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DETERMINATION OF GLUCOMANNAN CONTENT IN Amorphophallus hewittii

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DETERMINATION OF GLUCOMANNAN CONTENT IN *Amorphophallus hewittii*

By

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

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Dedication

To my beloved parents,
Mr. Awing b. Mohd and Mrs. Khamisah bt. Muhd,
who had always been supportive and encouraging
in my final year research project.

"I've missed more than 9,000 shots in my career. I've lost almost 300 games. Twenty-six times I've been trusted to take the game winning shot and missed, I've failed over and over and over again in my life. And that is why I succeed"

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ABSTRACT

Flour from corm of *Amorphophallus hewittii* was processed and evaluated for glucomannan contents. Glucomannan is a polysaccharide consisting of linked β–1,4 D–glucose and D–mannose was beneficial used as derivatives in pharmaceutical, biotechnology and fine chemical field. The major changes in glucomannan concerned the decrease and increase of drying temperature and also storage period. Flours derived from dried corm slice under 40, 50, 60, 70 and 80 ℃ was tested for glucomannan content and to produce a good quality of flour with analytical characteristics; moisture, arranging between 10 to 11%. Two old days corm produced higher glucomannan content in flour moisture content (11 to 10%) and increasing with drying temperature, from 14.93 to 33.90% at 40 to 60 ℃ but decreased after 70 to 80 ℃ with 25.56 to 24.61% in every 100 g of flour. Besides this value, better temperature 60 °C were used for second's treatment between storage period and glucomannan content. In one week, the percentage of availability glucomannan decreases compare sampling week; 32.40% or 0.88/100 g followed with, two weeks; 29.70% or 0.81/100 g, three weeks; 27.20% or 0.74/100 g and lastly, fourth weeks; 21.30% or 0.58/100 g of flour and the losses in glucomannan content parallel with increasing period storage and flour moisture, with no significance differences ($p \leq 0.05$) between the samples. After analysis by using biochemical method, flour with drying temperature 60 °C show a higher percentage of glucomannan, 33.90% or 0.92/100 g in flour and processing flour from different storage periods of corms starting with one, two, three and lastly with fourth weeks were used with these temperature, 60 °C. The changes of glucomannan content depend on increase and decrease of drying temperature also period storage. Again, flour from sampling period (two old days corms from sampling corms) showed the higher content of glucomannan compare another week; one, two, three and fourth weeks. Flour of 250 µm mesh size with moisture content of 10 to 11% derived from grounded dry corm slices were analysed for glucomannan contents using Glucomannan Megazyme Assay procedure. Drying of slices under 60 ℃ produced flour with higher glucomannan content of 33.9% (0.92/100 g) compared to other drying temperature for every 100 g of flour. It was also found that storage period (under ambient condition) are significantly affect the glucomannan content.

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ABSTRAK

Tepung dari umbisi *Amorphophallus hewittii* telah diproses dan dinilai untuk mengetahui kandungan glucomannan. Glucomannan adalah polisakarida yang terdiri daripada β–1,4 D–glukosa dan D–mannose dan digunakan sebagai bahan penambah dalam industri farmaseutikal, bioteknologi dan bidang kimia. Perubahan utama glucomannan berkait rapat dengan peningkatan dan penurunan suhu pengeringan serta tempoh penyimpanan umbisi. Tepung yang dihasilkan dari kepingan umbisi di bawah suhu pengeringan 40, 50, 60, 70 dan 80 ºC diuji untuk mengetahui kandungan glucomannan dan menghasilkan tepung berkualiti dengan ciri analitik; iaitu lembapan tepung, antara 10 hingga 11%. Ketersedian glucomannan yang terdapat di dalam tepung meningkat dengan lembapan tepung (11 kepada 10%) dan peningkatan suhu pengeringan, dari 14.93 kepada 33.90% pada 40 kepada 60 ºC tetapi menurun selepas suhu 70 kepada 80 ºC dengan 25.56 kepada 24.61% setiap 100 g tepung. Analisis ini dijalankan pada umbisi berusia dua hari selepas dituai. Berdasarkan kepada nilai ini, suhu pengeringan yang paling sesuai dipilih iaitu 60 ºC dan digunakan untuk analisis seterusnya bagi mengetahui perkaitan antara tempoh penyimpanan dan kandungan glucomannan. Pada minggu pertama, peratusan glucomannan yang tersedia menurun, jika dibandingkan dengan minggu pungutan; 32.40% atau 0.88/100 g diikuti dengan minggu kedua; 29.70% atau 0.81/100 g, minggu ketiga; 27.20% atau 0.74/100 g dan akhir sekali minggu keempat; 21.30% atau 0.58/100 g tepung dan penurunan kandungan glucomannan selanjar dengan peningkatan tempoh penyimpanan dan lembapan tepung, dengan tiada signifikan ($p \leq 0.05$) antara sampel. Selepas analisis menggunakan kaedah biokimia, tepung dengan suhu penyimpanan 60 ºC menunjukkan peratus kandungan glucomannan tertinggi, 33.90% atau 0.92 g setiap 100 g tepung dan pemprosesan tepung dari perbezaan tempoh penyimpanan umbisi bermula dengan minggu pertama, kedua, ketiga dan keempat juga menggunakan suhu yang sama (60 ºC). Perubahan kandungan glucomannan bergantung kepada peningkatan dan penurunan suhu pengeringan dan tempoh penyimpanan umbisi. Secara kesimpulannya, tepung dari umbisi yang berusia dua hari dari hari pungutan menunjukkan kandungan glucomannan tertinggi berbanding minggu pertama, kedua, ketiga dan keempat. Tepung dengan kehalusan bersaiz 250 µm dan kandungan lembapan 10 hingga 11% hasil dengan mengisar hirisan kering umbisi dianalisis untuk mengenalpasti kandungan glucomannan berdasarkan kaedah Glucomannan Megazyme Assay. Pengeringan kepingan di bawah suhu 60 ºC menghasilkan kandungan glucomannan tertinggi iaitu 33.9% (0.92/100 g) setiap 100 g tepung berbanding suhu pengeringan yang lain. Tempoh penyimpanan (di bawah suhu persekitaran) juga menunjukkan kesan terhadap kandungan glucomannan.

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APPROVAL

I certify that this research project entitled "Determination of Glucomannan Content in *Amorphophallus hewittii*" has been examined and approved as a partial fulfillment of the requirement for the degree of Bachelor of Bioindustry Science in the Faculty of Agriculture and Food Sciences, Universiti Putra Malaysia Bintulu Sarawak Campus.

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LIST OF ABBREVIATIONS

GlcMan	glucomannan
Ac–GlcMan	Acetylated–glucomannan
Ac–GlcManol	Acetylated–glucomanno–oligosaccharides
DP	Degree of Polymerisation
β–Gos	β–Glucosidase
β–Mos	β–Mannosidase
HK	Hexokinase
ATP	Adenosine–5'–Triphosphate
ADP	Adenosine–5'–Diphosphate
NADPH	Nicotinamide Adenine Dinucleotide Phosphate
G–6–P	Glucose–6–Phosphate
M–6–P	Mannose–6–Phosphate
F–6–P	Fructose–6–Phosphate
PMI	Phosphomannose Isomerise
PGI	Phosphoglucose Isomerise
glu	glucose
man	mannose
LDL	Low Density Lipoprotein
SCFA	Short Chain Fatty Acid
IPNs	Interpenetrating Polymer Networks

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CHAPTER 1

INTRODUCTION

Glucomannan presents in corms of several Araceae (Aroid) genus (e.g. in *Amorphophallus konjac*) and other plant families such as in Amaryllidaceae (e.g. in *Narcissus tazetta*), Orchidaceae (e.g. in *Blettilla striata*), Liliaceae, Iridaceae and Agavaceae (Michael and Bewley, 2000). The fresh corms are a good source of complex carbohydrates and easily digestible starch which contains an average of 18% dry matter, of which 55 to 60% is glucomannan (Susheela, 2007; Lind *et al.*, 2006; Catharina *et al.*, 1999). The corms of these species have hard and horny endosperms with thick walls (Michael and Bewley, 2000). Before drying or further processing corms of *A. hewittii* needs to be peeled sliced and repeated washing to remove toxic and irritating calcium oxalate crystals (Dietrich and Hans, 2004; Zakaria, 2000; Clare, 1998; David, 1993; Richard and Robert, 1990; George, 1951). In order to produce flour, the slices needs to be dehydrated, heated and grounded to enable glucomannan to be extracted (Nishinari and Gao, 2007; John and Leo, 2004; Naomi, 1978 and Birch *et al.*, 1973).

Glucomannan are the acetylated polysaccharides, predominant hemicelluloses and heteropolymers comprising β–1, 4 linked D–glucose and D–mannose (David, 2005; David, 2006; Ann–Charlotie, 1996; Arnold and David, 1995) residues in proportions ranging from 2.5 to 4.0:1 (Steve, 2005). For *Amorphophallus konjac*, *Aloe vera*, and *Orchis mureo*, glucose:mannose (Glc:Man) ratios of, 6:1, 2:1, and 4:1 respectively are commonly found (Hui, 2006; Stephen, 2006; Megazyme, 2004). Piet *et al.* (2002) have noted that the distribution of the glucose units over the mannose backbone is

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believed to be important for the functional properties of the glucomannan (polysaccharide) when applied in food products for example, the effect of glucomannan on the gel–forming properties of κ–carrageenan agar and xanthan gum (Xanthan–Glucomannan mixtures) (Stephen, 2006; Atkins, 1985; Karla, 1984).

The genus *Amorphophallus* consists of about 200 species in tropical and subtropical region. Four species have been cultivated especially in Asean countries, such as *Amorphophallus konjac*, *Amorphophallus muelleri*, *Amorphophallus paeoniifolius* and *Amorphophallus variabilis* for making flour prepared from corms and as ornamental plant (Punithavathy, 2008; Ipor *et al*., 2004; Cescutti *et al*., 2002; Bogner *et al*., 1993). Ipor *et al*. (2004) have recorded that sixteenth species are endemic to Borneo Islands (nine species in Sarawak, five species in Sabah and eight species in Kalimantan). *Amorphophallus* spp. grows well in tropical climate that have good soil condition (well drained and higher humus soil), good air humidity and circulation, with temperature range from 25 to 30°C. The plants can be found naturally in mineral, sandstone, limestone and shale soils of dipterocarp, kerangas, secondary and riverine forest (Kristian, 2000; Gerald and Junaidi, 1999; Susan, 1995; Whitmore, 1990; Daisy and Gooding, 1987; Henderson, 1954).

The corm, young leaves and the fruits of several *Amorphophallus* spp. are used as a vegetable (John and Blanca, 1999). In Philippines and India, all parts are used as food for farm animals (fodder) such as broiler chickens. Besides its value as food, the flour prepared from the corm is used industrially in China and Japan, example the glucomannan flour in *Amorphophallus konjac* has commonly useful as an ingredient (health food), additive (food) and non–food (Nishinari and Gao, 2007). In meat

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products, pet food, sauces and dressings, bakery, ice cream and dairy, jams and jellies, drinks beverages, reformed vegetables, confectionery, diet and health food, fruit, colourings, flavourings, encapsulation, veterinary and non–food (preparing acid and alcohol–alcoholic fermentation) (Susheela, 2007; James and Bogenshutz-Godwin, 2002; Kanna *et al.*, 2002; Zdzisław, 2002; Flach and Rumawas, 1996; Onwueme, 1978).

Glucomannan extracts from *Amorphophallus* spp. (e.g. *Amorphophallus albus*, *Amorphophallus campanulatus*, *Amorphophallus konjac*, *Amorphophallus muelleri*, *Amorphophallus oncophyllus*, *Amorphophallus paeoniifolius*, and *Amorphophallus variabilis*) have been approved as an ingredient for human consumption by the Food and Drug Administration (FDA) in the United Stated of America (US) and appeared in early 1997, with a tentative E425 agreement number in the modification of the 92/2/CE European Authorised Food Additives list (Kanna *et al.*, 2002).

Glucomannan flour from *Amorphophallus konjac* is a health product (e.g. for capsules) widely used in temperate Asian countries, (China, Japan and Philippines), mountainous regions (Thailand and Indonesia), India, Africa and the United States of America (Christophe, 2000). Many uses are reported in traditional medicine: for two types of diabetes; hypoglycaemia (low blood sugar) and hyperglycaemia (high blood sugar) (Paul *et al.*, 2002; Tim, 2002; Vladimir *et al.*, 1999), high blood pressure, high cholesterol (obesity) and digestion problem (Hermelin and Grimshaw, 2003; James and Bogenshutz-Godwin, 2002; Amy, 2000), dysentery, earache, cholera, respiratory problems, cancer, heart disease (Vladimir *et al.*, 1999), osteoporosis (Stanley, 2003),

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chronic stomach disease (Wu and Shen, 2001) and to cure rheumatic pains (Flach and Rumawas, 1996).

In India, *Amorphophallus* spp. is often grown mixed with other crops, such as arecanuts (*Areca catechu*), ginger (*Zingiber officinale*), methi (*Trigonellafoenum graecum*), cluster bean (*Cyamopsis tetragonoloba*), taro (*Colocasia* spp.), banana (*Musa* spp.) and grain crops, maize (*Zea mays*) and sorghum (*Sorghum* L.) (Flach and Rumawas, 1996; Daisy and Gooding, 1987). *Amorphophallus* spp. is also recommended for intercropping with coconuts. In India, the average yield of *Amorphophallus* spp. ranges between 12 to 22 t/ha; as an intercrop with coconuts. However, under experimental conditions over 60 t/ha has been reported, and 36 t/ha are in mixed cropping system. In parts of India, e.g. Bombay, the corms are usually dug at the end of each seasons, stored and then replanted, but in other areas, such as Japan, where *Amorphophallus* spp. is grown, the corms are left in the ground for the whole growth cycle (Daisy and Gooding, 1987).

In Sarawak, currently there are nine species of *Amorphophallus* has been identified. Some statement, species has been recorded as edible (Flach and Rumawas, 1996). Based on this the species probably has the potential to content glucomannan as in other species like *Amorphophallus konjac*, *Amorphophallus muelleri*, *Amorphophallus paeoniifolius* and *Amorphophallus variabilis*. Further, there has been no research or literature has been found on the glucomannan properties of the local *Amorphophallus* spp.

