

## Evaluation of *Piper nigrum* L. as a Prebiotic Ingredient Using In Vitro Colon Model

SITI MAISARAH NASHRI<sup>1</sup>, TAN HUI-YAN<sup>1</sup> & SHAHRUL RAZID SARBINI\*<sup>1,2</sup>

<sup>1</sup>Department of Crop Science, Faculty of Agricultural Science and Forestry, Universiti Putra Malaysia Bintulu Campus, Sarawak, Malaysia; <sup>2</sup>Halal Product Research Institute, Universiti Putra Malaysia, Putra Infoport, Serdang, Selangor, Malaysia.

\*Corresponding author: [shahrulrazid@upm.edu.my](mailto:shahrulrazid@upm.edu.my)

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### ABSTRACT

Black and white pepper of the species *Piper nigrum* L. is regarded as the king of spices, and Malaysia is the second largest producer of peppercorns, after Indonesia. This spice contains abundant bioactive compounds that are capable of enhancing human health. However, the prebiotic potential of *P. nigrum* L. as a food ingredient has not yet been explored. Therefore, this research studied *P. nigrum* L. through *in vitro* gastrointestinal digestion and colonic fermentation using human faecal slurry. Samples were analysed for the colonic bacterial changes and its metabolites production using HPLC. Both black and white pepper showed prebiotic responses similar to those in inulin, particularly in stimulating the growth of human gastrointestinal microbiota. This study finds that *P. nigrum* L. promotes the growth of probiotic strains such as *Bifidobacterium* spp., and *Lactobacillus* / *Enterococcus*. Both black and white pepper also showed the ability to suppress colonic pathogen strain like *Clostridium histolyticum*. *In vitro* colonic fermentation of *P. nigrum* L. also significantly stimulate production of health beneficial metabolites. The production of short-chain fatty acids like acetate and propionate were observed to be particularly abundant. This is the contribution of piperine in both black and white pepper. In which, piperine provide both anti-bacterial and anti-inflammatory properties. Overall, *P. nigrum* L. showed appreciable prebiotic value similar to the commercial prebiotic, inulin. Thus, black and white pepper from Sarawak, Malaysia helps in promoting human gastrointestinal health. This finding may contribute to the value-added of Sarawak pepper as a functional food that can be involved in daily meals as spices.

Keywords: Black pepper, digestibility, functional food, gut microbiota, short-chain fatty acids

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### INTRODUCTION

A dietary prebiotic is a selectively fermented ingredient that results in specific beneficial changes in the composition and/or activity of colonic microbiota which gives out benefit(s) to the host health (Shen *et al.*, 2011). When the substrate enters the large bowel, it should selectively change the activity or composition of the good microbiome, which leads to demonstrable health benefits (Gibson *et al.*, 2004). An interesting Malaysian local food source that may hold prebiotic potential source is the king of spice, Sarawak's *Piper nigrum* L. Malaysia is one of the biggest producers of peppercorns after Indonesia (Joy *et al.*, 2007). According to the Malaysian Pepper Board, Sarawak state is the largest *P. nigrum* L. producer with an annual contribution as much as 95% in 2016. This spice contains high bioactive compounds in enhancing human health and

contains up to 33% dietary fibre (Pradeep *et al.*, 1993). Other than that, the presence of alkaloids, polyphenols and flavonoids also increases its ability to act as a prebiotic (Dreger *et al.*, 2014). Some studies conducted proved that these secondary metabolites might contribute to improving intestinal health by maintaining the microbial environment in the gut through stimulation of *Lactobacilli* and *Bifidobacteria*, and inhibiting the pathogenic bacteria population in the human gut, which exerting the prebiotic-like effects (Dreger *et al.*, 2014). Most of the recorded studies on *P. nigrum* L. showed promising result and its effectiveness in livestock production especially on appetite and antibiotic substitute (Alloui *et al.*, 2014). Therefore, this research aimed to extend the application of *P. nigrum* L. in its responses to human gut digestion using local Sarawak's peppercorns.

## MATERIALS AND METHODS

### Sample Preparation

Dried black and white *Piper nigrum* L. of Kuching variety were provided by the Malaysian Pepper Board (Sarikei branch). The black pepper used in this study was the Special Black grade, while the white pepper used was of the Fair Average Quality White grade. Both dried black and white pepper were ground using a grinder (Kuaofung Electronic and Machine, Taiwan) and sieved through a 0.30 mm mesh sieve. The samples were kept in an air-tight container then stored at room temperature to avoid light exposures. Food grade inulin (Orafti HP, Germany) was used as positive control.

