



**UNIVERSITI PUTRA MALAYSIA**

***EVALUATION OF PIPER NIGRUM L. AS A PREBIOTIC  
INGREDIENT USING AN IN VITRO COLON MODEL***

**SITI MAISARAH MOHD NASHRI**

**FSPM 2019 8**



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USING AN *IN VITRO* COLON MODEL**

By

**SITI MAISARAH BINTI MOHD NASHRI**

**Thesis Submitted to the School of Graduate Studies,  
Universiti Putra Malaysia, in Fulfilment of the  
Requirements for the Degree of Master of Science**

**September 2019**

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Abstract of thesis presented to the Senate of Universiti Putra Malaysia  
in fulfilment of the requirement for the degree of Master of Science

## EVALUATION OF *PIPER NIGRUM* L. AS A PREBIOTIC INGREDIENT USING AN *IN VITRO* COLON MODEL

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SITI MAISARAH BINTI MOHD NASHRI

September 2019

Chair: Assoc. Prof. Shahrul Razid Sarbini, PhD

Faculty: Agriculture and Food Sciences, Bintulu Campus

*Piper nigrum* L., commonly known as pepper and often referred to as the 'King of Spice', is a well-known spice that is extensively used around the world in fields such as culinary (for its taste and odour) and pharmaceutical (for its complex chemical composition containing alkaloids, flavonoids and polyphenols). However, study on exploring the potential of *Piper nigrum* L. as a prebiotic is very scarce. Recent studies also show that it has the ability to promote the growth of several beneficial colonic bacteria especially *Bifidobacterium* and *Lactobacillus*. Therefore, the objectives of this study were to evaluate the digestibility of *Piper nigrum* L. by digestive enzymes of the upper gastrointestinal digestion, determine the selectivity in activity and/or growth of one or finite amount of colon microbiota and analyse metabolites produced from the fermentation of *Piper nigrum* L. i.e. short chain fatty acids. Two *Piper nigrum* L. samples; black pepper, grade Special Black Pepper and white pepper, grade Fair Average Quality, were used as test substrates for prebiotic property with inulin being used as the positive control. To identify its digestibility characteristic, *in-vitro* gastric and duodenal digestion were conducted, and the total carbohydrate and piperine content were determined using phenol sulphuric acid method and ethylene dichloride extraction method respectively. It was found that white pepper has a significantly higher digestibility ( $6.78\% \pm 2.27$ ) in comparison to inulin ( $2.03\% \pm 0.20$ ) where there are no significant difference shown against black pepper ( $5.99\% \pm 2.22$ ). The piperine content of both test substrates remained the same before and after the digestion. Inoculated with faecal slurry, the pepper samples were then further examined in *in-vitro* colon model by a pH-controlled batch culture system. Samples were withdrawn at 0, 6, 12 and 24 hours and analysed for their bacterial count and SCFA content respectively. Both black and white pepper showed no significant difference in most of the tested bacteria population growth when compared to inulin especially in *Bifidobacterium* spp., *Eubacterium rectale*/ *Clostridium*, *Lactobacillus*/ *Enterococcus*, *Atopobium* cluster, *Feacalibacterium*



*prausnitzii*. Black and white pepper were found to accumulate a significantly higher amount of acetate ( $155.99 \text{ mM} + 5.61$ ) and ( $151.90 \text{ mM} \pm 4.04$ ) respectively compared to inulin ( $133.00 \text{ mM} \pm 5.37$ ). Butyrate concentration in white pepper across fermentation time only showed a significant increase after 24 h, but no significant difference was found in the concentration of butyrate with black pepper and inulin. Although some contradiction was observed which was by having higher propionate production against butyrate, but this study showed that *Piper nigrum* L. could be a promising and valuable contribution as a local prebiotic.



Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia  
sebagai memenuhi keperluan untuk Ijazah Master Sains

**PENILAIAN *PIPER NIGRUM* L. SEBAGAI BAHAN PREBIOTIK  
MENGUNAKAN MODEL KOLON *IN VITRO***

Oleh

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*Piper nigrum* L., yang dikenali sebagai lada dan sering dirujuk sebagai 'King of Spice', adalah rempah yang terkenal yang digunakan secara meluas di seluruh dunia dalam pelbagai bidang seperti masakan (rasa dan bau) dan farmaseutikal (komposisi kimia yang kompleks melalui kandungan alkaloid, flavonoid dan polifenol). Walau bagaimanapun, kajian mengenai penerokaan potensi *Piper nigrum* L. sebagai prebiotik adalah amat terhad. Terdapat kajian terbaru yang menunjukkan bahawa ia mempunyai keupayaan untuk mempertingkatkan pertumbuhan beberapa jenis bakteria baik terutamanya *Bifidobacterium* dan *Lactobacillus*. Oleh itu, objektif kajian ini adalah untuk menilai pencernaan *Piper nigrum* L. melalui pencernaan enzim yang terdapat pada sistem pencernaan gastrointestinal bahagian atas tubuh manusia, menentukan selektiviti dalam aktiviti dan / atau pertumbuhan satu atau sejumlah bakteria dan menganalisis metabolit yang terhasil daripada proses fermentasi daripada *Piper nigrum* L. iaitu asid lemak rantai pendek. Dua sampel *Piper nigrum* L.; Lada hitam dalam gred *Special Black* dan lada putih dalam gred *Fair Average Quality*, digunakan sebagai substrat ujian dengan inulin yang digunakan sebagai kawalan positif. Untuk mengenal pasti ciri pencernaannya, pencernaan gastrik dan duodenal secara *in-vitro* telah dijalankan, dan jumlah karbohidrat dan kandungan piperine ditentukan dengan menggunakan kaedah asid sulfurik dan kaedah pengekstrakan etilena diklorida. Hasil kajian ini telah mendapati bahawa lada putih mempunyai kadar pencernaan yang lebih tinggi ( $6.78\% \pm 2.27$ ) berbanding dengan inulin ( $2.03\% \pm 0.20$ ) dan tiada perbezaan yang signifikan terhadap apabila dibandingkan dengan lada hitam ( $5.99\% \pm 2.22$ ). Kandungan piperine kedua-dua substrat ujian ini kekal sama sebelum dan selepas proses pencernaan dijalankan. Inokulasi dengan cairan feses, sampel lada kemudiannya diperiksa lagi dalam model kolon *in-vitro* oleh sistem kumpulan dengan pH yang dikawal. Sampel hasil fermentasi diambil pada 0, 6, 12 dan 24 jam dan dianalisis untuk jumlah bakteria dan kandungan SCFA masing-masing. Lada hitam dan putih tidak menunjukkan

perbezaan dalam kebanyakan populasi bakteria yang diuji apabila dibandingkan dengan nuin terutama pada populasi bakteria *Bifidobacterium spp.*, *Eubacterium rectale* / *Clostridium*, *Lactobacillus* / *Enterococcus*, kluster *Atopobium* dan *Feacalibacterium prausnitzii*. Hasil fermentasi lada hitam dan lada putih didapati menghasilkan asetat ( $155.99 \text{ mM} \pm 5.61$ ) dan ( $151.90 \text{ mM} \pm 4.04$ ) yang banyak berbanding nuin ( $133.00 \text{ mM} \pm 5.37$ ). Kandungan asid butirat dalam lada putih juga menunjukkan penghasilan yang ketara selepas 24 jam proses fermentasi dilakukan, tetapi tidak terdapat perbezaan yang ketara dalam penghasilan butirat dari fermentasi lada hitam dan inulin. Walaupun beberapa percanggangan dapat dilihat iaitu hasil jumlah propionat yang lebih tinggi terhadap butirat, tetapi kajian ini menunjukkan bahawa *Piper nigrum* L. mampu menjadi sumber prebiotik setempat yang berharga.

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I certify that a Thesis Examination Committee has met on 26 September 2019 to conduct the final examination of Siti Maisarah binti Mohd Nasri on her thesis entitled "Evaluation of *Piper nigrum* L. as a Prebiotic ingredient using an 'In Vitro' Colon Model" in accordance with the Universities and University Colleges Act 1971 and the Constitution of the Universiti Putra Malaysia [P.U. (A) 106] 15 March 1998. The Committee recommends that the student be awarded the Master of Science.

