Advances in Engineering Research, volume 196
International Joint Conference on Science and Engineering (IJCSE 2020)

Fruktooligosaccharides of Yacon Tubers (*Smallanthus sonchifolia*) on Variation in Height of Planting Area, Harvest Time and Storage with Natural Inhibitors

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ABSTRACT
Yacon (*Smallanthus sonchifolia*) was a potential plant to be developed: its tuberswere as antidiabetic sweetener, anti hypercholesterolemic, contains antioxidant polyphenols especially chlorogenic acid and cancer prevention; the leaves contain high protein and as an anti-fungal. The main bioactive compounds of yacon tubers are fructooligosaccharide (FOS).The purpose of this research: to obtain the optimum level fructooligosaccharides (FOS) yacon tubers at variations in height, harvest time and storage. The height variations at the area 900, 1500, and 1800 mamsl, the harvest time 8 and 10 months. The 14 days shelf life at 5 and 15oC with the combination immersion in 3% sodium chloride; 3% sodium chloride followed 1% ascorbic acid; 3% sodium chloride followed 1% ascorbic acid and then 0.2% citric acid, for 5 minutes respectively. Results of the study: 1) Optimum FOS levels in the planting area of 1800 masl harvest time of 8 months. 2) At a 14-days shelf life of yacon tubers in the 1800 m above sea level planting area and 8 months of harvesting, the highest levels of FOS at a storage temperature of 5^{o}C with the use of inhibitors through immersion by sequence, NaCl, ascorbic acid and citric acid.

Keywords: *FOS, Yacon tubers, Mamsl,Harvest time, Natural inhibitors*

1. INTRODUCTION

Yacon (*Smallanthus sonchifolia*) was a potential plant to be developed: its tuberswere as antidiabetic sweetener, anti hypercholesterolemic, contains antioxidant polyphenols especially chlorogenic acid (942 mg per kg dry weight), and cancer prevention [1]; the leaves contain high protein (11-17% dry weight) and as an anti-fungal [2]. The main bioactive compounds of yacon tubers are fructooligosaccharide (FOS). FOS is an oligosaccharide in which fructosyl units bonded with glucose in β-2,1 position; 2-9 (glucose- (fructose) n) polymerization degree; the main components are 1-glucose (GF2), nystose (GF3), and 1-b-D-fructofuranosylnystose (GF4).

Yacon is an adaptive plant with high altitude, growing at an altitude of 500-2750 m above sea level [3]. As in [4], FOS of yacon tubers will be different if the height for cultivation of the plant is different. At planting altitudes of 2600, 2900 and 3200 (Peru), 2560 (Ecuador), 1900 (Argentina), 2100 (Bolivia), and 1800 masml (Bolivia), dried yacon tubers have a FOS content of 66 gr / 93 gr, 53 gr / 91 gr, 54 gr / 91 gr, 52 gr / 91 gr, 53 gr / 92 gr, 60 gr / 93 gr, and 60 gr / 93 gr. The altitude affects the exposure to solar radiation and environmental temperature. The air temperature not only affects tissue growth but also the process of respiration and biochemical processes of photosynthesis. CO_2 fixation in photosynthesis is controlled by enzymes and increases with increasing temperature. Consequently, the composition of the material contained in the yacon will be different.

As in [5], the chemicals content of yacon are influenced by the planting temperature, growing conditions, and harvest time. Asami et al. [6], explained that the content of fructose, glucose, sucrose, and FOS (GF2-GF9) changes during plant growth and storage of yacon tubers. The level of saccharide polymerization increases during the period of plant growth, then decreases during the shelf life. If yacon tubers are not harvested and remain in the soil, the FOS content decreases between 21-41% while the fructose, glucose, and sucrose content increases.

One of the factors that directly affects the levels of FOS tubers of yacon is enzyme activity. Itaya et al. [7]

obtained, the activity of sucrose 1-fructosyl transferase (1-SST) enzyme, fructose 1-fructosyl transferase (1-FFT) and fructan 1-eksohidrolase (1-FEH) in rhizome during yacon plant growth. The highest activity value is found at the beginning of tuber formation (3 months) and flower formation (7 months). 1-FEH catalyzes the breakdown of FOS to fructose [5], [8] and it is optimum at pH 4.5-5.5 and temperatures of 25-40°C [9].