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Hence, the objectives of this study are:

1. To determine the glucomannan content in *Amorphophallus hewittii*.
2. To determine the effect of drying temperature towards glucomannan content.
3. To determine the effect of storage period towards glucomannan content.

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CHAPTER 2

LITERATURE REVIEW

2.1 *Amorphophallus* Species

Amorphophallus spp. (Araceae) is commonly known as elephant foot yam (David, 1993), elephant bread, suran, sweet yam (Flach and Rumawas, 1996), corpse flower, mo–yu (China), konnyaku (Japan), mayak (Iban–Sarawak) and bunga bankai (Indonesia) (Ipor *et al.*, 2006; Umberto, 2000). A robust herbaceous plant, with an erect solitary stem usually 1.0 to 2.5 m in height (David, 1993) and bearing at the top one or two tripartite leaves, each part of which is deeply dissected into numerous segments (Michael, 2006; Van–Balgooy, 2001; Kubitzki, 1998). Towards the end of the plant's cycle (usually 4 to 6 years) a large terminal inflorescence is produced, consisting of a short stalk and spathe and a spadix, which emits a malodorous smell, reminiscent of rotten meat. The corms are large globose depressed tubers, usually dull–yellow or brownish–yellow in color and these produce 5 to 10 cormels at the end of each growing season (Philip, 2005; Pieter *et al.*, 1990; Daisy and Gooding, 1987; Yong, 1981).

The various species of *Amorphophallus* spp. corms contain a hydrocolloidal polysaccharides consisting of glucose and mannose polymers that known as glucomannan (David, 2005; David, 2006; Arnold and David, 1995). Corms usually peeled, sliced, repeated washing to remove toxic and irritating calcium oxalate crystals because of this compound may cause temporary sterility and death of children and many experimental animals (Chia, 2004; Dietrich and Hans, 2004; Richard and Robert, 1990; Blanshard and Mitchell, 1979). Four of *Amorphophallus*

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spp. has been used for human consumption; *Amorphophallus konjac* originates from Southern and South–eastern China, Vietnam and Laos and cultivated in China, Japan, Indo–China, Philippines and Hawaii, *Amorphophallus paeoniifolius* are found wild and cultivated in Madagascar, India, South east Asia to Polynesia, including also Southern China and Northern Australia, *Amorphophallus muelleri* found wild from the Andaman Islands eastwards through Myanmar into Northern Thailand and South eastwards to Indonesia (Sumatra, Java, Flores and Timor) and it's occasionally cultivated (e.g. Java) and *Amorphophallus variabilis* is only known wild in Indonesia (Java, Madura, Kangean Islands) (Punithavathy, 2008; Ipor *et al*., 2006; Hetterscheid and Ittenbach, 2001; Flach and Rumawas, 1996; Susan, 1995; Whitmore, 1990).

2.2 *Amorphophallus* Species in Malaysia

Presently, overall number of *Amorphophallus* spp. in Malaysia includes Peninsular Malaysia is about 29 species as recorded, with nine species in Sarawak and five species in Sabah which are endemic to Borneo (Ipor *et al*., 2006; Henderson, 1954). One of the species, *Amorphophallus prainii* was found at Bukit Wang Recreational Park, 32 km from Alor Setar as well as in the Perlis State Park (Punithavathy, 2008). The corm from the species are yellow in colour, rich in starch and is a popular food among the Indians. The corms are used in curries, cakes, desserts, and puddings. The petiole is edible after boiling and the tuber juice extract is used as a cure for high blood pressure (Daisy and Gooding, 1987).

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2.3 *Amorphophallus hewittii*

Amorphophallus hewittii Alderw also known as Borneo Titan Aroid or Giant Voodoo Lily; evergreen species with a very tall, tree–like, white spotted, fresh green stem and a broad, umbrella–like single leaf–single deeply dissected leaf large spathe, green outside and reddish inside and a rose spadix up to 150 cm in height and with in this weight that species known as a second largest flower in Aroid genus (Michael, 2006; Van–Balgooy, 2001; Kubitzki, 1998). The corms of this species are subglobose or depressed globose and may gain up to 60 kg and more which becoming a desire characteristics for cultivation in producing glucomannan for healthy food that have been operated in Japan and China 2,000 years ago (Flach and Rumawas, 1996). This species may grow well with a high air humidity, good air circulation, rich humus soil, drained soil and soil temperature of 25 to 30 ºC. *Amorphophallus hewittii* Alderw are associated with mineral and sandstone soils in dipterocarp, kerangas, secondary and riverine forest (Ipor *et al.*, 2006; Gerald and Junaidi, 1999; Susan, 1995; Whitmore, 1990).

2.4 Glucomannan

2.4.1 Structure and Chemical Properties

Glucomannan are linear polymers of both (1→4) linked β–D–glucose and (1→4) linked β–D–mannose residues (Figure 2.1) and it classified in reserve polysaccharides group (Southgate, 1976) and obtained from dried and pulverized corm of the perennial herb *Amorphophallus* spp. Acetyl groups scattered randomly along the glucomannan backbone promote water solubility. Glucomannan is a high molecular weight polymer which can form viscous pseudoplastic solutions. It can form a gel in presence of strong elastic, heat–stable gels when heated with mild alkali

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(Ruszova *et al.*, 2008; Peter and Victor, 2007; Robert *et al.*, 2006; Lakshmi, 2002; Teeri *et al.*, 2002; Michael and Bewley, 2000).

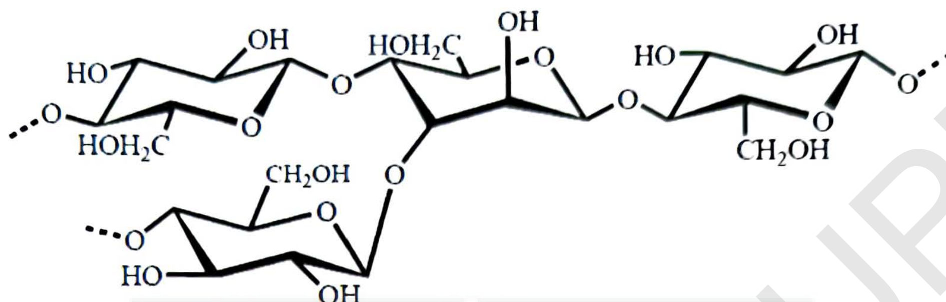

Figure 2.1: Glucomannan structure from *Amorphophallus konjac* showing Glu–Glu–Man–Man units
(Source : Anekant *et al.,* 2007; Villanueva–Suàrez *et al*., 2003)

In solid state, glucomannan has a crystalline structure. X–ray diffraction showed that it crystallizes into a structure related to mannan. Glucomannan is water–soluble and produces highly viscous, pseudoplastic solutions (Chandrasekaran *et al.*, 2003; Frederick, 2000). It forms strong, elastic gels when treated with strong alkali as to remove acetyl substituents (Stephen, 2006). The solution is a typical non–Newtonian system (food in which the viscosity changes rate of shear) showing shear thinning behaviour (Fellows, 2005). A thermo–irreversible gel is formed when glucomannan solution is heated in alkali conditions. A possible gelling mechanism is that hydrolysis of the acetyl groups under alkali conditions promotes intra– and inter–molecular hydrogen bonding leading to "cross–linking" of the glucomannan molecules (Steve, 2005; Berk, 1976).

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Another interesting property of glucomannan is its synergism with other hydrocolloids. Glucomannan, like locust bean and guar galactomannans (Piet *et al.*, 2002), have synergism with xanthan gum (*Xanthomonas campestris*) (Amy, 2000; Alais and Linden, 1991) and κ–carrageenans and can form thermally reversible gels (Daniel, 2006; Frederick, 2000). The mixture can form gels at a total polymer concentration as low as 0.1% (Steve, 2005). Thermo–reversible gels can be prepared when glucomannnan is mixed with agarose or carrageenan solutions (Hui, 2006). Thermally irreversible gel prepared from corms flour in alkali conditions is a popular traditional Japanese food (*Kon–nyaku*) item while the thermally reversible gels formed by the synergistic interactions between glucomannan and other gum applications are found in products such as health food (Steve, 2005; Tom and Emery, 2003).

Glucomannan is suitable as a texturing, gelling, fat substitute, water binding, suspension, gelatine substitute, fiber and low calories (Susan, 1999), and film forming agent in blenders (polysaccharides), meat products, pet food, sauces and dressings, bakery, ice cream and dairy, jams and jellies, diet and health food (Christophe, 2000), drinks beverages, reformed vegetables, confectionary, fruit, colourings, flavourings, encapsulation, veterinary and research centres sectors (Kanna *et al.*, 2002; Zdzisław, 2002).

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2.4.2 Factors Influencing Glucomannan Content

Corms of *Amorphophallus* spp. can lose as much as 25% of their initial weight in four weeks of storage, but can be successfully stored at cool conditions (10 °C) for several weeks (Daisy and Gooding, 1987; Ikuzo and Edilberto, 1984). Glucomannan contents is positively correlated with the weight lose of the corms. Hence, measures must be taken to reduce the weight loss/moisture loss in the corms by storing under approximately temperature and humidity.

Drying of the corms in an atmosphere of oxygen, as well as cooling then in the same atmosphere, creates the best conditions for carbohydrate transformation (Ikuzo and Edilberto, 1984; Birch *et al.*, 1973). Kanna *et al.* (2004) reported that drying of the corms slices under 60 °C produced better materials for extracting glucomannan. High amount glucomannan will be extracted from the corms when leaves of the plant wither and independent of the corms size (Flach and Rumawas, 1996).

2.4.3 Applications in the Pharmaceutical Area

2.4.3.1 Dietary Fiber and Cardiovascular Disease

Dietary fiber or non–starch polysaccharides are the edible parts of plants or analogous carbohydrates that are resistant to breakdown by enzyme in small intestine and they pass unchanged into the large intestine (bowel) (Gaman and Sherrington, 2004; Paul *et al.*, 2002; Brian and Allan, 1995). Dietary fiber includes polysaccharides (glucomannan), oligosaccharides, lignin and associated plant substances are classified by two groups; a soluble fiber and insoluble fiber (John and Leo, 2004). Soluble fibre refers to fibers form a viscous gel when mixed with a liquid such as glucomannan. Glucomannan as a dietary fiber provides little energy but has

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several beneficial effects. First, it adds bulk to the diet which is it can absorb 10 to 15 times its own weight in water, drawing fluid into the lumen of the intestine and increasing bowel motility. Glucomannan also can be delays gastric emptying and results in a sensation of fullness. This delayed emptying also results in reduced peaks of bloods glucose following a meal (Steven and Sandra, 2000). Second, consumption of glucomannan flour has now been shown to lower Low Density Lipoprotein (LDL) cholesterol levels by increasing fecal bile acid excretion and interfering with bile acid re–absorption. For example, diets rich in the soluble fiber of glucomannan flour (25 to 50 g/day) are associated with a modest, but significant, reduction in risk for cardiovascular disease by lowering total and LDL cholesterol levels. One study of twenty obese patients, those who took two capsules of 500 mg of purified glucomannan, one hour before meals, lost an average 5.5 pounds after eight weeks (James and Bogenshutz-Godwin, 2002).

Glucomannan–rich diets also can decrease the risk of haemorrhoids (piles), cancer of the colon and diverticular disease (small bulges in the wall of the large intestine). These conditions are associated with constipation and the slow transit of food through the alimentary canal. In some rural communities in Africa these disease are virtually unknown (Gaman and Sherrington, 2004). The recommended daily intake (AI) of fibre is 25 g/day for women and 38 g/day for men. However, most American diets are far lower in fiber approximately 11 g/day (Pamela *et al.*, 2008) but in Japan and China communities, glucomannan has been used 2,000 years ago in their diet (John and Leo, 2004).

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2.4.3.2 Constipation and Colon Cancer

Constipation is the ways that suggested in our body that make some minor adjustments in diet or exercise. However, constipation can also be a symptom of a serious illness like colon cancer, depression, or organ failure. Constipation also can be prevented by increasing the content of dietary fiber in normal diet such as vegetables, fruit, cereals and bread, the hemicellulose (Wong, 1989) with cellulose and lignin like glucomannan flour (Blanshard and Mitchell, 1979). Glucomannan has high water–binding capacity; its volume can be expanded for up 10 to 15 times its own weight through water–absorbing so and wet weight of dung and water content is increased after the meal (James and Bogenshutz–Godwin, 2002).

Moreover, when glucomannan is decomposed by colon bacteria (Anekant *et al.*, 2007), short–chain fatty acid (SCFA) such as acetic, butyric and propionic acid produced to stimulate intestine wriggling, and the interval of dung defecating and duration of each dung defecating are thereby shortened. Fibre of this type is now known to reduce the frequency of disease, including cancer, associated with the colon in man (Gail, 2003; Amy, 2000). The carcinogen in intestine can be diluted by glucomannan as a dietary fibre, and the passing time through intestine can be shortened, the duration of the irritation caused by poison to intestine is reduced to improve the excretion of the poison from intestine, the irritation on intestine cause by carcinogen is so reduced (Meimei *et al.*, 2007; Villanueva–Suàrez *et al.*, 2003; Paul *et al.*, 2002; Mahmood, 1998; Brian and Allan, 1995).

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2.4.3.3 Blood Triglyceride and Cholesterol Level

A triglyceride is a type of fat and stored in a body. Blood also has triglyceride that circulates in lipoproteins. As most of the fats in food are triglycerides, the food can affects blood level of triglycerides and it can be increased two to four times and reaches to maximum value in about five hours after the meal. The triglyceride concentrations in the blood vary considerably throughout the day, depending upon how much fat is eaten and how fast the body removes fat from the blood. Cholesterol and triglycerides are necessary for our life that it provides the fuel needed for body cells to function, but the elevated levels of cholesterol and triglycerides can make a health problem. A research reported that people with high triglycerides and elevated Low Density Lipoprotein (LDL) cholesterol are become to have a stroke. High triglycerides may be associated with insulin resistance, low HDL cholesterol, high LDL cholesterol, liver disease and obesity. Increase dietary fiber intake like glucomannan flour, legumes, vegetables and fruits can be reduced triglyceride level in body (Robert, 2007; Paul *et al.*, 2002).

