UNIVERSITI PUTRA MALAYSIA

PREBIOTIC POTENTIAL OF BANANA (Musa sapientum) PEELS ON THE GROWTH OF PROBIOTICS

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PREBIOTIC POTENTIAL OF BANANA (*Musa sapientum*) PEELS

ON THE GROWTH OF PROBIOTICS

BY

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A project submitted as a partial fulfilment of the requirement for the degree of Bachelor of Science (Nutrition and Community Health) from the Faculty of Medicine and Health Sciences, Universiti Putra Malaysia

ACKNOWLEDGEMENTS

In the name of Allah, the Most Gracious and the Most Merciful

Alhamdulillah, all praises and thanks to Allah, the Almighty, for the strengths and His blessing in completing this thesis. Special appreciation goes to my supervisor, Dr. Mohd Redzwan Sabran, for his supervision and constant support. His invaluable help of constructive comments and suggestions throughout the experimental and thesis works have contributed to the success of this research. Not forgotten, my appreciation to course coordinator of our final year project, Dr. Siti Raihanah Binti Shafie for her support and guidance along our project progression.

I would like to express my appreciation to lab instructors and demonstrators who gave a lot of guidance and advises while in the process of completing this project. They opened wide my eyes to the world of microorganisms and to understand the importance of this field. Sincere thanks to all my friends who always been there, especially final year students from Nutrition and Community Health course for their kindness and moral support during my study. Thanks for the friendship and memories.

Last but not least, my deepest gratitude goes to my beloved parents; Mr. Abdul Gaffar bin Esa and Mrs. Masturah binti Manab and all my family members for their support on me in completing this project and their endless love, prayers and encouragement. Without helps of the particular that mentioned above, I would face many difficulties while doing this. Also, to those who indirectly contributed in this research, your kindness means a lot to me. Thank you very much.

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ABSTRACT

PREBIOTIC POTENTIAL OF BANANA (*Musa sapientum*) PEELS ON THE GROWTH OF PROBIOTICS

Nur Farizah binti Abdul Gaffar

Banana (*Musa sapientum*) is an edible fruit and it has become the most important fruit crop in the world. Agro industrial by-products of banana has yielded its own by-products which mainly consist of peel and pulp. Banana peel is the outer layer of banana fruits which accounts for about one third of the whole fruit weight. Utilization of banana peels can provide another alternative, rather than disposing them as wastes. Therefore, this study aimed to assess the prebiotic potential of banana peels on the growth of probiotic *Lactobacillus casei* strain Shirota (LcS) using an *in vitro* experimental model. Two parameters were assessed; the growth performance of LcS (log CFU/mL, final pH, mean growth rate and mean duplication time) and the prebiotic activity score. This experiment used banana peels powder with different concentrations (1%, 2% and 3%) as samples. Two type of banana peels were used as samples; *Pisang Abu* and *Pisang Tanduk* peels powder. These samples were collected from producers of banana chips industries in Banting and Petaling Jaya, Selangor. The peels were collected by using purposive sampling method. Briefly, the banana peels were cleaned, freeze-dried, homogenized and divided into different concentrations. Then, the customized culture media was prepared by substituting glucose with *Pisang Abu* and *Pisang Tanduk* peels powder with concentrations of 1%, 2% and 3% respectively ($MRSa_1$, $MRSa_2$, $MRSa_3$, $MRSt_1$, $MRSt_2$ and $MRSt_3$). Commercial MRS (MRSc) broth was used as a positive control. After 48 hours of fermentation, the LcS growth was significantly difference ($p<0.001$) between the media. Further analysis indicated that the probiotic's growth at higher concentration (3%) ($MRSa_3$ and $MRSt_3$) showed insignificant difference to glucose and MRS broth, whereas others ($MRSa_1$, $MRSa_2$, $MRSt_1$ and $MRSt_2$) which less than 3% concentration, were significantly difference. The media's pH was reduced following the fermentation, but the final pH was not significantly different for all modified broths except for glucose media (4.56 ± 0.22) and commercial MRS broth (4.40 ± 0.18). There was a significant difference on the mean growth rate ($p<0.001$) and the mean duplication time ($p<0.001$) for the fermentation of LcS in $MRSa_1$, $MRSa_2$, $MRSa_3$, $MRSt_1$, $MRSt_2$, $MRSt_3$, MRS and MRSc after 24 hours of incubation. Besides, the prebiotic activity scores of $MRSa_1$, $MRSa_2$, $MRSa_3$, $MRSt_1$, $MRSt_2$ and $MRSt_3$ are comparable with other agro industrial by-products reported in a literature. From these findings, the by-products generated from the banana peels agro-industrial processing might be sources of compounds which capable of promoting the selective growth of probiotic LcS due to their potential prebiotic properties.

ABSTRAK

POTENSI PREBIOTIK KULIT PISANG (*Musa sapientum*) KE ATAS TUMBESARAN PROBIOTIK

Nur Farizah binti Abdul Gaffar

Pisang (*Musa sapientum*) adalah buah yang boleh dimakan dan ia menjadi tanaman buah yang paling penting di dunia. Industri pertanian pisang telah menghasilkan produk sampingannya sendiri yang keutamaannya terdiri daripada kulit dan pulpa. Kulit pisang adalah lapisan luar buah pisang yang menyumbang kira-kira satu pertiga daripada berat buah keseluruhan. Penggunaan kulit pisang dapat menjadi antara salah satu alternatif yang lain, di mana ianya adalah lebih bagus daripada menjadikannya sebagai bahan buangan. Oleh itu, kajian ini bertujuan untuk menilai potensi prebiotik pisang ke atas pertumbuhan probiotik *Lactobacillus casei* strain Shirota (LcS) menggunakan model eksperimen *in vitro*. Terdapat dua parameter yang telah dikaji; prestasi pertumbuhan LcS (log CFU/mL, pH akhir, purata kadar pertumbuhan dan purata masa penggandaan) dan juga kiraan aktiviti prebiotik. Kajian ini menggunakan serbuk kulit pisang dengan kepekatan yang berbeza (1%, 2% dan 3%) sebagai sampel. Dua jenis kulit pisang iaitu Pisang Abu dan Pisang Tanduk dan sampel ini telah digunakan sebagai sampel. Sampel ini dikumpul daripada dua pengeluar industri kerepek pisang yang terletak di Banting dan Petaling Jaya, Selangor. Sampel ini juga telah dikumpul dengan menggunakan kaedah pensampelan bertujuan. Secara ringkas, kulit pisang telah dibersihkan, menjalani pengeringan sejuk beku, melalui proses homogenisasi dan jumlah kandungan serbuk dibahagikan kepada kepekatan yang berbeza. Kemudian, media kultur yang telah disesuaikan disediakan dengan menggantikan glukosa dengan serbuk Pisang Abu dan serbuk Pisang Tanduk ($MRSa_1$, $MRSa_2$, $MRSa_3$, $MRSt_1$, $MRSt_2$ dan $MRSt_3$) masing-masing dengan kepekatan yang berbeza (1%, 2% dan 3%). Media MRS komersial (cMRS) digunakan sebagai kawalan positif. Selepas 48 jam proses fermentasi, terdapat perbezaan ketara ($p < 0.001$) pada tumbesaran LcS di antara media. Analisis seterusnya mendapati bahawa terdapat pertumbuhan probiotik pada kepekatan serbuk kulit pisang yang tinggi, iaitu 3% ($MRSa_3$ and $MRSt_3$) meunjukkan tiada perbezaan yang ketara berbanding pada tumbesaran probiotik di dalam media glukosa dan MRS, dimana media yang lain ($MRSa_1$, $MRSa_2$, $MRSt_1$ dan $MRSt_2$) adalah kurang daripada 3% kepekatan, semuanya terdapat perbezaan yang ketara. Nilai pH media mengalami penurunan semasa proses fermentasi, tetapi nilai pH akhir tidak terdapat perbezaan yang ketara untuk semua media yang telah diubahsuai kecuali media glukosa (4.56 ± 0.22) dan media MRS komersial (4.40 ± 0.18). Terdapat perbezaan yang ketara pada purata tumbesaran probiotik ($p < 0.001$) dan juga purata masa penggandaan ($p < 0.001$) bagi fermentasi LcS di dalam media $MRSa_1$, $MRSa_2$, $MRSa_3$, $MRSt_1$, $MRSt_2$, $MRSt_3$, MRS dan MRSc selepas 24 jam inkubasi. Selain itu, kiraan aktiviti prebiotik bagi media $MRSa_1$, $MRSa_2$, $MRSa_3$, $MRSt_1$, $MRSt_2$, dan $MRSt_3$ adalah setanding dengan bahan buangan industri pertanian yang lain berdasarkan rekod daripada kajian-kajian sebelumnya. Daripada keputusan kajian ini, bahan buangan yang dihasilkan daripada industri pertanian pemprosesan kulit pisang boleh menjadi sumber bahan yang mampu menggalakkan tumbesaran terpilih probiotik LcS yang disebabkan oleh ciri-ciri potensi prebiotik di dalam bahan tersebut.

CHAPTER 1

INTRODUCTION

1.1 Research Background

Banana is an edible fruit. It has become the most important fruit crop in the world due to its immense nutritional aspects and medicinal value. The banana belongs to the *Musaceae* family and there are two wild species which are *Musa acuminata*, and *Musa balbisiana*. Tchobanoglous et al. (1993) stated that banana or plantain peels composed of 40% of the total weight of fresh banana or plantain. According to Food and Agriculture Organization of the United Nations (FAO) data, the world's banana production had reached the mark of 114.3 million tons in 2014. The production of bananas is mostly produced in Asia, Latin America and Africa. In fact, the countries with the largest producers of bananas are India, which produced annually about 29 million tonnes, on average between 2010 and 2017, and China (mainland) at 11 million tonnes. Other large producers are the Philippines which produced 7.5 million tonnes per year on average between 2010 and 2017, and Ecuador and Brazil both at an average of 7 million tonnes. In Malaysia, banana is the second most widely cultivated fruit (Roff, Malik, & Sharif, 2012). It covers about 10% of the total fruit area with a total production of 535 000 tonnes. The popular local varieties grown for consumption as fresh fruits are the *Pisang Mas, Pisang Berangan, Pisang Rastali*, while the cooking or processing varieties are *Pisang Tanduk, Pisang Raja, Pisang Abu, Pisang Tanduk, Pisang Nangka* (Abd Shukor et al., 2001).

Agro-industrial processing of banana has yielded its own by-products which mainly consist of peel and pulp. Banana peel is the outer layer of banana fruits which accounts for about one third of the whole fruit weight (Vu, Scarlett, & Vuong, 2016). Recently, banana peel has been effectively used for many different industrial applications such as bio-absorbents, bio-fuel production, environmental clean-up, organic fertilizer and biotechnology related processes (Morton, 1987; Gunaseelan, 2004; Bori et al., 2007). In addition, there are some studies reported that the by-products in agro-industrial processing of banana can be used as a natural preservative agent in order to improve the quality of food as the banana peel has antimicrobial and antioxidant properties. For instance, the quality of food products such as fish oil can be improved when the banana peel extracts are added to suppress lipid oxidation. (Anal, Jaisanti, & Noomhorm, 2012; Devatkal, Kumboj, & Paul, 2014).

Gibson et.al (2017) through the International Scientific Association for Probiotics and Prebiotics (ISAPP) consensus panel recently proposes the following definition of a prebiotic: "a substrate that is selectively utilized by host microorganisms conferring a health benefit". Today, only a few compounds fully meet the definition of prebiotics which gives beneficial to the intestinal flora balance. The compounds are including fructo-oligosaccharides (FOS) (Hustoft et al., 2016), galacto-oligosaccharides (GOS) (Moro et al., 2002; Wilson & Whelan, 2017) and gluco-oligosaccharides (Møller, Yong, Viborg, & Andersen, 2014; Rastall, 2013). Moreover, the pectin oligosaccharides (POS) has become one of the compounds due to its functional aspects. This can be shown in several studies which have concluded that POS is a better prebiotic example than pectin (Chen et al., 2013; Gomez, Gullon, Yanez, Schols, & Alonso, 2016; Olano-Martin, Gibson, & Rastall, 2002). In general, the prebiotics such as galacto-oligosaccharides (GOS), fructo-oligosaccharides (FOS) or inulin are naturally present in certain plants such as in bananas, onions, garlic, asparagus, soybeans and whole-wheat foods.

Prebiotics act as a food for human microflora and typically they are made up from high-fiber foods while probiotics are live microorganisms that can help to enhance the "good" bacteria (normal microflora) in the body. Thus, the components in prebiotics can promote the selective growth or activity of beneficial probiotic bacteria such as *Lactobacillus* and *Bifidobacterium* species in the colon that will exert a beneficial physiological effect to the host. The benefits of prebiotic including an improvement in gut barrier function and host immunity, pathogenic bacteria such as clostridia, salmonella and listeria which capable of causing illness in host health can be reduced and short chain fatty acids will be highly produced (Slavin, 2013). Apart from that, Muhammad et al., (2018) stated that most of the probiotic bacteria strains are belonging to the genus *Lactobacillus* due to its non-pathogenic nature which found in most of the environments.

1.2 Problem Statements

According to Padam et al. (2014), agro industrial wastes especially from the food crop processing wastes such as sugarcane bagasse, wheat bran, and corn cob have increased tremendously and the amount of residues produced from the industries has become one of the crucial world issues from year to year. When the residues are released to the environment without any control and proper way of disposal, they can cause environmental pollution and thus, give harmful effects on both human and animal health. In fact, most of the agro-industrial producers are practising improper disposal procedures such as dumping, burning or unplanned landfilling which cause untreated and underutilized wastes. As the result, serious disposal problems can be occurred (Rodríguez-Couto, 2008). For example, there is a huge amount of wastes such as peels produced by the juice industries, coffee pulps produced by the coffee industry and husks waste from the cereal industries and they can produce major environmental problem if the wastes are not processed properly.

On the global scale, 998 million tonnes of agricultural waste are produced whereas in Malaysia, 1.2 million tonnes of agricultural waste are disposed into landfills every year. It is estimated that about 15% of the total waste generated in Asia are agro-industrial wastes. In Malaysia, approximately 0.122 (kg/cap/day) of agricultural waste was generated in 2009 which is projected to reach 0.210 (kg/cap/day) by 2025 (Agamuthu, Khidzir & Hamid, 2009). Even though the agro-based industry produced many different types of waste, these wastes are mostly composed of organic matter, which have high potential to be converted into some added value products. Hence, it is important to adopt and consider the new methods for treating agro-residues in order to achieve sustainable management of agricultural waste.

