IDENTIFICATION, GROWTH STUDY, ENCAPSULATION AND CHARACTERIZATION OF POTENTIAL PREBIOTIC AND PROBIOTIC EXTRACTED FROM SELECTED FRUIT PEELS

BEAUTY AKTER

UNIVERSITI SAINS MALAYSIA

2024

IDENTIFICATION, GROWTH STUDY, ENCAPSULATION AND CHARACTERIZATION OF POTENTIAL PREBIOTIC AND PROBIOTIC EXTRACTED FROM SELECTED FRUIT PEELS

by

BEAUTY AKTER

Thesis submitted in fulfilment of the requirements
for the degree of
Doctor of Philosophy

March 2024

ACKNOWLEDGEMENT

I would like to express our gratitude to Allah SWT for giving me opportunity and courage endlessly in finishing the PhD. journey. First and foremost, I would like to express my heartiest gratitude to my supervisor Dr. Rabeta Mohd Salleh and co-supervisors Dr. Mohamad Hafizi Abu Bakar, Assoc. Prof. Dr. Cheng Lai Hoong, Universiti Sains Malaysia. Without their valued directions, intensive support, patience, and encouragement, this work would not have been possible. I would also like to express my cordial appreciation to all the staff of Food Technology Division, School of Industrial Technology, Universiti Sains Malaysia. I would also thanks to Assoc. Prof. Dr. Husnul Azan Tajarudin including all lab stuff of bioprocess division for their enormous support.

I would like to thank from the bottom of my heart, those who have supported me during my stay at USM through their true friendship and co-operations. A special thanks to my parents and siblings. Your prayer helps me sustain thus far. At the end, I would like to express my heartiest appreciation to my beloved and co-operative husband Dr. Ferdous Hossain and our daughter Falaq Ferdous for their unconditional love, endless support, encouragement, positive motivations, and numerous sacrifices over the candidature. Last but not least, thanks to all the researchers included in the references. Their previous work and ideas guided me in this research work.

I would like to express our sincere thanks to School of Industrial Technology, Universiti Sains Malaysia which supported our project from its beginning. The financial support for the GA scheme from Universiti Sains Malaysia was gratefully acknowledged.

Beauty Akter, March 2024

TABLE OF CONTENTS

LIST OF TABLES

Page

LIST OF FIGURES

Page

LIST OF SYMBOLS

α	Alpha
^{o}C	Degree celsius
$\pm$	Plus minus
+	Positive
$\leq$	Less than or equal
%	Percent
μ	Specific growth rate
N	Number of bacteria after drying
N_0	Number of bacteria before drying
t_0	Initial time
t_1	Time each hour
X_0	Initial optical density
X_1	Optical density of each hour

LIST OF ABBREVIATIONS

ANOVA	Analysis of variance
BP	Banana peel
BPE	Banana peel extract
B. bifidum	*Bifidobacterium bifidum*
BAM	Bacteriological Analytical Manual
BPW	Buffered peptone water
CFU	Colony-forming unit
CCB	Centre for Chemical Biology
DF	Dieteary Fibre
DNS	Dinitrosalicylic acid
DNA	Deoxyribonucleic acid
EDTA	Ethylenediaminetetraacetic acid
ELSD	Evaporative Light Scattering
FAO	Food and Agriculture Organization
FOS	Fructooligosaccharide
GOS	Galactooligosaccharide
g	gram
GIT	gastrointestinal tract
GA	Gum arabic
h	hour
HPLC	High-Performance Liquid Chromatography
IN	Inulin
ITS	Internal transcribed spacer
IgA	Immunoglobulin A
ISAPP	International Scientific Association of Probiotics and Prebiotics
Lb. plantarum	*Lactobacillus plantarum*
Lb. rhamnosus	*Lactobacillus rhamnosus*
MRS	De Man, Rogosa and Sharpe
M. caribbica	*Meyerozyma caribbica*
MOS	Mannan-oligosaccharides
μL	microlitre

μm	micrometer
mBar	millibar
min	minute
mg	milligram
mL	millilitre
mm	millimeter
mM	millimolar
nm	nanometer
OD	Optical density
PCR	Polymerase chain reaction
PP	Pineapple peel
PPE	Pineapple peel extract
POS	Pectic oligosaccharides
RI	Refractive Index
rpm	rotation per minute
rRNA	Ribosomal ribonucleic acid
SEM	Scanning electron microscope
SCFAs	Short-chain fatty acids
TOS	trans-galacto-oligosaccharides
USM	University Sains Malaysia
UV	Ultraviolet
WP	Watermelon peel
WPE	Watermelon peel extract
WHO	World Health Organization
XOS	Xylooligosaccharide

LIST OF APPENDICES

PENGENALPASTIAN, KAJIAN PERTUMBUHAN, ENKAPSULASI DAN PENCIRIAN POTENSI PREBIOTIK DAN PROBIOTIK YANG DIEKSTRAK DARIPADA KULIT BUAH TERPILIH

ABSTRAK

Kajian ini memberi tumpuan kepada penggunaan sisa untuk sumber semulajadi prebiotik dan probiotik yang berpotensi secara ekonomi dan alternatif. Selain itu, kajian juga melibatkan pembungkusan biopolimerik berasaskan prebiotik untuk membangunkan serbuk sinbiotik bagi meningkatkan kelangsungan hidup *Lactobacillus plantarum* CAM 4 di bawah keadaan gastrointestinal dan penyimpanan. Fasa 1 kajian bertujuan untuk menilai potensi prebiotik kulit tembikai, kulit nanas, dan kulit pisang dengan menganalisis kesan kulit-kulit tersebut terhadap kadar pertumbuhan probiotik. Untuk menilai keboleh hidupan sel probiotik, pelbagai strain termasuk *Lb. rhamnosus* JCM 1136, *Lb. plantarum* CAM 4, dan Bifidobacterium bifidum JCM 1255 digunakan. Pertumbuhan *Lb. plantarum* CAM 4 dan *Lb. rhamnosus* JCM 1136 dalam ekstrak kulit nanas (PPE) menunjukkan nilai yang lebih tinggi secara signifikan pada 8.27 dan 7.53 log CFU/mL masing-masing ($p < 0.05$). Kandungan polisakarida yang tidak boleh dicerna tertinggi diperhatikan dalam PPE dengan 351.13 mg/g berbanding dengan sampel lain. Ekstrak kulit nanas dan kulit pisang menunjukkan kadar hidrolisis tertinggi pada 9.21 dan 8.74%, serta 32.95 dan 30.63%, masing-masing apabila dikenakan kepada jus gastrik dan rawatan α-amilase. Kajian ini menyoroti potensi kulit tembikai, kulit nanas, dan kulit pisang sebagai prebiotik yang berkesan untuk menggalakkan pertumbuhan mikrob yang baik. Fasa 2, dimulakan dengan mengasingkan, mengenal pasti, dan menilai pertumbuhan probiotik yang berpotensi yang diperoleh daripada kulit nanas, kulit tembikai, dan kulit pisang.

