Chemical Characteristics of Physical Pretreated Oil Palm Frond

By

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DECLARATION

I hereby declare that the work embodied in this report is the result of the original research and has not been submitted for a higher degree to any universities or institutions.

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I certify that the report of this final year project entitled “Chemical Characteristics of Physical Pretreated Oil Palm Frond” by Nur Athirah Binti Mohamad Zaini, matric number F14A0221 has been examined and all the correction recommended by examiners have been done for the degree of Bachelor of Applied Science (Animal Husbandry Science), Faculty of Agro Based Industry, University Malaysia Kelantan.

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Pencirian Pelepah Kelapa Sawit Melalui Rawatan Fizikal

Abstrak

Pelepah kelapa sawit mempunyai potensi yang tinggi untuk diguna pakai sepenuhnya sebagai bahan ataupun suplemen di dalam pembuatan makanan haiwan. Dengan menjalani rawatan fizikal ke atas pelepah kelapa sawit dengan memberikan tekanan ke atasnya menggunakan mesin tebu kovensional untuk mendapatkan gentian pelepah kelapa sawit dan juga jus pelepah kelapa sawit dianggap satu teknik yang berkesan. Rawatan fizikal mampu untuk melonggarkan struktur lignoselulosa di dalam kelapa sawit lalu menghasilkan amaun gula yang tinggi, dimana mampu meleraikan pelepah kelapa sawit. Kajian ini dijalankan untuk mencirikan pelepah kelapa sawit yang segar, gentian pelepah kelapa sawit dan juga jus pelepah kelapa sawit yang disejuk-keringkan dengan menentukan kandungan nutrient, kandungan lignoselulosa dan juga kandungan gula yang terdapat di dalam pelepah kelapa sawit. Kajian ini menunjukkan kandungan bahan kering (DM), abu, protein mentah (CP), ekstrek eter (EE) dan serat mentah (CF) daripada batang pelepah kelapa sawit tanpa daun tidak jauh berbeza dengan jus pelepah kelapa sawit daripada keseleruhan pelepah kelapa sawit. Kandungan lignoselulosa di antara hemiselulosa (HC) dan juga selulosa (CE) berbeza di antara pelepah kelapa sawit yang segar (21.06% HC; 31.27% CE), gentian pelepah kelapa sawit (9.03% HC; 45.32% CE) dan juga jus pelepah kelapa sawit yang disejuk-keringkan (0.72% HC; 0.33% CE). Kandungan lignin adalah tertinggi di dalam pelepah kelapa sawit yang segar dan juga batang pelepah kelapa sawit tanpa daun (19% and 17.1%), diikuti dengan gentian pelepah kelapa sawit (13.38% and 10.42%) dan yang paling kurang kandungan lignin ialah di dalam jus pelepah kelapa sawit (1.31% and 0.23%). Peleraian lignin mampu meningkatkan percernaan dan juga kadar pengambilan makanan oleh haiwan ruminan. Untuk kandungan gula di dalam jus pelepah kelapa sawit daripada keseluruhan pelepah kelapa sawit, terdapat 60.5% glukos, 18.5% fruktos dan juga 21% sukros. Jus pelepah kelapa sawit mampu memberikan wawasan di dalam penghasilan makanan haiwan ternakan dan juga sebagai suplemen dan ianya juga boleh digunakan sebagai komponen di dalam penghasilan silaj.

Kata kunci : Rawatan fizikal, gentian pelepah kelapa sawit, jus pelepah kelapa sawit, kandungan nutrient, analisa gula

Characterisation of Oil Palm Frond Following Physical Pretreatments

ABSTRACT

Oil palm frond has a great potential to be fully utilised as ingredients or supplements in animal feed. Physical pretreatment by pressing the OPF using conventional sugarcane pressing machine to obtain the pressed OPF fibre and OPF juice is considered to be a promising technique. Physical pretreatment is able to loosen up the lignocellulose structure in the OPF and thus release high amount of sugars, which further improve the degradability of OPF. This study aims to characterise the fresh OPF, pressed OPF fibre and OPF juice by determining their proximate composition, lignocellulose composition and also free sugar content in the OPF. The results shows contents of dry matter (DM), ash, crude protein (CP), ether extract (EE) and crude fibre (CF) of OPF juice (87.01% DM, 7.03% ash, 1.37% CP, 0.44% EE and 0.14% CF) from petiole are not statistically different with OPF juice from whole OPF (88.50% DM, 6.58% ash, 1.97% CP, 0.54% EE and 0.34% CF). The lignocellulose compositions between hemicellulose (HC) and also cellulose (CE) were significantly different between whole fresh OPF (21.06% HC; 31.27% CE), pressed OPF fibre (9.03% HC; 45.32% CE) and lyophilised OPF juice (0.72% HC; 0.33% CE). Lignin content was significantly highest in whole and petiole of fresh OPF (19% and 17.1%), followed by pressed OPF fibre (13.38% and 10.42%) and least in OPF juice (1.31% and 0.23%). The improving of lignin degradation would increase the digestibility and feed intake of the ruminant. For the free sugar composition in the OPF juice from the whole OPF was 60.5% glucose, 18.5% of fructose and 21% of sucrose. The OPF juice will provide important insight for the development animal feed as well as supplement and it can serve as component in silage making.

Keywords : physical pretreatment, pressed OPF fibre, OPF juice, nutrient composition and sugar analysis

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LIST OF ABBREVIATIONS

ADF	Acid Detergent Fibre
ADL	Acid Detergent Lignin
CE	Cellulose
CF	Crude Fibre
CP	Crude Protein
DM	Dry Matter
EE	Ether Extract
GE	Gross Energy
HC	Hemicellulose
HCi	Hydrochloric Acid
HPLC	High Performance Liquid Chromatography (HPLC)
H_2SO_4	Sulphuric Acid
H_3BO_3	Boric Acid
NaOH	Sodium Hydroxide
NDF	Neutral Detergent Fibre
OPF	Oil Palm Fronds
SPSS	Statistical Package for Social Science

LIST OF SYMBOLS

˚C	Degree Celsius
%	Percentage
M	Molarity
Mm	Millimetre
G	Gram
L	Litre
Kg	Kilogram
Mg	Milligram
Ml	Millilitre
µm	Micrometre
Cm	Centimetre
Lm	Lumen
Min	Minute

CHAPTER 1

INTRODUCTION

1.1 Background of Study

Malaysia has been one of world's largest producers and exporter of oil palm, accounting 60% of world's oil and fat production (Palm Oil Facts & Figures, 2013). The planted areas are estimated around 4.69 million hectares and it contributed 83 million tons (wet weight) of oil palm fronds (OPF) on each year (MPOC, 2010). These figures are expected to increase substantially year by year. Currently, the continuous availability of OPF have been widely used in ruminant diets in Malaysia. However, OPF cannot be used as a sole source of ruminant feed mainly due to its poor nutritive values and low degradability in the rumen of ruminant animals. In addition, feeding OPF would affect the rumen microbial populations and thus, the digestibility of OPF decreased and the feed intake would fall. It is mainly because of high lignin-(hemi)cellulose complex within the OPF.

This indicates challenges to include this by-product in the ruminant diets. However, these OPF can be treated by various pretreatment processes such as physical, biological and chemical pretreatments to improve the digestibility of fibrous by-product through an improvement in the breakdown of lignocellulosic bonds. Physical pretreatment commonly involved with the changes in the physical structure of the by-product and it includes the pressing method by using conventional sugarcane pressing machine, chopping, grinding and etc. While for the biological pretreatment, it involved with the use of metabolite microorganisms such as white, brown and soft rot fungi which help in breakdown of lignocellulosic bonds. Biological pretreatment consumed

less energy and less damage to the environment. For the chemical pretreatment normally involved the use of acidic or bases medium for the disruption of the by-product structure.

Therefore, the aim of the present study was to characterise OPF following physical pretreatment to evaluate its potential as an improved feedstuff by analysing its chemical and sugar compositions.

1.2 Problem Statement

Malaysia ruminant industry is still left behind compared to poultry industry. The most crucial factor that play important role in the development of this industry is high quality feed with adequate nutrients. Common diet available for ruminant is poor in nutritive value especially in term of protein level. Thus, high feeding cost becomes a constraint that limits ruminant production. It becomes a concern among local farmers to find alternatives for cost effective feed sources that have the potential to enhance animal performance. Since OPF are the major waste generated in oil palm plantation, it can be fully utilised as roughage sources to ruminant animals.

However, it is necessary to determine the dry matter degradability and digestibility in the rumen on different pretreatment technique. Characterisation of OPF following physical pretreatment was done by undergone proximate analysis and lignocellulose analysis to determine its nutritional contents from OPF, pressed OPF and OPF juice. In addition, sugar analysis was also done to analyse sugar composition in OPF juice.

1.3 Hypotheses

1. Fresh OPF, pressed OPF fibre and OPF juice have different nutritive values and OPF have the potential as an improved feedstuff in ruminant industry.
2. OPF contains higher amount of free sugars.
3. The nutritive value of fresh OPF were used as a control treatment to the nutritive value of pressed OPF fibre and OPF juice and pressed OPF fibre has the possibility higher nutrition value compare to the control treatments.

1.4 Objectives of Study

1. To compare the proximate composition between fresh OPF, pressed OPF fibre and lyophilised OPF juice as improved feedstuff.
2. To determine the lignocellulosic content of fresh OPF, pressed OPF fibre and lyophilised OPF juice.
3. To determine the free sugar contents in the whole OPF following physical pretreatment.

