PINK GUAVA (*Psidium guajava* L.) BY-PRODUCTS:

HEALTH-PROMOTING POTENTIALS

By

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A project report submitted to the Department of Chemical Science

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ABSTRACT

PINK GUAVA (*Psidium guajava* L.) BY-PRODUCTS: HEALTH-PROMOTING POTENTIALS

Heng Wooi Shin

Pink guava (*Psidium guajava* L.) is well-known for its rich content of fiber, phenolics and vitamins, hence, it is predicted that its co-products may possess these beneficial properties as well. In this study, three types of by-products collected from the pink guava puree processing line: refiner, siever and decanter were used for the evaluation of their health-related properties. Pre-analysis treatments including freeze-drying, grinding, sieving and determination of moisture content were done prior to ethanol (80%, v/v) extraction. The moisture content for all the freeze-dried samples was less than 10% for better chemical and microbial stability throughout the study. Through the simple maceration technique, dry ethanolic extract (from supernatant) was employed for the antioxidant assays and the α-amylase inhibitory assay; while dry pellet was used for the glucose diffusion assay. The health-promoting potentials of these guava by-products were evaluated in terms of their antioxidant capacity through DPPH assay, reducing power, anti-amylase activity and glucose dialysis retardation index. Results revealed that the pink guava by-products exhibit high levels of antioxidant activities. Among the three samples, siever was found to possess the highest antioxidant capacity with EC_{50} of 0.317 mg/ml, significantly ($p < 0.05$), followed by

decanter (EC_{50} = 0.389 mg/ml) and refiner (EC_{50} = 0.394 mg/ml), in DPPH assay. These results were consistent with the findings in reducing power assay. However, in terms of the α-amylase inhibitory potential and glucose retardation potential, decanter showed the highest activity as compared to siever and refiner. These findings could be attributed to the presence of fibers in the samples, which can enhance their hypoglycemic potentials. Thus, further study should be done on the composition of fibers in guava by-products, to correlate their compositional and functional properties. Through the present study, the guava by-products could be value-added due to their high antioxidant and antidiabetic potentials.

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DECLARATION

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

_____

HENG WOOI SHIN

APPROVAL SHEET

The project report entitled "**PINK GUAVA (*Psidium guajava* L.) BY-PRODUCTS: HEALTH-PROMOTING POTENTIALS**" was prepared by HENG WOOI SHIN and submitted as partial fulfilment of the requirements for the degree of Bachelor of Science (Hons) Biochemistry at Universiti Tunku Abdul Rahman.

Approved by:

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PERMISSION SHEET

It is hereby certified that HENG WOOI SHIN (ID No: 13ADB07272) has completed this final year project entitled "PINK GUAVA (*Psidium guajava* L.) BY-PRODUCTS: HEALTH-PROMOTING POTENTIALS" supervised by DR. CHANG YING PING from the Department of Chemical Science, Faculty of Science.

I hereby give permission to my supervisor to write and prepare manuscripts of these research findings for publishing in any form, if I do not prepare it within six (6) months from this date, provided that my name is included as one of the authors for this article. The arrangement of the name depends on my supervisor.

Yours truly,

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(HENG WOOI SHIN)

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LIST OF ABBREVATIONS

Abs	Absorbance
DPPH	2,2-Diphenyl-1-picrylhydrazyl
EC	Enzyme Commission
EC_{50}	Half maximal effective concentration
Eds	Editors
et al.	et alia (and others)
GAE	Gallic acid equivalents
GDRI	Glucose dialysis retardation index
GOPOD	Glucose oxidase/peroxidase
HCl	Hydrochloric acid
HPLC	High performance liquid chromatography
min	Minutes
MWCO	Molecular weight cut-off
Psidium guajava L.	*Psidium guajava* Linn.
R^2	Regression coefficient
SD	Standard deviation
v/v	Volume/volume
w/v	Weight/volume
α	Alpha
β	Beta
γ	Gamma

CHAPTER 1

INTRODUCTION

1.1 Background of Study

It was reported that the current world population has reached around seven billion and the number was believed to strike nine billion by year 2050, according to Hofstrand (2012). Generally, the rising population shows a close association with the food demand and supply, whereby the Food and Agricultural Organization (FAO) projected that 70% increment in food production will be required to sustain the needs in year 2050 (Hofstrand, 2012). As a consequence, the increase in food production will lead to environmental problems, since 33% of all food was squandered throughout the food supply chain, ranging from initial processing to the end products consumption (FAO, 2011). Many waste management issues have been arouse, including improper management of disposal sites, health and safety issues as well as limited landfills (Bartelings and Sterner, 1999). Thus, the exploitation of existing underutilized resources such as by-products is of utmost importance in coping with these critical issues.

Guava (*Psidium guajava* L.), is a highly nutritious tropical fruit which can be found all-year-round in Malaysia, in two major types: white-fleshed and pink-fleshed guava. Basically, white-fleshed guava is consumed freshly whereas pink-fleshed guava is normally used in puree and juice production in Malaysia. According to Kong and Ismail (2011), approximately 25% of the pink guava fruits are disposed of as by-products during puree processing, through cutting

and crushing, sieving and decanting step. Large amount of wastes have been produced through the processing of fruits and vegetables in the food processing industry. This can create environmental problems due to microbial spoilage on the liquid and pulp wastes (Sukeksi and Sarah, 2016). Thus, the present study was undertaken in order to explore the health-promoting potentials of various guava by-products obtained from the pink guava puree processing industry while minimizing the waste management issue and disposal cost at the same time.

Next, the cases of diabetes (especially Type 2 diabetes mellitus) have become the alarming issues among human populations in recent years. According to Wild et al. (2004), World Health Organization (WHO) estimated that the worldwide prevalence of diabetes for all age groups will be elevated from 171 million to 366 million (year 2000 to 2030). Basically, Type 2 diabetes mellitus is a systemic metabolic disease which closely associated with hyperglycemia, insulin insensitivity as well as obesity (characterized by hyperlipidemia and hypertension), and the coexistence of these diseases will eventually lead to cardiovascular diseases (Kaplan, 1989).

Due to the adverse effects caused by the prolonged usage of insulin and other hypoglycemic drugs such as biguanides, sulfonylureas and thiazolidinediones (Mary and Gayathri, 2015), there is an increasing demand for natural products with high anti-hyperglycemic activity (Choudhary et al., 2013). According to Mehta (2005), through the consumption of adequate dietary fiber, the risk of chronic diseases including bowel cancer, gastrointestinal disorders, obesity,

diabetes and other cardiovascular diseases can be lowered down. Also, fiber was believed to aid in the reduction of blood cholesterol and glucose attenuation, promoting optimum physiological functions in the body.

Besides, the potential risk of getting cancer, atherosclerosis, diabetes and other common human diseases can be reduced via the dietary intake of phytochemicals-rich foods containing high levels of antioxidant activities (Temple, 2000). Many plants serve as the exemplary natural sources of antioxidant and thus studies have been carried out for the isolation, extraction and characterization of the bioactive components from different plant parts and species (Musa et al., 2011). According to Gupta and Sharma (2006), several compounds present in plants, for instances, flavonoids (flavones, isoflavones, anthocyanins, catechins), vitamins (vitamin C, vitamin E, carotenoids) as well as polyphenols (ellagic acid, gallic acid, tannins), are important antioxidants.

In addition, Larrauri (1999) said that by-products from fruits and vegetables typically serve as the promising sources for dietary fibers as well as other functional compounds. This was further proven by other studies, which showed that many fruit peels contain polyphenolic compounds, flavonoids, ascorbic acid and other bioactive components contributing to various medicinal and pharmacological properties (Kim et al., 2010; Leontowicz et al., 2003; Zhao et al., 2007). Also, since the disposal of guava wastes could be a potential environmental issue, alternatives for reducing the amount of wastes produced are of great importance (Uchôa-thomaz et al., 2014).

1.2 Objectives

The general aim of this study is to value-add the guava by-products through evaluating their potential health-promoting properties.

The following are the specific objectives of this study:

1. To determine the antioxidant properties of guava by-products through DPPH free radical scavenging assay and reducing power assay.

2. To evaluate the α-amylase inhibitory potential of guava by-products using starch-iodine colour assay.

3. To determine the hypoglycemic effect of guava by-products through glucose diffusion assay.

CHAPTER 2

LITERATURE REVIEW

2.1 Selected Plant: Pink Guava (*Psidium guajava* L.)

Psidium guajava L. (Family: Myrtaceae) as shown in Figure 2.1, which is commonly called guava or "jambu batu" in Malaysia, originated from a wide variety of cultivars. Figure 2.2 shows Sungkai and Semenyih varieties of pink guava which are widely produced in Malaysia. Often, guava is known as "apple of the tropics", due to its year round availability as one of the important commercial fruit crops in the tropical and subtropical countries (Musa, Abdullah and Subramaniam, 2015).

Pink guava exhibits a characteristic sweet, musky smell upon ripening, with a layer of thick, pink flesh underneath the light-yellow peel, whereas numerous yellowish seeds can be found in the central pulp of the fruit (Purdue University, n.d.). The fruit can be consumed freshly and it is commonly used in the food industry to be further processed into juice and jam. In addition, guava possesses medicinal properties and is used as folk remedies to treat dermatosis, diarrhoea, epilepsy, hysteria and menstrual disorders (Taylor and County, 2012).

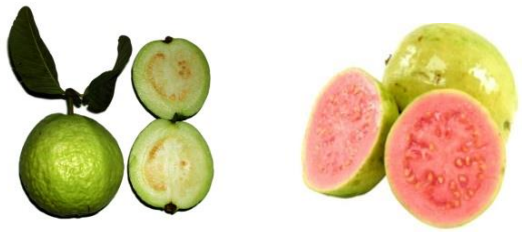


Figure 2.1: White and pink guava (Wikipedia, 2016; Ginting, 2014)

Figure 2.2: Varieties of pink guava in Malaysia

2.1.1 Guava By-products

There are three main wastes: refiner, siever and decanter discarded as by-products in the process of producing the pink guava puree (as shown in Figure 2.3). During the first step of processing, which involves cutting and crushing, 12% of the guava fruits in the form of refiner, comprising guava seeds and coarse pulp, are disposed of. Next, siever (8%) which contains a mixture of pulpy bits and fruit peels are formed through the sieving process. Decanter (5%), with a slight pinkish colour (containing lycopene), are formed as another by-product in the final stage (decanting step) of puree processing line, wherein the guava stone cells are found (Kong and Ismail, 2011). The guava stone cells (scleroids) are removed from the flesh so as to get rid of its gritty texture in the guava end products (Hui, 2006).

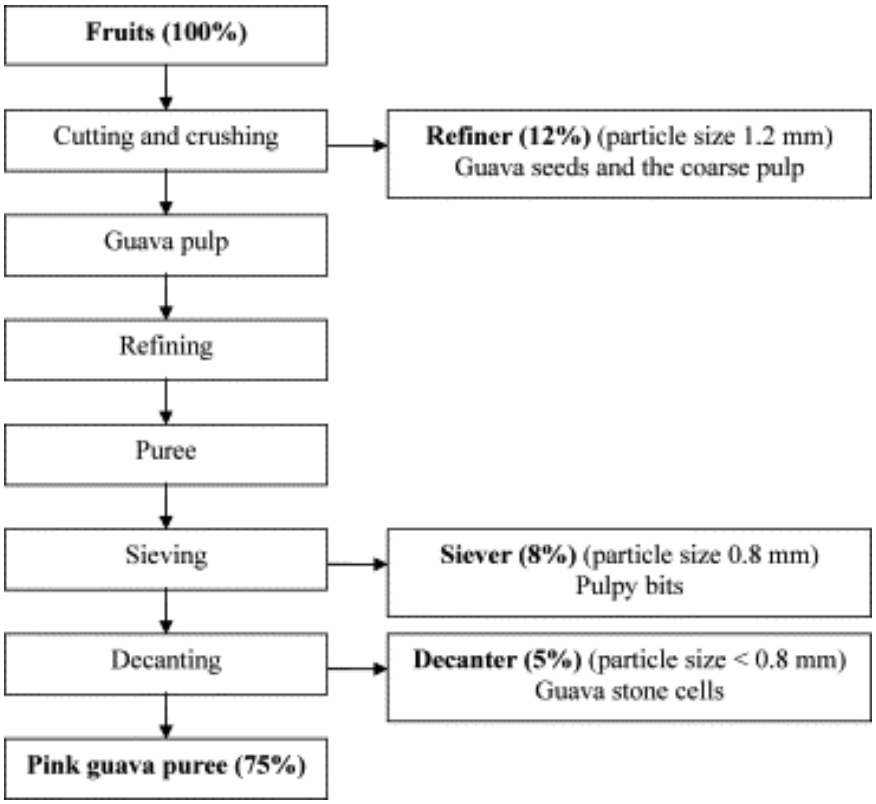


Figure 2.3: Pink guava puree processing (Kong and Ismail, 2011)

2.2 Nutritional Values of Pink Guava

Pink guava is known as "super fruit" due to its great health-promoting properties. It serves as the source of vitamins (vitamin C), minerals (potassium, calcium, phosphorus and iron), antioxidants, dietary fibers (pectin), lycopene and other bioactive compounds (Hui, 2006; Rudrappa, n.d.). Since the guava fruit is highly nutritious, the guava wastes may also contain rich amount of phytochemicals and fibers, therefore possess potential health-promoting qualities as well.

2.2.1 Nutrient Contents of Guava Fruits

In the study done by Thaipong et al. (2006), guava fruit was found to contain high amount of vitamin C, ranging from 174.2 to 396.7 mg/100 g fresh fruit in the pink pulp varieties. Beside exhibiting excellent radical scavenging activity and reducing power, guava powder also contains high dietary fiber content (5.4 g/100 g of fruit) (Rudrappa, n.d.) as well as phenolics content (44.04 mg GAE/g) (Verma et al., 2013). Phenolic compounds that are present in high amounts include myricetin, apigenin (Miean and Mohamed, 2011), ellagic acid and anthocyanins (Misra and Seshadri, 1968). According to Musa, Abdullah and Subramaniam (2015), kaempferol was found to be the predominant flavonoid in the pink guava. In addition, this highly nutritious fruit possesses some carotenoids such as β-carotene, γ-carotene, phytofluene, lycopene, β-cryptoxanthin, rubixanthin, lutein, cryptoflavin and neochrome (Mercadante, Steck and Pfander, 1999). These compounds are well-known antioxidants and thus are essential for maintaining optimal health upon consumption by the host (Rudrappa, n.d.).

2.2.2 Nutrient Contents of Guava Wastes

Based on the study carried out by Maniyan, John and Mathew (2015), guava peels showed the highest carbohydrate content (75 mg/ml) as compared to pomegranate, apple, banana and grapes. Besides, it also possesses considerable amount of protein (0.19 mg/ml) and reducing activity (0.47%) as well as low amount of antinutritional factors such as phytic acid and tannin. In addition, guava peel extract possesses high phenolic content (7.21 g GAE/100 g crude extract) (Marina and Noriham, 2014) and reducing power. Next, the guava

seeds are rich in vitamin C, carotenoids and insoluble dietary fiber, according to the study done by Uchôa-thomaz et al. (2014). On the other hand, pink guava contains high amount of dietary fiber (5.4 g/100 g fruit) in the flesh, as reported by Siddiq (2012). Also, Kong and Ismail (2011) suggested that the by-products of pink guava puree industry are rich in lycopene and antioxidant compounds. Hence, guava by-products serve as potential sources for nutrient enrichment.