Sampel-sampel ini mengalami pemencilan, pengenalpastian, dan analisis urutan gen. Berdasarkan analisis ciri koloni, morfologi sel, dan urutan, isolat dikenalpasti sebagai strain yis, iaitu *Meyerozyma caribbica* MT251155 dari kulit nanas dan *Candida orthopsilosis* MG373564 dari kulit tembikai dan kulit pisang. Kajian pertumbuhan dijalankan di bawah keadaan penapaian 30°C dan 150 rpm untuk tempoh 24 jam. Secara luar biasa, *M. caribbica* MT251155 menunjukkan pengeluaran biomassa tertinggi, mencapai 2.32 g/L. Dari segi kadar pertumbuhan spesifik, tidak ada perbezaan yang signifikan ($p > 0.05$) antara isolat dari sampel yang dipilih. Sel mengalami pertumbuhan secara eksponen semasa 12 jam pertama penapaian dan seterusnya sel berkembang pada kadar yang sangat rendah sehingga akhir tempoh penapaian. *M. caribbica* MT251155 yang diasingkan dari kulit nanas termasuk dalam kumpulan yis probiotik yang berpotensi, yang merupakan calon yang baik untuk kajian lanjut bagi menentukan sifat probiotiknya. Semasa fasa 3, serbuk sinbiotik dibangunkan untuk membungkus bakteria probiotik menggunakan matriks prebiotik sebagai dinding perlindungan. Proses mikroperisian melibatkan penyejatan beku *Lb. plantarum* CAM 4 dengan gabungan matriks yang berbeza: ekstrak kulit nanas (PPE), inulin (IN), ekstrak kulit nanas dengan gum arabic (PPE+GA), dan inulin dengan gam arab (IN+GA). Sifat fizikal sel mikropartikel, keboleh hidupan, dan ketahanan terhadap keadaan gastrointestinal dinilai. Imej mikroskopik serbuk sinbiotik menunjukkan kapsul yang utuh tanpa retakan atau kerosakan, menunjukkan kesan perlindungan positif pada sistem dinding. Semua mikropartikel menunjukkan keberkesanan enkapsulasi yang tinggi (88.43 hingga 91.47%), dan kadar kelangsungan hidup sel yang dikapsul adalah lebih tinggi secara signifikan ($p < 0.05$) daripada sel bebas apabila terdedah kepada keadaan asid dan garam hempedu. Kapsul PPE+GA menunjukkan kesan perlindungan terbaik, dengan pengurangan log minimum 3.71

CFU/mL dalam keadaan asid dan 3.91 CFU/mL dalam keadaan garam hempedu. Selain itu, pemilihan bahan pelapis secara signifikan mempengaruhi kelangsungan hidup *Lb. plantarum* CAM 4, mengekalkan kelangsungan hidup sehingga 7 log CFU/mL walaupun selepas tempoh penyimpanan 45 hari. Ekstrak kulit nanas menunjukkan hasil yang paling besar mengandungi kandungan polisakarida yang tidak boleh dicerna dan bukan kanji tertinggi, ketahanan terhadap pencernaan enzim asid, dan kesan pertumbuhan yang baik pada probiotik. Selain itu, serbuk sinbiotik *Lb. plantarum* CAM 4 yang dikapsul dengan ekstrak kulit nanas yang mengandungi gam arab menunjukkan kelangsungan hidup yang lebih baik di bawah keadaan asid, garam hempedu, dan tempoh penyimpanan. Kombinasi bahan dinding yang berbeza memberikan peluang untuk membangunkan mikropartikel dengan struktur dan perlindungan yang lebih baik. Sisa kulit nanas boleh dimanfaatkan sebagai sumber probiotik berasaskan yis yang berpotensi dan boleh disertakan dalam pelbagai produk makanan sebagai bahan pembungkusan yang berkesan bagi probiotik.

IDENTIFICATION, GROWTH STUDY, ENCAPSULATION AND CHARACTERIZATION OF POTENTIAL PREBIOTIC AND PROBIOTIC EXTRACTED FROM SELECTED FRUIT PEELS

ABSTRACT

This study focused on waste utilization for an economical and alternative natural source of potential prebiotic and probiotic production. Moreover, prebiotic-based biopolymeric encapsulation was also conducted to develop synbiotic powder for improved survival of *Lactobacillus plantarum* CAM 4 under gastrointestinal and storage conditions. Phase 1 of the study aimed to assess the prebiotic potential of watermelon rind/peel, pineapple, and banana peels by analysing their impact on the growth rate of probiotics. To evaluate the viability of the probiotic cells, different strains, including *Lb. rhamnosus* JCM 1136, Lb. plantarum CAM 4, and *Bifidobacterium bifidum* JCM 1255, were employed. Notably, the growth of *Lb. plantarum* CAM 4 and *Lb. rhamnosus* JCM 1136 in pineapple peel extract (PPE) exhibited significantly higher values of 8.27 and 7.53 log CFU/mL, respectively ($p<0.05$). The maximum amount of indigestible polysaccharides was observed in PPE containing 351.13 mg/g compared to other samples. Pineapple and banana peel extracts displayed the highest hydrolysis rates of 9.21 and 8.74%, as well as 32.95 and 30.63%, respectively, when subjected to gastric juice and α-amylase treatment. This study highlights the potential of watermelon rind, pineapple and banana peel extracts as effective prebiotics for promoting the growth of beneficial microbes. Phase 2, was initiated to isolate, identify, and evaluate the growth of potential probiotics obtained from pineapple peel (PP), watermelon peel (WP), and banana peel (BP). The samples underwent isolation, identification, and gene sequencing analysis. Based on an

analysis of colony characteristics, cell morphology, and sequencing, the isolates were detected as yeast strains namely *Meyerozyma caribbica* MT251155 from pineapple peel and *Candida orthopsilosis* MG373564 from watermelon rind and banana peel. Growth studies were conducted under incubation conditions of 30°C and 150 rpm for a duration of 24 hour (h). Remarkably, *M. caribbica* MT251155 exhibited the highest biomass production, reaching 2.32 g/L. In terms of specific growth rate, there was no significant difference ($p > 0.05$) between the isolates from the selected samples. The cells grew exponentially during the first 12 hr of incubation and thereafter cells grew at a very low rate until the end of the incubation period. *M. caribbica* MT251155 isolated from pineapple peel belongs to the group of potential yeast probiotics which is a good candidate for further studies to determine its probiotic properties. During phase 3, a synbiotic powder was developed to encapsulate probiotic bacteria using prebiotic matrixes as protective walls. The microencapsulation process involved freeze-drying *Lb. plantarum* CAM 4 with different matrix combinations: pineapple peel extract (PPE), inulin (IN), pineapple peel extract with gum arabic (PPE+GA), and inulin with gum arabic (IN+GA). The encapsulated cells' physical properties, viability, and resistance to gastrointestinal conditions were evaluated. Microscopic images of the synbiotic powder revealed intact capsules without cracks or damages, indicating a positive protective effect on the wall system. All microparticles demonstrated high encapsulation efficiency (88.43 to 91.47%), and the survival rate of encapsulated cells was significantly higher ($p<0.05$) than that of free cells when exposed to acidic and bile salt conditions. The PPE+GA capsules exhibited the best protective effect, with a minimal log reduction of 3.71 CFU/mL in acidic conditions and 3.91 CFU/mL in bile salt conditions. Furthermore, the choice of coating materials significantly influenced the survival of *Lb. plantarum* CAM 4, maintaining a viability of up to 7 log CFU/mL

even after 45 days of storage periods. Pineapple peel extract shows the most considerable results containing the highest indigestible & non-starch polysaccharide contents, excellent resistance to acid enzymatic digestion, and improved growth effect on probiotics. In addition, the synbiotic powder of *Lb. plantarum* CAM 4 encapsulated with pineapple peel extract containing gum arabic showed improved viability under acidic, bile salt, and storage period. The different combinations of wall materials provided an opportunity to develop microparticles with better structure and protection. Pineapple peel waste can be utilized as a potential source of yeast-based probiotics and can be incorporated into different food products as an effective encapsulation material for probiotics.