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## LIST OF ABBREVIATIONS

AMG	Anaerobic mixture gas
cfu/g	Colony-forming units per gram
Cy3	Cyanine 3
DNA	Deoxyribonucleic acid
FISH	Fluorescent in situ hybridization
FOA	Food and agriculture organization
G	Gravity
GC	Gas chromatography
GI	Gastrointestinal
g/ml	Gram per millilitre
g/l	Gram per litre
HPLC	High performance liquid chromatography
IBD	Inflammatory bowel disease
kDa	Kilodalton
mg/ml	Milligram per millilitre
Mm	Millimetre
mM	Millimolar
ml/min	Millilitre per minute
MT	Metric ton
mmol/l	Millimoles per litre
NADH	Nicotinamide adenine dinucleotide
qPCR	Quantitative polymerase chain reaction
Rpm	Revolutions per minute
rRNA	Ribosomal ribonucleic acid
SCFA	Short chain fatty acid

U/ml	Units per millilitre
v/v	Volume per volume
w/w	Weight per weight
w/v	Weight per volume
µl/l	Microlitre per litre
Mm	Micrometre





## CHAPTER 1

### INTRODUCTION

Due to consumers' demand and preference on what they are taking, the food production industry has been changed considerable. Today, consumers are preferring food products that will not only satisfy their hunger and nutrient needs but also foods that prevent diseases and improving health (Menrad, 2003). Prebiotic is a type of functional food that benefits health and wellness. 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). For a food to be recognized as a prebiotic, it must be able to resist the digestion and absorption by the small bowel. When the substrate enters the large bowel, it should selectively change the activity or composition of the good microbiome, which leads in demonstrable health benefits (Gibson et al., 2004). However, studies on potential prebiotic presence in local food sources is very scarce in Malaysia. Thus, it is important to explore their potentials in order to promote the potential prebiotic value in local food sources. One interesting local food sources and important exports that may hold prebiotic potential source is the king of spice, Sarawak Pepper.

Malaysia is one of Asian countries that has been blessed with various types of spices, and one of them is *Piper nigrum* L., commonly known as peppercorn. Belonging to the family Piperaceae, *Piper nigrum* L. has been extensively cultivated throughout the world, and Malaysia is one of the biggest producer of black pepper and white pepper after Indonesia (Joy, Abraham, & Soniya, 2007). According to Malaysia Pepper Board (MPB), in Malaysia, Sarawak is the largest *Piper nigrum* L. producer with annual contribution up to 95% in 2016. This spice is well-known for the availability of high bioactive compounds which are capable of enhancing human health and contains up to 33% dietary fibre (Pradeep, Geervani, & Eggum, 1993(a)). As dietary fibre could resist the upper part of human gastrointestinal digestion (Bellei, & Haslberger, 2013), *Piper nigrum* L. may have the potential in being a local prebiotic source. Other than that, the presence of alkaloids, polyphenols and flavonoids also increases its ability to act as a prebiotic. These chemical components have a broad range of beneficial health effects (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 a beneficial microbes namely *lactobacilli* and *bifidobacteria*, and inhibiting the pathogenic bacteria population in human gut, which exerting the prebiotic-like effects (Dreger, et al., 2014).

*Piper nigrum* L. is used in many kinds of applications such as in culinary and medicinal formulations. The consumption of food with addition of spices could lead to the alterations in the large bowel microbiota population. Pigs



fed with herb extract mixture (HEM) supplemented diets containing peppercorns had reduced faecal *Escherichia coli* (*E. coli*) (Yan, Meng, & Kim, 2012a). This finding is similar to another finding that found that thyme herb supplement could also decrease faecal concentration of *E. coli* in piglets (Jugl et al., 2005). Another analysis conducted on black pepper application in broilers diet observed that the antioxidants and phenolic substances may be the reason on the positive development of broilers carcass as the antimicrobial substance may reduce harmful bacteria population (Alloui, Alloui, & Agabou, 2014). Other than that, *Piper nigrum* L. also contains several polysaccharides i.e. arabinogalactan and acidic polysaccharides, of which one of them showed a strong effect as an immune enhancer because its polymer is an anti-complementary polysaccharide (Barsett et al., 2005). From this we can see that *Piper nigrum* L. may have the potential in becoming a dietary prebiotic. These promising results demonstrated from the present show that *Piper nigrum* L. has a valuable contribution to the prebiotic intake by improving the bacterial accumulation and SCFA production in the colon. In addition, *Piper nigrum* L. are also well-known to help in reducing systemic inflammation, but their prebiotic potential has not being explored (Babu et al., 2018), as most of recorded studies on *Piper nigrum* L. were only focus on its effectiveness in livestock production on how to increase animal's appetite, and as an antibiotic substitute. Therefore, this research would like to extend the application of *Piper nigrum* L. in its responses to human gut digestion.

