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The growth profile of *Lactobacillus plantarum*, *Bifidobacterium bifidum* and *Escherichia coli* during *in-vitro* fermentation of breadfruit resistant starch

[1]Zarinah Zakaria, [1]Nur Jannah Mohd Najeeb, *[2]Napisah Hussin, [1]Norshazila Shahidan and [1]Siti Nuriah Mohd Noor

[1]Faculty of Bioresources and Food Industry, Universiti Sultan Zainal Abidin, Terengganu, **Malaysia**
[2]Faculty of Health Sciences, Universiti Sultan Zainal Abidin, Terengganu, **Malaysia**.

***Correspondence: zarinah@unisza.edu.my** **Revised: 06 Oct. 2019, Accepted: 08 Oct. 2019 e-Published: 11 Nov. 2019**
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Breadfruit (BF) or *Artocarpus altilis* is a neglected fruit that has many nutritional values which has not been commercialized widely. Breadfruit resistant-starch (BFRS) is defined as a type of starch that resists digestion in the small intestine as they will be fermented by the gut microbiota to produce a variety of products, including short chain fatty acids that can provide a range of physiological benefits via fermentation. As such, this study determined the growth profile of *Lactobacillus plantarum* (ATCC 8014), *Bifidobacterium bifidum* (ATCC 11863), and *Escherichia coli* (ATCC 10536) during *in-vitro* fermentation of carbohydrate sources (BFRS, glucose, inulin, BF flour) and control for 0, 6, 12, 24, 48, and 72 hours. The growth changes for *L. plantarum*, *B. bifidum,* and *E. coli* displayed statistically significant differences ($p<0.05$) between BFRS and other carbohydrates sources after incubation at varied fermentation time (0, 6, 12, 24, 48, and 72 hours). As a conclusion, BFRS has potential to be used as a prebiotic substance to increase the amount of probiotic bacteria in terms of producing metabolites, such as acids, which can benefits the host.

Keywords: Growth Changes , *Lactobacillus plantarum*, *Bifidobacterium*, *Escherichia coli*, *In-vitro* Fermentation, Breadfruit Resistant Starch

INTRODUCTION

Food-deficit countries, such as South Pacific and the Caribbean are need to fully utilise all existing foodstuff, wherein one effective way to minimise post-harvest losses. An example of existing foodstuff is our starch rich crop, breadfruit (BF). Breadfruit is a perishable fruit, thus the conversion into flour has provided more stable and intermediate product (Adepeju et al., 2011). Previous research has focused on the production of resistant starch (RS) from readily accessible starch sources such as maize, wheat, rice and potato (Zi Ni et.al., 2015). Less research has been reported on the production of RS from breadfruit which considered as neglected fruit in Malaysia.

The significance of breadfruit RS (BFRS) was selected for this study because it has been gain a lot of attention for its benefits to one's overall health (Horstmann et al., 2017). Resistant-starch is defined as the total amount of starch and the products of starch degradation that resist digestion in the gastrointestinal tract. This type of starch can resist digestion upon arriving at the colon, where they will be fermented by the gut microbiota to produce a variety of acids that offer a range of physiological benefits (Zaman and Sarbini, 2015). Several other studies have demonstrated that, as the ratio of amylose to

amylopectin increases, the enzymatic digestibility of the starch decreases (Zaman and Sarbini, 2015). Due to this fact, RS has functional properties and positive effects on diabetes, obesity and osteoporosis.

The human's large intestine is inhibited by numerous and diverse species of microorganisms, making them a dense and complex microflora gut population. Colonic microflora plays a crucial role in influencing the health and the proper track of intestinal functions, which determines the physiological status of the host's health. Several strategies have been adapted to regulate a balance and proper density of microbial gut populations. Thus, this study aimed to determine some important attributes of breadfruit resistant starch (BFRS) and their significance as potential prebiotics in the growth profile of *L. plantarum, B. bifidum*, and *E. coli* by using *in-vitro* fermentation method for 6, 12, 24, 48, and 72 hours.

MATERIALS AND METHODS

Materials

Pure cultures *(L. plantarum, B. bifidum* and *E. coli)*

L. plantarum (ATCC 8014) and *B. bifidum* (ATCC 11863) were obtained from the teaching laboratory at UniSZA City Campus, while *E. coli* (ATCC 10536) was obtained from the teaching laboratory at UniSZA Besut Campus.

Resistant Starch, Chemicals and Microbiological

BFRS was produced from gelatinization and enzymatic treatment of breadfruit starch with one cycle of retrogradation process which involves heat (121°C) and cool methods in order to produce resistant-starch type III (RS3).

