EFFECTS OF FIBER-DEGRADING ENZYMATIC TREATMENTS ON STRUCTURAL PROPERTIES AND FERMENTABILITY OF PINK GUAVA (*Psidium guajava* Linn.) WASTES

By

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ABSTRACT

EFFECTS OF FIBER-DEGRADING ENZYMATIC TREATMENTS ON STRUCTURAL PROPERTIES AND FERMENTABILITY OF PINK GUAVA (*Psidium guajava* Linn.) WASTES

HUI CHOY YEE

Pink guava (*Psidium guajava* Linn.) is a well-known tropical fruit as it is fiber-rich material which contributes health-related benefits upon human consumption. There are large quantities of pink guava wastes (decanter and refiner) generated throughout pink guava puree processing. These plant-based waste materials have high portions of fiber and non-starch polysaccharides. They could not be utilized directly inclusive in one's diet for consumption, and usually be disposed of as un-utilizable wastes. Foremost, this research study aimed to hydrolyze pink guava wastes (decanter and refiner) through fiber-degrading enzymatic treatments; 150 U cellulase (150CE), 150 U xylanase (150XY) and 150 U cellulase-xylanase (150CX) have been employed to treat pink guava waste. It was aimed to analyze structural properties of untreated and enzymatic-treated pink guava wastes based on the attenuated total reflectance (ATR), X-ray diffraction (XRD) and scanning electron microscopy (SEM). The untreated and enzymatic-treated pink guava wastes were washed and extracted by using 80% (v/v) ethanol, to obtain alcohol

insoluble solid (AIS) and alcohol soluble carbohydrates (ASC). Besides, the sugar profiles of untreated and enzymatic-treated pink guava wastes were quantified, and functional properties were determined based on glucose dialysis retardation index (GDRI) of AIS material and prebiotic activity score of ASC. The suitability of whole untreated and enzymatic-treated pink guava wastes as potential prebiotic sources in yogurts was evaluated. The cellulase-xylanase combined enzymatic treatment (2: 1) hydrolyzed synergistically and converted pink guava wastes into simple carbohydrates (rhamnose, xylose, glucose, and fructose) and increased the sugar amount significantly, which were analyzed by High Performance Liquid Chromatography (HPLC). The hypoglycemic effects of combined enzymatic-treated pink guava wastes had been improved; it delayed glucose diffusion across dialysis tube. This is due to physical obstacle attributed by fiber particles towards glucose entrapment within fiber network, as evidenced with outcomes of SEM analysis. The prebiotic activity score of ASC derived from combined enzymatic-treated pink guava wastes had been significantly enhanced, using *Lactobacillus plantarum* as a probiotic strain. About 1% (w/w) of untreated and enzymatic-treated pink guava waste was added into yogurts to evaluate suitability of pink guava wastes as prebiotic sources, acidity and texture profile of yogurts. The CFU/mL of yogurts added with combined enzymatic-treated decanter and refiner was enhanced by 76.92% and 77.58%, respectively. Therefore, the synergistic hydrolytic action exerted by cellulase-xylanase enzymatic treatment is a feasible approach to hydrolyze and valorize both pink guava wastes into more value-added materials, with enhanced structural and functional properties.

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It is hereby certified that **HUI CHOY YEE** (ID No.: **18ADM01417**) has completed this thesis dissertation entitled "EFFECTS OF FIBER-DEGRADING ENZYMATIC TREATMENTS ON STRUCTURAL PROPERTIES AND FERMENTABILITY OF PINK GUAVA (*Psidium guajava* Linn.) WASTES" under the supervision of Dr. Chang Ying Ping (Supervisor) from the Department of Agricultural and Food Science, Faculty of Science, and Dr. Lee Kok Chang (Co-Supervisor) from the Department of Biological Science, Faculty of Science.

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This dissertation entitled "EFFECTS OF FIBER-DEGRADING ENZYMATIC TREATMENTS ON STRUCTURAL PROPERTIES AND FERMENTABILITY OF PINK GUAVA (*Psidium guajava* Linn.) WASTES" was prepared by HUI CHOY YEE and submitted as partial fulfillment of the requirements for the degree of Master of Science at Universiti Tunku Abdul Rahman.

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DECLARATION

I, Hui Choy Yee hereby declare that the dissertation 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.

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

×	Times
×*g*	Relative centrifugal force
≈	Approximately
150CE	150 U cellulase treatment
150CED	150 U cellulase-treated decanter
150CER	150 U cellulase-treated refiner
150CX	150 U cellulase-xylanase treatment
150CXD	150 U cellulase-xylanase-treated decanter
150CXR	150 U cellulase-xylanase-treated refiner
150XY	150 U xylanase treatment
150XYD	150 U xylanase-treated decanter
150XYR	150 U xylanase-treated refiner
1^{st}	First
2^{nd}	Second
AACC	American Association for Clinical Chemistry
AIS	Alcohol insoluble solid
ANOVA	One-Way Analysis of Variance
ASC	Alcohol soluble carbohydrates
ATCC	American Type Culture Collection
atm	Atmospheric pressure
ATR	Attenuated total reflectance

C – H	Carbon-hydrogen bond
C – O – C	Ether group
C – O	Carbon-oxygen bond
C = C	Carbon-carbon double bond
C = O	Carbonyl group
CFU	Colony forming unit
CHD	Coronary heart disease
cm	Centimeter
cm^{-1}	Wavelength
COOH	Carboxylic acid
CrI	Crystallinity index
CTDD	Untreated decanter
CTDR	Untreated refiner
deg	Degree
deg/min	Degree per minute
DNS	3,5-dinitrosalicylic acid
E. coli	*Escherichia coli*
et al.	et alia (and others)
FAO	Food and Agriculture Organization
ft.	Feet
g	Gram
GAE	Gallic Acid Equivalents
GalUA	α-D-galacturonic acid

GDRI	Glucose dialysis retardation index
GI	Glycemic index
Glu	Sugar content
GOPOD	Glucose oxidase/peroxidase
h	Hour
H_2SO_4	Sulfuric acid
HCl	Hydrochloric acid
HG	Homogalacturonic acid
RG	Rhamnogalacturonic
HPLC	High Performance Liquid Chromatography
I	Peak intensity
IDF	Insoluble dietary fiber
KCTC	Korean Collection for Type Cultures
KH_2PO_4	Potassium phosphate monobasic
$KNaC_4H_4O_6 \cdot 4H_2O$	Potassium sodium tartrate
kV	Kilovolt
L. brevis	*Lactobacillus brevis*
L. plantarum	*Lactobacillus plantarum*
L. rhamnosus	*Lactobacillus rhamnosus*
LB	Luria-Bertani
mA	Milliampere
mg	Milligram
mg/g	Milligram per gram

mg/mL	Milligram per milliliter
mg/mL	Milligram per milliliter
min	Minute
mL	Milliliter
mL/min	Milliliter per min
mm	Millimeter
mM	Millimolar
MRS	De Man, Rogosa and Sharpe agar
MSW	Municipal solid waste
MWCO	Molecular weight cut-off
n.d	Not detected
N/A	Not applicable
$NaHPO_4 \cdot 7H_2O$	Sodium phosphate dibasic heptahydrate
NaOH	Sodium hydroxide
nm	Nanometer
O – H	Hydroxyl bond
P	*P*-value
psi	Pound per square inch
R^2	Regression coefficient
Rha	α-rhamnose
RID	Refractive index detector
rpm	Revolutions per minute
SCFAs	Short-chain fatty acids

SDF	Soluble dietary fiber
SDF	Soluble dietary fiber
SEM	Scanning electron microscopy
SmF	Submerged fermentation
SPSS	Statistical Package for the Social Science
SSF	Solid state fermentation
TDF	Total dietary fiber
TVC	Total viable count
U	Unit
UHT	Ultra-high temperature
UV-VIS	Ultraviolet-visible
v/v	Volume per volume
w/v	Weight per volume
WSH	Washed
XRD	X-ray diffraction
α	Alpha
β	Beta
θ	Theta
μg	Microgram
μg/mL	Microgram per milliliter
μL	Microliter
μm	Micrometer

CHAPTER 1

INTRODUCTION

There are large quantities of biodegradable wastes produced by the agricultural and food industries, and this becomes a challenge to food industries (Kader et al., 2016). FAO (2011) stated that the increment of food production leads to various environmental problems since there are about 33% of all foods were squandered through the food supply chain, from the initial processing to the consumptions of end food products (FAO, 2011).

The residues produced by food industries are mainly organic substances, and they should be disposed of and well-managed (Pelizer et al., 2007). Those improper disposals of biodegradable wastes as landfills leads excessive release of carbon dioxide (50%), methane gases (50%) and trace amount of non-methane organic compounds (Plazotta et al., 2017). The emission of greenhouse gases may absorb infrared radiation, which directly results in global warming to take place (Latake and Pawar, 2015; Plazzotta et al., 2017). Meanwhile, improper management of perishable plant-based or animal-based wastes may lead to the formation of off-odors and microbiological instability and results in the losses of valuable biomass and nutrients (Pelizer et al., 2007; Plazzotta et al., 2017). In other words, improper disposal of biodegradable wastes cause various potential environmental problems

to take place, and eventually trigger the losses of energy and raw materials. These problems require high investments to manage and control (Sousa and Correria, 2010).

Valorization is one of the workable options to manage biodegradable wastes and prevent them from being disposed of as landfills (Rick et al., 2013). Waste valorization is defined as a process of converting waste materials into more value-added and applicable products such as chemicals, ingredients and fuels for better utilization (Rick et al., 2013). Wastes generated from food processing industries comprised of lignocellulosic constituents and provide appropriate structure and sustainable slow release of carbon source in supporting the growth of microbial community (Mhd Abd Kader et al., 2016; Yang et al., 2015). There are numerous research ideas focusing on implementing and converting various solid wastes into more marketable, utilizable and value-added functional products. For instance, some agricultural waste materials have been employed and converted into other more applicable value-added products such as bio-surfactant, biodiesel, bioactive natural products, fertilizers and animal feeds (Mhd Abd Kader et al., 2016).

Guava (*Psidium guajava* Linn.) is a well-known tropical fruit. Guava are gaining visibility in agro-food business, which is due to the attractive characteristics such as high nutritious content, excellent exhibition of antioxidant capacity, functional elements, health-promoting benefits, appearance, and flavor (Wang et al., 2014).

Guava fruit is often known as “apple of the tropics”, as it is a non-seasonal fruit and its year round availability as an exotic tropical fruit crop with high nutritive values which are found to be similar to apple (Musa, Abdullah and Subramaniam, 2015; Navin et al., 2018; Shishir et al., 2014). Guavas can be categorized into two major types: white-fleshed guava and pink-fleshed guava. White-fleshed guavas are consumed freshly; while pink-fleshed guavas can be either consumed freshly or used for the production of guava juice and puree (Kong and Ismail, 2011).

Pink guava fruits are cultivated widely in the tropical and subtropical countries, especially in Malaysia, that is described as the largest pink guava fruits plantation in Asia (Shishir et al., 2014). In Malaysia, Golden Hope Food & Beverages Sdn. Bhd. Malaysia is the largest pink guava fruits producer in Asia (Amri et al., 2013). The pink guava variety is commercially cultivated only in Sitiawan, Perak, which is mainly for the production of pink guava puree (Zahidah, Noriham and Zainon, 2013). There are high demands on production of pink guava processed products such as jellies, beverages, jams, and ice-cream, due to excellent health-promoting benefits of pink guavas and longer shelf life as compared with fresh pink guava fruits (Kong and Ismail, 2011). This leads to large quantities of pink guava wastes (by-products) generated from fruit puree and juice processing.

Approximately 25% of the pink guava fruits are disposed of as pink guava wastes from the puree processing line, which involves the steps of cutting and crushing, sieving, and decanting. These pink guava waste materials are contributed by 12% of refiner which is rich in seeds and coarse pulps; 8% of siever that contains pulpy bits; and another 5% of decanter with stone cells (Kong and Ismail, 2011). These fruit wastes are rich in phytochemical compounds and dietary fiber, and possess functional properties as good as the fruit (Khan et al., 2015; Kong and Ismail, 2011; Lim et al., 2018). Hence, the beneficiation of fruit wastes has been studied and implemented lately, with the purpose to reutilize them as more applicable and value-added products (Khan et al., 2015).

Pink guava wastes are rich in dietary fiber contents. Based on the previous study by Mhd Abd Kader et al. (2016), refiner contained the highest total dietary fiber (TDF) content which is approximately 29.86%; decanter has about 23.30% of TDF, and siever has the lowest TDF value that is only 18.63%. These fiber-rich food materials bring health-promoting benefits to host upon consumption, such as enhancing cardiac health, maintaining bowel health and achieving healthy weight, normalizing bowel movements, lowering cholesterol levels, and aid in controlling blood sugar levels (Mayo, 2015). Yet, fiber-rich food materials are non-digestible components and resistant to digestion and absorption in the human small intestine, as human digestive enzymes are not capable digesting these high portions of non-starch polysaccharides (AACC International, 2001; Hipsley, 1953; Ling and Chang, 2017; Lunn and Buttriss, 2007). Hence, fiber-rich food material cannot be

utilized directly or included wholly into one's diet. We suggest the lignocellulosic constituents of pink guava wastes (cellulose, xylose, lignin, and pectin) should be modified to improve digestibility and functional properties in the human digestive system.

Enzymes, specifically hydrolases are suitable to be used in waste valorization, due to they are effective in hydrolyzing food wastes into fermentative chemical or biofuel products in a more environmental friendly manner. For instance, cellulase, xylanase, protease and lipase are common and effective enzymes to be utilized in food wastes valorization (Lam, Kwan and Lin, 2015). Cellulase is an effective fiber-degrading enzyme, which has capable to convert lignocellulose into glucose and other simple soluble sugars via hydrolyzing the β-(1,4)-glycosidic linkages in the cellulose chain (Bhat, 2000). Therefore, insoluble fibers can be hydrolyzed and converted into soluble fibers and to enhance the prebiotic-associated health benefits of fruit wastes (Charalampopoulos and Rastall, 2012). Besides, xylanase is also effective as a fiber-degrading enzyme in hydrolyzing the 1,4-glycosidic bonding in xylan (Ratnadewi et al., 2016). Xylanase is a hemicellulolytic enzyme, as it could degrade heteroxylans, one of the lignocellulosic constituents found in plant cell wall (Gabriel, Jean-Guy and Johnny, 2012). Mrabet et al. (2016) have documented that enzymatic treatments can convert insoluble fibers into soluble fibers through hydrolytic action by Viscozyme® L, which is a commercial fiber-degrading enzyme, in which it has capable to produce a value-added functional

ingredient from fiber-rich waste materials with higher concentrations of prebiotic oligosaccharides and enhanced antioxidant activity.

In this research project, it postulates that fiber-degrading enzymes treatments (cellulase, xylanase, and combined cellulase-xylanase) on plant-based biomass help to liberate the phytochemicals entrapped in the dietary fiber structure through hydrolysis on complex lignocellulosic cell wall material and results in producing simple carbohydrates. In addition, the digestibility of fiber-rich plant material can be enhanced and improved by fiber-degrading enzyme. Hence, it could modify the functional properties of fiber-rich plant material, such as soluble dietary fiber content, glucose dialysis retardation index (GDRI), and prebiotic activity score. This research aims to hydrolyze the pink guava wastes (decanter and refiner) with cellulase, xylanase and combined cellulase-xylanase treatments. It aims to analyze structural properties of untreated and enzymatic-treated pink guava wastes based on the attenuated total reflectance (ATR), X-ray diffraction (XRD) and scanning electron microscopy (SEM). It also targets to quantify sugar profiles of untreated and enzymatic-treated pink guava wastes, and to determine functional properties based on glucose dialysis retardation index (GDRI) and prebiotic activity score. Besides, it intends to relate effects of enzymatic hydrolysis on selected functional and structural properties of pink guava wastes. Whole untreated and enzymatic-treated pink guava wastes were added directly in UHT fresh milk with a starter culture to evaluate the suitability as the source of prebiotic in yogurts.

CHAPTER 2

LITERATURE REVIEW

2.1 Pink Guava (*Psidium guajava* Linn.) Fruits

Guava (*Psidium guajava* Linn.) fruit is a kind of tropical fruit, commonly known as "Jambu Batu" in Malaysia (Mishra et al., 2017). It may be consumed freshly and in processed forms such as ice cream, jellies, beverages, dehydrated products or in pickles (Jiménez et al., 2011).

It is a well-known traditional medicinal plant, belongs to the family of Myrtaccae which is represented by approximately 120 – 150 species. It is a native plant of tropical America, but it has been cultivated widely in the tropical and subtropical countries as they could thrive in various soils, dry climate, propagate easily, and eventually bear fruits quickly (Gupta, Chahal and Arora, 2011; Kamath et al., 2014; Mishra et al., 2017; Wang et al., 2014). Guava plant is an evergreen shrub or small shade tree with about 33 ft. of height with spreading branches (Figure 2.1) (Joseph and Priya, 2011; Mishra et al., 2017). It has unsophisticated leaves which face one other with short petiole and absented stipules (Orwa et al., 2009). It has about 4 – 6 petals of guava flowers, and yellow color anthers where pollination takes place through insects as the pollinators (Figure 2.2) (Naseer et al., 2018).

Figure 2.1: Guava plant (Johnson, 2013).

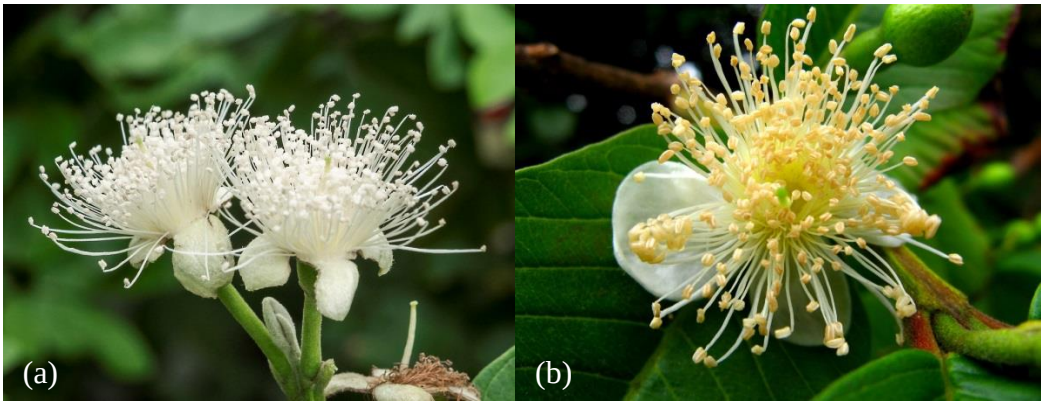


Figure 2.2: (a) Guava flowers with white anthers (Yeo, 2017) and (b) yellowish anthers (Mauroguanandi, 2008).

Guava fruit is often known as “apple of the tropics”, since it is a non-seasonal fruit and its year-round availability as an important exotic tropical fruit crop in the tropical and subtropical countries, and its high nutritive values are nearly similar

as those of commercially important temperate fruit apples (Musa, Abdullah and Subramaniam, 2015; Navin et al., 2018; Shishir et al., 2014). The guavas have strong, musky odor and taste sweet during ripening, and either ovoid (egg-shaped), round (globose) or pear-shaped (pyriform) with diameter of about 4 – 12 cm. It consists of a thin and yellowish edible outer layer with slight sour flavor (Mishra et al., 2017; Novriyanti, Riska and Wulan, 2017; Reddy et al., 2014; Uzzaman et al., 2018). There are numerous yellow seeds with more than 3 to 5 mm of length within a juicy yellowish or pinkish pulp (Novriyanti, Riska and Wulan, 2017).

Guavas can be classified into two major types, which including white-fleshed and pink-fleshed guavas. The contents of polyphenol, pro-vitamin A, and carotenoid vary between the white-fleshed guava (Figure 2.3; a) and pink-fleshed guava (Figure 2.3; b), where greater amount of these nutrients are found in pink-fleshed guavas (Reddy, 2014). Pink guava fruits exhibit a characteristic sweet and musky smell during ripening, with a thick and pinkish layer of flesh underneath the light-yellow fruit peel, numerous of yellowish seeds can be found in the central pulp of fruit (Heng, 2016). They have distinctive, strong, and acceptable aroma, therefore they may either be consumed freshly or processed into pickles, beverages, jams, and dehydrated guavas via clarification, concentration, canning and blending with other juices in local food industries (Heng, 2016; Sukeksi and Sarah, 2016).

Figure 2.3: (a) White-fleshed guava fruits (Staughton, 2018) and (b) pink-fleshed guava fruits (Tadimalla, 2015).

2.1.1 Nutritional Compositions of Pink Guava Fruits

Pink guava is known as “superfruit” as it is rich in dietary fiber content, vitamin A, vitamin C (ascorbic acid), folic acid, copper, potassium, manganese, tri-terpenes, flavonoids, phenols, tannins, and essential oils (Joseph and Priya, 2011; Kamath et al., 2014). A guava fruit contains about 86.0 g of calories, 1.0 g of crude fiber content, 0.5 g of protein, 17 mg of carbohydrates, 0.70 g of fat, and 10 g of ash content (Kamath et al., 2014). It serves as a good source of vitamin (vitamin C), antioxidants, dietary fiber contents (pectin) and essential minerals (calcium, iron, potassium and phosphorus) (Heng, 2016). In accordance to article by Hassimotto et al. (2005), a single guava fruit contains four times higher quantity of vitamin C (≈ 80 mg of vitamin C in 100 g of fresh fruit), as compared to an orange. Guava fruit contains high levels of carotenoids and polyphenols, thus it exhibits great

antioxidant capacities among plant-based foods (Joseph and Priya, 2011). The phenolic compounds (44.04 mg GAE per gram of guava fruit) such as apigenin and myricetin, anthocyanins, and ellagic acid have been quantified in pink guava fruit (Miean and Mohammed, 2011; Misra and Seshadri, 1968; Verma et al., 2013). Lycopene is the principle carotenoid, making up more than 80% of the total carotenoids of a single fresh pink guava fruit which contributes to pinkish color of guava pulp (Athapol, Imran and Anil, 2014; Haniza and Aminah, 2014; Shishir et al., 2014). It is the most potent carotene in family of carotenoid. It has excellent ability to exert physical quenching rate twice as high as that of β-carotent and ten times as high as that of α-tocopherol, and it has great antitumor properties which can protect against prostate cancer (Haniza and Aminah, 2014; Uzzaman et al, 2018).

Table 2.1: Nutritional compositions of pink guava fruit.

No.	Nutritional Composition	No.	Nutritional Composition
1	Vitamin A	**6**	Manganese
2	Vitamin C	**7**	Flavonoids
3	Folic Acid	**8**	Tri-terpenes
4	Copper	**9**	Essential oils
5	Potassium	**10**	Lycopene

2.1.2 Health-Promoting Benefits of Pink Guava Fruits

Guava fruit has high dietary fiber content, which aids in reducing the sugar levels in the body system and in the prevention of diabetes, colon cancer, hypertension, and other types of gastrointestinal disorder (Anderson et al., 2009; Uzzaman et al., 2018). Asmah et al. (2006) showed that the consumption of guava may improve the lipid profile and mitigate oxidative stress of the host. Therefore, it can reduce risks of diseases caused by free radical activities and even high blood cholesterol (Ayub and Norazmir, 2011). Ayub et al. (2010) reported that pink guavas exerted anti-hypertensive properties to the host.

The consumption of bioactive phytochemicals such as carotenoids and phenolics could promote great health benefits such as lowering the risk of chronic diseases (Liu, 2004). Fruits and vegetables contain high phenolics (such as flavonoids) and vitamin contents (vitamin C or ascorbic acid) could aid in reducing the risks of cancer and diabetes or the development of cardiac disorders (Liu, 2013). Fruit and vegetables in yellow or orange-red are good sources of carotenoids like lycopene and carotene (Liu, 2013; Uzzaman et al., 2018). For instance, orange-red guava fruits have more pigment contents such as pro-vitamin A, carotenoids, polyphenol, and retinoid as compared with those of yellow-green guava fruits (Joseph and Priya, 2011).

2.2 Pink Guava (*Psidium guajava* Linn.) Wastes

There are high demands on pink guava processed products, because pink guava fruits are rich in dietary fiber, essential minerals, and vitamins. Pink guava puree processing generates three types of waste materials: decanter, refiner, and siever (Kong and Ismail, 2011). These pink guava wastes are separated from the pink guava puree processing lines to produce puree products with the best quality and appearance (Kader et al., 2016).

The first step of puree processing involves cutting and crushing, at which 12% of pink guava fruits are removed from the guava pulp and disposed of as refiner with particle size approximately 1.2 mm (comprised of guava seeds and coarse pulp). Approximately 8% of pink guava fruits are disposed of as siever (particle size ≈ 0.8 mm, consists of a mixture of guava fruit peels and pulpy pits) from the sieving process (Kong and Ismail, 2011). About 5% of pink guava fruits are discarded as decanter with stone cells and particle size less than 0.8 mm from the decanting process (final stage). It appears in slightly pinkish color due to the presence of lycopene (Kong and Ismail, 2011). Stone cells or scleroids are eliminated from the flesh, in order to avoid any gritty texture within the pink guava final products (Hui, 2006).

Kong and Ismail (2011) reported that pink guava wastes have high amount of sugar, mineral, and vitamin contents which are nearly similar to those found in the pink guava processed products. Nevertheless, the presence of fibrous components: cellulose, hemicellulose and lignin make these wastes to have low digestibility of nutrients (Lousada et al., 2005). Thus, the structure of fibrous components should be modified by biochemical treatments (enzymes), and expectably to enhance the functional properties of pink guava wastes for value-added purposes (Anil, 2017).

2.2.1 Nutritional Compositions of Pink Guava Wastes

In accordance to the previous study carried out by Maniyan, John and Mathew (2015), guava peels have the highest carbohydrate content (75 mg/mL) among fiber-rich fruits (bananas, apples, pomegranate, and grapes). Besides, guava peels exhibit excellent reducing activity (0.47%), and protein (0.19 mg/mL), and low quantity of anti-nutritional factors like tannin and phytic acid (Maniyan, John and Mathew, 2015). In addition, guava peel extracts possess relatively high phenolic content (7.21 g GAE/100 g crude extract) and reducing power (Maniyan, John and Mathew, 2015; Marina and Noriham, 2014). According to previous research performed by Uchôa-thomaz et al. (2014), guava seeds are excellent sources of antioxidant dietary fiber, as they consist of lignin and cellulose, vitamin C, carotenoids, and protein (Keith, Graham and Craig, 2010).

The pink guava wastes especially decanter collected from the pink guava puree production had been reported to be rich in lycopene contents which contribute excellent antioxidant properties (Kong and Ismail, 2011). According to Kong and Ismail (2011), pink guava wastes have high lycopene contents and antioxidant activities; therefore they serve as great potential sources of nutrient enrichments.

2.2.1.1 Carbohydrates Constituents in Pink Guava Wastes

Basically, agricultural wastes are composed of non-starch polysaccharides such as lignin, hemicellulose, and cellulose, in which they are unpalatable due to limited digestion upon consumption, but they support a sustainable slow release of carbon source (Mhd Abd Kader et al., 2016; Yang et al., 2015), and a physical structure for the growth of microbial community (Yang et al., 2015). Pink guava wastes (decanter, refiner, and siever) had been reported to have carbohydrates, which ranged from 11.82 to 12.18%, hence they have great potential to be carbon source as good substrates for fermentation (Mhd Abd Kader et al., 2016).

2.2.2 Limitations of Directly Apply Pink Guava Wastes in Human Diet

The highly complex and variant compositions of agricultural wastes become the major challenge in waste valorization, as it could restrict the values and functional properties of waste material for reutilization (Lim et al., 2018). Guava wastes are rich in total and soluble dietary fiber contents (Lim et al., 2018). Just like guava fruits, fiber-rich characteristics are important in promoting hypoglycemic and hypocholesterol effect (Chang, Sit and Dhanapal, 2017). This is due to the fiber-rich foods often possess high capacity in cation binding, absorption of bile acids and water-holding (Dziedzic et al., 2012). However, there are huge quantity of cellulose content in guava wastes, which are insoluble dietary fiber contents and indigestible (Lim et al., 2018; Mhd Abd Kader et al., 2016), which could lower its digestibility, and constraint bioavailability of other bioactive compounds in guava wastes (Lim et al., 2018; Lunn and Buttriss, 2007).

2.3 Dietary Fiber Constituents

2.3.1 Background Review on Dietary Fiber

The term “dietary fiber” was originated by Hipsley (1953), is referring to non-digestible lignocellulosic constituent in the framework of plant cell wall (Figure 2.4) (Dhingra et al., 2012). Later, dietary fiber was defined as a plant-originated polysaccharide which cannot be digested and absorbed in gastrointestinal tract upon consumption (Van et al., 1983). Based on AACC International (2001), the definition of “dietary fiber” is updated as analogous carbohydrate and edible part

which is resistant to digestion and absorption in human small intestine with partial or complete fermentation in the large intestine.

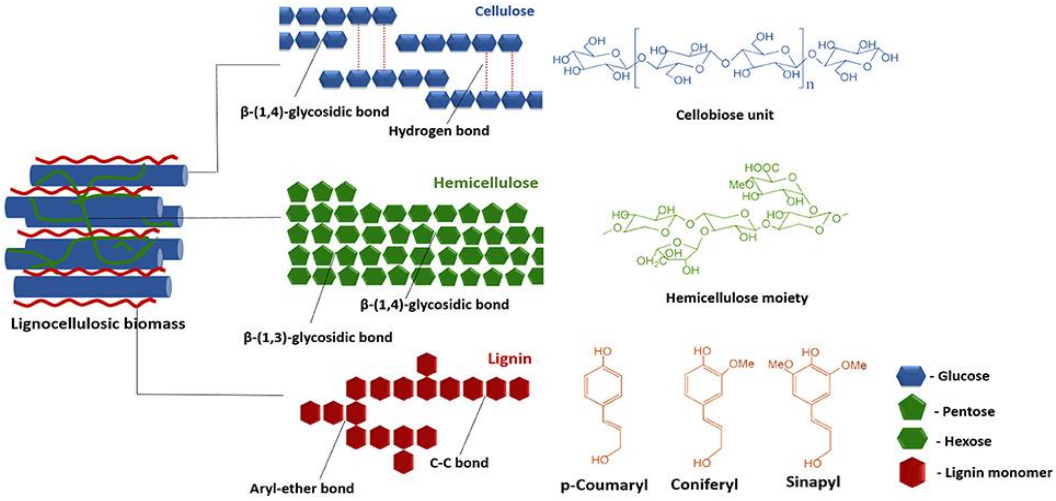


Figure 2.4: The structure of lignocellulose (Baruah et al., 2018).

Basically, all plant-based foods contain fiber-rich cell wall materials, but they are inconsumable and indigestible, since these fiber-rich materials fiber-rich materials cannot be decomposed in the human gut and influence moisture absorption in human digestive system (Lunn and Buttriss, 2007; Manzoni, Castelnuova and Molinari, 2008). Dietary fiber can be classified into two major types: insoluble dietary fiber (IDF) and soluble dietary fiber (SDF). Insoluble dietary fiber (IDF) (cellulose, hemicellulose and lignin) is also known as water-insoluble fiber which cannot be digested or absorbed. Meanwhile, soluble dietary fiber such as glucan, pectin and gum is also cannot be digested or absorbed by human bodies, but it is partial soluble in water (Yang et al., 2017).

2.3.2 Cellulose

Cellulose is an insoluble dietary fiber and classified as non-starch polysaccharide. It contains an un-branched linear chain along with over ten thousands of glucose units with β-(1,4)-glycosidic linkages (Figure 2.5) (Dhinga et al., 2012; Klemm et al., 2005; Lunn and Buttriss, 2007; Yang et al., 2017). Cellulose contributes about 25% of total fiber content in fruits and grains, and approximately 33.33% of total fiber content in nuts and vegetables (Zaragoza et al., 2010). Native cellulose is naturally difficult to be dissolved due to thermodynamics and high degree of polymerization, in which the entropic gain is reduced in the dissolution process (Jedvert and Heinze, 2017).

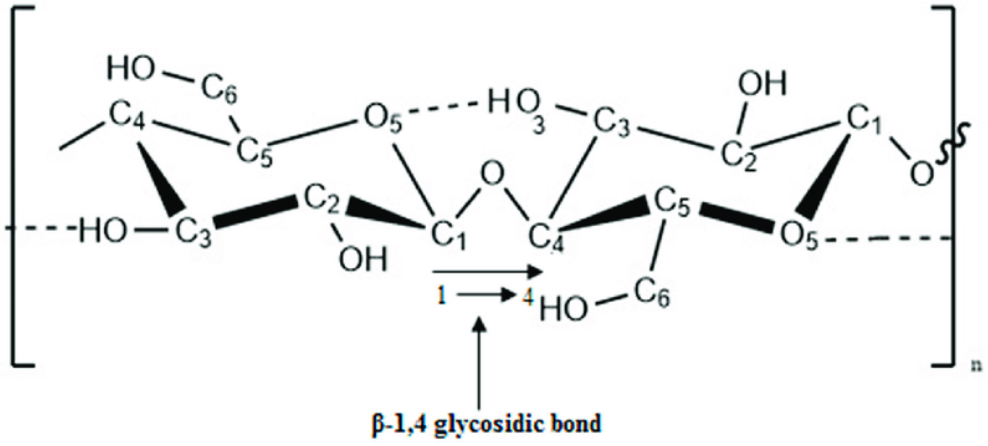


Figure 2.5: The structure of cellulose (Sampath et al., 2016).

Cellulose usually can be obtained from natural resources, such as annual plants, trees, algae, fungi and bacteria (Jedvert and Heinze, 2017). It can be isolated from woods through pulping: the sulfite process and the Kraft or sulfate process (Sixta, 2006). During pulping, celluloses may be liberated from lignins and other plant-based components like hemicelluloses, and results in the production of pulps with various fiber purities and strengths. For instance, paper-grade pulps are used for printing paper while dissolving grade pulp to produce regenerated cellulose and cellulose derivatives (Jedvert and Heinze, 2017).

2.3.3 Hemicellulose and Xylan

Hemicellulose is a non-starch polysaccharide and associated with cellulose in the framework of plant cell wall (Zaragoza et al., 2010). It represents up to 50% of the total biomass of perennial and annual plants (Albertsson and Edlund, 2011). Hemicellulose presents in both water insoluble and soluble forms (Zaragoza et al., 2010), as it can be solubilized by aqueous alkali solvent after the separation of water soluble and pectic polysaccharides (Kay, 1982). Besides, hemicelluloses are heteropolysaccharides which built up by backbones of glucose units via β-1,4 glycosidic linkages (Figure 2.6) (Kay, 1982; Sárossy, 2011). As compared with cellulose, hemicelluloses are smaller in size with a variety of sugars in branched-structure (Kay, 1982).

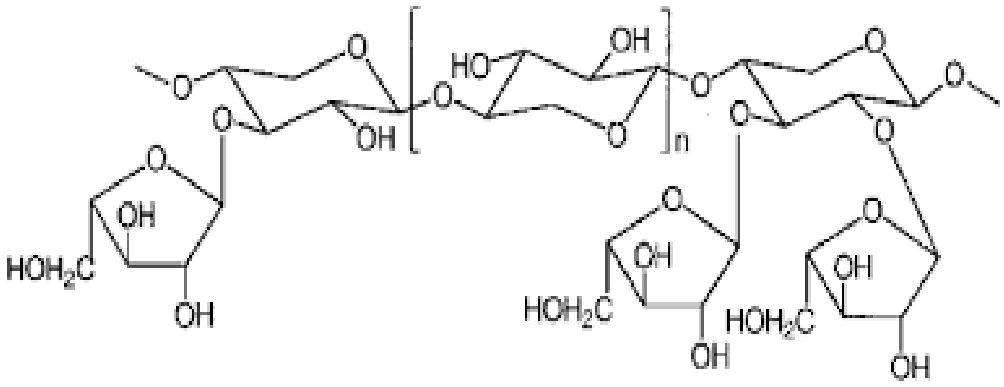


Figure 2.6: The structure of hemicellulose (Kontturi, 2015).

Hemicellulose is a complex polymeric carbohydrate which is mainly composed of pentose sugars. According to Harris and Ramalingam (2010), xylan is the main component in hemicellulose that holds the plant cell walls together. In the hemicellulose structure, xylose is the major constituent, while mannose, arabinose, galactose and other sugars are present in lower concentration (Anita and Abraham, 1997; Prajapati et al., 2018; Tabañag and Tsai, 2018). In human digestive system, hemicelluloses are partially fermented by the colon microorganisms, and results in the production of volatile fatty acids. They function with other insoluble dietary fibers, which results in reducing the time of intestinal transition (Mudgil, 2017).

Xylan is a complex polysaccharide comprised of a backbone of xylose residues connected by β-1,4-glycosidic linkages (Figure 2.7) (Harris and Ramalingam, 2010). Xylan is often known as heteropolysaccharide which contains substituent groups of acetyl, α-arabinofuranosyl, and 4-o-methyl-D-glucuronosyl residues.

These substituent groups are connected to the backbone β-1,4-glycosidic linked with xylopyranose units (Kanimozhi and Nagalakshimi, 2014). Xylan has great binding properties mediated with cellulose, lignin, and other polymers by the covalent and non-covalent interactions, in which lignin is attached and bound to xylan by an ester linkage to the 4-o-methyl-D-glucuronosyl residues (Kanimozhi and Nagalakshimi, 2014).

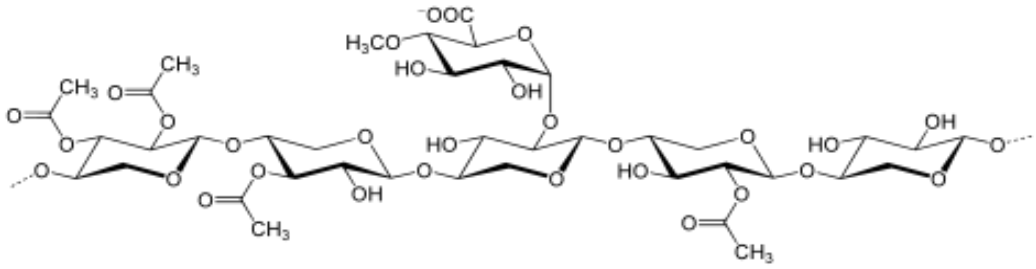


Figure 2.7: The structure of xylan (Nimz et al., 2000).

2.3.4 Lignin

Lignin is not a polysaccharide (Figure 2.8). However, it is a complex cross-linked random polymer that contains about 40 oxygenated phenylpropane units, such as sinapyl, coniferyl, and *p*-coumaryl alcohols (Braums, 1952; Schubert, 1956; Theander and Aman, 1979). Lignin could be varied in methoxyl contents and molecular weights, where the formation of strong carbon-carbon intramolecular bonding results in lignin to be inert (Dhingra et al., 2012). It is the most abundant renewable source with aromatic units in nature; in which the chemical structure of lignin enables itself to be a more sustainable option as a feedstock for aromatic chemicals (Li et al., 2015). However, the existing markets for lignin products are

limited to lower value products such as binder or dispenser applications in cement and polymers. There were only approximately 2% of the lignins available to be collected from the pulp and paper industry for commercial applications, in which those remaining lignins were burned as low value fuels (Gosselink et al., 2004).