### *In vitro* Gastrointestinal Digestion

Samples underwent *in vitro* digestion using enzymes and chemicals to mimic the upper part of human gastrointestinal digestion (Mandalari *et al.*, 2008). The test substrates were digested in three stages by mimicking the mouth, gastric and duodenal digestions in 100 mL Schott bottles.

#### *Oral digestion phase*

Sample (1.5 g) was added with 0.1 M sodium chloride solution at pH 7.0. Oral digestion was initiated through incubation using heat stable  $\alpha$ -amylase (500 U/500  $\mu$ L) for 5 minutes. The process was done in an orbital shaking incubator at 170 rpm under a controlled temperature of 37 °C.

#### *Gastric digestion phase*

For gastric digestion, acidic saline (150 mM NaCl, pH 2.5) was well mixed into the oral digested sample suspension. The pH of the mixture was then adjusted using hydrochloric acid. Pepsin (146 U/mL) and gastric lipase (0.56 mg/mL) were added next and incubated for 2 hours. Similarly, the digestion was carried out in an orbital shaking incubator at 170 rpm and temperature of 37 °C.

#### *Duodenal-intestinal digestion phase*

Sample was carried forward for duodenal-intestinal digestion. The pH of the gastric digesta was first adjusted to pH 6.5 using sodium hydroxide (0.5 M). The suspension was added

with bile salt (4.0 mmol/L sodium taurocholate, 4.0 mmol/L sodium lycopodeoxycholate), calcium chloride (11.7 mmol/L), bis-tris buffer (0.73 mmol/L, pH 6.5), alpha-chymotrypsin (5.9 U/mL), trypsin (104 U/mL), colipase (3104 U/mL) and pancreatic lipase (54 U/mL). The mixture was well homogenised and further incubated in orbital shaking incubator for 1 hour at 37 °C.

At the end of the digestion process, the digesta was filtered using filter paper (Whatman No.1). Both filtrate and residue were dried at 50 °C and stored at room temperature for further analyses.

### *In vitro* Colon Fermentation Using Human Faecal

#### *Preparation of Colonic Basal Nutrient Media (BNM)*

Sterile basal nutrient medium (BNM) was prepared by mixing peptone water (2.0 g/L), yeast extract (2.0 g/L), sodium chloride (0.1 g/L), di-potassium hydrogen phosphate (0.04 g/L), Potassium dihydrogen phosphate (0.04 g/L), Magnesium sulphate hepta-hydrate (0.01 g/L), calcium chloride hexahydrate (0.01 g/L), sodium bicarbonate (2.0 g/L), L-cysteine HCl (0.5 g/L), bile salts (0.5 g/L), 4.0 mL of Resazurin (0.025%), and Tween 80 (2.0 mL). Haemin (0.02 g/L) was dissolved by using 1.0 M sodium hydroxide then further added into the mixture. The BNM was sterilised using autoclave for 120 °C for 15 minutes. The medium was adjusted to pH 6.8 using 1.0 M sodium hydroxide and hydrochloric acid. Vitamin K1 (10.0  $\mu$ L/L) was added aseptically after sterilisation to avoid oxidation after the media was cooled down.

#### *Human faecal inoculate*

Faecal sample was obtained from healthy human volunteers based on criteria listed in the study of Sarbini *et al.* (2011). Faecal slurry was prepared by diluting the faecal sample (20 – 25 g) with phosphate-buffered saline (0.1 M, pH 7.4) at a ratio of 1:10. The faecal slurry sample was then homogenized for 2 minutes in a stomacher at 210 rpm (Stomacher 400, Seward, West Sussex, UK). Faecal inoculate from one healthy volunteer is considered as one replication of the

study. The faecal sampling process was repeated thrice for a three replications study.

Studies using faecal donations from healthy volunteer does not require any medical ethical committee approval as they are considered non-invasive. Volunteers who donated faecal samples were informed before the study was initiated and their participation were considered based on the criteria stated above.

#### *In vitro colonic fermentation*

A 100 mL working size of customized glass fermentation vessels (Soham Scientific, Fordham, UK) was used as a fermenter vessel in this study. The BNM (45 mL) was added aseptically and then gassed overnight with nitrogen (15.0 mL/min) to eliminate oxygen. The vessels were kept stirred using a magnetic stirrer for constantly for a homogenous and anaerobic media along the fermentation process.

