

UNIVERSITI PUTRA MALAYSIA

PHYSICO-CHEMICAL AND HEALTH-PROMOTING PROPERTIES OF DIETARY FIBRE POWDER FROM PINK GUAVA BY-PRODUCTS

AIDA HAMIMI BINTI IBRAHIM

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DOCTOR OF PHILOSOPHY UNIVERSITI PUTRA MALAYA

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By

AIDA HAMIMI BINTI IBRAHIM

Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfilment of the Requirements for the Degree of Doctor of Philosophy

November, 2009

DEDICATION

To my husband, daughters, parents, teachers and friends

who have always been with me



Abstract of thesis to the Senate of Universiti Putra Malaysia in fulfilment of the requirement for the degree of Doctor of Philosophy

PHYSICO-CHEMICAL AND HEALTH- PROMOTING PROPERTIES OF DIETARY FIBRE POWDER FROM PINK GUAVA BY-PRODUCTS

By

AIDA HAMIMI IBRAHIM November 2009

Chair: Associate Professor Dr. Amin Ismail, PhD Faculty: Faculty of Medicine and Health Sciences

Fruits by-products and their residues are usually available in large quantities and although costly to dispose, are not fully exploited commercially for lack of research. The pink guava by-product dietary fibre posses the physico-chemical and health-promoting properties, potentially a new natural ingredient for the health food industry. The objectives of the study were to determine the dietary fibre composition of pink guava by-product, to develop dietary fibre powder and to evaluate the functional properties and health benefits of the dietary fibre powder. The processing wastes from the pink guava industry were analysed for dietary fibre content (soluble, insoluble and total) and dietary fibre composition (hemicellulose, cellulose and lignin). The resultant dietary fibre powder (DFP)

was analysed for dietary fibre content, proximate composition (caloric value, moisture content, fat, protein, and carbohydrate); structure, colour and functional properties (water- retention capacity (WRC), oil-retention capacity (ORC), swelling-capacity (SWC), and particle size distribution. Fructooligosaccharides were also identified in the DFP. The dietary powder was evaluated for its healthpromotion properties (total antioxidant and polyphenol contents, prebiotic and hypocholesterolemic effects). Pink guava by-products were found to have high total dietary fibre content (68.4 - 78.8 % dry matter) with high proportion of insoluble fibres. The types of insoluble fibres determined were cellulose (25 - 44 % dry matter), hemicellulose (12 - 25 % dry matter) and lignin (19 - 46 % dry matter). On the other hand, soluble fibre represents about 3.4% - 4.4% dry matter of total dietary fibres. The prepared powder had a high total dietary fibre content (56.6% – 76.1% DM) and almost similar SDF:IDF ratios with cereal brans, and low caloric value (97.1 – 249.1 kcal/100 g). The DFP was light brown in colour with scale type structure. Due to their water- retention ability (3.75 – 12.17 g of water/ g of fibre), oil - retention (2.20 - 6.88 g of oil/g of fibre) and swelling (11.8 - 14.2 mL of water/g of fiber DM), the DFP may be used not only for dietary fibre enrichment and reduction of energy value, but also as functional ingredients in many food products. This study has shown that DFP of pink guava by-product contained fructooligosaccharides (FOS), known as prebiotic agent. A full separation of all FOS components (fructose, sucrose, 1-ketose and nytose) was

achieved for dietary fibre powder. This product was found high in antioxidant activities (52 - 91.4 % AOA), radical scavenging effects (85.4 - 91.7 %) and total phenolic content (156 - 227.6 FAE mg/g). The study demonstrates that the dietary fibre powder is prebiotic food due to the evident that the mesophilic bacteria decreased and bifidobacteria increased *in vivo* and *in vitro* conditions. It was evident that the dietary fibre powder had very pronounced hypocholesterolemic effects as it could significantly (p< 0.05) decrease the levels of serum total cholesterol (43%) and LDL (51%) in rats. The dietary fibre powder from pink guava by-products was identified to have high antioxidant activity, prebiotic and hypocholesterolemic effects, the health-promotion properties that could boost its potential as a functional ingredient for food industry.

Abstrak tesis yang dikemukan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Doktor Falsafah

CIRI -CIRI FIZIKAL - KIMIA DAN FAEDAH - KESIHATAN SERBUK SERABUT DIET DARIPADA PRODUK SAMPINGAN JAMBU BATU MERAH JAMBU

Oleh AIDA HAMIMI IBRAHIM

November, 2009

Pengerusi: Prof Madya Dr Amin Ismail Fakulti: Fakulti Perubatan dan Sains Kesihatan

Industri jus buah seperti jambu batu merah menghasilkan jumlah produk sampingan yang banyak dan mempunyai potensi sebagai ramuan sumber serat baru untuk industri makanan. Namun produk sampingan ini tidak dieksploitasi sepenuhny untuk tujuan komersil. Objektif kajian adalah untuk menentukan komposisi serat diet dari hasil sampingan jambu batu merah, membangunkan serbuk serat diet dan menilai ciri-ciri fungsian dan faedah kesihatan serbuk serat diet. Pemprosesan hasil sampingan daripada industri jambu batu merah dianalisis kandungan serat diet (dalam bentuk larut, tak larut dan jumlah) dan komposisi serat diet (DFP) daripada hasil sampingan industri jambu batu merah dibangunkan. Analisis yang dilakukan ke atas DFP adalah penentuan kandungan serat diet, komposisi makanan (nilai kalori, kelembapan, lemak, protein, dan karbohidrat), analisis struktur, warna, dan ciri kefungsian (keupayaan membendung air, keupayaan membendung keupayaan mengembang, taburan saiz partikel). minyak, dan Fruktooligosakarid juga dikenalpasti di dalam serbuk DFP. Serbuk serat diet juga dinilai dari segi ciri-ciri faedah kesihatan (kandungan jumlah antioksidan, kandungan polifenol, kesan prebiotik dan hipokolesterolemik). Serat diet jambu batu merah mempunyai jumlah kandungan serat pemakanan yang tinggi (68.4 - 78.8% berat kering) dengan tinggi kandungan serat tak larut. Serat tak larut terdiri daripada selulosa (25 - 44% kering), hemiselulosa (12 - 25% berat kering) dan lignin (19 - 46 % berat kering). Serat larut pula mewakili 3.4 - 4.4 % berat kering jumlah serat diet. Bagi serbuk serat diet ia mengandungi jumlah kandungan serat pemakanan diantara 56.6 - 76.1% berat kering dengan nisbah serat pemakanan larut: terhadap tidak larut menyamai nisbah yang terdapat didalam bijirin, dan mengandungi nilai kalori yang rendah (97.1 - 249 kcal/100 g). Produk ini berwarna perang cerah dengan struktur berbentuk sisik. Produk ini berkeupayaan membendung air (3.75 -12.17 g air/ g serat), minyak (2.20 - 6.88 g minyak/g serat) dan mengembang (11.8 – 14.2 mL of air/g serat) dengan baik. Produk ini bukan sahaja boleh memperkaya serat diet dan mengurangkan nilai tenaga dalam makanan,

tetapi juga boleh digunakan sebagai bahan-bahan fungsian dalam pelbagai produk makanan. Kajian menunjukkan serbuk serat diet mengandungi fruktooligosakarid (FOS) yang merupakan agen prebiotik. Pemisahan komponen FOS (fruktos, sukros, 1-ketose and nytose) telah dicapai untuk serbuk serat diet ini. Disamping itu, produk ini mempunyai kandungan aktiviti antioksidan (52 – 91.4 AOA%) kesan radikal scavenging (85.4 – 91.7%), dan fenolik (156 – 227.6 FAE mg/g) yang tinggi . Kajian in - vitro dan in-vivo menunjukkan serbuk serat diet daripada hasil sampingan jambu batu merah bersifat prebiotik, hasil kajian menunjukkan terdapat pengurangan mesofili bakteria dan penambahan bifidobakteria. Kajian juga menunjukkan serbuk serat diet mempunyai kesan hipokolesterolemik yang amat ketara, (p < 0.05) dalam mengurangkan paras serum jumlah kolesterol (43%) dan LDL (51%) dalam tikus. Aktiviti antioksidan yang tinggi dan kesan prebiotik dan, hipokolesterolemik; serbuk serat diet ini berpotensi digunakan sebagai ramuan fungsian untuk menghasilkan makanan fungsian.

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Lastly, my sincere appreciation goes to my husband, daughter and my parents for their support, love and understanding throughout my study life. I certify that an Examination Committee met on _______ to conduct the final examination of Aida Hamimi Ibrahim on her Doctorate of Philosophy thesis entitled "Physico-Chemical And Health-Promoting Properties of Dietary Fibre Powder from Pink Guava By-Products" in accordance with Universiti Pertanian Malaysia (Higher Degree) Act 1980 and Universiti Pertanian (Higher Degree) Regulation 1981. The Committee recommends that the candidate be awarded the relevant degree. Members of the Examination Committee are as follows:

Chairman, PhD Professor Faculty Graduate Studies Universiti Putra Malaysia (Chairman 1)

Examiner 1, PhD Professor Faculty Graduate Studies Universiti Putra Malaysia (Member)

Examiner 2, PhD

Professor Faculty Graduate Studies Universiti Putra Malaysia (Member)

Independent Examiner, PhD Professor Faculty Graduate Studies Universiti Putra Malaysia (Independent Examiner)

> Prof Dr Bujang Kim Huat Professor/Deputy Dean School of Graduate School Universiti Putra Malaysia

This thesis submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfilment of the requirement for the degree of Doctorate of Philosophy. The members of the Supervisor Committee are as follows:

Amin Ismail, PhD

Associate Professor Faculty of Medicine and Health Sciences University Putra Malaysia (Chairman)

Mohd Yazid Manap, PhD

Professor, Faculty Food Science and Technology University Putra Malaysia (Member)

Norhaizan Mohd Esa, PhD

Lecturer Faculty of Medicine and Health Sciences University Putra Malaysia (Member)

HASANAH MOHD GHAZALI, PhD

Professor and Dean School of Graduate Studies Universiti Putra Malaysia

Date: 8 April 2010

DECLARATION

I hereby declare that the thesis is based on my original work except for quotations and cititations which been duly acknowledged. I also declare that it has not been previously or concurrently submitted for any other degree at UPM or other institutions.



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LIST OF ABBREVIATIONS

β	beta
g	gram
μg	microgram
μL	microlitre
mg	milligram
mM	millimolar
mL	millilitre
mmol	millimolar
М	molar
Rpm	revolution per minute
°C	degree of Celsius
%	percentage
DP	degree of polymerisation
CFU	colony-forming unit
hr	hour
HCI	hydrochloric acid
NaOH	natrium hydroxide
H_2SO_4	Sulfuric acid
v/v	volume per volume
w/v	weight per volume

CHAPTER 1

INTRODUCTION

1.1. Background of dietary fibre

Trowell, (1974) defined the term "dietary fibre" to denote edible parts of plant substances that are resistant to hydrolysis by digestive enzymes in humans, and contain membrane components, as well as endocellular polysaccharides. The American Association of Cereal Chemist (2001) defined "dietary fibre as the edible part of plants or analogue carbohydrates that are resistant to digestion and absorption through the small bowel, with complete or partial fermentation in the large bowel. Dietary fibre components are usually grouped into two major classes: water-soluble (pectins, gums) and waterinsoluble (cellulose, lignin, some of the hemicellulose) (Thebaudin and Lefebvre, 1997; Grigelmo-Miguel *et al.*, 1999).

Dietary fibre is not biologically active as, for example, vitamins or mineral components, however they noticeably affect the metabolic and physiological processes that occur in human organisms. Dietary fibre has the ability to increase the fecal bulk, stimulates intestinal peristalsis, and provides a favorable environment for the growth of the desired intestinal flora. It is in the digestive system that the dietary fibre components bind a number of substances, including cholesterol and gastric juices (Veldman *et al.*, 1997; Jenkins *et al.*, 1998; Jiménez-Escrig and Sánchez-Muniz, 2000).

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Due to these specific properties, dietary fibres may play an important role in both prevention and treatment of obesity, atherosclerosis, coronary heart diseases, colon cancer and diabetes (Schweizer and Würsch, 1986; Topping, 1991; Davidson and McDdonald, 1998; Schneeman, 1998; Terry *et al.*, 2001; Wang *et al.*, 2002; Ferguson and Harris, 2003; Peters *et al.*, 2003; Bingham *et al.*, 2003). The results of epidemiological investigations have made it possible to relate the incidence of civilization-induced diseases to insufficient dietary fibre intake from fruit and vegetables (Burkitt & Trowell, 1975; Cummings, 1978; Grigelmo-Miguel *et al.*, 1999; Grigelmo-Miguel & Martín-Belloso, 1999; Jiménez-Escrig and Sánchez-Muniz, 2000). Available local data has indicated that the average urban Malaysian diet contains only about 180 g of vegetables and fruits, and 13–16 g of total dietary fibre (Ng, 1995). This total fibre intake is of course far below the 27–40 g recommended by WHO (1990), contributed in part by the fact the breakfast cereals are not widely consumed by Malaysian adults.

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To meet the WHO recommended intake of 27 -40 g of total dietary fibre, the average Malaysian adult would have to double his intake of vegetables and fruits, which is a formidable task indeed. Considering the practical implications and the current estimated total fibre intake of Malaysians, the expert panel on Malaysian Dietary Guidelines (1998) has recommended a population dietary goal of 20 – 30 g as consistent with "healthy eating" (Ng, 1997).

The key to obtaining the recommended level of fibre intake is the availability of high quality food with high dietary fibre content. The common sources of dietary fibres are the cereals added into commercial foods (juices, bakery products, snacks and dairy products. Recently, however, there has been an increase in the demand for by-products from fruit and vegetables as a source of dietary fibre, because these sources present higher nutritional quality, higher quantity of total and soluble dietary fibres, lower caloric content, strong antioxidant capacity, and higher levels of fermentability and water retention (Rodíguez *et al.*, 2006). Published data indicated that approximately 12% of the by-products obtained from fruit processing were sent to landfills for storage, where the total by-products volume was irreparably lost, although its health-promoting components and other valuable ingredients could be reused (e.g., dietary fibre, lipids, proteins, minerals, polyphenols and flavours). Larrauri (1999) reported that dietary fibre from by-products may provide health-promoting properties. The nondigestible components of foods are regarded as ballast substances (Asp, 1985) but, since then, increasing attention has been on their beneficial physiological effects on humans and animals.

By-products may also become cheap raw materials for food and fodder production (Fronc and Nawirska, 1994). From economic perspective, the potential reuse of a by-product as a raw material for production of new products has made it possible to reduce the troublesome seasonal pattern from which some food industries suffer. Fructooligosaccharide (FOS) is another component often related to fruit dietary fibre. FOS acts like dietary fibre in the gut system when it is fermented by microflora in the intestinal. The growing interest in the role of fructooligosaccharides compounds from natural sources in human health has prompted research in the fields of horticulture and food science. Fructooligosaccharides is inulin-type fructans with a degree of polymerisation (DP) lower than 9 (average DP = 4.8). FOS is considered as **(a)** prebiotics because it improves the intestinal microflora balance and promotes the growth of beneficial organisms (Delzenne and Roberfroid, 1994; Pedreschi *et al.*, 2003). FOS is present in onions, Jerusalem artichokes, asparagus, garlic (Bornet *et al.*, 2002) and Andean yacon root (Pedreschi *et al.*, 2003). However, there is a lack of information on the identification of FOS in fruit by-products.

1.2 Problem Statement

The re-use of fruits by-products of the food industries is of high economic interest because of its commercial viability. Fruits by-products and their residues are usually available in large quantities and although costly to dispose, are not fully exploited commercially for lack of research. While many recent researches focused mainly on the antioxidant capacities and biological activities of the fruits, not many investigations have been conducted to exploit the by-products of fruits processing.

Malaysia's pink guava puree industry has had produced a significant quantity of by-products. The average quantity of residue obtained was about 24.5% constituting of skins and seeds. Presently, the residues were disposed because they were not suitable for animal feeding. As significant amount of residues were produced, it caused problems for their disposal. Thus, instead of continuing with the wastage, an exploitation of these by-products for a new source of dietary fibre as functional compounds for food applications may be a significant option.

Furthermore, the commercial viability of the pink guava by-products dietary fibre is enhanced by its potential physico-chemical and health promoting properties. Therefore a study is warranted to evaluate the pink guava byproducts dietary fibre, through experimentation and analyses of its content, powder, fructooligosaccharides and health-promoting properties for it to be viable for commercial health food applications.

1.3 Significance of the Study

This study is significant both from the perspective of food science investigation and commercial application. Given the lack of investigation or experimentation on the viability of the pink guava by-products and its healing properties, the study will provide the methodological information to researchers. Scientific study is needed for evidence-based product, thus, people can consume functional food products without any doubt in terms of their effectiveness.

From the perspective of commercial application, the food industries shall benefit from the formulation and production of the product, and eventually the consumers. The dietary fibre from pink guava by-products has healthbenefiting quality due to its high total and soluble fibre contents, good functional properties, good colonic fermentability and low caloric content. The pink guava fruit juice industry produces significant amount of byproducts which through its re-use as fibre sources, may turn it into a potential

new natural ingredient for the food industry. Thus this study on the physicochemical and health promoting properties of the pink guava dietary fibre byproducts is both significant as a scientific investigation and potential commercial application.

1.4 Objectives

The objectives of this study are as follows:

(1) To determine the dietary fibre content (soluble and insoluble) of pink guava fruit by-products,

(2) To develop dietary fibre powder from pink guava by-products,

(3) To identify the fructooligosaccharides in dietary fibre powder of pink guava fruit by-products,

(4) To study the health-promoting properties of dietary fibre powder of pink guava fruit by-products.

CHAPTER 2

LITERATURE REVIEW

2.1. Guava

Guava (Psidium guajava L.,), a member of the dicotyledon family Myrtaceae, is a native tropical fruit. It is the most important fruit in a family which includes strawberry guava, rose apple, Surinam cherry, mountain apple, Java plum; and spices such as cinnamon, clove, allspice and nutmeg (Jagtiani *et al.*, 1988). Guava is classified under the genus Psidium, which contains 150 species; however it is mostly *Psidium guajava* that has been exploited commercially.

Guava are known by many other names all over the world, such as goyave (French), guave (German), guaiva (Italian), guayaba (Spanish), goiaba (Portuguese), guyava (Hebrew), gawafa (Arabic), amrud (Hindi), koya (Tamil), malaka (Burmese), farang/ma kuai (Thai), jambu batu (Malay), jambu biji or jambu klutuk (Indonesian), bayabas (Philippines), fan shi liu (Chinese), banjiro/guaba (Japanese), kuawa (Hawaiian), guave de Chine (Burkill, 1997). Botanically, the fruit is a berry which may be round, oval or pear-shaped. The fruits vary from 2 to 8 cm in diameter and from 50 to 500 g in weight (Salmah, 1993). When ripe, the fruit has either pale green or bright yellow skin, while the flesh colour may be whitish, deep pink or salmon-red (Ali and Lazan, 1997). The fruits may be thick flesh with only a few seeds embedded in large mass of the pulp. Guava fruits have a characteristic gritty texture due to the presence of stone cells or seeds (Wilson, 1980).

The flavour of the fruit may range from quite sweet in some types to sour and highly acidic in others. The characteristics musky guava aroma and flavour are quite evident in most forms, but in some types they are mild and pleasant (Ali and Lazan, 1997). In some other guava, the aroma and flavour are quite strong and penetrating. During its development, guava has two phases of fruit expansion, the first phase is from the time of fruit set to the sixth week; and the second is from the twelfth week to the time of ripening (Salmah, 1993).

2.1.1. Varieties of Guava

There are 150 varieties of guava, which can be as small as an egg or as large as a pear, with greenish-white, yellow, or red skins which can be either smooth or pitted. Each has its own subtly distinct flavour. Commercial production of guava, either for fresh consumption or for processing, requires careful consideration with respect to the choice of the cultivar. The different characteristics of each cultivar determine their end use. Cultivars planted for fresh consumption are normally those that are mildly acid, sweet and texturally less gritty, whereas for processing purposes, cultivar with high acid content, and deep pink or salmon-red flesh are selected (Ali and Lazan, 1997; Salmah, 1993). A variety, the 'Beaumont', looks like a pale yellow lemon with smooth skin. It has a shocking pink to salmon-colored flesh and a juicy, and sweet flowery flavor. The Beaumont is a favorite for making pink guava juice.

2.1.2. Nutrient Composition

Guava has relatively low seed content (1.6 – 4.4%), its edible portion is relatively high which not only increases its yield for processing but also increases its nutrient. As in most fruits, the moisture contents constitutes a

fairly large proportion of guava fruit (84%), the energy content is relatively high, protein and fats contents are low at 0.28% and 0.1% respectively (Wenkam and Miller, 1965).

Carbohydrate is the principal non-aqueous constituent of guava. Of the total carbohydrate (14.8 g per 100 g), 5.82 g are the sugars, fructose, glucose and sucrose. Fructose is the predominant sugar, constituting about 55.9% and 52.8% of the sugar in white and pink cultivars respectively (Mowlah and Itoo, 1982) followed by glucose, 35.7% and sucrose, 5.3% (Chan and Kwok, 1975). The fibre and ash contents are considered high, with values of 2.38 and 0.48 g per 100 g of fruit, respectively.

The fruit contains vitamin C, vitamin A, iron, calcium and phosphorus (Iwu, 1993; Burkill, 1997). Guavas are up to 5 times richer in vitamin C than oranges (Conway, 2001). Ascorbic acid is mainly in the skin, secondarily in the firm flesh, and little in the central pulp varies from 56 to 600 mg and may range to

350 – 450 mg in nearly ripe fruit (Conway, 2001). Canning or other heat processing destroys about 50% of the ascorbic acid (Dweck, 2001).

Manganese is also present in the plant in combination with phosphoric, oxalic and malic acids (Nadkarni and Nadkarni, 1999). The fruit contains saponin combined with oleanolic acid, morin-3-O-α-L-lyxopyranoside, morin-3-O-α-Larabopyranoside, flavonoids, guaijavarin and quercetin (Arima and Danno, 2002).

In pink fruit, the commercial essence is characterized to present volatile compounds with low molecular weight, especially alcohols, estersaldehydes, whereas in the fresh fruit puree terpenic hydrocarbons and 3-hydroxy-2-butanone are the most abundant components (Jordan *et al.*, 2003). New components are described for the first time as active aromatic constituents in pink guava fruit is 3-penten-2-ol and 2-butenyl acetate.

Principal differences between the aroma of the commercial guava essence and the fresh fruit puree could be attributed to acetic acid, 3-hydroxy-2-butano3methyl-1-butanol, 2,3-butanediol, 3-methylbutanoic acid, (Z)-3-hexen-1-ol, 6-
methyl-5-hepten-2-one, limonene, octanol, ethyl octanoate, 3-phenylpropanol, cinnamyl alcohol, α-copaene, and other unknown component (Bassols and Demole, 1994; Arima and Danno, 2002,).

Guava contained a considerable amount of stone cells which contribute an undesirable gritty texture to the processed puree. There are two types of stone cell in guava: an irregular-shaped type, abundant under the epidermis, and a smooth type, found in the core region of the fruit (Batten, 1983).

