



UNIVERSITI PUTRA MALAYSIA

***THE EFFECT OF DIFFERENT EXTRACTION METHOD
TOWARD EXTRACTED (1,3;1,4)- β -GLUCAN FINE
STRUCTURE FROM RICE GRAIN, LEAF AND STEM***

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FSPM 2015 4**

**The Effect of Different Extraction Method Toward Extracted (1,3;1,4)- β -Glucan
Fine Structure from Rice Grain, Leaf and Stem.**



By

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**A Project Report Submitted in Partial Fulfilment of the Requirement
for the Degree of Bachelor of Science Bioindustry in the
Faculty of Agriculture and Food Sciences
Universiti Putra Malaysia Bintulu Sarawak Campus**

2015

DEDICATION

*I dedicated this research project to my parents and
family.*

Thanks for the love and support.

ABSTRACT

The (1,3;1,4)- β -glucan is polysaccharide found in the cell wall of commercial cereal and grasses. It has been reported to accumulate highly in the barley and oat grain. Depend on the fine structure at the respective (1,3;1,4)- β -glucan, certain type of (1,3;1,4)- β -glucan are more soluble. Higher concentration of alkali is needed to extract the less soluble (1,3;1,4)- β -glucan as the streamlined method using aqueous buffer is only able to extract soluble (1,3;1,4)- β -glucan. The different extraction method will give different effect toward the total extracted (1,3;1,4)- β -glucan content in different rice tissue. Two types of extraction buffer were used in this work: the potassium hydroxide and aqueous extraction method. Both methods are modified to permit the analysis of small amount of sample. The Sarawak rice cultivars yield different concentration of the (1,3;1,4)- β -glucan using both method on different rice tissue. Stem has the highest concentration of (1,3;1,4)- β -glucan compare to other rice tissue. In grain, the highest concentration of (1,3;1,4)- β -glucan is 0.59 (% w/w) obtained at *Bario banjal (Setiam)*. 0.848 (% w/w) of (1,3;1,4)- β -glucan is the highest concentration obtained at leaf of *Celum halus stumbin* while in stem, the concentration of 4.768 (% w/w) is obtained at *Bario banjal (Setiam)*. All the highest concentration of (1,3;1,4)- β -glucan is obtained through the KOH extraction method.

ABSTRAK

(1,3;1,4)- β -glucan merupakan polisakarida yang terdapat dalam dinding sel tanaman bijirin komersial and rumput. Ianya banyak dilaporkan untuk terkumpul di bijirin pada barli dan oat. Berdasarkan kepada struktur halus (1,3;1,4)- β -glucan yang berkenaan, sesetengah jenis (1,3;1,4)- β -glucan lebih mudah larut. Kepekatan alkali yang lebih tinggi diperlukan untuk mengekstrak (1,3;1,4)- β -glucan yang kurang larut oleh kerana kaedah yang terdapat di pasaran yang menggunakan larutan akuoes hanya mampu mengekstrak (1,3;1,4)- β -glucan yang larut. Kaedah mengekstrak yang berlainan akan memberikan kesan yang berlainan terhadap kandungan penuh (1,3;1,4)- β -glucan yang diekstrak pada tisu pokok padi yang berlainan. Dua kaedah proses ekstraksi yang berlainan digunakan dalam projek ini: kalium hidroksida dan proses ekstraksi secara akueous. Kedua-dua kaedah ini diubahsuai untuk membolehkan proses ekstraksi dilakukan menggunakan jumlah sampel yang kecil. Kultivar padi Sarawak menghasilkan kepekatan (1,3;1,4)- β -glucan yang berbeza menggunakan kedua-dua kaedah ekstraksi tersebut pada bahagian yang berlainan. Bahagian batang mengandungi lebih banyak (1,3;1,4)- β -glucan berbanding dengan tisu yang lain. Dalam bijirin, kepekatan yang tertinggi ialah 0.59 (% w/w) yang diperolehi di *Bario banjal (Setiam)*. 0.848 (% w/w) ialah konsentrasi (1,3;1,4)- β -glucan yang tertinggi yang diperolehi pada bahagian daun di *Celum halus stumbin* manakala pada bahagian batang, sebanyak 4.768 (% w/w) konsentrasi didapati pada *Bario banjal (Setiam)*. Kesemua konsentrasi (1,3;1,4)- β -glucan yang tinggi diperolehi melalui kaedah ekstraksi KOH.

ACKNOWLEDGEMENTS

First and foremost, I am thankful to God for the good health and well being that are necessary to complete this Final Year Project. I also would like to express my sincere gratitude to my supervisor, Dr Wong Sie Chuong for the guidance, patience and knowledge that enable me to finish up the project. I also wish to express my deepest gratitude to my co-supervisor, Assc. Prof. Dr. Yiu Pang Hung for the support that he gave. I also would like to thanks to the postgraduate students for helping me with the initial understanding of the research work.

Special thanks also to my friends that have gave me lots of helps and moral supports. Lastly, millions thank to my family especially my parents for supportive words and encouragement to finish up my project and siblings who always gave me lots of love during my hard time to finish this project.

APPROVAL SHEET

I certify that this research project entitled “Effect of Different Extraction Toward Extracted (1,3;1,4)- β -glucan Content in Rice Grain, Leaf and Stem” has been examined and approved as a partial fulfilment of the requirement for the degree of Bachelor Science Bioindustry in the Faculty of Agriculture and Food Science, Universiti Putra Malaysia Bintulu Sarawak Campus.

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LIST OF ABBREVIATIONS

ANOVA	Analysis of variance
KOH	Potassium hydroxide
w/w	Weight over weight
v/v	Volume over volume
μ l	Micro litre
rpm	Rotation per minute



CHAPTER 1

INTRODUCTION

1.1 Background

The (1,3;1,4)- β -glucan is a linear glucose polymer with (1,3) and (1,4) linkage. It has been found widely in species of Poaceae family which include barley, oat and wheat. Recently, there are also studies that found the (1,3;1,4)- β -glucan in the walls of some algae, lichen, fungi and horsetail.

In the grain, the (1,3;1,4)- β -glucan serve as the energy sources for the germination process. Recently, there is also study that have identified high (1,3;1,4)- β -glucan level in mature stem of the rice. It may indicate that (1,3;1,4)- β -glucan have potential role in strengthening the cell wall of the rice stems. (1,3;1,4)- β -glucan found in the leaves of the cereals plants. This also showed that the (1,3;1,4)- β -glucan may act as the energy reserves by supplying additional glucose to the plants when the plants under glucose starvation. Such condition might occur when the plants are not exposed to the sunlight thus making it unable to do photosynthesis or when it is in prolonged night time.

The (1,3;1,4)- β -glucan have been found to give several health benefits to the human as it can reduce the serum low-density lipoprotein (LDL) - cholesterol level, reduce the time for bowel transit and also reduce the risk of obesity. However, the (1,3;1,4)- β -glucan have negative effect on the brewing industry as the (1,3;1,4)- β -glucan can impede the filtration and form haze in beer. In poultry, the high (1,3;1,4)- β -glucan

level in the feed could cause the sticky dropping and also reduced the growth performance of the animals.

1.2 Problem Statement

The study of (1,3;1,4)- β -glucan has been done widely on the barley, oat and other cereals and grasses species. There are many data collected regarding the (1,3;1,4)- β -glucan analysis in those plants. In rice, the data collection available from the analysis of other rice cultivar that cannot be found in Sarawak. Therefore, this study will provide the analysis data of (1,3;1,4)- β -glucan from the Sarawak rice cultivar which will help in future research about the Sarawak rice cultivar.

Besides that, the structure of (1,3;1,4)- β -glucan that consist of soluble and less soluble also is the main factor of this project. Due to the different in the solubility of the (1,3;1,4)- β -glucan in plant cell wall, the vigorous extraction method is required to extract all (1,3;1,4)- β -glucan inside it. The different part of the plant may have different ratio of the DP3:DP4 affecting its solubility therefore it is important to have extraction method that may extract the maximum concentration of the (1,3;1,4)- β -glucan.

1.3 Objective

Therefore, this study aimed:

- To determine the effect of different extraction method toward extracted (1,3;1,4)- β -glucan content in rice grain, stem and leaves.

CHAPTER 2

LITERATURE REVIEW

2.1 Introduction To (1,3;1,4)- β -Glucan

The (1,3;1,4)- β -glucan that made of from (1,3) and (1,4) mixed linkage of glucose units which are non- starchy polysaccharide (Benito-Román *et al.* 2011). The (1,3;1,4)- β -glucan can be found in the cell wall of the endosperm of oats and barley as well as other cereal grains (Irakli *et al.* 2004). Recently, the (1,3;1,4)- β -glucan also can be found in the *Equisetum* (horsetail) (Fry *et al.* 2004) and also in bryophytes (Popper and Fry 2003). Besides that, it also reported that the (1,3;1,4)- β -glucan can also be found in the lichen and other fungi.

The (1,3;1,4)- β -glucan is made up from the unsubstituted, unbranched chains that consist of the (1,4)- and (1,3)- β -glucosyl residues and are arranged in such a way to generate large, asymmetrical polysaccharide that is often soluble in water and forms solutions of high viscosity (Fincher and Stone 2004; Fincher 2009). The (1,4)- and (1,3)- branched are highly variable across the species with the (1,4)-linkages being more abundant (Burton and Fincher 2012). The (1,3;1,4)- β -glucan also consist of randomly arranged cellotriosyl and cellotetraosyl units linked with single (1,3)- β -linkages (Burton *et al.* 2011). The cellotriosyl consists of two consecutive (1,4)-linkages and cellotetraosyl is the three consecutive (1,4)-linkages. In some cereals, (1,3;1,4)- β -glucan can be found in longer block up to 12 or 14 adjacent (1,4)- β -glucosyl residues and this further contribute to the conformational irregularity (Woodward *et al.* 1983).

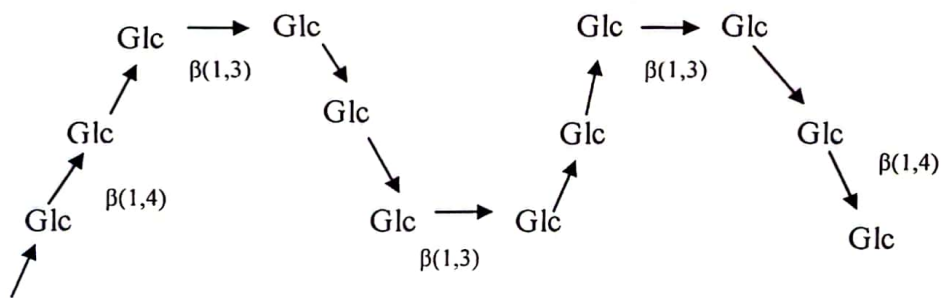


Figure 2. 1 The unbranched polymer of mixed linkage glucan. (Vogel 2008).