To mimic the human colon, a circulating water-bath was used to run through the water-jacketed vessels to maintain the temperature at 37 °C. After 30 minutes, test substrate 0.5 g (1% w/v) was added into the oxygen-free BNM prior to the 10% w/v faecal slurry. Throughout the fermentation period, the colon model vessels were kept stirring and constantly supplied with oxygen-free nitrogen gas at a flowrate of 15 mL/min. The pH of the fermentation sample was

maintained at pH 6.8 (distal colon: rapid and shorter incubation period) by using automatic pH controller (Fermac 260, Electrolab, UK). The pH controller system was supplied with 0.25 mM NaOH and HCl for an automatic adjustment of any pH fluctuation.

The fermentation process was performed for a period of 24 hours. In which, sample (5 mL) was taken at 0, 6, 12, and 24 hours for analyses of short chain fatty acids profiling and bacteria enumeration. The fermentation was done with three replications whereby each replication has a different supply of faecal slurry from volunteers.

#### **Bacterial Enumeration**

Bacterial population changes during *in vitro* colonic fermentation were observed via fluorescent *in situ* hybridization technique by using oligonucleotide probes designed to target specific diagnostic regions (Table 1) (Sarbini *et al.*, 2013).

Fermentation sample (10 µL) was diluted with PBS/SDS solution at suitable dilution to obtain 20 – 100 fluorescent cell counts in each field of microscopic view. Whereby, the PBS/SDS solution was prepared by 1 x phosphate-buffered saline added with 100 µL of 10% SDS (PBS/SDS).

**Table 1.** 16S rRNA oligonucleotide probes used in the present study

Probe code	Specificity	Sequence (5' to 3')
Chis150	Most of the bacteria in the <i>Clostridium histolyticum</i> group ( <i>Clostridium</i> clusters I and II)	TTATGCGGTATTAATCTYCCTTT
Lab158	<i>Lactobacillus-Enterococcus</i>	GGTATTAGCAYCTGTTTCCA
Erec482	Most of the bacteria in the <i>Clostridium coccooides-Eubacteriumrectale</i> group ( <i>Clostridium</i> clusters XIVa and XIVb)	GCTTCTTAGTCARGTACCG
Bif164	<i>Bifidobacterium</i> spp.	CATCCGGCATTACCACCC
Bac303	Most Bacteroidaceae and Prevotellaceae, some Porphyromonadaceae	CCAATGTGGGGGACCTT

Sample (20 µL) was spread evenly on the well of polytetrafluoroethylene/poly-L-lysine-coated glass slide (Tekdon Inc., Florida, US). Glass slide with sample were then dried on a slide dryer for 15 minutes. The slide then underwent dehydration using alcohol series solution of 50, 80, and 96% ethanol for 3 min

each. Excess ethanol on the slide was then dried off on the slide dryer for 2 min.

Hybridisation buffer was prepared according to the report of Sarbini *et al.* (2013). Probe-Hybridization mixture (PHM) was prepared by adding 45 µL of hybridization buffer with 5.0 µL

of targeted probe (diluted to 50 µg/L). The PHM was added onto the dried sample well on the glass slide. The sample mixture was left to hybridize for four hours in a microarray hybridization incubator (Grant-Boeckel, Cambridge, UK).

Washing buffer was also prepared according to the report of Sarbini *et al.* (2013). After the 4 hours of hybridisation, glass slide was then transferred into a pre-warmed 50 mL of washing buffer and soaked for 15 min at 50 °C. Glass slides were then dipped into cold water for a few seconds to stop the washing process and then air-dried in a dark condition. Polyvinyl alcohol mounting medium [1,4-diazabicyclo (2.2.2) octane (DABCO), 5 µL] was added, then covered with a glass coverslip (20 mm; thickness no. 1; VWR, Lutterworth, UK). Glass slides was viewed under a fluorescence microscope (CX31; Olympus; Tokyo; Japan) with CX-RFL-2 reflected fluorescence attachment for 15 different fields for each well (Rawi *et al.*, 2021). Fluorescent bacterial cells were enumerated and an average number was obtained.