Blanshard and Mitchell (1979) reported that rats and chickens showed a response to glucomannan flour (*Amorphophallus konjac*). Incorporation of glucomannan flour in hypercholesterolaemic diets of adult rats has been found to result in the lowering of serum and liver cholesterol levels by interfering with the transport of cholesterol in the jejunum and bile acids in the ileum. This hypocholesterolaemic activity of the hemicellulosic mannans was furthermore shown to be associated with certain structural and physical features (high molecular weight (MW) and viscosity, water solubility) and the activity was lost when the carbohydrate was subjected to acid or enzymic hydrolysis (Villanueva-Suàrez *et al.*, 2003).

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2.4.3.4 Cure Rheumatic Pains

According to Flach and Rumawas (1996), the corms of *Amorphophallus* spp. are caustic and are employed in anti-rheumatic poultices as rubefacients when applied externally. Flach and Rumawas (1996) have reported in India, the corm is stomachic and tonic and is used to treat haemorrhoids and given as a restorative in dyspepsia and debility. The roots also are used for boils and ophthalmia and also as an emmenagogue (Flach and Rumawas, 1996).

Corms are boiled and given to treat piles, liver and spleen enlargement, dysentery and stomach disorders; it is used aperient, expectorant, anthelmintic and carminative. Corm paste is boiled in mustard oil, cooled and dropped into the ear to treat ear ache. Dried corm flour is given to treat abdominal tumours. Leaf juice us used as antiseptic to clean wounds (Raveendra and Martin, 2006).

2.4.3.5 Drug Delivery

Glucomannan were used in drug delivery which is, in one glucomannan capsule was contain several amount of glucomannan, 0.4% of pyridoxine (vitamin B_6) and 5% of silicophosphate buffer for chronic stomach disease (Wu and Shen, 2001).

Wang and He (2002) reported that a kind of alginate–glucomannan–chitosan beads used as controlled release matrix. It was observed that glucomannan contained within beads, and faintness hydrogen binding and electrostatic interaction existed between alginate and glucomannan by infrared spectra.

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Hermelin and Grimshaw (2003) invented compositions and methods for the enhancement of iron uptake or the treatment of iron deficiency by enhancing the rate and extent of dissolution in a subject in need thereof. The composition contained at least two iron–providing materials in a single dosage form wherein at least one of the iron–providing materials contained a modified release mechanism, matrix, or coating. Glucomannan was used as a matrix. The iron–providing materials included within the composition have different rates of release. Following administration to the animal, the iron–providing materials were released in the gastrointestinal tract over a period of up to 24 hours (Meimei *et al.*, 2007).

Gel–forming macromers including at least four polymeric blocks, two of which were hydrophobic and at least one of which was hydrophilic, and including a cross–linkable group, which are glucomannan was used as a hydrophilic block. The macromers can be covalently cross–linked to form a gel on a tissue surface in vivo. The gels formed from the macromers have a combination of properties including thermosensitivity and lipophilicity, and are useful in a variety of medical applications including drug delivery and tissue coating (Pathak *et al.*, 2003).

The combination of an alginate, xanthan–gum and glucomannan improving bio–adhesive properties in pharmaceutical composition provide both a protecting and a healing effect on mucosal surface for treatment of disorders of the esophagus (Dettmar *et al.*, 2000).

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2.4.4 Applications in Biotechnology Area

Chen and Zhang (2000) provided a method for immobilization of cells using carrageenan and glucomannan. The composite for immobilizing cells was composed of 40 to 99% carageenan and 60 to 10% glucomannan. The cell was immobilized by dissolving the composite in water at 80 °C, cooling, adding cells, and dropping into 2 to 3% potassium chloride solution. The recombinant *Escherichia coli*, *Saccharomyces* spp., and *Bifidus bacillus* were immobilized using this method.

Husks of mannan contained in the conventional agent to improved qualities and measure that a biopsy sample embeded in the agent nicely thin–sliced with a microtome without destroying the cells on the cutting section due to the damage on the blade caused by the impurities. The novel agent exhibited the improved agglutination force and transparency, and it was less stained with many kinds of staining dyes. The fixation support agent for biopsy sample consisted of a different type of modified derivative of glucomannan obtained from the refined flour of *Amorphophallus rivieri* (Takezaki, 2000).

Nussinovitch (2004) invented a temperature–stable droplet containing a temperature–stable hydrocolloid membrane. The membrane encapsulated a liquid that contained at least one enzyme, a cell, a biological agent, a pharmaceutical agent, an immunological agent, or mixtures thereof. Glucomannan was used as one of the hydrocolloid materials. The hydrocolloid membrane encapsulating the liquid was a thickness capable of holding the liquid without bursting through a temperature range of about negative 20 °C to about 90 °C.

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2.4.5 Applications in the Fine Chemical Area

2.4.5.1 Films and Membranes

Konjac glucomannan has very good film–forming ability. Several kinds of transparent blend films of konjac glucomannan with polyacylamide, gelatin, sodium carboxymethylcellulose, polyvinylpyrrolidone, chitosan, sodium aginate and cellulose, were invented, respectively (Yang *et al.*, 2002; Xiao *et al.*, 2000; Xiao *et al.*, 2001). The results indicated that the occurrence of intra– and inter–molecular interaction of the pure components, as well as the inter–molecular interactions between glucomannan and these substance through hydrogen bond formation. The thermal stability and mechanic properties of both tensile strength and elongation at the break of the films were improved by blending glucomannan with these substances. Some semi–interpenetrating polymer networks (semi–IPNs) from glucomannan derivatives were also reported (Yang *et al.*, 2004; Lu and Zhang, 2002; Xiao *et al.*, 2002; Gao and Zhang, 2001a,b; Gao *et al.*, 2003).

Chen *et al.* (2003) prepared biodegradable plastics based on soy dreg (SD) for enhanced water resistivity of the SD plastics; thermoplastic benzyl konjac glucomannan (B–K–GLUM) films were covered on SD sheets in a lamination compression process, and SD/B–K–GLUM (SB) composite sheets. Tang *et al.* (2003) reported soy protein isolate–carboxymethylated konjac glucomannan blend films. The thermostability and mechanical and water vapor barrier properties of blend films were greatly enhanced due to the strong inter–molecular hydrogen bonding between SPI and CMKGLUM.

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2.4.5.2 Coating Materials

Coating materials containing glucomannan for preserving fresh produce (e.g. cantaloupes, apples) was effective to control respiratory exchange, i.e. the passage of gases, particularly oxygen, ethylene, carbon dioxide and water vapor, into and out of the produce, thereby controlling maturation and ripening of the produce (Yang *et al.*, 2001). In the patent of Kawano (2001), rice grains were coated with a gel of glucomannan hydrate, and treated with heat to obtain grains coated with dietary fibers or irreversible gel. The product can use in a variety of ways; it could be cooked in conventional ways, manufactured as retort or pressurized food. The cooked rice grains were not readily disintegrated and made into gruel.

Yasuda and Kawamura (2000) invented steam–supplying food packaging materials for microwave cooking and cooking of food. The packaging materials, which release steam toward the inside upon microwave heating to steam food such as steamed buns, had a steam–supplying material which comprised a shape retention material and a steam generator containing H_2O and a water retention agent such as konjac glucomannan, agar, carrageenan, gelatins, caseins, acrylic polymers, contained therein or supported thereon.

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2.4.5.3 Cosmetics

This field has been well studied in Japan which a hair composition containing glucomannan and/or keratose quaternary ammonium derivatives which provide excellent conditioning effect and moisture–retaining effect without causing stickiness. Some kinds of hair–styling preparations containing glucomannan with less stickness and giving natural gloss and smoothness to hair (Omura *et al.*, 2001a,b,c,d). Some kinds of cosmetic oil–in–water emulsions containing konjac glucomannan were also invented (Omura *et al.*, 2001b,e).

New technologies in dermatologist's kind of water–insoluble glucomannan dried gel particles did not damage the skin and tooth surface and they were effective as mild scrubbing agents for cosmetics (Takada, 2000). Another product is cosmetics containing pigments coated with water–soluble glucomannan that showed good skin–moisturizing effect, give long makeup effect, and had no sticky skin feel (Saito, 2000).

The quick–drying disinfecting gels for hand were invented by Shimizu and Ohshiba (2000). The gels were obtained by mixing EtOH solutions with gel–forming polymers and glucomannan as a thickener. The gels were uniformly applied to hands and were removed by rubbing without rinsing with water. Arminas and Calello (2002) invented an organic solvent based cosmetic remover composition which gelled to a viscosity of 25 to 500,000 centipoise with a synthetic metal silicate gelling agent.

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2.4.5.4 Emulsifier and Surfactants

New surfactant from refined konjac powder was hydrolyzed, oxidized and esterified with stearic acid, then neutralized with NaOH and the final product can be used widely in pharmaceutical, food and chemical industry (Gan, 1999). Four series of konjac glucomannan esters of long fatty acid were also prepared by heterogeneous method. Results showed that in the proper range of degree of substitution, konjac glucomannan esters had good emulsifying ability in clove oil–water (O/W) emulsion, even in high salt concentration and acidic pH. Clove oil–water emulsion had good stability within four weeks at room temperature, and the breakage of emulsion was not brought about (Tian *et al*., 1999).

Ma *et al.* (1999) studied the emulsifying properties of isolated soy protein–konjac glucomannan conjugate. By comparing the emulsifying properties of the samples prepared with different buffers at various pH with that of the sample made in water, phosphate and acetate in the buffers were assumed to have catalytic function to the conjugation. The reaction was carried out with isolated soy protein to konjac in weight ratio of 1:1 presented the best emulsifying ability. The research suggested that the conjugate could be used as an effective emulsifier for O/W emulsions.

2.4.6 Applications in the Other Areas

Glucomannan also can be used as biodegradable resin compositions (Tokiwa and Tsuchiya, 2003), soil modifier (Wakisaka *et al.*, 2000), soil amendment (Takada–Oikawa *et al.*, 2000), a surface size composition for the surface sizing of paper and board (Kimpimaki *et al.*, 2001) and as fish lure (Igarashi *et al.*, 2001). In addition, glucomannan powder can be mixed with powdery or granular charcoal and water and press–molded to charcoal shaped products having excellent properties in dehumidification, deodorization, sound absorption and electromagnetic shielding and are suitable for building materials, water treatment, pet care products, health products (Morita *et al.*, 2001).

2.5 Potential Aroids in Malaysia

The Araceae (Aroids) genus has some two thousand species and one hundred and fifteen genera (Chandra, 1984) and is widely distributed in all parts of the tropical and subtropical regions of the world. The Old World genera *Amorphophallus* spp. has long been dispersed with the migration of the seafaring peoples of the Indo–Malaysian–Pacific region (Hetterscheid and Ittenbach, 2001). Evidence of the early dispersals may be found in the irrigation terraces in South east Asia which are believed to predate the culture of rice (Daisy and Gooding, 1987). Today, edible aroids together with other corms provide the staple food for 400 to 500 million people (Bradbury and Holloway, 1988; Donald, 1970a,b). The edible aroids alone make up a major portion of the diet of the people in the Pacific and Oceanic Islands and are a widely consumed food in Africa, South America, and many parts of Asia (Kay, 1973).

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Although the *Amorphophallus* spp. to be a popular root crop in parts of India and Eastern Asia, production is limited mainly because of the four year crop cycle. However, in Japan, breeding and selection aimed at improved disease resistance, higher yields, earlier maturity and higher mannan contents, are in progress (Flach and Rumawas, 1996).

The vision to make this crop the global food system by the year 2020 is; "By 2020, roots and tubers will be integrated into emerging markets through the efficient and environmentally sound production of a diversified range of high–quality, competitive products for food, feed and industry. These crops adaptation to marginal environments, their contribution to household food security, and their great flexibility in mixed farming systems make them an important component of a targeted strategy that seeks to improve the welfare of the rural poor and to link smallholder farmers with these emerging growth market (Consultative Group on International Agricultural Research Center–CGIAR) (Scott *et al*., 2000; Terry *et al*., 1987).

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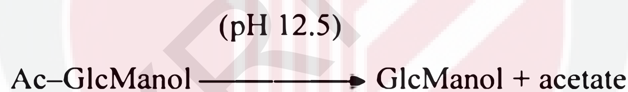
2.6 Glucomannan Extraction/Identification

The quantification of glucomannan requires several enzyme reactions and a treatment at high pH to remove the acetyl–groups from the polysaccharide. The first enzymic reaction involves depolymerisation of acetylated–glucomannan (Ac–GlcMan) by endo–β–mannanase to produce acetylated glucomanno–oligosaccharides (Ac–GlcManol).

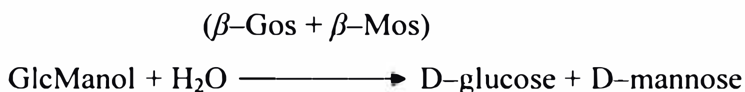
$$\text{Ac–GlcMan} + H_2O \xrightarrow{(\beta\text{–mannanase})} \text{Ac–GlcManol}$$

After depolymerisation into acetylated glucomanno–oligosaccharides of degree of polymerisation (DP) 2–6, the oligosaccharides are deacetylated by increasing the pH to 12.5.



$$\text{Ac–GlcManol} \xrightarrow{(\text{pH } 12.5)} \text{GlcManol} + \text{acetate}$$

After the acetyl–groups have been removed, the glucomanno–oligosaccharides are quantitatively hydrolysed to D–glucose and D–mannose by the combined action of β–glucosidase (β–Gos) and β–mannosidase (β–Mos).



$$\text{GlcManol} + H_2O \xrightarrow{(\beta\text{–Gos} + \beta\text{–Mos})} \text{D–glucose} + \text{D–mannose}$$

D–Glucose and D–mannose are phosphorylated by the enzyme hexokinase (HK) (Southgate, 1976) and adenosine–5'–triphosphate (ATP), to glucose–6–phosphate

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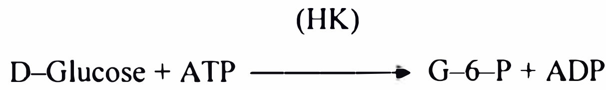
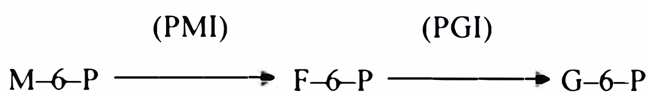
(G–6–P) and mannose–6–phosphate (M–6–P), respectively, with the similtaneous formation of adenosine–5'–diphosphate (ADP).



