Characterization of Non-Structural Carbohydrate in Two Cultivars of *Dioscorea hispida* Dennst

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DECLARATION

I hereby acknowledge that the work embodied in the 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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LIST OF SYMBOLS

		Page
%	Percentage	xiii
μm	Micrometer	13
ºC	Degree Celcius	17
α	Alpha	17
nm	Nanometer	19
keV	Kiloelectron volt	19
kV	Kilovolt	19
ϑ	Tetha	21
mL	Milliliter	27
cm	Centimeter	29
L*	Lightness	33
a*	Red-green colour	33
b*	Blue-yellow colour	33
g	Gram	34
L	Liter	34
M	Molar	34
µL	Microliter	36

LIST OF ABBREVIATIONS

		Page
D.	*Dioscorea*	xiii
NSC	Non-structural carbohydrate	xiii
XRD	X-ray Diffraction	xiii
SEM	Scanning Electron Microscope	xiii
EDX	Energy Dispersive X-ray	xiii
et al.	and others	2
$NaOH$	Sodium hydroxide	34
$CaCl_2$	Calcium chloride	34
GOPOD	Glucose / peroxidase	34
v/v	Volume over volume	36
CI	Crystallinity Index	49
AI	Amorphous Index	49

ABSTRAK

Dioscorea hispida Dennst (*D. hispida*) atau atau lebih dikenali sebagai ubi gadong di Malaysia adalah salah satu sumber karbohidrat yang berperanan sebagai sumber makanan di kalangan penduduk tempatan. Ianya boleh ditemui dengan banyak di kawasan pantai timur semenanjung Malaysia. Terdapat dua kultivar *D. hispida* boleh ditemui di Malaysia iaitu *D. hispida* kuning dan *D. hispida* putih. *D. hispida* kurang dikenali sebagai sumber makanan disebabkan kandungan toksiknya yang menjadikan ianya sumber alternatif yang baik untuk pembangunan bioproduk. Karbohidrat tidak berstruktur (NSC) adalah merujuk kepada kanji dan gula yang boleh digunakan dalam proses fermentasi untuk penghasilan bioetanol. Sebagai permulaan untuk mengaplikasi, maklumat yang sistematik bagi NSC adalah penting bagi tujuan formulasi oleh industri. Matlamat projek kajian ini adalah fokus pada pencirian kanji dan gula pada bahagian ubi dua jenis kultivar *D. hispida*. Kaedah enzimatik hidrolisis menunjukkan *D. hispida* kuning mengandungi peratusan kanji yang tinggi iaitu 25.58% berbanding *D. hispida* putih yang hanya 8.66%. Analisis kepekatan gula dilakukan menggunakan refraktometer pada sap menunjukkan dua kultivar *D. hispida* yang dikaji mengandungi kepekatan gula yang rendah, dimana *D. hispida* kuning adalah lebih tinggi sedikit iaitu 6.2% berbanding *D. hispida* putih yang hanya 6.0%. Pencirian kanji dilanjutkan menggunakan Pengimbas Mikroskop Elektron – Sinar-X Penyebaran Tenaga (SEM-EDX) dan Difraksi Sinar-X (XRD). Selain pencirian karbohidrat tidak berstruktur, analisis fizikal juga dilakukan iaitu pengukuran warna menggunakan kolorimeter.

Katakunci: *Dioscorea hispida* Dennst, kanji, gula.

Characterization of Non-Structural Carbohydrate of Two Cultivars of *Dioscorea hispida* Dennst

ABSTRACT

Dioscorea hispida Dennst (*D. hispida*) or commonly known as ubi gadong in Malaysia is one of carbohydrate source which is act as food source among local people. *D. hispida* is abundantly found in the east coast of peninsular Malaysia. There are two varieties of *D. hispida* can be found in Malaysia which is yellow *D. hispida* and white *D. hispida*. *D. hispida* is unpopular food source due to its toxic content which makes it a good source alternative for bioproduct development. Non-structural carbohydrate (NSC) which refer to starch and sugar can be used in fermentation process in bioethanol production. As the first step of application, systematic information of NSC is importance for formulation purpose by industry. The aims of this research project were focus on characterize starch and sugar on tuber part of two varieties of *D. hispida* cultivar. Enzymatic hydrolysis methods shows that yellow *D. hispida* contain higher starch percentage which is 25.58% compared to white *D. hispida* that is only 8.66%. Sugar concentration analysis using refractometer on *D. hispida* sap show that both cultivar studied have low sugar concentration, which yellow *D. hispida* was slightly higher which is 6.2% whereas white *D. hispida* was only 6.0%. The starch were further characterize by using Scanning Electron Microscope – Energy Dispersive X-ray (SEM-EDX) and X-ray Diffraction (XRD). Apart from NSC characterization, the physical analysis was also done which is colour measurement by colorimeter.

Keyword: *Dioscorea hispida* Dennst, starch, sugar.

CHAPTER 1

INTRODUCTION

1.1 Background of Study

Dioscorea hispida (*D. hispida*) Dennst or locally known by Malaysian as "ubi gadong" is a wild and toxic type of yam plant. Its tuber contains a toxic compounds known as dioscorine which is a soluble alkaloid in water (Andri and Indah, 2011). *D. hispida* grown inside the soil like cassava and tapioca. It has hard thin tuber skin with brown yellowish and sometimes a bit darker in colour, depending on the type of soil. It is harvested as many rounded shape individual tubers which are clump together, with fibrous root on all over tuber skin. It is consumed as an exotic food which means it is an unpopular food source due to the complicated intoxicated process of its tuber (Tattiyakul, Naksriarporn, & Pradipasena, 2012).

There are two types of *D. hispida* cultivar can be found in Malaysia which is "ubi gadong pulut" (yellow *D. hispida*) and "ubi gadong beras" (white *D. hispida*). The differences between both cultivars can obviously seen through its tuber flesh colour. "Ubi gadong pulut" has more intense yellow colour rather than "ubi gadong beras". Local people in Kelantan just simply name both cultivar base on its tuber flesh colour. The more yellowish tuber colour called as "ubi gadong kuning" or yellow *D. hispida* whereas tuber

that less yellowish called as “ubi gadong putih” or white *D. hispida*. This type of yam can be found grown abundantly grown in the east coast of peninsular Malaysia especially in Terengganu and Kelantan (Nashriyah, Yusoff, Tajuddin, Ngah & Rejab, 2010).

Non-structural carbohydrate (NSC) is a product from the photosynthesis process which is stored inside the plant as a substrate for growth and metabolism (Quentin *et al*., 2015). Sugar and starch are known as NSC. Within any given *Dioscorea* species and cultivar, a wide range of NSC studies especially starch have been reported previously. The diversity of NSC between *Dioscorea* species and cultivar show that it is a good characterization analysis to be considered to differentiate those two *Dioscorea* species in Malaysia.

Current knowledge on NSC composition of *D. hispida* is very limited especially on those two cultivars in Malaysia. Thus, in the present study investigation was focused on the NSC properties of two cultivars of *D. hispida* which is yellow *D. hispida* and white *D. hispida*. SEM-EDX were used to analyse the starch morphology. XRD was used to evaluate the crystalline properties of the tuber sample. Enzymatic hydrolysis method which is adopted from Megazyme assay to determine the starch percentage in *D. hispida* tuber. Refractometer were used to measure the sugar concentration on *D. hispida* tuber sap. Apart from NSC characterization, the physical analysis was also analysed.

1.2 Problem Statement

Among the yam species, *D. hispida* was considered as unpopular species due to the presence of the poisonous alkaloids known as dioscorine in its tuber (Kumoro & Hartati, 2015). However due to abundant amount of starch in its tuber, *D. hispida* shows

the potential to be used as a starchy biobased product. The limited previous study has been reported on the existence of *D. hispida* cultivar. Thus this study was carried out to explore the potential of two different cultivars of *D. hispida* to be a good renewable source for bioethanol production in the future. By discovering this, the new source of starch can be introduced to replace the chemically modified starch (Alcázar and Meireles, 2015). This study also helps in attracting the interest of the biofuel industry as well as other related industry, which in turn can help small former to commercialized this food material.

1.3 Objectives

The objectives of this research are:

1) To determine the concentration of non-structural carbohydrate (sugar and starch) content in two different cultivars of *D. hispida*.
2) To characterize the non-structural carbohydrate (sugar and starch) content in two different cultivars of *D. hispida*.
3) To compare the physical properties in two different cultivars of *D. hispida*.

1.4 Scope of Study

In this study, the *D. hispida* was prepared in four different form, which are dried tuber powder, fresh tuber flesh, sap and dried tuber cube shape, to investigate its NSC characteristic. The starch on *D. hispida* tuber was observed on SEM for the starch morphology identification, incorporated with EDX to justify the image observed is starch,

as it can detect the element involved. Enzymatic hydrolysis was conducted to determine the starch percentage. XRD analysis is conducted to determine the percentage crystallinity on the of *D. hispida* tuber sample respectively. A refractometer is used to analyse *D. hispida* sugar content from its sap. The colorimeter is used for systematic differentiation between the two cultivars of *D. hispida* tuber flesh colour.

1.5 Significant of Research

The significance of this research is to find out the NSC properties of *D. hispida*. This study focused more on the tuber part of *D. hispida* and specifically analyse the NSC content of sugar and starch. Since *D. hispida* was not popular food source, so is a perfect renewable source for bioproduct development. Industry are more preferred to use food as renewable source as it need less step for converting the feedstock into sugar compared to using agricultural waste but for industrial purpose, specific information on NSC is importance for formulation purpose. The utilization of NSC from this tuber can develop into a new renewable source of energy in the biofuel industry and may promote economic development in the agricultural sector since sugar and starch can be used in the fermentation process in bioethanol production. This research also proves the importance of being the third generation of the bioproduct development source.

CHAPTER 2

LITERATURE REVIEW

2.1 *Dioscorea*

Researchers has identified 1,137 *Dioscorea* genus can be found around the world, especially in the tropical and subtropical country (Zhu, 2015). From total, 600 of them are edible, and only 10 of it was used as staple food for daily life. The rest of the wild *Dioscorea* yam is only used during the food scarcity (Salda, 1999, Lebot, 2008). *Dioscorea* differs from each other which can be identified through its physical appearance. *Dioscorea* species are variable in term of shape, flesh colour either white, yellow or purple (Fauziah, Mas'udah, & Hendrian., 2016). *Dioscorea* growing cycle was in the range of 8 to 36 month, depend on the species involved (Lebot, 2008). *Dioscorea* is tuberous herbaceous lianas which can grow up to 12 meters and the leaf is spirally arranged (Razali, Muhammad, Mohd, & Ismail, 2011). Some of the *Dioscorea* species are monoecious, which mean its male and female vegetative part exist on the same plant (Zhu, 2015).

2.2 *Dioscorea hispida*

Dioscorea hispida or D. hispida is an exotic food source which locally known by indigenous communities as "ubi gadong". It is a wild yam which rarely use by indigenous people as a staple food source due to its toxic content (Bhandari, 2005). Excessive eating of *D. hispida* without proper detoxification can cause diarrhea, vomiting, nausea, stomach pain and other serious health complication (Hudzairi *et al*., 2011). In the worst case reported, excessive accumulation of alkaloid in the body can cause fatal which led to death (Webster, 1984). Figure 2.1 shows the *D. hispida* tuber.

Figure 2.1: *D. hispida* tuber.

Source: (Ashri, 2017).

The toxic content of *D. hispida* probably the main factor that leads to the unpopularity among people, especially as food. Based on the survey conducted in Terengganu Malaysia, it shows that 96% of the respondent does not use it in daily life (Nashriyah *et al*., 2012). Yam is a starchy material which can be used in industry for any

related bioproduct development, mainly in bioethanol production. The unpopularity of *D. hispida* as food intention make it has a potential to be commercialised for industrial purpose.

2.3 Cultivar of *Dioscorea hispida* in Malaysia

Researcher from University Sultan Zainal Abidin (UniSZa) Malaysia was identified that there are two different cultivars of *D. hispida* can be found in Malaysia, which is "ubi gadong pulut" or yellow *D. hispida* and "ubi gadong beras" or white *D. hispida*. It can be differentiate easily based on the tuber flesh colour intensity. The cultivar of *D. hispida* shared the same characteristic, which has toxic content and hairy fibrous root on the surface of the yam tuber. In term of taste, yellow *D. hispida* is preferable compared to white *D. hispida* (Nashriyah, Yusoff, Tajuddin, Ngah & Rejab, 2010).

2.4 Carbohydrate

Carbohydrate is a biomolecule of sugar which is made up of carbon, oxygen and hydrogen (Hanessian, 1997). Carbohydrate is synonym of "saccharide" which a group that include starch, sugar and cellulose or fiber (James and Bemiller, 2009). It was classify based on its structure or amount of sugar molecule that are linked together (Gravatar, 2012). Carbohydrate can be divided into two categories which are structural carbohydrate and NSC (NutrientReview, 2016). Structural carbohydrate is referred to cellulose and

fiber which making a bulk of plant cell wall while NSC is referred to the sugar and starch which is photosynthesis product that makes up a large energy component in the plant (Quentin *et al*., 2015). This study emphasized on determination NSC properties on *D. hispida* cultivar.

2.5 Non-Structural Carbohydrate

NSC which is starch and sugar has potential to be the raw material for bioproduct development especially in bioethanol production. Systematic information of NSC is importance for formulation purpose by industry (Fauziah, Mas'udah, & Hendrian., 2016). Table 2.1 show the NSC concentration determination between *Dioscorea* species.

Table 2.1: NSC concentration determination between *Dioscorea* species.

Dioscorea	**NSC**	
	Starch %	**Sugar %**
D. hispida	11.46 ± 0.08	None
D. opposita	69.90 ± 0.02	None
D. bulbifera	62.70 ± 0.01	None
D. nipponica	35.40 ± 0.03	None
D. alata	41.90 ± 0.01	None

*The result is mean triplicates determination ± standard deviation.

Source: (Jiang *et al*., 2012).

By referring Table 2.1, it shows that *D. hispida* has low starch content compared to other *Dioscorea* species. No studies were done to determine sugar content on *Dioscorea* species.

2.6 Characterization and Analysis of *Dioscorea hispida*

2.6(a) Study of Starch Morphology by Scanning Electron Microscope

SEM is a type of electron microscope focused on the surface morphology of the solid sample by using the high beam of electron energy. This will result in a variety of signal on the surface of the solid sample at various depth. So, at the end of the process, the surface topography and composition of specimen use is being determined. The result was in the form of image that can be screen out on the computer. Figure 2.2 shows the working principle of SEM.