Glucose, inulin and chemicals were used by the fermentation media (protease peptone, beef extract, yeast extract, sodium acetate, ammonium citrate, di-potassium hydrogen phosphate, magnesium sulphate, manganese sulphate, Tween 80 and L-cysteine). These chemicals were purchased from Merck Milipore (Germany), Bendosen Laboratory Chemicals (Norway), and Sigma-Aldrich (Steinheim Germany). Selective Media De Man, Rogosa, Sharpe (MRS) broth, De Man, Rogosa, Sharpe (MRS) agar, as well as nutrient broth and nutrient agar used in this study were obtained from HiMedia (India).

In-vitro Fermentation of Breadfruit Resistant Starch by Pure Culture of bacteria

Inoculum preparation

L. plantarum, *B. bifidum* and *E.coli*

L. plantarum and *B. bifidum* pure culture from glycerol stock were cultured in MRS broth and incubated at 37˚C for 24 hours. Next, the bacteria were subcultured on De Man, Rogosa, Sharpe (MRS) agar by streak plate method which then incubated at 37˚C for 24 hours and 48 hours. A single colony of *L. plantarum* and *B.bifidum* pure cultures from MRS agar was transferred into De Man, Rogosa, Sharpe (MRS) broth for optimisation process. *L. plantarum* and *B. bifidum* were cultured in aerobic and anaerobic condition respectively.

E. coli from glycerol stock was activated in nutrient broth and incubated at 37˚C for 24 hours. Then, the bacteria was subcultured on nutrient agar using streak plate method and incubated at 37˚C for 24 hours. Next, single colony of *E. coli* from the nutrient broth was transferred into nutrient broth for optimisation process. The target optimisation value of enumeration bacteria was ranged between 10^5 and 10^7 CFU/mL. The bacteria load of the sample was within the acceptable limit because to confer health benefits, probiotic bacteria must be viable at the time of consumption with concentration of $>10^6$ CFU/g (klare and Shah, 2008). Later, the samples were serially diluted and incubated at different hours, which started from 6, 8, 10, 12, 14, 16, 18, 20, 22 and 24 hours to obtain working culture containing 10^5-10^7 CFU/mL cells, as determined by plate counts.

After that, the incubation time for *E. coli*, *L. plantarum* and *B. bifidum* were selected at 8, 10 and 12 hours respectively. Then, these bacteria were sub-cultured again in 30 mL of nutrient broth, incubated at 37˚C under optimized incubation time. The incubation process was carried out in anaerobic condition by placing the anaerobic pack (Merck Milipore, Germany) in an anaerobic glass jar *B. bifidum* meanwhile *E. coli* and *L. plantarum* and was in aerobic condition (Klare et. al., 2005).

Fermentation media preparation

The fermentation media was prepared by weighing all the chemical substances, which were 10 g of protease peptone, 10 g of beef extract, 5 g of yeast extract, 5 g of sodium acetate, 2 g of ammonium citrate, 2 g of di-potassium hydrogen phosphate, 0.1 g of magnesium sulphate, 0.05 g

of manganese sulphate, 1 mL of Tween 80, and 1.5 g of L-cysteine (Hi-Media, India). Each fermentation medium substituted different types of carbohydrate sources (glucose, inulin, BFRS and BF flour) at 10 g/L (or 1% w/v). Fermentation medium without any carbohydrate or prebiotic source was served as control in this study. The pH was adjusted to 6.2 ± 0.2 by adding a small amount of 0.1 molar sodium hydroxide or hydrochloric acid. The pH of medium was measured by using Mettler Toledo pH Meter and autoclaved at 121˚C for 15 minutes.

Fermentation condition

The fermentation process was carried out in 20 mL of media in universal bottles. The growth media without carbohydrate or prebiotic source served as blank media, while the other growth media were added with 1% (w/v) of glucose, inulin, BFRS, and BF flour. One mL inoculum 1% (w/v) was added into each fermentation medium that consisted of different types of carbohydrates or prebiotic sources and then incubated at 37˚C for different fermentation durations (6, 12, 24, 48, and 72 hours) in anaerobic condition by using an anaerobic container for *B. bifidum* while *L. plantarum* and *E. coli* in aerobic condition. The fermentation medium of pure cultures was frozen with label and placed in a deep freezer (Thermo Scientific) at -20˚C. Analysis was performed on the next day.

Analyses

Enumeration of Bacterial Growth

The viable counts of bacterial cultures were enumerated by using the spread plate method with MRS agar for *L. plantarum* and *B. bifidum* pure cultures, while nutrient agar for *E. coli* in triplicate analysis. The samples (1 mL) was directly pipetted into 9 mL of sterile saline water for serial dilution technique which is to produce 10^1 until dilution 10^8. The serial dilution technique was used to identify the viability of bacteria.