2.3 Health-Promoting Factors

In the presence of bioactive phytochemicals (Figure 2.4) such as phenolics and carotenoids, health-promoting effects can be achieved through lowering down the risk of chronic diseases (Liu, 2004). Generally, fruits and vegetables possess rich amount of vitamins (such as vitamin C) as well as dietary phenolics (such as flavonoids) which involve in the reduction of cancer, diabetes and cardiac diseases development. On the other hand, orange-red and yellow fruits and vegetables serve as a good source for carotenoids such as β-carotene and lycopene. Briefly, all these bioactive components possess antioxidant potentials, due to their abilities in scavenging free radicals for cancer prevention (Liu, 2013). Next, adequate intake of dietary fibers aids in the prevention of diabetes, hypertension, colon cancer and other gastrointestinal disorders (Anderson et al., 2009), thus promoting and enhancing the health status of the consumers.

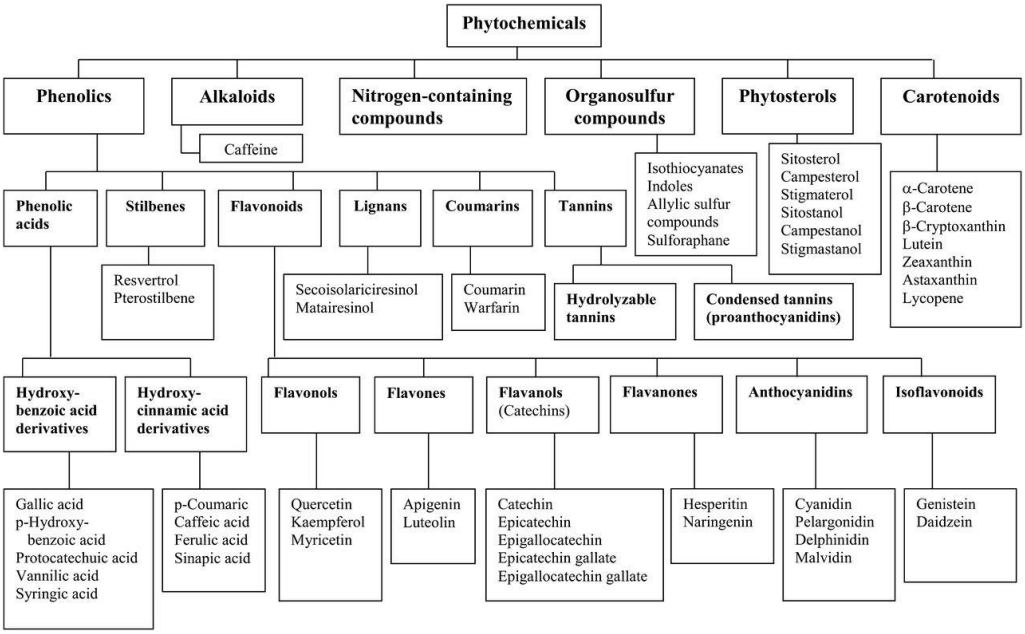


Figure 2.4: Classification of dietary phytochemicals (Liu, 2013)

2.4 Antioxidant Potentials

Basically, antioxidants are microconstituents which involve in the scavenging of free radicals as well as inhibition of lipid oxidation via prohibiting the oxidizing chain reactions (Irshad et al., 2012). According to Halliwell (1996), ascorbic acid and phenolics (hydrophilic antioxidants) as well as carotenoids (lipophilic antioxidants) are the major groups for defensive mechanism in body against cellular damage induced by reactive oxygen species (Harold et al., 2007). Antioxidants work by neutralizing the free radicals interactively and synergistically (Jayanthi and Lalitha, 2011), thus are crucial for optimal health condition. The harmful effects resulted by the usage of various synthetic antioxidants (butylated hydroxytoluene and butylated hydroxyanisole) necessitate the applications of natural antioxidants such as flavonoids, vitamin C, vitamin E and carotenoids from plant sources (Gupta and Sharma, 2006).

2.4.1 Antioxidant Properties of Flavonoids

Flavonoids are a group of unique plant secondary metabolites, characterized by the red, blue and purple anthocyanin pigments in plant tissues (Winkel-Shirley, 2001). According to Prior and Cao (2000), many flavonoids have stronger antioxidant capacities than that of vitamins. Basically, flavonoids can prevent damages induced by free radicals through several mechanisms, for examples: direct scavenging of reactive oxygen species (Figure 2.5) (Procházková, Boušová and Wilhelmová, 2011), activation of antioxidant enzymes (Nijveldt, et al., 2001), metal chelation (Ferrali et al., 1997) and so forth.

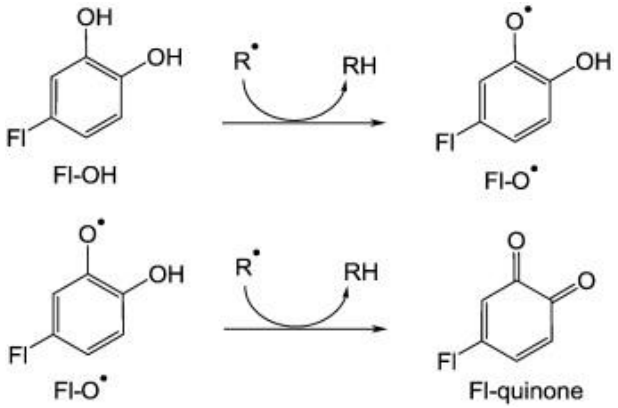


Figure 2.5: Scavenging action of reactive oxygen species (R·) by flavonoid (Procházková, Boušová and Wilhelmová, 2011)

2.4.2 Antioxidant Properties of Vitamins

Vitamins C and E are two crucial vitamins well-known for their antioxidant properties. Vitamin C (ascorbic acid) is a water soluble scavenger of free radicals, which causes direct neutralization on reactive oxygen species like the hydroxyl radicals, superoxide, hydrogen peroxide and also singlet oxygen (Smirnoff, 2000). Briefly, ascorbic acid reacts with various radical species, forming ascorbyl radical (as shown in Figure 2.6) so as to terminate the lipid

peroxidation chain reaction induced specifically by lipid radicals (Nimse and Pal, 2015).

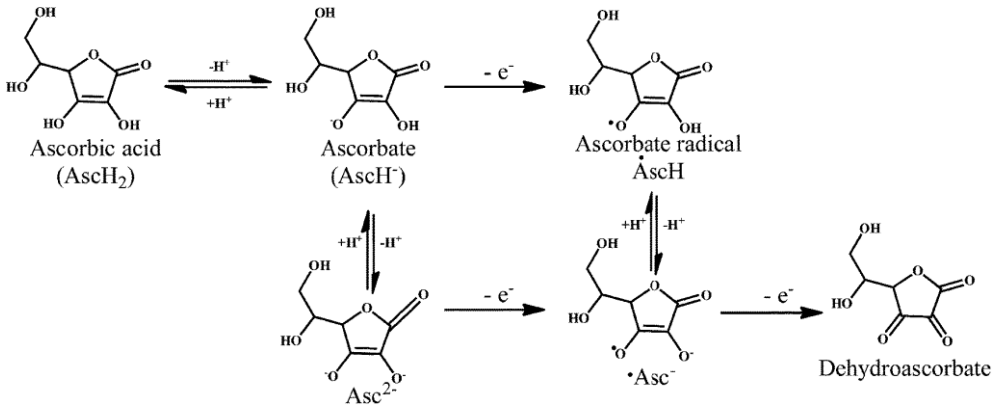


Figure 2.6: Radical scavenging mechanism of ascorbic acid (Nimse and Pal, 2015)

On the other hand, vitamin E (α-tocopherol) is a lipid soluble antioxidant, whereby it scavenges the lipid peroxyl radicals (Figure 2.7) to prevent damage to unsaturated lipids as well as membrane constituents in the body tissues (Christie, 2015). This involves the termination of lipid peroxidation chain reactions by preventing the propagation step of free radicals (Nimse and Pal, 2015).

Scavenging reaction: LOO˙ + α-tocopherol–OH → LOOH + α-tocopherol–O˙

(where LOO˙ represents lipid peroxyl radicals)

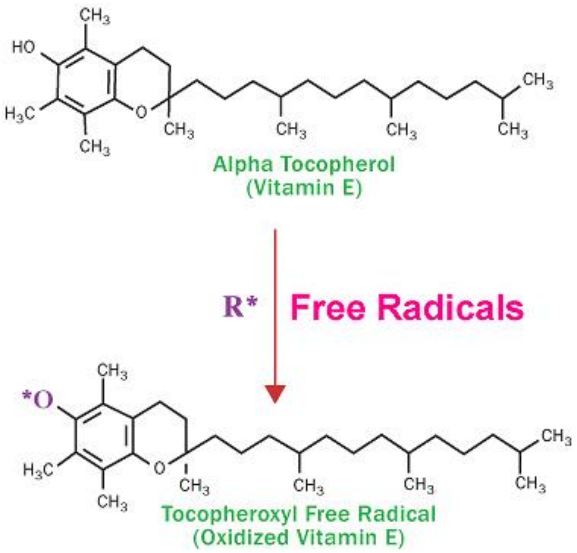


Figure 2.7: Lipid radical scavenging by α-tocopherol (Block, 2010)

2.4.3 Antioxidant Properties of Carotenoids

Carotenoids function as antioxidants for the protection of plants against the photooxidative processes. Most of the time, carotenoids are indulged in the scavenging of singlet molecular oxygen and peroxyl radical, hence providing a strong defence system for human against the damaging effects of these prooxidants (Stahl and Sies, 2003). Three general scavenging reactions by carotenoids include: formation of carotenoyl radical cation or anion through electron transfer between the free radicals and carotenoids, formation of radical adduct and formation of neutral carotenoyl radical through the transfer of hydrogen atom (Fiedor and Burda, 2014).

2.5 Antidiabetic Potentials

2.5.1 Action of α-amylase on Starch Hydrolysis

Starch, which is a complex carbohydrate, is broken down by α-amylase (EC 3.2.1.1) to form maltose, maltotriose and free glucose for the absorption by body (Srichamroen and Chavasit, 2011). Specifically, the α-1,4-glycosidic bonds in starch are hydrolysed by α-amylase, which is a digestive enzyme present abundantly in the salivary, intestinal mucosa and pancreatic secretions. Thus, the action of α-amylase (as shown in Figure 2.8) causes an increase in the glucose bioavailability in the blood (Ashok Kumar et al., 2011).

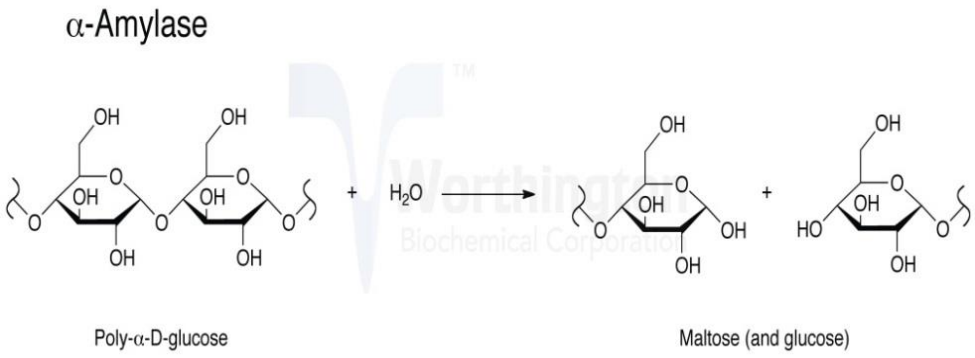


Figure 2.8: Starch hydrolysis by α-amylase (Worthington, n.d.)

Some drugs to treat diabetes mellitus such as acarbose and miglitol possess anti-amylase properties due to their abilities to delay the absorption of dietary starch into the body, through blocking the action of α-amylase in the small intestine (Lefebvre and Scheen, 1994). When the action of α-amylase is inhibited, the starch cannot be digested and absorbed by the body (Sousa and Correia, 2012). Thus, the hypoglycemic agents help in the control of postprandial increase of blood glucose for diabetic patients.

2.5.2 Hypoglycemic Effect of Dietary Fiber

Generally, dietary fiber can be classified into two major categories: soluble dietary fiber and insoluble dietary fiber; each with different physiological roles in body. According to Gohil and Lele (2014), insoluble dietary fiber is responsible for several health promoting properties, including hypoglycemic, hypocholesterolemic as well as anti-constipating effect. On the other hand, the fully hydrated chains of soluble dietary fibers are responsible in the reduction of the rate of carbohydrate digestion and absorption through increasing the viscosity of digestive system in the gastrointestinal tract (Torsdottir et al., 1989). However, the effect of insoluble fibers on GDRI was shown to be greater than soluble fibers (López et al., 1996).

Ou et al. (2001) and Chau, Huang and Lee (2003) revealed that dietary fiber could reduce the postprandial serum glucose levels through several mechanisms. First, through increasing the viscosity of digestive juices in small intestine and thus delaying glucose diffusion; second, through binding with glucose which causes the decreased availability of glucose in small intestine; and lastly, through retarding α-amylase action by capsuling starch and the enzyme respectively. Also, Srichamroen and Chavasit (2011) suggested that the higher the concentration of gum (a unique soluble fiber), the higher the retardation rate on glucose diffusion.

2.6 Antioxidant Assays

2.6.1 DPPH (2,2-Diphenyl-1-picrylhydrazyl) Radical Scavenging Assay

Figure 2.9 illustrates the reaction involves in DPPH assay. The presence of antioxidative compound scavenges the purple-coloured stable free radical (2,2-diphenyl-1-picrylhydrazyl), to form the yellow-coloured diphenylpicryl hydrazine (Ashok Kumar et al., 2011). Thus, the degree of colour fading (absorbance measured at 517 nm) indicates the scavenging power of the antioxidant for its hydrogen-donating ability. The proton radical scavenging reaction is a well-known mechanism for antioxidant activity (Irshad et al., 2012). Decrease in absorbance of the reaction mixture is an indicative of increase scavenging potential of the extract.

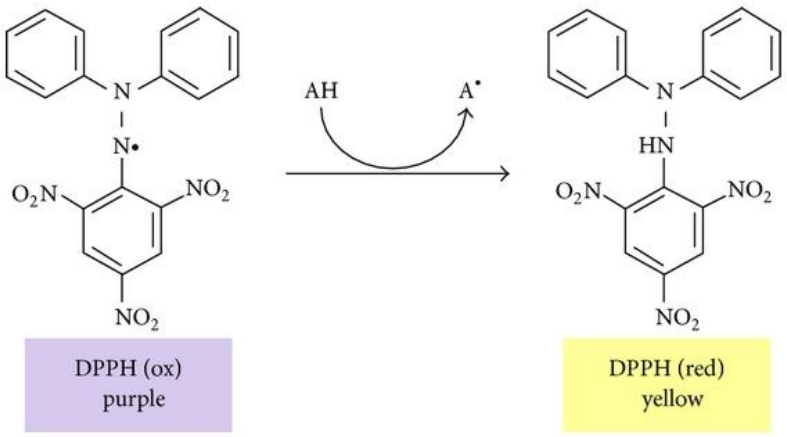


Figure 2.9: DPPH free radical scavenging reaction (Teixeira et al., 2013)

2.6.2 Reducing Power Assay

Potassium ferricyanide (Fe^{3+}) (which is also known as potassium hexacyanoferrate (III)) can be reduced to form potassium ferrocyanide (Fe^{2+}), in the presence of substance with reduction potential. The resulting potassium ferrocyanide will then produce a ferric-ferrous complex after the addition of ferric chloride. The stronger the reducing strength of a substance, the higher the absorbance reading at 700 nm (Jayanthi and Lalitha, 2011).