CHAPTER 1

INTRODUCTION

1.1 Research Background

The most commonly used horticultural crops are fruits and vegetables among other goods which can consume as fresh, processed, or minimally processed form due to nutritious components. Due to the changing pattern of diet and increasing population, consumer demand for fruits has increased drastically (Sagar et al., 2018). However, it has been estimated that approximately one-third of the global food supply is wasted or lost annually, amounting to nearly 1.3 billion tons of food (Irfanoglu et al., 2014). Actually, losses and wastage are high (reaching up to 60%) in fruit and vegetable processing, which become a critical issue for human nutrition and habitat (Sagar et al., 2018). Scarcely a small quantity of these is put to use as fertiliser and pet food (Hernández et al., 2016). Moreover, these by-product decrements are not only the losses of food products, but also indicate the losses of other assets, i.e., energy, field, water, compost chemicals, and manpower (Sagar et al., 2018).

The nutritional and functional characteristics of fruit byproducts make them a viable alternative source of functional compounds. Fruit peels, in particular, have been identified as containing various valuable and functional compounds, including polysaccharides, dietary fibres, antioxidants, and phenolic components (Zahid et al., 2021). The economic viability of recovering these highly nutritious and valuable components is significant, as it can serve as cost-effective sources of potential prebiotics and other bioactive compounds that offer various benefits to the human body. Moreover, these components hold potential for application in the food and pharmaceutical sectors (Bharat & Sahoo, 2016a; Šelo et al., 2021). For example, watermelon (*Citrullus lanatus*), pineapple (*Ananas comosus*), and banana (*Musa*

sapientum) fruit peels have gained significant usage as functional food ingredients across various industrial formulations, including dairy and bakery products. (Hao et al., 2021; Van et al., 2021; Zahid et al., 2021).

Additionally, fruit by-products are recognized for substantial content of dietary fibre, which can range from 40 to 90% of dry weight. The consumption of these fibres has been linked to a decreased risk of specific cardiovascular and cancer-related diseases (Garcia et al., 2018a). Moreover, the dietary fibre found in fruit by-products holds promising potential as prebiotics, facilitating the enhancement of growth, viability, and metabolic functions of beneficial microorganisms. Notably, probiotic bacteria, such as *Lactobacillus spp.* and *Bifidobacterium spp.*, which have been extensively studied, can benefit from the utilization of these prebiotics (Hossain et al., 2017). Pineapple peel can be used as a natural source of dietary fibre containing a large amount of carbohydrates, cellulose, and hemicellulose. Usually, nondigestible oligosaccharides are utilized as prebiotics which is directly linked to gut microflora modification and enhance the stability of probiotics by controlling the proliferation of pathogens (Patel et al., 2020).

Generally, bacteria and yeasts are considered probiotics added to various food products. The maintenance of the survival rate of probiotics must be established during the processing, and storage until reaching the target area (Azam et al., 2020). The viability of these probiotics is influenced by various environmental stress conditions, including temperature variations, oxygen levels, acidity levels, and storage conditions (Rascón et al., 2018). The synergistic effect of probiotic and prebiotic is known as synbiotic. The current definition of synbiotics are described as “A mixture consisting of live microbes and prebiotics selectively used by host microorganisms, that presents a health benefit on the host” (Dahiya & Nigam, 2022).

The process of microencapsulation of probiotics involves enclosing these beneficial microbes within protective materials, which can significantly improve their survival and stability throughout various stages until they reach the gut. This technique is widely employed in the field of food and pharmaceutical industries to enhance the effectiveness of probiotic delivery systems (Chan & Liu, 2022). By combining wall materials, such as polymers and oligosaccharides, it becomes possible to address the potential limitations of each component. Notably, one of the significant advantages of prebiotics is their ability to form gels, which surpasses the crystallization of fats. This gel-forming ability enhances the viability of probiotics while concurrently functioning as prebiotics (Pereira et al., 2018b). Moreover, oligosaccharides are capable to reduce the pore size of the encapsulating particles and increase the stability of the beneficial bacteria. On the other hand, coating materials such as gum arabic and sodium alginate can help to reduce the stickiness occurred by sugar content (Silva et al., 2018). In summary, microencapsulation of probiotics involves a thoughtful selection and combination of materials to provide optimal protection, stability, and functionality for the encapsulated beneficial microbes. This approach allows for the enhancement of probiotic viability and delivery to the gut, ultimately improving their potential health benefits.

Despite the existence of numerous studies demonstrating the positive impacts of prebiotics on human health, there is currently a lack of information regarding the specific prebiotic components present in fruit peels and their comprehensive investigation in conjunction with lactic acid bacteria. Therefore, it is imperative to conduct further research focusing on the identification of new strains for synbiotic production, clinical trials, and safety assessment to obtain accurate and reliable evidence.

1.2 Problem Statement

The unconsumed parts of fruit are known as by-products which are simply discarded for multiple reasons, i.e., inadequate handling operations and morphological characteristics of the product. The quantity of fruit waste changes based on product and morphological materials, including peels, pulp, seeds, kernel, pomace, and roots (Sagar et al., 2018). Fruit peels often contain different types of valuable compounds such as dietary fibre, phenolic compounds, and antioxidants which can serve as substrates for the growth of beneficial microbes (Ruiz et al., 2021a). However, there is a lack of comprehensive research on utilization, identifying, studying the application, and characterizing these potential components derived from fruit peels. Addressing this gap is crucial for understanding the potential health benefits and developing new value-added products for these extracts. By conducting a systematic investigation, can unlock the potential of fruit peel-derived synbiotics as functional properties in the food and medicine industries, promoting sustainable and value-added utilization of agricultural byproducts. The problem lies in the underutilization of fruit peel and the limited knowledge regarding their suitability as a source of probiotics. Despite the increasing demand for probiotic products, current sources are primarily derived from dairy or are synthetically produced (Iriondo & Miguel, 2018). The exploration of fruit peel as an alternative source of probiotics offers several advantages, including sustainability, cost-effectiveness, and potential allergen-free options for individuals with dairy intolerances (Iriondo & Miguel, 2018). However, several challenges need to be addressed including identifying and isolating specific strains, determining their viability, and evaluating their potential health benefits. Probiotics failed in targeted delivery under different stress factors during processing, storage, and digestion. Pre-treatment, selective pressure treatment, and genetic modifications can improve the stability.