After that, 0.1 mL of the sample from dilution series was pipetted into agar plate and spread evenly. Then, the MRS agar plate was incubated at 37˚C for 24 hours in anaerobic condition by placing the anaerobic pack in the anaerobic glass jar, while the nutrient agar plate was incubated at 37˚C for 24 hours. The incubator was sterile with 70% alcohol to prevent contamination from occurring on the agar plates. The enumeration of bacteria growth was based on colony counts by using colony counter. The results were reported as colony forming unit per millilitre suspension (CFU/mL) in duplicate reading. The formulation of growth percentage of bacterial cultures expressed as follow:

$$N\ (CFU/mL) = \frac{C}{vd(n1+0.1\ n2)}$$

N = Enumeration of bacteria colony
C = Sum of colonies count on n1 and n2
v = Volume of transfer dilution
d = Dilution selected from serial dilution

Measurement of pH Change

After completing the fermentation durations, 4 mL of sample was taken from the fermentation medium to determine the pH value using Mettler Toledo pH Meter. The value of pH was determined in the triplicate readings.

Statistical Analysis

The statistical analysis of one-way analysis of variance (ANOVA) was performed to compare the differences between samples on different fermentation durations by using IBM SPSS Statistics Data Editor, Edition 20. The results were recorded in mean and standard deviation. Significant difference is noted when ($p<0.05$), while insignificant difference if ($p>0.05$). All significant differences between the mean values were assessed at a significant level of $\alpha = 0.05$ (95% confidence level).

RESULTS AND DISCUSSION

Growth profile of *L. plantarum* on different substrates and different fermentation time.

The growth profiles of *L. plantarum* at different fermentation time from 0 hour to 72 hours with varied types of carbon supply and control medium was shown in Figure 1. The blank sample used in this study was considered as control because it did not contain any carbohydrate source. Generally, all carbohydrate substrates and control medium without carbon source utilised in *L. plantarum* cultures ($p<0.05$) were displayed by the increasing trends of growth over certain fermentation durations. Based on the result, *L.*

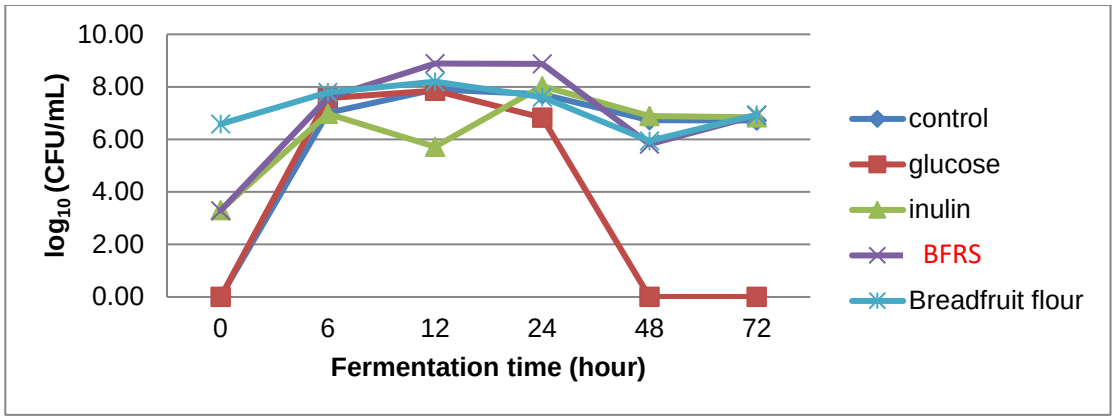

Figure 1; Effect of fermentation time on growth of control media and media containing glucose, inulin, BFRS and BF flour by *L. plantarum*

plantarum was able to grow in control media (blank) up to 7 log_{10} CFU/mL, while additional carbohydrate sources stimulated higher growths until up to about 8 log_{10} CFU/mL. At 0-hour fermentation, the growth of *L. plantarum* was varied between all carbohydrates sources. This is because; intrinsic properties of enzyme product or the conditions might differ in responses under which the experiment was carried out (Biggs et al., 2007). However, there was no growth of *L. plantarum* observed at 0-hour of fermentation in media containing glucose. This might be due to this bacteria still in the dormant state and takes time to adapt as well as multiply in a new conditions given.

The death phase of *L. plantarum* gradually started from 24 hours until 72 hours of fermentation duration, which resulted from 7.71 log_{10} CFU/mL to 6.72 log_{10} CFU/mL, respectively. This is attributed to lack of energy sources supply from the fermentation media. Meanwhile, the glucose affected the growth of *L. plantarum* very well ($p<0.05$). Buruleanu et al., (2010) suggested that it is important that the initial amount of sugars is adequate for lactic acid bacteria growth and multiplication. Although *L. plantarum* is a homo-fermentative bacterium, studies have reported that cultures of *L. plantarum* can alter their metabolism in response to different environmental changes (Sangwan et al., 2014).

