Full Length Research Paper

Effect of inulin isolated from lesser yam (*Dioscorea esculenta*) on the growth of probiotics bacteria and SCFA formation during fermentation

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ABSTRACT

Research on effect of inulin isolated from Lesser Yam (Dioscorea esculenta; Jv. Gembili) and SCFA (Short Chain Fatty Acid) formation during fermentation was performed. The objective of the research was to evaluate the effect of Lesser Yam inulin and SCFA formation during fermentation using Bifidobacterium bifidum BRL-130, Bifidobacterium breve BRL-131, Bifidobacterium longum ATCC-15707, Lactobacillus casei FNCC-90, Lactobacillus acidophillus FNCC-0051 and E.coli FNCC-195 (as an enteric bacteria). Effect of Lesser Yam inulin on the growth of bacteria and SCFA formation were observed at the incubation time of 0, 24, 48 and 72 hours comparison with commercial inulin (from Cichory) and glucose. The number of total bacteria was analyzed using total plate count method while SCFA profile were analyzed using GC (Gas Chromatography). The results showed that Lesser Yam inulin stimulated the growth of Bifidobacterium breve BRL-131, Bifidobacterium bifidum BRL-130, Bifidobacterium longum ATCC-15707 and Lactobacillus casei FNCC-90, but it did not effect on the growth of Lactobacillus acidophillus FNCC-0051. Lesser Yam inulin inhibited the growth of E.coli FNCC-195 more than 1 log cycles. The fermentation with Lesser Yam inulin medium had higher pH values (from 6.8 to 5.46) compared with glucose (from 6.8 to 3.89). Lactic acid produced by Bifidobacteria and Lactobacillus in medium Lesser Yam inulin were smaller (0.54 to 1.05%) than that of glucose medium (2.24%). Fermentation using Lesser Yam inulin by Bifidobacterium longum ATCC-15707 produced the highest acetic acid (113.794 mMol) and propionic acid (9.217 mMol). High butyric acid was produced by Bifidobacterium breve BRL-131 (3.262 mMol). The results indicated that Lesser Yam inulin had prebiotic effect and increased the amount of SCFA, better than commercial inulin.

Keywords: Inulin isolated, lesser Yam, prebiotic, probiotic, Dioscorea.

INTRODUCTION

The role of gastrointestinal microbiota or bacteria is essential for health of humans and animals. Some of degenerative diseases are strongly influenced by intestinal microbiota ecosystem. The majority of gastrointestinal illness is caused by bacterial pathogens

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that infected in the human gut. Factors that influence the composition of gut bacteria is food consumed (diet), beside factors in the digestive tract itself (Manning and Gibson, 2004).

Bacterial populations in the ecosystem digestive tract of healthy people who eat a balanced diet are generally sTab.. Changes in lifestyle, diet and health conditions change the stability of ecosystem digestive tract. Accordingly, to achieve optimal health and performance (well-being) we must Increased the proportion of beneficial bacteria, and reduced amount of harmful bacteria, by consuming probiotics and provide the appropriate nutrients for probiotic bacteria (Pompei et al., 2008). Nutrients which stimulate the growth of probiotic bacteria are called prebiotics.

Prebiotics are food components that can not be digested and can selectively stimulate the growth and activity of beneficial bacteria in digestive tract, specifically bifidobacteria and lactobacilli (Gibson 2004; Pompei et al., 2008; Gaggia et al., 2010). Prebiotics are carbohydrates that generally can not be digested, but it has good effects on ecosystems of probiotic microbiota in the gut so it can provide health effects on humans and animals. In the large intestine, prebiotic ingredients are fermented by probiotic bacteria, especially *Bifidobacteria* and *Lactobacillus*, and produce short chain fatty acids (SCFA), that are acetic, propionic, butyric, and lactic acid. SCFA can be used as an energy source by the body. One prebiotic component widely used in food formulation is inulin.