1.5 Significant of Study

In this current study, OPF show as an excellent source of fibrous by-product that can be fully utilised as roughage to the ruminant animals. The potential of OPF as a substitution feedstuff would aid in cost effective feed sources. It also would leads to the development of high quality feed with adequate nutrients and thus helps the ruminant industry in Malaysia. Proximate analysis were used to determine dry matter (DM), ash, crude fibre (CF), crude protein (CP), ether extract (EE) and gross energy (GE) while acid detergent fibre (ADF), neutral detergent fibre (NDF) and acid detergent lignin (ADL) analyses were used to determine cellulose, hemicellulose and also lignin content in the OPF. Sugar analysis was used to determine sugar component in the OPF.

1.6 Scope of Study

The OPF was characterised by following physical pretreatment to determine and compare the nutritional value of different treatments. OPF juice was undergo sugar analysis to evaluate the sugar compositions.

1.7 Limitation of Study

1. The OPF should be processed within 24 hours after harvesting to keep them in fresh condition. The delay between transportation and processing could reduce OPF juice recovery and yield.

2. Proximate and sugar analysis equipment are not available in UMK Jeli Campus and therefore the analyses were done in FPV, UMK Kota Campus.

CHAPTER 2

LITERATURE REVIEW

2.1 Current Issues on the Use of Agricultural By-product as Animal Feed

Agricultural by-products are growing in volume these days and fully utilised as animal feedstuff. The substitution of the agricultural by-product as feedstuff component in feed ration helps in mitigate the increase of potential input cost faced by ruminant and poultry producers. There are many examples of by-product that can be used in producing ruminant feeds as an alternative to substitute the current feedstuff component. These include sugar industry by-product, animal by-product, fruits and vegetables by-product, forest by-product, marine wastes and aquatic plants by-product and miscellaneous by-products.

The major factor that contributing to the usage of agricultural by-products occur when there are issues between the livestock animals and humans compete for land or crops as the rapid rate of human population growth. Thus, the increasing number of human population will reduce the percentage of land used for crops. It will be leading to the increasing prices of crops that have been used for manufacturing animal feeds as well as human consumption. When this issue occurs, respectively, it will affect the small countries that have imported those crops for animals and human consumption (Capper et al., 2013). For examples, Malaysia needs to import soybean from the major supplier, United States where the United States remains as the top supplier of soybean commanding 44% of the world market share (Rittgers & Hoh, 2016). Besides, feed quantity and quality are the primary constraints of sustainable and an effective production of ruminant animals. This sector has been suffering from the inability to

produce the feed resources for the ruminant animals. It becomes a concern to small countries to find an alternative for cost effective feed sources that have the potential to substitute as feedstuff component.

2.2 Availability of Oil Palm Fronds

The average economic life-span of the palm tree is about 25 years. The total oil palm planted in Malaysia grown by 2.8% to 4.17 million hectares in year 2006. The enlargement areas mainly take place in Sarawak and Sabah with a combined growth of 4.5% compared to 1.6% in Peninsular Malaysia (MPOB, 2006). Most of the fronds are left rotting between the rows of palm tree, mainly for soil conservation, erosion control and ultimately for long-term benefit of nutrient cycling. The need to increase the net return per hectare, has resulted in fronds being utilised as starting resource material for extraction of vitamin E, paper pulp and animal feed. The large quantity of fronds produced by a plantation each year makes this biomass a very promising source of roughage feed for ruminant animals.

2.3 Plant Morphology of Oil Palm Frond (OPF)

OPF is one of the most abundantly available and renewable lignocellulosic biomass. It consists of mainly three primary polymers that are composed in plant cell walls, with the structural carbohydrates cellulose and hemicellulose as well as heterogeneous phenolic lignin with small amounts of protein, pectin, extractives which are nonstructural materials such as chlorophyll and finally is ash (Pei et al., 2010).

2.3.1 Plant Cell Wall

Plant cell wall encloses all parts of the plant cell that known as a protoplast. OPF's cell wall composed of an intercellular layer, a primary and a secondary cell wall as illustrated in Figure 1 (Pei et al., 2010).

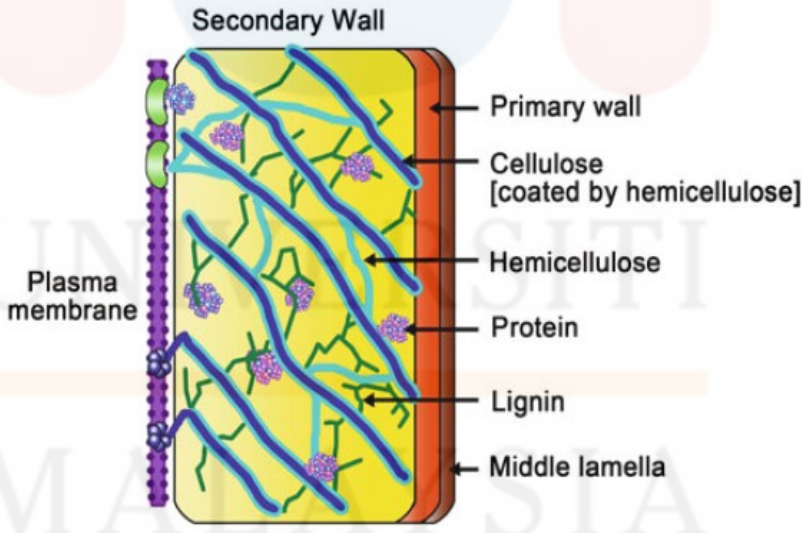

Figure 1. Schematic diagram of plant cell walls (Pei et al., 2010).

2.3.1.1 Primary Cell Wall

The primary cell wall is a thin layer developed with cell division and proliferated during cell growth to a fibre glass-like structure with crystalline cellulose micro fibrils, extremely fine fibres embedded in a matrix of polysaccharides such as hemicelluloses. The primary wall appeared as a solid boundary of the cell (Khalil et al., 2006). It is formed with an outer layer, a middle layer and an inner layer each with a different orientation of extremely fine cellulose micro fibrils. A sticky layer called middle lamella that is composed of pectin that glue together with the adjacent cells of the primary cell wall layers to form the conducting tissue system arranged in numerous vascular bundles (Pei et al., 2010).

2.3.1.2 Secondary Cell Wall

A second wall is gradually deposited inside the primary wall between the plasma membrane when the cells surcease to develop and the primary cell wall will take action by toughening them for a better mechanical strength and structural reinforcement of lignin into xylem fibres. (Pei et al., 2010; Khalil et al., 2006). A critical process occurs in the evolution of the plant during the development of the conducting tissue system with the rigid secondary cell wall which not only functioning in facilitating the transport of water and nutrients as well as extensive upright growth, but also increases its resistance to degradation due to the interaction and cross-linking of cellulose, hemicellulose and lignin (Himmel et al., 2007).

2.3.1.3 Importance of Cell Wall

The cell wall has been described as a network of micro fibrils that is embedded in a matrix of structural carbohydrates, phenolic lignin as well as the proteins. It is highly ordered providing a strong structural shape and often plays an important role in plant-microbe interaction including the defense responses against herbivores, pathogen, bacteria, fungal or any harmful microorganisms (Levetin & McMahon, 2008).

2.3.2 Carbohydrate Polymers

2.3.2.1 Cellulose

Cellulose is a major structural component in plant cell wall. It is the main constituents and it is a polymer of glucose that comprises the most abundant polysaccharide component in plant cell wall. Cellulose is found in an organised fibrous structure and it provides mechanical strength as well as chemical stability to the OPF. Solar energy is absorbed through the process of photosynthesis and it is stored in the form of cellulose (Evert & Eichhorn, 2013). It is composed of 1.10% of linear glucan chains that interact closely through hydrogen bonding into tightly packed and a highly crystalline structure that is resistance to depolymerisation and insoluble in water. Cellulose is linked by β-1,4-glycosidic bond with a degree of depolymerisation in the primary cell wall and held together by intramolecular hydrogen bonds as well as intermolecular Van der Waals forces which responsible to packed them into micro fibrils.

The cellulose micro fibrils are mostly an independent structure but the ultrastructure of the cellulose is largely due to the presence of covalent bonds, hydrogen bonding and Van der Waals forces (Sindhu et al., 2016). The hydrogen bonding in the plant cell wall determines the straightness of the cellulose micro fibrils. These bonds determine the straightness of the cellulose micro fibrils. Crystalline cellulose consists the major proportion of cellulose while amorphous cellulose is unorganised micro fibrils cellulose chains that are more susceptible to enzymatic degradation than crystalline cellulose (Fatin & Fauzi, 2015).

2.3.2.2 Hemicellulose

Hemicellulose is found in primary and secondary cell walls of OPF plant tissues (Waldron et al., 2003). They only occupied 16.4% from the total percentage of plants depending on their age (Zahari et al., 2009). They are a heterogeneous group of polysaccharide with β – (1,4) linked backbone structure of pentose sugar with 5 carbons and hemicellulose has the structural similarity to β – 1,4-glycosidic bonds of cellulose molecules. Hemicellulose plays an important role as they are thought to coat the cellulose micro fibrils and in order to increase the cellulose digestibility, 50% of the hemicellulose content should be removed by undergoing pretreatment (Fatin & Fauzi, 2015).

These impart in conformational homology which leads to a strong non-covalent bond association with cellulose micro fibrils. Besides, hemicellulose interacts and coated the cellulose micro fibrils by hydrogen bonds to provide the structural backbones and that will impart to the tensile strength of the plant cell walls. Hemicellulose is random and formless which it will easily hydrolyse to monomer

sugars. Xylan and xyloglucan are major hemicelluloses in plants including oil palm frond (Waldron et al., 2003; Pei et al., 2010; Sindhu et al., 2016).