Stolz et al., (1995) reported that in the presence of oxygen, the metabolism of obligate homo fermentative and facultative homo fermentative strains switch from producing lactic acid to acetic acid, thereby increasing the amount of ATP and hindering the growth proliferation of *L. plantarum* at 0 hour. However, the exponential phase started at the 6th hour until the 12th hour, which was 7.58 log_{10} CFU/mL and 7.86 log_{10} CFU/mL, respectively. In this study, the growth profile of *L. plantarum* slightly decreased to 6.83 log_{10} CFU/mL at 24 hours. Extending the fermentation period beyond 24 hours resulted in a significant decrease in the viable cell counts of *L. plantarum* ($p<0.05$). No growth was identified at 48 and 72 hours of fermentation time. This might due to insufficient of carbohydrate sources to support the bacterial growth such as glucose, which is a simple sugar, stored as polymer (monosaccharide) that can be directly used for bacterial growth.

Proliferation growth of *L. plantarum* on RS showed no significant difference on varied fermentation durations ($p>0.05$). Nevertheless, a significant difference at 48 and 72 hours of fermentation duration was noted for difference carbon sources. The growth trends of BFRS started with 3.28 log_{10} CFU/mL and underwent log phase with 8.89 log_{10} CFU/mL, which later gradually dropped to 8.87 log_{10} CFU/mL and 5.81 log_{10} CFU/mL at 24 and 48 hours, respectively. There was a slight leap by 1.1 log_{10} at 72 hours of fermentation time. Beyond 24 hours of fermentation time, the growth of *L. plantarum* gradually increased until 72 hours, when compared to the growth phase on glucose.

Roberfroid (2007) reported that in order to prove the potential of RS as prebiotics, it must fulfil three criteria, which are resistance to upper gastrointestinal environment, fermentation by intestinal microbiota and selective stimulation of growth or activity of beneficial bacteria. Hence, RS met the requirement as prebiotic in support of the growth of *L. plantarum* throughout 72 hours,

when compared to glucose. Moreover, at 72 hours of fermentation time, there was a significant difference of the growth *L. plantarum* between BFRS and control, which is similar to BFRS and glucose ($p<0.05$). From Figure 1, there was no significant difference in terms of growth profile of *L. plantarum* when supplemented with BFRS and inulin ($p>0.05$).

The type of bond link in the component monomers, in view of specific cleavage enzymes being required for fermentation of the carbohydrate, may affect fermentation rate and thereby determine the speed at which potential inhibitory metabolic end-products are released. In addition, the chain length of the carbohydrate is also likely to be a contributory factor, since long chain oligosaccharides, with multiple branching, require more enzymatic hydrolysis by the organisms before its complete fermentation (Fooks and Gibson, 2002).

Growth profile of *B. bifidum* on different substrates and different fermentation time.

Figure 2 shows the effect of fermentation time on growth profile of *B. bifidum* in control and different media containing glucose, inulin, BFRS and BF flour. The trends of *B. bifidum* on control media showed gradually decrease from 7.79 $\log_{10}$ CFU/mL to 6.42 $\log_{10}$ CFU/mL at 0 hour and 48 hours, respectively. At 72 hours, the ultimate growth occurred with 8.29 $\log_{10}$ CFU/mL, which could be due to utilisation of prebiotics source by *B. bifidum*. This is was in agreement with the finding of Huebner et al. (2007) where the lactic acid and other probiotic bacteria required the presence of specific hydrolysis and transport systems of prebiotics.

Fermentation media containing glucose showed fluctuation of *B. bifidum* growth profile. At 6th hour of fermentation time, the growth of *B. bifidum* was 1.26 $\log_{10}$. However, at the 12th hour, the growth started to decline to 6.63 $\log_{10}$ CFU/mL due to depletion of energy sources. Based on the result in Figure 2, at 6, 12 and 72 hours of fermentation time, there were significant differences ($p<0.05$) on glucose ingestion by *B. bifidum* compared to other carbon sources, which proved that glucose as simple sugar functioned as rapid energy sources, as required by bacteria.

