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Bifidum*)**

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**Keywords:**

*Lactobacillus plantarum*  
*Bifidobacterium bifidum*  
*Escherichia coli*  
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Prebiotics Activity Score

## ABSTRACT

The prebiotics activity score was a quantitative method to measure to which extent prebiotics (Breadfruit Resistant starch, Breadfruit flour and Inulin) to support the growth of *L.plantarum* and *B.bifidum*. The changes in growth after fermentation (0, 6, 12, 24, 48 and 72 h) of bacteria strains on prebiotics and glucose in relation to changes in *Escherichia coli* grown under same condition was used to determine prebiotics score. The fermentation of the different carbon sources (Breadfruit Resistant starch, Breadfruit flour and Inulin) by *L.plantarum* and *B.bifidum* produce short chain fatty acids that determined by changes of pH throughout 72 h of fermentation. The highest score (0.45) was obtained for *L.plantarum* that utilised breadfruit resistant starch after 12 h of fermentation time. However, the highest score (0.65) for *B.bifidum* also utilised breadfruit resistant starch as carbon sources after 48 h of fermentation. It was found that there was significant difference in pH changes for both strains. Greatest pH reduction for *L.plantarum* and *B.bifidum* for all samples after 6 h of fermentation. In conclusion, breadfruit resistant starch has potential as prebiotics as it meets the requirement as a prebiotic.

**Keywords:** *Lactobacillus plantarum*, *Bifidobacterium bifidum*, *Escherichia coli*, Breadfruit Resistant Starch, Prebiotics Activity Score

## ABSTRAK

Skor aktiviti prebiotik adalah kaedah kuantitatif untuk mengukur prebiotik (kanji rintang sukun, Tepung sukun dan Inulin) untuk menyokong pertumbuhan *L.plantarum* dan *B.bifidum*. Perubahan dalam pertumbuhan selepas penapaian (0 jam, 6 jam, 12 jam, 24 jam, 48 jam dan 72 jam) bakteria pada prebiotik dan glukosa berkaitan dengan perubahan dalam *Escherichia coli* yang tumbuh dalam keadaan yang sama digunakan untuk menentukan skor prebiotik. Fermentasi sumber karbon yang berbeza oleh *L.plantarum* dan *B.bifidum* menghasilkan asid lemak rantai pendek yang ditentukan oleh perubahan pH sepanjang 72 jam fermentasi. Skor tertinggi (0.45) diperolehi untuk *L.plantarum* yang menggunakan kanji rintang sukun sebagai sumber karbon selepas 12 jam masa fermentasi. Sementara itu, skor tertinggi (0.65) untuk *B.bifidum* juga menggunakan kanji rintang sukun sebagai sumber karbon selepas 48 jam fermentasi. Ia mendapati bahawa terdapat perbezaan yang signifikan dalam perubahan pH untuk kedua-dua mikrob ini. Pengurangan pH yang banyak bagi *L.plantarum* dan *B.bifidum* untuk semua sampel selepas 6 jam penapaian. Kesimpulannya,, kanji rintang sukun mempunyai potensi sebagai prebiotik kerana ia memenuhi syarat sebagai prebiotik.

**Kata Kunci:** *Lactobacillus plantarum*, *Bifidobacterium bifidum*, *Escherichia coli*, Kanji Rintang Sukun, Skor aktiviti prebiotik

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## INTRODUCTION

Breadfruit is staple food in some developing countries of the world and also categorizes as carbohydrate rich food. Previous studies reported that breadfruit has 80% of carbohydrates content and 77% of starch content but depends on breadfruits maturity (Topping et al., 2001). Non-digestible oligosaccharides are one form of starch that present in breadfruit. The non-digestible oligosaccharides in breadfruit act as resistant starch. Resistant Starch (RS) can be explain as sum of starch and the product of starch degradation that not fully broken down and in small intestine of healthy people. Resistant starch is important for human because resistant starch is function as substrate to stimulate colonic fermentation. It promotes the production of short chain fatty acids (SCFA). Probiotics can explain as live microbial food supplement that beneficial to the host animal by improving its host intestinal microbial balance (Fuller, 1991). World Health Organization (WHO) confirmed that when probiotics ingested in sufficient amount ( $10^6$  cfu/mL) can be beneficially effects the human health. According to Vasiljevic and Shah (2008), in order to claim the food has prebiotics benefits, it should meet the criteria which the prebiotics microorganism number should more than  $10^6$  cfu/g at the time of consumption.

