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# THE EFFECT OF STORAGE AND BOILING TIME OF YACON TUBERS TO SCFA AND LACTIC ACID CONCENTRATIONS BY *Bifidobacteriumlongum* Reuter ATCC 15707 AND *Lactobacillus acidophilus* IFO 13951

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**ABSTRACT**

This study aimed to describe the effect of storage (raw, 7 and 14 days) and boiling time (raw and 60 minutes) of yacon tubers to short-chain fatty acids (SCFA) and lactic acid concentrations in media supplemented yacon tubers extracts, as fermentation results of *Bifidobacteriumlongum* Reuter ATCC 15707 and *Lactobacillus acidophilus* IFO 13951. High-Performance Liquid Chromatography (HPLC) was applied for analysis the concentration of 1) fructooligosaccharide (1-kestosa, nystose, and $1^F$-fructofuranosylnystose), saccharides (glucose, fructose, and sucrose) yacon tubers, 2) SCFA (acetic acid, propionic acid, and butiric acid) and lactic acid in fermentation media. Increasing storage and boiling time, would increase of saccharides and decreasefructooligosaccharideconcentrations. In the media supplemented with yacon tubers extracts, *Bifidobacteriumlongum* Reuter ATCC 15707 produces SCFA and lactic acid higher than *Lactobacillus acidophilus* IFO 13951. Duration of storage and boiling affects the levels of SCFA- lactic acid. The highest concentrations of SCFA-lactic total on media with supplemented 14 days storage and boiling 60 minutes or raw/fresh yacon tubers extracts.

**Keywords:** SCFA, lactic acid, yacon, *Bifidobacteriumlongum* Reuter ATCC 15707, *Lactobacillus acidophilus* IFO 13951.



## INTRODUCTION

Fructooligosaccharide (FOS) are oligosaccharides of the glucose-fructose compound, consist glucose monomer (G) linked α-1,2 to two or more β-2,1-linked fructosyl units (F); the degree of polymerization 2-9 (glucose-(fructose) n) and the main components are 1-kestosa (GF2), nystose (GF3), and $1^F$-fructofuranosylnystose (GF4). That are found in fruits and vegetables such as yacon, bananas, onions, garlic. FOS can be produced by degradation of inulin, a mixture of poly and oligosaccharides structures α-D-Glu-(1-2)-[(β-D-Fru-(2-1)-]$^n$, or GFn (G = glucosyl units, F = fructosyl units, n = number of fructosyl units). FOS and inulin serves as a substrate for microflora in the large intestine, increases gastrointestinal tract health and bioavailability of minerals colon[1], as well as reducing the accumulation of harmful carcinogenic compounds and metabolites[2].

Yacon tubers, as opposed to most tuberous and root crops which deposit saccharides in the form of starch, cumulates saccharides in the form of FOS. The tuberous roots contain 0.3-3.7% protein and 70-80% saccharides (dry weight) mainly fructooligosaccharides[3]. Yacon tubers saccharides composed of fructose 350.1, glucose 158.3, sucrose 74.5, FOS (GF2-GF9) 206.4, and inulin 13.5 mg/g dry weight[4].In addition, it was reported that the content of FOS in yacon tubers vary widely, between 2.1-70.8 g/100 g dry weight[5]; 100 grams of fresh yacon tuber contains 6-12 grams FOS[6]. It has been identified that fructose, glucose, sucrose and FOS is changing during plant growth and yacon tubers storage[7].


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The changing is caused by the role of the fructan 1-exohydrolase (1-FEH), invertase and sucrose 1-fructosyltransferase (1-SST) enzymes. Yacon FOS decreased markedly by storage at 4 °C for 1 month, and FOS decreased by heating at 100 °C after peeling and grating[8].Decreasing activities of the enzyme invertase and 1-SST occurs during storage for one month[9]. The researchers also stated that cooking processes reduced the prebiotic contents until 25 to 77%.

The fermentation of FOS-inulin in the cecum by some strains of bacteria including *Bifidobacterium* and *Lactobacillus*, so as rapid and selectively stimulate its growth; this is due to the production of β-fructofuranosidase by *Bifidobacterium* and *Lactobacillus*[10]. Fermentation of intestinal microflora against FOS-inulin simultaneously will produce SCFA (short chain fatty acids: acetic, propionic, butyric, valerate), $CO_2$, $CH_4$, $H_2$, lactic acid, so that it is lowering the pH of the large intestine. In a consequence, it will inhibit the growth of pathogenic bacteria group and colon cancer cells in-vivo. Yacon tubers as a nutraceutical product for reducing triglycerides, reducing LDL cholesterol, and improving insulin levels[11], can be consumed in raw form or juice, processed into syrup and jam, dried by the sun or oven. Usage of yacon tubers with decreasing of boiling and storage time variation (60, 30, 0 minute/raw; 14, 7, 0 days/fresh) will improved the nutritional value of lipids and carbohydrates through decreasing level of triglycerides, cholesterol, and blood glucose[12].

