การผลิตแป้งบุกบริสุทธิ์จากหัวบุกพันธุ์เนื้อทราย *Amorphophallus muelleri*

นางสาวรริศรา อิ่มภาประเสริฐ

วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาวิทยาศาสตรดุษฎีบัณฑิต
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PRODUCTION OF PURIFIED KONJAC FLOUR FROM CORMS OF BUK NUEA SAI
Amorphophallus muelleri

Miss Rarisara Impaprasert

A Dissertation Submitted in Partial Fulfillment of the Requirements
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รริศรา อิ่มภาประเสริฐ : การผลิตแป้งบุกบริสุทธิ์จากหัวบุกพันธุ์เนื้อทราย *Amorphophallus muelleri*. (PRODUCTION OF PURIFIED KONJAC FLOUR FROM CORMS OF BUK NUEA SAI *Amorphophallus muelleri*) อ.ที่ปรึกษาวิทยานิพนธ์หลัก: ผศ. ดร.ชาลีดา บรมพิชัยชาติกุล, อ.ที่ปรึกษาวิทยานิพนธ์ร่วม: George Srzednicki Ph.D., 222 หน้า.

งานวิจัยนี้ศึกษากระบวนการผลิตผงบุกกลูโคแมนแนนจากหัวบุกพันธุ์เนื้อทราย *(Amorphophallus muelleri)* งานวิจัยนี้ แบ่งการทดลองออกเป็น 4 ส่วน ได้แก่ การศึกษาผลของชนิดและความเข้มข้นที่เหมาะสมของสารยับยั้งการเกิดสีน้ำตาล (Anti-browning agent) และเวลาที่เหมาะสมในการแช่ต่อการเกิดสีน้ำตาลของชิ้นบุกอบแห้ง, การศึกษาชนิดและความเข้มข้นที่เหมาะสมของสารยับยั้งการดูดซับน้ำของกลูโคแมนแนน (Anti-swelling agent) ต่อสมบัติทางกายภาพและเคมีบางประการของผงบุกกลูโคแมนแนน การศึกษาผลของวิธีการทำแห้ง ได้แก่ การทำแห้งด้วยลมร้อน (Hot air drying) การทำแห้งด้วยลมร้อนแบบหลายขั้นตอน (Multistage hot air drying) การทำแห้งด้วยเครื่องอบแห้งแบบแช่เยือกแข็ง (Freeze drying) และการทำแห้งด้วยเครื่องอบแห้งไมโครเวฟสุญญากาศ (Microwave-Vacuum drying) ต่อสมบัติทางเคมี กายภาพ และโครงสร้างบางประการของผงบุกกลูโคแมนแนน และการศึกษาสภาวะที่เหมาะสมที่สุดสำหรับการผลิตผงบุกกลูโคแมนแนนและความเป็นไปได้ในการนำไปใช้ในอุตสาหกรรมการผลิตผงบุกกลูโคแมนแนน จากการศึกษาพบว่าการใช้โซเดียมเมตาไบซัลไฟต์ที่ความเข้มข้น 500 ppm เวลาในการแช่ 10 นาที เป็นสภาวะที่เหมาะสมที่สุดของการยับยั้งการเกิดสีน้ำตาลในชิ้นบุกอบแห้ง และมีปริมาณซัลไฟต์ตกค้างต่ำอยู่ในระดับที่ไม่เกินมาตรฐาน เช่นเดียวกับการใช้เกลือโซเดียมคลอไรด์ที่ความเข้มข้น 10,000 ppm เป็นเวลา 20 นาที พบว่าสามารถป้องกันการเกิดสีน้ำตาลของชิ้นบุกอบแห้งได้ และมีประสิทธิภาพใกล้เคียงการใช้โซเดียมเมตาไบซัลไฟต์ ในขั้นตอนการสกัดผงบุกกลูโคแมนแนน พบว่าการใช้เอทานอลสามารถเพิ่มประสิทธิภาพในการสกัดกลูโคแมนแนนได้ดีกว่าการใช้โซเดียมเตตระบอเรต แต่ทำให้ค่าดัชนีความขาวและความหนืดของสารละลายบุกกลูโคแมนแนนมีค่าต่ำกว่าบุกกลูโคแมนแนนที่สกัดโดยใช้โซเดียมเตตระบอเรต อย่างไรก็ตามการใช้เอทานอลที่ความเข้มข้น 50% โดยปริมาตร เป็นความเข้มข้นที่เหมาะสมที่สุด สำหรับการยับยั้งการดูดซับน้ำของกลูโคแมนแนนและเพิ่มประสิทธิภาพในการสกัดกลูโคแมนแนนกรณีที่นำไปใช้กับอาหาร ภายหลังกระบวนการสกัดกลูโคแมนแนนจากหัวบุก พบว่าวิธีการอบแห้งมีผลต่อสี ความหนาแน่น ความเป็นรูพรุน ความหนืด ลักษณะอนุภาค และปริมาณซัลเฟอร์ตกค้างในผงบุก อย่างไรก็ตามวิธีการอบแห้ง เวลาที่ใช้ในการอบแห้ง และอุณหภูมิในการอบแห้งไม่มีผลต่อปริมาณกลูโคแมนแนนและลักษณะแถบสเปกตรัมของกลูโคแมนแนน แต่ปัจจัยที่มีผลต่อปริมาณกลูโคแมนแนน คือ สารที่ใช้ในการสกัดและวิธีการสกัดกลูโคแมนแนน เพื่อที่จะได้ผงบุกกลูโคแมนแนนที่มีคุณภาพดีจะต้องควบคุมปัจจัยต่างๆ ได้แก่ ควบคุมวิธีและอุณหภูมิในการอบแห้ง โดยให้มีอุณหภูมิมากกว่า 60 องศาเซลเซียสเพื่อยับยั้งการทำงานของเอนไซม์ที่ทำให้เกิดสีน้ำตาล เลือกใช้วิธีการอบแห้งที่ทำให้ความหนาแน่นของผงบุกต่ำและมีความเป็นรูพรุนมาก เพื่อช่วยการดูดซับน้ำและส่งผลต่อความหนืดของสารละลายจากผงบุก และวิธีการอบแห้งที่ใช้ความร้อนและระบบสุญญากาศจะช่วยกระตุ้นการระเหยของซัลเฟอร์ไดออกไซด์ เพื่อลดปริมาณซัลเฟอร์ดออกไซด์ตกค้างในผงบุก การอบแห้งด้วยไมโครเวฟสุญญากาศที่กำลังไมโครเวฟ 1440 W นาน 7.5 นาที เป็นวิธีที่มีความเป็นไปได้ในการนำมาประยุกต์ใช้ในอุตสาหกรรมการผลิตผงบุกกลูโคแมนแนน

ภาควิชา เทคโนโลยีทางอาหาร ลายมือชื่อนิสิต .....

สาขาวิชา เทคโนโลยีทางอาหาร ลายมือชื่อ อ.ที่ปรึกษาวิทยานิพนธ์หลัก .....

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5173843223 : MAJOR FOOD TECHNOLOGY
KEYWORDS: AMORPHOPHALLUS MUELLERI / KONJAC GLUCOMANNAN / ANTI-BROWNING AGENTS / ANTI-SWELLING AGENTS / EXTRACTION / MICROWAVE VACUUM DRYING / VISCOSITY

RARISARA IMPAPRASERT: PRODUCTION OF PURIFIED KONJAC FLOUR FROM CORMS OF BUK NUEA SAI *Amorphophallus muelleri*. ADVISOR: ASST. PROF. CHALEEDA BOROMPICHAICHARTKUL, Ph.D., CO-ADVISOR: GEORGE SRZEDNICKI, Ph.D., 222 pp.

The goal of this research is to devise a new processing method for production of purified konjac glucomannan (KGM) flour from corms of Buk Nuea Sai (*Amorphophallus muelleri*). The research on production of KGM flour from konjac corms was divided into following four parts: selection of the most appropriate anti-browning agent and soaking time for browning control of sliced corms; investigation the wet extraction process by using anti-swelling agent to improve glucomannan extraction and to study the effects of anti-swelling agent on physical and chemical properties of KGM flour; study of effects of different drying methods including hot air drying, multistage hot air drying, freeze drying, and microwave-vacuum drying on physical, chemical, and structural properties of KGM flour; and selection and optimisation of the most appropriate drying method for production of purified KGM flour for application in konjac industry. The results showed that using sodium metabisulphite as an anti-browning agent at 500 ppm with a soaking time of 10 min, was the most effective agent to retard browning reaction in konjac slices with low residual sulphite content as a requirement. In comparison, using sodium chloride at 10,000 ppm with a soaking time of 20 min, showed a performance comparable to sodium metabisulphite. In extraction process, using ethanol could provide higher extraction yield than sodium tetraborate. Meanwhile the whiteness index value and viscosity of konjac samples extracted with sodium tetraborate were higher than those extracted with ethanol. However, 50% ethanol was the most suitable anti-swelling agent for using in KGM extraction in case for safe consumption. After extraction process, the results showed that drying technique is the main factor that influences to the several important properties of KGM flour such as color change, bulk density, particle density, porosity, viscosity, morphology and sulphur dioxide residue. However, drying methods, drying temperature and drying time in this experiment did not affect the glucomannan content and FTIR spectra of KGM flour. The main factors that affect the glucomannan content of the KGM flour is the extraction medium and extraction technique. In order to meet the criteria of good quality KGM flour, the following factor are needed: the higher whiteness index of KGM flour can be obtained when the product temperature is above 60 °C in order to inactivate PPO activity for control the browning of KGM flour; choose the drying method in which provided low density and high porosity to promote the water absorption and viscosity of KGM flour; and thermal drying process and vacuum system can reduce the sulphur dioxide residue content in KGM flour. Using microwave vacuum drying at power level of 1440W for 7.5 min is an alternative drying method for KGM flour industry.

Department:	Food Technology	Student's Signature	.....
Field of Study:	Food Technology	Advisor's Signature	.....
Academic Year:	2013	Co-Advisor's Signature	.....

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CHAPTER 1

INTRODUCTION

1.1 Research background

Konjac is an indigenous crop found in hilly areas in subtropical regions, particularly in East and South East Asia such as China, Japan, Burma, Indonesia, and Thailand. Scientific name of konjac is *Amorphophallus* sp. This genus belongs to the Araceae family. There are 46 species of *Amorphophallus* found in Thailand (Sukumalanand, 2005), mostly in the northern and western part of the country. A widespread and valuable species that is used in food industry is "Buk Nuea Sai" or "Buk Khai" (*Amorphophallus muelleri*) which has high glucomannan content in its globulous tubers called corms. It is a native species in Thailand which is found mostly in northern part of the country namely provinces of Chiang Mai, Chiang Rai, Lam Pang, and Mae Hong Son and western part of the country such as Kanchanaburi and Tak province (Sukumalanand, 2005). They are becoming valuable commercial species since they show high resistance against elevated temperatures, water stress and soil borne diseases. They also have a higher propagation coefficient, higher growth rate than other species and higher konjac glucomannan content (Zhao, Zhang, Srzednicki, Kanlayanarat, & Borompichaichartkul, 2009)

Several researchers investigated the chemical properties of KGM. It is well known that KGM is a water soluble fibre which is extracted from konjac corm tissues, their molecules are rich in hydroxyl group that makes it easy to dissolve them in water, leads to high viscosity and forms thick hydrocolloid even if used at low

concentration (Li, Xie, & Kennedy, 2006c). Thus, it has been applied in food, cosmetics, fine chemicals, petroleum, medicine, and coating industry (Y. Q. Zhang, Xie, & Gan, 2005). The high viscosity of KGM solution is also required for those industries that use it as a thickener, stabilizer, emulsifier, carrier, gelling agent, glazing agent, and humectant (Codex, 2014a). Moreover, USFDA and Codex General Standard for Food Additives (GSFA) Online Database allowed konjac four (E 425) on food additives list and several scientific studies proved that KGM is a hydrophilic dietary fibre that is not digested by enzymes in human body and thus does not provide the energy and has positive effects on human metabolism and health (Sibbel, 2008). Thus, KGM is in high demand among Asian, American, and European food processors for industrial applications.

KGM is generally commercialised as purified KGM flour obtained from processed konjac corms. KGM content in *A. muelleri* is in excess of 50% depending on location, soil, weather and age of corms. The production yield also depends on extraction technique, purification and drying process (Kishida, 1979); (Fang & Wu, 2004). Production of KGM flour involves both dry and wet extraction processes including five main processing steps namely pre-treatment, primary drying process, extraction or separation, purification and secondary drying process which affect the quality of KGM flour. However, there are three major causes which affect the quality of KGM flour including the change of colour, KGM content, and viscosity of its solution. Konjac corms can be harvested only once a year namely from September until December. For safe storage of konjac slices in the mass production, the primary drying process is required to remove water to a level of 8-10% (d.b.) at which microbial growth will no longer occur. However, high temperature during drying

process of wet konjac cut slices can trigger browning reaction. Browning of konjac dried slices is one of the major causes of quality deterioration of KGM flour. This browning reaction is caused by enzymatic browning and related factors such as oxygen, drying method and drying temperature (Walker & Ferrar, 1998). Thus, anti-browning agents such as sodium metabisulfite are commonly used to control browning reaction of konjac slices. Drawbacks of using sodium metabisulfite are sulfur residues which are of concern to consumers. Thereby several studies have been devoted to use the non-sulfite anti-browning agents such as ascorbic acid, citric acid and sodium chloride in many fruits and vegetables. Nevertheless, no systematic studies have been carried out to investigate the effectiveness of anti-browning agents to control browning of konjac *A. muelleri* slices.

Another important step for producing KGM flour is extraction process which is needed to eliminate the impurities in order to produce purified KGM flour. Wet extraction method is widely used to produce KGM flour since it can provide a high extraction yield and at the same time deliver KGM flour of good quality (Zhao, Zhang, Srzednicki, Kanlayanarat, & Borompichaichartkul, 2010). However, the ability of KGM to absorb water becomes a problem during wet extraction by water, as impurities are absorbed onto the granules of KGM, making it difficult to separate and purify the latter. To overcome this problem, anti-swelling agents are trialled in this study. However, no systematically research has been conducted on the effects of anti-swelling agents in wet extraction process of *A. muelleri* so far. Thus, this study attempted to use organic and inorganic substances instead of water in wet extraction to improve KGM extraction from *A. muelleri* corm.

After extraction and purification, drying process is finally needed to dry the KGM flour to the desired final moisture content for safe storage. However, improper handling during the drying process can significantly reduce the quality of KGM flour. Drying method and related conditions are the main factors affecting the quality of final product. However, there is no evidence of published work on the effects of drying methods on the physical, chemical and structural properties of KGM flour. Thus, conventional hot air drying, multistage hot air drying, freeze drying, and microwave-vacuum drying will be tested regarding their effects on some physical, chemical, and structural properties of KGM flour. Then, the most appropriate drying method will be selected and optimised.

1.2 Objectives

In order to overcome the current constraints in the production of purified KGM flour, following objectives were defined in this thesis:

- to optimise the primary processing by selecting the most appropriate anti-browning agent and soaking time for browning control of konjac dried slices;
- to optimise process of wet extraction by using anti-swelling agents and studying the effects of anti-swelling agent on some physical, chemical, and structural properties of KGM flour;
- to study effects of different drying methods on quality of KGM flour;
- to select and optimise the most appropriate drying method

1.3 Scope

Thess research tasks have been divided into 4 main parts.

(i) Determination of chemical composition of fresh konjac corms.

(ii) Primary treatment of konjac slices.

- Determination of anti-browning agents and soaking time for browning control of konjac slices.
- Determination of anti-swelling agents for wet extraction process.

(iii) Study of the effects of different drying methods (hot air drying, multistage hot air drying, freeze drying and microwave vacuum drying) on selected physical and chemical properties of purified KGM flour.

(iv) Optimisation of the most appropriate drying method for production of purified KGM flour.

The total duration of this research was four years (May 2009–May 2013).

1.4 Hypothesis

The most appropriate anti-browning agent to control browning of konjac dried slices can be determined. The use of organic or inorganic substances instead of water in wet extraction process can reduce more impurities than water and improve KGM extraction. The most adequate drying method which can improve the quality of KGM flour can be devised. This drying method would have potential to become adopted in the industrial KGM flour production.

1.5 Expected outcomes

This research will introduce a new technique for production of purified KGM flour which minimises production cost as well as energy consumption. The outcome of this research will benefit farmers by providing them with a potentially high value-added cash crop. Moreover, this research will develop technology to produce locally a valuable raw material for Thai food and pharmaceutical industry.