Recently, growing interest has been increased in the development of functional foods that provide health benefits beyond basic nutrition. Synbiotics, which combine probiotics (beneficial live microorganisms) with prebiotics (substances that selectively increase the growth and function of these microorganisms), have gained considerable attention for their potential to promote gut health and overall well-being (Iriondo & Miguel, 2018). Probiotic bacteria need to be protected against different environmental stress conditions and the negative sensory impacts of addition into foods (Moayyedi et al., 2018). Stressful treatment may alter the potential of probiotics and are not well accepted for food applications. Microencapsulation can increase the protective effects against adverse conditions such as simulated gastric conditions, pH level, oxidation, and enzymatic degradation. Additionally, the encapsulation matrix helps to increase the viability of the core material during processing within the gastrointestinal tract (GIT). Therefore, the incorporation of biopolymer is crucial to improve the shelf-life throughout the encapsulation process and the storage period. The widely used polymer matrixes are mainly polysaccharides, different types of gum, proteins and their mixtures during the encapsulation of probiotic cell (Gul & Atalar, 2019). Changes in the structural and physical state of probiotic cell can occur during different process. The incorporation of biopolymers, specifically polysaccharides, gums, proteins, and their mixtures, is highlighted as a critical strategy to enhance the shelf-life of probiotics during both the encapsulation process and subsequent storage. The choice of biopolymer matrices is a key consideration in optimizing the effectiveness and stability of encapsulated probiotic products.

Addressing these research gaps will contribute to the development of functional components extracted from fruit peels that have the potential to enhance gut health and overall well-being. By understanding the encapsulation efficiency, survival rate and

characterization of the microparticles can unlock their full potential as new functional products in the food, medicine, and nutraceutical industries. Additionally, this research promotes the sustainable utilization of fruit peels, which are often discarded as waste, leading to a more environmentally friendly and economically viable approach in the agro-food sector. Hence, this study was conducted to develop synbiotic powder with prebiotic matrix extracted from pineapple peel using freeze drying encapsulation. The chapters of the thesis will represent the phases of the study conducted.

1.3 Objectives

1.3.1 Main objective

The main objective of this research is to develop the potential synbiotic extracted from selected fruit peels using freeze drying encapsulation.

1.3.2 Specific objectives

- To determine the potential prebiotic from watermelon rind (*Citrullus lanatus*), pineapple (*Ananas comosus*), and banana (*Musa sapientum*) peels including the effect on the growth of probiotic.
- To identify the potential probiotic isolated from the selected fruit peels and studying growth rate.
- To study the viability of encapsulated *Lb. plantarum* CAM 4 under acidic and bile salt conditions.
- To examine the physiochemical properties and storage stability of the microparticles.

CHAPTER 2

LITERATURE REVIEW

Biologically active compounds and phenolic components are present in peels, seeds, and pomace of fruits. The concentrations of these components present in the peels and rind/pomace of many fruits such as watermelon, banana, pineapple, dragon fruit, berries, and mangoes are considerably higher than those found in their edible parts (Wadhwa et al., 2016). This finding suggested that these wastes have the potential for isolating bioactive compounds.

Table 2.1 represents the applications of food by-product as sustainable ingredients. Studies have shown that the pomace of orange, raspberry, pear, watermelon, peach, banana, apple, cherry, durian seeds, date pits and mango peels are used as functional ingredients in processed food because of their pectin carotenoids and bound antioxidant contents (Wadhwa et al., 2016).

Table 2.1 Applications of food by-products as sustainable ingredients.

Byproduct	**Dose**	**Function**	**Bioactive compounds**	**References**
Chestnut waste flour	2%	Promote health	Source of phenolic compound and probiotic	(Ozcan et al., 2016)
Peel of banana	1%	Promote health	Source of fibre and probiotic and prebiotic	(Espírito et al., 2012)
Powder made by pineapple peel	1%	Promote health	Act as prebiotic	(Iriondo et al., 2018)
Peel of Passion fruit	0.7%	Promote health	Rich source of fibre	(Espírito et al., 2012)

Grape peel flour	0.167-1 g/100 g	Promote health	Source of polyphenol	(Karnopp et al., 2017)
Date waste	0.5, 1, and 2 ratios of dry powder/date syrup	Technological and health-promoting benefit	Source of phenolic compound	(Jridi et al., 2015)
Almond skin	100 to 400 mg/L	Technological benefit	Source of antioxidant	(Nadeem et al., 2014)
Pomegranate skin	100 mL/25 g	Technological benefit	Antioxidant and antimicrobial activities	(Shan et al., 2011)

This table provides an overview of various food by-products and their applications as sustainable ingredients, including the recommended dosage, primary function, bioactive compounds, and relevant references. According to (Hernández et al., 2016), fruit (e.g., banana, apple) peels and carrot pulp residue have been found to be good sources of fibre and antioxidants which functioning as prebiotics for the growth of bacteria. . This compilation underscores the diverse applications of food by-products, ranging from promoting health through the supply of beneficial compounds to providing technological advantages in various food processes. The dosage, function, and bioactive compounds highlighted in the table offer valuable insights for incorporating these sustainable ingredients into food production and formulation. The referenced studies provide further details and scientific evidence supporting the utilization of these by-products in the food industry.

Table 2.2 demonstrated the amount of edible part and the by-product of different fruits produced during processing in the food industry (Barbulova et al., 2015; Goni & Hernandez, 2012). This table provides insights into the quantities of by-products generated during the processing of various fruits. It details the sources, specific by-products, and the percentage distribution of edible and non-edible parts. Pineapple by-product can be utilised as alternative cheapest source for probiotic cultivation in

comparison to expensive MRS (De Man, Rogosa and Sharpe agar) medium. Moreover, after fermentation, these waste recoveries can develop the overall economic processing unit in a country (Pyar et al., 2014).

Table 2.2 Quantities of some fruit by-product produced in food processing industries.

Sources	By-Products	Edible Part
Apple	Pulp, seed core (11%)	89%
Banana	Peel (above 30%)	70%
Watermelon	Peel (30 –40%)	70–60%
Mandarin	Peels (16%)	84%
Mango	Seeds, peels, unusable pulp (42%)	58%
Citrus fruits	Peel (66%)	44%
Papaya	Seeds, peels, unusable pulp (47%)	53%
Passion fruit	Rind, seeds (75%)	25%
Pineapple	Core, peels, upper part (52%)	48%
Jackfruit	Peel, Seeds (50 –70%)	30–50%
Grapes	Peel, seeds, stalk (20%)	80%
Durian	Skin, seeds (60 -70%)	30 – 40%
Dragon fruit	Skin, seeds (30 -45%)	55 -70%

Source: (Barbulova et al., 2015; Fabani et al., 2021; Goni & Hernandez, 2012; Sagar et al., 2018).

This comprehensive table highlights the significant quantities of by-products generated from fruit processing, showcasing the percentages of edible and non-edible parts. The data is crucial for understanding the potential for by-product utilization in various industries, promoting sustainability and reducing waste. The information presented here can guide strategies for optimizing resource use in the food processing sector and foster the development of innovative solutions for by-product management.

2.1 Watermelon (*Citrullus lanatus*)

Watermelon (*Citrullus lanatus*) is a refreshing fruit with juicy red flesh and a thick green rind. Originating from Africa, it is renowned for its high-water content, nutritional benefits, and culinary versatility. Whether eaten on its own or in various dishes, watermelon is a delightful and hydrating treat.