According to Food and Agriculture Organization of the United Nations (FAO), prebiotic is defined as "a non-viable food component that confers a health benefit on the host which associated with modulation of the microbiota". In fact, some dietary material that enters the large intestine can be considered as potentially prebiotic, however the widely known prebiotics are non-digestible oligosaccharides (Gibson et al., 2000). Generally, the non-digestible oligosaccharides and polysaccharides exhibit prebiotic properties. Inulin, fructooligosaccharides (FOS), galactooligosaccharides and lactulose are some of the different types of oligosaccharides with prebiotic properties. Currently, these oligosaccharides can be derived from the plants and fruits sources have been used widely (Yin, Du, & Dong, 2016). For example, banana is a good source of dietary fructo-oligosaccharides and thus, can be a functional components of foods (Singh et al., 2016).

To date, there are many industries and researchers are at increasing interest in exploring and developing of new prebiotic compounds with added functionalities. For instance, many agricultural co-products such as fruits, vegetables and cereals residues are rich in fibre which have commercial values, sustainability and beneficial to health. Several studies have sought in order to evaluate either these co-products such as banana peel, apple peel, and carrot bagasse can be used or not as prebiotic potential by enhancing the growth of probiotic bacteria (Hernández, Totosaus & Pérez, 2016).

Due to serious environmental problems and shortage of natural resource, the high value of agricultural co-products has received growing attention as many co-products contain valuable substances such as pigments, sugars, organic acids, and bioactive compounds with antioxidant activity; good sources of fibres (Martínez et al., 2012). Thus, these co-products commonly can be produced into commercial products either as raw materials for secondary processes or as new products ingredients (Sánchez-Zapata et al., 2009). In this perspective by considering those aspects, the aim of this study is to determine the prebiotic potential of banana peel as agro-industrial by-product on the growth of a selected probiotic, *Lactobacillus* species.

1.3 Significance of the study

There are few studies on prebiotic potential on different agro- industrial by-products such as apple peel, pineapple peel, and carrot bagasse on the selected growth of *Lactobacillus* species. Nonetheless, there is a lack of study on prebiotic potential on agro-industrial by-products of banana peel (*Musa spp.*). In general, the organic waste decomposers such as fungi, have ability to hydrolyse the complex organic compounds as a major source of energy. This

potential has been used for many functions such as biomass production, organic waste disposal and its conversion into bio fertilizers (Moreira, Phillips, & Humphrey, 1981).

Based on a study by Smith et al. (1989), many filamentous fungi become major sources of industrial enzymes which can lead in bioconversion of organic wastes to protein rich cell mass (biomass). In addition, banana peel has been applied in the food industry as a source of carbohydrates which is mainly dietary fibre or thickening agent (Alkarkhi, Ramli, Yong, & Easa, 2011; Ramli et al., 2009). Besides, there were studies on mould growth using waste banana peel which analyse that the moulds grew comparatively well on the banana peel substrate (Essien et al., 2005). However, this study is using probiotic and banana peels as potential source of prebiotic and such research is limited. Thus, this research can provide a better understanding of banana peels' utilization by probiotic bacteria and the important findings such as probiotic growth performance parameter and prebiotic scores of agro industrial by-products of the banana peels can substantiate the current literature of this study on the prebiotic potential of agro-industrial by-product and thus, can make it as a baseline data for future researches.

Recently, there is an increasing demand in utilizing the low cost renewable agricultural waste as raw material in purpose of converting them into some added value products so that the land degradation can be well-managed, increasing the productivity of agriculture and reduce the waste (Mohammadi, 2006). In addition, banana by-products which found abundantly in local and global scale, have commercial values, sustainability and beneficial to health. Therefore, the utilization of agricultural wastes such as the banana by-products can be seen as an environmental friendly approach which then reduce the environmental problems due

to uncontrolled and improper way of disposal of the wastes. This also can increase an opportunity for a country in continuation of innovation and development of products from agricultural by-products and wastes. Developing and converting the waste such as from banana by-products should be considered as one of the ways to form an eco-friendly environment (Padam, Tin, Chye, & Abdullah, 2014b).

The banana peel is rich in several nutrients and bioactive compounds such as phenolic acids, vitamins, minerals and these by-products will be used as a prebiotic, practically can be a source of nutrient for probiotic growth in human gastrointestinal tract by stimulating indigenous beneficial flora. Therefore, the findings of this study can add to the existing body of knowledge on prebiotic potential of agro industrial by-products on probiotic growth and regarding the benefits of banana peels that to be used by agro industrial producers.

1.4 Objectives

1.4.1 General objective

To study prebiotic potential of *Pisang Tanduk* and *Pisang Abu* peels and their influence on selected probiotic growth parameters.

1.4.2 Specific objectives

1) To determine and compare the prebiotic potential of *Pisang Tanduk* and *Pisang Abu* peels.
2) To determine and compare the growth of *Lactobacillus casei* strain shirota (LcS) in modified media with *Pisang Tanduk* and *Pisang Abu* peels as the source of carbon.
3) To determine and compare the growth performance parameters of LcS in modified media with *Pisang Tanduk* and *Pisang Abu* peels.

1.5 Research hypothesis

1) To determine and compare the prebiotic potential of *Pisang Tanduk* and *Pisang Abu* peels.
2) To determine and compare the growth of *Lactobacillus casei* strain Shirota (LcS) in modified media with *Pisang Tanduk* and *Pisang Abu* peels as the source of carbon.
3) To determine and compare the growth performance parameters of LcS in modified media with *Pisang Tanduk* and *Pisang Abu* peels.

CHAPTER 2

LITERATURE REVIEW

2.1 Banana

Banana (*Musa spp.)* is a herbaceous plant of Musaceae family, one of the oldest cultivated plants crop dating to 4000 BCE in New Guinea (Denham et al. 2003, 2004). Bananas are grown in almost every country in the world especially in the tropical area and to a limited extent, in the subtropical. In particular, 37% of banana production is found in south and southeast Asia and Pacific, 30% in tropical Africa, 26% in Central and South America and Caribbean, and the remaining 7% can be found other regions and countries (FAOSTAT). In the prehistoric times, bananas are associated with the history, heritage and cultures of several societies and civilization of south and southeast Asia. This has been specifically related to past countries and regions which had been influenced by Hindu and Buddhist cultures (Reynolds, 1951). Historically, the bananas were found in southeast Asia, mainly in India. They were brought to the west by Arab conquerors in 327 B.C and then migrated from Asia Minor to the Africa. Later, first explorers and missionaries had carried the bananas to the New World (U.S, New Zealand, Argentina, Chile, Australia and South Africa) and Caribbean. Bananas are tall in size (2-8m), robust, suckering herbs with gigantic leaves, massive cormlike rhizomatous stems and the leaf sheaths form a pseudostem around the inflorescence scape (Anderson, 1998). Banana has many uses, either in cooking or many agricultural and food industries. For example, banana has become essential in food industry production since by-products of banana have

been used for wrapping foods, clothes and in various ceremonial occasions and expands the usage through cultural diversification (Kennedy, 2009).

2.1.1 Peels

Peel (fruit) is the outer layer of that protect the fruit or vegetable which can be peeled off. Banana peel is outer skin covering of the banana fruit (figure 1: c) and consists of fruit fibres from cluster of banana fruit (figure 1: a). The banana fruit comes from the banana plant (figure 1: b) which is a tree-like perennial herb that springs from underground stem. The banana fruit is protected by its peel which is discarded as wastes after the inner fleshy portion is eaten or process into food products in agro-based industries.

The chemical composition of banana peels commonly consist of dietary fibre fractions such as lignin, cellulose and hemicellulose. In general, the plantain peels are considered more robust than those of dessert bananas (Burdon et al., 1993). Emaga et.al stated that the plantain peels are richer in lignin (14.3-16.8%) than the banana variety (6.0-12.1%) while cellulose fraction for both plantain and banana varieties are similar (6.4-9.6%). Differs from the lignin, hemicelluloses content in the banana (6.4-8.4%) is higher than in plantain (0.6-2.0%) and this may be due to different genotype of those bananas (Emaga et al., 2007). Microorganisms such as bacteria and fungi produce commercial cellulases and they are grouped into important enzymes which are used for the industrial scale cellulose processing. For example, these components have been utilized as a potential economical substrate to support the microorganisms growth for production of cellulase in a solid-state fermentation system (Padam, Tin, Chye, & Abdullah, 2014).

a) Banana fruit (plantain)

b) Banana plant

c) Banana peel (plantain)

Figure 2.1. Banana plants with their parts

(Source: Google, 2018)

2.2 Nutritional Contents of Banana Peel

Several studies on the proximate properties of banana peel have been conducted in many countries including the banana peels that are cultivated in Malaysia. Commonly, the proximate properties specifically focusing on the determination of moisture content, ash, protein, crude fat, crude fibre and crude carbohydrate whereas the functional properties include the determination on characteristic of foods during processing, production, consumption and storage. Apart of the proximate properties, the functional properties such as water and oil holding capacity, viscosity and gelation are essential aspects in ascertaining the banana peel application especially in food industry (Dehnad, Jafari, & Afrasiabi, 2016).

Agro-waste products can be further analysed in order to determine their proximate composition. The parameters such as total ash, fibre, lipid, protein, moisture, nitrogen and carbohydrate were determined for proximate analysis of banana peel. In Malaysia, a study for proximate analysis of banana peel was carried out using method by Speight. Compared to another study from Nigeria, the proximate analysis was determined using standard methods of analysis of Association of Official Analytical Chemists (AOAC, 1990). The proximate properties of banana peel are vary according to different countries. This is might be due to different banana varieties and geographical factors with climatic changes which are then affected the proximate properties of banana peel (Pyar & K.K., 2018). Table 2.1 showed the comparison of proximate analysis of banana peel in Malaysia and Nigeria.

Table 2.1: Comparison of proximate properties of banana peel in Malaysia and Nigeria

Proximate Properties	**Percentage (%)**	
Country	**Malaysia**	**Nigeria**
Moisture content	50.5 ± 2.7	13.49 ± 0.17
Protein	5.3 ± 0.02	5.53 ± 0.11
Lipid	1.6 ± 0.14	23.93 ± 0.68
Fibre	19.2 ± 0.54	14.83 ± 0.28
Ash	8.8 ± 0.54	9.83 ± 0.06
Carbohydrate	14.6	32.39 ± 0.70

Adapted from (Pyar et al., 2018 and Abubakar et al., 2016)

As shown in Table 2.1, Pyar et al. (2018) and Abubakar et al. (2016) concluded that the nutritional compositions of banana peel *(Musa sapientum)* fruits cultivated in Malaysia has relatively low lipid (1.6 percent) in contrast to lipid content of banana peel from another country, Nigeria which is 23.93 percent. However, the banana peel fruits cultivated in Malaysia has high moisture content (50.5 percent) and fibre (19.2 percent) compared to the study from Nigeria, 13.49 percent and 14.83 percent respectively. The differences in terms of moisture content between the studies in different countries might be due to genetic makeup of varieties and geographical factors as well as the climate changes (Butt et al., 1997). Ash is an inorganic residue remaining after organic matter was burnt away and it indicated the mineral elements in the food.

According to Pyar et al. (2018) and Abubakar et al. (2016) findings, the ash contents of banana peel in Malaysia and Nigeria were 8.8 percent and 9.83 respectively. The findings in this research were in line with the study by Emaga et al. (2007) who reported that the ash contents in different banana peels varied from 6.4 to 12.8 percent. Besides, the authors also

suggested that those parameters that being used in proximate analysis can contribute to new idea of the nutritional value of agro industrial by-products in those studies. However, further research should be carried out in order to incorporate the banana peel as the agro industrial by-products that can be utilized into food systems and evaluate the quality of new food products.

2.3 Prebiotic

Prebiotics is a non-digestible food ingredient that confers benefits upon host well-being and health by selectively stimulating both composition and/or activity in the gastrointestinal microbiota (Roberfroid, 2007). Gibson et al. (2017) through the International Scientific Association for Probiotics and Prebiotics (ISAPP) consensus panel recently proposes the definition of a prebiotic: "a substrate that is selectively utilized by host microorganisms conferring a health benefit". Prebiotics can exert antagonistic to pathogenic organisms such as *Listeria, Salmonella sp.* or *Escheria coli* which limiting their proliferation. Globally, the world demand for prebiotics has been increasing and is estimated about 167,000 tons and 390 million Euro (Siró et al., 2008). This is because prebiotics have the ability to improve the growth, metabolism and beneficial health activities of probiotics in the digestive system. Prebiotics have properties of withstand acid hydrolysis in the stomach, so that they are capable of passing to large intestine without being absorbed and digested in the small intestine and then further utilized by the indigenous microflora to enhance their growth and metabolism in the large intestine (Gibson et al., 2004). For example, prebiotics can enhance the absorption of minerals and nutrients such as calcium, manganese and iron or decreasing the incidence of colon cancer in the large intestine.

2.3.1 Oligosaccharide as prebiotic

Oligomers are short chains of monomer units. A molecule that consists a few monosaccharide units which joined together is known as oligosaccharide. Commonly, oligosaccharides are defined as molecules that comprised of 2 to 10 sugar units joined by glycosidic linkages. The generic prefix indicating a carbohydrate-containing compound is *glyco-* whereas the generic term for a monosaccharide unit of an oligo- or polysaccharide is named as *glycosyl* unit. Oligosaccharides have much lower molecular weight than the polysaccharides. Hence, they have high solubility in water. According to term of IUB-IUPAC nomenclature, oligosaccharides are saccharides with Degree of Polymerization (DP) value ranging from 3 to 10 (Wang et al., 2018). In addition, oligosaccharides have similar functional effects to soluble dietary fiber which can enhance a healthy gastrointestinal tract. These oligosaccharides are known as non-digestible oligosaccharides (NDO). Non-digestible oligosaccharides (NDO) exert many important roles in term of physiological and physicochemical properties, which provide a prebiotic activity and can promote the host health by increasing the number of beneficial microbes. Commonly, the prebiotic oligosaccharides are fructooligosaccharides (FOS), galactooligosaccharides (GOS) and xylooligosaccharides (XOS). All of these prebiotic oligosaccharides have been proven to increase the population of beneficial microbes such as *Lactobacilli* and *Bifidobacteria*. When the gut microbes ferment the prebiotic oligosaccharides in the colon, the short chain fatty acids (SCFAs) such as acetate, propionate and butyrate were produced (Rivière et al., 2016). The production of these short chain fatty acids (SCFAs) lowering the pH value in the colon which can inhibit the growth of pathogenic bacteria.