CHAPTER 2

LITERATURE REVIEW

2.1 Characteristics of konjac

Konjac, or Buk in Thai, also known as Konjak, Konjaku, Konnyaku, Voodoo lily, Devil's tongue, Snake palm, Elephant yam, Elephant foot yam, Elephant bread, Sweet yam, Telinga potato (this name is mostly used for *Amorphophallus paeoniifolius*) (Kadprasert, 2004), are edible plants of the genus *Amorphophallus* in the Araceae family. They are perennial plants, native to warm subtropical to tropical eastern and southeastern Asia, such as Japan, south of China, Burma, Lao, Vietnam, Thailand, India and Indonesia (Anonymous, 2014a). There are also species growing throughout tropical areas in Indo-Pacific region and in Africa. Figure 2.1 shows the plant, fruit and flower of *Amorphophallus paeoniifolius*.

There are around 163 species of genus *Amorphophallus* around the world (Hetterscheid & Ittenbach, 1996) while 46 species of *Amorphophallus* are found in Thailand, mostly in the northern part of the country (Sukumalanand, 2005). Konjac plants consist of subterranean and aerial parts. The underground part is called tuber or corm which is shaped like globose, subglobose, depressed globose, saucer shaped, or rhizome (Sukumalanand, 2005). A large corm can reach a diameter of up to 25 cm (Figure 2.2).

(a) **(b)** **(c)**

Figure 2. 1 *A. paeoniifolius* (Dennst.) Nicolson (a) Konjac plant; (b) konjac fruit; (c) konjac flower.

Source: author's own photographs and Anonymous (2014b)

(a) **(b)**

Figure 2. 2 The corm of (a) *A. muelleri* and (b) *A. bulbifer*.

Source: author's own photographs.

The aerial part is a thick petiole which is sprouts from the terminal bud of corm. At the other end of the petiole grows a single divided leaf. The leaf comprises a number of compound leaflets that are supported by a rachis. Some species, such as *A. muelleri* and *A. bulbifer* have small to medium sized bulbils with a reproductive function formed between leaflets (Figure 2.3). The flowers are produced on a spathe enclosed by a spadix (Figure 2.4).

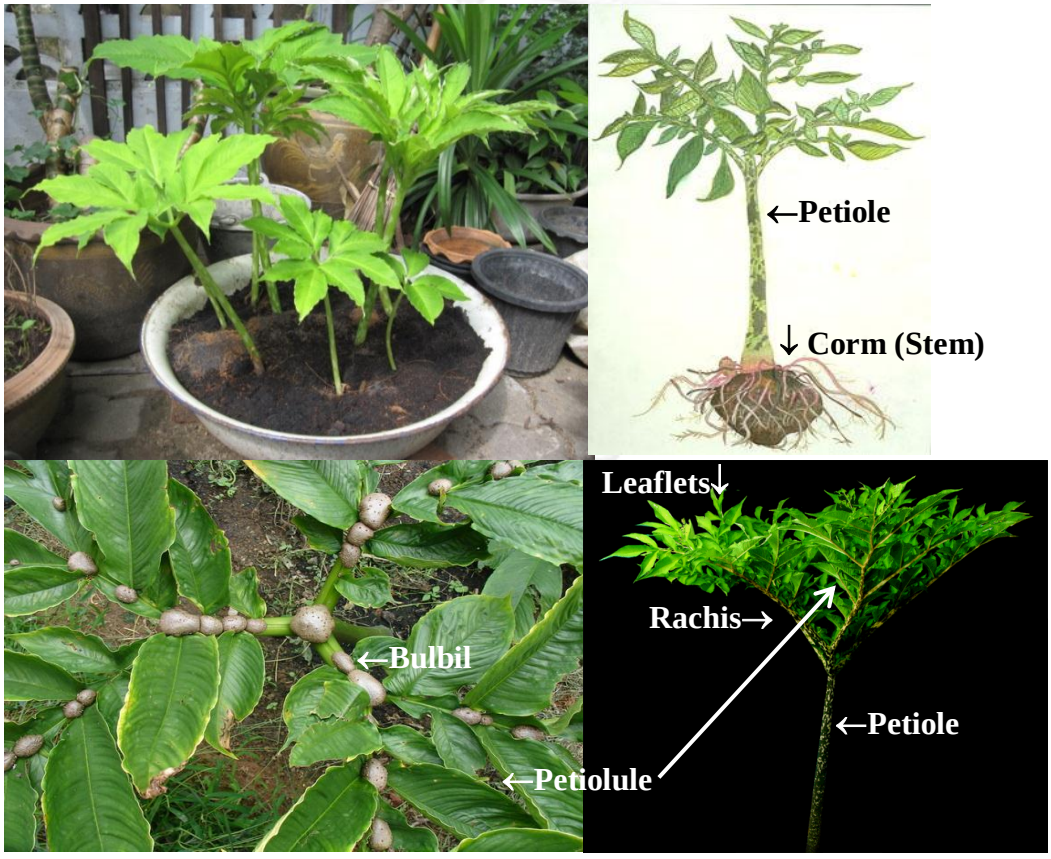


Figure 2. 3 Konjac plant with the compound leaf.

Source: author's own photographs and The International Aroid Society (2014)

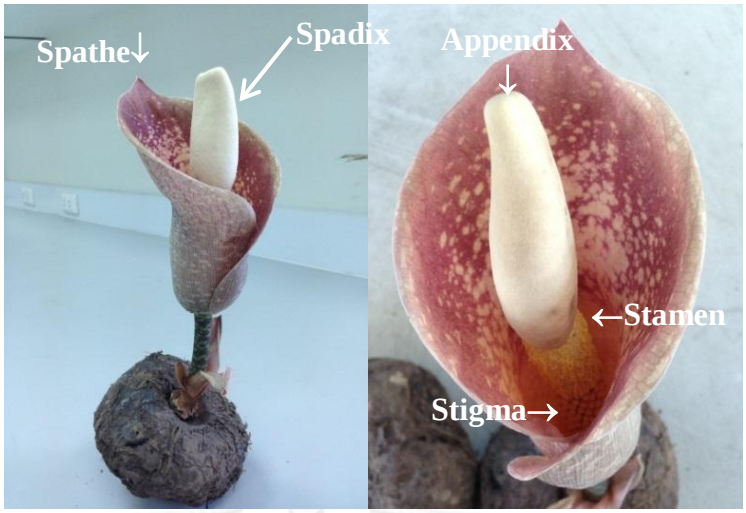
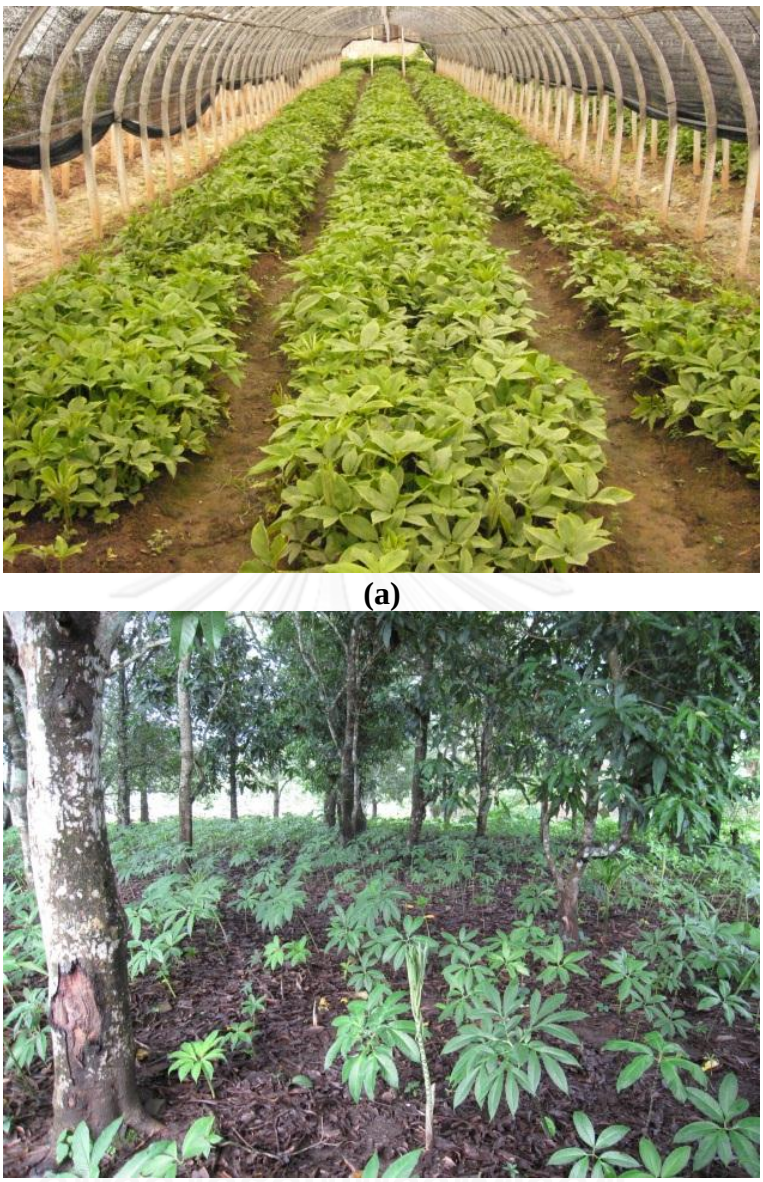


Figure 2. 4 Konjac flower of *A. muelleri*.
Source: author's own photographs.

For konjac cultivation, most species are easily grown in any humus-rich, rather organic, well drained and well aerated soil. The majority of *Amorphophallus* species can be grown at about 20 °C (optimal 25 °C) during summer in a semi-moist soil in a partially shaded area with protection from direct sunlight, especially during hours at midday (Anonymous, 2014c) (Figure 2.5).



(a)

(b)

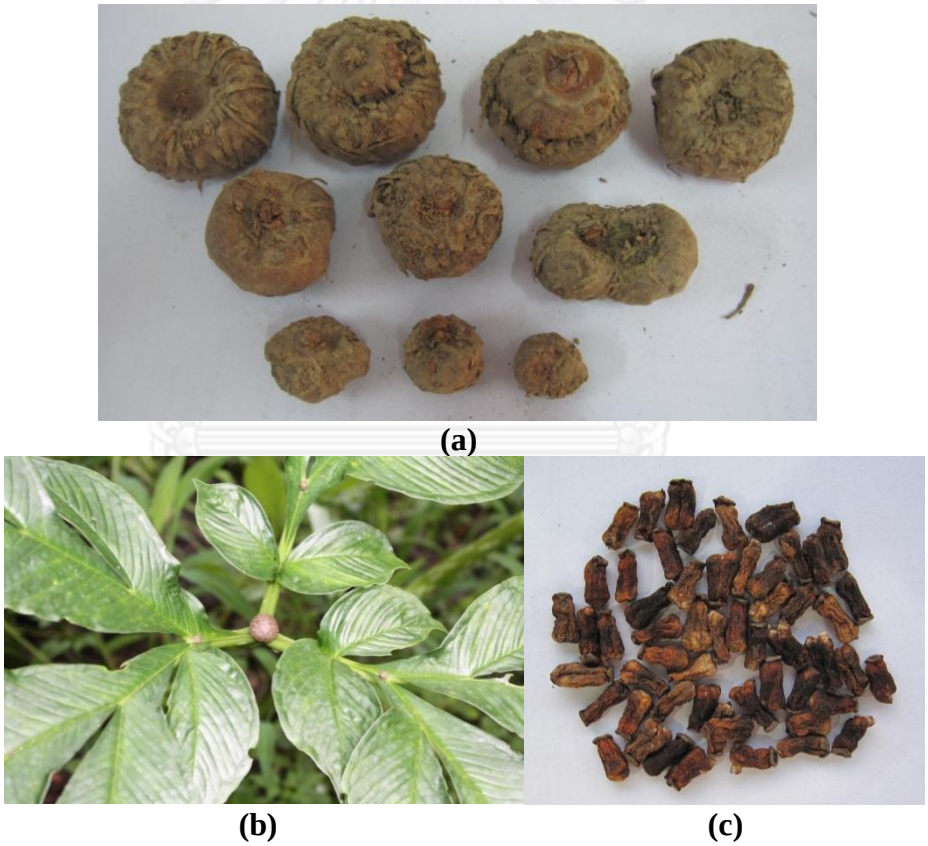
Figure 2. 5 Konjac plantation in (a) Dongchaun, China and (b) Tak province, Thailand.

Source: author's own photographs.

Most of the propagating organs (e.g. corm, bulbil, or seed – see Figure 2.6) will start growing in early rainy season (May-July in Thailand). The increase in moisture stimulates the growth of the new leaf appearing in the center of the corm. The corm in immature specimens may increase its weight threefold in a single season. Then the

leaf starts to yellow and die in the dry season (October-December) and the corm stays dormant for some 3 to 7 months until rainy season again.

Three-years old konjac corms are suitable for the production of konjac flour before flowering. Most *Amorphophallus* species flower before the leaf unfolds, whereas some may rest for the entire year. The terminal bud of a more than three-year old corm may produce a flower-bud. After differentiation the latter will develop into a spathe and a spadix and later on produce seeds (Figure 2.7).



(a) **(b)** **(c)**

Figure 2. 6 The propagating organs (a) corm; (b) bulbil; (c) seed.

Source: author's own photographs.

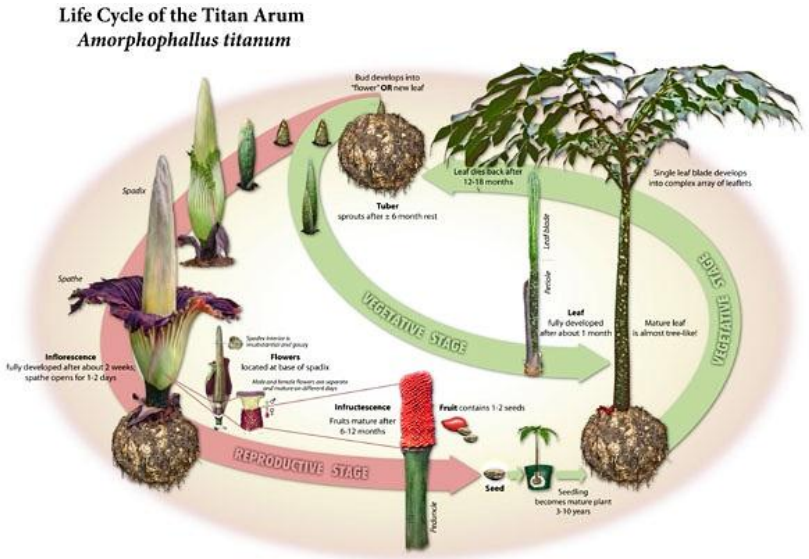


Figure 2. 7 Life cycle of *Amorphophallus titanium*.

Source: Anonymous (2014d)

From among the 163 species in genus *Amorphophallus*, some are inedible because the rhizome tissue is coarse and the formation of the edible compounds (glucomannan and starch) is very limited. Moreover, many species have no value as food because that there are large amounts of alkaloids and other toxic substances (e.g., oxalic acid) in konjac fresh corms, which are hard to remove in the processing. So there are just about 20 species that are considered as edible at present (Zhao, 2010). P. Liu (2004) listed the edible species as follows (see Table 2.1).

Table 2. 1 Known edible plants of genus *Amorphophallus*.

Scientific Name	Distribution Location
A. *aphyllus* Hutch	Senegal, Sudan
A. *albus* P. Y. Liu et J. F. Chen	Endemic to Southern Sichuan and Northern Yunnan, China
A *bulbifer* Br.	India, Java
A. *corrugatus* N.E.Brown	Yunnan and Guangxi, China, Thailand, Burma
A. *dracotioides* N. E. Br	From Madagascar to Polynesia
A. *harmandii* Engl. & Grhrm	Central African Republic, Ivory Coast
A. *kachinensis* Engl. & Gehrm	Yunnan (China), Myanmar, Thailand, Laos
A. *kiusianus* Makino	Eastern China, Taiwan, Southern Japan
A. *krausei* Engl.	Yunnan (China), Myanmar, Southern Thailand
A. *konjac* K. Koch	China, Japan, Indonesia, Philippines
A. *nanus* H. Li et C. L. Long	Endemic to Southeast Yunnan, China
A. *oncophyllus* Prain et Hook. f. (syn. A. *muelleri*)	Indochina, Southeast Asia
A. *odoratus* Hett. et H. Li	Endemic to Hongkong, China
A. *paeoniifolius* (Dennst.) Nicolson	Cambodia, Vietnam, Thailand, India
A. *prainii* Hook	Thailand, western Malaysia, eastern Kalimantan
A. *titanum* (Becc.) Becc.ex Arcang.	Sumatra, Myanmar
A. *tonkinensis* Engl.et Gehrm.	Yunnan (China), Vietnam
A. *variabilis* Blume	Philippines, Java, Malaysia
A. *yuloensis* H. Li	Endemic to Southern Yunnan (China)
A. *yunnanenis* Engl.	Yunnan (China), Thailand, Laos, Vietnam

Source: P. Liu (2004)

Some species deserve particular attention as economically important crops (Zhao, 2010). They include:

- ***A. konjac* K. Koch** (syn.: *A. rivieri* Durieu ex Carrierre, *A. mairei* Leveille) **and *A. albus*** are common crops in China and are widely used in KGM flour industry because of their white flesh. However, their production is limited due to serious incidence of soil borne diseases and low propagation rate (D. Zhang, Wang, & Srzednicki, 2010).