Reaction:

$$\text{Ferric chloride} + \text{Potassium ferricyanide} \xrightarrow{\text{Antioxidant}} \text{Ferrous chloride} + \text{Potassium ferrocyanide}$$

2.7 α-amylase Inhibitory Assay: Starch-iodine Colour Assay

Screening of α-amylase inhibition activities can be performed in either way: 3,5-dinitrosalicylic acid (DNSA) method and starch-iodine colour assay. The latter was adopted in this study. In the presence of α-amylase inhibitor, the starch (substrate) in the reaction mixture will not be degraded and thus forming a dark-blue complex with iodine reagent. On the other hand, in the absence of α-amylase inhibitor, no colour complex will be formed in the assay mixture since the starch is completely hydrolysed by α-amylase. The colour formed indicates the extent of inhibition by the extract: the highest (dark-blue; presence of starch), medium (brownish; partially degraded starch) and the lowest (yellow; absence of starch). The resulting starch-iodine complex absorbed maximally at wavelength of 620 nm (Sudha et al., 2011). Thus, the absorbance reading is directly proportional to the α-amylase inhibitory activity.

2.8 Glucose Diffusion Assay

2.8.1 Principle of GOPOD Assay Kit

In this study, GOPOD assay kit was used for the quantitation of D-glucose in dialysate. The GOPOD reagent contains high purity of glucose oxidase and peroxidase which can react specifically with D-glucose. This reaction results in colour formation which is stable at room temperature for a minimum of two hours after development (Megazyme, 2015).

The reactions are:

$$\text{D-glucose} + O_2 + H_2O \xrightarrow{\text{glucose oxidase}} \text{D-gluconate} + H_2O_2$$

$$2\ H_2O_2 + \textit{para}\text{-hydroxybenzoic acid} + \text{4-aminoantipyrine} \xrightarrow{\text{peroxidase}} \text{quinoneimine dye} + 4\ H_2O$$

CHAPTER 3

MATERIALS AND METHODS

3.1 Materials

3.1.1 Guava By-products

Pink guava (*Psidium guajava* L.) puree processing by-products namely refiner, siever and decanter were obtained from Sime Darby Beverages Sdn. Bhd. in Sitiawan, Perak, Malaysia. The by-products were collected at different processing points: cutting and crushing (refiner); sieving (siever); and decanting (decanter). They were kept in a frozen form at -20^{o}C prior to pre-analysis treatments.

3.1.2 Chemicals

The sources of all the chemicals used are listed in Table 3.1.

Table 3.1: List of chemicals used for sample preparation and analysis

Chemical	Manufacturer
1% Iodine Solution	Prochem Chemicals
2,2-Diphenyl-1-picrylhydrazyl (DPPH)	Sigma-Aldrich
95% Ethanol	HmbG® Chemicals
Acarbose 50 mg tablets	Glucobay
Alpha-amylase (3,000 Units/ml)	Megazyme
D(+)-glucose anhydrous, extra pure	Scharlau
D-Glucose (GOPOD) Assay Kit	Megazyme

Table 3.1: (continued)

Chemical	Manufacturer
di-Sodium hydrogen phosphate anhydrous	QRëC™
Hydrochloric Acid 37%	Fisher Scientific
Iron (III) Chloride	R&M Chemicals
L(+)-Ascorbic Acid	HmbG® Chemicals
Potassium Hexacyanoferrate (III)	R&M Chemicals
SnakeSkin® Dialysis Tubing (10,000 MWCO)	Thermo Fisher Scientific
Sodium Chloride	J. Kollin Chemicals
Sodium dihydrogen phosphate anhydrous	QRëC™
Starch Soluble	ChemAR-SYSTERM®
Trichloroacetic Acid	R&M Chemicals

3.1.3 Equipments

The equipments used are listed in Table 3.2.

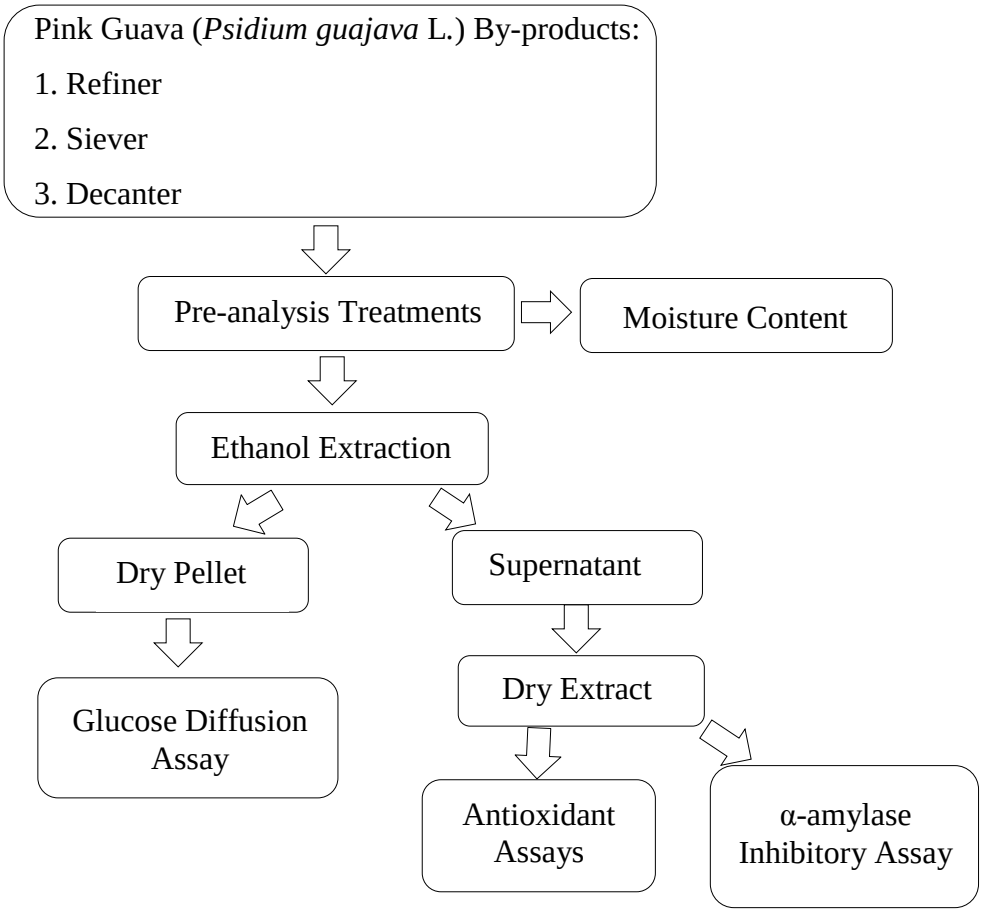
Table 3.2: List of equipments used for sample preparation and analysis

Equipment	Manufacturer
Analytical Balance	Sartorius
Blender	Waring® Commercial
Centrifuge Machine	Eppendorf
Electronic Balance	BEL Engineering
Freeze Dryer	ScanVac
Hot Plate	Stuart
Moisture Analyzer	A&D Instruments

Table 3.2: (continued)

Equipment	Manufacturer
Orbital Shaker	INFORS HT
Oven	Memmert
pH Meter	Mettler Toledo
Rotary Evaporator	BÜCHI
Shaking Water Bath	Memmert
Spectrophotometer	Biochrom
Vibratory Sieve Shaker	Retsch
Vortex Mixer	Scientific Industries
Water Bath	SASTEC™

3.1.4 Methodology



3.2 Pre-analysis Treatments

The samples (refiner, siever and decanter) were freeze-dried for about 48 hours to get rid of excess water. The dried samples were then finely ground to a particle size of less than 1.2 mm before passing through sieve sizes of 1 mm, 500 μm and 250 μm, using a vibratory sieve shaker (Retsch, Germany). In this study, the particle sizes selected for extractions were ranged from 250 μm to 500 μm. The pulverized samples were stored in capped bottles at room temperature prior to further analysis or extractions.

3.3 Determination of Moisture Content

Approximately 2 g of pulverized sample was weighed and spread evenly on a sample pan. The sample pan was then placed in a moisture analyzer (A&D Instruments Ltd, United Kingdom), with a pre-set drying temperature of 105^{o}C. Once the measurement was done, the heater cover was opened and the sample was removed from the moisture analyser by using the pan handle.

3.4 Ethanol Extractions

3.4.1 Simple Maceration

The ethanolic extraction of guava by-product was performed based on the method of Farrant et al. (2003), with some modifications. Approximately 2.5 g of sample was extracted in 100 ml of ethanol (80%, v/v) using two 50 ml centrifuge tubes. The mixture was then heated for 1 hour at 80^{o}C in a shaking water bath (Memmert, Germany). After heating, the mixture was centrifuged for 20 minutes at 7,800 rpm. The supernatant was decanted, whereas the pellet was re-suspended and re-extracted for another time. Again, the supernatant

was decanted, while the pellet was added with 50 ml of ethanol (80%, v/v), before heating (1 hour at 80^{o}C in a shaking water bath) and centrifugation (20 minutes at 7,800 rpm). All the three supernatants collected after centrifugations were combined. This extract was then concentrated through evaporation using a rotary evaporator at 50^{o}C with a water aspirator. The dry extract was then kept in a desiccator. On the other hand, the final pellet obtained was dried at 50^{o}C in an oven overnight and stored at room temperature for further analysis. The yield of the extract and dry pellet were calculated based on the following equation:

Yield (%) = (Weight of extract or pellet / 2.5 g of sample used) × 100%

3.4.2 Soxhlet Extraction

Soxhlet extraction of guava by-product was adapted from Pandey, Pandey and Singh (2014), with some modifications. The Soxhlet apparatus was set up as shown in Figure 3.1. Approximately 2.5 g of sample was weighed and wrapped with a few layers of cheese cloth. It was then placed into the extraction chamber and extracted for 7 hours with 250 ml of ethanol (80%, v/v) through hot continuous percolation method using Soxhlet apparatus so as to eliminate some coloured materials, oligosaccharides and other low molecular weight compounds (Boual et al., 2012). After that, the residue remained in the extraction chamber was allowed to dry out at 50^{o}C in an oven overnight and stored at room temperature for further analysis.

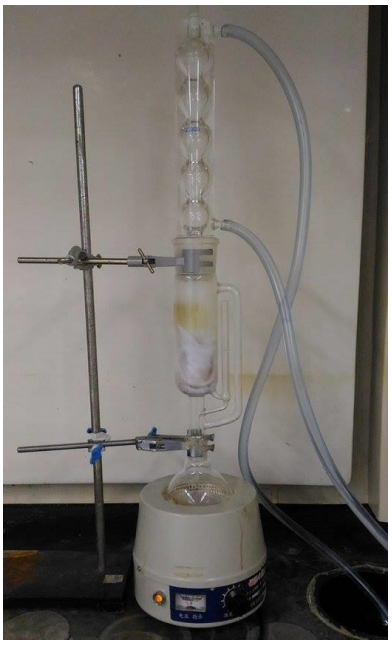


Figure 3.1: Soxhlet apparatus setup

3.5 Antioxidant Assays

3.5.1 DPPH (2,2-Diphenyl-1-picrylhydrazyl) Radical Scavenging Assay

DPPH (2,2-Diphenyl-1-picrylhydrazyl) free radical scavenging potential of the extract was determined according to the method by Siow and Hui (2013), with slight modifications. Various concentrations of the extract were prepared from the stock of 1 mg/ml, through a serial dilutions using ethanol (80%, v/v), as in Table 3.3.

Table 3.3: Serial dilutions for extract in antioxidant assay

Tube	1	2	3	4	5	6
Extract (1 mg/ml) (ml)	0.00	0.20	0.40	0.60	0.80	1.00
80% Ethanol (ml)	1.00	0.80	0.60	0.40	0.20	0.00
Final Concentration (mg/ml)	0.00	0.20	0.40	0.60	0.80	1.00

This was then followed by the addition of 0.15 mM DPPH reagent (2 ml) into separate test tubes containing different sample concentrations as shown in Table 3.3. After that, the solution mixtures were left to stand at room temperature in the dark for 30 minutes, before measuring the absorbance at 517 nm. Ethanol (80%, v/v) was used as the blank while the control (Tube 1) was prepared by replacing extract with ethanol (80%, v/v). Ascorbic acid was employed as a standard in this assay. All determinations were performed in triplicates. The antioxidant activity was determined by the following equation and the EC_{50} was evaluated.

$$\text{DPPH Radical Scavenging Power (\%)} = \frac{\text{Abs of Control} - \text{Abs of Sample}}{\text{Abs of Control}} \times 100\%$$

3.5.2 Reducing Power Assay

The ferric ions reducing capacity of extract was determined using the method of Irshad et al. (2012), with appropriate modifications. The extract (1 ml) (each with different concentration) was prepared as in Table 3.3. Then, 1 ml of 0.2 M sodium phosphate buffer (pH 6.6) and 1 ml of potassium hexacyanoferrate (III) ($K_3Fe(CN)_6$) (1%, w/v) were added to each tube, followed by incubation in a water bath (50^oC , 20 minutes). The reaction was terminated by adding 1 ml of trichloroacetic acid (10%, w/v). After that, 1 ml of the mixture was mixed with distilled water (1 ml) and 200 µl of ferric chloride ($FeCl_3$) solution (0.1%, w/v). The absorbance of the reaction mixture was then measured at 700 nm. A blank was prepared in a similar manner as the content in Tube 1 (Control), but with the replacement of $K_3Fe(CN)_6$ by distilled water. Ascorbic acid at various concentrations was used as standard.

All determinations were performed in triplicates. The reducing power for each extract can be expressed as:

$$\text{Reducing Power (\%)} = \frac{\text{Abs of Sample} - \text{Abs of Control for Sample}}{\text{Abs of Standard} - \text{Abs of Control for Standard}} \times 100\%$$

3.6 α-amylase Inhibitory Assay: Starch-iodine Colour Assay

α-amylase inhibition potential of the extract was determined based on the starch-iodine colour test by Ashok Kumar et al. (2013), with some modifications. A serial dilutions was carried out using 0.02 M sodium phosphate buffer (pH 6.9 with 6 mM sodium chloride), in the preparation of extract with various concentrations from the stock of 1 mg/ml, as shown in Table 3.4.

Table 3.4: Serial dilutions for extract in α-amylase inhibitory assay

Tube	1	2	3	4	5	6
Extract (1 mg/ml) (ml)	0.00	0.10	0.20	0.30	0.40	0.50
Sodium Phosphate Buffer (ml)	0.50	0.40	0.30	0.20	0.10	0.00
Final Concentration (mg/ml)	0.00	0.20	0.40	0.60	0.80	1.00

The extract (500 µl) of each tube was added with 500 µl of α-amylase solution (5 U/ml) dissolved in the same buffer as the extract. The mixture was then incubated at 37^{o}C for 10 minutes. After the addition of 500 µl of starch solution (1%, w/v) (dissolving 0.5 g of soluble starch in 50 ml of distilled water with boiling and stirring for 10 minutes), the reaction mixture was re-incubated

at 37^{o}C for 15 minutes. Then, 20 µl of 1 M HCl was added to terminate the enzymatic reaction in each tube, followed by the addition of 1% iodine reagent (100 µl). The absorbance was then read at 620 nm against buffer for the measurement of starch-iodine complex. Two controls were used in the assay, namely inhibition control (100% inhibition) and enzyme control (0% inhibition). The inhibition control contained 50 µg/ml acarbose whereas the enzyme control was the standard reaction with no inhibitors (Tube 1). All determinations were done in triplicates. Inhibition of α-amylase activity was evaluated as:

$$\text{Inhibition of } \alpha\text{-amylase activity (\%)} = \left(1 - \frac{S-I}{E-I}\right) \times 100\%$$

where:

S = Absorbance of extract

I = Absorbance of inhibition control

E = Absorbance of enzyme control

3.7 Determination of Hypoglycemic Potential

3.7.1 Quantitation of D-glucose

The quantitation of D-glucose was carried out by using the D-Glucose (glucose oxidase/peroxidase; GOPOD) Assay Kit K-GLUC 10/15 (Megazyme International Ireland Ltd.) based on enzymatic-spectrophotometric principles, through reactions of D-glucose with glucose oxidase and peroxidase (Megazyme, 2015).