Table 2.2: Sources of naturally occurring oligosaccharides

Naturally occurring oligosaccharides	Sources of food
Fructooligosaccharides	Asparagus, sugar beet, garlic, chicory, onion, Jerusalem artichoke, wheat, honey, banana
Xylooligosaccharides	Bamboo shoots, fruits, vegetable, milk, honey
Galactooligosaccharides	Human milk
Raffinose oligosaccharides	Seeds of legumes, lentils, peas, beans, chickpeas, mallow, composite and mustard
Cyclodextrins	Water soluble glycans

Adapted from (Wang et al., 2018)

As shown in Table 2.3, there are many sources of food which are naturally occurring oligosaccharides. Fructooligosaccharides (FOS) are oligosaccharides that usually present in plants such as onion, garlic, chicory, asparagus, banana, artichoke and many others (Sabater-Molina et al., 2009). Other examples of non-digestible oligosaccharides are xylooligosaccharides, galactooligosaccharides, raffinose oligosaccharides and cyclodextrins. Xylooligosaccharides provide naturally in bamboo shoots, fruits, vegetables, milk and honey (Vázquez et al., 2000). Galactose-containing oligosaccharides are appear naturally in human milk, which can be produced from lactose and the use of GOS (5-15g per day) may relieve the symptoms of constipation especially among adults and elderly people. Raffinose oligosaccharides are found naturally in leguminous seeds, lentils, peas, beans, chickpeas, mallow, composite and mustard (Johansen, Glitsø, & Knudsen, 1996) whereas water-soluble glucans are rich in cyclodextrins (M. Singh, Sharma, & Banerjee, 2002). Cyclodextrins are produced from starch using a group of amylolytic enzymes, cyclodextrin glucosyltranferases

that produced naturally from different strains of bacteria. In addition, an array of prebiotics have been existed with some origin and their chemical properties. Stowell (2007) reviewed the existing prebiotics and grouped them based on a set of common characteristic as shown in Table 2.3.

Table 2.3: Prebiotic carbohydrates

Category	Compound of saccharides
Established Prebiotics	Inulin Fructooligosaccharides (FOS) Galactooligosaccharides (GOS) Lactulose Polydextrose
Emerging Prebiotics	Isomatooligosaccharides (IMO) Xylooligosaccharides (XOS) Lacticol

Adapted from (Patel & Goyal, 2012)

Based on the Table 2.3, inulin, fructooligosaccharides (FOS), galactooligosaccharides (GOS), lactulose and polydextose are categorized as the established prebiotics, while isomaltooligosaccharides (IMO), xylooligosaccahrides (XOS), and lactitol are recognized as emerging prebiotics. Gibson et al. (2004) stated that the established prebiotics including fructooligosaccharides (FOS), galactooligosaccharides (GOS) and inulin are commonly used in the European market. However, some of compounds are considered as "emerging prebiotics" which need further investigation as they are not well-established. Some of them are oligomers of soya and xylan, isomalto-oligosaccharides (IMO), xylooligosaccharides and honey oligosaccharides. Although there is still lack of studies on their fermentation properties, these compounds are tend to have high possibility of antimicrobial activity which consist same or more desirable properties compared to established ones.

Of these prebiotics, inulin and FOS are commercially available and chicory root is the major source of both prebiotics (Sabater-Molina et al., 2009). Inulin is the most naturally found form of stored carbohydrates in plants. In term of chemical structure, it consists of fructose units' chains with a terminal glucose unit linked by β-glycosidic bonds (2→1). This means that they cannot be hydrolysed by the human digestive enzymes, as they are only capable of hydrolysis α-glycosidic bonds. According to Roberfroid (2002), both fructo-oligosaccharides and inulin are recognized as the prebiotics model and the consumption of 5-15g/day of them for many weeks, evidently show the prebiotic activity. For example, as the prebiotic carbohydrates were consumed, they become available to the intestinal bacteria in order to grow, proliferating in numbers and its volume which can enhance a faecal bulking effect to the host. In scientific publications, the inulin and fructooligosaccharides have become the most interest and achieved the prebiotic status. Therefore, Morris (2012) reviewed that those two polymers were focused which mainly differ by their Degree of Polymerization (DP). Figure 2.2 shows the chemical structures of fructooligosaccharides and inulin.

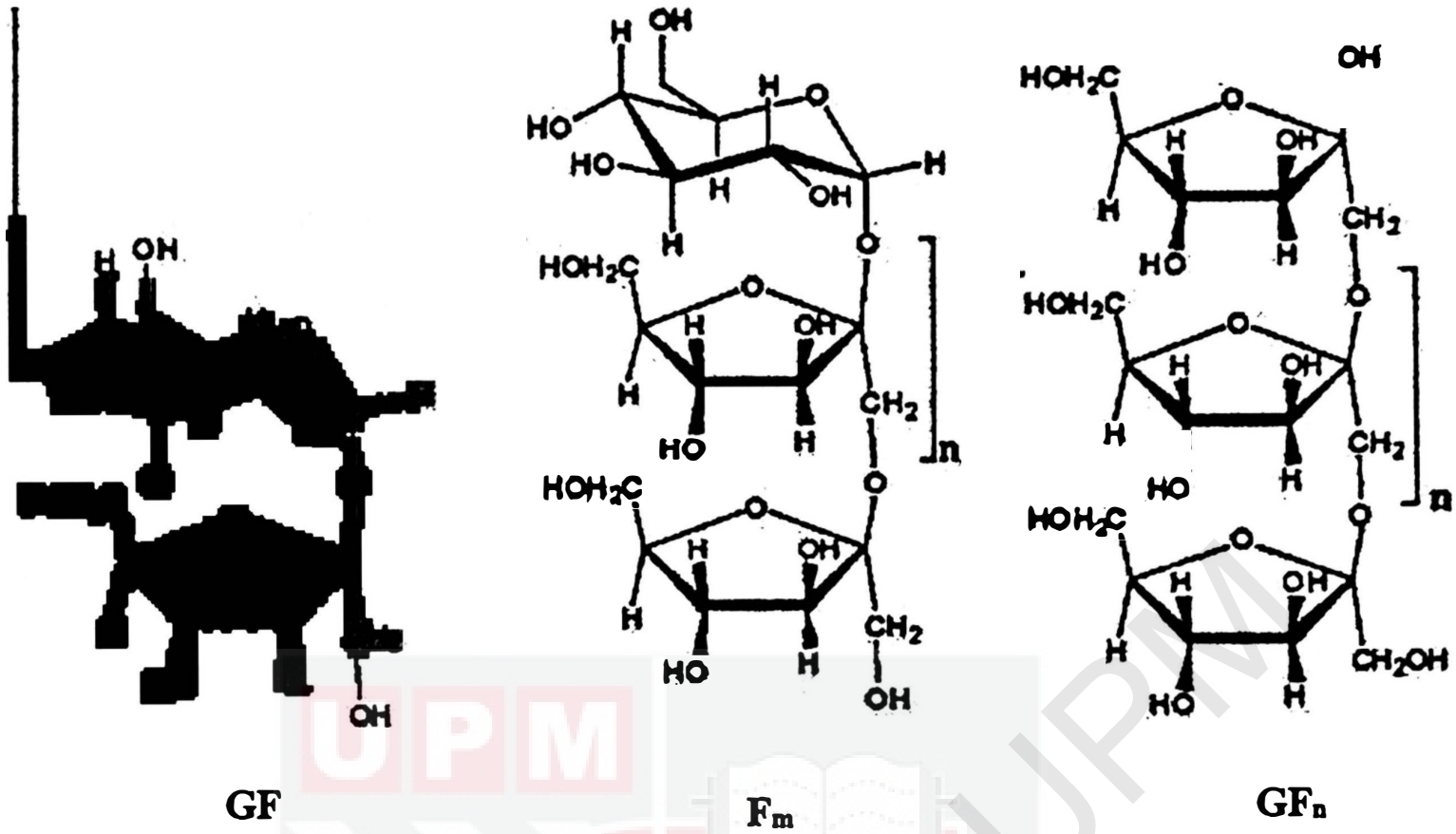

Figure 2.2 Chemical structures of sucrose, GF (left), GF_n-type inulin (centre) and F_m-type fructo-oligosaccharides (right). G: glucose; F-fructose.

(Source: Morris, 2012)

2.4 Probiotics

A probiotic has been defined by Food and Agricultural Organization of the United Nation (FAO) and World Health Organization (WHO) in 2006, as "live microorganisms which when administered in adequate amounts, confer a health benefit on the host". Tuohy et al., (2003) stated most of probiotics belong to genera of *Lactobacillus* (naturally found in small intestine) and *Bifidobacterium* (naturally found in large intestine). Lactobacilli are present in fermented dairy products and can give beneficial effects which include control of antibiotic-related diarrhea, prevention of urinary tract infections and control of intestinal inflammation (Bernardeau et al., 2008). On the other hand, bifidobacteria are normal inhabitants of gastrointestinal tract of humans (Picard et al., 2005). Some positive effects that given by

bifidobacterium are anti-carcinogenic capabilities, protection against infectious diarrhea and alleviation of lactose intolerance (Russell et al., 2011).

For an efficient probiotic strain to be considered as a beneficial probiotic, the bacteria must be able to withstand low pH and bile salts, have antimicrobial activity. Importantly, the bacteria must be non-pathogenic microorganism. A comprehensive approach is needed for the selection and assessment of new probiotic candidates due to target functions and technological applications in the probiotic range. There was no international regulation and guidelines in order to affirm the efficacy and safety of the probiotic microorganisms before the year of 2002. Then, the Food and Agriculture Organization of the United Nations/World Health Organization (FAO/WHO) has established the effectiveness and safety standards for probiotics - "Guidelines for Evaluation of Probiotics in Food" (Araya et al., 2002). The guidelines provides with several criteria for choosing the probiotics which include the capability of epithelium adhesion, resistance to adverse conditions that imposed by human body, antimicrobial activity and safety evaluation (Vinícius et al., 2018).

Table 2.4: Commercial probiotic microorganisms

Microorganisms	Strain	Company (sources)
Bifidobacterium animalis	DN173 010	Dannon (Tarrytown,NY)
Bifidobacterium breve	Yakult	Yakult (Tokyo, Japan)
Bifidobacterium infantis	35, 264	Procter and Gamble (Mason, OH)
Bifidobacterium lactis	HN019™ BB-12	DuPont™ Danisco® Chr. Hansen
Bifidobacterium longum	BB536	Morinaga Milk Industry Co. Ltd.
Bifidobacterium	CRL 431	Snow Brand Milk
Lactobacillus acidophilus	LB LA5 NCFM	Lacteol Laboratory Chr. Hansen Danisco
Lactobacillus johnsonii	LC1	Nestle
Lactobacillus casei	Immunitass®	Danone®
Lactobacillus casei	Shirota	Yakult
Lactobacillus plantarum	299 V	Probi AB, NextFoods
Lactobacillus fermentum	VRI003 (PCC)	Probiomics
Lactobacillus lactis	Lafti™ L1A	DSM
Lactobacillus paracasei	F19 33	Medipharm GenMont Biotech
Lactobacillus reuteri	RC-14® ATCC 55 730	Chr. Hansens Biogaia
Lactobacillus rhamnosus	GG	Valio Dairy The Dannon Company

Adapted from Yeung et al. (2002), Ciorba (2012), Fijan (2014) and Eskesen et al. (2015).

2.4.1 Lactic acid bacteria (LAB)

Lactic acid bacteria (LAB) are non-aerobic, non-sporulating, non-motile and Gram-positive bacteria which made up of rod- and coccus-shaped. Besides, LAB are the organisms which are used for fermentation especially in the preservation of foods (Muhammad et al., 2018). These microaerophilic bacteria typically are catalase-negative in the absence of porphorinoids and producing lactic acid as their major fermentation product of carbohydrates.

LAB require energy from substrate-level phosphorylation that includes two basic fermentative pathways, homofermentative and heterofermentative ways. The pathway that produces only lactic acid via glycolysis is homofermentative pathway whereas the pathway which yield lactic acid with presence of carbon dioxide and ethanol via 6-phosphogluconate or phosphoketolase is heterofermentative pathway (Fugelsang & Edwards, 2007). *Lactobacilli, Streptococci, Leuconostoc, Aerococci* and *Pediococci* are the genera that most likely shows the characteristic of lactic acid bacteria.

Lactobacilli is the result of the compounds including lactic acids, propionic acids, H_2O_2, CO_2, bacteriocins and other antimicrobial compounds. As all lactic acid bacteria, species which make up the genus *Lactobacillus* are chemoheterotrophic as they get their energy from fermentation. Commonly, the lactobacilli are found in many different foods and used as starter cultures in food fermentation which acts as probiotics. Probiotics usually administered in probiotic foods such as yogurts and its regional varieties, cheeses and fermented milks. (Yang et al., 2010) reviewed lactobacilli are present throughout the gastrointestinal tract of humans, from the oral cavity to fecal material. *Lactobacilli* species including *L.rhamnosus*, *L. gasseri* and *L. casei*, among others are found in the oral cavity of the humans.

2.5 Health benefits of probiotic and prebiotic

Probiotics and prebiotics are two main substances that have some positive effects in terms of nutritional health and therapeutic effects against diseases such as gastrointestinal diseases, liver diseases and respiratory infections in host. Even their composition and metabolism are different, they are more likely to share their common mechanisms of action especially modulation of gut microbiome that beneficial on the host health and well-being (Rodríguez et al., 2015). Prebiotics can result in improved health of the host as they have a selective effect on the microbiota. Thus, when a substance fulfills the following characteristics such as resistance to digestion, fermentation by the large intestinal microbiota, and a selective effect of the microbiota, it can be considered as a prebiotic. Currently, for probiotics, the most widely used are lactobacilli, bifidobacteria and some non-pathogenic strains. When they were administered in adequate amounts, they confer a beneficial effects on the host health that can enhance the prevention or improvement of some diseases (Ross & Preedy, 2016). The combination of prebiotics and probiotics, result in synbiotics, work best to enhance the growth of the gut microflora. Table 2.5 shows the evidence of prebiotics on its mechanisms of action with health benefits and Table 2.6 shows the evidence of health benefits of probiotics.