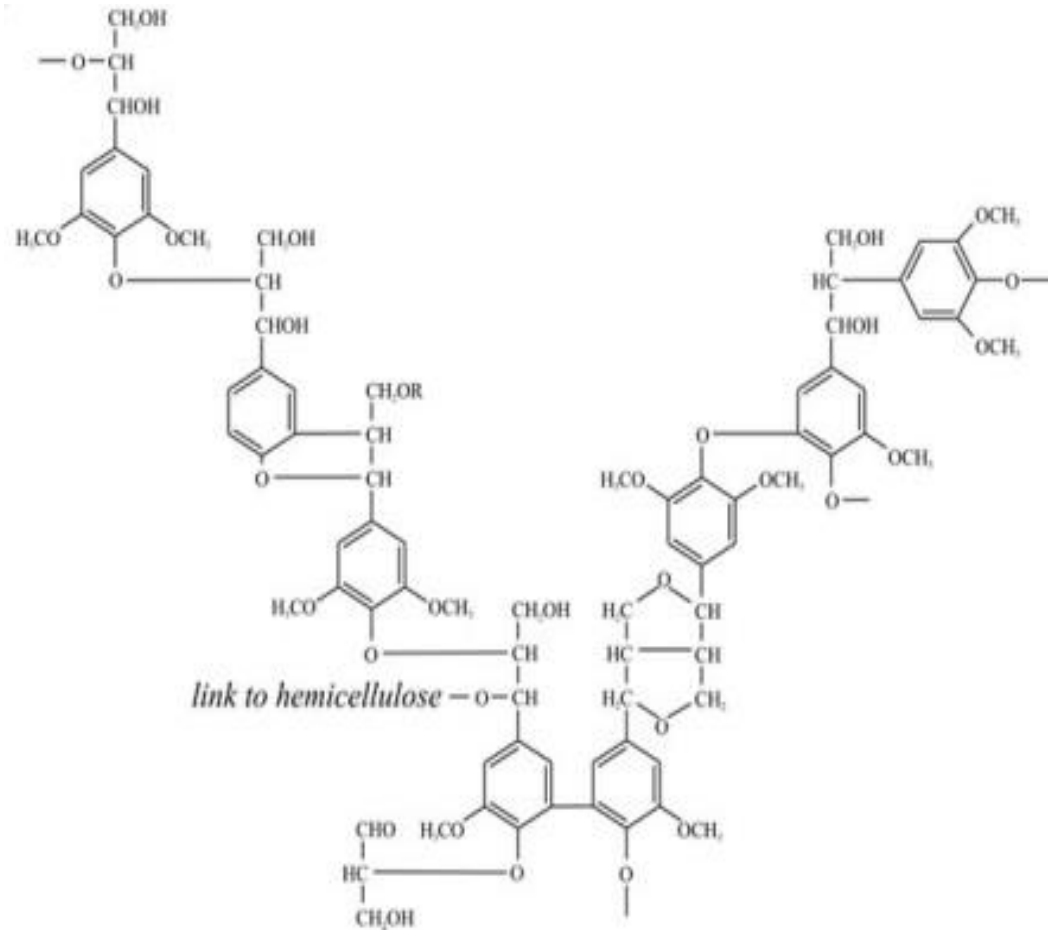


Figure 2.8: The structure of lignin (Moore, Robson and Trinci, 2016).

2.3.5 Pectin

Pectin is a heteropolysaccharide available in the primary cell walls of various plant-based materials (Figure 2.9) (Sulieman, Khodari and Salih, 2013; Hafeez et al., 2014). Pectin is often associated with other important cell wall components (cellulose, hemicellulose, and lignin) (Lara-Espinoza et al., 2018). It contributes to the structure and firmness of plant tissues, and also involves in the mechanical resistance and intercellular adhesion of plant cells, and enhancing turgidity (Lara-Espinoza et al., 2018). Pectin is built up by a backbone of (1→ 4)-linked α-D-galacturonic acid (GalUA) residues, in which the homogalacturonic (HG) regions are often interrupted by those rhamnogalacturonic (RG) regions containing (1 → 2)-linked α-rhamnose (Rha) residues (V. Smirnov et al., 2017).

Pectin is mainly composed of galacturonic acid units with variations in structures, compositions and molecular weights (Lara-Espinoza et al., 2018). In fact, it is an important plant cell wall component which can be obtained via aqueous extraction of the plant-based materials and commonly can be extracted from apple pomace and citrus fruit peels, followed by a salt or alcohol-induced precipitation (Kumar, Sharma and Singh, 2018). It is a powder-formed product which appears in white or light-brown, and widely used as gelling-agent in jams and jellies, stabilizer in fruit juice and dairy beverages, and even medicines (Sulieman, Khodari and Salih, 2013). There are several advantages of employing pectins as potential additives in food and pharmaceutical preparations. Pectins have excellent gel forming ability

due to the presence of divalent cations, which enables them as appropriate carriers to deliver bioactive agents (Kumar, Sharma and Singh, 2018). Besides, pectins can be applied for delivering drugs to consumers via vaginal route, oral and nasal, as they have long-standing reputation without toxicity and high availability with low production cost (Kumar, Sharma and Singh, 2018).

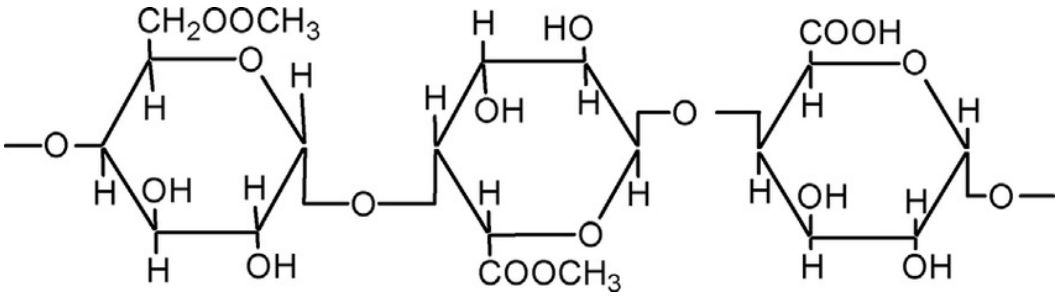


Figure 2.9: The structure of pectin (Hassan et al., 2017).

2.3.6 Health Effects of Dietary Fiber

Based on Lairon and Arnault and Bertrais (2005), the high levels of dietary fiber intakes are associated with the reduction of prevalence rates for cardiovascular diseases such as coronary heart disease (CHD), peripheral vascular diseases and stroke. High concentrations of dietary fiber consumptions can minimize the risk of metabolic disorders such as diabetes, obesity, hypertension, and dyslipidemia (Anderson et al., 2009; Kaczmarczyk, Miller and Freund, 2012). Dietary fibers are more supportive in the gastrointestinal microbial community as compared to refined diets, as they consist of numerous monosaccharides unit with α- and β-

linkages (Cummings and Macfarlane, 1991; Kaotari et al., 2013). Dietary fiber consumption is beneficial to the human digestive system, mainly due to it aids in relieving constipation. Insoluble dietary fiber can withdraw water from the human digestive system and increases the bulk, which lead to food mass in the intestine to be soften. Insoluble dietary fiber can minimize the transit time for it to travel through the digestive system, and makes the elimination to be easier (Rana et al., 2011). Meanwhile, when soluble dietary fiber (pectins, inulin-type fructans and gums) by-pass through human small intestine, it will be fermented easily by the microflora of large intestine (Wong and Jenkins, 2007). It can be fermented by bacteria produced by short-chain fatty acids, which can promote the fermentable fibers to reach the host colon (Dahl and Stewart, 2015). The fermentable fibers can lower the pH condition in the colonic lumen, thus enhance the bioavailability of minerals and inhibit the growth of pathogenic bacteria (Hosseini et al., 2011).

2.3.7 Applications of Dietary Fiber in Foods

Large quantities of food processing wastes are generated and not being utilized, but disposed of at landfills. If these food processing wastes could be used as a source of dietary fiber, this would reduce environmental pollution problems, and a variety of food additives or food ingredients may be developed to produce value-added food products (Dhingra et al., 2012; Figuerola et al., 2005; Jamie et al., 2002; Sharma et al., 2016).

Dietary fiber component has excellent water-binding capacity and gel forming ability, thickening, fat mimetic, texturizing, and anti-clumping effects. Thus, the addition of dietary fiber can improve sensory characteristics, texture and prolong shelf life of foods (Yangilar, 2013). Dietary fiber also imposes desired functional properties in food. It acts as a bulking agent to minimize sugar content of food products and to hold moisture content in food for fat mimic (Yangilar, 2013).

For instance, the addition of dietary fibers in beverages and drinks can enhance their overall stability and viscosity, in which soluble dietary fiber (SDF) is the most commonly used for this application as SDF is more dispersible in water as compared to insoluble dietary fiber (IDF) (Bollinger, 2001; Rodrigues et al., 2006). Besides, dietary fibers are also introduced in dairy products (Yangilar, 2013). For instance, dietary fiber incorporation in diary product aids to improve the mouth-feel and body in ice cream or cheese analogues (Blecker et al., 2001). Dietary fibers have been added as a functional ingredient in yogurt, to enhance the nutritive value and minimize the syneresis formed in yogurt products (Blecker et al., 2001; Staffolo, et al., 2004).

Dietary fibers incorporation in bakery products has been developed and found to enhance nutritional quality, higher water retention, greater antioxidant capacity, and higher extent of fermentability (Yangilar, 2013). For instance, Kurek and Wyrwisz (2015) states the application of dietary fibers in bread making process

can enhance water and oil holding capacities, improve texture properties and shelf life. Besides, dietary fiber addition in meat products also enhanced the overall acceptability, improved the yield and processing characteristics, and prolong shelf life of meat products (Galanakis, Tornberg and Gekas, 2010; Talukder, 2015).

2.3.8 Prebiotics

The term of "prebiotics" was defined as the non-digestible food ingredients which beneficially influence host by selectively stimulating the growth and/or activity of one or a limited number of bacteria in the colon, results in improving the host's health (Gibson, 1995). Later, it had been updated and revised as a selectively fermented ingredient allows specific changes, both in composition and/or activity in the gastrointestinal microflora which confers health benefits (Gibson, 2004). Prebiotics are also referred as unique dietary fibers. They are often employed in promoting growth and/or metabolic activities of species, and eventually contribute in promoting health benefits to the consumers upon consumption (Bellei and Haslberger, 2012).

Prebiotics are highly stimulated in the bacterial fermentation, and bring about the replication of the target genera, eventually the formation of short-chain fatty acids (SCFAs) (Venter, 2007). Lactobacilli and bifidobacteria are the common target genera for prebiotics, at which the changes in bifidobacteria are more likely to be

investigated as compared with lactobacilli. This is because more bifidobacteria reside in the human colon as compared to lactobacilli, and act as stable adherent microbiota to maintain the mucosal barrier; therefore they can exhibit preference for prebiotics (Slavin, 2013; Fahey, 2010).

Prebiotic fibers are indigestible food ingredients. They can be fermented in the colon by specific health-promoting bacteria (Neill, Orafti and Malvern, 2007). Prebiotics (fermentable dietary fiber) are carbon source in promoting the growth of beneficial taxa and to deliver a selective to confer the host health related to its metabolism (Carlson et al., 2018; Poeker et al., 2017). Prebiotic dietary fibers are indigestible, but they can be metabolized by beneficial gut microbes throughout fermentation, which depends on the physicochemical properties of carbohydrates, fiber dosage and bacterial community composition on the individual consuming prebiotic dietary fiber (Holscher, 2017). In accordance to Gibson et al. (2010), the primary mechanism of action for dietary fiber and prebiotics is fermentation. The consumption of prebiotic dietary fibers can reduce protein fermentation, improve immune system defense and minimize allergy risk, enhance calcium absorption and lower pathogenic bacteria populations in human gastrointestinal tract (Carlos et al., 2018).

2.3.9.1 Applications of Prebiotics in Food Products

Prebiotics can be found naturally in foods such as breast milk, garlics, wheat, soybeans, and onions (Cook et al., 2016; Loo et al., 1995; Pandrey, Naik and Vakil, 2015). Prebiotics such as inulin, oligofructose, and galactooligosaccharides allow nutrient content and structure claims, therefore prebiotics have been used as functional ingredients or "invisible" fiber sources to formulate and produce fiber-enriched products such as yogurt, bread, and beverages without causing negative effects on texture, mouthfeel, and appearance of food (Brownawell et al., 2012; Cook et al., 2016; Neil, Orafti and Malvern, 2007; Ozcan et al., 2016). In order to exert function as a prebiotic, the food ingredient should be resistant to digestive enzymes in upper gastrointestinal tract, fermentable by gastrointestinal microbiota, and selective in the stimulation of growth or activity of beneficial bacteria (Bellei and Haslberger, 2012). Based on Neill, Orafti and Malvern (2007), prebiotics are good natural choices to be added into food products which are eaten daily, so that there is a continual health benefit exerted by prebiotics to the consumers upon consumption. Particularly, prebiotics have been introduced as food additives in dairy products (milk, yogurts, and etc.) to enhance nutritional claims. For instance, inulin inclusion in fermented dairy products has been claimed, to enhance calcium absorption, and to improve digestive system (Neil, Orafti and Malvern, 2007). Consumption of prebiotic fortified food such as yogurt is claimed to be capable to minimize the risks of obesity, cancer, colonic disorders, and constipation, and aid in promoting gastrointestinal immunity and intestinal microflora growth (Dabija et al., 2018).

2.4 Plant-Based Wastes Valorization

2.4.1 Plant-Based Wastes

Food waste exists as a critical environmental issue in developed and developing countries, as it causes environmental pollution and huge loss of valuable nutrients (Jin et al., 2017). Food wastes are end products obtained from the food processing industries which have not been utilized for other purposes and recycled (Garcia, et al., 2015). Food waste sources are highly diverted, and can be classified based on the origins of waste materials: animal-based wastes and plant-based wastes (FAO, 2011; Galanakis, 2012), in which plant-based waste fills larger portion; nearly 63% of the whole food supply chain (Pfaltzgraff et al., 2013).

Plant-based wastes consist of kernels, peels, seeds, skin, pomace, stone, stem, rind, and other parts of plant materials (Joshi and Sharma, 2011). They are rich in dietary fiber (McKeee and Latner, 2000), mainly hemicelluloses (75%), cellulose (9%), lignin (5%), pectin, other essential nutrients (proteins and lipids), and phytochemical (Jin et al., 2017; Mirabella, Castellani and Sala, 2013). Thus, there is a great potential to recover plant-based wastes with high nutritive values or convert them into higher-value and novel products (Jin et al., 2017). For instance, cellulose can be used to produce organic acids and biofuel. Hemicellulose can be hydrolyzed into xylose, then further converting into xylite and furfural which are useful chemical products; while lignin can be utilized as natural adhesives and binders, and further derivatization to phenols (Kamm and Kamm, 2004).

2.4.2 Factors to be Considered in Wastes Disposal

Waste materials are disposed of from desired final products; because there is no appreciable value after processing (Brown and Brown, 2014). Waste disposal or the clearance of un-utilizable waste materials, especially in the food industry, is complicated as there are several factors should be considered for waste disposal planning (Oreopoulou and Russ, 2007).

The vast amount of water content in organic wastes is a critical issue in terms of disposal costing and microbial contamination. For instance, higher transportation cost is required to dispose meat and vegetable wastes which have high water content, between 70% and 95% by mass. On the other hand, the elimination of water content mechanically can cause further problems with the disposal of waste water. This is due to there are high levels of organic materials in the waste water, and could provide a favorable medium for microbial growth (Oreopoulou and Russ, 2007). Structure alteration caused by enzymatic activity is also a critical factor to perform waste disposal. Enzymes remain in active status in the structure of plant-based and animal-based wastes with relatively high water activity, if the wastes are not subjected to proper treatment. Enzyme-catalyzed reaction proceed in the organic wastes could results in increased spoilage rate (Oreopoulou and Russ, 2007).

Biological stability of waste materials and possible potential growth of pathogens are critical issues to be deliberated prior to any waste disposal. The plant-based or animal-based waste materials might have been contaminated by large number of microbes with expeditious microbial activity, and results in arising environmental hygiene problem (Oreopoulou and Russ, 2007). The breakdown of proteins which takes place within the structures of waste materials could generate strong and unfavorable odors. Moreover, rapid autoxidation (β-oxidation) could take place within these waste materials which contain high fat content, and results in the release of foul-smelling fatty acids caused by breakdown of fatty acid compounds (Oreopoulou and Russ, 2007; Tham, 2016). Therefore, management of the waste materials should be diligent handled with additional operating costs, to get rid of environmental pollution and spreading of infectious diseases (Oreopoulou and Russ, 2007; Tham, 2016).

2.4.3 Methods to Manage Waste Materials

There are four waste management methods to be practiced and implemented by the industries based on the characteristics of waste materials (Arancon et al., 2013; Oreopoulou and Russ, 2007). Incineration is one of the methods to be used in the waste disposal management. It can be applied and utilizable on waste materials with less than 50% of water content by mass. During incineration, heat energy can be generated and emitted which can be utilized for heating processes and to run steam turbines (Oreopoulou and Russ, 2007).

If the waste materials have more than 50% of water content by mass, anaerobic fermentation is the most applicable method to convert them into utilizable energy (Arancon et al., 2013; Oreopoulou and Russ, 2007). Besides, composting should be implemented to degrade cellulose and hemicellulose, instead of incineration or anaerobic fermentation. Composting can be carried out above ground and on the disposal site (Oreopoulou and Russ, 2007), where it involves microorganisms to breakdown lignocellulosic constituents of fiber-rich wastes (Oreopoulou and Russ, 2007). Food wastes rich in essential minerals can be reutilized as applicable organic fertilizers, as they are resistant to be converted them into animal feeds (Kirchgessner, 1997; Oreopoulou and Russ, 2007; Westendorf, 2000). For instance, pomace and pulp wastes should be utilized as organic fertilizer instead of animal feeds, due to presence of pesticide residues and phenolic compounds in the pomace and pulp (Kirchgessner, 1997; Westendorf, 2000).

In addition, food wastes disposal may be integrated in the farming of livestocks (Oreopoulou and Russ, 2007). For instance, food wastes with great amounts of protein and fat content can be employed as animal feeds for omnivores, since omnivores can digest protein and fat contents efficiently. However, these food wastes are susceptible to microbial spoilage or easily contaminated by pathogenic microbes. Thus, they should be sterilized before being utilized as animal feeds for omnivores, to ensure they are safe to be consumed (Oreopoulou and Russ, 2007). Among the livestock, ruminants can be fed with food wastes rich in hemicellulose and cellulose directly, as ruminants possess the enzymes required to break down

both lignocellulosic constituents in the plant-based wastes (Oreopoulou and Russ, 2007). In accordance to Chandrasekaran (2013), feeding food waste materials as animal feeds directly to livestock is a great way to reutilize them, and minimize the amount of solid and liquid wastes as landfills.

Waste valorization is a newly developed method which focuses on the food waste materials with high concentrations of fibrous materials (Oreopoulou and Russ, 2007). For instance, insoluble and soluble fiber constituents from agriculture wastes such as peels from citrus fruits can be used as binding agents in foods, as they have great abilities and absorptive properties in gel formations (Oreopoulou and Russ, 2007). The fibrous materials sourced from spent grains used to produce building or structural materials and fillers in the fiber boards (Chandrasekaran, 2013; Oreopoulou and Russ, 2007).

2.4.4 The Concepts of Waste Valorization

In view of waste disposal in huge quantities can create environmental pollution issues (Jin et al., 2017; Loizidou, 2016), environmental awareness emphasized on wastes reduction and promotion in reusing, recycling and energy recovery from waste materials, has been well-intensified since decades ago. Waste valorization is the process of converting any waste materials into more applicable products (fuels, materials and chemicals) (Arancon et al., 2013). Based on Chandrasekaran

(2013), the term of “valorization” is defined as the progress of creating value from knowledge towards less-valued residues or wastes or by-product into a product which is more suitable and appreciable (Chandrasekaran, 2013).

Food processing waste valorization is essential due to the increase of population growth. Based on the Population Division of the DESA of the United Nations Secretariat, there will be approximately nine billion world human population as it approaches to year 2050 (Tham, 2016). There are various challenges in fulfilling global demands and preserving healthy society in terms of shelters, food supplies increment, food resource sustainability, livelihoods availabilities (Chandrasekaran, 2013; Tham, 2016). Waste valorization is one of the solutions in coping with the challenges. It results in the recovery of potential biomolecules and minimizes the wastage of natural resources (Chandrasekaran, 2013; Galanakis, 2015).

Apart from these, high industrial production expenses trigger to explore waste valorization which is feasible, due to the abundancy of agricultural by-products or agro-industrial waste materials which are potential feedstock and raw material. Public listed corporations invest in exploring for raw materials at lower expenses and invent cost-effective non-complicated technologies to fulfill the necessities of food processing and pharmaceuticals industries (Chandrasekaran, 2013). Thus, derivatives of waste materials can be valorized and added into new developed food products or used as natural functional ingredients to prolong food shelf life

or boosting the nutritional contents (Galanakis, 2015). For instance, carotenoids and phenols from fruit wastes can be valorized and applicable as natural food preservatives, as they can prolong shelf life by delaying deterioration and enhance antioxidant activity (Chandrasekaran, 2013; Galanakis, 2015).

2.4.5 Applications of Fruit Wastes Valorization

Large amount of fruit and vegetable wastes are generated during the processing, packing, distribution, and consumption stages (Wadhwa and Bakshi, 2013). In Malaysia, there was about 0.68 million tons of wastes originated from fruits and vegetables (Tham, 2016). The overall waste composition in Malaysia is mainly built up by about 64% of municipal solid waste (MSW) with 50% of food waste materials (Ghafar, 2017). In Malaysia, these solid food waste materials can be managed by destruction, incineration, decomposing or depositing, according to the Malaysia Solid Waste and Public Cleansing Management Act 2007 (ACT 672) (Lim et al., 2016; Ngapan et al., 2012). An alternative method to diligent manages various agricultural waste materials is "wastes to opportunities for development": to valorize them as a source for feedstock or used as value-added products (Tham, 2016). Based on Khan et al. (2015), these -waste are rich in bioactive compounds to be extracted for further utilization. They are known as lignocellulosic-rich and nutrient-rich materials; therefore they can be converted into value-added products via enzymatic treatments, and fermentations such as solid state fermentation (SSF) or submerged fermentation (SmF) or used as ideal substrates for microbial growth.

Enzymatic treatment is an environmental friendly method to hydrolyze food waste materials into other fermentable or biofuel products (Lam, Kwan and Lin, 2015). The enzyme-assisted waste valorization can be applied in the waste management by food processing industries, as these food waste materials have great nutritional properties such as proteins, sugars and lipids which can be converted to value-added products by enzymes or to be utilized by microbes (Chandrasekaran, 2013).

Fruit wastes such as peels, pomace, bagasse, brans, shells, and seeds account for over 50% of respective fresh fruits. Most of the fruit waste materials have higher nutritious values with excellent functional properties as compared to those of final products (Ayala et al., 2011). These agricultural waste materials can be valorized as raw materials or food additives (Chandrasekaran, 2013; Torres-León et al., 2018). This could generate economic gains for the industries, contributes health-promoting benefits for human consumptions, and minimize environmental issues caused by improper waste management (Torres-León et al., 2018). For instance, apple pomace is the most common fruit waste generated from puree processing lines. Apple pomace comprises of soft tissues, cores, peels, calyxes, seeds, and stem with high water content, and rich in simple sugars, insoluble carbohydrates, proteins, minerals, and polyphenols. Based on previous research, apple pomace exhibited higher radical scavenging power than apple juice (Chandrasekaran, 2013). Apple peels are rich in polyphenols with antioxidant properties, which aid to inhibit the growth of cancer cells and act as potential protection agents against cardiovascular diseases (Chandrasekaran, 2013). Citrus fruits (orange, mandarin,

grapefruit, lemon, and lime) are consumed freshly due to abundance amount of vitamin C (antioxidant), fibers, and minerals (Rezzadori, Benedetti and Amante, 2012; Torres-León et al., 2018). There are large quantities of citrus wastes mainly composed of citrus peels and pressed pulp, generated from puree processing lines (Köse and Bayraktar, 2018; Moraes et al., 2013). These citrus wastes have low pH value, high water content (80 – 90%), and high organic matter content with about 95% of total solids (Ruiz and Flotats, 2014). A small percentage of citrus wastes can be valorized to obtain citrus essential oils, and reutilized in the cosmetic products, pharmaceutical and food products (Torres-Leôn et al., 2018). According to John, Muthukumar and Arunagiri (2017), citrus peel is valuable lignocellulosic feedstock to be utilized in producing bioethanol due to its richness in fermentable sugars and low lignin contents.

Researches related to waste valorization has been explored to study the possibility of valorizing potential waste materials for animal feeds and human consumption, with escalating production of food wastes (Tham, 2016). Based on Oreopoulou and Russ (2007), spent grains from brewery and wastes from carrots are fiber-rich materials, which can be converted to produce fibers as functional ingredients for binding water in food products due to gelling abilities and absorptive properties. The variability and complexity in composition of these waste materials are the major challenges in waste valorization. Thus, further researches on valorization of fibrous materials are critically essential (Tham, 2016).

2.5 Fiber-degrading Enzymatic Treatments

Figure 2.10 shows the enzymatic degradation of common lignocellulosic biomass such as sugarcane, cotton, wood, and corn residues. Lignocelluloses are mainly comprised by cellulose, hemicellulose, lignin, and pectin, which are the critical polymeric constituents to build up the cell wall structure of plant-based materials. Lignocelluloses found in plant-based materials may be hydrolyzed, degraded, and converted into value-added and utilizable products such as animal feeds, animal foods, biofuel and etc. through pretreatments.

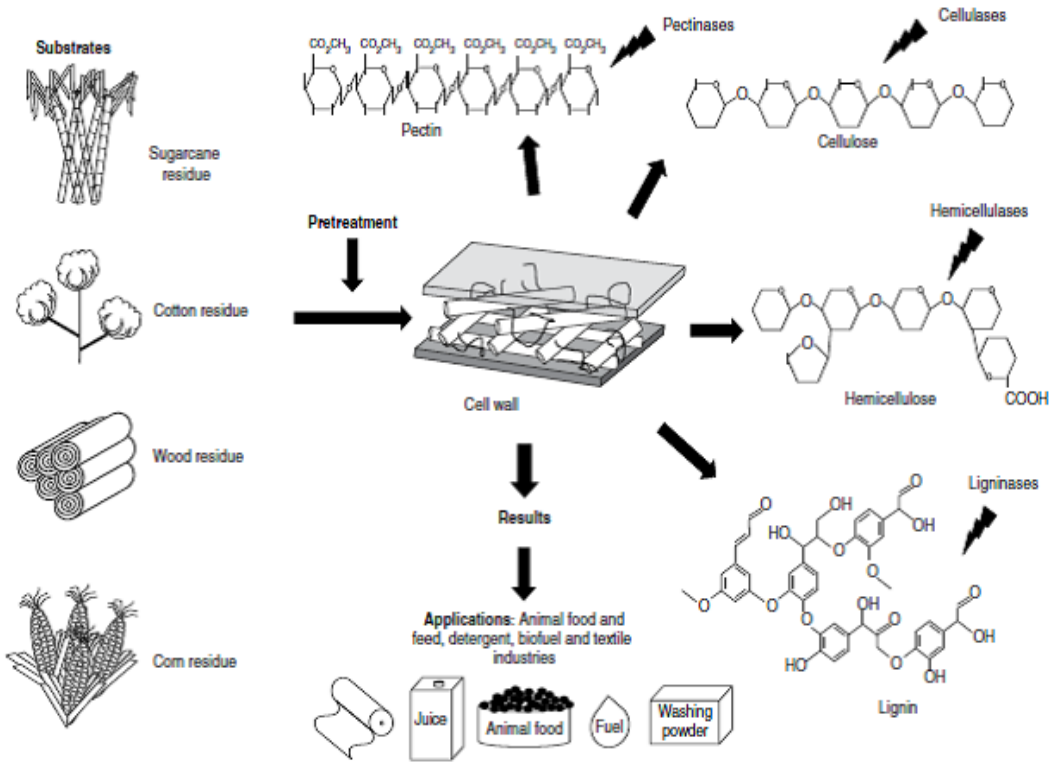


Figure 2.10: The enzymatic degradation of common lignocellulosic biomass (D. Jaramillo et al., 2015).

Acid hydrolysis is a common chemical method to degrade polymeric constituents in plant-based waste materials, as they are indigestible for animal and human consumption. Nevertheless, it generates hazardous acidic wastes and causes more technical difficulties to recover sugar from acid solution (Prajapati et al., 2018). Besides, acid hydrolysis has lower product yield via high process temperature (140 – 160℃), and neutralization is required after acid hydrolysis (Rozenfelde et al., 2017; Taher-zadeh and Karimi, 2007). Meanwhile, enzymatic hydrolysis is an environmental-friendly method to valorize plant-based wastes without producing any toxic waste during hydrolysis (Bahseer, Haq and Zahoor, 2014; Prajapati et al., 2018). Cellulase and xylanase are common fiber-degrading enzymes used to degrade lignocellulosic constituents by hydrolyzing β-1,4-glycosidic linkages in the plant structural polysaccharides (cellulose and xylan) (Figure 2.11 and Figure 2.12) (Gilbert and Hazlewood, 1993; Rozenfelde et al., 2017).

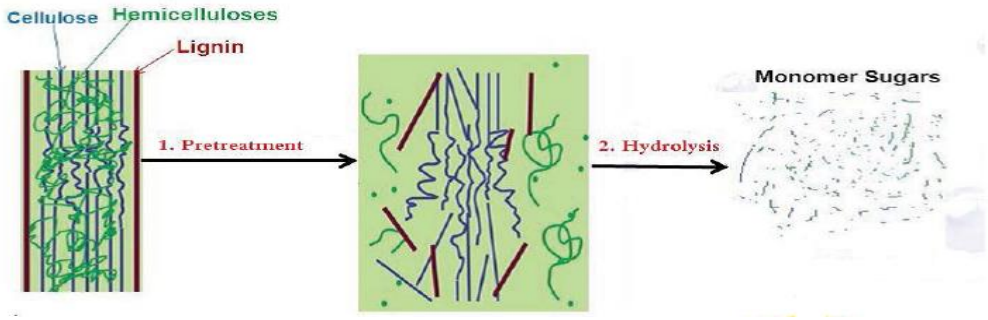


Figure 2.11: Degradation of lignocellulose (cellulose, hemicellulose and lignin) through fiber-degrading enzymatic treatments, and converted into monomer sugars (Madadi, Tu and Abbas, 2017).

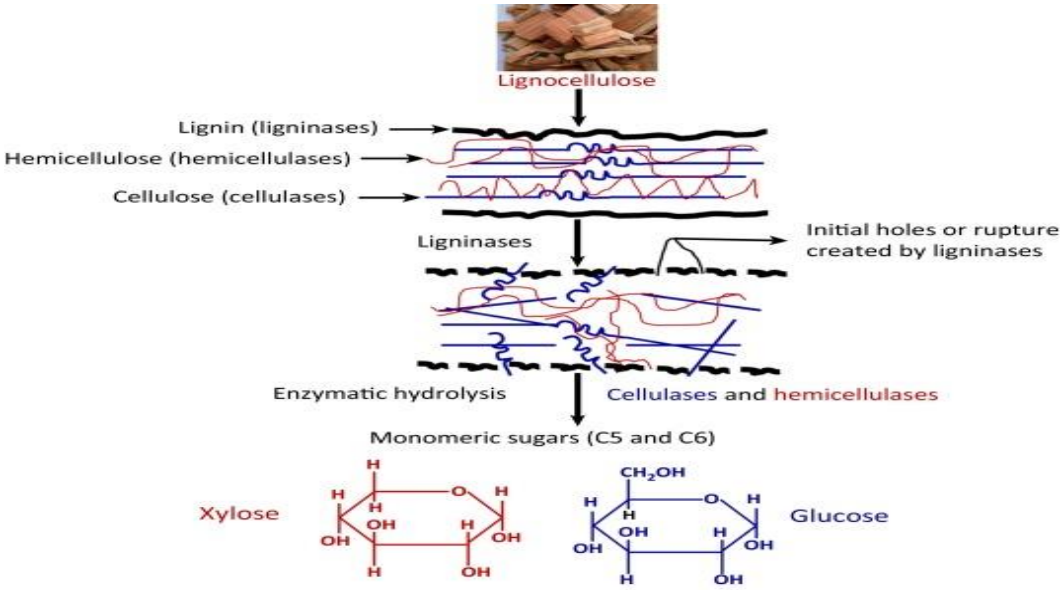


Figure 2.12: The enzymatic hydrolysis of lignin, hemicellulose and cellulose via the hydrolytic action exerted by cellulase and hemicellulase (Gupta et al., 2016).

To convert lignocellulose into fermentable sugars, it requires three critical steps in sequence: size reduction, perform pretreatment or fractionation and followed by enzymatic treatment (Jadhav, Chitanand and Shete, 2013). According to Jadhav, Chitanand and Shete (2013), lignocellulosic bio-wastes (rice husks, maize cobs, millet husks, wheat straws and leaves) were pretreated by sun-drying method, size reduction and soaking in 1% sodium hydroxide solution for 2 hours respectively. They were washed by using distilled water to eliminate any unwanted chemical, and followed by autoclaving for an hour, prior to enzymatic hydrolysis and microbial fermentation through *Aspergillus niger*. According to Martina et al. (2012), orange peel wastes were enzymatic hydrolyzed by using fungal cellulase and pectinases, to produce sugar-rich stream appropriate for reutilization based on the biorefinery philosophy.

Cellulases and xylanases can be used to pre-treat forage crops and other cellulosic biomass, to improve the nutritional quality of cellulosic biomass and enhance the digestibility of ruminant feeds (Gilbert and Hazlewood, 1991). Moreover, both enzymes can be added into the cereal-based diets for pig and poultry, as they can improve the nutrient reutilization through hydrolyzing arabinoxylans and barley β-glucan. The hydrolytic actions by cellulases and xylanases are alternative ways to manage reduction of landfill disposal by promoting enzymatic digestion on the industrial wastes (Gilbert and Hazlewood, 1991). Therefore, in this research study, cellulase and xylanase had been employed to treat and hydrolyze both pink guava wastes (decanter and refiner), in which they have capable to modify functional properties of plant-based waste materials and convert them into more value-added functional ingredients with higher concentrations of prebiotic oligosaccharides.

2.5.1 Cellulase Treatments

Based on Fariq (2016), cellulase is a derivable fiber-degrading enzyme produced by microorganism capable to catalyze the hydrolysis of β-1,4-glycosidic linkage which joins two glucose molecules in a cellulose chain (Figure 2.12). Cellulase producers can be found in various bacterial genera (*Cellulomonas*, *Bacillus* and *Microbispora*); and in the fungal genera (*Aspergillus*, *Penicillium*, *Rhizopus* and *Fusarium*) (Fariq, 2016; Pachauri et al., 2017; Petlamul et al., 2017; Santos et al., 2016). Cellulases are different from other fiber-degrading enzymes in the way which they degrade and hydrolyze insoluble substrates into soluble ones (Fariq,

2016). To hydrolyze cellulose into glucose efficiently, cellulase systems should consist of three main hydrolytic enzymes (endoglucanase, exoglucanase, and β-glucosidase (Fariq, 2016; Jadhav, Chitanand and Shete, 2013). Endoglucanase is capable to split the long chains of cellulose into shorter molecules by hydrolyzing the β-1,4-glycosidic linkages. Hence, it produces oligosaccharides with different lengths and subsequently forms new chain ends through cutting amorphous sites and soluble derivatives of cellulose units (Fariq, 2016; Rozenfelde et al., 2017). Exoglucanase can break down either reducing or non-reducing ends of crystalline substrates, cello-oligosaccharides and amorphous celluloses. From that, it results in eliminating units of two linked glucose molecules (Rozenfelde et al., 2017; Sadhu et al., 2014). Thus, it produces cellobiose which is cleaved into glucose monomer by β-glucosidase (Rozenfelde et al., 2017).

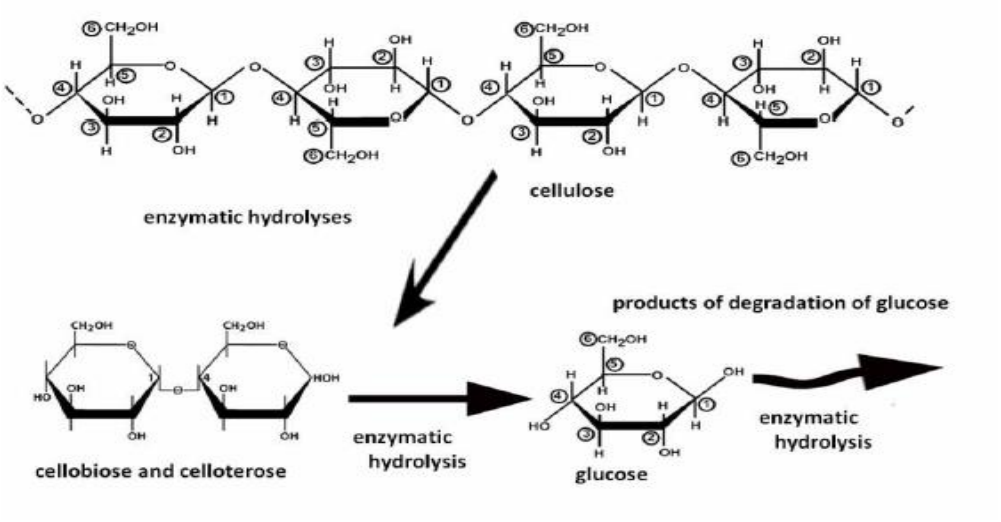


Figure 2.13: Mechanism of cellulose degradation via enzymatic hydrolysis (Mackul'ak et al., 2010).

The combined catalytic activity of a set of three hydrolytic enzymes is stronger as compared to a single cellulase (Wilson, 2011). The mechanism action of cellulase can be initiated by acting promptly on the cellulosic substrates (Fariq, 2016). Next, the recognition of free ends of cellulose chain takes place, and it is incorporated into the active site. As a result, a catalytically active complex will be formed and the hydrolysis reaction will be initiated. The product obtained from the hydrolysis reaction is expelled; therefore another catalytically active complex is formed by acting on of another cellobiose unit (Beckham et al., 2011; Fariq, 2016).

2.5.2 Applications of Cellulase Enzyme

Cellulases have been implemented widely in various industrial applications over many decades. For instance, cellulase is widely applied in textile wet processing, as it can knit and woven the cellulosic fabrics (cotton and linen) and results in improving the appearance of cellulose-based textiles such as brighter luminosity of colors (Hebeish and Ibrahim, 2007; Karmakar and Ray, 2011). Cellulases are also used for bio-stoning and bio-polishing on jeans, cotton, and other cellulosic fabrics (Kuhad, Gupta and Singh, 2011; Shah, 2013). Cellulases act on cotton fabric and break down small fiber ends on the surface of yarns during bio-stoning. The cellulose-based treatment can replace pumice stones, since it contributes less damage impacts to fiber structures, enhance machines productivity, environmental friendly, and lower work-intensive (Kuhad, Gupta and Singh, 2011).

Cellulase is often employed with pectinases and hemicellulase in food processing, such as to increase the yield of fruit and vegetable juice (Menendez, Garcia-Fraile and Rivas, 2015). Nowadays, cellulase application has been extended to produce animal feeds, as cellulases can improve and enhance the digestibility of cereal-based foods as animal feeds with enhanced nutritive values (Menendez, Garcia-Fraile and Rivas, 2015). The combination of cellulases with other enzymes can hydrolyze celluloses, lignins, β-glucans, oligosaccharides, and pectins present in the feed grains. Therefore, it could enhance nutritional values of feed grains and promotes health benefits to animals upon consumptions (Asmare, 2014; Azzaz, 2010; Kuhad et al., 2011; Murad and; Sharada et al., 2014). According to Phitsuwan et al. (2013), cellulases could liberate organic matters by releasing nutrients such as phosphorus and nitrogen, to improve and maintain the stability of soils for agricultural activities. Meanwhile, it can enhance microbial activities within the soils and buffer the pH values of soils. Therefore, it could enhance seed germination, improve root systems and promote plant growth (Fariq, 2016; Kuhad, Gupta and Singh, 2011; Phitsuwan et al., 2013). Cellulases are effective bio-control agents against pathogenic organisms such as *Phytophthoraparasitica*, *Phythium ultimum* and *Oomycetes* found in soils because the cellulosic walls of pathogen are susceptible to be penetrated and damaged by cellulolytic activities (Phitsuwan et al., 2013).