2.3.2.3 Lignin

Lignin is a non-sugar-based polymer and it is the second most abundant aromatic polymer in plants after cellulose (Pei et al., 2010). It is formed from an oxidative cross-linking of phenolic alcohols and it forms an integral part of secondary walls in the plant (Waldron et al., 2003). Lignin plays an important role in enhancing the efficiency of water conduction in vascular plants and provides compressive strength that is important in supporting tissues. It is known for its toughness and also provides protection against attack by pathogens. Lignin varies with concern to the stage of growth, age and other condition. It is part of non-phenolic and phenolic structures (Pei et al., 2010; Sindhu et al., 2016; Fauzi et al., 2016; Levetin & McMahon, 2008).

2.4 Oil Palm Frond as Animal Feed

The primary constraints on an efficient and sustainable production of ruminant livestock are feed quality and also its quantity produced. Inability to produce the required feed resources for ruminant causes this sector to be affected. The usage of plant residues as ruminant livestock feed has been recommended to reduce the feed cost and reutilise the biomass thus decrease the number of pollution occurs. Oil palm, *Elaeis guinensis* is an important crop in Malaysia and other countries. In addition, OPF contributes the largest portion of the solid biomass which makes up to 70% of the total residues generated from palm oil industry (Fauzi et al., 2016). OPF has been widely utilised as feedstuff and has a high potency as roughage that can replace grass especially for the ruminant animals.

2.4.1 Nutritive Value of OPF

An OPF is made up of three main components i.e. a petiole, rachis and leaflets. The ash, crude fibre (CF), crude protein (CP), ether extract (EE), acid detergent fibre (ADF) and neutral detergent fibre (NDF) are 3.2%, 38.5%, 4.7%, 2.1%, 55.6% and 78.7% (% of dry matter) respectively (Wan Zahari et.al., 2000). OPF comprised of about 15.5% lignin, 41.7% cellulose, and 16.4% hemicellulose (Hermiati et al., 2013).

However, some improvement in terms of nutritive value is needed to increase the degradability level further. The characteristics of rumen degradation, digestibility, voluntary intake and palatability of several types of processed OPF have been reported (Kawamoto et al., 1999).

2.4.2 Nutritive Value Improvement

These limitations can be overcome by undergoing pretreatment. Pretreatment is a process that involves the alteration of lignocellulosic structure so that the enzymatic hydrolysis of cellulose and hemicellulose can be achieved more rapidly and with greater yield (Ishida & Abu Hassan, 1997). One of the available pretreatment is physical and/or mechanical processing such as pressing, drying, chopping and grinding. It also includes through chemical and biological treatment by predigestion of fibre and stimulation of rumen microbes by supplementation with energy and protein-rich ingredients such as urea and molasses. The addition of supplementation with essential minerals such as calcium will also help to overcome this problem. Besides, there are several processing techniques have been developed to improve the nutritive value and feeding quality of OPF. The processing techniques include the alkali treatment, preservation as silage and steaming under high temperature, enzymatic degradation and also high pressure palletising to completely utilise the OPF as a ruminants feedstuff (Zahari et al., 2003).

2.5 Pretreatment Strategies of OPF

Pretreatment is an essential step and has a large impact on digestibility of cellulose. In oil palm plant structure, cellulose is protected by hemicellulose and lignin and that causes the availability of surface area for enzymatic saccharification reduced. Thus, pretreatment is required to alter the macroscopic and microscopic structure, size and as well as the plant's sub-microscopic chemical composition and structure so that the hydrolysis process of the carbohydrate fraction to monomeric sugars can occur more rapidly with greater yields. The effect of pretreatment has been shown in Figure 2. Increasing the concentration of fermentable sugars after the process of enzymatic saccharification and thus increasing overall process efficiency can be achieved with a proper pretreatment (Sindhu et al., 2016). Pretreatment should meet the following requirements: (1) increasing of the surface area and porosity, (2) modification of lignin structure, (3) removal of lignin, (4) partially depolymerisation of hemicellulose, (5) removal of hemicellulose and (6) reducing the crystallinity of cellulose (Kumar et al., 2009) .

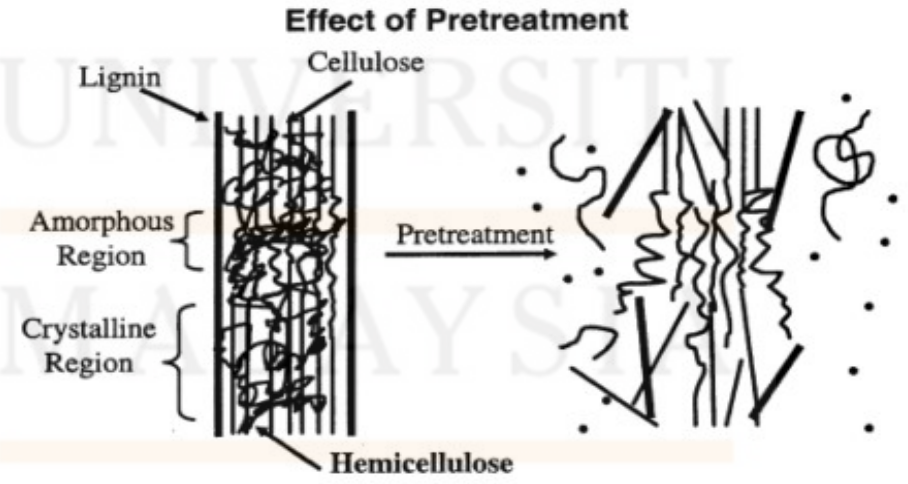

Figure 2. The structure of lignin before and after the pretreatment (Paulien Harmsen et al., 2010).

2.5.1 Physical Pretreatment

Physical pretreatment is non-biological pretreatment method that does not involve any microbial treatments and does not use any chemicals. Reduction in size by mechanical methods will result in the higher total surface area of the biomass and the degree of polymerisation and crystallinity of cellulose are decrease (Kumar & Sharma, 2017). Below are the examples of physical pretreatment that have recently used by the industry:

2.5.1.1 Pressed By-Product

It is a simple method of physical pretreatment. The by-product is cut into certain lengths that are required and then were pressed by using conventional sugarcane machine to remove the water content in the plant residues. The bagasse obtained during the pretreatment process undergo another physical pretreatment to increase the total surface area of the plants (Fauzi et al., 2016).

2.5.1.2 Mechanical Extrusion

Mechanical extrusion is the most conventional pretreatment method. The feedstock materials undergo heating process up to 300ºC. These will result in a production of gaseous products and charcoal from the pretreated agricultural by-product residues. The amorphous and crystalline cellulose matrix of the by-product is disrupted due to the combined effects of high temperatures and the forces generated by the screw blades. This method required a high amount of energy that causes it

costly intensive method and difficult to scale up for the industrial purposes (Zhu & Pan, 2010).

2.5.1.3 Milling

Milling is the grinding process that has been used for reducing the crystallinity of the cellulose. It is included chipping, milling and grinding process. The differences between chipping, milling and grinding are the reductions of plant residues size. Chipping is capable of reducing the size to 10-30 mm only while milling and grinding are capable of reducing the plant residues size up to 0.2 mm. The further reduction of the plant's residues below than 0.4 mm has no effect on the rate and yield of hydrolysis (Chang et al., 1997).

2.5.1.4 Microwave

Microwave irradiation is a broadly used method for feedstock pretreatment because of several reasons such as easy operation, low energy requirement, high heating capacity in short period of time, least generation of inhibitors, and degrades structural organisation of cellulose fraction. Also, an addition of mild alkali reagents is preferred for more effective breakdown (Kumar & Sharma, 2017).

2.5.2 Chemical Pretreatment

Chemical pretreatment involves the use of any chemical that involves in the alteration of biomass to ensure the enzymatic hydrolysis of cellulose and hemicellulose can be achieved more rapidly and with greater yields (Harmsen et al., 2010).

2.5.2.1 Mild-Alkali

Alkali pretreatment methods are in commonly performed at ambient temperature and pressure. Sodium, potassium, calcium and also ammonium salts are the most generally hydroxyl derivatives that have been used. Sodium hydroxide was the most effective alkali reagent between these hydroxyl derivatives (Kumar & Wyman, 2009). Degradation of the side chain of the esters and glycosides lead to the structural modification of the lignin, cellulose swelling, cellulose de-crystallisation and also hemicellulose solvation (Cheng et al., 2010).

2.5.2.2 Ozonolysis

Ozone treatment is mostly used for reducing the lignin content of plant residues as it mainly degrades lignin but slightly affects hemicellulose and cellulose. It has been used for removal of lignin in various agricultural by-products in feedstock industry (Kumar & Sharma, 2017).

2.5.2.3 Ionic Liquids

Ionic liquids have received great attention in last decade for the pretreatment of plant by-products. Ionic liquids are comparatively a new class of solvents which are entirely made of ions which involved cations and anions, have low melting points which are less than 100°C, negligible vapor pressure, high thermal stabilities, and high polarities (Zavrel et al., 2009). Imidazolium salts are the most normally used ionic liquids. Ionic liquids are assumed to compete with agricultural by-product components for hydrogen bonding thereby disrupting its network (Moulthrop et al., 2005).

2.5.3 Biological Pretreatment

Biological pretreatment is considered as an eco-friendly process and there is no inhibitor generation during the process. Biological pretreatment process use metabolite of microorganisms is an economically viable for enhancing enzymatic saccharification rate. Since there no chemical has been used in this pretreatment, there is no need for recycling of chemical and does not release toxic compound to the environment. Biological pretreatment is done by the used of brown, white and soft-rot fungi that play an important role in degrading lignin, hemicellulose and a small amount of cellulose (Sánchez, 2009). The presence of peroxidases and laccases which are the lignin degrading enzymes causes the degradation of the lignin to occur. The white-rot fungi species commonly engaged for pretreatment are *Phanerochaete chrysosporium, Ceriporia lacerata, Cyathus stercolerus, Ceriporiopsis subvermispora, Pycnoporus cinnarbarinus* and *Pleurotus ostreaus*.