Probiotics are called effective when it can exert beneficial effects on the host, non-pathogenic and nontoxic and also contain a large number number of viable cells. *Lactobacilli* and *Bifidobacteria* genera was the most studied probiotics. Recently, those genera has been used in probiotic foods due to its safety records within fermented foods industry where has been used for many years (Tuohy et al., 2003).

Prebiotics can be defined as foods ingredient that is non-digestible by intestine that give benefits to the host by selectively stimulating the growth, activity or both of one or limited number of bacterial species that already have in the colon (Gibson & Roberfroid, 1995). Prebiotics was expecting to modify the intestinal microbiota by stimulated the bacterial activities that advantageous to the host health and suppressed the bacterial activities that adverse host health. To have the effects of the flora composition in such a way that can impact host health, prebiotics must be resistant towards digestive processes until it reaches large intestine (Gibson et al., 2004). Resistance towards digestive processes means prebiotics must resist to gastric acidity which hydrolysed by mammalian enzymes and also resist to gastrointestinal absorption. Then, to allows food ingredient to act as prebiotics, the food ingredient must be selectively fermented by potentially bacterial in the colon. Furthermore, the required criteria of food to be as prebiotics were it must able to alter the colonic microflora become healthier composition as well as able to give substrate to supports the growth of beneficial bacteria comparable with others organism and non-prebiotics substrate such as glucose.

Resistant starch (oligosaccharides) also has potential to act as prebiotics. In order to enhance the survival and colonization of probiotics bacteria. SCFA is used by the colonic microflora such as *bifidobacteria* and *lactobacilli* as metabolic fuels (Topping, 2001; Rycroft et al., 2001). The growth of *bifidobacteria* and *lactobacilli* population in the colon increased with the consumption of inulin and resistant starch. The potential of the food component as prebiotics can be determined through prebiotics activity score. Prebiotics activity score is a quantative method to measure the prebiotics in supporting the growth of bacteria strains relative to others microorganism and relative to growth on non-prebiotics substrates as carbon sources. Huebner et al., (2007) has suggested this method because *Lactobacilli* and *Bifidobacteria* strain can ferment prebiotics carbohydrate as a substrate but which prebiotics is suitable for the strains to successfully exerts the benefits. Several studies states that different strains even from same genera has different ability to ferment prebiotics carbohydrates (Huebner et al., 2007; Gopal et al., 2001). Therefore, the objectives of this study was to determine the prebiotics activity score of the breadfruit resistant starch, inulin and breadfruit flour. Moreover, the pH changes during *in-vitro* fermentation were also determined.

## MATERIAL AND METHODS

### Bacterial Strains

Pure cultures of lactic acids bacteria (*Lactobacillus plantarum*, ATCC 8014), a culture of *Bifidobacteria bifidum*, ATCC 11863 and a strain of *Escherichia coli*, ATCC 10536. They were obtained from microbiology laboratory at Kota Campus and Faculty Bioresources and Food Industry, Besut Campus, Universiti Sultan Zainal Abidin respectively. The stock was sustained in sterile glycerol (40% v/v) at - 20°C until used.

### Resistant starch

Breadfruit resistant starch was extracted from breadfruit flour. The resistant starch being was type III (RS3) that obtained from chemically processing.

### Chemicals

Glucose and chemicals used in fermentation media ( protease peptone, beef extract, yeast extract, sodium actate, ammonium citrate, di-potassium hydrogen phosphate, magnesium sulphate, manganese sulphate, Tween 80 and L-cysteine) were purchased from Merck Milipore (Germany) and inulin was from Sigma-Aldrich (Steinheim Germany).

### Microbiological media

Selective Media De Man Ragosa, Sharph (MRS) broth, De Man Ragosa, Sharph (MRS) agar, Nutrient broth and Nutrient agar used in this study from Hi-Media®, India.

### In vitro Fermentation of Breadfruit Resistant Starch by Pure cultures

The ability of breadfruit resistant starch to support the growth of *Lactobacillus plantarum*, ATCC 8014, *Bifidobacteria bifidum*, ATCC 11863 and *Escherichia coli*, ATCC 10536 was study in this research. The selection of

media, limit amount of bacteria ( $10^6 - 10^7$  cfu/mL) and incubation time for each bacteria was determined during optimization prior fermentation.