Inulin is a dietary fiber chemically composed of a mixture of oligo- and/or polysaccharides constituted of fructose unit chains (linked by β -(2,1)-D-fructosyl-fructose bonds) of various length, generally terminated by a single glucose unit (linked by an α -D-glucopyranosoyl bond) (French, 1993; Roberfroid and Delzenne, 1998). Inulin is a group of fructan β -(2-1) consisting of a mixture of oligoand polysaccharides, in which almost every linear chains of fructose have GFn structure (with G=glucose unit, F=fruktose unit and n=number of units of the chain fructosil each other). Inulin is a soluble dietary fiber very beneficial to digestion and health (Sardesai, 2003). Inulin is soluble in water but can not be digested by enzymes in the digestive tract of mammals. Inulin in the colon is fermented by colon bacteria, so resulting in positive effect on the health of its host. Several types of Bifidobakteria can utilize inulin as an energy source caus by producing extracellular inulinase enzyme that can hydrolyze the bond β-(2-1-D-fructose-fruktosil to fructose (Robertfroid, 2005).

Inulin is widely used in food industry in Europe, USA, Canada and Indonesia as a component (ingredient) in the variouse of food products. Import value and volume of inulin in 2011 in Indonesia was US\$13.549.265 and 3.780.045 kg. Therefore, it is important to find the potential source of inulin from local raw materials. One of many types *Dioscorea spp*. that grows in Indonesia and its potentially contains in the highest amount of inulin is *Dioscorea esculenta*. *Dioscorea sp*. is very important as an alternative source of carbohydrate in the countryside. Isolation and characterization of inulin from *Dioscorea esculenta* (Lesser Yam) have been carried out, which has inulin rendement 21.33%, the purity 69.25%, the average solubility 76.77%, the average water content 13.68% and the degree of polymerization (DP) 6 (Winarti et al., 2011).

The objective of the research was to evaluate prebiotic

effect of inulin extracted from Lesser Yam (*Dioscorea* esculenta) and its SCFA profile during fermentation.

METHODOLOGY

Materials and Equipments

Raw materials used in research were Lesser Yam tubers (*Dioscorea esculenta*) from the traditional market in East Java, Indonesia. Commercial inulin (Februline Instant, native chicory inulin) is produced by Cosucra Warcoing Groupe SA, Belgium.

Probiotics bacteria used in this research, were Bifidobacterium bifidum BRL-130, Bifidobacterium breve BRL-131, Bifidobacterium longum ATCC-15707, Lactobacillus casei FNCC-90 and Lactobacillus acidophillus FNCC-195. The enteric bacterium is E. coli FNCC-0051 as an indicator pathogenic. The bacteria put from Food and Nutrition Culture Collection, Center for the Study of Food and Nutrition, Universitas Gadjah Mada, Yogyakarta.

The medium for bacterial growth was the MRS (Man Rogosa Soyprotein) medium broth and agar used for the growth *Bifidobacteria* and *Lactobacillus*. MRS medium formulation was used with replacing glucose with inulin for treating media. TSA (Trypticase Soy Agar) and TSB (Trypticase Soy Broth) were purchase for the growth of *E.coli*. Media M-9, for treating *E.Coli*, with glucose and inulin as an energy sources.

The equipment used were GC 8A Shimadzu with GP 10% SP column 1200/1% H3PO4 on 80/100 Cromosob WAW in a 3 mm diameter, 2 m length and detector type FID, autoclave, cabinet dryer, centrifuge, shaker waterbath, incubator and colony counter.

Isolation of Inulin from Lesser Yam

The isolation of inulin followed the method Park et al., 2006; Toneli, et al., 2008. Lesser yam tubers cleaned, washed, peeled and cut into small pieces, then blended with the addition of hot water at temperature $80-90^{\circ}$ C 1:10 (tubers: water). Slurry was performed in the shaker water bath at temperature 90° C for 1 hour. Filtered and cooled, then frozen at -20 °C for 24 hours. The frozen filtrate was thawed and then centrifuged at 1500 rpm, for 15 minutes until we got a white precipitate and be separated. White precipitate dried at 60 °C, for 5 hours, crushed and sieved. Crude inulin stored in an airtight container prior to further testing.

Bacterial cultures storage

Six species of bacteria that used in this research were in

the form of ampoules. Furthermore, bacteria were cultured in the MRS broth medium for *Bifidobacteria* and *Lactobacilli* and in the TS broth for *E. coli*. After a 24-hour-old culture, drawn 1 ml included in sterile evendorf, and then centrifuged at 3000 rpm for 15 minutes. Medium broth removed, sediment cells then added 1 ml of a mixture skim milk 10% and 20% sterile glycerol, vortexed and stored at -20 °C.