2.5.3 Xylanase Treatments

Xylanase is an extracellular enzyme produced by microorganisms such as bacteria (saprophytic and phytopathogenous), fungi (*Aspergillus niger* and *Trichoderma viride*), and yeasts (Harris and Ramalingam, 2010; Kanimozhi and Nagalakshmi, 2014). Microbial xylanase is more preferred as compared with plant-based or animal-based sources, because microbial xylanase has greater availability and structural stability with easier genetic manipulation (Harris and Ramalingam, 2010). Xylanase can break down plant cell walls held by xylan and hemicellulose, as it catalyzes the hydrolysis of β-1,4-glycosidic linkages of xylosides, and produces xylose (Figure 2.14). It results in the formation of sugar hemiacetal with the corresponding to free aglycone (Harris and Ramalingam, 2010; Hatanaka, 2012). Based on the previous research by Walia et al. (2017), xylanases show optimum enzymatic activities at mesophilic temperatures (between 40 and 60℃) in slightly acidic pH (fungal xylanase) and neutral pH (bacterial xylanase) (Harris and Ramalingam, 2010). Xylan is heterogeneity with relatively high complexity of chemical natures. It requires efficient hydrolytic actions exerted by repertoire xylanolytic system to achieve complete breakdown on xylan. (Motta, Andrade and Santana, 2013; Shahi et al., 2016). An efficient repertoire xylanolytic system consists of endoxylanase, β-xylosidase, α-glucuronidase, α-arabinofuranosidase, and acetylxylan esterase, to hydrolyze and convert xylan into its constituent sugars (Motta, Andrade and Santana, 2013).

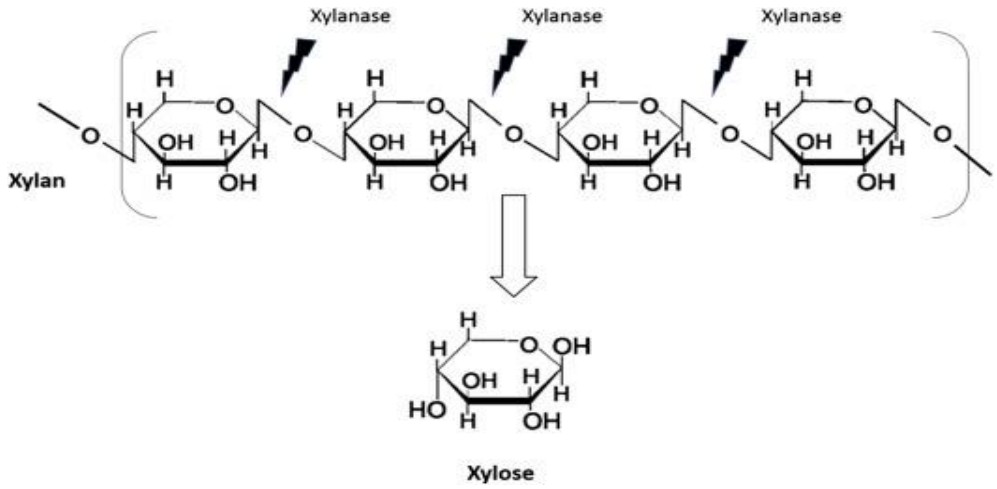


Figure 2.14: Mechanism of xylan degradation through the hydrolytic action exerted by xylanase (Godoy et al., 2018).

Endoxylanase (E.C. 3.2.1.8) cleaves the β-1,4-glycosidic linkages of xylan selectively based on the chain length, presence of xylan constituents and degree of substrate branching (Kirikyali and Connerton, 2015; Verma and Satyanarayana, 2012; Walia et al., 2017). In result, it produces xylose and xylo-oligosaccharides which inhibit endoxylanase to perform further xylanolytic action (Harris and Ramalingam, 2010; Kamble and Jadhav, 2012; Kanimozhi and Nagalakshmi, 2014). The cause of inhibition can be eliminated by β-xylosidase (E.C. 2.3.1.37), where it enhances the efficiency on hydrolyzing xylose and xylo-oligosaccharides (Andrade et al., 2004). β-xylosidase varies in thermo-stability and optimum hydrolytic action at 60℃ (Harris and Ramalingam, 2010). According to previous research by Rizzatti et al. (2001), β-xylosidase produced by *Aspergillus phoenics* shows stable enzymatic activity, as it can fully retain its activity after 21 days at room temperature or 4 h at 60℃.

2.5.4 Applications of Xylanase Enzyme

In recent years, xylanases are used widely for industrial applications. For instance, xylanase is applied as cost-effective fiber-degrading enzyme in the pulp bleaching process, as it could minimize lignin constituents, enhance fiber qualities in terms of brightness of the final white pulp (Motto, Andrade and Santana, 2013; Walia et al., 2017). In the pulp bleaching, xylanase must possess the characteristics such as alkaline-tolerance, thermo-stable and cellulase-free with lower molecular weight. This allows xylanase to penetrate easily into xylan on the surface of pulp particles (Valls et al., 2010), which is essential to remove lignin from pulps, as the presence of lignin residues and its derivatives can increase brown intensity of resultant pulp (Motta, Andrade and Santana, 2013). Application of xylanases in the pulp bleaching process can minimize the consumption of bleaching chemicals, reduce the toxic effluent and pollution load, and lower the absorbable organic halogen compounds (Walia et al., 2017).

Besides, xylanases can exert potential hydrolytic action to degrade and hydrolyze and degrade the lignocellulosic constituents in agricultural wastes, especially to act on hemicelluloses and xylans for further utilization. According to Gokhale, Patil and Bastawde (1997), yeast cellulase-free xylanase was utilized to treat different agricultural waste residues such as raw jute fibers, corn cob powder and sugarcane bagasse pulp. Cellulase-free xylanase produced reducing sugar (xylose) from the agricultural wastes to be the major end product with trace amounts of

xylobiose and xylotriose at the initial stage of xylanase-hydrolysis (Gokhale, Patil and Bastawde, 1997). In addition, xylanase was reported as capable to degrade and hydrolyze the lignocellulosic structures of agricultural wastes (corn cob and wheat bran) with the corresponding release of reducing sugar, which can be re-utilized as fermentative substrate for biofuel production (Kocabas, Guder and Ozben, 2015). In the feed industries, xylanases are applied as fiber-degrading enzymes in the pre-treatment of forage crops to enhance nutrition properties and digestibility of agricultural silages as ruminant feeds (Harris and Ramalingam, 2010; Motta, Andrade and Santana, 2013). Xylanases are often applied in a mixture of feed enzymes (amylase, lipase, cellulase, protease, galactosidase, glucanase, and phytase), to minimize the viscosity of raw materials as ruminant feeds (Twomey et al., 2013). The arabinoxylans in forage crops such as cereals and barleys are partially water soluble polysaccharides, and form highly viscous aqueous solution when reacted with water. This characteristic is unfavorable for its inclusion in animal feeds. Hence, xylanases and other biotechnological feed enzymes are required to hydrolyze the polysaccharide, thereby it minimizes the viscosity (Motta, Andrade and Santana, 2013; Shahi et al., 2016; Twomey et al., 2013). Xylanases are also effectively applied in commercial beverage industries, particularly to clarify wines and fruit juices. Besides, they are often incorporated with amylases, pectinases and cellulases, to improve structures stability of food pulp, minimize viscosity of raw materials, and break down substances hinder physical and chemical clearness of juice (Polizeli et al., 2005; Shahi et al., 2016).

CHAPTER 3

MATERIALS AND METHODS

3.1 Sample Materials

3.1.1 Pink Guava (*Psidium guajava* Linn.) Wastes

Pink guava (*Psidium guajava* Linn.) puree processing wastes known as decanter and refiner were obtained from Golden Hope Food and Beverages, a subsidiary company belongs to Sime Darby Sdn. Bhd., located at Sitiawan, Perak, Malaysia. They were collected at different processing points: cutting and crushing (refiner), and decanting (decanter). Decanter and refiner were packed separately and stored in a freezer (Hesstar, Malaysia) at -18℃ before being subjected for freeze-drying process.

3.1.2 List of Chemicals and Reagents

Table 3.1: List of chemicals and reagents with their respective sources.

Chemicals / reagents	**Sources**
3,5-dinitrosalicylic acid (DNS)	ACROS
Acetonitrile, HPLC grade	Fisher Scientific
Ammonium chloride	System ®
Ammonium heptamolybdate	Fisher Scientific
Calcium chloride	Techno Pharmchem
Cellulase	Bio-Cat
Citric acid	Fisher Scientific
Copper (II) sulphite	R&M Chemical
D(-)-arabinose	HIMEDIA
D(+)-Galactose	BioBasic
D(+)-glucose anhydrous	Scharlau
D(+)-Mannose	HIMEDIA
Di-sodium hydrogen arsenate	Bendosen
Escherichia coli	Microbiologics
Ethanol, 95 %	J.Kollin Chemicals
Fructose	HmbG Chemicals
Glucose determination (GOPOD) reagent	Megazyme
Glycerol	QRëC ™
Hydrochloric acid, 37 %	Fisher Scientific

Table 3.1: (continued)

Chemicals / reagents	Sources
L(+)-rhamnose	HIMEDIA
Lactobacillus brevis KCTC 3102	Choice Care
Lactobacillus MRS agar	HIMEDIA
Lactobacillus MRS broth	HIMEDIA
Lactobacillus plantarum ATCC 14917	Microbiologics
Lactobacillus rhamnosus ATCC 9595	Microbiologics
Lactobacillus yogurt starter culture	ChuanXiu
Luria-Bertani (LB) agar	Merck
Magnesium sulphate	System ®
Phenol	Merck
Potassium phosphate monobasic	Bendosen
Potassium sodium tartrate	GENE
Sodium bicarbonate	Rdeh
Sodium carbonate anhydrous	QRëC ™
Sodium chloride	Fisher Scientific
Sodium hydroxide	R & M Chemicals
Sodium phosphate dibasic heptahydrate	Fisher Scientific
Sodium sulphite	Fisher Scientific
Sucrose	Fisher Scientific
Sulfuric acid, 95 – 97 %	QRëC ™

Table 3.1: (continued)

Chemicals / reagents	Sources
Tri-sodium citrate dihydrate	DuLab
Tryptic soy agar	Merck
Tryptic soy broth	Merck
Ultra-high temperature (UHT) milk	Dutch Lady
Xylanase	Bio-Cat
Xylose	BioBasic

3.1.3 List of Equipment and Instruments

Table 3.2: List of equipment and instrument with their respective brand or model and manufacturing countries.

Equipment / instrument	Brand / model	Country
Attenuated total reflectance (ATR) spectroscopy	Thermo Fisher Scientific	United States
Autoclave	Hirayama	Japan
Centrifuge machine	Eppendorf Centrifuge 5430	Germany
Freeze-dryer	Scan Vac / CoolSafe ™	Denmark
Freezer	Hesstar	Malaysia
Grinder with siever	Fritsch	Germany

Table 3.2: (continued)

Equipment / instrument	Brand / model	Country
High performance liquid chromatography (HPLC)	Shimadzu	Japan
HPLC column	Merck	United States
Hot air drying oven	Memmert ™	Germany
Incubator	Binder	Germany
Moisture balance analyzer	A&D MX-50	Japan
Shaking incubator	Daihan Lab Tech	Korea
Texture analyzer	Stable Micro Systems	United Kingdom
UV-VIS spectrophotometer	Biochrom Libra S4	United Kingdom
Vortex	Scientific Industries Genie 2	United States
Water bath	Memmert ™	Germany
Weighing balance	Sartorius ® / CP224S	North America
X-ray diffractormetter	X'PertPro	United Kingdom

3.2 Overview of Research Study

Figure 3.1 shows the overview of entire research study, which mainly focuses on the effects of fiber-degrading enzymatic treatments (150 U cellulase treatment (150CE), 150 U xylanase treatment (150XY), and 150 U cellulase-xylanase treatment (150CX)) on the structural, functional, and fermentability properties of pink guava (*Psidium guajava* Linn.) waste materials. Foremost, both raw pink guava wastes (decanter and refiner) were freeze-dried for 120 h continuously, and grounded into smaller particle ($\leq 0.45\ \mu$m). Both freeze-dried samples were washed by using distilled water at 50 ± 5℃. The reducing sugar content released by both freeze-dried pink guava wastes during the sample washing process was determined by using 3,5-dinitrosalicylic acid (DNS) assay. After sample washing, both washed samples were subjected to fiber-degrading enzymatic treatments for 24 h at 55℃. Meanwhile, untreated sample was prepared by using 50mM citrate buffer (pH 4.5), and acted as control. All untreated and enzymatic-treated pink guava wastes were underwent 80% (v/v) ethanolic extraction, to separate alcohol insoluble solid (AIS) and alcohol soluble carbohydrates (ASC).

The AIS of untreated and enzymatic-treated pink guava wastes were analyzed based on attenuated total reflectance (ATR), scanning electron microscopy (SEM), X-ray diffraction (XRD), and glucose dialysis retardation index (GDRI) analysis. Meanwhile, ASC derived from untreated and enzymatic-treated pink guava waste materials were analyzed based on total soluble carbohydrates, High Performance

Liquid Chromatography (HPLC) analysis, and prebiotic activity score. In addition, we also investigated the suitability of whole untreated and enzymatic-treated pink guava wastes as potential prebiotic sources in yogurts. It was then evaluated based on CFU/mL, pH measurements and texture profiles of yogurts.

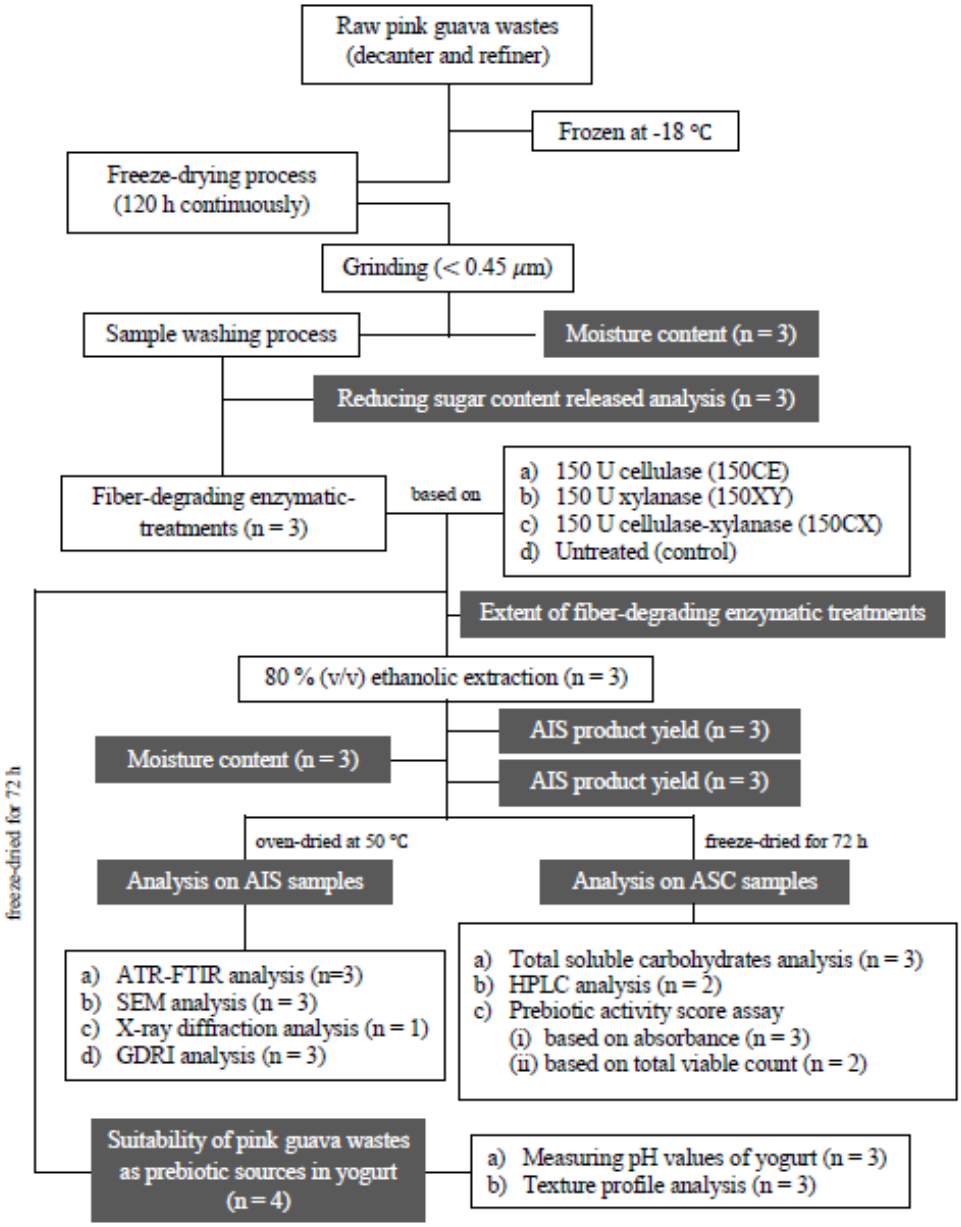


Figure 3.1: Overview of research study.

Besides, Figure 3.2 illustrates the overview on the relationship between structural and functional properties of untreated and enzymatic-treated pink guava wastes (decanter and refiner). As mentioned, AIS and ASC derived from untreated and enzymatic-treated pink guava wastes had been subjected for further analysis on determining respective structural and functional properties, and fermentability, upon after 24 h of fiber-degrading enzymatic treatments and solvent extraction. In this research, the results obtained from HPLC analysis was linked with those of ATR-FTIR and GDRI analysis. Each microscopy image of AIS material derived from untreated and enzymatic-treated pink guava wastes was then discussed and related to those obtained based on XRD and GDRI analysis. The results based on total soluble carbohydrates analysis were linked with XRD and prebiotic activity score assay. Besides, the prebiotic activity score assay estimation was also related with the suitability of untreated and enzymatic-treated pink guava wastes as prebiotic sources in yogurts. Furthermore, suitability of untreated and enzymatic-treated pink guava wastes were studied and determined based on the CFU/mL, acidity (pH) and texture profile of each yogurt sample.

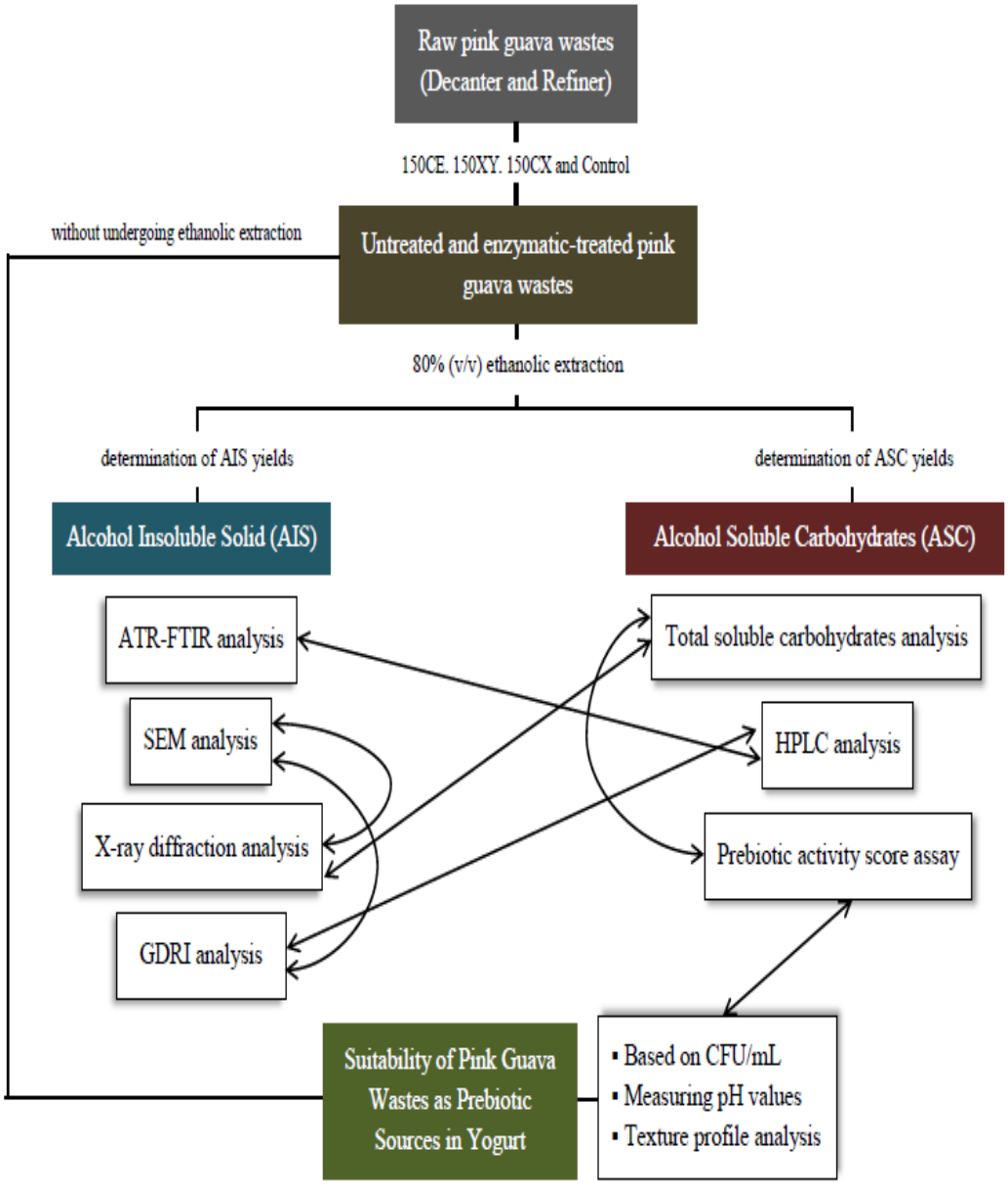


Figure 3.2: Overview on the relationship between structural and functional properties of untreated and enzymatic-treated pink guava wastes.

3.3 Sample Preparation

3.3.1 Pre-Analysis Treatment

Frozen pink guava wastes (decanter and refiner) were thawed at room temperature and freeze-dried for 120 h continuously (Figure 3.3), to remove moisture from the samples. These freeze-dried samples were ground by using a food-grade grinder equipped with a 0.45 μm-screen (Fritsch, Germany). The moisture content of each type of dried and sieved sample was then measured by using a moisture balance analyzer (A&D MX-50, Japan). Other samples were kept in respective air-tight glass jars and stored at chilled temperature (5℃) prior to further analysis.

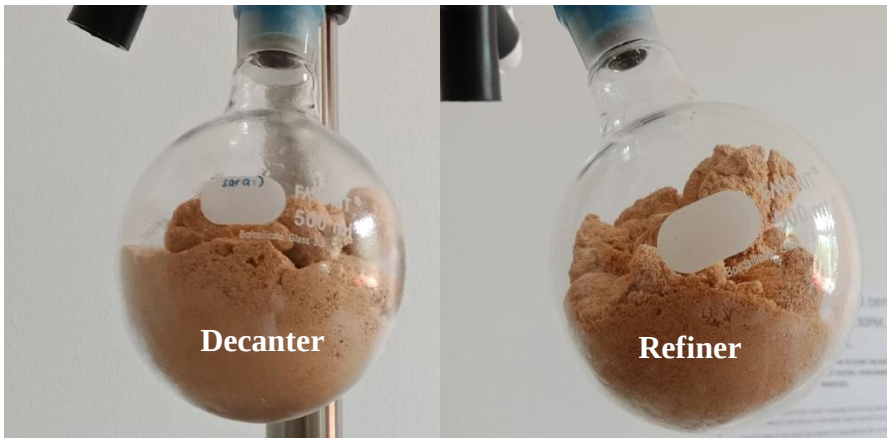


Figure 3.3: Frozen pink guava wastes (decanter and refiner) were freeze-dried for 120 h continuously to remove excessive moisture from the samples.

3.3.2 Sample Washing Process

The sample washing process was performed based on the procedures described by Sabajanes et al. (2012) with slight modifications. Pink guava wastes are rich in sugars, the products of enzymatic treatments on pink guava wastes are mainly sugars as well. Thus, the native sugars in pink guava wastes require to be removed through washing, to prevent its inhibition on the succeeding enzymatic treatment.

The freeze-dried sample was suspended in distilled water at a ratio of 1: 30 and it was then incubated at 50 ± 5℃ for 15 min with continuous stirring. The heated sample was centrifuged at 7,800 rpm (5850 ×*g*) for 15 min, by using a centrifuge machine (Eppendorf Centrifuge 5430, Germany). Subsequently, the supernatant was decanted while the pellet was then resuspended in distilled water at the same solid: liquid ratio and sample washing process was repeated. The supernatant obtained from the first and second washing process was decanted for reducing sugar content released analysis, with the aim to evaluate the efficiency of sample washing process. The washed sample pellet was collected and stored at chilled temperature (5℃) prior to enzymatic treatments.

3.3.2.1 Reducing Sugar Content Released Analysis

The reducing sugar content released during sample washing was determined by using the 3,5-dinitrosalicylic acid (DNS) assay according to Miller (1959) with some modifications. The 3,5-dinitrosalicylic acid (DNS) reagent was prepared by dissolving 30 g of potassium sodium tartrate ($KNaC_4H_4O_6 \cdot 4H_2O$) in 50 mL of distilled water, and it was then mixed with 1 g of 3,5-dinitrosalicylic acid (DNS) in 20 mL of 2 N sodium hydroxide (NaOH). The solution was topped up to a total volume of 1000 mL.

Supernatant sample collected from the washing process (as described in 3.2.2) was added with DNS reagent at a ratio of 1: 2, to form a total volume of 3 mL and mixed thoroughly. The mixture was incubated at 85 $\pm$ 5℃ in a hot water bath (Memmert ^{TM}, Germany) for 15 min, to ensure stable color development. After that, the incubated solution was allowed to cool to room temperature, and it was added with 9 mL of distilled water to form a total volume of 12 mL solution. The absorbance at 575 nm of the reaction mixture was measured by using a UV-VIS spectrophotometer (Biochrom Libra S4, United Kingdom). A series of glucose standard solutions with concentrations ranged from 0 to 1 mg was prepared and underwent similar steps before its absorbance at 575 nm was measured. A glucose standard curve was constructed, to estimate the reducing sugar content (expressed in mg/g) released from freeze-dried pink guava wastes during the first and second washing processes.

3.4 Fiber-Degrading Enzymatic Treatments

Each of the washed pink guava wastes (decanter and refiner) was treated with 150 U cellulase, 150 U xylanase, and 150 U cellulase-xylanase respectively. It was determined based on the actual enzymatic activity of commercial cellulase and xylanase, which are reported as 100,000 U and 150,000 U, respectively. First, each sample was treated with respective fiber-degrading enzyme (150 U cellulase, 150 U xylanase, and 150 U cellulase-xylanase) for 24 h at 55℃, with the purpose to convert the recalcitrant lignocellulosic materials of pink guava wastes into other soluble carbohydrates. The washed pink guava waste was added with 50 mM citrate buffer, pH 4.5 at a ratio of 1: 2 in a 200 mL Erlenmeyer flask. Next, 150 U of fiber-degrading enzyme was added into it. Meanwhile, a set of control experiment was also prepared which only contained washed sample with 50 mM citrate buffer, without any addition of fiber degrading enzyme. All samples were incubated in a shaking incubator (Daihan Lab Tech, Korea) at 55℃, 150 rpm for 24 h which were the optimum conditions for the selected fiber degrading enzymes on washed pink guava wastes. The incubated sample was placed in a hot water bath (Memmert ^{TM}, Germany) at 90℃ for 5 min, to terminate enzymatic activity. After that, the sample was centrifuged at 7,800 rpm (5850 $\times g$) for 15 min. The supernatant was collected and subjected to determine extent of fiber-degrading enzymatic treatments as described in the section 3.4.1 based on reducing sugar content produced by all enzymatic-treated pink guava wastes by using Nelson Somogyi method; while the pellet was kept in a 50 mL centrifuge tube and stored in a freezer at -18℃ prior to 80% (v/v) ethanolic extraction.

3.4.1 Extent of Fiber-Degrading Enzymatic Hydrolysis

The degree of hydrolysis was determined to evaluate the efficiency of each fiber degrading enzymatic treatment on both pink guava wastes (decanter and refiner), by using Nelson Somogyi method based on Nelson (1944) and Alexander, Elena and Arkady (2011) with some modifications.

To prepare Somogyi copper reagent, 24 g of sodium carbonate anhydrous and 12 g of sodium potassium tartrate were dissolved in 250 mL of distilled water. Then, 10% (w/v) of copper (II) sulphite solution was added into the mixture, followed by the addition of 16 g sodium bicarbonate to form solution (A). To prepare solution (B), 180 g of sodium sulphite was dissolved in 500 mL of distilled water, and boiled to expel air trapped within the solution. After that, solution (A) was mixed with the solution (B) thoroughly, and topped up to 1000 mL to form the Somogyi copper reagent. Meanwhile, Nelson Arsenomolybdate reagent was then prepared by combining the following two solutions: First solution was prepared by dissolving 25 g of ammonium heptamolybdate in 450 mL of distilled water, followed by adding 21 mL of concentrated hydrochloric acid (HCl). Meanwhile, second solution was prepared by dissolving 3 g of di-sodium hydrogen arsenate in 25 mL of distilled water. Both solutions was mixed thoroughly, and incubated at 37℃ for 24 h (Nelson, 1944).

To determine amount of reducing sugar content produced, supernatant collected from each fiber-degrading enzymatic-treatment was subjected to Nelson Somogyi analysis. Each sample supernatant was diluted 10 times prior to Nelson Somogyi analysis. An aliquot of 1 mL of sample was added with 1 mL of Somogyi copper reagent and then incubated at 80℃ for 20 min. After it was cooled to room temperature, 1 mL of Nelson Arsenomolybdate reagent was added into it, and the reaction mixture was incubated at room temperature for 10 min. The volume of reaction mixture was then topped-up to 10 mL with distilled water before its absorbance at 520 nm was measured spectrophotometrically. A series of glucose standard solutions with concentrations ranged from 0 to 10 mg/mL was prepared and underwent similar steps, thus a standard curve can be constructed to estimate sugar content in samples. The extent of enzymatic hydrolysis was denoted by the amount of reducing sugar content produced from each enzymatic-treated pink guava wastes, and expressed in μg/g.

3.5 Ethanolic Extraction

The ethanolic extraction of untreated and enzymatic-treated pink guava wastes were performed according to the method described by Farrant et al. (2003) with some modifications. About 2.5 g of sample was extracted in 100 mL of ethanol (80%, v/v) in a 200 mL Erlenmeyer flask. It was then heated in a hot water bath (Memmert ^{TM}, Germany) at 80℃ for 1 h. The heated sample was centrifuged at 7,800 rpm (5850 $\times g$) for 20 min. The supernatant was decanted; while the pellet was resuspended and re-extracted once by applying similar steps. After extraction, each sample supernatant was then collected and combined together, followed by concentration through vacuum-evaporation, by using a rotatory evaporator (Büchi, Switzerland) at 50℃, 102 atm with a water aspirator. The extract obtained was freeze-dried for 72 h continuously to achieve a constant weight. The freeze-dried extract was known as alcohol soluble carbohdyrates (ASC) sample. While, the final pellet was dried at 50℃ in a hot air drying oven (Memmert ^{TM}, Germany) for 24 h. The oven-dried pellet was collected and used as alcohol insoluble solid (AIS) sample. The ASC and AIS samples were kept in screwed-cap universal bottles at chilled temperature prior to further analysis. The product yields of dried extracts and pellets were calculated and expressed as percentage in dry weight basis based on the following equation:

$$\text{Product Yield (\%)} = \frac{(Weight\ of\ dried\ extract\ or\ pellet)}{2.5\ g\ of\ sample} \text{ x } 100\%$$

3.6 Moisture Content Analysis

The moisture content of pre-analysis treatment, alcohol insoluble solid (AIS) and alcohol soluble carbohydrates (ASC) samples were measured by using a moisture balance analyzer (A&D MX-50, Japan). Approximately 2 g of sample was weighed and spread evenly on the sample pan of moisture balance analyzer. The temperature set for moisture content analysis was 105℃ with the standard MID mode. The drying process was then initiated to achieve a constant weight. The moisture content of sample was displayed as percentage.

3.7 Analysis on Alcohol insoluble solid (AIS) Samples

3.7.1 Attenuated Total Reflectance (ATR) Spectroscopy Analysis

The functional groups of untreated and enzymatic-treated alcohol insoluble solid (AIS) samples were measured by using an attenuated total reflectance (ATR) spectroscopy (Thermo Fisher Scientific, United States). Approximately 0.5 g of AIS sample was placed in contact with the cleaned diamond cell. The pressure controller was screwed down until an optimum pressure level (80%) was achieved, and the ATR analysis was initiated. Subsequently, an ATR spectrum was then generated with the wavenumbers expressed as cm^{-1} which indicated the functional groups in the subjected sample. The generated ATR spectrum of AIS derived from enzymatic-treated sample was compared with that of the AIS of untreated sample.

3.7.2 Scanning Electron Microscopy (SEM) Analysis

The surface structural features of untreated and treated AIS sample of decanter and refiner were examined under scanning electron microscope (SEM) (JEOL JSM-7610F, United States). The AIS sample was loaded over conducting double side carbon tape. It was then sputter coated with platinum. Subsequently, the structural image of AIS was scanned under SEM with 1000 × the magnifications. Hence, the structural images of AIS derived from enzymatic-treated sample was compared with those of the AIS derived from untreated sample, washed sample, and raw pink guava waste.

3.7.3 X-ray Diffraction (XRD) Analysis

The X-ray crystallinity patterns of untreated and enzymatic-treated AIS sample was determined by using an X-ray diffractometter (X'PertPRO, United Kingdom). The X-ray diffraction (XRD) analysis was performed based on the measurement conditions shown in Table 3.3. The crystallinity index (CrI) of an AIS sample was determined by using the peak intensity method with the equation shown as below based on the method described by Palme, Theliander and Brelid (2016):

$$\text{Crystallinity index (CrI) (\%)} = ((I_{002} - I_{AM}) / I_{002}) \times 100\%$$

Where, I_{002} represents the peak intensity at 2 θ of 22.5 °;

I_{AM} represents the peak intensity at 2 θ of 18 °.

Table 3.3: Measurement conditions for X-ray diffraction (XRD) analysis.

X-ray diffraction (XRD) measurement conditions		
X-ray tube	Target	Cu
	Voltage	40.0 kV
	Current	30.0 mA
Slits	Divergence slit	1.00000 deg
	Scatter slit	1.00000 deg
	Receiving slit	0.30000 mm
	Drive axis	Theta-2 Theta
Scanning	Scan range	10.0000 – 80.0000 deg
	Scan mode	Continuous scan
	Scan speed	2.0000 deg/min
	Sampling pitch	0.0200 deg

3.7.4 Glucose Dialysis Retardation Index (GDRI) Analysis

Glucose dialysis retardation index (GDRI) analysis was performed according to Fuentes-Alventosa et al. (2009) with slight modifications, to determine the hypoglycemic potentials of pink guava wastes (decanter and refiner). About 200 mg of AIS sample was hydrated with 15 mL of glucose solution (10 mg/mL) in a 15 mL centrifuge tube. It was then placed on an orbital shaker for 2 h at room temperature. It was transferred into a 15 cm portion of a dialysis tube (10,000 MWCO, Thermo Fisher Scientific Inc., United States). A control sample was then prepared by using 10 mg/mL of glucose solution, without any sample, and named as control without AIS sample. All tubes were put into separate reservoirs, which contained 200 mL of distilled water. The set-up was incubated at 37℃, 100 rpm for 90 min. The glucose concentration in reservoir which may be diffused from the inner part of dialysis tube was measured by using the Megazyme glucose test kit. At 15 min of time intervals, 0.1 mL of dialysate was collected, and added with 3 mL of glucose oxidase/peroxidase (GOPOD) reagent. The sample was then incubated at 45 $\pm$ 5℃ for 20 min. The absorbance at 510 nm of incubated sample was measured spectrophotometrically. A series of glucose standard solutions was prepared with concentrations ranged from 0 to 10 mg/mL to construct a standard curve for estimating glucose concentration diffused into reservoir. The glucose dialysis retardation index (GDRI) value was then calculated and expressed as percentage according to the following equation:

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

3.8 Analysis on Alcohol soluble carbohydrates (ASC) Samples

3.8.1 Total Soluble Carbohydrates Analysis

Total soluble carbohydrate contents in each untreated and enzymatic-treated pink guava waste was determined by using phenol sulfuric acid method according to Dubios et al. (1956) with slight modifications. Approximately 0.05 g of ASC sample was re-dissolved in 5 mL ethanol (80%, v/v), and it was then diluted 100 times. The diluted ASC sample was added with 0.2 mL of 5% (w/v) phenol solution. After that, 1 mL of concentrated sulfuric acid (H_2SO_4) was added into it quickly and mixed thoroughly. It was placed in a dark area for 20 min at room temperature for further color development. Its absorbance at 490 nm was then measured against 80% (v/v) ethanol as a blank, spectrophotometrically. A series of glucose standard solutions with concentrations ranged from 0 to 10 μg/mL was prepared and underwent similar steps to construct a glucose standard curve for estimating total soluble carbohydrate content in ASC samples, expressed in mg/g of dried sample.

3.8.2 High Performance Liquid Chromatography (HPLC) Analysis

The sugar profiles of untreated and enzymatic-treated ASC samples of pink guava wastes were analyzed by using high performance liquid chromatography (HPLC) (Shimadzu, Japan), based on the method by Mhd Abd Kader et al. (2016) with slight modifications. The HPLC system was equipped with Shimadzu LC-20AD pump, a refractive index detector (RID-10A) and a manual injector. The mobile phase used was 80% of acetonitrile and 20% of distilled water; while the flow rate was 0.8 mL/min and 0.2 mL/min for Pump A and B, respectively. The HPLC column (Merck, United States) was operated at 30℃.

Sugar standard solutions with concentrations ranged from 0 mg to 1.20 mg were prepared for each sugar standard (glucose, fructose, sucrose, galactose, arabinose, rhamnose, mannose, and xylose). After analysis, a sugar calibration curve was constructed for each sugar standard to quantify sugars present in untreated and enzymatic-treated pink guava wastes. To determine the sugar profiles of untreated and enzymatic-treated pink guava wastes, 0.2 g of the ASC sample was dissolved in 1 mL of 80 % (v/v) acetonitrile, and it was then sonicated for 30 min. After sonication, it was filtered through a Sep-pak C18 cartridge with a 0.45 μm membrane filter by using a syringe. An aliquot of 10 mL filtrate was then injected into the HPLC column manually for sugar profile analysis. A HPLC spectrum was generated to quantify sugars in the ASC sample based on peak areas. The amount of sugar found in the sample was expressed as mg/g in dried weight basis.

3.8.3 Prebiotic Activity Score Analysis

3.8.3.1 Preparation of Media

The prebiotic activity score assay was performed to quantitatively determine the potential of untreated and enzymatic-treated pink guava wastes (decanter and refiner) as prebiotics sources to promote the growth of probiotics. *Lactobacillus rhamnosus* ATCC 9595, *Lactobacillus plantarum* ATCC 14917 and *Lactobacillus brevis* KCTC 3102 were used as probiotic strains; while the enteric strain was the *Escherichia coli* ATCC 25922. The growth of *L. rhamnosus*, *L. plantarum*, and *L. brevis* required both MRS agar and broth, while Tryptic Soy broth and agar were used to grow the *E. coli*.

M9 salt was prepared by adding 12.8 g of sodium phosphate dibasic heptahydrate ($NaHPO_4 \cdot 7H_2O$) with 1 g of ammonium chloride, 0.5 g of sodium chloride, and 3 g of potassium phosphate monobasic (KH_2PO_4) (Tham, 2016). It was made up to 200 mL with deionized water. To prepare M9 Minimal medium broth, 50 mL of 5× M9 salt was added with 0.5 mL of 1 M magnesium sulphate and 0.5 mL of 1 M calcium chloride, and it was then made up to 250 mL with deionized water. All media were autoclaved separately and aseptic technique was utilized. Glucose stock solution (10%) was prepared by using filter-sterilization; with the aid of a 0.45 μm syringe filter pore size (Tham, 2016). Meanwhile, each ASC sample was re-dissolved in deionized water, to prepare a 10% of sample extract and filter-sterilized.