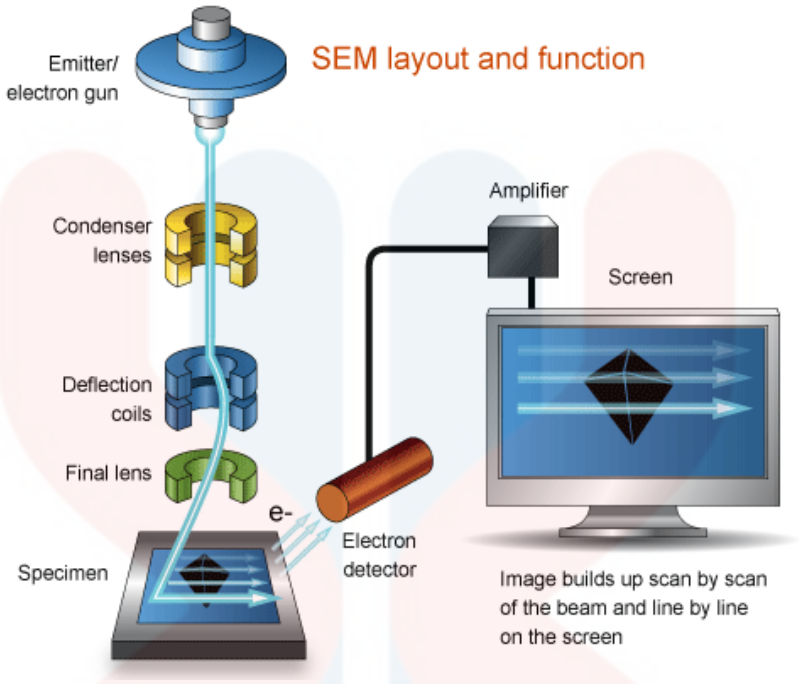

Figure 2.2: Working principle of SEM.

Source: (Australian Microscopy and Microanalysis Research Facility, 2014).

Identification of starch granule shape is one of the important starch analysis since it shows the plant identity and help in understanding the plant taxanomy. Starch granule shape are varies between *Dioscorea* species (Srichuwong, 2007). In term of *D. hispida* species, previous studies show that all variety was found dominantly polygonal shape, except triangular shape which has not been reported before (Fauziah, Mas'udah, & Hendrian., 2016). Table 2.2 show the starch granule shape between *Dioscorea* species and its varieties while Figure 2.3 show the scanning electron micrograph of native *D. hispida* starch with polyhedral shape.

Table 2.2: Starch granule shape between *Dioscorea* species and its varieties.

Species	Yam cultivars	Starch granule shape	Dominant shape between cultivar	Source
D. opposita	-	Spherical or oval	-	(Nadia *et al*., 2014)
D. alata	-	Spherical or oval	-	
D. nipponica	-	Lenticular-shape	-	
D. bulbifera	-	Irregular or polygonal flat	-	
D. septemloba	-	Oval or elongated	-	
D. opposita	Jichengerhao	Oval or ellipsoid	Oval or cake shape	(Shujun *et al*., 2006)
	Jiangxiangxichangmao	Oval or cake-shaped		
	Jingchengyihao	Oval or cake-shaped		
	Baiyu	Oval or cake-shaped		
D. alata	Sweet yam	Ellipsoid	Ellipsoid	(Riley, Wheatley, & Asemota, 2006)
	White yam	Polyhedral		
	Renta yam	Triangular, ellipsoid		
	Moonshine	Ellipsoid		
	Darknight	Ellipsoid		
	Barbados	Triangular, Ellipsoid		
	Purple/white	Ellipsoid, rod-like		

	Calabash	Ellipsoid		
D. hispida	Gadung	Triangular-polygonal	Polygonal	(Fauziah, Mas'udah, & Hendrian., 2016)
	Gadung Kuning	Polygonal		
	Gadung Jahe	Polygonal		
	Gadung mentega	Polygonal		
	Gadung Kebo	Polygonal		
	Gadung Kripik	Polygonal		
D. hispida	-	Polygonal	-	(Tattiyakul, Naksriarporn, & Pradipasena, 2012)

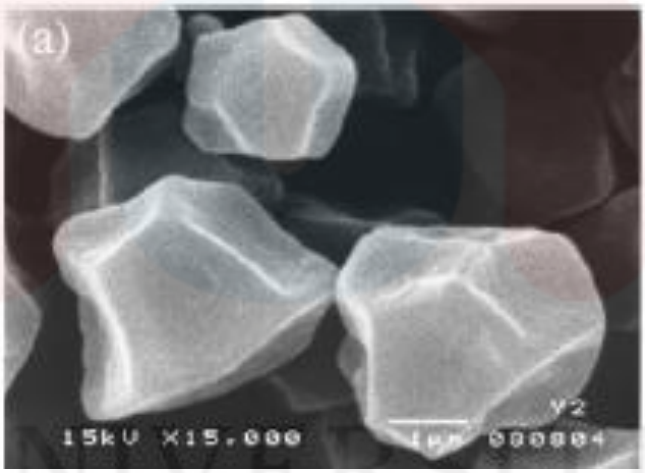

Figure 2.3: Scanning electron micrograph of native *D. hispida* starch with polyhedral shape.

Source: (Tattiyakul, Naksriarporn, & Pradipasena, 2012).

2.6(b) Starch Granule Size by Cell Sens

Starch granule size can be determined from the SEM picture through the application such as Cell Sens, by using size scale given as a reference. Starch granule size are varies between *Dioscorea* species and also its cultivar. The variety size of the starch granule are mainly because of its biochemical properties of the starch granule. The properties such as water binding capacity, swelling power, total amylose content and light transmittance are the physical factor that can affect the average granule size itself (Ali, Wani, and Masoodi, 2016). Table 2.3 show the starch granule size between *Dioscorea* species.

Table 2.3: Starch granule size between *Dioscorea* species.

***Dioscorea* species**	**Cultivar**	**Place Origin collected**	**Average diameter Granule (µm)**	**Source**
D. hispida	Gadung	Distric of Parusuan, Indonesia	3.7±1.30	(Fauziah, Mas'udah, & Hendrian., 2016)
	Gadung Kuning	Distric of Parusuan, Indonesia	4.0±1.20	
	Gadung Jahe	Distric of Parusuan, Indonesia	6.6±1.50	
	Gadung Mentega	Distric of Parusuan, Indonesia	3.6±1.30	
	Gadung Kebo	District of Parusuan, Indonesia	4.8±1.50	
	Gadung Kripik	Distric of Parusuan, Indonesia	3.4±1.20	

D. abyssinica	None	None	29.2	(Gebre-Mariam and Schmidt, 1998)
D. dumetorum	None	None	2.2	(Amani, Buleon, Kamenan, & Colonna, 2004)
D. nipponica	None	None	9.5	(Yuan, Zhang, Dai, & Yu, 2007)
D. zingiberensis	None	None	21.9	(Zhu, 2015)

2.6(c) Identification of Starch Element by Energy Dispersive X-Ray

In this study, SEM which coupled with EDX is used. EDX is being used to analyse the element present on the surface of the sample. This analysis helps to determine the identity of the sample. Figure 2.4 shows the working principle of EDX.

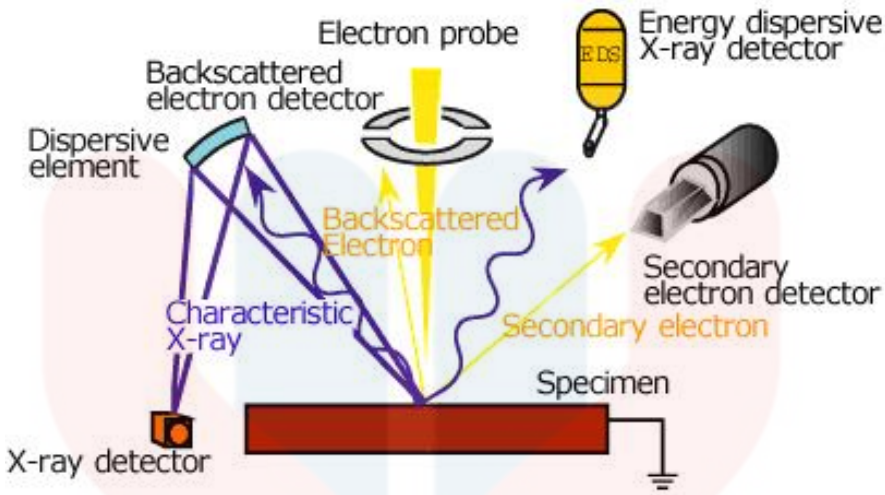

Figure 2.4: Working principle of EDX.

Source: (Jeol Ltd., 1996).

The *D. hispida* cultivar in dry form of tuber can be analysed by using EDX. The EDX analysis help in the detection of any major contaminates that exist on the sample due to the presence of particulate matter which could be an error, especially during sample preparation. This such impurity may lead to this research not be able to get the desired result as compared to the previous research that has been done (Bell, 2003). This impurity on the starch sample could lead quality and also safety problem or even lead to the existence of suspension for any further analysis that being carried out (John and Russ, 1984).

It is expected that the graph of *D. hispida* cultivar starch show carbon and oxygen as major constituent on graph peak of EDX analysis result since the basic chemical formula of the starch molecule is $(C_6H_{10}O_5)_{n.}$. Previous study SEM-EDX analysis on starch granule exist on chestnut wood indicates that it is composed of carbon and oxygen (Gunduz *et al.*, 2016). Figure 2.5 show the result of the elemental analysis on starch granule exist on chestnut wood that were conducted by SEM-EDX.

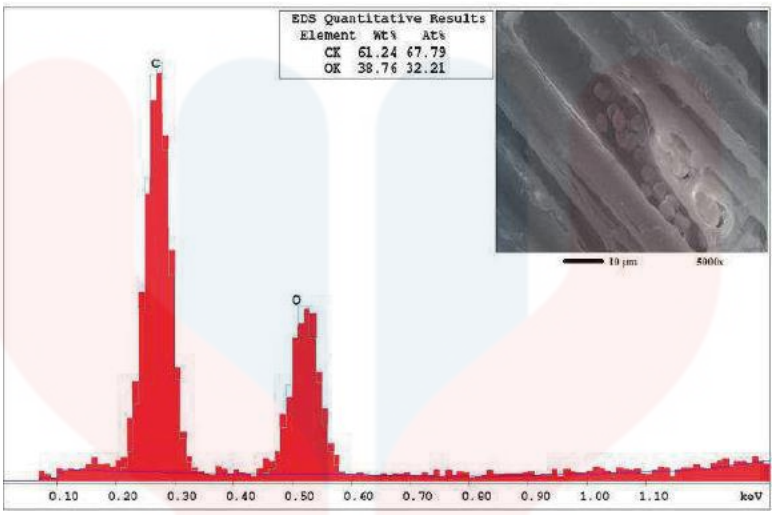

Figure 2.5: Result of the elemental analysis on starch granule exist on chestnut wood that were conducted by SEM-EDX.

Source: (Gunduz *et al*., 2016).

2.6(d) Assay of Starch Content by Enzymatic Hydrolysis

It is impossible to calculate the starch in granular form, so it needs to be converted into simple sugar (Megazyme, 2017). There is two ways to hydrolyses the granular starch into simple sugar which is either through acid or enzymatic hydrolysis. Compared using enzyme for hydrolyzing, acid hydrolysis has much more limitation which only can be applied on the pure starch sample (Azmi, Malek, & Puad, 2017). The main enzymatic procedure includes the pre-treatment step, gelatinization, liquefaction, dextrinization, hydrolysis of dextrin into glucose and lastly qualitative process for glucose measurement (Megazyme, 2017). Figure 2.6 shows the summary of enzymatic hydrolysis of starch.

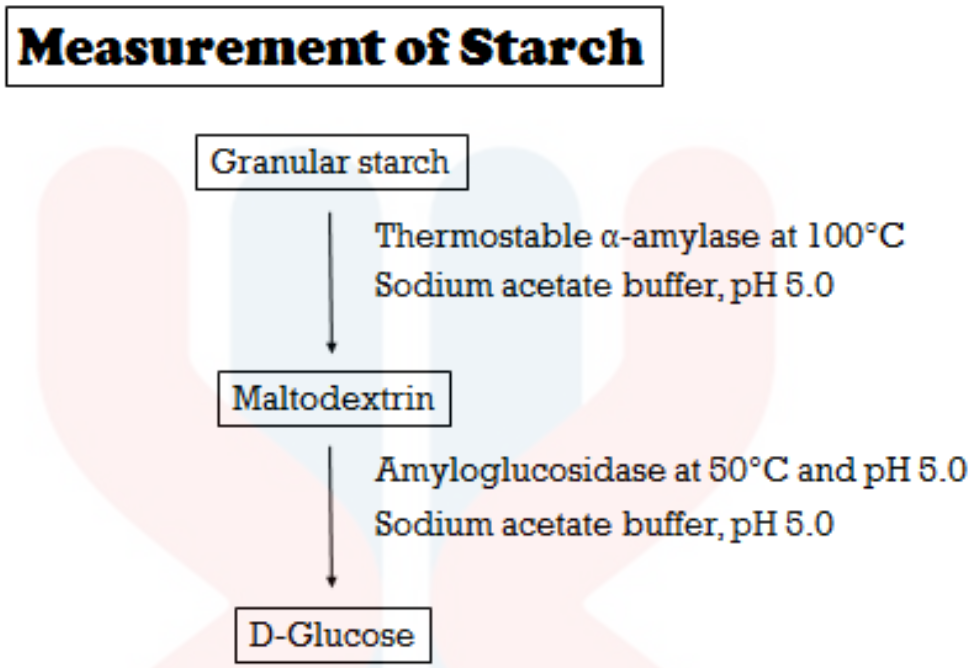

Figure 2.6: Summary of enzymatic hydrolysis of starch.

Source: (Megazyme, 2017).

The starch sample is being treated first with thermostable α-amylase before undergo starch gelatinization process step. The thermostable α-amylase is being used since it is active and stable which not degrade even in low pH which is acidic.