Preparation of Growth Media

MRS formulation was prepared by the replaced glucose with inulin. MRS was used for testing the bacterial growth of *Bifidobacteria* and *Lactobacilli*. TS media agar and broth were made from: triptone 15 g, 5 g soya peptone, 5 g, sodium clorite with and without 15 gr agar, diluted in one liter water. This media was used for the preparation, reculture and calculations of *E.coli*.

M-9 medium were made from Na₂HPO₄.7H₂O 64 g, 15 g KH₂PO₄, 2.5 g NaCl, 5.0 g NH₄Cl, diluted in one liter water and then sterilized. For testing/growth of *E.coli*, 200 ml solution was added with 700 ml sterile distilled water, 2 ml 1 M MgSO₄ sterile, 20 ml 20% glucose (other carbon sources) sterile, 100 μ l 1 M CaCl₂ sterile and added distilled water sterile to 1000 ml. This media is used for the testing the growth of *E. coli* with glucose and inulin as an energy sources.

Bacteria Treatment

Bifidobakterium bifidum BRL-130, *Bifidobakterium breve* BRL-131, *Bifidobakterium longum* ATCC-15707, *Lactobacillus casei* FNCC-90, *Lactobacillus acidophillus* FNCC-195 were grown in MRS broth for 24 hours. Then, it was diluted with physiological saline sterile of the cell number approximately 10⁵/ml. This culture is inoculated in the treating medium and incubated for 0, 24, 48 and 72 hours.

Analysis of SCFA (acetate, propionate and butyrate)

Profiles and levels of acetic, propionic and butyric acid during fermentation was analyzed at 72 hours using a Shimadzu GC 8A with a column of GP 10% SP 1200/1% H_3PO_4 on 80/100 Cromosob WAW in a 3 mm diameter, 2 m long column and FID detector type with test conditions at 140 °C column temperature, 240 °C detector temperature, carrier is gas N₂, 1.5 kg/cm² pressure and sample injected 1 µl.

RESULT AND DISCUSSION

Effect Lesser Yam Inulin on the Growth of Bifidobacteria

The results of the effect of Lesser Yam inulin on the growth of *Bifidobacteria* are presented in Fig. 1. Lesser Yam inulin could stimulate the growth of *Bifidobacterium breve* BRL-131 (Fig. 1), *Bifidobacterium bifidum* BRL-130 (Fig. 2) and *Bifidobacterium longum* ATCC-15707 (Fig. 3). This indicates that inulin can be used as an energy source similar with glucose. Roberfroid, (2005), said that several types of *Bifidobacteria* produce inulinase extraseluler enzyme that can hydrolyze inulin to fructose, then the fructose is used as an energy source for growth. Akin et al., (2007) that the addition of inulin in ice cream can stimulate the growth of probiotic bacteria *Bifidobacterium lactis* and *Lacctobacillus acidophilus* and increase viability at the storage for 90 days.

Lesser Yam inulin can be used as an energy source longer than that of glucose. In the medium with Lesser Yam inulin can increase the number of bacteria cells and still constant until 72 hours of incubation. Whereas with glucosa the number of cells declined at 72 hours after incubation, even at this time the Bifidobacterium longum still increase. This is probably due to that the molecular weight of Lesser Yam inulin is higher than glucose (degree of polymerization is 6), so it is more durable than glucose as an energy source. Pompei et al. (2008) explain that inulin can increase the growth of Bifidobacterium adolesentis, Bifidobacterium infantis, Bifidobacterium Bifidobacterium breve, longum, Lactobacillus plantarum. Lactobacillus rhamnosus, Lactobacillus reuteri, Lactobacillus delbruechii and it can inhibit the growth of E. coli and Clostridia. Influence of inulin on the growth of Lactobacillus and Bifidobakteria depends on degree of polymerization. High Soluble Inulin (HSI) can increase the growth of Bifidobacteria (7.0 log cfu/ml) and Lactobacillus (7.1 log cfu/ml) on fermentation of 48 hours, whereas the growth of E. coli only increased 5.1 log cfu/ml (Pompei et al., 2008). The commercial inulin has a degree of polymerization 12, can also increase the growth of Bifidobacteria and the increasing is almost the same as Lesser Yam inulin.