Yacon tubers quickly turn dark green or black at room temperature cutting. Browning reaction is related to polyphenol oxidase (PPO) activity. Optimum activity of PPO yacon tubers at 30ºC and pH 6.4 [10], relatively stable at 60-70ºC and inactive at 80-90ºC. Inactivation of 1-FEH and PPO can be done, among others, through the temperature of denaturation, reducing, chelating, acidulant and firmness agents, namely through the use of natural inhibitors.

The focus of this study is to obtain optimal FOS content through the use of natural inhibitors in the variation in height of the planting area and optimal harvest time.

1.1. Experimental

The study consisted of two stages. Stage 1 was to determine the optimum conditions for variations in the height of the planting area and harvest time for the bioactive component of yacon tuber. Yacon tubers were obtained from three different heights namely 900, 1500, and 1800 masl, at harvest time of 8 months and 10 months. Stage II was the use of natural inhibitors for the 1-FEH enzyme and PPO in the shelf life. From the results of phase 1 research, one optimum condition was selected and used for the second phase of the study. Yacon tuber pieces are soaked sequentially in a 3% NaCl inhibitor solution, 1% ascorbic acid and 0.2% citric acid [11]; each for 5 minutes and drained. The shelf life is 14 days, temperatures 5ºC and 15ºC. Treatment of yacon tubers was extracted, then HPLC analysis for FOS content.

The filtered extracts from yacon tubers were concentrated in vacuo at 30-35°C to dryness. The dry concentrate were redissolved in 1 ml of distilled water, filtered through 0.22 µm millipore membrane and analyzed by High Pressure Liquid Chromatography Agilent 1100 series, Zorbax Carbohydrate column (5µm, 4.6x150mm) at room temperature, and ELSD detector. A mixture of acetonitrile and water (75/25 v/v) as eluent was used as mobile phase at flow rate of 1.4 ml/min at 30ºC. The total yield of FOS was calculated as the sum of 1-kestosa (GF2), nystose (GF3), and 1F-fructofuranosylnystose (GF4).

2. RESULTS AND DISCUSSION

2.1. Fructooligosacharides Of Yacon Tubers At Variations Of High Altitude Planting And Harvest Time

The results of the analysis of yacon tuber FOS content on variations in planting height and harvest time are shown in Table 1

Table 1. Yacon tubers fos content (%)

High Alt. Plant. (masl)	Harvest Time (mounth)	GF2	Nystose (GF3)	Fructosylnystose (GF4)	X FOS (mg)	FOS mg/mg (dw)	%FOS (dw)
1800	8	146.085	131.1	168.6	445.785	0.429	42.982
1500	8	66.515	50.160	68.185	184.860	0.205	20.534
900	8	103.98	15.758	24.037	143.775	0.133	13.336
1800	10	97.415	70.185	92.875	260.475	0.250	25.043
1500	10	60.910	45.835	63.545	170.290	0.246	24.592
900	10	57.895	56.040	44.880	158.815	0.146	14.611

The yacon tubers FOS content at an altitude of 1800 masml with 8 months harvest time is the optimum condition. Enzymes play a role in fructant formation: β-fructosiltransferase namely 1-SST (sucrose fructosyltransferase), 1-FFT and 6-FFT (fructanfructosyltransferase). Asami et al. [6]: [fructose], [glucose], [sucrose], and FOS (GF2-GF9) undergo changes during plant growth and storage of yacon tubers. The level of saccharide polymerization has increased during the plant growth period (8 months), then decreased during the storage period. According to Oliveira & Nishimoto [3], in the tropics, the best period for yacon harvest related to the concentration of fructans and the proportion of mono-disaccharides is between 31

and 35 weeks. Itaya et al. [7] identified 1-SST and 1-FFT activity in yaconrizom during the growth of yacon plants. The highest activity value at the beginning of tuber formation (3 months old plant) and flower formation phase (7 months old). Yacon plants have high hydrolytic activity in the ripening phase of roots/tubers, so that low polymerization (fructan) will exceed the high degree of polymerization (inulin). Hydrolysis of the fructan compound by 1-FEH (fructanexohydrolase).