There are few main steps in principle of enzymatic hydrolysis of starch (Association of Official Agricultural Chemist, 1995). First, the starch is being hydrolyze into maltodextrin by addition of α-amylase which the enzyme that helps to hydrolyze α-linked polysaccharide. See equation 2.1.

$$Starch + H_2O \xrightarrow{\alpha-amylase,\ pH\ 7.0\ or\ 5.0,\ 100°C} maltodextrin \qquad (2.1)$$

Amyloglucosidase which is the enzyme that helps in removing glucose unit from liquefied starch will then hydrolyses the maltodextrin (partial starch hydrolysis form) compound into D-glucose. See equation 2.2.

$$Maltodextrin + H_2O \xrightarrow{AMG} D - glucose \quad (2.2)$$

D-glucose is then being oxidised into D-gluconate by the release of one mole of hydrogen peroxide (H_2O_2). It is used for quantitative measurement through a calorimetric reaction which employed peroxidase and also the production of a quinoneimine dye. See equation 2.3 and equation 2.4.

$$D - glucose + O_2 + H_2O \xrightarrow{(glucose\ oxidase)} D - gluconate + H_2O_2 \quad (2.3)$$

$$2H_2O_2 + \rho - hydroxybenzoic + 4 - aminoantipyrine \xrightarrow{(peroxidase)} quinoneimine\ dye + 4H_2O \quad (2.4)$$

2.6(e) Structural Properties of Starch by X-Ray Diffraction

Diffraction effect is observed when electromagnetic radiation gives an effect on periodic structures with geometrical variations on the length scale of the wavelength of the radiation. The interatomic distances in crystals and molecules amount to 0.15 to

0.4 nm which correspond in the electromagnetic spectrum with the wavelength of X-rays having proton energy between 3 and 8 keV. When crystalline and molecular structures are exposed to x-rays, phenomena like constructive and destructive interference should be observed.

X-rays are generated when electrons with kinetic energies in the keV range above influence on the matter. The emission spectrum comprises a continuous part called Bremsstrahlung and some discrete lines indicative of the chemicals elements of the target materials. In laboratory x-ray tubes, an anode plate made from specific metal of high purity is where electrons accelerated. The electrons are emitted from the cathode filament and accelerated towards the anode plate. The anode is typically fabricated from copper, chromium and molybdenum or another metal.

By turning the filament current in the range of some 10 mA, the electron current between filament and anode may be adjusted. When impinging upon the anode, the electrons are decelerated by their interactions with the target plate atoms leading to the emission of x-rays. The energy of the characteristic radiation must be lower to the emission of x-rays. The energy of the characteristic radiation must be lower than the acceleration voltage (in kV) (Birkholz, 2006). Figure 2.7 show the schematic and photograph of laboratory X-ray tubes: Conventional X-ray tube type and Figure 2.8 show the ceramic X-ray tubes for less laborious change from line to point focus mode and vice versa.

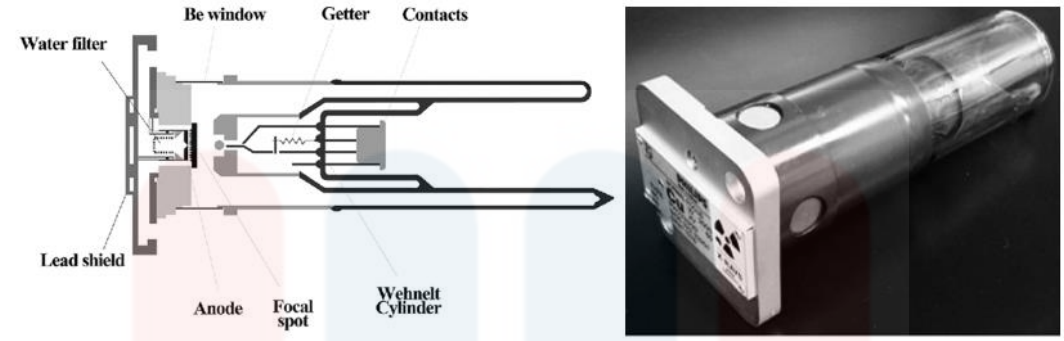

Figure 2.7: Schematic and photograph of laboratory X-ray tubes: Conventional X-ray tube type.

Source: (Birkholz, 2006).

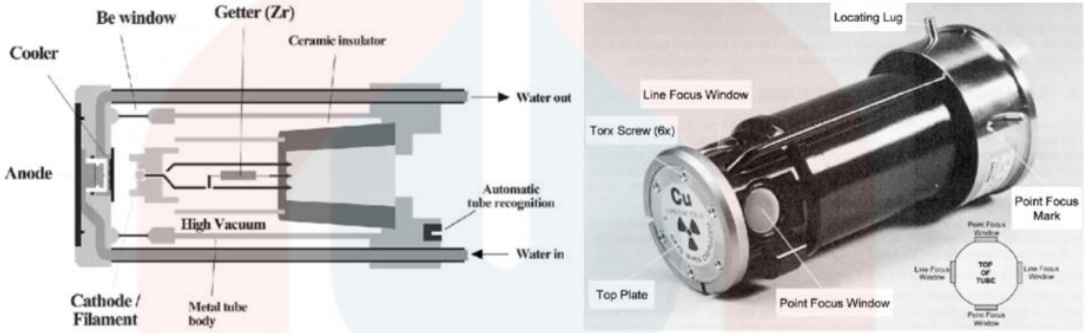

Figure 2.8: Ceramic X-ray tubes for less laborious change from line to point focus mode and vice versa.

Source: (Birkholz, 2006).

Starch are composed of two polysaccharides which are amylose and amylopectin. Amylose is made by an linear chain of α −D-glucose unit and bonded to each other by $\alpha - 1{,}4$ −glycosidic bond. The long linear chain of amylose contributes to its characteristic which are crystalline. In contrast, amylopectin is branched chain of α −D-glucose unit that are linked by $\alpha - 1{,}4$ −glycosidic bond. Its branched at $\alpha -$ 1,6 −glycosidic bond. The short branched chain of amylopectin contributes to its characteristic which are amorphous. In term of XRD crystallography pattern, the

crystallinity shown are due to existence of amylose in starch. Figure 2.9 show the crystallographic pattern of native starch of *D. hispida* from $2\vartheta = 0°$ to $2\vartheta = 35°$, with highest peak shown at $2\vartheta = 17.2°$.

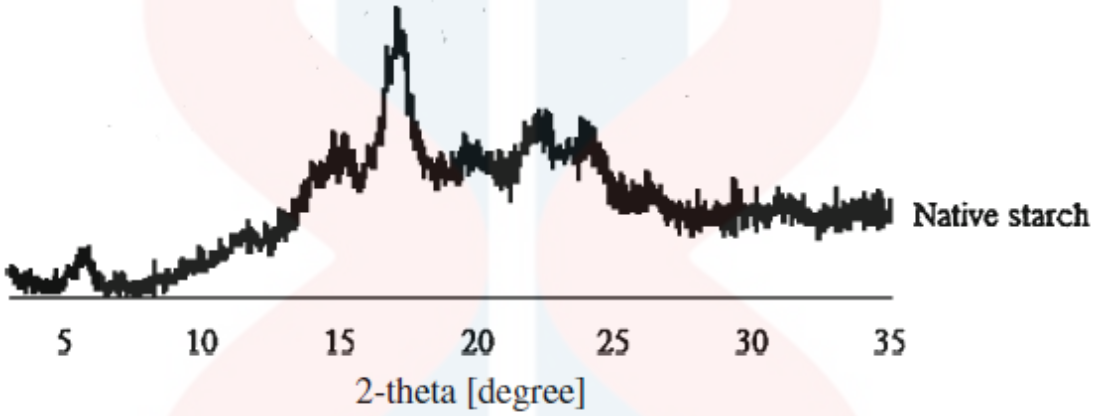

Figure 2.9: Crystallographic pattern of native starch of *D. hispida* from $2\vartheta = 0°$ to $2\vartheta = 35°$, with highest peak shown at $2\vartheta = 17.2°$.

Source: (Tattiyakul, Naksriarporn, & Pradipasena, 2012).

The previous study shows the crystallinity percentage varies between *Dioscorea* species. The main factor which contribute to these phenomena is due to the different ratio of amylose and amylopectin contain in the starch. Table 2.4 show crystallinity percentage between *Dioscorea* species.

Table 2.4: Crystallinity percentage between *Dioscorea* species.

Species	Percentage of crystallinity (%)	References
D. alata	31.0-41.0	(Amani, Buleon, Kamenan, & Colonna, 2004)
	43.0	(Jayakody, Hoover, Liu, & Donner, 2006)
D. bulbifera	27.1-53.0	(Jiang *et al*, 2012)
D. cayenensis-rotundata	27.0-45.0	(Amani, Buleon, Kamenan, & Colonna, 2004)
D. dumetorum	37.0	(Amani, Buleon, Kamenan, & Colonna, 2004)
D. esculenta	26.0-35.0	(Amani, Buleon, Kamenan, & Colonna, 2004)
	49.0-53.0	(Jayakody, Hoover, Liu, & Donner, 2006)
	25.1	(Bertoft, Piyachomkwan, Chatakanonda, & Sriroth, 2008)
D. nipponica	48.5	(Yuan, Zhang, Dai, & Yu, 2007)
D. opposita	31.0-34.9	(Zhou, Wang, Fang, Sun, & Dou, 2012)
	31.5-50.5	(Wang *et al.*, 2006)
D. persimilis	28.1	(Jiang *et al*, 2012)
D. septemloba	53.4	(Jiang *et al*, 2012)
D. trifida	24-33	(Perez *et al.*, 2013)

2.6(f) Sugar Analysis by Refractometer

A refractometer is a complicated instrument but is it easy to be used. It is handheld equipment which functions in the determination of sugar concentration which widely used in the food industry. The refractometer work through the concept of refraction of light. This equipment needs the sample in liquid form. The observer needs to put some

drop of sample on the prism assembly, peek on the screen and check out a chart of Brix level (Pedley, 2018).

Refractometer help in determining sugar concentration of *D. hispida* cultivar sap. The refractometer will give the reading in Brix %. The Brix % tell the sugar content of an aqueous solution. 1% of Brix is equivalent to the 1 g of sucrose in 100 g of solution. The advantages of using refractometer are it is extremely easy to use and gives an accurate reading in clean fluid. (Cole-Parmer Instrument Company, 2018). Figure 2.10 shows the refractometer working principle.

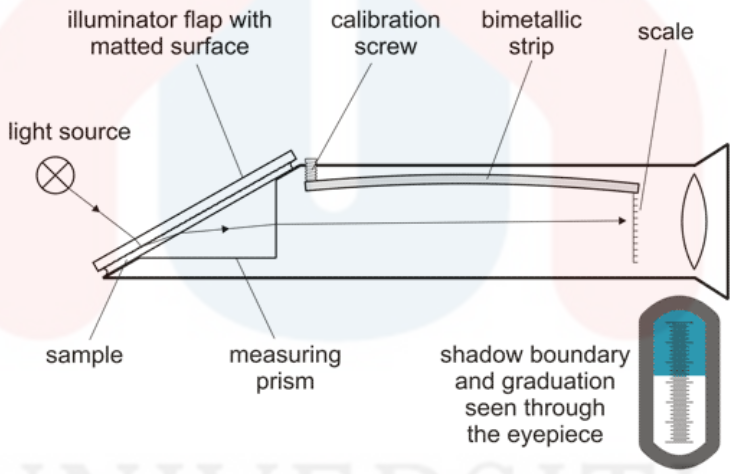

Figure 2.10: Refractometer working principle.

Source: (Kealey and Haines, 2011).

2.6(g) Colour Analysis by Colorimeter

The colour measurement is often used to determine the physical characteristics of the sample studied. Sometimes, the colour differences is too subtle to detect using human

eyes. A machine is used to determine the colour accurately and in a more detail result. Colorimeter is one of the machines to detect colour.

Colour characteristics can be divided into three which are hue, saturation and brightness. The definition of hue is the most dominant wavelength of light waves, saturation is the amount of white light plus a hue and brightness is the chromatic notion intensity. Colour standards in the world are used from The Commission de International de l'Eclairage (CIE), Munsell System and atlas. CIE uses three primary colour which is red, blue and green because of human eyes structure. Colour scales are developed based on how humans perceive colours such as the famous L*, a*, b* colour scales. The L is lightness, a is red-green, and b is blue-yellow (Jha, 2010).

There is some previous study which is specifically listed the tuber flesh colour to discover this *Dioscorea* species diversity. Table 2.5 shows the *Dioscorea* fresh flesh tuber colour and its origin.