Stone cells are developed from parenchyma cells by secondary thickening of the wall. As the fruits increased in maturity, cell wall thickening appeared to be more prominent (Batten, 1983). The presence of stone cells in the flesh of fruits has no specific functions; however, stone cells may help to give rigidity to the plant part or may act as a protection in the testa of seeds (Salmah, 1993).

The composition of stone cells was reported to be as follows: fat, 0.92%; ash, 1.05%; protein, 1.50%; lignin, 37.1%; cellulose, 53.9%; soluble carbohydrates 5.49% (Batten, 1983). The seeds which are very small but abundant in the fruit have been reported to contain 14% oil, 15% proteins and 13% starch (Burkhill, 1997).

The colour of guava flesh is attributed to the pigment content. The pink coloration in Beumont guava is due to the presence of lycopene, which amounts to 5.87% of the fruit (Jagtiani *et al.*, 1988). The colour of the white or yellowish-white fleshed guavas is associated with anthocyanins (Salmah, 1993).

2.2. Dietary fibre

Dietary fibre generally refers to parts of fruits, vegetables, grains, nuts and legumes that can not be digested by humans and are resistant to digestive enzymes. The dietary fibre is predominantly found in plant cell wall with the main components are cellulose, hemicellulose, pectin and lignin (Dreher and Cho, 2001).

The dietary fiber (DF) is a complex mixture of carbohydrate polymers associated with a number of other, non-carbohydrate components. The DF is originally defined as the skeletal remains of plants cells in the diet, which are resistant to hydrolysis by the digestive enzymes of man (Trowell, 1974). These excluded the polysaccharides such as plants gums and modified cellulose. The

one proposed in 1999 was different from Trowell's definition only in its physiological description of how DF acts in the human organism (Prosky,

1999).

According to this definition, the term "dietary fibre" is used to denote edible parts of plant substances that are resistant to hydrolysis by digestive enzymes in humans, contain membrane components, as well as endocellular polysaccharides (AACC, 2001 ; Asp, 2004).

2.2.1. Sources of dietary fibre

Current recommendations from the United States National Academy of Sciences, Institute of Medicine, suggest that adults should consume 20-35 grams of dietary fibre per day, but the average American's daily intake of dietary fibre is only 12-18 grams (Fuchs *et al.*, 1999; FDA, 2001). The American Dietetic Association recommends consuming a variety of fiber-rich foods. Soluble fibre is found in varying quantities in all plant foods, including: legumes (peas, soybeans, and other beans); oats, rye, chia, and barley, some fruits and fruit juices (particularly prune juice, plums and berries); certain vegetables such as broccoli, carrots and Jerusalem artichokes; root vegetables such as potatoes, sweet potatoes, and onions (skins of these vegetables are sources of insoluble fiber), psyllium seed husk (a mucilage soluble fiber) (FDA, 2001; Anon, 2008).

Sources of insoluble fibre include whole grain foods, bran, nuts and seeds, vegetables such as green beans, cauliflower, zucchini (courgette), and celery, the skins of some fruits, including tomatoes (FDA, 2001; Anon, 2008). Other sources of insoluble fibre include whole wheat, wheat and corn bran, flax seed and vegetables such as celery, nopal, green beans, potato skins and tomato peel (Alvarado *et al.*, 2001)

2.2.2. Dietary Fibre Components

Dietary fibre derives mainly from the plant cell wall that consists of a series of polysaccharides, often associated and/or substituted with proteins and

phenolic compounds in some cells, together with the phenolic polymer lignin (Bacic *et al.*, 1988; Theander *et al.*, 1989; Knudsen, 2001). DF components are usually grouped into two major classes: water-soluble (pectins, gums) and water-insoluble (cellulose, lignin, some of the hemicellulose) (Thebaudin and Lefebvre, 1997; Grigelmo-Miguel *et al.*, 1999). The absorption properties of the DF depend on the chemical structure and mass fraction of the components. Both types of fibre are present in all plant foods, with varying degrees of each according to a plant's characteristics (Saenz, 2007).

Insoluble fibre possesses passive water-attracting properties that help to increase bulk, soften stool and shorten transit time through the intestinal tract (Suter, 2005; Anon, 2008). Soluble fibre undergoes metabolic processing via fermentation, yielding end-products with broad health effects (Stacewicz *et al.*, 2001, Anon, 2008).

Soluble dietary fibre

In this study, only pectin was elaborated as a source of soluble dietary fibre as it was the major soluble dietary fibre in guava. Pectin means "congealed,

curdled" in Greek, a white to light brown powder, is a heteropolysaccharide derived from the cell wall of higher terrestrial plants. It was first isolated and described in 1825 by Henri Braconnot. Pectin is found in primary cell wall and intercellular layer. The amount, structure and chemical composition of the pectin differs between plants, within a plant and in different parts of a plant over time. During ripening, pectin is broken down by the pectinase and pectinesterase enzymes; in this process the fruit becomes softer as the cell walls break down. Pectin changes from an insoluble material in the unripe fruit to more water-soluble substances in the ripe fruit (Asp, 2004).

The characteristic structure of pectin is a linear chain of α -(1-4)-linked Dgalacturonic acid that forms the pectin-backbone, a homogalacturonan (Figure 2.1).There are regions where galacturonic acid is replaced by (1-2)-linked Lrhamnose. From rhamnose, sidechains of various neutral sugars branch off. This type of pectin is called rhamnogalacturonan I. The neutral sugars are mainly D-galactose, L-arabinose and D-xylose; the types and proportions of neutral sugars vary with the origin of pectin (Belitz *et al.*, 2004; Pornsak, 2007).



Figure 2.1: Structure of Pectin (Adapted from Belitz et al., 2004)

Isolated pectin has a molecular weight of typically 60 - 130 000 g/mol, varying with origin and extraction conditions. In nature, around 80% of carboxyl groups of galacturonic acid are esterified with methanol. This proportion is decreased more or less during pectin extraction. The ratio of esterified to non-esterified galacturonic acid determines the behavior of pectin in food applications (Asp, 1987; Asp, 2004).

The main use of pectin is as a gelling, thickening agent and stabilizer in food. In human digestion, pectin passes through the small intestine more or less intact. In the large intestine, microorganisms degrade pectin and liberate short-chain fatty acids that have positive influence on health (Lee *et al.*, 1999). In medicine, pectin increases viscosity and volume of stool so that it is used against constipation and diarrhea. Consumption of pectin has been shown to reduce blood cholesterol in intestinal tract, leading to a reduced absorption of cholesterol from bile or food (Pornsak *et al.*, 2007).

Insoluble dietary fibres

a. Cellulose

Cellulose is an organic compound with the formula of $(C_6H_{10}O_5)_n$, a polysaccharide consisting of a linear chain of several hundreds to over ten thousands $\beta(1\rightarrow 4)$ linked D-glucose units (Crawford, 1981; Young, 1986). Cellulose is derived from D-glucose units, which condense through $\beta(1\rightarrow 4)$ -glycosidic bonds. This linkage contrasts with that for $\alpha(1\rightarrow 4)$ -glycosidic bonds present in starch, glycogen, and other carbohydrates (David *et al.*, 2008). Cellulose is a straight chain polymer (Figure 2.2).



Figure 2.2: Structure of Cellullose (Adapted from Young, 1986)

The multiple hydroxyl groups on the glucose residues from one chain form hydrogen bonds with oxygen molecules on another chain, holding the chains firmly together side-by-side and forming microfibrils with high tensile strength (Young, 1986). This strength is important in cell walls in order to mesh into a carbohydrate matrix, conferring rigidity to plant cells. Cellulose has no taste, odourless, hydrophilic, and insoluble in water and most organic solvents, chiral and biodegradable (Klemn *et al.*, 2005). Cellulose is not digestible by humans and is often referred to as 'dietary fibre' or 'roughage', acting as a hydrophilic bulking agent for faeces. Cellulose has a property to take up water (0.4 g water/gram of cellulose), and these explain its ability to increase fecal weight when added to the diet (Klemn *et al.*, 2005;

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David *et al.*, 2008). Chemically, cellulose can be broken down into its glucose units by treating it with concentrated acids at high temperature (Peng *et al.*, 2002; David *et al.*, 2008). Many properties of cellulose depend on its degree of polymerization or chain length, the number of glucose units that make up one polymer molecule (David et al., 2008).

b. Hemicellulose

A heterogenic group of polysaccharides, defined originally as those soluble in alkali but not in water. Hemicellulose contains many different sugar monomers. For instance, besides glucose, sugar monomers in hemicellulose can include xylose, mannose, galactose, rhamnose, and arabinose (Spiller, 2001; Suter, 2005) (Figure 2.3). Hemicelluloses contain most of the D-pentose, and occasionally small amount of L-sugars as well. Xylose is often the sugar monomer present in the largest amount, but mannuronic acid and galacturonic acid also tend to be present (Spiller, 2001). Hemicelluloses also include xylan, glucuronoxylan, arabinoxylan, glucomannan, and xyloglucan.



Unlike cellulose, hemicellulose (also a polysaccharide) consists of shorter chains with 500-3000 sugar units as opposed to 7,000 - 15,000 glucose molecules per polymer in cellulose (Asp, 1987). In addition, hemicellulose is a branched polymer, while cellulose is unbranched. Hemicellulose is

represented by the difference between neutral detergent fiber (NDF) and acid detergent fiber (ADF) (Nawirska and Kwasniewska, 2008).

c. Lignin

Lignin is a complex chemical compound most commonly derived from wood and an integral part of the cell walls of plants (Lebo *et al.,* 2001). The term was

introduced in 1819 by de Candolle and is derived from the Latin word lignum meaning wood (Sjostrom, 1993). Lignin is not a carbohydrate. It is a highly cross-linked, complex three-dimensional structure based on phenylpropane units (Figure 2.4).



Figure 2.4: Structure of Lignin (adapted from David, 2005)

Lignin is a large, cross-linked, racemic macromolecule with molecular masses in excess of 10,000 units. It is relatively hydrophobic and aromatic in nature.

The degree of polymerisation in nature is difficult to measure, since it is fragmented during extraction and the molecule consists of various types of substructures which appear to repeat in a haphazard manner (Lebo *et al.*, 2001). There are three monolignol monomers; p-coumaryl alcohol, coniferyl

alcohol, and sinapyl alcohol (Lebo *et al.*, 2001). These are incorporated into lignin in the form of the phenylpropanoids p-hydroxyphenyl (H), guaiacyl (G), and syringal (S) respectively.

Lignin fills the spaces in the cell walls between cellulose, hemicellulose and pectin components, especially in tracheids, sclereids and xylem. It is covalently linked to hemicellulose, it confers mechanical strength to the cell walls and by extension the plant as a whole (Chabannes *et al.*, 2001) There are several reasons for including lignin in the dietary fibre concepts; it intimates structural relationship to dietary fibre polysaccharides (lignin is covalently linked to hemicellulose), its importance for digestibility of animal feeds and the physiological properties in human, and as a binder of bile salts in the human gastrointestinal tract (Boerjan *et al.*, 2003; David *et al.*, 2005).

2.2.3. Fermentable Fibre

The American Association of Cereal Chemists defined soluble fibre as "the edible parts of plants or similar carbohydrates resistant to digestion and absorption in the human small intestine with complete or partial fermentation in the large intestine". Fibre can be divided into two categories of fermentability. First, fibre components with high fermentability: pectin, naturals gums, oligosaccharides, and polysaccharides. Second, fibre components with partial or low fermentability: cellulose, hemicellulose, lignin, plants waxes; resistant starches (Tundland and Meyer, 2002).

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Consistent intake of fermentable fibre through foods is reported to reduce the risk of degenerative disease suh as diabetes, cardiovascular disease and numerous gastrointestinal disorders (Venn and Mann, 2004; Lee et al., 2008; Theuwissen and Mensink, 2008). Fermentable fibre can also provide healthful benefits to all disorders of the intestinal tract such as constipation, inflammatory bowel disease, hemorrhoids and colon cancer (Tungland and Mayer, 2002).

2.2.4. Physical Properties of Dietary Fibre

Dietary fibre has several significant physical properties that are related to its physiological effects. According to its water solubility, dietary fibre can be classified as being insoluble and soluble. The insoluble lignin and polysaccharides are mainly responsible for water retention capacity while the ions binding properties of fibre are attributed to the uronic acid content. Soluble fibre on the other hand, can be used to control the rheological properties of foods.

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Soluble fibre can be used as a gelling agent, emulsifier and thickening agent. The amount of fibre added to the foods is commonly less than 10% because above these levels, it decreases the sensory quality characteristics of the products (Carmen, 1997). The main physical properties of dietary fibre to be discovered in this study are the hydration properties, swelling capacity, oil retention capacity, bulk density, and particle size.

Hydration Properties

Hydration properties of dietary fibre refer to its ability to retain water within its matrix. These properties are related to porous matrix structure formed by polysaccharides chains which can bind to water through hydrogen bonds. Fibre with strong hydration properties could increase stool weight and potentially slow the rate of nutrient absorption from the intestine (Figuerola *et al.*, 2005). The hydration properties are described by three different measurable properties such as swelling capacity, water holding capacity and water retention capacity.

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Swelling capacity is a measure of the ratio of volume occupied when the sample is immersed in an excess of water and after equilibration to the actual weight (Raghavendra *et. al.,* 2006). The water holding capacity is defined by the quantity of water that is bound to the fibres without the application of any external force, except for gravity and atmospheric pressure (Robertson *et al.,* 2000; Raghavendra *et al.,* 2006).

It is calculated as the ratio of the quantity of water held up to the initial dry weight of the residue. Water retention capacity is defined as the quantity of water that remains bound to the hydrated fibre following the application of an external force such as pressure or centrifugation (Raghavendra *et al.,* 2005; Raghavendra *et al.,* 2006).

Soluble and insoluble fibres have the ability to hold water. The ability of soluble fibre to hold water is the phenomenon of gelation where water is entrapped in three-dimensional network of polysaccharide molecules (Oakenfull, 2001). In soluble fiber, water is held within the polysaccharides matrix, unable to flow away. The system has the semisolid properties characteristic of a gel.

Insoluble fiber can also absorb water, but more in manner of a sponge. They form a hydrophilic matrix in which water was entrapped, where the quasicrystallinity of the polysaccharide remains and water fills the interstices, often causing considerable swelling (Dreher and Cho, 2001).

The effect of gel formation in polysaccharides may slow absorption by trapping nutrients, digestive enzymes, or bile acids in the matrix and, by slowing mixing and diffusion in the intestine (Oakenfull, 2001). Processing factors, such as grinding, drying, heating or extrusion cooking modified the physical properties of the fibre matrix, and affect the hydration properties (Thibault *et al.*, 1992).

Oil- Retention Capacity

Oil retention capacity of fibre is related to its chemical composition, but is more largely a function of the porosity of the fiber structure rather than the affinity of the fiber molecule to oil (Tungland and Mayer, 2002). Sosulski and Cadden (1982) in studying the different sources of dietary fibre found that lignin-rich samples had more oil absorption capacity. In a study conducted by Lopez *et al.* (1996), the insoluble fractions had higher oil retention capacity levels than soluble fractions, due to their high percentage of large particles size, and lignin found in their chemical composition. Oil retention capacity has also been associated with oil, fat, and cholesterol absorption in the intestinal tract and thus may exert such health benefits (Kuan and Liong, 2008).

In food application, fibre with high oil retention capacity is used successfully with batters, breading and film coatings to reduce the oil up-take during frying operations, and reduces the total fat content of the final food product, enhancing crispiness (Tungland and Mayer, 2002). According to Trinidad *et al.* (2001), insoluble dietary fibre when added to any formulation can absorb oil present and the absorption is measured as fat absorption capacity. The higher the fat absorption capacity of the fibre, the higher will be the flavour retention in the product (Raghavendra *et al.*, 2006).

On the other hand, by hydrating a fibre with water, the water occupies the fiber pores, significantly reducing oil-binding. Physical processing such as grinding will result in an increase in the physical structure and surface area, also an increase of fat absorption capacity (Trinidad *et al.*, 2001; Raghavendra *et al.*, 2005).

Particle Size

The interest in particle size lies in the recognition of its role in controlling a number of events occurring in the digestive tract (transit time, fermentation,

faecal excretion). The range of particle size depends on the type of cell walls present in the foods and on their degree of processing.

Particle size of fibre may vary during transit in the digestive tract as a result of chewing, grinding in the stomach and bacterial degradation in the large intestine. Some components involved in the cohesiveness of the fibre matrix may be solubilized. Larger, coarser particles increase fecal bulk and have a stronger effect on bowel function, whereas smaller particles are denser and have decreased water holding capacity (Dreher, 2001).

The form of the fibres, wet or dry is of importance as some fibres may swell in water solution. The measurement of particle size in a wet form may be more relevant when comparing the bulk volume of fibre in the digestive tract. In any case, when giving particle size values, the methods used and the form of the fibre must be indicated.

2.3. Development Of Dietary Fibre Powder

By-products from the fruits and green industries are inexpensive and are available in large quantities. Many agriculture by-products are commonly used as animal feeds or fertilizers; however, some could also be useful in food industry. Dietary fibre is a significant constituent of many fruits and greens. In comparing the dietary fibre from cereals and those from fruit, fruit fibres have better quality due to higher total and soluble fiber contents, water and oil holding capacities and colonic fermentability, as well as a lower phytic acid and caloric value contents (Saura-Calixto *et al.*, 1996; Figuerola *et al.*, 2005).

There are many fruits, for example orange, apple, peach and olive, that originate a waste during their processing and this by-products contain both soluble and insoluble fibre compounds that can be used for designing new 'functional foods' (Rodriguenz *et al.*, 2006). For example, orange and lemon by-products, which are abundant and cheap, constitute as sources of fibre since they are rich in pectin (Alaska, 1998). A quantity of pectins and polyphenols can be recovered from apple by-products (Carle *et al.*, 2001); and different types of fibres are isolated from grapes, after the extraction of their juice, as well as from as from guava skin and pulp (Schieber *et al.*, 2002).

Pineapple shell has a high percentage of insoluble fibre (70% total dietary fibre), and presents a great antioxidant capacity (Larrauri *et al.*, 1997;

Prakongpan *et al.*, 2002). Other fibres of interest are those rich in highly branched pectins that can be isolated from the mango skin (Sudahakar and Miani, 2000).

According to Saura-Calixto *et al.* (1996) and Larrauri (1999), the ideal dietary fibre should meet the following requirements; no nutritionally objectionable components; as concentrated as possible so that minimum amount can have a maximum physiological effect. It should be bland in taste, colour, texture and odour; balanced composition (insoluble and soluble fractions) and adequate amount of associated bioactive compounds. The dietary fibre should have a good shelf life that does not adversely affect the quality of food to be added and compatibility with food processing.

Furthermore, it should also have the right and, positive image in the eyes of the consumers with regard to sources and wholesomeness. Ultimately, the dietary fibre should have the expected pysiological affect and be reasonable in

(1999) suggested for the total dietary fibre content to be higher than 50%,

moisture lower than 9%, low content of lipids, a low caloric value (lower than 8.36 kJ/g) and neutral flavour and taste.

The Processing Steps for Dietary Fibre Powder

There are four major steps involved in preparing dietary fibre powder which includes washing, wet milling, drying, dry milling

Washing

Washing is used to remove undesirable compounds associated to dietary fibre (such as sugar) and, to remove potential pathogenic microorganism. Losses of some soluble fibre components that contribute to the water holding capacity of the fibre, such as pectin, may also occur (Molla, *et al.*, 1994; Lario *et al.*, 2004). Studies by Larrauri (1999) and Lario *et al.*, (2004) found that washing dramatically increased water-holding capacity of dietary fibre powder in lemons and oranges, due to the removal of sugars. Sugar removal from the

raw material contributes to the drying process, avoiding a dark colour in the dried product and a lower caloric value is also obtainable (Larrauri, 1999).

Unwashed sample contain high amount of total free sugars and significantly lower the water-holding capacity.

Water holding capacity of wheat bran (2.8 to 3.6 ml/g) and apple fibre (5.1 to 6.2 ml/g) products was slightly increased after boiling for 15 minutes compared to the unwashed samples (Thibault *et al.*, 1994). Boiling produces losses in the dietary fibre components, especially in the low molecular weight carbohydrates; due mainly to thermal degradation, leaching into the process water and solubilization of insoluble dietary fibre components (Svanberg *et al.*, 1994; Rodriguez *et al.*, 2006).

The losses of dietary fibre components depend on the type of sample and its processing, and, in this sense, a higher loss in the dietary fibre content of different vegetables has been observed when they were processed (blanching, cooking and canning) (Rodriguez *et al.*, 2006; Agrieszka and Ceculia, 2008).

Wet milling

Wet milling is to control particle sizes. If a particle size is too small, high amount of water can be held during the washing step which, in turn, is detrimental for the drying process (Larrauri, 1999). Lower yields during the separation of water may also occur. On the other hand, big particle sizes do not facilitate the removal of the undesirable components such as sugar during the washing step, because of this; a longer drying time is needed. Hammer mill with a variety of screen size is preferred to colloidal mills in order to obtain a good control of particle size.

Drying

Drying is the main and most expensive step in dietary fibre production. It improves the fibre shelf life without the addition of any chemical preservative and reduces both the size of packaging and transportation costs. Different drying methods are used in the food industry such as rotary kiln, drum dryer, cabinet dryer and tunnel belt. There are six criteria for the selection of drying method; physical and chemical properties of the products, conservation of energy, optimization of space, good utilization of attendant, abatement of air

or other pollution and acceptable return on capital cost (Ferguson and Fox, 1978; Larrauri, 1999)

Little information regarding the influence of drying process on fibre quality are available, but, in general, severe heat treatment breaks down the cell membrane and releases the cell contents, affecting the stability of polysaccharides such as pectin (Bernardo *et al.*, 1990; Selvendran and Robertson, 1994; Femenia *et al.*, 1997).

Drying has significant effect on the water-holding capacity of the dried fibre of orange peels (Marin *et al.*, 2005). The higher the drying rate (110 °C, 8 kg/m²) the lower the water-holding capacity of the sample (Larrauri, 1999). Regarding the effects of the drying temperature on the bioactive compound in dietary fibre products, a significant decomposition may occur giving a number of breakdown products (Esposito *et al.*, 2005).

Dry milling

Most fibres are milled to improve acceptability in the final food products and the fraction obtained can have a different chemical composition, depending on the origin and cell wall material. Grinding may effects hydration characteristics of the fibres as well as texture, aspect and the quality of the food, depending on their chemical composition and physical structure (Larrauri, 1999; Raghavendra *et al.*, 2005).

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Dry milling affects dietary fibre powders physical structure by breaking the pores, resulting in the increase of fibre density and reduction of water-holding capacity (Raghavendra *et al.*, 2005; Esposito *et al.*, 2005). The decrease in dietary fibre particle sizes was associated with a reduction in water-holding capacity and oil-holding capacity (Prakongpan et al., 2002; Sanghark and Noohorn, 2003). Typical particle sizes distribution of commercial high dietary fibre powders are between 0.43 and 0.15mm. (Larrauri, 1999; Prakongpan *et al.*, 2002; Raghavendra *et al.*, 2005).