Besides these other basidiomycetes species were also considered for the breakdown of several lignocellulosic feedstocks. Among these *Bjerkandera adusta, Fomes fomentarius, Ganoderma resinaceum, Irpex lacteus, Phanerochaete chrysosporium, Trametes versicolor*, and *Lepista nuda* are well studied. These species have been conveyed to show high delignification efficiency (Shi et al., 2009).

CHAPTER 3

METHODOLOGY

3.1 Experimental Design

The flow chart of the experiment carried out is as below.

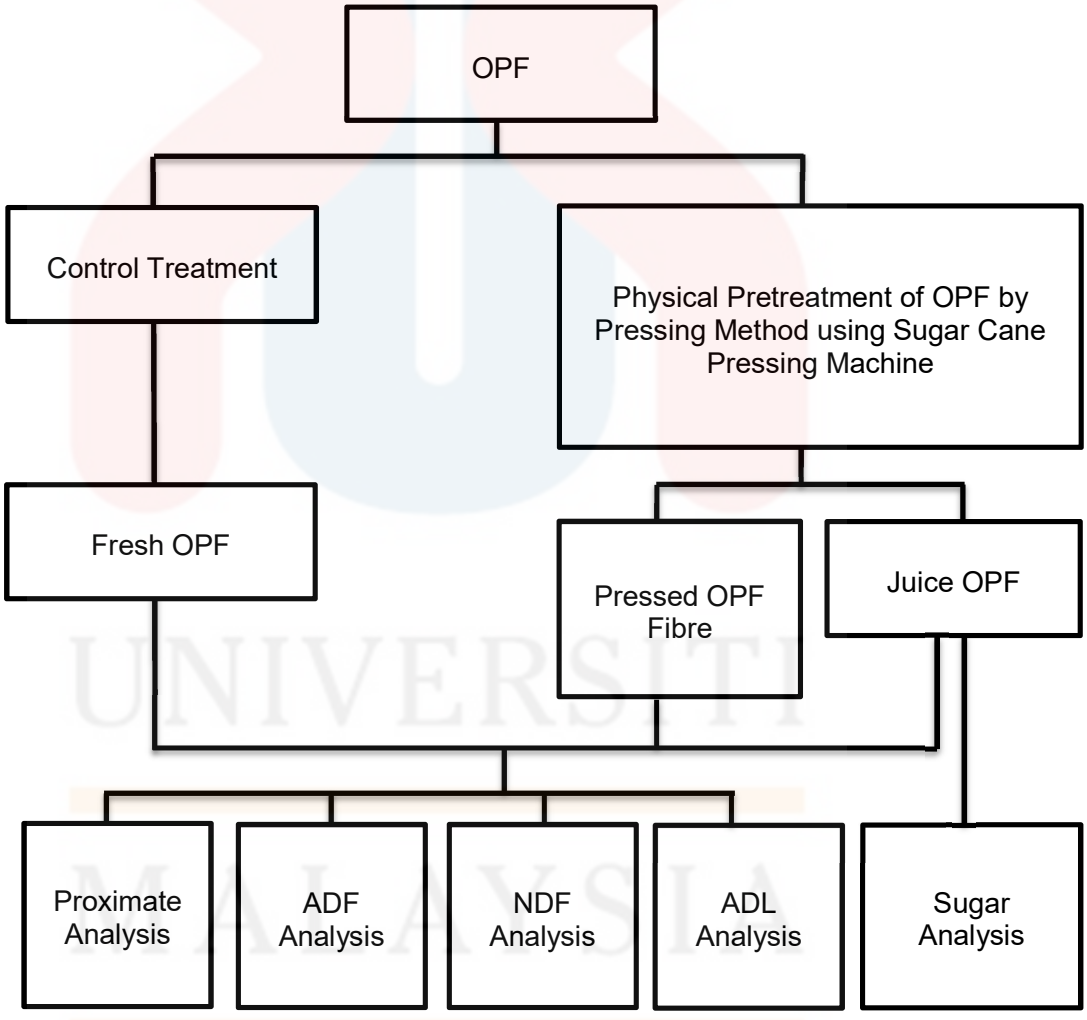

Figure 3. Flow Chart of Experimental Design

3.2 Extraction of OPF Juice

Fresh whole OPF and petioles were obtained from Universiti Malaysia Kelantan. Freshness of harvested OPF was kept assured by collecting and processing them on the same day. The OPF were pruned at both edges before pressing to remove dirt on the fronds. Then, the OPF were divided into whole OPF (petiole with leaflet) and petiole (without leaflet). The whole OPF were taken with the leaflets while petioles were chopped into 2 metre which is one third of the original OPF length in order to avoid mechanical failure during pressing, the harvested OPF were cut into small pieces. The whole OPF and petioles were extracted separately using conventional sugarcane pressing machine (Zahari et al., 2012) to obtain the OPF juice. However, both juices were kept to undergo proximate analysis and only OPF juice from the whole OPF was taken to undergo sugar analysis. The pressed OPF fibres were also collected for proximate analysis. All the fibre samples were kept in sealed plastic bags at ambient temperature (28 – 30ºC) to prevent the moisture leaked out and to ensure the samples were not altered to the sunlight or temperature before analysis. The OPF juice was kept in a container before being process. The OPF juice was centrifuged at 15,000x*g* for 15 minutes at 4ºC (Thermo Fisher Scientific, NC, USA) and then the supernatant was filtered using a mixed cellulose ester membrane filter (3 to 5µm) (Cole Parmer, Illinois, USA). The filtrate was lyophilised on a Labconco FreeZone 12L freeze-drier (Kansas City, Mo., USA) prior to chemical analysis.

3.3 Determination of Sugar Content in OPF Juice

3.3.1 Freeze Drying of OPF Juice

Freeze drying is a process whereby water is removed from frozen materials by converting the frozen water directly into vapour without the intermediated formation of liquid water. All the samples were run using Labconco FreeZone 12L freeze-drier (Kansas City, Mo., USA). Samples were frozen prior to freeze drying under -80ºC for 24 hours. Proper size of container should always be at least two to three times bigger than sample size. The lyophilised OPF were stored before being used.

3.3.2 Preparation of Standard Sugar

A standard mixed comprising of glucose, sucrose and fructose were prepared individually in a concentration of 1mg/ml. The standard solution was passed through ultra-filter in order to collect 2ml before injecting them into HPLC system. The standard solutions were stored in refrigerator at 4ºC before being used.

3.3.3 Determination of Filtrate OPF Juice

The OPF juice were centrifuged at 15,000g for 15 minutes at 4ºC (Thermo Fisher Scientific, NC, USA) and the supernatant was filtered using a mixed cellulose ester membrane filter with the pore size between 0.2µm (Thermo Scientific, Germany). The filtrates were stored at -20ºC before being used.

3.3.4 Determination of Sugar Content of OPF Juice by HPLC Refractive Index (RI) Detector

The sugar content in the OPF juice was determined by a high performance liquid chromatography (HPLC) (Agilent Series 1200, USA). The column used was Supelcosil LC-NH2 (Sigma Aldrich) (25cm_4.6mm ID, 5 Im particles) with a RI detector operated at 30ºC. The mobile phase was 70% of Acetonitrile and 30% of de-ionized water at a flow rate of 1.0ml/min. The components of the sugars were identified by comparing the retention times with those of authentic standards under analytical conditions and quantified by an external standard method (Kafkas et al., 2006). 10 minutes equilibrium time allowed between the injections. The reproducibility of the chromatographic separation of the components will be determined by making 2 injections of standard solutions and OPF juice sample. The external standards sugar used were glucose, fructose and sucrose in order to test the sugar content in the OPF.

3.4 Proximate Analysis on Fresh OPF, Pressed OPF Fibre and Lyophilised OPF Juice

Proximate analysis is a quantitative method for the determination of dry matter (DM), ash, crude protein (CP), ether extract (EE), crude fibre (CF) and also gross energy (GE). The samples were grind using hand blender to pass 1mm screen for proximate analysis (AOAC, 1995). Several methods and equipment were used following the standard methods of AOAC (1995).

3.4.1 Determination of Dry Matter (DM) and Moisture Content

DM and moisture content were determined by drying the fresh OPF (petiole, leaflet and whole OPF) and OPF pressed fibre of petiole and whole OPF overnight at 105ºC (AOAC, 2000). By using electronic balance, the samples were weighed separately between fresh OPF, OPF pressed fibre as well as OPF juice before the drying process take place. The DM and moisture content can be determined by using this formula:

$$\% \text{ DM} = W_f / W_i \times 100$$

Where,

% DM = Percentage of DM

W_f = Final weight of sample

W_i = Initial weight of sample

3.4.2 Determination of Gross Energy (GE) Content

By using IKA Calorimeter system C 200, the gross energy of the sample was determined. The sample was weighed approximately 1g using electronic balance and pelleting using IKA pelleting press C 21. A jug of water was prepared and placed in the ice box with ice pack and thermometer to ensure the temperature is between 18 - 25ºC. The calorimeter device and CalWin 2.25 software were turned on. 50J cotton thread was tied to the ignition wire at the vessel cover. Next, the incineration crucible was placed at the crucible holder under the ignition wire and the tied thread was inserted into the crucible in a way that was touched the base of the crucible. Then, the pelleted sample was placed on top of the thread and allowed to touch the thread.