Table 2.5: Mechanisms of action of prebiotics with health benefits

Prebiotics	Health benefits	Mechanism of actions
1. Fructo-oligosaccharides (FOS)	1. Antipathogenic effect	- Inhibits of human and animal pathogens - Increases SCFA in large bowel - Reduces damage of ulcerative colitis
	2. Improvement of gut health	- Stimulates *lactobacilli* and *bifidobacteria*
2. Galacto-oligosaccharides	1. Antipathogenic effect	- Reduces incidence of diarrhea
	2. Reduction of cardiovascular disease	- Reduced LDL-cholesterol
	3. Prevention of cancer	- Induces apoptosis - Increases immunogenicity of cancer cells
	4. Mineral absorption	- Stimulates intestinal calcium and magnesium absorption
3. Xylo-oligosaccharides (XOS)	1. Antipathogenic effect	- Increases SCFA
4. Inulin	1. Reduces gastroenteritis	- Relieves from colitis
	2. Improvement of gut health	- Stimulates *Lactobacilli* and *Bifidobacteria*
	3. Mineral absorption	- Stimulates intestinal calcium and magnesium absorption
5. Arabinoxylo-oligosaccharides	1. Prevention of cancer	- Effect on colonic cancer lesions

Adapted from (Al-Sheraji et al., 2013)

Table 2.6: Health benefits of probiotics strains

Strains	Health benefits
L. acidophilus	Treatment of travellers' diarrhea; reduction of hospital stay of children with acute diarrhoea
L. acidophilus ATCC-4495	Antifungal activity
L. casei Shirota	Treatment of functional constipation in adults; reduction of diarrhoea duration of antibiotic-associated diarrhoea in geriatric patients; immunomodulatory mechanisms
L. casei Lcr35	Restoration of vaginal flora of patient with bacterial vaginosis
L. rhamnosus GG	Prevention and reduction of severity of atopic dermatitis in children; reduction of risk for developing allergic diseases; treatment of acute gastroenteritis in children; protection of human colonic muscle from lipopolysaccharide-induced damage
L. rhamnosus CRL1505	Reduction of viral-associated pulmonary damage
L. plantarum	Reduction of irritable bowel syndrome symptoms
L. reuteri DSM 17938	Management of infant colic; reduction of onset of gastrointestinal disorders in infants; reduction of frequency of proven sepsis; feeding intolerance and duration of hospital stay in preterm infants
B.infantis	Reduction of irritable bowel syndrome symptoms; reduction of necrotizing enterocolitis in preterm infants
B. animalis subsp. Lactis DN-173 010	Treatment of functional constipation in adults; reduction of total microbial counts in dental plaque
B. bifidum	Reduction of hospital stay of children with acute diarrhea
B. longum	Prevention and treatment of necrotizing enterocolitis in newborns; reduction of radiation induced diarrhoea
Saccharomyces. boulardi	Treatment of travellers' diarrhoea; treatment of irritable bowel syndrome; treatment of moderate ulcerative colitis; and treatment of acute gastroenteritis in children

Adapted from (Fijan, 2014)

2.6 Prebiotic properties

As the world population increases over the years, the demand for food products, animal feeds, and biofuels have become high, and therefore many industries and researchers are at increasing interest in exploring and developing of agro-industrial by products with added functionalities. According to Clarke et al. (2008), several studies have sought to improve the utilization of banana by-products to overcome the greater supply in some manufacturers. The researchers had considered few methods for creating some value added products. For instance, the agricultural wastes such as banana peels, are available in most of the banana producing countries, are utilized to reduce the reliance on the costly imported animal feed (Ulloa et al., 2004). By recycling these underutilized wastes, it shows an effort to find for new sources of animal feed and to sustain the animal products market.

High-fiber foods which mainly composed in vegetables and fruits that probably provided in agro industrial by-products such as banana peels, can act as prebiotic. According to Kurtoğlu and Yildiz (2011), the banana peel contains high content of fructo-oligosaccharides, a prebiotic that act as food for human microflora and promote the selective growth of probiotic bacteria such as *Lactobacillus* and *Bifidobacterium* to improve the nutrients absorption in the colon. These beneficial bacteria will produce substantial vitamins and digestive enzymes which can increase the capability of nutrients to be adsorbed in the host health. Therefore, the banana peel has been used to improve the human health conditions and shows its great potential by functioning as prebiotics.

2.6.1 Probiotic properties of agro-industrial by-products

In general, most probiotics such as *Lactobacillus*, *Leuconostoc* and *Pediococcus* species have been used widely especially in food processing. *Lactobacilli*, *Bifidobacteria* and other non-pathogenic strains probiotics give many health benefits to the host and these probiotics must retain their viability during storage, process of manufacture and transit along the passages of stomach and small intestine. Based on current literature, the prebiotic potential or activity has been assessed in agro industrial by-products which involving the banana peel, apple peel and carrot bagasse (Hernández et al., 2016). For example, different by-products flours of banana peel, apple peel and carrot bagasse as the substitution of glucose (carbon sources) to determine the probiotic growth of *Lactobacillus rhamnosus GG*. The authors found a significantly ($P<0.05$) higher values of mean rate of growth and significantly ($P<0.05$) lower mean duplication times values in fermentations with glucose and apple peel flour. However, fermentation with banana flour as the carbon source did not produce a significant observation on the growth of probiotic (Hernández-Alcántara, Hernández, et al., 2016).

In another study by Espírito Santo et al. (2012), both apple and banana fiber yoghurts co-fermented by *L. acidophilus* and *B. animalis subsp. lactis* showed significantly higher of probiotic counts than passion fruit fiber yoghurts. This is because both apple and banana fiber showed positive effect on probiotics growth due to their high pectins and fructo-oligosaccharides contents (Happi Emaga et al., 2008). With regard to the net probiotic growth, Sah et al. (2016) also reported the viable counts of *S. thermophilus* and *L. bulgaricus* in the yogurts which supplemented with pineapple powders showed same value when compared to those in yogurts without supplementation. In fact, the pineapple waste powder that present in probiotic yogurts had enhanced the probiotic growth. For example, *L. acidophilus* (8.13-8.71 log cfu/g), *L. casei* and *L. paracasei* (8.12-8.72 log cfu/g) had shown greater counts than the

non-supplemented probiotic yogurts (7.34 log cfu/g and 7.83 log cfu/g) in the study (Sah et al., 2014).

Moreover, other studies had involved the agro industrial by-products of cashew apple to determine its prebiotic effects on different potentially probiotic *Lactobacillus* strains. The probiotics that were used are *L. acidophilus LA-05, L. casei L-26* and *L. paracasei L-10* in the basal broth. During the incubation period, significant ($P<0.05$) growth-promoting effects on *L. acidophilus LA-05* and *L. paracasei L-10* were observed in broth that contained glucose, followed by broth containing fructo-oligosaccharides (FOS) and cashew apple by-product powder (CAP). This study also reconfirmed that by using concentration of 20 g/L of cashew apple powder in substitution of glucose in cultivation media, there was greater growth-promoting effects on *L. acidophilus LA-05* and *L. paracasei L-10*, indicating the added value of agricultural product to promote the growth of probiotic (Duarte et al., 2017). These results showed positive prebiotic activity score, an indicator that can be used to determine the potential use of agro industrial waste as prebiotic to promote the growth of the probiotic.

2.6.2 Acidification (final pH) performance of probiotic on agro-industrial by-products

Fermentation is a process in which anaerobic microbial breakdown of organic matter undergo a variety of reactions and metabolic processes (Riggio et al., 2019). During fermentation, the short chain of fatty acids (SCFA) such as lactic, acetic, propionic and butyric acids are produced. Probiotics use prebiotics as source of food and produce metabolites such SCFA. Of many prebiotics, Mandalari et al. (2008) added that FOS had showed the highest total short-chain fatty acid production. Commonly, in terms of inhibiting pathogenic bacteria, the production of short chain fatty acids can lower the pH, thus improving the host's health.

This subsequently, increases the absorption of minerals and nutrients, reliefs of constipation, decreases colon cancer incidence and strengthens immune systems (Duarte et al., 2017).

The higher rate of acidification has been linked to an increased carbohydrate metabolism (Ogunbanwo & Okanlawon, 2009). Based on a study conducted by Hernández-Alcántara et al., (2016), the acidification parameters in *P. pentosaceus* and *L. rhamnosus* fermentations showed significantly ($P<0.05$) higher maximum acidification rate (V_{max}) values, pH at V_{max} and significantly ($P<0.05$) lower for the time to reach the maximum acidification rate employed in glucose or apple peel flour followed by the carrot bagasse as the carbon source. However, the banana peel result in lower of acidification for *P. pentosaceus* and no growth was observed when ferment in *L. rhomnasus*. The ability of depolymerizing capacity can enhance one prebiotic to be utilized into strain-dependent (Yeo & Liong, 2010).

Parra-Matadamas et al. (2015) stated that the growth rate of grapefruit peel flour is higher when it employed as carbon source. In context of carbohydrates utilization, the disaccharides and oligosaccharides provide maximum specific growth rate when compared to monosaccharide as their abilities and kinetics are different by various strains. According to Diaz-Vela et al. (2013), the strains *P.pentosaceus, A. viridans,* and *L.rhamnosus* were used in order to determine their acidification parameter on cactus pear peel flour and pineapple peel flour as the carbon sources. As the results, the maximum acidification rate values of *Pediococcus pentosaceus* were significantly ($P<0.05$) higher, and the lowest were for *E.coli*. The time needed to reach the maximum acidification was significantly ($P<0.05$) lower for *P. pentosaceus* and *A. viridans*. Therefore, the pH at this point was significantly ($P<0.05$) lower for *L. rhamnosus*. The cactus pear flour fermentations has obtained significantly ($P<0.05$) higher maximum acidification and the pH value at maximum acidification was not significantly ($P>0.05$) different for the different carbon sources used.

Moreover, a study from Duarte et al. (2017) showed that the pH values of cultivation media decreased, when culturing *Lactobacillus* strains in broth containing glucose, FOS and cashew apple waste product especially during first 24 hours. However, it was observed that there is no significant difference ($P>0.05$) in pH values in the cultivation media for the same inoculated strains. In fact, high viable cell counts of probiotic bacteria which produced low pH values of growth media indicates a rapid metabolic activities in the broth which inoculated with *Lactobacillus* strains. As the results, there was observed that the bacterial metabolic activity in broth containing cashew apple waste product is high and this shows a good result because the survival and growth of probiotics bacteria has been associated with decreased in pH values and increased production of organic acids in the colon of the host (Duarte et al., 2017).

2.6.3 Prebiotic potential: Prebiotic activity score on agro-industrial by-products

Prebiotic activity reflects the ability of a given substrate to support the growth of microorganisms in comparison with other microorganisms and to growth on a non- prebiotic such as glucose substrate. The prebiotic activity score was determined according to the relationship introduced by Huebner et al. (2007). It was considering the growth of each bacteria during fermentation and using the cactus pear peel or pineapple peel flours and glucose as carbon source (Diaz-Vela et al., 2013). In fact, substrates with a high prebiotic high prebiotic activity score support good growth of the probiotic bacteria, with cellular growth comparable to that observed when grown on glucose. The cellular growth of the enteric strains grown on the prebiotics should, in theory, be very low in relation to growth on glucose.

In agro-industrial by-products, the prebiotic activity has been assessed for evaluating and developing in their added functionalities. Based on a study conducted by Zhang et al. (2018), *L. paracasei* LPC-37 grown on MRS broth with added pectin oligosaccharide (POS)

and *L. paracasei* 1195 which paired with commercial prebiotic fructooligosaccharides (FOS) product-NutraFlora P-95 scored higher values of prebiotic activity. However, the pectin from citrus peel scored lower of prebiotic activity. The POS samples were used as substrates for *L. paracasei* LPC-37 scored higher than *L.paracasei* 1195 grown on purified galactooligosaccharides (GOS), but lower than *L. paracasei* 1195 grown on inulin-products and Raftiline HP (Huebner, Wehling, & Hutkins, 2007). As a result, the prebiotic activity of POS for *L. paracasei* showed higher than purified GOS, which similar to FOS product (NutraFlora P-95) but lower than inulin products (Inulin-S and Raftiline HP). The highest prebiotic scores were observed for *B. bifidum* ATCC 29521 with other POS scores which significantly lower ($P<0.05$). According to Barrangou et al. (2003), the utilization of prebiotics by lactic acid and specific bacteria requires the presence of specific enzyme hydrolysis and transport systems.

Diaz-Vela et al. (2013) reported there were significantly ($P<0.05$) higher values in prebiotic activity scores for *P. pentosaceus*, followed by *L. rhamnosus*. However, *A. viridans* obtained the lowest scores. For the cactus pear peel and pineapple peel flours as carbon source, both of them showed no significant ($P>0.05$) difference which had same effect on lactic acid bacteria growth. According to Huebner et al. (2007), it was reported that different microorganisms have different prebiotic activity scores. The result obtained from this study was *P.pentosaceus* showed higher prebiotic activity which indicates that a better assimilation of fermentable carbohydrates present in the peel flours. However, *A. viridans* has lower scores due to its lower performance during fermentations. Apart from that, the result was good because both of cactus pear and pineapple flours had a positive prebiotic effect although there was differences in terms of specific growth and acidification parameters (Hopkins et al., 1998; Cardelle-Cobas et al., 2011).

CHAPTER 3

METHODOLOGY

3.1 Substances and chemicals

Customized De Man Rogosa and Sharpe (MRS) broth and agar were prepared by using these chemicals and reagents: glucose (20g/L), yeast extract (6g/L), peptone from meat (30g/L), sodium acetate (8.29g/L), magnesium sulfate heptahydrate (0.2g/L), tween 80 (1.0g/L), manganese sulfate heptahydrate (0.05g/L), ammonia citrate (2g/L), disodium phosphate (2g/L), and agar powder (15g/L). They were mixed together with sterile distilled water. Besides, the commercial MRS powder for nutrient broth and agar were prepared based on this ratio, 52.2g/L and 68.2g/L respectively. The cultured milk drink, Yakult was used as a source of probiotic.

3.2 Propagation of cultures

Lactobacillus casei strain Shirota (LcS) was enumerated from Yakult cultured milk drink. The drink was purchased at a supermarket in Putrajaya, Malaysia. For the isolation of LcS, the spread plate technique was used. Approximately 1 mL of Yakult cultured milk that contains only LcS was spread over the surface on the MRS agar using a sterile spreading rod and incubated in the incubator at 37°C, for 48 hours (Waoo & Dixit, 2018). After that, a single

colony of bacteria from LcS was further grown in MRS broth for 24 hours at 250 rev min^{-1} (rpm) in an incubator shaker and at 37°C. The growth of LcS was monitored optically at the wavelength of 600 nm every 2 h for 24 h. The reading measurement of optical density (OD) was recorded and plotted. A serial dilution was prepared, and streak dilution plate was used to grow the LcS after a specific optical density (OD) reading measurement was recorded. The growth of LcS was calculated using colony forming unit (cfu/ml) formula.