This research aims to describe the effect of storage and boiling time of yacon tubers on: 1) the concentration of FOS bioactive compound and saccharides of yacon tubers, 2) fermentation activity of *Bifidobacteriumlongum* Reuter ATCC 15707 and *Lactobacillus acidophilus* IFO 13951in yacon tubers supplement media, through measurement of SCFA (acetic acid, propionic acid, and butiric acid) and lactic acid concentrations in the media. For this purpose, a combination of storage time 0/fresh, 7, and 14 days with boiling time of 0/raw and 60 minutes are used. The length of boiling time as it begins to boil, and storage in the shade.

## EXPERIMENITAL

The research was conducted through two stages involving (1) determining the concentration of FOS and saccharides yacon tubers and (2) determining fermentation activity of *Bifidobacteriumlongum* Reuter ATCC 15707 and *Lactobacillus acidophilus* IFO 13951 in media supplemented with yacon tubers. There were six variations of yacon tubers that have been treated with storage and boiling time, namely, T1 (fresh/raw yacon tubers and no boiling), T2 (fresh and 60 minutes of boiling time), T3 (7 days of storage time and no boiling), T4 (7 days of storage time and 60 minutes of boiling time), T5 (14 days of storage time and no boiling), and T6 (14 days of storage time and 60 minutes of boiling time).All sample yacon tubers were lyophilized (Martin Christ, Alpha 1-2 LDp) and milled to pass a sieve of 100 mesh.

The first stage (determining the concentration of FOS and saccharides yacon tubers) was water content analysis at 70ºC of freeze dried yacon tubers sample. FOS and saccharides were extracted by modification of Shiomi method[13]. Yacon tubers (10 g) were homogenized in 80 ml of ethanol (70%) and calcium carbonate (0.5g/L); immediately warmed up in ultrasonication water bath for 30 minutes at 50-60°C, then centrifuged at 11.000 rpm for 15 minutes. FOS is extracted in water with ultrasonication at 50-60°C because it dissolves in hot water but degraded at 90-10°C[14]. The residues were extracted 3 times with hot water (80ºC, 100 ml). 25 ml of filtered extracts were concentrated in a vacuum 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 FOS and saccharides concentrations were determined from standard curves made with known concentrations of each compound. Chromatograms were analyzed quantitative descriptively. The total yield of FOS was calculated as the sum of 1-kestosa (GF2), nystose (GF3), and $1^F$-fructofuranosylnystose (GF4); while saccharides as the sum of glucose, fructose and sucrose.

The second stage was determining fermentation activity of *Bifidobacteriumlongum* Reuter ATCC 15707 and *Lactobacillus acidophilus* IFO 13951 in a media supplemented with yacon tubers. Six kinds of yacon tubers extract as supplement of growth media of *Bifidobacteriumlongum Reuter ATCC* 15707 and *Lactobacillus acidophilus* IFO 13951.De Man, Rogosa and Sharpe broth/agar media (MRS) is used for *Lactobacillus acidophilus*, while MRS-cysteine HCl 0.05% for *Bifidobacteriumlongum* Reuter ATCC

15707. FOS concentration of yacon tubers suplemented is 2.0%[15,16]. FOS total of yacon tubers was 0.3730 g/g dry weight (Table-2). The yacon tubers extracts made from yacon powder was 2/0.3730, equal to 5.3619 gram per 100 ml media. Incubation time of anaerob fermentation was 24 hours, 37°C in anaerobic bath fermenter. The SCFA and lactic acid concentrations of fermentation media were determined using HPLC Hewlett Packard Series 1050, Zorbax 300 SB-C18 column (5µm) and UV detector. Components of SCFA measured in the research involved acetic, propionic, and butyric acid. A mixture of phosphate buffer (pH 2.20) and acetonitrile (70/20 v/v) was used as mobile phase at flow rate of 1.0 ml/min. Data were analyzed by Anova one-way ($\alpha = 5\%$).

## RESULTS AND DISCUSSION

### The Effect of Storage and Boiling Time to Yacon Tubers Oligosaccharides Content

The water content of freeze driedyacon tubers that have been treated with storage and boiling time (T1-T6) was presented in Table-1.