Table 2.5: *Dioscorea* fresh flesh tuber colour and its origin

***Dioscorea* species**	**Cultivar**	**Origin**	**Fresh flesh tuber colour**
D. alata L.	Uwi Kelopo	Purwodadi / Rembang	White-offwhite
	Uwi Putih	Purwodadi	White-offwhite
	Uwi Bangkulit	Purwodadi	White
	Uwi Jaran	Purwodadi / Wonosari	Offwhite
	Uwi Ungu	Purwodadi	White with purple blotches
	Uwi Ulo / Jero	Purwodadi / Prigen / Kejayan	White, offwhite
	Uwi Perti	Purwodadi	White
	Uwi Gedek	Purwodadi	Offwhite

	Uwi Ratu	Purwodadi	Offwhite, white with purple
	Uwi Jaran Ungu	Rembang	White with purples
	Uwi Elos	Rembang	Offwhite
	Uwi Soso'an	Rembang	Offwhite
	Uwi Cemeng		Offwhite with purples blotches
	Uwi Talas	Wonosari	Offwhite
	Uwi Tanduk Rusa	Puspo	White with purples blotches
	Uwi Alang-alang	Prigen	Offwhite
D. hispida Densst	Gadung	Pasrepan	Yellowish
	Gadung Jahe	Prigen / Wonosari	Yellowish-yellow
	Gadung Ketan	Purwodadi	Yellowish
	Gadung Kuning	Tutur / Wonosari	Yellowish-yellow
	Gadung Kripik	Rembang / Prigen	Yellow
	Gadung Lumut	Rembang	Yellowish
	Gadung Kebo	Purwosari	Yellow
	Gadung Mentega	Kejayan	Yellow
D. esculeta (Lour.) Burkill	Gembolo	Purwodadi	White
	Gembili	Wonosari	White
D. pentaphylla L.	Uwi Sosohan	Prigen	White
D. bulbifera L.	Uwi Gantung / Kentang Gedebug	Kejayan	Yellow

Source: (Fauziah, Mas'udah, & Hendrian, 2015)

CHAPTER 3

MATERIAL AND METHOD

3.1 Experimental Design

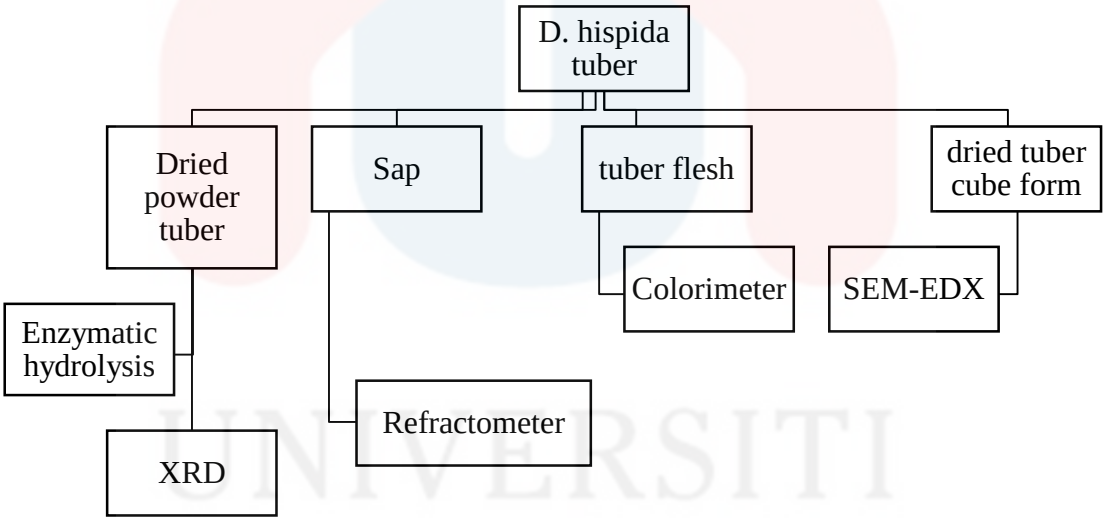

Figure 3.1: The procedure characterizes the differences between the two cultivars of *D. hispida*

3.2 Sample Preparation

The material used is the fresh sample of two cultivars of *D. hispida* namely as "ubi gadong kuning" and "ubi gadong putih", collected from Tanah Merah, Kelantan, Malaysia. The apparatus that were used was knife, slicer, wash basin, aluminium foil, oven, zipper bag, grinder, garlic press, 2 mL microcentrifuge tube, dropper, 50 mL beaker and desiccator. Figure 3.1 shows process of digging up the soil to obtain *D. hispida* tuber, location at Tanah Merah, Kelantan, Figure 3.2 shows *D. hispida* plant morphology, Figure 3.3 show yellow *D. hispida* tuber in clump form, and Figure 3.4 shows white *D. hispida* that has large size of tuber and less fibrous root on the tuber skin.

Figure 3.2 Process of digging up the soil to obtain *D. hispida* tuber, location at Tanah Merah, Kelantan.

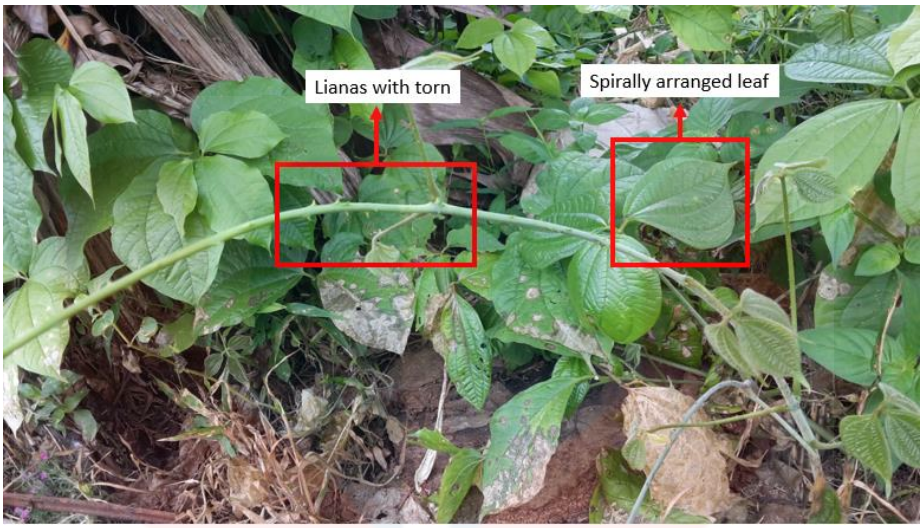

Figure 3.3: *D. hispida* plant morphology.

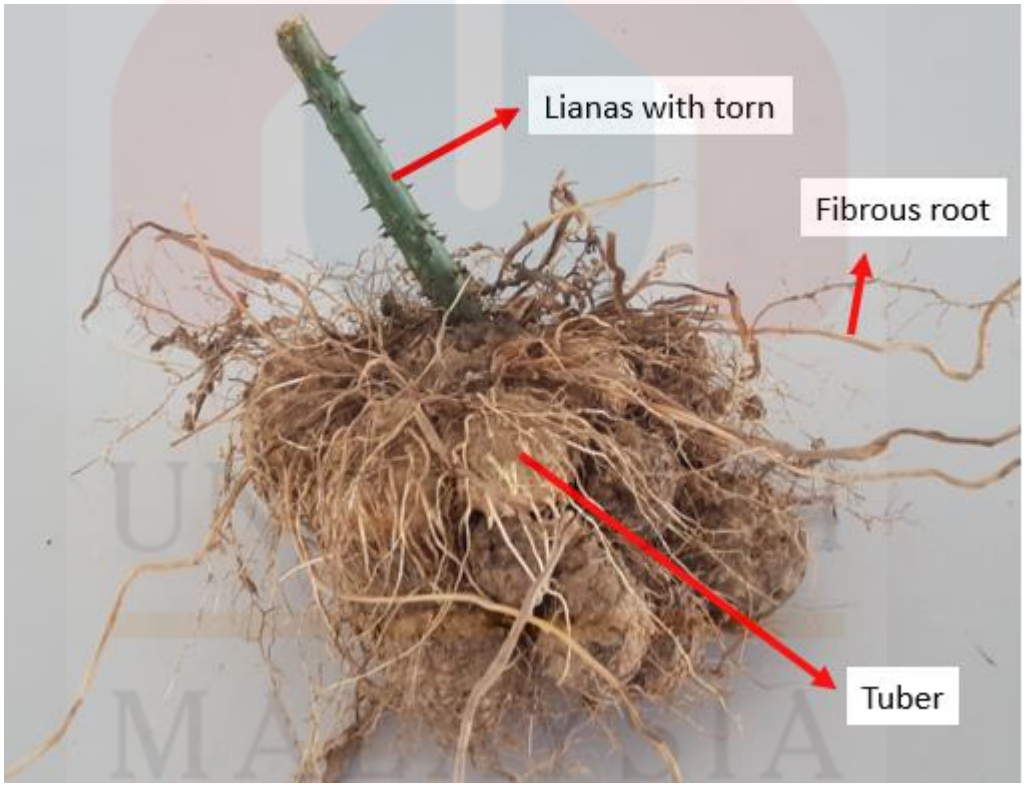

Figure 3.4: Yellow *D. hispida* tuber in clump form.

Figure 3.5: White *D. hispida* that has large size of tuber and less fibrous root on the tuber skin

There are three form of sample need to be prepared. The first one was dried tuber powder form, which was used in XRD analysis for starch structural properties determination, also enzymatic hydrolysis for starch concentration analysis. Next was dried tuber in cube form, in size of $1\ cm \times 1\ cm \times 1\ cm$ for SEM-EDX analysis. The third one was the tuber sap which was used for sugar concentration determination. Figure 3.6 show the three form of sample to be analysed from tuber yellow *D. hispida* and white *D. hispida*.

Figure 3.6: Three form of sample to be analyse from tuber yellow *D. hispida* and white *D. hispida*. (a) Dried powdered sample of yellow *D. hispida*; (b) Dried tuber powdered sample of white *D. hispida*; (c) Dried tuber cube form sample of yellow *D. hispida*; (d) Dried tuber cube form sample of white *D. hispida*; (e) Tuber sap of white *D. hispida*; (f) Tuber sap of yellow *D. hispida*

First, the fresh tuber was washed, peeled and cut into slices. Then, it was left to be dried overnight in the oven at 60℃ until it is fully dessicated. The dried form of tuber which is in cracker form was being grind into powder form. The tuber powder was being put into the zipper bag with proper labelling. It was left in the desiccator until further usage.

The second form of sample just needed to cut the tuber in cube form, left to be dried overnight in the oven at 60℃ until it is fully dessicated. The dried form of tuber which was in cube form. The dried cube form of tuber was being put into the zipper bag with proper labelling. It was left in the desiccator until further usage.

To obtain the *D. hispida* tuber sap, first, it was being cut into cube form. The cube form of tuber was pressed by using garlic press. The liquid is being collected in the beaker. The liquid was sieve to ensure there are no flesh exist. The liquid was being transferred into aliquot, which in microfuge tube. It was then centrifuged at 12,000 rpm at 4℃ for 20 minutes, and the supernatant was collected. The supernatant was filtered using Whatman syringe filter 0.45 μm TF. It was stored at -20℃ until further usage. Figure 3.7 show the sap of two cultivars of *D. hispida*.

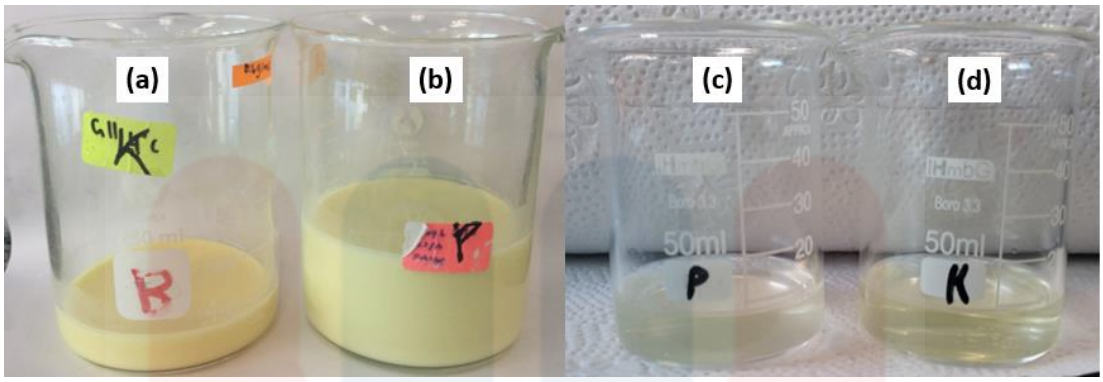

Figure 3.7: Sap of two cultivars of *D. hispida*. (a) Sap of yellow *D. hispida* before centrifuged; (b) Sap of white *D. hispida* before centrifuged; (c) Supernatant of sap of white *D. hispida* after centrifuge; (d) Supernatant of sap of yellow *D. hispida* after centrifuge.

3.3 Characterization and Analysis Procedure

3.3(a) Starch Morphology Analysis by Scanning Electron Microscope

The dried cube tuber form of *D. hispida* was being flattened its bottom first with knife to ensure that it able to have a good attachment on the double-sided tape placed on the metal sub. Then, it was coated with gold under vacuum to make the sample conductive so that electron will be able to reflect the specimen which resulting in the desired image obtained. The image was taken at ×50, ×100, ×500, ×1,000 and ×2,000 magnifications. The average starch granule size was determine using Scanning Electron Micrograph of two cultivars of *D. hispida* on magnification × 2000 respectively through application Cell Sens. Figure 3.8 show sputtering the dried tuber cube form of two *D. hispida* cultivar with gold at low vacuum, Figure 3.9 show SEM-EDX analysis on two cultivars *D. hispida* dried tuber cube form and Figure 3.10 show sizing starch granule using application Cell Sens.

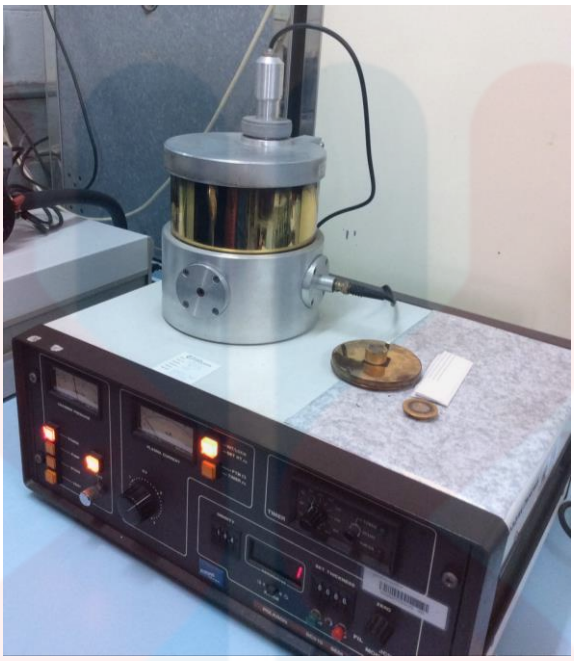

Figure 3.8: Sputtering the dried tuber cube form of two *D. hispida* cultivar with gold at low vacuum.

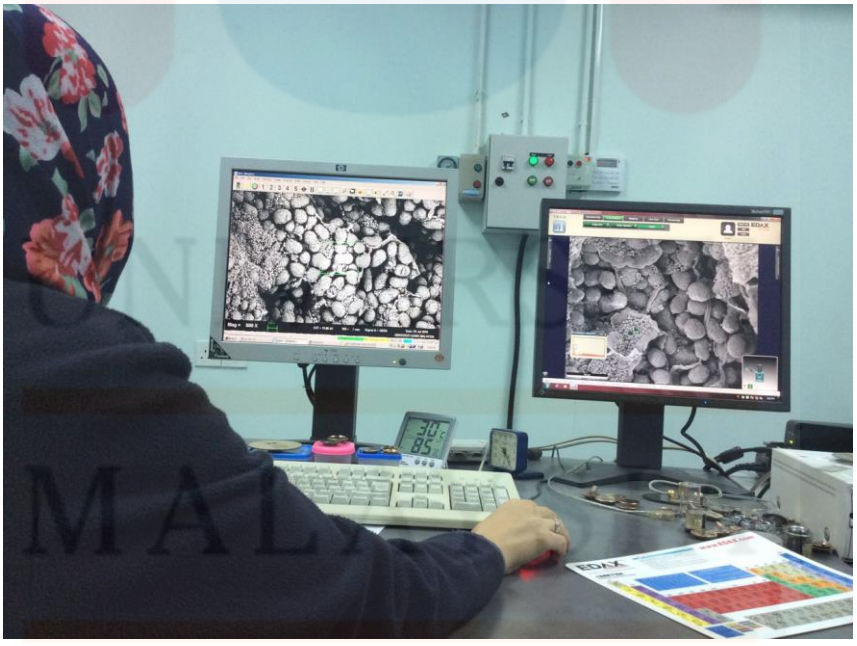

Figure 3.9: SEM-EDX analysis on two cultivars *D. hispida* dried tuber cube form.