An aliquot of 0.1 ml of sample solution (containing D-glucose) was added with 3 ml of GOPOD reagent, followed by incubation at 50^{o}C for 20 minutes. The absorbance was then read at 510 nm against the reagent blank (0.1 ml distilled water + 3 ml GOPOD reagent) (Megazyme, 2015).

3.7.2 Standard Curve of D-glucose

Serial dilutions of standard D-glucose solution (from the stock of 1 mg/ml) were performed according to Table 3.5:

Table 3.5: Serial dilutions for D-glucose

Tube	1	2	3	4	5	6
D-glucose (1 mg/ml) (µl)	0	20	40	60	80	100
Distilled Water (µl)	100	80	60	40	20	0
Final Concentration (mg/ml)	0.00	0.20	0.40	0.60	0.80	1.00

This was followed by the addition of 3 ml of GOPOD reagent to each tube. After incubation at 50^{o}C in a water bath for 20 minutes, the absorbance was read at 510 nm against Tube 1 (blank). All determinations were done in duplicates.

3.7.3 Suitability of Samples for Glucose Diffusion Assay

Selections of appropriate sample forms for glucose diffusion assay were carried out by measuring the intrinsic D-glucose content present in dry samples as well as in the dry pellets. The best form of samples should have negligible amount of D-glucose, so as to minimize the interference with the assay conditions which involve the addition of D-glucose to the samples.

Briefly, 400 mg of each sample (listed in Table 3.6) was added with 15 ml of distilled water in a 50 ml conical flask, followed by 1-hour stirring at room temperature on an orbital shaker (INFORS HT, Switzerland). After that, 0.1 ml of the solution was mixed with 3 ml of GOPOD reagent, which was then incubated at 50^{o}C in a water bath for 20 minutes, prior to the absorbance measurement at 510 nm. All determinations were performed in triplicates.

Table 3.6: Various sample forms for D-glucose determination

Tube	Sample Form
1	Refiner (Dry Pellet)
2	Siever (Dry Pellet)
3	Decanter (Dry Pellet)
4	Refiner (Dry Sample)
5	Siever (Dry Sample)
6	Decanter (Dry Sample)
7	Decanter (Soxhlet Residue)

3.7.4 Optimum Conditions for Glucose Diffusion Assay

The conditions for glucose diffusion assay were optimized from the method of Fuentes-Alventosa et al. (2009). An aliquot of 15 ml of glucose solution of various concentrations (as in Table 3.7) was poured into respective small conical flask before subjected to continuous stirring for 1 hour. After that, the solutions were transferred to 13 cm portions of dialysis tubes (10,000 MWCO, Thermo Fisher Scientific Inc., United States). The tubes were then put into separate reservoirs of distilled water and held in a shaking water bath at 37^{o}C for 1 hour. At 20 minutes intervals, 0.1 ml of dialysate was collected from each reservoir and the glucose concentration was determined spectrophotometrically using the Megazyme glucose test kit as described in Section 3.7.1.

Table 3.7: Various conditions for glucose diffusion assay

Condition	Glucose Concentration (mg/ml)	Reservoir Volume (ml)
1	2	200
2	5	200
3	10	200
4	10	400

3.7.5 Glucose Diffusion Assay

The hypoglycemic potential of guava by-product was evaluated in terms of the Glucose Dialysis Retardation Index (GDRI). The glucose diffusion assay was performed as described by Fuentes-Alventosa et al. (2009), with considerable modifications. Approximately 400 mg dry pellet was thoroughly hydrated with 15 ml of glucose solution (10 mg/ml) in a conical flask. After 1 hour of continuous stirring at room temperature, the mixture was transferred to 13 cm portions of dialysis tubes (10,000 MWCO, Thermo Fisher Scientific Inc., United States). Each tube and a control tube (with glucose, but without sample) were then put into separate reservoirs (each contained 200 ml of distilled water) and held in a thermostatic shaking water bath at 37^{o}C for 1.5 hours. At 15 minutes intervals, 0.1 ml of dialysate was collected and the concentration of glucose was measured and determined spectrophotometrically using the Megazyme glucose test kit as described in Section 3.7.1. All determinations were performed in triplicates. GDRI for each sample was calculated using the following equation:

$$\text{GDRI} = 100 - \left(\frac{\text{Total glucose in dialysate,sample}}{\text{Total glucose in dialysate,control}} \times 100\right)$$

3.8 Statistical Analysis

The experimental data were expressed as mean ± standard deviation and statistical analysis was performed through the one-way analysis of variance (ANOVA). The *p* value less than 0.05 ($p < 0.05$) was regarded as a significant difference. The Statistical Analysis System (SAS) programme version 9.3 was employed for the data analysis.

CHAPTER 4

RESULTS

4.1 Moisture Contents of Freeze-Dried Guava By-products

Table 4.1 shows the moisture contents of freeze-dried samples (with particle sizes of 250 μm to 500 μm) prior to extractions and further analysis. Refiner had the highest moisture content (4.81%), followed by siever (3.31%) and decanter (3.17%).

Table 4.1: The moisture content in freeze-dried guava by-products

Sample	Moisture Content (%)
Refiner	4.81
Siever	3.31
Decanter	3.17

4.2 Yield of Extraction

Figure 4.1 depicts the yield of extracts and dry pellets after ethanol extraction using the simple maceration method. Generally, no remarkable differences could be observed among the three samples in comparison of their yield of extracts and dry pellets respectively, at which the differences were within the range of approximately 3.00% (for extracts) and 1.30% (for dry pellets).

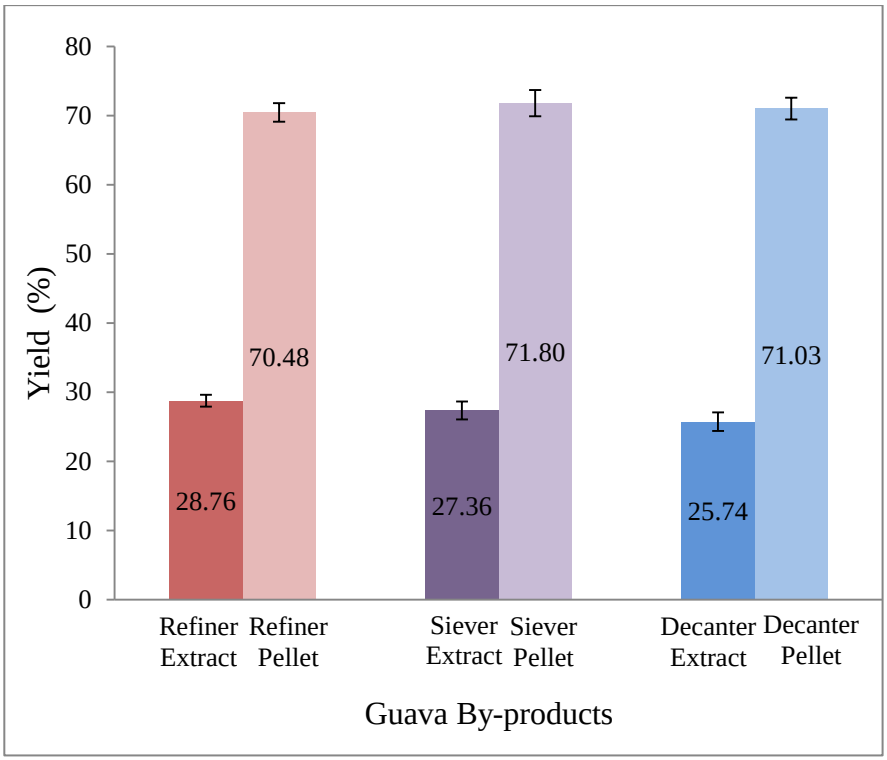


Figure 4.1: The extraction yield in guava by-products. Values represent means ± SD (n=2).

4.3 Antioxidant Properties of Ethanolic Extracts

4.3.1 DPPH (2,2-Diphenyl-1-picrylhydrazyl) Radical Scavenging Activity

Figure 4.2 shows the DPPH free radical scavenging activity for each extract (0.2 mg/ml – 1.0 mg/ml) while Figure 4.3 illustrates the EC_{50} values for the different extracts. The scavenging activities for all the samples were comparable to that of the standard (ascorbic acid) and among the samples, siever showed the highest scavenging power with the lowest EC_{50} value (0.317 mg/ml).

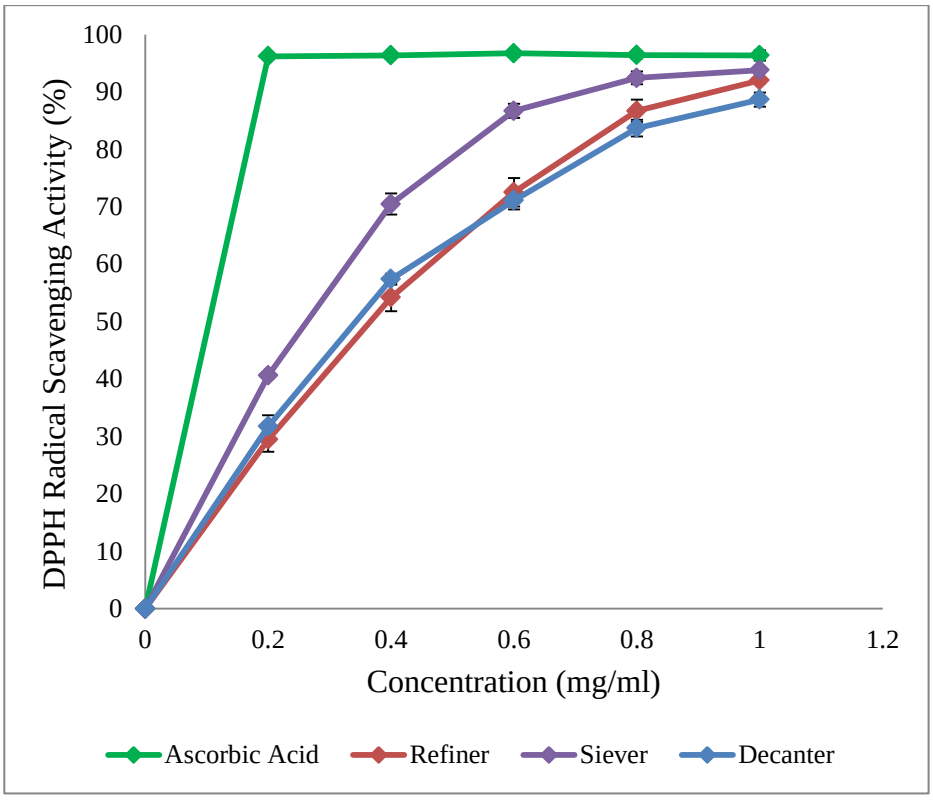


Figure 4.2: DPPH free radical scavenging activity for ethanolic extracts. Values represent means ± SD (n=3).

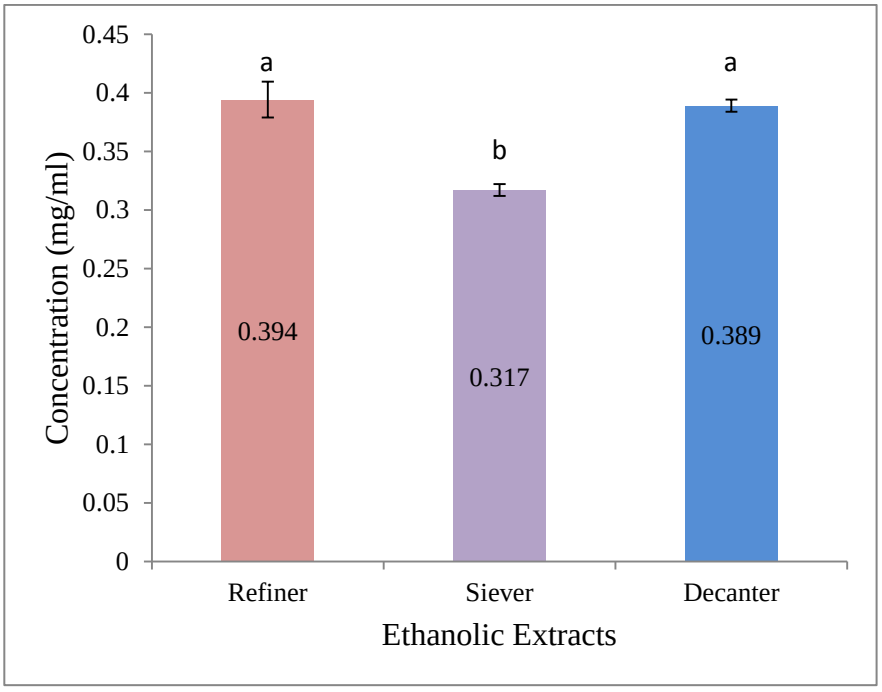


Figure 4.3: EC_{50} values for ethanolic extracts. Values represent means ± SD (n=3). Different letters denote a significant difference ($p < 0.05$).

4.3.2 Reducing Power

Reducing power of the ethanolic extracts is shown in Figure 4.4. The extracts showed a concentration-based reducing activity. The reducing potential of extracts increased in the order: refiner, decanter, siever.

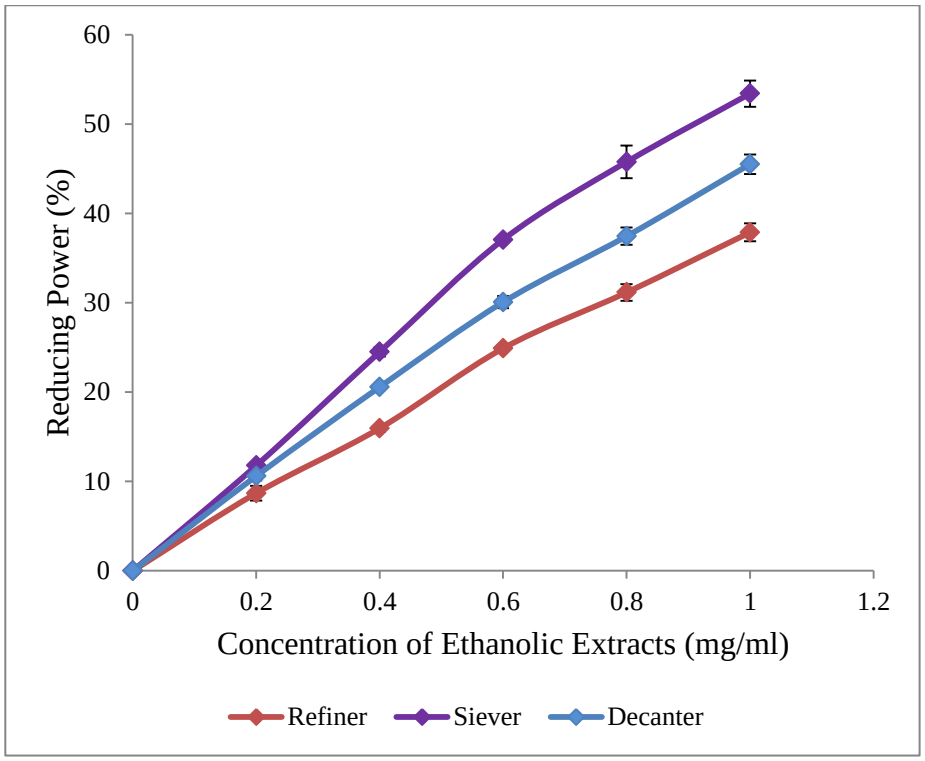


Figure 4.4: Reducing power of ethanolic extracts. Values represent means ± SD (n=3).