Table-1: Water Content of Freeze dried Yacon Tubers (Boiling and Storage Time)

| Sample Code | Water Content (%) |
|---|---|
| T1 | 9.0605 |
| T2 | 4.5455 |
| T3 | 1.2903 |
| T4 | 8.0378 |
| T5 | 0.2121 |
| T6 | 1.3468 |

Where, T1: fresh and no boiling, T2: fresh and 60 minutes boiling time, T3: 7 days storage time and no boiling, T4: 7 days storage time and 60 minutes boiling time, T5: 14 days storage time and no boiling, T6: 14 days storage time and 60 minutes boiling time

The supernatant resulted from milliporemembrane filtering was analyzed in terms of saccharides, glucose (G), fructose (F), and sucrose (S), as well as components of FOS including kestose (GF2), nystose (GF3), and kestopentaose (GF4). The results of T1 (Fig.-1), T2 (Fig.-2), T3 (Fig.-3), T4 (Fig.-4), T5 (Fig.-5), and T6 (Fig.-6) chromatogram showed different significantly.

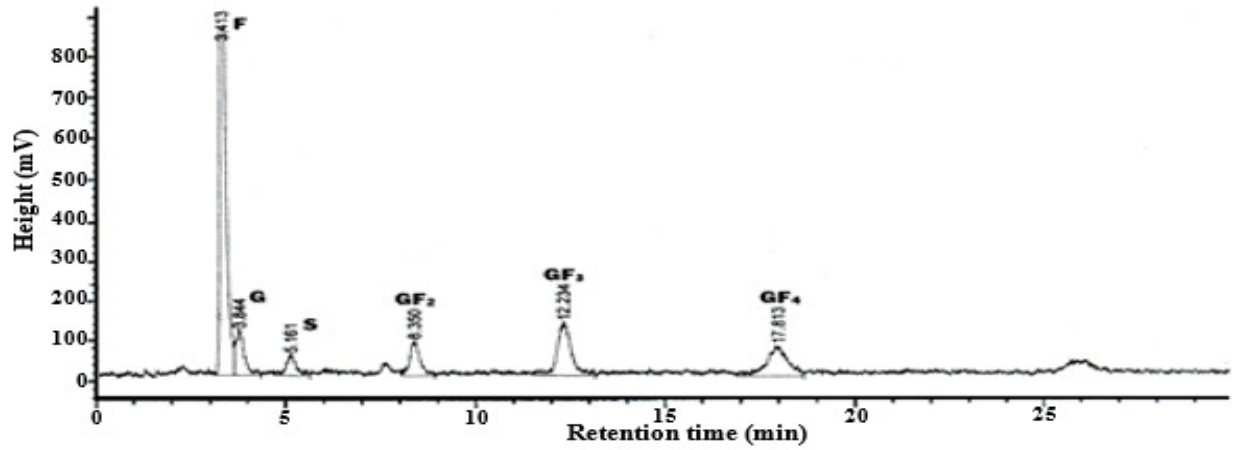


Fig.-1

Analysis of the chromatogram of the standard solution shows the retention time in a sequence of fructose, glucose, sucrose, GF2, GF3, and GF4 compound. Fructose, glucose, sucrose (2% w/v) at 3.497, 3.959, and 5.381, while GF2 (1:30% w/v), GF3 (1:28% w/v), and GF4 (2:20% w/v) 8.884, 13.131, and 17. 847 minutes respectively. Based on the water content of samples and HPLC chromatogram, consentration of FOS and saccharides yacon tubers samples can be identified, as showed in Table-2.

The Table-2 shows the effect of storage and boiling time variations on FOS and saccharides concentrations of yacon tubers. Increasing storage (0, 7, 14 days) and boiling time (0, 30, 60 minutes)

would decrease FOS and increase saccharides concentrations. The highest FOS percentage is shown by T1 (37.30), while saccharides by T6 yacon tubers (60.34).