The vessel clover was placed into the decomposition vessel properly and the union nut was screwed on the vessels. The decomposition vessel was placed on the oxygen station and 30 bar of oxygen was inserted. The ignition adapter was attached on the top of the vessel before inserting the vessel into the loading bay inside the calorimeter system and the cell cover was closed. Two litre of water that has been placed in the ice box was inserted into the tank filters. New measurements were set up on CalWin 2.25 software and the weight of the sample was inserted into the software. As the weight of the sample has been inserted into the software, the calorimeter device was allowed to start and the result of the combustion process appeared within 16 to 20 minutes. The GE of the sample was determined by using this formula:

$$\mathbf{H_o} = \frac{(C \times DT) - QExt1 - QExt2}{M}$$

Where,

H_o = Gross energy (GE)

C = Heat capacity (C-value) of calorimeter

DT = Calculated temperature increase in inner vessel of measuring cell

QExt1 = Correction value for the heat energy generated by the cotton thread as ignition aid

QExt2 = Correction value from the heat energy from other burning aids

M = Weight of pelleted sample

3.4.3 Determination of Crude Protein (CP) Content

Kjeldahl method was used to analyse crude protein and Gerhardt Kjeldatherm as well as Gerhardt Vapodest were used in this experiment. Kjeldahl methods were divided onto three different methods which are; (1) Digestion (2) Distillation and (3) Titration.

3.4.3.1 Digestion

About 1g of samples were weighed and inserted into each of the digestion tubes. Each of the tubes was filled with 10ml of distilled water and a piece of Kjeltab tablet. Next, 12ml of concentrated H_2SO_4 solution was added into each tube inside the fume chamber and then placed inside the digestion rack. The digestion block of Gerhardt Kjeldatherm was turned on and heated up to 400ºC for the pre-heating process. Then, the fume manifold was tightly attached on the top of the digestion tube. The H_2SO_4 aspirator was turned on to prevent the vaporisation of H_2SO_4. The pre-heated digestion was reset from 400ºC to 250ºC for 30 minutes. After 60 minutes, the digestion rack was removed into the rack holder inside the fume chamber and allowed to cool.

3.4.3.2 Distillation

The distillation unit was run 3 times in order to clean up the system. 40% of NaOH was placed in the alkali tank of Gerhardt Vapodest distillation unit. The digested samples were diluted with 80ml of distilled water and 50ml of 45% NaOH. 30ml of receiver solution was added to the receiver flask. The reaction was allowed to settle.

250ml Erlenmeyer titration flask was placed on receiving platform and filled with 4% of boric acid (H_3BO_3) along with the indicator and they were added into receiver solution tank. The dilution tube that contained diluted digested samples was attached to the distillation unit and the samples were then distilled for 5 minutes. Steam distillated in green colour was collected and the receiving flask was removed from the unit for titration process.

3.4.3.3 Titration

The H_3BO_3, a receiving solution was then titrated with the standard 0.1M HCl (Hydrochloric acid) until it reached pink colourisation end point. The volume of HCl used for the titration was recorded. The CP content of the samples was calculated by using this formula:

$$\% N = \frac{(T - B) \times N \times 1.4007 \times 100}{Weight\ of\ sample\ (mg)}$$

$$\% CP = \% N \times F$$

Where,

% N = Percentage of nitrogen content in the sample

T = Volume of titrant used for end point with sample

B = Volume of titrant used for end point without sample

N = Normality of titrant

% CP = Percentage of CP

F = Conversion factor for nitrogen to protein

3.4.4 Determination of Crude Fibre (CF) Content

Gerhardt Fibretherm was used to determine crude fibre content from the samples. The FibreBags were dried for an hour at 105ºC in Memmert oven and then cooled off in desiccator for 30 minutes. Then, the FibreBags were inserted into carousel with glass spacers. About 1g of samples was weighed with Fibrebags along with the glass spacers to obtain the m2 value. The samples along with glass spacers and carousel were defatted by washing the sample thrice with 40ml petroleum ether and the FibreBags were dried for about 2 minutes. Defatting is necessary if the fat content exceeded 10%. Usually, the washing phase consists of 2 phases.

In the first phase, the FibreBags with the samples were boiled in 260ml of 0.13M H_2SO_4 for 30 minutes right after the solution starts to boil. Then, the acid was removed by rinsed thrice with hot water. For the second phase of washing, the samples were boiled with 330ml of 0.11M NaOH solution for 30 minutes right after the solution starts to boil. Again, the alkali was removed by rinsing thrice with hot water. The FibreBags were removed from the carousel. The glass spacers were removed out carefully from the FibreBags without bringing out any sample. The FibreBags were allowed to dry in the Memmert oven for 4 hours at 105ºC and were cooled off in desiccator.

The crucibles were incinerated in Carbolite furnace for 30 minutes at 600ºC in order to prepare the incineration process. Then, the crucibles were cooled off in desiccator for 30 minutes. The FibreBags were placed into each crucible and weighed to determine m3 value. The crucible for blank FibreBags was weighed to get the value of m6. All the crucibles with the FibreBags were incinerated for 4 hours at 600ºC. After that, the crucibles were left for one whole night to cool off. The crucibles with ash were

weighed to get the m4 value. The crucible and blank FibreBag ash were weighed to get the value of m7. The value of ash from blank FibreBag was recorded as m5. The following formula was used to determine the percentage of crude fibre from the sample:

m5 = m7 – m6

% CF = (m3 – m1 – m4 – m5) × 100 / m2

Where,

% CF = Percentage of crude fibre content in the sample

m1 = Weight of FibreBag (g)

m2 = Initial sample weight (g)

m3 = Incinerating crucible and dried FibreBag after digestion (g)

m4 = Incinerating crucible and ash (g)

m5 = Blank value of empty FibreBag (g)

m6 = Incinerating crucible (g)

m7 = Incinerating crucible and ash of empty FibreBag (g)

3.4.5 Determination of Ether Extract (EE) Content

Soxtec method was used in this experiment to determine EE from the sample. The equipment used for this method was FOSS Soxtec 2055 Fat Extraction Systems. In preparing the extraction unit, the temperature was set up according to the suitability of the solvent used to achieve 3 to 5 drops per second. Next, the proper program was selected from 1 to 9 and the duration setting was checked for boiling, rinsing, recovery and pre-drying on the control unit of the extraction unit.

The tap water was open up as the reflux condensers to prevent the solvent (petroleum ether) evaporated from the condensers. Next, thimbles were prepared and attached to the adapters. The samples were then weighed according to the recommended sample weight based on their fat content. As the fat content of the samples were predicted 0 to 10%, 2 to 3g of the samples were weighed into each of the thimbles. As the fat content of the samples between 10 to 25%, then, 1 to 2g of the samples were weighed into each of the thimbles. And if the fat content was more than 25%, the samples were then weighed in the range of 0.5 to 1g.

The samples were weighed with a precision of ±0.1mg and the data was recorded. A layer of defatted cotton was inserted into each of the thimbles. The thimbles were then attached to the thimbles support and inserted to attach the magnetic holder inside the extraction system. Next, the aluminium cups were heated up to 103ºC for 30 minutes in the Memmert oven and allowed to dry in desiccator for 20 minutes to cool off. The aluminium cups were then weighed up to 4 decimal places. 70 to 90ml of petroleum ether was filled up in each of the aluminium cups and then was inserted into the extraction unit with the cup holder.

The extraction unit was then run and the temperature slowly increased until it ready for boiling process, which was notified by the buzzer from the control unit. The thimbles were lowered and immersed with petroleum ether inside the aluminium cups. The thimbles were boiled according to the boiling duration. At this time, the condenser valves must be opened and the control unit was timed for the boiling. After the boiling period ended, the thimbles were moved to rinsing position and timed again. After the rinsing period ended, the extraction unit was set to recovery position and timed for the last time. After that, all the aluminium cups were removed and heated up to 103ºC for 30 minutes. Then, the aluminium cups undergo cooling process in the desiccator for 20 minutes. The aluminium cups were weighed and the weight of the fat content was calculated for each samples. The following formula was used to determine the percentage of ether extract:

$$\% \text{ EE} = W_f - W_i / W_s \times 100$$

Where,

% EE = Percentage of ether extract content

W_f = Final weight of aluminium cup

W_i = Initial weight of aluminium cup

W_s = Weight of the sample

3.4.6 Determination of Ash Content

For the ash content analysis, the empty crucibles were cleaned and placed into Carbolite furnace for an hour at 600ºC. The crucibles were allowed to cool in the desiccator before being weighed. Approximately 1g of the samples were placed in the crucibles and they were incinerated at 550ºC for 4 hours. After 4 hours, the crucibles were allowed to cool overnight. The samples turned up to gray white ash which indicated complete oxidation of all organic matters. The crucibles along with the ash were weighed to obtain the result. The following formula was used to determine ash content in the samples:

$$\textbf{\% Ash} = \textbf{W}i - \textbf{W}f / \textbf{W}s \times 100$$

Where,

% Ash = Percentage of ash content

Wf = Weight of crucible with ash

Wi = Weight of crucible with sample

Ws = Weight of the sample

3.5 Determination of Lignocellulose Content

3.5.1 Determination of Acid Detergent Fibre (ADF) Content

The ADF analysis was done to determine the percentage of cellulose, hemicellulose and lignin content from the samples. Stock solution was prepared by diluting 20g of N-cetyl-N.N.N-trimethyl-ammoniumbromide in 1L of 0.5M H_2SO_4. The FibreBags were dried for an hour at 105ºC in Memmert oven and then placed in desiccator for 30 minutes for cooling process. FibreBags were then weighed to determine m1 value. After being weighed, the FibreBags were inserted into carousel with glass spacers. The initial weight of the samples which was around 1g was recorded as m2 value. After that, the samples along with glass spacers and carousel were defatted by washing the samples thrice with 40ml of petroleum ether. Defatting is necessary if the fat content exceeded 10%. After the washing process, the FibreBags were dried for 2 minutes. The FibreBags with the samples were boiled for an hour in 360ml of ADF solution. Antifoam agent and boiling chip were added while the boiling process took placed. After an hour, the acid was removed by rinsing them thrice with hot water. The FibreBags were then removed from the carousel and dried in Memmert oven at 105ºC for 4 hours. The FibreBags were placed in desiccator for cooling process. Next, the crucibles were incinerated in Carbolite Furnace for 30 minutes at 600ºC for the incineration process. The crucibles were then placed in the desiccator for 30 minutes to allow them to cool off.