4.4 α-amylase Inhibition Properties of Ethanolic Extracts

The α-amylase inhibition potential increased in correspondence to the concentration of ethanolic extract for every sample, as depicted in Figure 4.5. Based on the results, decanter possessed the highest anti-amylase potential compared to siever and refiner.

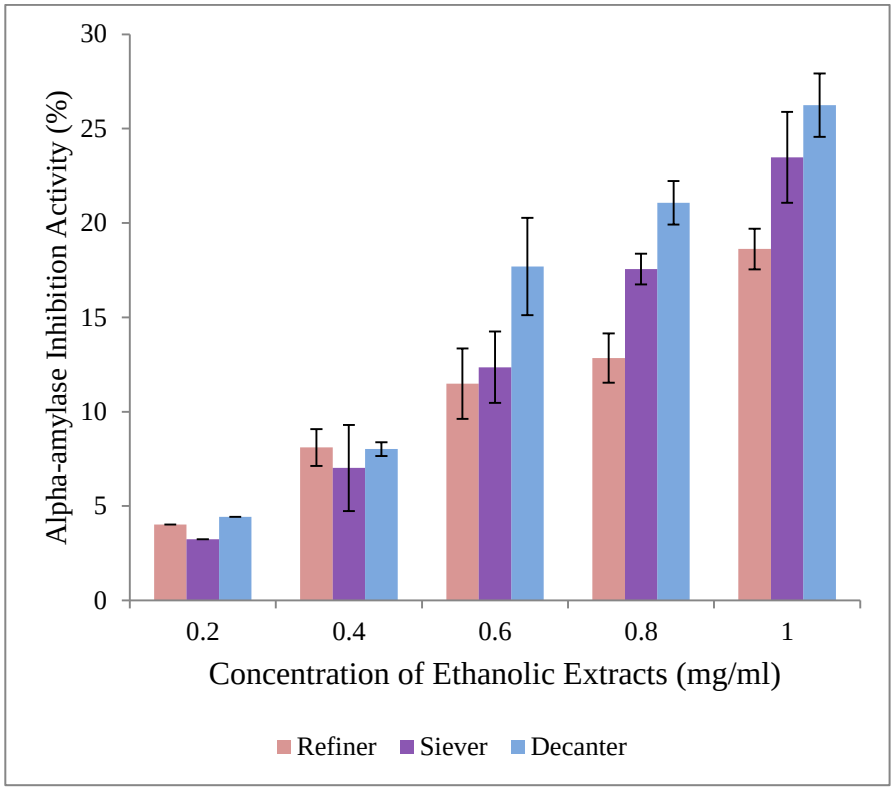


Figure 4.5: α-amylase inhibition activity of ethanolic extracts. Values represent means ± SD (n=3).

4.5 Hypoglycemic Potentials of Dry Pellets

4.5.1 Standard Curve of D-glucose

Figure 4.6 shows the standard curve of D-glucose (0.2 mg/ml – 1.0 mg/ml) employed in the determination of hypoglycemic potential for dry pellets of different guava by-products.

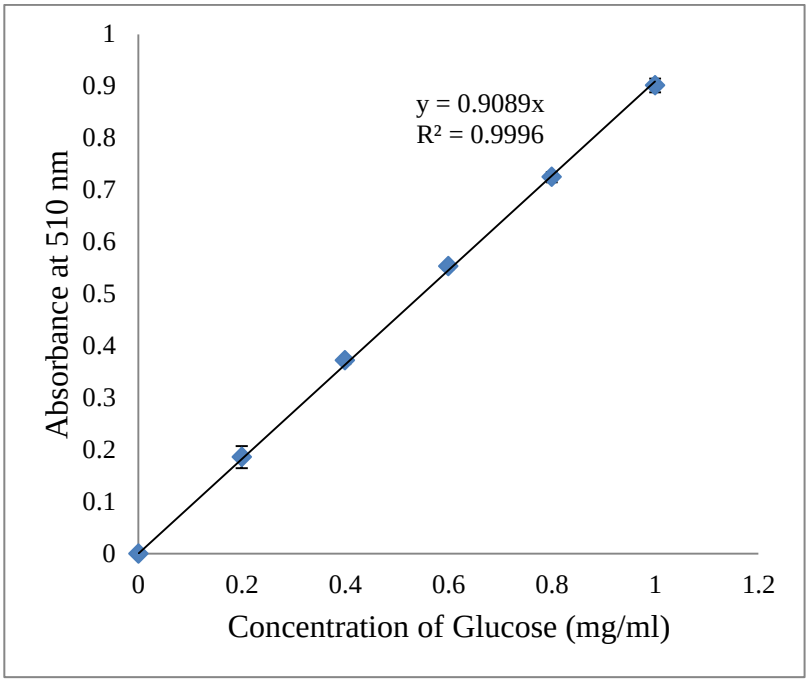


Figure 4.6: Standard curve of D-glucose. Values represent means ± SD (n=2).

4.5.2 Suitability of Samples for Glucose Diffusion Assay

Table 4.2 shows the intrinsic glucose concentrations in various forms for different samples. The results showed that, the intrinsic glucose concentrations in dry pellet form were basically negligible compared to that of direct sample form for all samples. Besides, decanter in dry pellet form had lower intrinsic glucose concentration (approximately 0.009 mg/ml) than in soxhlet residue form (approximately 0.014 mg/ml). Thus, it can be deduced that the dry pellet

served to be the most suitable sample form for the glucose diffusion assay, since it had negligible interference to the assay condition.

Table 4.2: The intrinsic glucose concentration in various sample forms

Sample Form	Glucose Concentration (mg/ml)
Refiner (Dry Pellet)	0.023 ± 0.002
Siever (Dry Pellet)	0.004 ± 0.002
Decanter (Dry Pellet)	0.009 ± 0.002
Refiner (Dry Sample)	1.550 ± 0.045
Siever (Dry Sample)	1.481 ± 0.031
Decanter (Dry Sample)	1.298 ± 0.018
Decanter (Soxhlet Residue)	0.014 ± 0.004

Values represent means ± SD (n=3).

4.5.3 Optimum Conditions for Glucose Diffusion Assay

Table 4.3 depicts the glucose concentration in dialysate over time (20 minutes, 40 minutes and 60 minutes) for different test conditions (prepared as in Table 3.7, Section 3.7.3.2). Based on the results, only condition 3 (glucose concentration: 10 mg/ml; reservoir volume: 200 ml) showed steady increment in glucose concentrations in dialysate from 20 minutes to 60 minutes. So, this serves to be the most optimal condition for glucose diffusion assay.

Table 4.3: Glucose concentration in dialysate over time for different test conditions

Condition	Glucose Concentration in Dialysate (mg/ml)		
	20 min	40 min	60 min
1	0.047	0.085	0.089
2	0.101	0.179	0.191
3	0.261	0.345	0.448
4	0.108	0.327	0.277

4.5.4 Glucose Diffusion Assay

Figure 4.7 illustrates the glucose concentrations of dialysate at different time interval (from 15 minutes to 90 minutes) for samples (in dry pellet form) and glucose control, which elevated over time. Generally, glucose diffusion was the lowest in decanter, as compared to refiner and siever. According to Table 4.4, decanter showed the highest Glucose Dialysis Retardation Index (GDRI) throughout the assay duration, whereby the highest GDRI value was 10.79%, at the first 15 minutes.

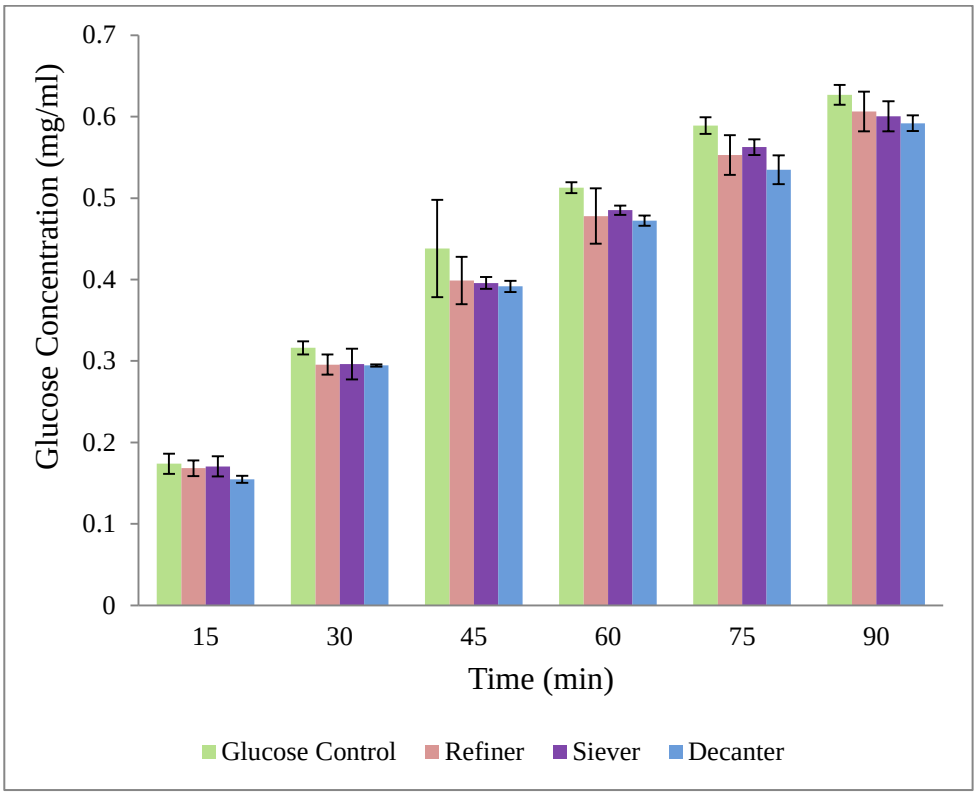


Figure 4.7: Glucose concentration of dialysate at different time interval. Values represent means ± SD (n=3).

Table 4.4: Glucose concentration of dialysate at different time interval

Time (min)	Glucose Concentration In Dialysate (mg/ml)			
	Glucose Control	Refiner	Siever	Decanter
15	0.174 ± 0.012 (0.00)	0.168 ± 0.010 (3.10)	0.171 ± 0.012 (1.90)	0.155 ± 0.004 (10.79)
30	0.316 ± 0.008 (0.00)	0.296 ± 0.012 (6.51)	0.296 ± 0.019 (6.30)	0.294 ± 0.001 (6.80)
45	0.438 ± 0.060 (0.00)	0.399 ± 0.029 (8.51)	0.396 ± 0.007 (8.80)	0.392 ± 0.007 (9.50)
60	0.513 ± 0.007 (0.00)	0.478 ± 0.034 (6.75)	0.485 ± 0.006 (5.35)	0.472 ± 0.006 (7.87)
75	0.589 ± 0.010 (0.00)	0.553 ± 0.024 (6.17)	0.563 ± 0.010 (4.48)	0.535 ± 0.018 (9.20)
90	0.627 ± 0.012 (0.00)	0.606 ± 0.024 (3.28)	0.600 ± 0.019 (4.22)	0.592 ± 0.010 (5.55)

Values represent means ± SD (n=3).

Data in parentheses represent the glucose dialysis retardation indexes of various samples.

CHAPTER 5

DISCUSSION

5.1 Moisture Contents of Freeze-Dried Guava By-products

In order to achieve higher accuracy for sample analysis, moisture content of sample has to be taken into account during the calculation of dry weight at the beginning of the assays. Based on the results (Table 4.1), the moisture content of freeze-dried guava by-products was in the order of: refiner (4.81%) > siever (3.31%) > decanter (3.17%). Surface area plays an important role in affecting the absorption of water molecules, as mentioned by Bridgeman et al. (2007). Basically, sample with larger particle size possesses smaller surface area, and thus the moisture content is lower. In this study, although the range of particle size selected was the same (250 μm to 500 μm) for all samples, the size distribution might not be uniform among the samples, thus resulting in slight deviation for their moisture contents.

According to Delgado and Barbosa de Lima (2014), the drying process causes the sample to shrink and change in size, due to the loss of water. The factors causing variations among samples in terms of their moisture content include different drying rate, density, size and shape, as well as physical properties such as water holding capacity. However, more replicates should be done in order to further confirm the effect of pre-analysis treatments (freeze-drying, grinding and sieving) on samples. Also, in this study, since the moisture contents of all samples were lower than 10%, they are suitable for long term storage at room temperature. This is supported by Procházková and

Bezděčková (2008), whereby the beechnuts dried to a moisture content of 8 to 9% can be stored for three to four years without affecting their germination.

5.2 Ethanol Extraction of Guava By-products

For the respective yield of ethanolic extract and dry pellet (Figure 4.1), no observable difference was found among different samples. Thus, the yield of extraction was irrespective to the moisture content as well as particle size. This is in agreement with Naidu et al. (2007), whereby no effect of particle size of ground corn on the yield of thin stillage soluble solids could be observed. Based on the results, the yield of ethanolic extract and dry pellet for all the samples were in the range of 25% - 29% and 70% - 72% respectively.

In this study, 80% (v/v) ethanol was employed in the extraction of bioactive compounds for the determination of their antioxidant as well as anti-amylase activities. According to Anwar and Przybylski (2012), high amount of flavonoids was extracted out using 80% ethanol. Also, in their study, extraction using 80% ethanol gave rise to better antioxidant capacity for the flaxseed sample, as compared to extraction using 80% methanol, 100% methanol and 100% ethanol. Hence, 80% ethanol is the most effective solvent which can be used to recover the antioxidant components from the samples.

Next, for the determination of hypoglycemic potential of guava by-products, dry pellet from ethanol extraction was used instead of the ethanolic extract. According to Farrant et al. (2003), the simple maceration method (as described in Section 3.4.1) was employed for the extraction of soluble carbohydrates. In

other words, the leftovers from the ethanol extraction (dry pellet) will be mainly comprised of insoluble carbohydrates including the insoluble fiber. Hence, dry pellet was used so as to examine the effect of insoluble dietary fiber on the hypoglycemic potential of guava by-products. Next, based on Table 4.2, the intrinsic concentration of glucose (soluble carbohydrate) for decanter was found to be higher in soxhlet residue form (0.014 mg/ml) than in dry pellet form (0.009 mg/ml). Thus, simple maceration method was proven to be more effective compared to the soxhlet extraction method in the ethanol extraction.

5.3 Health-Promoting Potentials of Guava By-products

5.3.1 Antioxidant Properties

The expression of EC_{50} can be defined as the effective concentration of sample extract required to achieve 50% antioxidant effect. The lower the EC_{50} value, the better the antioxidant potential of sample extract. In DPPH assay, the EC_{50} for siever (0.317 mg/ml) was significantly ($p < 0.05$) lower than decanter (0.389 mg/ml) and refiner (0.394 mg/ml) while there was no significant difference in terms of EC_{50} exhibited by decanter and refiner (Figure 4.3).