### Inoculum Preparation

*Lactobacillus plantarum*, ATCC 8014, *Bifidobacteria bifidum*, ATCC 11863 pure cultures from glycerol stock was activated into 10 mL de Man, Rogosa, Sharpe broth (MRS) for 24 h. A loop of the broth was streak in MRS agar and incubated at 37°C for 24 h and 48 h respectively. Next, the bacteria were subcultures in MRS broth by taking single colony. In order to getting same amount of bacteria in the broth within the range  $10^6 - 10^7$  cfu/mL, both bacteria was incubate at different time (Table 1). *Lactobacillus plantarum*, ATCC 8014 and *Bifidobacteria bifidum*, ATCC 11863 was incubated at 37°C for 10 h and 12 h respectively. One (1) mL of bacteria in their specific broth (Table 1) was transferred into universal bottles containing different carbon sources which is glucose, breadfruit resistant starch, inulin and breadfruit flour prior to fermentation process. The *Lactobacillus plantarum*, ATCC 8014 and *Bifidobacteria bifidum*, ATCC 11863 was incubated in anaerobic condition. Meanwhile, *Escherichia coli* ATCC 10536 was activated in nutrient broth. A loop of this *E.coli* cultures transferred into 10 mL of nutrient broth to reactivate. Then, a loop of the broth was streaked onto nutrient agar and incubated at 37°C for 24 h. Next, the bacteria then subculture in 35 mL of nutrient broth to obtained the amount of bacteria within the range  $10^6 - 10^7$  cfu/mL then incubated at 37°C for 8 h. One (1) mL of broth transfer into universal bottles with addition of different carbon sources which is glucose, breadfruit resistant starch, inulin and breadfruit flour prior to fermentation process.

**Table 1** Type, incubation condition and incubation period of selective pure cultures

Bacterial Groups	Broth	Incubation condition	Incubation period (h)
<i>Lactobacilli</i>	De Man, Rogosa, Sharpe (MRS) broth	Anaerobic	10
<i>Bifidobacteria</i>	De Man, Rogosa, Sharpe (MRS) broth	Anaerobic	12
<i>Escherichia coli</i>	Nutrient broth	Aerobic	8

### Fermentation medium

The fermentation medium was similar for MRS broth except that replacing dextrose with others carbohydrates source with glucose, breadfruit resistant starch, inulin and breadfruit flour. The fermentation medium consisted of (per L) protease peptone, 10.0 g beef extract, 10.0 g yeast extract, 5.0 g sodium acetate, 5.0 ammonium citrate, 2.0 g di-potassium hydrogen phosphate, 2.0 g magnesium sulphate, 0.1 g manganese sulphate and 1 mL L-cysteine. The pH was adjusted to  $6.2 \pm 0.2$  by added small of 0.1M sodium hydroxide and hydrochloric acid and then autoclaved at 121°C for 15 min. The pH was measured by using Mettler Toledo pH meter.

### Fermentation condition

The fermentation was carried out in 20 mL of media in universal bottles. There were growth media without carbohydrate source as blank media, growth media with 1% (w/v) glucose as positive control, growth media with 1% (w/v) of breadfruit resistant starch, growth media with 1% (w/v) of inulin, and growth media with 1% (w/v) of breadfruit flour. One (1) mL inoculum (5%,v/v) was added into fermentation medium and then incubated at 37 °C for 0, 6, 24, 48 and 72 h in anaerobic condition. The anaerobic condition was prepared by placing the anaerobic pack hang freely in anaerobic container. The aerobic condition was provided for *Escherichia coli*, ATCC 10536. Different amount of bacteria cultures in different fermentation time was taken for analysis. After 0, 6, 12, 24,48 and 72 h of fermentation, 4 mL sample was taken for pH determination using Mettler Toledo pH meter. One (1) mL of sample was diluted in serial dilution for growth enumeration. The fermentation media of pure cultures was freezing with label in deep freezer (Thermo Scientific) at -20°C.

## Analyses

### Enumeration of bacterial growth

Serial dilution was done immediately after sampling by using saline water. One (1) mL fermentation media was taken for serial dilution at tenth fold dilution ( $10^{-4}$  to  $10^{-8}$ ) using sterile saline water. The viable counts of bacterial cultures were enumerated by spread plate method using MRS agar (Hi-Media®, India) in duplicate analysis. The results were reported as colony forming per millimeter suspension (CFU/mL). The MRS agar and nutrient agar was prepared according to the instructions.