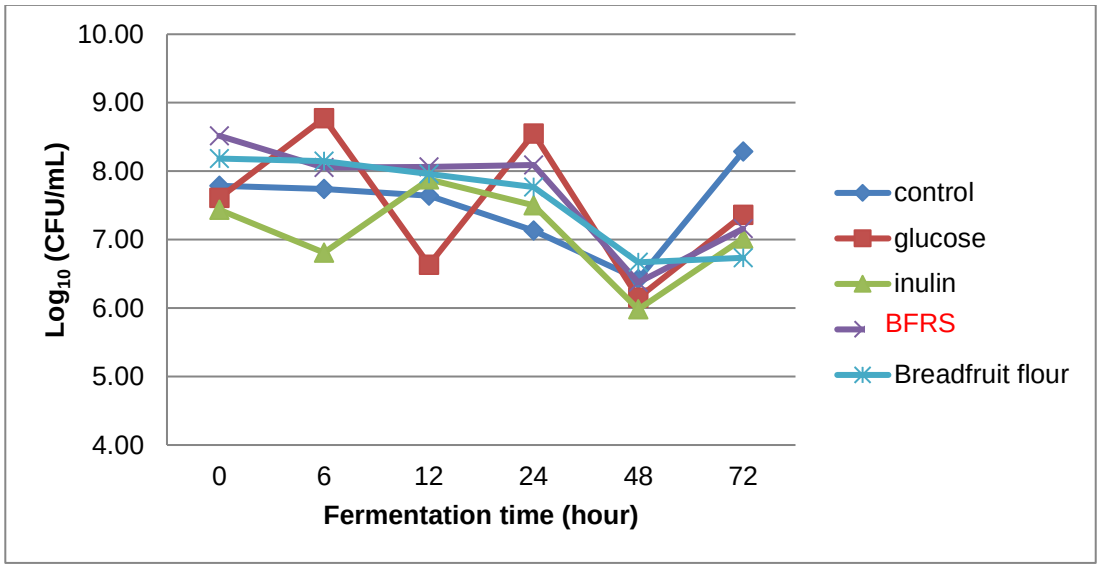

Figure 2; Effect of fermentation time on growth of control media and media containing glucose, inulin, BFRS and BF flour by *B. bifidum*.

Among the carbon sources throughout the varied fermentation time, significant difference ($p<0.05$) was recorded for BFRS with the highest growth rate of *B. bifidum* (8.52 $\log_{10}$ CFU/mL) at 0 hour. The growth pattern of *B. bifidum* had no significant difference ($p>0.05$) with glucose at 6 hours of fermentation time. This result was supported by Hopkins et al. (1998), stated that the fermentation of pure cultures commonly starts to digest after 24 hours.

The results may derive from the rapid growth of the strain within a very short period during incubation, giving an equivocal impression of the substrate's efficacy. Therefore, it is necessary to look at bacterial growth rate data in conjunction with results on cell yields. Metabolism of BFRS had significant difference ($p<0.05$) on *L. plantarum* (Figure 1), while no significant difference ($p>0.05$) *for B. bifidum* (Figure 2) at 24 hours of fermentation time. Hopkins et al., (1998) stated that the carbohydrate utilisation pattern has been reported to differ greatly between *Bifidobacterium* and *Lactobacillus* genera and within them, among different species and strains.

The other important characteristic of a prebiotic substrate is that it should selectively support the growth of probiotic bacteria and at the same time, should not be fermented by commensal organisms (Huebner et al., 2007). Kaplan and Hutkins (2003) stated that probiotic organisms behave quite differently in their capacity to utilise prebiotics, which depend largely on their system. Utilisation of prebiotics by bacteria requires the presence of specific hydrolysis and transport systems for a particular prebiotic. Prebiotics have been suggested to have several beneficial effects, including promotion of beneficial bacterial growth, stimulation of intestinal peristalsis, and production of short chain fatty acids (Cummings et al., 2001).

Growth profile of *E. coli* on different substrates and different fermentation time.

Growth profile of *E. coli* on different substrates (glucose, inulin, BFRS, and BF flour) and control at different fermentation time was illustrated in Figure 3. It would be suggest that based on result, the growth profile of *E. coli* in different media containing carbohydrate source including prebiotic was fluctuate. From the result, the growth of *E. coli* was not detected during *in vitro* fermentation at 0, 6 and 12 hours in control and media containing carbohydrate source (glucose and inulin). However, there was a growth under media containing BFRS and breadfruit flour. This condition might happen due to the growth rate of *E. coli* are different when it supplemented with different carbohydrate source media.

The rate of proliferation cell detected only in control media which was 5.39 $\log_{10}$ CFU/mL at 6 hours. The growth of this commensal pathogen

(*E. coli*) had significant difference ($p<0.05$) at 6, 12 and 72 hours between control and other carbohydrate sources. Apart from some well-known probiotic bacteria in the gut, *bacteroides* and other commodity members of intestinal microflora have the metabolic capacity to metabolise these substrates (Van der Meulen et al., 2006).

