UNIVERSITI PUTRA MALAYSIA

DIETARY FIBER OF MD2 PINEAPPLE (Ananas comosus (L.) Merr.) WASTES AND ITS RESISTANCE TOWARDS α-AMYLASE AND STOMACH ACID JUICE IN-VITRO

FEADLIND DAMMIA RADUAN

This project entitled "Dietary fiber of MD2 pineapple (*Ananas comosus (L.) Merr*.) wastes and its resistance towards α-amylase and stomach acid juice in-vitro" was prepared by Feadlind Dammia Raduan and submitted to the Faculty of Medicine and Health Sciences 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, University Putra Malaysia.

Received and examined by:

____

(Dr. Mohd Redzwan Sabran)

Date: ___________________

ACKNOWLEDGEMENT

First and foremost, praises and thanks to Allah Almighty for His showers of blessings throughout the journey in completion of this research project. I would like to express my sincere gratitude to my supervisor, Dr. Mohd Redzwan Sabran for the continuous support of my final year project, for his patience, motivation, enthusiasm, and immense knowledge. His guidance really helped me in all the time of research and writing of this thesis. I could not have imagined having a better supervisor for my final year project. Besides my supervisor, I would like to thank all the lab instructors and demonstrators who helped me so much in handling all the lab instruments and for making things clearer for me whenever I felt so lost and confused during my lab session. My sincere thanks also goes to my parents for their love, prayers, caring, and sacrifices for educating and preparing me for my future, especially for being so understanding with my busy schedule all along and not to mention, in being a good support system for me during the completion of my project. Also, thank you to all my classmates and friends for always being there to give such beneficial feedbacks and suggestions on how to improve my project work. I am so grateful for having constant guidance, encouragement and support from my final year project supervisor, laboratory staff, family and also my beloved friends. Without all of these precious people, I believe that I would have not been able to complete this project successfully.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

ABSTRACT

DIETARY FIBER OF MD2 PINEAPPLE (*Ananas comosus (L.) Merr.)* WASTES AND ITS RESISTANCE TOWARDS α-AMYLASE AND STOMACH ACID JUICE IN-VITRO

Feadlind Dammia Raduan

Pineapple (Ananas comosus (L.) Merr.) is the only important fruit crop in the family of Bromeliaceae as it is beneficial from the economical perspective. However, up to 80% of pineapple fruit is discarded as waste which resulted in high biological oxygen demand (BOD) and chemical oxygen demand (COD) values. However, pineapple wastes such as crowns and peels can be advantageous according to the literature reviews. One of them was due to their nutritional value, they can eventually be incorporated into food products. So, this study was conducted to determine the dietary fiber of MD2 pineapple's crown and peels and its resistance towards α-amylase and stomach acid juice in-vitro. Both pineapple crowns and peels were collected at the Revenue Collection Center, Serdang Agriculture Department. Next, they were cut, cleaned, oven-dried, homogenized and analyzed for the dietary fiber content by using enzymatic-gravimetric method and the digestibility of sugar by using phenol-sulphuric acid. The total dietary fiber (TDF), insoluble dietary fiber (IDF) contents and α-amylase enzyme digestibility were significantly different ($p<0.05$) between both samples where crowns had higher amount than the peels. However, the soluble dietary fiber (SDF) content and simulated gastric juice digestibility were not significantly different ($p>0.05$) between both samples. As for the correlation between dietary fiber and digestibility, IDF and TDF were significantly and negatively correlated with the sample resistance towards α-amylase enzyme activity. So, the higher the IDF and TDF content, the lower the digestibility indicated that the samples had higher ability to resist the enzymatic activities. Therefore, both samples should be taken into consideration for incorporation into foods as they contain dietary fiber, specifically IDF which is higher in the pineapple wastes. Also, both samples would be a good source of prebiotics as they fulfilled the first criteria of a prebiotic which is the ability to resist the host digestion, as more than 80% of the samples were resistant towards the α-amylase enzyme and the simulated gastric juice. Overall, this study shows that the pineapple wastes have a tremendous potential to be converted into value-added food products.

ABSTRAK

SERAT SISA NENAS MD2 (*Ananas comosus* (*L.*) *Merr.*) DAN KETAHANANNYA TERHADAP ENZIM α-AMILASE DAN JUS ASID PERUT SECARA *IN-VITRO*

Feadlind Dammia Raduan

Nenas (*Ananas comosus (L.) Merr.*) adalah satu-satunya tanaman buah yang penting dalam keluarga *Bromeliaceae* kerana ia memberikan manfaat dari sudut ekonomi. Namun, lebih kurang 80% buah nenas dibuang sebagai sisa mempunyai nilai permintaan oksigen biologi (BOD) dan permintaan oksigen kimia (COD) yang tinggi walaupun dari tinjauan literatur, sisa nenas seperti mahkota dan kulitnya dapat memberikan manfaat kepada kesihatan tubuh badan. Disebabkan potensi nilai pemakanannya, sisa-sisa ini boleh dimasukkan ke dalam produk makanan. Oleh itu, kajian ini dilakukan untuk mengetahui serat makanan dari mahkota dan kulit nenas MD2 dan ketahanannya terhadap enzim α-amilase dan jus asid perut secara *in-vitro*. Kedua-dua mahkota nenas dan kulit diambil dari Pusat Pengumpulan Hasil, Jabatan Pertanian Serdang, Selangor. Seterusnya, sisa nenas ini dipotong, dibersihkan, dikeringkan di dalam ketuhar, dihomogenkan dan dianalisis untuk kandungan serat makanan dengan menggunakan kaedah enzimatik-gravimetri dan pencernaan gula dengan kaedah asid fenol-sulfurik. Jumlah kandungan serat makanan (TDF), serat makanan tidak larut (IDF) dan pencernaan enzim α-amilase jauh berbeza ($p < 0.05$) antara kedua-dua sampel di mana mahkota mempunyai jumlah yang lebih tinggi berbanding kulitnya. Namun begitu, kandungan serat makanan larut (SDF) dan simulasi pencernaan jus gastrik tidak berbeza secara signifikan ($p > 0.05$) antara kedua-dua sampel. Bagi korelasi antara serat makanan dan pencernaan, IDF dan TDF berkorelasi secara signifikan dan negatif dengan ketahanan sampel terhadap aktiviti enzim α-amilase. Jadi, semakin tinggi kandungan IDF dan TDF, semakin rendah tahap penghadaman menunjukkan bahawa sampel mempunyai keupayaan yang lebih tinggi untuk menentang aktiviti enzimatik. Oleh itu, kedua-dua sampel harus dipertimbangkan untuk dimasukkan ke dalam makanan kerana mengandungi serat makanan, khususnya IDF yang lebih tinggi dalam sisa nenas. Juga, kedua-dua sampel akan menjadi sumber prebiotik yang baik kerana sisa-sisa ini memenuhi kriteria pertama prebiotik iaitu kemampuan untuk menahan pencernaan manusia, kerana lebih dari 80% sampel tahan terhadap enzim α-amilase dan simulasi jus gastrik. Secara keseluruhan, kajian ini menunjukkan bahawa sisa nenas berpotensi untuk diubah menjadi produk makanan yang mempunyai kualiti yang lebih baik.

CHAPTER 1

INTRODUCTION

1.1 Background

Pineapple or its scientific name, *Ananas comosus (L.) Merr.* is one of the tropical plant species, where the fruits are edible. The word "pineapple" in English is used initially to describe the conifer trees' reproductive organs which later is referred to as the pine cone. The scientific name of pineapple, i.e. *Ananas* comes from the Tupi word, *nanas* which has the meaning of "excellent fruits" as stated by André Teve in 1555 (Davidson, 2002). As for *comosus*, it means "tufted" which refers to the stem of the fruit (Davidson, 2002). According to Thalip et al. (2015), pineapple is the only important fruit crop in the family of Bromeliaceae. This is due to the significance of this tropical plant from the economic perspective as it can be seen that pineapple comes second after bananas in the ranking of the production of commercial tropical fruits at international level, according to the United Nations Conference on Trade and Development (UNCTAD). Pineapple may be cultivated from its discarded crown

(Morton, 1987). Once it is cultivated, it would probably take about five to ten months to bloom and the plant will start to produce fruits after six months of being flowered.

According to Jaji et al., (2018), among the production of tropical fruits in the world, the pineapple industry contributes to more than 20%. In the year of 2016, the global pineapple market grew up to $14.9 billion in wholesale prices. As for the volumes, the total production of pineapples that was marketed during that time was about 26.2 metric tonnes. In fact, this market has grown on average by 3.3% per year over the past decade. In 2017, the top three nations which produced pineapples were Costa Rica, Philippine and Brazil. Statistically, 3.06 million metric tonnes of pineapples had been produced by Costa Rica in 2017. In 2017, the production of pineapples had amounted to 27.4 million metric tonnes. Hence, there is about 1.2 metric tonnes increment of the production of pineapples worldwide in 2017 as compared to in 2016.

Malaysia is also one of the pineapples-producing countries. Pineapple is the first crop grown as a commodity crop in Malaysia. Then, this raised the nation's position to a very significant level in the world between the late 60's and early 70's (Jaji et al., 2018). This is due to the contribution of Malaysia's economic development and growth of other supporting economic activities such as packaging, transportation, and other value addition activities, specifically in Johor (Jaji et al., 2018). Abd-Halim (2016) stated that pineapple constitutes 6.3% agro-food in Malaysia, which is the fourth largest area after durian, banana, and rambutan. According to the Malaysian Pineapple Industry Board (MPIB) in 2011, Johor is known as the largest pineapple producer in Malaysia with the production's quantity approximately at 80,389.22 metric tonnes. An increment can be observed after four years. As in 2015, pineapples were grown in the area of around 10,847 hectares with an estimated production of 272,570 metric tonnes in Malaysia (Abd-Halim, 2016).

There are many pineapple cultivars that have been known. As reported by Joy and Anjana (2013), the numerous pineapple cultivars are grouped in four main classes. They are ‘Smooth Cayenne’, ‘Red Spanish’, ‘Queen’ and ‘Abaxaci’. Also, there are a lot of variations in the types within each class. For example, MD2 which is a hybrid pineapple of a seedling numbered 73-114 (Joy and Anjana, 2013). In fact, MD2 is one of the nine pineapple varieties that can be found in Malaysia apart from MORIS, Josepine, N36, Sarawak, Moris Gajah, Gandul, Yankee, and Masapine. Presently, the MD2 variety, which originated from Hawaii is planted and marketed in Malaysia as well as for international markets (Abd-Halim, 2016). MD2 was developed for its exceptional sweetness in taste as well as its uniformity and consistency in size and ripeness (Thalip et al., 2015). Other than that, MD2 pineapples are resistant to internal browning too. This hybrid pineapple is also better in certain quality aspects which are having uniform bright gold color, sweeter taste, and a longer shelf life.

A research conducted by Hossain et al. (2015), found that pineapple has a good amount of calcium, carbohydrates, crude fiber, water, and different minerals. These nutritional contents are beneficial for the digestive system. They help in maintaining ideal weight and balanced nutrition. Furthermore, the main function of vitamin C is to overcome viral and bacterial infections while dietary fiber can aid in improving the bowel movements.

Bromelain is an enzyme that can be extracted and purified from the pineapple wastes (Hebbar et al., 2008). From a medical perspective, Hossain (2016) mentioned that the enzyme is also used to tenderize meat and it aids in digestion and enhances the performance of small intestines, and kidneys. This enzyme is also good for the gastrointestinal system because it helps in preventing constipation, alleviating hemorrhoid, and normalizing the colonic flora (Lobo and Paull, 2017).

Of great interest, the waste products of pineapple, i.e. crown and peels are of remarkable relevance. The reason being is because there are already researchers that have published their studies on the pineapple waste products. Based on a research conducted by Larrauri et al., (1997), they found that the peels of pineapple are a promising source of dietary fibre which contains polyphenols. However, there are no studies about the dietary fibre in the pineapple's crowns yet. Hence, further research and study on the pineapple's waste products, specifically its crown and the peels could be carried out in order to discover the content of fibre and its prebiotic functions. The dietary fibre includes the soluble and insoluble fibre too. As for the prebiotic functions, the digestibility of sugar in both samples is experimented. In this study, the pineapple used is MD2 and the by-products are its crown and peel as mentioned above.

1.2 Problem statement

As stated by Agamuthu et al. (2009), 998 million tonnes of agricultural waste is produced per year globally and 1.2 million tonnes of agricultural waste is disposed of into landfills manually in Malaysia. In Asia, around 15% of the total waste generated is agro-waste, with agricultural waste generation in Malaysia at approximately 0.122 (kg/cap/day) in the year of 2009 which is projected to reach 0.210 (kg/cap/day) by the year of 2025 (Agamuthu et al., 2009). Though the agro-based industry produces different types of waste, these wastes are mostly composed of organic matter - have a high potential to be converted into value-added products (Al-Busaidi, 2010). If no actions are taken to overcome these issues, these can bring harm and damage to the environment over time.

Some of the agricultural wastes or by-products that can be studied are the pineapple's crown and peels. According to a study conducted by Schieber (2001), it is found that tropical and subtropical fruits processing have considerably higher ratios of by-products than the temperate fruits. This happened due to the selection and elimination of certain components that are unsuitable for human consumption (Upadhyay et al., 2010). In addition, reports have shown that 40% to 80% of pineapple fruit is discarded as waste having high biological oxygen demand (BOD) and chemical oxygen demand (COD) values (Ban-koffi and Han, 1990). Then, the problem of disposing by-products is further aggravated by legal restrictions. A high level of BOD and COD in pineapple wastes add to further difficulties in disposal (Upadhyay et al., 2010). This really can bring a problem in our environment which affects the Sustainable Development Goal (SDG) 12 which is responsible consumption and production. This is because according to the United Nation (UN), one of the main aims of SDG 12 is to encourage industries, businesses and consumers to recycle and reduce waste in order to reduce air, water and land pollution.

As claimed by Larrauri et al. (1997), dietary fiber powder prepared from the pineapple's peels contains 70.6% of total dietary fiber with better sensory properties than commercial dietary fibers from apple and citrus fruits. Also, some dietary fibers can be classified as prebiotics as well. As an example, oligosaccharides. In fact, oligosaccharides are included as fiber in food labels in the United States. Oligosaccharides are considered as the best known prebiotics due to one of their main criteria which is a selectively fermented ingredient that allows specific changes, both in the composition and/or activity in the gastrointestinal microflora that confers benefits upon host well-being and health (Slavin, 2013).

However, the studies of dietary fiber and prebiotics in pineapple's peels and crowns are quite limited in number, especially the ones that have been carried out within the past 3 to 5 years. Therefore, to make full use of the pineapple's peels and crown, this study aims to determine the total dietary fiber, soluble fiber, insoluble fiber, and the prebiotic effects through the digestibility of sugar in agricultural by-products, pineapple's peels and crown.

1.3 Significance of study

Dietary fiber is considered essential and fundamental to human health due to its benefits and advantages. As examples, dietary fiber helps in normalizing the bowel movements which brings about the maintenance of bowel health and it aids in achieving normal weight especially for those people who are overweight or obese. Currently, there is a bulk of research that is focusing on the dietary fiber content in various types of fruits but not their by-products. Hence, this makes the dietary fiber sources limited up to a certain extent when in fact, there is limited research claiming that dietary fiber does exist in the agricultural waste. To sum up, this study can widen the resources of dietary fiber for human beings to consume as people are becoming more health-conscious nowadays.