Effect Lesser Yam Inulin on the Growth of Lactobacillus

The results of the effects of Lesser Yam inulin and commercial inulin on the growth of *Lactobacillus* are presented in Fig. 4 and Fig. 5. Lesser Yam inulin

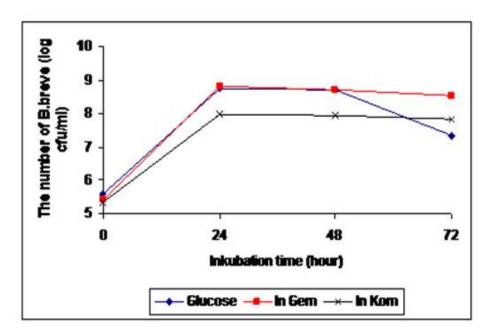


Fig. 1. Effect of medium and incubation time on the growth of *Bifidobacterium breve* BRL-131

Note : Glucose = MRS medium with glucose as an energy source In Gem = MRS medium with Lesser Yam inulin as an energy source In Kom = MRS medium with commercial inulin as an energy source

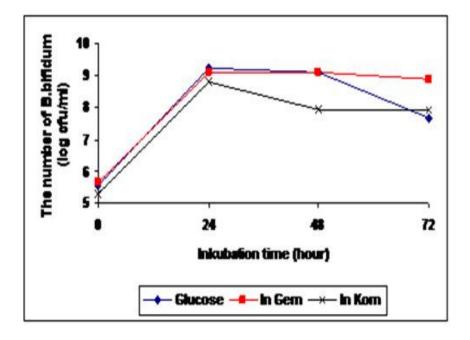


Fig. 2. Effect of medium and incubation time on the growth of *Bifidobacterium bifidum* BRL-130 (B)

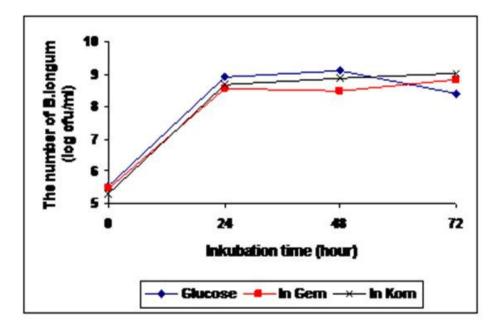


Fig. 3. Effect of medium and incubation time on the growth of *Bifidobacterium longum* ATCC-15 707.

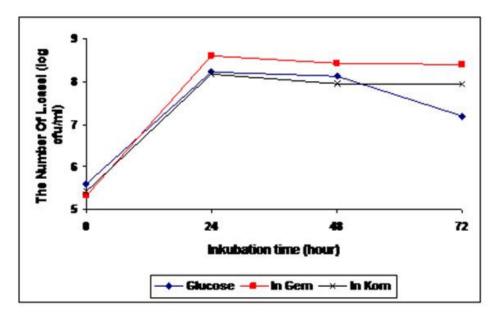


Fig. 4. Effect of medium and incubation time on the growth of Lactobacillus casei FNCC-90

can stimulate the growth of *Lactobacillus casei* FNCC-90 better than glucose and commercial inulin from cichory, but it did no effect on the growth of *Lactobacillus acidophillus* FNCC-0051. This indicates that inulin is a

prebiotic, defined as material that can be metabolized and selectively stimulated the growth of microbiota in the digestive tract (gastrointestinal tract) (Huebner et al., 2007). Similar to Lesser Yam inulin can be metabolized

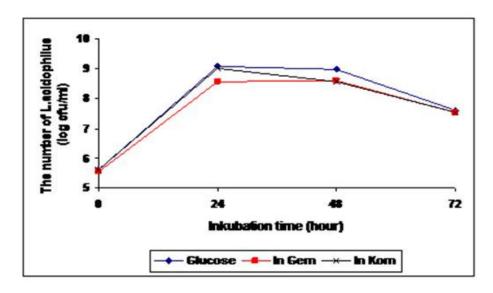


Fig. 5. Effect of medium and incubation time on the growth of *Lactobacillus acidophillus* FNCC-0051.

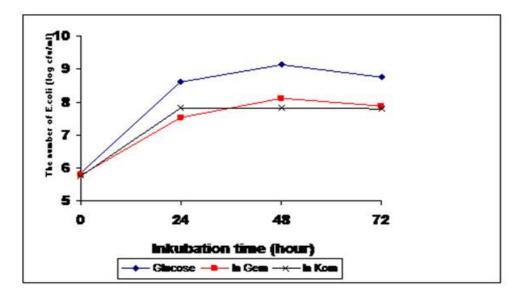


Fig. 6. Effect of medium and incubation time on the growth of Escherichia coli FNCC-195

and stimulated the growth of *Bifidobacteria* and *Lactobacilli* in different score for each bacterium.