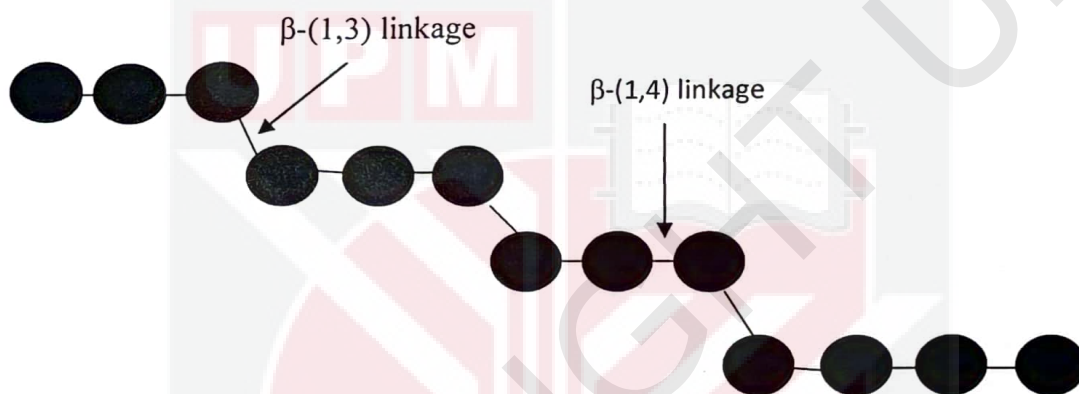


Figure 2.2 Schematic diagram of the (1,3;1,4)- β -glucan molecule (Tosh *et al.* 2004).

From the figure 2.1 and 2.2, the (1,3)- linkages of the (1,3;1,4)- β -glucan are arranged in between the cellotriosyl (DP3) and cellotetraosyl (DP4). The (1,3) linkages interrupted cause the polymer to bend. Figure 2.3 shows another ways of viewing the (1,3;1,4)- β -glucan structure with the cellotriosyl known as DP3 and cellotetraosyl known as DP4 (Burton and Fincher 2012). The Markov chain analyses indicated that the arrangement of cellotriosyl and cellotetraosyl units is essentially random (Staudte *et al.* 1983).

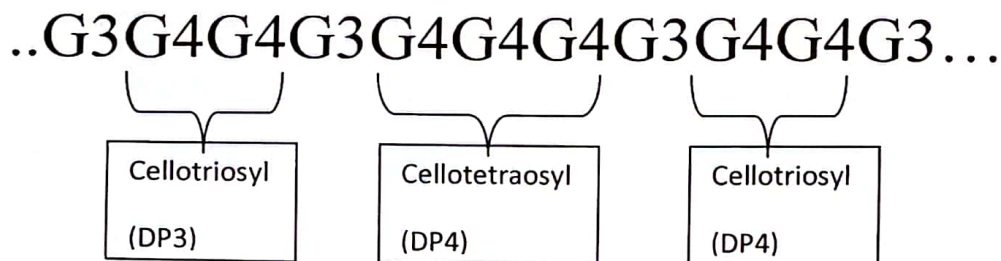


Figure 2.3 Arrangement of the cellotriosyl and cellotetraosyl in (1,3;1,4)- β -glucan.

Due to high abundance in the cell wall of the grains, it is believed that (1,3;1,4)- β -glucan is serving as the energy storage polysaccharide particularly in barley and oats grain (Fincher 2009; Vega-Sanchez *et al.* 2013). In vegetative tissues, (1,3;1,4)- β -glucan can also be found in secondary cell walls and mature stem tissue (Vega-Sanchez *et al.* 2013). The stem also might have functions as the carbohydrate storage and the (1,3;1,4)- β -glucan is important during the grain filling stages as demonstrate by the Arai-Sanoh *et al.* (2013). Roulin *et al* (2002) also found that the leaves tissue of barley plants contain (1,3;1,4)- β -glucan and it serve as energy storage for plants. (1,3;1,4)- β -glucan been used up when the plants under sugar depletion condition. The easier way of the hydrolysis of (1,3;1,4)- β -glucan to glucose form by only required two enzyme has made it possible for the energy storage in plants (Fincher 2009).

2.2: Importance of (1,3;1,4)- β -glucan

(1,3;1,4)- β -glucan have several importance and benefits to the human and also to the animals. The high fibre content of (1,3;1,4)- β -glucan have benefit the human in reducing the coronary heart disease. According to the US Food and Drug administration (FDA 2008), daily intake of 3 g or more of (1,3;1,4)- β -glucan soluble fibre could reduce the risk of coronary heart disease. The major contributors to the

total dietary fiber are the grain non starch polysaccharides, resistant starch, and oligosaccharides (Collins *et al.* 2010). (1,3;1,4)- β -glucan is dietary fibre that have several benefits to human including reducing high serum cholesterol and cardiovascular disease (Brennan and Cleary 2005). The lignocelluloses which are the cereal crop residues are the potential sources for the production of the bioethanol and other biofuels (McLaren 2005). (1,3;1,4)- β -glucan also important in providing the mechanical support to the plant as the cell wall of the stem contain (1,3;1,4)- β -glucan.

The fine structure of the polysaccharide known as DP3:DP4 ratio will allow prediction of the physiochemical properties of (1,3;1,4)- β -glucan (Lazaridau and Biliaderis 2004; Burton *et al.* 2010). Burton *et al.* (2010) also stated that when the DP3:DP4 ratio of the (1,3;1,4)- β -glucan are in the range of 2.0-3.0, some of the p(1,3;1,4)- β -glucan will be soluble in aqueous media and if the values move away from this, the (1,3;1,4)- β -glucan will made up of more DP3 or DP4 units. The ratio of the (1,4)- to (1,3)- linkages in the water soluble (1,3;1,4)- β -glucan from barley ranges from 2.2:1 to 2.6:1 (Fincher 2009). Lazaridau *et al.* (2004) also had found that with the increasing of the DP3:DP4 ratio, the strength of the (1,3;1,4)- β -glucan is also increased and the brittleness is decreased after the large deformation mechanical test.

Table 2.1 Levels of (1,3;1,4)- β -glucan in various type of grasses (Burton and Fincher 2012).

Species	(1,3;1,4)- β -glucan (% w/w)	DP3:DP4 ratio	Solubility in water	Starch (% w/w)
Oats	6-8	1.5-2.3:1	~80	~60
Barley	4-10	2.6:1	~20	~57
Wheat	1	3.2:1	0	~57
Brachypodium	40	5.9:1	Insoluble	<10
Rice	<0.06	1:1.4	?	80
Maize	Trace	2.5:1	?	70-80
Sorghum	0.07-0.2	2.8:1	?	70-80
Transgenic barley (AsGlo: CslF6)	7.8	2.1	?	44
Transgenic barley (AsGlo:CslF4)	6.0	2.8:1	?	?

Another research conducted by other researcher also shows the similar result obtain from the previous research. The result from other research is shown below.

Table 2.2 The average β -glucan content of cereal grains grown in Turkey (Demirbas 2005).

Grain	β -glucan content of grain (%)	
	The lowest	The highest
Barley	3.2	4.6
Beans	2.4	3.5
Canary seed	1.1	2.3
Corn/maize	0.5	1.3
Flax	0.3	0.7
Lentils	0.4	1.1
Millet	0.5	1.0
Oat	3.9	5.7
Peas	0.3	0.7
Rice	0.4	0.9
Rye	0.7	1.5
Spelt	0.6	1.2
Spring wheat	0.6	1.1
Winter wheat	0.5	1.0

From the Table 2.1, the levels of (1,3;1,4)- β -glucan in the rice grain is low and only trace amounts can be detected. For the Table 2.2, Demirbas (2005) had detected low amount of β -glucan in rice grain grown in Turkey. Barley and oats have the highest number of (1,3;1,4)- β -glucan content and have more water-soluble fraction inside the plants. The lichenan also have higher DP3:DPP4 ratio compared to the others plants following the molar ratio as the order of lichenan (24.5) > wheat (3.7) > barley (2.8-3.0) > oat (2.1) and thus making the lichenan less soluble (Lazaridau *et al.* 2004).

In the synthesis of (1,3;1,4)- β -glucan, there are a few genes that may responsible for the synthesis in the plants. From the Figure 2.4, there are three genes families which are CslF, CslH and CslJ found in the taxa of poaceae. For rice, there are six *CslF* genes that have been identify to be responsible for (1,3;1,4)- β -glucan synthesis which are the *OsCslF1*, *OsCslF2*, *OsCslF3*, *OsCslF4*, *OsCslF8* and *OsCslF9* (Burton *et al.* 2006). The most widely expressed gene in rice is the *OsCslF6* (Wang *et al.* 2010). To test the function of the *CslF* family, *Arabidopsis thaliana* had been used as cell wall of this species do not contain (1,3;1,4)- β -glucan. The transgenic *Arabidopsis* able to deposit the (1,3;1,4)- β -glucans and expressing various *CslF* gene families (Burton *et al.* 2006).

Recently *CslH* families also have been detected to be able to direct synthesis of the (1,3;1,4)- β -glucan synthesis in the barley (Doblin *et al.* 2009). There are three *CslH* families in rice but only one in barley which is the *HvCslH1* and the study done showed that the gene was transcribed most highly at the tips of the leaf (Doblin *et al.* 2009).