Besides, studies about the nutritional content of agricultural waste and by-products are limited in Malaysia. This study could help in establishing the nutritional content, which is specialized in dietary fiber for agricultural waste products instead of the fruits. It is hoped that this study can be a future reference for all the researchers who might be interested in understanding and studying the dietary fiber content and prebiotics properties of agricultural waste, the pineapple's crowns and peels specifically. Moreover, the studies about the pineapple generally, especially its crown, are very limited. Hence, the study is also not so significant in Malaysia. Hence, from this study, researchers can use this study as a reference for further investigation in terms of its utilization for food productions. Consequently, once the baseline of nutrition information of pineapple's crown has been achieved, the data can be used for a healthier food production which in the end, will affect the consumers positively in terms of their health. This study could also help in the production of organic food by using agricultural waste products.

Finally, from the environmental perspective, this study could be beneficial for Malaysia's environment because of the full utilization of the agricultural waste and by-products instead of being accumulated which could bring harm to the environment be it air pollution, water pollution, and even the land pollution. Overall, this study could help in achieving the Sustainable Development Goal (SDG) 12, responsible consumption and production.

1.4 Objectives

1.4.1 General objective

To investigate dietary fiber of MD2 pineapple's crown and peels and their influence on digestibility.

1.4.2 Specific objectives

1. To determine the total dietary fibre, soluble fibre and insoluble fibre of MD2 pineapple crown and peels powder.
2. To compare the total dietary fibre, soluble fibre and insoluble fibre between MD2 pineapple crown powder and peels powder.
3. To determine the digestibility of MD2 pineapple crown and peel powder towards α-amylase and stomach acid juice *in-vitro*.
4. To correlate the dietary fiber content of pineapple crown and peel power and its digestibility towards α-amylase and stomach acid juice *in-vitro*.

1.5 Hypothesis

1. There is a significant difference in the total dietary fibre, soluble fibre, and insoluble fibre between the MD2 pineapple crown powder and MD2 pineapple peels powder respectively.
2. There is a significant difference between MD2 pineapple peel and crown powders' digestibility towards α-amylase and gastric juice *in-vitro*.

3. There is a negative correlation between dietary fiber and digestibility towards α-amylase and gastric juice *in-vitro*.

CHAPTER 2

LITERATURE REVIEW

2.1 Pineapple

Pineapple or its scientific name *Ananas comosus* is one of herbaceous perennials. The roots or the underground parts of the plant can survive despite the death of the plant itself annually. This family of *Bromeliaceae* has about 3,590 known species (Gouda and Butcher, 2016). Most of them are epiphytic and also strikingly ornamental. Other than that, this fruit also obtains quite numerous vernacular names. For example, this fruit is called pina in Spanish, *abacaxi* in Portuguese and *nanas* in Southern Asia and the East Indes. Further details of the botanical classification of pineapple can be seen in Figure 1.

Kingdom:	Plantae	Plants
Subkingdom:	Tracheobionta	Vascular plants
Superdivision:	Spermatophyta	Seed plants
Division:	Magnoliophyta	Flowering plants
Class:	Liliopsida	Monocotyledons
Subclass:	Zingiberidae	
Order:	Bromeliales	
Family:	Bromeliaceae	Bromeliad family
Genus:	*Ananas* Mill.	Pineapple
Species:	*Ananas comosus* (L.) Merr.	Pineapple

Source: Lobo and Paull (2017)
Figure 1: Botanical description and classification of pineapples.

Pineapple is a terrestrial herb, which eventually grows up to 1 to 1.5 m. At times, it could be much taller than this. The roots, the stem, the peduncle, the shoots, the leaves, the compound multiple fruit, and the crown would be its main morphological structures. According to Joy (2013), in terms of its appearance, the plants generally have a very short and stout stem, a rosette of waxy, strap-like leaves, long-pointed (around 50 cm to 180 cm long); most of the time with a needle tipped and generally bearing sharp, upcurved spines on the margins. 200 flowers will be produced to make its fruits. However, some of the large-fruited cultivars can exceed this number. Consequently, the ovaries of the plant will develop into berries. Then, it will merge into a large, compact, multiple fruits. The pineapple's fruit is arranged in two interlocking helices, eight in one direction, 13 in the other, each being a Fibonacci number (Jones and Wilson, 2006). After that, the side shoots, which are called 'suckers' by the commercial growers are generated in the leaf axils of the main stem once the very first fruit is created. According to Nassr and Abu-Naser (2018), these side shoots will be removed for propagation or it could also produce some additional fruits on the original plant.

Other than that, pineapple consists of five botanical varieties which formerly regarded as separate species (Sanewski et al., 2018). The five botanical varieties mentioned are *Ananas comosus var. ananassoides*, *Ananas comosus var. bracteatus*, *Ananas comosus var. comosus*, *Ananas comosus var. erectifolius* and *Ananas comosus var. Parguazensis*. As stated by Lobo and Paull (2017), the most important cultivars are very incompatible while other cultivars have weaker compatibility. Fundamentally, the pineapple is diploid with 25-minute chromosomes in *Ananas comosus var. ananassoides* and *Ananas comosus var. comosus* (Coppens d'Eeckenbrugge et al., 2007). On the other hand, there are also triploid plants with 75 chromosomes. They can be found naturally in F1 hybrids. Triploids come from the conjugation between an unreduced egg cell with about 50 chromosomes and the normal haploid pollen. One of the examples for a commercial cultivar with a natural triploid of 75 chromosomes is called 'Cabezona' (Collins, 1933). Besides that, 'Gigante de Tarauaca' is also a natural triploid with weight around 4.4 kg (Scherer et al., 2015).

In Malaysia, there are nine major pineapple varieties planted, namely Moris, N36, Sarawak, Moris Gajah, Gandul, Yankee, Josapine, Masapine, and the most recent would be MD2. Certain cultivars like Josapine and N36 are locally developed for the local fresh fruit market and they are not grown outside of Malaysia (Thalip et al., 2015). The supply of pineapples has been projected by the Malaysian Pineapple Industry Board (MPIB) to increase from 350,000 metric tonnes in 2013 and up to

700,000 metric tonnes in the year 2020. Of the nine cultivars, MPIB has chosen MD2 cultivar to be promoted for industrial planting due to its sweetness and uniformity and consistency in size and its ripeness (Thalip et al., 2015).

2.1.1 Pineapple crown

Pineapple's crown is a part of agricultural-waste products that is inedible and usually will be discarded from the fruit itself. There are farmers in certain countries that would detach the crown from the plant for propagation at harvest. In terms of cultivation, once the crown is cut from the fruit, it could be cultivated in the next 20 to 24 months, when it is flowered. Subsequently, it will start fruiting in the next six months (Prasenjit et al., 2012). If the pineapples' crowns are not needed for planting, they will be fed to horses and some will have an ornamental value too (Morton, 1987). From the pineapple's waste products perspective, the crowns are considered as a burden for the canned pineapple industry as the need for them to be replanted is relatively small compared to the amount of the refuse generated (Tran, 2006). Fortunately, the pineapple's crown is rich with a fruitful enzyme namely as bromelain (Hebbar et al., 2008).

Bromelain is an enzyme that is useful for tenderizing meat, brewing, baking, as well as for the production of protein hydrolysates (Ketnawa et al., 2011; Walsh, 2002). Other than that, from the non-food industries, this enzyme is applied in tanning for both leather and textile industries, hair removal, wool, skin softening, and also detergent formulations (Uhlig, 1998; Subhabrata and Mayura, 2006). According to

Koh et al. (2006), from the medical perspective, it can be used as a folk medicine, a wound healer, an anti-inflammatory, and an anti-diarrhea and digestive aid. A study conducted by Dutta and Bhattacharyya (2013) found that pineapple's crown is a non-toxic bromelain enzyme-containing leaf which has properties in tissue-damaged repairing, wound healing and possibly prevents the secondary infections from microbial organisms. The bromelain content in pineapple's crown is quite stable as well. This is because it is found that the bromelain activity in the pineapple's crown is not affected by the acidity of the crown (Nadzirah et al., 2012). Though it is handy and convenient, the commercial bromelain can cost up to 2,400 USD/kg due to its usefulness (Ketnawa et al., 2012).

Although pineapple's crown contains a good amount of bromelain enzyme, this part of the plant is also playing an important role in maintaining the quality of harvested pineapple. Before that, one of the most popular postharvest disorders of pineapples is internal browning as most pineapple varieties are susceptible to blackheart disorder (Hassan et al., 2010). Then, this could bring about a severe economical loss (Hong et al., 2013). However, it is found that postharvest detachment of pineapple's crown significantly aggravated the internal browning of the fruit and increased the ripening of the fruit which makes the crown necessary for extending shelf-life of the pineapple (Liu et al., 2016). Once the crown is detached from the fruit, reactive oxygen species (ROS), malondialdehyde (MDA), and phenolic levels will be increased while the polyphenol oxidase (PPO) and phenylalanine ammonia-lyase (PAL) gene expression and activity will be upregulated (Liu et al., 2016). Hence, keeping the crown from a detachment of its fruit can prevent the generation of ROS and peroxidation of lipids while the biosynthesis and oxidation of the phenolic will be inhibited overall. Thus, decrowning the pineapple after it is harvested can deteriorate the quality of the fruit itself.

2.1.2 Pineapple peel

Pineapple's peels or also called as pineapple's shell is considered as one of the pineapple's waste products. In general, the pineapple's peel is the largest waste portion followed by the core, stem, and crown (Ketnawa et al., 2012). Due to the largest waste proportion of pineapple, the peels also have the richest amount of bromelain proteolytic enzyme which also existed in the crown. (Ketnawa et al., 2012). Other than that, the pineapple's peel is rich in cellulose, hemicelluloses, and other carbohydrates. This could lead to the production of paper, banknotes, and cloth (Sanewski et al., 2018). In the canned pineapple industry, all the residual parts cores, the peels, and the fruits' ends would be crushed first. After that, the juice obtained from the first pressing would be prepared as syrups to fill the cans (Morton, 1987). It also can be utilized in confectionery and beverages or even converted into powdered pineapple extract which has various roles in the food industry itself (Chaudhary et al., 2019).

Generally, the pineapple's peel has been used as a reducing agent or antioxidant and substrate for the production of bio-ethanol (Erukainure et al., 2011). Also, as claimed by Saraswaty et al. (2017), pineapple's peel contained phenolic compounds, ferulic acid, and vitamin A and C as the antioxidants. As for antioxidants, this substance is very fruitful in both the health sector and the chemical industry. However, certain types of antioxidants can bring harm to the human being's health such as butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT). These chemically synthesized antioxidants can bring about a growth of tumor when the dose is high (Kahl and Kappus, 1993). Based on a study conducted by Saraswaty et al. (2017), the pineapple's peels can be converted into a potential source of antioxidant whereby the ethanol/water of 55% (v/v) solvent for extraction provided the highest yield of antioxidant and it also has avoided microbial contamination.

Apart from that, dietary fiber could also be found from the pineapple's peel. This is because according to Quirós-Sauceda et al. (2014), pineapple's peels are reported as a promising source of dietary fiber, which is about 70.6%. A study conducted by Li et al. (2010), it also contains a high concentration of associated phenolics which are mainly gallic acid (31.76 mg/100 g dry extracts), catechin (58.51 mg/100 g dry extracts), epicatechin (50.00 mg/100 g dry extracts), and ferulic acid (19.50 mg/100 g dry extracts). Then, the pineapple's peel contains an appreciable amount (about 42%, wt/wt) of insoluble fiber-rich fraction which primarily consisted of cellulose, pectin substances, hemicellulose, and notable proportions of lignin (Huang et al., 2011). The fact that pineapple's peel is rich in dietary fiber with a high concentration of associated phenolics, is suitable fiber for a wide range of applications as a food ingredient as it comes together with the neutral color and flavor of it (Larrauri et al., 1997).

2.2 Total dietary fiber

Dietary fiber is one of the macronutrients that can be found in the diet aside from carbohydrates, protein, and fats. Macronutrients are nutrients that are needed in a large amount compared to the micronutrients like vitamins and minerals. Dietary fiber is also known as roughage. It is the plant-derived food that cannot be broken down completely by the human digestive enzymes (Ötles and Ozgoz, 2014). Dietary fiber and whole grains contain a unique blend of bioactive components such as resistant starch, vitamins, minerals, phytochemicals and antioxidants (Ötles and Ozgoz, 2014). The definition of dietary fiber has widened to include the oligosaccharides like inulin and resistant starches at a recent time (Jones and Wilson, 2006). According to Anderson et al. (2009), fibers can be divided into two main components which are soluble and insoluble. As for the soluble fiber, it will be fermented in the colon such as viscous or fermentable fibers like pectin. Then, as for insoluble fiber, it has bulking action but may only be fermented to a limited extent in the colon. An example of insoluble fiber is wheat bran. The components of dietary fiber are shown in Figure 2.

Non Starch polysaccharides and oligosaccharides	Analagous carbohydrates	Lignin substances associated with the NSP and lignin complex
Cellulose	indigestible dextrins	waxes
Hemicellulose	resistant maltodextrins	phytate
Arabinoxylans	resistant potato dextrins	cutin
Arabinogalactans	synthesized carbohydrates compounds	saponins
Polyfructoses	polydextrose	suberin
Inulin	methyl cellulose	tannin
Oligofructans	hydroxypropylmethyl cellulose	
Galacto-oligosaccharides	resistant starches	
Gums, mucilages, pectins		

Source: Ötles and Ozgoz (2014)
Figure 2: Components of dietary fiber

In addition, dietary fibers also can provide a lot of benefits and advantages to the human's health condition. In fact, over these past few years, the relationship between dietary fiber intake and the risk of getting cardiovascular diseases has been studied (Satija and Hu, 2012). The 'dietary fiber hypothesis' was first proposed during the year of 1972 to 1976 which indicated that when the consumption of dietary fiber is increased, the risks of getting diseases like chronic bowel disease, colon cancer, heart disease, and diabetes will be decreased (Burkitt et al, 1971; Trowell, 1976). Today, the researchers certainly found that this hypothesis can be accepted because there was an epidemiologic and randomized intervention study conducted by Satija and Hu (2012) found that higher consumption of dietary fiber has beneficial effects on CVD risk reduction. Furthermore, dietary fiber can help in overcoming obesity. This is due to the increment of dietary fiber consumption that can bring about the decrease of energy absorption by way of diluting a diet's energy availability while maintaining other important nutrients (Lattimer and Haub, 2010). The other health benefits of dietary fiber consumption are reducing the risk of getting diabetes mellitus (Yao et al., 2014), maintaining the health of the gastrointestinal system (Ötles and Ozgoz, 2014) and reducing the risk of getting cancers such as breast cancer among young adults (Farvid et al., 2016) and colorectal adenoma (Ben et al., 2014).

Fundamentally, enzymatic-gravimetric methods will be used to analyze the dietary fiber in foods. Not to mention, the other traditional method that can be used to analyze fiber (consists of soluble fiber and insoluble fiber) in foods would be the Total Dietary Fiber Assay Kit (K-TDFR). The enzymatic-gravimetric method is a method that uses different types of enzymes to help in the process of analyzing the fiber. This method was used in this study and further explanation can be referred to in Chapter 3.