### Short-Chain Fatty Acids (SCFA) Analysis

High-performance liquid chromatography (HPLC) was used to evaluate the short chain fatty acids content of the fermented samples with modification (Lee *et al.*, 2022a). The HPLC system (Shimadzu Corporation, Kyoto, Japan) was equipped with a pump (Shimadzu LC20-AT), a UV detector (Shimadzu SPD-20A), an autosampler (Shimadzu SIL-20A) and an oven (Shimadzu CTO-10ASvp). Stationary phase was set up using ion-exclusion column (Rezex ROA-Organic Acid H+ (8%), 300 x 7.80 mm, Phenomenex Cheshire, UK). The 0.25 mM sulphuric acid in HPLC grade water was used as mobile phase as the eluent. Samples from different fermentation time (0 h, 6 h, 12 h and 24 h) were centrifuged at 1500 x g for 15 min and filtered through a 0.22 µm filter unit. An aliquot of 20 µL filtered sample was injected into the HPLC system and eluted at a flow rate of 0.5 mL/min, 40 °C heated column, UV detector of 210 nm, at an isocratic flow for 35-50 minutes of running time. Metabolites quantification was done using lactate, acetate, propionate and butyrate as calibration standard from a concentration series of 12.5, 25, 50, 75, 100, 125, 150, 175 and 200 and 250 mM.

### Statistical Analysis

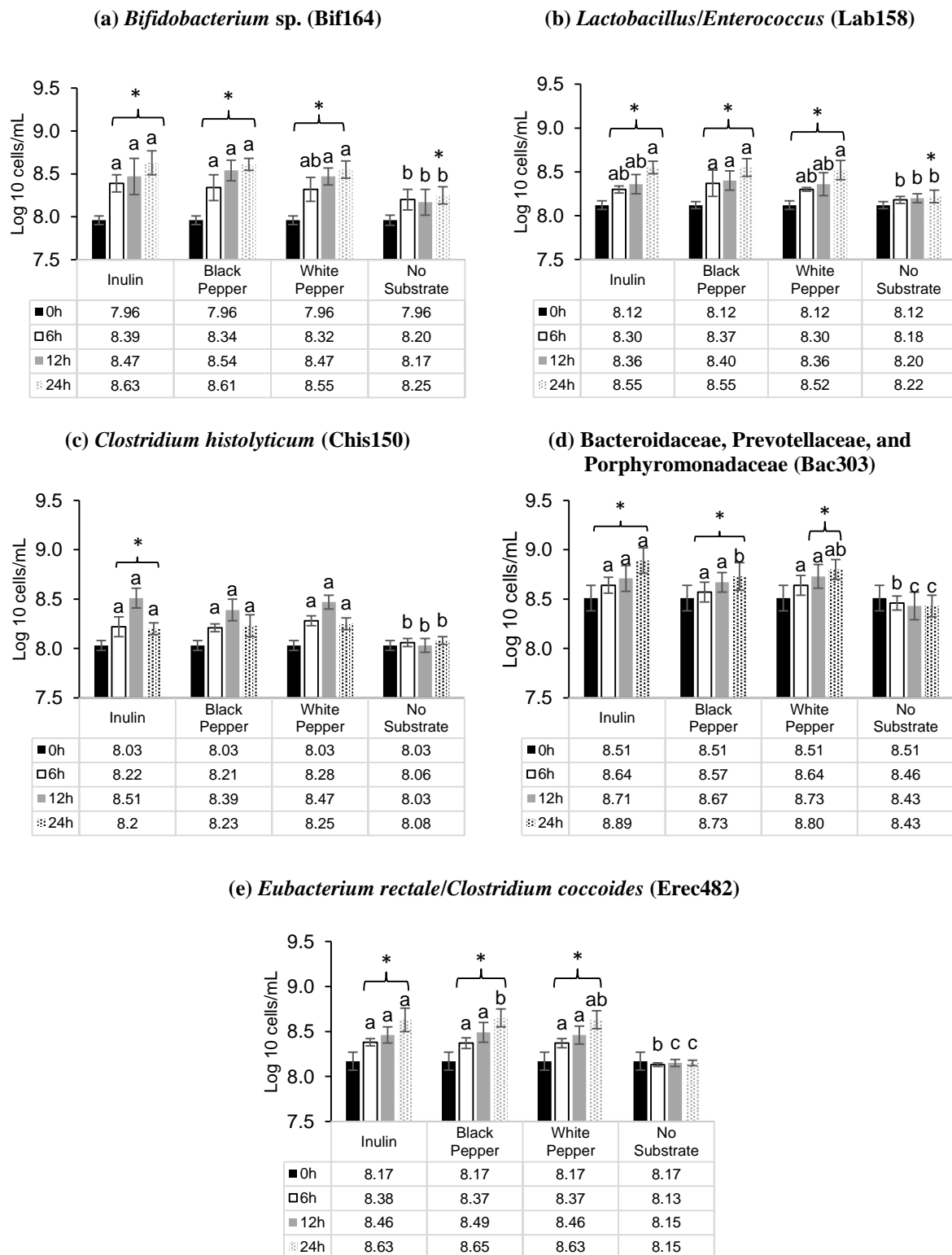
Factorial in Random Common Block Design was used to analyse the differences of bacterial enumeration and SCFA quantification between the substrate. Blocking effect was applied to the replication of different faecal sample. The data were subjected to ANOVA and treatment means were statistically compared by Tukey's test at 95% level of significance using SAS version 9.3.