Generally, DPPH scavenging power increased with the concentration of the sample extract (as shown in Figure 4.2). In the case of ascorbic acid (as standard), maximum scavenging effect was achieved at low concentration (96.19% at 0.2 mg/ml), due to its high antioxidant power. All samples showed remarkable DPPH scavenging activity at 1.0 mg/ml (88.67% to 93.80%), probably due to the contributions of their rich phenolics contents (Irshad et al., 2012). Thus, all the guava by-products possess great health-promoting

potential in terms of their antioxidant properties, which are especially crucial in the cancer prevention. Based on the results, it can be deduced that siever has the strongest antioxidant power among the samples, due to the lowest EC_{50} value.

In reducing power assay, due to the reduction of Fe^{3+} into Fe^{2+}, the colour of reaction mixture changes from yellow to green or blue-green, depending on the reducing capability of the extract (Ferreira et al., 2007). Based on the results (Figure 4.4), reducing power of ethanolic extracts decreased in the order: siever (53.40%), decanter (45.50%), refiner (37.87%), at the concentration of 1.0 mg/ml respectively. These findings are comparable to the study done by Marina and Noriham (2014) on the guava peel extract. According to the authors, high phenolic content was found in guava peel and this may contributes to the plant protection against UV radiation.

According to Shimada et al. (1992), the reducing potential of a compound is highly associated with its hydrogen-donating ability. This was further supported by Amarowicz et al. (2004), whereby a direct correlation was observed among antioxidant activities and reducing power of plant extracts. Hence, this could explain the consistency of data on the DPPH scavenging assay and reducing power assay, whereby siever showed the highest activity in both assay. The differences in antioxidative potential among the extracts might be influenced by several reasons, such as structure and antioxidative mechanisms of different phenolic compounds as well as the presence of synergistic effects of different components in the extracts (Abdul Hamid et al.,

2002). Furthermore, Torres de Pinedo, Penalver and Morales (2007) stated that the antioxidant strength is enhanced in the presence of primary alcohol and also increase in number of phenolic hydroxyl groups.

Also, the results are in consistent with the findings from Purdue University (n.d.), whereby the ascorbic acid of guava was found to be mainly distributed in the peel (siever), followed by flesh (decanter) and trace amount in the central pulp (refiner) of the fruit. Besides, Yahia (2011) also stated that higher amount of ascorbic acid can be found in the guava peel compared to the flesh. However, the results are slightly different from the study by Kong and Ismail (2011), whereby the decanter were reported to exhibit the highest lycopene content and lipophilic antioxidant capacity, followed by siever and refiner. This indicates that the antioxidant activity of guava waste is not solely contributed by lycopene. Instead, the presences of other phenolic compounds have to be taken into account for the study on their antioxidant potential.

5.3.2 α-amylase Inhibition Activities

Gohil and Lele (2014) stated that the action of α-amylase is affected by the presence of fibers. In other words, the increase in anti-amylase activity by fibers will result in the reduction of glucose formation through starch hydrolysis. Also, the phenolics present in the fruit parts may contain some phytochemical constituents which contribute to their hypoglycemic activity (Oboh et al., 2014). McCue and Shetty (2004) stated that phenolic compounds have the potential to inhibit amylase action and thus contribute to the control of Type 2 diabetes mellitus. Comparatively, according to Figure 4.5, decanter

showed a stronger inhibitory effect on α-amylase than siever and refiner. Further study needs to be done in order to correlate the composition of fiber and phenolic compounds in different guava by-products to their anti-amylase activities.

In general, the α-amylase inhibition potentials of the extracts (18.62% to 26.25%, at 1.0 mg/ml) were comparable to that of *Nisamalaki churna* (antidiabetic formulation in India), which showed about 45% inhibition at 50 μg/ml, as reported by Ashok Kumar et al., 2013. Thus, the guava by-products serve as the potential sources for antidiabetic function. This is in accordance to the findings by Krentz and Bailey (2005), whereby the mild inhibition of pancreatic α-amylase can be one of the effective management for Type 2 diabetes mellitus. Based on the study of Afrisham et al. (2015), plants phenolic content possesses therapeutic potential for the treatment of postprandial hyperglycemia due to their inhibitory effect on α-amylase enzyme.

However, the detailed mechanisms of hypoglycemic activity by guava by-products are yet to be determined. According to Algariri et al. (2013) and Malviya, Jain and Malviya (2010), several mechanisms involved in the functional restoration of pancreatic tissues: through increasing insulin output, prohibiting the intestinal absorption of glucose or facilitating metabolites which are involved in the insulin-dependent reactions. The variations in inhibitory strength exhibited by different samples could be attributed to several reasons, such as compositional difference of insoluble fiber, the presence of fiber inhibitors, fiber capsulation of starch and α-amylase as well as direct

adsorption of α-amylase on fiber, which then lowering the enzyme accessibility to starch (Bisoi et al., 2012; Chau, Chen and Lee, 2004). Also, the anti-amylase potential relies on the concentration, the number and position of the hydroxyl groups of the phenolic compounds (Rohn, Rawel and Kroll, 2002).

5.3.3 Hypoglycemic Potentials: Glucose Diffusion

In this study, effect of guava by-products on glucose diffusion was investigated in comparison with that of control from 15 minutes to 90 minutes, before the system attained equilibrium. The dialysis assay simulates the events happening along the gastrointestinal tract, specifically the jejunum (Bisoi et al., 2012; Daou and Zhang, 2012). In Figure 4.7, it can be clearly observed that all the samples showed elevated glucose concentration in dialysate from 0.155 – 0.171 mg/ml (at 15 minutes) to 0.592 – 0.606 mg/ml (at 90 minutes), but with lower amount compared to control at each time interval. This indicates a positive result, whereby the samples are capable to retard movement of glucose into external solution across the dialysis tubes (Mary and Gayathri, 2015).

Glucose diffusion was the lowest in decanter compared to control and other samples at corresponding time intervals. According to Chau, Chen and Lin (2004), the delay of glucose diffusion across the membrane could be due to the glucose adsorption abilities and complex meshwork of fibers present in the samples, which bind to the glucose and thus reduce its availability for diffusion. In addition, Ahmed, Sairam and Urooj (2011) further correlated the glucose diffusion to α-amylase inhibition, by suggesting that the inhibitors of α-amylase could have potential hypoglycemic effect through prolonging the

glucose release from starch. Thus, the consistency of findings for the anti-amylase assay and glucose diffusion assay could be explained, since decanter showed the highest activity in both assays.

5.3.4 Glucose Dialysis Retardation Index (GDRI)

Glucose Dialysis Retardation Index (GDRI) can be defined as the *in vitro* index which is employed in evaluating the effect of fiber on the delay in absorption of glucose in the gastrointestinal tract (López et al., 1996). Based on the results as shown in Table 4.4, decanter had the highest GDRI value at every time interval among the samples, with the maximum value of 10.79% (at 15 minutes). While the maximum GDRI value occurred at 45 minutes for both refiner and siever, which was 8.51% and 8.80% respectively. After 45 minutes, both refiner and siever showed considerable decrease in GDRI values. This phenomenon could be explained by Gupta and Premavalli (2011), whereby complete inhibitions and saturation had been achieved by samples and so further glucose retention did not happen, as the duration increased.

Based on the study regarding the effect of crystalline cellulose on plasma glucose concentration in rats done by Takahashi et al. (2005), the ingestion of cellulose may delay glucose diffusion and retard glucose absorption in the lumen, through inducing the increase in digesta viscosity. Also, cellulose could control the postprandial plasma glucose concentration and thus reducing the risk of getting diarrhoea associated with enteral nutrition. When comparing the GDRI values of various guava by-products to the findings from Gohil and Lele (2014), decanter (7.87%) and refiner (6.75%) showed higher glucose

retardation potential than cellulose (5.81%), at 60 minutes interval. On the other hand, siever (5.35%) showed a slightly lower GDRI value than cellulose at that time. Thus, the insoluble fiber from decanter and refiner serve to possess higher hypoglycemic potential than that of siever and cellulose.

According to Guillon et al. (1998), physicochemical properties of fiber might be influenced by porosity, available surface as well as the regiochemistry of the surface layer. Also, decanter fiber particles might be smaller than refiner and siever, and thus the resulting larger surface area facilitate glucose entrapment within the fiber meshwork. This causes slower diffusion rate, thus higher GDRI value (Peerajit, Chiewchan and Devahastin, 2012). Furthermore, the variations among samples in terms of their GDRI values could be attributed to their differences in fibers viscosity (Adiotomre et al., 1990). In this case, decanter might have higher viscosity of fibers which contributed to the delay in glucose diffusion across dialysis tubes. Therefore decanter probably could aid in the control of postprandial blood glucose level due to its fiber enrichment and hypoglycemic properties.

5.4 Future Study

This study can be further extended to:

1. Quantification of ascorbic acids and phenolics compounds in the guava by-products using reversed-phase HPLC.

2. Compositional study on the types of fiber in guava by-products.

3. Study on the mode of α-amylase inhibition by guava by-products.

4. Comparative study using different solvent systems for the extraction of bioactive compounds in guava by-products.

CHAPTER 6

CONCLUSION

From the results, it can be concluded that the peel (siever), flesh (decanter) and seed (refiner) of pink guava have crucial health-related functional properties. Their antioxidant potentials were evaluated in terms of DPPH free radical scavenging assay as well as reducing power assay. Both of these assays showed consistent results whereby siever demonstrated the highest antioxidant activity, followed by decanter and refiner. In DPPH assay, the EC_{50} for siever (0.317 mg/ml) was significantly ($p < 0.05$) lower than decanter (0.389 mg/ml) and refiner (0.394 mg/ml), indicating that lesser amount of siever is needed to achieve 50% antioxidant effect, and hence siever could serve as the natural antioxidant source, to lower down the risk of getting cancer, atherosclerosis, diabetes and so on. The ascorbic acid and phenolic contents were believed to be the major components causing the variation of antioxidant strength among different samples.

Also, the results revealed that the pink guava by-products (especially decanter, with GDRI of 10.79% at 15 minutes) could effectively hinder the α-amylase activity and retard glucose diffusion. The presence of dietary fiber could contribute to the anti-hyperglycemic effect. Thus, decanter can be included in the diet for its promising hypoglycemic potential. This aids in the prevention of chronic diseases such as Type 2 diabetes mellitus, cardiovascular diseases, hypertension and other gastrointestinal diseases. However, the findings should be further confirmed by *in vivo* animal studies, in order to establish the

possibility of the health-promoting applications of the guava by-products for their incorporation into dietary and pharmaceutical products. Also, further structural elucidation and functional group characterization should be done for the identification of the bioactive compounds present in the guava by-products.

REFERENCES

Abdul Hamid, A., Shah, Z., Muse, R. and Mohamed, S., 2002. Characterization of antioxidative activities of various extracts of *Centella asiatica* (L) Urban. *Food Chemistry*, 77(4), pp. 465-469.

Adiotomre, J., Eastwood, M., Edwards, C. A. and Brydon, W., 1990. Dietary fiber: *in vitro* methods that anticipate nutrition and metabolic activity in humans. *American Journal of Clinical Nutrition*, 52(1), pp. 128-134.

Afrisham, R., Aberomand, M., Ghaffari, M. A., Siahpoosh, A. and Jamalan, M., 2015. Inhibitory effect of *Heracleum persicum* and *Ziziphus jujube* on activity of alpha-amylase. *Journal of Botany*, 2015, pp. 1-8.

Ahmed, F., Sairam, S. and Urooj, A., 2011. *In vitro* hypoglycemic effects of selected dietary fiber sources. *Journal of Food Science and Technology*, 48(3), pp. 285-289.

Algariri, K., Meng, K. Y., Atangwho, I. J., Asmawi, M. Z., Sadikun, A., Murugaiyah, V. and Ismail, N., 2013. Hypoglycemic and anti-hyperglycemic study of *Gynura procumbens* leaf extracts. *Asian Pacific Journal of Tropical Biomedicine*, 3(5), pp. 358-366.

Anderson, J. W., Baird, P., Davis, R. H., Ferreri, S., Knudtson, M., Koraym, A., Waters, V. and Williams, C. L., 2009. Health benefits of dietary fiber. *Nutrition Reviews*, 67(4), pp. 188-205.

Anwar, F. and Przybylski, R., 2012. Effect of solvents extraction on total phenolics and antioxidant activity of extracts from flaxseed (*Linum usitatissimum* L.). *Acta Scientiarum Polonorum*, 11(3), pp. 293-301.

Ashok Kumar, B. S., Khan, S., Saran, G. S., Nandeesh, R. and Manjunath, N. K., 2013. *In vitro* antidiabetic activity of *Nisamalaki churna*. *Sains Malaysiana*, 42(5), pp. 625-628.

Ashok Kumar, B. S., Lakshman, K., Jayaveera, K. N., Sheshadri Shekar, D., Narayan Swamy, V. B., Khan, S. and Velumurga, C., 2011. *In vitro* α-amylase inhibition and antioxidant activities of methanolic extract of *Amaranthus caudatus* L*inn*. *Omam Medical Journal*, 26(3), pp. 166-170.

Bartelings, H. and Sterner, T., 1999. Household waste management in a swedish municipality: determinants of waste disposal, recycling and composting. *Environmental and Resource Economics*, 13(4), pp. 473-491.

Bisoi, P. C., Sahoo, G., Mishra, S. K., Das, C. and Das, K. L., 2012. Hypoglycemic effects of insoluble fiber rich fraction of different cereals and millets. *Journal of Food Processing and Technology*, 3(7), pp. 1-11.

Block, W., 2010. *Break the bonds of dementia*. [online] Available at: <http://www.life-enhancement.com/magazine/article/2274-break-the-bonds-of-dementia> [Accessed 16 August 2016].

Boual, Z., Kemassi, A., Ould El Hadj Khelil, A., Michaud, P. and DidiOuld El Hadj, M., 2012. Partial characterization and hydrolysis procedure of water soluble polysaccharides extracted from onesaharian medicinal plant: *Malvaaegyptiaca L.*. *International Journal of Bioscience, Biochemistry and Bioinformatics*, 2(2), pp. 100-103.

Bridgeman, T. G., Darvell, L. I., Jones, J. M., Williams, P. T., Fahmi, R., Bridgwater, A. V., Barraclough, T., Shield, I., Yates, N., Thain, S. C. and Donnison, I. S., 2007. Influence of particle size on the analytical and chemical properties of two energy crops. *Fuel*, 86(1), pp. 60-72.

Chau, C., Chen, C. and Lin, C., 2004. Insoluble fiber-rich fractions derived from *Averrhoa carambola*: hypoglycemic effects determined by *in vitro* methods. *LWT-Food Science and Technology*, 37, pp. 331-335.

Chau, C. F., Huang, Y. L. and Lee, M. H., 2003. *In vitro* hypoglycemic effects of different insoluble fiber-rich fractions prepared from the peel of *Citrus sinensis* L. cv. Liucheng. *Journal of Agricultural and Food Chemistry*, 51(22), pp. 6623-6626.

Choudhary, S., Sharma, M., Tripathi, J. and Mishra, P., 2013. Antihyperglycemic activity of *Vernonia cinera* L. on alloxan-induced diabetic mice. *International Journal of Advanced Research*, 1(2), pp. 35-42.

Christie, W. W., 2015. *Isoprenoids: 1. tocopherols and tocotrienols*. [online] Available at: <http://www.lipidhome.co.uk/lipids/simple/tocol/index.htm> [Accessed 16 August 2016].