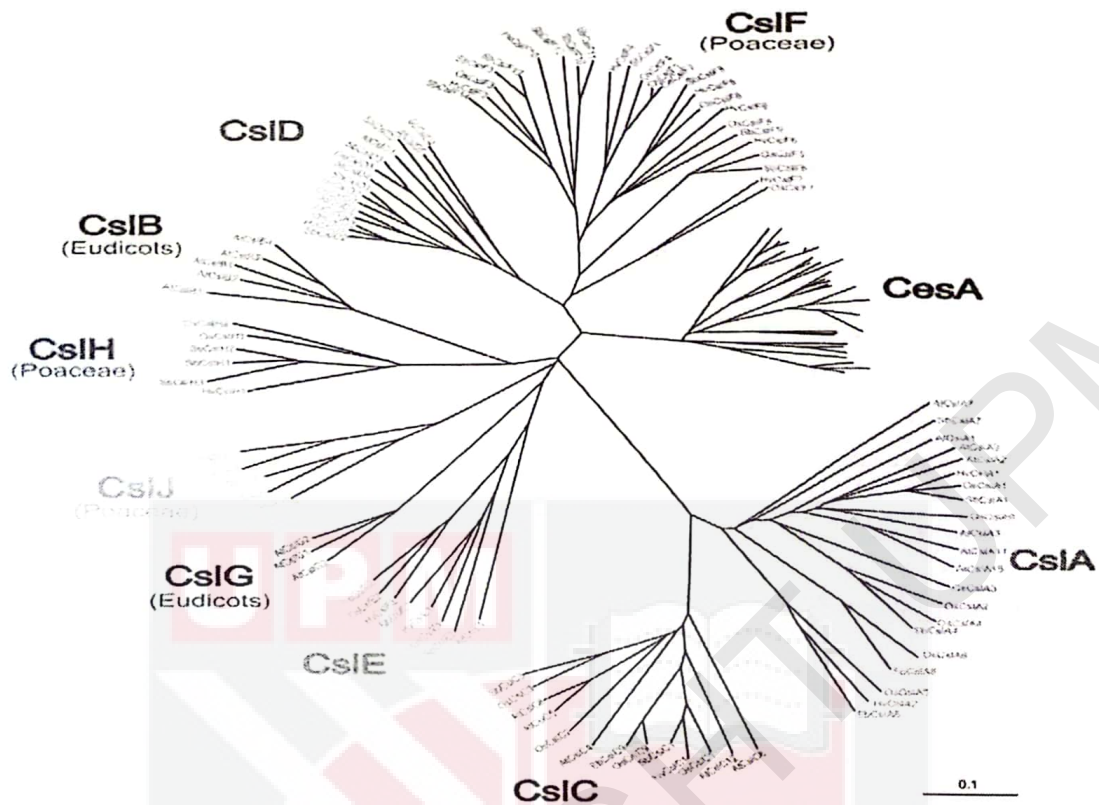


Figure 2.4 Updated phylogenetic tree of cellulose synthase and cellulose synthase-like gene families in higher plants (Fincher 2009).

2.3 The extraction method of (1,3;1,4)- β -glucan

The extraction process is vital for the maximum extraction of the (1,3;1,4)- β -glucan in the plants as the (1,3;1,4)- β -glucan occur in the cell wall of the plants. It is also important in the plant breeding programme as the breeder that produce barley plants for the beer production industry require barley grain that have low amount of (1,3;1,4)- β -glucan content. The extraction procedures that optimise the yield, purity and retained integrity of the (1,3;1,4)- β -glucan require the study of the physiochemical properties of the isolated β -glucan (Brennan and Cleary 2004). Following to the McCleary and Codd (1991), the enzymatic extraction process has been simplified to allow analysis up to 10 grain in samples in 70 minutes. The enzymatic extraction has the highest recovery of β -glucan due to the removal of the

protein and starch from the samples as the starch and protein appeared to be the major impurities in the samples (Ahmad *et al.* 2010). The extraction process of the (1,3;1,4)- β -glucan generally involve three basic steps which are (1) inactivation of the endogenous enzyme, (2) extraction of the (1,3;1,4)- β -glucan, (3) precipitation of the (1,3;1,4)- β -glucans (Brennan and Cleary 2004).

The use of the appropriate enzymes in the enzymatic process could reduce the intermolecular association of β -glucan with other components that result in high extractability of the (1,3;1,4)- β -glucan (Ahmad *et al.* 2010). The endogenous enzyme refer to the β -glucanase need to be inactivated as they are responsible for the degradation that could lead to the decrease in molecular weight and the functional properties of the extracted β -glucan (Irakli *et al.* 2004). The (1,3;1,4)- β -glucan is specifically depolymerised with a highly purified (1,3;1,4)- β -glucanase (lichenase) to tri-, tetra-, and higher degree of polymerisation oligosaccharides (McCleary and Glennie-Holmes 1985). The β -glucosidase also used in the enzymatic extraction process and it specifically and quantitatively hydrolysed the oligosaccharides to glucose (McCleary and Glennie-Holmes 1985).

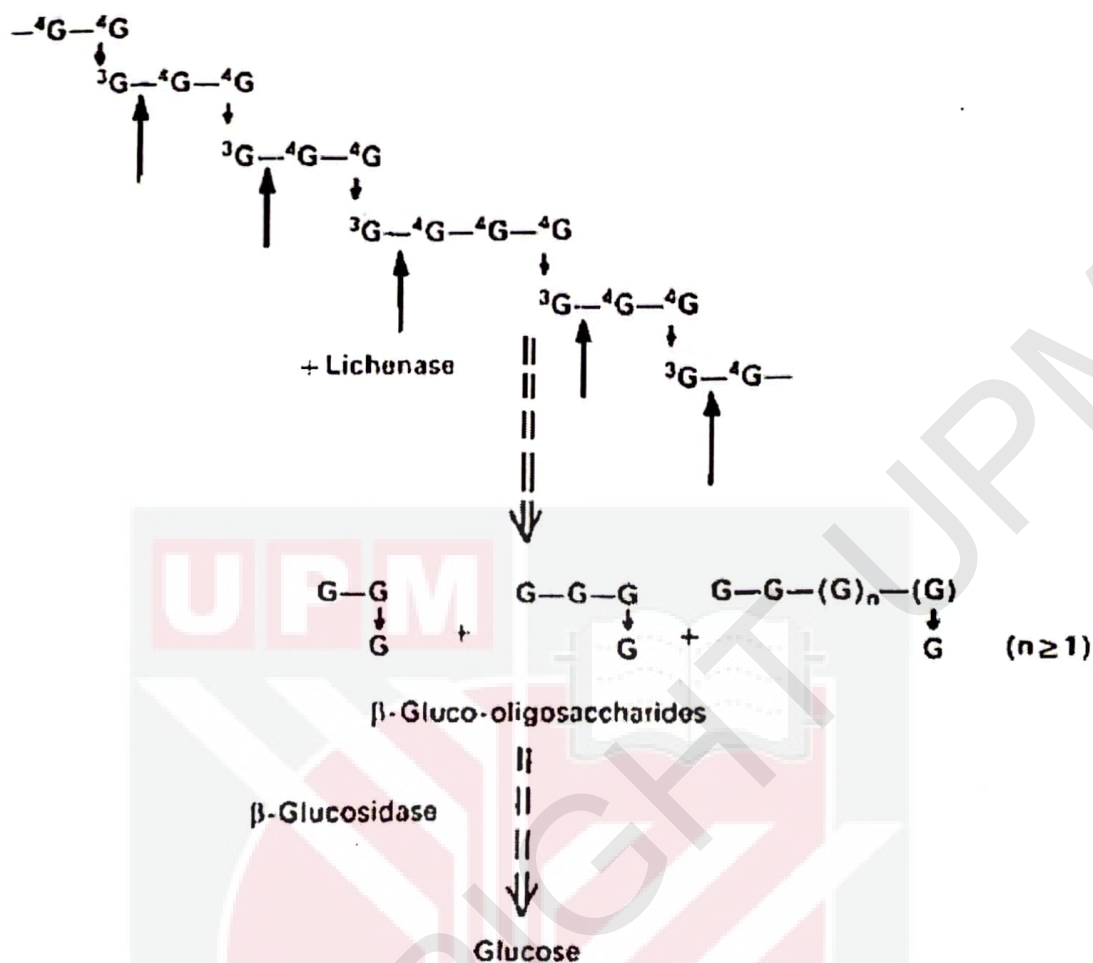


Figure 2.5 Diagram shows the theoretical basis of the (1,3;1,4)-β-glucan assay procedure (McCleary and Glennie-Holmes 1985).

Using the glucose oxidase/ peroxidase reagent, the glucose content can be measured (McCleary and Glennie-Holmes 1985). Vega-sanchez *et al.* (2013) had used the potassium hydroxide (KOH) to extract the (1,3;1,4)-β-glucan from the rice cell wall. Combination of 4 M KOH and enzyme-linked immunosorbent (ELISA) assay is able to extract more (1,3;1,4)-β-glucan from the plant cell wall. Studies revealed that the pH and temperature may have effect on the the extraction and functional properties of the (1,3;1,4)-β-glucan. Ahmad *et al.* (2010) also concluded that the enzymatic extraction method would extract (1,3;1,4)-β-glucan that have fair amount of mineral contents, have good water binding capacity, viscosity and whippability properties thus make it a good potential candidate for industrial usage.

CHAPTER 3

MATERIALS AND METHOD

3.1: Rice Cultivars

This study was conducted on five cultivars of Sarawak rice collected from various areas in Sarawak (Table 3.1).

Table 3. 1 The five cultivars used in this research project.

Cultivar	Cultivar's name
1	<i>Pulut bario asal</i>
2	<i>Bario banjal (setiam)</i>
3	<i>Boria (betong)</i>
4	<i>Ukong</i>
5	<i>Celum halus stumbin</i>

3.2: Sample Preparation

Before the extractions process started, rice cultivations were done to obtain the rice grain, stems and leaves. The seeds were undergoing sterilization using the 10% (v/v) Clorox (Clorox company, USA) and fungicide (Imas-Thiram 80) before planting process. The seeds were soaked with 10% v/v commercial Clorox solution for surface sterilization purpose while fungicide used to kill the fungus from infected the seeds after being sown. The seeds were then soaked in distilled water for overnight and then left to germinate on the moist tissue paper. The seeds were given adequate amount of water during the germination process. After being left for two days, the germinated seeds were sown into the pots that have measurement of 1.3 x 1.59 m. The pots were filled with soil that mixed with sand in the ratio of 3:1. For each pot, eight seeds were sown and two seeds will form one tiller. Sufficient amount of water

was provided for the plant growth and maintenance such as fertilizing and pesticide application were done to sustain the normal growth of the rice. The collections of sample were done after the grain had matured together with the stems and leaves.

3.3: (1,3;1,4)- β -glucan Extraction Process

The extraction process done using the method describe by the McCleary and Codd (1991) with the modifications to enable the analysis of small amount of the samples in 2-mL centrifuge tubes. For leaves and stems, 50-mg of the samples was required while 20-mg of grain samples were used in the extraction process. The collected samples were ground into fine powders using the liquid nitrogen and undergo freeze dried.

The extraction of (1,3;1,4)- β -glucan started with the washing samples using 75% (v/v) ethanol for 10 min at 95 °C with continues shaking of 1000 rpm using thermoshaker (Boeco, Germany). The samples were then centrifuge for 2 minutes at 1000 g and the step was repeated for three times. This process was to remove the free sugars and chlorophyll from the samples and only polysaccharides can be obtained. The (1,3;1,4)- β -glucan content were extracted using the Megazyme Mixed-Linkage Beta-Glucan assay kit (Megazyme International, Ireland). For the extraction process, 400 μ L (pH 6.5, 20 mM) of sodium phosphate buffer were added to samples. Then, the samples were stir and incubate using the thermoshaker at 97 °C with continues shaking at 1000 rpm for 30 minutes. Incubate the samples again at 50 °C and allow equilibrating for 15 minutes. The lichenase (20 μ L, 10 U) were added to each samples and incubated for another 90 minutes at 50 °C (1000 rpm). An amount of 400 μ L of sodium acetate buffer (200 mM, pH 4.0) was added and the samples were

stirred using the vortex mixer. The samples were equilibrated at room temperature for 5 minutes then centrifuged for 10 min at 16,000 g.