A reference strain, enteropathogen strain *Escherichia coli* K12 was used and then reactivated in nutrient broth for 24 h at 37°C in order to obtain a similar optical density responses. The growth of *E.coli* was also monitored optically at the wavelength of 600 nm every 2 h for 24 h. After reading measurement of optical density (OD) was recorded, the growth of *E.coli* was calculated using colony forming unit (CFU/ml) formula.

3.3 Instruments

In this study, the instruments used were freeze-dryer (The VIRTIS Company, Inc Route 208, Gardiner, New York), incubator orbital shaker (716, Protech electronic), incubator (716, Protech electronic), blender 21 (Torrington Ct, USA), centrifuge machine (Universal 32R, Hettich Zentrifugen, England), weighing scale (AC Adapter, Japan) and vortex (EVM-V1000, Erla, Jayu, Korea).

3.4 Sample collection and preparation

Two different types of banana peels which are *Pisang Tanduk* and *Pisang Abu* were collected from producers of small and medium-sized banana chips enterprises in Banting and Petaling Jaya, Selangor area. The peels were collected by using purposive sampling method. Briefly, the banana peels were cleaned and dried using 300 freeze dryer (The Virtis Company, Inc Route 208, Gardiner, New York). Then, the dried banana peels were ground and milled to fine powder. After that, the fine powder of banana peels was weighed, standardized to less than 180µm using sieves and then put into the plastic vacuum bags. These samples were stored at -20 °C for further analysis.

3.5 Experimental design

Pisang Tanduk and *Pisang Abu* peels were used in this study. Estimation method was used in order to determine the different concentration (1%, 2% and 3%) of the banana peels powder before fermentation process. The estimation method was conducted by weighing the banana peels powder according to their different concentrations; 1% (2g), 2% (4g) and 3% (6g). Later, two different assessments which are bacterial growth performance and prebiotic activity score parameter were conducted. Figure 3.1 shows experimental procedures that were conducted in this study.

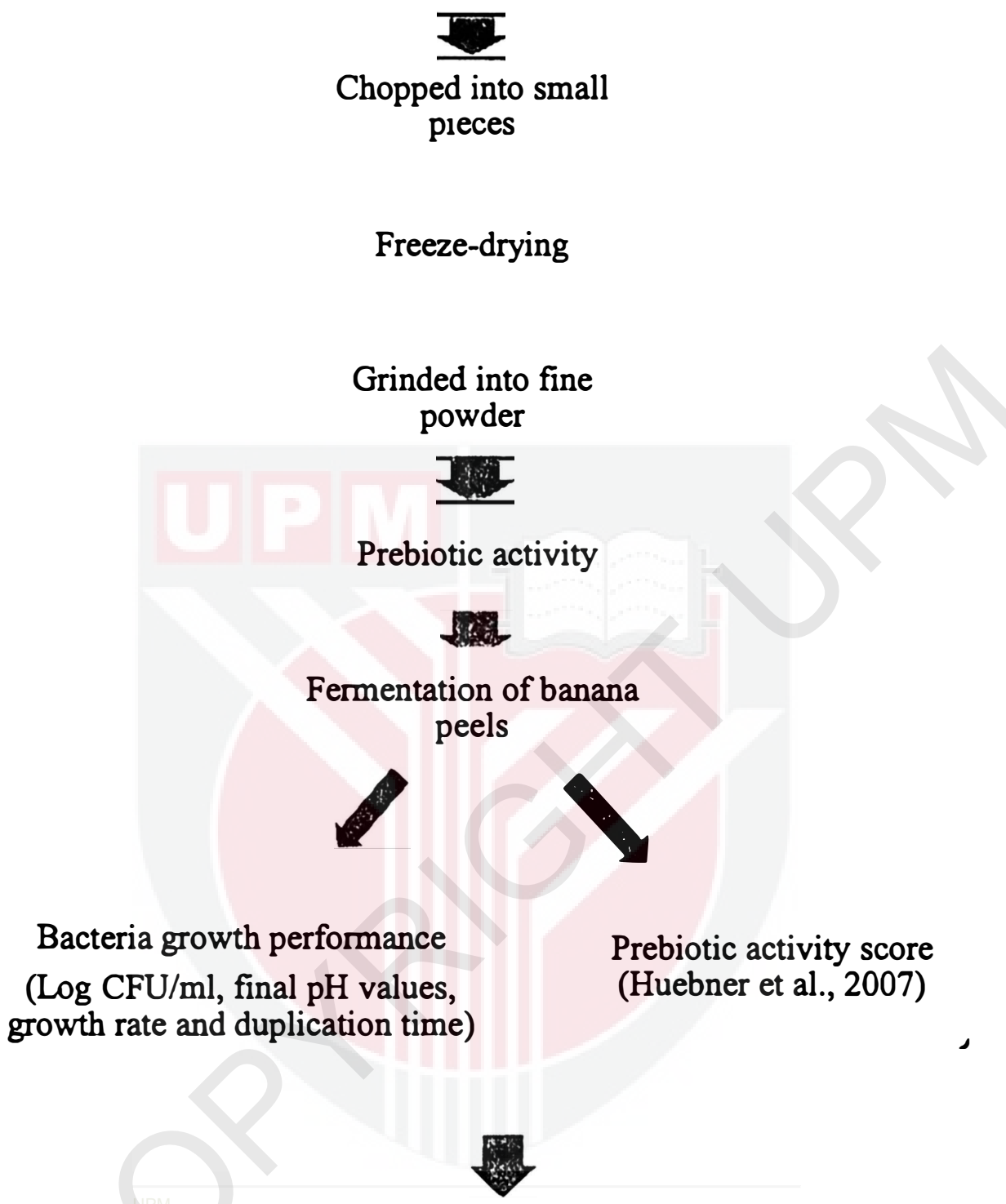

Figure 3.1. Experimental design of prebiotic potential of *Pisang Tanduk* and *Pisang Abu* peels

3.6 Assessment of probiotic growth performance

3.6.1 Modification of cultured media

The probiotic growth performance was determined to indicate as a probiotic performance and it was assessed through the growth of *Lactobacillus casei* with some modification. Table 3.1 below shows the different substances and chemicals that were used for some modification in MRS broth.

	Commercial MRS broth (MRS_c) – control	**MRS broth without glucose (MRS_g)**	**MRS broth of *Pisang Tanduk* (MRS_t)**	**MRS broth of *Pisang Abu* (MRS_a)**
Substances and chemicals used	1) MRS agar powder	1) Yeast extract (6 g/L) 2) Peptone from meat (30g/L) 3) Sodium acetate (8.29g/L) 4) Magnesium sulfate heptahydrate (0.2g/L) 5) Tween 80 (1.0g/L) 6) Manganese sulfate heptahydrate (0.05g/L) 7) Ammonia citrate (2g/L) 8) Disodium phosphate (2g/L)	1) Yeast extract (6 g/L) 2) Peptone from meat (30g/L) 3) Sodium acetate (8.29g/L) 4) Magnesium sulfate heptahydrate (0.2g/L) 5) Tween 80 (1.0g/L) 6) Manganese sulfate heptahydrate (0.05g/L) 7) Ammonia citrate (2g/L) 8) Disodium phosphate (2g/L) 9) *Pisang Tanduk* peels powder (20g/L)*	1) Yeast extract (6 g/L) 2) Peptone from meat (30g/L) 3) Sodium acetate (8.29g/L) 4) Magnesium sulfate heptahydrate (0.2g/L) 5) Tween 80 (1.0g/L) 6) Manganese sulfate heptahydrate (0.05g/L) 7) Ammonia citrate (2g/L) 8) Disodium phosphate (2g/L) 9) *Pisang Abu* peels powder (20g/L)*

Table 3.1. List of different substances and chemicals that were used for the modification in MRS broth.

The pH was adjusted to 6.7 ± 0.2 at 25°C. The amount of *Pisang Tanduk* and *Pisang Abu* peels were divided into different concentration, 1% (2g), 2% (4g), 3% (6g) which had been determined by using estimation method. The amount were mixed separately into customized MRS broth at the rate of 20g/L and then autoclaved at 121°C for 15 minutes (Sah et al., 2016). The modified and MRS broth were aseptically inoculated with 1% of LcS (1-2 x 10^9 cfu/ml) and then incubated for 24 hours at 37°C with agitation (120rpm).

After aerobic incubation for 24 hours at temperature of 37°C, colonies of LcS were enumerated on MRS agar. To determine the net growth of LcS, colony forming unit was measured at 0 h and 24 h using a formula given at 3.6.1. Then, the number of colony forming unit was converted into log cfu. The differences of colony forming unit at 0 h and 24 h were measured.

3.6.2 Enumeration of probiotic LcS

The enumeration of probiotic LcS, was conducted by spreading 0.1 ml of the appropriate dilutions of the sample in sterile distilled water onto MRS agar. The MRS agar was then incubated at temperature of 37°C and placed at inverted position in order to prevent the accumulation of water on agars due to condensation process. After 24 hours of incubation, the bacterial colonies were counted based on the formula below.

$$\text{Colony forming unit } \left(\frac{CFU}{ml}\right) = \frac{(Number\ of\ colonies)}{(Dilution \times amount\ plated)}$$

3.6.3 Determination of final pH of broth

According to the AOAC official method 947.05 (Horwitz & Latimer, 2006), the pH of modified MRS broth samples was measured using pH 720 precision pH meter (WTW inoLab®, Weilheim, Germany).

3.6.4 Bacteria growth parameter

To determine the bacterial growth parameter, the standard plate count was used in their respective culture medium with pertinent dilutions and incubating for 24 hours at 37°C under aerobic conditions. According to Wiley et al. (2008), the equations of mean bacterial growth rate constant (k) and mean duplication time (g) were determined by formula below:

$$k = \frac{\log N_{24} - \log N_0}{\log 2 \times 10}$$

Mean duplication time (g): $$g = \frac{1}{k}$$

Where N_{24} = CFU/ml at the end of fermentation (24 hours) and N_0 = CFU/ml at the start of fermentation (0 h).

3.7 Assessment of prebiotic potential: Prebiotic Activity Score

Huebner et al., (2007) described that the prebiotic activity score can be determined by considering the growth of each bacteria during fermentation using banana peel as a carbon source as follows:

$$\textbf{Prebiotic Activity} = LAB\ \frac{\Delta N\ prebiotic}{\Delta N\ glucose}\ -\ Enteric\ \frac{\Delta N\ prebiotic}{\Delta N\ glucose}$$

Where LAB (lactic acid bacteria) refers to *Lactobacillus casei*, and Enteric is *E.coli*. Prebiotic activity reflects the ability of a given substrate to support the probiotic growth in relation to other microorganism which is called a pathogen. Substrates which obtained higher prebiotic activity score can give better result of growth bacterial population (*Lactobacillus casei*) with good cellular growth when grown on glucose (Huebner et al., 2007). Theoretically, the cellular growth of the enteric strains, *E.coli* grown on the prebiotics should be very low in relation to growth on glucose.

3.8 Statistical analysis

All of the results were expressed as means ± standard deviation values. To differentiate among all of these means, one way analysis of variance (ANOVA) with post-hoc test (Tukey HSD) was used. P- at 0.05 was regarded to be significant. Statistical analysis was conducted by using IBM SPSS Statistics 24.

CHAPTER 4

RESULTS AND DISCUSSION

4.1 *Lactobacillus casei* strain Shirota (LcS) growth curve

Before the preparation of the media began, the probiotic *Lactobacillus casei* strain Shirota (LcS) was grown in de Man Rogosa Sharpe (MRS) broth. In order to determine the number of probiotic LcS that sufficient to be fermented in the media, a growth curve of the LcS was constructed and the result of the graph was presented in Figure 4.2. Besides, the LcS growth curve in Figure 4.2 can be compared and could be fully explained by using standard bacterial population growth curve in Figure 4.1.

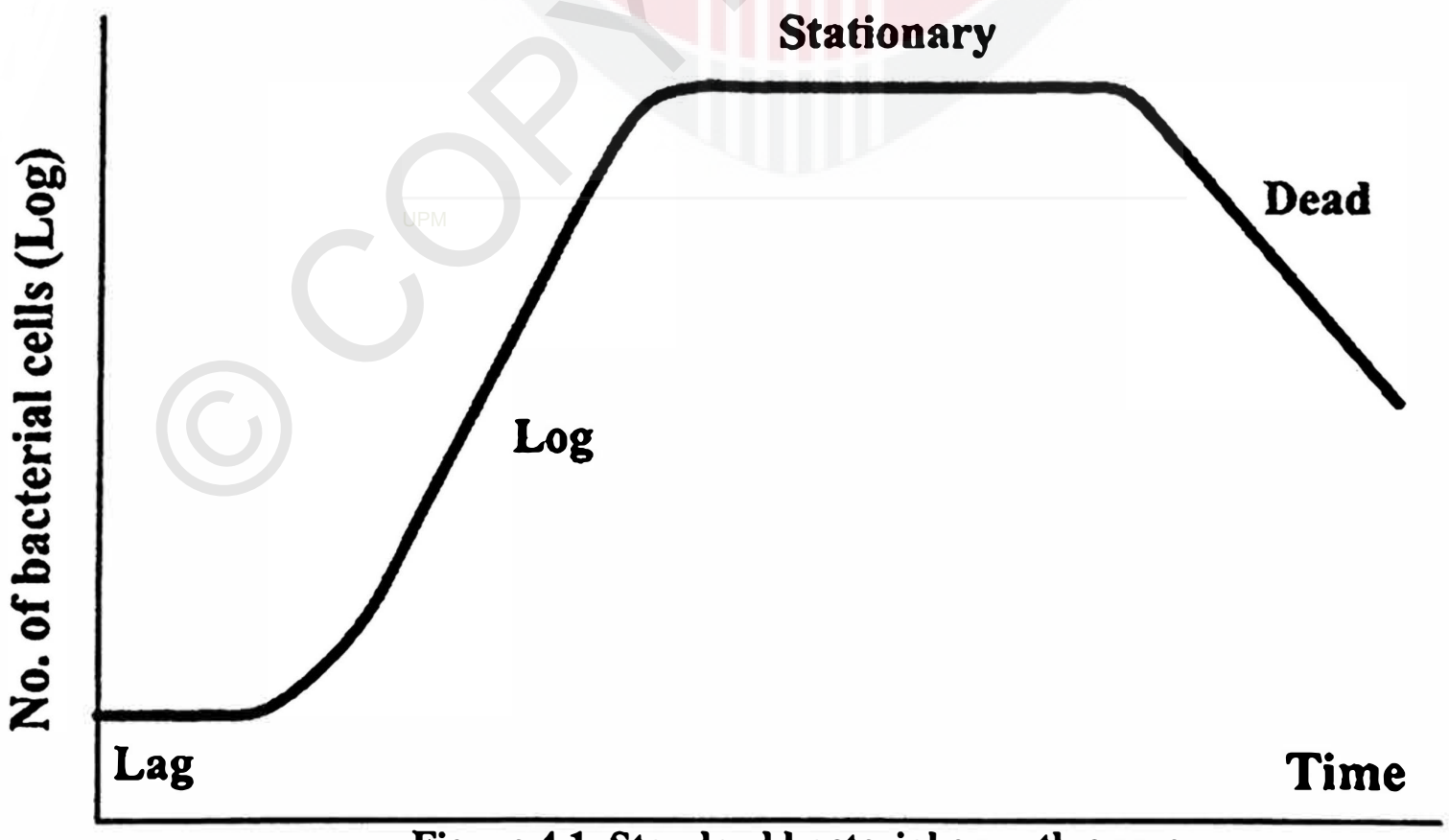

Figure 4.1. Standard bacterial growth curve

(Source: Scientific reports, 2011)

Figure 4.1 shows the standard bacterial growth curve which indicates the changes in number of live cells' size in a bacterial population over a period of time. Basically, there are four distinct phases of the growth curve; lag phase, log phase, stationary phase and dead phase. The bacteria need certain conditions such as oxygen, suitable temperature, pH and moisture availability factors which can influence the microbial growth.