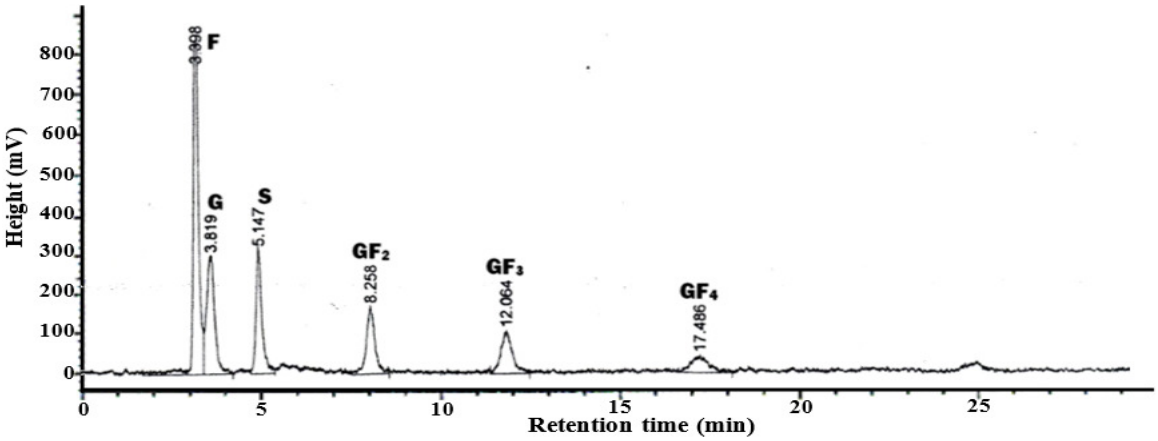


Fig.-2

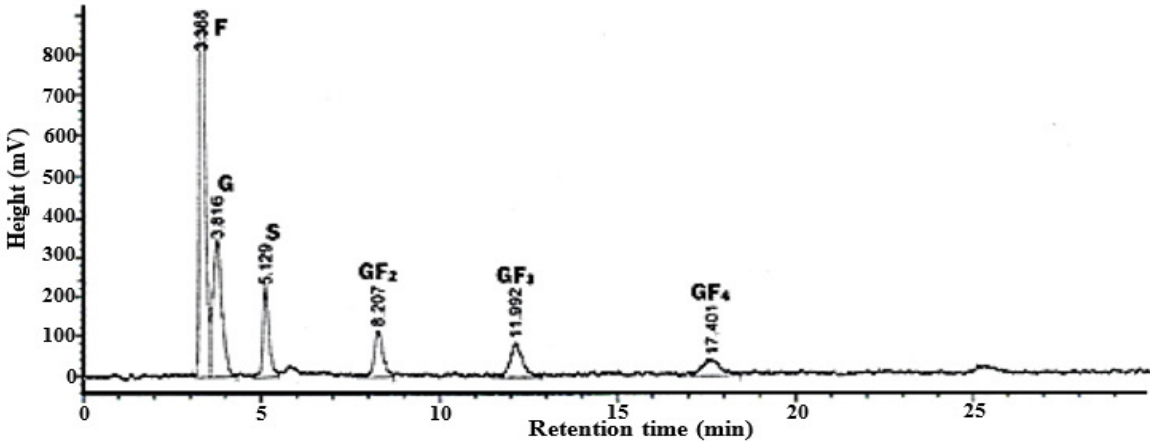


Fig.-3

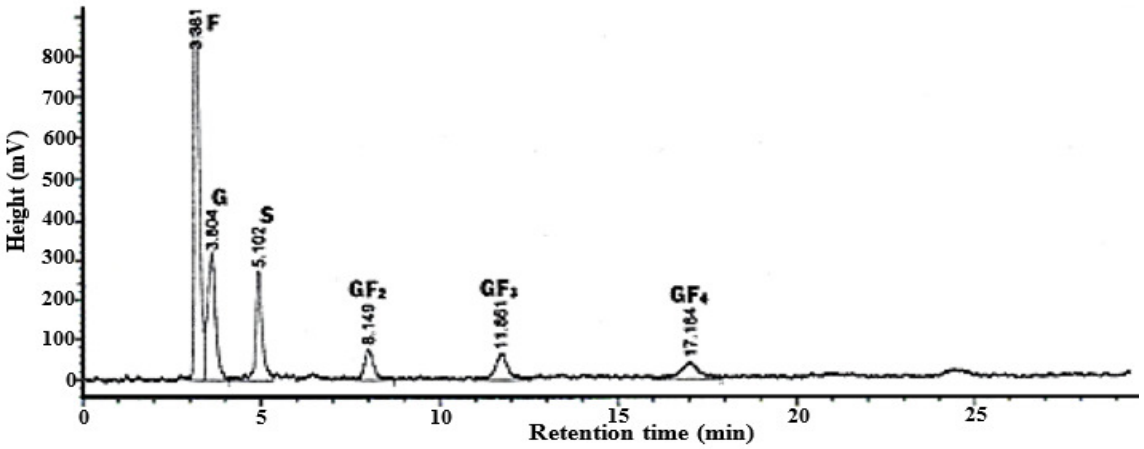


Fig.-4

There was a research finding that, after 12 days of shade storage, FOS concentration of yacon tubers were decreased from 50-62 to 27-37% of dry weight, while the concentration of saccharides increased from 29-34 to 48-52%. During the six days sunlight exposure, FOS concentrations were decreased from 50–62 to 29–44% and saccharides increased from 29–34 to 45–51% of dry weight[17].