For (1,3;1,4)- β -glucan analysis, the supernatant will be divided into three 2-mL centrifuge tubes. Each centrifuge tubes contain 50 μ L of supernatant. First two tubes were added with the β -glucosidase and the third tube were act as control and added with the 50 μ L of 50 mM acetate buffer (pH 4.0). The samples were incubated for 30 minutes (1000 rpm) at 50 °C using the thermoshaker. Then, the glucose oxidase/ peroxidase (GOPOD) reagent were added to all the tubes and incubated again for 20 minutes at 50 °C. The absorbances were read at wavelength of 510 nm using the ELISA plate reader (Sunrise, Tecan).

For the potassium hydroxide (KOH) extraction method, the similar process will be done except that the sodium phosphate buffer will be replaced with 4 M potassium hydroxide (R&M chemicals, United Kingdom) with the amount of 400 μ l. The potassium hydroxide was then neutralised using the 400 μ l of 25 % (v/v) acetic acid (HmBG chemicals) before the lichenase were added to the samples.

The reading from ELISA plate reader was calculated using the formula stated as following,

For aqueous extraction method:

$$\begin{aligned}\beta - glucan \left(\% \frac{w}{w} \right) &= \Delta A \times F \times 18.4 \times \frac{1}{1000} \times \frac{100}{W} \times \frac{162}{180} \\ &= \Delta A \times \frac{F}{W} \times 1.656\end{aligned}$$

For potassium hydroxide (KOH) extraction method:

$$\begin{aligned}\beta - glucan \left(\% \frac{w}{w} \right) &= \Delta A \times F \times 26.4 \times \frac{1}{1000} \times \frac{100}{W} \times \frac{162}{180} \\ &= \Delta A \times \frac{F}{W} \times 2.376\end{aligned}$$

Where:

ΔA = Absorbance after β -glucosidase treatment (reaction) minus reaction blank absorbance.

F = A factor for the conversion of absorbance values to μg of glucose.

$$= \frac{[12.5 (\mu\text{g of } D\text{-glucose})]}{\text{absorbance of } 100 \mu\text{g of } D\text{-glucose}}$$

18.4 = Volume correction factor (0.05 mL out of 0.92 mL was analysed for samples).

26.4 = Volume correction factor (0.05 mL out of 1.32 mL was analysed for samples).

1/1000 = Conversion from μg to mg.

100/W = Factor to express β -glucan content as a percentage of dry flour weight.

W = The calculated dry weight of the samples analysed in mg.

162/180 = A factor to convert from free D-glucose, as determined, to anhydro-D-glucose, as occurs in β -glucan.

3.4: Statistical Analysis

The statistical analysis of this study was done using the Statistical Analysis System (SAS) version 9.2. Analysis of variance (ANOVA) and Completely Randomized Design (CRD) was used to compared the total (1,3;1,4)- β -glucan concentration extracted from both extraction method. The mean comparison using Tukey at $\alpha=0.05$ also was done to determine the comparison.

CHAPTER 4

RESULT

4.1 (1,3;1,4)- β -glucan Concentration in Different Rice cultivar.

The Table 4.1 show the mean of (1,3;1,4)- β -glucan concentration (% w/w) in different Sarawak rice cultivar extracted using different extraction method. The Figure 4.1- 4.3 show the concentration of (1,3;1,4)- β -glucan (% w/w) for different rice tissue in all rice cultivar. Across all rice tissue, there is variation on the concentration of (1,3;1,4)- β -glucan (% w/w) for both type of extraction method.

Different concentrations of (1,3;1,4)- β -glucan were extracted using different extraction method. In the grain, the highest concentration of (1,3;1,4)- β -glucan is 0.590 (% w/w), extracted at rice cv. *Bario banjal (Setiam)* while the lowest is *Ukong* 0.09 (% w/w) extracted at rice cv. *Bario banjal (Setiam)*. The KOH extraction method extract the highest (1,3;1,4)- β -glucan concentration in the grain and for the lowest concentration is extracted by aqueous extraction method. Overall, KOH extraction extract more (1,3;1,4)- β -glucan in comparison to aqueous extraction buffer.

Table 4.1 Mean of (1,3;1,4)- β -glucan concentration (% w/w) (\pm standard error) extracted at different rice tissue across all rice cultivar.

	Grain			Leaf			Stem		
	Aqueous	KOH		Aqueous	KOH		Aqueous	KOH	
Pulut bario asal	0.234 ^a \pm 0.114	0.510 ^a \pm 0.284		0.208 ^a \pm 0.043	0.163 ^a \pm 0.007		1.075 ^b \pm 0.050	3.554 ^a \pm 0.290	
Bario banjal(Setiam)	0.096 ^a \pm 0.017	0.590 ^a \pm 0.068		0.191 ^a \pm 0.015	0.216 ^a \pm 0.051		1.180 ^b \pm 0.305	4.768 ^a \pm 0.063	
Boria (Betong)	0.126 ^b \pm 0.003	0.556 ^a \pm 0.079		0.247 ^b \pm 0.044	0.788 ^a \pm 0.129		1.469 ^b \pm 0.079	4.637 ^a \pm 0.792	
Ukong	0.090 ^a \pm 0.053	0.567 ^a \pm 0.340		0.116 ^b \pm 0.040	0.729 ^a \pm 0.046		1.403 ^b \pm 0.003	3.478 ^a \pm 0.061	
Celum halus stumbin	0.126 ^a \pm 0.036	0.552 ^a \pm 0.283		0.071 ^a \pm 0.018	0.848 ^a \pm 0.399		1.112 ^b \pm 0.144	4.580 ^a \pm 0.885	

Means with the same letter in the same row are not significantly different using tukey test at $\alpha=0.0$

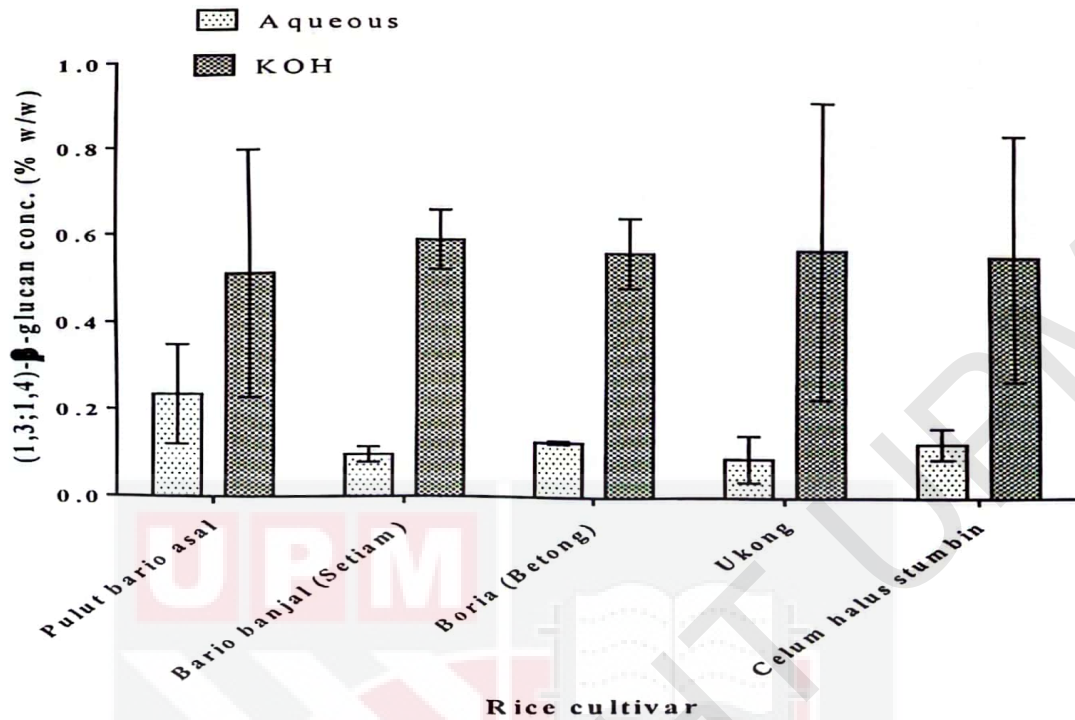


Figure 4.1 Mean (\pm standard error) for (1,3;1,4)- β -glucan concentration (% w/w) extracted in grain using different method across the Sarawak rice cultivar.

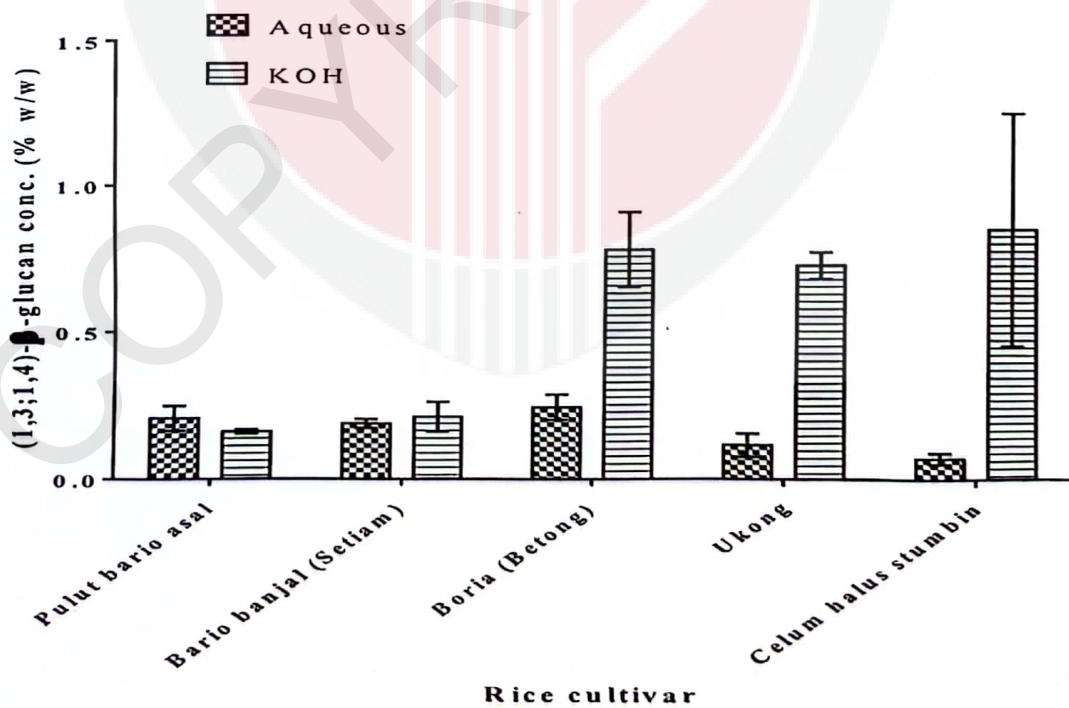


Figure 4.2 Mean (\pm standard error) for (1,3;1,4)- β -glucan concentration (% w/w) extracted in leaf using different method across different Sarawak rice cultivar.