2.2.1 Soluble dietary fiber

Dietary fiber can be classified into two, namely soluble dietary fiber and insoluble dietary fiber. Based on a research by Chawla and Patil (2010), they discovered that soluble dietary fiber (SDF) is usually present in small quantities. Then, the soluble dietary fiber is combined with the insoluble dietary fiber (IDF) to make up the total dietary fiber (TDF). Most of the foods will contain both types of fiber, approximately one-third soluble and two-third of insoluble dietary fiber (Wong and Jenkins, 2007). According to Surampudi et al. (2016), there are some crucial properties or characteristics of soluble dietary fiber that should be noted. It is soluble in water, able to form viscous solutions and ferments well. The examples of soluble dietary fiber are β-glucan, psyllium, pectins, guar gum, arabinoxylans, and inulins. The soluble dietary fiber can bypass the digestion process in the small intestine where they can be fermented by the microflora that existed in the large intestine (Surampudi et al., 2016). Besides, added that soluble dietary fiber beneficially affects human metabolism, fiber supplementation may be a feasible approach to improve body composition and glycemia in adults with overweight or obesity (Thompson et al., 2017).

As maintained by Surampudi et al. (2016), soluble dietary fiber can be found in certain foods, like whole grains, peas and beans, certain fruits and vegetables and seeds and nuts. One example of soluble dietary fiber is β-Glucan. β-Glucan is a linear polysaccharide of glucose monomers with β(1→4) and β(1→3) linkages. These linkages contain around 250,000 glucose units in the endosperm of cereal grains, primarily oats and barley, and also present in yeast, bacteria, and fungi. Its concentration in oats and barley can be varied from 3.9% up to 6.8% (Wood et al., 2007). The US FDA, Health Canada, and European Food Safety Authority have approved the health claims saying that the cholesterol-lowering effects of soluble dietary fiber from oat products or oat β-glucan, and several other studies have indicated that the β-glucan can reduce the levels of total cholesterols and LDL-cholesterols by about 5% to 10% (Brown et al., 2018; Othman et al., 2011; Ripsin et al., 1992; Mann et al., 2007). The other example of soluble dietary fiber is psyllium. Psyllium is a bulk-forming laxative. It will not be absorbed by the small intestine but it absorbs water in the gut and facilitates the bowel movement (Surampudi et al., 2016). “Diets low in saturated fat and cholesterol that include 7 g/d of soluble dietary fiber from psyllium may reduce the risk of getting heart disease.” - this health claim has been approved by the FDA (Surampudi et al., 2016).

Besides that, pectin is also one of the examples of soluble dietary fiber. Pectin is defined by methylated ester of polygalacturonic acid which composed of 300 to 1,000 galacturonic acid moieties with neutral sugars such as L-rhamnose, D-galactose, and L- arabinose units in 1α→4 linkages (Surampudi et al., 2016). Pectin fibers are highly present in citrus fruits, in which the amount would range from 0.5% to 3.5% (Mudgil, 2017). Also, the European Food Safety Authority (EFSA) Panel on Dietetic Products, Nutrition, and Allergies (2010) claimed that maintenance of cholesterol levels can be achieved by the consumption of 6 g of pectin per day. Next would be gums. Gum is a natural gum made of the hardened sap of various species of the acacia or seyal trees. Several studies claimed that gum contains a lipid-lowering effect (Bazzano, 2003).

According to Surampudi et al. (2016), the consumption of dietary fiber is linked to several health benefits such as lipid levels reduction, blood pressure lowered, improved blood glucose control, weight loss, immune function improvement, and inflammation reduction. Indeed, it is supported by a study conducted by Gulati et al. (2017), where daily consumption of 3 g soluble dietary fiber from 70 g of oats can lead to beneficial effects on the lipid parameters. This is specifically referred to the total cholesterol and low-density lipoprotein (LDL) cholesterol in hypercholesterolemic Asian Indians. Then, it is also found that isolated soluble fiber supplementation can improve anthropometric and metabolic outcomes in overweight and obese adults. Hence, this indicated that supplementation may improve fiber intake and health in these individuals (Thompson et al., 2017). As for diabetic patients, there is a study that showed that higher soluble dietary fiber intake was associated with lower postprandial glucose and breakfast (De Carvalho et al., 2017). Finally, soluble dietary fiber could also aid in a decrement of hunger and increment of satiety hormones in humans when it is ingested with a meal. According to Ye et al. (2015), fibersol-2, an example of a soluble dietary fiber significantly increased the satiety hormones PYY and GLP-1 of the participants in the study, where the authors perceived postprandial increases satiety. This could help overweight and obese people to lose their weight.

2.2.2 Insoluble dietary fiber

Insoluble dietary fiber is the other class or type of fiber that can be found in the food. Naturally, insoluble dietary fiber is the exact opposite of the soluble dietary fiber. Insoluble fiber does not dissolve in water nor it could absorb water. As a result, insoluble dietary fiber would usually just pass through the gastrointestinal tract without being affected, nearly to its original form. There are three general properties of insoluble dietary fiber, which are decrements of fermentability activity, water-insoluble, and can form stool bulk. Cellulose, lignin, and hemicellulose are the main examples of insoluble dietary fiber (Lattimer and Haub, 2010). Not to mention as well, foods that might contain insoluble dietary fiber are wheat bran, brown rice, nuts, beans, some vegetables like cabbage, cauliflower and celery, etc. (Surampudi et al., 2016). Besides that, a study conducted by Sahoo et al. (2012) which had used six millets and cereals as samples revealed that these samples contained a potent hypoglycemic effect. To sum up, these samples could be included in a diet with low carbohydrate and high fiber content - favorable for managing diabetes in both humans and pets.

Fundamentally, insoluble dietary fiber is proven in giving health advantages like weight management, maintaining the health of the gastrointestinal system, decrementing the incidence of diabetes mellitus and so much more. In terms of weight management, it is found that both soluble and insoluble dietary fiber could result in weight loss. But then, the types of diets and types of fiber consumed could be the main factors of this relationship as well. The insoluble dietary fiber could be playing a more significant role during the consumption of high-fat diets (Ötles and Ozgoz, 2014). According to Ötles and Ozgoz (2014), resistant starch is one of the constituents of the dietary fiber which would be subjected to the same digestion as insoluble dietary fiber. Hence, a good understanding of the mechanism of how insoluble dietary fiber could affect weight loss can be achieved through the comparison of insoluble dietary fiber and resistant starch. Additionally, Koh-Banerjee et al. (2004) also stated that numerous studies can be found with the findings of such an inverse relationship between dietary fiber and weight gain.

In addition, a study conducted by Deschasaux et al. (2014) found that dietary fiber, specifically insoluble dietary fiber and legume fiber could reduce the risk of getting prostate cancer. One of the reasons could be from the anti-inflammatory properties. According to Guillon and Champ (2000), insoluble dietary fiber can produce a good amount of butyrate. Therefore, the butyrate will enter the circulation system and exert anti-inflammatory activity (Nilsson et al., 2010). Furthermore, insulin resistance could be lessened with the intake of soluble dietary fiber, insoluble dietary fiber and resistant starch (Manning et al., 2008). This might be due to the decrement of carbohydrate absorption (Higgins et al., 2012) and increment of insulin-like growth factor-binding protein 3 (IGFBP-3) (Probst-Hensch et al., 2003) which could result in decrement of insulin-like growth factor (IGF) activity overall (Arcidiacono et al., 2012). Finally, according to Bosland (2000), a higher risk of getting prostate cancer is associated with the increment of circulating androgenic and estrogenic hormones' concentrations. Therefore, dietary fiber may reduce these hormones' concentrations by increasing the sex hormone-binding globulin (SHBG) and increased excretion of fecal of those hormones which could result from binding to insoluble fibers (Longcope et al., 2000; Ross et al, 1990).

2.3 Prebiotics

Back in the year of 1995, prebiotics was defined as a non-digestible food substance that can affect the consumer's health positively and selectively by stimulating the growth and/or activity of one or a limited number of bacteria in the colon, subsequently improve the consumer's health (Glenn and Roberfroid, 1995). However, there are some conflicts and arguments about this definition as it only allows a few compounds of the carbohydrate groups established as prebiotics like short and long chain of β-fructans, lactulose, and galactooligosaccharide (GOS) (Davanfi-Davari et al., 2019). Therefore, according to Gibson et al. (2010), in the year of 2008, dietary prebiotics is defined 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" by the 6th Meeting of the International Scientific Association of Probiotics and Prebiotics (ISAPP). With the latest definition, prebiotics could be beneficial not only in the colon area but also in the oral cavity, urogenital tract and on the skin (Valcheva and Dieleman, 2016).

Food substance or ingredients could only be established as prebiotics if they have these criteria: (i) it should be resistant to acidic pH of the stomach, cannot be hydrolyzed by mammalian enzymes, and also should not be absorbed in the gastrointestinal tract, (ii) it can be fermented by intestinal microbiota, and (iii) the growth and/or activity of intestinal bacteria can be selectively stimulated by this compound and this process improves host's health (Gibson et al., 2010). So, based on these criteria, all the prebiotics should be resisting hydrolysis by any kind of digestive enzymes and/or should not be absorbed by the upper part of the gastrointestinal system (Roberfroid, 2002). He had also maintained that all the prebiotics must be passed into the large bowel whereby they can modify the colonic microbiota. This is to ensure the prebiotics could help in health-enhancing bacteria, especially lactobacilli and bifidobacteria in becoming numerically and/or metabolically predominant. By this, it could fit the proposed a broader definition of prebiotics by Bindels et al. (2015) which shifted the focus towards ecological and functional characteristics of the microbiota that are likely more relevant for host physiology, such as ecosystem diversity, the support of broad consortia of microorganisms and production of short-chain fatty acids (SCFAs). Examples of prebiotics are resistant starches, pectin, arabinoxylan, whole grains and non- carbohydrate compounds such as polyphenols (Valcheva and Dieleman, 2016).

By the latest, there are quite numerous types of prebiotics currently. Some of them are fructans, galactooligosaccharides (GOS), starch and glucose-derived oligosaccharides, other oligosaccharides and non-carbohydrate oligosaccharides (Davani-Davari et al., 2019). The summary of each type of prebiotic and its explanation is shown in Table 1.

Table 1: Types of prebiotics and their explanations

Types of prebiotics	Explanation	Reference
Fructans	❑ Consist of inulin and fructooligosaccharides. ❑ Can selectively stimulate lactic acid bacteria at an earlier time. ❑ Their chain length is one of the most important criteria to determine which bacteria can ferment them.	Scott et al. (2014)
Galacto-oligo-saccharides (GOS)	❑ Product of lactose extension which can be categorized into two subgroups: - the GOS with excess galactose at C3, C4 or C6. - the GOS manufactured from lactose through enzymatic trans-glycosylation. ❑ Can stimulate *Bifidobacteria* and *Lactobacilli.* ❑ Some GOSs are derived from lactulose which is lactose's isomer. ❑ This lactulose-derived GOS can be considered as prebiotics.	Gibson et al. (2010)
Starch and glucose-derived oligo-saccharides	❑ Resistant starch (RS) is a type of starch that will be resistant to the digestion of the upper gut. ❑ RS could generate a high level of butyrate which can help in promoting health. Hence, it can be classified as prebiotics.	Fuentes-Zaragoza et al. (2011)
Non-carbo-hydrate oligo-saccharides	❑ Cocoa-derived flavanols. ❑ Able to stimulate lactic acid bacteria.	Tzounis et al. (2010)

There are also some studies that have shown that prebiotics could give health benefits to human beings. According to Davani-Davari et al. (2019), prebiotics do not protect against the gastrointestinal tract only, they are useful to the other parts of the body such as the central nervous system, immune system, and cardiovascular system as well. For the immune system, the most common types of cells that will be associated with prebiotics are dendritic cells and epithelial cells (Frei et al., 2015). Moreover, the prebiotics themselves, or the short-chained fatty acids (SCFAs) could modulate the epithelial barrier function, produce antimicrobial peptides and secrete pro-inflammatory mediators (Johnson-Henry et al., 2014; Jiang et al., 2013). As stated by Liu et al. (2010) and Kim et al. (2014), the beneficial effect of prebiotics, probiotics, and synbiotics against diseases such as allergies or colitis is often associated with enhancement of TREG cells.

Prebiotics are also associated with colorectal cancer prevention. On the report of Ambalam et al. (2016), in these past few years, any kind of food supplement or functional food or nutraceutical that are rich in probiotics, prebiotics, and synbiotics have gained enough attention due to their effectiveness as chemopreventive agent especially in the removal of food-borne mutagens and carcinogens. One of the crucial determining factors in colorectal cancer development is the distorted microbial composition in the gut (Ambalam et al., 2016). Evidence suggested that *S. gallolyticus*, *Enterococcus* spp., *B. fragilis*, *F. nucleatum*, and *E. coli* are involved in colorectal cancer initiation and progression. They also found that modulation of gut microbiota with beneficial microbes by probiotics and fecal 2 microbiota transplantation (FMT) and prebiotics would positively influence the cross-talk between the immune system and microbiota and would be beneficial to prevent inflammation and colorectal cancer (Ambalam et al., 2016).

Last but not least, prebiotics are both non-digestible and can be fermented by intestinal microbiota. Hence, what can be observed is their ability to alter the intestinal microbiota composition towards enrichment with microbial groups which can use the prebiotics as a source of energy during their fermentation process (Valcheva and Dieleman, 2016). According to Thibault et al. (2007), patients with inflammatory bowel disease (IBD) might face a problem with their butyrate oxidation due to the impairment in the colonic mucosa. This could be due to the malfunctioning butyrate transport into the epithelial cells during the colonic inflammation. So, prebiotics that particularly aim to improve the butyrate production must be beneficial in reducing colonic inflammation (Valcheva and Dieleman, 2016).

2.4 Pineapple waste powder as a functional ingredient and its beneficial health effects

The massive agricultural-waste products have been generated due to the increment of the pineapple processed items' production because of the selection and elimination of components that are inedible by human beings (Upadhyay et al., 2010). In addition, Nunes et al. (2009) also reported that 55% of product waste could be produced if there were rough handling of fruits and exposure to bad environmental conditions during transportation and storage. Usually, these waste products will be much more exposed to spoilage of microorganisms hence can result in limiting further exploitation (Upadhyay et al., 2010). As stated by Sako et al. (1999), a high amount of unusable waste products that will be produced during pineapple processing. Around 40% up to 80% of pineapple fruit would be discarded as waste (Ban-koffi and Han, 1990).

Furthermore, serious environmental problems, especially for disposal, could happen in the canning industries because they produce large quantities of solid and liquid wastes (Idris and Suzana, 2006).

To combat this environmental problem and support the 12th Sustainable Development Goal (SDG), responsible consumption and production, these pineapple's waste shall be utilized as much as it could be. Not to mention, Upadhyay et al. (2010) stated that discarded fruits, as well as their waste materials, are expected to be utilized for further industrial processes such as fermentation, bioactive component extraction and so much more. There are already numerous works on the utilization of by-products obtained from fruits and vegetables, dairy and meat industries as well. Besides that, isolation of bromelain from pineapple peels could also be one of the alternatives (Ketnawa et al., 2011). In addition, wastes from the pineapple canneries have been used as substrates as well. The substrates mentioned are bromelain, organic acids, ethanol, etc. as these are potential sources of sugars, vitamin and growth factors (Larrauri et al., 1997; Nigam, 1999; Dacera et al., 2009). Beohner and Mindler (1949) had a study conducted whereby they found out that sugar has been gotten from pineapple effluent by ion exchange and further use in syrup for canning pineapple slices.