In the presence of the enzyme glucose–6–phosphate dehydrogenase (G6P–DH), G–6–P is oxidised by nicotinamide–adenine dinucleotide phosphate ($NADP^+$) to gluconate–6–phosphate with the formation of reduced nicotinamide–adenine dinucleotide phosphate (NADPH).

The amount of NADPH formed in this reaction is stoichiometric with the amount of D–glucose. It is the NADPH which is measured by the increase in absorbance at 340 nm. On completion of reaction, M–6–P is converted to fructose–6–phosphate (F–6–P) and then to G–6–P by the sequential action of phosphomannose isomerase (PMI) and phosphoglucose isomerase (PGI).



G–6–P reacts in turn with $NADP^+$ forming gluconate–6–phosphate and NADPH, leading to a further rise in absorbance that is stoichiometric with the amount of D–mannose (Megazyme, 2004).

Chemical analysis of carbohydrates polysaccharides make use of some of the same techniques used for protein analysis. Full determination of the structure of complex heteropolysaccharides involves many steps not only because there are a variety of linkages possible. Spectrophotometer at 340 nm wavelength is equipment there is used to characterize carbohydrates in each samples (Maray and Karen, 2005).

CHAPTER 3

METHODOLOGY

3.1 Material Preparation

3.1.1 Corm Source

One species from Araceae (Ariod) genus was selected for this study (*Amorphophallus hewittii*) because it was suspected that contain considerable amount of glucomannan in their corms. Samples were taken from limestone area in Bau and Batu Niah, Miri, Sarawak (Ipor *et al*., 2006; Gerald and Junaidi, 1999).

Figure 3.1: Sampling site at Batu Niah, Miri, Sarawak, Malaysia

3.1.2 Corms Preparation

Preparing samples is an extremely important preliminary to carbohydrates determination (John and Leo, 2004; Suzanne and James, 2003). When collecting corms, unbroken skin was choose and it were keep for several weeks if stored in a cool (10 °C) and dark place (Clare, 1998; Ikuzo and Edilberto, 1984). Corms were taken up by using hand tools, garden trowel to reduce the damage of corms, because fungi or molds tend to grow, especially through the cracked or damaged corms. Outer layer of two old days *Amorphophallus hewittii* corms was removed and wash with distilled water. The corms were slices to 2 mm thickness as suggested by Clare, 1998.

Sliced sample were mixed with 20 ppm salt solution (20 g NaCl mixing in 1 L distilled water) as an alternative of sodium bisulfite or sodium hydrogen sulfite ($NaHSO_3$) (Kanna *et al.*, 2002) to reduced the concentration of oxalate crystals, prevent browning (caused by oxidation) and preserve apparent freshness of slices. All samples were repeatedly washed with distilled water to remove oxalate crystals (poisonous component) (Dietrich and Hans, 2004; Richard and Robert, 1990). All samples were air dried under ambient conditions for before drying process in oven. One hundred grams wet weight of sliced sample was taken before sealed. All samples were prepared for three replicates. The samples were dried in oven at 40, 50, 60, 70 and 80 °C for 24 hours. The dried samples then ground using home blender to produce 250 μm mesh size flour for further experiment.

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The moisture content of flour for each replicate were taken by using moisture analyzer at 200 °C, where one gram of sample are used for a eight minutes, with a moisture content in any type of flour not more than to 11%. Dried samples (flour) were stored in clean air tight plastic vials and close the cover with parafilm (Fellows, 2005; Gustavo *et al*., 2005; Jeffrey, 2004; Marie and Dana, 2002; Michael and Bewley, 2000; Morris, 1998; Norman and Joseph, 1998; Gladys, 1974; Yeshajahu and Shellenberger, 1971).

3.1.3 Test Kit/Use

The kits from Megazyme Assay (K–GLUM), Ireland contain reagents that are used to determine the glucomannan content. Each reagents contain sodium azide, NaN_3 (0.02% w/v) as preservative to stable the solutions for more than two years approximately.

The kit contains:

i. Sodium acetate buffer (25 ml, 1 M, pH 4.5)
ii. TEA buffer (12.5 ml, 1 M, pH 7.6) plus magnesium chloride (100 mM)
iii. $NADP^+$ (150 mg) plus ATP (440 mg)
iv. *β*–Mannanase suspension (1.2 ml, 450 U/ml)
v. *β*–Glucosidase (100 U/ml) plus *β*–mannosidase (100 U/ml) suspension, 1.1 ml
vi. Hexokinase (425 U/ml) plus glucose–6–phoshate dehydrogenase (212 U/ml) suspension, 1.1 ml

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vii. Phosphoglucose isomerase (1,000 U/ml) plus phosphomannose isomerase (1,000 U/ml) suspension, 1.1 ml

viii. D–Glucose plus D–mannose standard solution (0.2 mg/ml of each)

3.2 Experimentation

3.2.1 Effect of Drying Temperatures on Glucomannan Content

Drying temperature is most important factors to produce desire flour which is contain the higher glucomannan. The two days old corms, were prepare (refer – 3.1.2 Corms Preparation) and dried at different drying temperatures, which were 40, 50, 60, 70 and 80 °C. For each temperature, three replications of samples were tested. After all samples were dried, blended and sieved, one gram of each samples were analyzed for their moisture content using moisture analyzer (Megazyme, 2004; Norman and Joseph, 1998).

3.2.2 Effect of Storage Periods on Glucomannan Contents

Upon determined the best drying temperature for extracting glucomannan, the temperature were used for further study. For this reasons, drying temperature of 60 °C were used for further determination of glucomannan in this study. The harvested corms were stored in ambient room condition for the periods of 7, 14, 21 and 28 days with replicates for each period (Catharina *et al*., 1999; Clare, 1998; Southgate, 1976).

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3.3 Glucomannan Determination Method

3.3.1 Determination of Glucomannan in *Amorphophallus hewittii* Flour

The 0.100 g mass of grounder flours with replicates from different drying temperature; 40, 50, 60, 70 and 80 °C were transfer into centrifuge tubes (15 ml) and the tube were tap to ensure of the sample falls to the bottom of the tube. Five millilitres of 80% (v/v) aqueous ethanol were added to each tube, and tubes were stirred in a vortex mixer and incubated at 85 to 90 °C for five minutes. The ethanol mixer were added a further, and stirred in a vortex mixer for five minutes and centrifuge in Bench Top Centrifuge; 1,500 *g* for ten minutes. Supernatant solutions were decanted and discard with carefully. The pellet were resuspend in 5 ml of 80% (v/v) aqueous ethanol, and the tubes were stirred and added with another 5 ml of 80% (v/v) aqueous ethanol. All tubes were stirred for five minutes and centrifuge at 1,500 *g* for 10 minutes for second rounds. A supernatant solution were decanted and discards with carefully and were allow excess liquid to drain onto Whatman GF/A glass fiber filter paper (9 cm). The washing steps are performed to remove low molecular weight sugars, polysaccharides to monosaccharide; D–glucose and D–fructose.

The pellet was resuspend in 8 ml of 50 mM sodium acetate buffer (pH 4.5) (11.55 ml glacial acetic acid per litre (0.2 M) in water mixed with 27.2 g sodium acetate ($NaC_2H_3O_2.3H_2O$) per litre (0.2 M) in water, then with 100 ml distilled water) (Ronald *et al.*, 2005) plus sodium azide (0.02% w/v) as a preservative and entire tubes were stirred vigorously in a vortex mixer to effect complete dispersion. The tubes were immediately place in a boiling water bath and incubate for 30 seconds and removed stirred vigorously in a vortex mixer for five minutes. The heating and

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stirring step was repeated. Finally, the tubes were incubate in the boiling water bath for four minutes to ensure full hydration of the glucomannan and tubes were removed, stirred vigorously in a vortex mixer for five minutes and place in a water bath at 40 °C. After five minutes, 20 µl of β–mannanase suspension (450 U/ml) were added onto tubes and stirred vigorously on a vortex mixer for 30 seconds. Tubes were incubated at 40 °C for 60 minutes with intermittent vigorous stirring (about 2 to 3 times) in a vortex mixer. Alternatively, tube contents were stirred continuously with magnetic stirrer (Megazyme, 2004).

The solutions were quantitatively transferred to beaker (200 ml) and the volumes were increased approximately 70 ml. The pH of the solutions was adjusted to approximately pH 12.5 by dropwise addition of 1 M NaOH and was leave at room temperature for 10 minutes to effect complete deacetylation of the glucomanno–oligosaccharides. The twenty millilitres of 200 mM sodium phosphate buffer (27.6 g $NaH_2PO_4.H_2O$ per litre (0.2 M final) in water mix with 53.65 g $Na_2HPO_4.7H_2O$ per litre (0.2 M) in water and then were dilute with water to 200 ml) (Ronald *et al.*, 2005) was added and the pH was adjusted to pH 6.5 by dropwise addition of 1 M HCl. The solution was quantitatively transferred to a 100 ml volumetric flask, and the volume was adjusted by using distilled water and mix well.

The 0.5 ml of sample solution from 40, 50, 60, 70 and 80 °C drying temperatures with replicate for each treatment was pipette using Micro–pipettors and applies in glass cuvettes for two parts; blank and sample cuvettes. For blank part, 0.02 ml of distilled water (at 35 °C) and 0.5 ml of sample solution were added in glass cuvettes and for sample part, no distilled water were added but 0.5 ml of sample solution and

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0.02 ml of reagents suspension number five (β–Glucosidase (100 U/ml) plus β–mannosidase (100 U/ml) were added. The entire solutions were incubated at 35 °C for 20 minutes. These step was continued by adding 2 ml of distilled water, 0.2 ml of reagents suspension number two (TEA buffer) and 0.1 ml of reagents suspension number three ($NADP^+$/ATP) for both, blank and sample part. All solution were incubate at 25 to 30 °C and read the absorbance (A_1) after approximately three minutes using spectrophotometer in optical density at 340 nm and reactions were start by adding 0.02 ml of reagents suspension number six (hexokinase enzyme plus glucose–6–phosphate dehydrogenase) for both part, blank and sample (Southgate, 1976). The mixture of solution were read the absorbance (A_2) at the end of the reaction (approximately five minutes) and if the reaction has not stopped after five minutes, reading the absorbance were continued at two minutes intervals until the absorbance remain the same over two minutes.

The entire solutions were continued by mixer the 0.2 ml of reagents suspension number seven [Phosphoglucose isomerise (PGI) plus phosphomannose isomerise (PMI)] and the solutions were read the absorbance (A_3) at the end of the reaction (approximately 20 minutes) and reading the absorbance were continued at two minutes intervals until the absorbance remain the same over two minutes if reaction not stopped after 20 minutes.

3.4 Calculation

The absorbance differences $(A_2–A_1)$ and $(A_3–A_2)$ for both, blank and sample were determined and the values of $^{\Delta A}$D–glucose, $^{\Delta A}$D–mannose and $^{\Delta A}$D–glucomannan were calculated:

Formula to Determined D–glucose from Glucomannan

$^{\Delta A}$D–glucose = $(A_2–A_1)$ sample – $(A_2–A_1)$ blank

Formula to Determined D–mannose from Glucomannan

$^{\Delta A}$D–mannose = $(A_3–A_2)$ sample – $(A_3–A_2)$ blank

Formula to Determined of Glucomannan

$^{\Delta A}$D–glucomannan = $(A_3–A_1)$ sample – $(A_3–A_1)$ blank

Formula to Determined the Content of Glucomannan as percentage (w/w)

$$= \frac{^{\Delta A}\text{glucomannan}}{6{,}300} \times \frac{V}{1{,}000} \times MW \times \frac{FEV}{v} \times \frac{100}{w} \times F$$

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Where,

ΔA glucomannan	=	(A_3-A_1) sample – (A_3-A_1) blank
6300	=	extinction coefficient of NADPH at 340 mm [$1 \times mol^{-1} \times cm^{-1}$]
ΔA glucomannan/6300	=	factor to determine molarity of NADPH
V	=	final volume in assay cuvette [ml]
V/1000	=	factor to convert molarity to moles of NADPH
MW	=	molecular weight of anhydro–D–glucose/D–mannose as occurs in glucomannan polysaccharides
FEV	=	final extraction volume
V	=	volume of sample added to the glass cuvette
FEV/v	=	factor to determine the number of grams of glucomannan in the final extract
w	=	weight of sample extracted (grams)
100/w	=	factor to express glucomannan content as a percentage of the sample
F	=	dilution factor

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3.5 Morphology of Glucomannan Granule

A drop of Iodine–KI reagent was added onto a microscope slide which contains a small amount of glucomannan sample suspended in distilled water. The Iodine stain was observed and photographed on a transmission electron microscope (Leica CME) at 10,000 times and operated at an accelerating voltage of 60 or 80 kV. Photographs were digitized and manipulated with Adobe Photoshop to prepare figures.

3.6 Experimental Design and Statistical Analysis

For the determination of the correlation between treatments, the experimental design was used for this study are Completely Randomized Design (CRD). The data were analyzed by using Analysis of Variance (ANOVA) and the treatment means were statistically compared by Duncan's Multiple Range test (DMRT) at the 5% level of significance. The data were analyzed using Statistical Analysis System (SAS) program version 9.1. A Microsoft Excel spread sheet program was employed for all calculations. A more detailed description of the analysis procedure is provided under Results and Discussion.

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CHAPTER 4

RESULTS

4.1 Comparison of Glucomannan Content Different Drying Temperatures

At 60 °C contain the highest mean concentration of glucomannan content compared to 40, 50, 70, and 80 °C (Table 4.1) with 0.92/100 g or 33.90% glucomannan of flour but this value least then *Amorphophallus konjac* flour; which consists an average of 18% dry matter, of which 55 to 60% is glucomannan, starch (20%), protein (10%), minerals and others secondary components (around 10%) (Lind *et al.*, 2006; Kanna *et al.*, 2002; Catharina *et al.*, 1999). Drying temperature were found significantly affect the content of glucomannan in the corms. It was found that drying or corms slices under 60 °C produced higher amount of glucomannan (0.92/100 g) compared to other drying temperatures.