2.4. Fructo-Oligosaccharide

A report issued by the Food and Agriculture Organisation and the World Health Organization (FAO/WHO, 1998) suggested that carbohydrates should be classified primarily by molecular sizes, according to the degree of polymerization (DP), such as the number of monosaccharides units. In this classification, the dietary carbohydrates are divided into sugars, oligosaccharides polysaccharides and hydrogenated carbohydrates (Polyols)(Table 2.1).

2.4.1. Oligosaccharides

The term "oligosaccharide" refers to a short chain of sugar molecules; "oligo" means "few" and "saccharide" means "sugar (Laurentin and Edwards, 2005). The generic term "oligosaccharides" is customarily used for saccharides having the degree of polymerization of 2 – 10 (Mussatto and Mancilla, 2007).

Structurally, oligosaccharides are composed of 2-10 monosaccharides residues linked by glycosidic bonds that are readily hydrolysed to their constituent monosaccharides either by acids or by specific enzymes (Nakakuki, 2002).

Class (DP*) and Subgroup	Food Application	
Sugar (1-2) Monosaccharides Disaccharides	Glucose, galactose, fructose, tagatose Sucrose, lactose, trehalose, maltose isomaltose	
Oligosaccharides (3-9) Maltooligosaccharides Other oligosaccharides	Maltodextrin Raffinose, stachyose, fructooligosaccharides, galactooligosaccharides	
Polysaccharides (>9) Starch Non-starch polysaccharides Hydrogenated carbohydrate (polyols)	Amylose, amylopectin, modified starch Cellulose, hemicellulose (example: galactans, arabinoxylans), pectins, inulin, hydrocolloids (example: guar)	
Monosaccharides types Disaccharides types Oligosacchardies types Polysaccharides types	Sorbitol, mannitol, xylitol, erythritol Isomalt,lactitol, maltitol Maltitol syrups, hydrogenated starch hydrolysates Polydextrose	

Table 2.1: The classification of the major dietary carbohydrates based	ł
on a degree of polymerization	

*DP = Degree of polymerization

Source: Adapted from FAO/WHO Expert Consultation (1998). Carbohydrates in human nutrition

Various types of oligosaccharides have been found as natural components in many common foods including fruits, vegetables, milk, and honey. Oligosaccharides are relatively new functional food ingredients that have great potential to improve the quality of many foods. At present, oligosaccharides have been widely utilized in foods, beverages, and confectionery (Yun, 1996).

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In addition to providing useful modifications to physicochemical properties of foods, it has been reported that these oligosaccharides have various physiological functions such as the improvement of intestinal microflora based on the selective proliferation of bifidobacteria, stimulation of mineral absorption, and the improvement of both plasma cholesterol and blood glucose level (Nakakuki, 1993)

2.4.2. Classification of Oligosaccharides

There are various ways of classifying oligosaccharides in plants. Kandler and Hopf (1980) grouped oligosachharides into two distinct classes: primary and secondary oligosaccharides. Primary oligosaccharides are those synthesized *in* *vivo* from a mono- and oligosaccharides and a glycosyl donor by the action of a glycosyl tranferase. Secondary oligosaccharides are those formed *in vivo* or *in vitro* through hydrolysis of higher oligosaccharides, polysaccharides, glycoprotein and glycolipids.

Southgate (1995) has classified oligosaccharides into three major foods classes: raffinose- series, maltose-series and fructose-series. Raffinose series consists of stachyose (tetraose) and verbascose (pentaose) which are widely distributed in vegetables, especially seed legumes. Maltose series are frequently found in glucose syrups. Fructo-oligosaccharides are found in some tubers and grasses.

The oligosaccharides of the raffinose series are not hydrolysed by smallintestinal enzymes and are poorly absorbed in the small intestine (Peterbauer *et al.*, 2001). The maltose series are hydrolysed by ezymes in the small intestines and absorbed as glucose very efficiently; they therefore are 'available carbohydrates'. The fructose series are poorly absorbed but are readily hydrolysed by acid. These oligosaccharides are also readily soluble in water and in aqueous alcohols but less readily than sugars (Laurentin and Edwards, 2005; Johnson, 2005). Wolf *et al.*, (2003) and Hirayama (2002), classified oligosaccharides into two categories: digestible and non-digestible (Table 2.3). Digestible oligosaccharide consists of maltooligosaccharides which is hydrolysed by amylase in the upper gastrointestinal. Non-digestible oligosaccharides are resistant to digestion in stomach and small intestine but remain intact as they enter the large bowel where they are fermented by the colonic microflora (Rivero-Urgell and Santamaria-Orleans, 2001).

Oligosaccharides		Functional
Digestible	Non-digestible	properties
Maltooligosacch <mark>aride</mark>	/altooligosaccharide	
	Fructooligosaccharides	Bifidogenic
Isomaltooligosaccharide	Raffinose	Bifidogenic
	Galactooligosaccharide	Bifidogenic
	Xylooligosaccharide	Bifidogenic
	Galactose-sucrose	

Table 2.2: Digestible and non-digestible oligosaccharides

(Source: Cumming *et al.*, 2004)

FOS is comprised of one molecule of D-glucose in the terminal position and from 2 to 4 D-fructose units. FOS are oligemers with β -(2-1)-fructosyl linkages and exist in natural plants, such as vegetables, fruits, and crops. FOS can be

derived from inulin, or synthesized from sucrose via fungal fructosyltranferase (Nakakuki, 2002).

FOS with other linkages, such as β -(2-6), can be produced from sucrose by thermolysis, or through the action of levansucrase (Nakakuki, 2002). Commercially available FOS are a mixture of ketose (GF₂), nytose (GF₃) and fructosylnystose (GF₄) produced from sucrose by the transfructosyl-lating activity of β -fructofranosidase, which can transfer a fructosyl group of sucrose to the terminal fructose of acceptors (Figure 2.5).



Studies have shown that FOS were fermented preferentially by bifidobacterium microflora and thus increased its population as well as the fructooligohydrolase activity of stools (Solange and Mancilha, 2007). Therefore FOS, like dietary fibres, enters the large intestine without any change in their structure. At this stage they are totally fermented by the resident microflora. However, AOAC Dietary Fiber analytical method does not measure FOS because of their ethanol and water mixture solubility (Katarina and Nemcove, 2006).

Structure-function relationships

Glycosidic linkage

The linkage between the monosaccharide residues is a crucial factor in determining both selectivity of fermentation and digestibility in the small intestine. Fermentation of FOS prebiotics is selective because of a cell-associated β -fructofuranosidase in the bifidobacteria (Manning and Gibson, 2004).

Molecular weight

Most of prebiotics are relatively of small degree polymerization, the exception being inulin. It is thought that the oligosaccharides must be hydrolysed by the cell-associated bacterial glycosidases prior the uptake of to monosaccharides (Kolida and Gibson, 2002). It is, therefore, reasonable to assume that the longer the oligosaccharide the slower the fermentation and hence the delay of the prebiotic effects throughout the colon. For example, the FOS, will be more quickly fermented in the saccharolytic proximal bowel compared to inulin.

2.4.3. Physiological Functions of Oligosaccharides

Hirayama (2002) described the differences in the metabolic pathway between digestible and ingestible oligosaccharides. Food moves from the mouth to the stomach, then to the small intestine, and then to the large intestine. In the small intestines, digestible saccharides such as sucrose and starch, undergo the digestion and absorption process. Digestible enzymes convert the saccharides to monosaccharides, which are eventually metabolised, and exhaled as breath carbon dioxide or excreted in urine.
On the other hand, non-digestible oligosaccharides pass through the small intestine tract, and later, fermented and undergo absorption process in the large intestine. In this process, the intestinal microbes transform the oligosaccharides into short-chain fatty acids (SCFAs). Acetate, propionate and butyrate are the most common components. Subsequently, the SCFAs are absorbed and metabolized into carbon dioxide (Tanaka and Sako, 2004).

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The non-digestible oligosaccharide serves several kinds of physiological functions, and the functions are classified into three types as shown in Figure 2.7 (Hirayama, 2002). The primary function encourages a good gastrointestinal condition, including a normal stool frequency, less constipation, and healthy intestinal microflora. The second is related to better mineral absorption, including an increase in bone density and relief of anaemia. The third function is immunomodulation, such as allergy and cancer prevention (Wolf *et al*, 2003).

2.4.4. Fructooligosaccharide and Prebiotic Effect

Fructooligosaccharides are currently garnering much attention, especially in their application as prebiotics: they escape digestion in human upper intestine and reach the colon where they are totally fermented, mostly to lactate and SCFA. The most important property of oligosaccharides is their ability to specifically stimulate bifidobacteria growth and to induce butyrate production (Hooner, 2004).



Figure 2.6: An Overview of Physiological Function of Non-Digestible Oligosaccharides (Fructooligosaccharide). (Adapted from Hirayama, 2002)

Prebiotics have been defined as non-digestible food ingredients that beneficially affect the animal by selectively stimulating the growth of certain bacteria in the colon which are advantageous to the host (Roberfroid, 2002). Most prebiotics are oligosaccharides, the best-known example being the β -(2-1)- linked fructooligosaccharides (FOS).

FOS is resistant to digestion in the stomach and small intestine. The reason for this is the presence of β -configuration of the anomeric C₂ in the D-fructose residue. The human digestive enzymes such as sucrase, maltase-isomaltase and α -glucosidase are specific for α -glycosidic linkages. FOS largely escapes digestion in the human upper intestine and reaches the colon where they are totally fermented by the indigenous microflora and, they stimulate bifidobacterial growth (Bronet and Brouns, 2002).

Bifidobacteria have a relatively high amount of β -fructosidase, which is selective for the β -(2-1) glycosidic bonds that present in FOS (Hoover, 2004). After FOS hydrolysis, fructose serves as an efficient growth substrate for the bifidus pathway of hexose fermentation, which is almost exclusively carried out by bifidobacteria (Bronet and Brouns, 2002).

The prebiotic effect of FOS is dose dependent. It is associated with a decrease of fecal pH and an increase in the production of short-chain fatty acids. Studies by Hirayama (2002) and Mussatto and Mancilla (2007) showed that taking 6 gm daily of FOS could increase the number of bifidobacteria within a week. FOS intake shows an increase in SCFA amount (acetate, propionate and butyrate). The production of butyrate is most interesting because it regulates the growth and differentiation of the coloncytes, stimulates the immunogenicity of cancerous cells, and generates immunogenic "apobodies". (Bournet and Brouns, 2002).

2.4.5. FOS analysis

Two of the methods available to analyse FOS in foods are the high performance thin layer chromatography (TLC) and high layer chromatography (HLC). For this study, TLC method was chosen due to the simplicity of operation; repeatability of detection; any-time quantification with changed parameters, for fractions of the entire samples are stored on the plate. This method is also known for its cost effectiveness because many samples can be analysed on a single plate with low solvent usage (Sherma, 2000). Park *et al.* (2001) reported on a quantitative analysis of FOS through TLC using the solvent systems consisted of isopropyl alcohol: ethyl acetate: water (2:2:1). FOS was obtained by heating the plates at 105 °C after spraying phenol sulfuric acid. A routine method has been proposed by Vaccari *et al.*, (2000) for the analysis of FOS through thin layer chromatography, which provides a rapid method for the detection and quantitative determination of the oligosaccharides in beet molasses and other products.

Other methods are also applied in the identification of FOS such as diol high performance thin layer chromatography plates using solvents acetonitrile and acetone. A nine-step gradient was performed by mixing the two solvents using a Camag Automated Multiple Development apparatus. A direct method of measuring FOS is by high performance liquid chromatography. FOS is separated on an ion-exchanges column which is connected to a refractive index detector (Prosky and Hoebregs, 1999).

2.5. Health-Promoting Properties

The analyses of the dietary fibre from pink guava by-products healthpromoting properties involve their antioxidant activity, prebiotic and hypocholesterolemic effects.

2.5.1. Antioxidant Activity

Antioxidant refers to a compound that can delay or inhibit the oxidation of lipids or other molecules by inhibiting the initiation or propagation of oxidative chain reaction, thus prevent damage in the cells by free radical species (Tachakittirungrod *et al.*, 2007). Antioxidants act by one or more of the following mechanism: reducing activity, free radical-scavenging potential complexion of pro-oxidant metals and quenching of singlet oxygen (Moure *et al.* 2001).

Several methods have been used for the evaluation of the antioxidant activities of plants. They are DPPH – scavenging assay (Gamez *et al.*, 1998), ABTS (Re *et al.*, 1999), FRAP (Benzies and Srain, 1996; Pulido *et al.*, 2000), and β -carotene

bleaching model (Dapkenvicius *et al.*, 1998; Jayaprakasha *et al.*, 2001). The antioxidant activities are related to a number of different mechanisms, such as free radical–scavenging, hydrogen–donation, singlet oxygen quenching, metal ion chelation, and acting as a substrate for radicals such as superoxide and hydroxyl (Barreira *et al.*, 2008).

Agricultural and industrial residues are attractive sources of natural antioxidants. Grape pomace (Lu & Foo, 1999; 2000, Llobera and Canellas, 2007), citrus seeds and peels (Bocco *et al.*, 1998; Guo *et al.*, 2003), carrot pulp waste (Chen & Tang, 1998), chestnut leaf, skins and fruit (Barreira *et al.*, 2008), and cocoa by-products (Azizah *et al.*, 1999) have been studied as cheap sources of antioxidants.

The antioxidant compounds from residual sources could be used for increasing the stability of foods by preventing lipid peroxidation and also for protecting oxidative damage in living systems by scavenging oxygen radicals (Moure *et al.*, 2001).

However, the organoleptic characteristics of the by-products must be suitable for incorporation into food products (Elleuch *et al.*, 2007). The search for cheap, renewable and abundant sources of antioxidant compounds is attracting worldwide interest. Further research is required to select raw materials whose residual origin is especially promising due to their costeffectiveness.

2.5.2. Prebiotic Effects

Dietary fibres consist of a large group of substances (mainly of plant origin) that are not hydrolysed by enzymes of the human small intestine (Drzikova *et al.*, 2005). The main sources of dietary fibre in human nutrition are cereals,

fruits and vegetables. Dietary fibres have several preventive medical and nutritional effects in the intestinal tract, depending on their structure and molecular weight, as well as on their solubility and their physicochemical properties such as water-binding and viscosity (Dongowski, 2007). They occur in isolated, more or less in soluble form (e.g., pectin, β -glucan, carrageenan, guaran) in the diet or as a part of the more or less intact complex cell wall architecture in plant materials. (van der Kamp *et al.*, 2004).

For the past decade, the use of prebiotics, such as non-digestible food ingredients that selectively stimulate growth and activity of particular gut microbiota considered beneficial to health, such as bifidobacteria and lactobacilli has grown rapidly (Gibson and Roberfroid, 1995; Holzapel and Schillinger, 2002).

Gibson and Roberfroid (1995) first described a prebiotic as a 'non-digestible food ingredient that beneficially affects the host by selectively stimulating the growth and/or activity of one or a limited number of bacteria in the colon, and thus improves host health'. For a dietary substrate to be classed as a prebiotic, at least three criteria are required: (1) the substrate must not be hydrolysed or absorbed in the stomach or small intestine, (2) it must be selective for beneficial commensal bacteria in the colon such as the bifidobacteria, (3) fermentation of the substrate should induce beneficial luminal/systemic effects within the host (Manning and Gibson, 2004). As diet is the main factor controlling the intestinal microflora, it is possible to modulate the composition of the microflora through foods. A prebiotic substrate is selectively utilized by beneficial components of the indigenous gut flora but does not promote potential pathogens (Manning and Gibson, 2004).

Many fruits and vegetables contain prebiotic component such as FOS and soluble dietary fibre. Examples are onions, garlic, bananas, asparagus, leeks, Jerusalem artichoke, chicory. However, the probable situation is that levels in these foods are too low to exert any significant effect. Some unpublished data recommended consumption of at least 4 g/days but more preferably 8 g/days of FOS would be needed to significantly (ca. one log10 value) elevate bifidobacteria in the human gut (Holzapfel and Schillinger, 2002; Frank, 2002).

2.5.3. Hypocholesterolemic Effect

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There is significant interest in the food industry to develop functional foods to modulate blood lipids such as cholesterol and triglycerides. It is widely believed that elevated cholesterol levels in the blood represent a risk factor for coronary heart disease, with low-density lipoproteins (LDL) being the utmost concern (Delzene and Kok, 1999).

The long-term cholesterol-lowering effect of several sources of dietary fibres have been fully documented (Lairo, 2001). Nevertheless, the mechanisms involved still open for discussion. Two different mechanisms of action for interaction of dietary fibre with cholesterol metabolism in the gut and liver are suggested.

One mechanism is suggested to deal with an increase in cholesterol and reduction of bile acid excretion (Micheal *et al.*, 1998; Marlett, 2001). Another mechanism is to promote the increase of bile acid excretion with citrus pectin and β -glucan from oat (Andersson, 1998). However, as mentioned by Fernandez (1998), the mechanism which plasma LDL cholesterol is lowered by dietary soluble fibre may vary depending on the fibre source and its physical characteristics.

The nature of a fibre can sometimes have an effect on the quantity of cholesterol reduction. In some cases the viscosity of the fiber in solution is vital. An investigation of beta-glucan with and without treatment by degrading enzymes supported the hypothesis that higher molecular weight beta-glucan is more effective than lower molecular weight beta-glucan in increasing bile acid excretion (Lia *et al.*, 1995). On the other hand, partially hydrolyzed psyllium had comparable effects on cholesterol metabolism in rats (Champ *et al.*, 2003). Insoluble dietary fiber fractions also lowered liver cholesterol, but not significantly.

Soluble fibre intake results in a consistent reduction of hepatic cholesterol generated by a decrease in delivery of cholesterol to the liver through the chylomicron remnant to the interruption of enterohepatic circulation of bile acids (Theuwissen and Mensink, 2008). Cholesterol-lowering effect of xanthan, guar gum and β -glucan (1–2% in the diet +0.25% cholesterol) in rats was recently described by Favier et al., (1998). Gullion and Champ (2000) concluded that a decrease of cholesterol absorption is due to the high viscosity of the fibre that is able to alter lipid emulsification and lipolysis.

Oat bran β -glucan (8.7 g/day) was shown to increase bile acid excretion by 83% after 24 hours ; and by 93% after taking an oat bran test meal (6 g β -glucan from oat bran, high in fat and cholesterol) (Rosamund, 2002). Oligofructose has also been shown by Kok et al., (1998) to decrease serum triglycerides in rats (10% FOS for 30 days). Trautwein *et al.*, (1998) demonstrated an hypocholestrolemic effect of inulin (16% of the diet) in hamsters. Feeding male Wistar rats on a carbohydrate rich diet containing 10% FOS significantly lowers serum triacylglycerol (TAG) and phospholipid concentration (Delzenne *et al.*, 2002).

Out of nine studies reported on the responses of blood lipids to inulin and FOS, three have shown no effects on blood levels of cholesterol or triacyl glycerol, three have shown significant reductions in TAG, whilst four have shown modest reductions in total and LDL cholesterol (Williams & Jackson, 2002). Feeding rats with 10% FOS significantly lowers serum triglycerides and phospholipids concentrations but does not modify free fatty acid concentration in the serum.

CHAPTER 3

DIETARY FIBRE COMPOSITION

3.1 Introduction

Dietary fibre is part of the plant that cannot be digested in the human body but it is partially digestible in the colon. Dietary fibre is often classified as soluble dietary fiber (SDF) and insoluble dietary fibre (IDF: cellulose, hemicellulose and lignin) depending on their solubility in water. The physiological effects of total dietary fibre, in the forms of insoluble and soluble fractions of foods have significant advantages for human nutrition and food applications.

The fruit juice industry produces significant amount of by-products which could cause problems for disposal. Usually, these products were used for animal feeding. However, their high amount of dietary fibre could permit its development as a novel natural ingredients for food application (Figuerola *et al.*, 2005).

In recent years, many studies have investigated dietary fibres from the byproducts such as apple pomace , citrus fruits, grapes skin and seeds, guavas, mangoes, pineapples and passion fruits to explore their potential applications and physiological activities (Gourgue *et al.*, 1992, Grigelmo-Miguela *et al.*, 1999a, Grigelmo-Miguela *et al.*, 1999b, Larrauri *et al.*, 1996, Larrauri *et al.*, 1997, Leontowitc *et al.*, 2001 and, Chau and Huang, 2004).

The Golden Hope Fruit and Beverages Sdn. Bhd., a local pink guava puree industry located in Setiawan Perak has been producing about 24.5% of byproducts per-day, constituted mainly of pulp and seed. The exploitation of pink guava by-products as a new source of dietary fibre for a functional food compound application is essential. This also serves the need for the industry to provide a proper solution for the pollution problem connected with the disposing process of the by-products. However, information on the dietary fibre contents prepared from the pink guava by-product was quite scarce. To initiate the analysis of the potential of pink guava by-products as a source of dietary fibre, first to be determined is the total dietary fibre (TDF), soluble dietary fibre (SDF) and insoluble dietary fibre (IDF) content of pink guava byproducts.

3.2 Materials and Methods

The by-products of pink guava puree industry for the study were collected from Golden Hope Food and Beverages Sdn. Bhd. in Setiawan , Perak. About 60 kg of by-products were collected three times for replication throughout the study. The pink guava by-products consisted of namely refiner (RW), siever (SW) and decanter (DW). The by-products were brought to Food Technology Processing Laboratory at MARDI, Serdang in sterilized plastics packaging and frozen at -20 °C until further analysis.

Among the pink guava by-products, refiner was the first by-product to be collected through the process followed by siever and decanter. The processing flow of pink guava puree produced by Golden Hope Food and Beverages Sdn Bhd is shown in Figure 3.1. Firstly, the fruit undergone sorting process once it reached the factory where the rotten and immature fruit were eliminated from the holding tank. The selected fruit were later washed in a tank consisted of 0.05% chlorine solution. Then the fruit was cut by a sharp chopper to small pieces. The resulting pieces including the pulps were put through three steps of processing namely refining, sieving and decanting.



Figure 3.1: The Processing Flow of Pink Guava Puree Production (Adapted from Fruit and Beverages, Golden Hope Sdn. Bhd., Manjung, Perak). RW – refiner waste, SW – siever waste, DW – decanter waste

Refining was the first step of screening where the pulp with particle size bigger than 1.2 mm was collected as by – product called refiner (RW). The (RW) pulp whose particle size smaller than 1.2 mm was later passed through sieving, the next screening machine. At sieving stage , the collected particle size bigger than 0.8 mm was termed as siever (SW). The pulp which had particle size smaller than 0.8 mm was collected in centrifuge machine with the speed of 1450 rpm. The end products were lighter pulp collected as pink guava puree and, heavier pulp collected as a decanter (DW).

3.2.1 Soluble and Insoluble Dietary Fiber Determination

Soluble dietary fibre (SDF) and insoluble dietary fibre (IDF) were determined according to the AOAC method 991.42 developed by Prosky *et al.* (1988). FIBRETEC, the dietary fibre extract equipment from FOSS, Switzerland was used in this procedure. The method is described as follows.

Chemicals

Celite, acid wash (Celite 545 AW), ethanol solutions; (85% and 78% of ethanol solution), and hydrochloric acid solution; (0.561 M and 0.325 M) were used. Phosphate buffer, (0.08 M), Na phosphate monobasic monohydrate and sodium hydroxide were also included. All chemicals used were of analytical grade from Sigma Chemical Co. (USA).