According to Larrauri et al. (1997), phenolic compounds such as myricetin, salicylic acid, tannic acid, *trans*-cinnamic acid, and *p*-coumaric acid have been identified in the high dietary fiber powder from pineapple shells. Polyphenolic compounds are secondary plant metabolites that exist extensively in the plant kingdom where they have a wide range of different structures (Kammerer et al., 2007). Polyphenolic compounds also have different physiological properties. Some of them are anti-allergenic, anti-atherogenic, anti-inflammatory, anti-microbial, antioxidant, anti-thrombotic, cardioprotective, and vasodilatory effects (Li et al., 2014). They also found that majority of polyphenolic compounds that exist in the pineapple peels are catechin (58.51 mg/100 g dry extracts), epicatechin (50.00 mg/100 g), gallic acid (31.76 mg/100 g), and ferulic acid (19.50 mg/100 g). To sum it up, these four polyphenolic compounds exhibited their antioxidant capacities with structure-activity relationships (Li et al., 2014).

Beyond that, pineapple waste powder, specifically the pineapple peel powder is also good for growth improvement and retention of viability of *L.acidophilus* (ATCC 4356), *L.casei* (ATCC 393), and *L.paracasei* spp. *paracasei* (ATCC BAA52) observed during their storage in the refrigerator at 4°C for 28 days straight in synbiotic yogurt formulation with added pineapple peel powder (Sah et al., 2015). This might be due to the faster lowering of pH by pineapple peel powder and selected probiotics that had been added to the milk. This suggested a much higher rate of acid production compared to the control yogurt. According to Damasceno et al. (2016), low pH might lead to a lower risk of food deterioration by the microorganisms, enzymes or any kind of non-enzymatic reactions. Not to mention, pineapple powders also contain dietary fibers, proteins, and minerals, including divalent cations, and serve as growth factors or growth promoters for probiotics in the yogurts (Diaz-Vela et. al, 2013). Higher proteolytic activities could be found due to the mineral-rich pineapple peel powder. This could likely stimulate bacterial enzymes (Sah et al., 2015). Finally, the water-soluble peptide extracts (WSPE) of the probiotic yogurt with the pineapple peel powder exhibited the most potent antimutagenic and antioxidant activities (Sah et al., 2015).

Last but not least, pineapple peel powder could also be a useful alternative as a raw material to produce cereal bars, as it has substantial amounts of crude fiber. This could lead to an improvement of the nutritional properties of the final products (Damasceno et al., 2016). Even though the pineapple waste mostly is hard and irregular, they are certainly fruitful for various culinary purposes as well - teas, juices, and candy. This is because they are very rich in vitamin A, C, B complex, calcium, iron, potassium, and fibers containing significant amounts of insoluble fibers. (Damasceno et al., 2016). Due to this, it could help in constipation, and promote feelings of satiety, thus being considered as a functional food (Slavin, 2013). Since pineapple waste (peels, stems, crowns, and pineapple cores) contain high amounts of pectin, it results in high sugar content. Then, the waste of pineapple, specifically its peel could also be used to make vinegar, alcohol and animal feed (Roda et al. 2014; Tropea et al. 2014). As for cereal bars, the pineapple peel flour provided good technological characteristics to the cereal bars, such as lower pH and higher acidity, contributing to the microbiological quality of the bars (Damasceno et al., 2016). Hence, this can make the product's shelf life increase.

CHAPTER 3

METHODOLOGY

3.1 Sample selection and sampling method

The pineapple's variety that had been used in this study was MD2. Two different types of pineapple by-products were analyzed in this study which were the crowns and the peels or shells. Basically, a bunch of harvested pineapples' peels and crowns were collected freshly from Revenue Collection Center, Serdang Agriculture Department, Selangor by using a convenient sampling. All the recent pineapples' peels and crowns were collected and transported to the Nutrition Laboratory in the Faculty of Medicine and Health Sciences, Universiti Putra Malaysia for storage. All data were recorded in the duplicates of three.

3.2 Reagents and chemicals

The reagents used in this experiment were dietary fiber assay kit (α-amylase, protease, amyloglucosidase), celite, 5% hydrochloric acid (HCl) solution, 5% sodium hydroxide (NaOH) solution, 0.56M hydrochloric acid (HCl) solution, 0.05% hydrochloric acid (HCl) solution, 95% ethanol, 78% ethanol, acetone, 5% phenol solution, 2 units of α-amylase solution, simulated gastric juice, glucose standard solution, concentrated sulphuric acid solution, and distilled water. Both the chemicals and reagents were obtained from the Nutrition Laboratory in the Faculty of Medicine and Health Sciences and some were purchased from authorized local resources.

3.3 Sample preparation

All the pineapple peels and crowns which were collected freshly were cut into smaller pieces by using knives. After doing so, the pineapple peels and crowns that had been cut were homogenised, whereby both pineapple peels and crowns had been placed separately on two different trays and mixed together according to their types. Then, the samples were kept in sealable bags and pre-stored at -20℃ for around a week before they were oven-dried at 50℃ for four days. The samples were then grounded into fine powders by using a laboratory grinder. All the samples were then weighed by using a weighing scale (AC Adapter, Japan) before they were all getting packed again into sealable bags and stored at -20℃ for further analysis. Afterwards, the oven-dried samples were weighed again and used for determination of total dietary fiber, insoluble fiber, soluble fiber and functional properties of banana peel powders as illustrated in Figure 3.

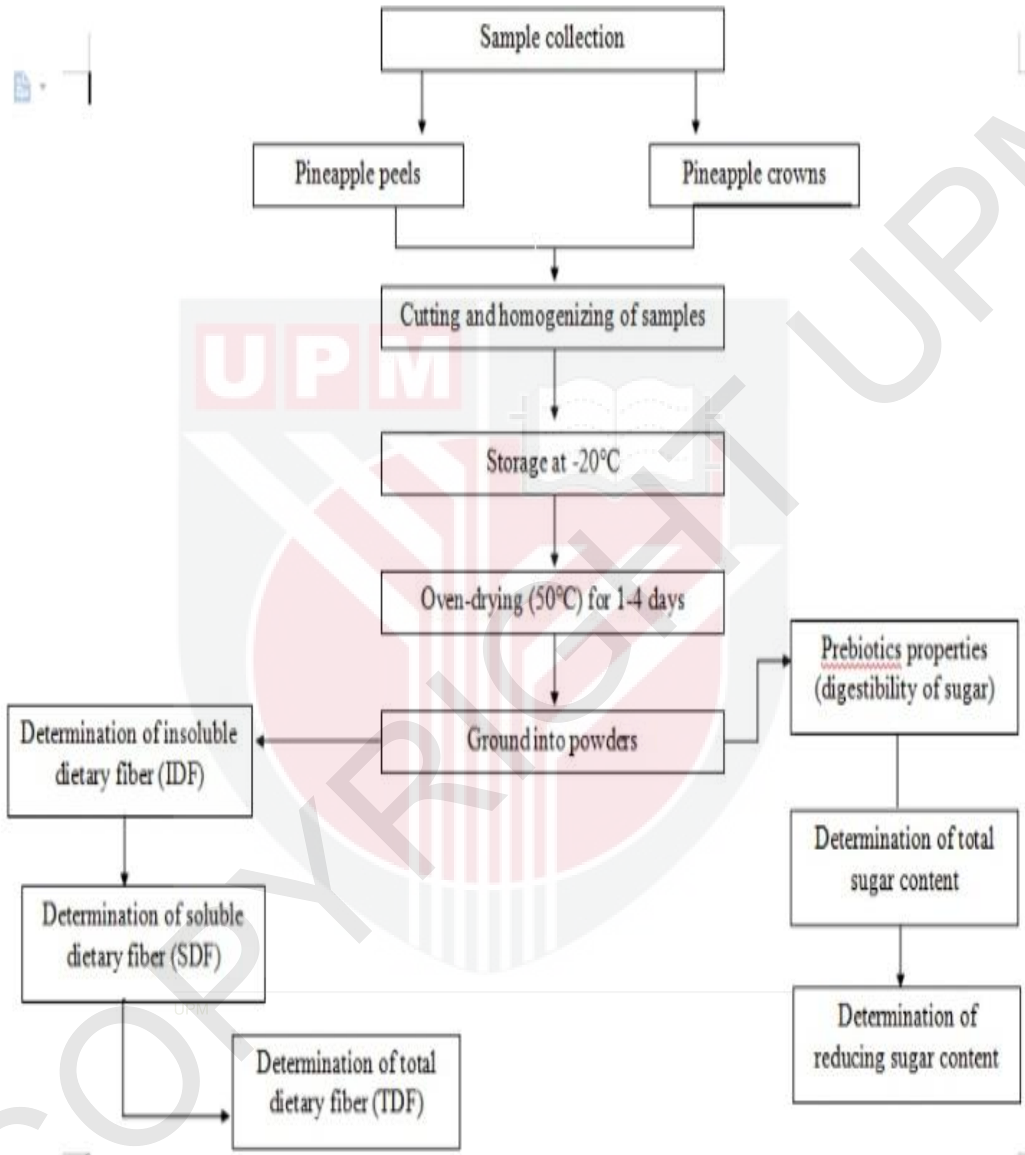

Figure 3: Sample preparations and nutrient analysis

3.4 Determination of dietary fiber content

3.4.1 Insoluble dietary fiber content

Fundamentally, enzymatic-gravimetric procedure by using Prosky and Lee method (AACC Method 32-07.01) was used in order to analyse the total dietary fiber (TDF), insoluble dietary fiber (IDF) and soluble dietary fiber (SDF) content in the pineapple peel and crown samples. A total dietary fiber assay kit (TDF-100A; Sigma-Aldrich, St. Louis, Missouri, USA)was used.

Briefly, 1g of pineapple waste powder was incubated with a heat-stable α-amylase solution for 30 minutes. After that, all the samples were cooled down to 60°C before they were incubated with protease solution for 30 minutes as well. 0.56M HCl solution was dispensed in each sample prior to checking the pH of each sample. Next, all the samples were incubated with amyloglucosidase. As for insoluble dietary fiber, all the enzyme mixtures were filtered into a filtration flask. Then, the residue was washed with 78% ethanol, 95% ethanol and acetone in a slight vacuum. Consequently, the filtrate and residue were transferred to new, different flasks which would be used to determine soluble dietary fiber soon. Then, all the residues in the crucible were dried in the oven overnight. Subsequently, the dried crucible would be cooled in a desiccator for approximately 1 hour before the protein and ash could be determined.

3.4.2 Soluble dietary fiber content

For soluble dietary fiber, the filtrates and residue from the previous insoluble dietary fiber were weighed to the nearest 0.1 g. After that, the combined solution was added with pre-heated 95% ethanol before it was precipitated for 1 hour at room temperature. Then, the precipitated enzyme digest was filtered by transferring to crucibles and washed with 95% ethanol, 78% ethanol and acetone in a slight vacuum. The crucible then dried overnight before it was cooled in a desiccator for 1 hour. Lastly, protein and ash content in the samples were determined.

3.4.3 Total dietary fiber content

Total dietary fiber for both pineapple peel powder and pineapple crown powder were obtained by addition of insoluble dietary fiber content in milligram (mg) with soluble dietary fiber content in milligram (mg).

3.4.4 Calculation

3.4.4.1 Insoluble and soluble dietary fiber content

$$\text{Dietary fiber (DF, g/100 g)} = \frac{R-P-A-B}{M} \times 100$$

Where:

R = residue weight in mg

P = average protein weight in mg

A = average ash weight in mg

B = average blank weight in mg

M = average sample weight

3.4.4.2 Total dietary fiber content

$$\text{Total dietary fiber (TDF, g/100 g)} = \text{IDF} + \text{SDF}$$

Where:

IDF = insoluble dietary fiber content in mg

SDF = soluble dietary fiber content in mg

3.5 Prebiotics functionalities

3.5.1 Enzyme preparation

3.5.1.1 2 units α-amylase solution

In order to prepare 2 units of α-amylase solution, 3.35 mg of α-amylase from 30 U/mg Aspergillus oryzae by Sigma-Aldrich was added to 50 ml of phosphate buffer solution. After that, the pH was adjusted to pH 6.0 by using 5% of NaOH solution and 5% of HCl solution.

3.5.1.2 Simulated gastric juice

Simulated gastric juice was prepared by using sodium phosphate buffer containing 4 g/l of sodium chloride (NaCl), 0.1 g/l of potassium chloride (KCl), 4.125 g/l of sodium phosphate dibasic dihydrate (Na2HPO4-2H2O), 7.175 g/l of disodium hydrogen phosphate (Na2HPO4), 0.05 g/l of calcium chloride dihydrate (CaCl2-2H2O), and 0.09 g/l of magnesium chloride hexahydrate (MgCl2-6H2O) (Korakli, Ganzle, and Vogel, 2002). Then, the pH was adjusted to pH 2.0 by using 5% of NaOH solution and 5% of HCl solution.

3.5.2 Digestibility of sugar content

Sample was dissolved with distilled water to make 1% (w/v) solution. Then, 5 ml of the enzyme solution was added to 5 ml of diluted sample solution. The mixture was then mixed by using vortex. Consequently, different procedures had to be used to determine initial reducing sugar, final reducing sugar, and total sugar contents.

3.5.2.1 Initial reducing sugar content

Once the mixture had been mixed with vortex, 1 ml of the solution from the previous mixture was taken into a test tube. The solution was then added with 1 ml of DNS acid solution followed by 1 ml of distilled water. After that, the mixture was mixed by using vortex again. Next, the solution was boiled in a 100°C water bath for 15 minutes. To avoid further reaction took place, the test tube was then cooled in ice for 2 minutes. 9 ml of distilled water was added prior to the spectrometer readings taken at 540 nm absorbance. Seven different concentrations of glucose standard solutions (0.015, 0.03125, 0.0625, 0.125, 0.25, 0.5, and 1mg glucose/mL) had been prepared to generate the standard curve for glucose equivalents.

3.5.2.2 Final reducing sugar content

After the mixture had been mixed by using a vortex, it had been incubated in a water bath at 37°C for about 6 hours. Next, 1 ml of the incubated solution would be taken into a new test tube to analyze the final reducing sugar content by using the DNS acid method. 1 ml of DNS acid solution and 1 ml of distilled water were then added to the same, new test tube before boiling it in the 100°C water bath for 15 minutes. Then, it would be cooled in ice for 2 minutes to avoid any kinds of further reactions. Subsequently, 9 ml of distilled water was added to dilute the solution. Finally, spectrometer reading was taken at the absorbance of 540 nm. Note that seven different concentrations of glucose standard solutions had been prepared to generate the standard curve for equivalents of glucose.

3.5.2.3 Total sugar content

Following the mixture by using a vortex, the solution would then be incubated in a 37°C water bath for 6 hours. After 6 hours, 1 ml of the sample solution was taken and added with 1 ml of 5% phenol solution. The solution would then be added with 5 ml of concentrated sulphuric acid solution. Then, the solution had been left for about 10 minutes prior to mixing it by using a vortex. Once it was being mixed with vortex, it would be left at room temperature for 20 minutes before the spectrometer reading was taken at 490 nm absorbance, whereby the blank used was 0.05% of HCl solution. Seven different concentrations of glucose standard solutions had been prepared to generate the standard curve for equivalents of glucose. Finally, the total sugar content was obtained by a comparison with the glucose' standard curve mentioned.