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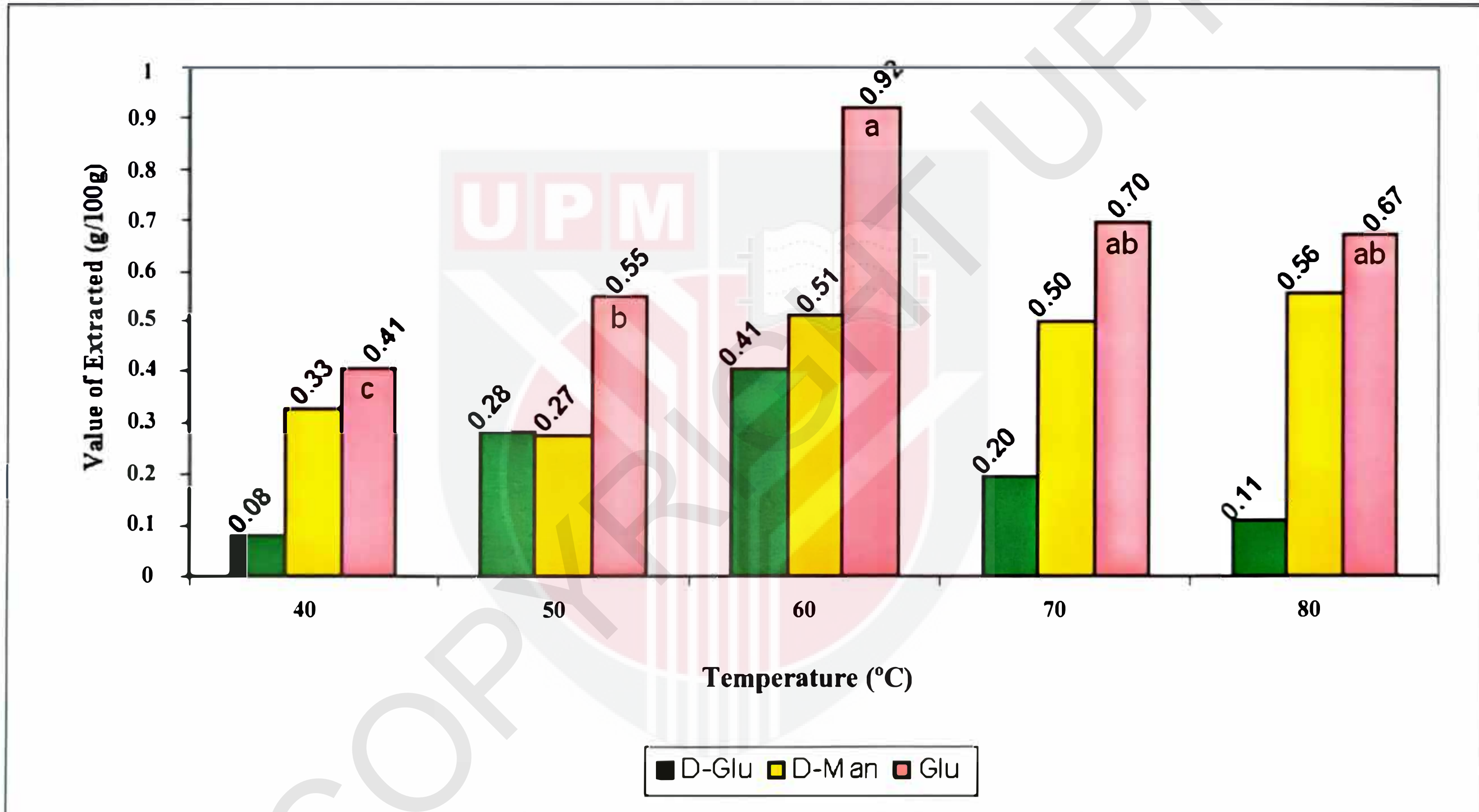

Means with same alphabets within each temperature are not significantly different at $p \leq 0.05$ (DMRT)

Figure 4.1: Concentration of glucomannan content in flour samples

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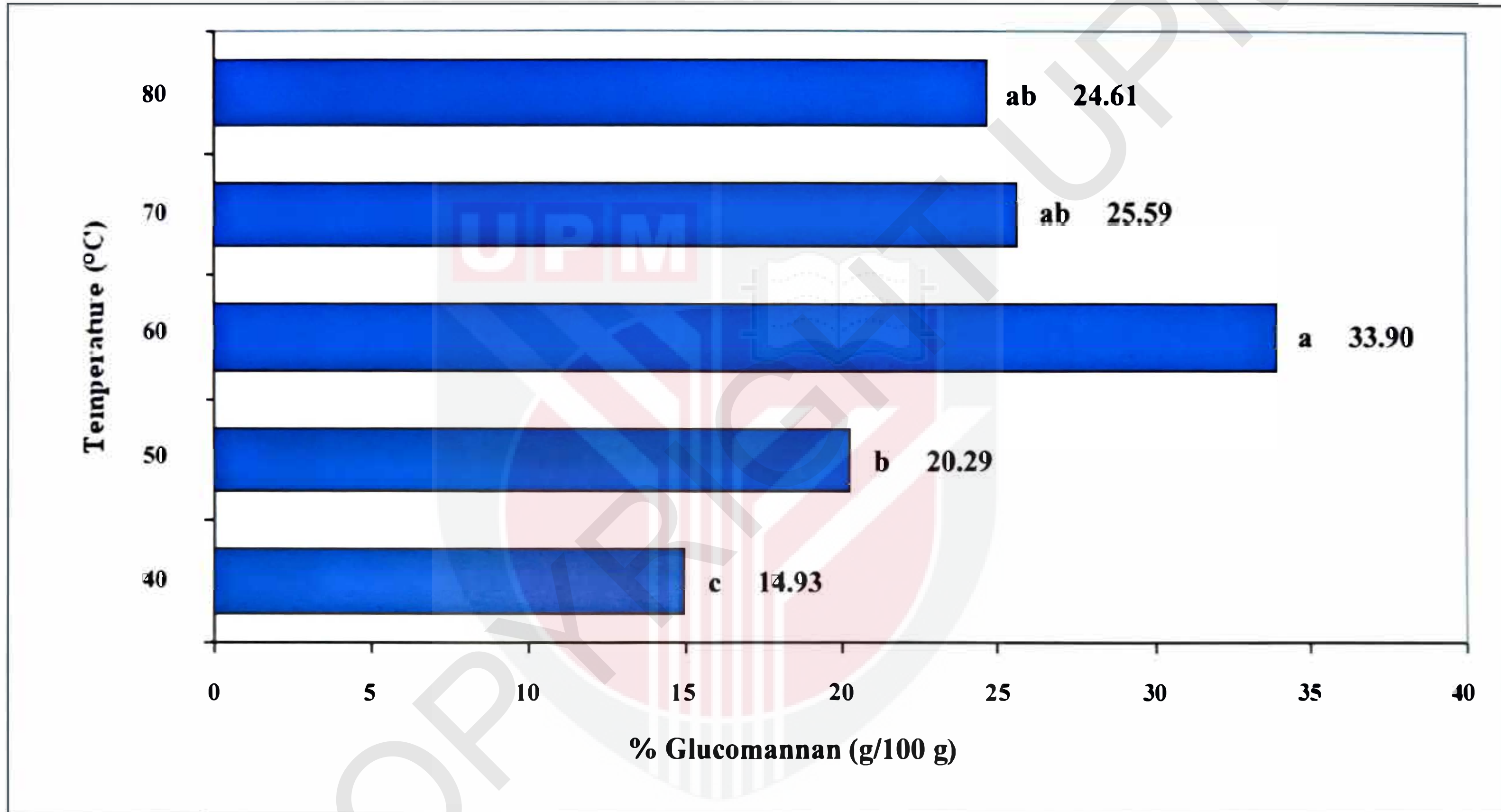

Figure 4.2: Percentage of glucomannan content in flour samples at different drying temperatures

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4.2 Comparison of Glucomannan Content Due to Different Storage Periods

Storage periods showed significant affect on the contents of glucomannan prolong storage periods under ambient condition was found to result on the reduction of glucomannan content in the corms (Table 4.1).

Table 4.1: Percentage of glucomannan content in the flour samples

Week	Percentage of Glucomannan (%/100 g)
0	33.90 [a]
1	32.40 [ab]
2	29.70 [ab]
3	27.20 [b]
4	21.30 [c]

The changes in percentage of glucomannan decreased with increasing flour moisture and period storage over four weeks of corm storage are shown in Figure 4.3. The percentage of glucomannan available in grounded flour with four weeks storage corm are 21.30%/100 g and 18.00% moisture showed no significant difference from two old days corms; 33.90%/100 g of glucomannan with 10.40% moisture. These treatments were doing under 60 °C, better temperature for producing higher glucomannan.

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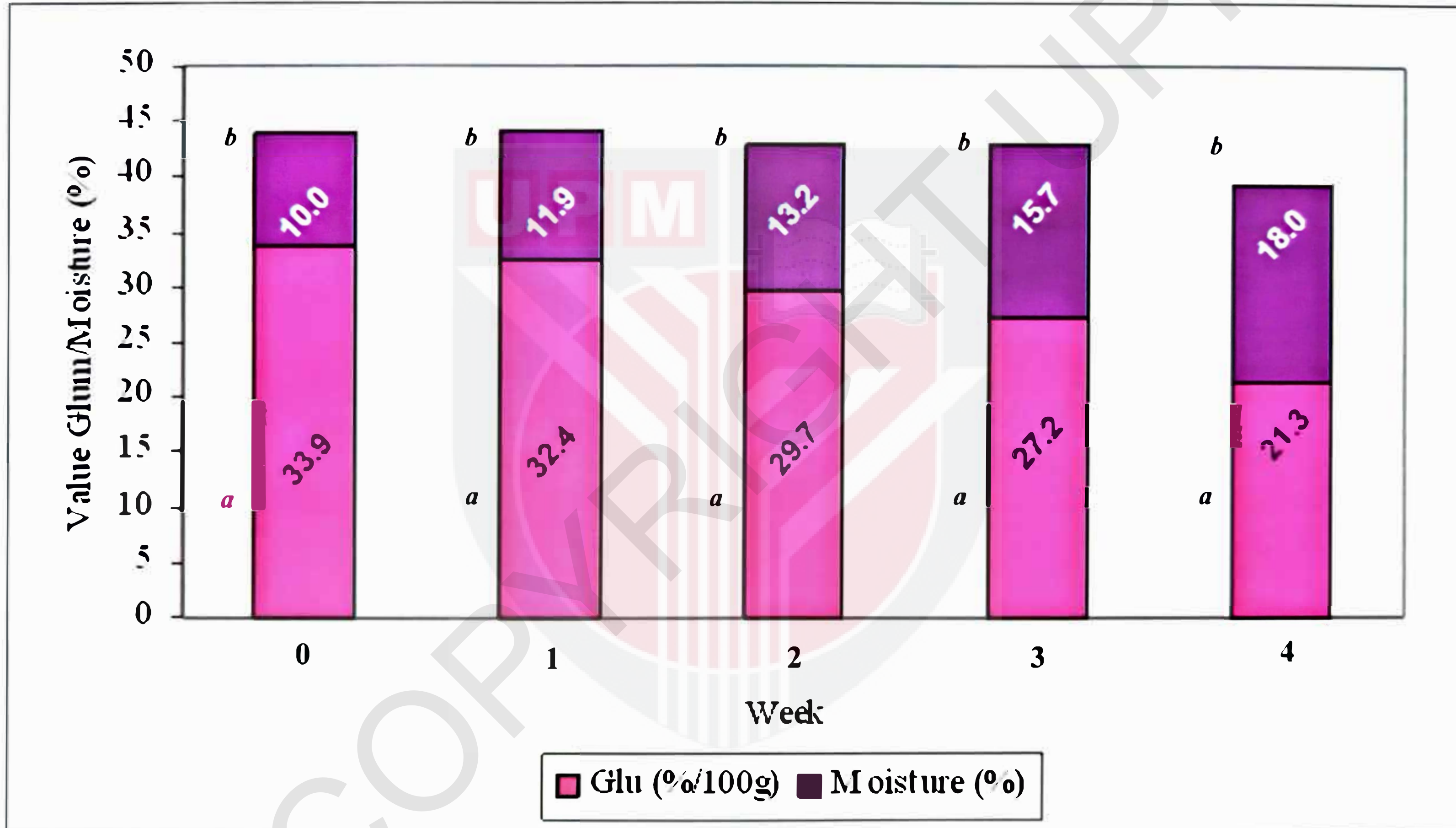

Figure 4.3: Changes in percentage of glucomannan and moisture content in flour samples over four weeks
[a] Values glucomannan availability in flour are expressed as 100/g in percentage
[b] Moisture in 100 g grounded flour are expressed in percentage

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CHAPTER 5

DISCUSSION

5.1 Availability of Glucomannan

The result showed (Figure 4.1) the availability of glucomannan in *Amorphophallus hewittii* obtained in this study were 10% of dry matter; 33.90% is glucomannan relatively low compare glucomannan available in *Amorphophallus konjac* were 18% dry matter; 55 to 60% glucomannan, 20% starch, 10% protein, and minerals with others secondary components, around 10% (Lind *et al.*, 2006; Catharina *et al.*, 1999). However, the percentage of glucomannan available in these species same with others species such as *A. albus*, *A. dunnii*, *A. muelleri*, *A. rivieri*, *A. variabilis*, *A. ximengensis* and *A. yuloensis* with more then 30% but *A. campanulatus* yield very low (Kanna *et al.*, 2002). The higher glucomannan available in *Amorphophallus konjac* become its most common species were used for producing food products and others such as in pharmaceutical, biotechnology, and fine chemical (Steve, 2005; Tom and Emery, 2003; Flach and Rumawas, 1996).

