same range. The effect of soaking of purple yam in NaCl solution through microscopic observation with photomicrograph on the number and length of Ca oxalate showed no significant difference. The number and size of the oxalate crystals, although decreased per treatment, microscopically this Ca oxalate crystallite has not been said to be reduced since both the quantity and size of the calculated and measured crystal oxalate are still intact in both shape and structure.

Based on research Kumalawati (2017) Uwi Ca oxalate crystal violet have shaped raphide with higher numbers and sizes longer when compared with other bulbs species of the same genus as wild yam and lesser yam. Calcium oxalate crystals are most commonly found in higher plants and are the end product of cell metabolism which is then stored in the lumen of cells or vacuoles. The crystalline Ca oxalate is formed from oxalic acid bound by calcium ions. Excessive oxalic acid can be toxic to plants, so oxalic acid is usually bound by calcium ions to form calcium oxalate. These crystals are generally present in the cortical parenchyma cells, phloem or pith (Sutrian, 2011). These calcium oxalate crystals are known to cause irritation to humans. Calcium oxalate compounds derived from calcium ions with oxalic ions. This compound is present in the form of a non-volatile solid crystalline, insoluble in water but soluble in a strong acid (Wahyudi, 2010).

3.2 Size of Amylum

Based on the result of research indicate that soaking of purple yam in NaCl solution with various concentration has no significant effect on length of amylum. The average yield of amylum length showed no significant difference; this is because the length of amylum of each treatment is almost the same. Based on observations made of purple yam amylum oval-shaped like an oval, eccentric type for hilum position located on edge and has a monoadelph arrangement because each grain of amylum has one hilum only. In accordance with Kumalawati (2017) which states that the amylum of purple yam tuber has type eccentrically with rounded oval shape and large size.

The amylum of purple yam was undamaged form, structure, and composition microscopically in all soaking treatment. This is following research Wiguna *et al*. (2014) that the cassava amylum solubility test in water does not indicate the occurrence of solubility, both natural cassava amylum and cassava amylum fully pregelatinized. In his research also performed a microscopic test (1000x) on the shape of cassava amylum which showed all samples of amylum did not change shape and hilum position. Based on research Wiguna *et al*. (2014) can be said if water does not affect the shape of cassava amylum, but the increase in warming temperature affect the composition of amylum. According to Purnobasuki (2011) in the amylum some lamellas surround hilum. The existence of these lamellas is due to the formation time of amylum. Each layer has different water content so that the refraction index is different. These lamellas will be lost if exposed alcohol because water in the starch will be absorbed by alcohol so that the index of refraction becomes the same.

The existence of the relations between the influence of salt to the content of amylum in the purple yam samples of each treatment, further testing is required that is the test of total carbohydrate content to find out. Salt and heat influence on Bogor taro starch content in research Mayasari (2010). The best taro soaking at 10% NaCl for 60 minutes and soaking in warm water for 3 hours tended to decrease the starch content compared with the sample without treatment.

3.3 Ca Oxalate Content (ppm) and Decrease Percentage

NaCl solution has a significant effect on Ca oxalate reduction. The treatment of soaking of purple yam in a solution of NaCl with various concentrations showed significantly different results. The results of the research content of Ca oxalate (ppm) can be seen that the different treatments have different Ca oxalate content and the percentage decrease in the content of Ca oxalate different. The highest average Ca oxalate content was in the sample without NaCl solution soaking or control sample (P0) of 102.44 ppm, while the lowest Ca oxalate content was obtained at P2 (soaking of 10% NaCl solution) of 78.92 ppm. The average percentage decrease of the highest Ca oxalate content was obtained from P2 of 22.89%, while the average decrease in the lowest Ca oxalate content was found at P1 13.61%.

The result showed that the optimal soaking in NaCl solution in reducing the Ca oxalate content in purple yam is on the soaking of 10% NaCl (P2) solution, which can reduce the highest oxalate content of 22.89%. Addition of NaCl concentration above 10%, i.e., 15% (P3) has no significant effect on the decrease percentage of oxalate content because the result of an average reduction of its content is lower than P2 which is 20,96%. This refers to the study of Mayasari (2010) in Bogor taro reducing oxalate content with the flouring process through soaking in 5% NaCl solution; 7.5% and 10% with two-time factors of 30 minutes and 60 minutes. The best results obtained on 10% NaCl soaking for 60 minutes were able to reduce the oxalate content of taro by 96.83%. Addition of salt concentration above 5%, i.e.. 7,5% for 30 minutes resulted in the percentage decrease of oxalate content which is not significantly different. While in soaking of NaCl 7.5% can decrease oxalate equal to 62,73%; the decrease was lower when compared with 5% NaCl soaking that is equal to 72,47%.

The daily consumption of oxalate allowed in the UK is 70-150 mg (Noonan and Savage 1999). According to Massey (2007) for patients with kidney disease, the *American Dietetic Association's Nutrition Care Manual* recommends taking oxalates less than 40-50 mg per day. Based on the limits of oxalate consumption above, the oxalate content of purple yam either without treatment or with treatment is still in the category of safe for consumption. However, according to Holmes and Kennedy's (2000) who studied the approximate levels of food oxalate and daily oxalate intake on oxalate absorption and urinary oxalate excretion, said the foods which contain oxalate >10 mg/100 g had a potential cause of kidney stones. According to Zimmerman et al. (2005), the excessive consumption of oxalate foods causes hyperoxaluria. Hyperoxaluria is a major risk factor for calcium oxalate urolithiasis which is a major risk for kidney stone formation. Consuming excess oxalate long term, increased absorption of oxalate intestiïnal. Based on the above statement the oxalate content of purple yam without treatment slightly exceeds the limit of 10 mg/100g; in other words, the intake of foods containing oxalate into the body every day should not exceed 100 ppm because it can be risky in the formation of kidney stones.