## RESULTS

### Microbial Population

Figure 1 shows the microbial population changes throughout the *in vitro* fermentation of the test substrates. Overall, most of the colonic microbial in all test substrates revealed population changes similar to those in inulin which act as the positive control. Particularly, the population of *Bifidobacterium* spp. (Bif164), *Lactobacillus* and *Enterococcus* group (Lab158), *Clostridium histolyticum* (Chis150), *Eubacterium rectale* and *Clostridium coccoides* (Erec482), Bacteroidaceae, Prevotellaceae, and Porphyromonadaceae (Bac303) in response to black pepper and white pepper at all period. Except for negative control, the bacteria population of all strains showed a different trend of changes when compare to the all-other sample treatment.

In general, the probiotic strains (i.e., *Bifidobacteria* and *Lactobacillus/Enterococcus* group) from both black and white pepper showed a significant increasing population from 6 h to 24 h of the fermentation period when compared to the start of the fermentation (i.e, 0 h). Similarly, the Bacteroidaceae, Prevotellaceae, and Porphyromonadaceae (Bac303) from the treatment of black and white pepper also have a significant increasing trend from the start until the end of the fermentation period. However, the population of Bac303 from the colonic fermentation of white pepper showed a slower significant treatment effect. Whereby, the treatment effect is only statistically significant from 12 h and above. Whereas, the growth of Bacteroidaceae and Prevotellaceae at 24 h in black pepper ( $\log_{10}$  8.73) does show a significant difference when compared to inulin ( $\log_{10}$  8.89)  $p < 0.05$ .



**Figure 1.** Microbial population of the *in vitro* colonic fermentation of black and white pepper at 0, 6, 12 and 24 hours

Note: <sup>a,b,c</sup> unlike superscript letters represents mean value with significantly higher / lower when compare between substrates within the same sampling hour ( $p < 0.05$ ). \*Mean value was significantly different from that of 0 h ( $p < 0.05$ ) ( $n = 4$ ).

For *Clostridium histolyticum* group, no significant growth of *C. histolyticum* was seen in the fermentation of black pepper, white pepper and no substrates at 6, 12 and 24 h ( $p < 0.05$ ). The population of all samples showed a reducing trend and/or constant population throughout the fermentation. In the fermentation of both black and white pepper, it is revealed that an insignificant reduce was observed, especially at 24 h. However, the *C. histolyticum* population in the no substrate group showed a significant lower population when compared to those in the fermentation of inulin, black pepper, and white pepper. The *Eubacterium rectale* and *Clostridium coccoides* (Erec482) from the fermentation of black and white pepper showed a similar increasing trend similar to those in the fermentation of inulin. However, statistical comparison of the population of Erec482 in the fermentation of black and white pepper revealed a significant lower population when compared to the positive control (inulin). Meanwhile, the population of Erec482 in the fermentation of no substrate remain constant throughout the 24 hours of fermentation.

### Short Chain Fatty Acids (SCFA)

Figure 2 shows the concentration of lactate, acetate, propionate and butyrate of the *in vitro* fermentation products at 0, 6, 12 and 24 h. Lactate was shown to be depleted significantly in all the samples, particularly from 6 h to 24 h. At the end of the fermentation (24 h), lactate production in the fermentation of inulin and black pepper was observed to be significantly higher at 12.10 mM and 9.50 mM respectively. White pepper was showing the lowest amount of lactate accumulation during the 24 h of fermentation at only 6.42 mM.