3.5.2.4 Calculation

$$\text{Hydrolysis yield} = \frac{R}{T - I} \times 100$$

Where:

R = reducing sugar released

T = total sugar content

I = initial reducing sugar content

3.6 Statistical analysis

Data entry and analysis of the dietary fiber contents and the digestibility of sugar content were executed by using Statistical Package for the Social Sciences (SPSS) Version 23.0. The objective of the analysis was to find out the difference in dietary fiber content and digestibility of sugar contents between the pineapple peel powder and the pineapple crown powder. Independent t-test was used to compare the nutrient content and dietary fiber content and digestibility of sugar contents between the pineapple peel powder and the pineapple crown powder. Significance level was set at $p<0.05$.

CHAPTER 4

RESULTS AND DISCUSSION

4.1 Total Dietary Fiber Content

Total dietary fiber (TDF) content was determined by using the gravimetric-method by the TDF assay kit (TDF-100A; Sigma-Aldrich, St. Louis, Missouri, USA). Starch and protein of both pineapple peel and crown powders were removed by digesting them with a heat-stable α-amylase (A 3306), protease (P 3910), and amyloglucosidase (A 9913) accordingly. As for insoluble dietary fiber (IDF), it was recovered by filtration method while soluble dietary fiber (SDF) was precipitated and filtered with ethanol. All dietary fibers were collected first for the drying process in the oven, before being corrected for residual protein, ash, and blank.

Table 2: Results of independent sample t-test on the total dietary fiber content

Sample	Mean (%) (n=2)	SD	*t*-value	*p*-value
Pineapple peel powder (PPP)	41.86	0.81	-8.039	0.015
Pineapple crown powder (PCP)	50.21	1.22		

Significantly different at *p*-value <0.05

As shown in Table 2, it is found that pineapple crown powder (PCP) yielded a higher total dietary fiber (TDF) content compared to pineapple peel powder (PPP) where the significant difference ($p<0.05$) was obtained. By comparison to studies in the literature, PCP had the highest amount of TDF compared to cabbage outer leaves and sweet potato leaves. The cabbage outer leaves contained 41.83% of TDF (Jongaroontaprangsee et al., 2007). On the contrary, Beniazuma sweet potato leaves yielded about only 6.90% and Koganesengan sweet potato leaves contained 5.94% of TDF (Ishida et al., 2000). Nonetheless, several studies found that PPP had a significant amount of dietary fiber, protein, minerals, and that were capable to enhance the growth of tested probiotic *Lactobacilli* (Diaz-Vela et al., 2013). In addition, PPP had a way better sensory properties compared to commercial dietary fibers from apple and citrus fruits (Upadhyay et al., 2010).

There are many studies that have shown the advantages of taking dietary fibers in everyday meals. Dietary fibers are defined as non-digestible carbohydrates that have monomeric units innate in food and secluded or synthetic fibers with proven physiological benefits (Thompson et al., 2017). According to Holscher (2017), dietary fibers are generally heterogenous. It means that they can be further classified differently to describe each one of them like its origin, chemical composition or even its physicochemical properties. The physicochemical properties mentioned include their physicochemical properties, such as their solubility, viscosity, and fermentability would bring about a therapeutic consumption effect (Mcrorie and k, 2013). Furthermore, dietary fiber could be categorized as soluble dietary fiber (SDF) and insoluble dietary fiber (IDF). SDF usually is fermented in the colon. For instance, pectin. On the other hand, IDF has a bulking action with a limited fermentation in the colon (Otles and Ozgoz, 2014).

Table 3: Dietary fiber content of pineapple peel from this study and few other studies

	References			
	Present study	**Larrauri et al. (1997)**	**Huang, Chow and Fang (2011)**	**Diaz-Vela et al. (2013)**
Origin	**Serdang, Selangor**	**Cuba**	**Taiwan**	**Ecatepec, Mexico**
Species/ variety	MD 2	Red Spanish	Tai-Nong-17	-
TDF content (%)	41.86	70.6	42.2	62.54

Based on Table 3, it was found that all the studies mentioned above had a varying TDF content which suggested that TDF content could be influenced by some factors such as the location, specifically the climatic conditions and the species of the pineapple used. For instance, Jonasson et al. (1986) concluded that the phenols and nutrient content of crops could be different in accordance with the climatic fluctuations of the location as all the studies had been conducted at different places. As for other species of fruits, Martí et al. (2011) reported that different varieties of tangerine might produce different dietary fiber contents where Oroval variety had a higher fiber content in the peel compared to its core while the Hernandina variety had a higher fiber content in the frit than in the peel. Therefore, both climatic conditions and the pineapple variety used may contribute to different TDF content and this explanation can further support our finding on TDF.

It is of great interest that both of these samples could be of significance and profitable to the food market in the view of their potential use as a functional ingredient in dietary fiber products, particularly PCP. Therefore, agricultural wastes of pineapple like the peel and the crown could be incorporated into foods, which is likely to be high-fiber foods as these wastes could be a potential source of ingredients for foods to enrich their nutritional value. Moreover, high dietary fiber intake could lower the risk of getting large bowel cancer associated with gut microbiota modulation as well as the production of butyrate (Chen and Vitetta, 2018).

4.2 Insoluble Dietary Fiber Content

Table 4: Results of independent sample t-test on the insoluble dietary fiber content

Sample	Mean (%) (n=2)	SD	*t*-value	*p*-value
Pineapple peel powder (PPP)	40.14	0.53	-15.820	0.004
Pineapple crown powder (PCP)	48.53	0.53		

Significantly different at *p*-value <0.05

Based on table 4, the IDF content of PCP was higher compared to PPP and the IDF content was significantly different (p<0.05) between both samples. Compared to the outer cabbage leaves, the IDF content of PCP was higher as the cabbage outer leaves only yielded around 37.19% of IDF (Jongaroontaprangsee et al., 2007). In another study, Larrauri et al. (1997) added that PPP had insoluble dietary fiber-rich fractions with considerable potential and application in food development, which were low in calories and could be used to enhance the quality of dietary fiber. Besides, the authors revealed that dietary fiber powder that was coming from the Red Spanish pineapple peels contained about 70.6% of TDF (Larrauri et al., 1997). Likewise, Gorinstein et al. (2001) reported that peels of some other fruits like citrus fruits had IDF as the largest fiber fraction. In fact, Sharoba et al. (2013) showed that orange waste, carrot pomace, potato peels, and green pea peels could also be utilized as good raw material to produce dietary fiber powder as they contained a good amount of IDF.

The high IDF content could be probably due to good amounts of cellulose, hemicellulose and lignin, which are the types of IDF that can be found in both PPP and PCP (Lattimer and Haub, 2010). Besides, pineapple crowns were mainly made up of leaves, where the leaves were found to have 19.6%, 71.0% and 2.2% hemicellulose, cellulose and lignin respectively while the peels were made up of 28.7% hemicellulose, 40.6% cellulose & 10.0% lignin (Asim et al., 2015; Pardo et al., 2014). As such, this could be a possible explanation of high IDF content in PCP compared to PPP. In terms of its function and beneficial health effects, IDF was found to be good in suppressing the increment of the post-prandial blood glucose level (Takano et al., 2013) and decrement of prostate cancer risk (Deschasaux et al. (2014). Hence, it is important for food industries to include this type of fiber in their food products nowadays.

4.3 Soluble Dietary Fiber Content

Table 5: Results of independent sample t-test on the soluble dietary fiber content

Sample	Mean (%) (n=2)	SD	*t*-value	*p*-value
Pineapple peel powder (PPP)	1.72	0.28	0.076	0.947
Pineapple crown powder (PCP)	1.68	0.69		

Significantly different at *p*-value <0.05

Table 5 shows a result of an independent t-test analysis and the mean difference of SDF contents was not significantly different (p>0.05) between PCP and PPP. Nevertheless, PPP yielded a slightly higher amount of SDF as opposed to PCP. It can be speculated that both samples had low SDF. A possible explanation could be due to lack of pectin, which is one of the types of SDF in both samples (Shin, 2012). In a study by Madhav and Pushpalatha (2002), pineapple peel and cocoa pod husk had low amounts of pectin. Although Asim et al. (2015) reported that pineapple leaves fiber could provide such a good amount of dietary fiber, the pectin content was only 2.3%, representing the SDF part of the dietary fiber. It is possible that the low content of SDF in pineapple is due to the low content of pectin as explained earlier.

The consumption of SDF is beneficial to health as it could aid in reducing lipid levels, lowering blood pressure, weight loss, and reducing inflammation (Surampudi et al., 2016). To the best of knowledge, numerous studies showed that fruit wastes like their peels and leaves usually contained a substantial amount of IDF in contrast to SDF fractions. In particular, a study by Ajila et al. (2010), showed that the TDF content in mango peels powder (MPP) was 51.2%, in which the SDF only constituted for about 19.0% while IDF contents of the MPP was 32.1% making it the majority fraction of dietary fiber. Hence, it is safe to say that fruit wastes generally contain higher amounts of IDF content in comparison to the SDF content. This is due to the higher composition of IDF, which are mainly composed of cellulose, hemicellulose and lignin in the fruits wastes in comparison to the SDF composition such as gums, pectins and mucilage. Therefore, the results of IDF and SDF in the present study were in accordance with the previous studies published in the literature.

4.4 Resistance towards α-Amylase Hydrolysis

In order to study one of the prebiotics' properties, which in this research is the ability to resist the host digestion, both PPP and PCP were mixed with the α-amylase enzyme. In this study, α-amylase from *Aspergillus oryzae* by Sigma was used. All the samples were mixed in the test tubes and agitated during the incubation to mimic the human's peristalsis movement in the gastrointestinal tract. The pH was set at pH 6.0 as instructed by the brand to get the most optimum activity despite the fact that the human's saliva has a normal pH range at 6.2 to 7.6 (Baliga et al., 2013). The temperature was set at 37 °C, which is the normal human's body temperature. In fact, the activity of α-amylase enzyme was found to be the most effective at a temperature of 37°C (Divakaran et al., 2011). The samples had been incubated for 6 hours as the human's digestion process would usually take about 6 to 8 hours. In fact, Wichienchot et al. (2010) concluded that maximum hydrolysis by α-amylase enzyme occurred after 4 hours before it went stagnant and saturated. In this experiment, the exposure of the samples towards α-amylase enzymes was done *in vitro* to simulate the environment of digestion process in the mouth.

Table 6: Results of independent sample t-test on the α-amylase enzyme

Sample	Mean (%) (n=2)	SD	*t*-value	*p*-value
Pineapple peel powder (PPP)	13.55	2.50	5.507	0.031
Pineapple crown powder (PCP)	3.46	0.67		

Significantly different at *p*-value <0.05

According to table 6, PCP had a lower amount of hydrolysis (3.46%) compared to PPP (13.55%) which yielded about 96.54% and 86.45% of the remaining products accordingly. The result was significantly different ($p<0.05$) between both samples. Based on a study conducted by Wichienchot et al. (2010), about 84% of the samples consumed shall reach the stomach as 16% of the samples would be hydrolyzed by the α-amylase enzyme. Besides, Cummings and Macfarlane (2002) also showed that about 88% of inulin and oligofructose would eventually reach the colon by using the ileostomy patients' model and the intubation model. Hence, the current study is in line with the previous study as the samples obtained lower amounts of hydrolysis.

4.5 Resistance towards Artificial Human Gastric Juice Hydrolysis

Table 7: Results of independent sample t-test on the simulated gastric juice

Sample	**Mean (%) (n=2)**	**SD**	***t*-value**	***p*-value**
Pineapple peel powder (PPP)	4.75	2.89	-1.412	0.293
Pineapple crown powder (PCP)	8.79	2.83		

Significantly different at *p*-value <0.05

Based on table 7, PPP had a lower amount of hydrolysis (4.75%) compared to PCP (8.79%) which yielded about 95.25% and 91.21% of the remaining products accordingly. However, the result was not significantly different ($p>0.05$) between the samples. It can be implied that both of the samples had similar resistance towards the simulated gastric juice. Both of the samples had less than 10.00% of digestibility which might be due to the presence of other biocompound in the samples like oligosaccharides and strong interlink bonds between the structures. Selani et al. (2014) reported that oligosaccharides could not be digested as they contained glycosidic linkages. To be more specific, Nakada et al. (2003) found that the high resistance of α(1-2) bonds to *in vitro* and *in vivo* gastrointestinal digestion had been shown in previous studies. Moreover, TDF could also be the key ancestral compounds that aid in the gut ecology preservation as most prebiotics typically consisted of dietary fiber and oligosaccharides (Makki et al., 2018; Patel and Goyal, 2012).

On the contrary, the simulated gastric juice represented the digestion process inside the human stomach. Human stomach has the function of storing and softening the food which would start at such early phases of the food digestion itself, basically (Haschek et al., 2010). Besides that, human stomach contains gastric juice that is responsible for microorganism's inactivity (Martinsen et al., 2019). Martinsenet al. (2019) also reported that gastric juice consisted of hydrochloric acid (HCl), pepsin, and lipase, which are essential ingredients for protein and fats hydrolysis, respectively.

In comparison to α-amylase enzymes, gastric juice, which contained the pepsin enzyme, tend to have the highest activity at temperatures ranging from 25°C to 30°C with the pH 1.6 (Kihara, 2015). However, in this study, the temperature was set at 37°C in order to imitate the real environment of the human digestion process in the stomach. Wichienchot et al. (2010) also found that maximum hydrolysis of the artificial gastric juice would occur after 2 hours of incubation at pH value of 1.0. This is due to the retention of food in human's stomach, whereby the gastric juice would be released in 2 hours-time. The incubation had been done for straight 6 hours in this study because the whole process of human digestion would take about 6 up to 8 hours that runs from mouth to the anus.

Table 8: Hydrolysis of samples towards simulated gastric juice from previous studies

			References		
	Present study		Nakada et al. (2003)	Wichienchot et al. (2006)	Wichienchot et al. (2010)
Types of prebiotics present	**Xylo oligo-saccharides**	**-**	**Kojio oligo-saccharides**	**Gluco oligo-saccharides**	**Oligo-saccharides**
Sources	PPP	PCP	Kojibiose phosphorylase	*Gluconobacter oxydans*	Dragon fruits flesh
Hydro-lysis amount (%)	4.75	8.79	0.0	1.60	2.43

According to table 8, Nakada et al. (2003) found that 100% of kojiooligosaccharide are resistant towards the artificial gastric acid. Other than that, 98.4% of gluco-oligosaccharides were resistant to the acidic conditions of the human stomach (Wichienchot et al., 2006). Then, Wichienchot et al. (2010) found that the Pitaya oligosaccharides in the dragon fruits flesh was resistant towards the simulated gastric juice by 97.57%.

Overall, the digestibility for PCP and PPP were 12.25% and 18.30% respectively. It means that 87.75% of the reminding PCP and 81.70% of the reminding PPP will reach the colon without being digested at all. Also, it was found that PCP had significantly higher resistance towards α-amylase enzymes compared to PPP generally. Therefore, both PCP and PPP could not be digested by α-amylase enzyme and the simulated gastric juice, which found to be one of the prebiotic criteria which it must be resistant to acidic pH of stomach, cannot be hydrolyzed by mammalian enzymes, and also cannot not be absorbed in the gastrointestinal tract (Davani- Davari et al., 2019). So, both samples had the potential to become sources of prebiotics.