2.1.1 Watermelon peel

Malaysia's watermelon production in 2017 was 172,275.36 metric tons (Department of Agriculture of Malaysia, 2017). The byproducts of watermelon consist with three main parts namely, rind/peel, seed, and flesh. Roughly 30% of the entire weight of a watermelon is made up of rinds, and Malaysia discards approximately 36 million tonnes of this by-product annually. Because of limited research work on utilisation of watermelon peel to new value added food, it is still considered as waste since it possesses no commercial value (Jawad et al., 2018). Watermelon has potential therapeutic effects due to the high content of flavonoids, glycoside, phenol, alkaloids and saponins in the fruit (Koocheki et al., 2007; Saad et al., 2020). Particularly, the antioxidant properties of citrulline in watermelon rind helps to protect humans from free radical damage. The waste from watermelon rind contains pectin, cellulose, proteins, and citrulline. These polymers are considered to have large amount of functional groups for example, hydroxyl, carboxyl, and amino groups, play essential roles in various biochemical processes, including antioxidant activity. The ability of these functional groups to scavenge free radicals and their potential protective effects against diseases make watermelon peel waste an interesting source for functional ingredients with health-promoting properties (Hao et al., 2021). Watermelon rind is purported to possess properties that can promote relaxation of blood vessels and potentially contribute to the management of cancer, cardiovascular diseases, as well as addressing erectile

dysfunction. Recent investigations have indicated that the incorporation of watermelon rind powder into bakery products, in conjunction with flour, has been employed in the preparation of cake batter (Al-Sayed & Ahmed, 2013).

2.2 Pineapple (*Ananas comosus*)

Pineapple (*Ananas comosus*) is a tropical fruit renowned for its sweet taste and unique texture. With a spiky exterior and juicy yellow flesh, it is packed with vitamin C, bromelain enzymes, and antioxidants. Originally from South America, pineapple is a versatile ingredient enjoyed in various culinary creations worldwide.

2.2.1 Pineapple Peel

In Malaysia, pineapple uses 6.3% agro-food area with an approximate production of 340,721.95 metric tons (Department of Agriculture, 2017). During pineapple processing, the stem, crown, core, and peel are extracted and disposed of as waste. Up to 50% of the entire fruit weight is discarded as waste in the canning process. It is approximated that over 150,000 kg of pineapple waste is generated annually in Malaysia . Pineapple peel, scientifically known as *Ananas comosus* peel, refers to the outer skin of the pineapple fruit. Often discarded, it contains valuable nutrients and enzymes. With potential health benefits, pineapple peel can be utilised in culinary endeavors, herbal teas, and natural remedies, offering a sustainable and resourceful approach. Pineapple by-product shows prebiotic characteristics which can be applied commercially in the formulations of new functional food (Sah et al., 2016b). According to (Pyar et al., 2014), pineapple waste is a possible source of carbon and other nutrition for the growth of lactic acid by microbial systems. During canning operation, around 30% of pineapples waste is produced which can be responsible for the hostile environment if not used properly due to low protein content, high amount of carbohydrates, and fibres

(Nadzirah, 2013; Pyar et al., 2014). Therefore, this fruit by-product could be the cheapest alternative nutrition growth source for probiotics.

2.3 Banana (Musa sapientum)

Banana (*Musa sapientum*) is a widely consumed fruit known for its curved shape and yellow color when ripe. Belonging to the Musaceae family, bananas are packed with essential nutrients like potassium, vitamin C, and dietary fibre. Their versatility and natural sweetness make them a favourite ingredient in desserts, smoothies, and healthy snacks.

2.3.1 Banana Peel

The total production of bananas in Malaysia was 350,492.59 metric tons in 2017 (Department of Agriculture, 2017). Research findings showed that peel fractions of bananas contain higher antioxidants and phenolic compounds than that of in their pulp (González et al., 2010). The primary antioxidant constituents present in banana peels include ascorbic acid, phenolic compounds, tocopherol, dopamine, beta carotene, and gallocatechol (Bankar & Kumar, 2010). Furthermore, due to its high antioxidant content, bananas are considered a viable option as a functional food in the prevention of cancer and heart disease (Ibrahim et al., 2017).

2.4 Probiotics

The term probiotic was explained by a Russian scientist namely Elie Metchnikoff for the first time, suggesting the possibility of replacing bad microbes with beneficial ones at the early stage of the 20th century (Park et al., 2008). Metchnikoff also mentioned about the consumption of fermented milk would "seed" the bacteria and helps to reduce the outgrowth of proteolytic bacteria. Probiotic is live microorganisms that promote the intestinal microbial status and provide the beneficial effect to the host

(Ohshima et al., 2016). “Food and Agriculture Organization” (FAO) and the “World Health Organization” (WHO) defined probiotics as “live microorganisms when administered in adequate amounts confer a health benefit on the host” (FAO & WHO, 2001). The mostly used probiotics genus is *Lactobacillus, Bifidobacterium, Saccharomyces, Enterococcus, Leuconostoc*, and *Bacillus* (Park et al., 2008). There are few characteristics of an ideal probiotic. First of all, the culture should be gram-positive, exhibit a protective effect on the host health, resistant to acid and bile salt, with a minimum colony-forming unit (CFU) of 30×10^9 per gram. Secondly, the culture should be strain-specific with longer survival rates and multiply rapidly in the digestive area. Lastly, they should be nontoxic to the host with the firm and faster adhesiveness ability of a microorganism (Park et al., 2008).

Yeast probiotics, exemplified by *Saccharomyces boulardii*, play a crucial role in maintaining gut health. Unlike traditional bacterial probiotics, yeast probiotics are resilient in the face of antibiotics, making them an excellent choice for individuals undergoing antibiotic therapy. They contribute to digestive balance by promoting the growth of beneficial bacteria, aiding in nutrient absorption, and mitigating issues like diarrhea. Additionally, yeast probiotics bolster the immune system, offering a comprehensive approach to overall well-being. Their versatility and resilience make them a valuable supplement for those seeking to support and optimize their gut microbiota, fostering a harmonious environment for digestive and immune functions.

2.4.1 *Lactobacillus plantarum*

Lactobacillus plantarum, a prevalent species within the *Lactiplantibacillus* genus, is commonly encountered in various fermented food products and anaerobic plant material (Zheng et al., 2020). This gram-positive bacterium exhibits a bacilli shape (Landete et al., 2010). Initially isolated from saliva, *Lb. plantarum* earned its distinction

as a nomadic organism due to its capacity for temporary colonization in plants, insect intestines, and the intestinal tracts of vertebrate animals (Duar et al., 2017; Martino et al., 2016). With one of the most extensive genomes observed among lactic acid bacteria, *Lb. plantarum* displays remarkable flexibility and versatility as a species. It is capable of growth within a pH range of 3.4 to 8.8 (Giraud et al., 1991). When stored under refrigerated conditions (4 °C), the viable counts of *Lb. plantarum* remained consistently high. Nevertheless, a notable reduction in counts was observed when the samples were stored under ambient conditions at room temperature (25 ± 1 °C) (Dhewa et al., 2014). *Lb. plantarum* is commonly found in a wide range of food products, including milk, meat, and various vegetable fermentations such as sauerkraut, pickles, brined olives, Korean kimchi, Nigerian Ogi, sourdough, and other fermented plant materials. It can also be detected in certain cheeses, fermented sausages, and stockfish. The prevalence of this microorganism in food underscores its promising suitability as a probiotic candidate for further exploration and development (Nybom et al., 2008).