- ***A. bulbifer* Roxb. Blume** is a wild species in Myanmar and Northeast India. It is similar to *A. muelleri* with high resistance to disease, water logging and drought, has a high propagation coefficient but it has a pink and dense flesh and does not have a very high KGM content (D. Zhang et al., 2010).

- ***A. muelleri*** (syn.: *A. planus* Teijsm. & Binn.; *A. blumei* (Schott) Engl.; *A. oncophyllus* Prain; *A. burmanicus* Hook. F.; *A. carnosus* Engl.; *A. timorensis* Alderw.). This species has high disease resistance, is tolerant to water logging and drought and has high propagation coefficient and high KGM content. It has higher potential for development as a commercial crop than *A. bulbifer* because of a higher KGM content than *A. bulbifer* (minimum 60% in *A. muelleri* vs. 40% in *A. bulbifer*). However, the flesh is yellowish.

- ***A. kerrii* N.E. Br. and *A. paeoniifolius*** grow wild widely in Thailand. The corms have a very high starch content but varying KGM content.

- ***A. corrugatus* N.E. Br.** grows wild widely in Thailand and has a high KGM content but has low propagation coefficient.

Among the species mentioned above, *A. muelleri* has maximum potential to be used in the KGM flour industry in Thailand. For this reason, *A. muelleri* was selected as a raw material in this study.

2.2 *Amorphophallus muelleri*

Among 46 species of *Amorphophallus* which are found in Thailand (Sukumalanand, 2005), *A. muelleri* which is a native species, grows wild widely in Thailand. It may become commercially important in KGM flour industry. “Buk Nuea Sai” or “Buk Khai” is the by-name of *A. muelleri* in Thai language. They are found mostly in northern part of the country namely provinces of Chiang Mai, Chiang Rai, Lam Pang, and Mae Hong Son and western part of the country such as Kanchanaburi and Tak province (Sukumalanand, 2005). Figure 2.8 shows various parts of *A. muelleri*.

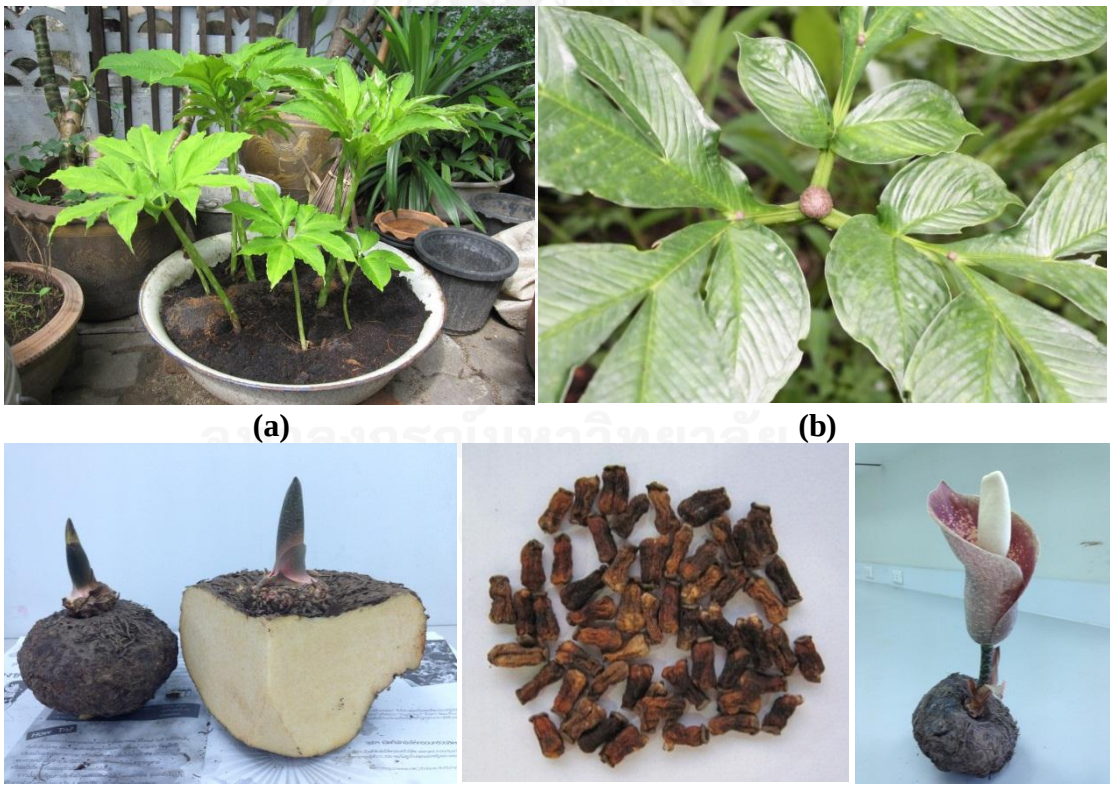


Figure 2. 8 Various parts of *A. muelleri* (a) young plants; (b) bulbil on the leaf; (c) underground corm; (d) seed; (e) flower.

Source: author’s own photographs.

This species becomes a valuable commercial crop since it has a high glucomannan content, high viscosity of its solution, and show high resistance against excessive temperatures, water stress and soil borne diseases. It also has a higher propagation coefficient and a higher growth rate than other species (Zhao et al., 2009).

2.3 Chemical composition of fresh konjac corms

Fresh konjac corms generally consist of 75-80% water and 20-25% total solids (Shimizu & Shimahara, 1984). In the portion, konjac corm contains a variety of insoluble materials as well as a major amount of desirable water-soluble substances. There are numerous impurities in crude (native, unclarified) konjac flour, principally insoluble starches, cellulose, and nitrogen-containing materials, including protein. Many of such impurities are derived from "sacs" which encapsulate the konjac flour in the corm (Ohashi, Shelso, Moirano, & Drinkwater, 2000).

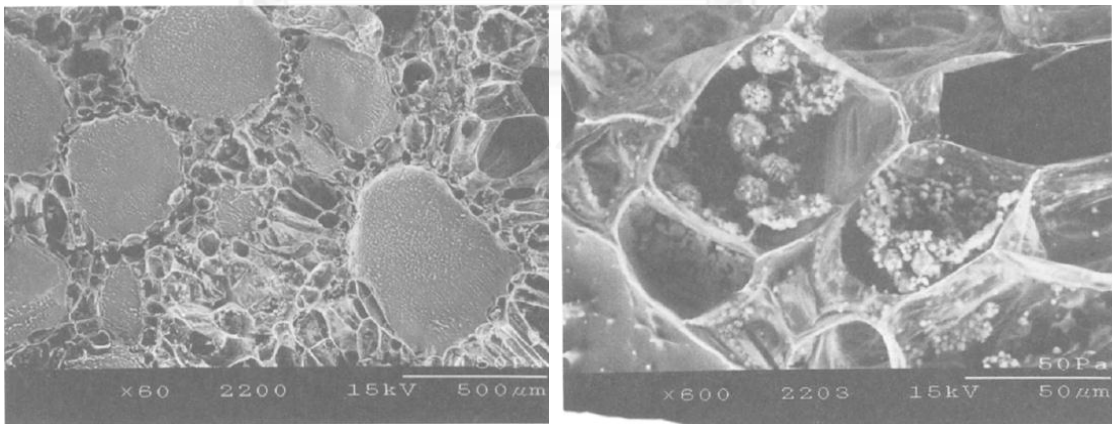
Shimizu and Shimahara (1973) studied method of selective separation of konjac flour from the corms of *A. konjac* and found there are the impurities called "Tachiko" which is the fine powder (0.01 mm), an impurities consisting of starch, protein, and calcium oxalate (less than 0.2%) which are to be eliminated for safe consumption.

Akesowan (1999) studied the chemical composition of *A. oncophyllus* (syn. *A. muelleri*) and found that fresh konjac consists of 81.9% moisture and 18.1% solids. The solid portion contains carbohydrates (90.2%), ash (4.0%), proteins (3.3%), fibre (1.7%), and lipids (0.7%).

P. Liu (2004); Li, Xia, Wang, and Xie (2005); USDA (2004); and Chua, Baldwin, Hocking, and Chan (2010) studied the chemical composition of *A. konjac*

and found that dried konjac consist of 49-60 % glucomannan, 10-30 % starch, 2.6-7 % inorganic elements (aluminium, calcium, chromium, cobalt, iron, magnesium, manganese, phosphorus, potassium, selenium, silicon, sodium, tin, and zinc), 5-14% crude protein, 3-5 % soluble sugars, 3.4-5.3 % ash and a small amount of alkaloids (trigonelline) and saponin. It can be concluded that glucomannan is the main storage carbohydrate in *A. konjac* corms.

Takigami, Takiguchi, and Phillips (1997) studied the tissue structure of 2-year-old corms of *A. konjac* by scanning electron microscopy and found that KGM granules accumulate in egg-shaped idioblasts within the parenchyma and that the size and number of these idioblasts increases with distance from the epidermis, reaching ~350 μm in diameter in the central region of the corm (Figure 2.9a). Other types of small particle were observed in honeycombed cells. The authors found and concluded that the spherical granules are starch (Figure 2.9b) and the needle-shaped crystals which are deposited in the parenchyma are calcium oxalate (Figure 2.10).



(a) **(b)**

Figure 2. 9 Scanning electron micrographs of a cross-section of a 2-year-old konjac corm. (a) egg-shaped idioblasts of KGM granule and the surrounding parenchyma; (b) starch granules in honeycombed cells.

Source: Takigami et al. (1997)

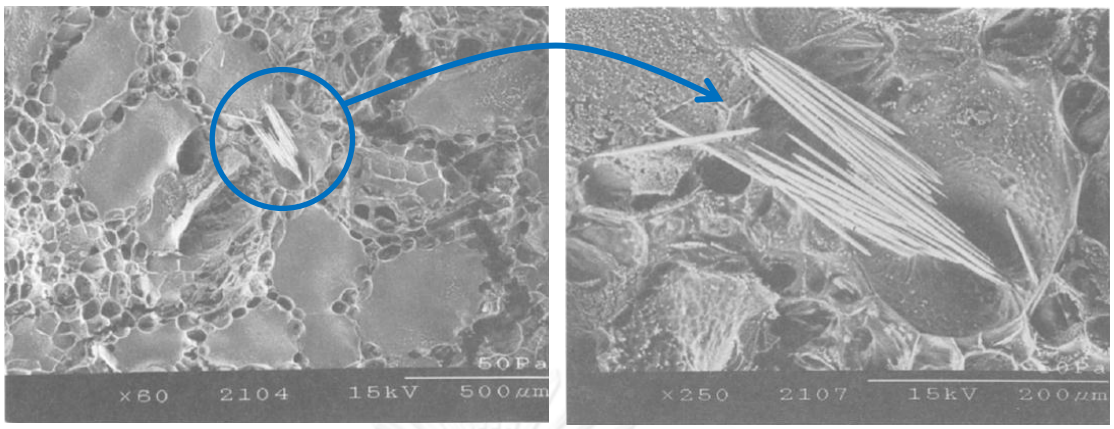


Figure 2. 10 Scanning electron micrographs of a cross-section of a 2-year-old konjac corm. The needle-like crystals are calcium oxalate.

Source: Takigami et al. (1997)

Zhao et al. (2010) developed the processing technique for production of purified konjac flour from *A. konjac* and *A. bulbifer* and reported the characteristics of some differences between idioblasts and ordinary cells are shown in Table 2.2.

Table 2. 2 Differences between idioblasts and ordinary cells in konjac corm tissue.

Attribute	Idioblast	Ordinary cell
Main cell content	Glucomannan	Starch
Hardness of the granules	High	Low, easily break to dust
Granule characteristics	Single granule	Agglomerated granules
Particle diameter (dry)	0.15-0.45 mm	Approx. 0.004 mm
Water-solubility	Easily soluble	Insoluble in cold water

Source: adapted from Zhao et al. (2010)

According to Table 2.2, it seems that the most important operation in the production of KGM flour is to separate starch granules and other impurities from konjac tissue. The processing of konjac corms is based on the differences between these characteristics.

2.4 Konjac glucomannan (KGM)

Maeda (1911), Maeda (1922), Nishida and Hashima (1930), and Nishida and Hashima (1932) found that when konjac flour was hydrolysed, the purified acid hydrolysate of konjac flour consisted of mannan and glucose in the ratio of 2: 1 and called it konjac mannan.

Smith and Srivasta (1959); Maeda, Shimahara, and Sugiyama (1980); and Nishinari, Williams, and Phillips (1992) identified D-glucose and D-mannose in purified KGM by paper chromatography of the acid hydrolysate. The results indicated that the mannose: glucose ratio as 3: 2 and concluded the main chain of KGM consisted of β-D-1, 4 linked glucose and mannose and there was no 1, 6 linkage present. After methanolysis, it can be concluded that branching in KGM occurred through 1, 3 linkages, approximately three for every 32 sugar units and the side chains were terminated by glucose and mannose.

Nakajima and Maekawa (1966) and Nakajima and Maekawa (1967) confirmed that branching occurs at the C-3 position of mannose and the length of the branched chain was about 11-16 hexose units.

Maekaji (1978); Tye (1991); and Williams, Foster, Martin, and Norton (2000) concluded that KGM is a linear random copolymer of D-mannose and D-glucose in the ratio 3: 2, joint by β-1,4 glycosidic linkage with ~1 acetyl group in every 19 sugar

units at the C-6 position. It is a high molecular weight polysaccharide (more than 300,000 D). It is water soluble and is characterized by high viscosity of its solution where the degree of water solubility is controlled by the presence of acetyl units. It is also insoluble in organic solvent (e.g. ethanol, methanol, dimethyl ether).

From the findings of several researchers, it can be concluded that the main component of konjac flour is a carbohydrate which consists of glucose and mannose which is called glucomannan or konjac glucomannan which is composed of D-mannose and D-glucose in the ratio of 1.6: 1 or 1.4: 1, linked by β-(1→4) glycosidic linkages with about 1 in every 17-19 sugar units being acetylated (Williams et al., 2000) (Figure 2.11). KGM may contain short side branches at the C-3 position of the mannoses, and acetyl groups (-$OCOCH_3$) that are randomly present at the C-6 position of sugar units (Kato and Matsuda (1969); Katsuraya et al. (2003), Maeda et al. (1980)), which contributes to the solubility and swelling properties of KGM (Alvarez-Mancenido, Mariana, & Martinez-Pacheco, 2008; Luo, He, & Lin, 2013). KGM is a kind of amorphous polymer and its relatively high molecular weight (Mw) is $10^5 – 10^6$ (Li & Xie, 2003).

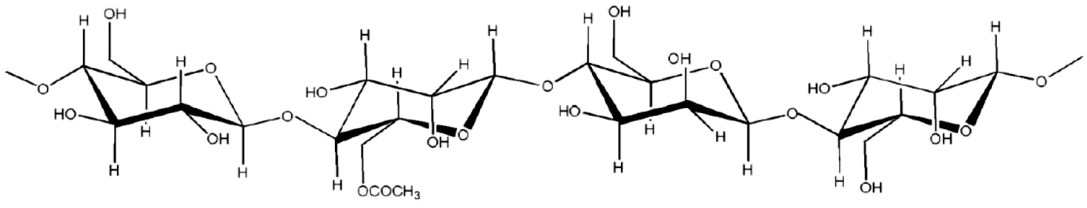


Figure 2. 11 Structure of konjac glucomannan.
Source: Kök, Abdelhameed, Ang, Morris, and Harding (2009)

Thus, it can be concluded that KGM is a high molecular weight water soluble non-ionic (neutral) polysaccharide extracted from konjac corm tissues (Fang & Wu, 2004; Nishinari, 2000; Nishinari et al., 1992). Its molecules are rich in hydroxyl groups that makes it easy to dissolve them in water, leads to high viscosity and forms thick hydrocolloid even if used at low concentration (Li et al., 2006c). Thus, it is used in food, cosmetics, fine chemicals, petroleum, pharmaceuticals and coating industry (Y. Q. Zhang et al., 2005). The high viscosity of KGM solution is required for those industries.