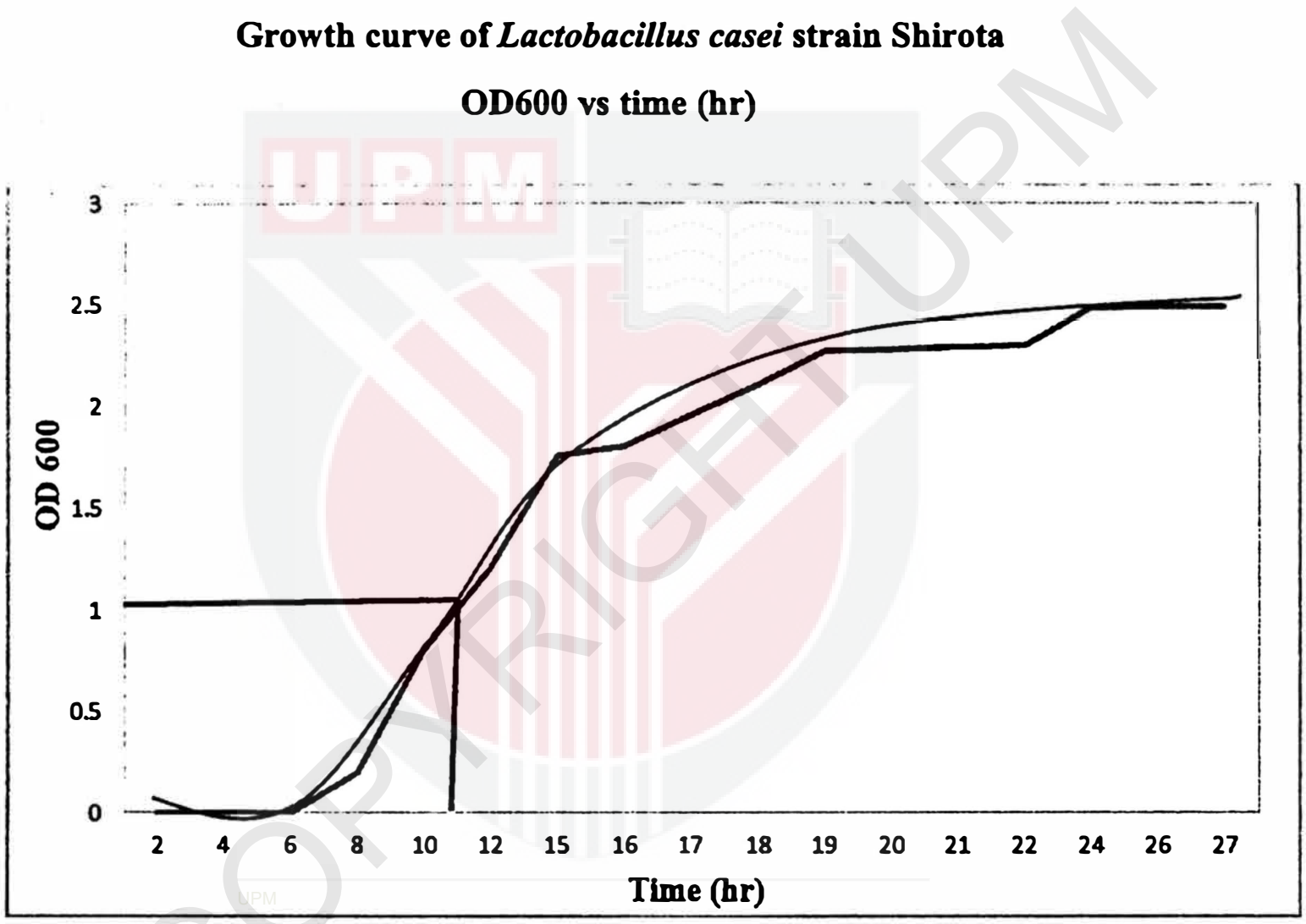

Figure 4.2 LcS growth curve

In order to determine the concentration of the probiotic LcS in this study, the absorbance of the fermented broth with LcS were measured every two hours in 24 hours of incubation. This means, the growth activity of the probiotic LcS in MRS broth was assessed by monitoring the optical density (OD) at 600nm absorbance by using spectrophotometer. The LcS growth curve as shown in Figure 4.2 was plotted by using Microsoft Excel 2013.

Figure 4.2 above shows the line graph at the beginning shows a plateau trend which obtained 0 reading OD of LcS at time between 2 hours to 6 hours. This initial phase was described as lag phase and usually, it allows the adaptation that needed for bacterial cells to begin to exploit new environmental conditions. In fact, a small group of cells are placed in a nutrient rich medium to synthesize the proteins for replication. Thus, the size of the cells increase, but no cell division occurs. Also, as the bacterium first undergoes an adaptation period during lag phase, it synthesizes certain enzymes and intermediate metabolites and a critical stage can be reached by the bacterium before multiplication takes place (T Neelesh, n.d.).

Besides, the OD values at time of 8 hours and 24 hours shows an increasing trend in Figure 4.2. This phenomena can be described that the probiotic LcS population had entered the log phase or exponential phase. In this phase, the bacteria will undergo cell division and their population number increase logarithmically. In other words, the bacteria cells double in number during a specified time period is known as the generation time. However, different organisms consist of different rate of the generation time. As an example, *E.coli* divides in every 20 minutes, hence its generation time is 20 minutes, and for *Staphylococcus aureus* it is 30 minutes, which takes longer generation time than *E.coli* (Devi A., 2011).

According to Nasrabadi et al. (2013), a single colony of bacteria required at least 16 hours growth in MRS broth in an incubator shaker at 250 revolution per minute and temperature of 37°C to achieve 10^8 CFU. This is because at time 16 hours, the LcS is in time exponential phase or log phase as they rapidly growing and in duplicating state. The metabolic activity of the cells increase because their DNA, RNA and other substances are necessary for the growth. However, in this study, it only took 11 hours for a single colony of LcS grow in the MRS broth in order to get an absorbance of 1.00 ± 0.02. In fact, an absorbance of 1.0 is equal to 10^8 (Nasrabadi et al., 2013). So, instead of 16 hours that required to achieve 10^8 CFU as established in the study by Nasrabadi et al. (2013), 11 hours was used in order to culture the probiotic bacteria so that it can be taken in the log phase for the fermentation process.

Other than that, the LcS growth curve in Figure 4.2 shows tremendously increased at time between 19 hours and 24 hours, and this explained that the bacterial population used up all the nutrients in the growth medium for their rapid multiplication as they continue to grow. This phase is called stationary phase and it also indicated that the bacterial cells grow at a constant state due to an absence of fresh nutrients in the medium and accumulation of inhibitory compounds. After that, some of the cells may continue to divide, however others begin to die. When the cells die, their growth rate eventually stop thus the bacterial population will not increase in their cells number which creates a horizontal growth curve as shown in Figure 4.2 above.

As the number of bacterial population remains constant during stationary phase, the nutrients will be depleted and the metabolic waste products will be accumulated subsequently. This can enhance the bacteria cells to move into death phase (Manisha Garg, n.d.) Due to unfavorable conditions during this phase, the cells loses its ability to reproduce when they begin to die. Then, the number of dead cells exceed the number of live cells and thus, the number of viable bacterial cells begins to be declined. In addition, the spore forming bacteria will produce endospores by resisting in this phase in order to survive long enough when exposed into an environment (Preiss et al., 2015).

4.2 LcS growth parameters

Two parameters were used in this experiment in order to assess and compare the prebiotic effects of the fermentable bacterial metabolic activities among different cultivation in the media. First parameter used was to assess the LcS growth performance including enumeration of LcS in log CFU/mL, monitoring the final pH values, analyzing the mean growth rate and mean duplication time. Accordingly, second parameter used was to assess and evaluate the prebiotic activity score. As mentioned in Chapter 3 previously, the modified MRS broths were prepared by substituting the glucose with samples of *Pisang Abu* and *Pisang Tanduk* peels powder. This study used different concentrations; 1% (2g), 2% (3g) and 3% (6g). Briefly, a single colony of probiotic LcS on MRS agar was inoculated into the modified MRS broths. Then, they were incubated in an incubator for 24 hours at temperature of 37°C. For the results of this study, they were analyzed and recorded in the subtopics 4.2.1 and 4.2.2 below for further explanation and discussion.

4.2.1 The enumeration of LcS in log CFU/mL

In general, bacteria are unicellular organisms and they can reproduce. The reproduction of bacteria can be called as microbial growth. In order to determine the rates of their microbial growth, it is necessary to enumerate the microorganisms which is to determine the microbial numbers (Bassiri, n.d.). In this experiment, the enumeration of probiotic bacteria which is LcS was determined and it involved the viable plate count method as it is the most common procedure. At this condition, it permits the microbial reproduction that lead to development of the colonies as they arise from a single cell which has undergone cell division during their stage or phase of bacterial growth.

Table 4.1: Final fermentation (24-h) of CFU/ml counts (N) for the LcS employed with different carbon sources and concentrations by using One-way ANOVA.

Strains	**Carbon sources**	***N* (log CFU/ml)**		
		Mean ± SD	***F*-value**	***p*-value**
Lactobacillus casei Strain Shirota (LcS)	*Pisang Abu* peel powder 1%	8.66 ± 0.03^{a}	26.49	<0.001
	Pisang Abu peel powder 2%	9.03 ± 0.02^{c}		
	Pisang Abu peel powder 3%	9.24 ± 0.02^{b}		
	Pisang Tanduk peel powder 1%	9.01 ± 0.02^{d}		
	Pisang Tanduk peel powder 2%	9.07 ± 0.01^{e}		
	Pisang Tanduk peel powder 3%	9.17 ± 0.02^{b}		
	Glucose	9.29 ± 0.15^{b}		
	Commercial MRS broth	9.36 ± 0.14^{ab}		

Results are expressed as mean ± standard deviation (n=8). Values with different letter in the same column are significant ($p < 0.05$) between the carbon sources in the cultured media.

Table 4.1 shows the result of the viable cell counts of LcS of commercial MRS broth and modified broth which containing glucose (MRS), *Pisang Abu* peels powder ($MRSa_1$, $MRSa_2$, $MRSa_3$) and *Pisang Tanduk* peels powder ($MRSt_1$, $MRSt_2$, $MRSt_3$) with different concentrations of 1%, 2% and 3% respectively. The result also shows that as the concentration of the peel powders increases, the viable cell counts of LcS also increases. LcS strains showed significant difference ($p = <0.001$) viable cell counts in MRS, $MRSa_1$, $MRSa_2$, $MRSa_3$, $MRSt_1$, $MRSt_2$, $MRSt_3$ and MRSc. Within the sample, the viable count was significantly different at higher concentration. Nonetheless, the concentration was not significantly different compared to glucose (9.29 ± 0.15) and MRS broths (9.36 ± 0.14). It can be postulated that at 3% concentration of banana peel powder, the growth performance of LcS was comparable to glucose and MRS broths.

Table 4.2: Final fermentation (24-h) of CFU/mL counts (N) for the LcS and other bacteria employed with different carbon sources in comparison with previous study.