Lb. plantarum, tested as a probiotic, exhibits noteworthy health effects. It demonstrates significant antioxidant activities and aids in maintaining intestinal permeability, offering potential benefits (Bested et al., 2013). Through the inhibition of gas-producing bacteria in the intestinal tract , it may provide relief for individuals with irritable bowel syndrome (Bixquert, 2009). Experimental evidence suggests that *Lb. plantarum* has the capacity to elevate hippocampal brain-derived neurotrophic factor levels, indicating a potential therapeutic application in the management of depression. Moreover, its ability to withstand the harsh conditions of the human gastrointestinal tract makes it a promising candidate for in vivo transportation of therapeutic compounds or proteins (Bested et al., 2013).

2.4.2 *Lactobacillus rhamnosus*

Lactobacillus rhamnosus is a gram-positive probiotic bacterium that exists naturally in the body, primarily in the intestines. It is the most popular probiotics which is commercially available and has been reported to colonise in the gut area of a new born when administered to a diet plan of pregnant mother (MacDonald & Sabatino, 2006; Schultz et al., 2004). *Lb. rhamnosus* was first isolated from faecal of healthy human adult and was recognized as a prospective probiotic strain because of the resistance to acid and bile, protective adhesion to the epithelial layer and excellent growth characteristics (Doron et al., 2005; Segers & Lebeer, 2014). Similar to most of the added probiotics, *Lb. rhamnosus* is utilized in the manufacture of fermented milk as a form of supplemental culture having excellent therapeutic value (Westerik et al., 2018).

Among the advantageous effect conferred by *Lb. rhamnosus* bacteria are the prevention of atopic diseases, nosocomial gastrointestinal infections, antibiotic-associated diarrhoea, decreases risk of respiratory tract infections and improved symptoms of atopic dermatitis (Ciszek et al., 2011; Vargas et al., 2015). Studies have suggested that the possible mechanisms underlying the beneficial effects brought by *Lb. rhamnosus* are due to the inhibitory effect of biofilm formation by the pathogen, modulation of the gut microbiome, management of host immune response and maintenance of intestinal barrier function (Berni et al., 2016; Durack et al., 2017; Johansson et al., 2016; Petrova et al., 2016)

2.4.3 *Bifidobacterium bifidum*

The probiotic *Bifidobacterium bifidum* is a gram-positive probiotic strain belongs to the Actinobacteria phylum (Wen et al., 2019). *B. bifidum* inhabits the human intestines as well as animals (Kailasapathy & Chin, 2000) and is one of the first

colonisers of the infant gut (Metchnikof, 1908). It is resistant to the antimicrobial enzyme lysozyme and can thrive at low surface tension resulting it able to withstand unfavourable conditions and grow in the gut area (Gilliland, 1979). The survival of *B. bifidum* is dependent on the surrounding pH, whereby low pH decreases their survival, and its growth is significantly retarded below pH 5.0 (Kailasapathy & Chin, 2000).

B. bifidum confers many health promoting effects which include immunomodulation, production of bacteriocin, pathogen exclusion and helps maintain health compatible microbiota (Fuller, 1989; Tamime & Robinson, 2007a). Thus, there has been a report that *B. bifidum* is able to prevent rotavirus diarrhoea, intestinal acute and chronic inflammation, including inflammation (Saavedra et al., 1994; Wen et al., 2019). *B. bifidum* is most commonly added into yoghurt to produce milk items having outstanding therapeutic value (Tamime & Robinson, 2007b).

2.4.4 Mechanism action of probiotics

Probiotics exhibit a lot of advantageous effects on the human body. For instance, probiotics can develop the existing microbial status in the gut area and balance the functional activities of good and bad bacteria (Ta, 2010). Furthermore, probiotics can reduce the growth activity of harmful bacteria such as *Clostridium perfringens*, *Escherichia coli*, *Campylobacter jejuni*, and *Salmonella enteritidis* efficiently (Markowiak & Ślizewska, 2017).

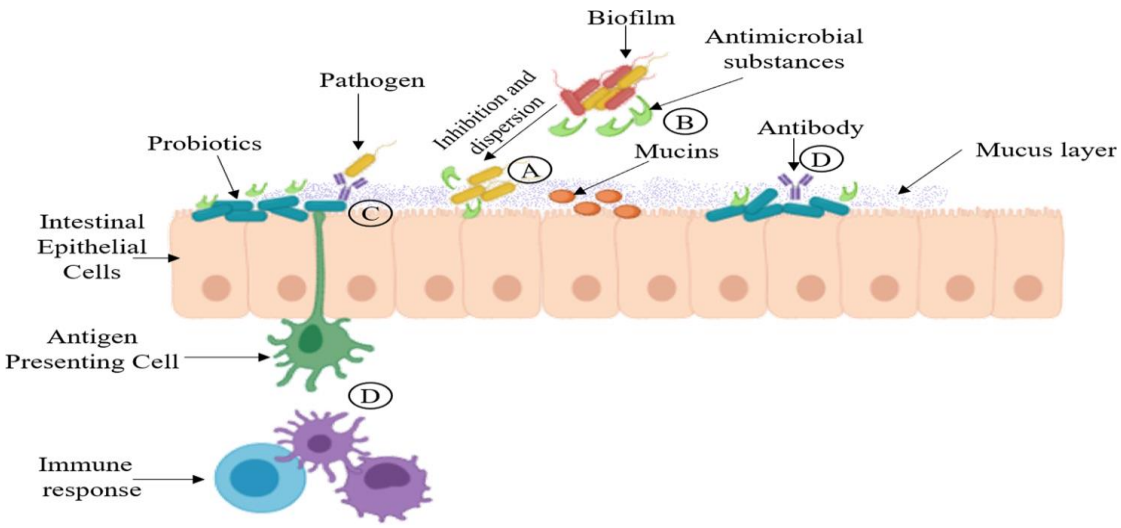

Figure 2.1 Delineates the diverse mechanisms through which probiotics operate, encompassing (A) competitive inhibition, (B) antimicrobial synthesis, (C) mucosal adhesion, and (D) immune system activation.

Figure 2.1 explain the way of action of probiotic includes strong epithelial barriers, mucosal adhesiveness properties, the prohibition of pathogen adhesion, competitive exclusion of pathogens, and immune system regulation. The intestinal barrier is the key defense system for controlling the epithelial integrity to save the microbes from unfavorable conditions. The factors responsible to disrupt the barrier function are peptides (antimicrobial), Immunoglobulin A (IgA), and the epithelial junction adhesion while antigens from bacteria and food can influence the inflammation that may cause the intestinal disease (Ohland & MacNaughton, 2010).The mechanisms of probiotics to enhance the function of the intestinal barrier still not identified clearly. Studies have revealed that promoting gene expression for tight junction signaling can be a probable mechanism to amplify the mucosal barrier unity (Anderson et al., 2010). Moreover, probiotics may have the ability to block pathogens by adhering with epithelial cells which exerts a beneficial effect for immune modulation by triggering the signaling cascade. The release of many soluble components from epithelial cells may confer to direct/indirect activation of immune cells to prevent contagious diseases and the

inflammation of the alimentary tract (Ta, 2010). Organic acids generated by probiotics namely acetic and lactic acid can exert strong inhibitory action towards gram-negative bacteria. The abovementioned acids are thought to be as principle antimicrobial components to fight against pathogens (Makras et al., 2006). One individual bacteria can decrease the activity of another species bacteria by subsequent mechanisms: formation of hostile microbial ecology, exclusion of attainable bacterial receptors, emission of antimicrobial components, and competitive reduction of necessary nutrients (Khare et al., 2018).