Effect Lesser Yam Inulin on the Growth of *Escherichia coli* FNCC-195

The results of the effect of Lesser Yam inulin and commercial inulin on the growth of *Escherichia coli* FNCC-195 in M-9 medium formulations are presented in

Fig. 6. Lesser Yam inulin and commercial inulin can inhibit the growth of *Escherichia coli* FNCC-195 more than 1 log cycles, whereas glucose can not inhibit growth of *Escherichia coli* FNCC-195.

This is because *Escherichia coli* can grow in medium with simple nutrient and ferment lactose (simple sugars) to produce acids and gas. However it is low in ability to degrade the complexes carbohydrate (Pelczar, 1988). The growth of enteric bacteria as *E. coli* on M-9 medium with inulin as an energy source is smaller than in M-9

Medium	Culture Bacteria	pH at incubation time (hour)					
		0	24	48	72		
Glucose	B. breve		4,89	4,65	4,43		
	B. bifidum		4,07	3,90	3,84		
	B. longum	6,81	4,88	4,65	3,93		
	L. casei		4,26	4,24	4,04		
	L. acidophilus		4,26	4,12	3,96		
	E. coli		4,07	4,00	3,97		
Lesser Yam Inulin	B. breve		5,75	5,75	5,49		
	B. bifidum		5,63	5,50	5,46		
	B. longum	6,80	5,75	5,75	5,58		
	L. casei		6,13	6,42	6,37		
	L. acidophilus		6,06	5,94	5,73		
	E. coli		5,81	5,91	5,72		
Commercial Inulin	B. breve		4,96	4,91	4,86		
	B. bifidum		4,81	4,80	4,77		
	B. longum	6,80	4,96	4,91	4,31		
	L. casei		5,69	5,83	5,85		
	L. acidophilus		5,66	5,51	5,27		
	E. coli		4,41	3,94	3,94		

Tabel 1. Changes of medium pH by *Bifidobacteria, Lactobacillus* and *E.coli* in MRS with glucose, Lesser Yam inulin and commercial inulin as an energy saurces

medium with glucose, shown by the cell density 0.81 on Inulin-S and 1.94 at the glucose (Huebner et al., 2007). High Soluble Inulin (HSI) can increase the growth of *Bifidobacteria* 7.0 log cfu/ml and *Lactobacillus* 7.1 log cfu/ml on fermentation at 48 hours, whereas the growth of *E. coli* only increased about 5.1 log cfu/ml (Pompei et al., 2008).

Changes of medium pH with Lesser Yam inulin during fermentation

Changes of medium pH during fermentation by Bifidobacteria, Lactobacillus and E. coli in the MRS medium with glucose, Lesser Yam inulin and commercial inulin as an energy sources are presented in Tab. 1. The Bifidobacteria, Lactobacillus and E.coli in glucose medium as an energy source can be lower of medium pH (from 6.81 to 4.0) at incubation time 24 hours and below from 4.0 at incubation time 72 hours. This is indicated that Bifidobacteria, Lactobacillus and E. coli can transform glucose into lactic acid and acetic acid so lowering pH on growth medium. The Lesser Yam inulin can decrease medium pH lower than with glucose. It is because inulin has more complex structure than glucose, so it is imperfectly fermented by Bifidobacteria, Lactobacillus and E. coli (Kaur et al., 2011). Robertfroid (2005) showed that fermentation of inulin by *Bifidobacterium* lower medium pH to 2.8, whereas by *Lactobacillus* only 0.3 and fermentation of glucose can lower medium pH between 2.0 to 3.1.

Commercial inulin from cichory can lower medium pH from 6.8 to 3.94 during fermentation by *E. coli* and between 4.31 to 5.85 by *Bifidobacteria* and *Lactobacillus*. That is caused by the differences of specific subtypes and different chain lengths of inulin that have effects on different the growth of *Bifidobacterium* and *Lactobacillus* and changes of medium pH during fermentation (Kaur et al., 2011).