3.8.3.2 Prebiotic Activity Based on Absorbance

The prebiotic activity score assay was performed based on the method described by Tan (2015) with slight modifications. Probiotic strains (*L. rhamnosus*, *L. plantarum* and *L. brevis*) were taken out from the package and directly streaked on the MRS agar plates separately, and incubated at 37℃ for 48 h. Meanwhile, the enteric strain (*E. coli*) was taken out and directly streaked on the Tryptic Soy agar plate, and incubated at 37℃ for 24 h.

Single colony from both MRS agar and Tryptic Soy agar plates was taken after incubation, and it was then inoculated into 10 mL of MRS broth and Tryptic Soy broth respectively. The MRS broths with *L. rhamnosus*, *L. plantarum* and *L. brevis* were incubated at 37℃ for 48 h separately. Meanwhile, the Tryptic Soy broth with *E. coli* was incubated for 24 h at 37 ℃. After incubation, 1% (v/v) of *E. coli* from the Tryptic Soy broth was transferred into 10 mL of M9 Minimal medium broth and incubated at 37℃ for 24 h.

The MRS broth containing respectively 1% of glucose and 1% of ASC was prepared accordingly, similar preparation steps were applied for M9 Minimal medium broth. Then, 10 μL of the probiotic strain (*L. rhamnosus*, *L. plantarum* and *L. brevis*) in MRS broth was inoculated to the MRS broth containing 1% of glucose and 1% of ASC respectively. A control sample was prepared by adding

probiotic strain into a MRS broth without addition of sample. Meanwhile, similar procedure was carried out to transfer the *E. coli* in the M9 Minimal medium prepared 24 h ago was inoculated into the M9 Minimal medium containing 1% of glucose and 1% of ASC sample, respectively. Cell density of the respective microorganism was measured at 600 nm spectrophotometrically at 0 h and after 24 h of incubation against a MRS broth (without any bacteria strain) as blank. The prebiotic activity score for ASC sample of untreated and enzymatic-treated pink guava wastes (decanter and refiner) was then calculated based on the following equation:

$$\text{Prebiotic activity score} = \left[\frac{(Probiotic\ O.D.on\ prebiotic\ at\ 24\ hours - Probiotic\ O.D.on\ prebiotic\ at\ 0\ hour)}{(Probiotic\ O.D.on\ glucose\ at\ 24\ hours - Probiotic\ O.D.on\ glucose\ at\ 0\ hour)} \right] - \left[\frac{(Enteric\ O.D.on\ prebiotic\ at\ 24\ hours - Enteric\ O.D.on\ prebiotic\ at\ 0\ hour)}{(Enteric\ O.D.on\ glucose\ at\ 24\ hours - Enteric\ O.D.on\ glucose\ at\ 0\ hour)} \right]$$

3.8.3.3 Prebiotic Activity Based on Total Viable Count

The prebiotic activity score for each ASC sample of untreated and enzymatic-treated pink guava waste was also determined based on total viable count by using pour plate method, based on Kusmiyati, Wahyuningsih and Widodo (2018), and Sanz, Gibson and Rastall (2005) with some modifications. Similar steps as described in 3.8.3.2 on MRS broth and M9 Minimal medium broth preparation and inoculation with probiotic strains and enteric strain were followed. However, pour plate technique to enumerate actual CFU was used to determine the prebiotic activity score instead of measuring the growth of microorganisms at 600 nm

spectrophotometrically. About 0.1 mL MRS broth or M9 Minimal Medium broth prepared with glucose or ASC with probiotic strain or enteric strain was added into 9.9 mL of saline solution (0.85 %, w/v), to achieve a series of hundredfold dilution (10^{-1}, 10^{-2}, 10^{-3}, 10^{-4}, 10^{-5}, 10^{-6}, 10^{-7}, 10^{-8} and 10^{-9}). Then, 1 mL aliquot was transferred aseptically into an empty petri dish and added with 15 mL of respective sterile molten nutrient agar. It was then mixed thoroughly to ensure the medium covered the plate evenly and allowed to solidify completely. All samples were incubated at 37℃ for 24 h. The colony count on each plate at 0 h was assumed as 1. After 24 h of incubation, each plate containing between 30 and 300 visible colonies was counted. The total viable count (TVC) of enteric strain or probiotic strain in each petri dish was determined. The prebiotic activity score for each ASC sample derived from untreated and enzymatic-treated pink guava wastes (decanter and refiner) was estimated based on the following equation:

$$\text{Prebiotic activity score} = \left[\frac{(Probiotic\ log\ TVC\ on\ prebiotic\ at\ 24\ hours - Probiotic\ log\ TVC\ on\ prebiotic\ at\ 0\ hour)}{(Probiotic \log TVC\ on\ glucose\ at\ 24\ hours - Probiotic\ log\ TVC\ on\ glucose\ at\ 0\ hour)} \right] - \left[\frac{(Enteric\ log\ TVC\ on\ prebiotic\ at\ 24\ hours - Enteric\ log\ TVC\ on\ prebiotic\ at\ 0\ hour)}{(Enteric\ log\ TVC\ on\ glucose\ at\ 24\ hours - Enteric\ log\ TVC\ on\ glucose\ at\ 0\ hour)} \right]$$

3.9 Suitability of Pink Guava Wastes as Prebiotic Sources in Yogurt

3.9.1 Yogurt Production

The yogurt production with the addition of untreated and enzymatic-treated pink guava wastes (decanter and refiner) was performed according to the procedures described by Park and Oh (2007) with slight modifications by using aseptic technique. The untreated and enzymatic-treated pink guava wastes were freeze-dried for 72 h continuously. Each sample was washed and sterilized by 70% (v/v) ethanol at a ratio of 1: 10, to eliminate any bacteria on the surfaces and lower the risk of contamination prior to yogurt production. Total viable count for each sterilized sample was determined based on pour plate method with sterile Luria-Bertani (LB) agar.

Approximately 1 g of commercial *Lactobacillus* yogurt starter culture (ChuanXiu, China) (Figure 3.4) was dissolved in 1000 mL of ultra-high temperature (UHT) fresh milk produced by Dutch Lady (Malaysia), and allowed to mix thoroughly on an orbital shaker (Tech-Lab, Malaysia) at 150 rpm for 30 min. Then, 1% of sterilized sample was inoculated into 100 mL of UHT fresh milk containing *Lactobacillus* yogurt starter culture. Meanwhile, a sample was prepared without any pink guava waste, and acted as control. All samples were then sealed tightly in sterilized glass containers and fermented at 37℃ for 24 h.

Figure 3.4: Commercial *Lactobacillus* yogurt starter culture produced and manufactured by ChuanXiu from Beijing, China.

3.9.2 Determination of Total Viable Count

Approximately 0.1 mL of yogurt sample was collected after incubation and diluted hundredfold, through serial dilution up to 10^{-9} with sterilized 0.85 % (v/v) saline solution. Then, 1 mL of aliquot was transferred aseptically into an empty petri dish and added with 15 mL of sterile Luria-Bertani (LB) agar. It was mixed thoroughly to ensure the medium covered the plate evenly and allowed to solidify completely. All samples were incubated at 37℃ for 24 h. Each sample plate containing between 30 and 300 visible colonies was counted and expressed as CFU/mL (Park and Oh, 2007).

3.9.3 Measuring pH Values

The pH value of each yogurt with untreated and enzymatic-treated pink guava waste (decanter and refiner) was measured by using a pH meter (Thermo Fisher Scientific, United States). The pH values of each yogurt added with enzymatic-treated samples were compared with the untreated ones.

3.9.4 Texture Profile Analysis

Texture profiles analysis of yogurts with untreated and enzymatic-treated pink guava wastes were determined by using a texture analyzer (TA.XTplus, Stable Micro Systems, United Kingdom) in accordance to the method described by Kose, Altun and Kose (2018) with slight modifications. The texture analyzer was equipped with a load cell of 5 kg and a cylindrical probe with 25 mm in diameter, and supplied with Texture Exponent Programs. Each sample was then left at room temperature for 2 min prior to texture profile analysis. It was compressed by using a probe which travelled 10 mm with the speed of 5 mm/s within the yogurt. The hardness (mm), stickiness (g), adhesiveness (g.s), and stringiness (mm) were determined by using Exponent software provided by Stable Micro Systems, as shown in Figure 3.5.

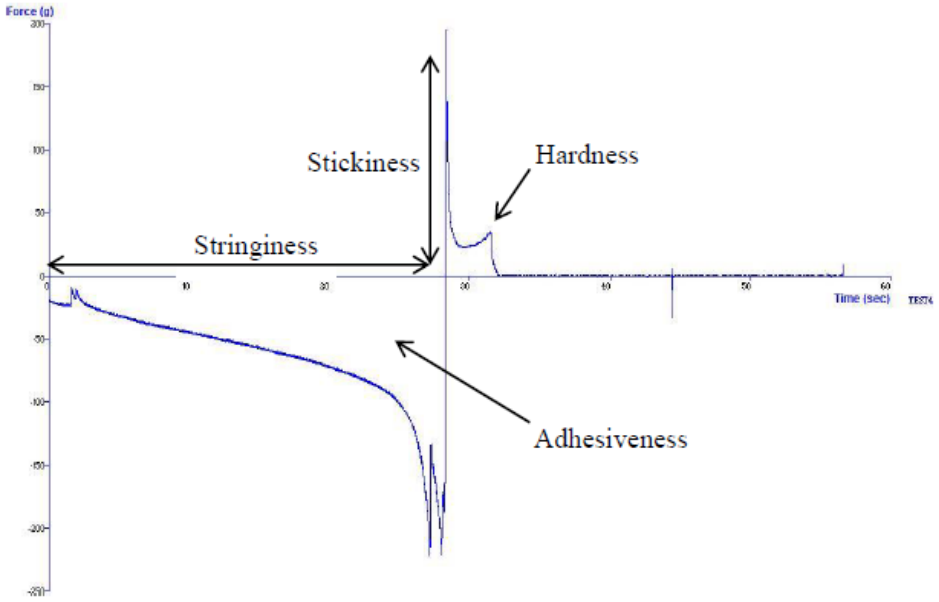


Figure 3.5: Texture profile analysis performed on yogurts with untreated and enzymatic-treated pink guava wastes based on hardness, stickiness, adhesiveness and stringiness.

3.10 Statistical Analysis

Raw pink guava wastes (decanter and refiner) were freeze-dried continuously for 3 batches, to obtain sufficient amount of sample for research study. The freeze-dried samples were used for moisture content analysis and reducing sugar content released analysis, and triplicate samples were analyzed. Besides, the enzymatic-treated samples were prepared in triplicate and analyzed based on the reducing sugar content produced by enzymatic-treated pink guava wastes during fiber-degrading enzymatic treatments. The moisture content and product yields for ethanolic extraction were also triplicated. Each analysis on all AIS samples were performed in triplicates, except for X-ray diffraction (XRD) analysis. At the same

time, sugar profile analysis through HPLC to analyze ASC samples was carried out in duplicate, except for total soluble carbohydrates analysis (in triplicate). Moreover, suitability of pink guava wastes as prebiotic sources in yogurt was analyzed in quadruplicate; but the pH analysis and texture profile analysis were performed in triplicate.

The collected data were tabulated and expressed as mean $\pm$ standard deviation. In this research, Statistical Package for the Social Science (SPSS) program (Version 20.0), and Microsoft Excel (Windows 8) were applied to perform One-Way Analysis of Variance (ANOVA) and paired *t*-test, respectively to identify the significant difference. If the *P*-value less than 0.05 ($P < 0.0.5$) indicated there was a significant difference found among enzymatic-treated and untreated pink guava wastes, or between both enzymatic-treated pink guava decanter and refiner.

CHAPTER 4

RESULTS

4.1 Reducing Sugar Content Released during Sample Washing

Table 4.1 shows the amount of reducing sugar released by freeze-dried pink guava wastes (decanter and refiner) during the first and second washing processes. The reducing sugar content released by freeze-dried decanter during the first and second sample washing processes was 27.70 mg/g and 3.47 mg/g, respectively. The amount of reducing sugar content released by freeze-dried refiner during 1^{st} and 2^{nd} sample washing processes was 21.89 mg/g and 2.04 mg/g, respectively. The reducing sugar content released by both freeze-dried decanter and refiner had been minimized significantly by 90.68% and 87.74% ($P < 0.05$), respectively, after second sample washing process. It reflects most of the free sugars had been washed off and released out from freeze-dried pink guava wastes into the effluent, prior to further fiber-degrading enzymatic treatments.

Table 4.1: Amount of reducing sugar released by each freeze-dried pink guava waste (decanter and refiner) during the 1st and 2nd sample washing processes.

Samples	Sample washing process	Reducing sugar released (mg/g)	Decrement (%)
Freeze-dried decanter	**First**	21.89 ± 0.00^{b}	90.68
	Second	2.04 ± 0.00^{a}	
Freeze-dried refiner	**First**	27.70 ± 0.00^{b}	87.74
	Second	3.47 ± 0.00^{a}	

Values represent means $\pm$ standard deviation (n = 3). Means with different letter in the same row is significantly different ($P < 0.05$).

4.2 Reducing Sugar Content Produced After Enzymatic Hydrolysis

Table 4.2 shows the amount of reducing sugar content released into the effluent obtained from 24 h of enzymatic-treated pink guava wastes (decanter and refiner): 150 U cellulase (150CE), 150 U xylanase (150XY), and 150 U cellulase-xylanase (150CX). The amount of reducing sugar produced by combined enzymatic-treated decanter was 335.8 μg/g and significantly higher ($P < 0.05$) than those of cellulase-treated (310.5 μg/g) and xylanase-treated (207.5 μg/g) decanters. On the other hand, the amount of reducing sugar content produced by the combined enzymatic-treated refiner was 337.8 μg/g, significantly higher ($P < 0.05$) than those of cellulase-treated (313.0 μg/g) and xylanase-treated (181.80 μg/g) refiners.

In a nutshell, the extent of fiber-degrading enzymatic-treated pink guava decanter and refiner followed the increasing order of 150 U xylanase < 150 U cellulase < 150 U cellulase-xylanase treatment.

Table 4.2: Amount of reducing sugar content released into the effluent of 24 h – enzymatic-treated pink guava wastes (decanter and refiner).

Samples	Amount of reducing sugar released (μg/g)		
	150CE	**150XY**	**150CX**
Decanter	310.50 ± 0.11^{b}	207.47 ± 0.86^{a}	335.80 ± 0.09^{c}
Refiner	313.00 ± 0.15^{b}	181.80 ± 0.48^{a}	337.80 ± 0.09^{c}

Values represent means $\pm$ standard deviation (n = 3). Means with different letter in the same row is significantly different ($P < 0.05$).

Table 4.3 shows the comparison on amount of reducing sugar produced after 24 h of fiber-degrading enzymatic treatments between both enzymatic-treated pink guava wastes (decanter and refiner) based on 150 U cellulase (150CE), 150 U xylanase (150XY), and 150 U cellulase-xylanase (150CX) treatments. For each enzymatic treatments, there was a significant difference ($P < 0.05$) between decanter and refiner. From this, refiner was more susceptible to be hydrolyzed by fiber-degrading enzymes in comparison to decanter, except in the case of 150 U xylanase.

Table 4.3.: Comparison between decanter and refiner on the amount of reducing sugar released into the effluent after 24 h enzymatic treatments.

Fiber-degrading enzymatic treatments	Amount of reducing sugar produced (μg/g)	
	Decanter	**Refiner**
150CE	310.50 ± 0.06^{a}	313.00 ± 0.15^{b}
150XY	207.47 ± 0.84^{b}	181.80 ± 0.48^{a}
150CX	335.80 ± 0.09^{a}	337.80 ± 0.09^{b}

Values represent means $\pm$ standard deviation (n = 3). Means with different letter in the same row is significantly different ($P < 0.05$).

4.3 Ethanolic Extraction

4.3.1 The Yield of Alcohol Insoluble Solid (AIS)

Table 4.4 shows the yield of AIS sample derived from untreated and enzymatic-treated pink guava wastes (decanter and refiner), which were expressed in dry matter basis. The AIS yield of the untreated decanter and refiner was 88.07% and 89.27%, respectively. Fiber-degrading enzymatic treatments have capable to increase solubility of the fibrous pink guava wastes and a decreased amount of alcohol insoluble solids was observed. Xylanase showed the least effect among the fiber-degrading enzymatic treatments. The AIS yield of xylanase-treated decanter (20.29%) was significantly higher ($P < 0.05$) than those of cellulase-treated (9.84%) and combined enzymatic-treated (11.74%) decanters. On the

other hand, the AIS yield of xylanase-treated refiner was 20.06%, which was significantly higher ($P < 0.05$) than those of cellulase-treated (11.95%) and combined enzymatic-treated (10.41%) refiners. Yet, there was no significant difference found between the AIS yield of cellulase-treated and combined enzymatic-treated decanters. Similar trends were observed on the enzymatic-treated refiners. The AIS yield of enzymatic-treated decanter was reduced by: 78.23% (150CE), 67.78% (150XY), and 76.33% (150CX). On the other hand, the AIS yield of each enzymatic-treated was reduced by: 77.32% (150CE), 69.21% (150XY), and 78.86% (150CX). In a nutshell, the AIS yield of decanter and refiner followed the order of untreated (CTD) < 150 U xylanase (150XY) < 150 U cellulase (150CE) < 150 U cellulase-xylanase treatment (150CX).

Table 4.4: The yields of alcohol insoluble solid (AIS) of each untreated and enzymatic-treated pink guava waste (decanter and refiner).

Samples	**AIS yield (%)**			
	150CE	**150XY**	**150CX**	**CTD**
Decanter	9.84 ± 0.32^{a}	20.29 ± 0.84^{b}	11.74 ± 0.52^{a}	88.07 ± 1.53^{c}
Refiner	11.95 ± 0.20^{a}	20.06 ± 1.05^{b}	10.41 ± 0.57^{a}	89.27 ± 2.30^{c}

Values represent means $\pm$ standard deviation (n = 3). Means with different letter in the same row is significantly different ($P < 0.05$).

4.3.2 The Yield of Alcohol Soluble Carbohydrates (ASC)

Table 4.5 shows the yields of alcohol soluble carbohydrates (ASC) of untreated and enzymatic-treated pink guava wastes, which were expressed in dry matter basis. The ASC yield of the untreated decanter and refiner was 11.92% and 10.67%, respectively. The ASC yield of cellulase-treated decanter (90.14%) was significantly higher ($P > 0.05$) than xylanase-treated decanter (79.69%). Yet, it was not significantly different from that of combined enzymatic-treated decanter (88.23%). The ASC yield of combined enzymatic-treated refiner was 89.50%, significantly higher ($P < 0.05$) than xylanase-treated (79.91%). Yet, it was not significantly different from that of cellulase-treated refiner (88.03%). Overall, the ASC yield of decanter and refiner had been enhanced significantly ($P < 0.05$) after fiber-degrading enzymatic treatments. The AIS yield of enzymatic-treated decanters was enhanced by: 78.22% (150CE), 67.77% (150XY), and 76.31% (150CX). Meanwhile, the AIS yield of each enzymatic-treated was enhanced by: 77.36% (150CE), 69.30% (150XY), and 78.83% (150CX). In a nutshell, the AIS yield of decanter and refiner followed the ascending order of untreated (control) $<$ 150 U xylanase $<$ 150 U cellulase $<$ 150 U cellulase-xylanase treatment.

Table 4.5: The yields of alcohol soluble carbohydrates (ASC) of each untreated and enzymatic-treated pink guava waste (decanter and refiner)

Samples	ASC yield (%)			
	150CE	**150XY**	**150CX**	**CTD**
Decanter	90.14 ± 0.31^{c}	79.69 ± 0.85^{b}	88.23 ± 0.42^{c}	11.92 ± 1.54^{a}
Refiner	88.03 ± 0.11^{c}	79.91 ± 1.05^{b}	89.50 ± 0.59^{c}	10.67 ± 2.30^{a}

Values represent means $\pm$ standard deviation (n = 3). Means with different letter in the same row is significantly different ($P < 0.05$).

4.4 Moisture Content

Table 4.6 reflects the moisture content of freeze-dried raw pink guava wastes (decanter and refiner) with particle size of $< 0.45\ \mu$m before being subjected to any enzymatic treatment. Freeze-dried decanter (3.69%) has higher moisture content than freeze-dried refiner (2.68%). Both freeze-dried samples had low moisture content ($< 10\%$) with stable shelf life at room temperature. There was a significant difference found between freeze-dried decanter and refiner as $P < 0.05$.

Table 4.6: Moisture content of freeze-dried raw pink guava wastes.

Samples	Moisture content (%)
Freeze-dried decanter	3.69 ± 0.22^{b}
Freeze-dried refiner	2.68 ± 0.27^{a}

Values represent means $\pm$ standard deviation (n = 3). Means with different letter in the same column is significantly different ($P < 0.05$).

Table 4.7 shows the moisture content of alcohol insoluble solid (AIS) of untreated and enzymatic-treated pink guava wastes (decanter and refiner). The moisture content shown by the moisture balance analyzer is on wet weight basis. AIS of the untreated decanter contained significantly higher ($P < 0.05$) than those AIS of cellulase-treated (3.45%), xylanase-treated (4.19%), and combined enzymatic-treated (3.40%) decanters. Nevertheless, there was no significant difference ($P > 0.05$) found between cellulase-treated and combined enzymatic-treated decanters. Besides, the moisture content of AIS sample of the untreated refiner (6.84%) significantly higher ($P < 0.05$) than those AIS derived from the cellulase-treated (4.78%), xylanase-treated (4.93%), and combined-enzymatic treated (4.76%) refiners. However, there was no significant difference ($P > 0.05$) exist among three enzymatic-treated refiners. Therefore, this suggests that fiber-degrading enzymatic treatments decreased the capacity of the AIS derived from pink guava decanter and refiner to retain moisture.

Table 4.7: Moisture content of alcohol insoluble solid (AIS) derived from the untreated and enzymatic-treated pink guava wastes.

AIS Samples	Moisture content (%)			
	150CE	**150XY**	**150CX**	**CTD**
Decanter	3.45 ± 0.08^{a}	4.19 ± 0.03^{b}	3.40 ± 0.07^{a}	5.74 ± 0.03^{c}
Refiner	4.78 ± 0.03^{a}	4.93 ± 0.05^{a}	4.76 ± 0.04^{a}	6.84 ± 0.07^{b}

Values represent means $\pm$ standard deviation (n = 3). Means with different letter in the same row is significantly different ($P < 0.05$).

Table 4.8 shows the moisture content of alcohol soluble carbohydrates (ASC) samples derived from untreated and enzymatic-treated pink guava wastes, which were expressed in dry weight basis. The moisture content of ASC sample of untreated decanter (5.93%) was significantly higher ($P < 0.05$) than those of the ASC derived from cellulase-treated (3.89%), xylanase-treated (4.39%), and combined enzymatic-treated (3.62%) decanters. Similarly, the ASC of untreated refiner had significantly higher ($P < 0.05$) moisture content (7.07%) than those of the AIS derived from cellulase-treated (4.90%), xylanase-treated (5.12%) and combined enzymatic-treated (4.84%) refiners. However, there was no significant difference exist between both cellulase-treated and combined enzymatic-treated refiners.

Table 4.8: Moisture content of alcohol soluble carbohydrates (ASC) derived from the untreated and enzymatic-treated pink guava wastes.

ASC Samples	Moisture content (%)			
	150CE	**150XY**	**150CX**	**CTD**
Decanter	3.89 ± 0.010^{b}	4.39 ± 0.029^{c}	3.62 ± 0.040^{a}	5.93 ± 0.130^{d}
Refiner	4.90 ± 0.029^{a}	5.12 ± 0.025^{b}	4.84 ± 0.031^{a}	4.84 ± 0.031^{a}

Values represent means $\pm$ standard deviation (n = 3). Means with different letter in the same row is significantly different ($P < 0.05$).

4.5 Analysis on Alcohol Insoluble Solid (AIS) Samples

4.5.1 Chemical Bond through Attenuated Total Reflectance (ATR) Analysis

Attenuated total reflectance (ATR) spectroscopy analysis was performed, with the objective to identify the chemical bonds and functional groups in the AIS samples of untreated and enzymatic-treated pink guava wastes (decanter and refiner). Figure 4.3 illustrates the ATR spectrums of AIS derived from untreated decanter compared with cellulase-treated (150CED), xylanase-treated (150XYD), and combined enzymatic-treated (150CXD) decanters. Meanwhile, Figure 4.4 show the ATR spectrums of AIS derived from untreated refiner compared with those derived from cellulase-treated (150CER), xylanase-treated (150XYR), and combined enzymatic-treated (150CXR) refiners. ATR spectrums were analyzed according to chemical bonds and functional groups as shown in the Table 4.9.

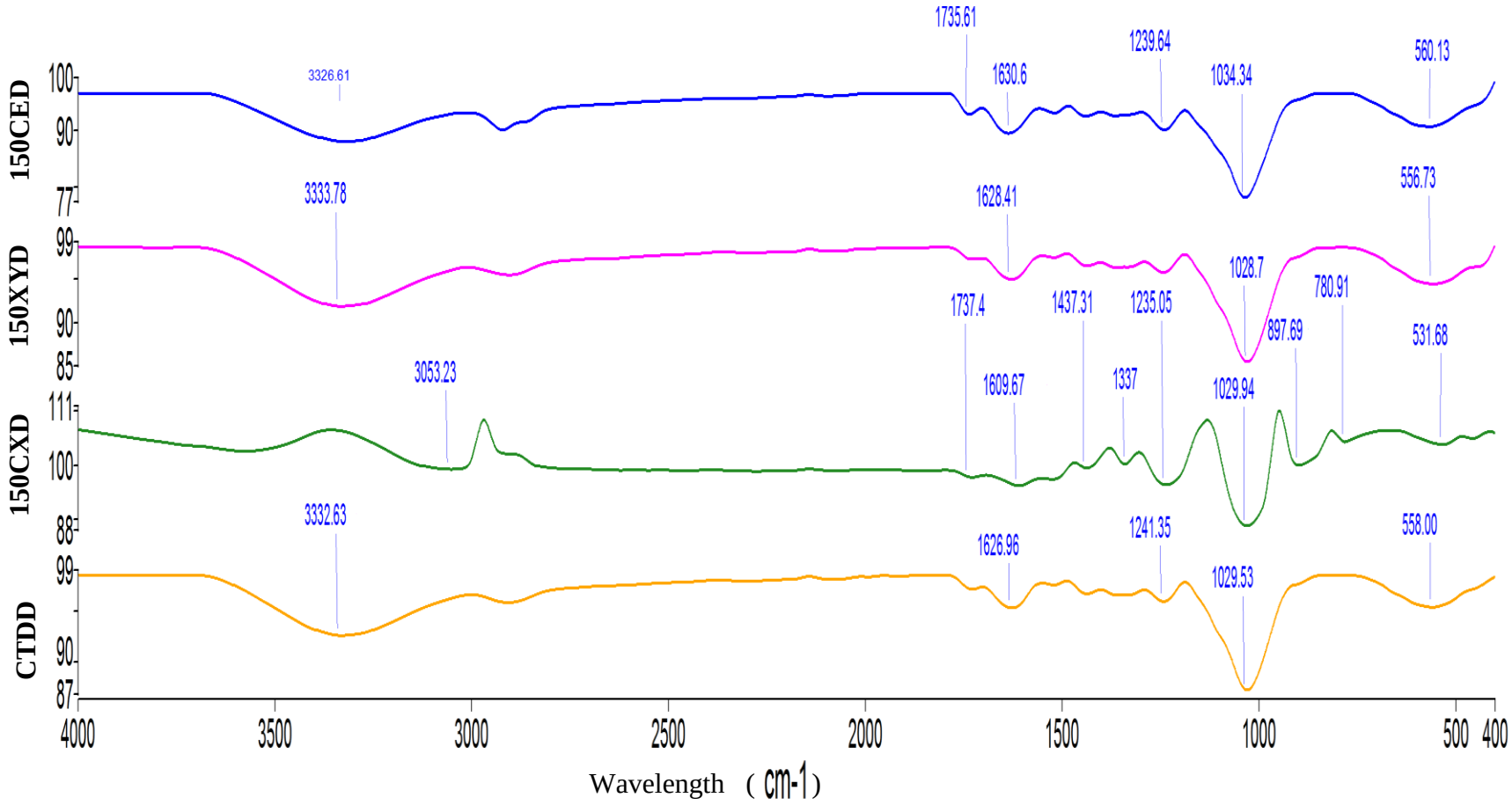


Figure 4.1: ATR spectrum of AIS derived from untreated and enzymatic-treated decanter.

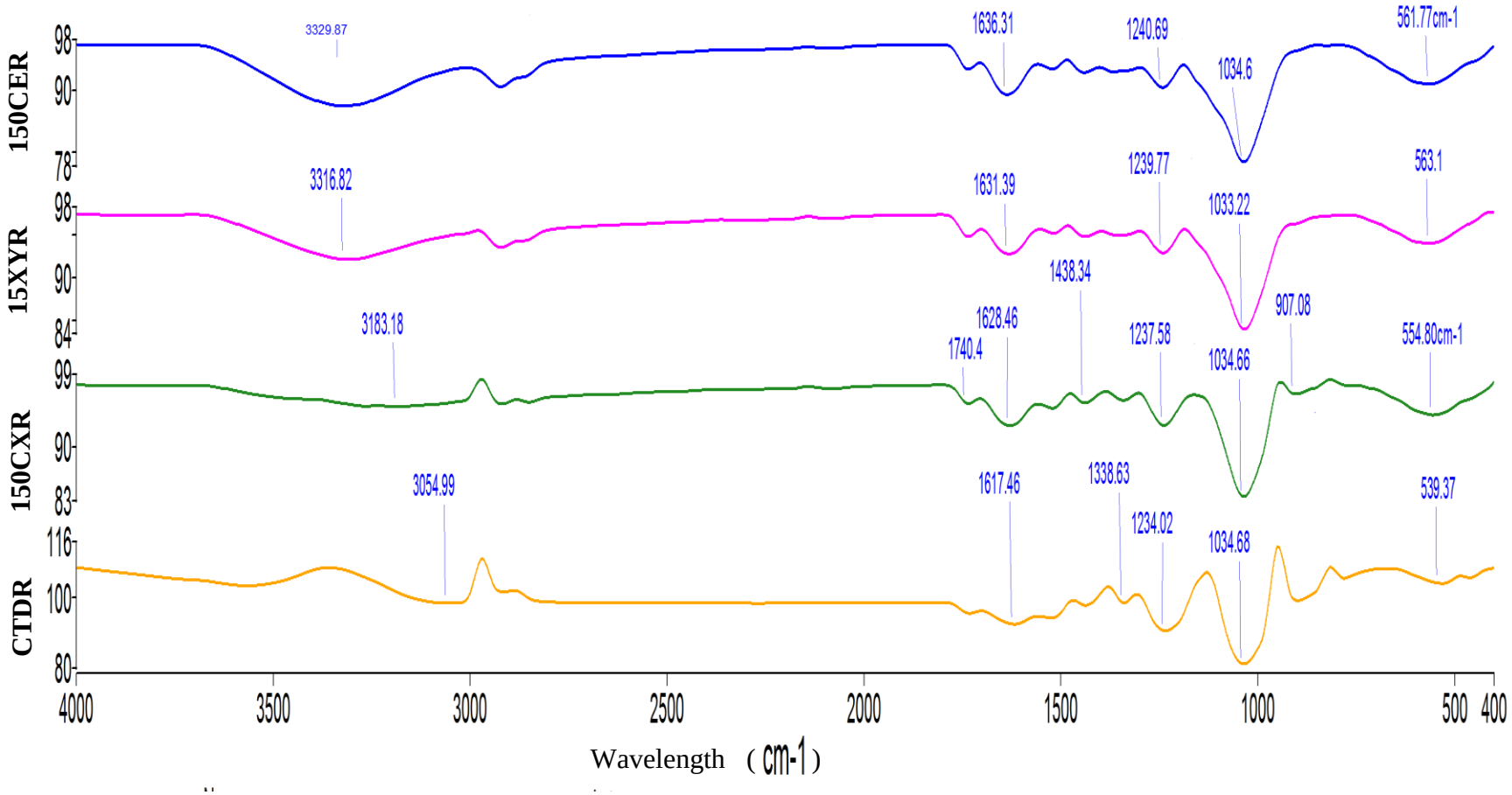


Figure 4.2: ATR spectrum of AIS derived from untreated and enzymatic-treated refiner.

Table 4.9 shows the analyzed information obtained from the ATR spectrums. The O – H stretching, O – H bending, C – H stretching, C = C bending, C = O stretching, C – O stretching, C – H bending, and C – O – C stretching were found in the AIS derived from each untreated and enzymatic-treated pink guava waste (decanter and refiner).

A broad hydroxyl (O – H) stretching, which is ranged between 3053.23 cm^{-1} and 3333.78 cm^{-1} was found in each AIS sample. It is mainly contributed by the presence of cellulose and hemicellulose moieties in both pink guava wastes. Meanwhile, medium O – H bending was only found in the AIS derived from both combined enzymatic-treated decanter (1437.31 cm^{-1}) and refiner (1438.34 cm^{-1}), which represents the presence of carboxylic acid (COOH). It is an indicator of the formation of acetic acid through the synergistic hydrolytic action exerted by combined enzymatic-treatment. On the other hand, medium carbon-carbon double bond (C = C) stretching, at the range between 1609.67 cm^{-1} and 1636.31 cm^{-1} was found in each AIS sample derived from untreated and enzymatic-treated pink guava wastes. Besides, there was also a strong C = C bending only found in the AIS derived from both combined enzymatic-treated decanter (897.09 cm^{-1}) and refiner (907.08 cm^{-1}), respectively. The bands detected with C = C bond were results from the presence of alkenes. Alkenes were produced from the degradation of lignocelluloses after treated with the combination of cellulase and xylanase.

Besides, a strong carbon-hydrogen bond (C –H) bending was only found in the AIS derived from combined enzymatic-treated decanter (780.91 cm^{-1}), where it reflects that the production of glucose from the degradation of lignocellulose compounds (cellulose, hemicellulose and lignin) during the combined enzymatic-treatment. This bending was not found on AIS derived from combined enzymatic-treated refiner. A strong carbon-oxygen (C – O) stretching, which was ranged between ranged between 1234.02 cm^{-1} and 1241.35 cm^{-1} was detected in each AIS sample, with the exception of 150XYD. In addition, there was a strong carbonyl (C = O) stretching, ranged from 1735.61 cm^{-1} and 1740.40 cm^{-1} detected in the AIS of 150CED, 150CXD and 150CXR, which indicates the presence of unsaturated ester. It is contributed by the aromatic hydrocarbons of lignin. The band became broader in both combined enzymatic-treated pink guava wastes, which is due to the hydrolysis of lignocelluloses through the hydrolytic action of cellulase and xylanase. Other than this, strong and broad ether (C – O – C) stretching, ranged between 1028.70 cm^{-1} and 1034.68 cm^{-1} was found in each AIS derived from untreated and enzymatic-treated pink guava wastes. It was resulted from the stretching of polymer backbone (β-1,4-glycosidic bond), which contain anhydride.

Table 4.9: Chemical bonds analyzed from ATR spectrums of AIS of untreated and enzymatic-treated decanter and refiner.

AIS sample		Chemical bond (cm^{-1})							
		O – H stretching	**C = O stretching**	**C = C stretching**	**O – H bending**	**C – O stretching**	**C – O – C stretching**	**C = C bending**	**C – H bending**
Decanter	**150CE**	3326.61	1735.61	1630.60	N/A	1239.64	1034.34	N/A	N/A
	150XY	3333.78	N/A	1628.41	N/A	N/A	1028.70	N/A	N/A
	150CX	3053.23	1737.40	1609.67	1437.31	1235.05	1029.94	897.09	780.91
	CTD	3332.63	N/A	1626.96	N/A	1241.35	1029.53	N/A	N/A

Table 4.9: (continued)

AIS sample		Chemical bond (cm^{-1})							
		O – H stretching	**C = O stretching**	**C = C stretching**	**O – H bending**	**C – O stretching**	**C – O – C stretching**	**C = C bending**	**C – H bending**
Refiner	**150CE**	3329.87	N/A	1636.31	N/A	1240.69	1034.60	N/A	N/A
	150XY	3316.28	N/A	1631.39	N/A	1239.77	1033.22	N/A	N/A
	150CX	3183.18	1740.40	1628.46	1438.34	1237.58	1034.66	907.08	N/A
	CTD	3054.99	N/A	1617.46	N/A	1234.02	1034.68	N/A	N/A

4.5.2 Scanning Electron Microscopy (SEM) Images

The scanning electron microscopy (SEM) analysis was performed to observe and compare the structural surface features of AIS samples of the washed, untreated and enzymatic-treated pink guava wastes (decanter and refiner). Table 4.10 illustrates the SEM images of untreated decanter compared with the images of the original, washed, cellulase-treated, xylanase-treated, and combined enzymatic-treated decanter, under 1000 times magnification. On the other hand, Table 4.11 show the SEM images of untreated refiner compared with the images of the original, washed, cellulase-treated, xylanase-treated, and combined enzymatic-treated refiners at the 1000 times magnification. The AIS surfaces of original pink guava decanter and refiner were uneven, rough with some fibers. Meanwhile, the washed samples were more even with less obvious fibers on their surfaces. Besides, the microscopic images consistently revealed that more rough and porous fibrous structures were formed on the AIS structures of enzymatic-treated pink guava wastes than those of the untreated samples.

Table 4.10: SEM images of untreated decanter compared with the images of the original, washed, cellulase-treated, xylanase-treated and combined enzymatic-treated decanter, under 1000 times magnification.