In the process of storage after harvesting, a decline FOS with a low polymerization degree will causefructose increase[9]. Decreased levels of FOS in storage process is mainly related to fructan 1-exohydrolase (1-FEH) activity, hydrolyzes FOS to fructose[18]. The pH optimum of 1-FEH activities varies between pH 4.5-5.5, the temperature optimum ranges from 25-40ºC, and declined slightly over the 8 day at 20ºC[19].

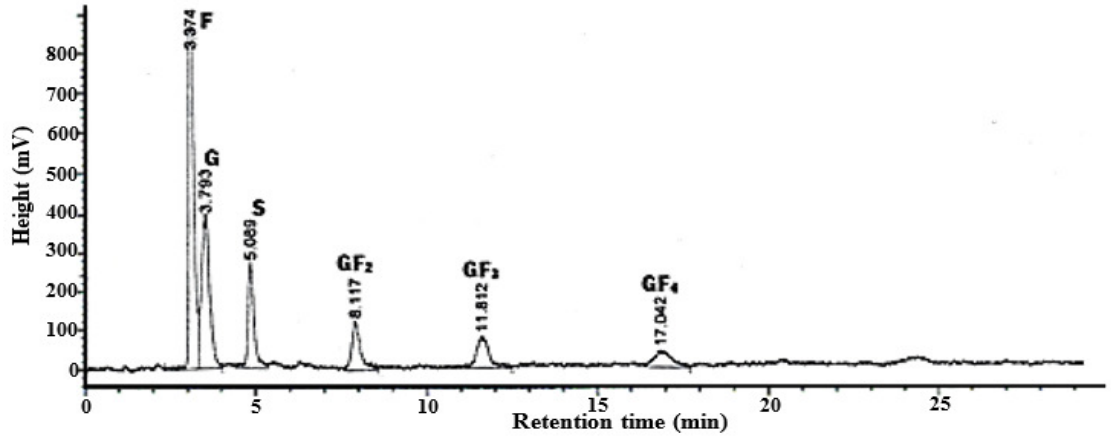


Fig.-5

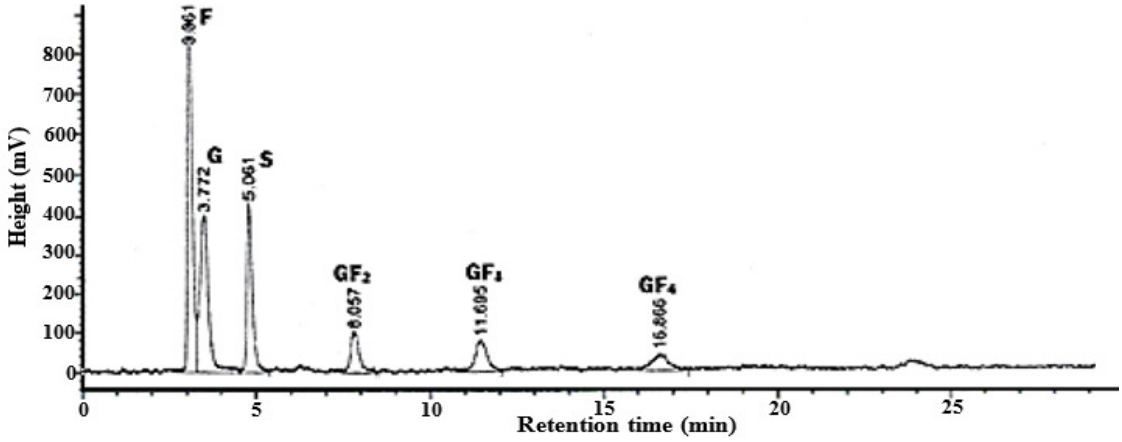


Fig.-6

Tabel-2: FOS and Saccharides Concentrations of Yacon Tubers

| Sample Code | FOS (% w/w) | | | | Saccharide (% w/w) | | | |
|---|---|---|---|---|---|---|---|---|
| | GF2 | GF3 | GF4 | Total | Fructose | Glucose | Sucrose | Total |
| T1 | 4.33 | 9.83 | 23.15 | 37.30 | 34.40 | 5.37 | 2.69 | 42.46 |
| T2 | 7.56 | 6.94 | 12.05 | 26.55 | 30.15 | 15.22 | 9.79 | 55.16 |
| T3 | 5.30 | 6.43 | 13.39 | 25.13 | 33.56 | 18.02 | 6.79 | 58.37 |
| T4 | 3.75 | 4.92 | 13.12 | 21.79 | 30.05 | 15.17 | 9.12 | 54.34 |
| T5 | 5.65 | 5.90 | 11.84 | 23.39 | 30.56 | 19.34 | 8.37 | 58.27 |
| T6 | 4.91 | 5.63 | 11.66 | 22.20 | 27.47 | 21.09 | 11.78 | 60.34 |

