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Chlorogenic Acid Yacon Tubers [*Smallanthus sonchifolia* (Poepp. Et Endl.) H. Robinson]. On the Use of Natural Inhibitors and Storage Temperature Variations

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***Abstract*— The aim of the study was to obtain the optimum content of chlorogenic acid (CA) yacon tubers on the use of natural inhibitors through a combination of immersion in natrium chloride, ascorbic acid and citric acid with 14 days storage at 5 and 15°C temperature variations. Yacon tubers are obtained from the planting area of 1800 meters above sea level (mdpl) with harvest ages of 8 and 10 months. Data were analyzed through two-way Anova (α = 5%). The results of the study: The optimum content CA of 8 months harvest yacon tubers was 113.10 mg/L, through natural immersion of inhibitors NaCl, ascorbic acid and citric acid respectively at 5℃ storage temperature.**

Keywords*— *chlorogenic acid (CA), harvest time, natural inhibitors, storage temperature

I. INTRODUCTION

Yacon has good potential to be developed, its tuber as an antidiabetic sweetener, anti-hypercholesterolemia, contains antioxidant polyphenols especially chlorogenic acid (CA) (942 mg per kg dry weight); the leaves contain high protein (11-17% dry weight) and as an antifungal [1]. The main bioactive compounds of yacon tubers are fructooligosaccharides (FOS) and CA. As an antioxidant, CA also has antiviral and bacterial functions. According to [2], in its role on carbohydrate metabolism, CA is able to inhibit glucose-6-phosphatase translocase-1 and decrease the sodium gradient that regulates glucose transport and stimulates insulin secretion. Against lipid metabolism, CA inhibits the activity of triglyceride-forming enzymes and cholesterol, namely fatty acid synthase, 3-hydroxy-3-methylglutarilCoA reductase, and Acyl-CoA cholesterol acyltransferase.

Yacon is an adaptive plant to regional heights, growing at an altitude of 500-2750 masl [3]. Climate is a major environmental factor affecting the composition of CA. According to Galani et al. [4], various factors affect levels of polyphenol compounds, such as growth conditions, processing and storage conditions after harvest. Air temperature not only affects tissue growth but also the process of respiration and the biochemical process of photosynthesis. Ierna and Melilli [5] found a decrease in the content of phenolic compounds in potatoes during the delay in harvest time. Madiwale et al. [6] obtained increased levels of CA, caffeic acid and synaptic acid in potatoes during 90-day storage at 3℃. Pek et al. [7] get external factors, such as season, rainfall, extreme temperatures, soil composition, significantly influence the content of CA bioactive compounds in broccoli.

CA is a main biphenyl compound from yacon tubers, which will be enzymatically oxidized by polyphenol oxidase or peroxidase, forming o-quinone and melanoidin resulting in brown color of the freshly cut tubers. The activity of peroxidase and polyphenol oxidase was maximum at pH 6.5 and 7.0. Peroxidase activity was highest at 70ºC and remained active for a period of 120 min at 70 and 80ºC. Polyphenol oxidase activity was highest at 20ºC and remained active for a period of 120 min at 40 and 50ºC and was inactivated after 10 min at 60ºC [8]. Various ingredients to prevent the effects of enzymatic browning; Ascorbic acid acts as a reducing agent and transfers oxygen to the reaction of o-quinone formation, citric acid as an acidulant agent and chelating enzyme, while sodium chloride inhibits the activity of peroxidase and polyphenol oxidase. Qiong Bo et al. [9] found that the interaction of citric acid with ascorbic acid was an

effective inhibitor. Ascorbic and citric acid found in lemons (pH ± 2.2) 64% and 5-6%, respectively, can function as natural inhibitors. Besides being oxidized, CA is also hydrolyzed by chlorogenic hydrolase to form caffeate and quinate, optimum pH 7.5 and temperature 37℃ [10]; however, caffeine inhibits the hydrolysis of α-amylase and α-glucosidase (the main enzyme for type 2 diabetes) significantly than CA [11].

The purpose of this study: to obtain optimum CA yacon tuber levels, through the use of natural sodium chloride/NaCl inhibitors, ascorbic acid and citric acid, as well as variations in storage temperatures of 5 and 15℃. Yacon bulbs are planted at an altitude of 1800 masl, harvest ages 8 and 10 months, with a storage time of 14 days.

II. METHODS

A. Tools and Materials

The tools used in the research are laboratory glass tools, TLC, and UV-VIS instrument. The main material was yacon tubers were taken from Argosari village, Senduro Lumajang, 1800 masl and harvest ages of 8 and 10 months. For ± 2 cm thick yacon tubers soaked in sequence in an inhibitor solution, namely: 3% NaCl (P1), 3% NaCl, 1% ascorbic acid (P2), and 3% NaCl, 1% ascorbic acid, 0.2% citric acid P3) for 5 minutes each and drain. Stored in a sealed plastic bag for 14 days, at 5 and 15ºC. Determination of the concentration of inhibitors based on research [12],[13].