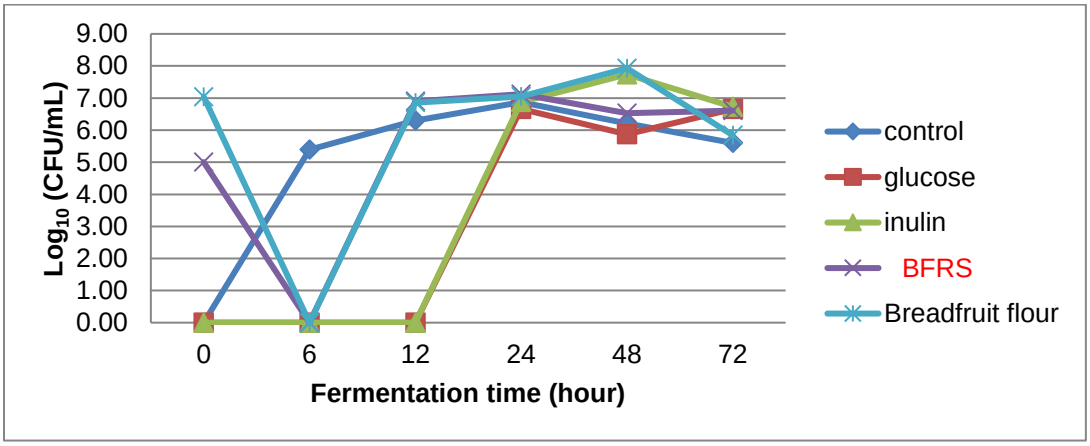

Figure 3; Effect of fermentation time on growth of control media and media containing glucose, inulin, BFRS and BF flour by *E. coli.*

Growth profile of *E. coli* on different substrates and different fermentation time.

Growth profile of *E. coli* on different substrates (glucose, inulin, BFRS, and BF flour) and control at different fermentation time was illustrated in Figure 3. It would be suggest that based on result, the growth profile of *E. coli* in different media containing carbohydrate source including prebiotic was fluctuate. From the result, the growth of *E. coli* was not detected during *in vitro* fermentation at 0, 6 and 12 hours in control and media containing carbohydrate source (glucose and inulin). However, there was a growth under media containing BFRS and breadfruit flour. This condition might happen due to the growth rate of *E. coli* are different when it supplemented with different carbohydrate source media.

The rate of proliferation cell detected only in control media which was 5.39 $\log_{10}$ CFU/mL at 6 hours. The growth of this commensal pathogen (*E. coli*) had significant difference ($p<0.05$) at 6, 12 and 72 hours between control and other carbohydrate sources. Apart from some well-known probiotic bacteria in the gut, *bacteroides* and other commodity members of intestinal microflora have the metabolic capacity to metabolise these substrates (Van der Meulen et al., 2006). There was a significant difference ($p<0.05$) between all carbohydrates sources throughout 72 hours of fermentation time. Based on result in Figure 3, the growth of *E. coli* varied on media containing BFRS at 0, 12, 24, 48 and 72 hours of incubation time which was 5.00, 6.90, 7.12, 6.52 and 6.60 $\log_{10}$ CFU/mL respectively. Based on the result, the growth of *E. coli* on glucose media (6.66 $\log_{10}$ CFU/mL) at 72 hours fermentation time was higher than media containing complex starch such as BFRS (6.60 $\log_{10}$ CFU/mL). This is because *E. coli* can grow in a medium with simple nutrient and ferment simple sugars to produce acids and gas.

Moreover, this result was supported with the finding of Huebner et al., (2007), the growth of enteric bacteria as *E. coli* on inulin which also known as complex carbohydrate is lower than in glucose media. From this study, it showed that *E. coli* could utilized the glucose as an energy source better than complex carbohydrate including BFRS. The other characteristic of a prebiotic substrate is that it should be selective and not fermented by commensal organisms (Sangwan et al., 2014). At 12th hour, the growth of *E. coli* on BFRS (6.90 $\log_{10}$ CFU/mL) had significant difference ($p<0.05$) with glucose (no growth).

pH changes of different pure cultures during *in-vitro* fermentation at different fermentation time.

Changes of pH on different pure cultures (*L. plantarum*, *B. bifidum*, and *E. coli*), during *in-vitro* fermentation were illustrated in Figure 4, 5 and 6. Decrease in pH was observed started from the first hour until 72 hours of *in vitro* fermentation for all the bacteria tested (*L. plantarum*, *B. bifidum*, and *E. coli*). It might be assumed that, the longer the fermentation time, more acids are produced by these three microorganisms during their proliferation in different carbohydrate source (control, glucose, inulin, BFRS and BF flour).

Chung et al., (2016) and Walker et al., (2005) reported that gut microorganisms use a variety of fermentative pathways to harvest energy and the pathways utilised depend on many factors, including pH and available fermentation substrates. In this study, glucose was successfully fermented by all three bacteria strains (*L. plantarum*, *B. bifidum*, and *E. coli*), and reduced the pH level ($p< 0.05$) throughout 72 hours of fermentation time compared to other fermentation medium (control, inulin, BFRS and BF flour). This may due to the characteristic of glucose as glucose as simple sugar that can be used directly for bacterial growth and this was demonstrated by bacteria proliferation in glucose medium.