Enzyme Solution

Three types of enzyme solutions were used in the analysis; α-amylase solution, heat stable, amyloglugosidase and protease. All the enzymes were bought from Sigma Chemical Co. (USA).

Sample Preparation

All samples were in pulp form at the initial pH of 3.35. All the three byproducts were dried at 105 °C using an air oven (Mermmet, Germany) until the weight was constant. The dried samples were finely grounded using a dry blender (Kenwood, U.K) into 0.5 mm particles sizes using dry mill for analyses. Dry weights of samples were determined according to AOAC (1980). The samples were weighed in duplicate $(1g \pm 0.1 mg)$ of homogenised sample with not more than 20 mg weight difference between the sample duplicates and transferred quantitatively to cleaned flasks. Then, the flasks were later covered with aluminium foil.

Enzymatic Digestion

For enzymatic digestion, the method described by Prosky *et al.* (1988) was replicated. About 1 g of sample was weighed and put in 400 mL beakers. Fifty mililitres of 0.08 M phosphate buffer with pH 6.0 was added. The pH was adjusted to pH 6.0 \pm 0.2. Then, 100 μ L α -amylase added into it. The sample was then incubated at 95 – 100° C in water bath for 15 minutes. The sample was cooled at room temperature (27 °C), before protease solution was added, and the pH sample adjusted to 7.5 \pm 0.2. Afterward, the 100 μ L protease solution was added and incubated at 60 °C for 30 minutes. The mixture was cooled at room temperature (27 °C). The mixture was adjusted to pH 4.0 – 4.6, before the addition of amyloglucosidase solutions. After 100 μ L amyloglucosidase solution was added, the mixture was incubated at 60 °C for 30 °C.

30 minutes. After incubation the mixture was cooled at room temperature (27 °C). The resultant sample was prepared for the subsequent IDF determination.

Determination of IDF

The celite was weighed to the nearest 0.1 mg and put into a crucible, then it was wet with water and redistributed by distilled water. Then, the suction was applied to a crucible to draw the celite into fritted glass. Next, precipitation from the enzyme digest was quantitatively transferred through crucible into a pre -weighed suction flask. The residue was later washed with 2×10 mL portions of water. The filtrate and waster washings were retained to determine soluble dietary fibre. The remaining residue then washed with 2×10 mL portions of 95% ethanol and then 2×10 mL portions of acetone. For washing the crucible, normal suction was applied.

The crucible containing the residue was put to dry overnight at 105 °C. The crucible contained celite and residue was later cooled in a desiccator. After cooling; the crucible was weighed to nearest 0.1 mg. In determining the

weight of the residue, the weight of crucible and celite was subtracted. For protein and ash determination AOAC method were used.

Determination of SDF

For soluble dietary fibre determination, filtrate and water washings from IDF procedure were combined. Then, the solution was transferred to the beaker with preheated 4 x 100 ml of 95% ethanol. Later , the suction flask was rinsed with some ethanol. The mixture was later kept at room temperature (27 °C) for 60 minutes. After that, the crucible containing celite was weighed to nearest 0.1 mg. The celite in crucible was wet with water and redistributed using 78% ethanol. The suction was applied to crucible to draw celite into fritted glass to form an even mat.

Next, the enzymes digest was filtered through crucible. The residue was then washed with 3 x 20 mL portion of 78% ethanol, 10 ml portion of 95% ethanol, and 2 x 10 mL portion of acetone. Normal suction was applied at washing. The crucible containing the residue was dried overnight in 105 °C air oven. Then, the crucible and residue was cooled in desiccator and weighed to nearest 0.1

mg. In determining the residue weight, the weight of the crucible and celite were subtracted. AOAC method (1990) was later to be used for protein and ash determination.

Determination of Protein

The protein content was determined according to AOAC method (1990). The automated Kjedhal system (Kjeltec System 2200, Tecator, Sweden) was used in identifying the amount of nitrogen in the sample. Protein was calculated by multiplying the nitrogen content with a factor of 6.25. Sample (2 g) and 2 Kjeltabs Cu 35 were placed in digestion tube. Then, 25 mL of concentrated H₂SO₄ was carefully added, and gently shaken to dilute the sample with the acid. The digestion tube was placed in an inclined position and heated gently until frothing ceased; the digestion continued until all samples became clear with a blue/green solution and then allowed to prolong for another 30 minutes.

The digestion solution was cooled at room temperature for 30 minutes, then 200 mL of water added into it. The solution was later cooled to 25 °C. The digestion tube containing the digestion solution was placed in the distillation unit and 50 mL of 40% natrium hydroxide was added into the solution. At the distillation cycle, the digestion solution turned green in colour, indicating the presence of an alkali-ammonia. At this point, titration of digestion solution was done with standardized hydrochloric acid until the blue/grey end point was achieved. The volume of acid consumed in the titration was determined.

In this analysis, Kjeltec System 2200 was used where the titration was done automatically and the result of titration was obtained after the analysis. For blank determination, full chemical blanks were run before each batch of analyses to compensate for any shortcoming from reagents used. In these procedures, 25 mL of H₂SO₄ and Kjeltabs Cu 35 were digested and subsequently treated similar as to the sample.

Calculations:

% N =
$$(T - B) \times N \times 14.007 \times 100$$

W

% P = % N x F

where,

T = titration volume fro sample (ml)

B = Titration volume fro blank (blank)

N = Normality of acid

F = Conversion factor for Nitrogen to Protein; 6.25

W = Weight of sample in mg

P = Protein

Determination of Ash

Total ash was determined according to the AOAC method (1995). Three grams of homogenised sample was put into a silica basin that had been heated, cooled in dessicator, and weighed soon after reaching room temperature. The sample in the silica basin was heated in a furnace at 550 °C until grayish ash was obtained. Then the sample was removed and cooled in the dessicator. The sample then weighted after it attained room temperature (27 °C). The sample was heated in the furnace until it reached constant weight.

Calculation:



The determination of IDF and SDF was calculated as follow:

% DF =
$$((R_1 + R_2) / 2) - (P(g) - A(g) - B)$$
 X 100

 $(M_1 + M_2) / 2$

where,

P: protein

A: Ash

B: Blank

M₁, M₂: sample weight

R₁, R₂: Weight of residue for sample

Total dietary fibre was calculated as the sum of SDF and IDF.

% TDF = % SDF + % IDF

3.2.2. Determination of Dietary Fibre Fractions (Neutral Dietary Fibre (NDF), Acid Dietary Fibre (ADF), Lignin, Cellulose and Hemicellulose)

Chemicals

Seventy two percent of sulfuric acid solution and acid detergent solution were used. For acid detergent solution, 20 g of acetyl trimethylammonium bromide in sulfuric acid was used. All chemicals used were of analytical grade from Sigma Chemical Co. (USA).

Determination of neutral dietary fibre (NDF)

Neutral dietary fibre was identified using the method developed by Van Soest *et al.* (1991). In this method five grams of sample was heated to boiling in 100 mL of neutral detergent containing 50 μ L of heat stable amylase. Sodium sulfite (5 g) was added to the mixture. The mixture was then boiled for 1 hour

and filtered through coarse sintered glass crucible. For this analysis, the samples were sieved through 1-mm screen, but not finer, because overgrinding could adversely affect the filtration step.

During the filtration step, the lowest possible vacuum pressure was applied. The sample was not to be added while vacuum pressure was on. The pressure was released when liquid was added. Sample was allowed to settle at least for 15 seconds before vacuum pressure resumed. This was to ensure, finer matter was filtered onto a settle mat. Boiling water was used to prevent crucible from cooling. If a crucible clogs, positive pressure was to be exerted from beneath to flush particles out of the filter plate.

Afterwards the residue was later dried at 130 ± 2 °C, then cooled in desiccator, and weighed to nearest 0.1 mg. The residue was corrected for nitrogen x 6.25 for protein. For ash, the residue was incinerated for 5 hours at 525 °C. Then, the crucible and residue were cooled in the desiccator and weighed to nearest 0.1 mg. The crucible containing the fibre preparation was analysed using a Tecator (Helsingborg, Sweden)

Determination of acid detergent fibre (ADF)

The ADF procedure followed the Van Soest method (1973). One gramme of dried sample with particle size 1 mm was weighed into reflux container. Then, 100 mL acid-detergent solution was added into it at room temperature (27 °C). The sample was heated for 5 – 10 minutes until boiling. Relux was set 60 minutes from the start until the onset of boiling. Afterward, container, swirled, and filtered was removed using minimum suction. Then, fritter glass crucible was weighed (W₁).

The filtered mat disintegrated when the crucible was filled with 2/3 of hot water (90 -100 °C). The sample was stirred and let to soak for 15 - 30 seconds. The sample was then washed with water. Afterward, the sample was washed twice with acetone. The sample was later washed repeatedly with acetone until no more colour was removed. Then, the sample was dried for three hours in 100 °C air oven and weighed (W₂).

Calculation:

% ADF = $100 (W_2 - W_1) / S$

where, $S = Ws \times W_{DM} / W_{DM}$

Ws = weight of sample

W_{DM} = weight of oven dried-matter

Determination of Lignin

The determination of lignin in analysed samples was through the use of Van Soest method (1973). 1 gramme of asbestos was added into the crucible containing fibre (from ADF analysis). Then, the crucible was placed in 50 mL beaker. Later, the mixture was added with 72% of cooled H_2 SO₄. The mixture was stirred with glass rod to smoothen it. Acid was added to about half of the crucible and stirred with glass rod. The mixture was once again refilled with 72% H_2 SO₄ and stirred hourly as the acid drained. The crucible was maintained at 20 – 23 °C through out the analysis.

After 3 hours the mixture was filtered completely. The mixture was later washed with hot water until acid-free. The mixture was then dried at 100 °C for 1 hour, and cooled in desiccator, and weighed (W₃). The obtained residue was heated at 500 °C using a furnace for 2 hours. Then, the crucible was transferred into 100 °C forced-draft oven for 1 hour. Finally , the crucible was put into the desiccators to be cooled and weighed (W₄).

To determine asbestos blank, 1 gram of asbestos was weighed into the crucible and proceed as above. The loss in weight was recorded (W_5). The determination of blank was discontinued when the asbestos was more than 0.0020 g.

Calculation:

% Acid-insoluble lignin -= $(W_3 - W_4 - W_5) / S$

Determination of Hemicellulose and Cellulose

The proportion of hemicellulose was calculated from the difference between

NDF and ADF, as shown bellow:

% H = % NDF - % ADF

where,

H = hemicellulose

NDF = neutral detergent fibre

ADF = acid detergent fibre

Cellulose was determined according to the calculation below;

% C = % ADF - % AIL

where,

C = cellulose

ADF = acid detergent fibre

AIL = acid-insoluble lignin

Determination of soluble fraction

The proportion of soluble fraction was calculated as follow:

 $S_r = 100 - NDF$

Where,

S_r = soluble fraction

NDF = neutral detergent fibre

3.3 Statistical Analysis

Three measurements were taken on each analysis. Results were expressed as mean of values \pm standard deviation of three separate determinations. Comparison of means was performed by one-way analysis of variance (ANOVA) followed by LSD test. ANOVA was performed at p < 0.05 to consider the significant difference. Statistical analyses were run using the SAS V. 9.1 software (SAS, USA).

3.4 Results and Discussion

Table 3.1 shows the total (TDF), insoluble (IDF) and soluble dietary fibre (SDF) contents of pink guava by-products and the IDF:SDF ratio. This study showed that pink guava by-products had high content of TDF in the range of 68 to 79%. According to Femenia *et al.* (1997) and Larrauri (1999), plant by-products that contained more than 60% of TDF could be considered as a rich source of dietary fibre. With this evidence, the pink guava by-products can be considered as a good source of dietary fibre.

Sample	Dietary Fibre (g/100 g dry matter)				
	Moisture content	Insoluble	Soluble	Total	Ratio SDF:IDF
RW	59.1 ± 0.81°	75.5 ±0.84 ^a	3.4 ± 0.18^{a}	78.8 ± 1.01^{a}	1:22
SW	73.8 ± 0.74^{b}	64.1 ± 0.51^{b}	4.4 ± 0.04^{b}	68.5 ± 0.47^{b}	1:15
DW	65.5±0.17ª	71.1 ± 0.41 ^c	4.0 ± 0.09^{ab}	75.7 ± 0.27^{a}	1:18

Table 3.1: Dietary fibre composition of pink guava by-products

Note: ^{a,b,c,} means of three replications; if different within a column, indicates significant difference at level p<0.05. RW: refiner waste; SW: siever waste; DW: decanter waste

Among the analysed by-products, RW showed the highest TDF content (78.8%) followed by DW (75.7%) and SW (68.5%). There was significantly different (p<0.05) TDF content among pink guava by-products. This could be due to the mechanical processes the sample had undergone. For processing the pink guava puree, the RW was screened using 1.2 mm screen size, whilst SW was screened through screen size 0.8 mm. The bigger the screen size, the more seeds and skins of pink guava were trapped in the screener. This contributed to the higher content of TDF in the sample.

This study has discovered that IDF was the predominant fibre fraction of the pink guava by-products which contained more than 90% of the TDF. In fruits and vegetables by-products, insoluble fibre was reported to be a major fibre fraction (Chau and Huang, 2004; Gorinstein *et al.*, 2001; Thomas *et al.*, 2000). Among the by-products, RW had significantly higher insoluble dietary fibre (75.5%) compared to SW and DW. This could be due to high quantity of seeds and skins. In addition, RW was derived from the first processing step in pink guava puree production, which any particle bigger than 1.2 mm was collected as a by - product. A study on artichoke by Lopez *et al.* (1996) showed that insoluble dietary fibre fractions had a particle size greater than the soluble fraction.

In pink guava by-products, the soluble fraction represented 3.4 to 4.4% of the TDF of the products. The soluble dietary fibre fraction in pink guava by-products was much higher than found in cereals bran; 2.9% in oat bran and 3.6% in wheat bran (Grogelmo-Miguel and Martin-Belloso, 1999). SW was found to be higher in soluble dietary fibre compared to DW and RW, at 4.0 and 3.4% respectively. These could be due to more skin and pulp was collected in SW due to the mechanical processes.

Pink guava by-products has higher IDF/SDF ratios compared to citrus byproducts (Marin et al., 2005) and asparagus by-products (Fuentes-Alventosa et al., 2009). The SDF/IDF ratios in the analysed by-products , ranged between 1:15 to 1:22, these values were in agreement with Grigelmo-Miguel et al. (1997) and Mollá et al. (1994) whose findings of the SDF: IDF ratios in cereals were in the range of 1:6 to 1:24. The relative amount of IDF of the pink guava byproducts (above 90%) obtained in the study were similar to the proportions reported for apple pomace (90%) and citrus peel (80%) (Figuerola et al.(2005), and in carrot (91.8%) and beet (82.1%) (Zambrano et al., 2001).
The concept of dietary fibre refers to the components derived from the plant cell wall which include cellulose, hemicellulose and pectin. However, this concept is now expanded to include lignin. Determination of these component fractions in sample study is important for better understanding of their function as dietary fibre, as the physiological effect of dietary fibre depends on the relative amount of individual fibre components.

The plant cell can be described as a highly ordered network of cellulosic microfibrils embedded in a matrix of non-carbohydrate, protein and with phenolic cross-links between the various polysaccharides (Wadron *et al.*, 2003). In the cell wall found in dicotyledonous plants including fruits, the major polyscharides are pectic polysaccharides, cellulose and xyloglucans, while, lignin is found in relatively small quantities in most edible fruit tissues (Serena and Knudsen, 2007).

For determination of fibre fraction, NDF and ADF the method used by Van Soest (1973) was employed. Under the NDF and ADF methods, the technique used was the hot detergent treatments to remove digestible material (soluble fibre). The methods discriminate the insoluble fibres in hemicellulose, cellulose and lignin. The comparison of the NDF and ADF values for pink guava by-products are summarized in Table 3.2.

It was evident that the dietary fibre content in pink guava by-product had high percentage of NDF and ADF. The NDF contained in pink guava byproducts was higher compared to those of apple pomace (24 to 31%) and carrot pomace (18%) (Nawirska and Uklanska, 2008). There was no significant difference (p>0.05) of NDF between RW and DW, but there was slightly lower NDF in SW. An ADF extraction system was developed to measure cellulose and lignin (Van Soest *et al.*, 1973). The ADF content was very high in RW (71 %) and slightly lower in SW (57%).

Among the analysed samples, RW had the highest quantity of NDF (83%) and ADF (71%). It also showed the highest content of cellulose (44%) and hemicellulose (25%). This result was confirmed by another method used where RW showed high dietary fibre composition: TDF, SDF and IDF amounted to 78, 3.4 and 76 % respectively.

Table 3.2: Porportion of NDF, ADF, cellulose, hemicellullose and lignin in
pink guava by-product, dry matter.

Sample	NDF (g/100 g)	ADF (g/100 g)	Cellulose (g/100 g)	Hemicellullose (g/100 g)	Lignin (g/100 g)
RW	83.8 ±0.48ª	71.3 ±5.21ª	44.3±1.20ª	24.8±0.57ª	45.90 ±1.64ª
SW	75.9 ±0.83 ^b	57.2 ±1.30 ^c	36.1±2.23b	18.7±0.77 ^b	19.25 ±0.70 ^b
DW	89.4 ± 1.34^{a}	64.6 ±0.46 ^b	25.4±2.76°	12.1±2.71°	20.21 ±1.22 ^b

Note: ^{a,b,c,} Means of three replications; if different within a column, indicates significant difference at the level p<0.05. NDF: Neutral dietary fibre, ADF: Acid dietary fibre, RW: refiner waste; SW: siever waste; DW: decanter waste

As shown in Figure 3.2, the proportion of soluble fractions (pectins, gum etc) was slightly different among the analysed by-products (11 to 24%). In the SW, soluble DF fractions accounted for 24% of the total volume. A slightly lower proportion was detected in RW and DW. These results were similar to the one found in enzymatic-gravity method where soluble dietary fibre was found

high in SW.

The soluble fraction found in pink guava by-products were comparatively higher compared to other dietary fibre by-products of apples (11.7%), pears (13.4%) and carrots (3.88%) (Nawirska and Kwaniewska, 2005). The results indicated that pink guava by-products could be a good source of pectin for functional health food ingredient.



Figure 3.2: Percentage of Soluble Fractions in Pink Guava By-Product

Results are means of triplicate analyses. ^{abc} Means in the same column with different letters indicate significant difference at the level p<0.05. RW: refiner waste; SW: siever waste; DW: decanter waste.



3.5 Conclusions

The results suggested that the processing methods would affect the dietary fibre composition of the analysed by-products. It was evident that refiner, the first by-product from the processing steps contained more seeds and skin due to the large sieve size. The effect of the small size sieve was that the sample contained more pulp than seed. The mechanical sieving process and the sizes of the sieves used may have affected the fractions and sizes of dietary fibre in pink guava by-products; where bigger IDF particle sizes (lignin, hemicellulose and cellulose) were found more in refiner compared to other by-products.

Pink guava by-products showed a high total dietary fibre content (68 - 79%) with high ratios of SDF to IDF. The results of the two methods of dietary fibre content determination showed that the pink guava by-products contained high dietary fibre content, with refiner had higher TDF compared to siever and decanter. Pink guava by-products found to be more useful as dietary fibre sources for food application due to high content of dietary fibres (more than 60% TDF). Further analysis to be carried out next, was to determine the physico-chemical properties of dietary fibre powder from pink guava by-products.

CHAPTER 4

PHYSICO-CHEMICAL PROPERTIES OF DIETARY FIBRE POWDER FROM PINK GUAVA BY – PRODUCTS

4.1 Introduction

Fibre is often classified as soluble dietary fibre (SDF) and insoluble dietary fibre (IDF) (Gorinstein *et al.*, 2001). The SDF/IDF ratio is important for both dietary and functional properties. It is generally accepted that those fibre sources suitable for use as food ingredient should have an SDF/IDF ratio close to 1:2 (Esposito *et al.*, 2005; Jaime *et al.*, 2002; Schneeman, 1987).

Plant fibres have shown some functional properties, such as water-holding capacity, oil-holding capacity and swelling capacity which have been more useful for understanding the physiological effects of dietary fibre, than the chemical composition alone (Femenia *et al.*, 1997;Gallaher and Schneeman, 2001). These properties are related to the porous matrix structure formed by polysaccharide chain which could hold large amount of water through hydrogen bonds (Dawkins *et al.*, 2001; Kethireddipalli *et al.*, 2002). Functional

properties of plant fibre depend on the IDF/SDF ratio, particle sizes, extraction conditions and its sources (Jaime *et al.*, 2002; Esposito *et al.*, 2005).

Currently, there is a great variety of raw materials, mainly processed byproducts, from which dietary fibre powders could be obtained (Femenia *et al.*, 1997; Lario *et al.*, 2004; Nawirska and Kwasnieska, 2005). The main characteristics of the commercialized fibre products are: total dietary fibre content above 50%, moisture content lower than 9%, low lipids content, a low calorie value and neutral in flavour and taste (Larrauri, 1999). To be acceptable, a dietary fibre added to a food product must function in a satisfactory manner as a food ingredient (Jamie *et al.*, 2002).

According to Larrauri (1999), the "ideal dietary fibre" should meet, among others, the following requirements; no nutritionally objectionable components, as concentrated as possible, bland in taste, colour and odour; balanced in composition, adequate amount of associated bioactive compounds; good shelf life; compatible with food processing; and produces the expected physiological effects. To get evidence on the benefits from the pink guava by-products high dietary fibre and functional properties, its dietary fibre powder (DFP) need to be developed and analysed. In this study, all the three pink guava by-products prepared were the DFP with more than 60 % of total dietary fibre content. The objectives of this procedure therefore were to evaluate the dietary fibre composition, its proximate composition and its major functional properties, in order to use them as a potential fibre source in the enrichment of foods. The first procedure began with decolourisation.

4.2. Materials and Methods

4.2.1 Decolourisation

Materials

Samples of pink guava by-products were collected from Golden Hope Fruit and Beverages Sdn Bhd, in Manjung, Perak. The three types of pink guava byproducts were refiner (RW), siever (SW) and decanter (DW). The analysed samples were taken three times throughout the study for replication. For decolorisation, 0.2% of sodium metabisulphite (Sigma, USA) and 15% of ascorbic acid (Merck, USA,) were used.

Methods

To develop dietary fibre powder (DFP), the product should be colourless. In order to produce colourless DFP of pink guava by-products, three different methods were employed. In the first method, the sample was washed with hot water (90 °C for 5 minutes) (Larrauri, 1999). The ratio between sample and hot water was 1:2. For the second method, the sample was immersed in 0.2% sodium metabisulfite for 2 hours at 30 °C (Salmah, 2005) and in the third method, the sample was immersed in 15% ascorbic acid for 15 minutes at 30 °C (Ewart *et al.*, 1988). Similar sample and hot water ratio was used for the second and third methods.