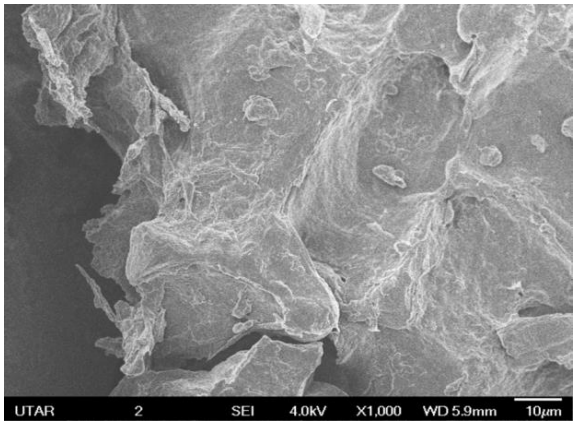
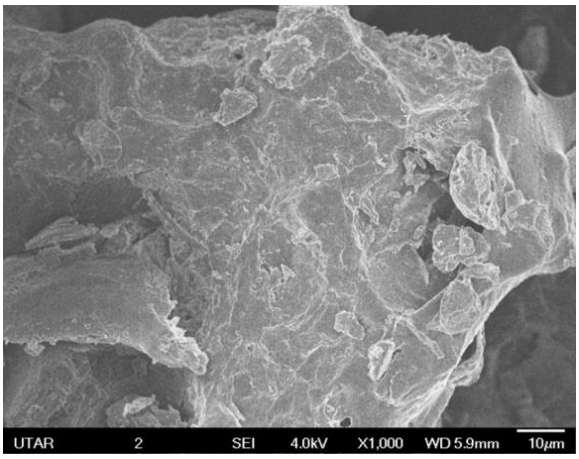
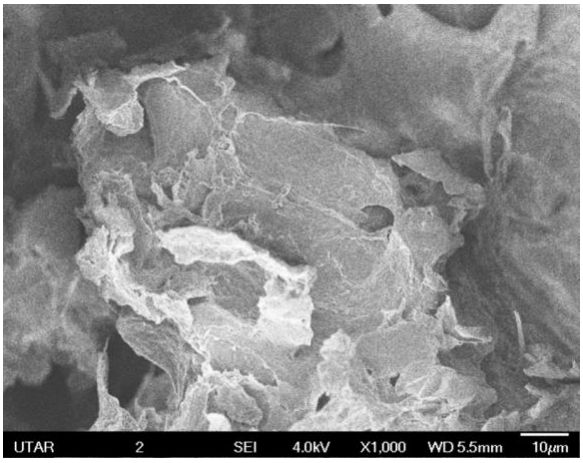
SEM image	Description
	AIS sample derived from untreated decanter
	AIS sampled derived from original decanter
	AIS sample derived from washed decanter

Table 4.10: (continued)

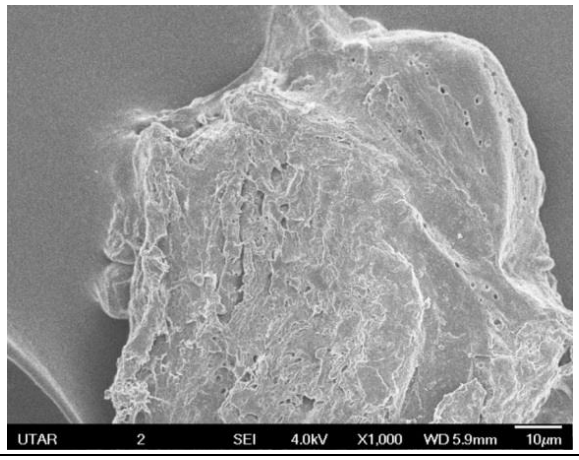
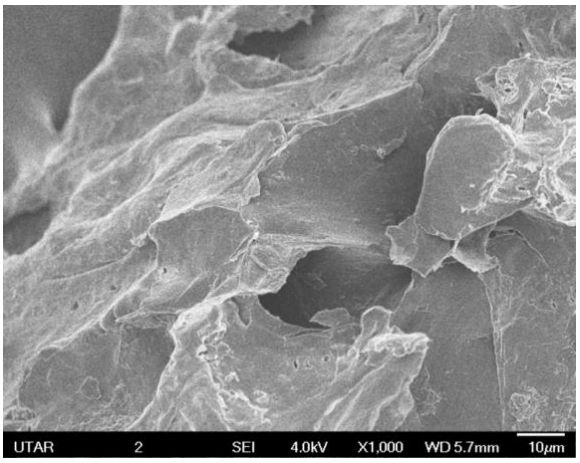
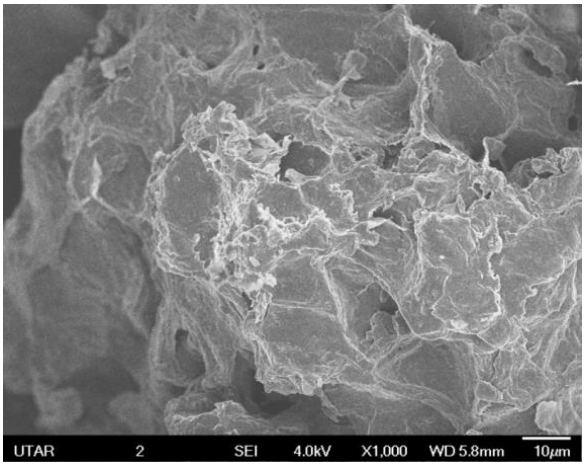
SEM image	Description
	AIS sample derived from cellulase-treated decanter
	AIS sampled derived from xylanase-treated decanter
	AIS sample derived from combined enzymatic-treated decanter

Table 4.11: SEM images of untreated refiner compared with the images of the original, washed, cellulase-treated, xylanase-treated and combined enzymatic-treated refiners at the 1000 times magnification.

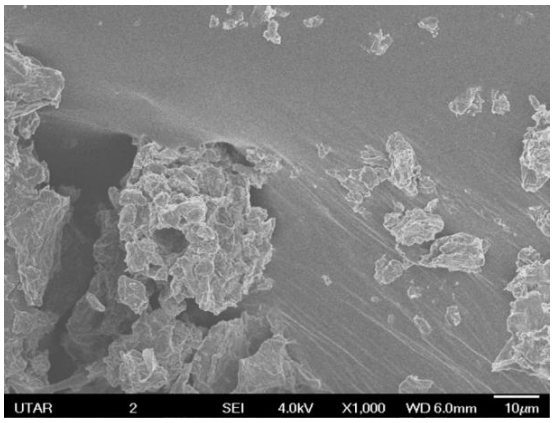
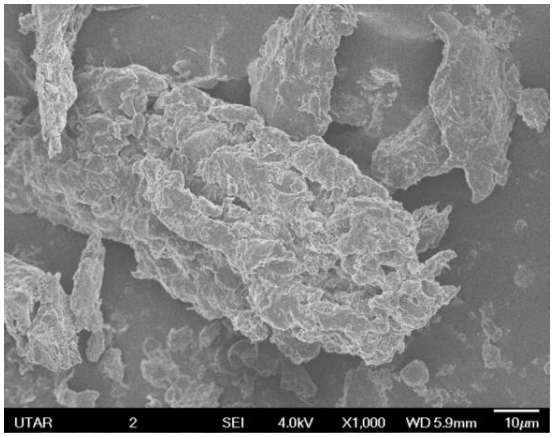
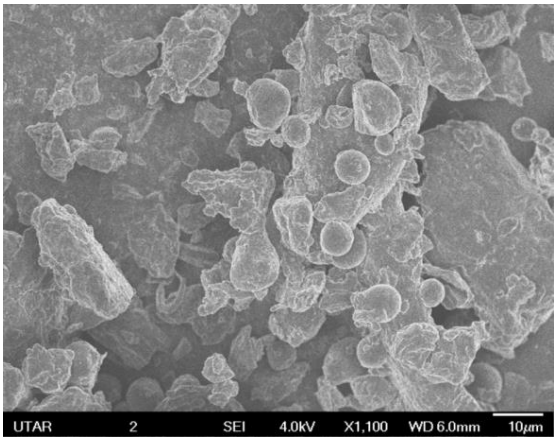
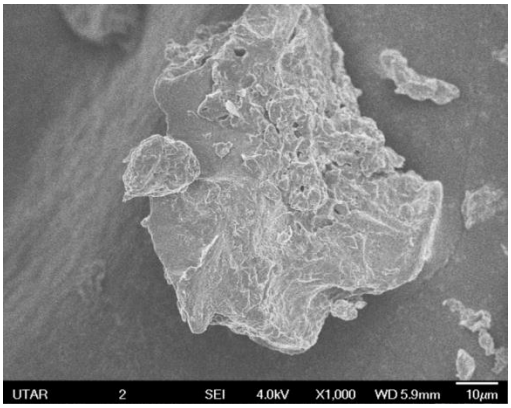
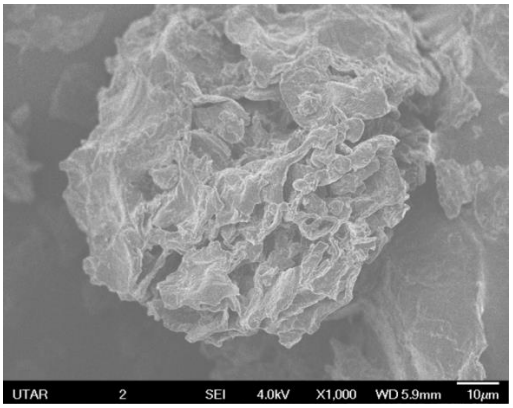
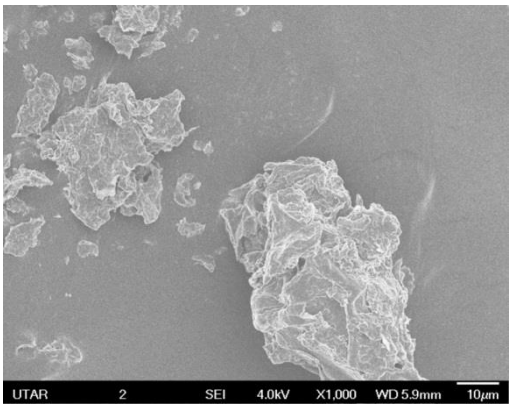
SEM image	Description
	AIS sample derived from untreated refiner
	AIS sampled derived from original refiner
	AIS sample derived from washed refiner

Table 4.11: (continued)

SEM image	Description
	AIS sample derived from cellulase-treated refiner
	AIS sampled derived from xylanase-treated refiner
	AIS sample derived from combined-enzymatic treated refiner

4.5.3 Crystallinity Index through X-ray Diffraction (XRD) Analysis

Table 4.12 shows the X-ray diffraction (XRD) analysis results and crystallinity index for the AIS samples of the untreated and enzymatic-treated decanter and refiner, respectively. Based on Table 4.12, 22.5 ° represents the maximum peak intensity at 2 θ; while 18 ° reflects the minimum peak intensity at 2 θ throughout the X-ray diffraction (XRD) analysis. The crystallinity index of AIS of cellulase-treated (150CE), xylanase-treated (150XY), combined enzymatic-treated (150CX) and untreated (CTD) decanter was reported as 66.15%, 62.96%, 63.47%, and 31.56% respectively. Meanwhile, the crystallinity index of AIS of the cellulase-treated (150CX), xylanase-treated (150XY), combined enzymatic-treated (150CX) and untreated (CTD) refiner was determined as 54.52%, 58.38%, 54.98%, and 49.11% respectively. There was a slight increase in the crystallinity of the AIS samples of pink guava wastes after 24 h of fiber-degrading enzymatic treatments. From this, it reflects that other lignocellulosic fibers in the AIS of pink guava decanter and refiner may influence their crystallinity and susceptibility towards fiber-degrading enzymatic treatments. Besides, untreated decanter exerted the lowest crystallinity index (31.56%); therefore it is more susceptible to enzymatic treatments, as compared to that of untreated refiner.

Table 4.12: Crystallinity index (CrI) for AIS derived from untreated and enzymatic-treated pink guava wastes (decanter and refiner).

Treatments	Decanter			Refiner		
	22.5 °	**18 °**	**Crystallinity index (%)**	**22.5 °**	**18 °**	**Crystallinity index (%)**
150CE	644	218	66.15	2546	1158	54.52
150XY	2454	909	62.96	2499	1040	58.38
150CX	2598	949	63.47	2268	1012	54.98
CTD	2370	1622	31.56	674	343	49.11

4.5.4 Glucose Dialysis Retardation Index (GDRI)

Glucose dialysis retardation index (GDRI) analysis was performed to determine the hypoglycemic potential of the AIS samples of untreated and enzymatic-treated pink guava wastes (decanter and refiner). Figure 4.3 shows the glucose standard curve used to estimate the glucose concentration in each AIS dialysate at different time intervals. Values in the glucose standard curve represent means of triplicate determination. The linear equation of standard curve is y = 0.8515x with the regression of R^2 = 0.9707. Both Table 4.13 and Table 4.14 show the extent of glucose diffuse from the dialysis tube containing glucose with or without AIS samples. From that, the AIS dialysate of untreated decanter exerted the highest extent of glucose diffuse (0.583 mg/mL) from the dialysis tube at 90th min; while the AIS dialysate of combined enzymatic-treated decanter exerted the lowest glucose concentration (0.370 mg/mL) at 90th min. Similar trend was observed on refiner (Table 4.14).

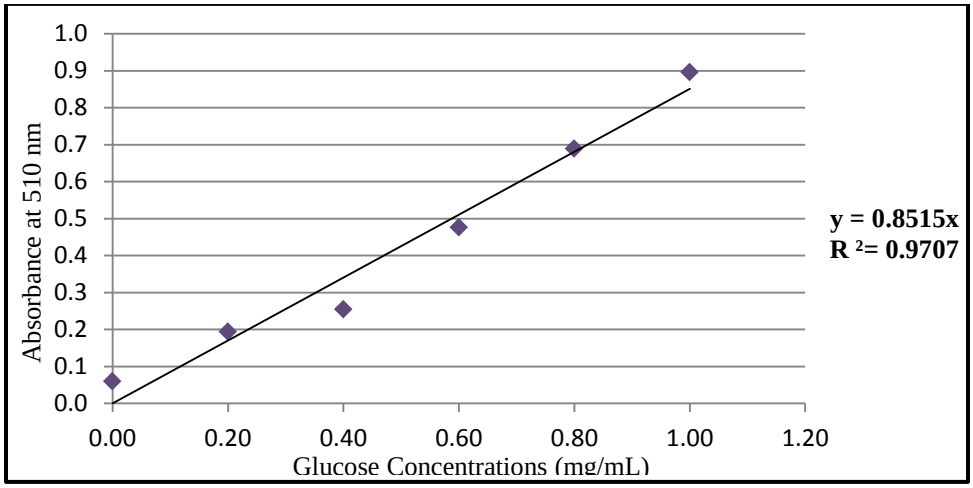


Figure 4.3: Standard curve constructed based on GDRI analysis.

Table 4.13: Glucose concentrations of the AIS dialysate of untreated and enzymatic-treated decanters at different time intervals.

Sample	Time (min)	Glucose concentration in AIS dialysate (mg/mL)				
		Control	**150CE**	**150XY**	**150CX**	**CTD**
Decanter	**15**	0.232 ± 0.004	0.172 ± 0.001	0.170 ± 0.005	0.123 ± 0.003	0.216 ± 0.001
	30	0.433 ± 0.001	0.256 ± 0.001	0.263 ± 0.000	0.209 ± 0.000	0.325 ± 0.004
	45	0.570 ± 0.004	0.323 ± 0.003	0.337 ± 0.000	0.240 ± 0.004	0.383 ± 0.003
	60	0.824 ± 0.011	0.345 ± 0.004	0.442 ± 0.001	0.274 ± 0.003	0.551 ± 0.003
	75	0.893 ± 0.004	0.422 ± 0.003	0.473 ± 0.002	0.321 ± 0.002	0.556 ± 0.002
	90	0.916 ± 0.003	0.445 ± 0.001	0.532 ± 0.002	0.370 ± 0.003	0.583 ± 0.004

Values represent means ± standard deviation, where n = 3.

Table 4.14: Glucose concentrations of the AIS dialysate of untreated and enzymatic-treated refiners at different time intervals.

Sample	Time (min)	Glucose concentration in AIS dialysate (mg/mL)				
		Control	**150CE**	**150XY**	**150CX**	**CTD**
Refiner	**15**	0.232 ± 0.004	0.190 ± 0.001	0.217 ± 0.003	0.147 ± 0.003	0.229 ± 0.003
	30	0.433 ± 0.001	0.230 ± 0.003	0.326 ± 0.000	0.204 ± 0.001	0.348 ± 0.005
	45	0.570 ± 0.004	0.261 ± 0.002	0.346 ± 0.004	0.248 ± 0.001	0.415 ± 0.004
	60	0.824 ± 0.011	0.349 ± 0.000	0.390 ± 0.004	0.310 ± 0.004	0.497 ± 0.006
	75	0.893 ± 0.004	0.403 ± 0.004	0.473 ± 0.004	0.423 ± 0.003	0.534 ± 0.003
	90	0.916 ± 0.003	0.494 ± 0.009	0.578 ± 0.003	0.456 ± 0.009	0.602 ± 0.001

Values represent means ± standard deviation, where n = 3.

Table 4.15 depicts the GDRI values of the AIS of untreated and enzymatic-treated decanter at different time intervals. There was a significant difference ($P < 0.05$) found among the untreated and enzymatic-treated decanter at each time interval. The AIS sample derived from combined enzymatic-treated decanter exerted the highest GDRI values throughout the assay duration, in comparing to those of AIS derived from untreated, cellulase-treated and xylanase-treated decanters. Hence, it indicates that combined enzymatic-treated decanter exerted the highest retardation effect on glucose diffusion, in comparing to those of untreated, cellulase-treated and xylanase-treated decanters. Meanwhile, the AIS of untreated decanter had the lowest GDRI throughout the diffusion. This suggests the hypoglycemic potential of pink guava decanter was enhanced after treated with fiber-degrading enzymes. Besides, the AIS of xylanase-treated and untreated decanter also peaked at longer time interval of diffusion (75^{th} min), which was 47.04% and 37.75%, respectively, as compared to others which peaked at the 60^{th} min.

Table 4.15: GDRI of the AIS derived from untreated and enzymatic-treated pink guava decanter at different time intervals.

Sample	Time (min)	GDRI (%)			
		150CE	**150XY**	**150CX**	**CTD**
Decanter	**15**	25.93 ± 0.59 [b]	26.94 ± 2.05 [b]	46.85 ± 1.34 [c]	6.86 ± 0.501 [a]
	30	40.96 ± 0.16 [c]	39.25 ± 0.00 [b]	51.72 ± 0.06 [d]	25.05 ± 1.03 [a]
	45	43.41 ± 0.52 [c]	40.87 ± 0.01 [b]	57.97 ± 0.74 [d]	32.76 ± 0.48 [a]
	60	58.15 ± 0.44 [c]	46.36 ± 0.08 [b]	66.70 ± 0.41 [d]	33.11 ± 0.36 [a]
	75	52.79 ± 0.35 [c]	47.04 ± 0.27 [b]	64.04 ± 0.20 [d]	37.75 ± 0.20 [a]
	90	51.45 ± 0.07 [c]	41.96 ± 0.27 [b]	59.66 ± 0.32 [d]	36.37 ± 0.39 [a]

Values represent means ± standard deviation (n = 3). Means with different letter in the same row is significantly different ($P < 0.05$).

Table 4.16 depicts the GDRI values of AIS of untreated and enzymatic-treated refiner at different time intervals. There was a significant difference ($P < 0.05$) found among the untreated and enzymatic-treated refiner at each time interval. The AIS sample of combined enzymatic-treated refiner showed the highest GDRI values throughout the assay duration in comparing to those of untreated, cellulase-treated, and xylanase-treated refiners. It reflects that combined enzymatic-treated refiner had the highest retardation effects on glucose diffusion as compared to those of untreated, cellulase-treated, and xylanase-treated refiners. On the other hand, the AIS derived from untreated refiner showed the lowest GDRI throughout the diffusion, which was similar to the trend observed on decanters. This suggests that the hypoglycemic potential of pink guava refiner was enhanced through fiber-degrading enzymatic treatments. In addition, the AIS derived from xylanase-treated and untreated refiners exerted the peak of GDRI at longer time interval of diffusion (75^{th} min), which was 47.00% and 40.21% respectively, in comparing to others which peaked at 60^{th} min.

Table 4.16: GDRI of the AIS derived from untreated and enzymatic-treated pink guava refiner at different time intervals.

Sample	Time (min)	GDRI (%)			
		150CE	**150XY**	**150CX**	**CTD**
Refiner	**15**	17.99 ± 0.51 [c]	6.52 ± 1.27 [b]	36.56 ± 1.46 [d]	1.46 ± 1.27 [a]
	30	46.84 ± 0.72 c	24.87 ± 0.00 [b]	52.81 ± 0.27 [d]	19.63 ± 1.10 [a]
	45	54.12 ± 0.32 [c]	39.29 ± 0.66 [b]	56.53 ± 0.00 [d]	27.13 ± 0.63 [a]
	60	57.67 ± 0.00 [c]	52.73 ± 0.44 [b]	62.42 ± 0.44 [d]	39.71 ± 0.75 [a]
	75	54.89 ± 0.47 [d]	47.00 ± 0.46 [b]	52.66 ± 0.35 [c]	40.21 ± 0.34 [a]
	90	46.07 ± 1.00 [c]	36.88 ± 0.32 [b]	50.21 ± 1.04 [d]	34.31 ± 0.07 [a]

Values represent means ± standard deviation (n = 3). Means with different letter in the same row is significantly different ($P < 0.05$).

Figure 4.4 illustrates comparison on GDRI between cellulase-treated decanter (150CED) and cellulase-treated refiner (150CER) at different time intervals. Both type of AIS showed the highest GDRI at the 60th min of diffusion assay, which was 58.15% and 57.67%, respectively. From this, it indicates cellulase-treated decanter exerted slower glucose diffusion as compared to that of cellulase-treated refiner. There was a significant difference ($P < 0.05$) between the GDRI values of cellulase-treated decanter and refiner at each time interval, with the exception of GDRI at the 60th min. After 60th min, the GDRI of AIS derived from cellulase-treated decanter decreased to 52.79% and 51.45% at the 75th and 90th min, respectively. Similar trend was observed on that of AIS derived from cellulase-treated refiner, the GDRI was reduced to 54.89% and 46.07% at the 75th and 90th min respectively.

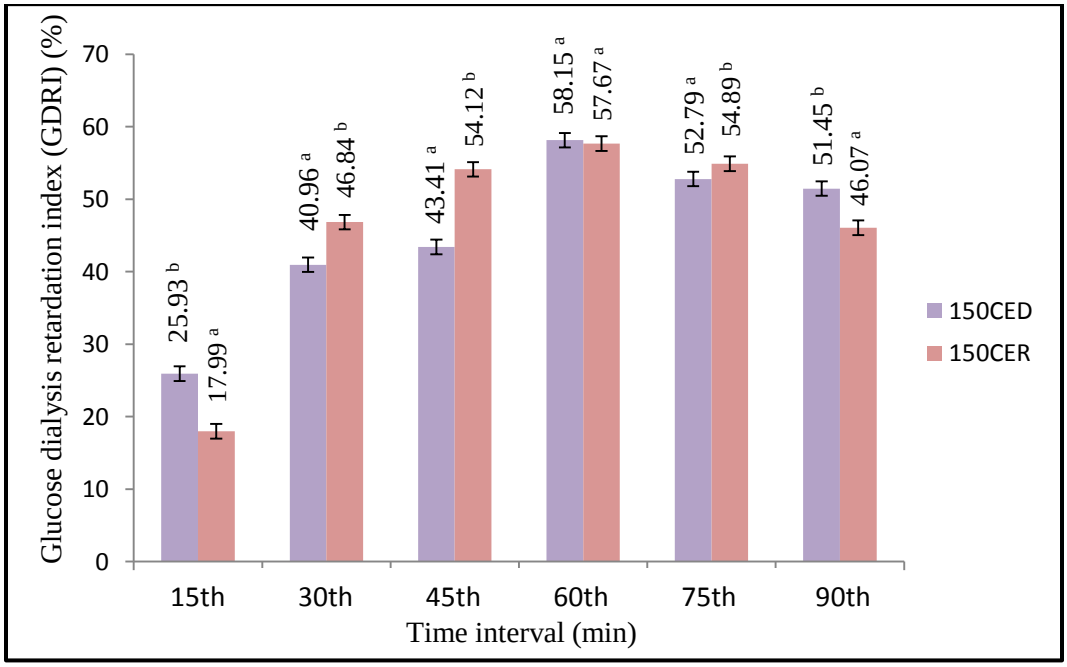


Figure 4.4: Comparison on GDRI between cellulase-treated decanter (150CED) and cellulase-treated refiner (150CER) at different time intervals.

Figure 4.5 illustrates comparison on GDRI between xylanase-treated decanter (150XYD) and xylanase-treated refiner (150XYR) at different time intervals. The AIS derived from xylanase-treated decanter exerted the highest GDRI (47.04%) at the 75th min; while that of AIS derived from xylanase-treated refiner exerted the highest GDRI (52.73%) at the 60th min. It reflects that xylanase-treated refiner exerted slower diffusion rate than that of xylanase-treated decanter. There was a significant difference ($P < 0.05$) between the GDRI of xylanase-treated decanter and xylanase-treated refiner at each time interval, with the exception of GDRI at the 75th min. After 75th min, the GDRI of AIS of xylanase-treated decanter was decreased to 41.96% at the 90th min. Meanwhile, that of AIS of xylanase-treated refiner was reduced to 47.00% and 36.88% at the 75th and 90th min, respectively.

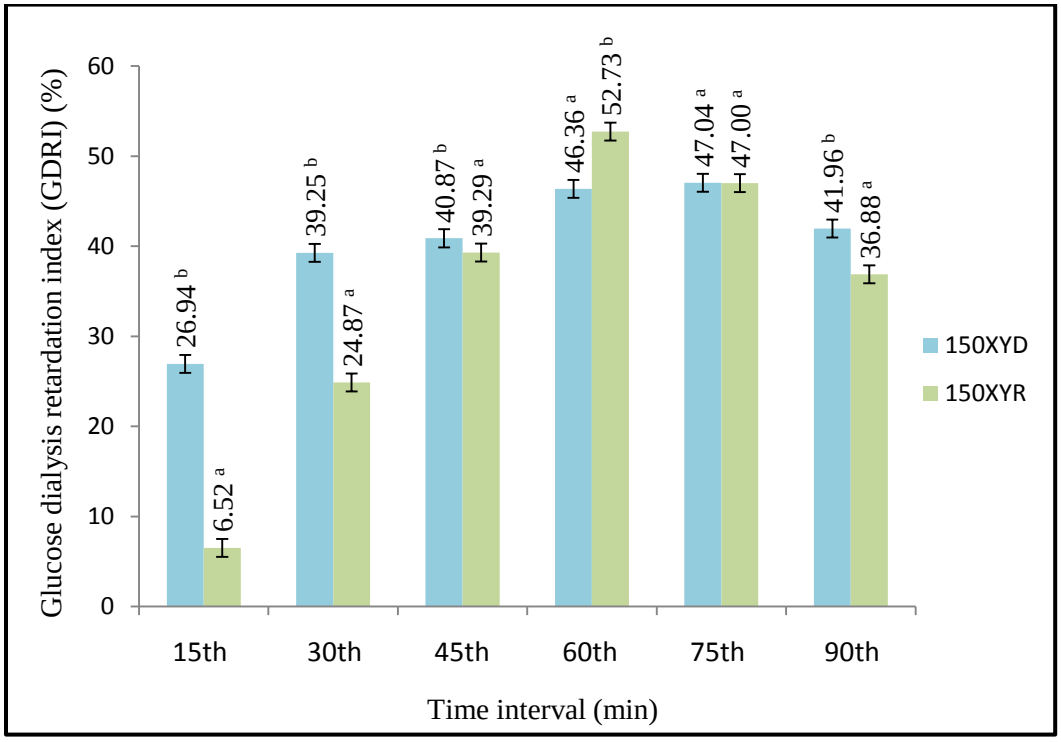


Figure 4.5: Comparison on GDRI between xylanase-treated decanter (150XYD) and xylanase-treated refiner (150XYR) at different time intervals.

Figure 4.6 shows the comparison on the GDRI between AIS of both combined enzymatic-treated decanter (150CXD) and refiner (150CXR) at different time intervals. There was a significant difference ($P < 0.05$) between the GDRI of combined enzymatic-treated decanter and refiner at each time interval. Both AIS derived from combined enzymatic-treated decanter and refiner exerted the highest GDRI at the 60th min of diffusion, which was 66.70% and 62.42%, respectively. It suggests that glucose diffusion rate in the presence of AIS derived from combined enzymatic-treated decanter was significantly slower ($P < 0.05$) than that in the presence of AIS derived from combined enzymatic-treated refiner. After the 60th min, the GDRI of AIS derived from combined enzymatic-treated decanter was reduced to 64.01% and 59.66% at the 75th and 95th min, respectively. Meanwhile, that of AIS derived from refiner was also decreased to 52.65% and 50.21% at the 75th and 90th min, respectively.

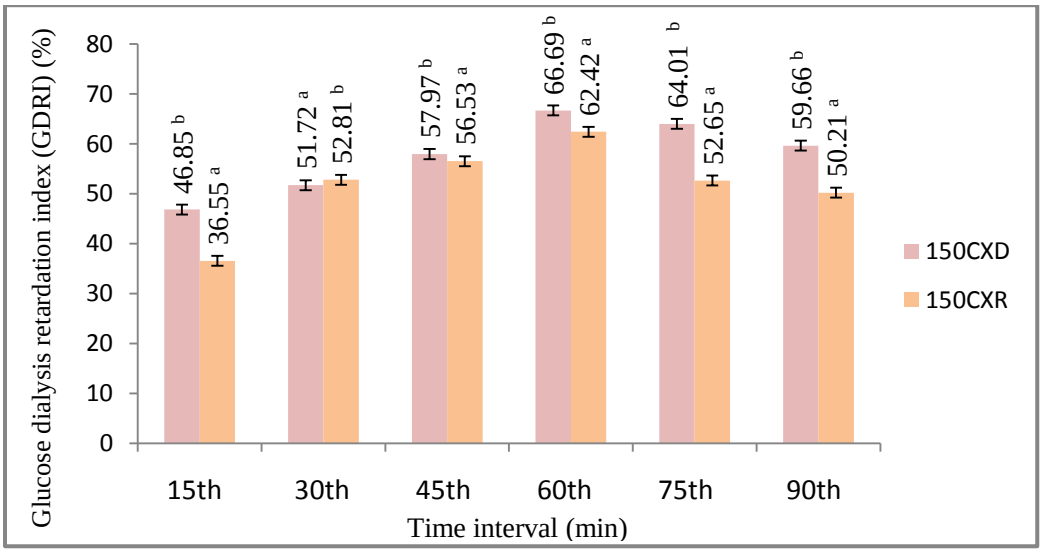


Figure 4.6: Comparison on GDRI between combined enzymatic-treated decanter (150CXD) and combined enzymatic-treated refiner (150CXR) at different time intervals.

Figure 4.7 illustrates comparison on the GDRI values between both AIS derived from untreated decanter (CTDD) and refiner (CTDR) at different time intervals. There was a significant difference ($P < 0.05$) between the GDRI exerted by both untreated decanter and refiner at each time interval. Both untreated decanter and refiner showed the highest GDRI values at the 75th min of diffusion, which was 37.75% and 40.21%, respectively. It reflects that the AIS derived from untreated refiner had significantly higher retardation effect on glucose diffusion rate ($P < 0.05$) in comparing to that of AIS derived from untreated decanter at the 75th min. After the 75th min, the GDRI of AIS derived from untreated decanter was reduced to 37.37%; while that of AIS derived from untreated refiner was decreased to 34.31% at the 90th min.

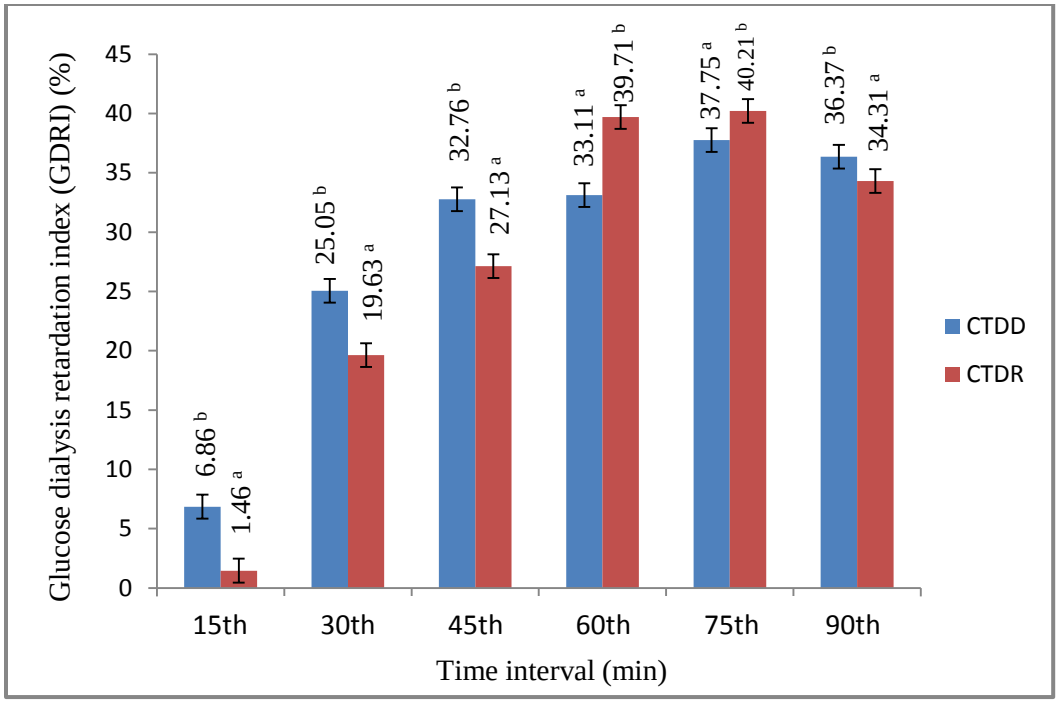


Figure 4.7: Comparison on GDRI between untreated decanter (CTDD) and untreated refiner (CTDR) at different time intervals.

4.6 Analysis on Alcohol soluble carbohydrates (ASC) Samples

4.6.1 Total Soluble Carbohydrates Content

Table 4.17 shows the amount of total soluble carbohydrate in ASC derived from the untreated and enzymatic-treated pink guava wastes (decanter and refiner). There was a significant difference ($P < 0.05$) among the ASC of untreated and enzymatic-treated decanters. The combined enzymatic-treated decanter contained the highest amount of total soluble carbohydrates (77.87 mg/g of dried matter); while the untreated decanter had the lowest total soluble carbohydrates content (3.74 mg/g of dried matter). Meanwhile, there was also a significant difference ($P < 0.05$) among the ASC derived from the untreated and enzymatic-treated refiners. The untreated refiner exhibited the lowest amount of total soluble carbohydrates (2.83 mg/g of dried matter); while the combined enzymatic-treated refiner had the highest total soluble carbohydrates content (91.27 mg/g of dried matter). Hence, it reflects that the total soluble carbohydrate of each pink guava waste was enhanced after treated with fiber-degrading enzymes.

Table 4.17: Total soluble carbohydrates for each ASC samples of untreated and enzymatic-treated pink guava wastes (decanter and refiner).

Samples	Total soluble carbohydrates (mg/g of dry matter)			
	150CE	**150XY**	**150CX**	**CTD**
Decanter	71.69 ± 0.19^{c}	49.17 ± 0.14^{b}	77.87 ± 0.14^{d}	3.74 ± 0.04^{a}
Refiner	87.64 ± 0.19^{c}	55.51 ± 0.05^{b}	91.27 ± 0.10^{d}	2.83 ± 0.11^{a}

Values represent means $\pm$ standard deviation (n = 3). Means with different letter in the same row is significantly different ($P < 0.05$).

Table 4.18 shows the comparison on the amount of total soluble carbohydrates between each ASC sample of decanter and refiner based on the types of fiber-degrading enzymatic treatments. There was a significant difference ($P < 0.05$) between each ASC sample extracted from decanter and refiner. The total soluble carbohydrates content of ASC derived from the enzymatic-treated refiners were significantly higher ($P < 0.05$), as compared with those of ASC derived from enzymatic-treated decanters. However, the ASC derived from untreated decanter had significantly higher total soluble carbohydrates content (3.74 mg/g of dried matter) than those of untreated refiner (2.83 mg/g of dried matter), at $P < 0.05$.

Table 4.18: Comparison on the total soluble carbohydrates content between decanter and refiner according to fiber-degrading enzymatic treatments.

Fiber-degrading enzymatic treatments	Total soluble carbohydrates (mg/g of dry matter)	
	Decanter	**Refiner**
150CE	71.69 ± 0.19^{a}	87.64 ± 0.19^{b}
150XY	49.17 ± 0.14^{a}	55.51 ± 0.05^{b}
150CX	77.87 ± 0.14^{a}	91.27 ± 0.10^{b}
CTD	3.74 ± 0.04^{b}	2.83 ± 0.11^{a}

Values represent means $\pm$ standard deviation (n = 3). Means with different letter in the same row is significantly different ($P < 0.05$).

4.6.2 Sugar Profile through High Performance Liquid Chromatography

Sugar profiles of the alcohol soluble carbohydrates (ASC) samples derived from untreated and enzymatic-treated pink guava wastes (decanter and refiner) were determined by using HPLC analysis. Table 4.19 shows the retention time (Rt), linear equation and regression (R^2) for each sugar standard (glucose, fructose, sucrose, galactose, arabinose, rhamnose, mannose and xylose). Values shown represent the mean of duplicated data. It was used to identify and estimate the sugar presented in each ASC material derived from untreated and enzymatic-treated samples after HPLC analysis.

Table 4.19: Retention time, linear equation and regression for each sugar standard in High Performance Liquid Chromatography (HPLC) analysis.

Sugar standards	Min retention time (min)	Max retention time (min)	Linear equation	Regression (R^2)
Glucose	7.468	7.921	y = 696344.63x	0.97
Fructose	6.488	6.958	y = 1360585.75x	0.96
Sucrose	10.881	11.549	y = 1715855.13x	0.98
Galactose	8.424	9.561	y = 1087852.68x	0.97
Arabinose	6.341	6.942	y = 1675315.28x	0.99
Rhamnose	5.036	5.108	y = 1172632.33x	0.97
Mannose	7.418	8.141	y = 952679.40x	0.96
Xylose	5.738	5.981	y = 1020812.92	0.93

According to Table 4.20, it shows the amount of each identified sugar presented in each ASC derived from the untreated and enzymatic-treated pink guava wastes (decanter and refiner), which was expressed as μg/g of dry matter. The ASC of untreated decanter and refiner had lower amount of rhamnose, xylose, arabinose and mannose as compared to those of ASC of enzymatic-treated. It suggests that the fiber-degrading enzymes exhibited effective hydrolytic action and hydrolyzed

complex lignocellulosic constituents (cellulose and hemicellulose) found in both pink guava wastes into simple sugars, which included glucose, fructose, pentose (arabinose and xylose), and hexose (rhamnose and mannose). In overall, galactose (monosaccharide) and sucrose (disaccharide) were not found in the ASC derived from untreated and enzymatic-treated pink guava wastes. This is most probably due to these sugars had been washed off during the sample washing processes.

Table 4.20: Amount of each identified sugar in ASC of untreated and enzymatic-treated pink guava wastes (decanter and refiner).

Identified sugars	Amount of sugar (μg/g of dry matter)							
	Decanter				Refiner			
	150CE	150XY	150CX	CTD	150CE	150XY	150CX	CTD
Rhamnose	16.49 ^{a}	12.21 ^{a}	5.89 ^{a}	2.05 ^{a}	10.85 ^{a}	24.30 ^{b}	3.34 ^{a}	2.67 ^{a}
Xylose	142.03 ^{b}	110.10 ^{a}	202.58 ^{c}	13.77 ^{b}	252.54 ^{c}	252.72 ^{c}	104.03 ^{b}	65.02^{b}
Arabinose	7.93 ^{a}	n.d	n.d	0.80 ^{a}	70.36 ^{b}	n.d	10.58 ^{a}	9.14 ^{a}
Fructose	n.d	n.d	29.95 ^{ab}	n.d	n.d	n.d	n.d	n.d
Mannose	21.90 ^{a}	29.19 ^{a}	n.d	1.15 ^{a}	n.d	9.64 ^{a}	n.d	n.d
Glucose	n.d	n.d	65.04 ^{b}	n.d	n.d	n.d	n.d	n.d

Values represent means (n = 2). Means in the same column with different letter is significantly different ($P < 0.05$). The n.d denotes as not found.

4.6.3 Prebiotic Activity Score Analysis

4.6.3.1 Prebiotic Activity Based on Absorbance

Table 4.21 shows the prebiotic activity score for each ASC samples of enzymatic-treated and untreated pink guava wastes in supporting the growth or probiotic strain such as *Lactobacillus rhamnosus*, *Lactobacillus plantarum*, *Lactobacillus brevis*, which compared with a control sample (without ASC). It was determined spectrophotometrically. Based on Table 4.21, *Lactobacillus plantarum* apparently exhibited the highest growth if compared with those of *L. rhamnosus* and *L. brevis*. From that, the ASC derived from combined enzymatic-treated decanter had the significant highest prebiotic activity score (4.28) if compared with those of ASC of untreated and other enzymatic-treated decanters. It was enhanced by 95.86 % (from 0.18 to 4.28). Meanwhile, the prebiotic activity score for ASC of the combined enzymatic-treated refiner to support the growth of *L. plantarum* was enhanced by 96.07 % (from 0.18 to 4.51). It showed the significant highest prebiotic activity score (4.51) as compared to those of ASC derived from the cellulase-treated (2.29), xylanase-treated (1.16) and untreated (1.12) refiners ($P < 0.05$).

Table 4.21: Prebiotic activity score (determined based on absorbance) for the ASC samples of enzymatic-treated and untreated pink guava wastes (decanter and refiner), and a control (without ASC), in supporting the growth of different probiotic strain.