The production of acetate in all samples showed an increasing trend. Throughout the fermentation period, both black and white pepper have a statistically higher acetate production than those in the fermentation of inulin. Whereas, the acetate production in the fermentation of no substrate is the least. At the end of the fermentation period (24 h), the black and white pepper showed the highest concentration at 155.99 mM and 151.90 mM respectively. Meanwhile, the fermentation of positive control, inulin revealed a significantly lower acetate production when compared to the

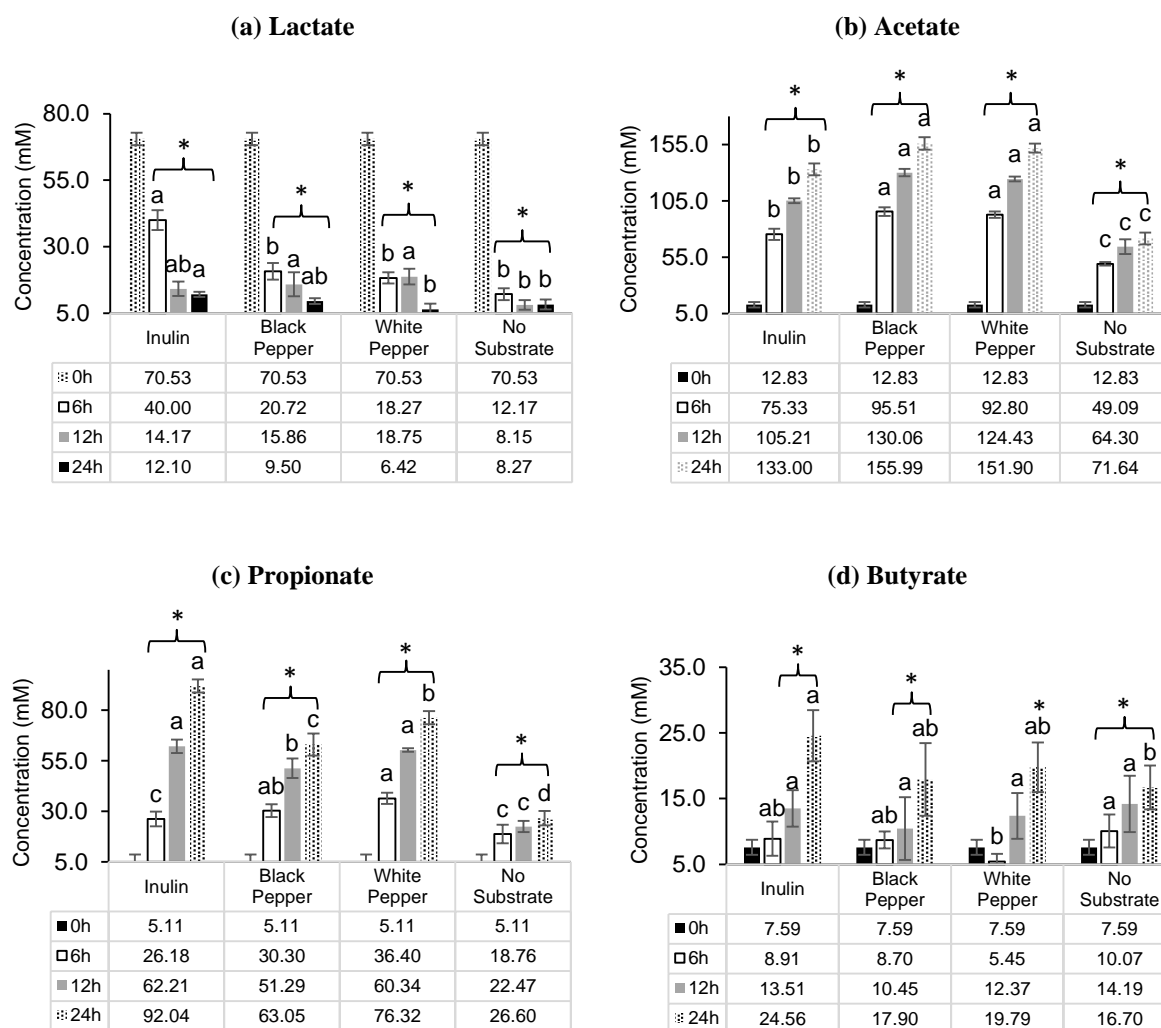
fermentation of black and white pepper (133.00 mM) at ( $p < 0.05$ ).

Propionate production in the fermentation of all test substrates have a significant increasing trend throughout the 24 hours' fermentation. In which, propionate production from all treatment substrates showed a significant difference in the final hour (24 h). Throughout the fermentation period, inulin was observed to have the highest production of propionate. Particularly at the 24 h, the propionate production from the fermentation of inulin is 92.04 mM; followed by white pepper and black pepper at 76.32 mM and 63.05 mM respectively. Whereas for the treatment of no substrate, the production of propionate is the least of all samples.

In terms of butyrate production, all samples revealed an increasing trend throughout the 24 h fermentation. In which, all samples showed the highest concentration of butyrate at the end of the fermentation (24 h). Statistically, fermentation of black pepper and inulin significantly produces butyrate at 12 h to 24 h when compared to the beginning of the fermentation (0 h). While, the butyrate production from the fermentation of white pepper has a slower treatment effect. In which, the butyrate production is only significant at 24 h.