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

N= Enumeration of bacteria colony

C= Sum of colony count in n1 and n2

v= Volume of transfer dilution

d= Dilution selected from serial dilution

### Prebiotic Activity Score

Prebiotic activity score (PAS) by different cultures of *Lactobacilli* and *Bifidobacteria* were calculated against *Escherichia coli* strains as reported by Huebner et al. (2007). The changes in cell density were calculated as the differences in  $\log_{10}$  CFU/mL between viable count at 24 h and the viable count at 0, 6, 12, 24, 48 and 72 h.

$$\text{PAS} = \frac{\text{Changes in cell density on prebiotic}}{\text{Changes in cell density on glucose}} - \frac{\text{Changes in cell density of pathogen on prebiotic}}{\text{Changes in cell density of pathogen on glucose}}$$

\*\* Changes in cell density were calculated as differences in the differences in  $\log_{10}$  CFU/mL between viable count at 0 hrs and the viable count at 24 h.

### Measurement of pH Changes

Determination of pH of the sample was measured by using Mettler Toledo pH meter. Hydrogen ion present in the sample was detected by special bulb in the pH meter. The signal produce by the bulb was being amplified and sent to an electronic meter connected to the bulb which measured and display the pH reading. The pH meter (Mettler Toledo) was using standard buffer solution. Then, 4 mL of sample was pipette into universal bottles and value of pH was display after immersing the bulb of pH meter into the sample in duplicate analysis.

### Statistical Analysis

Statistical analysis will be performed using one-way ANOVA (IBM SPSS Statistics Data Editor, Edition 20) to compare the mean values of effects of different cultivation and different cultures for bacterial growth and prebiotic activity score. Subsequently, mean comparison will compute using Turkey's test. All significant differences between means will assessed at significant level of  $\alpha = 0.05$  (95% confident level). Analysis of variance (ANOVA) and Duncan's multiple range test was used to compare any significant different between sample, where  $p < 0.05$  was considered statistically significant.

## RESULTS AND DISCUSSION

### Prebiotic Activity Score

The measurement of prebiotic activity score of *L. plantarum* and *B. bifidum* to metabolized different substrates rather than glucose was performed according to Huebner et al., 2007. The prebiotics activity score was obtained between difference in the growth of the probiotics at certain fermentation time on prebiotics and glucose as carbon sources and the differences in the growth of pathogen or enteric mixture at certain fermentation time on prebiotics and glucose as carbon sources. The results of the prebiotic activity score were presented in Table 2

and Table 3. The rate at which a targeted organism can growth on a specific carbon source is a key factor in the choice of a prebiotic and probiotic combination, as this will impact its capacity to compete for carbon sources with other microflora organisms in the colon (Hopkins et al., 1998). From the results, the highest prebiotics score for *L. plantarum* were grown on breadfruit resistant starch as a carbon source at 12 h ( $p < 0.05$ ) which is 0.48. In contrast with *B. bifidum*, the highest prebiotic activity score was also grown on breadfruit resistant starch but at 48 h which is 0.65, but there was no significant difference ( $p > 0.05$ ) between others carbohydrates sources for all fermentation time. This showed that breadfruit resistant starch has ability to support the growth of targeted organism after 12 h and 48 h of consumption respectively.

*L. plantarum* has high rate of cell proliferation within short period of incubation time which is 12 h compare with *B. bifidum* which need 48 h. Marotti et al. (2012) stated that *Bifidobacteria* need 48 h of incubation to utilized prebiotics carbohydrates. Eventhough *L. plantarum* has highest rate of proliferation compare with *B. bifidum* but the prebiotic activity score of *B. bifidum* (mean 0.65) achieved higher than *L. plantarum* (0.48). Huebener et al., (2007) have proved that different strains of microorganism have different metabolic capacity that caused variations on prebiotic activity. Lactic acid bacteria and other bacteria need the presence of specific hydrolysis and transport system for utilization of prebiotics. So, some strains may be present or absent of these gene coding transport system that cause variation in prebiotics score. Resistant starch showed more its potential as prebiotics on *B. bifidum* compare with *L. plantarum* since it has higher score. This results were in agreement with the previous studies (Rubel et al., 2014) which proved that, the higher prebiotics activity score showed that the higher the growth rate probiotics microorganism, the lower the growth of pathogen. These indices the more selective used the prebiotics by probiotics microorganism in relation to glucose and limited used of prebiotics in relation to glucose by pathogen microorganism.