However, other factors must be considered to obtain the availability of glucomannan in species such as size and aged of corms, processing of corms, storage of corms in suitable temperature, fresh corms without any damage and infected of fungi and insect (Clare, 1998; Ikuzo and Edilberto, 1984).

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5.2 Drying Temperatures

The higher of glucomannan content were produced at 60 °C (Catharina *et al.*, 1999). At 60 °C drying temperature of sliced for processes flour (Figure 4.1), 0.92 g with 10.4% moisture content glucomannan can react from 100 g of flour compare 0.70 g at 70 °C for two old days corms. The increasing with 10 °C of drying temperature gives bigger affects with reducing around 0.23 g per 100 g flour or 8.25% in glucomannan concentration even moisture content in flour at 70 °C drying temperature lower then 60 °C. The enzymes in these kits can be useful functioning when find of flour, 250 mesh approximately, suitable temperature between processed and moisture in the flour because in the certain level, enzymes were stop to breakdown the flours cause by limited factors. For analysis total carbohydrate, the best percentage of moisture is arranging between 10.0 to 11.0% (corn flour, wheat, and rice flour) (Ronald *et al.*, 2005). In this case, moisture content and drying temperature are limited factor at 70 °C and same reasons for 40, 50 and 80 °C drying temperatures. Drying temperature significantly affect the availability of glucomannan.

Increase of drying temperature above to 70 and 80 °C inhibits carbohydrate transformations in dried flours (Ikuzo and Edilberto, 1984; Birch *et al.*, 1973). The best combination occurred for stating temperature of 60 °C during 24 hours drying slices from two old days corm; causing a rapid formation of an outer crust on the corms sliced a preventing agglomeration. The final dry matter obtained was 10%, which is considerable as a good starting point for another step, grinding with nice and off–white slices with produce off–white flour (Kanna *et al.*, 2002).

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In these conditions the inactivation of enzymes decomposing the starch is likely to have occurred, and a slight decrease in content of polysaccharides should result from the action of enzymes in the initial phase of drying when the temperature is rising to the fixed level.

5.3 Storage Periods under Ambient Conditions

Corms from this Aroid genus have a short shelf life because of their high moisture content (13 to 25%) and high metabolic activity after harvesting. Higher availability of glucomannan was reacted at two old days storage and experimentation was followed with 7, 14, 21, and 28 days showed decreasing of glucomannan available in grounder flour after seven days. Immediate processing after collecting is suggested method for further experimentation. The principal corms are high moisture content and their parenchyma cells are packed with starch grain an these materials show variable degrees of inherent keeping life which remains dormant for three to four months to other species such as; *Manihot esculenta* in which there is no natural dormancy (Daisy and Gooding, 1987). When such material is stored in the fresh state, respiration and transpiration continue with inevitable losses of water and dry matter. Prolonged high levels of transpiration result in a change of texture affecting quality and weight loss.

The dry matter loss, caused by superation constitutes a real food value as distinct from the moisture loss which only has an economic value. Sprouting at the end of dormancy can result in dramatic loss as the physiological state is altered. At this stage the stored starch is transformed to sugars and utilized by the elongating shoots with appreciable loss of both food value and moisture. Storage of fresh corms is

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important for marketing, to free farmland for new cropping, and to ensure the availability of seed cormels in the next planting season (Daisy and Gooding, 1987).

In particular, storage at ambient temperatures is considered impossible due to very high incidence of fungal decay. Corms are stored in a variety of traditional storage in India, China and Japan; low–cost structures such as shade, hut and underground pits, placed in boxes or directly on the storage floor (Nishinari and Goa, 2007). Corms may also be stored in heaps in a shade and/or covered with straw or plantain leaves. These traditional storage conditions reduce moisture loss and promote the curing of wounds. Under these conditions, the storage life of corms has been extended for up to four weeks with no beneficial effect on the storage and fungal infection is also reduced. In this study, corms were sealed with aluminium foil and then tightly in plastic bag before placed in box to reduced the damaged, hibernate the corms and maintain the natural moisture but these methods still not efficiency because carbohydrates transformations in corms also reduce with increasing of storage periods with 33.90% in two old days storage to 21.30% around 20 to 30 days.

Traditional storage systems are mainly suited for short–term storage and have limited success with long–term storage, which is necessary for marketing beyond the harvest period. Existing results are largely variable and in many instances the corms decay and become unfit for human consumption after a short period. Different levels of corm wastage and losses have been reported for different lengths of storage. It has been shown in Figure 4.3, that the corms can be stored at tropical ambient conditions (7 to 10 °C with 86 to 98% relative humidity) for at least two weeks without no significant changes in nutritional values such as crude protein content and total

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amino acids but significant reduction in carbohydrates available (glucomannan) and increase in total sugar content (Daisy and Gooding, 1987; Ikuzo and Edilberto, 1984).

The limitations of traditional storage structures have resulted in the search for improved storage systems refrigerated storage. Corms can lose as much as 25% of their initial weight in four weeks of storage, but can be successfully stored at cool conditions (10 °C) for several weeks (Daisy and Gooding, 1987; Ikuzo and Edilberto, 1984). When their weight of corms is lower, the glucomannan content also reduced. The results show that the percentages of glucomannan in flour varied from storage period of corms were reduced consistent from two old days to four weeks (Table 4.1). It is because of carbohydrate transformation in flour are weak comparing with flour processes from sampling and first week (Ikuzo and Edilberto, 1984; Birch *et al.*, 1973).

Corms in refrigerated containers and storage at 3 to 5 °C is a common practise can remain in good condition for up to six weeks, but once they are exposed to ambient conditions they deteriorate rapidly after 24 hours. Plastic bags are successful storage of corms and the conditions created inside the bag reduce moisture loss and facilitate the curing of wounds. Packing corms in plastic bags and closely tying the open end with rubber bands reduced the decay severity and percentage weight loss. For commercial handling purposes, packing in polyethylene bags often follows the selection of good quality corms, fungicide application and draining, and air–drying (Kanna *et al.*, 2002).

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It was reported that the storage life of corms in such bags was 26 to 40 days over those packed in cartons (Kay, 1973). Corms stored in polyethylene bags showed a 6% loss in fresh weight and 50% decay, without any treatment loss 25% of initial weight. Dipping corms in one percentage of NaCl before storage in polythene bags provided additional protection against fungal infection (Kanna *et al*., 2002). Other storage environments such as coir dust and hull ash can increase storage life and reduce the severity of decay of corms when corms were stored in boxes with containing moist coir, showed a weight loss and decay were only 7 and 5%, respectively. For best results, it is important to ensure that the moisture content of the coir is damp and not wet as the latter would facilitate the decay of corms. The combination of this method should practice and applied for corms of *Amorphophallus hewittii* for extend shelf–life, two or three months.

Flour is a very hygroscopic material and its moisture changes with changes in temperature and humidity of the store environments. In fourth weeks, the characteristics of flour are not very fine compare sampling and first week because the corms already absorb much moisture from air. Flour changes become less pronounced during storage at low temperatures. Besides that, the proper methods for storage of flour also must be replaced in airtight container and sealed with safety cover (parafilm) at top of container to prevent any microbe and fungus with can affect enzyme to breakdown product for small chain of saccharide carbohydrates (Marie and Dana, 2002).

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However, the results could have been influenced by others factors. Corms are susceptible to damage during harvesting (Kanna *et al.*, 2002). Damage can occur as a result of the harvesting tool injuring the corm, or as a consequence of rough handling (e.g. corm–to–corm impact by throwing harvested corms into a pile). Physical damage such as punctures, cuts or abrasion lead to high rates of moisture loss and provide avenues for microbial infection. These conditions lead to high incidence of shrinkage and postharvest losses down the handling chain.

Fresh corms without any infected of fungus or injured cause equipments when sampling collection have differentiations of water content in sap cell tissues which is higher then non–fresh corms. These were effects the sliced amount in 100 g mass of wet weight if wet weight of fresh sliced corms higher then sliced of wilt corm. It's because fresh corms contain more water compare non–fresh (Clare, 1998). Harvesting is carried out by lifting the corms by hand. Simple tools such as trowel are used to remove the soil around the corm. Although experimental mechanical lifters have been studied, there is no commercial equipment dedicated to harvesting aroids (Kanna *et al.*, 2002).

Another factor will be decreased or increased the glucomannan content with mannan content of the corms is highest when the leaves wither and is independent of the corms size. The condition of the leaves is a good maturity index for assessing the readiness of corms for harvest. Corms are mature for harvesting when the leaves begin to turn yellow and start to wither. A mature corm produces a large edible main corm and a few lateral cormels, about 4 to 10 in number. The main tuber is often harvested with the smaller corms left to develop later. Its means independent fresh

corms with no shade of leave were collected to produce higher glucomannan flour (Flach and Rumawas, 1996).

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CHAPTER 6

CONCLUSION

In line with the objectives of the project, the percentages of glucomannan available in five different storage periods were determined. The scope of analysis focuses on the availability of glucomannan in different of temperatures sample reading and their changes over a period of fourth weeks. From these studies, glucomannan was found in grounded flour of *A. hewittii* slices dried corms. The availability of glucomannan was the highest in dried sliced from fresh corm at 60 °C drying temperature. Post-harvest are critical point in controlled fresh corms from damage and lower of glucomannan available. Under ambient conditions of high temperature and high relative humidity common in most tropical regions, wet tissue provides conductive environment for microbial growth and spread to healthy produce and the proper methods results showed at two until seven days showed higher availability of glucomannan in grounded flour processes in 60 °C. The mean availability of glucomannan in most flour samples fluctuated throughout the fourth weeks because the changes of moisture in corms with higher of humidity of the store environment. The availability of glucomannan determined in these studies was same higher with others species. *A. hewittii* has the potential to be introduced as new crop for glucomannan flour producing and needs further investigation for create new methods for Aroids species for long term storage without affects any nutrient value available in corms.

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BIBLIOGRAPHY

Alais, C. and G. Linden. 1991. Food Biochemistry. Polysaccharides Hydrocolloids and Gelling Agents. England: Ellis Harwood Limited.

Amy, B. 2000. Understanding Food Principles and Preparation. Food Science and Nutrition. Chemistry of Food Composition. United State of America: Wadsworth Thomson Learning.

Anekant, J., G. Yashwant and K.J. Sanjay. 2007. Perspectives of Biodegradable Natural Polysaccharides for Site–Specific Drug Delivery to the Colon. Gour University, Sagar, India.

Ann–Charlotie, E. 1996. Carbohydrates in Food. New York: Marcel Dekker, Inc.

Arminas, S.A. and J.F. Calello. 2002. Gelled Cosmetic Remover Composition. United States of America: United States Patent.

Arnold, E.B. and A.B. David. 1995. A Dictionary of Food and Nutrition. New York: Oxford University Press, Inc.

Atkins, E.D.T. 1985. Polysaccharides: Topics in Structure and Morphology. Ultrastructure and Morphology. London: The Macmillan Press Ltd.

Berk, Z. 1976. Braverman's: Introduction to The Biochemistry of Foods. Amsterdam: Elsevier Scientific Publishing Company.

Belitz, H.D., W. Grosch and P. Schieberle. 2004. Food Chemistry. 4th ed. German: Springer–Verlag Berlin Heidelberg.

Birch, G.G., L.F. Brennan, R.J. Priestley and G. Sodah–Ayernor. 1973. Molecular Structure and Function of Carbohydrate. London: Applied Science Publishers Ltd.

Blanshard, J.M.V. and J. R. Mitchell. 1979. Polysaccharides in Food. London: Butterworths & Co (Publishers) Ltd.

Bogner, J. and W.L.A. Hetterscheid and S. Ittenbach. 1993. Notes on the Genus Amorphophallus (Areceae). Three New Species from Tropical Asia. Netherlands: Blumea.

Bradbury, J.H. and W.D. Holloway. 1988. Chemistry of Tropical Root Crops: Significance for Nutrition and Agriculture in the Pacific. Canberra: Australian Centre for International Agricultural Research (ACIAR).

Brain, A.F. and G.C. Allan. 1995. Food Science, Nutrition and Health. Sixth ed. London: ARNOLD.

© COPYRIGHT UPM

Catharina, Y.M.A., L. KeShun and H. Yao–Wen. 1999. Asian Foods. Science and Technology Cultural Aspects of Asian Dietary Habits–Japanese Foods. Pennsylvania, USA: Technomic Publishing Company, Inc.

Cescutti, P., C. Campa, F. Delben and R. Rizzo. 2002. Structure of the oligomers obtained by enzymatic hydrolysis of the glucomannan produced by the plant *Amorphophallus konjac. Journal of Science Direct Elsevier Ltd* **24**: 2505-2511

Chandrasekaran, R., J. Srinivas and J.M. Victor. 2003. Acetan: glucomannan interactions – A molecular modeling study. *Journal of Science Direct Elsevier Ltd* **24**: 2889-2898

Chandra, S. 1984. Edible Aroids. Oxford: Clarendon Press.

Chao–bo, X., G. Shan–jun, L. Guo–rong and Z. Qun–chao. 1999. Preparation of konjac glucomannan and acrylamide grafted konjac glucomannan. *Journal of Natural Sciences* **4**: 459-462

Chen, W.D. and S.C. Zhang. 2000. Faming. China: People's Republic of China Press.

Chen, Y., L.N. Zhang., Y.S. Lu., C.S. Ye and L.B. Du. 2003. Preparation and properties of water–resistant soy dreg/benzyl konjac glucomannan composite plastics. *Journal of Applied Polymer Science* **90**: 3790-3796

Chia, J.S. 2004. What's In Your Food? Your Healthy Lifestyle Guide. What You Need to Know About Food Ingredients, Additives, Toxins and Nutrients. Malaysia: Pelanduk Publications Sdn. Bhd.

Christophe, W. 2000. Medicinal Plants of Southeast Asia. Malaysia: Pelanduk Publications (M) Sdn. Bhd.

Clare, C. 1998. The Hamlyn Vegetable Book. Yam. London: Pelanduk Publications (M) Sdn. Bhd.

Daisy, E.K. and E.G.B. Gooding. 1987. Root Crops. Crop and Product Digest No. 2 – 2nd ed. London: Tropical Development and Research Institute.