2.4.1 KGM and its applications

For centuries KGM has been broadly used as food, food additive, and traditional Chinese medicine. KGM has also been consumed as a functional food in form of noodles, tofu and snacks since it is an excellent dietary fibre which cannot be hydrolyzed by digestive enzymes in humans (Nishinari & Gao, 2007). It is characterized by low calorie content and is considered as providing health benefits to people who suffer from obesity (N. Sugiyama & Shimahara, 1974). For food applications, the KGM is used as a thickening agent, gelling agent and water binding agent because of its high water-absorbing capacity (Ford & Chesey, 1986). It is also used as fat replacement in low-fat meat products (Tye, 1991). In addition, Codex General Standard for Food additives (GSFA) listed konjac flour as a carrier, emulsifier, gelling agent, glazing agent, humectant, stabilizer, and thickener (INS No. 425) for application in many kinds of food products (Codex, 2014a).

KGM has recently been marketed in capsule form, as a drink mix and in food products for the treatment of obesity, obesity-related dyslipidemia and diabetes.

The potential use of KGM as a prebiotic has also been suggested (Chua et al., 2010). Thus, KGM shows a potential development and application in agricultural, industrial and medical fields, such as nutraceutical food, functional food, cosmetic, and pharmaceuticals (Douglas, Follett, & Waller, 2005; Luo et al., 2013). Table 2.3 shows the application example and dosage of KGM flour in many field of food industry.

Table 2. 3 Application of konjac glucomannan in food industry.

Properties utilized	**Applications**	**Reference dosage**
Thermo-irreversible gelling dietary fibre source	Gel foods (konjac cake, konjac noodles, konjac curd and other konjac series animal food-imitating foods, konjac vegetarian foods)	2.5 – 4.0 %
Thermo-reversible gelling	Jelly, pudding	0.2 – 0.5 %
Water holding	Gummy candy	
	Meat products (ham, sausage)	
	Pet foods	
Thickening Emulsifying Suspending Stabilizing Water holding	Ice-cream	0.5 – 1.0 %
	Jam	
	Fruit juice, soup, flavoured drinks	
	Dairy products, yogurt	
	Suspending drinks, Fibre drinks	
Water holding Texture improver Dietary fibre source	Frozen foods	
	Noodles, pasta, bread, cakes, cookies	
Film-forming	Edible thin film material for food package, slurry for thin slice food, Slurry for thin film foods	1.0 – 1.5 %
	Medicine capsule, microcapsule, powder essence	0.5 – 1.5 %

Source: adapted from Anonymous (2014e)

2.4.2 Viscosity properties of KGM

KGM is water-soluble, and has a high viscosity even at low concentration. Dispersions of KGM in water exhibit pseudoplastic fluid or shear thinning behaviour (Dave & McCarthy, 1997) where the viscosity of KGM solution decreases with increasing shear rate. However, the viscosity of KGM solution depends on the concentration of KGM flour, shearing rate, shearing time, pH, concentration of solute (e.g. salt, sugar), temperature, and other polysaccharide in the solution. The viscosity of KGM solution increased with increasing amount of KGM flour, lower shear rate, pH more than 10, temperature not more than 50 °C, and mixing with other polysaccharide. The temperature at 50 °C provides a peak viscosity (Tye, 1991). However, concentration of solute (0-10 %), and pH (2-8) had no effect on the viscosity of KGM solution (Akesowan (2002); Dave, Sheth, McCarthy, Ratto, and Kaplan (1998); and Tye (1991)). Moreover, KGM has very high water absorbency, absorbing as much as 100 g of water per g of KGM sample, and the water absorbency of KGM decreases with increasing degree of acetylation on its chains (Koroskenyi & McCarthy, 2001).

2.5 Production of purified KGM flour

KGM is generally commercialised as purified flour obtained by processing konjac corms. KGM content in *A. muelleri* corms is in excess of 50% depending on location, soil, weather and age of corms. The production yield depends on extraction technique, purification and drying process (Kishida (1979) and Fang and Wu (2004)). From previous literature review (see 2.3), the most important concern in the production of KGM flour is the separation and extraction of KGM from corm tissue.

In recent decades, methods for the extraction and purification of KGM have been studied and developed. The traditional processing methods to obtain konjac flour consist either in dry (mechanical) processing, or in wet (chemical) processing which can separate starch granules and other impurities (tachiko) from larger KGM granules. The following sections describe traditional processing method, commonly used industrial processing method, and combined dry-wet processing method for production of KGM flour.

2.5.1 Traditional processing of konjac flour

Shimizu and Shimahara (1973) invented the method of separation of konjac flour from corm of *A. konjac* and found that to accomplish this process, the konjac corm must be comminuted. However, when the konjac corm is comminuted in its raw state, the comminuted product become exceedingly viscous and sticks to the pulverizer, resulting in the drop of the performance of the pulverizer making it difficult to withdraw the comminuted product out of the pulverizer. Thus, the dry method has been adopted. The dry processing method for production of konjac flour is shown in Figure 2.12.

Fresh konjac corm

↓

Washing and peeling

↓

Slicing into chips

↓

Drying in hot air or sun drying

↓

Crushing in a stamp mill to produce crude konjac flour

↓

Sifting or Air classification to remove starch and some impurities

↓

Konjac flour

Figure 2. 12 Dry processing of konjac flour.
Source: Shimizu and Shimahara (1973)

The disadvantage of this method is the difficulty to grind the dried konjac slices into a fine powder. Moreover, the konjac flour obtained from the dry method has a low extraction yield, low purity, low viscosity, and presents the difficulty to remove the impurities since the tachiko component sticks firmly to konjac flour. Thus, a long processing time is required for the comminution process (Shimizu & Shimahara, 1973). In addition, the konjac flour may be lost during air classification process, causing a decrease in production yield. As a result, the konjac flour cannot be directly used as a thickening agent and hence is sold as food commodity at a low price (Chua et al., 2012).

Zhao et al. (2010) also listed the following drawbacks of using traditional dry processing for the production of konjac flour:

The quality of the first stage of drying process (konjac dried slices) cannot be controlled when the sliced corms are dried on a sunning floor.

The combustion residues contaminate the dried product when using coal as a fuel in the dryer resulting in high sulphur dioxide residues in konjac flakes.

To eliminate the drawbacks of the dry process, a wet process has been developed. The principle of this method is to comminute raw konjac corm with a pulverizer in a liquid medium such as water or water-miscible organic solvent and then separate the large particles of konjac flour from the fine powder of tachiko by sifting (Figure 2.13). Since konjac flour swells and becomes viscous in a short period of time on contact with water, the step of separating water must be carried out in a very short period of time (less than 1 minute) in the method which uses water as the pulverizing medium. The water-miscible organic solvent may be an organic solvent such as methanol, ethanol, propanol, acetone, and 5 % of ethyl acetate-modified ethanol. N,N-dimethylformamide and ethylene glycol dimethyl ether can also be used (Shimizu & Shimahara, 1973).

Fresh konjac corm

↓

Washing and peeling

↓

Raw konjac corm crushed with a pulverizer in a liquid medium
(water or water-miscible organic solvent with concentration at least 30 % by volume)

↓

Sifting to remove “tachiko”

↓

Hot air drying (50-130 °C) to the final moisture content of 10-16 % wet basis

↓

Konjac flour

Figure 2. 13 Wet processing of konjac flour.
Source: (Shimizu & Shimahara, 1973)

Although the wet method is superior to the dry method in terms of production yield and shorter time required for production of konjac flour, this method is not the most used in the industry because of significant fluctuations in product quality as well as a much greater proportion of poor quality product (Shimizu & Shimahara, 1973).

2.5.2 Combined wet and dry processing method of KGM flour

In order to overcome the drawbacks of using dry or wet processing method alone, the combination of dry and wet processing method has been developed by several researchers following procedures shown in Figures 2.14-2.17.

Fang and Wu (2004) studied the variations of KGM from *A. konjac* and its refined powder in China. This procedure is used for preparing konjac samples before KGM determination (see Figure 2.14).

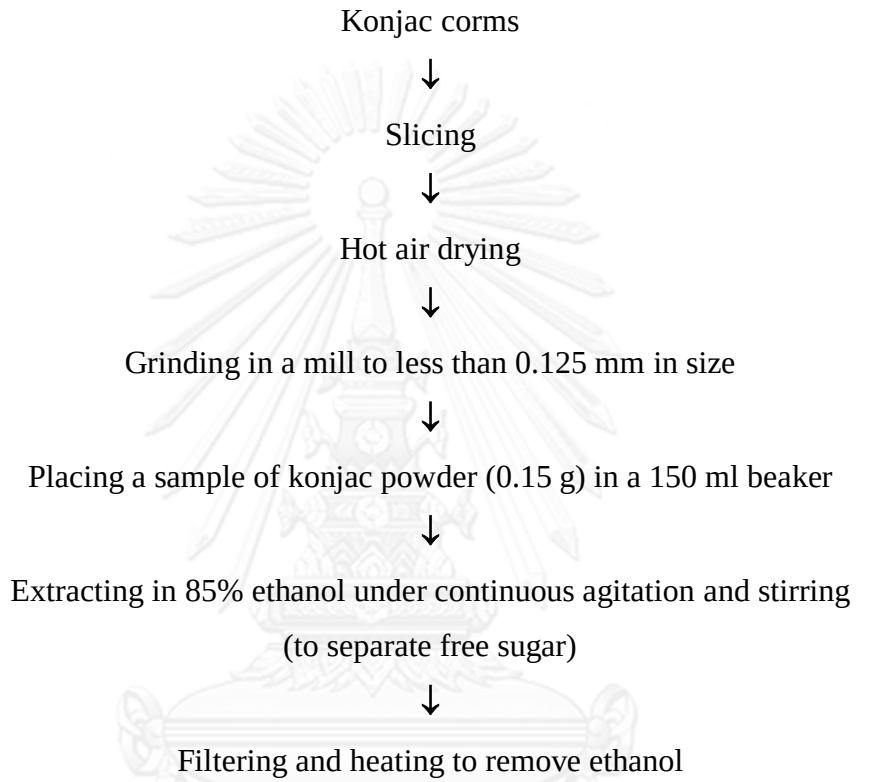


Figure 2. 14 KGM extraction methods for determination of KGM in konjac flour. Source: (Fang & Wu, 2004).

Li and Xie (2004); Ye, Kennedy, Li, and Xie (2006); Li and Xie (2006); and Li et al. (2006c) studied the molecular chain morphology of KGM and prepared KGM for film formation. The separation and purification of KGM which is used in their experiment are shown in Figure 2.15.

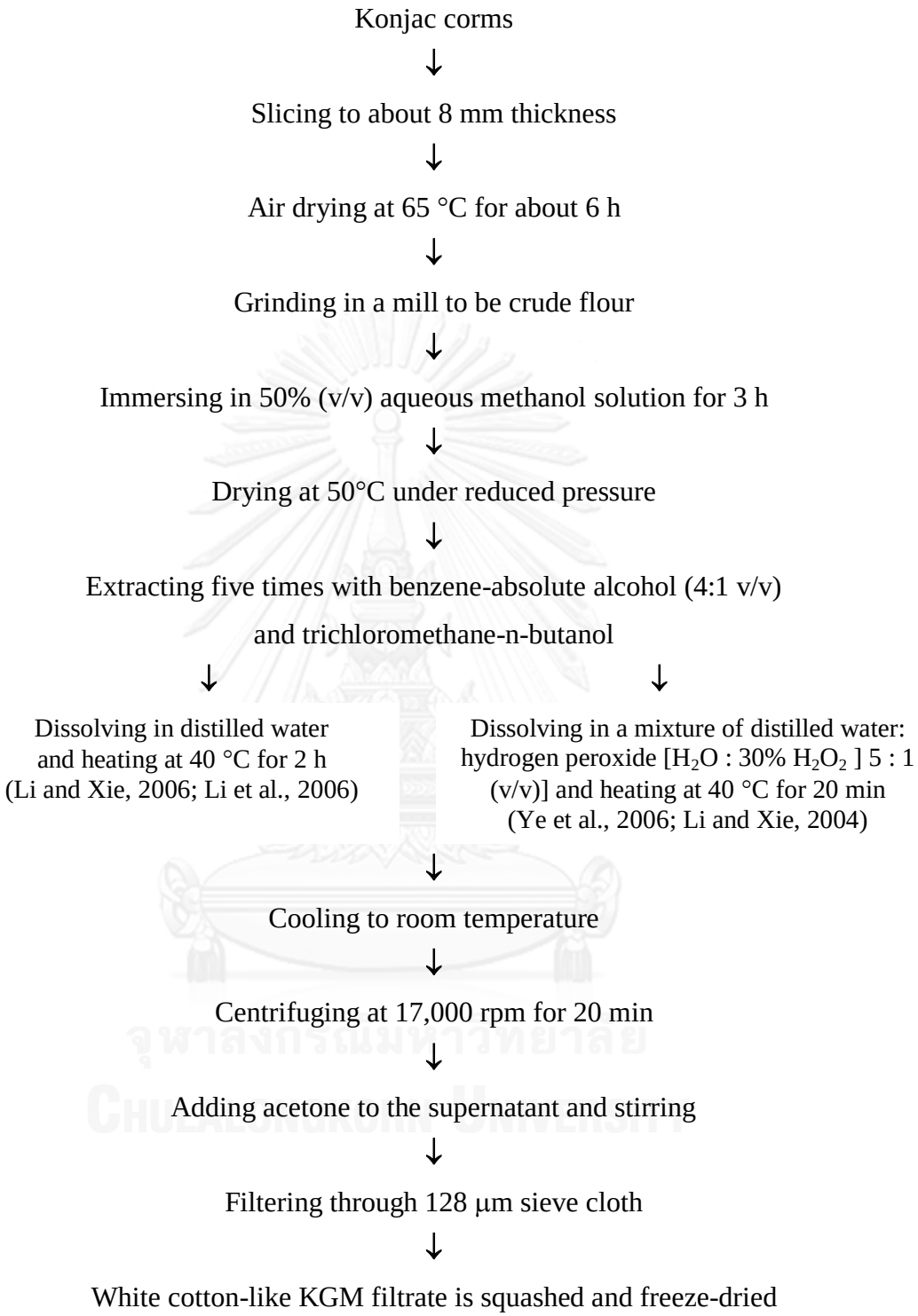


Figure 2. 15 The separation and purification of KGM.

Source: Li and Xie (2004); Ye et al. (2006); Li and Xie (2006); and Li et al. (2006c).

Zhao et al. (2010) developed a low-cost two-stage technique for production of low-sulphur purified konjac flour using the following procedure (Figure 2.16). The results show that the advantages of the combination method were improved the colour and higher viscosity of the product, speed of the operations, and a low production cost when compare with the traditional method used separately.