4.6 Correlation between Dietary Fiber and Enzymatic Resistance

Table 9: Results of Pearson's correlation analysis between dietary fiber with α-amylase enzyme and artificial gastric juice

Digestibility	TDF	IDF	SDF
	Pineapple Peel Powder (PPP)		
α-amylase	-0.986*	-0.984*	-0.099
Artificial gastric juice	0.576	0.641	-0.686
	Pineapple Crown Powder (PCP)		
α-amylase	-0.986*	-0.984*	-0.099
Artificial gastric juice	0.576	0.641	-0.686

*significantly different at p-value <0.05

Table 9 shows that IDF and TDF were significantly and negatively correlated with the sample resistance towards α-amylase enzyme activity. IDF originally does not dissolve in the water and it does not absorb water. This might result in IDF passing through the gastrointestinal tract without being affected at all. So, it would come out as its original form. Lattimer and Haub (2010) mentioned that cellulose, lignin and hemicellulose were the main examples of IDF which could never be digested by human's body. Hence, this might be the reason the samples could not be hydrolysed by both enzymes thoroughly as both of them contained high amounts of IDF. Importantly, what can be seen here is that the higher the IDF and TDF content, the lower the hydrolysis yield which means that it has a higher ability to resist the enzymatic activities. Therefore, both PCP and PPP have the potential to become good resources of prebiotics.

Nowadays people are more aware of the benefit of consuming dietary fiber. Consumers tend to search for the production of new foods with high dietary fiber content and today they are more feasible to find in the market (Elleuch et al., 2011). Besides, several studies had shown the link between dietary fiber and prebiotics potential. As an example, Slavin (2013) reported that not all dietary fibers are prebiotics but all prebiotics come from dietary fibers. Hence, dietary fiber consumption may provide prebiotics to one's diet.

Furthermore, the results of this study also showed that agricultural waste is a good potential source of prebiotics. Indeed, Sah et al. (2015) showed that PPP added to yogurt could affect the texture and rheological properties of it, which was similar to one of the established prebiotics, inulin. Besides that, Huang et al. (2014) reported that xylan and xyloglucan were the major parts of hemicellulose contained in pineapple peel. It is proven by a lot of clinical trials and lab studies that xylan could be beneficial for the guts and also its health benefits as a prebiotic in general (Singh et al., 2015). Also, xylooligosaccharide (XOS) seems to be a promising source of prebiotics as it could be obtained from the agricultural crop residues which found to be way cheaper, abundant, and of course, renewable in nature too (Samanta et al., 2015).

CHAPTER 5

CONCLUSION AND RECOMMENDATIONS

5.1 Conclusion

In this study, it was found that MD2 pineapple peel powder contained 41.86% TDF, 40.14% IDF, and 1.72% SDF. On the other hand, MD2 pineapple crown powder contained 50.21% TDF, 48.53% IDF, and 1.68% SDF. As for the hydrolysis by α-amylase enzyme, 3.46% of pineapple crown powder was hydrolyzed while 13.55% of pineapple peel powder had been hydrolyzed. In addition, for the hydrolysis by the simulated gastric juice, pineapple peel powder had been hydrolyzed by 4.75%, whereas pineapple crown powder had been hydrolyzed by 8.79%. Overall, 18.3% and 12.25% of PPP and PCP, respectively were digested by α-amylase and gastric juice, which means that more than 80% can reach colon for further fermentation by the commensal microbiota. Regardless of the samples, it was found that TDF and IDF were negatively and significantly associated between pineapple peel ($r = -0.986$, $p= 0.014$) and crown ($r = -0.984$, $p= 0.016$) respectively with the α-amylase digestibility.

The first hypothesis of this study can be accepted for TDF and IDF only as SDF was not significantly different between the pineapple peel powder and the pineapple crown powder. Studies in the literature showed that most agricultural wastes, including pineapple peel and crown had high amounts of cellulose, hemicellulose and lignin, where these are considered as IDF (Asim et al., 2015; Lattimer and Haub, 2010). In contrast, some studies reported that pectin, a type of SDF was found to be low in both pineapple peel powder and pineapple crown powder (Madhav and Pushpalatha, 2002; Asim et al., 2015). Hence, that might be why the content of SDF in both samples was not significantly different. Then, the second hypothesis could be accepted in this study because both of the samples tend to show a high resistance towards the activity of the α-amylase enzyme and the gastric juice in-vitro, as both of the samples only had less than 10% of digestibility after they had been exposed towards α-amylase enzyme and the artificial gastric juice. However, for the third hypothesis, it showed that there was a negative correlation between the TDF and IDF of both samples with the α-amylase enzyme, but not with simulated gastric juice. Hence, the third hypothesis is rejected. As for IDF, it might be due to the presence of cellulose, hemicellulose, and lignin in both samples and it is found that they could not be digested by the human's body (Lattimer and Haub, 2010).

Therefore, both pineapple peel powder and pineapple crown powder should be taken into consideration for incorporation into foods as both of them contained dietary fiber, specifically IDF which is more likely to be the high-fiber foods as these pineapple wastes had the potential of becoming a good source of ingredients in enriching and nourishing the nutritional value of a particular food. In addition, the consumption of IDF could aid in reducing colorectal and prostate cancer risk. Other than that, IDF could also help in suppressing the increment of post-prandial blood glucose levels as well. Furthermore, it appeared that both pineapple peel powder and pineapple crown powder would be a good source of prebiotics as well as they fulfilled the first criteria of a prebiotic which is the ability to resist the host digestion, since more than 80% of both samples had been resistance towards the α-amylase enzyme and the simulated gastric juice. Also, prebiotic consumption can help in the regulation of appetite and the reduction of circulating postprandial glucose and insulin concentrations. It also helps in the increment of the population of protective microorganisms in the host's body which consequently boosts the immune function overall. Hence, it is crucial for pineapple peel powder and the pineapple crown powder to be incorporated in the foods as people are becoming more health-conscious today.

5.2 Recommendations

One of the limitations of this study is that it only focuses on dietary fiber and prebiotics, whereby pineapple peel and pineapple crown have other beneficial nutrients. There are numerous studies regarding the bromelain enzyme contained in the pineapple crown that can be further utilized in the future study. Besides, findings in the literature showed that pineapple waste could be used as substrates for organic acids, ethanol, etc. since they are potential sources of growth factors, vitamins, and also sugars (Larrauri et al., 1997; Nigam, 1999; Dacera et al., 2009). Therefore, further research and analysis should be done on these particular aspects in order to provide additional nutritional information to the particular food products that might incorporate the pineapple waste powder. This study also does not extract the oligosaccharides that present in the samples. So, future research should have included the extraction of oligosaccharides in the samples since literature reviews found that the pineapple peel contains xylooligosaccharides (Huang et al., 2014).

Other than that, this study also focuses mainly on the first criteria of prebiotics only, the resistance towards the host's digestion whereby, prebiotics have other two criteria which are the ability to ferment by the intestinal microorganisms and also the ability to stimulate the growth or the activity of the intestinal bacteria selectively in association with health and well-being. Therefore, it is safe to say that both samples could not be established as prebiotics yet until further research on the other two criteria are conducted. Hence, it is strongly recommended that further analysis should be conducted to discover whether these pineapple wastes could be utilized as prebiotics or not.

Next, this study only focuses on the mouth and stomach compartments for the digestibility test and it did not include the small intestine part. So, this study does not comprise the complete human digestion process as there are presence of enzymes at the brush-borders of the small intestine (Wichienchot et al., 2010). Hence, future research should test on the digestion at the small intestine in order to get the overall enzymatic resistance of the samples. Moreover, the speed of the agitation process during the incubation for 6 hours does not stimulate the exact peristalsis process. Therefore, it is suggested that future research should use the model of in-vitro digestion as it could imitate the real human digestion process, like the Dynamic in vitro GastroIntestinal model, a high-end dissolution and digestion apparatus “TIM".

Finally, future studies should be conducted with more species or varieties of pineapple as there are about nine cultivars of pineapple in Malaysia. This causes the results obtained are not representable and cannot be generalized for all the pineapple compositions in this country. So, it is recommended that different varieties of pineapples across the country must be included in future research hence the results obtained can be used widely without being biased towards only a variety of pineapples. Besides that, the data collected can be considered representative data as well.

REFERENCES

Abd-Halim, N. (2016). Policy Intervention for the Development of the Pineapple Industry in Malaysia. *Food and Fertilizer Technology Center for the Asian and Pacific Region*, *2015*, 65.

Agamuthu, P., Khidzir, K. M., & Hamid, F. S. (2009). Drivers of sustainable waste management in Asia. *Waste Management and Research*, *27*(7), 625–633. https://doi.org/10.1177/0734242X09103191

Ajila, C. M., Aalami, M., Leelavathi, K., & Rao, U. J. S. P. (2010). Mango peel powder: A potential source of antioxidant and dietary fiber in macaroni preparations. *Innovative Food Science & Emerging Technologies, 11*(1), 219–224. doi:10.1016/j.ifset.2009.10.004

Al-Busaidi, K. A. (2010). *No Titleتوظيف المكتبات الأكاديمي. 2024*(45), 39. Retrieved from https://www.nber.org/papers/w15827.pdf

Ambalam, P., Raman, M., Purama, R. K., & Doble, M. (2016). Probiotics, prebiotics and colorectal cancer prevention. *Best Practice and Research: Clinical Gastroenterology*, *30*(1), 119–131. https://doi.org/10.1016/j.bpg.2016.02.009

Anderson, J. W., Baird, P., Davis, R. H., Ferreri, S., Knudtson, M., Koraym, A., … Williams, C. L. (2009). Health benefits of dietary fiber. *Nutrition Reviews, 67*(4), 188–205. https://doi.org/10.1111/j.1753-4887.2009.00189.x

Arcidiacono, B., Iiritano, S., Nocera, A., Possidente, K., Nevolo, M. T., Ventura, V., … Brunetti, A. (2012). Insulin resistance and cancer risk: An overview of the pathogenetic mechanisms. *Experimental Diabetes Research*, *2012*. https://doi.org/10.1155/2012/789174

Asim, M., Abdan, K., Jawaid, M., Nasir, M., Dashtizadeh, Z., Ishak, M. R., … Deng, Y. (2015). A review on pineapple leaves fibre and its composites. *International Journal of Polymer Science, 2015*. https://doi.org/10.1155/2015/950567

Baliga, S., Muglikar, S., & Kale, R. (2013). Salivary pH: A diagnostic biomarker. *Journal of Indian Society of Periodontology*, *17*(4), 461–465. https://doi.org/10.4103/0972-124X.118317

Ban-koffi, L. and Han, Y. W. (1990). Alcohol production from pineapple waste. *World J. of Micro. and Biotech., 6:* 281-284.

Bazzano, L. A., He, J., Ogden, L. G., Loria, C. M., & Whelton, P. K. (2003). Dietary Fiber Intake and Reduced Risk of Coronary Heart Disease in US Men and Women. *Archives of Internal Medicine*, *163*(16), 1897. https://doi.org/10.1001/archinte.163.16.1897

Ben, Q., Sun, Y., Chai, R., Qian, A., Xu, B., & Yuan, Y. (2014). Dietary fiber intake reduces risk for colorectal adenoma: A meta-analysis. *Gastroenterology*, *146*(3), 689-699.e6. https://doi.org/10.1053/j.gastro.2013.11.003

Beohner, H. L., & Mindler, A. B. (1949). Ion Exchange in Waste Treatment. *Industrial & Engineering Chemistry, 41*(3), 448–452. doi:10.1021/ie50471a004

Bindels, L. B., Delzenne, N. M., Cani, P. D., & Walter, J. (2015). Opinion: Towards a more comprehensive concept for prebiotics. *Nature Reviews Gastroenterology and Hepatology*, *12*(5), 303–310. https://doi.org/10.1038/nrgastro.2015.47

Bosland, M. C. (2000). The role of steroid hormones in prostate carcinogenesis. *Journal of the National Cancer Institute. Monographs*, *10016*(27), 39–66.

Brown, L., Rosner, B., Willett, W. W., & Sacks, F. M. (2018). *Cholesterol-lowering effects of dietary fiber : a meta-analysis 1 , 2*. (February), 30–42.

Burkitt, D. P. (1971). Epidemiology of cancer of the colon and rectum. *Epidemiology of Colon-Rectum Cancer, 28*(1), 3–11. https://doi.org/10.1007/BF02047303

Chaudhary, V., Kumar, V., Sunil, Vaishali, Singh, K., Kumar, R., & Kumar, V. (2019). Pineapple (Ananas cosmosus) product processing: A review. *Journal of Pharmacognosy and Phytochemistry*, *8*(3), 4642–4652.

Chawla, R., Patil, G. R. (2010). Soluble dietary fiber. *Comprehensive Reviews in Food Science and Food Safety*, *9*(2): 178–196.

Chen, J., & Vitetta, L. (2018). Inflammation-Modulating Effect of Butyrate in the Prevention of Colon Cancer by Dietary Fiber. *Clinical Colorectal Cancer, 17*(3), e541–e544. https://doi.org/10.1016/j.clcc.2018.05.001

Collins, J. L. (1933). Morphological and cytological characteristics of triploid pineapples. *Cytologia*, *4*(3), 248–256. https://doi.org/10.1508/cytologia.4.248

Coppensd'Eeckenbrugge, G., Carlier, J. D., & Leitão, J. M. (2007). Pineapple. *Genome Mapping and Molecular Breeding in Plants, 4,* 331–340. https://doi.org/10.1093/nq/s9-vi.139.154f

Cummings, J. H., & Macfarlane, G. T. (2002). Gastrointestinal effects of prebiotics. *British Journal of Nutrition, 87*(S2), S145–S151. https://doi.org/10.1079/bjn/2002530

Dacera, D., Babel, S., & Parkpian, P. (2009). Potential for land application of contaminated sewage sludge treated with fermented liquid from pineapple wastes.

Journal of Hazardous Materials, *167*(1–3), 866–872. https://doi.org/10.1016/j.jhazmat.2009.01.064

Damasceno, K. A., Alvarenga Gonçalves, C. A., Dos Santos Pereira, G., Lacerda Costa, L., Bastianello Campagnol, P. C., Leal De Almeida, P., & Arantes-Pereira, L. (2016). Development of Cereal Bars Containing Pineapple Peel Flour (Ananas comosus L. Merril). *Journal of Food Quality*, *39*(5), 417–424. https://doi.org/10.1111/jfq.12222

Davani-Davari, D., Negahdaripour, M., Karimzadeh, I., Seifan, M., Mohkam, M., Masoumi, S. J., … Ghasemi, Y. (2019). Prebiotics: Definition, types, sources, mechanisms, and clinical applications. *Foods*, *8*(3), 1–27. https://doi.org/10.3390/foods8030092

Davidson, A. (2002). *Penguin companion to food.* Penguin Books.