For the second method, modification was done on a preliminary analysis to set the optimum immersion time of the studied sample. The optimum time was indicated with the increase of lightness and reduction of red colouring in the sample. For third method, a preliminary analysis was carried out to determine the optimum percentage of ascorbic acid for immersion. The increase of lightness and reduction of red colouring was an indicator of the optimality of these two parameters. After the discolorisation procedure, the samples were milled using the Mass Colloidal (Masuko, Saitama, Japan). Later it was pressed to eliminate excess water with hydrolic pressure. Afterward, the sample was dried in the oven (Memmert, Germany) for 8 hours at 65 °C. After that it was dry-milled using Hammer mill (Lehman, Ohio, USA) and sieved using Retsch sieve shaker (Retsch 200, Germany) up to 600 µm particle size. Lastly, the sample was packed in laminated packaging material and kept at room temperature for further analysis. Two parameters namely colouring and water retention were analysed to determine the best method to produce DFP. To identify the best decolourisation method, samples (RW, SW and DW) were mixed together. The samples were mixed since it was collected from the same factory source and batch.

4.2.2 Proximate Analysis

The proximate analysis of DFP pink guava by-products were run to determine protein, fat, carbohydrate, total ash, moisture content and energy properties.

Materials

For protein analysis, sodium hydroxide (pellets forms), sulfuric acid (95% and 97% of concentration), boric acid (as receiver solution) were used. Kjeltabs was used as a catalyst. Methyl red indicator was the indicator. Whilst the petroleum ether was used for fat analysis. All chemicals used were of analytical grade from Sigma Chemical Co. (USA).

Methods

Moisture content

Moisture content was determined according to AOAC method (1990). Two grammes of homogenized sample were accurately weighed in an aluminum dish. The aluminium dish was provided with a lid cover and heated to 130 °C and then allowed to cool. Later, the dish with the sample was left heated without cover at 105 °C overnight. Following that, the lid was replaced before removing it from the oven. The dish was cooled in the dessicator and weighed after attaining the room temperature. The procedure was repeated until constant weight was attained.

Calculation

% MC=
$$\frac{W_2}{W_1} \times 100$$

where

MC: moisture content, W₂: loss of weigh in g of the sample, W₃: weigh in g of

the sample taken.



Carbohydrate content

Carbohydrate was calculated by subtracting the sum of the moisture, protein, fat, dietary fibre and ash from 100% (Chau and Huang, 2003).

Calculation

% C = 100 % - (%MC + % P + % F + % TA + % DF)

where, C: carbohydrate, MC: moisutre content, P: protein, F: fat, TA: total ash

and DF: dietary fibre

Protein content

The protein content was determined according to AOAC method (1990). The method as described in Chapter 3.

Fat content



Fat was determined according to AOAC (1990) with a Soxhlet apparatus and petroleum ether as an extraction solvent. The dried sample (10g ±0.1) was grounded and transferred into extraction thimble. The sample was covered with cotton to prevent it from spilling out. Petroleum ether was then put into a Soxhlet extractor with a weighed flask attached to it. A hundred and fifty mL (150 mL) of petroleum ether was added into the bottom flask. Then, an extraction apparatus was connected to the condenser for 8 hours. After the extraction completed, the flask containing petroleum ether was removed. Then, the flask was transferred into an oven for 1 hour to dry the extract. Later the flask was put immediately into a dessicator to cool and its weight recorded.

Calculations

 $\% F = \frac{F_1}{F_2} X 100$

where,

 F_1 = weight (g) fat in sample ((weight of flask + fat) – (weight of flask))

 F_2 = weight (g) the sample taken



Total Ash

Total ash was determined according to the AOAC method (1995). The method as described in Chapter 3.

Total energy

As 1 kcal was equivalent to 4.184 kJ (Royal Society, 1972), factors of 4, 4, and 9 were used for calculating energy from protein, carbohydrate and fat respectively. The total energy was present in kcal/100 g.

4.2.3. Dietary Fibre Composition

Samples were analysed for soluble and insoluble fibre contents according to AOAC 991.42, an enzymatic-gravimetric method (Prosky *et al.*, 1988). Total dietary fibre was calculated as the sum of soluble and insoluble dietary fibre. The method as described in Chapter 3.

4.2.4. Physical Properties Of Pink Guava By-Products

Materials

For physical properties, RW, SW and DW were sieved to get different particle sizes; 100, 140, 250, 425 and 600 μ m using Retsch sieve shaker (Retsch 200, Haan, Germany) to determine their physical properties (size distribution, bulk density, water-retention capacity, oil-retention capacity and swelling). For the electron microscopy scanning, the samples were divided into two groups based on particle sizes; 600 – 425 μ m and 250 – 140 μ m. Colour and pH was also determined on the studied sample.

Methods

Determination of particle size distribution

One kilogram of homogenized samples were shaken on a Retsch test sieve, with their respective sizes of 100, 140, 250, 425 and 600 μ m, stacked in the order of decreasing opening sizes. The weight of particle retained on each sieve was calculated as percentage of total weight. Triplicate analyses of DFP pink guava by-products were carried out. Particle size distribution was determined according to the method of Prakongpan *et al.* (2002)

Determination of bulk density

Bulk density of the samples was determined according to the method of Prakongpan *et al.* (2002). Fifty mL of pre - weighed graduated cylinder was filled with the sample and shaken slightly. The volume of the sample was recorded, and the content of the cylinder weighed and the resultant bulk density expressed as weight per volume.

Determination of water and oil- retention capacities

Water- retention capacity (WRC) and oil- retention capacity (ORC) of DFP pink guava by-products were determined following the method conducted by Ang (1991). Two grammes of samples were mixed with 30 mL of distilled water in a 50 mL weighed centrifuge tube. The slurry was allowed to stand for 10 minutes, and then centrifuged at 2,000 rpm using a table top centrifuge (Universal 32R, Hettich, Germany) for 15 minutes. Following centrifugation, the supernatant was discarded and the resultant precipitates weighed. The result was expressed as gramme of water per gramme of sample. For oilretention capacity, the procedure was similar to the one described for WRC except corn oil was used instead of water.

Determination of swelling capacity

Swelling capacity (SWC) of DFP pink guava by-products was analysed by the bed volume technique after equilibrating in excess solvent (Kuniak and Marchessault, 1972). Two hundred milligrammes (200 mg) of homogenized samples was put in a 50 mL measuring cylinder. Twenty mL (20 mL) of deionised water added and the mixture was then gently stirred and left to stand at room temperature overnight. Swelling volume was measured and expressed as millitres of swollen per sample.

Scanning Electron Microscopy (SEM)

Each of the DFP samples of dietary fibre was sprinkled onto a carbonconductive adhesive tape that was attached to the stub. Then, it was coated with gold dust by the SPI-sputter coater. The photos of the prepared samples were taken using a scanning electron microscope (JSM-6400, Japan). Scanning electron microscope has a magnification of x 750. Scanning electron microscopy procedures were according to the method of Prakongpan *et al.* (2002) with modification.

Colour

Samples in triplicate were transferred to a glass cuvette, and colour was measured using Spectrocolorimeter (Minolta C.M. 2002, Osaka, Japan). The instrument was calibrated to standard black and white prior to use. Hunter colour was determined: lightness (L^*), redness (a^* , ± red-green) and

yellowness (b*, \pm yellow- blue). L* refers to the relation between reflected and absorbed light. L* values equals to 0 for black and 100 for white. a* for the degree of redness (0 to 60) or greenness (0 to -60) and b* for the degree of yellowness (0 to 60) or blueness (0 to -60).

4.3 Statistical Analysis

Three measurements were taken on each analysis. The results were expressed as a mean of values ± standard deviation of three separate determinations. Comparison of means was performed by one-way analysis of variance (ANOVA) followed by LSD test. ANOVA procedure was performed at p<0.05 to study the variation. Statistical analyses were run using SAS V. 9.1 software (SAS, USA).

4.4. Results And Discussion

4.4.1. Effects of Decolourisation

From the preliminary study, for the second method; 2 hours immersed in 0.2% metabisulfite showed significant increased in lightness and reduced the red colour compared to one and zero hour immersion (Table 4.1). For third method; the preliminary study showed that 15% ascorbic acid gave lighter brown colour to the studied sample compared to 10 and 20% ascorbic acid. Based on this result 0.2% metabisulfite with 2 hour immersion and 15% ascorbic acid with 30 minutes immersion were choose for decolourisation techniques for comparison with hot water treatment.

In determination of optimum decoloring techniques for DFP three techniques of decolorisation were compared. The results showed that there were significant differences (p<0.05) on the colour effects between the decoloring techniques. For colour, all the three techniques of decolourisation had increased the lightness and reduced the redness of the samples as compared to control (Table 4.2).

Treatme	ent	0.2% Metabisulfite			Ascorbic acid with 30 min immersion		
		Immersion time (hour)			Percentage		
		0	1	2	10	15	20
L		54.5	66.9	74.8*	72.0	77.8	78.3
а		17.1	11.7	10.4*	14.3	9.71*	18.5
b		29.2	30.6*	25.4	26.0*	18.5	20.0

Table 4.1: Preliminary study on different decolourisation techniques onpink guava by-product

Note: Results are means of triplicate analyses. * Means in the same row with different letter indicate significant difference at level (p<0.05).

L* value is corresponds to black ($L^* = 0$) and white ($L^*=100$), whereas a positive a* value responds red and a negative value denotes green. A positive b* value corresponds to yellow, whereas a negative value indicates blue

Treatment	L*	a*	b*
Control	54.6 ± 3.83°	17.1 ± 0.09^{a}	29.2 ± 3.30 ^a
Hot water (90 °C, 5 min)	66.8 ± 0.06^{b}	11.7 ± 0.2 ^b	30.6 ± 0.18 ^a
0.2% sodium metabisulphite	74.8 ± 2.38^{a}	8.4 ± 0.32^{d}	25.4 ± 1.59 ^b
15% ascorbic acid	77.8 ±.014 ^a	$9.7 \pm 0.04^{\circ}$	18.5 ± 0.15 ^c

Table 4.2: Effects of different decolourisation methods on colour of pinkguava by-product

Note: Results are means of triplicate analyses. ^{abc} Means in the same column with different letter indicate significant difference at level (p<0.05).

L* value is corresponds to black $(L^* = 0)$ and white $(L^*=100)$, whereas a positive a* value responds red and a negative value denotes green. A positive b* value corresponds to yellow, whereas a negative value indicates blue

Sodium metabisulphite and ascorbic acid are known as reducing agent. Theoretically, polyphenol oxidase-copper enzyme combined with oxygen would catalyse the oxidation of phenolic compound present mainly in the skin to form o-quinones which then polymerise to produce brown, red or black colourings. The reducing agent will prevent o-quinones formation by inactivating the polyphenol oxidase activity through reduction of the quinones formation or by coupling the quinones to form a product which is not further oxidized and inhibited the enzymes (Mayer, 2006).

Treatment with of 15% of ascorbic acid gave more lightness to the colour of the powder compared to other techniques. This could be attributed to the prevention of the oxidation of polyphenol oxidase by ascorbic acid (Segovia-Bravo *et al.*, 2009). On the other hand, 0.2% sodium metabisulphite could significantly reduce the redness compared to hot water and ascorbic acid.

For hot water treatment, it had illustrated an increase of the lightest (L* value) and reduction of redness compared to the controlled sample. Hot water (> 70 °C) not only could inactivate the polyphenol oxidase enzyme, but also reduce Maillard reaction and caramelization through reduction of free sugar and ash

content (Lario *et al.*, 2004; Kuan and Liong, 2008). As DFP was brown in colour , this may be due to the Mallaird reaction compounds. The decolouring treatments had produced light brown DFP pink guava by-products as shown in Figure 4.1.





Table 4.3 shows the effects of different decolourisation methods on water retention capacity of DFP. Results showed that there was significant difference (p<0.05) in water retention capacity among decolourisation methods. Hot water treatment gave the highest water retention capacity (4.81 g of water/g of fibre) compared to other techniques. This may be due to the reduction of sugar content after the treatment which increased the ability of the by-products to retain water (Larrauri, 1999; Lario *et al.*, 2004).

Based on the above results, the hot water treatment (90 °C, 5 minutes) was found to be the best method in producing dietary fibre powder from pink guava by-products compared to sodium metabisulphite and ascorbic acid in term of colour and water- retention capacity.

Table 4.3: Effect of different decolorisation methods on water retentioncapacity

Treatment	WRC
	(g of water/g fiber)
Control	3.2 ± 0.08^{a}
Hot water (90 °C, 5 min)	4.8 ± 0.60^{b}
0.2% sodium metabisulphite	1.4 ± 0.22^{C}
15% ascorbic acid	2.2 ± 0.07^{d}

Note: Results are means of triplicate analyses. ^{abc} Means in the same column with different letters indicate significant difference at level p<0.05

4.4.2. Proximate Analysis

The results of proximate composition of dietary fibre powder of the pink guava by- products are shown in Table 4.4. DFP had low content of moisture which was between 2.42 - 3.68%. According to Larrauri (1999) the upper limit of moisture content for commercial fibre product was below 9%. Protein content ranged between 1.57 – 13 g/100 g in DW, SW and RW, respectively.

RW had shown significantly (p<0.05) higher protein content (13 g/100 g) compared to other by-products. The high amount of protein in RW was comparable to oat bran, 11.4 g/100 g (Grielmo-Miguel and Martin-Belloso, 1999) and higher than passion fruit seeds, 8.25 g/100g (Chau and Huang, 2004).

Fat content of DFP was low in SW and DW and, significantly higher in RW (p<0.05) (Table 4.4). The high content of fat in RW may due to the high amount of seed content in the sample as RW was the first by-products obtained in the process of pink guava puree industry. The high content of

Table 4.4: Proximate composition of DFP from pink guava by-products

Sample/ dry matter	Moisture (%)	Protein (g/100 g)	Fat (g/100 g)	Ash (g/100 g)	Carbohydrate (g/100 g)	Energy (kcal/100 g)
RW	3.67 ± 0.13 ^a	13 ± 0.25 ^a	11.43 ±0.55 ^a	1.86 ± 0.11 ^a	23.55 ± 1.51 ^a	249.07 ±12.03 ^a
SW	$2.89 \pm 0.18^{\text{b}}$	2.56 ± 0.13 ^b	0.47 ± 0.06^{b}	1.2 ± 0.11^{b}	31.32 ± 1.72 ^b	134.71 ±7.92 ^b
DW	$2.42 \pm 0.03^{\circ}$	$1.57 \pm 0.03^{\circ}$	0.17 ± 0.21 ^c	0.69 ± 0.06 ^c	22.31 ± 0.37 ^a	97.05 ±0.55 ^c

Note: Results are means of triplicate analyses . ^{abc} Means in the same column with different letter significantly differ at the level p<0.05. RW: Refiner, SW: Siever, DW: Decanter

lipid in the pink guava by-product containing seed was similar to that found in passion fruit seeds (24.5 g/100 g) (Chau and Huang, 2004).

Ash content ranged between 0.69 g/100g in DW and 1.86 g/100 g in RW (Table 4.4). There was a significant difference (p<0.05) in ash content among the pink guava by-products. RW showed high ash content compared to other by-products due to high content of lignin in RW (46%, Table 3.3). Lignin was less soluble and, therefore, hydroalcoholic solvents could not extract them; this produced a concentration effect on the studied by-products (Marin *et al.*, 2005).

Carbohydrate content of DFP was between 22.3 to 31.3 g/100g in DW, RW and in SW, respectively (Table 4.4). Calorie value of DFP varied widely between 97.1 kcal/100g in decanter to 249.1 kcal/100g in RW. RW had slightly higher calorie value, mainly due to their high fat content. According to Larrauri (1999), an adequate fibre concentrates should have a calorie value below 200 kcal/100 g limit which was met by DFP pink guava by-products. Overall, DFP pink guava by-products had high potential as dietary fibre

source as it had high amount of dietary fibre, and low in calorie and fat contents

4.4.3. Dietary Fibre Composition

Table 4.5 shows the dietary fibre composition of DFP from pink guava byproducts. Total dietary fibre constituted 56.6% - 76.1% of the DFP pink guava by-products. Among the products, the quantity of dietary fibre in DW was found higher (76.1%) compared to SW (64.3%) and RW (56.6%).

The quantity of total dietary fibre in pink guava by-products was higher compared to by-products of other processed fruits such as apple pomace, pears, oranges and peaches (Appendix 1). It was also found that total dietary fibre in pink guava by-products was higher than that of oat bran and wheat bran, 23.8 g/100 g and 44.0 g/100g respectively (Grigelmo-Miguel and Martin-Belloso, 1999).

Sample/ dry matter (DM)	IDF (g/100 g)	SDF (g/100 g)	TDF (g/100 g)	Ratio SDF: IDF
RW	48.7 ± 6.45^{a}	7.9 ± 0.18 ^a	56.6 ± 6.64^{a}	1:6
SW	58.1 ±0.96 ^b	5.6 ± 1.48 ^b	64.3 ± 0.35 ^b	1:10
DW	72.1 ± 1.20°	$4.0 \pm 0.02^{\circ}$	76.1 ± 1.22°	1:17

Table 4.5: Dietary fiber composition of DFP pink guava by-products

Note: Results are means of triplicate analyses. ^{abc} Means in the same column with different letters indicate significant difference at p<0.05.

IDF – Insoluble dietary fibre, SDF – Soluble dietary fibre, TDF – Total dietary fibre; RW – Refiner, SW – Siever, DW – Decanter

There was a significant decrease (p<0.05) of total dietary fibre content in RW after hot water treatment (from 78.8% to 56.6%), an increase of SDF (from 3.4% to 7.9%) and decrease of IDF (from 75.5% to 48.7%). The trend was also found in wheat bran and pureed carrot after boiling (Anderson and Clydesdale, 1980). A major effect appeared to be dramatic in pectic substances: wet heat tends to solubilised the pectins. For IDF, it was hemicelluloses broken down during processing, changed from insoluble to soluble hemicellulose (Rabe, 1999; Nawariska and Kwasniewska, 2005).

The insoluble fibre was found to be the major fraction in the DFP pink guava by-products with the range from 48.7 - 72.1%, which was more than 85% of total dietary fibre in pink guava by-products. On the other hand, the soluble fraction represented 4.0% - 7.9% of the total dietary fibre content. In pomace and agricultural by-products of many other fruits and vegetables, insoluble fibre was also reported to be the major fibre fraction (Grigelmo-Miguel and Martin-Belloso, 1999; Gorinstein *et al.*, 2001;). However, DFP pink guava byproducts showed higher soluble fraction compared to other fruits and vegetable processing wastes such as cherries (1.5%), blackcurrants (2.7%) and carrots (3.9%) (Nawriska and Kwasniewska, 2005). It was also found that soluble fraction in DFP was higher compared to cereals (Appendix 1).

The difference in SDF:IDF ratios of DFP pink guava by-products, ranging between 1:6 -1:17; was parallel to findings of Figuerola *et al.* (2005), who indicated the ratios of 1:4 to 1:13 in apple pomace and citrus peel respectively. Among the DFP, RW had the lowest SDF: IDF ratio and the higher SDF content.

4.4.4 Physical properties of Pink Guava By-Products

To evaluate the physical properties of DFP; its, particle size distribution, bulk density, water-retention capacity, oil-retention capacity, swelling capacity, particle structures, colour and pH were analysed.

Particle size distribution

Table 4.6 shows the percentage of particle size distribution of DFP using different mesh sizes of 600, 425, 250, 140 and 100 μ m. The pooled sample of DFP was sieved through Retsch test sieves stacked in order of decreasing opening size (600, 425, 250, 140 and 100 μ m).

The largest amount of DFP pink guava by-products was retained on the 425 μ m and 250 μ m screen sizes. These results were consistent with Larrauri (1994) and Sangnark and Noomhorn (2003), who stated that in general the products with high content of dietary fibre have particle sizes between 150 and 430 μ m. Except for RW where 66% of the particle size was more than 600 μ m. RW was mainly consists of seed that difficult to crash during grinding. The process of grinding was the major factor affecting particle size of fibres (Raghavengra *et al.,* 2005).

Sieve size (µm)	% Retained on Sieve					
	RW	SW	DW			
600	24.8 ±0.96 ^a	27.9 ±0.12 ^a	2.5 ±0.13 ^a			
425	7.1 ±0.37b	42.2 ±0.49b	49.8 ±0.78 ^b			
250	1.7 ±0.53 ^c	30.4 ±0.35 ^c	47.6 ±1.41 ^b			
140	NA	13.4 ±0.13 ^d	8.9 ±0.81 ^c			
100	NA	NA	NA			

Table 4.6: Particle size distribution of DFP pink guava by-products

Note: Results are means of triplicate analyses. ^{abc} Means in the same column with different letters indicate significant difference at p<0.05). NA: Not Applicable, RW – Refiner, SW – Siever, DW – Decanter
Scanning Electron Microscopy (SEM)

Figure 4.2 to 4.4 shows the images obtained by electron microscopy scanning of DFP pink guava by-products at x 750 magnification. The microstructure of DFP at 600 μ m (X) was compared to 250 μ m (Y). From the scanning electron micrograph, particle size 600 μ m DFP was shown to have scales, rough and hollow surfaces (X: A, C and E). The DFP was also of various sizes with irregular shapes (A, C and E).

The scanning electron micrograph showed the collapse of matrix structure and the surface area was increasing with the decrease in particle sizes (Y; B, D and F). The grinding process resulted in the rupture of the hollow physical structure of fibre matrix and in a scale type structure, thereby providing increased surface area for water and oil absorption (Sangnark and Noomhorn, 2003). Grinding did not only result in particle size reduction, but also a deep structural modification of the fibre (Raghavendra *et al.*, 2006). Resulting from the reduction of particle size, the DFP was made more porous than the bigger particle size. This opened structure, increased the surface area, trapping more water/oil molecules, therefore exhibited higher water/oil holding capacity (Kuan and Liong, 2008).



Firgure 4.2: Scanning Electron Micrograph of RW. Size mesh 600 μ m (A) and size mesh 250 μ m (B); Bar = 10 microns X: matrix structure at 600 μ m; Y: matrix structure at 250 μ m, RW – refiner, magnification at x 750.



Firgure 4.3: Scanning Electron Micrograph of SW. Size mesh 600 μ m (C), size mesh 250 μ m (D); Bar = 10 microns X: matrix structure at 600 μ m; Y: matrix structure at 250 μ m SW – siever, magnification at x 750.



Firgure 4.4: Scanning Electron Micrograph of DW.

Size mesh 600 μ m (E) size mesh 250 μ m (F); Bar = 10 microns X: matrix structure at 600 μ m; Y: matrix structure at 250 μ m DW – decantrer, magnification at x 750.

Bulk density

Bulk density of various sizes (140 to 600 μ m) of DFP pink guava by-products are shown in Figure 4.5. For RW, there was no significant difference in bulk density between the different particle sizes. However, the 250 μ m particle size showed higher density than those of other sizes. In SW, particle size 425 μ m had higher bulk density than other particle sizes. For DW, bulk density was higher at 250 μ m particle size. Normally, the bulk density of the fibre depends on their shapes and sizes. All large-size particles of DFP showed lower density than small-size ones. According to Robertson *et al.* (2000), smaller particles size would have a higher packing density due to the increase in porosity.

The SEM micrograph has shown that smaller particle sizes (250 μ m) have made DFP more porous than bigger particle sizes (600 μ m). The increase of porosity is related to the damage of matrix structure and the collapse of the pores during grinding of the products (Raghavendra *et al.,* 2006). The bulk density of fibre is depending on it's the particles sizes where the sizes could be modified in milling process (Saenz, 1997).