### DISCUSSION

Black pepper and white pepper revealed no significant difference in the development of *Bifidobacterium* population throughout the fermentation period when compared to inulin as a positive control. Inulin's best-known nutritional effect is its actions to promote *Bifidobacterium spp.* production in the gut. Inulin is one of the oligosaccharides that is extensively studied as a prebiotic ingredient. Whereby, inulin exhibit prebiotic properties such as resistance towards upper gastrointestinal digestion; and able to remain intact until reaching the large intestine (Zaporozhets *et al.*, 2014). A closely similar study, using culture-dependent method i.e. agar plating, reported that *P. nigrum* produced modest stimulatory activity in promoting the growth of *Bifidobacterium* when comparing with Mediterranean Oregano and other 5 spices using 17 strains of *Bifidobacterium* (Lu *et al.*, 2017).



**Figure 2.** The production of SCFA from the *in vitro* colonic fermentation of black and white pepper at 0, 6, 12 and 24 hours

Note: <sup>a,b,c</sup> Unlike superscript letters represents mean value with significantly higher / lower when compare between substrates within the same sampling hour ( $p < 0.05$ ). \*Mean value was significantly different from that of 0 h ( $p < 0.05$ ,  $n = 4$ ).

The abundance of dietary fibre content in *Piper nigrum* which is up to 30%, is a good source of potential prebiotic in order to promote the accumulation of beneficial microbes. By comparing it with inulin (commercial prebiotic), a similar effect in *Bifidobacterium* production could make an interesting value for black pepper and white pepper being a potential source of prebiotic. As *Bifidobacterium* are normal inhabitants in gastrointestinal tract, their composition, diversity, or relative abundance have been implicated in several intestinal disease conditions. Through their effects on the immune system, *Bifidobacterium* was seen to alleviate infectious diarrhoea (Azagra-Boronat *et al.*, 2020).

In this study, black pepper is seen to also have a good production of the *Lactobacillus* population across the fermentation hours. Similar findings were found that, black pepper was observed to enhance the development of most of the 11 strains *Lactobacillus* sp. (Lu *et al.*, 2017). As for the white pepper, studies on it against *Lactobacillus* sp. were unknown which make these findings valuable as an additional contribution on white pepper as a potential prebiotic ingredient. Also, a finding showed that probiotic bacteria (*L. reuteri*) at a 1:50 dilution give out growth in aqueous extractions of black pepper and garlic. This study also stated that, through *in vitro* extract, black pepper showed dual prebiotic and antibacterial activity (Peterson *et al.*, 2019; Ogwaro *et al.*, 2021). Presence of available polysaccharides in *P.*

*nigrum* L. i.e. galactose (34.7%), rhamnose (13.1%), arabinose (18.1%), galacturonic acid (8.5%) (Yogendrarajah *et al.*, 2015) and fructose-oligosaccharides in inulin can be the reason for the increase in *Lactobacillus* and *Bifidobacterium* population (Pokusaeva *et al.*, 2011). Black pepper has anti-complementary polysaccharides polymer which shows a strong effect as an immune enhancer (Sarker, 2012). Through glycosidic linkage analysis, *P. nigrum* has pectic polysaccharide with side chains of type II arabinogalactan (Khawas *et al.*, 2017). Type II arabinogalactan mainly contains a  $\beta$ -(1-3)-linked galactose backbone and is more branched because of galactose moieties  $\beta$ -(1-6) linkages (Munoz *et al.*, 2020). Human upper digestion is not able to breakdown arabinogalactan (Sun *et al.*, 2021; Cao *et al.*, 2023). Therefore, arabinogalactan is an interesting prebiotic potential ingredient (Sun *et al.*, 2021). It was found to be fermented with human colonic microbiome as well as promoting the development of beneficial metabolites (short chain fatty acids) and the growth of selective bacteria that are generally thought to improve the host health (Harris *et al.*, 2020; Wang *et al.*, 2021).

In addition, fibre (30.1%) and fats (6.77%) availability in *P. nigrum* L. might relate to the promotion of *Bacteroides-Prevotella* group. As these beneficial bacteria are largely modulated by diet intakes. The available piperine and volatile pepper oil in *P. nigrum* L. was demonstrated to have disease preventive functions such as antimicrobial properties, anti-inflammatory (Ashokkumar *et al.*, 2021) and improve the functionality of the gastrointestinal. Hence this might be closely related to the pathogenic bacteria count of *Clostridium histolyticum*, *Eubacterium rectale* and *Clostridium coccooides* which some of them decreases through the fermentation hours. Thus, this could support the ability of both white and black pepper in being potential prebiotic substrates.