Daou, C. and Zhang, H., 2012. Study on functional properties of physically modified dietary fibres derived from defatted rice bran. *Journal of Agricultural Science*, 4(9), pp. 85-97.

Delgado, J. M. P. Q. and Barbosa de Lima, A. G. eds., 2014. *Transport phenomena and drying of solids and particulate materials*. [e-book] Springer International Publishing Switzerland. Available at: Google Books <books.google.com> [Accessed 6 August 2016].

FAO, 2011. *Food loss and food waste*. [online] Available at: <http://www.fao.org/food-loss-and-food-waste/en/> [Accessed 23 August 2016].

Farrant, JM., Bailly, C., Leymarie, J., Hamman, B., Côme, D. and Corbineau, F., 2003. Wheat seedlings as a model to understand desiccation tolerance and sensitivity. *Physiologia Plantarium*, 120, pp. 563-574.

Ferrali, M., Signorini, C., Caciotti, B., Sugherini, L., Ciccoli, L., Giachetti, D. and Comporti, M., 1997. Protection against oxidative damage of erythrocyte membranes by the flavonoid quercetin and its relation to iron chelating activity. *FEBS Letters*, 416, pp. 123-129.

Ferreira, I. C. F. R., Baptista, P., Vilas-Boas, M. and Barros, L., 2007. Free-radical scavenging capacity and reducing power of wild edible mushrooms from northeast Portugal: Individual cap and stipe activity. *Food Chemistry*, 100, pp. 1511-1516.

Fiedor, J. and Burda, K., 2014. Potential role of carotenoids as antioxidants in human health and disease. *Nutrients*, 6, pp. 466-488.

Fuentes-Alventosa, J. M., Rodríguez-Gutiérrez, G., Jaramillo-Carmona, S., Espejo-Calvo, J. A., Rodríguez-Arcos, R., Fernández-Bolaños, J., Guillén-Bejarano, R. and Jiménez-Araujo, A., 2009. Effect of extraction method on chemical composition and functional characteristics of high dietary fibre powders obtained from asparagus by-products. *Food Chemistry*, 113, pp. 665-671.

Ginting, M., 2014. *10 benefits of red guava for health*. [photograph] Available at: <http://www.medicineofherbs.com/2015/12/10-benefits-of-red-guava-for-health.html> [Accessed 15 August 2016].

Gohil, D. D. and Lele, S. S., 2014. Insoluble vegetable fiber as a potential functional ingredient: *In vitro* studies on hypoglycemic and hypocholesterolemic effect. *American Journal of Food Science and Technology*, 2(2), pp. 48-52.

Guillon, F., Auffret, A., Robertson, J. A. and Barry, J. L., 1998. Relationship between physical characteristics of sugar beet fibre and its fermentability by human faecal flora. *Carbohydrate Polymers*, 37(2), pp. 185-197.

Gupta, P. and Premavalli, K. S., 2011. *In-vitro* studies on functional properties of selected natural dietary fibers. *International Journal of Food Properties*, 14(2), pp. 397-410.

Gupta, V. K. and Sharma, S. K., 2006. Plants as natural antioxidants. *Natural Product Radiance*, 5(4), pp. 326-334.

Haliwell, B., 1996. Antioxidants in human and disease. *Annual Review of Nutrition*, 16, pp. 33-50.

Harold, E. S., Darrell, E. A., Evan, I. F. and John, A. M., 2007. A review of the interaction among dietary antioxidants and reactive oxygen species. *The Journal of Nutritional Biochemistry*, 18, pp. 567-579.

Hofstrand, D., 2012. *More on feeding nine billion people by 2050*. [online] Available at: <http://www.agmrc.org/renewable-energy/renewable-energy/more-on-feeding-nine-billion-people-by-2050/> [Accessed 22 August 2016].

Hui, Y. H., ed., 2006. *Handbook of fruits and fruit processing*. Iowa, USA: Blackwell publishing.

Irshad, M., Zafaryab, M., Singh, M. and Rizvi, M. M. A., 2012. Comparative analysis of the antioxidant activity of *Cassia fistula* extracts. *International Journal of Medicinal Chemistry*, 2012, pp. 1-6.

Jayanthi, P. and Lalitha, P., 2011. Reducing power of the solvent extracts of *Eichhornia crassipes* (Mart.) Solms. *International Journal of Pharmacy and Pharmaceutical Sciences*, 3(3), pp. 126-128.

Kaplan, N. M., 1989. The deadly quartet. upper-body obesity, glucose intolerance, hypertriglyceridemia, and hypertension. *Archives of Internal Medicine*, 149(7), pp. 1514-1520.

Kim, H., Moon, J. Y., Kim, H., Lee, D., Cho, M., Choi, H., Kim, Y. S., Mosaddik, A. and Cho, S. K., 2010. Antioxidant and antiproliferative activities of mango (*Mangifera indica* L.) flesh and peel. *Food Chemistry*, 121, pp. 429-436.

Kong, K. W. and Ismail, A., 2011. Lycopene content and lipophilic antioxidant capacity of by-products from *Psidium guajava* fruits produced during puree production industry. *Food and Bioproducts Processing*, 89, pp. 53-61.

Larrauri, J. A., 1999. New approaches in the preparation of high dietary fibre powders from fruit by-products. *Trends in Food Science and Technology*, 10(1), pp. 3-8.

Lefebvre, P. J. and Scheen, A. J., 1994. The use of acarbose in the prevention and treatment of hypoglycaemia. *European Journal of Clinical Investigation*, 24(3), pp. 40-44.

Leontowicz, M., Gorinstein, S., Leontowicz, H., Krzeminski, R., Lojek, A., Katrich, E., Cíž, M., Martin-Belloso, O., Soliva-Fortuny, R., Haruenkit, R. and Trakhtenberg, S., 2003. Apple and pear peel and pulp and their influence on plasma lipids and antioxidant potentials in rats fed cholesterol-containing diets. *Journal of Agricultural and Food Chemistry*, 51(19), pp. 5780-5785.

Liu, R. H., 2004. Potential synergy of phytochemicals in cancer prevention: mechanism of action. *The American Society for Nutritional Sciences*, 134, pp. 3479S-3485S.

Liu, R. H., 2013. Health-promoting components of fruits and vegetables in the diet. *Advances in Nutrition*, 4, pp. 384S-392S.

López, G., Ros, G., Rincón, F., Periago, M. J., Martínez, M. C., and Ortuño, J., 1996. Relationship between physical and hydration properties of soluble and insoluble fiber of artichoke. *Journal of Agricultural and Food Chemistry*, 44, pp. 2773–2778.

Malviya, N., Jain, S. and Malviya, S., 2010. Antidiabetic potential of medicinal plants. *Acta Poloniae Pharmaceutica*, 67(2), pp. 113-118.

Maniyan, A., John, R. and Mathew, A., 2015. Evaluation of fruit peels for some selected nutritional and anti-nutritional factors. *Emer Life Sci Res*, 1(2), pp. 13-19.

Marina, Z. and Noriham, A., 2014. Quantification of total phenolic compound and *in vitro* antioxidant of fruit peel extracts. *International Food Research Journal*, 21(5), pp. 1925-1929.

Mary, S. D. C. and Gayathri, D. S., 2015. *In vitro* glucose binding activity of *Terminalia bellirica*. *Asian Journal of Pharmaceutical and Clinical Research*, 8(2), pp. 320-323.

McCue, P. P. and Shetty, K., 2004. Inhibitory effects of rosmarinic acid extracts on porcine pancreatic amylase *in vitro*. *Asia Pacific Journal Clinical Nutrition*, 13(1), pp. 101-106.

Megazyme, 2015. *D-glucose assay procedure (GOPOD-FORMAT)*. [online] Available at: <https://secure.megazyme.com/files/Booklet/K-GLUC_DATA.pdf> [Accessed 30 July 2016].

Mehta, R., 2005. Dietary fiber benefits. *Cereal Foods World*, 50(2), pp. 66-71.

Mercadante, A. Z., Steck, A. and Pfander, H., 1999. Carotenoids from guava (*Psidium guajava* L.): isolation and structure elucidation. *Journal of Agricultural and Food Chemistry*, 47(1), pp. 145-151.

Miean K. H. and Mohamed, S., 2001. Flavonoid (myricetin, quercetin, kaempferol, luteolin, and apigenin) content of edible tropical plants. *Journal of Agricultural and Food Chemistry*, 49(6), pp. 3106-3112.

Misra, K. and Seshadri, T. R., 1968. Chemical components of the fruits of *Psidium guava*. *Phytochemistry*, 7(4), pp. 641-645.

Musa, K. H., Abdullah, A. and Subramaniam, V., 2015. Flavonoid profile and antioxidant activity of pink guava. *ScienceAsia*, 41, pp. 149-154.

Musa, K. H., Abdullah, A., Jusoh, K. and Subramaniam, V., 2011. Antioxidant activity of pink-flesh guava (*Psidium guajava* L.): effect of extraction techniques and solvents. *Food Analytical Methods*, 4(1), pp. 100-107.

Naidu, K., Singh, V., Johnston, D. B., Rausch, K. D. and Tumbleson, M. E., 2007. Effects of ground corn particle size on ethanol yield and thin stillage soluble solids. *Cereal Chemistry*, 84(1), pp. 6-9.

Nijveldt, R. J., Van Nood, E., Van Hoorn, D. E., Boelens, P. G., Van Norren, K. and Van Leeuwen, P. A., 2001. Flavonoids: a review of probable mechanisms of action and potential applications. *The American Journal of Clinical Nutrition*, 74(4), pp. 418-425.

Nimse, S. B. and Pal, D., 2015. Free radicals, natural antioxidants, and their reaction mechanisms. *RSC Advances*, 5, pp. 27986-28006.

Oboh, G., Isaac, A. T., Akinyemi, A. J. and Ajani, R. A., 2014. Inhibition of key enzymes linked to type 2 diabetes and sodium nitroprusside induced lipid peroxidation in rats' pancreas by phenolic extracts of avocado pear leaves and fruit. *International Journal of Biomedical Science*, 10(3), pp. 208-216.

Ou, S., Kwok, K., Li, Y. and Fu, L., 2001. *In vitro* study of possible role of dietary fiber in lowering postprandial serum glucose. *Journal of Agricultural and Food Chemistry*, 49(2), pp. 1026-1029.

Pandey, S., Dr. Pandey, R. and Singh, R., 2014. Phytochemical screening of selected medicinal plant *Cinnamon zeylanicum* bark extract, area of research; Uttarakhand, India. *International Journal of Scientific and Research Publications*, 4(6), pp. 1-6.

Peerajit, P., Chiewchan, N. and Devahastin, S., 2012. Effect of pretreatment methods on health-related functional properties of high dietary fiber powder from lime residues. *Food Chemistry*, 132, pp. 1891-1898.

Procházková, D., Boušová, I. and Wilhelmová, N., 2011. Antioxidant and prooxidant properties of flavonoids. *Fitoterapia*, 82(4), pp. 513-523.

Procházková, Z. and Bezděčková, L., 2008. Effects of moisture content, storage temperature and type of storage bag on the germination and viability of stored European beech (*Fagus sylvatica* L.) seeds. *Journal of Forest Science*, 54(7), pp. 287-293.

Purdue University, n.d. *Guava*. [online] Available at: < https://www.hort.purdue.edu/newcrop/morton/guava.html> [Accessed 6 August 2016].

Rohn, S., Rawel, M. and Kroll, J., 2002. Inhibitory effects of plant phenols on the activity of selected enzymes. *Journal Agricultural Food Chemistry*, 50(12), pp. 3566-3571.

Rudrappa, U., n.d. *Guava nutrition facts*. [online] Available at: http://www.nutrition-and-you.com/guava.html [Accessed 6 August 2016].

Shimada, K., Fujikawa, K., Yahara, K. and Nakamura, T., 1992. Antioxidative properties of xanthan on the autoxidation of soybean oil in cyclodextrin emulsion. *Journal of Agricultural and Food Chemistry*, 40(6), pp. 945-948.

Siddiq, M., ed., 2012. *Tropical and subtropical fruits: postharvest physiology, processing and packaging*. John Wiley & Sons.

Siow, L. F. and Hui, Y. W., 2013. Comparison on the antioxidant properties of fresh and convection oven-dried guava (*Psidium guajava* L.). *International Food Research Journal*, 20(2), pp. 639-644.

Smirnoff, N., 2000. Ascorbic acid: metabolism and functions of a multi-facetted molecule. *Current Opinion in Plant Biology*, 3, pp. 229-235.

Sousa, B. A. and Correia, R. T. P., 2012. Phenolic content, antioxidant activity and antiamylolytic activity of extracts obtained from bioprocessed pineapple and guava wastes. *Brazilian Journal of Chemical Engineering*, 29(01), pp. 25-30.

Srichamroen, A. and Chavasit, V., 2011. *In vitro* retardation of glucose diffusion with gum extracted from malva nut seeds produced in Thailand. *Food Chemistry*, 127, pp. 455-460.

Stahl, W. and Sies, H., 2003. Antioxidant activity of carotenoids. *Molecular Aspects of Medicine*, 24, pp. 345-351.

Sudha, P., Zinjarde, S. S., Bhargava, S. Y. and Kumar, A. R., 2011. Potent α-amylase inhibitory activity of Indian Ayurvedic medicinal plants. *BMC Complementary and Alternative Medicine*, 11(5), pp. 1-10.

Sukeksi, L. and Sarah, M., 2016. Characterizations and extraction of polyphenols from residual pulp of pink guava as source of antioxidants. *Asian Research Publishing Network (ARPN)*, 11(8), pp. 5209-5216.

Takahashi, T., Karita, S., Ogawa, N. and Goto, M., 2005. Crystalline cellulose reduces plasma glucose concentrations and stimulates water absorption by increasing the digesta viscosity in rats. *The Journal of Nutrition*, 135, pp. 2405-2410.

Taylor, L. and County, M., 2012. *Tropical plant database*. [online] Available at: <http://www.rain-tree.com/guava.htm#.V7EqLfnnako> [Accessed 15 August 2016].

Teixeira, J., Gaspar, A., Garrido, M. E., Garrido, J. and Borges, F., 2013. Hydroxycinnamic acid antioxidants: an electrochemical overview. *BioMed Research International*, 2013, pp. 1-11.

Temple, N. J., 2000. Antioxidants and disease: more questions than answers. *Nutrition Research*, 20(3), pp. 449-459.

Thaipong, K., Boonprakob, U., Crosby, K., Cisneros-Zevallos, L. and Byrne, D. H., 2006. Comparison of ABTS, DPPH, FRAP, and ORAC assays for estimating antioxidant activity from guava fruit extracts. *Journal of Food Composition and Analysis*, 19, pp. 669-675.

Torres de Pinedo, A., Penalver, P. and Morales, J. C., 2007. Synthesis and evaluation of new phenolic-based antioxidants: structure-activity relationship. *Food Chemistry*, 103(1), pp. 55-61.

Torsdottir, I., Alpsten, M., Andersson, H. and Einarsson, S., 1989. Dietary guar gum effects on postprandial blood glucose, insulin and hydroxyproline in humans. *Journal of Nutrition*, 119(12), pp. 1925-1931.

Uchôa-thomaz, A. M. A., Sousa, E. C., Carioca, J. O. B., De Morais, S. M., De Lima, A.., Martins, C. G., Alexandrino, C. D., Ferreira, P. A. T., Rodrigues, A. L. M., Rodrigues, S. P., De Albuquerque Thomaz, J. C., Silva, J. D. N. and Rodrigues, L. L., 2014. Chemical composition, fatty acid profile and bioactive compounds of guava seeds (*Psidium guajava* L.). *Food Science and Technology*, 34(3), pp. 485-492.