Figure 4.5: Bulk Density of DFP Pink Guava By-Products

Note: Results are means of triplicate analyses. ^{abc} Means in the same column with different letters indicate significant difference at level p<0.05. RW- Refiner; SW – Siever; DW – Decanter.



Water-retention capacity (WRC)

Water exists in dietary fibre in three forms: it is bound to the hydrophilic polysaccharides; held within the fibre matrix; or trapped within the cell wall lumen. WRC, determined by the centrifugation method represented all three types of water associated with the fibre as reported by Wong and Cheung (2000) and Fluery and Lahaye (1991).

As shown in Table 4.7, WRC of the DFP pink guava by-products ranged between 3.75 to 5.8 g of water/g of fibre for RW, 4.8 to 12.4 g of water/g of fibre for SW and 3.8 to 9.9 g of water/g of fibre for DW. The highest values were found in SW and DW at 140 µm particle size, which could be associated with their high amount of insoluble dietary fibre, 58.1% in SW and 72.1% in DW compared to 48.7% in RW (Table 4.4). According to Saenz (1997) and Femenia *et al.* (1997) the insoluble dietary fibre was responsible for the water holding properties in the fibre as hemicelluloses; and lignin and IDF components water affinity. WRC has been used to measure the amount of water associated with only the insoluble fibre matrix (Femenia *et al.*, 1997).

Particle	WRC (g of water/g of fiber)				
Size (µm)	RW	SW	DW		
600	5.82 ±0.21 ^a	4.82 ±0.15 ^a	3.77 ±0.07 ^a		
425	5.43 ±0.48 ^a	6.21 ±0.24 ^b	4.17 ±0.12 ^b		
250	3.75 ±0.17 ^b	12.17 ±0.11 ^c	8.20 ±0.03 ^c		
140	NA	12.39 ±0.43 ^c	9.92 ±0.18 ^d		
100	NA	NA	NA		

Table 4.7: Water retention capacity (WRC) of DFP pink guava by-products

Note: Results are means of triplicate analyses. ^{abc} Means in the same column with different letters indicate significant difference at level p<0.05. NA: Not Applicable, RW- Refiner , SW – Siever, DW – Decanter

Among the analysed sample, the RW showed low value of WRC. The low WRC in RW may be attributed to the high fat content (Table 4.4). This may be due to the fat getting trapped inside the fibre matrix, thus restricting the entry of water molecules and resulting in low hydration properties (Sowbhagya *et al.*, 2007).

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The study also indicated that the decrease in particle size from 600 to 140 μ m resulted in an increase in WRC for SW and DW (Table 4.6). Statistically, there was a significant difference (p<0.05) of water retention ability for different particle sizes among the DFP.

An increase in SW and DW particle sizes was associated with reduction in WRC (Table 4.7). The increase of WRC in SW and DW and the decrease of particle sizes may be due to the shearing of the cell wall and collapse of matrix structure. Upon grinding, an increase in the theoretical surface area and total pore volume could be the reason in the increase of WRC in SW and DW (Raghavendra *et al.*, 2006). The scale type structure in DFP (Figure A,B,C,D,E, and F) gave more porous structure to the samples, this has increased the density, and increase its ability to retain water.

On the other hand, a decrease in particle size of RW had decreased the ability of WRC in DFP. This finding was in line with Prakongpan *et al.* (2002), who reported that, as the particle size of pineapple core dietary fibre and pineapple core cellulose fibre were reduced as a result of mechanical milling, the WRC was also reduced. The WRC value of DFP pink guava by-products was found to be higher compared to apple pomace and citrus peel (Figuerola *et al.*, 2005) (Appendix B). From the study, it was shown that DFP had good hydration properties that could be used in food product as a food ingredient.

Oil- Retention Capacity (ORC)

A similar trend of oil-retention capacity (ORC) of DFP and its water-retention capacity was evident. ORC was found to increase with smaller particle size for SW and DW and no significant difference (p>0.05) for RW (Table 4.8). According to Femenia *et al.* (1997), and Prakongpan *et al.* (2002), ORC was related to the particle size, surface properties, overall charge density and hydrophilic nature of the individual particles, whereby those particles with the greatest surface area posses greater capacity for absorbing and binding component of an oily nature (Kuan and Liong, 2008). The mechanism of ORC was mainly due to the physical entrapment of oil by capillary attraction.

The increase of ORC in SW (from 3.5 to 7.0 g of oil/ g of fibre) and DW (from 3.2 to 6.7 g of oil/g of fibre) was due to the decrease of the particle size. It was related to the nature of the surface, and the density of the particles. The greatest surface areas theoretically present a greater capacity to absorb and bind component of an oily nature (Lopez *et al.*, 1996; Kuan and Liong, 2008). The capacity of a fibre to bind fat depends more on its porosity. For this reason, the ability of fibre in ORC would be reduced when the fibre was put in the water where the pores absorbed the water and prevent the entry of fat (Borderias *et al.*, 2005).

In addition, the high amount of IDF in SW (58%) and DW (72%) may contribute to the ability of oil retention in the sample. This was in line with Sosulski and Cadden (1982) who evaluated different sources of dietary fibre and found that lignin-rich sample had higher ORC. Lignin and cellulose are the types of IDF are commonly used as functional ingredients in food product.

On the other hand, the low value of ORC in RW may be due to the high content of carbohydrates in the sample (Table 4.4). According to Kuan and Liong (2008), sample that contained higher amount of starches, had lower

ORC compared to those samples which was low in carbohydrate but high in fibre. Native starches have been found to be poor oil absorbers as the granular structure remains intact. The ORC had also been reported to correlate well with protein and lipids contents, the high content of protein and lipid (Table 4.4) in the sample will enable the sample to act as a good oil absorbers (Rodriguez *et al.*, 2006).

The ORC obtained from DFP was higher compared to citrus peel (0.15 – 0.35 g of oil/g of fibre) (Marin *et al.*, 2005), apple pomace (0.60 – 1.81 g of oil/g of fibre) (Figuerola *et al.*, 2005) and pineapple wastes (2.15 – 3.91 g of oil/g of fibre) (Prakongpan *et al.*, 2002).

Particle		ORC (g of oil/g of fiber)				
Size (µm)	RW	SW	DW			
600	2.79 ±0.35a	3.52 ±0.18 ^a	3.18 ±0.10 ^a			
425	2.28 ±0.19 ^a	3.16 ±0.18a	2.62 ±0.07 ^b			
250	2.20 ±0.07 ^a	6.88 ±0.04 ^b	6.19 ±0.01 ^c			
140	NA	6.99 ±0.08 ^b	6.78 ±0.18 ^d			
100	NA	NA	NA			

Table 4.8: Oil retention capacity (ORC) of DFP pink guava by-products

Note: Results are means of triplicate analyses. ^{abc} Means in the same column with different letters indicate significant difference at level p<0.05. NA: Not Applicable, RW – Refiner, SW – Siever, DW – Decanter

Swelling Capacity

The swelling capacity (SWC) for DFP pink guava by-products ranged from 10.87-15.0 ml water/ g (Table 4.9). Among DFP, DW was shown to be higher in swelling capacity (10.8 – 15.0 ml of water/g of fibre dry matter) followed by SW (11.7 – 13.3 ml of water/g of fibre dry matter) and RW (10.3 – 13.3 ml of water/g of fibre dry matter) and RW (10.3 – 13.3 ml of water/g of fibre dry matter). As a whole, SWC of DFP pink guava by-products were remarkably higher compared to citrus by-products (6.11 – 9.19 ml water/g DM) and apple pomace (6.50 – 6.89 g water/ g DM) reported previously (Figeurola *et al.*, 2005).

The values obtained could be attributed to the quantity of IDF found in the DFP pink guava by-products. According to Figuerola *et al.* (2005), the structural characteristic and chemical composition of the fibre (water affinity of IDF component) played important roles in the kinetics of water uptake in fibre.

Particle	SWC (n	nL of water/g of fil	per DM)
Size (µm)	RW	SW	DW
600	10.83 ± 3.82 ^a	13.33 ± 2.89 ^a	15.00 ± 0.00^{a}
425	13.33 ± 1.44^{b}	13.33 ± 2.89 ^a	14.17 ± 1.44 ^b
250	13.33 ± 1.44 ^b	11.67 ± 1.44 ^b	14.17 ± 1.44 ^b
140	NA	11.67 ± 1.44 ^b	$10.83 \pm 1.44^{\circ}$
100	NA	NA	NA

Table 4.9: Swelling capacity (SWC) of DFP pink guava by-products

Note: Results are means of triplicate analyses. ^{abc} Means in the same column with different letters indicate significant difference at level p<0.05. NA: Not Applicable, RW – Refiner , SW – Siever , DW – Decanter.

Colour

Table 4.10 shows the L, a* and b* values of DFP pink guava by-products of different particles sizes (600, 425, 250, 140, and 100 μ m). The colours of DFP pink guava by-products were all light brown (see Appendix C). DFP from RW was darker in colour than that of SW and DW. Size of fibre is one of the factors that could affect the colouration. As 66% percent of RW consists of particle size more than 600 μ m this constributed to the darker colouring in RW compared other samples. Overall, small-size particle had lighter colour than larger particle size.

Pigment and colour precursors of fruits were found in celluler plastid (Potter, 1986). When the tissue was damaged in the preparation and grinding process, most of the pigments were eliminated and fibre particles were much lighter after drying. This could contribute to the lighter colour of the DFP pink guava by-products. Furthermore, hot water treatment (90 °C for 5 min) would prevent DFP browning, probably due to the removal of sugar; these will reduce Mallaird and caramelisation during drying (Lario *et al.*, 2004).

Particle		L			a*			b*	
Size									
(um)	RW	SW	DW	RW	SW	DW	RW	SW	DW
(perc)		511	DII		511			011	211
600	63.8	67.6	70.2	6.40	7.88	9.13	23.9	25.7	30.2
	±1.29a	±0.18a	±0.38a	±0.08a	±0.11a	±0.25a	±0.81a	<u>+2.49a</u>	±0.64a
425	63.7	67.7	72.0	6.47	7.64	8.28	24.5	27.5	30.2
	+0 21a	+1 18a	+0.09b	+0.03a	+0 68a	+0.28b	+0.84a	+0.21b	+0 15a
	_0.21	_1.10	_0.07	_0.00	_0.00	20.20	_0.01	-0.21	20.10
250	63.8	69.6	74 3 +0 1C	6.52	6.61	7.28	24.9	24.5	28.6
	+1 29a	+1 02b	74.5 ±0.1	+0.18a	+0.61b	+0.030	+0.79a	+0 36a	+0.42b
140	1.29" NIA			NIA	6 20	7.45	NIA	25.20	10. 1 2~
140	INA	$70.4 \pm 0.16^{\circ}$	$74.2 \pm 0.27^{\circ}$	INA	0.39	7.45	INA	23.2	20,4
					±0.20b	±0.16 ^c		±0.32a	±0.32b
100	NA	NA	NA	NA	NA	NA	NA	NA	NA

Table 4.10: Colour of DFP pink guava by-products at different particle size

Note: ^{abc} Means in the same column with different letters were significantly differ at the level p<0.05. NA: Not Applicable, RW – Refiner waste, SW – Siever waste, DW – Decanter waste

4.5 Conclusion

Results of the study showed that the DFP pink guava by-products may be useful in the food industry as high dietary fibre ingredients. DFP pink guava by products showed a high total DF content and almost similar SDF:IDF ratios with cereal brans. In addition, DFP was also low in fat content and calorie value.

Particle size distribution played an important part in the physical characteristics of dietary fibre powder. The largest amount of DFP was retained on the 250 and 425 μ m. Except for RW where most of a particle size was more than 600 μ m. From the SEM results, it was shown that mechanical process such as grinding affected the porosity and surface area of DFP, where grinding resulted in particle size reduction and structural modification of the fibre.

The DFP showed potential physical properties such as water, oil retention and swelling capacities. These may be due to high IDF in DFP, as IDF components such as lignin, hemicellulose and cellulose have water or oil affinity. Furthermore, the scale type structure of DFP had increased the porosity and the ability to entrap water or oil. Due to their water retention, oil retention and swelling capacities, DFP pink guava could be used not only for dietary fibre enrichment and reduction of energy value, but also as functional ingredients in many food products.



CHAPTER 5

IDENTIFICATION OF FRUCTOOLIGOSACCHARIDES AND PREBIOTIC EFFECT

5.1. Introduction

Dietary carbohydrates, particularly the fructooligosaccharides (FOS) has been gaining a lot of health and commercial attention. The possible health benefits associated with the consumption of these compounds have led to their increased popularity as food ingredients and their promotion as alternative sweeteners for diabetic formulations. In general, FOS; the non-digestible oligosaccharides known as bifidogenic compounds favour the development of intestinal bacteria (Losada and Olleros, 2002). FOS is used selectively by certain types of acid-producing bacteria such as bifidobacteria which are habitual inhabitants of the intestine and are considered beneficial. In turn, FOS increases the growth of beneficial microorganism.

FOSs are a group of linear glucosyl α (1 \rightarrow 2) (fructosyl)_n β (2 \rightarrow 1) fructose polymers with a degree of polymerization ranging from n = 1 to up 5. The β -

2,1 linkage in FOS renders them resistant to hydrolysis by digestive enzyme and thereby imparts a dietary fibre effect (Sangeetha et al., 2005). Previous study on physico - chemical properties of pink guava by-products powder had shown significant characteristics of dietary fibre of the products (Chapter 4).

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This chapter describes the thin layer chromatography (TLC) technique to investigate the pink guava by-products powder fructooligosacharide component and its prebiotic effects. The procedures detailed the application of rapid and simple TLC for identification of FOS in the DFP. The TLC method was particularly well suited as an analytical method for analysis of FOS. This was concurred by Reiffova *et al.* (2006), Reiffova *et al.* (2003), Park *et al* (2001), and Vaccari *et al.* (2000) for the analysis of FOS utilizing TLC. The main advantage of this method was the possibility of simultaneous analyses of many samples and the possibility of analyzing crude samples with minimal preparation and sequential detection methods to identify and confirm the samples without time constraint (Reiffova *et al.*, 2006).

5.2. Materials and Methods

The thin layer chromatography (TLC) technique used to identify FOS was similar to the one employed by Reiffova *et al.* (2006) with some modifications. The prebiotic study followed the method conducted by Lopez-Molina *et al.*

(2005).

5.2.1 Materials

The three types of samples from pink guava by-products used in these procedures were refiner (RW), seiver (SW) and decanter (DW). The standards used were fructose, sucrose, 1-ketose, and nytose. The commercial fructozym was also included. All the standards and enzyme were brought in from Fluka, Switzerland. For spray reagent, p-anisaldehyde and sulfuric acid (95% -97%) was used. The mobile solution for FOS identification was mixture of 1-butanol, 95% ethanol and deionised water. All chemicals used were of analytical grades from Sigma Chemical Co. (USA). Silica gel 60, Kiesel Gel, TLC glass sheets, with size 20 x 20 cm (code 1.05721.0001, Merck, USA) was used.

Two types of *Bifidobacterium* spp.; *Bifidobacterium bifidum* (ATCC 29521, source - MARDI) and *Bifidobacterium longum* (BB536, source Moringa Milk Industry, Japan) were used in the prebiotic study. BSM Broth and BSM Agar for *Bifidobacterium* spp. used as the media were purchased from Fluka, Switzerland. Glucose from Sigma Chemical Co. (USA) was used as control. Whilst for obtaining the aerobic condition, gas pack CO₂ system from BBL, USA was employed.

5.2.2. Sample preparation

The sample was prepared according to the method by Texeira *et al.*, (1997) with modifications. Five grammes of sample were boiled in 120 mL of 80% aqueous ethanol for 3 minutes for enzyme denaturation. The mixture was then grounded using mortar and pestle. The homogenate was placed in a water bath at 80 °C for 15 minutes and centrifuged at 1000 x g for 15 minutes. The residue was re-extracted as the preceding procedure and then submitted twice for water extraction (120 mL) for 30 minutes at 60 °C. Then the supernantant was cooled at room temperature (27 °C). Later, 0.056 g of enzyme fructozym was added in to the pooled supernatant. The sample solution was incubated at

60 °C for 30 minutes , then allowed to cool at room temperature (27 °C) before further analysis.

5.2.3. Preparations of Standard Solutions and Detection Reagent

Four standards solutions of fructose, sucrose, 1-ketose (GF₂), and nytose (GF₃), (2 mg of each) were prepared by dissolving each one individually in 2 mL of 80% methanol. Three sample solutions of DFP pink guava by-products (RW, SW, DW) were applied at the start of the thin layer, 2 cm from the bottom of the plates. The primary detection reagent was prepared by mixing 1 ml of panisaldehyde, and 1 ml of 97% sulfuric acid in 18 ml of ethanol.

5.2.4. Methods

Thin-layer Chromatography

TLC analysis was performed using 20 x 20 cm vertical twin glass developing chambers (CAMAG, Switzerland) by the solvent vapour saturation. Prior to TLC analysis, silica gel was pre-treated with 1.2% boric acid in ethanol. Then

the plates were dried out at 100 °C in an oven for 1 hour. Stock solutions of fructose, sucrose, 1-ketose, nytose and three sample solutions of DFP pink guava by-products were applied at the start of the plated process using sample application, semi-automatic (Linomat 5, Camag, Switzerland) by means of a micro syringe in volume of 0.2 μ L. The layer with applied standards solution and sample solution were developed with butanol-ethanol-water (5:3:2, v/v) as mobile phase at laboratory temperature (27 °C). The process continued until the solvent reached the 12 cm line. The plates were then removed, and dried on plate heater (Camag, Switzerland) at 60 °C for 5 minutes, and FOS was identified by means of p-anisaldehyde spray reagent. The violet colouring was produced by heating at 110 °C for 10 minutes.

Measuring R_f values

The relative mobility (R_f) for each spot was then calculated using the formula:

 $R_f = \frac{\text{distance travelled by component}}{\text{distance travelled by solvent}}$

Prebiotic Study

The prebiotic effect of DFP from pink guava by-products was determined according to the method developed by Lopez-Molina *et al.* (2005). Two types of *Bifidobacterium* spp.; *Bifidobacterium bifidum* and *Bifidobacterium longum* were used to study the prebiotic effect of pink guava by-products DFP.

Bifidobacterium spp was inoculated onto BSM Agar about 96 hours prior to use. The culture medium used was BSM broth containing peptone and meat extract as a source of carbon, nitrogen, vitamins and minerals. The sources of carbohydrate were dextrose and sodium choride.

The medium had a final pH of 6.8 \pm 0.2. Glucose or the analysed DFP was added individually before inoculation to give final concentration of 2%. Sterile bottles containing 25 mL of BSM Broth medium were inoculated with 250 μ L of a solution of *Bifidobacterium* spp mixed and capped before introducing into the anaerobic jars within anaerobic sachets. The anaerobic jars were incubated at 37 °C. Samples were removed initially at the zero hour and subsequently at 24th, 48th, 72th and 96th hours to enumerate the bacteria. BSM broth with glucose was used as the control. For bacteria enumeration, samples were serially diluted to 10⁻⁷ in an anerobic jar with pre-rediced tryptone water and inoculated onto BSM agar. The dishes were introduced into anaerobic jars at 37 °C, and CFU were counted after 48 hours of incubation.

5.3. Results and Discussion

5.3.1. Fructooligosaccharides

The individual components of oligosaccharides were separated in order to increase molecular mass (Gasparic *et al.*, 1981Reiffova *et al.*, 2003; Reiffova *et al.*, 2006;). Selection of the optimum mobile phases (butanol-ethanol-water with 5:3:2 ratio, v/v) on thin layer was based on the migration distance for fructose, because it was the basic units of FOS with the lowest molecular masses.

Chromatograms obtained through the analysis of standard solutions of fructose (F), sucrose (S), nytose (N), 1-ketose (K) and samples (RW, SW, DW) on thin layer chromatography are shown in Figure 5.1. The full separation of all components of FOS was achieved for standards and samples RW and SW. For DW, there was no spot present, except for fructose which was very weakly visible. In RW and SW three spots (fructose, sucrose and nytose) were clearly detected, but the second spot (1-ketose) was weakly visible on plates. The degree of polymerization ranged from two to three units as reported by Texeira *et al.* (1997). The degree of polymerization indicated the number of the fructose units in the sample.

The first position on chromatograms (the utmost spots from start) could be fructose (monosaccharide) with the lowest molecule masses, then sucrose (S, including G + F, DP1) and gradually 1-ketose (DP2) and nytose (DP3) with increasing molecules mass.

FOS belong to the fructan group, which has the same basic structure of linear chains of fructose units connected by β -2,1 linkage with inulin. FOS is composed of only small molecules, with a chain length between two or eight

fructose units. In this study, fructozym hydrolysis was carried out to break down the β -2,1 linkage in identification of FOS components in the dietary fibre of the pink guava processing waste.

In accordance with their R_f values, spot 1 belongs to nytose, spot 2 was 1ketose, spot 3 was sucrose and spot 4 was frustose. Data of R_f values for each separated spot in standard solution and studied samples are given in Table 5.1.



Figure 5.1: Separation of fructo-oligosaccharide.

Standard solution (A) and pink guava by-products; (B) = RW, and (C) = SW. Standard labels: F= fructose, S = sucrose ,1-K = 1-ketose and N = nytose; stationary phase; glass sheet silica gel, mobile phase; butanol-ethanol-water (5:3:2 v/v), detection reagent; p-anisaldehyde, volume of sample; 0.2 μ L.



1	R _f v		
	Standard	RW	SW
1	0.30	0.30	0.31
2	0.38	0.36	0.38
3	0.44	0.45	0.44
1	0.49	0.50	0.49

Table 5.1: R_f values for each detected spot in standard and DFP

5.3.2. Prebiotic Effects

Prebiotic carbohydrates are, by definition, metabolized only selected members of the gastrointestinal tract. The effectiveness of a prebiotic depends, therefore on its ability to be selectively fermented by and to support growth of specific targeted organism (Huebner *et al.* 2007). The aim of this experiment was to quantify the extent to which prebiotic express this activity using selected strains of *Bifidobacterium; Bifidobacterium bifidium* and *Bifidobacterium longum*. In this study, prebiotic indices was based on population growth of *Bifidobacterium bifidium* and *Bifidobacterium longum* with the presence of glucose, RW, SW and DW.

The results showed that the growth behaviour of *Bifidobacterium* was increased with the addition of the sampled by-products (RW, SW and DW) and decreased with glucose as a control (Figures 5.2 and 5.4). A pH decreased correlates with the population growth (Figure 5.3 and 5.5). The increase of *Bifidobacterium* growth may be due to the existence of fructooligosaccharide (FOS) in the samples. FOS was non-digestible carbohydrate that fermented *in vitro* by a limited range of micro-organisms that include most species of bifidobacteria (Bornet *et al*, 2002).

Bifidobacteria has relatively high amounts of β -fructosidase, which is selective for the β -(1-2) glycosidic bonds present in FOS. After FOS hydrolysis, fructose serves as efficient growth substrate for the bifidus pathway of hexose fermentation, which is almost exclusively carried out by bifidobacteria (Bornet *et al.*, 2002; Scardovi, 1986). Numerous *in vivo* studied showed that FOS ingestion led to increase bifidobacteria growth (Roberfroid, 2002; Huebner *et al.*, 2007; Huebner *et al.*, 2008).