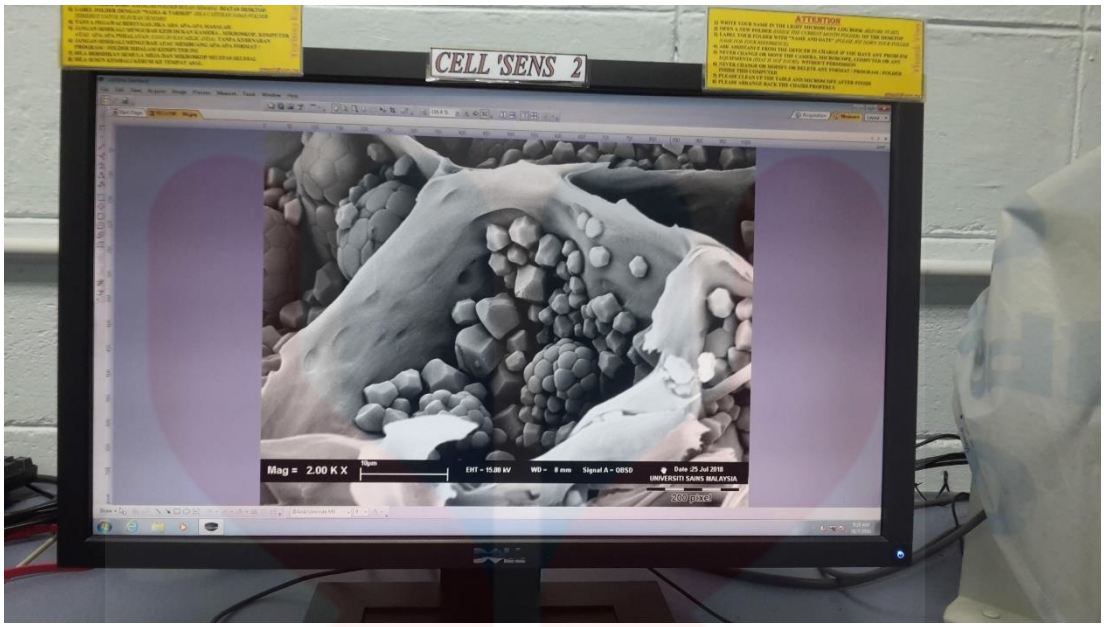

Figure 3.10: Sizing starch granule using application Cell Sens.

3.3(b) Elemental Analysis of Starch by Energy Dispersive X-Ray

Once SEM analysis finished, the experimental process was further with EDX analysis. The analysis is focusing on the starch granule at $\times 2000$ magnification. This analysis is done to confirm the element on starch, and no major contaminant exists.

3.3(c) Colour Analysis by Colorimeter

The two cultivars of *D. hispida* fresh tuber flesh was being analysed by using Konica Minolta Chroma Meter CR-400/410. The Lightness (L*), Red- Green colour (a*), and Blue-Yellow colour (b*) reading is taken and recorded.

3.3(d) Sugar Concentration Determination by Refractometer

The filtered *D. hispida* sap that was prepared earlier were analysed by using Atago digital hand-held pocket refractometer. The brix index reading is taken and recorded.

3.3(e) Enzymatic Hydrolysis

3.3(e)i Preparation of Glucose / Peroxidase Reagent

GOPOD reagent enzyme was diluted with distilled water until reached 1 L into reagent bottle. Then, GOPOD reagent buffer was added to the solution. The reagent bottle was covered with aluminium foil and stored in the chiller at 4℃.

3.3(e)ii Preparation of Sodium Acetate Buffer

5.8 mL of glacial acetic acid were mixed with 1 M of $NaOH$ (pH 5.0), 0.74 g of $CaCl_2$ and 900 mL of distilled water. The distilled water was added until reached 1 L and the solution was transferred into reagent bottle. The reagent bottle then was stored in the chiller at 4℃.

3.3(e)iii Preparation of α −amylase Solution

1 mL of α −amylase was mixed with 30 mL sodium acetate buffer. The solution was the stored in the chiller at 4℃.

3.3(e)iv Moisture Content

Moisture content was calculated as it was used in Mega-Calc of Total starch by Megazyme for starch percentage determination. First, 1 g of dried powder tuber sample was fully covered with aluminium foil. It was placed at drying oven at 105℃ for 24 hours. After being removed from the oven, it was left to be cool in the desiccator. The moisture content for each sample were then determined by different in weight and expressed in percentage. The percentage of moisture content was calculated. See equation 3.1.

$$Moisture\ content\ (\%) = \frac{W_i - W_f}{W_i} \times 100 \qquad (3.1)$$

Where:

W_i= Initial weight of tuber sample before dried

W_f= Final weight of tuber sample after dried

3.3(e)v Determination of Starch Concentration Procedure

0.1 g of dried *D. hispida* tuber powder was put into the 15 mL Falcon tube. Using 100 μL micropipettor, 0.2 mL of aqueous ethanol (80% v/v) was added to wet the sample and also the aid the dispersion. Next, the 3 mL of thermostable $\alpha-$amylase was immediately added, stirred using vortex mixer and the tube were incubate in boiling water bath for 12 minutes (stirred vigorously after 4, 8 and 12 minutes). Using 100 μL micropipettor, 0.1 μL amyloglucosidase was added on the tube. The tube were stir on the vortex mixer until it was homogenous, and then left to be incubate at 50 °C for 30 minutes. All test tube content was then transferred to the 100 mL volumetric flask. Distilled water was used in order to rinse the tube content thoroughly. The volume were adjusted with distilled water and the flask was inverted few times to mix it thoroughly. 30 mL of the volumetric flask content was transferred into 50 mL Falcon tube. Then, it was centrifuge at 3,000 rpm for 10 minutes (Megazyme, 2017). Using 100 μL micropipettor, 0.1 mL of the clear supernatant was transferred into 15 mL Falcon tube. Then, 3 mL GOPOD Reagent was added to each tube, and incubated at 50 °C for 20 minutes. Reagent blank solution used contain 0.1 mL of water and 3 mL of GOPOD reagent whereas the D-glucose control consist 0.1 mL D-glucose standard and 3 mL of GOPOD reagent. The absorbance value for each sample was recorded included control at 510 nm against reagent blank. All absorbance value of sample, D-glucose and moisture content was insert into Mega-Calc of Total starch by Megazyme to obtain the starch percentage value.

3.3(f) Starch Crystallinity by Energy Dispersive X-Ray Analysis

XRD analysis of prepared sample of *D. hispida* dried tuber powder was done by Bruker D2-Phaser machine. Data was taken for the 2θ range 10° to 90°. Through application Diffrac Eva version 3.2, it interpret the XRD analysis degree of crystallinity and amorphous of the sample. Figure 3.11 show Bruker D2-Phaser machine used for XRD analysis of crystallinity percentage of dried tuber powder of two *D. hispida* cultivar.

Figure 3.11: Bruker D2-Phaser machine used for XRD analysis of crystallinity percentage of dried tuber powder of two *D. hispida* cultivar.

CHAPTER 4

RESULTS AND DISCUSSION

4.1 Introduction

This chapter presents the results and discussions of the research from six different analyses that were done on two cultivars of *D. hispida* for characterization purposes.

4.2 Colour Analysis of Two Cultivars of *Dioscorea hispida* by Colorimeter

D. hispida fresh tuber was cut in slices. The chroma meter was put on the slices surface, and then L, a, and b reading were taken. Figure 4.1 shows two cultivars of *D. hispida* fresh tuber flesh and Figure 4.2 show the colour map of fresh tuber of two cultivars of *D. hispida*.

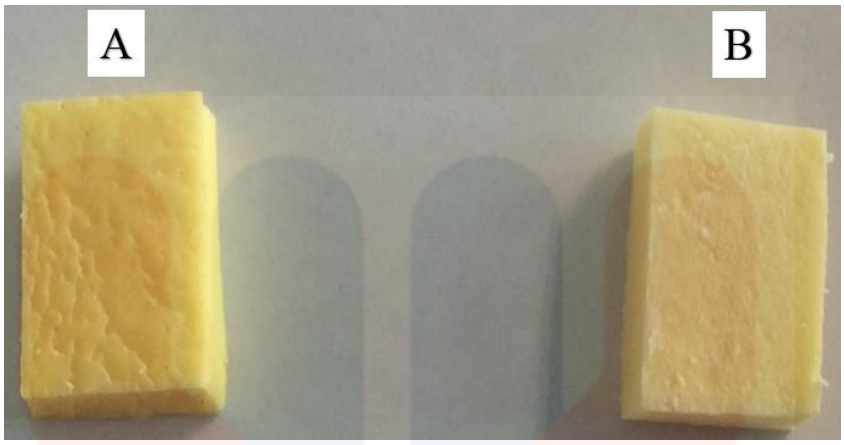

Figure 4.1: Two cultivars of *D. hispida* fresh tuber flesh. (a)Yellow *D. hispida*; (b)White *D. hispida*

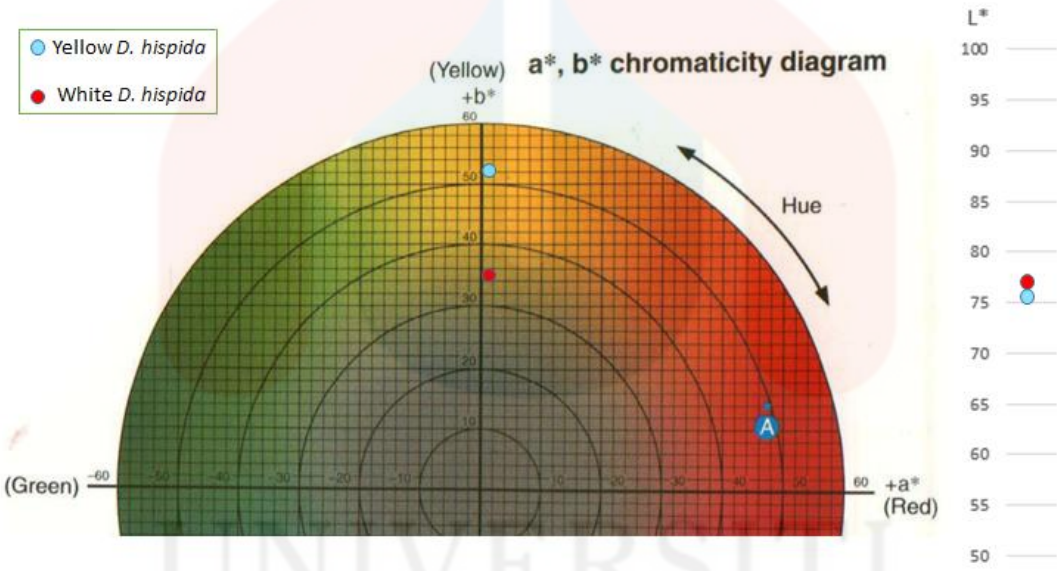

Figure 4.2: The colour map of fresh tuber of two cultivars of *D. hispida*

From the result, it showed that L* of colour between fresh flesh tuber of two types of *D. hispida* was different which is where the yellow *D. hispida* was 76.10 and white D. hispida was 77.97. From these lightness reading, the white *D. hispida* was lighter compared to yellow *D. hispida*.. The yellowness colour (b*) indicate the yellow colour of yellow *D. hispida* was 52.88 and white *D. hispida* was 34.16. These explain that the yellow *D. hispida* was more yellowish than the white D. hispida. For the redness of colour

(a*), the flesh of fresh tuber yellow *D. hispida* was 1.32 whereas white *D. hispida* was 0.41 where the detected red colour for both cultivars was very low. The differences in a* of both cultivars were too minimal to notice with naked eyes and can only be detected using the colorimeter.

It can be concluded that the L*, a* and b* of this two cultivars does not match in colour. Figure 4.2 show that the yellow *D. hispida* is light and more yellow. Different from white *D. hispida*, it also lights in colour but less yellow compared to yellow *D. hispida*.

The result is one of the information that can be used in further research which helps to differentiate between this two cultivars. Colorimeter help detect the minor changes that human eyes cannot detect. The colorimeter work by analysing the two light sources which first light radiation is the light emitted and second light radiation is the reflected light. It replicates the three different cones in retina which are light sources of illumination, modification of reflected light filtered and photoelectric that convert light into electrical output (Pathare, Opara, & Al-Said, 2013).

4.3 Starch Observation of Two Cultivars of *Dioscorea hispida* by Scanning Electron Microscope-Energy Dispersive X-Ray Analysis

The starch exists on the *D. hispida* cultivar was examined by using SEM. The picture was taken at magnification $\times 100$, $\times 1000$, and $\times 2000$. Figure 4.3, 4.4 and 4.5 show the Scanning Electron Micrograph of two cultivars of *D. hispida* on magnification $\times 100$, $\times 100$, and $\times 2000$ respectively.