Changes of lactic acid in the medium Lesser Yam inulin

Changes of total lactic acid during fermentation by *Bifidobacteria*, *Lactobacillus* and *E.coli* in the MRS medium with glucose, Lesser Yam inulin and commercial inulin as an energy sources are presented in Tab. 2. The *Bifidobacteria*, *Lactobacillus* and *E. coli* growth in medium glucose as an energy source can produce lactic acid 1.09% to 2.24% during fermentation. The longer of fermentation time, the greater acids were produced. The lactic acid, which is produced by *Bifidobacteria* and *Lactobacillus* in medium Lesser Yam inulin and commercial inulin were lower than in glucose that was 0.54% to 1.05%. This is because *Bifidobacteria*,

Medium	Culture Bacteria	Total lactic acid (%) during fermentation			
		0	24	48	72
	B. breve		1,16	1,18	1,17
	B. bifidum		1,19	1,99	2,24
Glucose	B. longum	0	1,09	1,59	2,22
	L. casei		1,62	1,86	1,94
	L. acidophilus		1,67	1,75	1,87
	E. coli		1,78	1,80	1,80
Lesser Yam Inulin	B. breve		0,71	0,71	0,72
	B. bifidum		0,72	0,63	0,74
	B. longum	0	0,54	0,62	0,65
	L. casei		0,54	0,55	0,54
	L. acidophilus		0,63	0,63	0,63
	E. coli		0,57	0,60	0,65
	B. breve		0,95	0,99	1,02
	B. bifidum		1,05	0,93	0,99
Commercial Inulin	B. longum	0	0,53	0,84	1,27
	L. casei		0,63	0,65	0,69
	L. acidophilus		0,66	0,66	0,69
	E. coli		1,23	1,26	1,29

Tab. 2. Changes of lactic acid by *Bifidobacteria, Lactobacillus* and *E.coli* in the MRS medium with glucose, Lesser Yam inulin and commercial inulin as an energy saurces

Lactobacillus and E. coli can transforme glucose into lactic and acetic acid, it is easier than transformed to inulin. It is probably that the inulin has higher molecular weight than glucose, so can not be fermented perfectly so that smaller acid produced (Kaur et al., 2011). According to Oliveira *et al.* (2009), fermentation oligofruktosa for 24 hours by mixed cultures of *Streptococcus thermophillus-L.acidophillus* produce lactic acid of 0.98% and mixed cultures of *Streptococcus thermophillus-Bifidobacterium lactis* produce lactic acid of 0.91%.

Profile SCFA (Short Chain Fatty Acid)

The profile of acetic acid formation on fermentation by *Bifidobacteria, Lactobacillus* and *E. coli* with the media Lesser Yam inulin, glucose and commercial inulin as an energy sources at incubation time 72 hours are presented in Fig. 7. The highest acetic acid 113.794 mMol was produced by *Bifidobacterium longum* ATCC-15707 in medium Lesser Yam inulin, followed by *Bifidobacterium bifidum* BRL-131 of 107.441 mmol in medium glucose and 100.811 mmol in madium Lesser Yam inulin.

The profile of propionic acid formation on fermentation by *Bifidobacteria*, *Lactobacillus* and *E. coli* with the media Lesser Yam inulin, glucose and commercial inulin as an energy sources at incubation time 72 hours are presented in Fig. 8. The highest propionic acid 19,217 mMol was produced by *Bifidobacterium longum* ATCC-15707 in medium Lesser Yam inulin, followed in glucose 14,229 mMol and in commercial inulin 12,057 mMol.

The profile of butyric acid formation on fermentation by *Bifidobacteria, Lactobacillus* and *E. coli* with the media Lesser Yam inulin, glucose and commercial inulin as an energy sources at incubation time 72 hours are presented in Fig. 9. The highest butyric acid 3,262 mMol was produced by *Bifidobacterium breve* BRL-131 in medium Lesser Yam inulin. In the medium commercial inulin *Bifidobacterium breve* BRL-131 produce acetic acid 3,252 mMol, whereas *Bifidobacterium bifidum* BRL-130 produce 2,854 mMol in medium Lesser Yam inulin.