Under acidic condition (pH 2.7-3.3) and at 70-80ºCfructooligosacharides is so considerable for hydrolysis, but no significant at 60ºC[19]. All of the oligomers were degraded in 1-1.5 hours at 90-100ºC.The primary reaction of sucrose thermal degradation at 185ºC is the cleavage of glycosidic bond to produce glucose and fructose[20]. FOS is non-digestable saccharides in the digestive tract, because humans do not have the β-fruktofuranosidase/fruktanase enzyme. The bacteria in the large intestine have the enzymes need to break down FOS. FOS from yacon tubers as prebiotic, is a preferential energy source for these bacteria, including *Bifidobacteriumlongum* Reuter ATCC 15707 and *Lactobacillus acidophilus* IFO 13951. In this research, *Bifidobacteriumlongum* Reuter ATCC 15707 and *Lactobacillus acidophilus* IFO

13951have been grown in the media supplemented with yacon tubers extract. SCFA and lactic acid as fermentation results in media were analyzed by HPLC in Table-3.

Table-3: Content of Short Chain Fatty Acid and Lactic Acid (%) by *Bifidobacteriumlongum* Reuter ATCC 15707 and *Lactobacillus acidophilus* IFO 13951 In media supplemented with Yacon Tubers Extract.

| Bacteria and Suplement | SCFA Content (%) | | | Lactic Acid (%) |
|---|---|---|---|---|
| | Acetic Acid | Propionic Acid | Butiric Acid | |
| *B. Longum* | | | | |
| Control*) | $6.85^{a}$±0.17 | 0.34±0.01 | $0.11^{ac}$±0.03 | $2.64^{a}$±0.34 |
| + T1 yacon | $8.79^{b}$±0.11 | 0.21±0.01 | $0.20^{b}$±0.05 | $3.48^{be}$±0.13 |
| + T2 yacon | $8.14^{c}$±0.06 | 0.12±0.01 | $0.13^{ac}$±0.04 | $3.09^{c}$±0.08 |
| + T3 yacon | $7.92^{cd}$±0.27 | 0.14±0.01 | $0.10^{c}$±0.04 | $3.25^{bc}$±0.16 |
| + T4 yacon | $7.41^{e}$±0.28 | 0.13±0.02 | $0.12^{ac}$±0.04 | $2.74^{a}$±0.12 |
| + T5 yacon | $7.79^{de}$±0.08 | 0.25±0.08 | $0.11^{ac}$±0.01 | $3.53^{e}$±0.06 |
| + T6 yacon | $8.85^{bf}$±0.07 | 0.12±0.18 | $0.16^{ab}$±0.02 | $3.92^{d}$±0.04 |
| Significances | p=0.000 | p=0.340 | p=0.016 | p=0.000 |
| *L. acidophilus* | | | | |
| Control*) | $6.22^{a}$±0.04 | $0.31^{be}$±0.01 | 0.08±0.02 | $0.34^{ad}$±0.02 |
| + T1 yacon | $9.75^{b}$ ±0.15 | $0.35^{ad}$±0.02 | 0.08±0.02 | $1.60^{b}$±0.39 |
| + T2 yacon | $6.94^{cd}$±0.22 | $0.33^{abcd}$±0.01 | 0.12±0.02 | $1.02^{c}$±0.04 |
| + T3 yacon | $7.00^{d}$±0.15 | $0.33^{abcd}$±0.03 | 0.10±0.04 | $0.52^{a}$±0.01 |
| + T4 yacon | $7.86^{e}$±0.21 | $0.31^{ce}$±0.01 | 0.10±0.03 | $0.58^{ae}$±0.25 |
| + T5 yacon | $9.16^{f}$±0.16 | $0.35^{d}$±0.01 | 0.14±0.02 | $0.51^{af}$±0.01 |
| + T6 yacon | $8.92^{fg}$±0.20 | $0.33^{de}$±0.02 | 0.14±0.02 | $4.10^{g}$±0.16 |
| Significances | p=0.000 | p=0.037 | p=0.103 | p=0.000 |

Control*): media without Yacon Tubers Extract.