Furthermore, the lower pH in this studied may indicating the production of acetic acid and lactic acids (Scardovi, 1986). The lower pH has potentially more effect because the production of these acids reduces intestinal pH and restricts or prohibits the growth of many potential pathogens and putrefactive bacteria (Lopez-Molina *et al.*, 2005). The synbiotic effect of *Bifidobacterium* and DFP may be a way of stabilization and/or improvement of the probiotic effect.

There was also a significant difference (p < 0.05) on the growth rate of *Bifidobacterium* among RW, SW and DW. The results showed that there was higher growth of *Bifidobacterium* in RW compared to those in SW and DW. These may be due to the high content of soluble dietary fibre and the existence

of FOS in the RW. However, there was no significant difference (p < 0.05) on pH decreased of *Bifidobacterium* in studied samples.

Dietary fibre displays different degrees of solubility. Soluble dietary fibre and FOS are readily soluble in water. This leads to the formation of gels in the gastrointestinal tract. This aids their fermentability by the gut microflora by virtue of an increased surface area available for enzymatic attack (Gibson, 2004). A study done by Lopez-Moline *et al.* (2005) indicated that the growth of bifidobacterium in human gut system was due to symbiosis relationship between soluble dietary fibre and FOS in artichoke. Bifidogenic effect of dietary fibre and non-digestible polysaccharide on the intestinal microbiota of rats was reported by Queiroz-Monici *et al.* (2005).



Figure 5.2: Population Growth of *Bifidobacterium bifidum* (ATCC 26521) Means \pm S.D (vertical lines). Values with superscripts (*) are statistically different at level $p \le 0.05$ according to LSD test. RW –refiner, SW – siever, DW – decanter, glucose as a control.



Figure 5.3: pH decrease of *Bifidobacterium bifidum* (ATCC 26521) Means ± S.D (vertical lines). RW –refiner, SW – siever, DW – decanter, glucose as a control.



Figure 5.4: Population Growth of *Bifidobacterium longum* (BB536)

Means \pm S.D (vertical lines). Values with superscripts (*) are statistically different at level p \leq 0.05 according to LSD test. RW –refiner, SW – siever, DW – decanter, glucose as a control.



Figure 5.5: pH decrease of *Bifidobacterium longum* (BB536)

Means ± S.D (vertical lines). RW –refiner, SW – siever, DW – decanter, glucose as a control.
5.4 Conclusions

This study has shown that the dietary fibre powder prepared from pink guava by-products contained fructooligosaccharides. A full separation of FOS components namely fructose, sucrose (DP1), nytose (DP2) and 1-ketose (DP3) was achieved for RW and SW, except for DW. The study indicated that RW consisted of FOS component and soluble dietary fibre (7.9 %, see Chapter 4) had significant effects of prebiotic. On the other hand, DW which contains low soluble dietary fibre and poor separation of FOS showed low prebiotic effect.

The study also indicates that there was synbiotic effect between *Bifidobacterium* and DFP, where there was a decreased of pH with the growth of *Bifidobacterium*. The decreased of pH may protect gastrointestinal system against pathogenic bacteria. This could indicate that the dietary fibre powder might possess the health-promoting properties due to FOS and soluble dietary fibre. Further study on the determination of health benefits of the investigated sample will be described in the following chapter.

CHAPTER 6

HEALTH- PROMOTING PROPERTIES OF DIETARY FIBRE POWDER

6.1 Introduction

In recent decades consumer demands in the field of food production has changed considerably. Consumers believe that foods contribute directly to their health (Young, 2000; Mollet and Rowland, 2002). Foods are not intended to only satisfy hunger and provide necessary nutrients for humans but also to prevent nutrition-related diseases (Roberfroid, 2000; Menrad, 2003).

The design of food products that confer health benefits is a relatively new trend, and recognized as a growing acceptance of the role of diet in disease prevention, treatment and well-being. This change in attitude for product design and development has compelled food industry to formulate food for health benefits (Sangeetha *et al.*, 2005).

It has becoming increasingly clear that there is a strong relationship between the food we consume and the state of our health. Scientific knowledge of the health benefits of various nutrients of food ingredients for prevention of specific diseases is rapidly accumulating.

The previous chapters 4 and 5 have shown that pink guava by-products dietary fibre powder contained high dietary fibre with suitable functional properties as food ingredient. This study has also identified the FOS components such as fructose, sucrose, nytose and 1-ketose and their prebiotic effects.

This chapter shall describe the procedures to determine the health promoting properties (antioxidant activity, total phenolic content, prebiotic and hypocholesterolemic effects) of the dietary fibre powder (DFP) prepared from pink guava by-products.

6.2 Materials and Methods

To evaluate the health promoting properties of DFP from pink guava byproducts four analysis were carried out namely antioxidant activity, total phenolic, prebiotic studies and hypocholesterolemic effect.



6.2.1. Materials

The samples used in this study were the RW, SW and DW from pink guava by-products collected from the farms of Golden Hope Fruit and Beverages Sdn. Bhd. Manjung, Perak, Malaysia.

Chemicals

For antioxidant activity, α -tocopherol, vitamin C and BHT were used as the standard. The reagents used in antioxidant activity analysis were β -carotene, chloroform, linoleic acid, Tween 20, nitroblue tetrazolium (NBT), R- \mathcal{R} ic, R,Rdiphenyl- \hat{a} -picrylhydrazyl (DPPH), hydrocholic acid, trichloroacetic acid

and methanol purchased from Sigma Chemical Co. (USA). All other chemicals were of reagent grades.

For total phenolics analysis, the reagents used were Folin-Ciocalteu, methanol, hydrochloric acid solution, and sodium bicarbonate. All chemicals used were of analytical grade from Sigma Chemical Co. (USA).

Microbiological assay was carried out using specific selective media. For the growth of *Bifidobacteruim*, BSM agar was used. MRS agar was used for the growth of *Lactobacillus*. For the growth of *Enterobacter*, Mac Conkey agar was used, whilst RCM agar was used for *Clostridium*,. For total *anaerobes* count, plate count agar was used. All the media were purchased from Sigma Chemical Co. (USA).

6.2.2 Methods

6.2.2.1 Determination of Antioxidant Activity

Determination of antioxidant activity was carried out using two methods namely β -carotene bleaching and 2,2'-diphenyl-1-piccryhydrazyl (DDPH).

β -carotene bleaching method

Antioxidant activity of DFP was determined according to β -carotene bleaching method (Velioglu *et al.*,1998). The antioxidant activity of the sample was measured as percentage inhibition of lipid peroxidation in the β -carotene-linoleic acid system, and was compared with the most common used standard antioxidants, α -tocopherol.

The oxidative losses of β -carotene in a β -carotene/linoleic acid emulsion were used to assess the antioxidation ability of the studied DFP. One ml of β -carotene (0.2 mg/mL) dissolved in chloroform was pipetted into a small

round-bottom flask. After removing the chloroform by using a rotary evaporator (Buchi, Italy), 20 mg of linoleic acid, 200 mg of Tween 20 and 50 mL of aerated distilled water were added to the flask with vigorous stirring. Aliquots (5 mL) of the prepared emulsion were transferred to a series of test tubes containing 2 mg of samples or standard or 80% methanol (control).

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Each type of sample was prepared in triplicate. The test systems were placed in water bath at 50 °C for 2 hours. The absorbance of each sample was measured using a spectrophotometer (Perkin Elmer, USA) at 470 nm, immediately after sample preparation (t = 0 minutes) and at 20 minutes intervals until the end (t = 120 minutes) of the experiment.

The rate of β -carotene bleaching was calculated according to first-order kinetics, as described by Al-Saikhan, Howard and Miller (1995) shown as an equation below.

Rate of β -carotene bleaching

 $= In (A_{t=0} / A_{t=t}) \times 1/t, \qquad (eq. 1)$

Where A $_{t=0}$ was the initial absorbance of the emulsion at time 0; A $_{t=t}$ was absorbance at 40, 60, and 80 minutes; and t was the time in minute. Based on the rates determined at the 40, 60, 80 minutes time intervals. An average rate was calculated. The antioxidant activity (AOA) was expressed as percentage inhibition of the rate of β -carotene bleaching relative to the control using the following equation:

% AOA = $100 \times (R_{control} - R_{sample}) / R_{control}$, (eq. 2)

where $R_{control}$ and R_{sample} were the average bleaching rates of β -carotene in the emulsion without antioxidant and with the analysed extract, respectively.

The 2,2'-diphenyl-1-piccryhydrazyl (DDPH) method

The sample of vitamin C and BHT were prepared as follows: 1 g of sample was mixed with 20 mL of 60% methanol solution and left overnight at 5° C. Positive control (vitamin C and BHT) was freshly prepared by dissolving 0.02g with 10 mL of absolute methanol solution. All samples were filtered using 0.4 μ m Whatman filter paper and kept in an amber bottle to prevent oxidation.

The effect of dietary fibre powdered from pink guava by-products on the DPPH radical was estimated according to the Lai *et al.* (2001) method. An aliquot of DFP (200 μ L, 3mg/mL), BHT (0.2 mg/mL), vitamin C (0.2 mg/mL) was mixed with the 100 mM Tris-HCl buffer (800 μ L, pH 7.4) and then added to 1 mL of 500 μ m DPPH of ethanol (final concentration of 250 μ m). The mixture was shaken vigorously and left to stand for 20 minutes at room temperature (27 °C) in the dark. The absorption of the resulting solution was measured spectrophometrically at 517 nm. The capability to scavenge the DPPH radical was calculated using the following equation:

Scavenging effect (%) = 1 - <u>absorbance of sample at 517 nm</u> x 100 absorbance of control at 517 nm

6.2.2.2. Determination of Total Polyphenols Content

Two hundred milligrammes of sample was extracted for 2 hours with 2 mL of 80% methanol containing 1% hydrochloric acid at room temperature on an orbital shaker set at 200 rpm. The mixture was centrifuged at 1000 x g for 15 minutes and the supernatant decanted into 4 mL vials. The pellets were reextracted under identical conditions. Supernatants were combined and used for total phenolics assay. One hundred microlitres of extract was mixed with 0.75 mL of Folin-Ciocalteu reagent (previously diluted 10-fold with distilled water) and allowed to stand at room temperature (32 °C) for 5 minutes and 0.75 mL of sodium bicarbonate (60 g/L) solution was added to the mixture. After 90 minutes at room temperature, absorbance was measured at 725 nm. Results were expressed as ferulic acid equivalents (FAE) per 100 g DFP.

6.2.2.3. Hypocholesterolemic Study Subject and location of the study

Twenty four male Sparague-Dawley rats, aged approximately 10 weeks with average initial body weight in the range of 150 ± 20 g were purchased from Perniagaan Usaha Cahaya, Batu Caves, Selangor, Malaysia. The animals were kept individually in a cage with wire mesh bottom at room temperature with a 12:12 hour light: dark cycle. The animals were acclimated for a week and given free access to a commercial diet and distilled water *ad libitium*. After acclimation period, body weight and blood sample were taken and regarded as zero (0) day data.

The rats (n=24) were grouped by stratified allocation, based on body weight, and placed into 3 groups (control, cholesterol, and 10% RW) of 8 rats each.

Rats in each group were similar in initial body weight. RW of pink guava byproduct was chosen in this study because it showed high prebiotic effects, antioxidant activity and potential functional properties (water retention capacity, oil retention capacity, bulk density, swelling capacity).

Preparation of diets

Three different diets were prepared , normal diet (control group), cholesterol diet (hypercholesterol group) and 10% RW diet (10% RW group). The normal diet (cholesterol-free diet) did not contain cholesterol or cholic acid; however 1% cholesterol and 0.2% cholic acid were added to other diets to increase serum and liver cholesterol (Anderson *et al.*, 1994; Chau *et.al.*, 2004), (Table 6.1). The 10% dietary fibre powder was added into the 10% RW diets, for above of these levels, it would decrease the sensory quality characteristic of the products (Carmen, 1997). All diets were introduced in the form of powder.

Basal diet was in pellet form which was grounded into powder using stainless steel grinder (Hammer Mill, USA) and passed through a sieve the size of 20 μ m. Then, diets were prepared as required (Table 6.1). The basal diet was consisted of wheat, lupins, barley, soya meal, fish meal, mixed vegetable oils, canola oil, salt, calcium carbonate, dicalcium phosphate, magnesium oxide and a vitamin and trace mineral premix. The nutritional composition of basal diet is tabulated in Table 6.2.

Experimental Diets						
Ingredients	Control	Hypercholesterol	10% RW			
Basal	1000	988	888			
Cholesterol	-	10	10			
Cholic acid		2	2			
DFP DW			100			

Table 6.1: Formulation of experimental diets

Note: Ingredient is expressed as grammes per kilogramme of diets.

Table 6.2: Nutrient composition of basal diets					
Nutrient	Nutritional value				
Protein	19%				
Total fat	4.6%				
Crude fibre	4.5%				
Energy	14.3 MJ/kg				
Vitamin A (Retinol)	10,000 IU/kg				
Vitamin D3 (Cholecalciferol)	2,000 IU/kg				
Vitamin K (Menadione)	2 mg/kg				
Vitamin E (α-tocopher <mark>ol ac</mark> etate)	20mg/kg				
Thiamine	6 mg/kg				
Riboflavin	6 mg/kg				
Nicotinic acid	25 mg/kg				
Pyrodoxine	6 mg/kg				
Calcium pantothenate	20 mg/kg				
Biotin	100 µg/kg				
Folic acid	2 mg/kg				
Vitamin B12	30 µg/kg				
Magnesium	100mg/kg				
Iron	70 mg/kg				
Copper	16mg/kg				
Iodine	0.5mg/kg				
Manganese	70 mg/kg				
Zinc	60mg/kg				

Table 6.2: Nutrient composition of basal diets

Source: Perniagaan Usaha Cahaya, Selangor Malaysia

Animal study

After one week of acclimation period, the hypercholesterol and 10% RW groups were subjected to a diet composed of the basal diet with additional 1% (w/w)cholesterol and 0.2% cholic acid induce alimentary to hypercholesterolemia in the rats (Anderson et al., 1994; Chau et al., 2004). All animals had free access to food and water for 30 days. Feed consumption was measured every 48 hours. The body weight was recorded daily. On the 15th day, about 4 – 5 mL of blood samples were drawn from the animals by cardiac puncture into plain vaccutainer, centrifuged at 3000 x g for 10 min at 4 °C to obtain serum samples and kept at -80 °C for biochemical analysis.

At the end of the experiment period (30 days), food was removed 16 hours before the animals were anesthetized. Water was provided after food removal. After the animals were anesthetized with ether, blood was withdrawn by cardiac puncture. The cecal materials (length: 7 cm of large intestine) of rats were removed, and immediately placed in the sterile flask, and inserted into an anaerobic jar, and the atmosphere was maintained by commercial system



Acclimation (1 weeks)



using carbon dioxide Gas Pack System (BBL; 270609, USA) and kept at 0 °C until further analysis (Queiroz-Monici *et al.*, 2004). The flow of the experimental study is as shown in Figure 6.1

Determination of lipid profile in serum

Lipid profile in rats serum was determined using commercially available assay kits, concentrations of total cholesterol (CH200, Randox, UK), highdensity lipoprotein (HDL) cholesterol (CH2652, Randox, UK), HDL/LDL Cholesterol calibrator (CH2673, Randox, UK) and triglyceride (TR210, Randox, UK), were enzymatically determined using Chemistry Analyzer (Selectra E, Vitalab, Italy).

Microbiology Assay of Cecal

Microbiologic assay of rat cecal was determined using the method developed by Queiroz-Monici *et al.* (2004). Tissues and cecal contents were removed from anaerobic jar and blended in peptone water, serial dilution (10⁻¹ to 10⁻⁷) were prepared, and inoculation was made into specific selective media for the growth of *Bifidobacteruim* (incubation period was at 37 °C for 48 hours), *Lactobacillus* (incubation at 37 °C for 48 hours), *Enterobacter* (incubation at 37 °C for 24 hours), *Clostridium* (incubation at 37 °C for 48 hours) and *total anaerobes* (incubation at 37 °C for 48 hours).

6.3. Statistical analysis

Three measurements were taken on each analysis. The results were expressed as mean of values \pm standard deviation of three separate determinations. Comparison of means was performed by one-way analysis of variance (ANOVA) followed by LSD test and t- test. ANOVA procedure was performed at p = 0.05 to study the variation. The statistical analyses were run using a computer SAS V. 9.1 software (SAS, USA).

6.4. Results and Discussion

6.4.1 Antioxidant Activities

β-carotene bleaching activity

Heat-induced oxidation of an aqueous emulsion system of β -carotene and linoleic acid was employed as an antioxidant test reaction (Figure 6.2). The linoleic acid free radical attacks the highly unsaturated β -carotene. The presence of different antioxidants can hinder the extent of β -carotene bleaching by neutralizing the linoleate-free radical and other free radicals formed in the systems (Jayaprakasha *et al.,* 2001).

Accordingly, the absorbance decreased rapidly without antioxidant whereas, in the presence of antioxidant, absorbance was sustained for a longer time. Antioxidant activities were observed in the ethanolic extract of DFP pink guava by-products and synthetic antioxidant (α -tocopherol as the control). In this study, the greatest antioxidative efficacy found was from α -tocopherol. The relative inhibitions of β -carotene consumption, after 60 minutes of incubation, by the ethanolic extracts of RW, SW and DW were 80.3%, 64% and 52.0% respectively (Table 6.3). After 120 minutes of incubation, the percentage activities observed were 74.5%, 37.4% and 29.4%, respectively.



Figure 6.2: Antioxidant Activities of DFP Pink Guava By-Products.

Assayed by the β -carotene bleaching method, vitamin E at 50 mg/L was used as a reference.

The differences in the antioxidant content in DFP pink guava by-products were statistically significant (p<0.05). Among the DFP pink by-products, RW had shown higher percentage (80.3%) of antioxidant activities followed by SW and DW. RW was the first step of the process which majority of the waste being collected were skin, pulp and seed. On the other hand, SW mainly contained skin and pulp and, DW mostly contained pulp.

A study by Barreira *et al.* (2007), on antioxidant activities of the extracts from chestnut flower, leaf, skin and fruit indicated that skins exhibited the highest antioxidant activity compared to other parts. A similar finding was by Guo *et al.* (2003), where most of fruit skin and seed fractions in 28 fruits (hawthorn, date, guava, kiwifruit, purple mulberry, strawberry, white pomegranate, lukan tangerine, honey tangerine, orange, lemon, cherry, logan, banana, pineapple, plum, lychee, kumquat, red rose grape, pamelo, mango, jiubao peach, apricot, hami melon, duck pear, jingxin melon, and persimmon) possess higher antioxidant activity than the pulp fractions. Similar findings were reported by Moure *et al.* (2001) and Shahidi *et al.* (1997) in tamarind seeds and coated peanut seeds. Therefore, the skin and seed fractions of fruit may potentially contain quantitatively more antioxidants than the pulp fractions.

Time (min)	40	60	80	120	
RW	73.4	80.3	76.8	74.5	
SW	67	64	63	37.4	
DW	59.1	52	49.5	29.4	

Table 6.3: Percentage (%) of antioxidant activities

Note: Analyses by β -carotene bleaching method at t= 40 min., 60 min., 80 min. and 120 min. Results are means of triplicate analyses.

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Scavenging activities on 2,2'-diphenyl-1-piccryhydrazyl (DDPH)

The proton radical scavenging action was known to be one of the various mechanisms for measuring antioxidant activity. DPPH was one of the compounds that posses a proton free radical and showed a maximum absorption at 517nm (Azizah *et al.*, 2007). This assay determined the scavenging of stable radical species of DPPH by antioxidants. Figure 6.3 shows the radical-scavenging activity of DFP, vitamin C and BHT by the DPPH coloring method. It was found that the scavenging percentage on the DPPH radical was found to be 91.7%, 91.5 % 85.4% for SW, DW and RW respectively.

The differences in the scavenging effects of DFP pink guava by-products were statistically significant (p<0.05). The highest scavenging effect was in SW and the lowest in RW. This may be due to the fraction of the SW which contained more skin compared to others sample. This finding was comparable with Barreira *et al.* (2008), who discovered that the outer and inner skin of chestnut exhibited higher scavenging effect. However, this result showed negative correlation ($r^2 = 0.92$) between β -carotene bleaching and DPPH methods. This may be due to the different method of measuring antioxidant activity that led



Figure 6.3: Scavenging Effects of Pink Guava By-Products on DPPH Radicals.

Means ± S.D (vertical lines). Bars with different letters indicate significant difference at level p<0.05. RW –refiner waste, SW – siever waste, DW – decanter, Vit C – Vitamin C

to differences in observation results. The measured antioxidant activities of the sample depend on which free radical or oxidant is used in the assay (Sun and Ho, 2004).

6.4.2. Total Polyphenols Content

Polyphenols are bioactive compounds believed to be involved in the defence process against deleterious oxidative damage, at least in part due to their antioxidant properties (Fresco *et al.*, 2006). The total polyphenol content in SW, DW and RW were 227.6, 171.7 and 156.0 mg FAE/g dry product respectively. Figure 6.4 shows a comparative content of total polyphenol content in DFP pink guava by-products. The differences in the content of total polyphenols in DFP pink guava by-products were statistically significant (p<0.05). The highest total polyphenols content was in SW and the lowest in RW.

The high value of polyphenols in this study was related to the high dietary fibre content of DFP (SW- 64.3 % total dietary fibre (TDF), DW – 76.1% TDF and RW – 56.6% TDF). This can be explained by the fact that the polyphenols



Figure 6.4: Total Polyphenol Content in Pink Guava By-Products.

Means ± S.D (vertical lines). Bars with different letters indicate significant difference (p<0.05). RW –refiner waste, SW – siever waste, DW - decanter



ere compound associated with dietary fibre (Larrauri *et al.*, 1996; Elleuch *et al.*, 2007).

There was a higher value of polyphenols found in SW compared to DW even though DW contained high total dietary fibre. This may be due to the different fractions of fibre in these two samples. SW contained mainly skin and pulp, whereas DW contained more pulp. The results indicated that skins presented the highest polyphenol contents.

It was reported that coats of vegetables seeds, coats of cereal grains and peels of fruits (characterized by the high dietary fibre content) contained higher amount of polyphenols than the cotyledon, the endosperm and the pulp fractions respectively (Duenas et al., 2002; Gorinstein et al., 2001; Shahidi et al., 2006).

This study found significantly negative linear correlation between the polyphenol contents and β -carotene bleaching method with correlated coefficient values (r² = 0.712). This negative correlation showed that the

samples with highest polyphenol content showed lower antioxidant activity, confirming that phenolics were unlikely to contribute to the antioxidant activity of the DFP pink guava by-products. A high antioxidant activity could be due to other compounds besides phenolics which were soluble in the ethanol (Azizah *et al.*, 2007). This result was in agreement with two previous reports on the antioxidant activity of buckwheat (Sun and Ho, 2005) and cocoa beans (Azizah *et al.*, 2007) which was inversely correlated with polyphenol content.