2.2 The Using of Natural Inhibitors for 1-FEH of Yacon Tubers in Shelf Life

The water content of yacon tubers which had been treated using the variations of inhibitors and storage temperatures are shown in Table 2. The concentration of NaCl, ascorbic acid (AA), and citric acid (CA) are 3%, 1%, and 0.2%

Storage Temprature (oC)	Natural Inhibitors		
	NaCl	*NaCl, AA*	*NaCL, AA, CA*
5	91.076 ± 1.081 ac	87.948 ± 3.193 b	87.877 ± 1.093 b
15	90.089 ± 1.169 abc	88.987 ± 2.372 ab	92.373 ± 0.852 c

Note: The alphabet (superscript position) indicates the value is significantly different.

The total of FOS on natural inhibitor use with 14 days storage at 5ºC and 15ºC were written in Table 3 and 4.

Table 2. Water content of yacon tubers with 8 months harvest time (%)

Table 3. Yacon tubers fos content (%)

Inhibitor	T (ºC)	Kestose (GF2)	Nystose (GF3)	Fructosylnystose (GF4)	X FOS mg	FOS mg/mg (dw)	%FOS (dw)
NaCl	5	155.045	136.705	188.68	480.430	0.241	24.095
NaCl, AA	5	433.930	51.440	*	485.370	0.257	25.678
NaCl, AA,CA	5	715.625	91.765	166.17	973.560	0.487	48.738
NaCl	15	84.670	84.8	94.445	263.915	0.131	13.112
NaCl, AA	15	90.130	87.11	112.485	289.725	0.145	14.538
NaCl, AA, CA	15	391.455	54.38	*	445.835	0.224	22.425

*) not detected

The highest content of FOS was at storage temperature of 5ºC with a inhibitors combination of NaCl, ascorbic acid and citric acid. 1-FEH hydrolyzes FOS at optimum conditions pH 4.5-5.5 and 25-40ºC. The degree of acidity of the NaCl solution is about 6-7, ascorbic acid ± 3 and at the addition of citrate to ± 2.3. The pH of the inhibitor solution used to soak the yacon tubers will affect the yacon tuber cells. Changes in pH affect the structure of enzyme ions, thus affecting the effectiveness of the active side of the enzyme against the enzyme-substrate complex. So at low pH the enzyme becomes inactive. At a temperature of 15ºC the enzyme works more actively than 5ºC so that the level of FOS at storage is 15ºC, lower than 5ºC. It is recommended, storing food at temperatures of -2ºC to 10ºC due to low temperatures slowing metabolic activity and inhibiting microbial growth. This can be seen in the results of storage that storage temperature of 5ºC is better (clearer) than 15ºC (changing smell and turbid).

Itaya et al. [7] obtained, the activity of sucrose 1-fructosyl transferase (1-SST) enzyme, fructose 1-fructosyl transferase (1-FFT) and fructan 1-eksohidrolase (1-FEH) in rhizome during yacon plant growth. The highest activity value is found at the beginning of tuber formation (3 months) and flower formation (7 months).

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3. CONCLUSION

In the variation in treatment of the height of the planting area and harvest time for yacon tubers, the optimum results were obtained: increasing the height of the planting area, increasing the level of FOS. The highest FOS content was obtained in the planting area of 1800 masml with harvest time of 8 months.

The use of natural inhibitors of NaCl, ascorbic acid and citric acid with a storage time of 14 days for 8 months harvest time of yacon tubers, were obtained: (1) FOS content at storage temperature of 5ºC is higher than storage temperature of 15ºC; (2) the most effective natural inhibitor to maintain the FOS bioactive components in the yacon tubers was a sequential soaking in 3% NaCl solution, then in 1% ascorbic acid, and continued in 0.2% citric acid with immersion time of 5 minutes for the each treatment.

Further research is recommended with varying ranges of planting area and harvest time, so that there is a complete picture of changes in the levels of bioactive components of FOS in yacon tubers.

ACKNOWLEDGMENT

The acknowledgment was addressed to Universitas Negeri Surabaya who have funded this research. We acknowledged to the laboratory staffs in Universitas Negeri Surabaya who supported the research. Also, we appreciated to the farmers who supported and helped the researchers for planting the yacon.

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