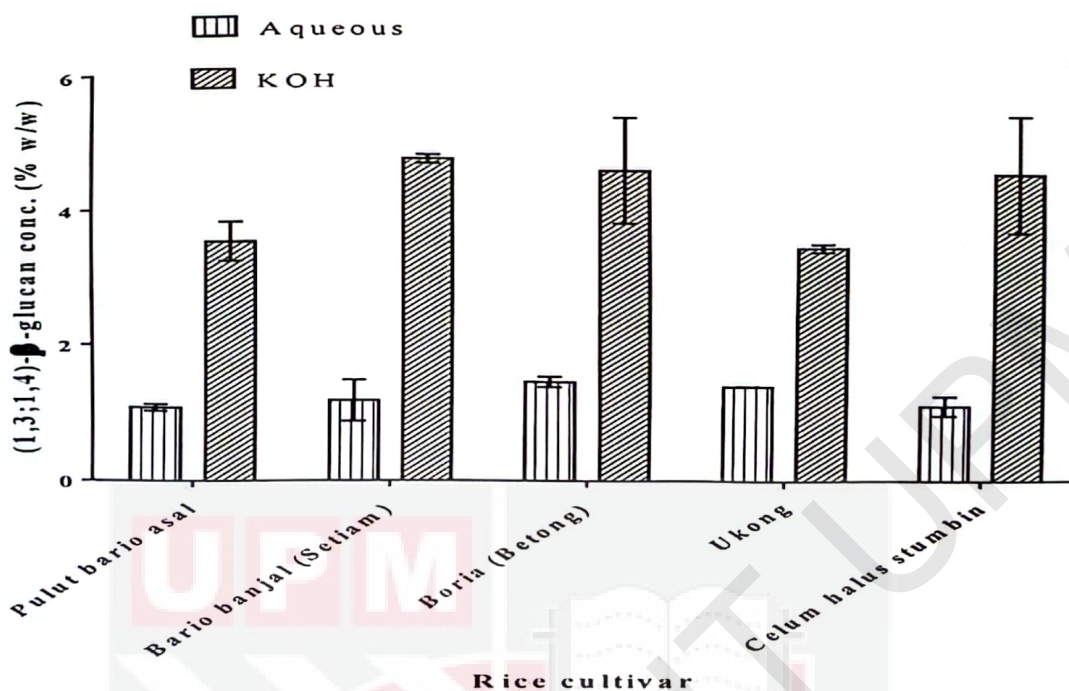


Figure 4.3 Mean (\pm standard error) for (1,3;1,4)- β -glucan concentration (% w/w) extracted in stem using different method across different rice cultivar.

In leaf, both extraction method have different effect toward concentration of (1,3;1,4)- β -glucan. For the rice cv. *Pulut bario asal*, the aqueous buffer extracted slightly higher concentration of (1,3;1,4)- β -glucan compared to KOH extraction method. However, the other cultivar shows high concentration of (1,3;1,4)- β -glucan extracted using KOH extraction. The highest concentration of (1,3;1,4)- β -glucan found in leaf is 0.848 % (w/w), extracted at rice cv. *Celum halus stumbin* through KOH extraction method and the lowest is 0.116 % (w/w) found in leaf of rice cv. *Ukong* extracted through aqueous extraction method.

In stem, the KOH extraction method extract the highest concentration of (1,3;1,4)- β -glucan in all of the rice cultivar used. The highest concentration is extracted at rice cv. *Bario banjal (Setiam)* with concentration of 4.768 (% w/w) while the lowest extracted at rice cv. *Pulut bario asal* with the concentration of 1.075 (% w/w).

4.2 (1,3;1,4)- β -glucan Concentration in Different Rice Tissue.

The Table 4.2 below show the mean of the (1,3;1,4)- β -glucan concentration (% w/w) extracted using different method for each Sarawak rice cultivar at different rice tissue. The Figure 4.4- 4.8 were plotted to show the different concentration of (1,3;1,4)- β -glucan (% w/w) for each rice tissue at rice cultivar.

For both extraction methods, different rice tissues in each cultivar give different effect toward the concentration of (1,3;1,4)- β -glucan. Stem has high concentration of (1,3;1,4)- β -glucan compared to grain and leaf. The highest concentration of (1,3;1,4)- β -glucan is in rice cv. *Bario banjal (Setiam)* which is 4.768 (% w/w) and the lowest is in stem of rice cv *Pulut bario asal* which is at 1.075 (% w/w). For the overall rice tissue, the KOH extraction method had extracted more concentration of (1,3;1,4)- β -glucan except for rice cv. *Pulut bario asal*. In this cultivar, leaf has more concentration of (1,3;1,4)- β -glucan when extracted using aqueous buffer. The high concentration of (1,3;1,4)- β -glucan in stem could be due functional properties of the (1,3;1,4)- β -glucan.

Table 4.2 Mean of (1,3;1,4)- β -glucan concentration (% w/w) (\pm standard error) extracted at different rice tissue using different method of extraction.

	Pulut bario asal		Bario banjal (Setiam)		Boria (Betong)		Ukong		Celum halus stumbin	
	Aqueous	KOH	Aqueous	KOH	Aqueous	KOH	Aqueous	KOH	Aqueous	KOH
Grain	0.234 ^a \pm 0.1	0.51 ^a \pm 0.28	0.096 ^a \pm 0.0	0.59 ^a \pm 0.06	0.126 ^b \pm 0.0	0.556 ^a \pm 0.0	0.09 ^a \pm 0.05	0.567 ^a \pm 0.3	0.126 ^a \pm 0.0	0.552 ^a \pm 0.2
	14	4	17	8	03	79	3	4	36	83
Leaf	0.208 ^a \pm 0.0	0.163 ^a \pm 0.0	0.191 ^a \pm 0.0	0.216 ^a \pm 0.0	0.247 ^b \pm 0.0	0.788 ^a \pm 0.1	0.116 ^b \pm 0.0	0.729 ^a \pm 0.0	0.071 ^a \pm 0.0	0.848 ^a \pm 0.3
	43	07	15	51	44	29	4	46	18	99
Stem	1.075 ^b \pm 0.0	3.554 ^a \pm 0.2	1.18 ^b \pm 0.30	4.768 ^a \pm 0.0	1.469 ^b \pm 0.0	4.637 ^a \pm 0.7	1.403 ^b \pm 0.0	3.478 ^a \pm 0.0	1.112 ^b \pm 0.1	4.58 ^a \pm 0.88
	5	9	5	63	79	92	03	61	44	5

Means with the same letter in the row are not significantly different using tukey test at $\alpha=0.05$.

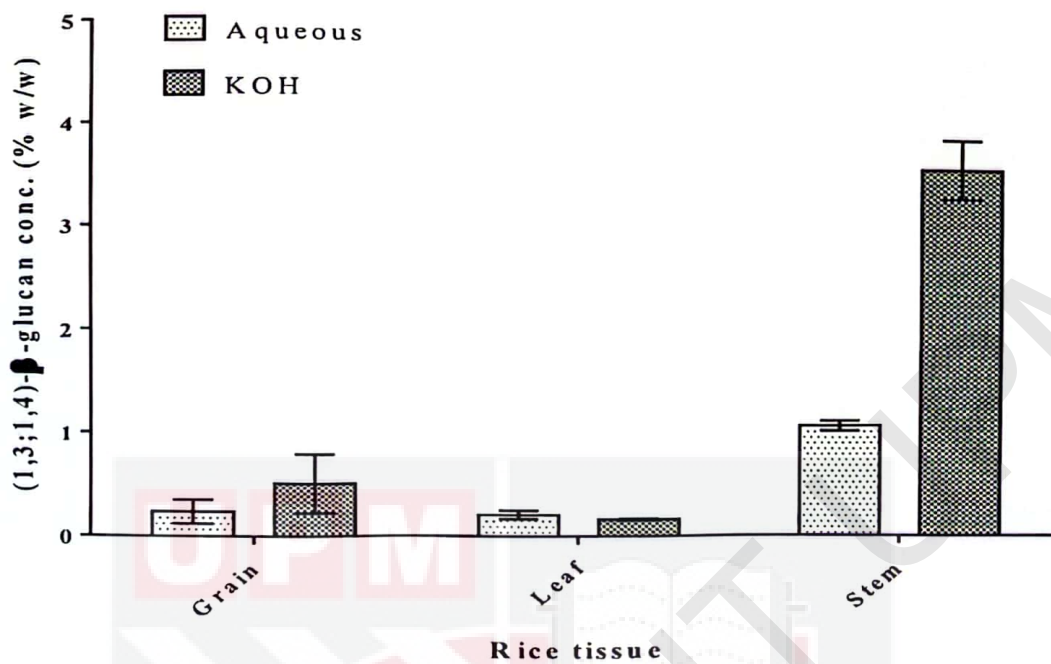


Figure 4.4 Mean (\pm standard error) for (1,3;1,4)- β -glucan concentration (% w/w) extracted using different method in rice tissue of *Pulut bario asal*.

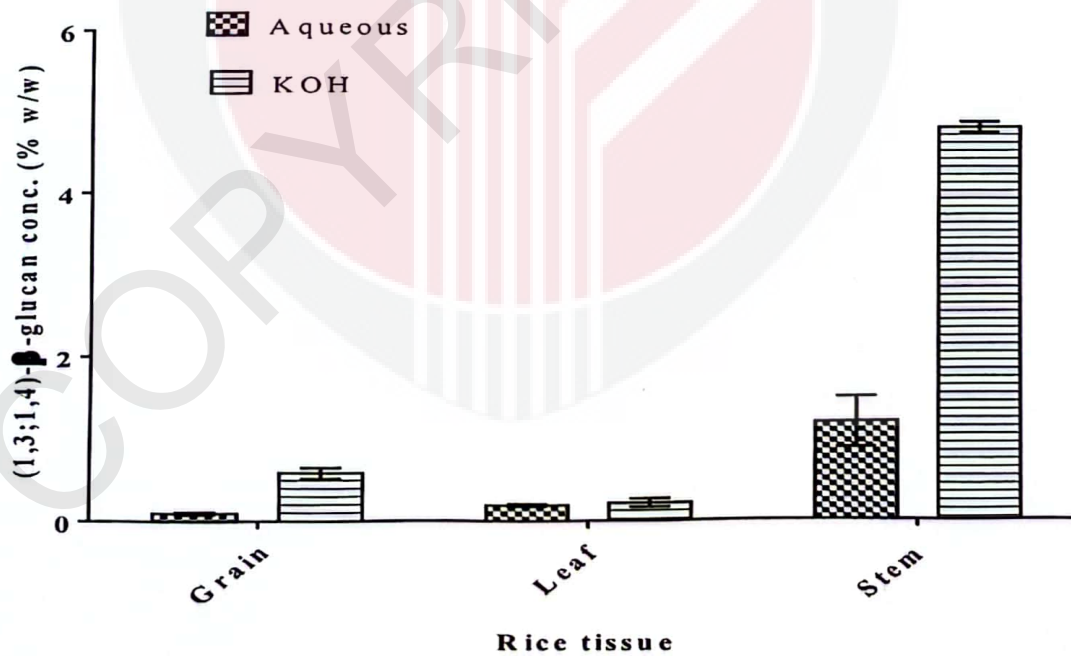


Figure 4.5 Mean (\pm standard error) for (1,3;1,4)- β -glucan concentration (% w/w) extracted using different method in rice tissue of *Bario banjal (Setiam)*.