The highest of SCFA namely acetic, propionic and butyric acid is produced from Lesser Yam inulin during fermentation. Highest production of acetic and propionic acid was by *Bifidobacterium longum* ATCC-15707, while butyric acid was by *Bifidobacterium breve* FNCC-130. These happened because each polysaccharide is broken and hydrolyzed at different rates by different microbes. Inulin is polyfructose which is metabolized by intestinal microbiota, including *Bifidobacteria* through the glycolytic pathway to produce pyruvate then pyruvate is converted to Acetyl-co-A, lactate and succinate. Acetil-co-A can be converted to acetate, butyrate, while succinate is converted to propionate and format is converted to gas

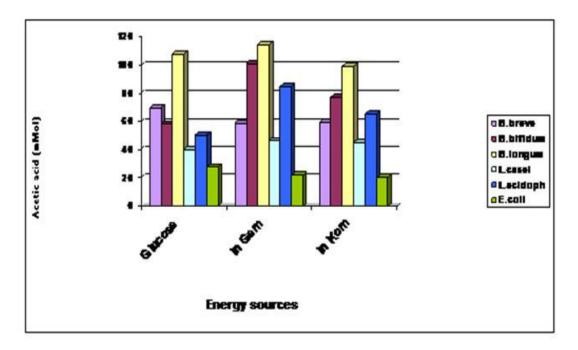


Fig. 7. Profile of acetic acid formation by *Bifidobacteria, Lactobacillus* and *E.coli* in medium glucose, Lesser Yam inulin and commercial inulin as an energy sources.

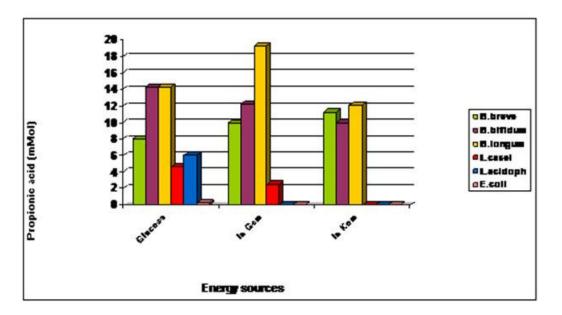


Fig. 8. Profile of propionic acid formation by *Bifidobacteria, Lactobacillus* and *E.coli* in medium glucose, Lesser Yam inulin and commercial inulin as an energy sources.

 H_2 , CH_4 and H_2S (Macfarlane and Macfarlane, 2003). Hexoses used by *Bifidobacteria* through an unusual metabolic pathway called "*bifid shunt*" (Robertfroid, 2005; Tannock, 2010). *Bifidobacteria* genus has a unique metabolic pathway that produces the fructose-6phosphate phosphoketolase enzyme to ferment oligosaccharides. The enzyme is a key enzyme to recognize the genus (Sela et al., 2010).

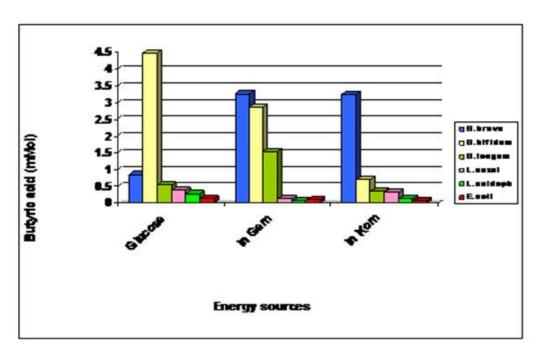


Fig. 9. Profile of butyric acid formation by *Bifidobacteria, Lactobacillus* and *E.coli* in medium glucose, Lesses Yam inulin and commercial inulin as an energy sources.

CONCLUSIONS

Lesser Yam Inulin can stimulate the growth of Bifidobacterium breve BRL-131, Bifidobacterium bifidum BRL-130, Bifidobacterium longum ATCC-15707, and Lactobacillus casei FNCC-90, but had a little effect on the growth of Lactobacillus acidophillus FNCC-0051, the number of cells is still constant until 72 hours. Lesser Yam inulin can inhibit the growth of E.coli FNCC-195 more than 1 log cycle. Lesser Yam inulin can lower of medium pH less than glucose. Lactic acid produced from Lesser Yam inulin by Bifidobacteria and Lactobacillus was lower than that from glucose. Acetic, propionic and butyric acid produced by Bifidobacteria, Lactobacillus and E. coli during fermentation of Lesser Yam inulin. The highest acetic acid and propionic acid produced by Bifidobacterium longum ATCC-15707 were 113.794 mmol and 9.217 mmol, respectively. The highest butyric acid produced by Bifidobacterium breve FNCC-131 was 3.262 mmol.

ACKNOWLEDGEMENTS

Thank to Directorate of Human Resources Directorate General of Higher Education Ministry of National Education via. BPPS Program was supported in this research.

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