A low or negative value of prebiotic activity score indicates that *L. plantarum* and *B. bifidum* less favored growth on the prebiotics medium without carbon sources that obtained from glucose at same fermentation time. The low prebiotics score also affected by the fact that the growth of enteric strains less restrict with carbon sources availability on the medium compare with probiotics strains (Rubel et al., 2014). The negative results obtained also shows that *L. plantarum* and *B. bifidum* exhibited the selectively fermentation on all prebiotics carbohydrates (inulin, breadfruit resistant starch and breadfruit flour) at incubation time others than 12 h and 48 h respectively. Resistant starch only effective as prebiotics at 12 h and 48 h after consumption since it has ability to ferment by *L. plantarum* and *B. bifidum* respectively and support their growth. The lower score for both bacteria strains at others fermentation time even their growth rates were high was due the growth of *E. coli* are also high. According Tsuda et al., (2010), *E. coli* has utilized the monosaccharides and lactose at first hours of fermentation time others than prebiotics. But the growth of the *E. coli* remains high even after 24 h of the fermentation time. This might be due to *E. coli* had some ability to ferment prebiotics carbohydrates (Hartemink et al., 1997). Low prebiotics activity scored for *Bifidobacteria* strain for all the fermentation time for inulin. This finding was in agreement with previous studies that report inulin exhibited the higher prebiotic score (Huebner et al., 2007; Gopal et al., 2001). Both lactic acid bacteria (LAB) strain tested showed negative values for breadfruit flour. Breadfruit flour contains non-digestible dietary fiber which has lower water holding capacity which difficult for the bacteria to penetrates the granules matrix and start degradation. So, *L. plantarum* and *B. bifidum* cannot utilizes the carbon sources in the breadfruit flour.

**Table 2** Prebiotic Activity Score of *L. plantarum* ( $\log_{10}$  CFU/mL) grown on different prebiotics.

Types of carbon source	In Vitro Fermentation Time (h)				
	6	12	24	48	72
Blank	-0.5595 <sup>a</sup>	-0.1135 <sup>b</sup>	-3.119 <sup>a</sup>	-1.5475 <sup>a</sup>	-1.2815 <sup>a</sup>
Inulin	-1.0348 <sup>a</sup>	-1.7802 <sup>a</sup>	-2.5237 <sup>a</sup>	-1.7227 <sup>a</sup>	-1.2972 <sup>a</sup>
Breadfruit Resistant Starch	0.0407 <sup>a</sup>	0.4849 <sup>b</sup>	-4.3077 <sup>a</sup>	0.1877 <sup>a</sup>	-1.9074 <sup>a</sup>
Breadfruit Flour	-0.304 <sup>a</sup>	-0.0628 <sup>b</sup>	-3.5103 <sup>a</sup>	-0.0263 <sup>a</sup>	-1.5669 <sup>a</sup>

Mean with different superscript are significantly ( $p < 0.05$ ) different

Gebruers *et al.*, (2008) reported that gastrointestinal tract microorganism easily and fully fermented digestible dietary fiber compare with non-digestible fiber. Higher fermentability digestible fiber contributed to high water holding capacity, making bacteria easily degrades the fiber (Morotti *et al.*, 2012).