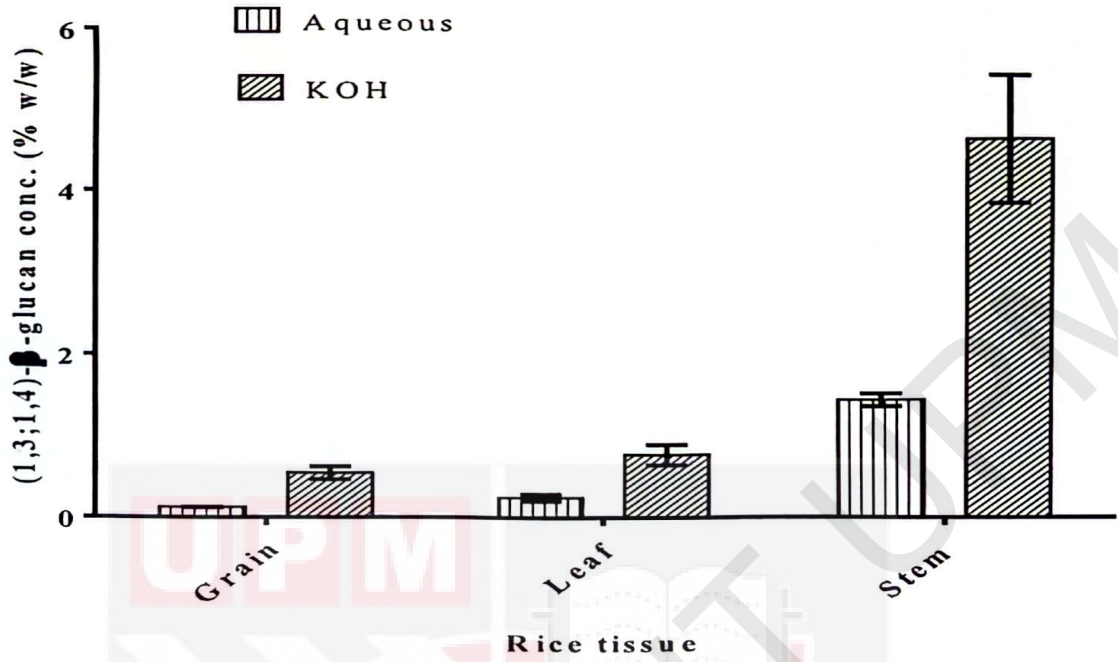


Figure 4.6 Mean (\pm standard error) for (1,3;1,4)- β -glucan concentration (% w/w) extracted using different method in rice tissue of *Boria (Betong)*.

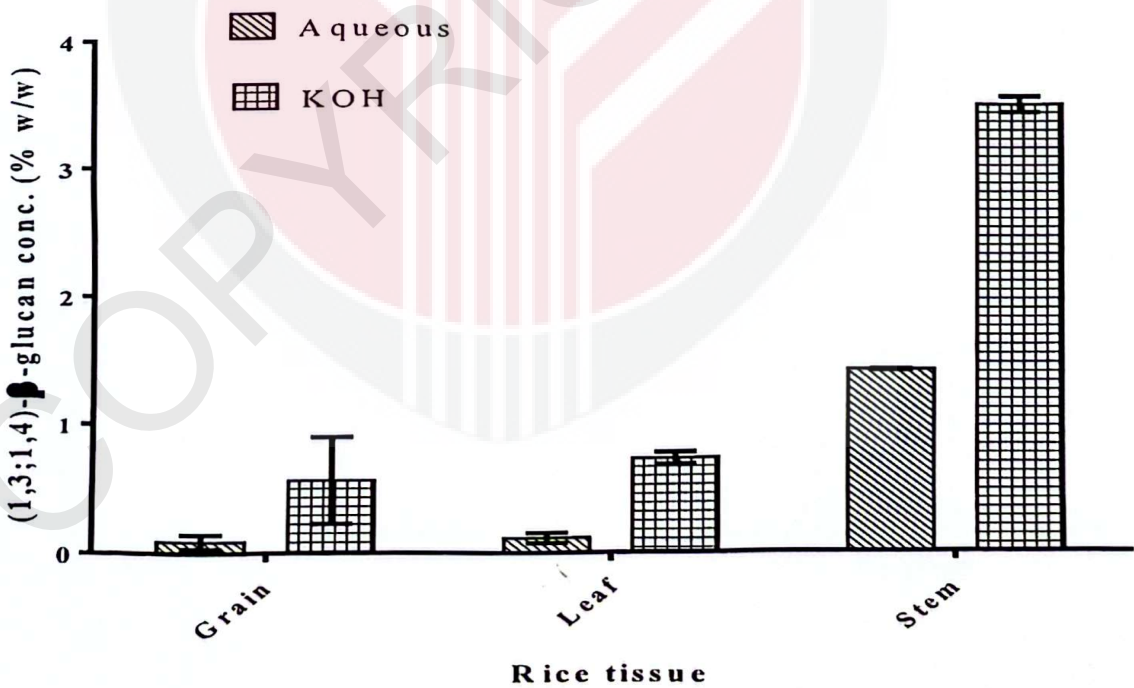


Figure 4.7 Mean (\pm standard error) for (1,3;1,4)- β -glucan concentration (% w/w) extracted using different method in rice tissue of *Ukong*.

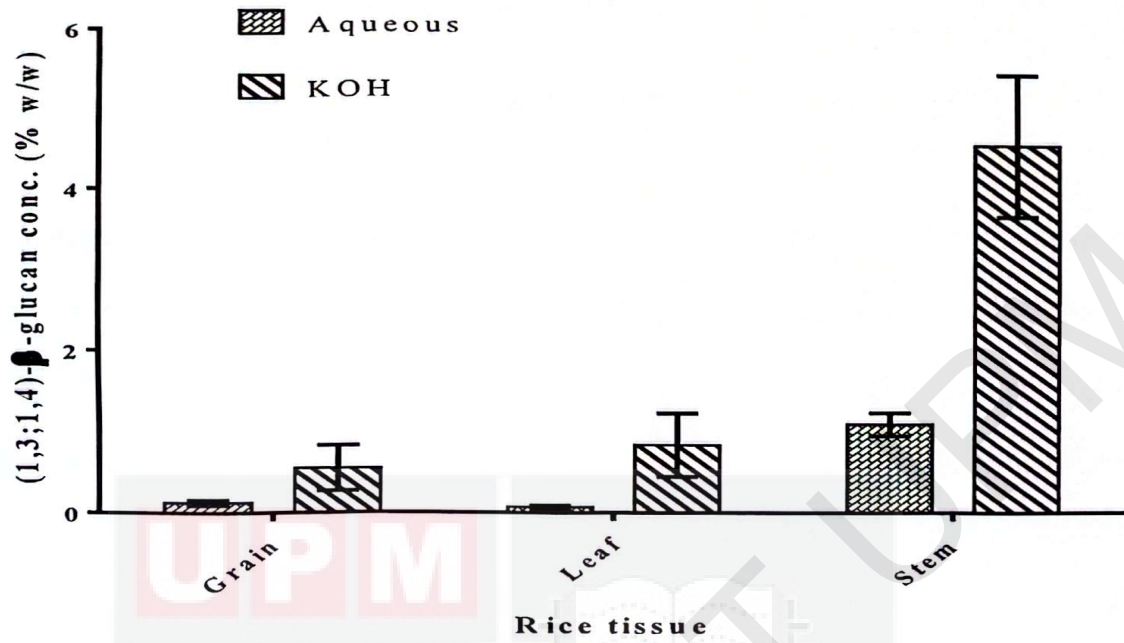


Figure 4.8 Mean (\pm standard error) for (1,3;1,4)- β -glucan concentration (% w/w) extracted using different method in rice tissue of *Celum halus stumbin*.

CHAPTER 5

DISCUSSION

From the result that had been obtained, in Figure 4.3, stem has shown that all cultivar has significant difference in concentration of (1,3;1,4)- β -glucan (% w/w) extracted from both type of extraction. The potassium hydroxide extraction method extract more concentration of (1,3;1,4)- β -glucan (% w/w) compared to the aqueous extraction method for all the stem samples. The range of (1,3;1,4)- β -glucan concentration (% w/w) extracted in the stem is at the range of 1.075 to 4.768. The high (1,3;1,4)- β -glucan content in stem indicates that the (1,3;1,4)- β -glucan can be presence in the secondary cell wall and mature tissue of stem in rice as stated by Vega-Sanchez *et al.* (2013). (1,3;1,4)- β -glucan may contribute to the flexibility properties of the cell wall and also to the cell wall strengthening properties in the mature stem (Vega-Sanchez *et al.* 2012). In this project, the KOH extraction method also had extracted more concentration of (1,3;1,4)- β -glucan compared to aqueous. This shows that the stem has high concentration of less soluble (1,3;1,4)- β -glucan. The less soluble of (1,3;1,4)- β -glucan can be extracted using KOH extraction as had been discussed earlier.

For the leaf, the highest concentration of (1,3;1,4)- β -glucan is obtained by the KOH extraction method. The content of extracted (1,3;1,4)- β -glucan in the leaf is at range of 0.071 % to 0.848 % (w/w). Vega-Sanchez *et al.* (2013) also has shown that the (1,3;1,4)- β -glucan is present in the mature, senescing tissue of leaves. The author also suggest that the presence of (1,3;1,4)- β -glucan in mature leaves might indicate the strengthening role of the cell wall. The presence of (1,3;1,4)- β -glucan in the leaf

also might indicate that the (1,3;1,4)- β -glucan may serve as an energy source for the plants when it is under sugar depletion as reported by the Roulin *et al.* (2002) in young barley leaves. Slightly higher concentration of (1,3;1,4)- β -glucan was extracted using the aqueous extraction method in leaf of the *Pulut bario asal* had extracted. This result could be due to technical factors as KOH extraction is more vigorous extraction method.