Probiotic strains	Prebiotic activity score								
	Decanter				Refiner				Control (without ASC)
	150CE	150XY	150CX	CTD	150CE	150XY	150CX	CTD	
L. rhamnosus	1.60 ± 0.06 [b]	1.26 ± 0.06 [a]	2.14 ± 0.06 [c]	1.14 ± 0.01 [a]	1.49 ± 0.05 [b]	1.08 ± 0.15 [a]	2.44 ± 0.04 [c]	1.05 ± 0.03 [a]	0.06 ± 0.03
L. plantarum	2.02 ± 0.05 [b]	1.13 ± 0.04 [a]	4.28 ± 0.09 [c]	1.04 ± 0.07 [a]	2.29 ± 0.06 [b]	1.16 ± 0.05 [a]	4.51 ± 0.11 [c]	1.12 ± 0.04 [a]	0.18 ± 0.02
L. brevis	0.75 ± 0.08 [b]	0.52 ± 0.05 [a]	1.22 ± 0.08 [c]	0.54 ± 0.02 [a]	0.78 ± 0.07 [b]	0.38 ± 0.06 [a]	1.47 ± 0.08 [c]	0.69 ± 0.03 [b]	0.10 ± 0.02

Values represent means ± standard deviation (n = 3). Means with different letter is in the same row significantly different ($P < 0.05$).

4.6.3.2 Prebiotic Activity Based on Total Viable Count

Table 4.22 shows the prebiotic activity score for each ASC sample derived from untreated and enzymatic-treated pink guava wastes (decanter and refiner) in supporting the growth of probiotic strains such as *Lactobacillus rhamnosus*, *Lactobacillus plantarum*, *Lactobacillus brevis*, which was compared to that of a control sample (without ASC). It was determined based on total viable count. *Lactobacillus plantarum* exhibited the highest growth, as compared with those of *L. rhamnosus* and *L. brevis*. This trend is similar as the prebiotic activity score determined in the previous section 4.19. Based on Table 4.22, the ASC of combined enzymatic-treated decanter showed the highest prebiotic activity score (0.486) to support the growth of *Lactobacillus plantarum*, as compared to those of ASC derived from untreated and enzymatic-treated decanters. It was enhanced by 97.12 % (from 0.014 to 0.486). However, there was no significant difference ($P \geq 0.05$) found among the ASC of enzymatic-treated decanters in supporting the growth of *Lactobacillus plantarum*. On the other hand, the prebiotic activity score exerted by ASC derived from combined enzymatic-treated refiner to support the growth of *L. plantarum* was enhanced by 97.20 % (from 0.014 to 0.50). It had the highest prebiotic activity score (0.50) as compared to those of ASC derived from cellulase-treated (0.48), xylanase-treated (0.46) and untreated (0.36) refiners, where there was no significant difference ($P \geq 0.05$) found among the ASC of enzymatic-treated refiners.

Table 4.22: Prebiotic activity score (determined based on total viable count) for the ASC samples of enzymatic-treated and untreated pink guava wastes (decanter and refiner), and a control (without ASC), in supporting the growth probiotic strains.

Probiotic strains	Prebiotic activity score								
	Decanter				Refiner				Control (without ASC)
	150CE	150XY	150CX	CTD	150CE	150XY	150CX	CTD	
L. rhamnosus	0.14 ± 0.01 ^{b}	0.15 ± 0.00 ^{b}	0.25 ± 0.00 ^{c}	0.02 ± 0.01 ^{a}	0.14 ± 0.00 ^{b}	0.13 ± 0.00 ^{b}	0.25 ± 0.08 ^{c}	0.02 ± 0.00 ^{a}	0.02 ± 0.01
L. plantarum	0.48 ± 0.01 ^{b}	0.45 ± 0.01 ^{b}	0.49 ± 0.01 ^{b}	0.33 ± 0.01 ^{a}	0.48 ± 0.02 ^{b}	0.46 ± 0.02 ^{b}	0.50 ± 0.01 ^{b}	0.35 ± 0.02 ^{a}	0.01 ± 0.00
L. brevis	0.10 ± 0.02 ^{a}	0.07 ± 0.01 ^{a}	0.23 ± 0.00 ^{b}	0.09 ± 0.00 ^{a}	0.10 ± 0.02 ^{a}	0.07 ± 0.00 ^{a}	0.23 ± 0.01 ^{b}	0.06 ± 0.06 ^{a}	0.01 ± 0.00

Values represent means ± standard deviation (n = 2). Means with different letter is in the same row significantly different ($P < 0.05$).

4.6.4 Suitability as Prebiotic Sources in Yogurt

We further analyzed the whole untreated and enzymatic-treated pink guava wastes (instead of ASC) on the growth of probiotic strains in yogurts. Table 4.23 shows the CFU/mL for each yogurt added with untreated and enzymatic-treated pink guava wastes (decanter and refiner), which was expressed in log and compared with the yogurt without any addition of sample (control). The *Lactobacillus* spp. growth in yogurt added with untreated and enzymatic-treated samples exerted similar trending as that of prebiotic activity score. Yogurt contained combined enzymatic-treated decanter showed the highest CFU/mL (8.05), as compared to those contained cellulase-treated (7.15), xylanase-treated (6.32), and untreated (6.02) ones. It was enhanced by 43.49 %, as compared to that of control (4.55). There was a significant difference ($P < 0.05$) among the yogurts added with untreated and enzymatic-treated decanters.

Similarly, yogurt contained combined enzymatic-treated refiner had the highest CFU/mL (8.08), which was increased by 43.71 %. Furthermore, the CFU/mL exhibited by yogurts contained cellulase-treated, xylanase-treated and untreated refiners was 7.17, 6.34 and 5.97, respectively. There was a significant difference ($P < 0.05$) among yogurts added with untreated and enzymatic-treated refiners. In a nutshell, enzymatic-treated pink guava wastes in yogurts supported significantly higher ($P < 0.05$) probiotic growth as compared to untreated pink guava wastes in yogurts.

Table 4.23: Total viable counts of each yogurt sample contained untreated and enzymatic-treated pink guava wastes (decanter and refiner), and a control (without sample).

Samples	Total viable counts (log CFU/mL)			
	150CE	**150XY**	**150CX**	**CTD**
Decanter	7.15 ± 0.03^{c}	6.32 ± 0.04^{b}	8.05 ± 0.03^{d}	6.02 ± 0.05^{a}
Refiner	7.17 ± 0.04^{c}	6.34 ± 0.01^{b}	8.08 ± 0.05^{d}	5.97 ± 0.01^{a}
Control	4.55 ± 0.05			

Values represent means $\pm$ standard deviation (n = 4). Means with different letter in the same row is significantly different ($P < 0.05$).

4.6.4.1 Determining pH Values of Yogurt

Table 4.24 shows the pH values of each yogurt sample added with untreated and enzymatic-treated pink guava wastes (decanter and refiner), which was compared with the yogurt without any sample (control). The yogurt added with combined enzymatic-treated decanter showed the lowest pH value (4.16), as compared with the yogurts added with cellulase-treated (4.34), xylanase-treated (4.51), untreated (4.60) decanters, and control (4.66). There was a significant difference ($P < 0.05$) found among the yogurts added with untreated and enzymatic-treated decanters. Meanwhile, the pH values of yogurts added with untreated and enzymatic-treated refiners were expressed in an ascending order: combined enzymatic-treated (4.06)

and cellulase-treated (4.24), xylanase-treated (4.46), and followed by untreated refiner (4.58), which were lower than that of control sample (4.66). There was a significant difference ($P < 0.05$) found among the yogurts added with untreated and enzymatic-treated refiners. Each yogurt contained untreated and enzymatic-treated pink guava wastes showed low pH values (less than 4.60), which achieved the ideal set point in between pH 4.0 and 4.6 required by most of the yogurt producers to cease yogurt incubation. The optimum and ideal pH range aids in yogurt thickening and contributes as natural preservative against any undesirable pathogen or bacteria strains (Masulli, 2016).

Table 4.24: pH values of yogurts contained untreated and enzymatic-treated pink guava wastes (decanter and refiner), and a control.

Samples	**pH values**			
	150CE	**150XY**	**150CX**	**CTD**
Decanter	4.34 ± 0.04^{b}	4.51 ± 0.04^{c}	4.16 ± 0.05^{a}	4.60 ± 0.05^{c}
Refiner	4.24 ± 0.04^{b}	4.46 ± 0.02^{c}	4.06 ± 0.02^{a}	4.58 ± 0.03^{d}
Control	4.66 ± 0.067			

Values represent means $\pm$ standard deviation (n = 3). Means with different letter in the same row is significantly different ($P < 0.05$).

4.6.4.2 Texture Profile of Yogurt

Table 4.25 shows the texture profile of yogurt contained untreated and enzymatic-treated pink guava wastes (decanter and refiner), and a control (without sample), based on hardness. The hardness of yogurt contained combined enzymatic-treated decanter was 41.54 mm, which was significantly higher ($P < 0.05$) in comparing with those contained cellulase-treated, xylanase-treated and untreated decanters. However, there was no significant difference ($P > 0.05$) between the hardness of yogurts added with cellulase-treated and xylanase-treated decanters. Meanwhile, the hardness of yogurt contained combined enzymatic-treated refiner was 43.50 mm, which was significantly higher ($P < 0.05$) as compared with those contained cellulase-treated, xylanase-treated and untreated refiners.

Table 4.25: Texture profile for each yogurt added with untreated and enzymatic-treated pink guava wastes (decanter and refiner), and a control (without any sample), based on hardness.

Samples	**Hardness (mm)**			
	150CE	**150XY**	**150CX**	**CTD**
Decanter	36.53 ± 0.01^{b}	36.50 ± 0.05^{b}	41.54 ± 0.04^{c}	34.12 ± 0.07^{a}
Refiner	36.65 ± 0.03^{b}	37.13 ± 0.11^{c}	43.50 ± 0.30^{d}	34.64 ± 0.03^{a}
Control	33.97 ± 0.40			

Values represent means $\pm$ standard deviation (n = 3). Means with different letter in the same row is significantly different ($P < 0.05$).

Table 4.26 shows the texture profile of yogurt contained untreated and enzymatic-treated pink guava wastes (decanter and refiner), and a control (without sample), based on stickiness. The stickiness of yogurt added with combined enzymatic-treated decanter was 125.00 g, which showed significantly higher ($P < 0.05$) as compared to the yogurts added with cellulase-treated (121.21 g), xylanase-treated (98.68 g), and untreated (96.54 g) decanters. On the other hand, the stickiness of yogurt contained combined enzymatic-treated refiner was 124.26 g, which was significantly higher ($P < 0.05$) in comparing to the yogurts added with cellulase-treated (121.13 g), xylanase-treated (100.68 g), and untreated (98.64 g) refiners.

Table 4.26: Texture profile for each yogurt added with untreated and enzymatic-treated pink guava wastes (decanter and refiner), and a control (without any sample), based on stickiness.

Samples	**Stickiness (g)**			
	150CE	**150XY**	**150CX**	**CTD**
Decanter	121.21 ± 0.20^{c}	98.68 ± 0.14^{b}	125.00 ± 0.49^{d}	96.54 ± 0.07^{a}
Refiner	121.13 ± 0.11^{c}	100.68 ± 1.05^{b}	124.26 ± 0.57^{d}	98.64 ± 0.03^{a}
Control	74.14 ± 0.78			

Values represent means $\pm$ standard deviation (n = 3). Means with different letter in the same row is significantly different ($P < 0.05$).

Table 4.27 shows the adhesiveness of yogurt contained untreated and enzymatic-treated pink guava wastes (decanter and refiner), and a control (without sample). The adhesiveness of yogurt contained combined enzymatic-treated decanter was 192.83 g.s, which showed significantly higher ($P < 0.05$) as compared to the yogurts added with cellulase-treated (170.90 g.s), xylanase-treated (157.77 g.s), and untreated (114.79 g.s) decanters. On the other hand, the adhesiveness of yogurt added with combined enzymatic-treated refiner was 190.43 g.s, which was significantly higher ($P < 0.05$) as compared to those of cellulase-treated (170.72 g.s), xylanase-treated (155.33 g.s) and untreated (115.65 g.s) refiners.

Table 4.27: Texture profile for each yogurt added with untreated and enzymatic-treated pink guava wastes (decanter and refiner), and a control (without any sample), based on adhesiveness.

Samples	**Adhesiveness (g.s)**			
	150CE	**150XY**	**150CX**	**CTD**
Decanter	170.90 ± 0.06^{c}	157.77 ± 0.30^{b}	192.83 ± 0.21^{d}	114.79 ± 0.33^{a}
Refiner	170.72 ± 0.19^{c}	155.33 ± 0.75^{b}	190.43 ± 0.09^{d}	115.65 ± 0.48^{a}
Control	103.42 ± 0.93			

Values represent means $\pm$ standard deviation (n = 3). Means with different letter in the same row is significantly different ($P < 0.05$).

Table 4.28 shows the texture profile of yogurt contained untreated and enzymatic-treated pink guava wastes (decanter and refiner), and a control (without sample), based on stringiness. The stringiness of yogurt added with combined enzymatic-treated decanter was 159.36 mm, which showed significantly higher ($P < 0.05$) as compared to the yogurts added with xylanase-treated (154.14 mm) and untreated (37.60 mm) decanters. However, it has no significantly difference ($P > 0.05$) with cellulase-treated (157.90 mm). On the other hand, the stringiness of yogurt added with combined enzymatic-treated refiner was 158.92 mm, and it was significantly higher ($P < 0.05$) as compared to the yogurts added with xylanase-treated (153.16 mm) and untreated (36.88 mm) refiners, but no significant difference ($P > 0.05$) with cellulase-treated (158.72 mm).

Table 4.28: Texture profile for each yogurt added with untreated and enzymatic-treated pink guava wastes (decanter and refiner), and a control (without any sample), based on stringiness.

Samples	**Stringiness (mm)**			
	150CE	**150XY**	**150CX**	**CTD**
Decanter	157.90 ± 0.01^{c}	154.14 ± 1.18^{b}	159.36 ± 0.85^{c}	37.60 ± 0.02^{a}
Refiner	158.72 ± 0.49^{c}	153.16 ± 0.03^{b}	158.92 ± 1.15^{c}	36.88 ± 0.11^{a}
Control	34.88 ± 0.50			

Values represent means $\pm$ standard deviation (n = 3). Means with different letter in the same row is significantly different ($P < 0.05$).

CHAPTER 5

DISCUSSION

5.1 Reducing Sugar Content Released during Sample Washing

The sample washing process was performed at 50 ± 5℃ before treated with any fiber-degrading enzyme to prevent any interferences caused by other chemical components present in both pink guava wastes (decanter and refiner) (Renata et al., 2012). Sample washing was required as part of sample preparation processes, as it has capable to induce the release of free sugars from fiber-rich materials at high temperature without the catalysis by fiber-degrading enzymes. This finding was supported by previous research study by Moiser et al. (2005). The presence of free sugars in plant-based material may have interference the hydrolytic action of fiber-degrading enzymes. These free sugars may occupy the active sites of enzymes, to hinder the enzyme's action on hydrolyzing fiber, thus the release of free sugars may be hindered after treatment and reduced efficiency in term of exerting hydrolytic action on substrates. Therefore, hot water treatment was applied to remove free sugars in plant-based material without solubilizing fibrous fractions such as cellulose and hemicellulose (Moiser et al., 2005).

The optimum temperature to eliminate free sugars in the plant-based sample was reported as 55℃, as it is similar as that of cellulase treatment. For this research, decanter and refiner were washed by using distilled water for twice, to wash off the free sugars and undesirable inorganic compounds from them efficiently. In addition, the freeze-dried sample was stirred continuously during sample washing process, to increase the rate of free sugars released and prevent sample clumping to take place. Centrifugation was also employed to achieve an efficient separation of the fiber-rich fraction (pellet) and the supernatant under gravity force.

The reducing sugar content released by each freeze-dried pink guava waste was determined to ensure previous sample washing processes were efficient to remove and wash off the free sugars before any fiber-degrading enzymatic treatments. In accordance to the Table 4.1, the reducing sugar content in the effluent released by the freeze-dried decanter during the first sample washing process was 21.89 mg/g, which was then decreased to 2.04 mg/g after the second sample washing process. It indicates that reducing sugar content released by freeze-dried decanter was reduced by 90.68% after the entire sample washing process, where there was a significant difference found as $P < 0.05$. Meanwhile, the reducing sugar content released by freeze-dried refiner during the first sample washing process was 27.70 mg/g, and it was minimized significantly ($P < 0.05$) by 87.74% after the second sample washing process (3.74 mg/g). In overall, the reduction of reducing sugar content in freeze-dried decanter (90.68%) was significantly higher ($P < 0.05$) than freeze-dried refiner (87.74%), as decanter consists of stone cells with higher

monosaccharides (glucose and fructose), as compared with that of refiner (Mhd Abd Kader et al., 2016). This suggests that the entire sample washing process was effective and efficient in removing most of the free sugar content in both freeze-dried pink guava wastes. In addition, the possibilities of interferences occurred in between free sugars and fiber-degrading enzymatic treatments may be decreased significantly ($P < 0.05$). Therefore, it assures the effectiveness of fiber-degrading enzymes (cellulase and xylanase) in hydrolyzing the lignocellulosic constituents (cellulose, hemicellulose, lignin and pectin) found in washed pink guava wastes.

5.2 Fiber-degrading Enzymatic Treatments

Cellulase is a derivable fiber-degrading enzyme produced by microorganism with the ability to catalyze in the hydrolysis of β-1,4-glycosidic linkage which joins two glucose monomers in a cellulose chain (Fariq, 2016). Cellulase has great ability to improve and enhance the structural properties of pink guava wastes by physical disruption of cellulose. To achieve better performance in hydrolyzing cellulose into glucose and other simple sugars, a cellulase system should consist of three hydrolytic enzymes (endoglucanase, exoglucanase, and β-glucosidase). The cellulose hydrolysis can be completed by the synergistic action performed by both endoglucanase, and exoglucanase, based on the findings stated by Fariq, 2016; Jadhav, Chitanand and Shete, 2013. The optimum parameters for cellulase to exert efficient enzymatic activity: dissolved in 50 mM citrate buffer (pH 4.5) at 55℃ for 24 h (Sreeranjini, 2017). On the other hand, xylanase performs effective

hydrolytic action to break down plant cell walls held by xylan and hemicellulose. It can break down the β-1,4-glycosidic linkages of xylosidase. Therefore, it forms sugar hemiacetal with the corresponding free aglycone (Harris and Ramalingam, 2010). Endo-xylanase, β-xylosidase, α-glucuronidas,α-arabinofuranosidase, and acetylxylan esterase are well combined together to achieve better efficiency in hydrolyzing xylan which is converted into its constituent sugars (Motta, Andrade and Santana, 2013; Shahi et al., 2016). Xylanase activity achieved the maximum at 55℃ with pH 4.5 – 8.0, which employed for industrial application (Ninawe et al., 2008; Walia et al., 2014).

5.2.1 Reducing Sugar Content Produced After Enzymatic Hydrolysis

The reducing sugar content produced after enzymatic hydrolysis was determined to reflect the extent of fiber-degrading enzymatic treatments workable on both pink guava wastes (decanter and refiner). It was determined by employing Nelson Somogyi method, which is applied for the quantitative determination of reducing sugar content according to the presence of aldehyde- or keto- groups (Alexander, Elena and Arkady, 2011). In accordance to Table 4.2, 335.80 μg/g of reducing sugar released by combined enzymatic-treated decanter into the effluent, which is significantly higher ($P < 0.05$) than cellulase-treated (310.50 μg/g) and xylanase-treated (207.47 μg/g) decanters. Meanwhile, 337.80 μg/g of reducing sugar released by combined enzymatic-treated refiner into the effluent, in which it is significantly higher ($P < 0.05$) than cellulase-treated (313.00 μg/g) and xylanase-

treated (181.80 μg/g) refiners. It reflects that refiner was more susceptible to be hydrolyzed via fiber-degrading enzymatic treatments, in comparing to decanter, except in the case of 150 U xylanase. This is due to refiner consists of seeds and coarse pulp which are rich in dietary fiber content and lignocelluloses (cellulose, hemicellulose, and lignin). These are literally target substrates for fiber-degrading enzymes to act on and exhibit hydrolytic action (Mhd Abd Kader et al., 2016). Fiber-degrading enzymes could break down the lignocellulosic constituents into reducing sugars such as glucose (Figure 5.1). Therefore, there was more reducing sugar content released by refiner when treated with fiber-degrading enzymes for 24 h at 55℃.

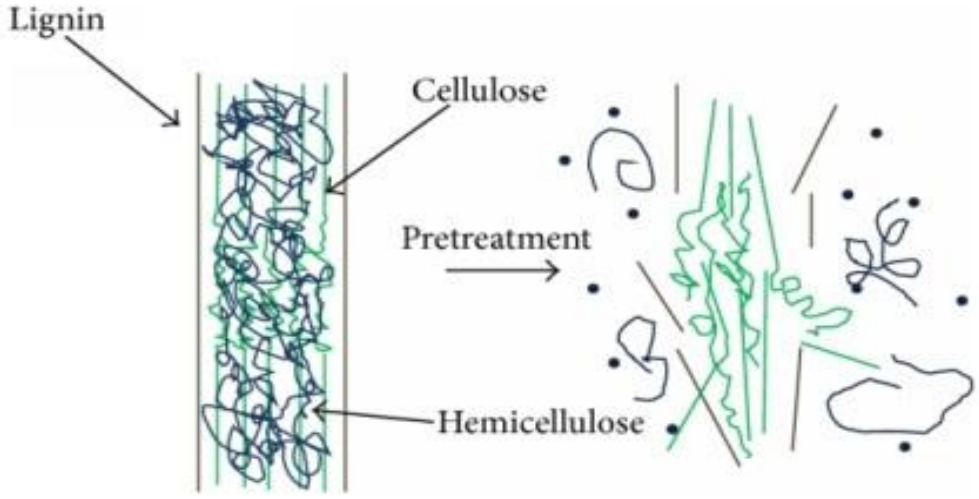


Figure 5.1: Deconstruction of lignocellulosic (cellulose, hemicellulose and lignin) (Lee, Bee Abd Hamid and Zain, 2014).

On the other hand, both combined enzymatic-treated pink guava wastes (decanter and refiner) released the highest amount of reducing sugar, as compared to those treated with single cellulase and xylanase only. This suggests that the combination of cellulase and xylanase exhibits effective hydrolytic action with a synergistic effect to hydrolyze and break down lignocelluloses (cellulose, hemicellulose, and lignin) of pink guava wastes. This finding was supported by Huang et al. (2018). The addition of xylanase into cellulase treatment can boost up and enhance the hydrolysis of lignocellulosic biomass by withdrawing cellulose, hemicellulose, and lignin. In addition, xylanase promotes cellulose hydrolysis from the inner part of lignocellulosic biomass through generating tiny holes in xylan-surface-covered cellulose, which results in the depolymerization and fragmentation of cellulose (Arantes et al., 2011). According to Song et al. (2016), the addition of xylanase promotes an additive effect against cellulase, which results in the formation of potential synergistic effects. The combination of cellulase and xylanase produced more reducing sugar content released by lignocellulosic biomass (corncob powder, rice straw, and corn stover), as compared with those treated with an individual cellulase. Besides, based on the previous research finding by Hu, Arantes and Saddler (2011), the addition of xylanase into cellulase treatment can overcome blocking effect caused by xylan. The combination of cellulase and xylanase was proven as have potential capability to perform synergistic interaction in improving cellulose accessibility, in which it could increase fiber porosity and fiber swelling (Hu, Arantes and Saddler, 2011). This could be evidenced with the outcomes of SEM analysis, as per discussed in the section of 4.5.2 and elaborated in 5.5.2.

5.3 The Yield of Ethanolic Extraction

Ethanolic extraction was carried out on all untreated and enzymatic-treated pink guava decanter and refiner by using 80% (v/v) ethanol, to obtain alcohol insoluble solid (AIS) and alcohol soluble carbohydrates (ASC) (Farrant et al., 2003). Based on the previous study by Thomas et al. (2000), 80% (v/v) boiling ethanol was employed to inactivate possible endogenous enzymes and separate alcohol soluble solids from alcohol insoluble solids effectively. Alcohol insoluble solid (AIS) is described as the fraction consists of lignin, cellulose, hemicellulose, and dietary fiber, which are the important components of cell wall polysaccharides (Yang et al., 2017). According to Table 4.4, the yield of alcohol insoluble solid (AIS) derived from the untreated, cellulase-treated, xylanase-treated, and combined enzymatic-treated decanters was determined as 88.07%, 9.84%, 20.29%, and 11.74%, respectively. On the other hand, the yield of AIS derived from the untreated, cellulase-treated, xylanase-treated, and combined enzymatic-treated refiners was 89.27%, 11.995%, 20.06%, and 10.41%, respectively. From this, it indicates that the AIS yields of pink guava wastes were decreased significantly ($P < 0.05$) after treated with fiber-degrading enzymes (cellulase and xylanase). This is due to cellulose, hemicellulose, lignin, and pectin substances in the AIS derived from pink guava wastes had been broken down and produced reducing sugars such as glucose and other simple sugars (Yang et al., 2017). These sugars may diffuse out from the AIS through porous structures, and results in the decrement of AIS yield.

In addition, alcohol soluble carbohydrates (ASC) are the fraction which rich in reducing sugars (glucose and fructose), lower molecular weight carbohydrates (lactose and sucrose), soluble lignin, and hemicellulose (Chen et al., 2007; Yang et al., 2017). The yield of ASC derived from each untreated and enzymatic-treated pink guava wastes was determined after freeze-dried for 72 h continuously. According to Table 4.5, the ASC yields of untreated, cellulase-treated, xylanase-treated, and combined enzymatic-treated decanters was determined as 11.92%, 90.14%, 79.69%, and 88.23%, respectively. Meanwhile, the yield of ASC derived from the untreated, cellulase-treated, xylanase-treated, and combined enzymatic-treated refiners was 10.67%, 88.03%, 79.91%, and 89.50%, respectively. ASC yield of both pink guava wastes had been significantly ($P < 0.05$) enhanced after fiber-degrading enzymatic treatments. Reducing sugars (glucose monomers) and lower molecular carbohydrates may be diffused out from the AIS and dissolved in the 80% (v/v) ethanol solvent, which results in an increase of ASC yield (Yang et al., 2017). This suggests fiber-degrading enzymatic modifications could reduce the molecular weight and improve the solubility of dietary fibers, which results in enhancing the ASC yields.

5.4 Moisture Content

Freeze-drying technique was employed to minimize and withdraw moisture from both pink guava wastes (decanter and refiner), therefore it can increase the sample shelf life and microbial stability at room temperature through limiting the water activity, a_w (Zhang et al., 2006). This enables us to use the same starting material throughout the entire research. Freeze-drying involves minimizing surrounding pressure, so that frozen water trapped within the food materials can be sublimated from solid phase to gas phase directly (Park, Lee and Eun, 2016). It dehydrates pink guava wastes I worked on without causing browning index and appearance changes (Park, Lee and Eun, 2016). It has better ability to preserve antioxidants and polyphenols in samples, as compared to other drying methods such as hot air-oven drying and sun drying techniques (Wolfe and Liu, 2003). Moisture content of dehydrated samples has to be determined because it is critical to eliminate any variations caused by the weight of moisture in samples. Thus, the constituents in dehydrated sample can be expressed in dried matter basis (Lim, 2016).

According to Table 4.6, freeze-dried decanter has significantly higher ($P < 0.05$) moisture content (3.69%) than that of freeze-dried refiner (2.68%). According to Bridgeman et al. (2007), surface area is a critical factor affects the absorption of water molecules, in which a sample with smaller particle size posses with larger surface area per volume. It absorbs more water vapor from the atmosphere, which results in containing higher moisture content (Bridgeman et al., 2007). Based on

Mhd Abd Kader et al. (2016), the residues in pink guava refiner contains more seeds as compared to decanter, and it leads to the variation in moisture content between both. Nevertheless, the size distribution might not be uniform throughout pre-treatment, which caused moisture content deviation had taken place between pink guava decanter and refiner.

Besides, the moisture content of alcohol insoluble solid (AIS) and alcohol soluble carbohdyrates (ASC) materials derived from each untreated and enzymatic-treated pink guava waste (decanter and refiner) were determined prior to further analysis. In accordance to Table 4.7, the moisture content of AIS derived from the untreated, cellulase-treated, xylanase-treated, and combined enzymatic-treated decanters was 5.74%, 3.45%, 4.19%, and 3.40%, respectively. Meanwhile, the moisture content of AIS derived from untreated, cellulase-treated, xylanase-treated, and combined enzymatic-treated refiners was 6.84%, 4.78%, 4.93%, and 4.76%, respectively. In overall, the AIS derived from all enzymatic-treated pink guava wastes had significantly ($P \leq 0.05$) lower moisture content as compared to those of untreated ones. The AIS derived from pink guava wastes are mainly composed of natural fibers (cellulose, hemicellulose, pectin, and lignin), to make it has great ability to absorb moisture from atmosphere and exhibit as hygroscopic (Faruk et al., 2012; Yang et al., 2017). These natural fibers had been hydrolyzed after treated with fiber-degrading enzymes, and results as the ability of moisture absorption may be minimized.

According to Table 4.8, the moisture content of ASC derived from the untreated, cellulase-treated, xylanase-treated and combined enzymatic-treated decanters was reported as 5.93%, 3.89%, 4.89%, and 3.62%, respectively. On the other hand, the moisture content of ASC derived from the untreated, cellulase-treated, xylanase-treated and combined enzymatic-treated refiners was determined as 7.07%, 4.90%, 5.12%, and 4.84%, respectively. The moisture content of ASC derived from pink guava wastes had been significantly ($P \leq 0.05$) decreased after treated with fiber-degrading enzymes, which is the similar trend as AIS derived from untreated and enzymatic-treated pink guava wastes, after freeze-drying process. In this research study, those freeze-dried, AIS and ASC derived from both pink guava wastes contained moisture content less than 10%. Thus, their shelf life stability could be prolonged and free from any risk of microbial spoilage (Afolabi et al., 2012). Each sample may be used as fresh sample for research analysis, under optimum chilled temperature (5℃). It is necessary to keep dehydrated samples in air-tight containers properly, to prevent them from absorbing unwanted water vapor from surrounding atmosphere (FAO, 2007). According to Westendorf (2000), only food wastes with relatively low moisture content are applicable and utilizable as commercial animal feeds. Food wastes with relatively high moisture content (more than 20%) have low amount of dry matter and nutrients to be consumed by animals. In addition, food wastes with high moisture content are favorable for microbial growth if they are kept in improper conditions. There is a concern on the risk of microbial spoilage to take place and affect the further utilization of

animal feeds and food consumptions if food wastes consist of high moisture content and unstable shelf life (Westendorf, 2000).

5.5 Analysis on Alcohol Insoluble Solid (AIS) Samples

The alcohol insoluble solid (AIS) derived from each untreated and enzymatic-treated pink guava waste (decanter and refiner) was analyzed based on attenuated total reflectance (ATR) spectroscopy analysis, X-ray diffraction (XRD) analysis, scanning electron microscopy (SEM) analysis, and glucose dialysis retardation index (GDRI) analysis.

5.5.1 Chemical Bond through Attenuated Total Reflectance (ATR) Analysis

Attenuated total reflectance (ATR) spectroscopy analysis was performed, with the aim to identify and compare the chemical bonds and functional groups in the AIS derived from untreated and enzymatic-treated pink guava wastes (decanter and refiner). It was performed to reveal structural changes on pink guava wastes after treated with fiber-degrading enzymes. ATR spectroscopy analysis was employed, instead of using fourier-transform infrared spectroscopy (FTIR) method, because it could analyze samples in their natural states with minimal sample preparation (without any sample grinding). Based on Table 4.9, a strong and broad hydroxyl (O – H) stretching, which is ranged between 3053.23 cm^{-1} and 3333.78 cm^{-1} was found in the AIS derived from untreated and enzymatic-treated samples. It is

contributed by the presence of cellulose and hemicellulose moieties in both pink guava wastes. By referring to Figure 4.1 and 4.2, the O – H absorption band of both pink guava wastes became weak and less sharp, after being treated with the combination of cellulase and xylanase. However, there was no distinct difference observed among the O – H absorption bands of cellulase-treated, xylanase-treated and untreated pink guava wastes. This suggests that the addition of xylanase into a cellulase treatment may promote, enhance and improve degradation of cellulose and hemicellulose synergistically, which is much better than the separate single cellulase and xylanase treatments. On the other hand, medium O – H bending was only found in AIS derived from both combined enzymatic-treated decanter (1437.31 cm^{-1}) and refiner (1438.34 cm^{-1}). It suggests the presence of carboxylic acid (COOH), which is an indicator of the formation of acetic acid via the synergistic hydrolytic action of combined enzymatic treatment. It was produced and released from hydrolysis of acetyl groups, due to synergistically degradation of hemicellulose backbone during combined enzymatic treatment (Leif and Carlos, 2015).

Besides, there was a medium carbon-carbon double bond (C = C) stretching, at the range between 1609.67 cm^{-1} and 1636.31 cm^{-1} was found in each AIS derived from untreated and enzymatic-treated pink guava wastes. There was also a strong C = C bending only found in the AIS sample of both combined enzymatic-treated decanter (897.09 cm^{-1}) and refiner (907.08 cm^{-1}), respectively. It results from the

presence of alkenes, which were produced from the degradation of lignocelluloses throughout the combined enzymatic treatment. Den et al. (2018) suggested that alkene is one of the common soluble organic products (hydrocarbons) produced and released from depolymerized materials extracted from aqueous solution. In addition, a strong carbon-hydrogen bond (C –H) bending was only found in the AIS derived from combined enzymatic-treated decanter (780.91 cm^{-1}), where it reflects that glucose monomers were produced and released from the degradation of lignocellulose compounds (cellulose, hemicellulose, pectin, and lignin) during the combined enzymatic-treatment. However, this C –H bending was not found in the AIS derived from combined enzymatic-treated refiner. It can be evidenced from the HPLC results (Table 4.20), in which glucose was only detected in the ASC derived from combined enzymatic-treated decanter. This suggests that the combination of cellulase and xylanase exhibited synergistic hydrolytic action, which degraded and converted part of the lignocelluloses into glucose monomers.

Moreover, a strong carbon-oxygen (C – O) stretching, which was ranged between 1234.02 cm^{-1} and 1241.35 cm^{-1} was detected in the AIS derived from untreated and enzymatic-treated pink guava wastes, except xylanase-treated decanter. The C – O band was contributed by the formation of aromatic compounds released from degradation of lignin through fiber-degrading enzymatic treatments (Deng et al., 2015). However, there was no C – O band detected on xylanase-treated decanter. This suggests that the lignins found in decanter were less susceptible to be broken

down by xylanase, as compared to those found in refiner, without the addition of cellulase. Hence, there was an increase of C – O band absorption on refiner after combined enzymatic treatment, from 1234.02 cm^{-1} to 1237.58 cm^{-1}. There were more aromatic compounds produced and released from refiner throughout the synergistic hydrolytic action of combined enzymatic treatment.

There was a strong carbonyl (C = O) stretching, ranged from 1735.61 cm^{-1} and 1740.40 cm^{-1} detected in the AIS derived from 150CED, 150CXD, and 150CXR. It indicates the presence of unsaturated ester, which was contributed by aromatic hydrocarbons of lignin. It became broader after the combined enzymatic treatment, due to the degradation of lignocelluloses via the synergistic hydrolytic action of combined enzymatic treatment. This reflects the lignin of both pink guava wastes were more susceptible to be degraded, and followed by the production of aromatic compounds (unsaturated ester), with the addition of xylanase in a cellulase system. Hence, it was not detectable in the AIS derived from cellulase-treated, xylanase-treated and untreated pink guava wastes.

In addition, strong broad ether (C – O – C) stretching, ranged between 1028.70 cm^{-1} and 1034.68 cm^{-1}, was detected in the AIS sample derived from untreated and enzymatic-treated pink guava wastes. It was formed due to the stretching of polymer backbone (β-1,4-glycosidic bond), which contain anhydride (Zhang et al.,

2011). The C – O – C bond in refiner is more susceptible to be broken down via fiber-degrading enzymatic treatments, in comparison to decanter. The breakdown of ether bond may lead to the lignin separation from the matrix of polysaccharides (cellulose and hemicellulose) and depolymerization (Myriam, Teresa and Jorge, 2017). Hence, higher amount of total soluble carbohydrates were produced from depolymerized polysaccharides in refiners (as shown in Table 4.16), as compared to those produced by decanters.

5.5.2 Scanning Electron Microscopy (SEM) Image

Scanning electron microscopy (SEM) analysis was performed to observe and compare the surface structural differences of AIS derived from original, washed, untreated, and enzymatic-treated pink guava wastes (decanter and refiner). Table 4.10 shows the AIS of untreated decanter compared with those of the AIS derived from original, washed, and enzymatic-treated decanters. On the other hand, Table 4.11 shows the AIS derived from untreated refiner compared to those of the AIS of original, washed, and enzymatic-treated refiners. The surfaces of AIS derived from both original pink guava wastes were uneven, rough, and containing some fibers. However, these surface features had been improved, in which they became smoother and less fouling with fibers after being washed at 50 ± 5℃. This is because most of the free sugars and undesirable organic compounds had been washed off and eliminated from both pink guava wastes, and hence clearer SEM images were observed on the AIS derived from both washed pink guava wastes.

On the other hand, the surfaces of AIS derived from the enzymatic-treated pink guava wastes were looked like more irregular and rough. There are even greater amount of pores spread out on the cellulose surfaces, as compared with those of AIS derived from untreated pink guava wastes. The irregular structures on AIS of enzymatic-treated samples were caused by lignin and hemicellulose which were amorphous. Small particulate size carbohydrates such as reducing sugars and lower molecular weight carbohydrates were diffused out from the hydrolyzed lignocelluloses in the AIS by hydrolyzing lignocelluloses in pink guava wastes after treated with fiber-degrading enzymes. As a result, there were higher amount of porous holes on the surface of AIS sample derived from enzymatic-treated pink guava wastes, as compared to those of AIS derived from untreated ones. This suggests that the hydrolytic action exerted by fiber-degrading enzymes not only affecting the functional groups in AIS derived from untreated and enzymatic-treated pink guava wastes, as revealed by the ATR result which was discussed in the section 5.5.1, and it also leads to modification on the physical surface. Among these, the cellulose surfaces of combined enzymatic-treated pink guava wastes were observed as asymmetrical and highly porous with the greatest amount of pores, as compared with the AIS of separate single cellulase-treated and xylanase-treated samples. It was observed that number of pores on the cellulose surfaces of cellulase-treated and xylanase-treated samples was irregular with tiny pores. This also suggests the cellulose structures of pink guava wastes are more susceptible to be hydrolyzed by combined enzymatic treatments, as compared to those of single cellulase and xylanase treatments.

The microscopic images of AIS derived from refiners were slightly different from those of AIS derived from decanters. The particles of AIS derived from untreated and enzymatic-treated refiners were observed as more spherical and long-flaked, as compared to those AIS derived from untreated and enzymatic-treated decanters. This is due to the composition differences between decanter and refiner. Based on Mhd Abd Kader et al. (2016), pink guava refiner contains coarse pulp with seeds, and exerted higher insoluble dietary fiber content (28.58%), as compared to that of pink guava decanter (19.19%), in which the AIS derived from refiners might be more compact with spherically flaked to each other.

5.5.3 Crystallinity Index through X-ray Diffraction (XRD) Analysis

X-ray diffraction (XRD) analysis was used to determine the crystalline structure of alcohol insoluble solid (AIS) derived from untreated and enzymatic-treated pink guava wastes (decanter and refiner). The crystalline region within insoluble dietary fiber (IDF) is mainly contributed by cellulose; while the amorphous region is comprised of non-crystalline cellulose, hemicellulose, and lignin (Ma and Mu, 2016). By referring to Table 4.12, the crystallinity index of AIS derived from enzymatic-treated pink guava wastes were relatively higher, as compared to those derived from untreated samples. The increment of crystallinity index after treated with fiber-degrading enzymes probably was caused by the degradation of lignin and hemicellulose within amorphous region of pink guava wastes (Rehman et al., 2014). It may be resulted from the hydrolysis of non-crystalline celluloses in the

amorphous region, which was easier to be broken down, in comparing to that of crystalline part. As a result, the remaining parts of AIS structures comprise of substances are resistance to be degraded or hydrolyzed crystalline celluloses. This finding was supported by Hamzah et al. (2016), in which the crystallinity index of celluloses found in empty fruit bunches was increased after underwent hydrolysis treatments. In addition, a high crystallinity index was found on AIS derived from enzymatic-treated pink guava wastes, possibly due to the elimination of lignin throughout fiber-degrading enzymatic treatments. This finding was supported by Flórez Pardo, Salcedo Mendoza and López Galán (2019), where the removal of lignin from enzymatic-hydrolyzed sugar cane residues lead to an increment of crystallinity index, which results from the increase of crystallinity region in the samples. It can be concluded that the crystalline structure is not the only factor affecting the enzymatic hydrolysis of pink guava wastes. Other factors such as lignin and hemicellulose are relatively critical in the enhancement of enzymatic hydrolysis on the lignocelluloses of both pink guava wastes. Thus, it results in the difference of crystallinity index between the AIS derived from enzymatic-treated and untreated pink guava wastes.