This research concluded that the soaking in NaCl solution did not have a microscopic effect on the number and size of crystals Ca oxalate and the size of purple yam amylum. Meanwhile, the soaking in NaCl solution was proved to decrease oxalate content of purple yam, with the best treatment on P2 (10% NaCl soaking).

REFERENCES

Abdillah, R. (2015). Perbaikan Ruang Tumbuh Umbi Uwi (*Dioscorea alata L*) dan Pengaruhnya terhadap Pertumbuhan dan Produktivitas Umbi. *Skripsi*. Departemen Agronomi dan Hortikultura Fakultas Pertanian Institut Pertanian Bogor, Bogor.

Chotimah, S. & D. T. Fajarini. (2013). Reduksi Kalsium Oksalat dengan Perebusan Menggunakan Larutan NaCl dan Penepungan untuk Meningkatkan Kualitas Sente (*Alocasia macrorrhiza*) sebagai Bahan Pangan. *Jurnal Teknologi Kimia dan Industri* 2(2): 76-83.

Fitriani, H., Nurlailah & D. Rakhmina. (2016). Kandungan Asam Oksalat Sayur Bayam. *Medical Laboratory Technology Journal* 2 (2): 51-55.

Holmes, R. P. & Kennedy, M. (2000). Estimation of The Oxalate Content of Foods and Daily Oxalate Intake. *International Society of Nephrology, Kidney International* 57: 1662–1667. https://doi.org/10.1046/j.1523-1755.2000.00010.x

Kumalawati, H. (2017). Aktivitas Antioksidan, Jumlah dan Ukuran Kristal Ca Oksalat serta Amilum Umbi Gadung, Gembili, Uwi Ungu, Porang dan Rimpang Ganyong. *Skripsi*. Departemen Biologi Fakultas Sains dan Matematika Universitas Diponegoro, Semarang.

Lukitaningsih, E. (2012). Analysis of Macronutrien Content, Glycemic Index and Calcium Oxalate Elimination in *Amorphophallus campanulatus (Roxb)*. *Jurnal Natural* 2(12).

Marliana, E. (2011). Karakterisasi dan Pengaruh NaCl terhadap Kandungan Oksalat dalam Pembuatan Tepung Talas Banten. *Skripsi*. Fakultas Teknologi Pertanian Institut Pertanian Bogor, Bogor.

Massey, K.L. (2007). Food Oxalate: Factors Affecting Measurement, Biological Variation, and Bioavailability. *Journal of the american dietetic Association* 107:1191-1194. https://doi.org/10.1016/j.jada.2007.04.007

Mayasari, N. (2010). Pengaruh Penambahan Larutan Asam dan Garam sebagai Upaya Reduksi Oksalat pada Tepung Talas (*Colocasia esculenta* (L.) Schott). *Skripsi*. Departemen Teknologi Industri Pertanian Fakultas Teknologi Pertanian Institut Pertanian Bogor, Bogor.

Nadia, L. & Hartari, A. (2012). Potensi Umbi Uwi Ungu sebagai Bahan Pangan dan Khasiatnya sebagai Pangan Fungsional. Laporan Penelitian Hibah Bersaing Tahap II, Universitas Terbuka.

Noonan, S. C. & Savage, G. P. (1999). Oxalate content of Food and Its Effect on Humans. Asia Pacific *Journal of Clinical Nutrition* 8(1): 64 – 74.

Purnobasuki, H. (2011). Inklusi Sel. http://skp.unair.ac.id/repository/Guru-Indonesia/InklusiSel_HeryPurnobasuki_242.pdf. Accessed 30 Juli 2017.

Purwaningsih, I. & Kuswiyanto. (2016). Perbandingan Perendaman Asam Sitrat dan Jeruk Nipis terhadap Penurunan Kadar Kalsium Oksalat pada Talas. *Jurnal Vokasi Kesehatan* 1(2): *293–297.*

Sutrian, Y. (2011). Pengantar Anatomi Tumbuh-tumbuhan: Sel dan Jaringan. Rineka Cipta, Jakarta.

Wahyudi, D. (2010). Pengaruh Suhu Perendaman terhadap Kandungan Oksalat dalam Talas pada Proses Pembuatan Tepung Talas. *Skripsi*. Departemen Teknologi Industri Pertanian Fakultas Teknologi Pertanian Institut Pertanian Bogor, Bogor.

Wibawa, I. P. A. H., A. Kurniawan & B. Adjie. (2011). Studi Keragaman Jenis, Kandungan Gizi Esensial dan Kalsium Oksalat *Dioscorea* di Pulau Bali dan Lombok. *Buletin Kebun Raya* 2 (14): 1-8.

Wiguna, I. P. G. S. D., Prasetia, I. G. N. J. A., and Arisanti, C. I. S. (2014). Pengaruh Variasi Suhu Pemanasan terhadap Spesifikasi Amilum Singkong *Fully Pregelatinized* sebagai Eksipien Tablet. Jurusan Farmasi Fakultas Matematika dan Ilmu Pengetahuan Alam Universitas Udayana, Bali.

Yalindua, A. (2014). Potensi Genetik Klon Tanaman Uwi (*Dioscorea alata* L.) asal Banggai Kepulauan sebagai Sumber Pangan dalam Menunjang Ketahanan Pangan Nasional. *Disertasi*. Program DoktorSekolah Pascasarjana Institut Pertanian Bogor, Bogor.

Zimmermann, D. J., Hesse, A. & Unruh, G. E. (2005). Influence of a high-oxalate diet on intestinal oxalate absorption. *World J Urol* 23: 324–329. https://doi.org/10.1007/s00345-005-0028-0