In grain parts, the range of (1,3;1,4)- β -glucan concentration extracted in grain is consistent with the report previously. According to Demirbas (2005), the (1,3;1,4)- β -glucan concentration (% w/w) in rice grain is on the range of 0.4 to 0.9. The highest concentration is 0.59 (% w/w) and lowest is 0.09 (% w/w). The KOH extraction method also had extracted more concentration of (1,3;1,4)- β -glucan in grain. The grain had been said to serve as an energy storage polysaccharide and utilise when seed germinate.

In all rice tissue analysed, there is high deviation of the concentration of (1,3;1,4)- β -glucan. This could be due to amount of sample used in the extraction process. This project had undergone scale of the measurement and only small amount of samples were being used. This could cause the less sensitive of detection of (1,3;1,4)- β -glucan content in the samples. Therefore, the amount of samples could be increase to further reducing the variation and increase the preciseness of the result. The use of ELISA plate reader to measure the concentration of (1,3;1,4)- β -glucan can also affect the reading. Using this method, only 150 μ l of extraction samples were analysed. The small amount may not be able to represent total concentration of (1,3;1,4)- β -glucan in samples. Thus, in the future study, the reading of (1,3;1,4)- β -glucan concentration

could be change by using UV-vis spectrophotometer. The UV-vis spectrophotometer can analysed higher amount of samples than ELISA plate reader. As this study was also preliminary study of (1,3;1,4)- β -glucan in Sarawak rice cultivar, the equipment used in the extraction process need to be further calibrated. The use of more appropriate equipment also should be considered in the project.

In the future, the research could be expanded for all Sarawak rice cultivar. This could help in better understanding about the Sarawak rice cultivar. The information regarding the amount of (1,3;1,4)- β -glucan and best extraction method for the rice cultivar could help in developing better rice quality. As been reported so far, the rice grain have low amount of (1,3;1,4)- β -glucan compared to other cereals, thus we may want to produce rice grain that have high (1,3;1,4)- β -glucan concentration. This could help in improving the health of consumer as the (1,3;1,4)- β -glucan is a dietary fiber that have health benefits towards the human.

CHAPTER 6

CONCLUSION

From this research, we had found that the (1,3;1,4)- β -glucan content in Sarawak rice cultivar accumulate in similar concentration with the other rice cultivar from the previous work. The different extraction method of the (1,3;1,4)- β -glucan also show different concentration of (1,3;1,4)- β -glucan in the cultivar. The (1,3;1,4)- β -glucan content across the cultivar show that stem has high amount of (1,3;1,4)- β -glucan extracted. In stem, the (1,3;1,4)- β -glucan is highly extracted through the KOH extraction method.

There is high variation in concentration of (1,3;1,4)- β -glucan in all samples. To alter this, several possible corrections in procedure could be done toward extraction process. The amount of samples play important role in obtaining more precise concentration of (1,3;1,4)- β -glucan. The scale down of measurement could have effect on the concentration of (1,3;1,4)- β -glucan in all rice tissue.

REFERENCES

Ahmad, A., Anjum, F. M., Zahoor, T., Nawaz, H., and Ahmad, Z. 2010. Extraction and characterization of B-D-glucan from oat for industrial utilization. *International Journal of Biological Macromolecules*, 46 (3): 304–9.

Arai-Sanoh, Y., Ida, M., Zhao, R., Nishitani, K., Yoshinaga, S., Takai, T., Nakano, H., Iwasawa, N., and Kondo, M. 2013. Varietal differences in cell wall Beta-(1,3),(1,4)-Glucan and nonstructural carbohydrate in rice stems during the grain filling stage. *Plant Production Science*, 16 (4): 335–41.

Benito-Román O., Alonso E., and Lucas S. 2011. Optimization of the B-Glucan extraction conditions from different waxy barley cultivars. *Journal of Cereal Science*, 53 (3): 271–76.

Benito-Román, ó., Alonso E., Palacio L., Pradanos P., and Cocero M. J. 2014. Purification and isolation of B-Glucans from barley: Downstream process intensification. *Chemical Engineering and Processing: Process Intensification*, 84 (October): 90–97.

Brennan, C. S., and Louise J. C. 2005. The potential use of cereal (1→3,1→4)-B-D-glucans as functional food ingredients. *Journal of Cereal Science*, 42 (1): 1–13.

Burton, R. A., and Fincher, G. B. 2012. Current challenges in cell wall biology in the cereals and grasses. *Frontiers in Plant Science*, 3: 130

Burton, R., A., Ma, G., Baumann, U., Harvey, A., J., Neil J. S., Taylor, J., Pettolino, F. 2010. A customized gene expression microarray reveals that the brittle stem phenotype fs2 of barley is attributable to a retroelement in the HvCesA4 Cellulose Synthase Gene. *Plant Physiology*, 153 (4): 1716–28.

Burton, R. A., Sarah, M. W., Maria, H., Andrew, J. H., Neil, J. S., Anne Medhurst, Bruce, A. S., Edward, J. N., Bacic A., and Geoffrey, B. F. 2006. Cellulose Synthase-Like CslF genes mediate the synthesis of cell wall (1,3;1,4)-β-D-Glucans. *Science*, 311 (5769): 1940–42.

Burton, R.A., Collins, H.M., Kibble, N.A.J., Smith, J.A., Shirley, N.J., Jobling, S.A., Henderson, M. 2011. Over-expression of specific HvCslF Cellulose Synthase-like

Genes in transgenic barley increases the levels of cell wall (1,3;1,4)-B-D-Glucans and alters their fine structure. *Plant Biotechnology Journal*, 9 (2): 117–35.

Burton, R. A., and G. B. Fincher. 2009. (1,3;1,4)-B -D-Glucans in cell walls of the Poaceae, lower plants, and fungi: A tale of two linkages. *Molecular Plant*, 2 (5): 873–82.

Collins H. M., Burton, R. A., David L. T., Liao M., Bacic A., and Ficher, G. B. 2010. Review: Variability in fine structures of noncellulosic cell wall polysaccharides from cereal grains: Potential Importance in human health and nutrition. *Cereal Chemistry Journal*, 87 (4): 272–82.

Danielson, M. E., Dauth, R., Elmasry, N. A., Langesley R. R., Magee, A. S., and Will, P. M. 2010. Enzymatic method to measure B-1,3-B-1,6-Glucan content in extracts and formulated products (GEM Assay). *Journal of Agricultural and Food Chemistry*, 58 (19): 10305–8.

Demirbas, A. 2005. B-Glucan and mineral nutrient contents of cereals grown in Turkey. *Food Chemistry*, 90 (4): 773–77.

Doblin, M. S., Pettolino, F. A., Wilson, S. M., Campbell, R., Burton, R. A., Fincher, G. B., Newbigin, E., and Bacic, A. 2009. A barley Cellulose Synthase-like CSLH gene mediates (1,3;1,4)- β -D-Glucan synthesis in transgenic Arabidopsis. *Proceedings of the National Academy of Sciences*, 106 (14): 5996–6001.

Fincher, G. B. 2009. Revolutionary times in our understanding of cell wall biosynthesis and remodeling in the grasses. *Plant Physiology*, 149 (1): 27–37.

Irakli, M., Biliaderis, C. G., Izydorczyk, M. S., and Papadoyannis, I. N. 2004. Isolation, structural features and rheological properties of water-extractable- β -Glucans from different greek barley cultivars. *Journal of the Science of Food and Agriculture*, 84 (10): 1170–78.

Lazaridou, A, Biliaderis, C.G., Micha-Screttas M., and Steele, B.R. 2004. A comparative study on structure–function relations of mixed-linkage (1→3), (1→4) inear B-D-Glucans. *Food Hydrocolloids*, 18 (5): 837–55.

McCleary, B. V., and Glennie-Holmes, M. 1985. Enzymic quantification of (1→3) (1→4)-B-D-Glucan in barley and malt. *Journal of the Institute of Brewing*, 91 (5): 285–95.

McCleary, B. V, and Codd, R. 1991. Measurement of (1 → 3),(1 → 4)-B-D-Glucan in barley and oats: A Streamlined Enzymic Procedure. *Journal of the Science of Food and Agriculture*, 55 (2): 303–12.

McLaren, J. S. 2005. Crop biotechnology provides an opportunity to develop a sustainable future. *Trends in Biotechnology*, 23(7): 339–42.

Schreiber, M., Wright, F. 2014. The barley genome sequence assembly reveals three additional members of the Cs1F (1,3;1,4)-B-Glucan Synthase Gene family. *PloS One*, 9 (3): e90888.

Popper, Z. A., and Fry S. C. 2003. Primary cell wall composition of Bryophytes and Charophytes. *Annals of Botany*, 91 (1): 1–12.

Roulin, S., Buchala, A., and Fincher, G. B. 2002. Induction of (1→3,1→4)-β-D-Glucan hydrolases in leaves of dark-incubated barley seedlings. *Planta*, 215 (1): 51–59.

Staudte, R.G., Woodward, J.R., Fincher, G.B., and Stone, B.A. 1983. Water-Soluble (1→3), (1→4)-B-D-Glucans from barley (*Hordeum Vulgare*) endosperm. III. Distribution of cellotriosyl and cellotetraosyl residues. *Carbohydrate Polymers*, 3 (4): 299–312.

Tosh, S. 2004. Structural characteristics and rheological properties of partially hydrolyzed oat B-Glucan: The effects of molecular weight and hydrolysis method. *Carbohydrate Polymers*, 55(4): 425–36.

Vega-Sanchez, M. E., Verherbruggen, Y., Christensen, U., Chen, X., Sharma, V., Varanasi, P., Jobling, S. A. 2012. Loss of cellulose Synthase-Like F6 function affects mixed-linkage glucan deposition, cell wall mechanical properties, and defense responses in vegetative tissues of rice. *Plant Physiology*, 159 (1): 56–69.

Vega-Sánchez, M. E., Verhertbruggen, Y., Scheller, H. V., and Ronald, P. C. 2013. Abundance of mixed linkage glucan in mature tissues and secondary cell walls of grasses. *Plant Signaling & Behavior*, 8 (2): e23143.

Wang, L., Guo, K., Li, Y., Tu, Y., Hu, H., Wang, B., Cui, X., and Peng, L. 2010. Expression profiling and integrative analysis of the CESA/CSL Superfamily in rice. *BMC Plant Biology*, 10 (1): 1–16.

Woodward, J.R., Fincher, G.B., and Stone, B.A. 1983. Water-soluble (1→3), (1→4)-B-D-Glucans from barley (*Hordeum Vulgare*) endosperm. II. fine structure. *Carbohydrate Polymers*, 3 (3): 207–25.



APPENDICES

Appendix 1 Table of raw data of aqueous extraction of grain, leaf and stem respectively.