Meanwhile, untreated decanter exhibited lower crystallinity index (31.56%) than that of untreated refiner (49.11%). AIS sample is referred as the residue retained after 80% (v/v) ethanolic extraction, in which the lignocellulosic constituents in AIS materials are resistance to fiber-degrading enzymatic treatments. This finding was supported by this research outcomes on the AIS product yield and total

soluble carbohydrates produced, as shown in both Table 4.4 and Table 4.17, respectively. In comparison to untreated refiner, untreated decanter showed lower AIS yield and produced higher total soluble carbohydrates content which diffused out from the AIS of decanter. According to Mhd Abd Kader et al. (2016), refiner consists of pink guava seeds and coarse pulp, which are rich in lignocelluloses: cellulose (10.77%), hemicellulose (9.72%), and lignin (40.02%). While, decanter contains only pink guava stone cells along with lower amount of cellulose (11.74%), hemicellulose (4.42%), and lignin (4.57%) (Mhd Abd Kader et al., 2016). This reflects that decanter is more susceptible to be hydrolyzed by fiber-degrading enzymes, as compared to refiner. Hence, untreated decanter had AIS and lower crystallinity index, which is attributed by the reduction of crystalline structure, which comprised of degraded hemicellulose and lignin in its amorphous region, as compared to that of untreated refiner.

5.5.4 Glucose Dialysis Retardation Index (GDRI)

Glucose dialysis retardation index (GDRI) is defined as the *in vitro* index which is employed to evaluate the effect of fiber on the delay of glucose absorption in the gastrointestinal tract (López et al., 1996). Based on Table 4.13, the GDRI of AIS derived from enzymatic-treated decanters were significantly higher ($P < 0.05$) than that of untreated decanter. The combined enzymatic-treated decanter showed the highest GDRI at each time interval among the samples, which achieved the maximum GDRI (66.70%) at the 60^{th} min. On the other hand, cellulase-treated

and xylanase-treated decanters reached the peak GDRI at the 60^{th} and 75^{th} min, which was 58.15% and 47.04%, respectively. Similarity, the GDRI of enzymatic-treated refiners were significantly higher ($P < 0.05$), as compared with that of untreated refiner, where the combined enzymatic-treated refiner achieved the peak GDRI (62.42%) at the 60^{th} min (as tabulated in Table 4.14). This indicates the hypoglycemic effects of both pink guava wastes had been enhanced due to the hydrolytic action exerted by fiber-degrading enzymes, which results in delaying glucose diffusion across the dialysis tubes, as compared with those derived from untreated pink guava wastes (decanter and refiner).

Based on Ahmed, Sairam and Urooj (2011), the retardation in glucose diffusion might be affected by the physical obstacle attributed by fiber particles towards the glucose molecules and glucose entrapment within the fiber network. This finding supports the outcomes of SEM analysis, as discussed in previous 5.5.2. The AIS surfaces of enzymatic-treated pink guava wastes were more irregular and rough. The fiber networks on AIS surfaces of enzymatic-treated samples were improved through hydrolytic action exerted by fiber-degrading enzymes. Therefore, the formation of porous AIS structure on enzymatic-treated pink guava wastes might possess stronger attractive forces on glucose molecules, which results in causing slower glucose diffusion across the glucose dialysis tubes, and results in higher GDRI. Untreated and enzymatic-treated decanters possessed higher GDRI, in comparison with those of untreated and enzymatic-treated refiners. This is due to the presence of hydrophobic/hydrophilic fibrous entities and high $_D$-galacturonic

contents of pink guava decanter, since it is a good source of pectin. Hence, it may delay glucose diffusion rate across the dialysis tubes (Lim et al., 2018).

5.6 Analysis on Alcohol Soluble Fiber (ASC) Samples

The alcohol soluble carbohydrates (ASC) derived from untreated and enzymatic-treated pink guava wastes (decanter and refiner) were analyzed based on the total soluble carbohydrates analysis through phenol sulfuric acid method, sugar profile through HPLC analysis, and prebiotic activity score assay estimation.

5.6.1 Total Soluble Carbohydrates Content

Phenol sulfuric acid method was used to determine the total soluble carbohydrates in the ASC samples derived from untreated and enzymatic-treated pink guava wastes (decanter and refiner), spectrophotometrically. It is a rapid, reliable and precise colorimetric method in detecting all categories of carbohydrates such as monosaccharides, disaccharides, oligosaccharides, and polysaccharides (Nelson, 2010). Based on Table 4.17, the ASC derived from combined enzymatic-treated pink guava decanter and refiner showed the highest total soluble carbohydrates, which were 77.87 mg/g of dry matter and 91.27 mg/g of dry matter, respectively, among the enzymatic-treated ASC. The total soluble carbohydrates of each ASC followed the ascending order of untreated < xylanase-treated < cellulase-treated < combined enzymatic-treated pink guava wastes. This suggests the total soluble

carbohydrates of both pink guava wastes can be significantly enhanced ($P < 0.05$) throughout fiber-degrading enzymatic treatments. In both pink guava wastes, the lignocelluloses are built up by cellulose, hemicellulose, pectin, and lignin, which may be converted into soluble sugars and diffuse out from the ASC material. Hence, it produces the highest total soluble carbohydrates content in combined enzymatic-treated pink guava wastes, in comparing with those of cellulase- and xylanase-treated ones. This trend is similar as the differences on reducing sugar content released by each enzymatic-treated decanter and refiner after treated with fiber-degrading enzymes, as discussed in previous section 5.2.1.

Based on Table 4.18, each ASC derived from the enzymatic-treated pink guava refiners had significantly higher ($P < 0.05$) total soluble carbohydrates content, in comparison with ASC derived from enzymatic-treated pink guava decanters. Yet, the ASC derived from untreated refiner contained significantly lower ($P < 0.05$) total soluble carbohydrates than that of ASC derived from untreated decanter. Pink guava refiner contains guava seeds and coarse pulp, with higher proportions of lignocelluloses than those of pink guava decanter, which are target substrates to be hydrolyzed and converted into simple sugars through the hydrolytic action exerted by fiber-degrading enzymes. Therefore, there are more total soluble carbohydrates and reducing sugars to be quantified in the pink guava refiner than that of pink guava decanter.

5.6.2 Sugar Profile through High Performance Liquid Chromatography

Sugar profiles of untreated and enzymatic-treated ASC derived from pink guava wastes (decanter and refiner) were analyzed through HPLC analysis, at which HPLC spectrums were generated to quantify sugars in the ASC based on peak areas. According to Table 4.20, rhamnose and xylose were identified in each ASC material derived from untreated and enzymatic-treated pink guava wastes, with the exceptions of ASC samples derived from xylanase-treated pink guava wastes and combined enzymatic-treated decanter. Rhamnose was detected in untreated and enzymatic-treated samples due to the presence of galacturonic acid. Basically, galacturonic acid chains in pectins and linked through α-1,2 bonds covalently, and along with the presence of hexose (rhamnose) (Moreno and Peinado, 2012). The presence of galacturonic acid in both pink guava wastes may be evidenced from the GDRI analysis, as discussed in previous section 5.5.4. Particularly, the amount of rhamnose found in combined enzymatic-treated decanter was 5.89 μg/g of dry matter, which is higher than that detected in combined enzymatic-treated refiner (3.34 μg/g of dry matter). This finding was supported by the outcomes of GDRI analysis. The degradation of pectin in both pink guava wastes may release rhamnose as primary product, via the synergistic hydrolytic action of combined enzymatic treatment. Decanter contains higher $_{D}$-galacturonic acid contents than refiner, which is attributed by relatively high pectin contents in decanter. Hence, higher amount of rhamnose was produced and released from pink guava decanter, in comparison with refiner, through combined enzymatic treatment.

Other than rhamnose, xylose was also detected in the ASC derived from untreated and enzymatic-treated pink guava wastes. It is possibly due to the degradation of hemicellulose and xylan through the hydrolytic action exerted by fiber-degrading enzymes. Xylose is one of the pentose components to build up the heterogeneous structure of hemicelluloses and xylan in the cell wall of fiber-rich materials. It is also a primary product to be released out from sample during the degradation of hemicellulose and xylan. Particularly, higher amount of xylose was detected in the ASC derived from combined enzymatic-treated decanter (202.58 μg/g of dry matter), as compared with that of ASC derived from combined enzymatic-treated refiner (104.03 μg/g of dry matter). This reflects both hemicellulose and xylan in the decanter are more susceptible to be hydrolyzed, and followed by releasing xylose during the combined enzymatic treatment, as compared to refiner. Besides, mannose is another hexose sugar only detected in the ASC derived from untreated and enzymatic-treated decanter (except 150CXD), and xylanase-treated refiner. It is one of the important sugar components to build up the structures of cellulose, hemicellulose and pectin in the cell wall of fiber-rich materials. Particularly, the quantities of mannose produced and released from cellulase-treated and xylanase-treated decanters, were higher than that of untreated decanter. This suggests that part of lignocelluloses is more susceptible to be degraded by single cellulase and xylanase, to produce and release mannose, as compared to combined enzymatic treatment.

Meanwhile, the sugar amount of identified sugars (rhamnose, xylose, arabinose, and mannose) in the untreated ASC was lower than those detected in the ASC derived from enzymatic-treated pink guava wastes. Therefore, this evidences the lignocelluloses in both pink guava wastes had been hydrolyzed and converted into simple sugars (rhamnose, xylose, arabinose, and mannose), through the hydrolytic action of fiber-degrading enzymes, at 55℃ for 24 h. On the other hand, hexoses such as glucose and fructose were only detectable in the ASC derived from combined enzymatic-treated decanter, which is unlikely on the other enzymatic-treated ASC. As mentioned, the ASC derived from combined enzymatic-treated decanter and refiner showed the highest amount of xylose, which was 202.58 μg/g of dry matter and 104.03 μg/g of dry matter, respectively, among the ASC derived from enzymatic-treated samples. This is possibly due to degradation of cellulose, hemicellulose and pectin in decanter via the synergistic hydrolytic action of combined enzymatic treatment, in which various simple sugars such as glucose, fructose, and xylose may be produced and diffused out from the AIS derived from pink guava wastes (Bhaumik and Dhepe, 2015; Binder and Raines, 2010). As mentioned, glucose, and fructose were only detected in the ASC derived from the combined enzymatic-treated decanter. This suggests stronger hydrolytic action is required to produce glucose and fructose from the hydrolyzed AIS of decanter. This is due to the strong hydrogen bond within the hydroxyl group. This finding was supported by the results from ATR analysis, as shown in Table 4.9, there was a reduction of O – H stretching of decanter after combined enzymatic treatment, but it was not valid for the AIS of combined enzymatic-treated refiner.

5.6.3 Prebiotic Activity Score Analysis

Prebiotic is a non-digestible food ingredient which contributes beneficially effects to the host upon consumptions by selectively stimulating growth and /or activity of bacteria (probiotic) in the colon, and hence to improve host's health (Gibson and Roberfroid, 1994). Based on Gibson et al. (2010), prebiotics are confined to non-digestible oligosaccharides, in which they can be produced by the hydrolysis of polysaccharides or catabolic enzymatic reactions from lower molecular weight sugars. In this research, three types of *Lactobacillus* spp. such as *Lactobacillus rhamnosus* ATCC 9595, *Lactobacillus plantarum* ATCC 14917 and *Lactobacillus brevis* KCTC 3102 were employed as probiotics to determine prebiotic activity scores of pink guava wastes (decanter and refiner), based on absorbance and total viable count. Pink guava wastes are fiber-rich organic waste materials, which are indigestible in human gastrointestinal tract. Yet, they could serve as potential food sources to stimulate the bacterial population growth, which results in balancing food absorption (Thuaytong and Anprung, 2011). Based on Table 4.21, the ASC derived from untreated and enzymatic-treated pink guava wastes showed greater capability in promoting probiotic growth than those of control (without any ASC sample). Similar trend was observed on the prebiotic activity score determinations based on total viable count, as shown in Table 4.22. From this, it evidences the presences of pink guava wastes may enhance the probiotic growth on MRS broth and agar after incubated at 37℃ for 24 h.

Based on Table 4.21, the probiotic growth supported by the ASC derived from untreated and enzymatic-treated pink guava wastes followed the ascending order of *Lactobacillus brevis* < *Lactobacillus rhamnosus* < *Lactobacillus plantarum*, based on absorbance and total viable count. The prebiotic activity scores of ASC derived from combined enzymatic-treated decanter (4.28) and refiner (4.51) in promoting *Lactobacillus plantarum* were the highest among the sample, in which they were enhanced by 75.61% and 75.08%, respectively, as compared with that of control sample (0.18). Similar trend of the growth of *Lactobacillus plantarum* supported by the ASC derived from combined enzymatic-treated pink guava wastes were observed on the prebiotic activity score determinations based on total viable count (Table 4.22). The lignocelluloses in both pink guava wastes had been hydrolyzed and converted into oligosaccharides which are built up by glucose, fructose, xylose, and other simple sugars, through the hydrolytic action of fiber-degrading enzymes. As discussed in previous 5.6.1, the ASC derived from both combined enzymatic-treated pink guava wastes contained the highest total soluble carbohydrates, among the samples. Therefore, there were more oligosaccharides produced and diffused out from the AIS derived from pink guava wastes through the synergistic hydrolytic action of combined enzymatic-treatments. These are food sources used to promote and stimulate the growth of probiotics, especially for *Lactobacillus plantarum*. Besides, ASC of combined enzymatic-treated refiner had greater ability in promoting growth of *L. plantarum*, as compared to ASC derived from the combined enzymatic-treated decanter. This is due to the former contained higher total soluble carbohydrates as compared to that of the latter.

5.7 Suitability of Pink Guava Wastes as Prebiotic Sources in Yogurt

According to Gibson et al. (1995), prebiotics are widely utilized as sub-category of functional food ingredients to be included in food products such as breads, ice-cream, cereals, milk and yogurts. In this research, untreated and enzymatic-treated pink guava wastes (decanter and refiner) were used as prebiotic sources and added with commercial *Lactobacillus* spp. (yogurt starter culture), to produce yogurts by fermentation. The suitability of each untreated and enzymatic-treated pink guava wastes as prebiotic sources in yogurts was determined based on colony forming unit (CFU) per mL. Each untreated and enzymatic-treated samples was washed and sterilized by 70% (v/v) ethanol. As a result, there was no single colony found on sterilized sample to grow on sterile Luria-Bertani (LB) agar after incubation (37℃ for 24 h), as shown in Figure 5.2. This proves most of the bacteria on the surfaces of both pink guava wastes had been eliminated by 70% (v/v) ethanol, and the risk of contamination had been eliminated prior to yogurt production.

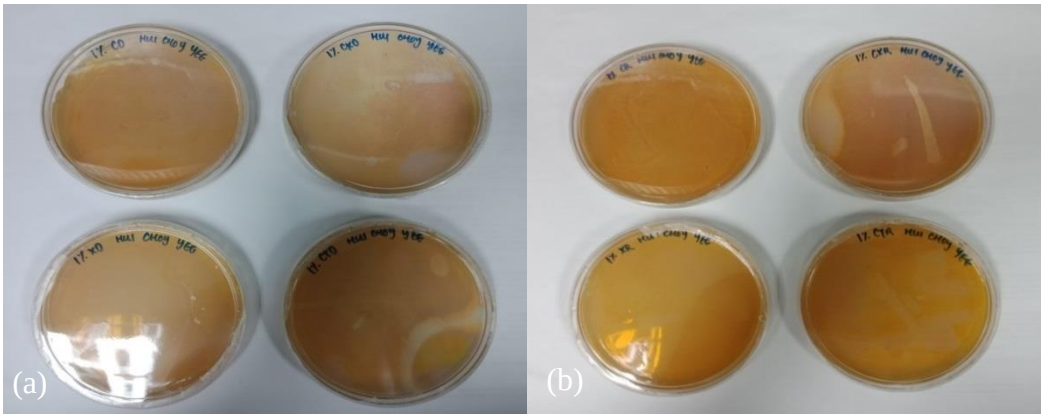


Figure 5.2: There was no single colony found on those sterilized untreated and enzymatic-treated pink guava decanter (a) and refiner (b).

According to Table 4.23, the CFU/mL of each yogurt added with untreated and enzymatic-treated pink guava wastes was higher than that of control (without any sample). For instance, the CFU/mL of both yogurts added with untreated decanter (6.02) and refiner (5.97) were enhanced by 25.25% and 23.80%, respectively, in comparison to that of control (4.55). This reflects that the addition of pink guava wastes may enhance the *Lactobacillus* growth in yogurts through fermentation at 37℃ for 24 h. Other than that, the CFU/mL of yogurts added with enzymatic-treated pink guava wastes was significantly higher ($P < 0.05$) in comparing with those of yogurts added with untreated ones. This suggests that enzymatic-treated pink guava wastes in yogurts supported higher probiotic growth, as compared to those of yogurts contained untreated pink guava wastes. Particularly, the CFU/mL of yogurts contained combined enzymatic-treated pink guava decanter and refiner were the highest, which were 8.05 and 8.08, respectively, as compared with others. Besides, the CFU/mL of combined enzymatic-treated refiner in yogurt was higher than that of combined enzymatic-treated decanter in yogurt. This is similar as that of prebiotic activity score, which was discussed in previous 5.6.3. Therefore, the combination of cellulase and xylanase exerted synergistic action to hydrolyze and convert lignocellulose in both pink guava wastes into utilizable oligosaccharides as food sources to promote and stimulate probiotic growth in yogurts. Based on Irionondo-DeHond, Miguel and del Castillo (2018), the discarded food processing by-products as sources of functional compounds in other foods, is much desirable in the waste management system, with biological purposes, which is aimed to promote health-beneficial effects to the host upon consumption.

5.7.1 pH Determinations on Yogurt

The pH of each yogurt added with untreated and enzymatic-treated pink guava wastes (decanter and refiner) was measured by using a pH meter. The pH study of yogurt is important as it is a critical factor to affect microbial growth (Mhd Abd Kader et al., 2016). In addition, pH measurements could also estimate the acid development of dairy products like yogurt (Masulli, 2016). According to Mhd Abd Kader et al. (2016), pink guava wastes are acidic waste materials, since they have pH value about 4.0. Based on Table 4.24, yogurts contained untreated and enzymatic-treated pink guava wastes showed lower pH values than that of control (without sample). In addition, yogurts added with combined enzymatic-treated pink guava decanter and refiner showed the lowest pH values, which were 4.16 and 4.06, respectively, among the samples. This reflects an inversely proportional relationship between the growth of probiotic strain and pH value of yogurt. Thus, it can be concluded that higher probiotic growth supported by pink guava wastes added in yogurt, the lower pH value of yogurt to be produced. In overall, yogurt added with untreated and enzymatic-treated pink guava wastes achieved the ideal set point (between pH 4.0 and 4.6) required by most of the yogurt producers nowadays, which aims to cease yogurt incubation. Low pH value (acidic) was detected in each yogurt as lactic acid was produced. The growth of *Lactobacillus* spp. was supported by untreated or enzymatic-treated pink guava wastes in yogurt, where it enhanced the reaction between UHT milk and probiotic, and results in converting lactose into lactic acid (Masulli, 2016).

5.7.2 Texture Profile of Yogurt

Texture is one of the important attributes to be evaluated in yogurts (Ozcan, 2013). Texture profile of yogurts added with untreated and enzymatic-treated pink guava wastes (decanter and refiner) were analyzed based on the parameters of hardness, stickiness, adhesiveness and stringiness. According to Mudgil, Barak and Khatkar (2017), hardness is related to the force required to achieve deformation (firmness), which is the peak force during the first compression cycle (Ozcan, 2013). Based on Table 4.25, the hardness of control (without any sample) was 33.97 mm. On the other hand, the hardness of yogurts added with untreated decanter and refiner were 34.12 mm and 34.63 mm, respectively. The hardness of yogurts may be slightly increased upon the addition of pink guava wastes by 1.91%. On the other hand, the hardness of yogurts with the addition of combined enzymatic-treated decanter and refiner were 41.54 mm and 43.50 mm, respectively, which were the highest among the samples.

Stickiness is the feeling perceivable to tongue and palate upon consumption (Adhikari et al., 2001). Based on Ozcan (2013), it is a tertiary characteristic to be evaluated in texture profile of yogurt. By referring to Table 4.26, the stickiness of control (without sample) was 74.14 g. Meanwhile, the stickiness of yogurts added with untreated pink guava decanter and refiner were determined as 96.54 g and 98.64 g, respectively. It reflects the stickiness of yogurts may be enhanced due to the addition of pink guava wastes in yogurt by up to 24.83%. Meanwhile, the

stickiness of yogurts with the addition of combined enzymatic-treated decanter and refiner were reported as 125.00 g and 124.26 g, respectively, which were the highest among the samples. This is similar as the trend observed on hardness, as making comparison among the enzymatic-treated pink guava wastes. Hence, the addition of whole combined enzymatic-treated pink guava wastes (decanter and refiner) could significantly increase ($P < 0.05$) the stickiness of yogurts.

Other than these, adhesiveness of yogurt was also evaluated as part of texture profile. Adhesiveness is the force required to remove the adhered material in the mouth upon consumption (Mudgil, Barak and Khatkar, 2017). It is also referred as the negative force area of the first compression cycle, as shown in Figure 3.4 (Ozcan, 2013). According to Table 4.26, the adhesiveness of control (without any sample) was 103.43 g.s. Meanwhile, the adhesiveness of yogurts added with un-treated decanter and refiner was 114.79 g.s and 115.65 g.s, respectively. Hence, this suggests that the adhesiveness of yogurts may be increased by the addition of pink guava wastes by up to 10.58%. The yogurts contained combined enzymatic-treated decanter and refiner was 192.83 g.s and 190.43 g.s, respectively. This trend is similar as observed on stickiness, in which the adhesiveness of yogurts may be significantly ($P < 0.05$) enhanced by the addition of combined enzymatic-treated pink guava wastes.

Stringiness is one of the tertiary characteristics of yogurt to be determined (Ozcan, 2013), which is defined as the distance travelled along by the probe during the negative force area (Trinh and Steve, 2018). A material tends to be stretched when it is pulled out as it adheres strongly to the surface on which it resists to, and this is known as "stringiness" (Trinh and Steve, 2018). In accordance to Table 4.28, the stringiness of control (without any sample) was 34.88 mm; while the stringiness of yogurts contained untreated pink guava decanter and refiner was 37.60 mm and 36.88 mm, respectively. This trend is also similar as those of evaluated based on hardness, stickiness and adhesiveness of yogurts contained whole untreated and enzymatic-treated pink guava wastes.

In a nutshell, there is a directly proportional relationship between the growth of probiotic strains and texture profile of yogurts. Hence, there was an increase of hardness, stickiness, adhesiveness and stringiness in yogurts, due to the increment of probiotic growth in yogurts, and it was directly impacted by the addition of whole pink guava wastes (decanter and refiner) in yogurts. Moreover, this trend may be enhanced through fiber-degrading enzymatic treatments, which followed the ascending order of xylanase treatment $<$ cellulase treatment $<$ combined enzymatic treatment. Similar trend as the CFU/mL of yogurt produced by adding enzymatic-treated pink guava wastes, as shown in Table 4.23. As a conclusion, the presence of enzymatic-treated pink guava wastes may enhance the growth of probiotic strain (CFU/mL); lactic acid produced and texture profile of yogurts.

5.8 Future Study Recommendations

Research related to functional properties may be further extended. For instance, *in vitro* bile acid binding, α-amylase inhibitory effect, pancreatic lipase inhibitory effect, and determination of glycemic index (GI) are suggested to be employed as future analysis on the functional characteristics of untreated and fiber-degrading enzymatic-treated pink guava wastes (decanter and refiner). Besides, information regarding the yogurt production which employed untreated and enzymatic-treated pink guava wastes is limited. Hence, viscosity analysis, sensory evaluation (taste, odor, mouthfeel and overall acceptability) and physicochemical analysis (ash, fat, protein, total carbohydrates and total soluble solid) are suggested to perform on the characterizations of yogurts added with untreated and enzymatic-treated pink guava wastes, for further research study. These further research studies are critical in term of determining modified functional and structural properties of enzymatic-treated pink guava wastes as potential functional ingredients to be employed for commercial applications.

CHAPTER 6

CONCLUSION

In a conclusion, the combination of cellulase and xylanase could exert synergistic hydrolytic action to hydrolyze and convert the lignocelluloses in both pink guava wastes (decanter and refiner) into simple sugar such as rhamnose, xylose, glucose, and fructose. In this research, it had proven that cellulase-xylanase treatment has great capability to convert pink guava wastes (decanter and refiner) into potential functional food ingredients. Combined enzymatic treatments on both pink guava wastes had been brought about structural modification and function enhancement based on the results obtained from ATR, X-ray diffraction, SEM analysis, *in vitro* hypoglycemic potential (GDRI analysis), and prebiotic activity score estimation. Besides, the incorporation of untreated and enzymatic-treated pink guava wastes to UHT milk for yogurt preparation has further proven the potential of combined enzymatic-treated pink guava wastes to be utilized as potential prebiotic sources to promote and enhance probiotic growth in yogurts. Furthermore, the addition of whole combined enzymatic-treated pink guava wastes in yogurts may increase the hardness, stickiness, adhesiveness, stringiness, and acidity of yogurts. Hence, the cellulase-xylanase treatment is a feasible approach to valorize pink guava wastes into more re-utilizable and value-added materials, such as applicable as functional food ingredients in the commercial productions of foods and beverages, health-promoting supplements, and dairy products with enhanced probiotic growth.

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APPENDICES

APPENDIX A

Table 1: Moisture content of each freeze-dried pink guava waste.

Samples	Moisture content (%)				Standard deviation
	1st	2nd	3rd	Average	
Freeze-dried decanter	3.890	3.460	3.717	3.689	0.216
Freeze-dried refiner	2.970	2.620	2.450	2.680	0.265

APPENDIX B

Figure 1 shows the glucose standard curve obtained by using the DNS assay. In the standard curve, the values shown represent the mean of triplicated data. The linear equation of glucose standard curve is y = 1.0182x with the regression coefficient of R^2 = 0.9884. It was then used to estimate the reducing sugar content released by freeze-dried pink guava wastes (decanter and refiner) during the first and second sample washing processes.

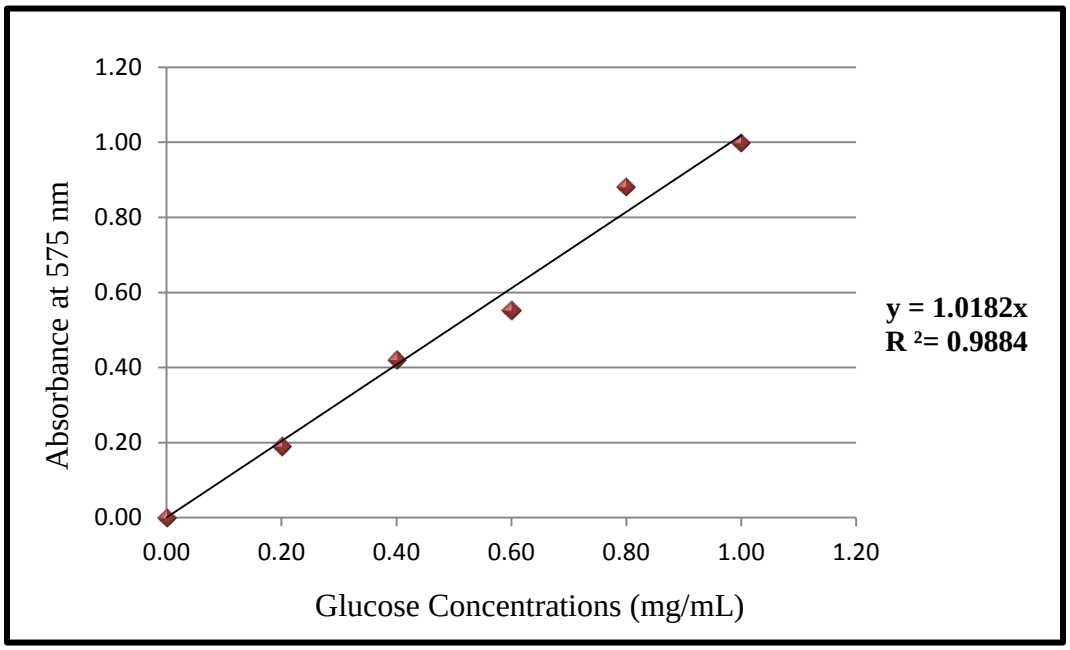


Figure 1: Standard curve of glucose constructed based on DNS assay.

Table 2: Reducing sugar content released by each freeze-dried pink guava waste during sample washing determined by using 3,5-dnitrosalicylic acid (DNS) assay.

Samples	Sample washing process	Reducing sugar content released (mg/g)				Standard Deviation
		1st	2nd	3rd	Average	
Freeze-dried decanter	1st	21.89	21.89	21.89	21.89	0.000
	2nd	2.04	2.04	2.04	2.04	0.000
Freeze-dried refiner	1st	27.70	27.70	27.70	27.70	0.000
	2nd	3.47	3.47	3.47	3.47	0.000

APPENDIX C

Figure 2 shows the glucose standard curve constructed based on Nelson Somogyi method. In the standard curve, values represent the mean of triplicated data. The linear equation of the standard curve is y = 0.0591x with the regression coefficient of R^2 = 0.9898. It was then used to estimate the glucose content produced by each pink guava wastes during enzymatic treatments, and the efficiency of hydrolytic action performed by fiber-degrading enzymes on both pink guava wastes was calculated.

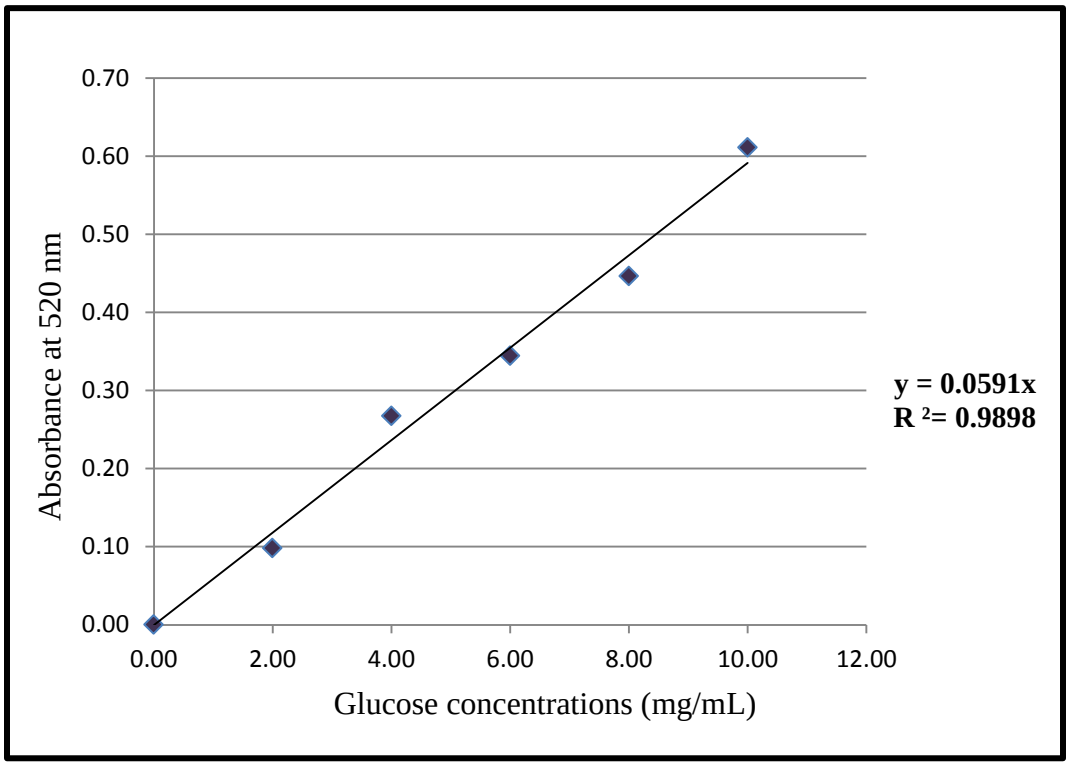


Figure 2: Standard curve of glucose constructed based on Nelson Somogyi method.

Table 3: Amount of reducing sugar produced by each enzymatic-treated decanter and refiner in the Nelson Somogyi (NS) assay.

Samples	Amount of reducing sugar produced (μg/g)				Standard deviation
	1st	2nd	3rd	Average	
150CED	310.40	310.40	310.60	310.50	0.001
150XYD	204.80	208.80	208.80	207.47	0.007
150CXD	335.80	335.80	335.80	335.80	0.000
150CER	312.40	313.40	313.00	313.00	0.001
150XYR	181.40	181.80	182.00	181.80	0.007
150CXR	337.80	338.00	337.80	337.80	0.000

APPENDIX D

Table 4: Weight and product yield of each AIS exact from 1 g of untreated and enzymatic-treated pink guava waste by using 80 % (v/v) ethanolic extraction.

AIS Samples	1st ethanolic extraction		2nd ethanolic extraction		3rd ethanolic extraction		Average AIS Yield (%)
	Weight (g)	AIS Yield (%)	Weight (g)	AIS Yield (%)	Weight (g)	AIS Yield (%)	
150CED	0.292	9.99	0.261	10.45	0.246	9.84	10.09
150XYD	0.501	19.07	0.521	20.67	0.511	20.29	20.22
150CXD	0.282	10.71	0.282	11.27	0.297	11.74	11.24
CTDD	2.313	90.72	2.313	90.71	2.272	88.07	89.83

Table 4: (continued)

AIS Samples	1st ethanolic extraction		2nd ethanolic extraction		3rd ethanolic extraction		Average AIS Yield (%)
	Weight (g)	AIS Yield (%)	Weight (g)	AIS Yield (%)	Weight (g)	AIS Yield (%)	
150CER	0.297	11.79	0.290	11.55	0.299	11.95	11.76
150XYR	0.567	22.05	0.512	20.47	0.501	20.06	20.86
150CXR	0.291	9.72	0.271	10.85	0.265	10.41	10.33
CTDR	2.350	93.25	2.350	93.25	2.312	89.27	91.92

Table 5: Weight and product yield of each ASC exact from 1 g of untreated and enzymatic-treated pink guava waste by using 80 % (v/v) ethanolic extraction.

ASC Samples	1st ethanolic extraction		2nd ethanolic extraction		3rd ethanolic extraction		Average ASC Yield (%)
	Weight (g)	ASC Yield (%)	Weight (g)	ASC Yield (%)	Weight (g)	ASC Yield (%)	
150CED	2.627	89.98	2.239	89.54	2.253	90.14	89.89
150XYD	2.129	80.94	1.999	79.31	2.008	79.69	79.98
150CXD	2.342	89.06	2.218	88.72	2.232	88.23	88.67
CTDD	0.236	9.24	0.236	9.27	0.307	11.92	10.14

Table 5: (continued)

ASC Samples	1st ethanolic extraction		2nd ethanolic extraction		3rd ethanolic extraction		Average ASC Yield (%)
	Weight (g)	ASC Yield (%)	Weight (g)	ASC Yield (%)	Weight (g)	ASC Yield (%)	
150CER	2.223	88.21	2.209	88.00	2.201	88.03	88.08
150XYR	2.003	77.93	1.988	79.51	1.998	79.91	79.12
150CXR	2.699	90.27	2.228	89.12	2.273	89.50	89.63
CTDR	0.169	6.70	0.169	6.70	0.276	10.67	8.02

Table 6: Moisture content determination on each AIS extract of untreated and enzymatic-treated pink guava waste.

AIS Samples	AIS Moisture content (%)				Standard deviation
	1st	2nd	3rd	Average	
150CED	3.41	3.54	3.39	3.45	0.081
150XYD	4.24	4.25	4.09	4.19	0.090
150CXD	3.46	3.41	3.33	3.40	0.066
CTDD	5.77	5.74	5.71	5.74	0.030
150CER	4.79	4.80	4.74	4.78	0.032
150XYR	4.91	4.99	4.89	4.93	0.053
150CXR	4.78	4.71	4.78	4.76	0.040
CTDR	6.84	6.78	6.91	6.84	0.065

Table 7: Moisture content determination on each ASC extract of untreated and enzymatic-treated pink guava waste.

ASC Samples	ASC Moisture content (%)				Standard deviation
	1st	2nd	3rd	Average	
150CED	3.88	3.90	3.89	3.89	0.010
150XYD	4.36	4.41	4.41	4.39	0.0289
150CXD	3.60	3.67	3.60	3.62	0.040
CTDD	5.93	6.06	5.80	5.93	0.130
150CER	4.87	4.92	4.92	4.90	0.029
150XYR	5.09	5.12	5.14	5.12	0.025
150CXR	4.85	4.87	4.81	4.84	0.031
CTDR	7.08	6.97	7.17	7.07	0.100

APPENDIX E

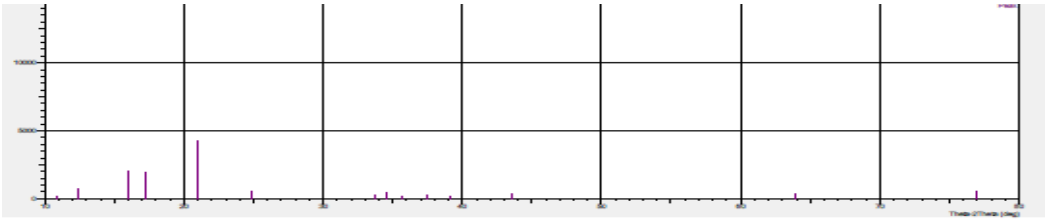


Figure 3: XRD spectrum of cellulase-treated decanter (150CED).

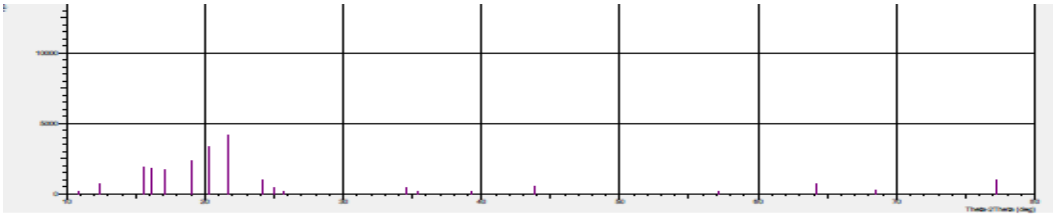


Figure 4: XRD spectrum of xylanase-treated decanter (150XYD).

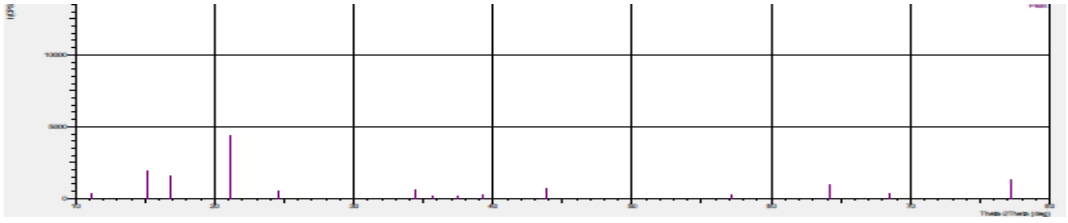


Figure 5: XRD spectrum of combined enzymatic-treated decanter (150CXD).