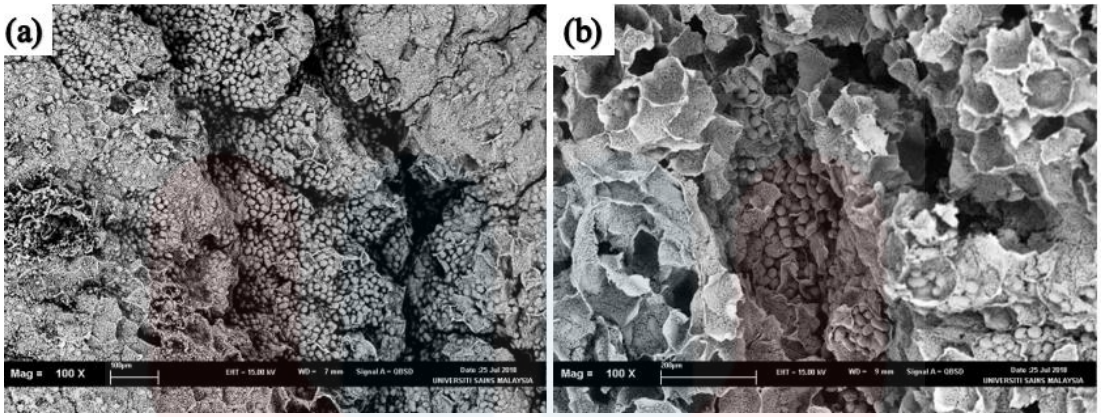

Figure 4.3: Scanning Electron Micrograph of two cultivars of *D. hispida* on magnification × 100. (a) white *D. hispida*; (b) yellow *D. hispida*.

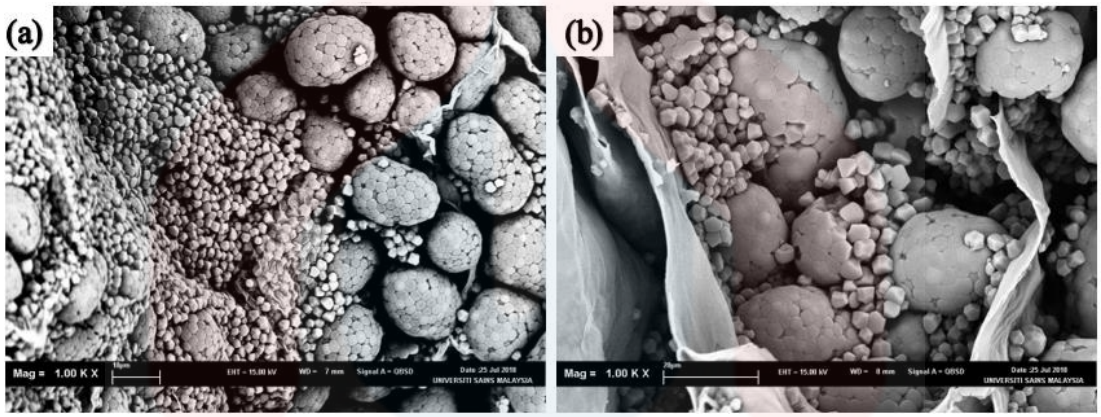

Figure 4.4: Scanning Electron Micrograph of two cultivars of *D. hispida* on magnification × 1000. (a) white *D. hispida*; (b) yellow *D. hispida*.

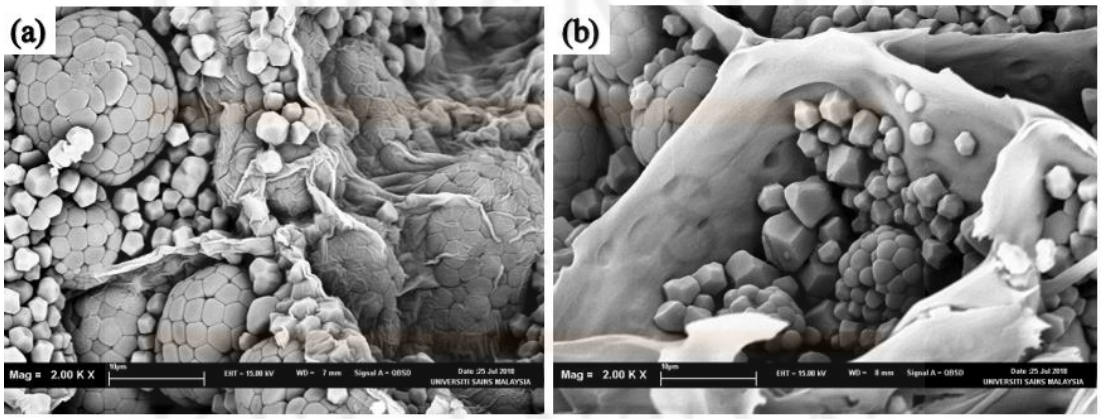

Figure 4.5: Scanning Electron Micrograph of two cultivars of *D. hispida* on magnification × 2000. (a) white *D. hispida*; (b) yellow *D. hispida*.

Based on Figure 4.3 which is picture taken by SEM on magnification × 100 show the starch granule distribution on all over tuber surface, so it proves that the *D. hispida* tuber is rich, starchy material. Figure 4.4 clearly shows the starch size comparison between both *D. hispida* cultivar. Figure 4.5 clearly show the shape of starch granule of both cultivar, which can be seen full of polygonal shape of starch. At glance, it is obvious that the size of starch granule yellow *D. hispida* is greater compared to white *D. hispida*.

Based on this study, both *D. hispida* cultivar found is categorized its starch granule as 3-dimensional (3D) polygonal shape, as it has many irregular flat sides which vary in shape and size. Its sides have a 2-dimensional shape (2D) formed with more than three straight lines which can be seen clearly on Figure 4.5.

The elemental analysis was done by using EDX analysis on the sample of *D. hispida* , under magnification × 500, the selected are is the section which has clump of polygonal shape, which is believed to be the starch granule. EDX analysis was done for starch confirmation. Figure 4.6 shows EDX graph of yellow *D. hispida* on the selected area, starch granule and Figure 4.7 show the EDX graph of white *D. hispida* on the selected area, starch granule.

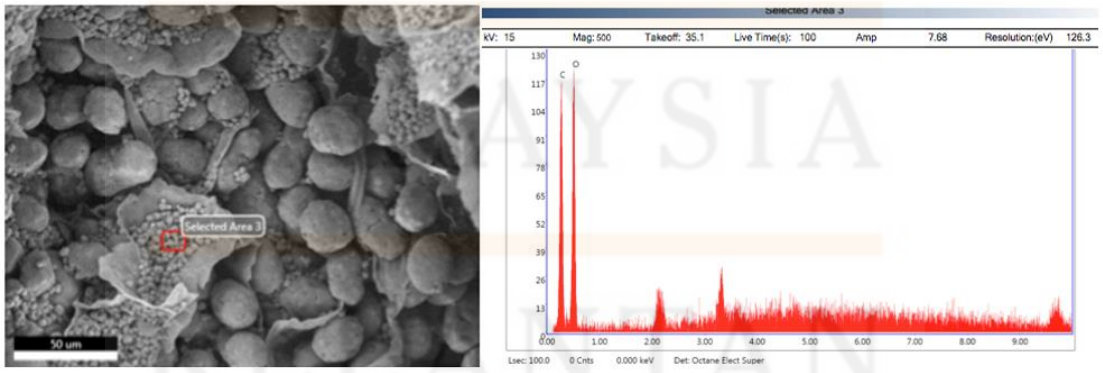

Figure 4.6: EDX graph of yellow *D. hispida* on the selected area, starch granule.

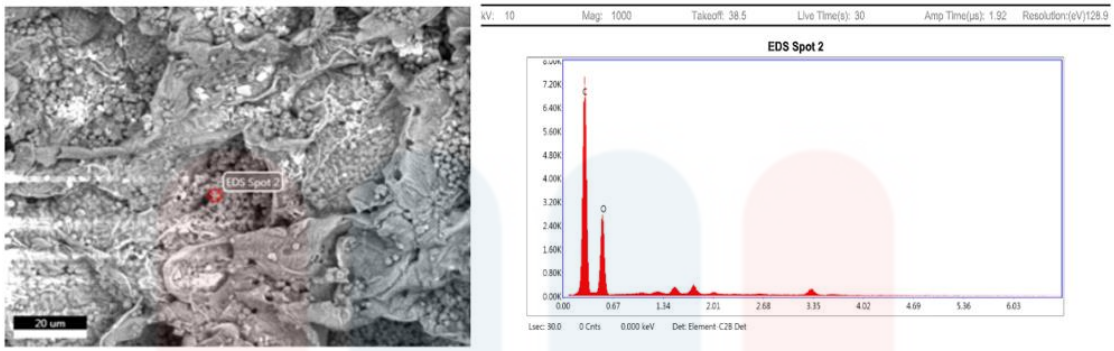

Figure 4.7: EDX graph of white *D. hispida* on the selected area, starch granule.

Based on the graph on Figure 4.6 and Figure 4.7, it is clearly shown that carbon and oxygen was the main constituent on the selected area since it shows the highest peak compared to another element which exists on the sample. This confirmed the starch identity as previous study by EDX analysis state that structure of starch granule is composed of carbon and oxygen (Gunduz *et al*., 2016).

4.4 Sizing Starch of Two *Dioscorea hispida* Cultivars

The starch size of *D. hispida* cultivar was determined by using SEM image on magnification $\times 2000$ through the application of Cell Sens. All potential individual starch in the figure was measure and taken its mean value. The result shows the average starch size of yellow *D. hispida* is $3.36 \pm 1.01\ \mu m$ whereas white *D. hispida* is $2.48 \pm 0.45\ \mu m$. At glance, we can see that the size of starch of yellow *D. hispida* is bigger compared to white *D. hispida* based on micrograph at magnification $\times 2000$. Figure 4.8 show the sizing starch granule using application Cell Sens on Scanning Electron Micrograph on magnification $\times 2000$ and Figure 4.9 show the graph of starch granule size of two cultivars of *D. hispida*.

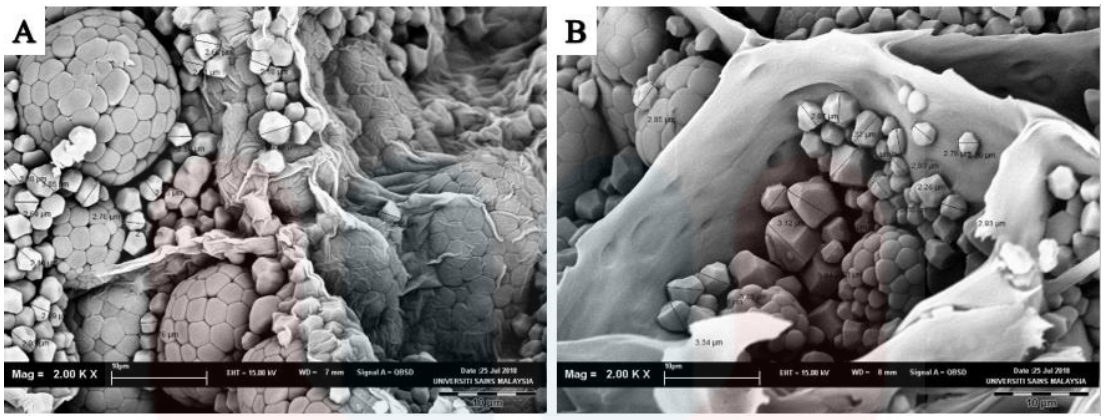

Figure 4.8: Sizing starch granule using application Cell Sens on Scanning Electron Micrograph on magnification × 2000. (a) white *D. hispida*; (b) yellow *D. hispida*.

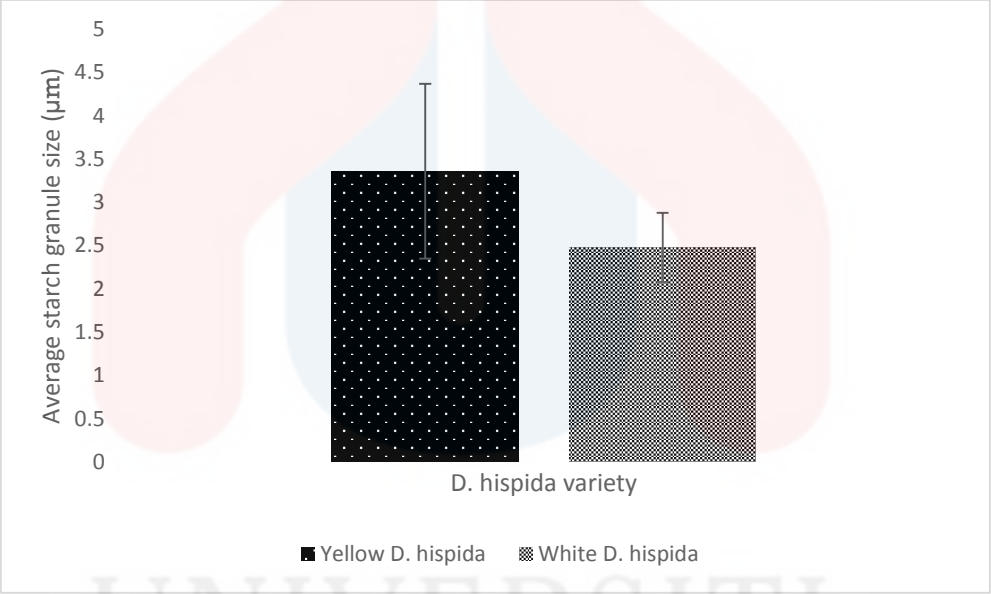

Figure 4.9: Graph of starch granule size of two cultivars of *D. hispida*.

Previous study of *Dioscorea* species shows that the biggest average starch granule size recorded is *D. abyssinica* which is 29.2 µm (Zhu, 2015) whereas the smallest is *D. dumetorum* that only 2.2 µm (Amani, Buleon, Kamenan, & Colonna, 2004). Based on those range, it can be see that the two cultivars of *D. hispida* studied has an average size or can be summarized as small size of starch granule. There are many factors which can contribute to this size granule differences which can be clearly seen on Figure 4.9.

The may due to the effect of the environmental condition where the yam growth (Akinoso and Abiodun, 2013). Besides that, it also this depends on the biochemistry mechanism of the plant that includes physiology, chloroplast and amyloplast of the plant (Alcázar and Meireles, 2015). The properties such as water binding capacity, swelling power, total amylose content and light transmittance are the physical factor that can affect the average granule size itself (Ali , Wani, and Masoodi, 2016).

Different pre-treatment need to be consider when dealing with different size of starch, especially to break it into a simple sugar. Bigger size of starch need high energy to break the starch into simple sugar, which as the result it will provide high content of sugar. Contrast with small size of starch, low energy needed to break the starch into simple sugar, but the result is it will provide low content of sugar (Subramoney and Moorthy, 2018).

4.5 Starch Concentration of Two Cultivars of *Dioscorea hispida* by Enzymatic Hydrolysis

The starch percentage contain in *D. hispida* tuber was determined by enzymatic hydrolysis. Figure 4.10 show the solution from enzymatic hydrolysis which is taken its absorbance value at 510 nm, Figure 4.11 show the calculation of starch percentage using Mega-Calc of Total Starch provided by Megazyme, and Figure 4.12 show graph of starch percentage on tuber sample both cultivar of *D. hispida* respectively.