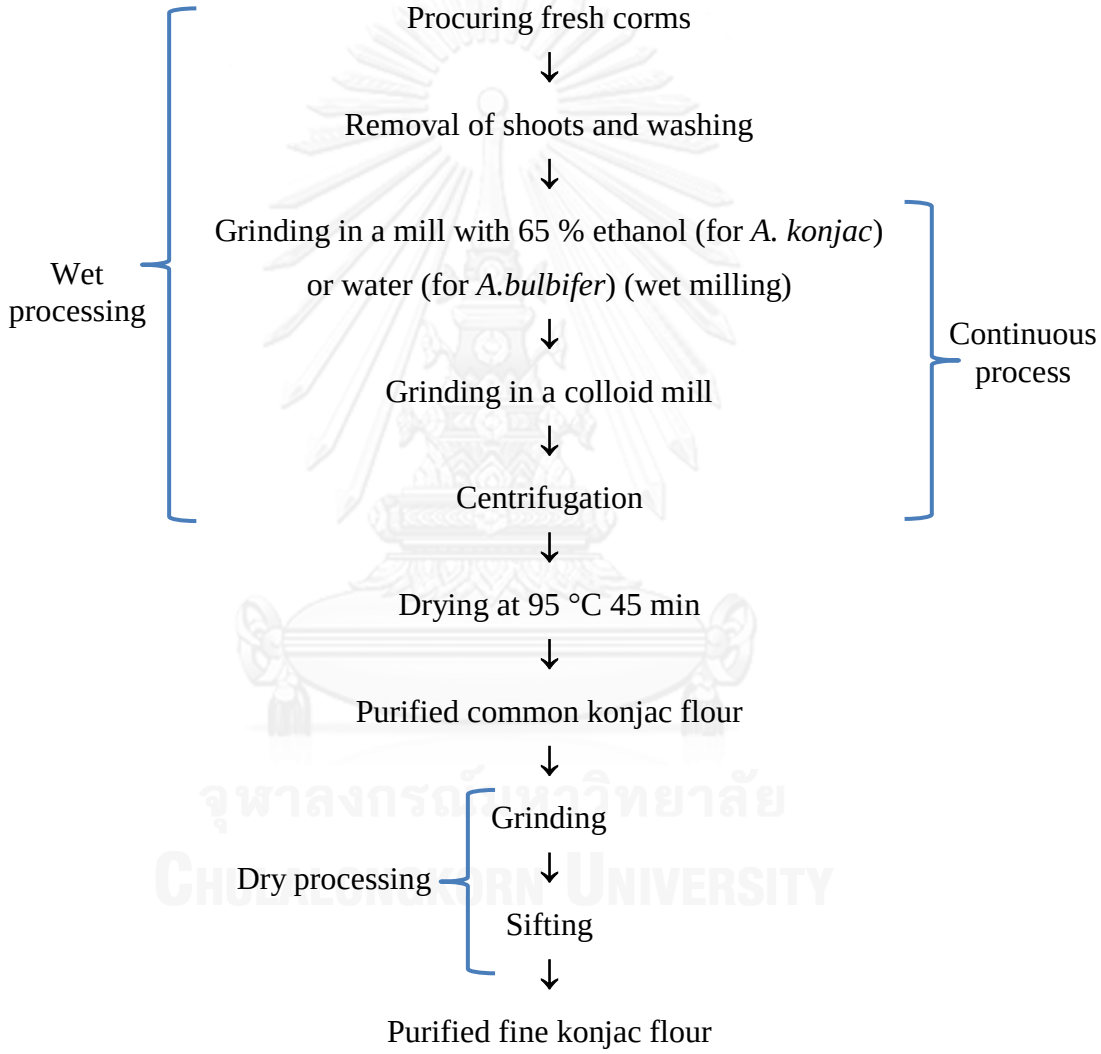


Figure 2. 16 The combination of wet and dry processing of konjac flour from *A. konjac* and *A. bulbifer*.

Source: Zhao et al. (2010).

Chua et al. (2012) studied the methodologies for the extraction and analysis of KGM from corms of *A. konjac*. They developed the alternative method to isolation of konjac flour from fresh corm material following procedure shown in Figure 2.17.

Fresh konjac corms

↓

Washing and removal of epidermis

↓

Slicing (2-3 mm thickness)

↓

Immersing in 1 % (w/v) sodium bisulphite for 1 min

↓

Oven-drying at 120 °C for 40 min then at 60 °C until constant weight

↓

Grinding and sifting (425 μm aperture)

↓

Crude konjac flour (CKF)

↓

Stirring 2.00 g of CKF in 50% (v/v) ethanol (200 mL)
for 90 min at room temperature

↓

Centrifugation (5000 × g, 30 min, 25 °C)

↓

Rotary evaporation to reduce the volume of the filtrate to ~1/3 the original volume

↓

KGM precipitation overnight with 95 % (v/v) ethanol (450 mL) at 4 °C

↓

Centrifugation (9000 × g, 40 min, 25 °C)

↓

Washing twice with anhydrous ethanol (Dehydration)

↓

Vacuum filtration

↓

Freeze drying for 48 h

↓

Grinding and sifting

↓

Purified konjac flour

Figure 2. 17 The production of konjac flour from *A. konjac*.
Source:Chua et al. (2012)

As for the wet processing, some organic and inorganic substances, such as lead acetate (Wu, Meng, Chai, & Wang, 2002), salt (e.g. aluminium sulphate) (Ohaski, Shelso, Moirano, & Drinkwater, 1993), 2-propanol coupled with starch hydrolysing enzymes (Wootton, Luker-Brown, Westcott, & Cheetham, 1993), ethanol (Fang and Wu (2004); Z. L. Wang, Wu, and Li (1998); Ogasawara, Yamazaki, and Nunomura (1987); M. Sugiyama, Shimahara, and Andoh (1972), methanol and acetone (Li and Xie (2006); Li et al. (2006c); Ye et al. (2006); Li and Xie (2004)), were used as an extraction medium for extracting KGM from crude konjac flour. KGM extracted by lead acetate is not edible, thus its applications are limited to the non-food industries (Chua et al., 2012).

However, the most common konjac flour purification method involves ethanol extraction of KGM, due to its simplicity and high efficiency (Takigami, 2000). The successful production of high quality purified konjac flour using ethanol

in extraction solvent has been reported by (Ogasawara et al. (1987); M. Sugiyama et al. (1972); Fang and Wu (2004); Zhao et al. (2010); and Chua et al. (2012)).

2.5.3 Combined dry-wet processing for KGM flour

From the above information, it seems that using combination of wet and dry extraction process was more effective than using the single method. However, most of the methods mentioned above are suitable for the production at a laboratory scale but not suitable for industrial-scale production. Moreover, the KGM content varies in different species and so does its solubility in the water-ethanol solution. Therefore, it is necessary to determine the appropriate concentration of ethanol in each species of konjac raw material. Since the fresh konjac is used as a raw material the moisture content of the fresh corms has to be considered, in order to calculate the amount of ethanol to be used in the extraction process. Since this study deals with *A. muelleri*, it is necessary to determine the processing conditions required by this species.

It can be concluded that the production of KGM flour from konjac corms is divided into five main processing steps namely pre-treatment, primary drying process, extraction or separation, purification and secondary drying process which has a major effect on the quality of KGM flour. The important properties that are used to evaluate the quality of konjac flour are glucomannan content, viscosity, and the colour of konjac flour. At the same time low moisture content and a minimum percentage of impurities such as sulphur, ash, sand, and heavy metal are required (P. Y. Liu et al., 2002). The Chinese standard for the different grades of konjac flour is shown in Tables 2.4 and 2.5.

Table 2. 4 Physical properties of different grade of konjac flour.

Property	**Common konjac flour**			**Purified konjac flour**	
	Superior	**First grade**	**Second grade**	**Superior**	**First grade**
Color	White	White, with a trace of brown permitted	White or yellow, with a little brown or dark permitted	White	White
Smell	The fishy smell innate to konjac and a slight SO_2 smell are allowable.			The slight fishy smell innate to konjac and a slight alcohol smell are allowable.	
Shape	Granulate, without lumping or molding				

Source: adapted from P. Y. Liu et al. (2002)

Table 2. 5 Physico-chemical characteristics of different grades of konjac flour.

Property		**Common konjac flour**			**Purified konjac flour**	
		Superior	**First grade**	**Second grade**	**Superior**	**First grade**
Viscosity (Pa·s) (#4 rotors; 12 rpm; 30°C; 1% solution)	≥	22	18	14	32	28
Glucomannan content (%)	≥	70	65	60	90	85
Sulphur content (mg/kg)	≤	1,600	1,800	2,000	300	500
Moisture content (%)	≤	11	12	13	10	10
Ash (%)	≤	4.5	4.5	5	3	3
Sand (%)	≤	0.04			0.04	
As content (mg/kg)	≤	3.0			2.0	
Pb content (mg/kg)	≤	1.0			1.0	
Compliance with particle size standard (%)	≥	90				

Source: adapted from P. Y. Liu et al. (2002)

2.5.3.1 Primary treatment and drying process of konjac slices

Konjac corms can be harvested once a year namely from the end of rainy season until end of winter (October – January). For safe storage of konjac chips for whole year in the industrial-scale production, their moisture content has to be reduced during the primary drying process to a safe storage level of 5-8% (d.b.). At this moisture level the microbial growth will no longer occur. One of the major causes of quality deterioration of konjac slices during dry processing is browning of cut slices. This browning reaction is caused by enzymatic browning and related factors such as oxygen, drying method and drying temperature (Walker & Ferrar, 1998). High temperature during drying process of the moist konjac cut slices can trigger browning reaction. Polyphenol oxidases (PPOs, EC 1.10.3.1) are a group of copper-proteins, widely distributed phylogenetically from bacteria to mammals, that catalyze the oxidation of phenolics to quinones which produce brown pigments in wounded tissues (Mayer, 1987). The mechanism of action proposed for PPO is based on their capacity to oxidize phenolic compounds. When the tissue is damaged, the rupture of plastids, the cellular compartment where PPO is located, leads to the enzyme coming into contact with the phenolic compounds released by rupture of the vacuole, the main storage organelle of these compounds. The active site of PPO consists of two copper atoms and the enzyme catalyzes two different reactions in the presence of molecular oxygen: the hydroxylation of monophenols (monophenolase activity) and the oxidation of *o*-diphenols to *o*-quinones (diphenolase activity), see Figure 2.18 and 2.19. This reaction is followed by non-enzymatic polymerization of the quinones giving rise to melanins, pigments of high molecular mass, and dark colour (Queiroz, Lopes, Fialho, & Valente-Mesquita, 2008).

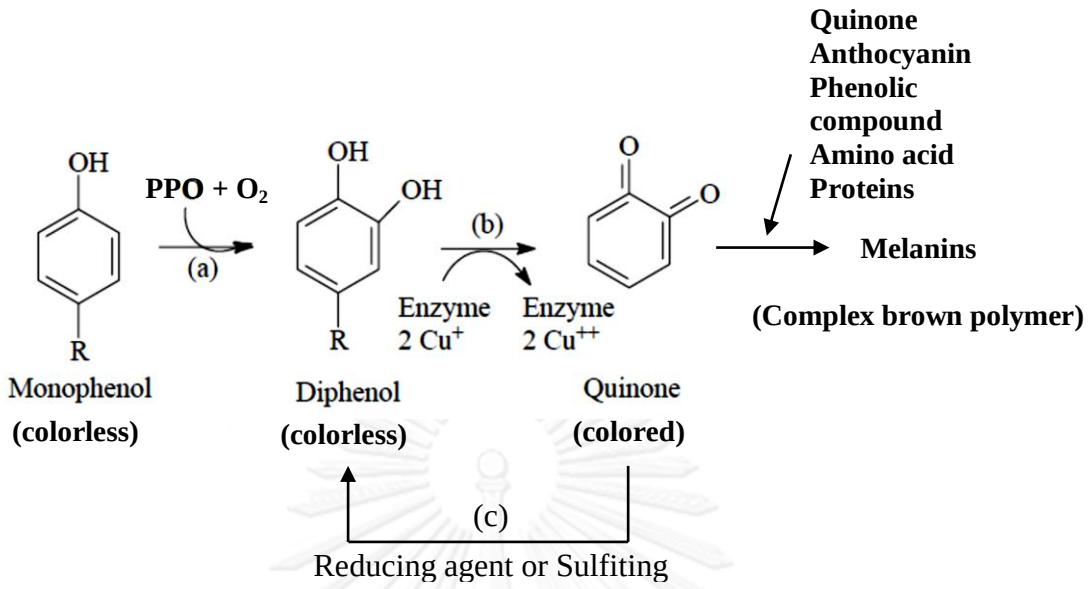


Figure 2. 18 Reactions of (a) hydroxylation, (b) oxidation catalyzed by PPO and (c) the inhibition of browning by polyphenol oxidase.

Source: adapted from Iyengar and McEvily (1992) and Queiroz et al. (2008)

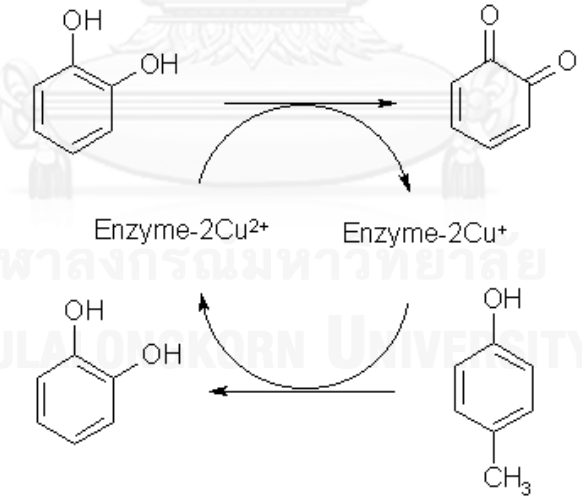


Figure 2. 19 Simplified mechanisms for the hydroxylation and oxidation of diphenol by phenoloxidase.

Source: adapted from Marshall, Kim, and Wei (2000)

Several methods and technologies, such as thermal processing, exclusion of oxygen and anti-browning agents, have been studied to inhibit polyphenol oxidase-related enzymatic browning (Loaiza-Velarde, Mangrich, Campos-Vargas, & Saltveit, 2003). A common approach for preventing enzymatic browning is the use of anti-browning agents (Arslan & Doğan, 2005). Sulphites are found to be the successful inorganic salts to control browning in fruits and vegetables since 1986 (Son, Moon, & Lee, 2001). They are currently applied for the inhibition of the browning in potatoes, mushrooms, apples, and other fruits and vegetables. Sulphite concentrations necessary for controlling enzymatic browning vary widely in accordance with the food material and the time required for inhibition of the browning reaction (Taylor, Higley, & Bush, 1986). Many compounds produce sulphite, called sulphiting agents including sulphur dioxide, sodium sulphite, sodium and potassium bisulphites and metabisulphites. Walker (1977) proposed that the primary role of sulphiting agents is to react with the pigment precursors (*o*-quinone) to produce stable and colourless, diphenols before they can undergo further reaction to form pigments. Ferrer, Otwell, and Marshall (1989) proposed that bisulphate inhibition was due to the reaction of sulphites with intermediate quinones, resulting in the formation of sulphoquinones, which irreversibly inhibited polyphenol oxidase, causing complete inactivation. On the other hand, Madero and Finne (1982) proposed that bisulphite exerted a competitive inhibitory effect on polyphenol oxidase, by binding a sulfhydryl group at the active site of the enzyme. The destruction of the disulphide bonds may cause the enzymes to denature or lose their shape because enzymes must have a specific three-dimensional structure to catalyze their biochemical reactions (Fu, Zhang, Wang, & Du, 2007).

However, Codex General Standard for Food Additives (GSFA) online database limited the maximum level use of sulphiting agent to 1,500 mg/kg food (1,500 ppm) or less than 500 mg/kg (500 ppm) as residual SO_2, in dried vegetables (including mushrooms and fungi, roots and tubers, pulses and legumes, and aloe vera), seaweeds, and nuts and seeds and 1,000 mg/kg (1,000 ppm) as residual SO_2, in dried fruits (Codex, 2014b). Although sulphite treatment levels in foods vary widely between applications, the residue levels should not usually exceed several hundred ppm (Sapers (1993); Taylor et al. (1986)). Moreover, the Joint Expert Committee on Food Additives (JECFA) of the World Health Organization (WHO) and the Food and Agriculture Organization (FAO) recommended an acceptable sulphite daily intake of sulphur dioxide in food stuffs as 0.7 mg/kg of body weight (Queiroz et al., 2008). In addition, these compounds have been restricted by the Food and Drug Administration (FDA) due to the possibility of their associated potential hazards to human health, especially in asthmatic patients (Simon, 1998). Hence several studies have been devoted to the non-sulphite anti-browning agents (e.g. ascorbic acid, citric acid and sodium chloride) in many fruits and vegetables.

Inorganic halides are well-known inhibitors of polyphenol oxidases (Vámos-Vigyázó, 1981). Janovitz-Klapp, Richard, Goupy, and Nicolas (1990) found that sodium fluoride was the most potent inhibitor of apple polyphenol oxidase, followed by sodium chloride, sodium bromide, and sodium iodide. Sodium chloride and calcium chloride at concentrations of ranging between 2 and 4 % (w/v) are most commonly used in the food industry for the inhibition of browning (Steiner & Rieth, 1989). Polyphenol oxidase activity was observed to decrease with increasing concentrations of sodium chloride for peach (Luh & Phithakpol, 1972), eggplant and

avocado (Knapp, 1965). Therefore, soaking konjac slices in sodium chloride solution before drying can be an alternative way to replace the use of sulphiting agents.

One of the most effective anti-browning agent is ascorbic acid which is a highly water-soluble and moderately strong reducing compound. It is acidic in nature and forms neutral salts with bases. Ascorbic acid, also called vitamin C and its derivatives have been widely used as an anti-browning agent for processing of fruits and vegetables. In addition, ascorbic acid also acts as an oxygen scavenger for the removal of molecular oxygen in polyphenol oxidase reactions. Polyphenol oxidase inhibition by ascorbic acid has been attributed to the reduction of enzymatically formed *o*-quinones of their precursor diphenols (Walker, 1977). Ascorbic acid is however irreversibly oxidized to dehydroascorbic acid during reduction process, thus allowing browning to occur when the reducing efficiency of ascorbic acid was depleted (Figure 2.20).