On the other hand, there was positive correlation coefficient value ($r^2 = 0.70$) between DPPH method and total polyphenol content, this showed that SW had higher total polyphenol content with high scavenging effect. The results indicated that high scavenging ability on DPPH radicals could be due to the phenolic compound in the DFP. Based on the antioxidant assays, it was suggested that phenolic compounds present in DFP had strong**er** scavenging ability compared to β -carotene bleaching activity. This may be due to the antioxidant mechanisms of phenolic compounds towards free radicals.

6.4.3. Hypocholesterolemic Effects

Body weight gain, food intake and fecal weights

After 30 days of feeding, the body weights of the rats increased from 19 g – 19.1 g (initial weight) to 32.6 g – 35 g (final weight) among the three diets groups (Figure 6.5). The results showed that the experimental diets, with or without the inclusion of fibre, did not interfere with food intake (12 – 19.6 g/30 days).

The experimental results showed that there was no significant difference (p>0.05) in the mean body weight gain (Figure 6.6) and food efficiency (Figure 6.7) between the rats fed with 10% RW diet and those rats in control and cholesterol diet. This suggested that the consumption of 10% RW did not affect the body weight of the experiment rats.

As expected, cholesterol-10% RW group had remarkable fecal bulking effect, with the fresh weights of faeces significantly (p<0.05) higher than in the control and cholesterol groups respectively within 30 days of experiment (Figure 6.8). The study showed that, at the initial experiment, there was no

significant difference (p>0.05) in faeces-fresh weight among the three diets group.

The results of the present study showed that total dietary fibre and its soluble and insoluble fractions of DFP can result in an increase in fecal weight, possibly due to the hypertrophy caused by the ingestion of such voluminous components. Comparative studies on the effect of different non-digestible carbohydrate on intestinal mucous tissues have shown that the type of fibre in the diet determines the nature of alterations in the growth and morphology of mucous (Cummings *et al.* 2002, Kleessen *et al.* 2003).



Figure 6.5: Effects of diets on body weight for 30 days

Values are presented as mean \pm SD (n = 8). Asterisk (*) indicates the significant difference at level p<0.05 between final values (30 days) and initial values (0 day) according to Student's t-test. Control group: basal diet: free cholesterol diet Hypercolesterol diet: basal diet + 1% cholesterol + 0.2% choline acid 10% RW diet: = basal diet + 10% RW + 1% cholesterol + 0.2% choline acid



Figure 6.6: Effects of Diets on Body Weight Gain within 30 days

Values are presented as mean \pm SD (n = 8). Control group: basal diet: free cholesterol diet. Hypercolesterol diet: basal diet + 1% cholesterol + 0.2% choline acid. 10% RW diet:- basal diet + 10% RW + 1% cholesterol + 0.2% choline acid. Body weight gain = final weight - initial weight



Figure 6.7: Effects of Diets on Food Efficiency for 30 days

Values are presented as mean \pm SD (n = 8). Values with same superscripts (^{a,b,c}) are not statistically different at level p < 0.05 according to LSD test Control group: basal diet: free cholesterol diet. Hypercolesterol diet: basal diet + 1% cholesterol + 0.2% choline acid 10% RW diet:- basal diet + 10% RW + 1% cholesterol + 0.2% choline acid Food efficiency = body weight gain x (food intake) ⁻¹



Figure 6.8: Effects of Diets on Faeces-Fresh Weight of Rats within 30 days

Values are presented as mean \pm SD (n = 8). Asterisk (*) indicates there were significant difference at level p<0.05 between final values (30 days) and initial values (0 day) according to Student's t-test. Values with same superscripts (^{a,b,c}) are not statistically different at level p < .05 according to LSD test Control group: basal diet: free cholesterol diet. Hypercolesterol diet: basal diet + 1% cholesterol + 0.2% choline acid .10% RW diet:- basal diet + 10% RW + 1% cholesterol + 0.2% choline acid

Soluble dietary fibre had little influence on the increase in weight of the faeces because during fermentation in large intestine, they were degraded, losing some of their physiochemical characteristics, such as their water-holding capacity. The water-holding capacity was physical properties contributing to the increase in weight of the faeces.

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In contrast, the insoluble fibre did not lose their water-holding capacity, which makes them resistant to bacteria fermentation, contributing to a great extent to the increase in weight and decrease in consistency of the faeces (Kolida *et al.*, 2002; Queiroz-Monici *et al.*, 2005).

From the previous analyses (Chapter 4), 48.7% of IDF and 7.9% of SDF was shown to be present in RW. The main fraction of IDF in RW was lignin and cellulose which had high water affinity that increased its water retention capacity. Due to this factor, RW had an ability to retain water in large intestine which contributed to the increase of weight stool.

Serum lipids and antherogenic index

The addition of 1% cholesterol and 0.2% choline acid to diets efficiently induced hypercholesterolemia in rats (Anderson *et al.*, 1994; Chau *et al.*, 2004). The group fed with the cholesterol-rich diets had altered serum lipid concentrations, causing a marked hyperlipidemia. Supplementation of 10% RW had reduced total serum cholesterol and low density lipoprotein levels, where as the high density lipoprotein and triglycerides levels were not affected within 30 days of experiment (Figure 6.9 and Figure 6.10).

However, there was a significant difference (p<0.05) of HDL concentration in control diets between the initial values and final values. The increase of HDL concentrate in control diet may be due to absence of 1% cholesterol in the diet. Nevertheless, there was no significant difference (p<0.05) of HDL final values between control diets and other diet groups at/on final day. The same trend was also shown for triglycerides level for 10% of control RW diets.



Figure 6.9: Effect of Diets on High Density Lipoprotein (HDL-C) of Rats within 30 days

Values are presented as mean \pm SD (n = 8). Asterisk (*) indicates there were significant difference at level p<0.05 between final values (30 days) and initial values (0 day) according to Student's t-test. Control group – basal diet: free cholesterol diet. Hypercolesterol diet – basal diet + 1% cholesterol + 0.2% choline acid. 10% RW diet – basal diet + 10% RW + 1% cholesterol + 0.2% choline acid


Figure 6.10: Effect of Diets on Triglycerides (TG) of Rats within 30 days Values are presented as mean \pm SD (n = 8). Asterisk (*) indicates significant difference at level p<0.05 between final values (30 days) and initial values (0 day) according to Student's t-test. Control group – basal diet: free cholesterol diet. Hypercolesterol diet – basal diet + 1% cholesterol + 0.2% choline acid 10% RW diet – basal diet + 10% RW + 1% cholesterol + 0.2% choline acid The present study showed that supplementation with 10% RW had significantly (p<0.05) lowered antherogenic index compared to hypercholesterol diet, but there was no significant difference with control diet (Figure 6.11). Dietary fibre, especially viscous soluble fibre, was well known for their effect in lowering total cholesterol, thus preventing hypercholesterol (Lecumberri *et al.*, 2007a). It has been shown that there was a positive correlation between antherogenic index and risk of coronary heart disease, the lower antherogenic index obtained with the consumption of DFP will be beneficial to heart patients.

The total serum cholesterol concentration was averaged at 2.15 mmol/L when cholesterol and choline acid were added to the basal diet (Figure 6.12). The supplementation of 10% RW had significantly (p < 0.05) lowered the serum total cholesterol by 43% which normalized to the level of control group. This showed that the inclusion of fibre DFP of RW in diets could effectively decrease the serum total cholesterol concentration, with the hypocholesterolemic effect.



Figure 6.11: Effect of Diets on Antherogenix Index of Rats within 30 days Values are presented as mean \pm SD (n = 8). Values with same superscripts (^{a,b,c}) are not statistically different between groups at level p < 0.05 according to LSD test . Control group – basal diet: free cholesterol diet. Hypercolesterol diet – basal diet + 1% cholesterol + 0.2% choline acid.10% RW diet – basal diet + 10% RW + 1% cholesterol + 0.2% choline acid. Antherogenic index = (total cholesterol - HDL) x (HDL) ⁻¹



Figure 6.12: Effects of Diets on Total Cholesterol of Rats within 30 days

Values are presented as mean \pm SD (n = 8). Asterisk (*) indicates the significant difference at level p<0.05 between final values (30 days) and initial values (0 day) according to Student's t-test. Values with same superscripts (^{a,b,c}) are not statistically different between group at level p < 0.05 according to LSD test * Control group – basal diet: free cholesterol diet. * Hypercolesterol diet – basal diet + 1% cholesterol + 0.2% choline acid. * 10% RW diet – basal diet + 1% cholesterol + 0.2% choline acid

Previous study has indicated that the fibres derived from guava pulp (Basumullik, 1994) and some other agriculture by-products (e.g., sugar beet pulp and apple pomace) possessed hypocholesterolemic properties (Leontowicz *et al.*, 2001). In this study, a significant (p<0.05) high bulk density (0.57 g/mL) and low water-retention capacity (3.75 g of water/g of fibre) of the analysed RW (Chapter 4; Figure 4.5 and Table 4.6) might lead to the reduction in the transit time, and the total time available for cholesterol absorption in the small intestine.

Thus, the influence of insoluble dietary fibre (RW contained 48.7% of insoluble dietary fibre) on serum cholesterol might be partially due to the reduction of cholesterol absorption by the concerted effects of these physico-properties of RW. Chau and Cheung (1999) reported the reduction of cholesterol absorption by legumes IDF due to their concerted effects on bulk density, water-holding capacity and cation-exchange capacity.

For LDL concentration, there was a significant increase (p<0.05) of the LDL within 30 days experiment for all the rats. However, the supplementation of 10% RW diet could lead to a decrease in LDL concentration (51%) compared to

hypercholesterol diet, while there was no significant difference (p>0.05) in the LDL concentration between the control and 10% RW groups (0.68 mmol/L versus 0.84 mmol/L respectively) after 30 days of experiment (Figure 6.13). The serum LDL lowering effect of the diet containing fibre could be corroborated with the findings by Lecumberri *et al.* (2007a) ; Martinez-Flores *et*

al., (2004).

Dietary fibre, especially soluble dietary fibre, was known for its effect in lowering total and LDL cholesterol, thus attenuating hypercholesterol (Lecumberri *et al.*, 2007a). A significant amount of soluble fibre present in RW hinders digestion and absorption of dietary fats, resulting in lower cholesterol delivery to the liver by chylomicron remnants, with a concomitant upregulation of LDL receptor and decreased lipoprotein secretion to maintain cholesterol homeostasis in liver (Jalili *et al.* 2001). Moreover, soluble fibres were fermented by the colonic microflora generating short-chain fatty acids (acetic, propionic and butyric acids) (Brighenti *et al.* (1999).



Figure 6.13: Effects of Diets on Low Density Lipoprotein (LDL-C) of Rats within 30 days

Values are presented as mean \pm SD (n = 8). Asterisk (*) indicates the significant difference at level p<0.05 between final values (30 days) and initial values (0 day) according to Student's t-test. Values with same superscripts (^{a,b,c}) are not statistically different between group at level p < 0.05 according to LSD test Control group – basal diet: free cholesterol diet.Hypercolesterol diet – basal diet + 1% cholesterol + 0.2% choline acid.10% RW diet – basal diet + 10% RW + 1% cholesterol + 0.2% choline acid.

Hypolipidemic effects of propionate through inhibition of cholesterol and fatty acids syntheses in the liver have been reported (Delzanne *et al.* 2001). Furthermore, insoluble fibre through its effect in diluting gasteriointestinal contents may hinder digestion and absorption of dietary fats, thus contributing to the effects of soluble dietary fibre (Lecumberri *et al.*, 2007a).

UPM

All these mechanisms lead to lower serum levels of cholesterol and LDL, subjacent to reduced risk of cardiovascular disease associated to dietary fibre intake (Lecumberri *et al.*, 2007b; Jalili *et al.*, 2001; Anderson *et al.*, 2000). From this study, it is evident that dietary fibre from pink guava processing waste has significant hypocholesterolemic effect.

In-vivo: Prebiotic effects

At the end of *in-vivo* experiment (hypochelesterolemic study) cecal materials of rats were collected for enumeration of *Bifidobacterium* and *Lactobacillus*. Results showed that *Bifidobacterium* was higher in the cecal material of rats fed with 10% RW than in the other rats. There was no statistically significant difference (p>0.05) between groups with respect to *Lactobacillus* count in rat cecal material.

The 10% RW group showed lower counts of *Enterobacter* and *Clostridium* which was significantly different (p<0.05) from the control group, which presented higher counts. With respect to the count of total anaerobes, there was a significant difference (p<0.05) between the 10% RW with control and hypocholesterolemic groups.

The intestinal microbiota of the animal was shown to be influenced by the items tabulated data evident (Figure 6.14, 6.15 and 6.16). The 10% RW group showed larger *Bifidobacterium* count than those of the control and hypocholesterolemic groups. With the conditions for the growth of this bacterium favourable, the counts of *Enterobacter* and *Clostridium* in the animals fed with 10% RW diets were lower than in the control group.

The ten percent (10%) RW diet has shown a modulating effect on the beneficial intestinal microbiota of the rats, where counts of total anaerobic were found to be significantly higher in 10% RW compared to other groups. The presence of dietetic components, especially soluble dietary fibre and FOS, could have a potential the bifidogenic effect of 10% RW diet.

UPM

Reports by Mussatto and Mancilha (2007), Queiroz-Monici *et al.*, (2005) and Cummings et al. (2004) that water soluble substances such as soluble dietary fibre and FOS may enhance resistance to pathogen invasion. The substances also could be a source of SCFAs and in conjuction with probiotic species reduce the risk of neoplastic change in gut epithelium.





Data are mean \pm standard deviation (n = 8). Asterisk (*) indicates the significant difference p < 0.05 by analysis of variance and LSD versus the control group. CFU, colony-forming unit.



Data are mean \pm standard deviation (n = 8). Asterisk (*) indicates the significant difference p < 0.05 by analysis of variance and LSD versus the control group. CFU, colony-forming unit



Figure 6.16: Cecal Concentration of *Clostridium* (E) in Rats Fed with Experimental Diets for 30 days.

Data are mean \pm standard deviation (n = 8). p < 0.05 by analysis of variance and LSD versus the control group. CFU, colony-forming unit.



This study has demonstrated that 10% RW diet offers sufficient amount of dietary fibre and FOS for the diets because these components influence intestinal microbiota of rats. The animals fed with the 10% RW diet showed higher counts of *Bifidobacterium*, demonstrating the bifidogenic effects of these components.

6.5. Conclusions

The present study demonstrates that the dietary fibre powder from pink guava by-products is not only high in dietary fibre content but also high in antioxidant activities (52 - 91.4 % AOA), radical scavenging effects (85.4 - 91.7 %) and total phenolic content (156 - 227.6 FAE mg/g). The study has also showed that the dietary fibre powder of RW prepared from pink guava by-product offered sufficient amount of dietary fibre and fructooligosaccharide for intestinal microbiota of rats. These results demonstrated that dietary fibre powder from pink guava by-product (RW) is a prebiotic food due to the evident of the decrease of mesophilic bacteria and an increase of bifidobacteria in rat intestine.

Dietary fibre powder from the pink guava by-products has had very pronounced hypocholesterolemic effects as it could significantly (p< 0.05) decrease the levels of serum total cholesterol (43%) and LDL levels (51%) in rats. Thus, dietary fibre from pink guava by-products especially RW could be a potential cholesterol-lowering ingredient in human diets, and offer the industry an opportunity to develop new formulation of fibre-rich functional foods from pink guava processing waste. However, human trials on this product should be carried out in future studies.

CHAPTER 7

CONCLUSIONS

7.1. Conclusions

The by-products from pink guava puree industry, namely refiner, siever and decanter had dietary fibre of 79%, 68% and 76% respectively. The dietary fibre consisted of 64 to 75 % of IDF and 3.4 to 4.4 % of SDF. Lignin and cellulose were the major fraction in IDF of the pink guava by-products. Refiner had the highest total dietary fibre followed by decanter and siever. Thus, the pink guava by-products could be classified as high source of dietary fibre as all the by-products contained more than 50% of dietary fibre.

The development of dietary fibre powder from the pink guava by-products (DFP) using the hot water treatment had produced potential functional food ingredient. The products had potential hydration properties (3.8 g to 12.2 g of water/g of fibre), oil binding capability (2.2 g to 6.9 g of oil/g of fibre), low calories (less than 250 kcal/100 g), light brown in colour and bland in taste.

From the electron micrograph scanning, the DFP was shown to have scales and surface which were rough and hollow. Milling process resulted in an open structure of DFP, thus increasing the surface area and trapping more water/oil molecules, therefore exhibiting high water/oil-holding capacity The present study has demonstrated that the dietary fibre powder was high in antioxidant activities (80%, 63% and 52% for RW, SW and DW, respectively), scavenging effect (91.7%, 91.5% and 85.4% for SW, DW and RW, respectively) and total phenolic content (between 156 mg/g to 228 mg/g dry basis). Dietary fibre powder from pink guava by-product offered sufficient amount of dietary fibre and fructooligosaccharides for the diets because it had influenced the intestinal microbiota of the rats and increased the growth of bifidobacterium in the culture. These results confirmed the dietary fibre powder as prebiotic food due to the evidence of the decrease of mesophilic bacteria and bifidobacteria increase in the rats' intestines.

This investigation has also indicated that dietary fibre powder from the pink guava by-products had highly pronounced hypocholesterolemic effects as it could significantly (p<0.05) decrease the levels of serum total cholesterol by 43%, LDL by 51% in rats. Therefore, this dietary fibre from the powder could be a potential cholesterol-lowering ingredient in human diets, and offer the industry an opportunity to develop new formulation of fibre-rich functional health foods from pink guava processing waste.

7.2. Recommendations for further research

The evidence of the prebiotic and hypocholesterolemic effects of DFP from pink guava by-products and its health benefits has underscored its high potential as functional and health food formulation. However, there are remaining assumptions about the present evidence that need further experimental verification through future research.

There are at least three research directions that can be taken from here. First, the need to elucidate the mechanisms underlying the action of dietary fibre which requires a further understanding of the structure–function relationships. The tertiary structure of the architecture of dietary fibre must be further investigated with the help of microphysical techniques such as NMR and microscopy tools. Second, more *in vivo* data are still required to progress in the formulation. The methods for the measurement of the physico-chemical properties relevant from a physiological perspective are very much needed. The microphysical methods will help reveal how the fibre matrix might behave *in vivo*, thus putting researchers in a vantage position to examine the link of physicochemical properties to physiological effects. However, there is still a need to refine and develop *in vitro* methods to evaluate the physicochemical properties in food and within the gut lumen. Furthermore, studies on some processing parameters like texture profile analysis, viscosity, viscoelastisity, stress relaxation are needed to discover more of the properties of guava by-products as food ingredient.

Lastly, the role of microflora and its metabolic activity on the gut and endocrine systems must be further investigated, for the metabolism in the large intestine has significant health implications. The study has also underlined a need for a more fundamental research in the adequation of raw materials and processing parameters to develop optimised products for both quality and nutritional aspects. Thus the present and future investigations on the physico-chemical and health-promoting properties of the pink guava by-products DFP are highly worthy scientific endeavour given the many scientific significance so far uncovered and yet to be revealed.



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APPENDIX A

Table: DF composition of selected cereals brans and IDF concentrates from processing by-products of fruits and greens (g/100 g)

Dietary fibre	TDF	IDF	SDF	
Apple	60.1	46.3	13.8	
Pear	36.1	22	14.1	
Orange	37.8	24.2	13.6	
Peach	35.8	26.1	9.7	
Wheat	44	41.1	2.9	
Oat	23.8	20.2	3.6	
Artichoke	58.8	44.5	14.3	
Asparagus	49	38.5	10.4	

Source: Grigelmo-Miguel and Martin-Belloso, 1999

TDF: total dietary fibre; IDF: insoluble dietary fibre; SDF: soluble dietary fibre

APPENDIX B

Table: Water retention capacity (WRC), and fat absorption capacity (FAC) of fruit fibre concentrate

Fibre concentrate	WRC	FAC
	(g water/g fibre)	(g oil/g fibre)
Grapefruit		
Ruby	2.09	1.52
Marsh	2.26	1.20
Lemon		
Eureka	1.85	1.30
Fino 49	1.74	1.48
Orange		
Valencia	1.65	1.81
Apple		
Royal gala	1.62	0.95
Granny smith	1.78	1.45
Liberty	1.87	0.60
-		

Source; Figeurola *et al.*, 2005





APPENDIX C

A. Effect of particle sizes (600 – 250 $\mu m)$ on colour in refiner by-products



B. Effect of particle sizes (600 – 250 µm) on colour in siever by-products



C. Effect of particle sizes (600 – 250 $\mu m)$ on colour in decanter by-products



BIODATA OF THE AUTHOR

Aida Hamimi Ibrahim was born in Kota Bharu, Kelantan on the 10th of April 1970. She had early education at Sekolah Rendah Jenis Kebangsaan (2) Batu Empat Jalan Ipoh, Kuala Lumpur (1977- 1980) and Sekolah Kebangsaan Segambut, Kuala Lumpur (1981 1982). She continued her secondary school at Maktab Rendah Sains MARA Seremban and completed her Sijil Pelajaran Malaysia (SPM) with first grade.

She continued her tertiary education as Matriculation student at Maktab Rendah Sains MARA Kulim for two years and continued for her degree at Universiti Pertanian Malaysia (1990 - 1994). She completed her Bachelor of Science degree in Food Science and Technology with second class honour. She obtained her Master of Technology degree in Food Technology from Massey University, Palmerston North, New Zealand.

Presently, she works as research officer at Food Technology Research Centre, Malaysia Agriculture Research Centre (MARDI), Serdang, Selangor. She is married to Wan Ahmad Fuad Wan Yusoff and they have three daughters named Wan Nur Fatina, Wan Nur Fadlina and Wan Nur Falishah.

PUBLICATIONS

International Referred Seminars

1 I. Aida Hamimi, I. Amin, M. Mohd Yazid, M.E. Norhaizan, H. Mahanum, A.S. Norhartini. 2008. Dietary fibre contents and antioxidant activity in tropical fruit by-products. 8th ASEAN S&T Week Technical and Scientific Conference, Manila, Philippine, 3 -4 Julai 2008

2. I. Aida Hamimi, I. Amin, M. Mohd Yazid, M.E. Norhaizan, H. Mahanum, A.S. Norhartini. 2007. Fibre concentrates from pink guava by-products as a potential fibre sources for food ingredient. International Conference on the premier food science and technology in 10th ASEAN Food Conference, Sheraton, Subang Jaya, Selangor, 21-23 August 2007

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Honours and Awards

1. Brussel-Innovation Expo, Belgium, 13 – 15 November 2008 – Gold medal

2. International Technology Expo, 9- 11 May 2008, KLCC Kuala Lumpur – Silver medal

3. Malaysia Technology Expo, 21 – 23 February 2008, PWTC Kuala Lumpur – Bronze medal

4. Mardi Science and Technology Expo, 2 – 5 September 2007, Persada Johor Bharu – Gold and Bronze medal

5. Pertandingan Reka Cipta Bahan Terpakai, Mardi Science and Technology Expo, 8 -9 August 2006, ESSET, Bangi – Third Place

6. Malaysia Technology Expo, 2003, PWTC Kuala Lumpur, Bronze medal

7. Malaysia Technology Expo, 2002, PWTC Kuala Lumpur, Bronze medal

8. Anugerah Khidmat Cemerlang MARDI – 2002