Verma, A. K., Rajkumar, V., Banerjee, R., Biswas, S. and Das, A. K., 2013. Guava (*Psidium guajava* L.) powder as an antioxidant dietary fibre in sheep meat nuggets. *Asian-Australasian Journal of Animal Sciences*, 26(6), pp. 886-895.

Wikipedia, 2016. *Green apple guava*. [photograph] Available at: < https://en.wikipedia.org/wiki/Guava> [Accessed 15 August 2016].

Wild, S., Roglic, G., Green, A., Sicree, R. and King, H., 2004. Global prevalence of diabetes: estimates for the year 2000 and projections for 2030. *Diabetes Care*, 27(5), pp. 1047-1053.

Winkel-Shirley, B., 2001. Flavonoid biosynthesis. a colorful model for genetics, biochemistry, cell biology, and biotechnology. *American Society of Plant Physiologists*, 126(2), pp. 485-493.

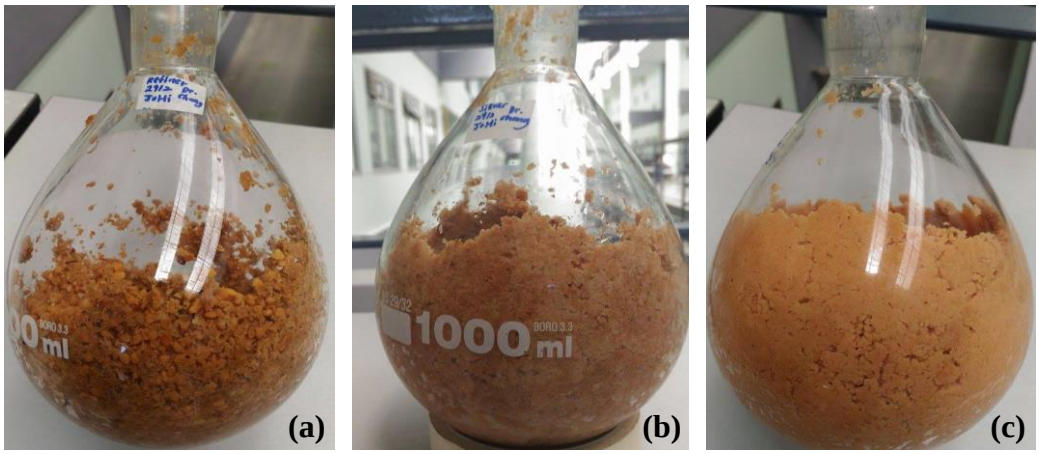
Worthington, n.d. *Amylase, alpha*. [image] Available at: < http://www.worthington-biochem.com/AA/default.html > [Accessed 11 August 2016].

Yahia, E.M., ed., 2011. *Postharvest biology and technology of tropical and subtropical fruits volume 3: cocona to mango*. Philadelphia: Woodhead Publishing Limited.

Zhao, M., Yang, B., Wang, J., Liu, Y., Yu, L. and Jiang, Y., 2007. Immunomodulatory and anticancer activities of flavonoids extracted from litchi (*Litchi chinensis* Sonn.) pericarp. *International Immunopharmacology*, 7, pp. 162-166.

APPENDICES

APPENDIX A



Guava by-products (before freeze-drying): (a) refiner (b) siever (c) decanter

Yield of ethanolic extracts of freeze-dried guava by-products

Sample	Yield (%)			Standard Deviation
	1	2	Average	
Refiner	29.36	28.15	28.76	0.856
Siever	28.27	26.45	27.36	1.287
Decanter	26.69	24.79	25.74	1.344

Yield of dry pellets of freeze-dried guava by-products

Sample	Yield (%)			Standard Deviation
	1	2	Average	
Refiner	69.53	71.43	70.48	1.344
Siever	70.46	73.14	71.80	1.895
Decanter	69.92	72.13	71.03	1.563

APPENDIX B

Absorbance at 517 nm for ethanolic extracts of freeze-dried guava by-products in DPPH assay

Standard/Sample	Absorbance at 517 nm					
	Concentration (mg/ml)					
	0.00	0.20	0.40	0.60	0.80	1.00
Ascorbic acid						
1	1.110	0.037	0.034	0.029	0.032	0.029
2	1.086	0.044	0.043	0.040	0.042	0.043
3	0.990	0.040	0.038	0.035	0.039	0.043
Refiner						
1	1.092	0.791	0.511	0.305	0.133	0.084
2	1.087	0.771	0.517	0.323	0.169	0.093
3	0.960	0.655	0.413	0.238	0.117	0.074
Siever						
1	1.081	0.636	0.333	0.149	0.072	0.061
2	1.090	0.648	0.331	0.155	0.096	0.072
3	0.975	0.584	0.267	0.116	0.070	0.062
Decanter						
1	1.089	0.737	0.468	0.300	0.182	0.119
2	1.094	0.729	0.474	0.311	0.191	0.139
3	0.927	0.653	0.385	0.284	0.136	0.096

DPPH radical scavenging activity for ethanolic extracts of freeze-dried guava by-products

Standard/Sample	DPPH Radical Scavenging Activity (%)					
	Concentration (mg/ml)					
	0.00	0.20	0.40	0.60	0.80	1.00
Ascorbic acid						
1	0	96.67	96.94	97.39	97.12	97.39
2	0	95.95	96.04	96.32	96.13	96.04
3	0	95.96	96.16	96.46	96.06	95.66
Average	0	96.19	96.38	96.72	96.44	96.36
SD	0	0.413	0.489	0.582	0.593	0.909
Refiner						
1	0	27.56	53.21	72.07	87.82	92.31
2	0	29.07	52.44	70.29	84.45	91.44
3	0	31.77	56.98	75.21	87.81	92.29
Average	0	29.47	54.21	72.52	86.69	92.01
SD	0	2.133	2.430	2.491	1.943	0.497
Siever						
1	0	41.17	69.20	86.22	93.34	94.36
2	0	40.55	69.63	85.78	91.19	93.39
3	0	40.10	72.62	88.10	92.82	93.64
Average	0	40.61	70.48	86.70	92.45	93.80
SD	0	0.537	1.863	1.232	1.122	0.504
Decanter						
1	0	32.32	57.02	72.45	83.29	89.07
2	0	33.36	56.67	71.57	82.54	87.29
3	0	29.56	58.47	69.36	85.33	89.64
Average	0	31.75	57.39	71.13	83.72	88.67
SD	0	1.964	0.954	1.592	1.444	1.226

EC_{50} values for ethanolic extracts of freeze-dried guava by-products in DPPH assay

Sample	EC_{50} value (mg/ml)				Standard Deviation
	1	2	3	Average	
Refiner	0.400	0.406	0.377	0.394	0.015
Siever	0.319	0.320	0.311	0.317	0.005
Decanter	0.385	0.387	0.395	0.389	0.005

APPENDIX C

Absorbance at 700 nm for ethanolic extracts of freeze-dried guava by-products in reducing power assay

Standard/Sample	Absorbance at 700 nm					
	Concentration (mg/ml)					
	0.00	0.20	0.40	0.60	0.80	1.00
Ascorbic acid						
1	0.191	1.683	1.766	1.776	1.795	1.855
2	0.179	1.693	1.707	1.769	1.822	1.827
3	0.195	1.726	1.776	1.837	1.891	1.942
Refiner						
1	0.126	0.266	0.384	0.523	0.635	0.770
2	0.193	0.311	0.433	0.592	0.713	0.822
3	0.182	0.318	0.431	0.585	0.692	0.824
Siever						
1	0.203	0.381	0.587	0.796	0.964	1.097
2	0.209	0.385	0.592	0.798	0.965	1.110
3	0.215	0.395	0.595	0.818	0.958	1.120
Decanter						
1	0.191	0.356	0.514	0.674	0.800	0.950
2	0.177	0.338	0.497	0.661	0.802	0.944
3	0.200	0.355	0.520	0.681	0.816	0.975

Reducing power for ethanolic extracts of freeze-dried guava by-products

Sample	Reducing Power (%)					
	Concentration (mg/ml)					
	0.00	0.20	0.40	0.60	0.80	1.00
Refiner						
1	0	9.38	16.38	25.05	31.73	38.70
2	0	7.79	15.71	25.09	31.65	38.17
3	0	8.88	15.75	24.54	30.07	36.75
Average	0	8.68	15.95	24.89	31.15	37.87
SD	0	0.813	0.376	0.307	0.936	1.008
Siever						
1	0	11.93	24.38	37.41	47.44	53.73
2	0	11.62	25.07	37.04	46.01	54.67
3	0	11.76	24.04	36.72	43.81	51.80
Average	0	11.77	24.50	37.06	45.75	53.40
SD	0	0.155	0.525	0.345	1.829	1.463
Decanter						
1	0	11.06	20.51	30.47	37.97	45.61
2	0	10.63	20.94	30.44	38.04	46.54
3	0	10.12	20.24	29.29	36.32	44.36
Average	0	10.60	20.56	30.07	37.44	45.50
SD	0	0.471	0.353	0.673	0.973	1.094

APPENDIX D

Absorbance at 620 nm for ethanolic extracts of freeze-dried guava by-products in α-amylase inhibitory assay

Sample	Absorbance at 620 nm					
	Concentration (mg/ml)					
	0.00	0.20	0.40	0.60	0.80	1.00
Refiner						
1	0.524	0.540	0.561	0.591	0.594	0.635
2	0.523	0.549	0.576	0.578	0.592	0.606
3	0.532	0.552	0.567	0.587	0.591	0.625
Siever						
1	0.393	0.399	0.430	0.472	0.489	0.534
2	0.420	0.454	0.477	0.502	0.534	0.560
3	0.438	0.458	0.475	0.508	0.555	0.595
Decanter						
1	0.394	0.420	0.428	0.505	0.518	0.565
2	0.448	0.475	0.498	0.548	0.576	0.598
3	0.509	0.534	0.564	0.610	0.628	0.652

Absorbance at 620 nm for inhibition control (50 μg/ml acarbose) in α-amylase inhibitory assay

Inhibition Control	Absorbance at 620 nm			
	1	2	3	Average
Acarbose	1.013	1.057	1.049	1.040

α-amylase inhibition activity for ethanolic extracts of freeze-dried guava by-products

Sample	α-amylase Inhibition Activity (%)					
	Concentration (mg/ml)					
	0.00	0.20	0.40	0.60	0.80	1.00
Refiner						
1	0	3.10	7.17	12.98	13.57	21.51
2	0	5.03	10.25	10.64	13.35	16.05
3	0	3.94	6.89	10.83	11.61	18.31
Average	0	4.02	8.10	11.48	12.84	18.62
SD	0	0.968	1.864	1.300	1.074	2.743
Siever						
1	0	0.93	5.72	12.21	14.84	21.79
2	0	5.48	9.19	13.23	18.39	22.58
3	0	3.32	6.15	11.63	19.44	26.08
Average	0	3.24	7.02	12.36	17.56	23.48
SD	0	2.276	1.892	0.810	2.411	2.283
Decanter						
1	0	4.02	5.26	17.18	19.20	26.47
2	0	4.56	8.45	16.89	21.62	25.34
3	0	4.71	10.36	19.02	22.41	26.93
Average	0	4.43	8.02	17.70	21.08	26.25
SD	0	0.363	2.577	1.155	1.673	0.818

APPENDIX E

D-glucose standard for glucose diffusion assay

Concentration (mg/ml)	Absorbance at 510 nm			Standard Deviation
	1	2	Average	
0.00	0	0	0	0
0.20	0.171	0.201	0.186	0.021
0.40	0.367	0.377	0.372	0.007
0.60	0.549	0.558	0.554	0.006
0.80	0.718	0.732	0.725	0.010
1.00	0.892	0.911	0.902	0.013

Absorbance at 510 nm for dialysates collected at different time intervals in glucose diffusion assay

Control/Sample	Absorbance at 510 nm					
	Time (min)					
	15	30	45	60	75	90
Glucose Control						
1	0.171	0.293	0.461	0.466	0.546	0.578
2	0.152	0.290	0.367	0.472	0.531	0.574
3	0.151	0.279	0.367	0.460	0.529	0.557
Refiner						
1	0.163	0.281	0.391	0.462	0.518	0.576
2	0.148	0.266	0.339	0.401	0.477	0.534
3	0.148	0.259	0.357	0.440	0.512	0.543
Siever						
1	0.168	0.289	0.367	0.444	0.521	0.565
2	0.148	0.262	0.354	0.435	0.509	0.538
3	0.149	0.257	0.358	0.444	0.504	0.534
Decanter						
1	0.145	0.267	0.351	0.426	0.488	0.544
2	0.137	0.267	0.363	0.436	0.469	0.542
3	0.140	0.269	0.354	0.426	0.501	0.528

Glucose concentration of dialysates collected at different time intervals in glucose diffusion assay

Control/Sample	Glucose Concentration (mg/ml)					
	Time (min)					
	15	30	45	60	75	90
Glucose Control						
1	0.188	0.322	0.507	0.513	0.601	0.636
2	0.167	0.319	0.404	0.519	0.584	0.632
3	0.166	0.307	0.404	0.506	0.582	0.613
Average	0.174	0.316	0.438	0.513	0.589	0.627
SD	0.012	0.008	0.060	0.007	0.010	0.012
Refiner						
1	0.179	0.309	0.430	0.508	0.570	0.634
2	0.163	0.293	0.373	0.441	0.525	0.588
3	0.163	0.285	0.393	0.484	0.563	0.597
Average	0.168	0.296	0.399	0.478	0.553	0.606
SD	0.010	0.012	0.029	0.034	0.024	0.024
Siever						
1	0.185	0.318	0.404	0.489	0.573	0.622
2	0.163	0.288	0.389	0.479	0.561	0.592
3	0.164	0.283	0.394	0.489	0.555	0.588
Average	0.171	0.296	0.396	0.485	0.563	0.600
SD	0.012	0.019	0.007	0.006	0.010	0.019
Decanter						
1	0.160	0.294	0.386	0.469	0.537	0.599
2	0.151	0.294	0.399	0.480	0.516	0.596
3	0.154	0.296	0.389	0.469	0.551	0.581
Average	0.155	0.294	0.392	0.472	0.535	0.592
SD	0.004	0.001	0.007	0.006	0.018	0.010

GDRI for dry pellets of freeze-dried guava by-products at different time intervals in glucose diffusion assay

Sample	GDRI (%)					
	Time (min)					
	15	30	45	60	75	90
Refiner						
1	4.68	4.10	15.18	0.86	5.13	0.35
2	2.63	8.28	7.63	15.04	10.17	6.97
3	1.99	7.17	2.72	4.35	3.21	2.51
Average	3.10	6.51	8.51	6.75	6.17	3.28
SD	1.405	2.166	6.28	7.391	3.593	3.377
Siever						
1	1.75	1.37	20.39	4.72	4.58	2.25
2	2.63	9.66	3.54	7.84	4.14	6.27
3	1.32	7.89	2.45	3.48	4.73	4.13
Average	1.90	6.30	8.80	5.35	4.48	4.22
SD	0.666	4.366	10.057	2.247	0.303	2.013
Decanter						
1	15.20	8.87	23.86	8.58	10.62	5.88
2	9.87	7.93	1.09	7.63	11.68	5.57
3	7.28	3.58	3.54	7.39	5.29	5.21
Average	10.79	6.80	9.50	7.87	9.20	5.55
SD	4.039	2.821	12.499	0.631	3.422	0.338