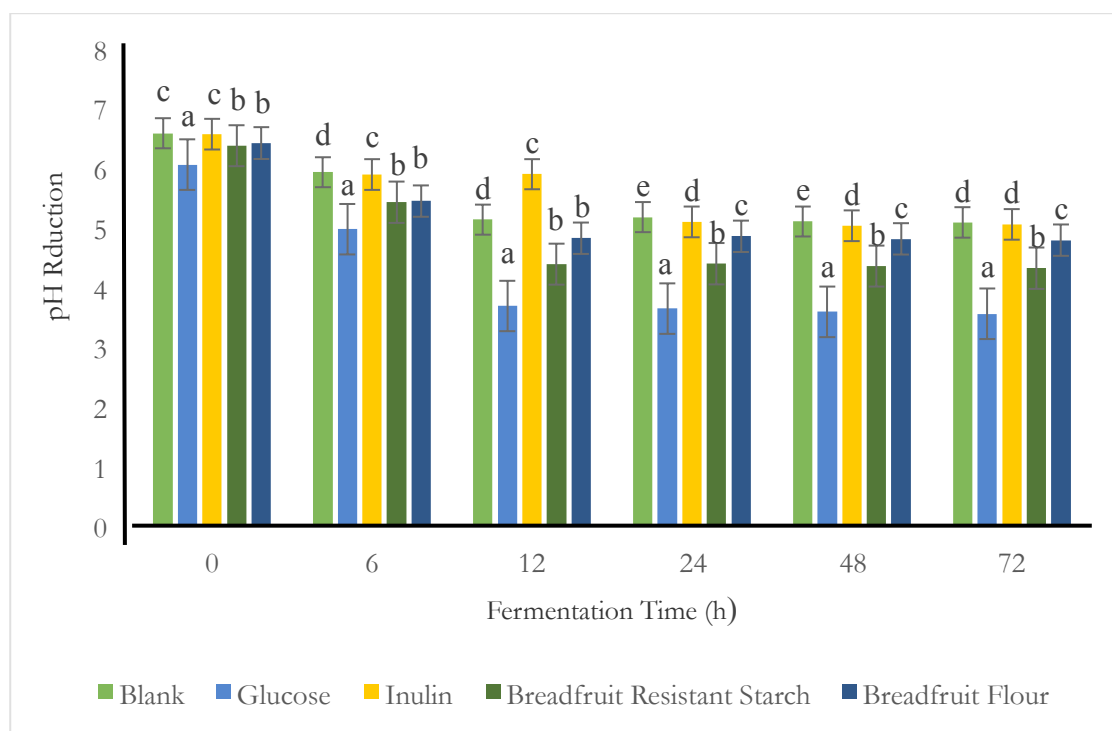
**Table 3** Prebiotic Activity Score of *B.bifidum* ( $\log_{10}$  CFU/mL) grown on different prebiotics.

Types of carbon source	In Vitro Fermentation Time (h)				
	6	12	24	48	72
Blank	-0.9648 <sup>a</sup>	-0.697 <sup>a</sup>	-1.7302 <sup>a</sup>	0.2097 <sup>a</sup>	-0.3951 <sup>a</sup>
Inulin	-2.0094 <sup>a</sup>	-1.9295 <sup>a</sup>	-1.0379 <sup>a</sup>	-0.3326 <sup>a</sup>	-1.32 <sup>a</sup>
Breadfruit Resistant Starch	-1.3714 <sup>a</sup>	-0.3472 <sup>b</sup>	-1.5107 <sup>a</sup>	0.6546 <sup>a</sup>	-2.5225 <sup>a</sup>
Breadfruit Flour	-1.1559 <sup>a</sup>	-0.6551 <sup>a</sup>	-1.426 <sup>a</sup>	0.1269 <sup>a</sup>	-2.8143 <sup>a</sup>