Strains	Carbon sources	N (log CFU/ml)	References
		Mean ± SD	
Lactobacillus casei Strain Shirota (LcS)	*Pisang Abu* peel powder 1%	8.66 ± 0.03	
	Pisang Abu peel powder 2%	9.03 ± 0.02	
	Pisang Abu peel powder 3%	9.24 ± 0.02	
	Pisang Tanduk peel powder 1%	9.01 ± 0.02	
	Pisang Tanduk peel powder 2%	9.07 ± 0.01	
	Pisang Tanduk peel powder 3%	9.17 ± 0.02	
Pediococcus pentosaceus	Cactus pear flour	9.65 ± 0.25	(Diaz-Vela et al., 2013)
	Pineapple flour	9.56 ± 0.31	
Aerococcus viridians	Cactus pear flour	8.73 ± 0.25	(Diaz-Vela et al., 2013)
	Pineapple flour	9.33 ± 0.13	
Lactobacillus rhamnosus	Cactus pear flour	9.24 ± 0.27	(Diaz-Vela et al., 2013)
	Pineapple flour	9.38 ± 0.26	
Escherichia coli	Cactus pear flour	8.63 ± 0.24	(Diaz-Vela et al., 2013)
	Pineapple flour	8.21 ± 0.22	
Lactobacillus enterococci	Lemon peel wastes (POS)	8.92 ± 0.08	(Gómez et al., 2016)
	Sugar beet pulp (POS)	8.59 ± 0.19	
Aerococcus viridans	Grapefruit albedo flour (0.5%)	7.94	(Parra-Matadamas et al., 2015)
	Grapefruit albedo flour (1%)	8.13	
	Grapefruit albedo flour (1.5%)	8.08	
	Grapefruit peel flour (0.5%)	8.60	
	Grapefruit peel flour (1%)	8.70	
	Grapefruit peel flour (1.5%)	8.90	
	Cactus pear peel flour (0.5%)	8.80	
	Cactus pear peel flour (1%)	8.80	
	Cactus pear peel flour (1.5%)	8.60	
	Pineapple peel flour (0.5%)	7.50	
	Pineapple peel flour (1%)	8.20	
	Pineapple peel flour (1.5%)	8.00	
Pediococcus pentosaceus	Grapefruit albedo flour (0.5%)	7.98	(Parra-Matadamas et al., 2015)
	Grapefruit albedo flour (1%)	7.96	
	Grapefruit albedo flour (1.5%)	8.60	
	Grapefruit peel flour (0.5%)	8.50	
	Grapefruit peel flour (1%)	9.02	
	Grapefruit peel flour (1.5%)	9.00	
	Cactus pear peel flour (0.5%)	8.78	
	Cactus pear peel flour (1%)	8.86	
	Cactus pear peel flour (1.5%)	8.90	
	Pineapple peel flour (0.5%)	8.30	
	Pineapple peel flour (1%)	8.20	
	Pineapple peel flour (1.5%)	7.90	

Table 4.2 shows the result of the final fermentation (in 24 hours) of CFU/mL counts (*N*) for the LcS and other bacteria which employed with different carbon sources that mainly from other agro industrial by-products such as peels and pulp, in comparison with previous study from Diaz-Vela et al. (2013), Sah et al. (2016) and Gómez et al. (2016). The study conducted by Diaz-Vela et al. (2013) used agro industrial of by-products, i.e cactus pear and pineapple and converted into flour form in order to substitute the glucose as carbon source used for probiotics fermentation. Different type of microorganisms such as *Pediococcus pentosaceus, Aerococcus viridans, Lactobacillus rhamnosus* and *Escherichia coli* were used. All of those bacteria will consume the carbon sources from the cactus pear peel and pineapple peel. The final viable count (*N*) of microorganisms was significantly ($p < 0.05$) higher for *P. pentosaceus* strain and lower for *E.coli*. However, for final viable cell count by carbon source type, there was no significant difference ($p > 0.05$).

Furthermore, Table 4.2 also shows the final fermentation (in 24 hours) of CFU/mL counts (*N*) for the LcS and other bacteria employed with different carbon sources using different concentrations in comparison with previous study. In this study, different concentration of *Pisang Abu* and *Pisang Tanduk* peels powder (1%, 2% and 3%) were used and added into modified broth in order to know and compare their bacterial growth rate at different condition. According to the study conducted by Parra-Matadamas et al. (2015), the agro industrial by-products such as grapefruit albedo, grapefruit peel, cactus pear peel and pineapple peel were employed as the carbon sources. In addition, there was a similarity in terms of using different concentration of peel flour or powder between in this study and the study conducted by Parra-Matadamas et al. (2015). However, the amount of concentration were different among of these two studies. This study was used concentration of 1%, 2% and 3%

whereas other study was used concentration of 0.5%, 1.0% and 1.5% as shown in Table 4.3 above.

For *P.pentosaceus* with 1.5% concentration of grapefruit albedo as carbon source showed a higher growth as compared to glucose. At final fermentation, the cellular growth for albedo grapefruit flour concentration of 1%, 2% and 3% obtained the values of 7.98, 7.96 and 8.6 log CFU/mL respectively. For *A. viridans* growth, it had reached 7.94, 8.13 and 8.08 log CFU/mL counts for of 0.5%, 1.0% and 1.5% of albedo grapefruit flour. The higher growth profile of *P. pentosaceus* when 1.0% concentration of grapefruit peel flour was employed as carbon source obtained was 9.02 CFU/mL whereas the higher growth of *A. viridans* observed with 1.5% of grapefruit peel flour was 8.9 CFU/mL. *P. pentosaceus* and *A. viridans* presented higher growth at 0.5% and 1.0% concentration of the cactus pear peel as carbon source, as compared with glucose or higher flour fermentation (1.5%). When pineapple peel was employed as carbon source, *P. pentosaceus* presented a higher growth. However, it showed no appreciable difference due to carbon source concentration. For *A. viridans*, the cellular growth was higher with 1.0% of pineapple peel flour (8.2 log CFU/mL) as compared to glucose as carbon source (Parra-Matadamas et al., 2015).

4.2.2 The final pH of prebiotic samples

For the assessment of probiotic growth performance, the final pH of prebiotic samples were determined by measuring pH of the modified broth after the cultivation of LcS in 24 hours. The initial pH values of all modified broth were set at pH of 6.70 ± 0.2. Riggio et al. (2019) stated that the fermentation is a process in which anaerobic microbial breakdown of organic matter undergo a variety of reactions and metabolic processes. During fermentation of probiotics, short chain of fatty acids (SCFA) such as lactic acid, acetic, propionic and butyric acids are produced. According to Juarez Tomás et al. (2002), the decrease in pH of the culture medium was parallel to growth. A decrease in pH with an optimum growth conditions, indicating pH reduction is directly related to the lactobacilli growth.

Table 4.3: Final pH of modified prebiotic samples at the end of the fermentation (24 h) for the LcS employed with the different carbon sources by using One-way ANOVA.

Strains	Carbon sources	Final pH		
		Mean ± SD	*F*-value	*p*-value
Lactobacillus casei strain Shirota (LcS)	*Pisang Abu* peel powder 1%	5.69 ± 0.34^{b}	18.57	<0.001
	Pisang Abu peel powder 2%	5.53 ± 0.09^{b}		
	Pisang Abu peel powder 3%	5.44 ± 0.22^{b}		
	Pisang Tanduk peel powder 1%	5.53 ± 0.13^{b}		
	Pisang Tanduk peel powder 2%	5.34 ± 0.12^{b}		
	Pisang Tanduk peel powder 3%	5.28 ± 0.06^{b}		
	Glucose	4.56 ± 0.22^{a}		
	Commercial MRS broth	4.40 ± 0.18^{ab}		

Results are expressed as mean ± standard error of mean (n=8). Values with different letter in the same column are significant ($p < 0.05$) between the carbon sources in the cultured media.

There was no significant different of the final pH of the broth for both banana peels. However, after 24 hours of incubation, the pH was reduced. The reduction of pH could be due to the production of fermentation end-metabolites such as short chain fatty acids (SCFA). During the fermentation, the bacteria used glucose and metabolized it into end products such as SCFA. Subsequently, it can cause the medium to become acidic and reduce the pH. The modified broths were compared to glucose because perhaps, the metabolism of glucose in banana powders yielded other end metabolites that can affect the growth of microorganisms.

Table 4.4 shows the result of the final pH in 24 hours of incubation for the LcS and other bacteria employed with different carbon sources from agro industrial by-products such as apple peel, carrot bagasse, banana peel, grapefruit albedo, grapefruit peel, cactus pear peel, pineapple peel and cashew apple in comparison with previous study. Further analysis indicated that the studies conducted by Duarte et al., (2017), Hernández-Alcántara et al., (2016) and Parra-Matadamas et al., (2015) used different type of bacteria. For example, the bacteria used in those studies were *Lactobacillus casei, Pediococcus pentosaceus* and *Aerococcus viridans*. Different bacteria will utilize the glucose as their carbon sources differently. However, certain probiotic bacteria will have low metabolism, thus the production of end metabolites such as short chain fatty acids (SCFA) is low and greatly impact the pH of the broth. Other than that, the type of agro industrial by-products as the carbon sources were different among those studies and this study. For instance, the cultivation of *Lactobacillus casei* strain Shirota (LcS) were used in this study whereas cultivation of *Lactobacillus casei, Pediococcus pentosaceus* and *Aerococcus viridans* strains were used in comparison with previous study. Different carbon source will be utilized by the microorganism. In general, glucose content in by-products will be used as the major carbon source. Nonetheless, certain agro industrial by-products have low

glucose but contain high monomeric unit of CHO such as xylose that can be utilized by the microorganisms.

Table 4.4: Final pH (24-h) of prebiotic samples for the LcS and other bacteria employed with different carbon sources in comparison with previous study.

Strains	Carbon sources	Final pH	References
		Mean ± SD	
Lactobacillus casei Strain Shirota (LcS)	*Pisang Abu* peel powder 1%	5.69 ± 0.34	
	Pisang Abu peel powder 2%	5.53 ± 0.09	
	Pisang Abu peel powder 3%	5.44 ± 0.22	
	Pisang Tanduk peel powder 1%	5.53 ± 0.13	
	Pisang Tanduk peel powder 2%	5.34 ± 0.12	
	Pisang Tanduk peel powder 3%	5.28 ± 0.06	
Lactobacillus casei	Glucose	4.00 ± 0.16	(Duarte et al., 2017)
	Fructooligosaccharides	4.08 ± 0.03	
	Cashew apple powder	4.19 ± 0.07	
P. pentosaceus	Glucose	5.72 ± 0.20	(Hernández et al., 2016)
	Apple peel	5.66 ± 0.30	
	Carrot bagasse	5.15 ± 0.20	
	Banana peel	4.85 ± 0.60	
P. pentosaceus	Grapefruit albedo flour (0.5%)	6.30 ± 0.10	(Parra-Matadamas et al., 2015)
	Grapefruit albedo flour (1%)	6.10 ± 0.10	
	Grapefruit albedo flour (1.5%)	6.40 ± 0.20	
	Grapefruit peel flour (0.5%)	5.70 ± 0.30	
	Grapefruit peel flour (1%)	5.10 ± 0.40	
	Grapefruit peel flour (1.5%)	4.80 ± 0.30	
	Cactus pear peel flour (0.5%)	4.90 ± 0.20	
	Cactus pear peel flour (1%)	4.40 ± 0.10	
	Cactus pear peel flour (1.5%)	4.40 ± 0.20	
	Pineapple peel flour (0.5%)	5.90 ± 0.20	
	Pineapple peel flour (1%)	4.90 ± 0.10	
	Pineapple peel flour (1.5%)	4.40 ± 0.20	
Aerococcus viridans	Grapefruit albedo flour (0.5%)	6.05 ± 0.20	(Parra-Matadamas et al., 2015)
	Grapefruit albedo flour (1%)	6.00 ± 0.20	
	Grapefruit albedo flour (1.5%)	5.80 ± 0.20	
	Grapefruit peel flour (0.5%)	5.40 ± 0.20	
	Grapefruit peel flour (1%)	4.90 ± 0.30	
	Grapefruit peel flour (1.5%)	4.70 ± 0.20	
	Cactus pear peel flour (0.5%)	4.80 ± 0.20	
	Cactus pear peel flour (1%)	4.30 ± 0.10	
	Cactus pear peel flour (1.5%)	4.20 ± 0.10	
	Pineapple peel flour (0.5%)	5.50 ± 0.10	
	Pineapple peel flour (1%)	4.80 ± 0.10	
	Pineapple peel flour (1.5%)	4.50 ± 0.10	

4.2.3 The mean growth rate constant (k) and mean duplication time (g) of LcS

For the last parameter on LcS growth performance, mean growth rate constant (k) and mean duplication time (g) were determined according to Willey et al. (2008) equations. In general, the growth of bacterial cultures is defined as an increase in the number of bacteria in a population rather than in the size of individual cells. Kenneth (2008) stated that under favorable conditions, a growing bacterial population doubles at regular intervals. Growth is by geometric progression: 2^0, 2^1, 2^2, 2^3, until, after n divisions, the number of cells is 2^n. This is called exponential growth which is only part of the bacterial life cycle. During exponential growth, the rate at which the bacteria increases is proportional to the number of bacteria at any given time. In other words, the rate of exponential growth of a bacterial culture is expressed as generation time, also the doubling time of the bacterial population. Generation time (g) is defined as the time (t) per generation and are calculated during the exponential phase of growth.

Table 4.5: Fermentation kinetics, k (mean growth rate constant) for LcS strains employed with the different carbon sources by using One-Way ANOVA.

Strains	**Carbon sources**	**Mean growth rate constant (k) h**		
		Mean ± SD	***F*-value**	***p*-value**
Lactobacillus casei strain Shirota (LcS)	*Pisang Abu* peel powder 1%	0.13 ± 0.01	26.24	<0.001
	Pisang Abu peel powder 2%	0.25 ± 0.01		
	Pisang Abu peel powder 3%	0.32 ± 0.01		
	Pisang Tanduk peel powder 1%	0.24 ± 0.01		
	Pisang Tanduk peel powder 2%	0.26 ± 0.01		
	Pisang Tanduk peel powder 3%	0.30 ± 0.01		
	Glucose	0.34 ± 0.05		
	Commercial MRS broth	0.36 ± 0.04		

*p-value significant at <0.05 (n=8)

In this study as shown in Table 4.5, there was a significant different of mean growth rate between the samples. In general, as the concentration of banana peel powders increase, the mean growth rate also increases. This observation reflects the previous finding on the viable count (Table 4.1). Between two banana peel powders, *Pisang Abu* peel powders show a superior results than the *Pisang Tanduk* peel powders. This is due an abundant of glucose content as the carbon sources in *Pisang Abu* peel powders compared to *Pisang Tanduk*. According to Chen et al. (2007); Yeo & Liong, (2010) reported that the prebiotic compounds resulted in a slower growth of lactic acid bacteria compared with monosaccharides (hexoses), but had similar final biomass. For examples, in the fruit peel flours used, the presence of free monomeric components in the culture medium with complex substrates resulted in lower growth rate, but the final biomasses were the same. This implies that the peel flours as prebiotic ingredients were metabolized by the employed strains as carbon source present in the culture medium (Kaplan & Hutkins, 2000; Rossi et al., 2005). Several mechanisms for the use of polymeric carbohydrates, either intracellularly or extracellularly, hydrolyzing monomeric components for easy assimilation (Amaretti et al., 2006). Besides, Parra-Matadamas et al. (2015) reported the maximum specific growth rates observed with disaccharides and oligosaccharides as compared to monosaccharide, confirming differences carbohydrate utilization abilities and kinetics by different strains. As an example, there is higher growth rate when grapefruit peel flour was employed as carbon source.

Table 4.6: Mean generation time, g (mean duplication time) for LcS strains employed with the different carbon sources by using One-Way ANOVA.