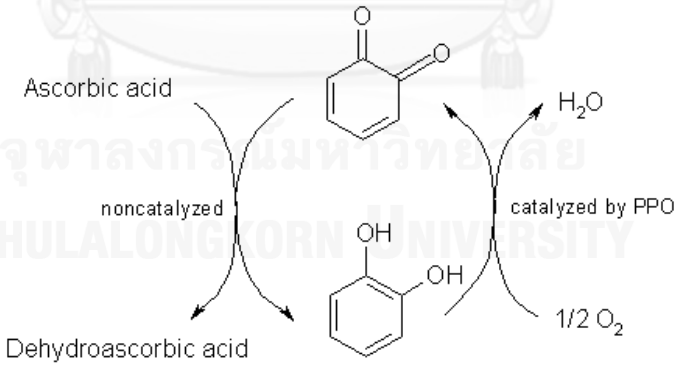


Figure 2. 20 Mechanism of enzymatic browning by PPO

Source: adapted from (Marshall et al., 2000)

The anti-browning activities of ascorbic acid and its derivatives were examined on the apple slices after treatment in a 1% dipping solution (10,000 ppm). Initial observation on treated apple slices revealed that 1% solutions of ascorbic acid and its derivatives appeared to effectively inhibit browning. When the reducing efficiency of ascorbic acid was depleted, within 20 min, the brown colour developed rapidly thereafter (Son et al., 2001).

The studies which are related to the browning control in konjac are showed as follow: Shimizu and Shimahara (1973) studied the extraction process of konjac flour and used sodium or potassium salt of sulphurous or hyposulfurous acid in an amount of 100 – 200 ppm as an anti-browning agent in the pulverizing medium. Chua et al. (2010) used sulphur dioxide as a bleaching agent to prevent darkening of konjac chips. Zhao et al. (2010) studied effects of using four SO_2 releasing agents including Na_2SO_3, K_2SO_3, $Na_2S_2O_5$, and $NaHSO_3$ at concentration of 0.1-0.4 % (1,000-4,000 ppm), and ascorbic acid at a concentration of 6 % (60,000 ppm) on discoloration of konjac flour in extraction process. They found that all four inorganic anti-browning agents gave good protection against discoloration of konjac flour at a concentration of 0.25 % (2,500 ppm). Moreover, at this concentration, the sulphurous acid radical remained below 40 ppm which complies with the Chinese food standards. Furthermore, using 6% of ascorbic acid resulted in a non-discolored, sulphur-free konjac flour. Chua et al. (2012) studied the methodologies for the extraction of KGM from corms of *A. konjac* and used 1 % (w/v) sodium bisulphite for 1 min as an anti-browning agent. The experimental results were satisfactory. In addition, the konjac flour manufacture commonly uses sulphur dioxide in sulphur fumigation during drying process of konjac chips. From previous information, it can

be concluded that sulphating agent is commonly used as an anti-browning agent to prevent browning in konjac flour production. Nevertheless, no systematic studies have been carried out investigating the effectiveness of anti-browning agents in browning control of konjac slices, especially for *A. muelleri* species.

2.5.3.2 Extraction and purification process

Extraction processes are needed to eliminate the impurities for producing purified konjac flour. The traditional extraction methods of konjac flour are dry or wet processing. Dry processing method is simple, less expensive and needs less equipment. Limitations of dry processing method usually include low percentage of separation, long processing time, low glucomannan content and low viscosity of final product (Shimizu & Shimahara, 1973). Thus, wet processing method is developed to improve quality of konjac flour.

Wet extraction method is widely used to produce konjac glucomannan flour because it can provide a high extraction yield and at the same time deliver KGM flour of good quality. However, the ability of glucomannan to absorb water becomes a problem during wet extraction by water, as KGM flour can absorb water as much as 100 times of its weight (Tatirat & Charoenrein, 2011). At the same time, other impurities are also diffused onto the glucomannan granules. Thus, it is difficult to separate the impurities from glucomannan and purify at a later stage. To overcome this problem, anti-swelling agents are trialled in the experiment. The use of anti-swelling agents in solution in water appears as an attractive option in wet extraction of KGM flour instead of pure water, to prevent or inhibit the water absorption by the glucomannan granules. Ethanol is commonly applied in the wet

processing method to provide high purity product, high percentage of extraction and shorter processing time. However, using ethanol increases production cost (Singhavanich & Patanawong, 1992) and creates workplace safety issues. Wet processing method using water-miscible organic solvent (e.g. sodium tetraborate) is introduced to solve these problems.

It is the fact that sodium tetraborate (borax), when in solution, would break down into borate ion [$B(OH)_4^-$] to form complexes with cis-diol groups (hydroxyl pairs) of different KGM molecules to form an inter-chain crosslink (Figure 2.21) and become network structure (Gao, Guo, & Nishinari, 2008). Therefore, the hydroxyl pairs of KGM molecules are not available for combining with water, thus KGM does not swell when in contact with water. Hence, this effect will enhance the efficiency of the extraction. In contrast, the impurities will be dissolved in water and separated from KGM molecules by filtration process.

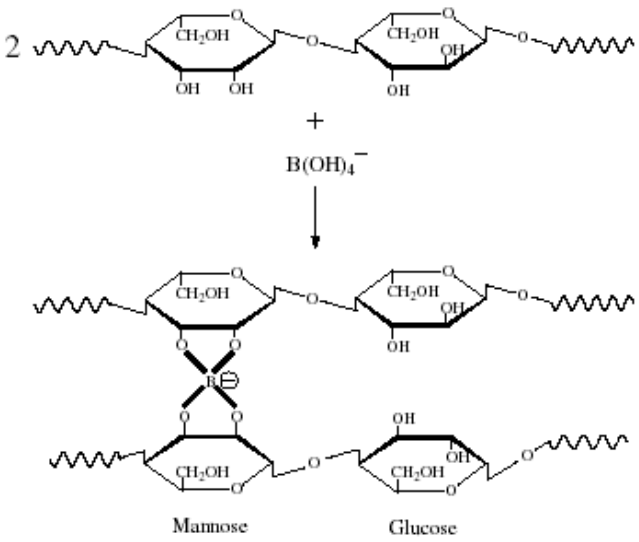


Figure 2. 21 The mechanism of the crosslinking reaction of borate ion with KGM molecules.

Source: Gao, Guo, and Nishinari (2008).

2.5.3.3 Secondary drying process

Finally, the moist KGM flour derived from wet extraction and purification step will undergo a drying process to become dried KGM flour. At this stage, improper handling of the drying process can significantly reduce the quality of konjac flour. Drying method, temperature and time are the main factors affecting the quality of final product. Therefore, several researchers studied effects of drying KGM with various degrees of success. There is no systematic evidence of published work on the effect of these drying processes on the physical and chemical properties of KGM. Thus, the different drying techniques will be investigated to find an appropriate drying method for improving quality of KGM flour.

a) Conventional hot air drying

The conventional hot air drying is one of the most frequently used methods for food dehydration. The objective of drying is to remove water to a safe storage level at which microbial growth will no longer occur. It also provides longer shelf-life, reduction in the volume of the final product for storage, and more convenience for transportation. However, the conventional drying methods normally require long processing time and high temperature (Vadivambal & Jayas, 2007). Thus, this technique can cause serious damage to the quality attributes of the product such as off-flavours, nutrient loss, and colour changes (Krokida, Tsami, and Maroulis (1998); Z. W. Cui, Xu, and Sun (2004); Nahimana and Zhang (2011)), loss of some volatile compounds (De Torres, Diaz-Maroto, Hermosin-Gutierrez, & Perez-Coello, 2010), and reduction in bulk density, porosity, and rehydration capacity of the dried product (Lin, Durance, and Scaman (1998) and Drouzas, Tsami, and Saravacos

(1999)). Thus, attempts are made to find an alternative efficient drying method for the food industry to process and preserve products of high quality. The desire to eliminate the existing problems in conventional drying and to achieve fast and effective thermal processing has resulted in the increasing interest in the use of microwaves for food drying (Vadivambal & Jayas, 2007).

b) Multistage drying

Since a material has both surface and internal moisture, the constant and falling rate periods exist in batch drying. Therefore, the optimal drying conditions and type of dryer should be different to remove these two distinctively different types of moisture. To remove the surface moisture, it requires generally a more rapid process requiring shorter residence time in the dryer. In contrast, internal moisture removal is a slower process requiring a longer residence time and often a larger capacity dryer. Normally, the first stage may be used simply to remove the surface moisture, so that the product becomes non-sticky and suitable for processing in the next step (Kudra & Mujumdar, 2009). These considerations are behind the concept of design and operation of a multistage drying system.

c) Freeze drying

Although freeze drying is known as a superior dehydration process for pharmaceutical and food products, protection against chemical decomposition, easy rehydration, retention of heat-sensitive and active ingredients in food, and excellent shape retention; it is a very energy intensive and also costly process (Nijhuis et al., 1996). Therefore, it is only suitable for high-value

products and not economical for bulk products. In this study, freeze drying is used as a reference to compare the quality of KGM flour with other drying techniques.

d) Microwave

Microwave heating is based on the transformation of alternating electromagnetic field energy into thermal energy by affecting the polar molecules of a material. The most important characteristic of microwave heating is volumetric heating (Mullin, 1995). Conventional heating occurs by convection followed by conduction where heat must diffuse from the surface of the material into the interior of the material which may cause the case hardening problem. Volumetric heating means that materials can absorb microwave energy directly and internally and convert it into heat. In microwave heating, heat is generated throughout the material, leading to faster heating rates, compared to conventional heating where heat is usually transferred from the surface to the interior (Gowen, Abu-Ghannam, Frias, & Oliveira, 2008). Microwave drying is caused by water vapour pressure differences between interior and surface regions, which provide a driving force for moisture transfer (Vadivambal & Jayas, 2007). Microwaves are electromagnetic waves like light and radiate outward from a source. The frequency is generally considered to range from 300 MHz to 300 GHz, hence the wavelength range from 1 m to 1 mm. The microwave frequency band in the electromagnetic spectrum is illustrated in Figure 2.22. The standard frequency used in domestic microwave ovens is 2450 MHz and its wavelength is 12.25 cm. The energy of microwaves comes from electrical energy that is converted by a power supply to high voltages which in turn are applied to the microwave power tube or generator to produce power at microwave frequencies.

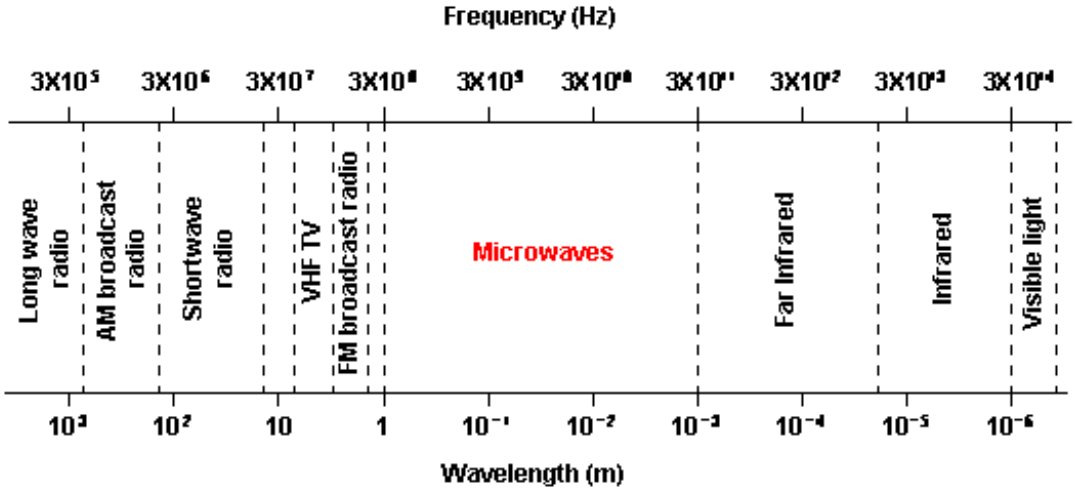


Figure 2. 22 The microwave frequency band in the electromagnetic spectrum. Source: (Anonymous, 2014f)

Microwave equipment consists of three major components: a microwave generator, a waveguide and an applicator. Other components such as a transformer, a rectifier and a device to control microwave energy supply are also necessary for smooth operation of equipment. Magnetrons use DC voltage to produce microwaves. The waveguide are used to transport the produce microwave from the microwave generator to the applicator. The waveguides are hollow rectangular metallic conduit. Applicators are constructed of metal and are of different types, of which cavity applicator is common for industrial applications. Microwave generators that are currently used for generation of microwaves are vacuum tubes classified by the trajectory of electron beam path, linear (type O) and crossfield (type M) (Saltiel & Datta, 1999). There are three types of microwave generators namely magnetron, klystron and traveling wave tubes (TWT). The most common microwave generator used in microwave oven is magnetron (Liamkaew,

2006). The major component of microwave and magnetron are illustrated in Figure 2.23 and 2.24.

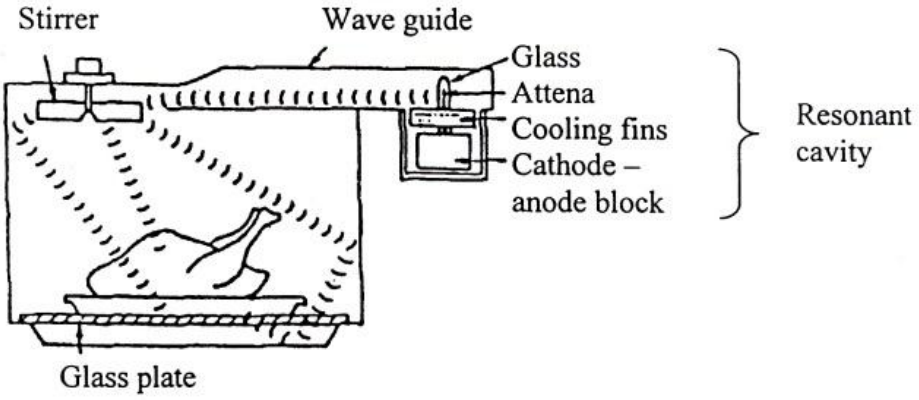


Figure 2. 23 The major components of microwave oven.
Source: Pradit-Duang (1996)

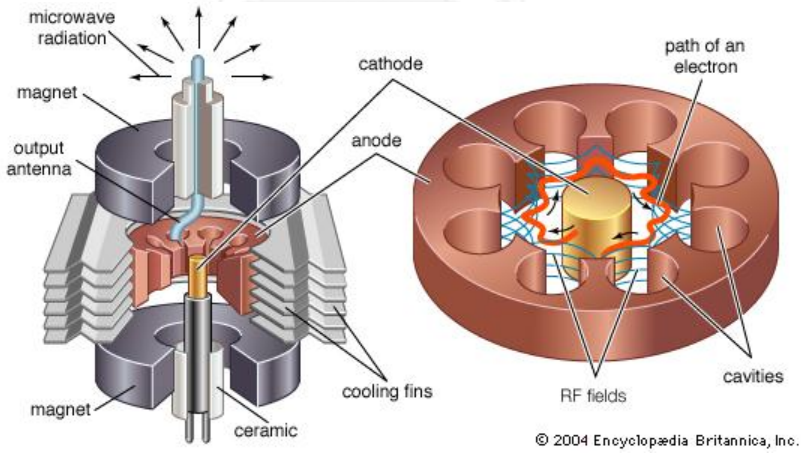


Figure 2. 24 The major components of microwave oven.
Source: Anonymous (2014g)

The mechanism of microwave heating involves two important mechanisms (Kantrong, 2012) that are as follows:

(1) Ionic polarization

After an electrical field is applied to food containing ions, the ions will be moved due to the electromagnetic field and collisions between the ions will occur causing conversion of kinetic energy of the moving ions into thermal energy (Figure 2.25a).

(2) Dipole rotation

Polar molecules such as water molecules inside the food generally have a random orientation. When an electrical field is applied, the molecules will be orienting themselves according to the polarity of the field. In microwave field, the polarity alternates rapidly so the polar molecules rotate to maintain alignment with the rapidly changing polarity as shown in Figure 2.25b. Thus, heat is generated according to the rotation of molecules which leads to friction with the surrounding medium.