Cultivar	Sample	Absorbance value			β -glucan (% w/w)	Adjusted % (w/w)	
		Blank	Sample	Δ Abs			
1	Grain (G1)	0.066	0.063	0.127	0.029	0.09421	0.3487
	Grain (G2)	0.071	0.067	0.095	0.01	0.03249	0.1203
	Grain (G3)	0.06	0.063	0.061	0.002	0.006497	0.02405
2	Grain(G1)	0.046	0.057	0.056	0.0105	0.03411	0.1263
	Grain (G2)	0.051	0.056	0.057	0.0055	0.01787	0.06614
	Grain (G3)	0.047	0.054	0.056	0.008	0.02599	0.0962
3	Grain(G1)	0.052	0.061	0.063	0.01	0.03249	0.1203
	Grain (G2)	0.05	0.061	0.06	0.0105	0.03411	0.1263
	Grain (G3)	0.049	0.053	0.067	0.011	0.03573	0.1322
4	Grain(G1)	0.063	0.069	0.068	0.0055	0.01787	0.06614
	Grain (G2)	0.059	0.071	0.079	0.016	0.05198	0.1924
	Grain (G3)	0.067	0.068	0.068	0.001	0.003249	0.01203
5	Grain(G1)	0.054	0.055	0.053	0	0	0
	Grain (G2)	0.048	0.058	0.065	0.0135	0.04386	0.1623
	Grain (G3)	0.048	0.051	0.06	0.0075	0.02436	0.0902
	Barley (B1)	0.041	0.385	0.372	0.3375	1.0964	
	Barley (B2)	0.044	0.391	0.381	0.342	1.111	
	Barley (B3)	0.046	0.372	0.357	0.3185	1.03468	
	D-glucose standard		0.297	0.411	0.354		

Cultivar	Sample	Absorbance value			β -glucan (% w/w)	Adjusted % (w/w)	
		Blank	Sample	Δ Abs			
1	Leaves (L1)	0.062	0.252	0.246	0.187	0.174	0.2893
	Leaves (L2)	0.065	0.191	0.185	0.123	0.1144	0.1902
	Leaves (L3)	0.085	0.178	0.179	0.0935	0.08699	0.1446
2	Leaves (L1)	0.068	0.199	0.188	0.1255	0.1168	0.1942
	Leaves (L2)	0.075	0.203	0.158	0.1055	0.09815	0.1632
	Leaves (L3)	0.096	0.233	0.239	0.14	0.1302	0.2165
3	Leaves (L1)	0.104	0.205	0.216	0.1065	0.09908	0.1647
	Leaves (L2)	0.058	0.257	0.265	0.203	0.1889	0.3140

	Leaves (L3)	0.051	0.212	0.231	0.1705	0.1586	0.2637
4	Leaves (L1)	0.09	0.213	0.22	0.1265	0.1177	0.1957
	Leaves (L2)	0.071	0.116	0.127	0.0505	0.04698	0.0781
	Leaves (L3)	0.066	0.11	0.118	0.048	0.04466	0.07425
5	Leaves (L1)	0.055	0.087	0.089	0.033	0.0307	0.05104
	Leaves (L2)	0.05	0.119	0.12	0.0695	0.06466	0.1075
	Leaves (L3)	0.061	0.094	0.097	0.0345	0.0321	0.05337
	Barley (B1)	0.043	1.082	1.056	1.0035	2.5933	
	Barley (B2)	0.049	0.891	0.903	0.848	2.1915	
	Barley (B3)	0.044	0.96	1.011	0.9415	2.4331	
	D-glucose standard		0.444	0.446	0.445		

Cultivar	Sample	Absorbance value			β -glucan (% w/w)	Adjusted % (w/w)
		Blank	Sample	Δ Abs		
1	Stem (S1)	0.178	0.889	0.879	0.706	1.0492
	Stem (S2)	0.257	1.056	1.035	0.7885	1.1719
	Stem (S3)	0.306	0.988	0.975	0.6755	1.00404
2	Stem (S1)	0.705	1.049	1.129	0.384	0.5708
	Stem (S2)	0.06	1.115	1.021	0.975	1.4491
	Stem (S3)	0.07	1.086	1.101	1.0235	1.5212
3	Stem (S1)	0.067	1.126	1.138	1.065	1.5829
	Stem (S2)	0.07	1.132	1.036	1.014	1.5070
	Stem (S3)	0.072	0.975	0.941	0.886	1.3168
4	Stem (S1)	0.069	1.045	0.979	0.943	1.4015
	Stem (S2)	0.066	1.018	0.996	0.941	1.3986
	Stem (S3)	0.061	1.01	1.009	0.9485	1.4097
5	Stem (S1)	0.071	0.765	0.603	0.613	0.9111
	Stem (S2)	0.06	0.997	0.996	0.9365	1.3918
	Stem (S3)	0.096	0.746	0.836	0.695	1.0329
	Barley (B1)	0.044	1.058	1.094	1.032	2.6403
	Barley (B2)	0.05	0.9623	0.956	0.9092	2.3261
	Barley (B3)	0.049	1.013	0.986	0.9655	2.4701
	D-glucose standard		0.453	0.446	0.4495	

Appendix 2 Table of raw data of KOH extraction of grain, leaf and stem respectively.

Cultivar	Sample	Absorbance value			β -glucan (% w/w)	Adjusted % (w/w)	
		Blank	Sample	Δ Abs			
1	Grain (G1)	0.085	0.1	0.08	0.005	0.01948	0.2268
	Grain (G2)	0.1	0.104	0.131	0.0175	0.06818	0.7939
	Grain (G3)	0.114	0.089	0.096	-0.0215	0	0
2	Grain(G1)	0.111	0.146	0.099	0.0115	0.04481	0.5218
	Grain (G2)	0.066	0.074	0.087	0.0145	0.05649	0.6578
	Grain (G3)	0.088	0.068	0.059	-0.0245	0	0
3	Grain(G1)	0.085	0.067	0.067	-0.018	0	0
	Grain (G2)	0.053	0.065	0.062	0.0105	0.04091	0.4764
	Grain (G3)	0.052	0.061	0.071	0.014	0.05455	0.6352
4	Grain(G1)	0.058	0.084	0.072	0.02	0.07792	0.9074
	Grain (G2)	0.064	0.064	0.074	0.005	0.01948	0.2268
	Grain (G3)	0.102	0.068	0.075	-0.0305	0	0
5	Grain(G1)	0.052	0.063	0.063	0.011	0.04286	0.4991
	Grain (G2)	0.051	0.091	0.058	0.0235	0.09156	1.0662
	Grain (G3)	0.053	0.054	0.056	0.002	0.007792	0.0904
	Barley (B1)	0.07	0.153	0.159	0.086	0.3351	
	Barley (B2)	0.064	0.121	0.14	0.0665	0.2591	
	Barley (B3)	0.058	0.173	0.167	0.112	0.4364	
	D-glucose standard		0.427	0.42	0.4235		

Cultivar	Sample	Absorbance value			β -glucan (% w/w)	Adjusted % (w/w)	
		Blank	Sample	Δ Abs			
1	Leaves (L1)	0.054	0.101	0.103	0.048	0.06954	0.1628
	Leaves (L2)	0.054	0.094	0.103	0.0445	0.06447	0.15095
	Leaves (L3)	0.069	0.113	0.128	0.0515	0.07461	0.1747
2	Leaves (L1)	0.066	0.104	0.111	0.0415	0.06012	0.1408
	Leaves (L2)	0.05	0.109	0.105	0.057	0.08258	0.1934
	Leaves (L3)	0.054	0.101	0.192	0.0925	0.134	0.3137
3	Leaves (L1)	0.045	0.334	0.344	0.294	0.4259	0.9972
	Leaves (L2)	0.053	0.296	0.29	0.24	0.3477	0.8141
	Leaves (L3)	0.054	0.212	0.222	0.163	0.2362	0.553
4	Leaves (L1)	0.076	0.304	0.314	0.233	0.3376	0.7905
	Leaves (L2)	0.061	0.291	0.278	0.2235	0.3238	0.7581
	Leaves (L3)	0.075	0.267	0.26	0.1885	0.2731	0.6394

5	Leaves (L1)	0.063	0.165	0.696	0.3675	0.5324	1.2466
	Leaves (L2)	0.063	0.19	0.201	0.1325	0.192	0.4495
	Leaves (L3)	1.074	0.189	0.121	-0.919	0	0
	Barley (B1)	0.08	0.423	0.451	0.357	1.4367	
	Barley (B2)	0.061	0.487	0.476	0.4205	1.6923	
	Barley (B3)	0.034	0.558	0.502	0.496	1.9961	
	D-glucose standard		0.441	0.379	0.41		

Cultivar	Sample	Absorbance value			Δ Abs	β -glucan (% w/w)	Adjusted % (w/w)
		Blank	Sample				
1	Stem (S1)	0.052	0.35	0.267	0.2565	0.3568	3.6633
	Stem (S2)	0.053	0.368	0.295	0.2795	0.3888	3.9918
	Stem (S3)	0.05	0.263	0.258	0.2105	0.2928	3.0062
2	Stem (S1)	0.062	0.393	0.381	0.325	0.4521	4.6417
	Stem (S2)	0.067	0.401	0.409	0.338	0.4702	4.8275
	Stem (S3)	0.061	0.363	0.436	0.3385	0.4709	4.8347
3	Stem (S1)	0.058	0.505	0.45	0.4195	0.5836	5.9918
	Stem (S2)	0.053	0.287	0.274	0.2275	0.3165	3.2495
	Stem (S3)	0.055	0.396	0.368	0.327	0.4549	4.6704
4	Stem (S1)	0.06	0.305	0.292	0.2385	0.3318	3.4066
	Stem (S2)	0.079	0.303	0.335	0.24	0.3339	3.4282
	Stem (S3)	0.091	0.298	0.388	0.252	0.3506	3.5996
5	Stem (S1)	0.059	0.401	0.366	0.3245	0.4514	4.6345
	Stem (S2)	0.138	0.443	0.256	0.2115	0.2942	3.0206
	Stem (S3)	0.067	0.562	0.424	0.426	0.5926	6.0842
	Barley (B1)	0.053	0.145	0.164	0.1015	0.3922	
	Barley (B2)	0.055	0.184	0.145	0.1095	0.4231	
	Barley (B3)	0.058	0.141	0.158	0.0915	0.3536	
	D-glucose standard		0.428	0.426	0.427		

PUBLICATION OF PROJECT UNDERTAKING

This is to certify that I have no objection to publish the project entitled “The Effect of Different Extraction Method Toward the Extracted (1,3;1,4)- β -glucan Content in Rice Grain, Leaf and Stem” by the supervisor in a joint authorship. However, it has to be evaluated by the Faculty of Agriculture and Food Sciences, Universiti Putra Malaysia Bintulu Sarawak Campus and published in the form approved by the faculty.



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DATE: 15-7-15