Daniel, M. 2006. Medicinal Plants: Chemistry and Properties. Gums and Mucilages. United States of America: Science Publishers, Enfield.

David, A.B. 2006. Benders' Dictionary of Nutrition and Food Technology. 18th ed. England: Woodhead Publishing In Food Science, Technology and Nutrition and CRC Press LLC.

David, A.B. 2005. Dictionary of Food and Nutrition. A hand, Non–Technical Guide on All Aspects of Diet and Health. 2nd ed. New York: Oxford University Press Inc.

© COPYRIGHT UPM

David, T.J. 1993. Flora of Malaysia: Illustrated. New York: Oxford University Press, Inc.

Dettmar, P.W., P.A. Dickson, F.C. Hampson and I.G. Jolliffe. 2000. Compositions containing alginate and gums to improve bio–adhesive properties for treatment of disorders of the esophagus, 35p. World Intellectual Property Organization.

Dietrich, F. and J.P. Hans. 2004. Poisonous Plants. A Handbook for Doctors, Pharmacists, Toxicologists, Biologists and Veterinarians. London: Manson Publishing Ltd.

Donald, L.P. 1970a. Tropical Root and Tuber Crops Tomorrow. University of Hawaii Press.

Donald, L.P. 1970b. Tropical Root and Tuber Crops Tomorrow. University of Hawaii Press.

Fellows, P.J. 2005. Food Processing Technology: Principles and Practices. 2nd ed. England: Woodhead Publishing in Food Science and Technology.

Flach, M. and F. Rumawas. 1996. Plant Resources of South–East Asia (Prosea). Plants Yielding Non-seed Carbohydrates–*Amorphophallus* spp. Indonesia: Backhuys Publishers, Leiden.

Frederick, J.F. 2000. Wiley Encyclopedia of Food Science and Technology. Gums. 2nd ed. Canada: John Wiley and Sons, Inc.

Gail, G. 2003. Plants: Diet and Health. Epidemiology Linking Consumption of Plant Foods and Their Constituents with Health. USA: Blackwell Science.

Gaman, P.M. and K.B. Sherrington. 2004. The Science of Food. Carbohydrates. 4th ed. Oxford, UK: Elsevier Butterworth–Heinemann.

Gan, X. 1999. Method for the production of glucomannan aldehydic acid fatty ester sodium salts from konjac, 9p. People's Republic of China, China.

Gao, S.J. and L.N. Zhang. 2001a. Molecular weight effects on properties of polyurethane/nitrokonjac glucomannan semi–interpenetrating polymer networks. *Journal of Applied Polymer Science* **34**: 2202-2207

Gao, S.J. and L.N. Zhang. 2001b. Semi–interpenetrating polymer networks from castor oil–based polyurethane and nitrokonjac glucomannan. *Journal of Applied Polymer Science* **81**: 2076-2083

Gao, S.J., L.N. Zhang and J.L. Cao. 2003. Synthesis and characterization of poly (Ester urethane) nitrokonjac glucomannan semi–interpenetrating polymer networks. *Journal of Applied Polymer Science* **90**: 2224-2228

© COPYRIGHT UPM

George, H.M.L. 1951. Taxonomy of Vascular Plants. Selected Families of Vascular Plants–Monocotyledoneae. New York: Macmillan Publishing Co., Inc.

Gerald, C. and P. Junaidi. 1999. Wild Malaysia. The Kelabit Highlands–Gunung Mulu National Park. UK: New Holland Publishers Ltd.

Gladys, C.P. 1974. Foundations of Food Preparation. Preparation of Food and Food Products. 3rd ed. New York: Macmillan Publishing Co., Inc.

Gustavo, V.B., O. Enrigue, J. Pablo and Y. Han. 2005. Food Powders. Physical Properties, Processing, and Functionality. Particle properties. New York: Kluwer Academic/Plenum Publishers.

Henderson, M.R. 1954. Malayan Wild Flowers: Monocotyledons. Kuala Lumpur: The Malayan Nature Society.

Hermelin, M.S. and M. Grimshaw. 2003. Modified Release Minerals. United States of America: United States Patent Application.

Hetterscheid, W.L.A. and S. Ittenbach. 2001. Notes on The Genus Amorphophallus (Areceae)–11 New and Obsolete Species from East Malaysia and Continental Southeast Asia. Netherlands: Blumea.

Hui, Y.H. 2006. Food Powders. Physical Properties, Processing, and Functionality Functional Properties of Carbohydrates: Polysaccharide Gums. London: CRC Press Book Taylor & Francis Group.

Igarashi, S., H. Saiki and T. Yamada. 2001. Biodegradable Resin Fishing Lures. Tokyo: Jpn. Kokai Koho Press.

Ikuzo, U. and D.R. Edilberto. 1984. Tropical Root Crops. Postharvest Physiology and Processing. Tokyo: Japan Scientific Societies Press, Tokyo Press.

Ipor, I.B., C.S. Tawan, J. Abai, K. Meekiong, A. Simon and P.C. Boyce. 2004 Preliminary Observations on The Diversity of *Amorphophallus* (Araceae:Thomsonieae) in Sarawak. Universiti Malaysia Sarawak: Universiti Press.

James, A.D., Bogenshutz–Godwin, JuCellier, Duke. 2002. Handbook of Medicinal Herbs 2nd ed. London: CRC Press Book Taylor & Francis Group.

Jeffrey, H. 2004. Bread. A Baker's Book of Techniques and Recipes. New Jersey, Canada: John Wiley & Sons, Inc.

John, A.M. and M.L.N. Leo. 2004. Handbook of Analysis. Revised and Expanded Physical Characterization and Nutrient Analysis. 2nd ed. New York: Library Congress Cataloging in Publication Data.

John, H.W. and L. Blanca. 1999. World Economic Plants: A Standard Reference. Boca Raton, Florida: CRC Press LLC.

© COPYRIGHT UPM

Kanna, S., R.F. Tester and R. Prentice. 2002. Konjac project consortium. Ph.D. Thesis, 3, 12, 26-27p. Glasgow Caledonian University, United Kingdom, London.

Karla, L.R. 1984. Carbohydrate Biochemistry and Metabolism. Specialized Uses of Carbohydrates. United States: Avi Publishing Company, Inc.

Kay, D.E. 1973. Root Crops. Crop and Product Digest. London: Tropical Products Institute.

Kawano, N. 2001. Rice Grains Coating with Dietary Fibers. Tokyo: Jpn. Kokai Koho Press.

Kimpimaki, T., M. Lindstrom and K. Nurmi. 2001. Surface size composition for the surface sizing of paper, board or other suchlike. 18p. World Intellectual Property Organization (WO), PCT Int. Appl.

Kristian, H.K. 2000. The herbaceous vegetation on limestone in Tabin Wildlife Reserve: Quantitative description and microhabitat preferences. In *Tabin Limestone Scientific Expedition 2000*, ed. M. Maryati, S. Menno and A. Mahedi, p. 79, 82-83. Malaysia: Universiti Malaysia Sabah.

Kubitzki, K. 1998. The Families and Genera of Vascular Plants Flowering Plants. Monocotyledons Alismatanae and Commelinanse (except Graminaea). United States: Springer–Verlag Berlin Heidelberg.

Lakshmi, A. 2002. CBS quick Review in Biochemistry. Reference biochemistry on university question papers (1st MBBS 1985 – 2000) 3rd Edition, 8, 46p. CBS Publishers & Distributors, New Delhi.

Lind, E.G., M.G. James and L.W. Wilcox. 2006. The Families and Genera of Vascular Plants Flowering Plants. Introduction to Plant Biology. New Jersey: Pearson Prentice Hall, Pearson Education, Inc.

Lu, Y.S. and L. Zhang. 2002. Interfacial structure and properties of regenerated cellulose films coated with superthin polyurethane/benzoyl konjac glucomannan coating. *Journal of American Chemical Society* **41**: 1234-1241

Ma, J.G., L. Wang and S.Y. Xu. 1999. Emulsifying properties of isolated soy protein–konjac glucomannan conjugate. 12-16p. Wuxi Qinggong Daxue Xuebao, China.

Mahmood, A.K. 1998. Nutrition for Foodservice Managers. Concepts, Applications and Management. Sources of Carbohydrates. Canada: John Wiley & Sons, Inc.

Maray, O. and O. Karen. 2005. The Absolute, Ultimate Guide to Lehninger Principles of Biochemistry. 4th ed.: Study Guide and Solutions Manual. Carbohydrates and Glycobiology. New York: W.H. Freeman and Company.

© COPYRIGHT UPM

Marie, H. and M. Dana. 2002. Changes of wheat flour properties during short term storage. *Czech Journal of Food Science* **4**:125-130

Megazyme Glucomannan Assay Procedure. 2004. Ireland: Megazyme International Ireland Ltd.

Meimei, L., F. Jiangyang, W. Kang and H. Zhimin. 2007. Pharmaceutical Science. Drug Delivery. Synthesis, Characterization, and Evaluation of Phosphated Cross–linked Konjac Glucomannan Hydrogels for Colon Targeted Drug Delivery. United States: Routledge Taylor & Francis Group.

Michael, B. and J.D. Bewley. 2000. Seed Technology and Its Biological Basic. Seed Carbohydrates. London: Sheffield Academic Press Ltd.

Michael, G.S. 2006. Plant Systematics. Evolution and Diversity of Plants Diversity and Classification of Flowering Plants, Systematic Evidence and Descriptive Terminology–Plant Morphology. United States: Elsevier Academic Press.

Morita, S., T. Niimura, K. Matsunaga and H. Uhara. 2001. Charcoal Shaped Products and Their Manufacture. Tokyo: Jpn. Kokai Koho Press.

Morris, C.F. 1998. Pacific People and Their Food. Evaluating the End–Use Cavality of Wheat Breeding Lines for Suitability in Asian Noodles, ed. A.B. Blakeney and L.O'. Brien, p. 91-99. Australia: American Association of Cereal Chemists, Inc.

Naomi, M.N. 1978. Food Drying at Home. Food Drying Round The World. Vancouver, Canada: David & Charles Ltd.

Norman, N.P. and H.H. Joseph. 1998. Food Science. Cereal Grains, Legumes, and Oilseeds. United States: Springer Science Business Media, Inc.

Nishinari, K. and S. Gao. 2007. Konjac glucomannan. In *Functional Food Carbohydrates*, ed. G.B. Costas and M.S. Izydorczyk, p. 97-123. Boca Raton, Florida: CRC Press Taylor & Francis Group.

Nussinovitch, A. 2004. Encapsulating liquid with hydrocolloid membrane stable from about -20 to 90 degrees celsius without bursting, 184p. United States Patent.

Omura, T., K. Abe and T. Nanba. 2001. High Water–Containing Water–In–Oil Cosmetic Emulsions. Tokyo: Jpn. Kokai Koho Press.

Omura, T. and T. Nanba. 2001a. Hair–Styling Preparations Containing Vinyl Alcohol–Vinylamine Copolymers, Glucomannan and Acids. Tokyo: Jpn. Kokai Koho Press.

Omura, T., T. Shida and T. Nanba. 2001a. Hair Compositions Containing Glucomannan and/or Keratose Quaternary Ammonium Derivatives. Tokyo: Jpn. Kokai Koho Press.

© COPYRIGHT UPM

Omura, T. and T. Nanba. 2001b. Cosmetic Emulsions Containing Alkyl–Modified Caroxyvinyl Polymers and Glucomannan. Tokyo: Jpn. Kokai Koho Press.

Omura, T., T. Shida and T. Nanba. 2001b. Hair–Styling Preparations Containing Glucomannan and Polyhydric Alcohol Alkylene Oxide Adducts. Tokyo: Jpn. Kokai Koho Press.

Omura, T., T. Shida and T. Nanba. 2001c. Hair Preparation Containing Glucomannan and Polysiloxanes, p. 10. Tokyo: Jpn. Kokai Koho Press.

Omura, T., T. Shida and T. Nanba. 2001d. Hair Styling Preparations Containing Surfactants, Waxes, and Glucomannan. Tokyo: Jpn. Kokai Koho Press.

Omura, T., T. Shida and T. Nanba. 2001e. Oil–In Water Hair Cosmetic Emulsions. Tokyo: Jpn. Kokai Koho Press.

Onwueme, I.C. 1978. The Tropical Tuber Crops. Yams, Cassava, Sweet Potato, and Cocoyams. New Jersey: John Wiley & Sons Ltd.

Pamela, C.C., H.A. Richard and F.R. Denise. 2008. Lippincott's Illustrated Reviews: Biochemistry. 4th ed. United States: Lippincott Williams & Wilkins.

Pathak, C.P., S.P. Barman, M.C. Philbrook, A.S. Sawhney, A.J. Coury, L.Z. Avila, and M.T. Kieras. 2003. Multiblock Biodegradable Hydrogels for Drug Delivery and Tissue Treatment. 639p. United States Patent.

Paul, I., R.E. Turner and D. Ross. 2002. Nutrition. Carbohydrates. Canada: Jones and Bartlett Publishers.

Peter, B. and J.M. Victor. 2007. The Chemical Physics of Food. Gels. London: Blackwell Publishing Ltd.

Philip, J.H. 2005. Diversity in plant cell walls. In *Plant Diversity and Evolution*: *Genotypic and Phenotypic Variation in Higher Plants*, ed. R.J. Henry, p. 203, 211. Cambridge: CABI Publishing.

Piet, J.H.D., G. Katja, V.V. Ton, A. S. Henk and H. J. Harmen. 2002. The recognition of structure–function relationship in galactomannans. *Journal of Agricultural and Food Chemistry* **15**: 4282

Pieter, B., K. Kalkman and R. Geesinie. 1990. The Plant Diversity of Malaysia. Netherlands: Klumer Academic Publishers.

Punithavathy, R. 2008. Rare 'Olympic torch' plant found in Kedah Park. 20p. The Star.

Radley, J.A. 1976. Physical Methods of Characterising Starch. Examination and Analysis of Starch and Starch Products. London: Applied Science Publishers Ltd.

© COPYRIGHT UPM

Raveendra, R.K. and P. Martin. 2006. Ethnomedicinal Plants. Araceae–Monocotyledons. India: Agrobois Press.