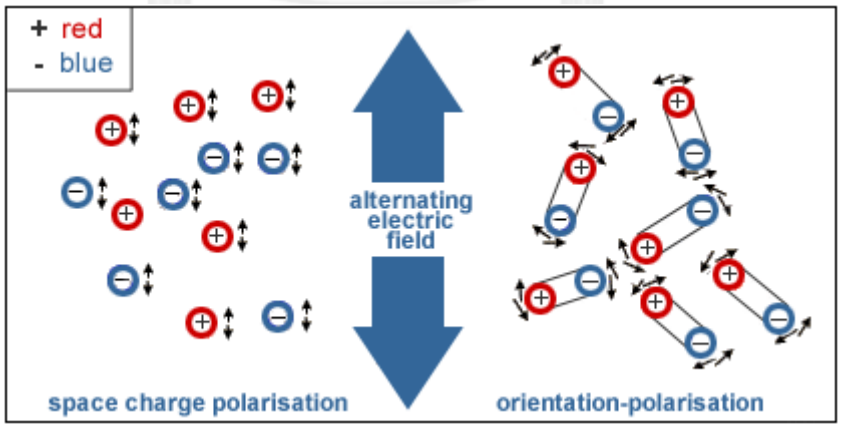


Figure 2. 25 A schematic representation of (a) ionic polarization and (b) orientation polarization under the influence of an alternating electric field.

Source: Püeschner (2014)

e) Microwave-vacuum drying

Microwave-vacuum drying offers an alternative way to improve the quality of dehydrated products which recently became used in the food industry. Heat generated by microwave energy occurs principally in the product, not in the oven walls or atmosphere. Therefore, heat losses from the oven to the surroundings are much lower, making for more comfortable working temperatures. Fast start-up and shut-down and precise process control are possible in microwave heating (Mullin (1995) and Vadivambal and Jayas (2007)). The low temperature and fast mass transfer conferred by vacuum, combined with rapid energy transfer by microwave heating, generate very rapid, low temperature drying (Yongsawatdigul and Gunasekaran (1996) and Zheng et al. (2013)). Moreover, the absence of air during drying may inhibit oxidation, and therefore, colour and nutrient content of products can be largely preserved (Nahimana and Zhang (2011); Z. W. Cui et al. (2004); Kelen, Ress, Nagy, Pallai, and Pintye-Hodi (2006); McLoughlin, McMinn, and Magee (2003); and Zielinska, Zapotoczny, Alves-Filho, Eikevik, and Blaszczak (2013)). Drying under application of microwave vacuum dryer can lead to a shorter drying time at lower temperature and thus results in a superior quality of the product. In contrast, in conventional hot air drying when low temperature is applied the drying time is long. A long drying time contributes to the reduction of quality of dried materials. Thus, the quality of materials dried in a microwave vacuum dryer would be higher than that of materials dried in conventional hot air dryers. Applying microwave energy under vacuum combines advantages of both vacuum drying and microwave drying as far as improved energy efficiency and product quality are concerned (Krokida & Maroulis, 1999). Most of the microwave-vacuum drying studies focus on

fruits and vegetables that need the ‘puffing’ characteristic to improve rehydration properties of the final product (M. Zhang, Tang, Mujumdar, & Wang, 2006). The quick microwave energy absorption by water molecules causes rapid evaporation of water from the interior of the product towards the surface of the product, creating a flux of rapidly escaping vapour which helps in preventing the shrinkage and case hardening and induces more porous and puffing structure, thus improving the rehydration properties of the dried materials. Markowski, Bondaruk, and Błaszczak (2009) found higher rehydration ability for potato cubes dried with microwaves under low pressure. Similar results are reported by Giri and Prasad (2007a) who found that the rehydration properties were improved by drying at lower system pressure and higher microwave power as indicated by higher values of rehydration ratio. The advantages of microwave vacuum drying, especially in food and agricultural products are listed below (Kanlapong, 2006);

- Efficiency: in most cases the energy couples into solvent, not the substrate.
- Nondestructive: Drying can be done at low ambient temperatures; no need to maintain high surface temperatures. This leads to lower thermal profiles.
- Reduction of migration: Solvent often mobilized as a vapor thereby not transporting other materials to the surface.
- Leveling effects: Coupling tends toward wetter areas.
- Speed: Drying time can be shortened by 50% and more.
- Uniformity of drying: By a combination of more uniform thermal profiles and leveling.
- Conveyor systems, less floor space, reduced handling: No need for batch processing in most cases.
- Product improvement in some cases: Eliminates case hardening, internal stresses, etc.

In particular, microwave-vacuum drying techniques are reported to be used successfully for the dehydration of many kinds of fruits and vegetables such as carrots (Z. W. Cui et al. (2004); Z. Cui, Xu, Sun, and Chen (2005); Nahimana and Zhang (2011)), bananas (Maskan (2000) and Mousa and Farid (2002)), wild cabbage (Yanyang, Min, Mujumdar, Le-qun, & Jin-cai, 2004), beetroot (Figiel, 2010), garlic (Z. W. Cui, Xu, and Sun (2003) and Figiel (2006)), mushrooms (Rodríguez, Lombraña, Kamel, and de Elvira (2005); Giri and Prasad (2007a) and (2007b)), potatoes (Setiady, Clary, Younce, and Rasco (2007); Song, Zhang, and Mujumdar (2007); Markowski et al. (2009) and Song, Zhang, Mujumdar, and Fan (2009)), mint leaves (Therdthai & Zhou, 2009), and green peas (Chauhan and Srivastava (2009) and (Zielinska et al., 2013)). These products possess excellent quality in terms of taste, aroma, texture, and appearance.

As for the limitations of microwave-vacuum drying (Kanlapong, 2006), non-uniformities in the microwave field and associated heating patterns can lead to non-uniformities in drying which can be a significant problem. Especially in regions dried earlier, non-uniformities in the microwave field can lead to high temperatures in some regions and cause product degradation (Lu, Tang, & Pan, 1999). However, various ways of averaging the microwave field to improve uniformity have been achieved by such means as mechanical movement (Torringa, Van DIJK, & Bartels, 1996), pneumatic agitation such as in a fluidize bed dryer (Kudra, 1989), or spouted bed dryers (Feng & Tang, 1998). Some of the limitations are specific sample size and shape (Liamkaew, 2006). For industrial applications, it is difficult to dry large size of food and agricultural products in the flow process because of microwave penetration and microwave leaking. The shape and size of objects

heated by microwave irradiation have much greater and completely different impact on temperature distribution than classical means of heating. Microwave energy is deposited directly in the heated material, so the interior of the object can be heated to a higher temperature than near the surface, especially for solids such as frozen meat with low thermal conductivity. A number of researchers studied effects of drying foodstuffs using this technique with various degrees of success. However, there is no systematically research has been conducted on the effects of different drying technique or even microwave-vacuum drying on the quality of KGM flour. From the above information, microwave-vacuum drying has various advantages and limitations. However, it seems possible to use this technique in the production of KGM flour.

CHAPTER 3

MATERIALS AND METHODS

3.1 Materials

3.1.1 Raw materials

The 2-3 years (~ 1 kg weight) old corms of *Amorphophallus muelleri* were collected from Tak province, Thailand (Figure 3.1). After harvesting, they were washed with water to remove soil and other contaminants. Excess water was removed with a cloth and then the konjac corms were shade dried to remove surface moisture. After that, they were stored at room temperature until needed for experiments.

Figure 3. 1 Pictures of Buk Nuea Sai or Buk Khai (*Amorphophallus muelleri*).

Source: Authors own pictures.

3.1.2 Chemicals and reagents

3.1.2.1 Chemicals for the preparation of the anti-browning agents

Name	Company	Country
Potassium hydroxide (A.R. grade)	APS Finechem	Australia
Sodium hypochlorite (A.R. grade)	Ajax Finechem	New Zealand
Sodium tetraborate (A.R. grade)	Ajax Finechem	New Zealand
Sodium chloride (A.R. grade)	Ajax Finechem	New Zealand
Ascorbic acid (A.R. grade)	Ajax Finechem	New Zealand
Citric acid (A.R. grade)	Ajax Finechem	New Zealand
Sodium metabisulphite (A.R. grade)	Ajax Finechem	New Zealand
Potassium Aluminium Sulphate (A.R. grade)	Ajax Finechem	New Zealand

3.1.2.2 Chemicals for the preparation of the anti-swelling agents

Name	Company	Country
95% Ethanol (A.R. grade)	Merck	Germany
Sodium tetraborate (A.R. grade)	Ajax Finechem	New Zealand

3.1.2.3 Chemicals for determination of konjac glucomannan

Name	Company	Country
Crystalline Phenol (A.R. grade)	Fluka	USA
Sodium hydroxide (A.R. grade)	Ajax Finechem	New Zealand
Sodium bisulfite (A.R. grade)	Ajax Finechem	New Zealand

Name	Company	Country
Sodium potassium tartrate (A.R. grade)	Ajax Finechem	New Zealand
3, 5-Dinitrosalicylic acid (A.R. grade)	Fluka	USA
D-Glucose monohydrate (A.R. grade)	Sigma	USA
95% Ethanol (A.R. grade)	Merck	Germany
Sulfuric acid (A.R. grade)	Merck	Germany

3.1.2.4 Chemicals for determination of sulfur dioxide residue

Name	Company	Country
Sodium hydroxide (A.R. grade)	Ajax Finechem	New Zealand
Sulfuric acid (A.R. grade)	Merck	Germany
p-Rosaniline Hydrochloride (A.R. grade)	Ajax Finechem	New Zealand
Hydrochloric acid (A.R. grade)	Merck	Germany
Sodium chloride (A.R. grade)	Ajax Finechem	New Zealand
Mercury (II) Chloride (A.R. grade)	Ajax Finechem	New Zealand
Formaldehyde (A.R. grade)	Ajax Finechem	New Zealand
Sodium bisulfite (A.R. grade)	Ajax Finechem	New Zealand

3.1.2.5 Chemical for particle density measurement

Name	Company	Country
Toluene (A.R. grade)	Merck	Germany

3.1.3 Apparatus

Instruments	Model	Company, Country
Try dryer	HA-100S	Yeoheng Co., Ltd., Thailand
Hot air oven	FD 240	Binder, Germany
Freeze dryer	FreeZone 6	Labconco, USA
Microwave vacuum dryer	-	MarchCool, Thailand
Thermocouple	Type K	Lega, Taiwan
Water activity meter	-	AquaLab, USA
Chromameter	CR-300	Minolta, Japan
Rheometer	C-VOR	Bohlin Rheometer, UK
Kjeldahl analysis	Vapodest 10	Pauley Equipment solution, UK
Soxhlet extraction	AV6AII/16	C. Gerhardt UK Ltd., UK
Muffle furnace	GF-03	Luoyang Gefei Co., Ltd., China
High speed blender	HGBTWT	Waring, USA
Image analyzer	SMZ1000	Nikon, Japan
Scanning electron microscope (SEM)	JEOL: JSM-5800LV	Jeol Ltd., Japan
High performance liquid chromatography (HPLC)	1100 series	Agilent Technology, USA
Fourier Transform Infrared Spectrometer (FTIR)	Spectrum One	Perkin Elmer, USA
Refrigerator	SBC-2DB	Sanyo, Japan
Electronic balance (2 digits)	BA 4100 S	Sartorius, Germany

Instruments	Model	Company, Country
Electronic balance (4 digits)	BSA 2245	Sartorius, Germany
pH meter	CyberScan pH 1000	Eutech, Singapore
Water bath	1083	GGFL, Germany

3.2 Methods

3.2.1 Chemical composition analysis of fresh konjac corms

Moisture content, protein, lipid, crude fibre, ash, and carbohydrate of fresh konjac corms were analysed as follows:

3.2.1.1 Moisture, protein, lipid, crude fibre, ash, and carbohydrate contents

Standard AOAC methods (AOAC, 2006) were used to determine moisture (AOAC 925.10), total nitrogen (AOAC 920.87), lipid (AOAC 920.39), ash (AOAC 942.05), and crude fibre (AOAC 920.86). Crude protein content was calculated from total nitrogen content using 6.25 as conversion factor. Total carbohydrate content was calculated from the difference between 100 and the sum of moisture, crude protein, total lipid, crude fibre and ash content. The analysis was performed in triplicate and mean values were reported.

3.2.1.2 KGM content determination

The quantitative analysis of the glucomannan content was performed by two methods. The first method was done by using the 3,5-

dinitrosalicilic acid (3,5-DNS) colorimetric assay (adapted from P. Y. Liu et al. (2002); Zhao et al. (2010); and Chua et al. (2012)) which was the most reproducible and accurate method for determination of glucomannan content in konjac samples. This method has been adopted by the Chinese Ministry of Agriculture (CMA) for the classification of konjac flour. The second method was done by using high performance liquid chromatography (HPLC) method (adapted from Cengiz, Dogan, and Karaman (2013)) (see in Appendix A, A.1-A.2). The analysis was performed in triplicate and mean values were reported.

3.2.2 Determination of anti-browning agents and soaking time for browning control of konjac slices

In order to prevent enzymatic browning reaction, anti-browning agents were trialed in this experiment. Konjac corms were peeled and cut into about 2 mm thick slices using a stainless steel hand slicer. Then they were immersed in anti-browning solution for times ranging between 5-30 min at room temperature (Figure 3.2). Anti-browning agents used in the experiment were divided into two groups namely sulphur containing compounds such as sodium metabisulphite and potassium aluminium sulphate and sulphur-free compounds namely potassium hydroxide, sodium hypochlorite, sodium tetraborate, sodium chloride, ascorbic acid, and citric acid. Samples treated with distilled water were used as the control. Concentration of each soaking solution was varied from 10 to 10,000 ppm. Concentration of each soaking solution is shown in Table 3.1.

Table 3. 1 Two major groups of anti-browning agents.

Anti-browning agent	Concentration (ppm)
Sulphur-free compounds	
Potassium hydroxide (KOH)	10; 50; 100; 150
Sodium hypochlorite (NaOCl)	500; 1,000; 1,500; 2,000
Sodium tetraborate ($Na_2B_4O_7.10H_2O$)	2,000; 4,000; 6,000; 8,000
Sodium chloride (NaCl)	5,000; 10,000; 20,000; 30,000
Ascorbic acid	2,000; 4,000; 6,000; 8,000; 10,000
Citric acid	2,000; 4,000; 6,000; 8,000; 10,000
Sulphur containing compounds	
Sodium metabisulphite ($Na_2S_2O_5$)	500; 1,000; 1,500; 2,000
Alum ($KAl(SO_4)_2.12H_2O$)	500; 1,000; 1,500; 2,000

Figure 3. 2 Buk Nuea Sai or Buk Khai (Amorphophallus muelleri) after peeling, cutting, and immersion in anti-browning solution.

After each treatment, the liquid was drained off. Then the konjac samples were dried in hot air dryer at 50 °C to remove moisture until 5-8% (d.b.) (about 6-8 hours) (see Appendix B, Figure B-1).

3.2.2.1 Whiteness index value measurement

The colour of the dried konjac slices was measured by a CR-300 Chromameter equipped with a CR-300 measuring head (Minolta, Japan). Hunter values, expressed as *L*, *a* and *b* values, were monitored on the surface of dried konjac slices samples. Browning of the konjac surface was analysed by conversion of measured Hunter values into whiteness index (WI) values (Albanese, Cinquanta, & Dimatteo, 2007). The analysis was performed in triplicate and mean values were calculated using equation 3.1. The most suitable anti-browning agent was determined by a high whiteness index value.

$$\text{Whiteness Index} = 100 - [(100 - L)^2 + (a)^2 + (b)^2]^{1/2} \quad (3.1)$$

3.2.3 Determination of anti-swelling agents for wet extraction process

The dried konjac slices obtained using the most suitable anti-browning agent as described in section 3.2.2 were subsequently ground into powder and sifted through 60 mesh (250 μm) sieve to separate the particles of konjac flour smaller than 250 μm. From that fraction, starch and other impurities were removed by sifting through a 120 mesh (125 μm) sieve. Thus, the particles of resulting crude konjac flour were between 125-250 μm. Then they were mixed with anti-swelling agent and blended in a hi-speed blender for 2 minutes. The ratio of the crude konjac flour to anti-swelling agent was 1:10 by weight per volume (w/v). Ethanol and sodium tetraborate were used as anti-swelling agent in concentration ranging between 10-95% and 0.5-3.0%, respectively. After this process, the wet konjac flour was filtered through a 80 mesh (180 μm) sieve to drain the anti-swelling agent with starch and

other impurities while the semi-dried konjac flour was still on the sieve. The semi-dried konjac flour was then blended with anti-swelling agent and sifted again 4 times. After extraction process, the semi-dried konjac flour was dried by using freeze dryer at -47 ºC, $46x10^{-3}$ mbar (4.6 Pa) until final moisture content 5-8% (d.b.) and subsequently ground into powder and sifted through a 120 mesh (125 μm) sieve. Thus, the particles of resulting konjac flour was between 180-250 μm. At this stage, crude konjac flour was more purified and could be called konjac glucomannan (KGM) flour (see in Appendix B, Figure B-2). The most suitable anti-swelling agent was determined by high extraction yield and low water uptake percentage and at the same time delivered konjac flour of good quality such as high whiteness index value, glucomannan content, and apparent viscosity.