De Carvalho, C. M., De Paula, T. P., Viana, L. V., Machado, V. M. T., De Almeida, J. C., & Azevedo, M. J. (2017). Plasma glucose and insulin responses after consumption of breakfasts with different sources of soluble fiber in type 2 diabetes patients: A randomized crossover clinical trial. *American Journal of Clinical Nutrition*, *106*(5), 1238–1245. https://doi.org/10.3945/ajcn.117.157263

Deschasaux, M., Pouchieu, C., His, M., Hercberg, S., Latino-Martel, P., & Touvier, M. (2014). Dietary Total and Insoluble Fiber Intakes Are Inversely Associated with Prostate Cancer Risk. *The Journal of Nutrition*, *144*(4), 504–510. https://doi.org/10.3945/jn.113.189670

Diaz-Vela, J., Totosaus, A., Cruz-Guerrero, A. E., & De Lourdes Pérez-Chabela, M. (2013). In vitro evaluation of the fermentation of added-value agroindustrial by-products: Cactus pear (Opuntia ficus-indica L.) peel and pineapple (Ananas comosus) peel as functional ingredients. *International Journal of Food Science and Technology*, *48*(7), 1460–1467. https://doi.org/10.1111/ijfs.12113

Divakaran, D., Chandran, A., & Pratap Chandran, R. (2011). Comparative study on production of α-amylase from Bacillus licheniformis strains. *Brazilian Journal of Microbiology*, *42*(4), 1397–1404. https://doi.org/10.1590/S1517-83822011000400022

Dutta, S., & Bhattacharyya, D. (2013). Enzymatic, antimicrobial and toxicity studies of the aqueous extract of Ananas comosus (pineapple) crown leaf. *Journal of Ethnopharmacology*, *150*(2), 451–457. https://doi.org/10.1016/j.jep.2013.08.024

Elleuch, M., Bedigian, D., Roiseux, O., Besbes, S., Blecker, C., & Attia, H. (2011). Dietary fibre and fibre-rich by-products of food processing: Characterisation, technological functionality and commercial applications: A review. *Food Chemistry*. https://doi.org/10.1016/j.foodchem.2010.06.077

Erukainure, O. L., Ajiboye, J. A., Adejobi, R. O., Okafor, O. Y., & Adenekan, S. O. (2011). Protective effect of pineapple (Ananas cosmosus) peel extract on alcohol-induced oxidative stress in brain tissues of male albino rats. *Asian Pacific Journal of Tropical Disease*, *1*(1), 5–9. https://doi.org/10.1016/S2222-1808(11)60002-9

Farvid, M. S., Eliassen, A. H., Cho, E., Liao, X., Chen, W. Y., & Willett, W. C. (2016). Dietary fiber intake in young adults and breast cancer risk. *Pediatrics*, *137*(3). https://doi.org/10.1542/peds.2015-1226

Frei, R., Akdis, M., & O'mahony, L. (2015). Prebiotics, probiotics, synbiotics, and the immune system: Experimental data and clinical evidence. *Current Opinion in Gastroenterology*, *31*(2), 153–158. https://doi.org/10.1097/MOG.0000000000000151

Fuentes-Zaragoza, E., Sánchez-Zapata, E., Sendra, E., Sayas, E., Navarro, C., Fernández-Lõpez, J., & Pérez-Alvarez, J. A. (2011). Resistant starch as prebiotic: A review. *Starch/Staerke*, *63*(7), 406–415. https://doi.org/10.1002/star.201000099

Gibson, G. R., Scott, K. P., Rastall, R. A., Tuohy, K. M., Hotchkiss, A., Dubert-Ferrandon, A., … Buddington, R. (2010). Dietary prebiotics: current status and new definition. *Food Science & Technology Bulletin: Functional Foods*, *7*(1), 1–19. https://doi.org/10.1616/1476-2137.15880

Gibson, G. R., & Roberfroid, M. B. (1995). Dietary modulation of the human colonic microbiota: Introducing the concept of prebiotics. *Journal of Nutrition*, *125*(6), 1401–1412. https://doi.org/10.1093/jn/125.6.1401

Gorinstein, S., Zachwieja, Z., Folta, M., Barton, H., Piotrowicz, J., Zemser, M., … Màrtín-Belloso, O. (2001). Comparative contents of dietary fiber, total phenolics, and minerals in persimmons and apples. *Journal of Agricultural and Food Chemistry*, *49*(2), 952–957. https://doi.org/10.1021/jf000947k

Gouda, E. J., & Butcher, D. (2016). A list of accepted bromeliaceae names.

Guillon, F., & Champ, M. (2000). Structural and physical properties of dietary fibres, and consequences of processing on human physiology. *Food Research International*, *33*(3–4), 233–245. https://doi.org/10.1016/S0963-9969(00)00038-7

Gulati, S., Misra, A., & Pandey, R. M. (2017). Effects of 3 g of soluble fiber from oats on lipid levels of Asian Indians - a randomized controlled, parallel arm study. *Lipids in Health and Disease*, *16*(1), 1–8. https://doi.org/10.1186/s12944-017-0460-3

Haschek, W. M., Rousseaux, C. G., & Wallig, M. A. (2010). Gastrointestinal tract. *Fundamentals of Toxicologic Pathology. Academic Press, San Diego*.

Hassan, A., Wills, R., Atan, R. M., Othman, Z., Fatt, L. P., & Smith, M. (2010). Blackheart disorder in fresh pineapple. *New Trends in Postharvest Management of Fresh Produce II. Fresh Produce, 4,* 29-35.

Hebbar, H. U., Sumana, B., & Raghavarao, K. S. M. S. (2008). Use of reverse micellar systems for the extraction and purification of bromelain from pineapple wastes. *Bioresource Technology*, *99*(11), 4896–4902. https://doi.org/10.1016/j.biortech.2007.09.038

Higgins, J. A. (2012). Whole grains, legumes, and the subsequent meal effect: Implications for blood glucose control and the role of fermentation. *Journal of Nutrition and Metabolism*, *2012*. https://doi.org/10.1155/2012/829238

Holscher, H. D. (2017). Dietary fiber and prebiotics and the gastrointestinal microbiota. Gut Microbes, 8(2), 172–184. https://doi.org/10.1080/19490976.2017.1290756

Hong, K., Xu, H., Wang, J., Zhang, L., Hu, H., Jia, Z., … Gong, D. (2013). Quality changes and internal browning developments of summer pineapple fruit during storage at different temperatures. *Scientia Horticulturae*, *151*, 68–74. https://doi.org/10.1016/j.scienta.2012.12.016

Hossain, M. F. (2016). World pineapple production: An overview. *African Journal of Food, Agriculture, Nutrition and Development*, *16*(4), 11443–11456. https://doi.org/10.18697/ajfand.76.15620

Hossain, M. F., Akhtar, S., & Anwar, M. (2015). Nutritional Value and Medicinal Benefits of Pineapple. *International Journal of Nutrition and Food Sciences*, *4*(1), 84. https://doi.org/10.11648/j.ijnfs.20150401.22

Huang, Y. L., Chow, C. J., & Fang, Y. J. (2011). Preparation and physicochemical properties of fiber-rich fraction from pineapple peels as a potential ingredient.

Journal of Food and Drug Analysis, *19*(3), 318–323. https://doi.org/10.38212/2224-6614.2179

Huang, Y. L., Tsai, Y. H., & Chow, C. J. (2014). Water-insoluble fiber-rich fraction from pineapple peel improves intestinal function in hamsters: Evidence from cecal and fecal indicators. *Nutrition Research*, *34*(4), 346–354. https://doi.org/10.1016/j.nutres.2014.03.001

Idris, A., & Suzana, W. (2006). Effect of sodium alginate concentration, bead diameter, initial pH and temperature on lactic acid production from pineapple waste using immobilized Lactobacillus delbrueckii. *Process Biochemistry*, *41*(5), 1117–1123. https://doi.org/10.1016/j.procbio.2005.12.002

Ishida, H., Suzuno, H., Sugiyama, N., Innami, S., Tadokoro, T., & Maekawa, A. (2000). Nutritive evaluation on chemical components of leaves, stalks and stems of sweet potatoes (Ipomoea batatas poir). *Food Chemistry*, *68*(3), 359–367. https://doi.org/10.1016/S0308-8146(99)00206-X

Jaji, K., Man, N., & Nawi, N. M. (2018). Factors affecting pineapple market supply in Johor, Malaysia. *International Food Research Journal*, *25*(1), 366–375.

Jiang, W., Sunkara, L. T., Zeng, X., Deng, Z., Myers, S. M., & Zhang, G. (2013). Differential regulation of human cathelicidin LL-37 by free fatty acids and their analogs. *Peptides*, *50*, 129–138. https://doi.org/10.1016/j.peptides.2013.10.008

Johnson-Henry, K. C., Pinnell, L. J., Waskow, A. M., Irrazabal, T., Martin, A., Hausner, M., & Sherman, P. M. (2014). Short-chain fructo-oligosaccharide and inulin modulate inflammatory responses and microbial communities in Caco2-bbe cells and in a mouse model of intestinal injury. *Journal of Nutrition*, *144*(11), 1725–1733. https://doi.org/10.3945/jn.114.195081

Jonasson, S., Bryant, J. P., Chapin III, F. S., & Andersson, M. (1986). Plant phenols and nutrients in relation to variations in climate and rodent grazing. *The American Naturalist*, *128*(3), 394-408.

Jones, J.; Wilson, W (2006). "Chapter 11: Science". *An Incomplete Education*. Ballantine. p. 544. ISBN 978-0-7394-7582-9.

Jongaroontaprangsee, S., Tritrong, W., Chokanaporn, W., Methacanon, P., Devahastin, S., & Chiewchan, N. (2007). Effects of drying temperature and particle size on hydration properties of dietary fiber powder from lime and cabbage by-products. *International Journal of Food Properties*, *10*(4), 887–897. https://doi.org/10.1080/10942910601183619

Joy, P. P., & Anjana, R. (2013). Pineapple varieties. *Queensl Fruit Veg News*, *3* (January 2013), 724.

Joy, P. P. (2013). Pineapple Research Station Report Vazhakulam 2012-13. *Kerala Agricultural University*, (March 2013). https://doi.org/10.13140/RG.2.1.1887.6403

Kahl, R., & Kappus, H. (1993). Toxikologie der synthetischen Antioxidantien BHA und BHT im Vergleich mit dem natürlichen Antioxidans Vitamin E. *Zeitschrift Für Lebensmittel-Untersuchung Und -Forschung*, *196*(4), 329–338. https://doi.org/10.1007/BF01197931

Kammerer, D. R., Saleh, Z. S., Carle, R., & Stanley, R. A. (2007). Adsorptive recovery of phenolic compounds from apple juice. *European Food Research and Technology*, *224*(5), 605–613. https://doi.org/10.1007/s00217-006-0346-5

Ketnawa, S., Chaiwut, P. & Rawdkuen, S. (2011). Extraction of bro- melain from pineapple peels. *Food Science and Technology Interna- tional*, *17*, 395–402.

Ketnawa, S., Chaiwut, P., & Rawdkuen, S. (2012). Pineapple wastes: A potential source for bromelain extraction. *Food and Bioproducts Processing*, *90*(3), 385–391. https://doi.org/10.1016/j.fbp.2011.12.006

Kihara, M. (2015). Temperature and pH dependency of pepsin activity in the gastric juice of farmed Pacific bluefin tuna Thunnus orientalis. *Aquaculture Science*, *63*(4), 459–461. Retrieved from https://www.jstage.jst.go.jp/article/aquaculturesci/63/4/63_459/_pdf

Kim, H. J., Kim, Y. J., Lee, S. H., Yu, J., Jeong, S. K., & Hong, S. J. (2014). Effects of Lactobacillus rhamnosus on allergic march model by suppressing Th2, Th17, and TSLP responses via CD4+CD25+Foxp3+ Tregs. *Clinical Immunology*, *153*(1), 178–186. https://doi.org/10.1016/j.clim.2014.04.008

Koh, J., Kang, S., Kim, S., Cha, M., & Kwon, Y. (2006). Dyeing Method Activity of Protease Protease Treatment of Silk and Wool Fibers. *Fibers and Polymers*, *7*(2), 180–185.

Koh-Banerjee, P., Franz, M., Sampson, L., Liu, S., Jacobs, D. R., Spiegelman, D., … Rimm, E. (2004). Changes in whole-grain, bran, and cereal fiber consumption in relation to 8-y weight gain among men. *American Journal of Clinical Nutrition*, *80*(5), 1237–1245. https://doi.org/10.1093/ajcn/80.5.1237

Larrauri, J. A., Rupérez, P., & Saura Calixto, F. (1997). Pineapple Shell as a Source of Dietary Fiber with Associated Polyphenols. *Journal of Agricultural and Food Chemistry*, *45*(10), 4028–4031. https://doi.org/10.1021/jf970450j

Lattimer, J. M., & Haub, M. D. (2010). Effects of dietary fiber and its components on metabolic health. *Nutrients,* *2*(12), 1266–1289. https://doi.org/10.3390/nu2121266

Liu, T. W., Cephas, K. D., Holscher, H. D., Kerr, K. R., Mangian, H. F., Tappenden, K. A., & Swanson, K. S. (2016). Nondigestible fructans alter gastrointestinal barrier function, gene expression, histomorphology, and themicrobiota profiles of diet-induced obese C57BL/6J Mice. *Journal of Nutrition, 146*(5), 949–956. https://doi.org/10.3945/jn.115.227504

Liu, Y., Fatheree, N. Y., Mangalat, N., & Rhoads, J. M. (2010). Human-derived probiotic Lactobacillus reuteri strains differentially reduce intestinal inflammation. *American Journal of Physiology - Gastrointestinal and Liver Physiology, 299*(5), 1087–1096. https://doi.org/10.1152/ajpgi.00124.2010

Li, T., Shen, P., Liu, W., Liu, C., Liang, R., Yan, N., & Chen, J. (2014). Major polyphenolics in pineapple peels and their antioxidant interactions. *International Journal of Food Properties, 17*(8), 1805–1817. https://doi.org/10.1080/10942912.2012.732168

Lobo, M. G., & Paull, R. E. (2017). Handbook of Pineapple Technology. In *Handbook of Pineapple Technology*. https://doi.org/10.1002/9781118967355

Longcope, C., Feldman, H. A., McKinlay, J. B., & Araujo, A. B. (2000). Diet and sex hormone-binding globulin. *Journal of Clinical Endocrinology and Metabolism, 85*(1), 293–296. https://doi.org/10.1210/jcem.85.1.6291

Madhav, A., & Pushpalatha, P. B. (2002). Characterization of Pectin Extracted From Different Fruit Wastes. *Journal of Tropical Agriculture, 40,* 53–55.

Malaysian Pineapple Industry Board (MPIB). 2011. Quantity of pineapple production. Retrieved on February 18, 2016 from *http://mpib.gov.my/en/ sejarah*.

Makki, K., Deehan, E. C., Walter, J., & Bäckhed, F. (2018). The Impact of Dietary Fiber on Gut Microbiota in Host Health and Disease. *Cell Host and Microbe*, *23*(6), 705–715. https://doi.org/10.1016/j.chom.2018.05.012

Mann, J., Cummings, J. H., Englyst, H. N., Key, T., Liu, S., Riccardi, G., … Wiseman, M. (2007). FAO/WHO Scientific Update on carbohydrates in human nutrition: Conclusions. *European Journal of Clinical Nutrition*, *61*, S132–S137. https://doi.org/10.1038/sj.ejcn.1602943

Manning, P. J., Sutherland, W. H. F., McGrath, M. M., De Jong, S. A., Walker, R. J., & Williams, M. J. A. (2008). Postprandial cytokine concentrations and meal composition in obese and lean women. *Obesity*, *16*(9), 2046–2052. https://doi.org/10.1038/oby.2008.334

Martí, N., Saura, D., Fuentes', E., Lizama, V., García, E., Mico-Ballester, M. J., & Lorente, J. (2011). Fiber from tangerine juice industry. *Industrial Crops and Products*, *33*(1), 94–98. https://doi.org/10.1016/j.indcrop.2010.09.004

Martinsen, T. C., Fossmark, R., & Waldum, H. L. (2019). The phylogeny and biological function of gastric juice—microbiological consequences of removing gastric acid. *International Journal of Molecular Sciences, 20*(23), 1–22. https://doi.org/10.3390/ijms20236031

McRorie, J. W., & Fahey, G. C. (2013). A review of gastrointestinal physiology and the mechanisms underlying the health benefits of dietary fiber: matching an effective fiber with specific patient needs. *Clin Nurs Stud*, *1*(4), 82-92. https://doi.org/ 10.5430/cns.v1n4p82

Morton, J. F. (1987). Fruits of warm climates. Creative Resource Systems. *Inc.*, *Winterville*, *NC,* 160-168.