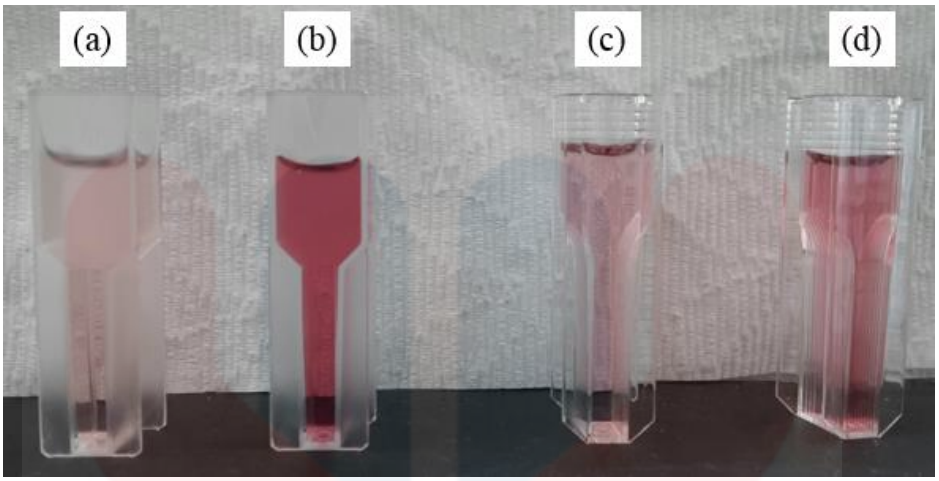

Figure 4.10: The solution from enzymatic hydrolysis which is taken its absorbance value at 510 nm. (a) Blank; (b) D-glucose standard; (c) White *D. hispida*; (d) Yellow *D. hispida*.

Megazyme

Setting new standards in test technology

Mega-Calc™

Total Starch (K-TSTA) Determination (Solids)

FEED ANALYSIS

FOOD ANALYSIS

BEVERAGE ANALYSIS

Sample details: Ubi Gadong

Absorbance values for 100 micrograms of D-glucose standard

Rep. 1	Rep. 2	Rep.3	Rep.4	Average
1.2970	1.4270	1.1400	1.0470	1.2278

81.4498 Factor [=100 (micrograms of D-glucose)/Absorbance for 100 micrograms of D-glucose]

	Sample identifier	Absorbance values		Sample volume (mL)	Dilution (-fold)	Starch (g/L)	Sample weight (mg)	Extract volume (mL)	Starch (g/100 g) "as is"	Moisture Content %	Starch (g/100 g) "dwb"
		Sample	ΔAbs								
1	Yellow D. hispida (Method A)	0.4750	0.4750	0.1	1	0.3482	150	100	23.2132	9.27	25.5849
2	White D. hispida (Method A)	0.1610	0.1610	0.1	1	0.1180	152	100	7.7645	10.29	8.6551

Figure 4.11: Calculation of starch percentage using Mega-Calc of Total Starch provided by Megazyme.

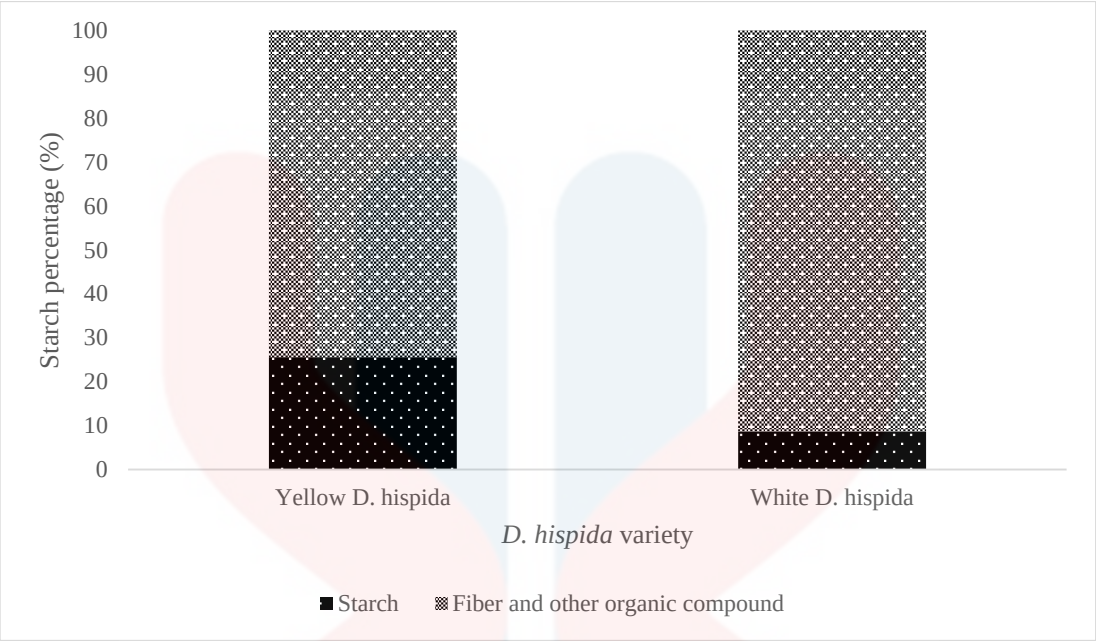

Figure 4.12: Graph of starch percentage on tuber sample both cultivar of *D. hispida* respectively.

Based on Figure 4.12, it is clearly shown that the starch percentage in yellow *D. hispida* are higher compared to white *D. hispida*. It can be seen that yellow *D. hispida* contain low starch content specifically less than 30% of starch whereas white *D. hispida* is tuber sample contains less than 10% of starch as constituent in every 100 g of tuber. Other than starch, yam tuber like *D. hispida* composed mainly fiber, and several organic materials.

The starch content plays important role in industry, especially for bioproduct development such as bioethanol production. The industry need to know the exact amount of starch contain in the plant source that they use, so that they can consider the option in choosing their raw material for bioethanol production. Criteria such as starch content, raw material cost, easy to found or abundant, plant grow cycle and intention of people to use it as food source are the list to be consider by industry in choosing the best raw material for industrial purpose (Otegbayo, 2014).

4.6 Starch Structural Properties of Two Cultivars of *Dioscorea hispida* by X-Ray Diffraction

The crystallinity of *D. hispida* dried tuber powder of both cultivars were examined by using XRD. Data was taken for the 2θ range 0° to 90°. Application Diffrac Eva version 3.2 use to interpret the XRD analysis degree of crystallinity and amorphous of the sample. Figure 4.13 show the XRD difractograms of *D. hispida* cultivar.

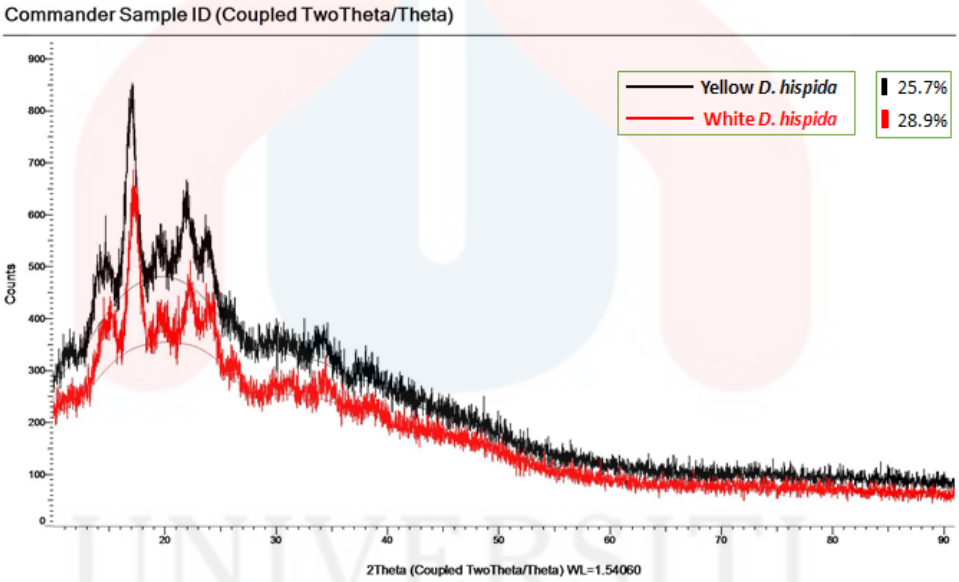

Figure 4.13: XRD diffractogram of two *D. hispida* cultivar.

Phase analysis (crystalline/ amorphous) of both samples was confirmed using XRD technique. It was shown in the figure that there is slightly significance crystalline plane of both cultivars of *D. hispida*. Based on the diffractogram, the diffraction pattern of both cultivar tuber dried powder show six peaks at $2\vartheta = 15°, 17°, 20°, 22°, 24°$ and 26° which peak showed start at $2\vartheta = 15°$, highest peak specifically at $2\vartheta = 17°$ and its

flatten at $2\vartheta = 62°$. The difference of the diffractogram between yellow *D. hispida* and white *D. hispida* were analysed to determine the crystallinity index (CI) and amorphous index (AI). Figure 4.14 show graph of starch structural properties of yellow *D. hispida* and white *D. hispida*.

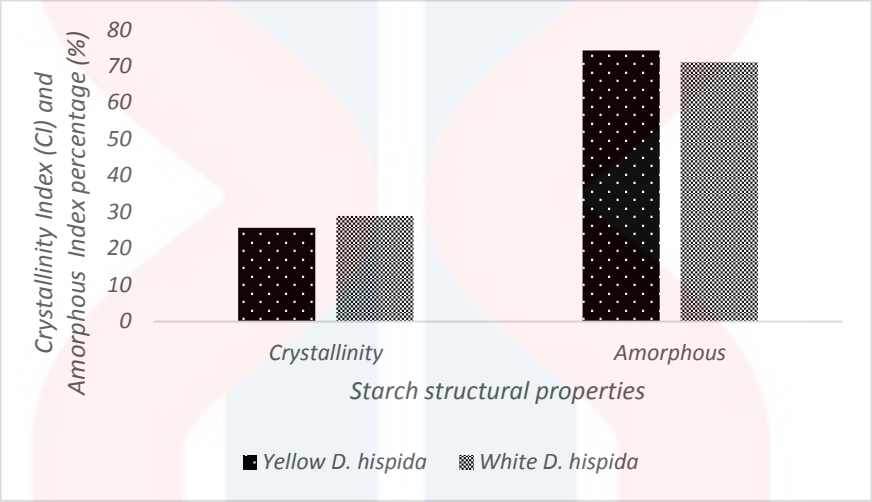

Figure 4.14: Graph of starch structural properties of yellow *D. hispida* and white *D. hispida*.

In Figure 4.14, it is obvious seen that there are only slight differences between CI and AI value of both cultivar of *D. hispida*. The CI value for white *D. hispida* was 28.9%, slightly higher than yellow *D. hispida* that is 25.7%. In term of AI, yellow *D. hispida* was 74.3%, slightly higher than white *D. hispida* that is 71.1%.

Yam tuber like *D. hispida* composed mainly fiber, starch and several organic materials. The main contributor to this crystalline and amorphous structure is due to existence of starch. Starch is a mixture of two polysaccharides which is amylose and amylopectin. Amylose is crystalline because it has linear structure amylopectin is amorphous due to its branched structure (Eliasson, 2004).

CI value of the sample was related to the proportion of amylose contain in the yam starch. High CI value indicates that there are high amylose content contain in its starch. Based on Figure 4.14, white *D. hispida* has slightly higher amylose content on its starch compare to yellow *D. hispida.* The same concept as CI, higher AI value of the substrate was related to the proportion of amylopectin contain in the yam starch. Thus, the high AI value indicates that there is high amylopectin content contain in its starch. Based on Figure 4.14, yellow *D. hispida* has slightly higher amylopectin content on its starch compare to white *D. hispida.* Generally, both cultivars has low CI value compared to AI value. Thus, its starch contains high amylopectin content compare to amylose content on its starch.

The highest crystallinity of *Dioscorea* species recorded is *D. septemloba* which is 53.4% (Jiang *et al*., 2012) whereas the lowest is *D. trifida* that is only 24% (Perez *et al.*, 2013). These range shows that cultivars of *D. hispida* studied tend to have low crystallinity percentage compared to those *Dioscorea* species. It can be see that the overall trend shows the crystallinity percentage of *D. hispida* cultivars less than 30%.

The amount of crystallinity which indicates the amylose concentration in starch are important information for industries especially when dealing with pre-treatment of starch, to break it into simple sugar. In enzymatic hydrolysis of starch method, its two enzyme was responsible for the process which is α −amylase and amyloglucosidase. α −amylase were responsible for hydrolyzing linear chain of amylose whereas amyloglucosidase responsible for hydrolysing both amylose and amylopectin. Starch with low amylose and high amylopectin content need high concentration of amyloglucosidase and low concentration of α −amylase for successful conversion of starch into simple sugar.

4.7 Sugar Concentration Analysis of Two Cultivars of *Dioscorea hispida* by Refractometer

The fresh tuber of *D. hispida* was cut into cube and being press using garlic press to obtain its sap. Then, the sap was centrifuge to remove the starch. Then, the few drops of the sap were used put on refractometer prism assembly. Figure 4.15 show graph of Brix percentage of sap two cultivars of *D. hispida*.