Products of *Piper nigrum* L. peppercorns that have been commercialized nowadays include black pepper, white pepper, green pepper and pepper in brine. Sarawak *Piper nigrum* L. is extensively commercialized as dried black and white pepper forms. Therefore, the present research has focused on the evaluation of Sarawak's black pepper and white pepper as potential prebiotics through *in vitro* human digestion and fermentation. The objectives of this study are:

1. To assess carbohydrate digestibility prior to digestion of *Piper nigrum* L. *in vitro* at the upper gastrointestinal.
2. To evaluate the selectivity of microbial growth during *in vitro* colon fermentation of *Piper nigrum* L. using fluorescent *in situ* hybridization (FISH) technique.
3. To profile short chain fatty acid production during *in vitro* colon fermentation of *Piper nigrum* L. using high performance liquid chromatography (HPLC).

### **Problem Statement**

Most studies on *Piper nigrum* L. were focussed in microbial interactions, livestock production and organic antibiotic substitute, however none were looking it as a potential prebiotic. The prebiotic potential of a food ingredient depends on the ability to resist digestion for reaching the colon, selectively



utilize by human microbiota as well as the production of beneficial metabolites from colonic fermentation. The information on the digestibility and fermentability of *Piper nigrum* L. is currently scarce. Thus, the prebiotic study of colonic microbiota towards *Piper nigrum* L. is yet to be studied, as well as its beneficial fermentation metabolites.

### Significance of Study

The findings of this study may extent the knowledge on functional food considering the demand of functional food and nutraceuticals have grown exponentially. High demand in functional food products such as prebiotic justifies the need for more research to improve and evaluate more potential ingredients namely *Piper nigrum* L.. Thus, by evaluating *Piper nigrum* L. as a prebiotic ingredient using in vitro colon model, could expand the knowledge in the beneficial reaction of this interesting spice towards human microbiota, ultimately human health. Since *Piper nigrum* L. is one of the major cultivated crop in Malaysia, this study could plays by adding value to the commodity. In addition, it is a hoped that this study will be the starting point of an ongoing research on *Piper nigrum* L. as a functional food ingredient.



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## BIODATA OF STUDENT

Siti Maisarah binti Mohd Nashri was born in 17<sup>th</sup> of January 1992 in Tapah Hospital, Perak, Malaysia. She holds a first-class honour Bachelor's Degree in Bioindustrial Sciences from Universiti Putra Malaysia. She is now expanding her research experience in Master of Science (Food Biotechnology) in the same university.

Her final year project was on the 'Isolation of resistant starch portion from various flour for prebiotic rvaluation'. The project was done successfully in exploring resistant starch availability in local flour products. Other than that, she had conducted two mini projects during her industrial training which are on 'Antimicrobial activity of rhizobacteria from Sarawaks' herbal plants against rice born disease' and the 'Receptibility of Sarawak local rice varieties against brown spot disease".

In addition, she co-authored a review in Food Reviews International (Impact Factor: 1.74, Q1) entitled 'Evaluation of potential prebiotics: A review'. Her research interest is in food biotechnology which involves functional food evaluation, gut microbiome and human nutrition.

## LIST OF PUBLICATION

### Articles

Bajury, D. M., **Nashri, S. M.**, King, P. J. H., & Sarbini, S. R. (2018). Evaluation of potential prebiotics: A review. *Food Reviews International*, 34(7), 639–664.

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### Poster presentation

**Nashri, S. M.** and Sarbini, S. R. (2018). Evaluation of *Piper nigrum* L. as prebiotic using *in vitro* colon model. Presented at *International Scientific Conference (ISCIC2018)*. Kuching, Malaysia.