2.4.5 Potential preventive and therapeutic role of probiotics

Studies have revealed the promising effect of probiotics on the development of strong immunity, protection against disease caused by infections or allergic substances, improvement in total antioxidant capacity (TAC) and plasma total glutathione (GSH) level (Gourbeyre & Bodinier, 2011; Roshan et al., 2019).

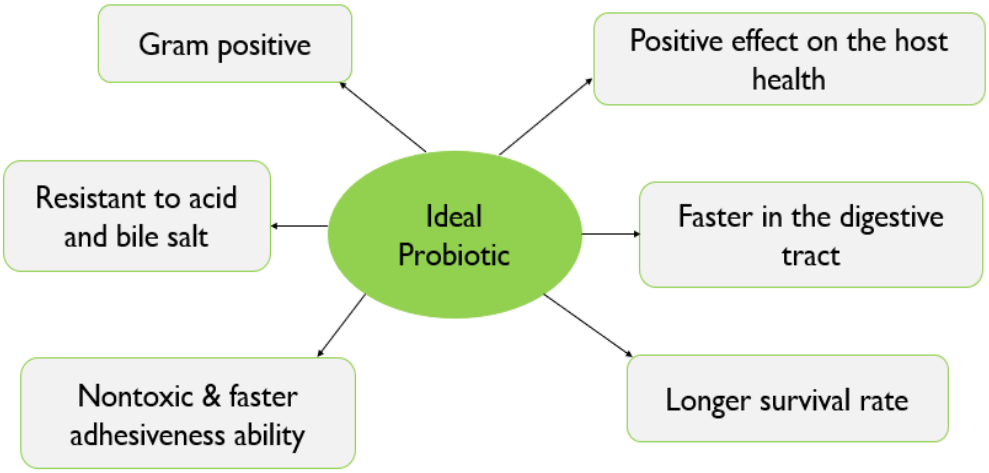

Figure 2.2 Properties of ideal probiotics.

Figure 2.2 shows some properties of ideal probiotics (Khare et al., 2018). The possible therapeutic application for disease prevention has been investigated by the researchers, such as, insulin resistance syndrome, diabetes, obeseness, and non-alcohol

diseases of probiotic microorganisms (George et al., 2018; Markowiak & Ślizewska, 2017). Probiotics show significant contributions in host metabolic processes, thus improve their health status by decreasing the risk of metabolic disorders, for example hypertension, obesity, heart diseases, arteriosclerosis, cancer, and ageing. Probiotics are also responsible for qualitative alterations in intestinal mucus which prevent pathogen binding (Moayyedi et al., 2010; Roy & Kumar, 2019). Moreover, fruit by-product increases the viability of probiotic bacteria and have been confirmed to have potential anti-obese properties. (Wang et al., 2019), studied probiotic effect on obese adults and reported that probiotics show beneficial effects on weight reduction with the changes in lipid profile and glucose metabolism. Another study revealed that probiotic diet plays a preventive role in obesity establishment and reduces the risk of problems associated with obesity.

2.5 Prebiotics

The word prebiotics was first commenced by Glenn Gibson and Marcel Roberfroid in 1995 and described as "a non-digestible food ingredient that beneficially affects the host by selectively stimulating the growth and activity of one or a limited number of bacteria in the colon, and thus improves host health" (Gibson & Roberfroid, 1995). Concerning the definition, only a limited number of carbohydrate groups can be categorized as prebiotics such as "fructo-oligosaccharides" (FOS), inulin, and "galacto-oligosaccharides" (GOS). In 2008, the "International Scientific Association of Probiotics and Prebiotics" (ISAPP) defined "dietary prebiotics" as "a selectively fermented ingredient that results in specific changes in the composition and/or activity of the gastrointestinal microbiota, thus conferring benefit(s) upon host health". The criteria for a compound to be considered as prebiotic: resistant to acidic pH, do not

absorb in the gut area, can be fermented by intestinal microbes, and enhance their viability to improve host's health. The popular prebiotics is FOS, GOS, trans-galacto-oligosaccharides (TOS), Mannan-oligosaccharides (MOS), and inulin (Davani et al., 2019). Fruit wastes are a natural source of carbohydrates with potential prebiotics (Ohshima et al., 2016); moreover, prebiotics can be synthesized from starch or carbohydrates utilizing apposite enzymes. Recently, the production of functional foods with prebiotic components has exhibited dominant features in food factories and promising market value for economic reasons and scientific evidence of its well-being. The result has shown that during bio-therapeutic formula preparation, appropriate microbial strains and prebiotic ingredients can increase the survival of probiotics in the target site (Ohshima et al., 2016). Therefore, the increasing demand of health consciousness among consumers will offer remunerative opportunities for the prebiotic producer.

2.5.1 Sources of Prebiotics

Traditionally, most of the prebiotics are found naturally in plant sources such as Jerusalem artichoke, leeks, asparagus, chicory roots, unrefined wheat, unrefined barley, yacon, garlic and onions. Fruits, being a plant source is also an excellent choice for prebiotics as they contain high amount of pectin (Pandey et al., 2015). The valuable pectin can form gels in the presence of sugar and acids under suitable environment, is often extracted as by products from fruit wastes which include the peels. On the other hand, animal sources only made up a small portion of prebiotic sources (Chandel et al., 2022). Raffinose, a non-digestible oligosaccharide is an example of prebiotics which can be found in many fruits and vegetables (Sayama et al., 1992). Extraction of soybean whey also produces oligosaccharides composing raffinose and stachyose (Blaut, 2002). The most commonly used prebiotics in food is inulin, which can be derived from

chicory root after undergoing enzymatic hydrolysis of polysaccharides (Singla & Chakkaravarthi, 2017). Raffinose is a trisaccharide found in vegetables, consisting of galactose, glucose, and fructose. It's less complex and may contribute to gas production. Inulin, a polysaccharide in plants like chicory, contains fructose units. It functions as a prebiotic, promoting beneficial gut bacteria growth, and has potential health benefits. Additional noteworthy prebiotics sourced from plants include xylans, hemicellulose, mannans, α-glucans, β-glucans, and galatians, which can be obtained from mushrooms like *Pleurotus ostreatus* and *Pleurotus eryngii*. (Singdevsachan et al., 2016; Synytsya et al., 2009), resistant starch from native starch or starch which is not hydrolysed in human digestive system (Carlson et al., 2018), guar gum from guar seeds (McCleary & Matheson, 1983) and FOS from extracted sucrose of beet. Moving on to prebiotics of animal origin, the most commonly found prebiotics from animal sources are GOS and lactulose. They are the products of cow's milk after undergoing isomerisation and transglycosylation, respectively (Singla & Chakkaravarthi, 2017). As the importance of prebiotics increases, microorganisms are used to produce prebiotics on a larger scale to reduce the extraction time and cost, while also enabling easier application in food products. For example, XOS are produced from xylanase derived from *Trichoderma* sp. (Fujikawa et al., 1990) and FOS is prepared using enzyme of plants and micro-organisms (Hidaka et al., 1988). Commercial production of GOS is also done using the enzyme β-galactosidase from *Aspergillus oryzae* and *Cryptococcus leurentii* OKN-4 (Ishikawa et al., 2005).