Mudgil, D. (2017). The Interaction Between Insoluble and Soluble Fiber. *Dietary Fiber for the Prevention of Cardiovascular Disease*, 35–59. doi:10.1016/b978-0-12-805130-6.00003-3

Nadzirah, K. Z., Zainal, S., Noriham, A., Normah, I., & Roha, A. M. S. (2012). Physico-Chemical Properties of Pineapple Crown Extract Variety N36 and Bromelain Activity in Different Forms. *APCBEE Procedia*, *4*(March 2016), 130–134. https://doi.org/10.1016/j.apcbee.2012.11.022

Nakada, T., Nishimoto, T., Chaen, H., & Fukuda, S. (2003). *Kojioligosaccharides: Application of Kojibiose Phosphorylase on the Formation of Various Kojioligosaccharides.* 104–117. https://doi.org/10.1021/bk-2003-0849.ch009

Nassr, M. S., & Abu-Naser, S. S. (2018). Knowledge Based System for Diagnosing Pineapple Diseases. *International Journal of Academic Pedagogical Research (IJAPR)*, *2*(7), 12–19. https://doi.org/10.1007/978-3-642-48706-4_93

Nigam, J. N. (2000). Continuous ethanol production from pineapple cannery waste using immobilized yeast cells. *Journal of Biotechnology*, *80*(2), 189–193. https://doi.org/10.1016/S0168-1656(00)00246-7

Nilsson, A. C., Östman, E. M., Knudsen, K. E. B., Holst, J. J., & Björck, I. M. E. (2010). A cereal-based evening meal rich in indigestible carbohydrates increases plasma butyrate the next morning. *Journal of Nutrition*, *140*(11), 1932–1936. https://doi.org/10.3945/jn.110.123604

Nunes, M. C. N., Emond, J. P., Rauth, M., Dea, S., & Chau, K. V. (2009). Environmental conditions encountered during typical consumer retail display affect fruit and vegetable quality and waste. *Postharvest Biology and Technology*, *51*(2), 232–241. https://doi.org/10.1016/j.postharvbio.2008.07.016

Othman, R. A., Moghadasian, M. H., & Jones, P. J. H. (2011). Cholesterol-lowering effects of oat β-glucan. *Nutrition Reviews*, *69*(6), 299–309. https://doi.org/10.1111/j.1753-4887.2011.00401.x

Ötles, S., & Ozgoz, S. (2014). Health effects of dietary fiber. *Acta Scientiarum Polonorum*, *Technologia Alimentaria*, *13*(2), 191–202. https://doi.org/10.17306/J.AFS.2014.2.8

Pardo, M. E. S., Cassellis, M. E. R., Escobedo, R. M., & García, E. J. (2014). Chemical Characterisation of the Industrial Residues of the Pineapple (Ananas comosus). *Journal of Agricultural Chemistry and Environment*, *03*(02), 53–56. https://doi.org/10.4236/jacen.2014.32b009

Patel, S., & Goyal, A. (2012). The current trends and future perspectives of prebiotics research: a review. *3 Biotech*, *2*(2), 115–125. https://doi.org/10.1007/s13205-012-0044-x

Prasenjit, D., Prasanta, D., Abhijit, C., & Tejendra, B. (2012). A survey on pineapple and its medicinal value. *Scholars Academic Journal of Pharmacy*, *1*(1), 24–29.

Probst-Hensch, N. M., Wang, H., Goh, V. H., Seow, A., Lee, H. P., & Mimi, C. Y. (2003). Determinants of circulating insulin-like growth factor I and insulin-like growth factor binding protein 3 concentrations in a cohort of Singapore men and women. *Cancer Epidemiology and Prevention Biomarkers*, *12*(8), 739-746.

Quirós-Sauceda, A. E., Palafox-Carlos, H., Sáyago-Ayerdi, S. G., Ayala-Zavala, J. F., Bello-Perez, L. A., Álvarez-Parrilla, E., … González-Aguilar, G. A. (2014). Dietary fiber and phenolic compounds as functional ingredients: Interaction and possible effect after ingestion. *Food and Function*, *5*(6), 1063–1072. https://doi.org/10.1039/c4fo00073k

Ripsin, C. M. (1992). Oat Products and Lipid Lowering. *JAMA*, *267*(24), 3317. doi:10.1001/jama.1992.03480240079039

Roberfroid, M. (2002). Functional food concept and its application to prebiotics. *Digestive and Liver Disease, 34*(SUPPL. 2). https://doi.org/10.1016/S1590-8658(02)80176-1

Roda, A., De Faveri, D. M., Dordoni, R., & Lambri, M. (2014). Vinegar production from pineapple wastes -preliminary saccharification trials. *Chemical Engineering Transactions, 37*, 607–612. https://doi.org/10.3303/CET1437102

Ross, J. K., Pusateri, D. J., & Shultz, T. D. (1990). Dietary and hormonal evaluation of men at different risks for prostate cancer: Fiber intake, excretion, and composition, with in vitro evidence for an association between steroid hormones and specific fiber components. *American Journal of Clinical Nutrition*, *51*(3), 365–370. https://doi.org/10.1093/ajcn/51.3.365

Sah, B. N. P., Vasiljevic, T., McKechnie, S., & Donkor, O. N. (2015). Effect of refrigerated storage on probiotic viability and the production and stability of antimutagenic and antioxidant peptides in yogurt supplemented with pineapple peel. *Journal of Dairy Science*, *98*(9), 5905–5916. https://doi.org/10.3168/jds.2015-9450

Sahoo, G., Bisoi, P., Mishra, S., Das, C., & Das, K. (2012). Hypoglycemic Effects of Insoluble Fiber Rich Fraction of Different Cereals and Millets. *Journal of Food Processing & Technology*, *03*(11). https://doi.org/10.4172/2157-7110.1000191

Sako, T., Matsumoto, K., & Tanaka, R. (1999). Recent progress on research and applications of non-digestible galacto-oligosaccharides. *International Dairy Journal*, *9*(1), 69–80. https://doi.org/10.1016/S0958-6946(99)00046-1

Samanta, A. K., Jayapal, N., Jayaram, C., Roy, S., Kolte, A. P., Senani, S., & Sridhar, M. (2015). Xylooligosaccharides as prebiotics from agricultural by-products: Production and applications. *Bioactive Carbohydrates and Dietary Fibre, 5*(1), 62–71. https://doi.org/10.1016/j.bcdf.2014.12.003

Sanewski, G. M., Bartholomew, D. P., & Paull, R. E. (Eds.). (2018). *The pineapple: botany, production and uses*. CABI.

Saraswaty, V., Risdian, C., Primadona, I., Andriyani, R., Andayani, D. G. S., & Mozef, T. (2017). Pineapple peel wastes as a potential source of antioxidant compounds. *IOP Conference Series: Earth and Environmental Science*, *60*(1). https://doi.org/10.1088/1755-1315/60/1/012013

Satija, A., & Hu, F. B. (2012). Cardiovascular benefits of dietary fiber. Current Atherosclerosis Reports, 14(6), 505–514. https://doi.org/10.1007/s11883-012-0275-7

Scherer, R. F., Olkoski, D., Souza, F. V.D., Nodari, R. O., & Guerra, M. P. (2015). Gigante de Tarauacá: A triploid pineapple from Brazilian Amazonia. *Scientia Horticulturae*, *181*, 1–3. https://doi.org/10.1016/j.scienta.2014.10.052

Schieber, A. (2001). By-Products of Plant Food Processing as a Source of Valuable Compounds. *Reference Module in Food Science*, *12*(2001), 401–413. https://doi.org/10.1016/b978-0-08-100596-5.21346-2

Scott, K. P., Martin, J. C., Duncan, S. H., & Flint, H. J. (2014). Prebiotic stimulation of human colonic butyrate-producing bacteria and bifidobacteria, in vitro. *FEMS Microbiology Ecology*, *87*(1), 30–40. https://doi.org/10.1111/1574-6941.12186

Selani, M. M., Brazaca, S. G. C., Dos Santos Dias, C. T., Ratnayake, W. S., Flores, R. A., & Bianchini, A. (2014). Characterisation and potential application of pineapple pomace in an extruded product for fibre enhancement. *Food Chemistry, 163*, 23–30. https://doi.org/10.1016/j.foodchem.2014.04.076

Shin, D. (2012). Analysis of dietary insoluble and soluble fiber contents in school meal. *Nutrition Research and Practice, 6*(1), 28–34. https://doi.org/10.4162/nrp.2012.6.1.28

Singh, R. D., Banerjee, J., & Arora, A. (2015). Prebiotic potential of oligosaccharides: A focus on xylan derived oligosaccharides. *Bioactive Carbohydrates and Dietary Fibre, 5*(1), 19–30. https://doi.org/10.1016/j.bcdf.2014.11.003

Slavin, J. (2013). Fiber and prebiotics: Mechanisms and health benefits. Nutrients, 5(4), 1417–1435.

Sharoba, A., Farrag, M., & Abd, E. (2013). Utilization of some fruits and vegetables waste as a source of dietary fiber and its effect on the cake making and its quality attributes. *Journal of Agroalimentary* …. Retrieved from https://doi.org/10.21608/jfds.2013.72084

Subhabrata, S., & Mayura, D. (2006). Industrial and clinical applications excluding diagnostic clinical. *Enzymology, 1*, 1-25.

Surampudi, P., Enkhmaa, B., Anuurad, E., & Berglund, L. (2016). Lipid Lowering with Soluble Dietary Fiber. *Current Atherosclerosis Reports*, *18*(12). https://doi.org/10.1007/s11883-016-0624-z

Takano, A., Kamiya, T., Tomozawa, H., Ueno, S., Tsubata, M., Ikeguchi, M., … Takahashi, T. (2013). Insoluble fiber in young barley leaf suppresses the increment of postprandial blood glucose level by increasing the digesta viscosity. *Evidence-Based Complementary and Alternative Medicine, 2013*. https://doi.org/10.1155/2013/137871

Thalip, A.A., Tong, P. S., & Ng, C. (2015). The MD2 “Super Sweet” pineapple (Ananas comosus). *Utar Agriculture Science Journal*, *1*(4), 14–17. Retrieved from http://eprints.utar.edu.my/1982/1/The_MD2_(Super_Sweet)_pineapple_(Ananas_comosus).pdf

Thibault, R., De Coppet, P., Daly, K., Bourreille, A., Cuff, M., Bonnet, C., … Segain, J. P. (2007). Down-Regulation of the Monocarboxylate Transporter 1 Is Involved in Butyrate Deficiency During Intestinal Inflammation. *Gastroenterology*, *133*(6), 1916–1927. https://doi.org/10.1053/j.gastro.2007.08.041

Thompson, S. V., Hannon, B. A., An, R., & Holscher, H. D. (2017). Effects of isolated soluble fiber supplementation on body weight, glycemia, and insulinemia in adults with overweight and obesity: A systematic review and meta-analysis of randomized controlled trials. *American Journal of Clinical Nutrition*, *106*(6), 1514–1528. https://doi.org/10.3945/ajcn.117.163246

Tran, A. Van. (2006). Chemical analysis and pulping study of pineapple crown leaves. *Industrial Crops and Products*, *24*(1), 66–74. https://doi.org/10.1016/j.indcrop.2006.03.003

Tropea, A., Wilson, D., Torre, L. G. La, Curto, R. B. Lo, Saugman, P., Troy-Davies, P., … Waldron, K. W. (2014). Bioethanol Production From Pineapple Wastes. *Journal of Food Research*, *3*(4), 60. https://doi.org/10.5539/jfr.v3n4p60

Trowell, H. (1976). Definition of dietary fiber and hypotheses that it is a protective factor in certain diseases. *American Journal of Clinical Nutrition*, (March). Retrieved from http://www.scopus.com/inward/record.url?eid=2-s2.0-0017228865&partnerID=40&md5=7aed7bbbb3ea60ea0a860d430f39b3d5

Tzounis, X., Rodriguez-Mateos, A., Vulevic, J., Gibson, G. R., Kwik-Uribe, C., & Spencer, J. P. (2010). Prebiotic evaluation of cocoa-derived flavanols in healthy humans by using a randomized, controlled, double-blind, crossover intervention study. *The American Journal of Clinical Nutrition*, *93*(1), 62–72. https://doi.org/doi:10.3945/ajcn.110.000075

Uhlig, H. (Ed.). (1998). *Industrial enzymes and their applications*. John Wiley & Sons.

Upadhyay, A., Lama, J. P., & Tawata, S. (2010). Journal of food science & technology, Nepal : JFSTN. *Journal of Food Science and Technology Nepal*, *6*(0), 10–18. Retrieved from https://www.nepjol.info/index.php/JFSTN/article/view/8255/6722

Valcheva, R., & Dieleman, L. A. (2016). Prebiotics: Definition and protective mechanisms. *Best Practice and Research: Clinical Gastroenterology*, *30*(1), 27–37. https://doi.org/10.1016/j.bpg.2016.02.008

Walsh, G. (2002). Biochemistry and Biotechnology. *John Willey & Sons*.

Wichienchot, S., Jatupornpipat, M., & Rastall, R. A. (2010). Oligosaccharides of pitaya (dragon fruit) flesh and their prebiotic properties. *Food Chemistry*, *120*(3), 850–857. https://doi.org/10.1016/j.foodchem.2009.11.026

Wichienchot, S., Prasertsan, P., Hongpattarakere, T., Gibson, G. R., & Rastall, R. A. (2006). *In vitro Fermentation of Mixed Linkage Gluco- oligosaccharides Produced by Gluconobacter Further Reading*. *01*, 7–12.

Wong, J. M. W., & Jenkins, D. J. A. (2007). Carbohydrate Digestibility and Metabolic Effects. *The Journal of Nutrition*, *137*(11), 2539S–2546S. Retrieved from https://doi.org/10.1093/jn/137.11.2539S

Wood, R. J., Fernandez, M. L., Sharman, M. J., Silvestre, R., Greene, C. M., Zern, T. L., … Volek, J. S. (2007). Effects of a carbohydrate-restricted diet with and without supplemental soluble fiber on plasma low-density lipoprotein cholesterol and other clinical markers of cardiovascular risk. *Metabolism: Clinical and Experimental*, *56*(1), 58–67. https://doi.org/10.1016/j.metabol.2006.08.021

Yao, B., Fang, H., Xu, W., Yan, Y., Xu, H., Liu, Y., … Zhao, Y. (2014). Dietary fiber intake and risk of type 2 diabetes: A dose-response analysis of prospective studies. *European Journal of Epidemiology*, *29*(2), 79–88. https://doi.org/10.1007/s10654-013-9876-x

Ye, Z., Arumugam, V., Haugabrooks, E., Williamson, P., & Hendrich, S. (2015). Soluble dietary fiber (Fibersol-2) decreased hunger and increased satiety hormones in humans when ingested with a meal. *Nutrition Research*, *35*(5), 393–400. https://doi.org/10.1016/j.nutres.2015.03.004