B. Procedures

Measurement of water level content of yacon tuber was conducted through the drying method. Determination of CA levels through the TLC densitometry method, at the Faculty of Pharmacy, Universitas Negeri Jember. The treated yacon tubers were extracted with methanol p.a and a yacon extract (100 µL) was prepared with the addition of 100 µL of standard CA solution (Sigma Aldrich). The stages of the densitometry method are the optimization of the analysis conditions, validation of the analysis method, determination of content. Optimization includes eluent optimization, determination of maximum wavelength and optimization of test concentrations. The validation stages of the analytical method include linearity test, sensitivity test (detection limit and quantitation limit), selectivity and specificity test, precision and accuracy test. Optimum conditions of the method: pa methanol solvent, silica Gel 60 F254 stationary phase, formic acid mobile phase: ethyl acetate: aquabides (v /v/v) = 1: 8: 1.5, test concentration of 50 ppm and slab analyzed with the win CATS densitometry scanner Camag uses a UV-VIS detector at a wavelength of 335 nm as well as an ascending development method. CA analysis method in yacon tubers extract provides linear analysis results with a correlation coefficient r = 0.998. Data were analyzed through t-test and ANOVA two way (α = 5%)

III. RESULTS AND DISCUSSION

Water levels and CA of yacon tubers without inhibitor treatment and storage are shown in Table 1.

TABLE I. WATER LEVELS AND CA OF YACON TUBERS WITH 8 AND 10 MONTHS OF HARVEST TIME.

Harvest Time (month)	Water Level (%)	CA (ppm)
8	89.769±1.774a	75.102±0,295a
10	93.280±1.051b	63.648±0.405b
Significance	p<0.05	p<0.05

Notes: values/numbers with different superscript letters mean their different significantly.

CA levels of harvest age of 8 months are higher than 10 months, significantly. Yacon tuber CA levels were also obtained at 12 months 1800 masl harvest time was 62.2 ppm. From the comparison of CA yacon tuber content, the harvest age is 8,10 and 12 months, so the good harvest age for CA is 8 months. After 8 months of harvest, CA levels decrease. A good harvest age showed the highest enzyme activity for CA formation at the age of 8-month yacon bulbs.

According to Slimestad and Verheul [14] there will be a decrease in CA after maturation and during storage after harvest; Likewise, according to Ierna and Melilli [5], there was a decrease in the content of phenolic compounds in potatoes during the postponement of harvest time. Koshiro et al. [15], in the analysis of CA biosynthesis in the growth and ripening of fruit from coffee plants, transcription of PAL1, C3´H and CCoAMT, three genes related to CA biosynthesis, was detected at each growth, but significantly less at full ripening stage. CA is formed through the amino acid phenyl alanine. The biosynthetic activity of 5-O-Caffeoylquinic acid is high through the incorporation of phenylalanine to form CA, obtained in young fruits at the pericarp stage, endosperm production and at the seed development stage; this stage comes before the ripening process. In yacon plants, Itaya et al. [16] obtained, the activity of fructan bioactive-forming enzymes in yacon tuber rhizomes during the growth of yacon plants; The highest activity is found in early tuber formation (3 month old plants) and flower formation phase (7 month old).

Water level of yacon tubers at 8 and 10 months of age that have been subjected to variations in the use of NaCl inhibitors, ascorbic acid (AA), citric acid (AS) and storage temperature, are shown in Table 2 and 3.

TABLE II. TABLE TYPE STYLES

Storage Temperature (℃)	Natural Inhibitor		
	NaCl	***NaCl, AA***	***NaCl, AA, AS***
5	91.076 ± 1.081^{ac}	87.877 ± 1.093^{b}	87.948 ± 3.193^{b}
15	90.089 ± 1.169^{abc}	88.987 ± 2.372^{ab}	92.373 ± 0.852^{c}

Notes: NaCl 3%; AA 1%; AS 0.2%; values/numbers with different superscript letters mean their different significantly.

TABLE III. TABLE TYPE STYLES

Storage Temperature (℃)	Natural Inhibitor		
	NaCl	***NaCl, AA***	***NaCl, AA, AS***
5	90.418 ± 0.658^{a}	92.024 ± 0.093^{b}	93.726 ± 0.226^{c}
15	93.278 ± 0.436^{cd}	92.234 ± 0.228^{eb}	95.018 ± 0.231^{f}

Notes: NaCl 3%; AA 1%; AS 0.2%; values/numbers with different superscript letters mean their different significantly.

At 10 months of harvest, water levels higher than 8 months were obtained. Tubers of 10-month-old harvest appear to begin to rot and runny. This is due to the longer age of harvest the softer the tissue, the tubers are damaged and easily attacked by diseases that rot. Water is formed due to the overhaul of sugar into simpler compounds and at the same time there will be evaporation of water through lenticels. The difference in the rate of formation of water in the tissue and the rate of evaporation will determine the water content in a material. Water in the cell affects the turgor pressure of the cell. If the water in the cell increases beyond the normal state, the cell will break and the cell contents will come out and the cell violence will disappear. Therefore, turgor affects the hardness of parenchymal cells and thus also affects the texture of the material. Tuber damage may also be caused by a decrease in tuber hardness. In fruit hardness caused by the content of pectin substances in the middle lamella of cells, which are not water soluble. During the maturation process various hydrolase enzymes play a role in pectin substances being a water-soluble component so that the total pectin substance will decrease in levels and the water-soluble component will increase in number and soften.