2.5.2 Mechanism action of prebiotics

The intestinal microorganism works as a protector against very harmful bacteria by generating antimicrobial components and compete for epithelial attachment or nutrients (Schley & Field, 2002). By the fermentation, process prebiotics generates propionic acid, lactic acid, and butyric acid which might have multiple advantages on human health (Davani et al., 2019).

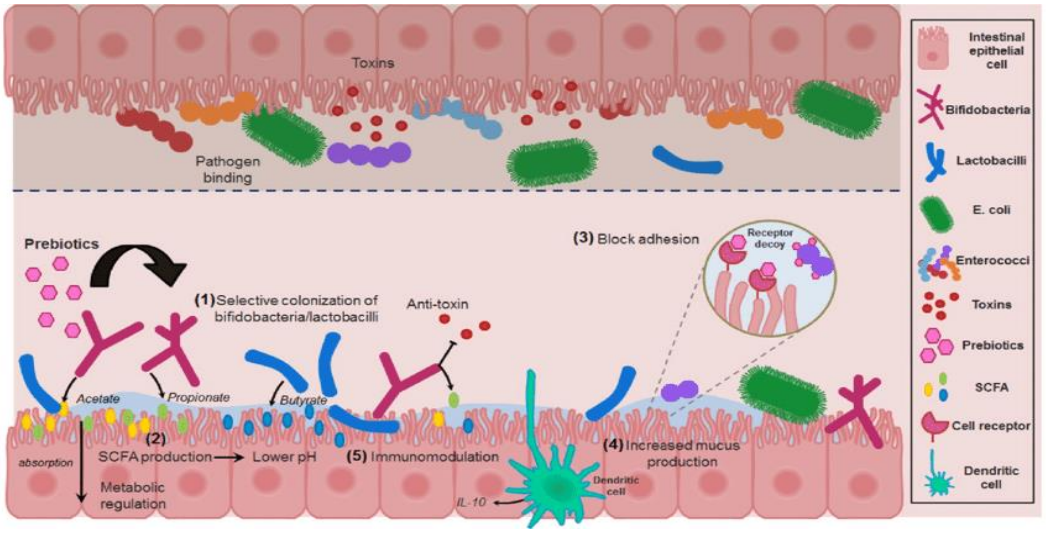

Figure 2.3 Diagram illustrating how prebiotics impact gut microbiota. Prebiotic effects are depicted below the dashed line. (1) Preferential colonization of beneficial bacteria through competition with pathogens for prebiotics. (2) Generation of SCFAs.

Figure 2.3 provides a summary of the proposed mechanisms underlying prebiotic action. For instance, butyrate can influence the development of intestinal epithelial. These acids are highly digestible substrates for bacteria. Besides, they show advantageous effects on metabolic activity, pH value, increase the length and number of the intestinal and epithelial villus, respectively (Khare et al., 2018). Multiple surface determinants are displayed by prebiotics involving in the interaction process with intestinal epithelial cells and mucous to restrain the adhesion of harmful bacteria (Khare et al., 2018). Mannan-oligosaccharides (MOS), act by agglutination *via* the collaboration process of mannose sensitive lectins that are found in the cell wall surface

of particular bacterial strain and restrain colonization of pathogens by the attachment of digestive tract sites (Heinrichs et al., 2003). Another commonly used prebiotic namely inulin is not absorbed in the small intestine but can be fermented rapidly in other parts of the alimentary canal agitating the proliferation of *Lactobacillus* and *Bifidobacterium* (Gibson & Roberfroid, 1995). A significant reduction of detrimental bacteria is also observed due to the changing effect of bifidogenic bacterial microbiota in the gut system. Short-chain fatty acids (SCFAs) produced by *Bifidobacteria* which can inhibit the proliferation and maintain low pH value with a concurrent hostile environment for pathogens (Khare et al., 2018). Furthermore, the proliferation caused by different species like *E. coli, Clostridium perfirgensis* and *Salmonella* can be repressed by several *Bifidobacteria* strains.

2.5.3 Potential preventive and therapeutic role of prebiotics

(Parra et al., 2015) investigated the prebiotic activity of flour obtained from grapefruit, pear and pineapple peel in association with probiotic bacteria. Orange peel extract contains pectic oligosaccharides (POS) which expressed the prebiotic properties of increasing the number of *Bifidobacteria* and *Eubacterium rectale* with higher butyrate concentrations (Suárez et al., 2010; Wan et al., 2018), reported that grape pomace extract shows a significant role in intestinal tract microbiota and improve gastrointestinal health with the presence of *Lactobacillus acidophilus*. Together with the polyphenol contents, grape seeds contain a particular number of oligosaccharides and prebiotic activity is the most well-demonstrated action of oligosaccharides (Bordiga et al., 2019). Agro-food by-product offers a golden opportunity in nutraceuticals and value-added foods. XOS produced from these by-product presents the prebiotic efficiency on host health by optimising the activity in the colon, metabolic process and immunomodulatory function (Samanta et al., 2015). In addition, XOS can abate pro-

carcinogenic enzymes, high pH value and nitrogenous waste. Resistant starch, which is unabsorbed in the gastrointestinal tract and oligosaccharides namely raffinose has been recognised as prebiotic carbohydrates that improve the growth activity of good bacteria (Dwivedi et al., 2014).

2.6 Synbiotics

A new concept has been introduced in biogenic known as synbiotics which is composed of probiotics and prebiotics. Synbiotics, combining probiotics and prebiotics, synergistically promote gut health. Probiotics, live microorganisms, enhance beneficial bacteria growth, while prebiotics provide nourishment. Applications span functional foods to clinical interventions. Mechanisms involve gut microbiota modulation and immunomodulation, aiding in gastrointestinal disorders and immune function. Potential benefits include improved nutrient absorption, immune response, and metabolic regulation. Synbiotics offer promise for gastrointestinal health enhancement and therapeutic intervention. The dietary intervention with synbiotics aimed at correcting the breakdown of gut microbiota or subsequent imbalanced diets which may offer benefits for health by simplifying the weight reduction process (Sergeev et al., 2020). Probiotics like *Lactobacilli, Bifidobacterium, Saccharomyces boulardii* and prebiotics such as galactooligosaccharides (GOS), xylooligosaccharides (XOS), fructo-oligosaccharides (FOS) and inulin are the widely used materials to produce synbiotics (Hernández et al., 2016). The study revealed that synbiotic consumption along with less calorie diet programs exhibits salutary effects on the congelation of serum irritant markers and on excess fluid volume in breast cancer with lymphedema (Vafa et al., 2020). The therapeutic potential of synbiotics includes antimicrobial and anticarcinogenic activity, antidiarrheal characteristics, defensive mechanisms against