Mean with different superscript are significantly ( $p < 0.05$ ) different

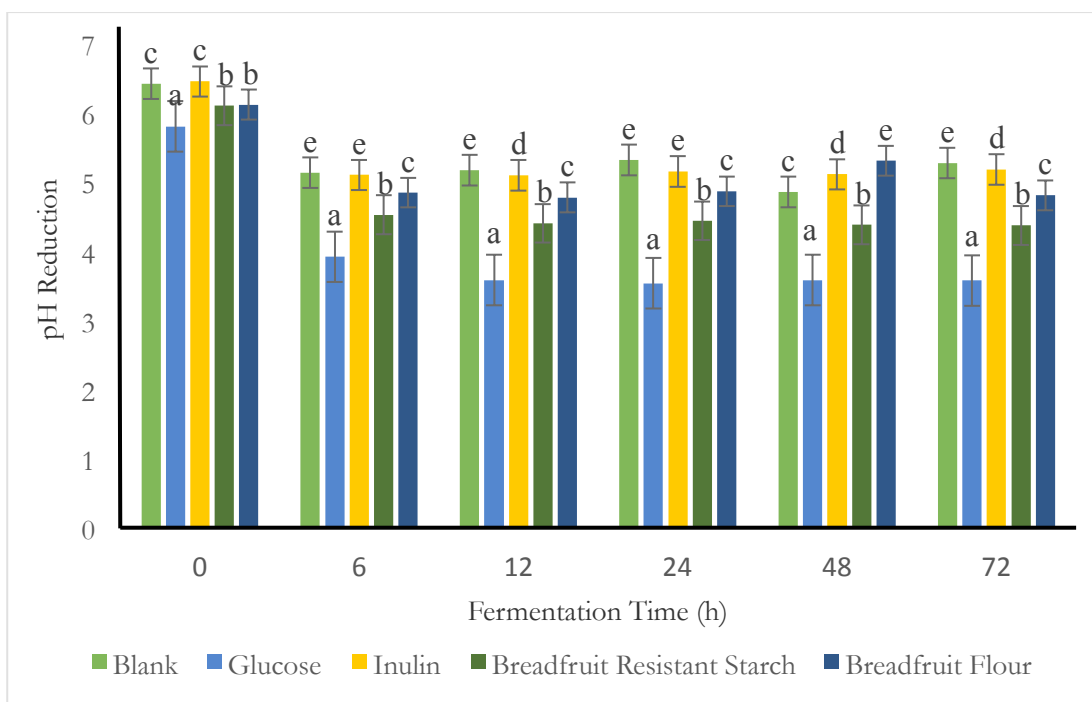
### pH Changes for Selected Pure Cultures

The pH was showed in Figure 1 for *L.plantarum* ATCC 8014, Figure 2 for *B.bifidum* ATCC 11863, and Figure 3 for *E.coli* ATCC 10536. Based on the results obtained, glucose showed pH was reduced significantly ( $p < 0.05$ ) for all three strains throughout the fermentation time compare to blank, inulin, breadfruit resistant starch and breadfruit flour. Glucose is a simple sugar and can be directly utilizes by the *L.plantarum*, *B.bifidum* and *E.coli*. Reduced in pH showed that glucose has been fully fermented thus indicated the production of organic acids. Low pH indicates that high amount of organic acids produced during fermentation process. Same pattern of pH reduction was shown for other fermentation medium (blank, inulin, breadfruit resistant starch and breadfruit flour).



**Figure 1** pH reductions of blank media and media containing glucose, inulin, breadfruit resistant starch and breadfruit flour by *L.plantarum* ATCC 8014 during 72 h fermentation. Mean with different superscript are significantly ( $p < 0.05$ ) different

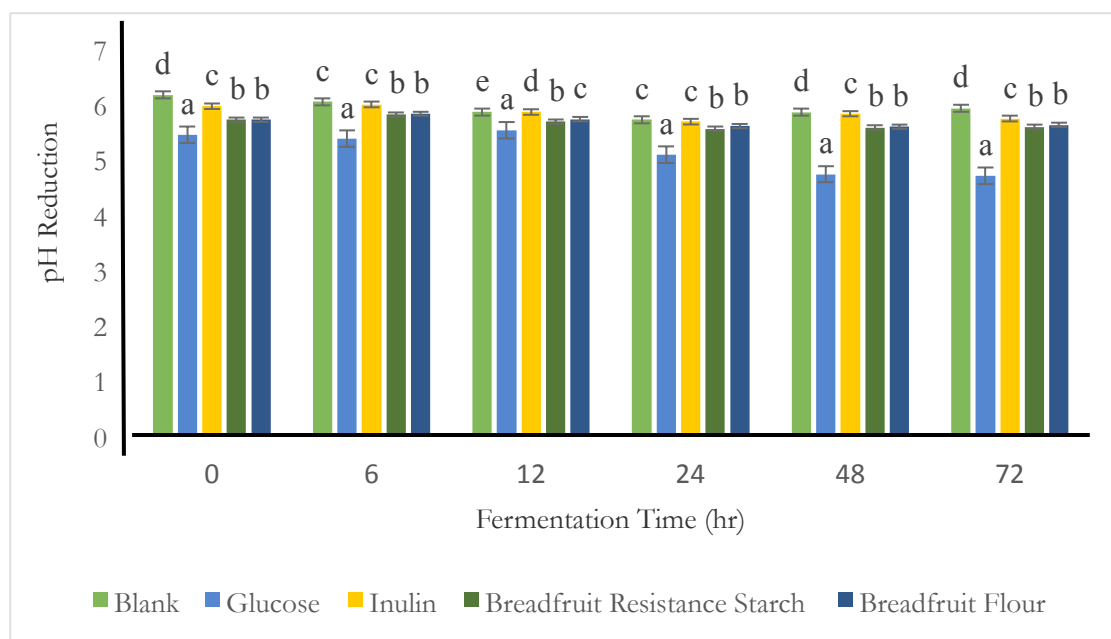
Generally, pH decreases was greater after 6 h fermentation for all samples for *L.plantarum* and *B.bifidum* strains excepts for *E.coli*. For *L.plantarum* and *B.bifidum*, breadfruit resistant starch has lowest pH from the beginning of fermentation until end fermentation ( $p < 0.05$ ). Meanwhile, breadfruit flour shows greatest reduction of pH after 12 h of fermentation. As expected, smallest pH changes throughout 72 h of *in-vitro* fermentation for inulin and without carbon sources. In contrast, *E.coli* only experienced small changes of pH reduction for all prebiotics samples (inulin, breadfruit resistant starch and breadfruit flour). Some *E.coli* strains can ferment prebiotics as carbon sources but a few can utilised others than glucose (Hartemink et al., 1997). The strains tested in this study unable to ferment prebiotics to support their growth thus produced end product acid that lead to decreased in pH. Previous studies has shown that short chain fatty acids produced during fermentation contributed to inhibition of *E.coli* at reduced pH (Wolin,1969; Roe et al., 1998). After 6 h incubation time of *B.bifidum*, breadfruit resistant starch and breadfruit flour experienced significantly decreased pH than glucose.