The total SCFA-lactic acid obtained in the control media by *Bifidobacteriumlongum* Reuter ATCC 15707 (9.94%) and *Lactobacillus acidophilus* IFO 13951 (6.95 %), was lower than in media supplemented with yacon tubers extract. However, it has been shown that control media is suitable for their growth. The probiotics bacteria, *Lactobacillus acidophilus* IFO 13951,ishomofermentative, while *Bifidobacteriumlongum* Reuter ATCC 15707 is heterofermentative bacteria. Hexoses will be degraded with the main production of lactic acid for homofermentative or to lactic acid, $CO_2$, and ethanol/acetic acid for heterofermentative fermentation. These bacteria are saccharolytic which highly adapted for growth on complex carbohydrates and capable of producing various polyhydrolases and glycosidases. According to Gibson and Roberfroid[21], the fermentation reaction stoichiometry described as follows:

$$1C_6H_{12}O_6 \rightarrow 1.5CH_3COOH + 0.33CH_3CH_2COOH + 0.15CH_3(CH_2)_2COOH + 0.33CH_3CHOHCOOH + 0.33CO_2 + 0.33H_2O$$

*Bifidobacteriumlongum* Reuter ATCC 15707 and *Lactobacillus acidophilus* IFO 13951produce the highest SCFA-lactic acid total in media supplemented with T6 yacon tubers extract (13.06, 13.49 %),and then followed by T1 (12.68;11.78%). This is due to T6yacon tubers contains the highest saccharides (60.34%) than T1-T5, can be used as carbon source for its growth. FOS in T1 fresh yacon tubers extract (37.30%) slightly hydrolyzed into glucose and fructose than T2-T6, as prebiotics can be used as a source of nutrients for probiotics bacteria that have β-fructofuranosidase. This phenomena is also possible due to chlorogenic acid in the fresh yacon tubers (T1) is higher than after storage or boiling (T2-T6). The content of chlorogenic acid decrease after 3 weeks storage at 20°C in darkness[22]. Chlorogenic acid is easily oxidized and polymerizes to form quinones that cases brown colors. While at boiling there is a significant

decrease chlorogenic acid content, chlorogenic acid could be isomerized to 4-O-caffeoylquinicacid (4-O-CA) and 5-O-caffeoylquinicacid (5-O-CA).[23,24] Chlorogenic acid can be used as a specific growth substrate[25]. The gut bacteria, including strains of *Bifidobacterium* and *Lactobacillus* will hydrolyze chlorogenic acid to form caffeic acid and quinic acid in metabolism process. Caffeic acid is further metabolized to form 3-coumaric acid, 3-hydroxyphenylacetic acid, and 3,4-dihydroxyphenylpropionic acid which available for absorption; while quinic acid to $CO_2$, cathecoland hipuric acid which excreted in the urine. In humans, as polyphenolic compounds, chlorogenic, caffeic, and quinic acids also to prevent inflammation, atherosclerosis, and cardiovascular diseases[26].

This research also showed that total SCFA and lactic acid of fermentation in media supplemented with yacon tubers extracts by *Bifidobacteriumlongum* Reuter ATCC 15707 is higher than *Lactobacillus acidophilus IFO 13951.*Combination offructose-oligosacharides and *Bifidobacterium* strains potentially effective as synbiotic, as well as use lactilol or lactulose with lactobacilli[27]. This indicates that specific substrates needed for the growth and activity of each of these microorganisms. *Lactobacillus* and *Bifidobacterium* were grown using FOS, then *Bifidobacterium* grew faster than *Lactobacillus*[28].In addition, chlorogenic acid significantly increases in the growth of *Bifidobacterium* spp[29].

## CONCLUSION

From the results can be stated that, the storage and boiling time of yacon tubers influence to FOS and saccharide content; the highest FOS in fresh and no boiling (T1), saccharides in 14 days storage and 60 minutes boiling time yacon tubers (T6). Total amount of SCFA-lactic acid fermentation yields in media supplemented yacon tubers by *Bifidobacteriumlongum* Reuter ATCC 15707 is higher than *Lactobacillus acidophilus* IFO 13951, the highest SCFA-lactic acid total in media supplemented with T6yacon tubers, and then followed by T1 yacon tubers extracts.