Yacon tubers stored at 15℃ produced higher water content than 5℃ storage. Cooling is one method to slow down biological reactions including the rate of respiration in food so that it can extend shelf life, reduce product damage; in addition, cold temperatures will prevent and slow down bacterial growth so that tissue decay or damage is greater at 15℃ than 5℃.

At the tuber age of 10 months of harvest, there were significant differences in water content between inhibitor treatments. In soaking with each inhibitor solution carried out for each 5 minutes. Water content data shows that there are a number of inhibitor solutions that enter the plant cell tissue, and the highest is the use of NaCl, ascorbic acid, and citric acid.

The results of CA identification in yacon tubers with certain conditions (harvest time, storage time, and storage temperature) are presented in Table 4 and 5.

TABLE IV. TABLE TYPE STYLES

Storage Temperature (℃)	**Natural Inhibitor**		
	NaCl	***NaCl, AA***	***NaCl, AA, AS***
5	96.60 ± 0.596^{a}	106.34 ± 0.619^{b}	113.10 ± 0.587^{c}
15	69.22 ± 0.804^{d}	98.74 ± 0.546^{e}	108.92 ± 0.349^{f}

Notes: NaCl 3%; AA 1%; AS 0.2%; values/numbers with different superscript letters mean their different significantly.

TABLE V. TABLE TYPE STYLES

Storage Temperature (℃)	**Natural Inhibitor**		
	NaCl	***NaCl, AA***	***NaCl, AA, AS***
5	65.86 ± 0.673^{a}	70.42 ± 0.389^{b}	77.9 ± 0.356^{c}
15	57.80 ± 0.570^{d}	61.74 ± 0.182^{e}	64.30 ± 0.596^{f}

Notes: NaCl 3%; AA 1%; AS 0.2%; values/numbers with different superscript letters mean their different significantly.

The data of Tables 4 and 5 shows that there was a significant difference between the use of natural inhibitors and the storage temperature ($p < 0.05$). The highest CA rates between yacon tubers 8 and 10 months old on 14-day storage treatments, as well as the use of natural inhibitors and storage temperature variations, were at yacon tubers at 8 months old, storage temperature of 5ºC, 113.10 ppm. The rate of CA after storage with the inhibitor, for an average yacon harvested at 8 months is higher than 10 months. As a result of the CA rate, the average 8 month old yacon harvest is better than 10 months.

At 15ºC storage CA levels are lower than 5ºC, harvesting time is 8 or 10 months. At 15ºC the enzyme becomes more active than 5ºC. According to Mizobutsi et al. [8], CA is oxidized enzymatically by polyphenol oxidase or peroxidase to its o-quinone; polyphenol oxidase activity was highest at 20ºC and remained active for a period of 120 min at 40 and 50ºC and was inactivated after 10 min at 60ºC. So the best storage for groceries is in a cool place. Cold temperatures will prevent and slow down bacterial growth and prevent tubular damage.

CA content of yacon tubers in storage, increases sequentially with NaCl (P1) inhibitors, NaCl and AA (P2) inhibitors, NaCl, AA and AS (P3) inhibitors, at 8 or 10 months of harvest. NaCl acts as an enzyme inhibitor (polyphenol oxidase and peroxidase); ascorbic acid as a reducing agent, reduces o-quinone back to biphenyl compounds, reduces Cu2+ the active side of the enzyme to Cu+, and transfers oxygen to the o-quinone formation reaction. Citric acid functions as an acidulant agent, reducing the pH and chelating agent against Cu on the active side of the enzyme. According to Suttirak and Manurakchinakorn [13], at a pH lower than 4, polyphenoloxidase activity is very low due to the release of Cu on the active site. Maximum activity of peroxidase and polyphenoloxidase at pH 6.5 and 7.0; although CA is also hydrolyzed by chlorogenate hydrolase to form caffeate and quinate, the optimum pH is 7.5 and the temperature is 37℃ [10]. The effectiveness of using three enzyme inhibitors simultaneously prevents changes in CA in the hydroxylation and oxidation reactions to form o-quinone compounds in browning reactions. Therefore, a good color is obtained on the product and the highest CA content.

IV. CONCLUSION

Yacon tuber harvested at an altitude of 1800 masl, the time of harvest that produces the highest CA level is 8 months. The highest CA content in 14 days storage of yacon tubers at 8 months of age, was obtained at 5ºC with the use of 3% NaCl natural inhibitors, 1% ascorbic acid and 0.2% citric acid with soaking time of each @ 5 minutes. It is recommended that further research with a range of height of the planting area and harvest time vary, so that a complete picture of changes in the levels of CA can be obtained.

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