The crucible was weighed to get the m6 value. The FibreBags were inserted into each crucible and was weighed to obtain m3 value. All the crucibles with the FibreBags were incinerated for 4 hours at 600ºC in Carbolite Furnace. The crucibles were left overnight to cool off. The crucible with ash was weighed to determine m4

value. The blank crucible with empty FibreBags ash was weighed to obtain m7 value. The value of ash from blank FibreBags was recorded as m5. The following was the formula used to determine the percentage of acid detergent fibre from the sample:

m5 = m7 – m6

% ADF = [(m3 – m1) – (m4 – m5)] × 100 / m2

Where,

% ADF = Percentage of acid detergent fibre in the sample

m1 = Weight of FibreBag (g)

m2 = Initial weight of sample (g)

m3 = Incinerating crucible and dry FibreBag (g)

m4 = Incinerating crucible and ash (g)

m5 = Value of empty FibreBag (g)

m6 = Incinerating crucible (g)

m7 = Incinerating crucible and ash of empty FibreBag (g)

3.5.2 Determination of Neutral Detergent Fibre (NDF) Content

FibreBags system was also done in analysed and determination of NDF content. Approximately 93g of disodium dihydrogen ethylene diamine tetraacetate and 34g of disodium tetra borate-decahydrate were dissolved in 2L of water. 150g of sodium laurylsulphate, 50ml of 2-ethoxyethanol and 22.8g of sodium dihydrogen phosphate was added in the solution. The mixture was then heated for an hour at the solvent boiling temperature.

The FibreBags were dried for an hour at 105ºC in Memmert oven and then placed in desiccator for 30 minutes to cool off. The FibreBags were then weighed to determine m1 value. Then, the FibreBags were inserted into the carousel with glass spacers. The initial weight of the samples which was around 1g was recorded as m2 value. After that, the samples along with glass spacers and carousel were defatted by washing the samples thrice with 40ml of petroleum ether. Defatting is necessary if the fat content exceeded 10%. After the washing process, the FibreBags were dried for 2 minutes. The FibreBags with the samples were boiled for an hour in 360ml of ADF solution. Antifoam agent and boiling chip were added while the boiling process took placed. 15µl of enzyme amylase was added after 10 minutes. The solution was removed by rinsing them thrice with hot water. The FibreBags were then removed from the carousel and dried in Memmert oven at 105ºC for 4 hours. The FibreBags were placed in desiccator for cooling process. Next, the crucibles were incinerated in Carbolite Furnace for 30 minutes at 600ºC for the incineration process. The crucibles were then placed in the desiccator for 30 minutes to allow them to cool off.

The crucibles were weighed to get the m6 value. The FibreBags were inserted into each crucible and were weighed to obtain m3 value. All the crucibles with the

FibreBags were incinerated for 4 hours at 600ºC in Carbolite Furnace. The crucibles were left overnight to cool off. The crucibles with ash were weighed to determine m4 value. The blank crucible with empty FibreBag ash was weighed to obtain m7 value. The value of ash from blank FibreBag was recorded as m5. The following was the formula used to determine the percentage of neutral detergent fibre from the sample:

m5 = m7 – m6

% NDF = [(m3 – m1) – (m4 – m5)] × 100 / m2

Where,

% NDF = Percentage of neutral detergent fibre in the sample

m1 = Weight of FibreBag (g)

m2 = Initial weight of sample (g)

m3 = Incinerating crucible and dry FibreBag (g)

m4 = Incinerating crucible and ash (g)

m5 = Value of empty FibreBag (g)

m6 = Incinerating crucible (g)

m7 = Incinerating crucible and ash of empty FibreBag (g)

3.5.3 Determination of Acid Detergent Lignin (ADL) Content

ADL analysis was used to determine the lignin percentage in the sample. The FibreBags were dried for an hour at 105ºC in Memmert oven and then cooled off in desiccator for 30 minutes. The FibreBags were then weighed to determine m1 value. Next, the FibreBags were inserted into carousel with glass spacers. The initial weight of the samples which was around 1g was recorded as m2 value. After that, the samples along with glass spacers and carousel were defatted by washing the samples thrice with 40ml of petroleum ether. Defatting is necessary if the fat content exceeded 10%. After the washing process, the FibreBags were dried for 2 minutes. The FibreBags with the samples were boiled for an hour in 360ml of ADL solution. Antifoam agent and boiling chip were added while the boiling process took placed. The solution was removed by rinsing them thrice with hot water. The FibreBags were washed in 360ml of 72% H_2SO_4. The FibreBags were then removed from the carousel and dried in Memmert oven at 105 ℃ for 4 hours. The FibreBags were placed in desiccator for cooling process. Next, the crucibles were incinerated in Carbolite furnace for 30 minutes at 600ºC for the incineration process. The crucibles were then placed in the desiccator for 30 minutes to allow them to cool off.

The crucibles were weighed to get the m6 value. The FibreBags were inserted into each crucible and were weighed to obtain m3 value. All the crucibles with the FibreBags were incinerated for 4 hours at 600ºC in Carbolite furnace. The crucibles were left overnight to cool off. The crucibles with ash were weighed to determine m4 value. The blank crucible with empty FibreBag ash was weighed to obtain m7 value. The value of ash from blank FibreBag was recorded as m5. The following was the formula used to determine the percentage of acid detergent lignin from the sample:

m5 = m7 – m6

% ADL = [(m3 – m1) – (m4 – m5)] × 100 / m2

Where,

% ADL = Percentage of acid detergent lignin in the sample

m1 = Weight of FibreBag (g)

m2 = Initial weight of sample (g)

m3 = Incinerating crucible and dry FibreBag (g)

m4 = Incinerating crucible and ash (g)

m5 = Value of empty FibreBag (g)

m6 = Incinerating crucible (g)

m7 = Incinerating crucible and ash of empty FibreBag (g)

The percentage (%) of cellulose, hemicellulose and lignin were then calculated using the equations below:

Cellulose (%) = ADF – ADL

Hemicellulose (%) = NDF – ADF

Lignin (%) = ADL

3.6 Statistical Analysis

Each parameter was repeated thrice. The results were presented with means and standard deviation using SPSS (Statistical Package for Social Science) version 25.0. The data of comparing the nutrients content of fresh OPF, pressed OPF fibre and OPF juice were determined by the Duncan multiple range test from one-way analysis of variance (ANOVA), and the significance of the differences between means was determined, where $p<0.005$ of sample was considered statistically significant.

CHAPTER 4

RESULTS

4.1 Sugar Components in OPF Juice

Table 1 summarises the components of free sugar in whole OPF. It was shown that total free sugar content in OPF juice is 119g/L consisting glucose (72g/L), fructose (22g/L) and sucrose (25g/L). Glucose was found to be the most major sugar (60.5%) in the OPF juice. Meanwhile, fructose and sucrose contents of OPF juice from whole OPF were 18.5% and 21% respectively.

Table 1. Sugar components in OPF juice from whole OPF

Components of Sugar	Amount of Sugar (g/L)	Percentage (%)
Glucose	72	60.5
Fructose	22	18.5
Sucrose	25	21.0
Total	119	100

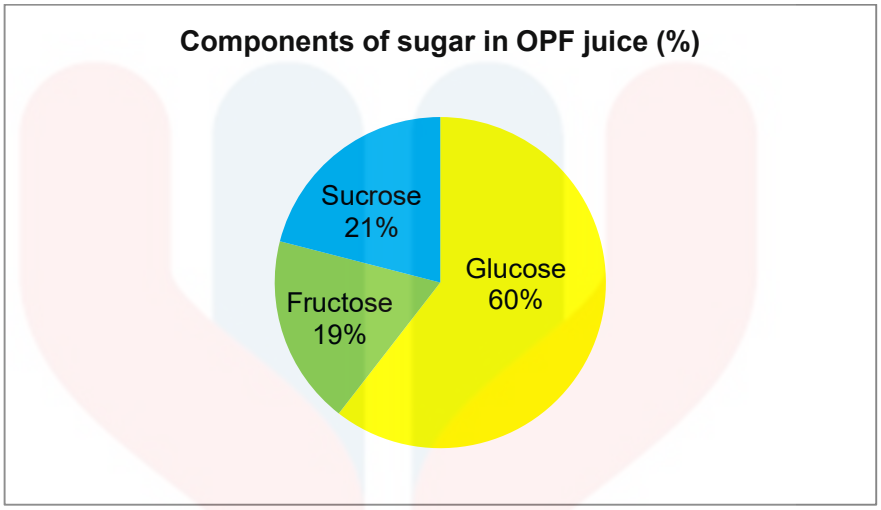

Figure 4. Proportion of free sugar in whole OPF juice.

4.2 Determining Proximate Composition in Fresh OPF, Pressed OPF Fibre and Lyophilised OPF Juice

Table 2 presented proximate compositions of different fractions of fresh OPF, pressed OPF fibre and lyophilised OPF juice. From the fresh OPF data, leaflet showed the highest ($p<0.05$) DM content (51.09%) followed by whole OPF (43.54%) and petiole (22.39%). Leaflet contained more CP (11.15%), EE (2.36%) and less CF (34%) than petiole and whole OPF. GE content of whole OPF was higher than petiole and leaflet. Ash content was highest in petiole (8.51%) followed by whole OPF and leaflet.