Richard, E. S. and F.R. Robert. 1990. The Healing Forest. Medicinal and Toxic Plants of the Northwest Amazonia. Medicinal and Toxic Plants of the Indians of Northwest Amazonia. Oregon, Portland: Dioscorides Press.

Robert, E.C.W. 2007. Handbook of Nutraceuticals and Functional Foods. Dietary Fibre and Coronary Heart Disease (CHD). Relationship Between Cholesterol Levels & CHD. 2nd ed. Boca Raton, Florida: CRC Press Taylor & Francis Group.

Robert, K.M., D.K. Granner and V.W. Rodwell. 2006. Harper's Illustrated Biochemistry. Bioenergetics: The Role of ATP. Twenth–seventh ed. India: Books Allied Publishers Pvt. Ltd.

Ronald, E.W., E.A. Terry, A.D. Eric, H.P. Michael, S.R. David, J.S. Steven, F.S. Charles, M.S. Denise and S. Peter. 2005. Handbook of Food Analytical Chemistry: Pigments, Colorants, Flavors, Texture, and Bioactive Food Components. Laboratory Stock Solutions, Equipment, and Guidelines Common Buffers and Stock Solutions. New Jersey: John Wiley and Sons, Inc.

Ruszova, E., S. Pavek, V. Hajkova, S. Jandora and V. Velebny. 2008. Photoprotective effects of glucomannan isolated from *Candida utilis*. *Journal of Science Direct Elsevier Ltd* 3: 501-511

Saito, M. 2000. Moisturizing Cosmetics Containing Glucomannan. Tokyo: Jpn. Kokai Koho Press.

Scott, G.J., R. Best, M. Rosegrant and M. Bokanga. 2000. Roots and Tubers in the Global Food System. A Vision Statement to the Year 2020. International Potato Center (CIP), Centro Internacional de Agricultura Tropical (CIAT), International Food Policy Research Institute (IFPRI), International Institute of Tropical Agricultural (IITA) and International Plant Genetic Resources Institute (IPGRI): CRC Press Taylor & Francis Group.

Shimizu, K. and M. Ohshiba. 2000. Quick–drying Disinfecting Gels for Hands. Tokyo: Jpn. Kokai Koho Press.

Southgate, D.A.T. 1976. Determination of Food Carbohydrates. Selected Methods. London: Applied Science Publishers Ltd. Essex.

Stanley, P.C. 2003. Bread Making. Improving Quality. The Chemistry and Biochemistry of Wheat. Boca Raton, United States: Woodhead Publishing Limited and CRC Press LLC.

© COPYRIGHT UPM

Stephen, P.W. 2006. Bacterial polysaccharides. In *Food Polysaccharides and Their Applications. Second Edition*, ed. M.S. Alistair, O.P. Glyn, and A.W. Peter, p. 439-441. Florida, United States: CRC Press Taylor & Francis Group.

Steven, J. and G. Sandra. 2000. 30 Secrets of the World's Healthiest Cuisines. Global Eating Tips and Recipes from China, France, Japan, the Mediterranean, Africa, and Scandinavia Canada: John Wiley & Sons, Inc.

Steve, W.C. 2005. Food Carbohydrates Chemistry, Physical Properties and Applications. United States: CRC Taylor & Francis Group.

Susan, M.P. 1995. Enchanted Gardens of Kinabalu: A Borneo Diary. Back of Beyond. Malaysia: Natural History Publications (Borneo) Sdn. Bhd.

Susan, S.C., P. Leon and D. Mark. 1999. Complex Carbohydrates in Foods. Sources and Uses of Fiber. New York: Marcel Dekker, Inc.

Susheela, R. 2007. Handbook of Spices, Seasonings, and Flavorings. Emerging Flour Contributors. 2nd ed. United States: CRC Press Taylor & Francis Group.

Suzanne, N.S. and N.B. James. 2003. Food Analysis. Compositional Analysis of Foods–Carbohydrate Analysis. 3rd ed. United States: Springer Science Business Media, LLC.

Takada–Oikawa, N., N. Katoh, T. Oshida, S. Kawanabe and T. Kaise. 2000. Studies on materials containing polysaccharides as soil amendments. Effects on animal waste water purification in a potting experiment. *Journal of Material Cycles and Waste Management* **2**: 138-142

Takada, T. 2000. Manufacture of Glucomannan Gel Particles as Mild Scrubbing Agents for Cosmetics. Tokyo: Jpn. Kokai Koho Press.

Takezaki, T. 2000. Fixation Support Agent Consisting of Modified Derivative of Glucomannan for Biopsy Sample, and It Manufacturing Method. Tokyo: Jpn. Kokai Koho Press.

Tang, R.P., M.Y. Du, H. Zheng and L.H. Fan. 2003. Preparation and characterization of soy protein isolate–carboxymethylated konjac glucomannan blend films. *Journal of Applied Polymer Science* **88**: 1095–1099

Teeri, T.T., B. Svensson, H.J. Gilbert and T. Feizi. 2002. Carbohydrate Bioengineering: Interdisciplinary Approaches. Domain Structure and Engineering. London: The Royal Science of Chemistry.

Terry, E.R., M.O. Akoroda and O.B. Arene. 1987. Tropical Root Crops. Root Crops and The African Food Crisis. Canada: International Development Research Centre.

Thain, M and M. Hickman. The Penguin Dictionary of Biology. London: Penguin Group.

© COPYRIGHT UPM

Tian, B.S., C.M. Dong, D.F. Luo and S.N. Yao. 1999. Preparation of konjac glucomannan esters of long fatty acid and studies on the emulsifying properties. 326-331p. Acta Polymcrica Sinica.

Tim, H. 2002. Food Chemical Composition: Dietary Significance in Food Manufacturing. Key Topics in Food Science Technology. Special Dietary Needs. London: Royal Society of Chemistry, U.K.

Tokiwa, Y. and A. Tsuchiya. 2003. Biodegradable Resin Compositions. United States: United States Patent.

Tom, S. and P. Emery. 2003. Molecular Basis of Human Nutrition. New York: Taylor Francis Inc.

Umberto, Q. 2000. CRC World Dictionary of Plant Names–Common Names, Scientific Names, Eponyms, Synonyms, and Etymology. New York: CRC Press LLC.

Van Balgooy, M.M.J. 2001. Malesian Seed plants: Portraits of non–tree families. 34p. Universiteit Leiden, Leiden, Netherlands.

Villanueva–Suàrez, M.J., A. Redondo–Cuence, M. D. Rodrĭguez–Sevilla and M. De Las Heras Martĭnez. 2003. Characterization of nonstarch polysaccharides content from different edible organs of some vegetables, determined by GC and HPLC: Comparative study. *Journal of Agricultural and Food Chemistry* **3**: 5950

Vladimir. V., J.A.J. David, S. Peter, L.S. John, O. Robin, V. Edward, B. Furio, A.L. Lawrence and B.T. Charles. 1999. Konjac–mannan (Glucomannan) improves glycemia and other associated risk factors for coronary heart disease in type 2 diabetes. A randomized controlled metabolic trial. Diabetes care. 913-919p. University of Milan, Milan, Italy.

Wakisaka, O., S. Uda, M. Katayama and T. Shimizu. 2000. Soil Modifier Having Good Workability. Tokyo: Jpn. Kokai Koho Press.

Wang, K. and Z. He. 2002. Alginate–konjac glucomannan–chitosan beads as controlled release matrix. *Journal of Pharmaceutics* **244**: 117-126

Whitmore, T.C. 1990. An Introduction to Tropical Rain Forests. Interconnections Between Plants and Animals–The Web of Life. New York: Oxford University Press.

Wong, W.S. 1989. Mechanism and Theory in Food Chemistry. Carbohydrates–Hemicellulose. New York: AVI Book.

Wu, C.Y. and D.Y. Shen. 2001. Konjak–glucomannan: Capsule for Chronic Stomach Disease. China: People's Republic of China Press.

© COPYRIGHT UPM

Xiao, C.B., S.J. Gao, H. Wang and L.N. Zhang. 2000. Blend films from chitosan and konjac glucomannan solutions. *Journal of Applied Polymer Science* **76**: 509-515

Xiao, C.B., H.J. Liu, Y.S. Lu and L.N. Zhang. 2001. Characterization of poly (Vinylpyrrolidone)–konjac glucomannan blend films. *Journal of Applied Polymer Science* **81**: 1049–1055

Xiao, C.B., L.H. Xiao, L.H. Weng and L.N. Zhang. 2002. Improvement of physical properties of cross-linked alginate and carboxymethyl konjac glucomannan blend films. *Journal of Applied Polymer Science* **84**: 2554–2560

Yang, L., X.M. Yang, R.J. Petcavich and L.J. Mao. 2001. Coating Materials for Preserving Fresh Produce. United States: United States Patent.

Yang, G., X.P. Xiong and L. Zhang. 2002. Microporous formation of blend membranes from cellulose/konjac glucomannan in NaOH/thiourea aqueous solution. *Journal of Membrane Science* **2**: 161-173

Yang, G., Q.L. Huang, L.N. Zhang, J.P. Zhou and S.J. Gao. 2004. Miscibility and properties of blend materials from waterborne polyurethane and carboxymethyl konjac glucomannan. *Journal of Applied Polymer Science* **92**: 77-83

Yasuda, M. and S. Kawamura. 2000. Steam–Supplying Food Packaging Materials for Microwave Cooking and Cooking of Food. Tokyo: Jpn. Kokai Koho Press.

Yong, H. 1981. Aroids: Magnificent Plants. Flowering Plants. The Spathe Bearers. Kuala Lumpur: Tropical Press Sdn. Bhd.

Yeshajahu, P. and J.A. Shellenberger. 1971. Bread Science and Technology. Wheat, Flour, and Bread Testing Around the World. London: The AVI Publishing Company, Inc.

Zakaria, M.A. 2000. Unique properties of calcium oxalate crystals–USM. In *Towards Bridging Science and Herbal Industry*, ed. Y.S. Chang, M. Mastura, S. Vimala and A.S. Zainon, p. 81. Kuala Lumpur: Forest Research Institute Malaysia (FRIM).

Zhenlin, X., Y. Youhui, J. Yueming, S. Yuanming, S. Yudong and P. Jie. 2008. Molecules: Synthesis and characterization of konjac glucomannan–graft–polyacrylamide via γ–Irradiation. 491p. Molecular Diversity Preservation International (MDPI), Basel, Switzerland.

Zdzislaw, F. S. 2002. Chemical and Functional Properties of Food Components. Carbohydrate Structure, Functional Properties of Carbohydrates. Saccharides. United States: Technomic Publishing Company, Inc.

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APPENDICES

Appendix A1: Raw data the values of absorption for different drying temperatures

Treat. (°C)	40	50	60	70	80
Smp					
A_1	0.0937	0.1166	0.1191	0.1329	0.1397
A_2	0.2610	0.6325	0.7327	0.4242	0.2948
A_3	0.7299	1.1455	1.4614	1.2169	1.1543
Blk					
A_1	0.0440	0.0600	0.0354	0.0395	0.0440
A_2	0.1337	0.2984	0.2417	0.1347	0.0578
A_3	0.2744	0.5370	0.4565	0.4289	0.3900
Smp					
$(A_2–A_1)$	0.1673	0.5159	0.6136	0.2913	0.1251
$(A_3–A_2)$	0.4689	0.5130	0.7287	0.7927	0.8895
$(A_3–A_1)$	0.6362	1.0289	1.3423	1.0840	1.0146
Blk					
$(A_2–A_1)$	0.0897	0.2384	0.2063	0.0952	0.0138
$(A_3–A_2)$	0.1407	0.2386	0.2148	0.2942	0.3322
$(A_3–A_1)$	0.2304	0.4770	0.4211	0.3894	0.3460
D–Glu	0.0776	0.2775	0.4073	0.1961	0.1113
D–Man	0.3282	0.2744	0.5139	0.4985	0.5573
Glu	0.4058	0.5513	0.9212	0.6946	0.6686
% Glu	14.93	20.29	33.90	25.56	24.61

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Appendix A2: Raw data the values of absorption for different storage periods

Week	Concentration of Glu. (%/100 g)	MC (%)
0	33.9	10.0
1	32.4	11.9
2	29.7	13.2
3	27.2	15.7
4	21.3	18.0

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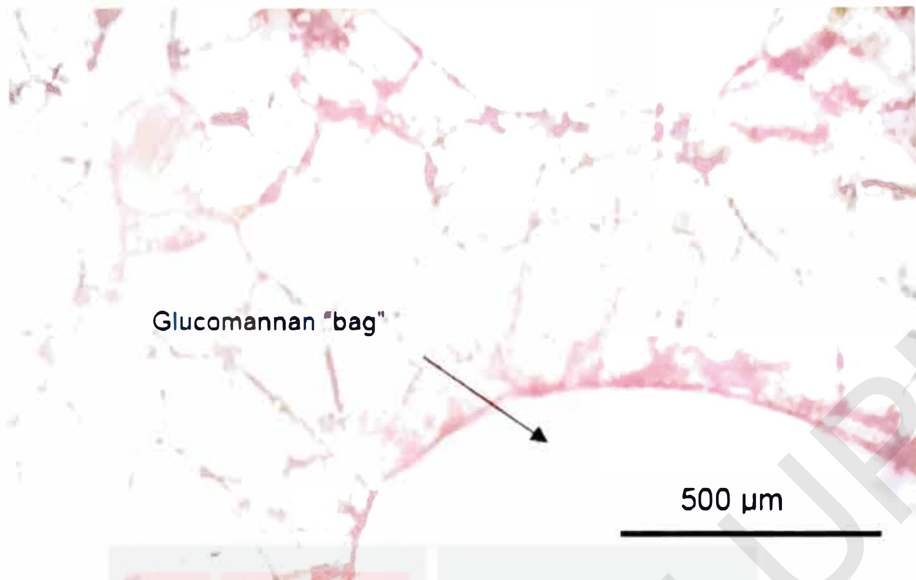

Appendix A3: The microphotography of glucomannan polymer is contained in large "bags" included in the parenchyma cells of the corm

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PUBLICATION OF THE PROJECT UNDERTAKING

This is to certify that I have no objection to publish the project entitled "Determination of Glucomannan Content in *Amorphophallus hewittii*" 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: 7/5/2009