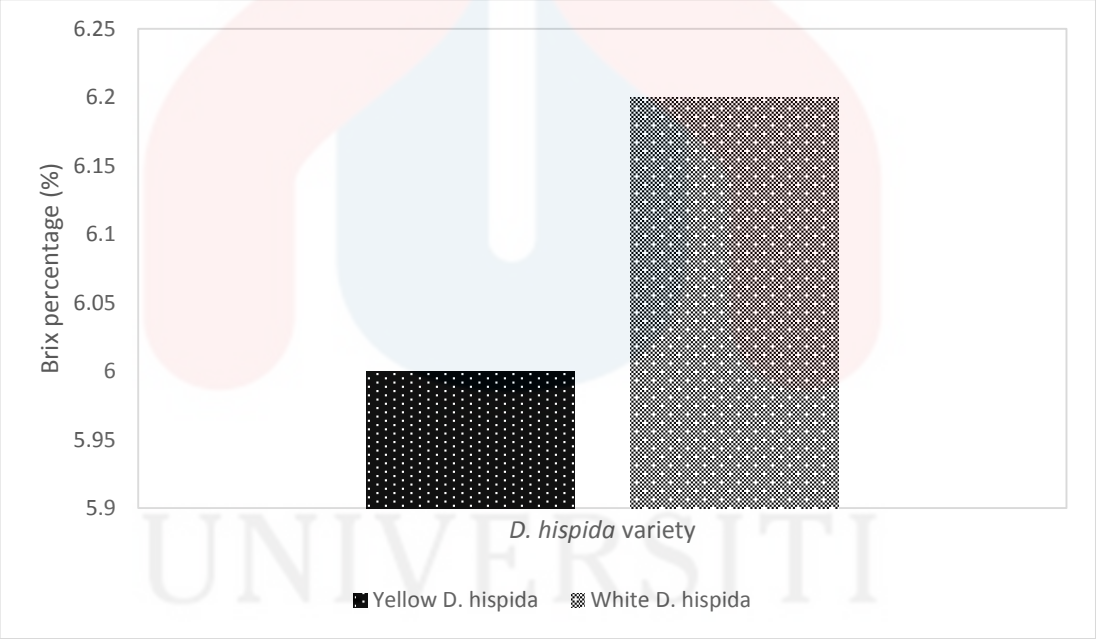

Figure 4.15: Graph of Brix percentage of sap two cultivars of *D. hispida*.

Based on Figure 4.15, it is obvious that the Brix percentage which indicates the sugar concentration contain in the *D. hispida* sap on both cultivar studies have only slightly differences between each other. The white *D. hispida* sap contain slightly higher sugar concentration, which is 6.2% compared to yellow *D. hispida* which is 6.0%. Table 4.1 show sugar concentration of feedstock derived from different crops.

Table 4.1: Sugar concentration of feedstock derived from different crops.

Feedstock	Sugar concentration (%)	Reference
Sugarcane juice	12.0-17.6	(Dhaliwal *et al*., 2011, Wheals, Basso, Alves, & Amorim, 2005, Quintero, Montoya, Sanchez, & Giraldo, 2008, Ogbonna, Mashina, & Tanaka, 2001)
Sugar beet juice	16.5	(Ratnavathi, Chakravarthy, Komala, Chavan, & Patil, 2011)
Sweet sorghum juice	16.0-21.8	(Mamma *et al*., 1995)
Watermelon juice	7.0-10.0	(Fish, Bruton, & Russo, 2009)

Sugar have been used in industry, especially in bioethanol production (Zabed H. *et al*, 2014). Sugar are more preferable compared to starch since it can be used directly in the fermentation process without any prior treatment. Statistic show that 60% of global ethanol production is produce from sugar crops, while remaining 40% is produced from starchy grain (Salassi, 2007). Industries are preferred to use free sugar as feedstock as it require non-costly step for such pre-treatment and hydrolysis to get fermentable sugar (Nikolov *et al*., 2000).

CHAPTER 5

CONCLUSION AND RECOMMENDATION

5.1 Conclusion

The study of NSC properties on *D. hispida* tuber were done between yellow *D hispida* and white *D. hispida* were done to determine the significance differences between this two cultivars which are obtained from Tanah Merah, Kelantan, Malaysia. This study determined the starch morphology and size between this two cultivars, with addition of EDX analysis as confirmation starch picture obtained are actually starch. Next is to XRD analysis were also done to differentiate the crystalline and amorphous percentage on that tuber sample on of two cultivars respectively. Besides that, this study also determines the starch percentage in dried weight basis on tuber part through enzymatic hydrolysis. Lastly, this study determines the colour differences on tuber by using colorimeter as addition to characterization which differentiate this two cultivars of *D. hispida*.

The findings shows that starch concentration in yellow *D. hispida* was higher which is 25.58% compared to white *D. hispida* that is only 8.66%. Low sugar concentration shown on both sap of *D. hispida* cultivar studied, with less significance

differences shown between cultivars, which yellow *D. hispida* is only 6.2% whereas white *D. hispida* is 6.0%. The starch of *D. hispida* are successfully characterize by SEM and XRD. SEM analysis show that two cultivars of *D. hispida* studied has same starch granule shape which is polygonal shape. In term of starch granule size, yellow *D. hispida* starch granule was bigger with average size $3.36 \pm 1.01\ \mu m$ compared to white *D. hispida* that is only $2.48 \pm 0.40\ \mu m$. XRD analysis show that white *D. hispida* were slightly more crystalline which is 28.9% compared to yellow *D. hispida* that is only 25.7%. The physical analysis which is colour measurement resulting that both cultivars studied shown less significance differences in term of lightness, but shown main gap differences in term of yellow colour intensity, with yellow *D. hispida* was more yellowish compared to the white *D. hispida* which is less yellowish. In conclusion, all the objectives which are characterize the NSC on two cultivars of *D. hispida* have been successfully achieved for this study.

5.2 Recommendations

The researcher would like to recommend several ideas for further study of *D. hispida*. First, for the sample collection, GPS should be used to determine the geographic coordinate that is location where *D. hispida* were taken for easier the next research to find its source. Next, the plant morphological study between this two cultivars of *D. hispida* should be done to this differentiate between this plant variety. The further work on *D. hispida* was hoping to get it commercialized and used widely in industry because of its huge potential.

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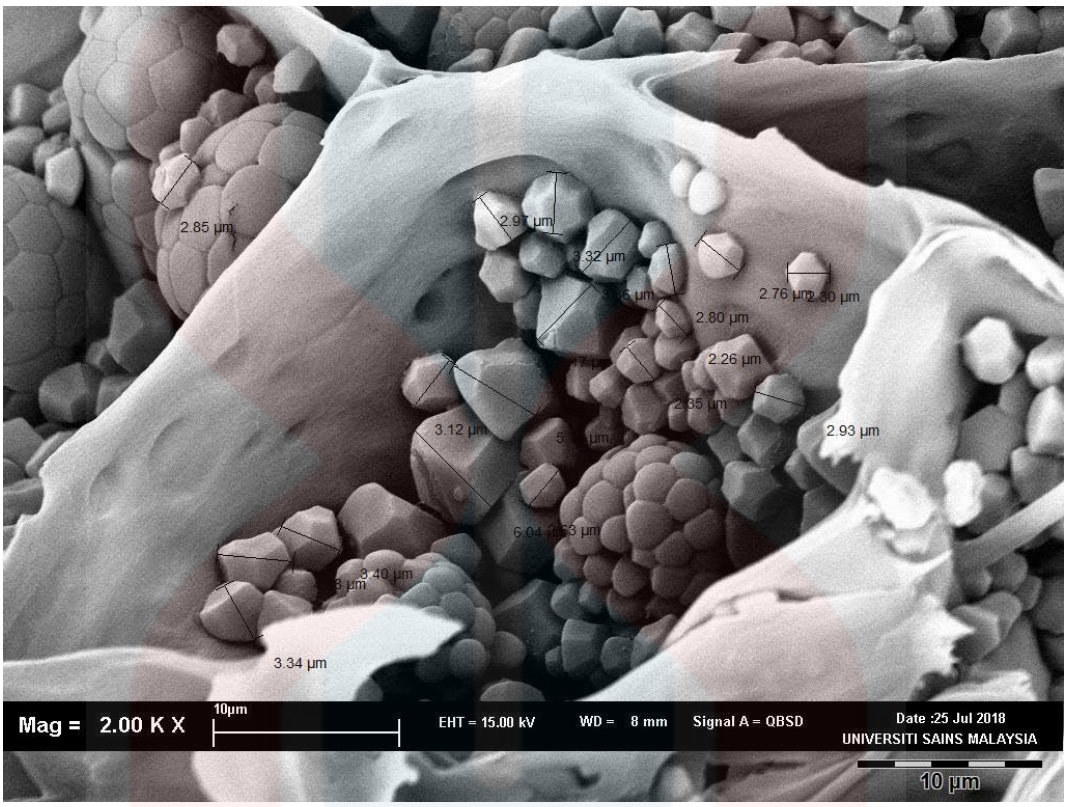
APPENDICES A

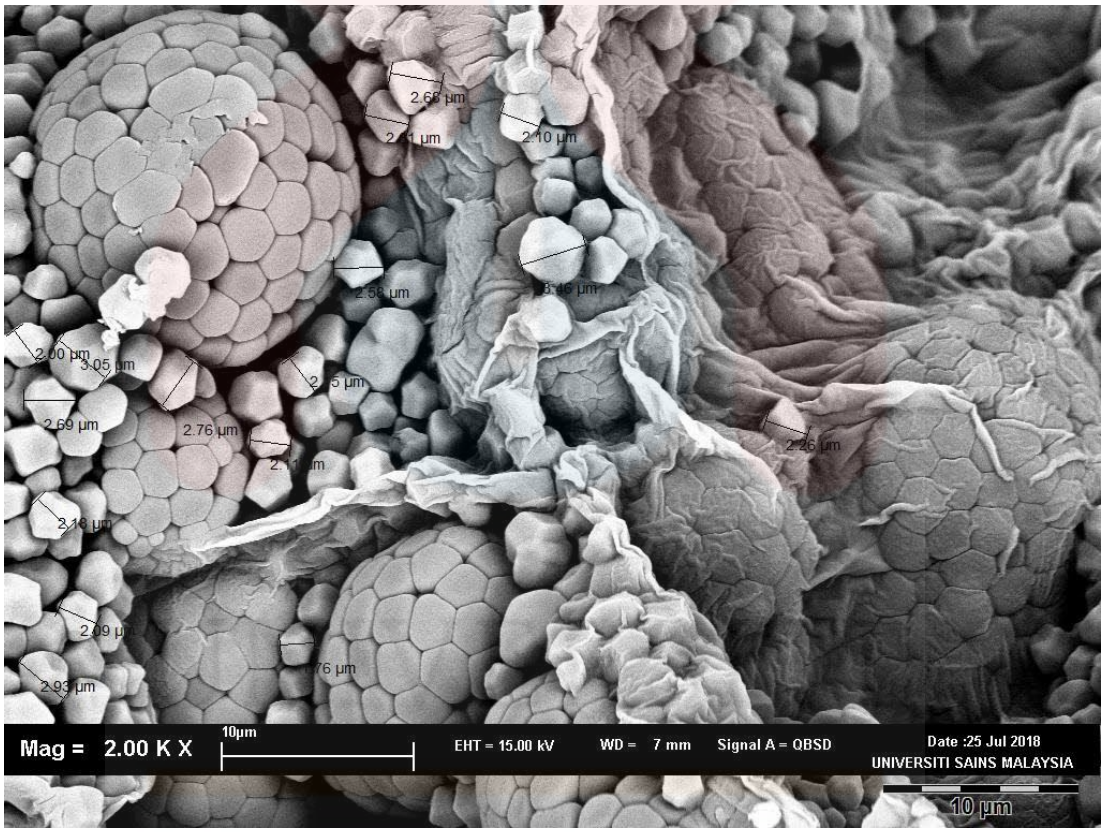
Table A.1: Sizing starch granule of yellow *D. hispida* by using application Cell Sens.



Type	Length (µm)
Arbitrary Line	5.19313
Arbitrary Line	3.123869
Arbitrary Line	2.966331
Arbitrary Line	3.318836
Arbitrary Line	3.855116
Arbitrary Line	2.755058
Arbitrary Line	2.303334
Arbitrary Line	3.401027
Arbitrary Line	4.175796
Arbitrary Line	3.344886
Arbitrary Line	2.854087
Arbitrary Line	2.264643
Arbitrary Line	2.352145

Arbitrary Line	2.632627
Arbitrary Line	6.03812
Arbitrary Line	4.171259
Arbitrary Line	2.932624
Arbitrary Line	2.798814
Standard Deviation	1.008683
Mean	3.360095

Table A.2: Sizing starch granule of white *D. hispida* by using application Cell Sens.



Type	Length (µm)
Arbitrary Line	3.046355
Arbitrary Line	2.00003
Arbitrary Line	2.1771
Arbitrary Line	2.759314
Arbitrary Line	2.154099
Arbitrary Line	3.458048
Arbitrary Line	2.306384

Arbitrary Line	2.10304
Arbitrary Line	2.105909
Arbitrary Line	1.761672
Arbitrary Line	2.926566
Arbitrary Line	2.092245
Arbitrary Line	2.25877
Arbitrary Line	2.583002
Arbitrary Line	2.683322
Arbitrary Line	2.692308
Standard Deviation	0.453664
Mean	2.44426

Table A.3: Moisture content of dried sample powder tuber after 24 hour dry at 105℃, used in calculation of starch percentage by Mega-Calc provided from Megazyme.

Sample	**Aluminium foil (g)**	**Powder tuber (before dried)-W_w(g)**	**Aluminium foil + powdered tuber (g)**	**After dried (Aluminium + sample) (g)**	**Dried sample (aluminium + sample before dried – aluminium foil)-W_d (g)**	**Moisture content $(\frac{W_w - W_d}{W_w}) \times 100\%$ (%)**
Yellow *D. hispida*	0.302	1.003	1.305	1.212	0.910	9.27
White *D. hispida*	0.383	1.001	1.383	1.281	0.898	10.29

Table A.4: EDX analysis of two cultivars of *D. hispida*, mainly focus on carbon and oxygen element.

Sample	**Element**	**Weight %**
Yellow *D. hispida*	Carbon	47.34
	Oxygen	52.66
White *D. hispida*	Carbon	60.05
	Oxygen	39.95

Table A.5: Refractometer reading of both *D. hispida* cultivar sap.

Sample	Brix %
Yellow *D. hispida* sap	6.0
White *D. hispida* sap	6.2

Table A.6: Colorimeter reading of fresh *D. hispida* tuber.

Yellow *D. hispida*	**White *D. hispida***
L*=76.10	L*=77.97
a*=1.32	a*=0.41
b*=52.88	b*=34.16

L*: Lightness, a*=Red-green colour, b*: blue-yellow colour.

Table A.7: Starch percentage of *D. hispida* cultivar.

Sample	Starch (%)
Yellow *D. hispida* dried tuber powder	25.58
White *D. hispida* dried tuber powder	8.66

Table A.8: Crystallinity and amorphous percentage of tuber two cultivars of *D. hispida*.

Sample	% Crystallinity	% Amorphous
Yellow *D. hispida*	25.7	74.3
White *D. hispida*	28.9	71.1