The whole pressed OPF has better nutritive value than pressed petiole as whole pressed OPF contained higher CP and EE than pressed petiole. Leaflet contributed high content of CP and EE in whole pressed OPF. However, there were no significant difference of CF and GE contents between whole pressed OPF and pressed petiole.

For OPF juice, there were no significant difference of nutrient contents between petiole and whole OPF ($p>0.05$). As shown in Table 1, OPF juice from petiole part contained 87.01% DM, 7.03% ash, 1.37% CP, 0.44% EE and 0.14% CF. Meanwhile OPF juice from whole OPF contained 88.50% DM, 6.58% ash, 1.97% CP, 0.54% EE and 0.34% CF.

Table 2. Proximate composition and nutritive values of OPF sections (% in DM)

OPF Section	DM (%)	Ash (%)	CP (%)	EE (%)	CF (%)	GE (cal/g)
Fresh OPF						
Petiole	22.39±1.05	8.51±0.60	2.49±0.08	0.57±0.02	45.34±0.24	1549.3±3.06
Leaflet	51.09±0.54	4.63±0.05	11.15±0.61	2.36±0.17	34.0±1.91	40.93±16.2
Whole OPF	43.54±2.89	3.64±0.58	6.12±0.5	1.41±0.21	35.74±1.11	4236±7.07
Pressed OPF Fibre						
Petiole	43.39±5.25	2.29±0.14	1.14±0.44	0.58±0.14	36.57±4.64	3131.75±79
Whole OPF	34.98±3.63	4.18±1.60	4.71±0.24	1.46±0.26	42.05±7.87	3919.75±22.05
Lyophilised OPF Juice						
Petiole	87.01±1.87	7.03±3.31	1.37±0.17	0.44±0.27	0.14±0.13	3182±75
Whole OPF	88.50±3.64	6.58±2.82	1.97±0.45	0.54±0.30	0.34±0.15	3305.25±80.5

Notes: Data shown are means±standard deviation (SD) of triplicates. DM: dry matter; CP: crude protein; EE: ether extract; CF: crude fibre; GE: gross energy.

4.3 Determining Lignocellulosic Composition in Fresh OPF, Pressed OPF Fibre and Lyophilised OPF Juice

Table 3 shows mean content of lignocellulosic composition in fresh OPF, pressed OPF fibre and lyophilised OPF juice. Hemicellulose (HC), cellulose (CE) and lignin contents varied with a significance difference ($p<0.05$) between different fractions of fresh OPF which consist of whole OPF, petiole and also leaflet.

Fresh OPF contained primarily 71.33% of lignocellulose with 21.06% HC, 31.27% CE and 19% lignin. Comparing the contents of HC and CE from whole OPF, they showed significant difference between fresh OPF (21.06% HC; 31.27% CE), pressed OPF fibre (9.03% HC; 45.32% CE) and lyophilised OPF juice (0.72% HC; 0.33% CE). For petiole fraction, pressed petiole fibre showed a higher ($p<0.05$) HC content (8.22%) followed by fresh petiole (5.98%) and lyophilised OPF juice (0.5%). The OPF juice obtained from the petiole contained significantly lowest ($p<0.05$) CE compared to fresh petiole and pressed petiole fibre. Both fresh whole OPF and fresh petiole were rich in lignin (19% and 17.1%) respectively compared to pressed whole OPF fibre and pressed petiole (13.38% and 10.42% respectively) as well as OPF juice from whole OPF and petiole (1.31% and 0.23% respectively).

Table 3. Lignocellulosic composition of OPF sections with different fractions

OPF Section	NDF	ADF	HC	CE	Lignin
Fresh OPF					
Petiole	69.3±1.05	63.05±0.91	5.98±0.59	45.95±1.18	17.1±0.53
Leaflet	60±1	43.21±0.77	16.79±1.74	16.87±1.20	26.34±0.48
Whole OPF	71.3±0.58	50.26±0.31	21.06±0.83	31.27±0.72	19±0.41
Pressed OPF Fibre					
Petiole	62.23±6.85	54±4.18	8.22±0.23	43.59±2.40	10.42±0.66
Whole OPF	67.73±8.45	58.7±6.46	9.03±0.40	45.32±1.35	13.38±2.59
Lyophilised OPF					
Juice					
Petiole	1.05±0.07	0.55±0.04	0.5±0.07	0.32±0.05	0.23±0.06
Whole OPF	2.36±0.02	1.64±0.03	0.72±0.02	0.33±0.04	1.31±0.18

Notes: Data shown were means±standard deviation (SD) of triplicates.

NDF: Neutral detergent fibre; ADF: Acid detergent fibre; HC: Hemicellulose; CE: Cellulose.

CHAPTER 5

DISCUSSION

5.1 Sugar Components in OPF Juice from Whole OPF

The current study is to determine the free sugar components in OPF juice from whole OPF as well as petiole only. Considering whole OPF has better nutritive value, whole OPF was chosen in this study to undergo pressing method using conventional sugarcane machine to extract OPF juice. In addition, this would minimise and reduce the cost involved in this study. On the other hand, by applying the physical method using conventional sugarcane pressing machine, the total potential of free sugar in OPF juice obtained from whole OPF was 119g/L. However, this recorded data was contradicted with the total potential of free sugar by Lee & Abdul Halim (2014) which is 647.76g/L.

The presence of sugars in OPF was due to the process of photosynthesis as OPF used carbon dioxide and water with the help of sunlight to produce glucose as their food source and also oxygen (Evert & Eichhorn, 2013). Glucose showed the highest percentage (60.5%) from whole OPF followed by sucrose (21.0%) and fructose (18.5%). These sugars are the common sugars found in OPF. Previous study showed the same pattern whereby glucose was the dominant sugar followed by sucrose and fructose (Zahari et al., 2012). This claim can also be supported by similar research done from OPF petiole by Srimachaia et al. (2015). Glucose was found to be the highest percentage in free sugar composition in whole OPF which contributed 60.5% and it was in agreement with report by Che Maail et al. (2014). However, it showed 9.5% less than glucose percentage recorded by Zahari et al. (2012) and 17.46% less

from data recorded by Lee & Abdul Halim (2014). This may due to numerous factors such as the difference in individual oil palm tree itself and the way of handling the juice during and after pressing using the conventional sugarcane machine.

However, the percentage of individual sugar components were contended with the previous studies that used similar physical method by using conventional sugarcane pressing machine by Zahari et al. (2012) and Lee & Abdul Halim (2014). In this current study, 21% of sucrose was recorded and it was higher than the study done by Lee & Abdul Halim (2014) which recorded 16.22% of sucrose. Besides, fructose obtained was 18.5% and 12.68% higher than the one recorded by the same researchers. The difference in the percentage of sucrose and fructose indicated that there is no breakdown of sucrose into its monomers occurs which will result in difference percentage of fructose and glucose in OPF juice. This may cause by some of the sugars from the juice could be trapped in the petiole fibres and it reduced the yield of OPF juice extraction. It was reported by Roslan et al. (2013) and experimentally verified that one-third of glucose were remained in the petiole fibres

5.2 Proximate Composition of Fresh OPF, Pressed OPF Fibre and Lyophilised OPF Juice

In this study, OPF leaflet showed the highest ($p<0.05$) DM content (51.09%) followed by whole OPF (43.54%) and petiole (22.39%). It showed that the nutrients left over after the removal of water in leaflet was much higher than whole OPF and petiole. DM is important for the ruminant as DM provide them for maintenance and consistent growth.

Leaflet in OPF contained more CP (11.15%), EE (2.36%) and less CF (34%) than petiole (2.49% CP; 0.57% EE; 45.34% CF) and whole OPF (6.12% CP; 1.41% EE; 35.74% CF). This claim was in line with the previous study by Islam et al. (2000) who showed 13.1% of CP in leaflet, followed by whole OPF (6.5%) and petiole (2.6%). According to Dahlan et al. (2000), most of the protein was accumulated in the leaflet and it has promising value for ruminants feeding as its CP content is far above the critical CP value (6.25%) required by the ruminants in order to maintain their normal intake.

In addition, in previous study by Islam et al. (2000), the finding has showed that petiole has lowest CP (6.5%) content compared to leaflet and whole OPF. In the digestibility trial done by the same researchers, the goats were given chopped OPF and almost entirely rejecting the petiole and select leaflet. It indicated that petiole is unacceptable feed. It cannot be used as maintenance feed for the ruminants as it has low digestibility. However, the leaflet alone cannot be given as feed as it contained high lignin which adversely affects degradability, digestibility and feed intake in ruminants.

GE content of whole OPF (4236 cal/g) was higher than petiole (1549.3 cal/g) and leaflet (4093.7 cal/g). Ash content was highest in petiole (8.51%) followed by whole OPF (3.64%) and leaflet (4.63%). However, the value of ash content in OPF is not in agreement with a previous study who reported that ash content was highest in leaflet (7.4%) followed by whole OPF (3.9%) and petiole (2.2%) (Islam et al., 2000). Another study reported that high content of ash (12.3%) in OPF is due to the presence of silica in OPF (Choo, 2002). OPF that contained considerable amount of silica could reduce its nutritive value when fed to the ruminants and caused it is less palatable. It may due to several factors such as the high temperature during handling the experiment and also the different cultivar used that may cause the results were contradicted from the other studies.