Other than the microbial accumulation, analysing the SCFA production also is a biomarker in determining prebiotic fermentation studies of a targeted food product. Lactate production was shown to be significantly depleting in all the test substrates including black and white pepper samples. This is expected as lactate was utilised by available bacteria through

cross-feeding (Rawi *et al.*, 2021) which then converted into acetate, butyrate and propionate (Lee *et al.*, 2022b). It was reported that lactate accumulates in certain types of gut disorders such as ulcerative colitis (Louis *et al.*, 2022). These show a positive indicator of the test substrates (black pepper and white pepper) to have a potential prebiotic as they did not produce significant amount of lactate throughout the fermentation process which they might be able to maintain the colon health by not contributing abundant lactate production.

Acetate is used for the process of lipogenesis in the cytosol of adipose and mammary glands, with the presence of acetyl-CoA. It is also the primary substrate for cholesterol synthesis and are seen to be used for butyrate production in the colon by the colonic microbes (Rawi *et al.*, 2000). In this study, black pepper and white pepper both showed a significantly higher concentration of their acetate productions which were at 155.99 mM and 151.90 mM during their 24 h of fermentation in comparison to inulin with 133.00 mM. These results can be synchronized with a positive increase in their *Bifidobacterium* and *Lactobacillus* population previously. The selectivity in bacterial promotions may be due to the presence of arabinogalactan as the side-chains of *P. nigrum* L.'s polysaccharides (Lu *et al.*, 2017). A similar result was seen in a study done on arabinogalactan containing media shows a growth of *B. longum* and *B. thetaiotaomicron* after 72 h of cultivation with pH of the media significantly decreased and both  $\alpha$ -lactic acid and acetic acid accumulated (Sun *et al.*, 2021).

Propionate concentration also increased in inulin, black pepper and white pepper across the fermentation hours. However, a significant difference was seen at 24 h of fermentation time. Fermentation of inulin was seen to be able to produce a higher concentration of propionate, at 92.0 mM followed by white pepper and black pepper by having 76.32 mM and 63.05 mM respectively. This can be justified by the growth of *Bacteroides-Prevotella* group in all substrates. In which, *Bacteroides-Prevotella* group is recognised to be the producer of propionate (Li *et al.*, 2023). For example, *Bacteroides fragilis* which stands out as one of prevalent group in the human gut that produces a substantial amount of propionate (Shon *et al.*, 2023). Besides, *Clostridia histolyticum* has also



been reported to be a propionate producing bacteria (Sarhini *et al.*, 2011) but the significant increase of this bacteria group only can be seen in inulin. Other than that, fermentation of arabinogalactans which present in *P. nigrum* L., could also show convincing production of propionate and n-butyrate (Aguirre *et al.*, 2016).

There was no significant difference in the production of butyrate from fermentation of inulin, black pepper and white pepper. Butyrate production in inulin fermentation shows a significant increase, from 0 to 24 h. As for black pepper, butyrate was significantly discovered during 12 h onwards. White pepper was seen to have slower development of butyrate as its accumulation was seen to be significantly increasing during its 24 h of fermentation. *Clostridium coccooides* – *Eubacterium rectale* group were the bacteria responsible for producing butyrate. In obese patients, the microbial concentration of arabinogalactan was seen to metabolically producing higher propionate than butyrate (Aguirre *et al.*, 2016). Lean microbiota will be generally producing more butyrate compared to obese microbiota fermentation. This statement may have been a contradiction towards the result obtained from this study as lean faecal samples were used. However, a mixed gut community will be producing a direct conversion of lactate into butyrate and/or propionate (Wang *et al.*, 2020; Peterson *et al.*, 2022). Therefore, their production might be similar or higher to each other based on the mixed gut community. Butyrate is the most preferred metabolites as an energy source of colonocytes and may demonstrate the preventive effect on colon cancer and adenoma development (Genua *et al.*, 2021; Clarke *et al.*, 2023).

## CONCLUSION

In conclusion, the present study indicates that *P. nigrum* L. has met up a few characteristics like the commercial prebiotic, inulin, in terms of bacterial growth and SCFA production. However, as this is just a preliminary study, it is recommended for an *in vivo* observation as an extension, in order to support this prebiotic characteristic in a real environment.

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