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## REFERENCES

1. A. Perry, *Agricultural Research Magazine,* **2**(2008).
2. B. S. Reddy, *J. Nutr.*,**129**, 1478S(1999), **DOI:** 10.1093/jn/129.7.1478S
3. http://www.tropentag.de/2007/abstracts/links/Milella_CMacday0.pdf
4. K. Valentova, J. Frcekand J. Ulrichova, *Chem. Listy.,* **95**, 594 (2001).
5. R. J. Singh, Genetic Resources, Chromosome Engineering, and Crop Improvement: Medicinal Plants, Taylor & Francis Group, CRC Press, USA (2012).
6. I. Manrique, M. Hermann, and T. Bernet, Yacon-Fact Sheet, International Potato Center (CIP) Lima, Peru(2004).
7. T. Asami, K. Minamisawa, T. Tsuchiya, K. Kano, I. Hori, T.Ohyama, M. Kuboya and T. Tsukihashi, *Jpn. J. Soil Sci. Plant Nutr.,* **65**, 621(1991).
8. Y. Miyaguchi and E. Inoue, *Nippon Shokuhin Kagaku Kaishi*, **59**, 4 (2012).
9. A. N. Kanayama, N. Tokita, and K. Aso, *Journal of Food Science*, **72**, S381 (2007), **DOI:** 10.1111/j.1750-3841.2007.00422.x
10. K. Swennen, C. M. Caurtin, and A. Delcour, *Critical Reviews in Food Science and Nutrition*, **46**, 459 (2006), **DOI:** 10.1080/10408390500215746
11. N. C. Habib, S. M. Honore, S. B. Gentaand S. S. Sanchez, *Chem. Biol. Interact.*, **194**, 31(2011). **DOI:**10.1016/j.cbi.2011.08.009
12. L. Yuanita, P. R. Wikandari andW. B. Sabtiawan, *Adv. Sci. Lett.*, **23**, 11982(2017), **DOI:**10.1166/asl.2017.10557
13. N. Shiomi, *New Phytol.*, **122**, 421 (1992), **DOI:** 10.1111/j.1469-8137.1992.tb00069.x
14. W. Puminat and C. Teangpook, *Journal of Food Sience and Engineering*, **3**, 141(2013).
15. H. Kaplan and R. W. Hutkins, *Applied and Environmental Microbiology*, **66**, 2682(2000), **DOI:**10.1128/AEM.66.6.2682-2684.2000

16. S. Graefe, M. Hermann, I. Manrique, S. Golombek, and A. Buerkert, *Field Crops Research*, **86**, 157 (2004), **DOI:** 10.1016/j.fcr.2003.08.003
17. N. Shiomi, N. Benkeblia, S. Onodera, T. Omori, N. Takahashi, M. Fujishima, T. Yoshihira, and S. Kosaka, *J. Appl. Glycosci*., **54**, 187 (2007).
18. R. J. Simpson, R. P. Walker, and C. J. Pollock, *New Phytol*.,**119**, 499 (1991), **DOI:** 10.1111/j.1469-8137.1991.tb01041.x
19. M. Aniko, M. Peter, L. Thi, and O. Ferenc, *European Food Research and Technology*, **228**, 355 (2009).
20. I. Simkovic, I. Surina, and M. Vrican, *J. Anal. Appl. Pyrolysis*, **70**, 493(2003), **DOI:** 10.1016/S0165-2370(03)00007-X
21. G. R. Gibson and M. B. Roberfroid, *J. Nutr.*, **125**, 1401(1995).
22. R. Slimestad and M. J. Verheul, *J. Agri. Food Chem*., **53**, 7251 (2005), **DOI:** 0.1021/jf050737d
23. S. Kan, M. W. M. Cheung, Y. Zhou, and W. S. Ho, *Journal of Food Science*, **79**, C147(2014), **DOI:**10.1111/1750-3841.12350
24. B. Xu and S. K. C. Chang, *J. Agric. Food. Chem.*,**56**, 7165 (2008), **DOI:**10.1021/jf8012234
25. D. Couteau, A. L. Mc. Cartney, G. R. Gibson, G. Williamson and C. B. Faulds, *Journal of Applied Microbiology*, **90**, 873 (2001), **DOI:** 10.1046/j.1365-2672.2001.01316.x
26. M. B. Hossain, N. P. Brunton, C. Barry-Ryan, A. B. Martin-Diana and M. Wilkinson, *Rasayan J. Chem.*,**4**, 751 (2008), **DOI:** 10.21427/D7105D
27. I. R. Rowland, 1992, Metabolic Interaction in the Gut. In: Probiotics. The Scientific Basis, R. Fuller (Eds.), Chapman & Hall, London.
28. A. Sghir, J. M. Chow, and R. I. Mackie, *J. Appl. Microbiol*., **85**, 769(1998), **DOI:** 10.21427/D7105D
29. C. E. Mills, X. Touzonis, M. Oruna-Concha, D. Mottram, G. Gibson, and J. Spencer,*British Journal of Nutrition*,**113**, 1220 (2015), **DOI:** 10.1017/S0007114514003948

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