**Figure 2** pH reductions of blank media and media containing glucose, inulin, breadfruit resistant starch and breadfruit flour by *B.bifidum* ATCC 11863 during 72 h fermentation. Mean with different superscript are significantly ( $p < 0.05$ ) different.

Thus, it might be *B.bifidum* more preferred to utilize resistant starch and breadfruit flour. This result was in agreement with some previous studies that show some *Bifidobacterium* strains preferred to ferment disaccharides or oligosaccharides compare monosaccharides (González-Rodríguez et al., 2013). Studies show *Bifidobacterium* strains cannot ferment inulin as carbon sources (Valdés-Varela et al., 2017). Results shows the pH reduction was very low changes over long incubation time as more organics acid produced. It due to inulin more preferred to ferment at more neutral pH (Palframan et al., 2002). In contrast with *L.plantarum*, the greatest pH reduction for all substrates as presented in Figure 1 which proved that *L.plantarum* can ferment breadfruit resistant starch, inulin and breadfruit flour to produce short chain fatty acids that lead to the pH reduction in the colon.





**Figure 3** pH reduction of blank media and media containing glucose, inulin, breadfruit resistant starch and breadfruit flour by *E.coli* ATCC 10536 during 72 h fermentation. Mean with different superscript are significantly ( $p < 0.05$ ) different

But, the carbohydrates types affect extend of pH reduction, the amounts and types of short chain fatty acids produced by *L.plantarum* (Zhou et al. 2012). The reduction of pH for *L.plantarum* and *B.bifidum* was due to the production of short chain fatty acids. Major end products for *L.plantarum* and *B.bifidum* was acetate and lactate which lead to decreased the pH value. There is some varying in pH reduction for both strains respectively because different strains have different ability to utilize carbon sources. Even within same genera, there is varying in pH due to different ability to degrade carbohydrates sources. De Vuyst and Leroy, (2011) has stated that *Bifidobacterium*, *Lactobacillus*, *Bacteriocides* and *Faecalibacterium* can ferment in vitro the short and medium oligofructose such as resistant starch but some strains can utilize long chain sugar fructans. So, this finding was in agreement with the results obtained since there was decrease in pH values for both strains that shows *L.plantarum* and *B.bifidum* can utilise the carbohydrates sources rather glucose.

## CONCLUSION

Prebiotics activity score describes the extent to which prebiotics can support the growth of lactic acid bacteria. The breadfruit resistant starch has significantly higher prebiotic activity score. Thus, breadfruit resistant starch might be has high potential as new prebiotics. The pH changes also have significantly difference in breadfruit resistant starch that might be due to the production of short chain fatty acids during in vitro fermentation process. This study showed that lactic acid bacteria strains has ability to utilizes resistant starch from breadfruit as carbon sources and can be another option as prebiotics.

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