Bacterial composition produced during fermentation on each carbohydrate source has affected the pH value (Palframan et al. 2002). Based on the result in Figure 4, 5 and 6, the pH values of media containing glucose for all pure culture bacterial tested (*L. plantarum*, *B. bifidum*, and *E. coli*), starting from 0 to 72 hours of fermentation time showed significantly decreased ($p<0.05$) in the pH value compared to other substrate (control, inulin, BFRS and BF flour). Based on the result in Figure 1, 2 and 3, all bacteria (*L. plantarum*, *B. bifidum*, and *E. coli*) have utilized the glucose rapidly to support their growth as energy source which then produced lactic acid during fermentation period.

During *in vitro* fermentation *L. plantarum*, *B. bifidum* and *E. coli* can transform glucose into lactic and acetic acids easier compared to inulin. This might be due to the different structure between glucose and inulin or other complex carbohydrate including BFRS. Inulin has more complex structure and has higher molecular weight than glucose, thus cannot be fermented perfectly as those produced in smaller acid (Kaur

et al., 2011). Moreover, Nugent (2005) reported that, RS is fermented in the large intestine which resulting the production of fermentation products such as carbon dioxide, methane, hydrogen, organic acid and short chain fatty acids. In this study, these three microorganisms *L. plantarum, B. bifidum,* and *E. coli*) may grew in media supplemented with RS, but does not reduce the pH value drastically compared to media supplemented with glucose. The extent of inhibition with these prebiotic carbohydrates was often correspondingly increased, suggesting that a possible mechanism of antimicrobial action that may be attributable to the low culture pH (Fooks and Gibson, 2002).

Based on Figure 6, the pH value produced by *E.coli* showed higher than *L. plantarum* (Figure 4) and *B. bifidum* (Figure 5) throughout the fermentation time in different carbohydrate source media. According to Kaplan and Hutkins (2000) both *L. plantarum* and *B. bifidum* are categorize as commercial probiotic which then could release lactic acid thus affect the pH value. Bai et al. (2004) stated that the drop in pH, especially in pH 6.0 cultures, resulted in more lactic-acid-producing bacteria (Lactobacillales). The major metabolic end-products of lactobacilli and bifidobacterial fermentations are acetate and lactate (Macfarlane et al., 1997). Furthermore, lactic-acid-producing bacteria exerted beneficial effects on host health, such as promoting cholesterol absorption (Pereira and Gibson, 2002) and reducing diarrhea (Hou et al., 2007).

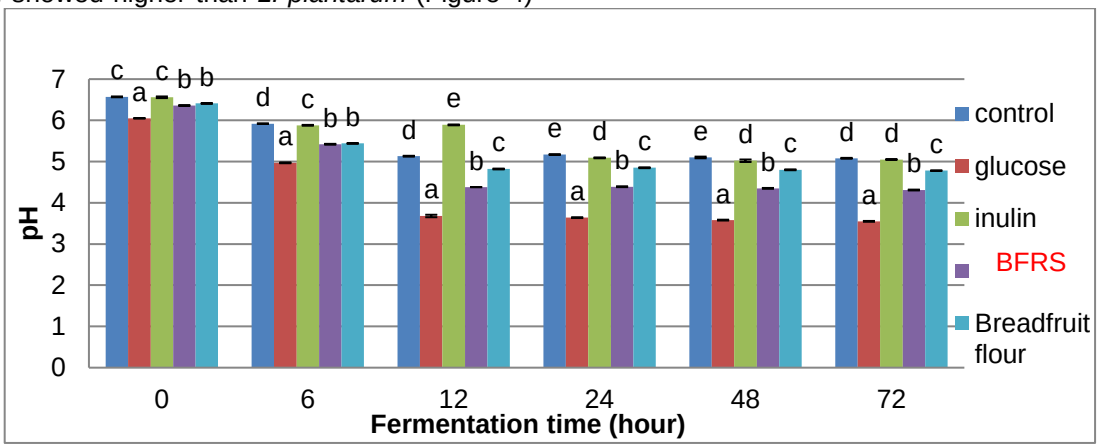

Figure 4; Effect of fermentation time on pH of control media and media containing glucose, inulin, BFRS and BF flour by *L. plantarum*.