By using pressing method, pressed OPF fibre and OPF juice were obtained from the petiole and also whole OPF. Based on Table 2, the whole pressed OPF fibre has better nutritive value than pressed petiole fibre as whole pressed OPF fibre contained higher CP and EE percentage. It may due to leaflet that contributed high content of CP and EE in whole pressed OPF fibre. The result obtained on leaflet has been demonstrated by Dahlan et al. (2000) and it stated that leaflet from whole OPF contained high content of CP and EE percentage. The physical pretreatment done using conventional sugarcane pressing machine helps in increasing the surface area and porosity of OPF besides it also helps in modification of lignocellulosic structure of OPF (Kumar et al., 2009). As the lignocellulosic structures have been altered, more chemical composition of OPF could be recovered.

In addition, the DM in whole pressed OPF fibre (34.98%) was lesser than pressed petiole fibre (43.39%). It showed that pressed petiole fibre has higher nutrients left over after the removal of water. However, there were no significant difference of CF

and GE contents between whole pressed OPF (42.05% CF; 3919.75 cal/g GE) and pressed petiole (36.57% CF; 3131.75 cal/g GE).

For OPF juice following freeze-drying process, there was no significant difference of nutrient contents between petiole and whole OPF ($p>0.05$). As shown in Table 2, OPF juice from whole OPF contained higher DM (88.50%) than petiole part (87.01%). Physical pretreatment by pressing the fresh OPF using conventional sugarcane pressing machine into OPF juice reduced almost 99% of the CF content. However, the EE and CP contents were still accumulated in the OPF juice especially from whole OPF. This may cause by some of the nutrients from the juice could be trapped in the petiole fibres and caused it was difficult to obtain a high yield of OPF juice extraction as reported by Roslan et al., (2013). The other nutrient contents between petiole and whole OPF have slightly similar percentage. The reasons underlying this observation may include the fact that the samples used in this study were whole OPF, not petiole (without leaves) and also the difference in the age of oil palm tree that was harvested gave different nutritive values. It was reported that older oil palm trunk will diminished the sugar content as the tree became matured and older (Murai & Kondo, 2010). Therefore, 7 to 10-year-old oil palm tree were harvested to run this study.

5.3 Lignocellulosic Composition of Fresh OPF, Pressed OPF Fibre and Lyophilised OPF Juice

Table 3 shows mean content of lignocellulosic composition in fresh OPF, pressed OPF fibre and lyophilised OPF juice. Hemicellulose (HC), cellulose (CE) and lignin contents varied with a significance difference ($p<0.05$) between different fractions of fresh OPF which consist of whole OPF, petiole and also leaflet.

Fresh whole OPF contained primarily 71.33% of lignocellulose with 21.06% HC, 31.27% CE and 19% lignin. Significantly, the current results were in line with earlier reports which demonstrated 20% lignin by Dahlan et al. (2000) and 31.5% CE and 19.2% HC by Sheng-Hong et al. (2012). Yet, the CE percentage was shown higher in all fractions of fresh OPF (whole OPF, petiole and leaflet) than HC which was opposed to Dahlan et al. (2000). Dahlan et al. (2000) stated that the CE level is usually lower than HC in both leaflet and petiole. A possible reason for higher percentage in CE may due to difference in individual oil palm tree itself where HC occupied in OPF is depending on their age (Zahari et al., 2009). Young oil palm tree has less amount of HC and they are not able to coat the CE micro fibrils and cause decrease in cellulose digestibility (Fatin & Fauzi, 2015).

Comparing the contents of HC and CE from whole OPF, they showed significant difference between fresh OPF, pressed OPF fibre and lyophilised OPF juice. For petiole fraction, pressed petiole fibre showed a higher ($p<0.05$) HC content followed by fresh petiole and lyophilised OPF juice. The difference between them was caused by the physical pretreatment on OPF by pressing the fresh OPF to obtain the OPF juice and also pressed OPF fibre. Such physical pretreatment disrupted the inter- and intra-molecular hydrogen bonds embedded within the matrix of HC and lignin,

increasing the surface area and porosity, modification or removal in lignin structure, depolymerisation or removal of HC and also reducing the crystallinity of CE (Kumar et al., 2009). Thus, the disruption of matrix of HC and lignin released CE. The amount of CE was significantly enhanced in pressed OPF fibre followed by fresh OPF and lesser in OPF juice.

The OPF juice obtained from the petiole contained significantly lowest ($p<0.05$) CE (0.32%) compared to fresh petiole (45.95%) and pressed petiole fibre (43.59%). Both fresh whole OPF and fresh petiole were rich in lignin (19% and 17.1% respectively) compared to pressed whole OPF fibre and pressed petiole (13.38% and 10.42% respectively) as well as OPF juice from whole OPF and petiole (1.31% and 0.23% respectively). This implied that fresh whole OPF and fresh petiole might be gradually degradable because of high content of lignin. Since lignin is a key element that limits forage digestibility, the physical pretreatment by pressing method has reduced the lignin level and therefore increases the ruminants' ability to digest OPF.

CHAPTER 6

CONCLUSION

The finding of the present study highlights the ability of the physical pretreatment using conventional sugarcane pressing machine to loosen up the lignocellulose structure of OPF and increase the surface area and porosity of OPF. It is considered that assessing the proximate composition, lignocellulose and sugar contents of pressed OPF juice will provide important insight for the development animal feed and ruminant production. Effective utilisation of OPF may provide a feedstuff for the ruminant industry. With the availability of cheaper and plentiful source of ruminant feed from oil palm plantations, the production costs of livestock rearing will be reduced and this will promote further development in ruminant industry. And OPF juice recovery was mainly dependent on time lapsing between OPF harvesting and pressing. Short time lapse would avoid the depletion in juice yield. The OPF juice is suggested for fermentation substrate as component in silage making and the production of bioethanol. While the nutritive value of pressed OPF fibre can be increased by combining physical and biological pretreatment to enhance enzymatic saccharification rate. It is considered as an eco-friendly process and does not involve any chemical which is safe for ruminant consumption.

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APPENDIX

Table 1. Sugar components in OPF juice from whole OPF

Components of Sugar	Amount of Sugar (g/L)	Percentage (%)
Glucose	72	60.5
Fructose	22	18.5
Sucrose	25	21.0
Total	119	100

Figure 4. Proportion of free sugar in whole OPF juice.

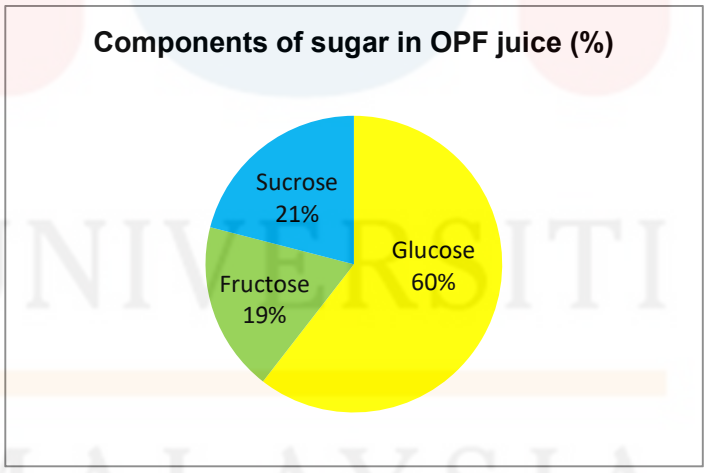

Table 2. Proximate composition and nutritive values of OPF sections (% in DM)

OPF Section	DM (%)	Ash (%)	CP (%)	EE (%)	CF (%)	GE (cal/g)
Fresh OPF						
Petiole	22.39±1.05	8.51±0.60	2.49±0.08	0.57±0.02	45.34±0.24	1549.3±3.06
Leaflet	51.09±0.54	4.63±0.05	11.15±0.61	2.36±0.17	34.0±1.91	40.93±16.2
Whole OPF	43.54±2.89	3.64±0.58	6.12±0.5	1.41±0.21	35.74±1.11	4236±7.07
Pressed OPF Fibre						
Petiole	43.39±5.25	2.29±0.14	1.14±0.44	0.58±0.14	36.57±4.64	3131.75±79
Whole OPF	34.98±3.63	4.18±1.60	4.71±0.24	1.46±0.26	42.05±7.87	3919.75±22.05
Lyophilised OPF Juice						
Petiole	87.01±1.87	7.03±3.31	1.37±0.17	0.44±0.27	0.14±0.13	3182±75
Whole OPF	88.50±3.64	6.58±2.82	1.97±0.45	0.54±0.30	0.34±0.15	3305.25±80.5

Notes: Data shown are means±standard deviation (SD) of triplicates. DM: dry matter; CP: crude protein; EE: ether extract; CF: crude fibre; GE: gross energy.

Table 3. Lignocellulosic composition of OPF sections with different fractions

OPF Section	NDF	ADF	HC	CE	Lignin
Fresh OPF					
Petiole	69.3±1.05	63.05±0.91	5.98±0.59	45.95±1.18	17.1±0.53
Leaflet	60±1	43.21±0.77	16.79±1.74	16.87±1.20	26.34±0.48
Whole OPF	71.3±0.58	50.26±0.31	21.06±0.83	31.27±0.72	19±0.41
Pressed OPF Fibre					
Petiole	62.23±6.85	54±4.18	8.22±0.23	43.59±2.40	10.42±0.66
Whole OPF	67.73±8.45	58.7±6.46	9.03±0.40	45.32±1.35	13.38±2.59
Lyophilised OPF					
Juice					
Petiole	1.05±0.07	0.55±0.04	0.5±0.07	0.32±0.05	0.23±0.06
Whole OPF	2.36±0.02	1.64±0.03	0.72±0.02	0.33±0.04	1.31±0.18

Notes: Data shown were means±standard deviation (SD) of triplicates.

NDF: Neutral detergent fibre; ADF: Acid detergent fibre; HC: Hemicellulose; CE: Cellulose.