Strains	Carbon sources	Mean generation time (g) h^{-1}		
		Mean ± SD	***F*-value**	***p*-value**
Lactobacillus casei strain Shirota (LcS)	*Pisang Abu* peel powder 1%	7.72 ± 0.60	80.30	<0.001
	Pisang Abu peel powder 2%	4.01 ± 0.16		
	Pisang Abu peel powder 3%	3.13 ± 0.10		
	Pisang Tanduk peel powder 1%	4.11 ± 0.10		
	Pisang Tanduk peel powder 2%	3.80 ± 0.09		
	Pisang Tanduk peel powder 3%	3.37 ± 0.07		
	Glucose	2.98 ± 0.45		
	Commercial MRS broth	2.81 ± 0.37		

**p*-value significant at <0.05 (n=8)

Table 4.6 shows the result of mean generation time for LcS strains employed with the different carbon sources. There was a significant different of mean duplication time between the samples. Based on this finding on the viable count in Table 4.1 previously, when the concentration of both banana peel powders increase, the mean generation time become lower. This shows that lower mean generation time was due to increasing in mean growth rate. The result also shows the same result as the mean growth rate in Table 4.5, which is, *Pisang Abu* peel powders show a superior results than the *Pisang Tanduk* peel powders. Higher mean growth rate and lower generation time can be described as two good parameters in carbon sources for the bacterial growth.

Table 4.7 shows the result of mean growth rate and mean duplication time for LcS and other bacteria employed with different carbon sources in comparison with previous study. Other study conducted by Hernández-Alcántara et al., (2016), agro industrial by-products such as apple peel, carrot bagasse and banana peel were used as carbon sources whereas grapefruit albedo, grapefruit peel, cactus pear peel and pineapple were used in another study that conducted by Parra-Matadamas et al., (2015). Both of these studies used different type of agro industrial by-products as carbon sources and different types of bacteria. The bacteria used were *Pediococcus pentosaceus* and *Aerococcus viridans*. Table 4.8 shows that the utilization of banana peel powders as carbon source for the growth LcS was inferior compared to others. This is because different types of bacteria and carbon sources were used when compared to previous study. Different types of bacteria used the carbon sources and produced the end-metabolites which can affect the bacteria growth. As for example, during fermentation, the bacteria used glucose and metabolized it into end products such as short chain fatty acids.

Furthermore, in comparison with the previous studies by Hernández-Alcántara et al. (2016) and Parra-Matadamas et al. (2015) as shown in Table 4.8, it also showed that different probiotic will produce different mean growth rate constant (k) and mean generation time (g). This is because different probiotic might use differently sources of carbon which from agro industrial by-products during fermentation process. Besides, the study from Parra-Matadamas et al. (2015) showed the different concentration (0.5%, 1.0% and 1.5%) of agro industrial by-products; grapefruit albedo, grapefruit peel, cactus pear peel and pineapple peel were used as the carbon sources (Parra-Matadamas et al., 2015). In other words, when compared to previous studies, there was not so much different because this study also use the different concentration (1%, 2% and 3%) of banana peels as the carbon sources. Between these two studies, it reported

the same result which is, when the concentration is higher, the mean growth rate (k) was higher and mean generation time (g) was lower.

Table 4.7: Fermentation kinetics, k (mean growth rate constant) and mean generation times (g) for LcS and other bacteria employed with different carbon sources in comparison with previous study.

Strains	Carbon sources	Growth rate constant (k) h	Mean generation time (g) h^{-1}	References
		Mean ± SD	Mean ± SD	
Lactobacillus casei Strain Shirota (LcS)	*Pisang Abu* peel powder 1%	0.13 ± 0.01	7.72±0.60	
	Pisang Abu peel powder 2%	0.25 ± 0.01	4.01±0.16	
	Pisang Abu peel powder 3%	0.32 ± 0.01	3.13±0.10	
	Pisang Tanduk peel powder 1%	0.24 ± 0.01	4.11±0.10	
	Pisang Tanduk peel powder 2%	0.26 ± 0.01	3.80±0.09	
	Pisang Tanduk peel powder 3%	0.30 ± 0.01	3.37±0.07	
Pediococcus pentosaceus	Glucose	1.40 ± 0.04	0.62±0.04	(Hernández-Alcántara et al., 2016)
	Apple peel	1.45 ± 0.04	0.65±0.04	
	Carrot bagasse	1.25 ± 0.01	0.70±0.01	
	Banana peel	1.23 ± 0.03	0.85±0.00	
Aerococcus viridans	Grapefruit albedo flour (0.5%)	1.30 ± 0.10	0.77±0.06	(Parra-Matadamas et al., 2015)
	Grapefruit albedo flour (1%)	1.32 ± 0.04	0.76±0.02	
	Grapefruit albedo flour (1.5%)	1.48 ± 0.08	0.68±0.04	
	Grapefruit peel flour (0.5%)	1.62 ± 0.07	0.62±0.02	
	Grapefruit peel flour (1%)	1.65 ± 0.04	0.61±0.02	
	Grapefruit peel flour (1.5%)	1.80 ± 0.10	0.56±0.03	
	Cactus pear peel flour (0.5%)	1.47 ± 0.07	0.62±0.02	
	Cactus pear peel flour (1%)	1.41 ± 0.10	0.61±0.02	
	Cactus pear peel flour (1.5%)	1.83 ± 0.08	0.56±0.03	
	Pineapple peel flour (0.5%)	1.09 ± 0.21	0.68±0.03	
	Pineapple peel flour (1%)	1.21 ± 0.17	0.72±0.11	
	Pineapple peel flour (1.5%)	1.15 ± 0.28	0.55±0.02	
Pediococcus pentosaceus	Grapefruit albedo flour (0.5%)	1.02 ± 0.09	0.99±0.09	(Parra-Matadamas et al., 2015)
	Grapefruit albedo flour (1%)	0.92 ± 0.16	1.11±0.19	
	Grapefruit albedo flour (1.5%)	1.59 ± 0.03	0.63±0.01	
	Grapefruit peel flour (0.5%)	1.73 ± 0.07	0.58±0.02	
	Grapefruit peel flour (1%)	1.80 ± 0.05	0.55±0.01	
	Grapefruit peel flour (1.5%)	1.66 ± 0.07	0.60±0.03	
	Cactus pear peel flour (0.5%)	1.36 ± 0.07	0.74±0.04	
	Cactus pear peel flour (1%)	1.34 ± 0.08	0.75±0.05	
	Cactus pear peel flour (1.5%)	1.64 ± 0.02	0.61±0.01	
	Pineapple peel flour (0.5%)	1.21 ± 0.04	0.83±0.03	
	Pineapple peel flour (1%)	1.31 ± 0.10	0.76±0.01	
	Pineapple peel flour (1.5%)	1.53 ± 0.11	0.66±0.04	

4.2.3 Prebiotic Potential: Prebiotic Activity Scores of LcS

Huebner et al., (2007) described the prebiotic activity score that was determined by considering the growth of each bacteria during fermentation using banana peel (*Pisang Abu* and *Pisang Tanduk*) and glucose as a carbon source. According to the study, the determination of prebiotic activity using an equation which involved the lactic acid bacteria (LcS) and enteric pathogen (*E.coli*). Prebiotic activity reflects the ability of a given substrate to support the probiotic growth in relation to other microorganisms which called a pathogen. Substrates which obtained higher prebiotic activity score can give better result of growth bacterial population (LcS) with good cellular growth when grown on glucose. Theoretically, the cellular growth of the enteric strains, *E.coli* grown on the prebiotics should be very low in relation to growth on glucose. For example, *E.coli* was used as enteric reference strain in this study and basically it would have very low growth on probiotic medium.

Table 4.8: Prebiotic activity score for LcS strains employed with the different carbon sources by using One-Way ANOVA.

Strains	**Carbon sources**	**Prebiotic activity score**		
		Mean ± SD	***F*-value**	***p*-value**
Lactobacillus casei strain Shirota (LcS)	*Pisang Abu* peel powder 1%	0.15 ± 0.02^{b}	7.14	0.001
	Pisang Abu peel powder 2%	0.12 ± 0.02^{b}		
	Pisang Abu peel powder 3%	0.07 ± 0.02^{b}		
	Pisang Tanduk peel powder 1%	0.21 ± 0.03^{a}		
	Pisang Tanduk peel powder 2%	0.07 ± 0.03^{b}		
	Pisang Tanduk peel powder 3%	0.06 ± 0.07^{b}		
	Commercial MRS broth	0.08 ± 0.10^{ab}		

Results are expressed as mean ± standard error of mean (n=7). Values with different letter in the same column are significant ($p < 0.05$) between the carbon sources in the cultured media.

As shown in Table 4.8, there was a significant difference ($p < 0.05$) for the growth of LcS on *Pisang Abu* and *Pisang Tanduk* at different concentration (1%, 2% and 3%) as carbon source and this described that banana by-products had same effect on LcS growth. However, further analysis indicated that there was no significant difference ($p > 0.05$) for the carbon sources, that is all modified broth which had the same effect on LcS except $MRSt_1$ (0.21 ± 0.03) and MRSc (0.08 ± 0.10). In other words, the results in this study were comparable with commercial MRS (MRSc), which has glucose. Thus, *Pisang Abu* and *Pisang Tanduk* peels powder can be a substituted ingredient for carbon source in order to grow the probiotic. In addition, as the concentration of banana peel powders increase, the prebiotic score become decreases. This means, low concentration (1%) of banana peel powders have to be used instead of high concentration during fermentation process in this study. This is because, if high concentration was used, the fermentation process can produce end-metabolite products which can inhibit the probiotics growth. The findings of this study was comparable with the previous study.

According to Duarte et al. (2017), Hernández-Alcántara et al. (2016) and Diaz Vela et al. (2013) show no significant difference growth of bacteria including probiotic bacteria on different carbon sources (cashew apple peel, apple peel, carrot bagasse, banana peel, cactus pear peel and pineapple peel powder). Anprung & Sangthawan (2012) stated that Lactobacilli and Bifidobacterium strains were used in order to test the prebiotic activity score because they are abundantly present in dairy foods and have good potential probiotic properties. According to Huebner et al. (2007), different bacterial strain affected to the different prebiotic activity score. This is due to differences in the metabolic capacity of related strains and utilization of prebiotics by these related bacteria. Table 4.9 shows the prebiotic activity scores for LcS and other bacteria employed with different carbon sources in comparison with previous study.

Table 4.9: Prebiotic activity scores of LcS strain and other bacteria employed with different carbon sources in comparison with previous study.

Strains	Carbon sources	Prebiotic activity scores	References
		Mean ± SD	
Lactobacillus casei Strain Shirota (LcS)	*Pisang Abu* peel powder 1%	0.15 ± 0.02	
	Pisang Abu peel powder 2%	0.12 ± 0.02	
	Pisang Abu peel powder 3%	0.07 ± 0.02	
	Pisang Tanduk peel powder 1%	0.21 ± 0.03	
	Pisang Tanduk peel powder 2%	0.07 ± 0.03	
	Pisang Tanduk peel powder 3%	0.06 ± 0.07	
Pediococcus pentosaceus	Apple peel	0.42 ± 0.04	(Hernández-Alcántara et al., 2016)
	Carrot bagasse	0.38 ± 0.07	
	Banana peel	0.28 ± 0.03	
Lactobacillus acidophilus	Cashew apple powder	0.89 ± 0.05	(Duarte et al., 2017)
	Fructooligosaccharide	0.95 ± 0.05	
Lactobacillus paracasei	Cashew apple powder	0.95 ± 0.04	(Duarte et al., 2017)
	Fructooligosaccharide	0.94 ± 0.03	
	Inulin	1.17 ± 0.04	
Pediococcus pentosaceus	Cactus pear flour	0.33 ± 0.03	(Diaz-Vela et al., 2013)
	Pineapple peel flour	0.32 ± 0.01	
Aerococcus viridans	Cactus pear flour	-0.03 ± 0.02	(Diaz-Vela et al., 2013)
	Pineapple peel flour	0.09 ± 0.02	
Lactobacillus rhamnosus	Cactus pear flour	0.19 ± 0.01	(Diaz-Vela et al., 2013)
	Pineapple peel flour	0.21 ± 0.02	

CHAPTER 5

CONCLUSION, LIMITATIONS AND RECOMMENDATIONS

5.1 Conclusion

Some main objectives of this study were determined in order to assess the prebiotic potential of banana peel agro industrial by-products on the growth of probiotic *Lactobacillus casei* strain Shirota (LcS) using an *in vitro* experimental model. The cultivation of LcS in modified broth containing banana peel powder as the sole of carbon source resulted in intense bacterial metabolic activities as observed by high viable cell counts in log CFU/mL, decreased in pH values and increase in mean growth rate constant (k) and lower mean generation time (g) besides obtaining positive effects in prebiotic properties. The modified broth containing *Pisang Abu* and *Pisang Tanduk* peels powder showed positive prebiotic scores towards LcS, indicating a desirable selective fermentable activity compared to the enteric microorganism (*E.coli*). However, in this study, as the concentration increased which is from 1% to 3%, the prebiotic activity score become lower. This is because as higher concentration was used, the fermentation process might produce the end-metabolite products such as short chain fatty acids (SCFA). The results were compared to glucose, perhaps, the metabolism of glucose in banana peel powders yielded other end metabolites that can affect the probiotics growth. These findings demonstrated that the by-products generated from the banana plant agro industrial processing might be sources of compounds which capable to promote the selective growth of probiotic LcS. The findings might stimulate the agro-industrial sector to utilize the by-products from plant, particularly from banana processing as added-value ingredients to the food industry.

5.2 Limitations

There are some limitations that were found in this study. When compared to other studies that used more than one probiotic and other microorganisms including pathogenic bacteria, this study was a lack types of strains used. Besides, only specific concentration (1%, 2% and 3%) of carbons sources used. Apart from that, there was a lack of parameter conducted such as determination of organic acid and carbohydrate consumption in the prebiotic after fermentation of the probiotic LcS. Last but not least, the glucose content in *Pisang Tanduk* and *Pisang Abu* peels powder are undefined which meant it only used estimation method by using different concentration.

5.3 Recommendations

The following recommendations have been made for the study of prebiotic potential of banana peel agro industrial by-products on the growth of probiotic. For the first recommendation, different types of probiotic should be used for future study so that different effects can be seen for the fermentation of probiotics based on type of species. Besides, different amount of carbon sources concentration which low and high amount should be incorporated in this study as for one of the recommendations. Moreover, more parameters could be added in this study such as the determination of organic acid and carbohydrates consumption. Lastly, an acid hydrolysis method must be conducted first to determine the exact glucose content of banana peels powder before the fermentation process.

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