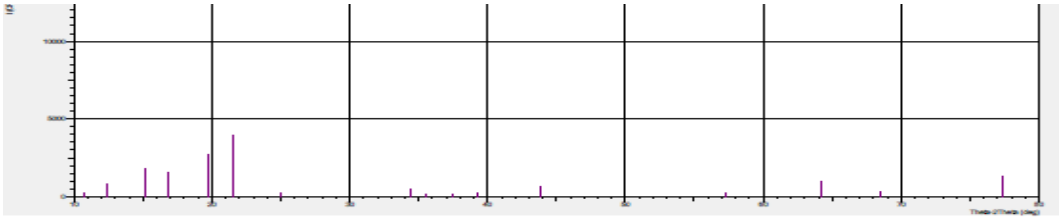


Figure 6: XRD spectrum of untreated decanter (CTDD).

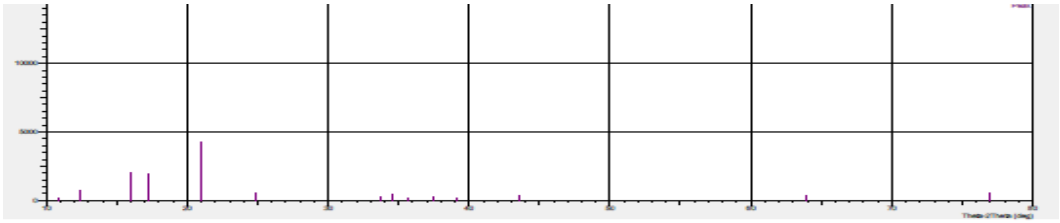


Figure 7: XRD spectrum of cellulase-treated refiner (150CER).

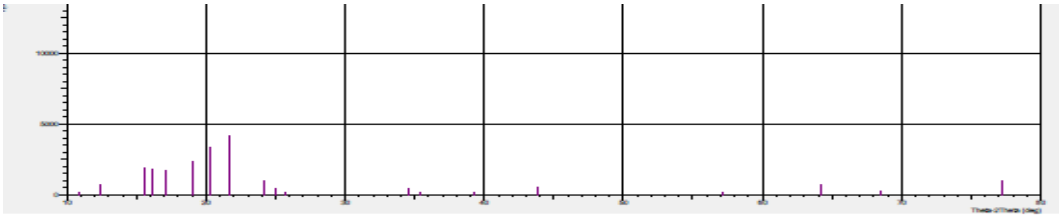


Figure 8: XRD spectrum of xylanase-treated refiner (150XYR).

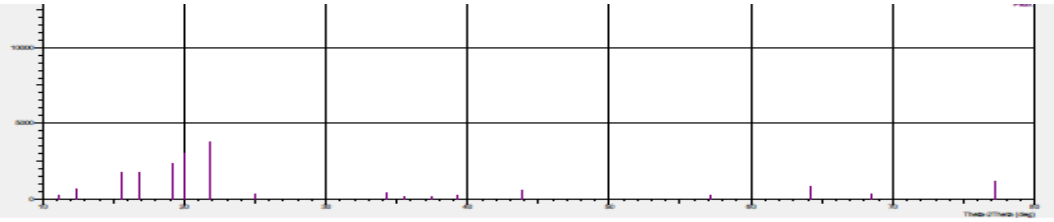


Figure 9: XRD spectrum of combined enzymatic-treated refiner (150CXR).

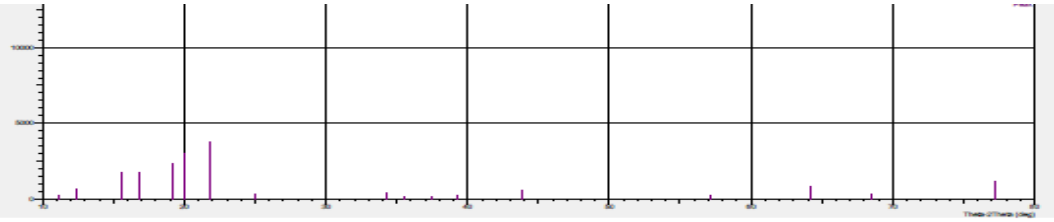


Figure 10: XRD spectrum of untreated refiner (CTDR).

APPENDIX F

Table 8: Glucose concentrations of glucose controls at different time intervals in Glucose Dialysis Retardation Index (GDRI) analysis.

Sample	Time interval (min)	Glucose concentration (mg/mL)				Standard deviation
		1st	2nd	3rd	Average	
Glucose control	15	0.229	0.231	0.236	0.232	0.00
	30	0.433	0.435	0.432	0.433	0.00
	45	0.568	0.567	0.575	0.570	0.00
	60	0.814	0.835	0.823	0.824	0.01
	75	0.889	0.896	0.893	0.893	0.00
	90	0.914	0.920	0.916	0.916	0.00

Table 9: Glucose concentrations of AIS extracts of untreated and enzymatic-treated decanters at different time intervals in GDRI analysis.

AIS Samples	Time interval (min)	Glucose concentration (mg/mL)				Standard deviation
		1st	2nd	3rd	Average	
150CED	15	0.173	0.173	0.170	0.172	0.00
	30	0.256	0.256	0.255	0.256	0.00
	45	0.323	0.319	0.325	0.323	0.00
	60	0.349	0.344	0.342	0.345	0.00
	75	0.419	0.420	0.425	0.422	0.00
	90	0.444	0.445	0.445	0.445	0.00
150XYD	15	0.174	0.170	0.164	0.170	0.01
	30	0.263	0.263	0.263	0.263	0.00
	45	0.337	0.337	0.337	0.337	0.00
	60	0.442	0.443	0.442	0.442	0.00
	75	0.472	0.471	0.476	0.473	0.00
	90	0.530	0.531	0.534	0.532	0.00

Table 9: (continued)

AIS Samples	Time interval (min)	Glucose concentration (mg/mL)				Standard deviation
		1st	2nd	3rd	Average	
150CXD	15	0.124	0.126	0.120	0.123	0.00
	30	0.209	0.209	0.209	0.209	0.00
	45	0.243	0.241	0.235	0.240	0.00
	60	0.278	0.272	0.272	0.274	0.00
	75	0.322	0.319	0.323	0.321	0.00
	90	0.366	0.370	0.372	0.370	0.00
CTDD	15	0.215	0.216	0.217	0.216	0.00
	30	0.328	0.326	0.319	0.325	0.00
	45	0.382	0.382	0.386	0.383	0.00
	60	0.548	0.551	0.554	0.551	0.00
	75	0.554	0.555	0.558	0.556	0.00
	90	0.579	0.584	0.586	0.583	0.00

Table 10: Glucose concentrations of AIS extracts of untreated and enzymatic-treated refiners at different time intervals in GDRI analysis.

AIS Samples	Time interval (min)	Glucose concentration (mg/mL)				Standard deviation
		1st	2nd	3rd	Average	
150CER	15	0.191	0.190	0.189	0.190	0.00
	30	0.231	0.227	0.233	0.230	0.00
	45	0.260	0.262	0.263	0.261	0.00
	60	0.349	0.349	0.349	0.349	0.00
	75	0.408	0.402	0.399	0.403	0.00
	90	0.484	0.501	0.497	0.494	0.01
150XYR	15	0.214	0.220	0.217	0.217	0.00
	30	0.325	0.325	0.326	0.326	0.00
	45	0.342	0.349	0.348	0.346	0.00
	60	0.389	0.386	0.393	0.390	0.00
	75	0.471	0.478	0.471	0.473	0.00
	90	0.575	0.578	0.581	0.578	0.00

Table 10: (continued)

AIS Samples	Time interval (min)	Glucose concentration (mg/mL)				Standard deviation
		1st	2nd	3rd	Average	
150CXR	15	0.149	0.149	0.143	0.147	0.00
	30	0.206	0.203	0.204	0.204	0.00
	45	0.248	0.248	0.249	0.248	0.00
	60	0.314	0.309	0.307	0.310	0.00
	75	0.425	0.424	0.419	0.423	0.00
	90	0.466	0.447	0.454	0.456	0.01
CTDR	15	0.229	0.231	0.225	0.229	0.00
	30	0.349	0.352	0.343	0.348	0.01
	45	0.412	0.419	0.415	0.415	0.00
	60	0.501	0.490	0.499	0.497	0.01
	75	0.531	0.534	0.537	0.534	0.00
	90	0.601	0.602	0.601	0.602	0.00

Table 11: GDRI values of AIS extracts of untreated and enzymatic-treated decanters at different time intervals.

AIS Samples	Time interval (min)	GDRI value (%)				Standard deviation
		1st	2nd	3rd	Average	
150CED	15	25.59	25.59	26.60	25.93	0.585
	30	40.87	40.87	41.14	40.96	0.157
	45	43.34	43.96	42.93	43.41	0.519
	60	57.67	58.24	58.53	58.15	0.435
	75	53.05	52.92	52.39	52.79	0.348
	90	51.54	51.41	51.41	51.45	0.074
150XYD	15	25.08	26.60	29.13	26.94	2.046
	30	39.25	39.25	39.25	39.25	0.000
	45	40.87	40.87	40.88	40.87	0.007
	60	46.41	46.27	46.41	46.36	0.082
	75	47.13	47.25	46.74	47.04	0.274
	90	42.18	42.05	41.66	41.96	0.267

Table 11: (continued)

AIS Samples	Time interval (min)	GDRI value (%)				Standard deviation
		1st	2nd	3rd	Average	
150CXD	15	46.34	45.84	48.37	46.85	1.339
	30	51.72	51.72	51.73	51.72	0.005
	45	57.35	57.76	58.79	57.97	0.743
	60	66.22	66.93	66.93	66.70	0.411
	75	63.97	64.23	63.83	64.04	0.201
	90	60.00	59.61	59.36	59.66	0.323
CTDD	15	7.36	6.86	6.35	6.86	0.506
	30	24.33	24.60	26.23	25.05	1.027
	45	33.04	33.04	32.21	32.76	0.476
	60	33.04	33.16	32.73	33.11	0.359
	75	37.93	37.80	37.53	37.75	0.201
	90	36.79	36.28	36.02	36.37	0.392

Table 12: GDRI values of AIS extracts of untreated and enzymatic-treated refiners at different time intervals.

AIS Samples	Time interval (min)	GDRI value (%)				Standard deviation
		1st	2nd	3rd	Average	
150CER	15	17.49	17.99	18.50	17.99	0.506
	30	46.57	47.65	46.30	46.84	0.718
	45	54.47	54.05	53.85	54.12	0.315
	60	57.67	57.67	57.67	57.67	0.000
	75	54.37	55.02	55.29	54.89	0.474
	90	47.18	45.25	45.77	46.07	0.996
150XYR	15	7.87	5.34	6.35	6.52	1.274
	30	24.87	24.87	24.88	24.87	0.005
	45	40.04	38.81	39.01	39.29	0.662
	60	52.82	53.11	52.25	52.73	0.435
	75	47.26	46.47	47.26	47.00	0.456
	90	37.18	36.92	36.54	36.88	0.323

Table 12: (continued)

AIS Samples	Time interval (min)	GDRI value (%)				Standard deviation
		1st	2nd	3rd	Average	
150CXR	15	35.71	35.71	38.24	36.56	1.461
	30	52.54	53.08	52.81	52.81	0.271
	45	56.53	56.53	56.24	56.43	0.166
	60	61.95	62.52	62.80	62.42	0.435
	75	52.39	52.52	53.05	52.66	0.348
	90	49.10	51.15	50.38	50.21	1.036
CTDR	15	1.29	0.28	2.81	1.46	1.274
	30	19.45	18.63	20.80	19.63	1.096
	45	27.68	26.45	27.27	27.13	0.629
	60	39.14	40.57	39.43	39.71	0.754
	75	40.56	40.16	39.90	40.21	0.331
	90	34.36	34.23	34.36	34.31	0.074

APPENDIX G

Figure 12 shows glucose standard curve obtained by phenol sulfuric acid method, which was constructed and employed to estimate the amount of total soluble carbohdyrates of the alcohol soluble carbohydrates (ASC) samples of untreated and enzymatic-treated pink guava wastes (decanter and refiner). In the standard curve, values were shown represent the means of triplicated data. The linear equation of the curve is y = 0.0932x with the regression coefficient of R^2 = 0.9956.

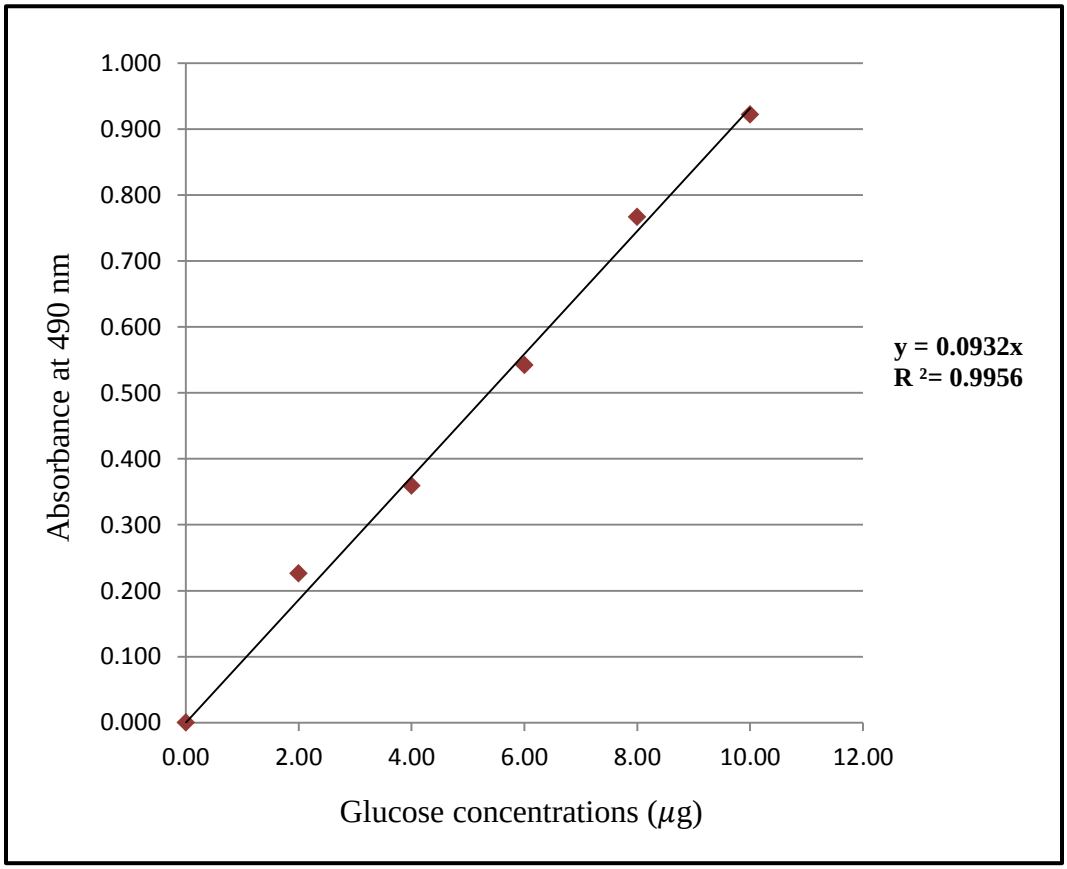


Figure 12: Standard curve constructed based on phenol sulfuric acid method.

Table 13: Total soluble carbohydrates in each ASC extract of pink guava waste by using phenol sulfuric acid method.

Samples	Total soluble carbohdyrates (mg/g)				Standard deviation
	1st	2nd	3rd	Average	
150CED	71.84	71.75	71.47	71.69	0.193
150XYD	49.09	49.09	49.34	49.18	0.144
150CXD	77.84	77.75	78.02	77.87	0.137
CTDD	3.74	3.78	3.71	3.74	0.035
150CER	87.58	87.49	87.85	87.64	0.187
150XYR	55.56	55.48	55.45	55.51	0.046
150CXR	91.33	91.33	91.15	91.27	0.104
CTDR	2.95	2.75	2.79	2.83	0.106

APPENDIX H

Table 14: Retention time, peak area and peak height of glucose standards with different concentrations in HPLC analysis.

Glucose concentrations (mg)	Retention time (min)	Peak area	Peak height
0.00	0.000	0.000	0.000
0.05	7.886	23571	1418
0.10	7.921	96482	5494
0.20	7.890	251494	14320
0.40	7.722	314432	20978
0.60	7.724	432742	28777
0.80	7.468	602658	40698
1.00	7.469	691732	49235
1.20	7.567	769164	49235

Table 15: Retention time, peak area and peak height of fructose standards with different concentrations in HPLC analysis.

Fructose concentrations (mg)	Retention time (min)	Peak area	Peak height
0.00	0.000	0.000	0.000
0.05	6.925	114297	9093
0.10	6.949	308494	26631
0.20	6.592	506511	45740
0.40	6.958	607437	69786
0.60	6.495	773955	69786
0.80	6.529	1031400	90164
1.00	6.488	1425494	123545
1.20	6.523	1561426	99969

Table 16: Retention time, peak area and peak height of sucrose standards with different concentrations in HPLC analysis.

Sucrose concentrations (mg)	Retention time (min)	Peak area	Peak height
0.00	0.000	0.000	0.000
0.05	11.524	201895	11588
0.10	11.549	257599	14447
0.20	10.881	537603	29747
0.40	10.914	833520	41589
0.60	11.085	9739793	45578
0.80	11.280	1385956	56598
1.00	11.369	1712187	64272
1.20	11.421	1987625	73519

Table 17: Retention time, peak area and peak height of galactose standards with different concentrations in HPLC analysis.

Galactose concentrations (mg)	Retention time (min)	Peak area	Peak height
0.00	0.000	0.000	0.000
0.05	8.424	80712	3162
0.10	8.461	165369	5832
0.20	8.609	345873	10072
0.40	8.661	552043	17517
0.60	8.643	688115	19759
0.80	8.597	794057	24393
1.00	8.667	1021565	30732
1.20	9.561	1327615	40562

Table 18: Retention time, peak area and peak height of arabinose standards with different concentrations in HPLC analysis.

Arabinose concentrations (mg)	Retention time (min)	Peak area	Peak height
0.000	0.000	0.000	0.000
0.05	6.942	62509	5589
0.10	6.341	154688	10262
0.20	6.416	293129	13399
0.40	6.391	568006	25169
0.60	6.381	905708	39045
0.80	6.394	1478146	63812
1.00	6.395	1685047	73646
1.20	6.613	2003065	88467

Table 19: Retention time, peak area and peak height of rhamnose standards with different concentrations in HPLC analysis.

Rhamnose concentrations (mg)	Retention time (min)	Peak area	Peak height
0.00	0.000	0.000	0.000
0.05	5.066	122772	10715
0.10	5.074	218850	19274
0.20	5.108	280338	23382
0.40	5.097	589966	50860
0.60	5.086	674864	57066
0.80	5.075	926050	79410
1.00	5.074	1250234	109866
1.20	5.036	1305808	112340

Table 20: Retention time, peak area and peak height of mannose standards with different concentrations in HPLC analysis.

Mannose concentrations (mg)	Retention time (min)	Peak area	Peak height
0.00	0.000	0.000	0.000
0.05	7.531	121400	6845
0.10	7.522	199127	7982
0.20	7.431	235320	12103
0.40	7.418	408953	21994
0.60	7.454	650476	34993
0.80	7.445	777995	41597
1.00	7.460	808532	59469
1.20	8.141	1184850	67974

Table 21: Retention time, peak area and peak height of xylose standards with different concentrations in HPLC analysis.

Xylose concentrations (mg)	Retention time (min)	Peak area	Peak height
0.00	0.000	0.000	0.000
0.05	5.738	140510	12932
0.10	5.981	228261	23624
0.20	5.746	255165	19596
0.40	5.757	400916	35492
0.60	5.758	410047	36668
0.80	5.755	774947	68132
1.00	5.757	944172	117287
1.20	5.981	1397592	162359

Table 22: Structural images and range of retention time for each sugar standard.

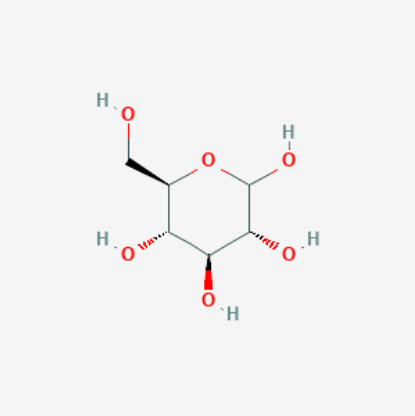
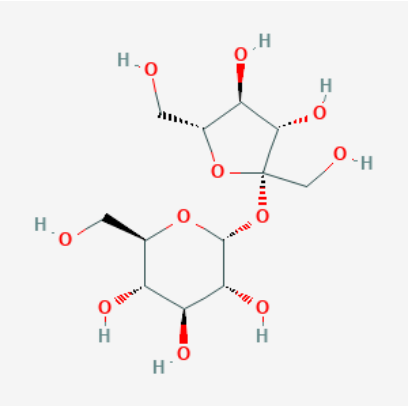
Sugar standards	retention time (min)	Structures
Glucose	7.468 – 7.921	
Fructose	6.488 – 6.958	
Sucrose	10.881 – 11.549	

Table 22: (continued)

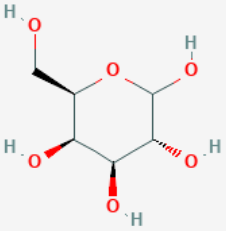
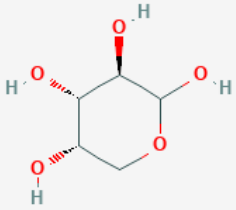
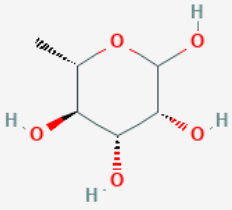
Sugar standards	retention time (min)	Structures
Galactose	8.424 – 9.561	
Arabinose	6.341 – 6.942	
Rhamnose	5.036 – 5.108	

Table 22: (continued)

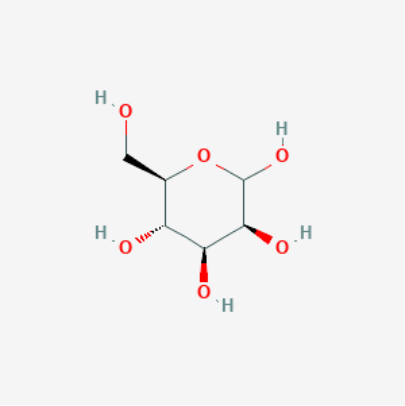
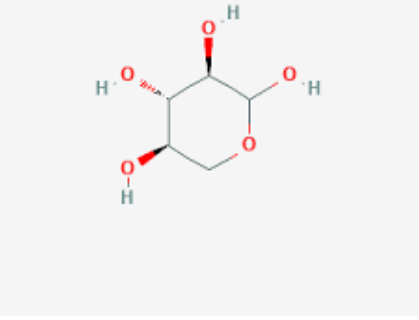
Sugar standards	retention time (min)	Structures
Mannose	7.418 – 8.141	
Xylose	5.738 – 5.981	

Table 23: Retention time, peak area and peak height of 150 U cellulase-treated decanter in HPLC analysis.

Peak	Retention Time (min)		Peak Area		Peak Height	
	1st	2nd	1st	2nd	1st	2nd
1	4.642	4.687	5302	2935	366	286
2	5.465	5.472	26789	34978	1328	1314
3	7.015	6.905	3289	2367	239	210
4	7.202	7.211	2115	6776	139	511
Total			37496	47055	2072	2324

Table 24: Retention time, peak area and peak height of 150 U xylanase-treated decanter in HPLC analysis.

Peak	Retention Time (min)		Peak Area		Peak Height	
	1st	2nd	1st	2nd	1st	2nd
1	5.054	5.128	6317	572	359	47
2	5.522	5.556	36116	17972	1124	791
3	7.368	7.403	6301	7082	353	346
Total			48734	25626	1836	1184

Table 25: Retention time, peak area and peak height of 150 U combined enzymatic-treated decanter in HPLC analysis.

Peak	Retention time (min)		Peak area		Peak height	
	1st	2nd	1st	2nd	1st	2nd
1	4.583	4.604	6329	6246	436	434
2	5.026	5.031	1601	1437	154	147
3	5.509	5.508	46965	44102	2082	1988
4	6.854	6.859	7615	10331	482	541
5	7.912	7.941	11443	8500	344	256
Total			73954	70616	3497	3366

Table 26: Retention time, peak area and peak height of untreated decanter in HPLC analysis.

Peak	Retention time (min)		Peak area		Peak height	
	1st	2nd	1st	2nd	1st	2nd
1	4.473	4.453	6239	6207	473	463
2	5.532	5.542	38143	34691	1532	1441
3	6.943	6.913	3022	3915	197	261
4	7.308	7.316	2886	2794	224	209
Total			50290	47607	2426	2374

Table 27: Retention time, peak area and peak height of 150 U cellulase-treated refiner in HPLC analysis.

Peak	Retention time (min)		Peak area		Peak height	
	1st	2nd	1st	2nd	1st	2nd
1	4.440	4.420	6545	5968	491	464
2	5.021	5.002	3022	2433	194	173
3	5.694	5.707	57702	52832	2209	2052
4	6.804	6.787	26210	24331	1562	1488
Total			93478	85564	4456	4178

Table 28: Retention time, peak area and peak height of 150 U xylanase-treated refiner in HPLC analysis.

Peak	Retention time (min)		Peak area		Peak height	
	1st	2nd	1st	2nd	1st	2nd
1	4.440	4.397	6633	6978	487	518
2	5.619	5.632	61928	61321	2907	2879
3	7.489	7.464	2361	2027	221	179
Total			70922	70326	3615	3576

Table 29: Retention time, peak area and peak height of 150 U combined enzymatic-treated refiner in HPLC analysis.

Peak	Retention time (min)		Peak area		Peak height	
	1st	2nd	1st	2nd	1st	2nd
1	4.381	4.320	4664	3945	326	288
2	5.084	5.038	932	721	72	67
3	5.691	5.692	19901	25019	896	1052
4	6.908	6.872	2410	5084	169	282
5	7.433	7.700	1603	1346	140	2
Total			29510	36114	1602	1691

Table 30: Retention time, peak area and peak height of untreated refiner in HPLC analysis.

Peak	Retention time (min)		Peak area		Peak height	
	1st	2nd	1st	2nd	1st	2nd
1	4.629	4.673	8568	6405	667	567
2	5.390	5.411	168754	148806	8847	7855
3	6.868	6.871	5942	6737	408	440
Total			183264	161948	9922	8862

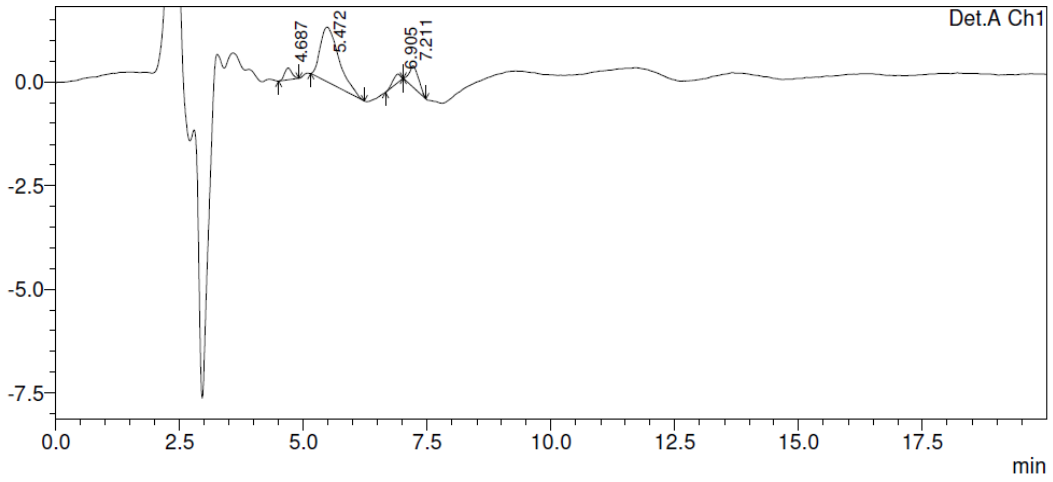


Figure 13: HPLC chromatogram of cellulase-treated decanter (150CED) with identified peaks.

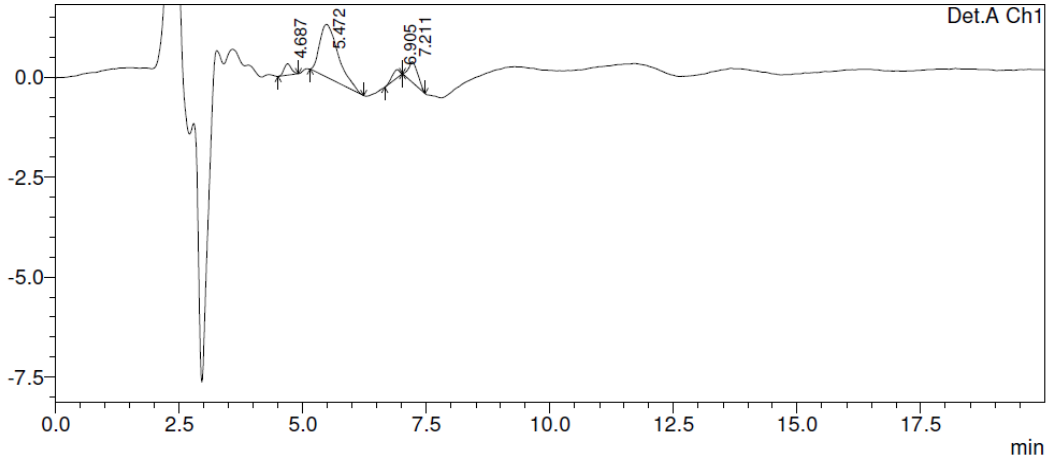


Figure 14: HPLC chromatogram of xylanase-treated decanter (150XYD) with identified peaks.

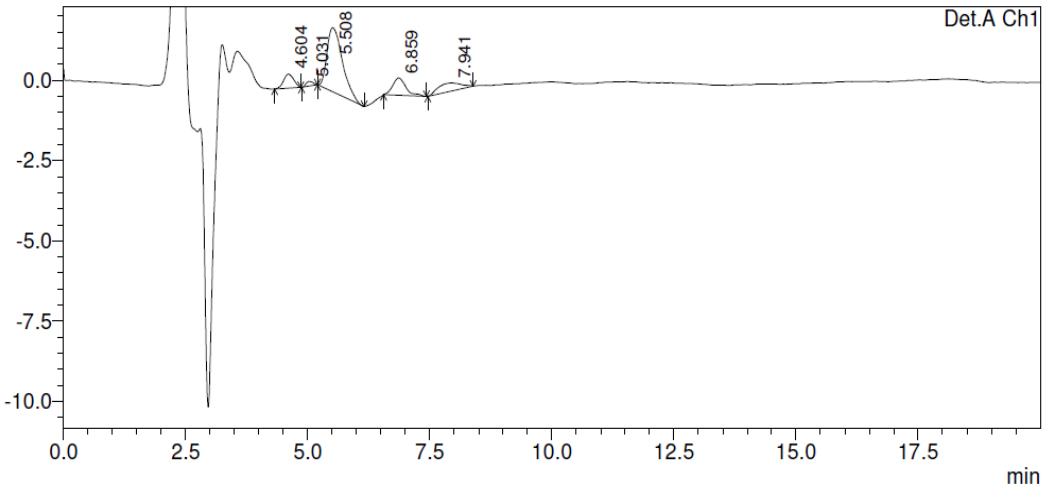


Figure 15: HPLC chromatogram of combined enzymatic-treated decanter (150CXD) with identified peaks.

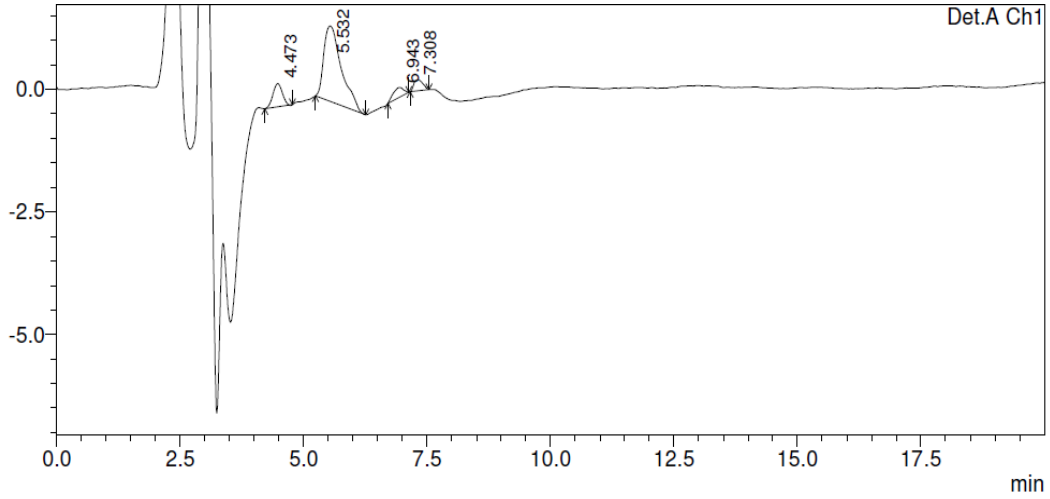


Figure 16: HPLC chromatogram of untreated decanter (CTDD) with identified peaks.

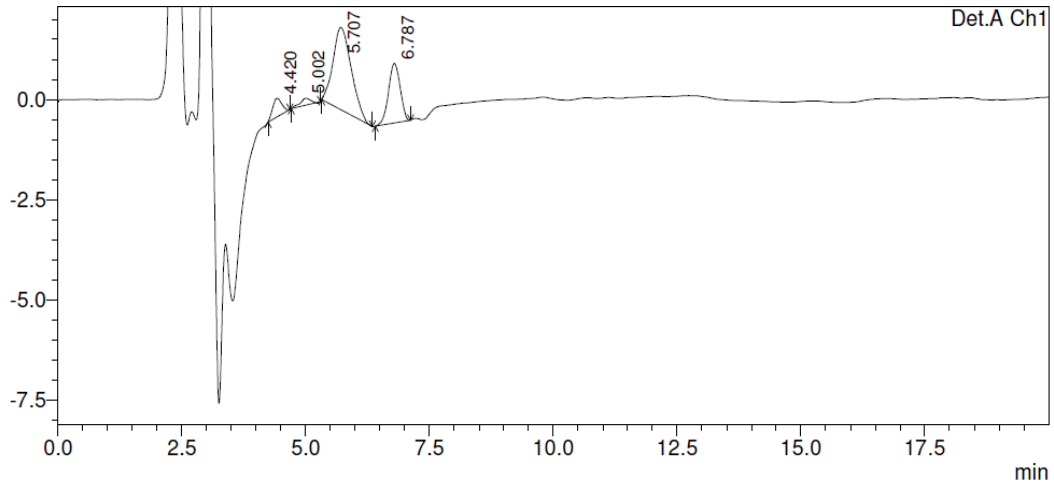


Figure 17: HPLC chromatogram of cellulase-treated refiner (150CER) with identified peaks.

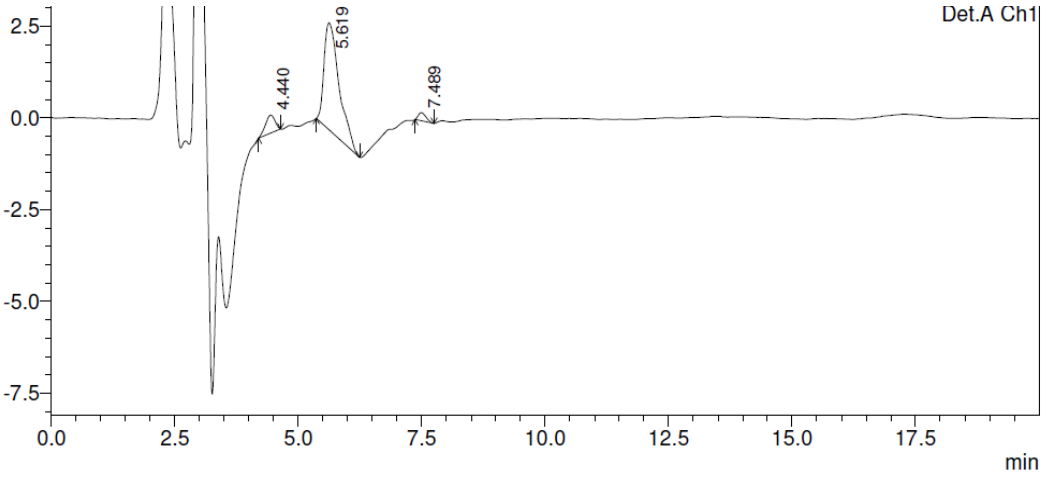


Figure 18: HPLC chromatogram of xylanase-treated refiner (150XYR) with identified peaks.

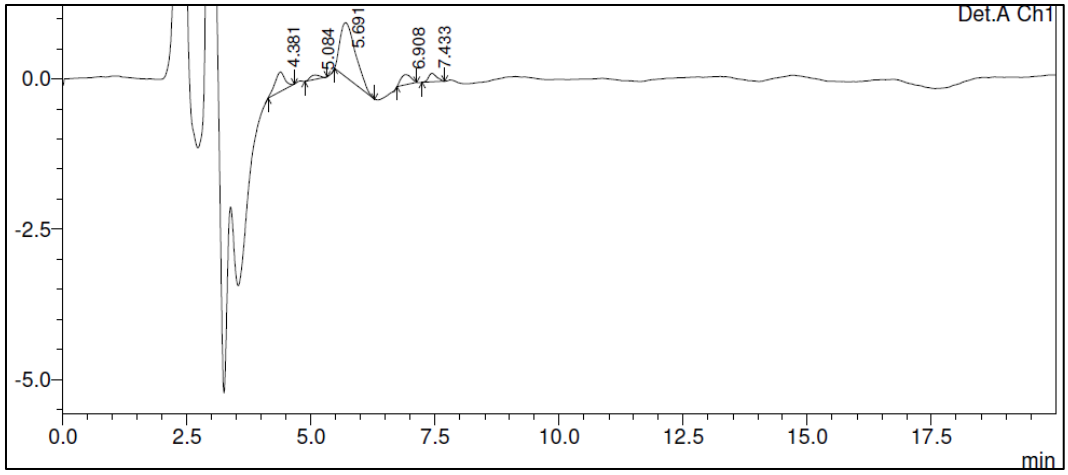


Figure 19: HPLC chromatogram of combined enzymatic-treated refiner (150CXR) with identified peaks.

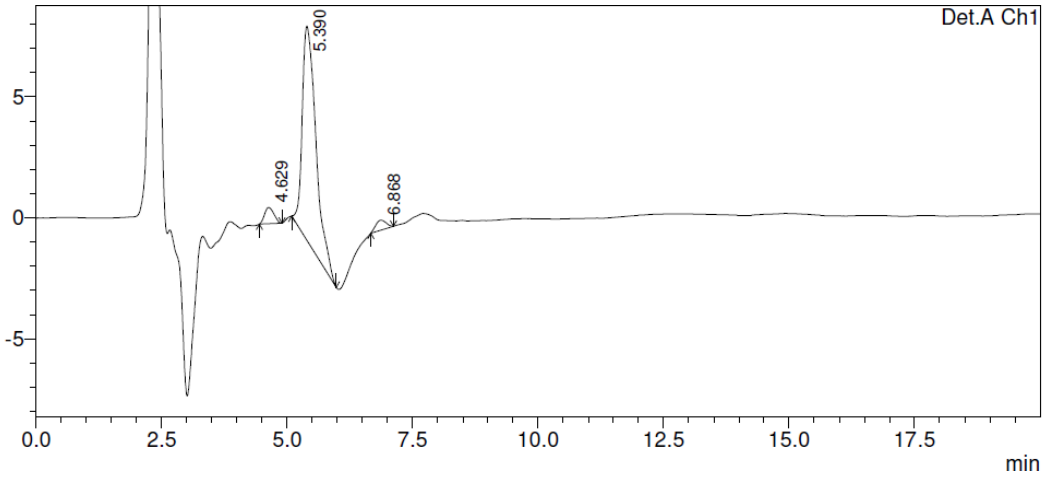


Figure 20: HPLC chromatogram of untreated refiner (CTDR) with identified peaks.