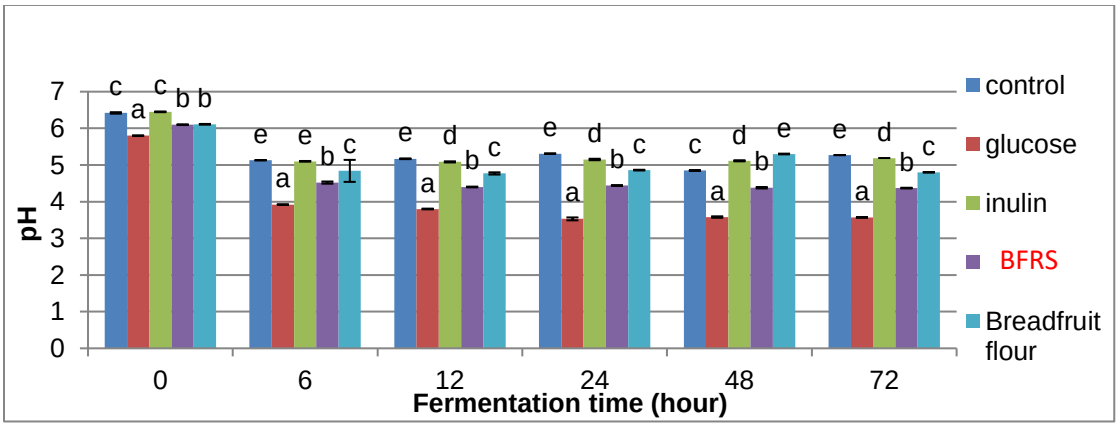

Figure 5; Effect of fermentation time on pH of control media and media containing glucose, inulin, BFRS and BF flour by *B.bifidum,*

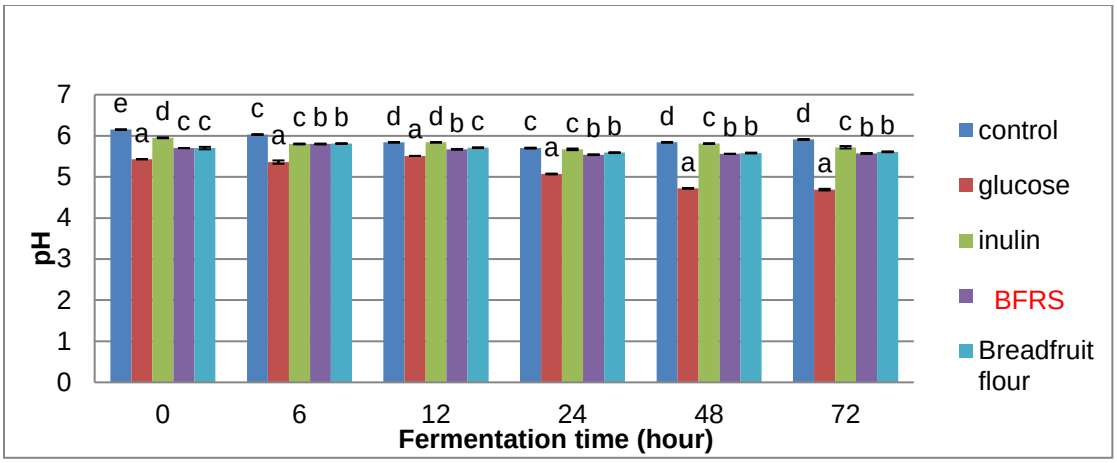

Figure 6; Effect of fermentation time on pH of control media and media containing glucose, inulin, BFRS and BF flour by *E. coli*

CONCLUSION

In conclusion, the growth profile and pH changes of *L. plantarum, B. bifidum* and *E. coli* during *in-vitro* fermentation of carbohydrate sources (glucose, inulin, BFRS and BF flour) at 0, 6, 12, 24, 48,and 72 hours were observed. The growth profiles of *L. plantarum*, *B. bifidum* and *E. coli* showed a different pattern when supplied with different carbohydrate sources. In present study, these three microorganism tested may utilized glucose rapidly during *in-vitro* fermentation process and this might be due to its simple structure compared to BFRS (complex carbohydrate). During *in-vitro* fermentation process, the growth of *L. plantarum*, *B. bifidum* and *E. coli* (72 hours) in media containing different carbohydrate sources reduced the pH value due to the release of, organic acid (lactic acid, acetic acid) as well as short chain fatty acid. The *in vitro* fermentation process done in this study was mimics to the microbial ecology of human intestinal tract. From this study, it is clear that BFRS has a potential to support the growth of beneficial probiotics thus give lots of health benefits. BFRS has partially met the criteria as prebiotic which it could be fermented and be utilized by gut microbiota, selectively stimulated activity and/or growth of one or a limited number of gut bacteria that contribute to host health. This critical work will maximize the potential for breadfruit to become a much more widely grown and utilized crop throughout the tropics.

CONFLICT OF INTEREST

The authors declared that present study was performed in absence of any conflict of interest.

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AUTHOR CONTRIBUTIONS

NJMN performed the experiments and wrote the draft while ZZ designed, supervised and reviewed the manuscript. NH, SS and SNMN approved the final version.

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