3.2.3.1 Extraction yield determination

The extraction yield percentage of KGM flour after extraction process was determined by weighing konjac flour before extraction and KGM flour after extraction, drying, and sifting. The analysis was performed in triplicate and mean values were calculated using equation 3.2 (adapted from Sriamornsak and Kennedy (2008).

$$\%\ \text{yield of extraction} = \frac{W_f}{W_0} \times 100 \qquad (3.2)$$

In the equation:

W_f = the final dry weight of KGM flour after extraction, drying, and sifting

W_0 = the initial dry weight of konjac flour before extraction

3.2.3.2 Water uptake determination

The molecules of glucomannan in konjac flour have the ability to absorb water, swell and become dense paste when in contact with water during wet extraction. Hence, the impurities are absorbed onto the granules of glucomannan, making it difficult to separate and purify the latter. To overcome this problem, anti-swelling agents were trialled in this experiment to prevent konjac granules from combining with water. The most effective anti-swelling agents should show a low water uptake level which is determined by weighing konjac flour before and after extraction. The analysis was performed in triplicate and mean values were calculated using equation 3.3 (adapted from Sriamornsak and Kennedy (2008).

$$\% \text{ water uptake} = \frac{W_t}{W_0} \times 100 \qquad (3.3)$$

In the equation:

W_t = the wet weight of konjac flour after extraction

W_0 = the initial dry weight of konjac flour before extraction

3.2.3.3 Whiteness index value measurement

The colour of the dried KGM flour was measured by a CR-300 Chromameter equipped with a CR-300 measuring head (Minolta, Japan). Hunter values, expressed as *L*, *a* and *b* values, were monitored on the surface of dried KGM flour samples. Browning of KGM flour was analysed by conversion of measured Hunter values into whiteness index (WI) values (Albanese et al., 2007). The analysis was performed in triplicate and mean values were calculated using equation 3.1.

3.2.3.4 Apparent viscosity measurement

The viscosity of 1% w/v KGM solution was obtained by using an advanced stress/strain controlled rheometer (C-VOR Bohlin Rheometer, UK) equipped with a cone-plate geometry (40 mm of diameter, 4 ° cone angle, and 150 μm gap) in shear rate ranging from 0.1-100 s^{-1}. The apparent viscosity result showed in shear rate 10 s^{-1}. The viscosity was measured after stirring KGM solution with a magnetic stirrer at constant speed for 3 hours until the sample became fully rehydrated (as per results from a preliminary study). For all the test samples, the temperature was set to 25 °C and controlled by a Peltier device. The viscosity of each sample was averaged from 5 measurements.

3.2.3.5 KGM content determination

The quantitative analysis of the glucomannan content was performed by two methods as described in section 3.2.1.2. The analysis was performed in triplicate and mean values were calculated using equation A1 and A2 (see in Appendix A, A.1-A.2). Both glucose and mannose calibration curves were constructed for the 3,5-DNS and HPLC methods, in order to compare the sensitivity of the assay systems to each reducing sugars.

3.2.3.6 Morphology of KGM flour

The morphology of the KGM flour was investigated using an image analyzer (Nikon SMZ1000, Japan). The KGM flour was initially examined at 120× magnification. The microstructure of the KGM flour was studied using a scanning electron microscope with EDS attachment (SEM-EDS) (JEOL: JSM-

5800LV, Jeol Ltd., Tokyo, Japan) with accelerating voltage of 15 kV. The KGM flour was examined at 350× magnification. All measurements were performed in triplicate.

3.2.4 Drying experiment

From previous section (3.2.2 and 3.2.3), the most suitable anti-browning agent and anti-swelling agent were selected and the extraction procedure repeated as follows: The konjac corms were peeled and cut into 2 mm thick slices. Then, the konjac slices were immersed in a 0.05% sodium metabisulphite solution for 10 minutes (anti-browning agent as a result obtained from step 3.2.2), followed by hot air drying at 50 ºC to reduce the moisture content to 5-6% (d.b.) (about 6-8 hours). The dried konjac slices were subsequently ground into powder and sifted through 60 mesh (250 μm) sieve to limit the particle size of konjac flour to less than 250 μm. Starch and other impurities were removed by sifting through 120 mesh (125 μm) sieve. Thus, the particle size of resulting crude konjac flour was between 125-250 μm. Then they were mixed with 50 % ethanol (anti-swelling agent obtained from step 3.2.3) and blended in a hi-speed blender for 2 minutes. The ratio of the crude konjac flour to anti-swelling agent was 1:3 by weight per volume (w/v). After this process, the wet konjac flour was filtered through 80 mesh (180 μm) sieves to drain anti-swelling agent with starch and other impurities while the semi-dried konjac flour was still on the sieve. The semi-dried konjac flour was then blended with anti-swelling agent and sifted again 4 times. Subsequently, semi-dried KGM flour resulting from wet extraction process was dried by different drying techniques until the final moisture content was reduced to 5-6 % (d.b.) in order to study the effect of drying methods on physicochemical properties of KGM flour. The drying methods used in this

experiment were hot air drying, multistage hot air drying, freeze drying, and microwave-vacuum drying. A completely randomised design (CRD) experimental design was used in this study. All drying experiments were performed in duplicate.

The change of moisture content in KGM flour during drying was expressed as moisture ratio (MR) as defined in the equation (3.4) (Zielinska & Cenkowski, 2012). The equilibrium moisture content in this experiment was 1.50±0.05% (d.b.).

$$\text{Moistureratio} = \frac{M_t - M_e}{M_0 - M_e} \quad (3.4)$$

Where:

M_t is the moisture content (g water/g dry solid) at time t = t

M_0 is the moisture content at time t = 0

M_e is the equilibrium moisture content

However, in the calculation of moisture ratio in this experiment, the equilibrium moisture content of KGM flour was very low (1.50% d.b.) and the initial moisture content of KGM flour was more than 100% (d.b.). Thus, the calculation of moisture ratio was evaluated by the equation 3.5

$$\text{Moisture ratio} = \frac{M_t}{M_i} \quad (3.5)$$

Where:

M_t is the moisture on a percentage of dry basis (% d.b.) at any time *t* during drying

M_i is the initial moisture content (% d.b.)

3.2.4.1 Hot air drying

The hot air drying was conducted in a hot air oven (FD 240-model; Binder, Germany) at 50, 60, 70, and 80 °C. After wet extraction, the semi-dried KGM flour (100 ± 0.05 g) with an average moisture content of 100 % (d.b.) was placed on the aluminum tray in a 1 cm thick layer. All drying experiments were performed in duplicate. The characteristics of all samples were compared at the same final moisture content 5-6 % (d.b.).

3.2.4.2 Multistage hot air drying

Using experimental data from hot air drying curves helps developing a strategy for multistage drying process aiming at reducing drying time and improving quality of KGM flour. It could be clearly seen that the colour of KGM flour was changing from yellowish to white once the moisture ratio was reduced to about 0.40 or temperature of KGM flour reached about 38 °C. After this period, the colour of KGM flour continued changing slightly during drying process. Based on this observation, the multistage can be developed. Drying process can be divided into two periods including before and after the change of colour. The possible multistage hot air drying conditions namely 50+80 °C, 60+80 °C, and 70+80 °C. The semi-dried KGM flour (100 ± 0.05 g) with an average moisture content of 100 % (d.b.) was placed on the aluminum tray in a 1 cm thick layer. All drying experiments were performed in duplicate. The characteristics of all samples were compared at the same final moisture content 5-6% (d.b.).

3.2.4.3 Freeze drying

The semi-dried KGM flour was placed on the polyethylene plastic tray in a 1 cm thick layer and frozen at -40 ºC in an freezer overnight, then freeze dried by using freeze dryer at -47 ºC under vacuum pressure at $46x10^{-3}$ mbar (4.6 Pa) until final moisture content 5-6% (d.b.).

3.2.4.4 Microwave-vacuum drying

The semi-dried KGM flour with the moisture content in average of 100 % (d.b.), was dried using a pulsed microwave vacuum dryer (MarchCool, Thailand). The microwave vacuum dryer consisted of six magnetrons with a 360 ° rotating polyethylene basket. The velocity of rotating polyethylene basket was 15 rpm (Figure 3.3). For the drying experiment, an amount of about 100±0.05 g of the semi-dried KGM flour was placed in a nylon bag with 125 μm opening so that the moisture could move out from the sample during drying process.

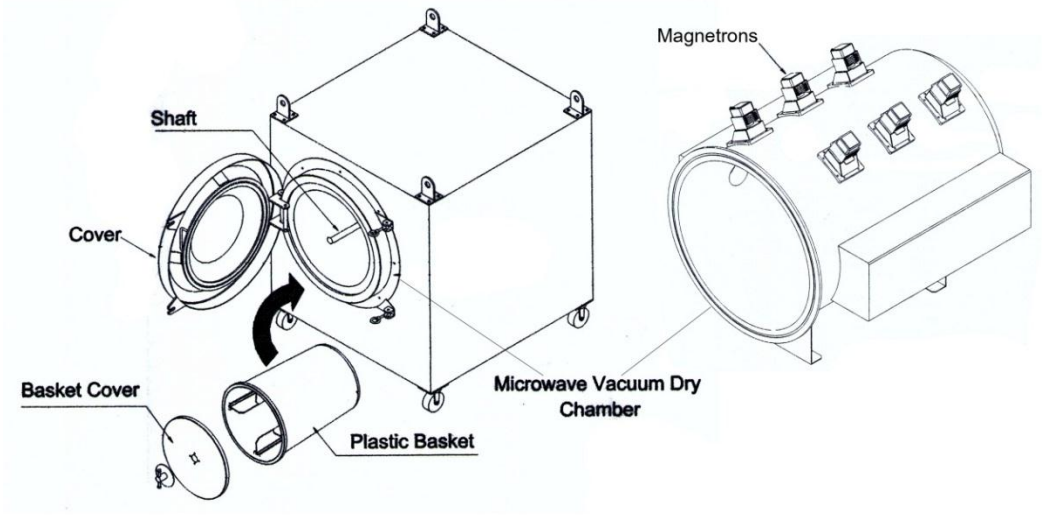


Figure 3. 3 Components of a microwave vacuum dryer.
(MarchCool, Thailand)

The microwave vacuum dryer was operated at three microwave power outputs namely 960 W, 1200 W, and 1440 W or microwave intensities of 9.6 W g^{-1}, 12 W g^{-1}, and 14 W g^{-1}, all with controlled pressure of 80 kPa (600 mm Hg) and controlled frequency of 2450 MHz for up to 12 minutes. All drying experiments were performed in duplicate. The characteristics of all samples were compared at the same final moisture content 5-6% (d.b.).

3.2.5 Study the effects of different drying methods on some physical and chemical properties of purified KGM flour.

After all drying treatments, the KGM flour from each treatment was ground and sifted through 120 mesh (125 μm) sieve to remove starch and other impurities again (see the experimental procedures in Appendix B, Figure B-3). The physicochemical properties of KGM flour were determined as follows:

3.2.5.1 Moisture content determination

The moisture content of KGM flour was determined by the hot air oven drying method according to the AOAC standards AOAC (2006).

3.2.5.2 Water activity determination

The water activity of the KGM flour was determined by using a water activity meter (AquaLab, USA) at 25 °C.

3.2.5.3 Product temperature measurement

The product temperature after drying was measured using a thermocouple type K (Lega, Taiwan). After drying, the sample was immediately placed into a thermally insulated incubator chamber where the product temperature was measured.

3.2.5.4 Whiteness index value measurement

The colour of KGM flour was measured by a CR-300 Chromameter equipped with a CR-300 measuring head (Minolta, Japan). Hunter values, expressed as *L, a,* and *b* values, were monitored on the surface of KGM flour. Browning of the KGM flour was analyzed by conversion of measured Hunter values into whiteness index (WI) (Albanese et al., 2007). The analysis was performed in triplicate and mean values were calculated using equation 3.1.

3.2.5.5 Bulk density, particle density, and porosity determination

The bulk density (ρ_{bulk}) of the KGM flour was determined by using the standard test weight procedure (Gotoh, Masuda, & Higashitani, 1997). After grinding and sifting through 80-120 mesh sieves, the KGM flour with particle sizes between 125-180 μm was gently loaded into a 50 cm^3 graduated cylinder until the KGM flour reached a volume of 10 cm^3. Thus, the volume of KGM flour for bulk density determination was 10 cm^3. The sample weight was used to calculate the bulk density according to the relationship of mass and volume as shown in the equation (3.6).

$$\rho_{bulk} = \text{Weight of KGM flour / Volume of KGM flour} \quad (3.6)$$

The measurement of the particle density (ρ_p) and the volume of KGM flour were carried out by standard liquid pycnometric method according to the AOAC standards (AOAC, 2006). The volume of particles is determined from the liquid volume increase upon adding the particles into a liquid, which was toluene (density of toluene is 0.8625 g/cm^3). A calibrated glass pycnometer of approx. 25 cm^3 was used in this experiment. The particle density was calculated by the equation (3.7) (Gotoh et al., 1997).

$$\rho_{particle} = \frac{\rho_l(m_s - m_0)}{(m_l - m_0) - (m_{sl} - m_s)} \quad (3.7)$$

Where:

ρ_l is the toluene density, m_0 is weight of empty pycnometer, m_l is weight of pycnometer containing toluene, m_s is weight of pycnometer including sample particles, and m_{sl} is weight of pycnometer including sample and toluene.

The porosity (ε) of KGM flour was calculated by the equation (3.8) (Gotoh et al., 1997). All measurements were performed in triplicate.

$$\varepsilon = \frac{1 - \rho_{bulk}}{\rho_{particle}} \quad (3.8)$$

3.2.5.6 Apparent viscosity measurement

The viscosity of 1% w/v KGM solution was determined by using an advanced stress/strain controlled rheometer (C-VOR Bohlin Rheometer, UK)

equipped with a cone-plate geometry (40 mm of diameter, 4 ° cone angle, and 150 μm gap) in shear rate ranging from 0.1-100 s^{-1}. The apparent viscosity result showed in shear rate 10 s^{-1}. The viscosity was measured after stirring with a magnetic stirrer at constant speed for 3 hours until the sample became fully rehydrated (as per results from a preliminary study). For all the test samples, the temperature was set to 25 °C and controlled by a Peltier device. The viscosity of each sample was averaged from 5 measurements.

3.2.5.7 Structure characterization

a) Microstructure characterization (Scanning Electron Microscopy-SEM)

The microstructure of the KGM flour was observed using a scanning electron microscope with EDS attachment (SEM-EDS) (JEOL: JSM-5800LV, Jeol Ltd., Tokyo, Japan) using an accelerating voltage of 15 kV. The KGM flour was evaluated at 350× magnification.

b) Morphological characterization (Image analyser)

The morphology of the KGM flour was studied using an image analyzer (Nikon SMZ1000, Japan). The KGM flour was examined at 120× magnification.

c) Fourier Transform Infrared spectroscopy (FT-IR)

The FT-IR spectra were generated directly from the KGM flour samples using a Fourier Transform Infrared Spectroscopy: Spectrum One

(Perkin Elmer, USA). The spectra were obtained in the wavenumber range 400-4000 cm^{-1} using a KBr-pellet method.

3.2.5.8 KGM content determination

The quantitative analysis of the glucomannan content was performed using the 3,5-dinitrosalicilic acid (3,5-DNS) colorimetric assay (adapted from (P. Y. Liu et al. (2002); Zhao et al. (2010) and Chua et al. (2012)). The analysis was performed in triplicate and mean values were calculated using equation A1 and A2 (see in Appendix A, A.1-A.2). Comparing with high performance liquid chromatography (HPLC) method (adapted from Cengiz et al. (2013)). The analysis was performed in triplicate and mean values were calculated the peak area of glucose and mannose standard curve (see in Appendix A).

3.2.5.9 Sulphur dioxide residue determination

The residual sulphite content of KGM flour was determined in each sample according to the AOAC standards 963.20 (AOAC, 2006) (see Appendix A, A.3). All measurements were performed in triplicate.

Finally, the most appropriate drying technique for production of purified KGM flour was selected on the basis of the amplitude of high apparent viscosity, whiteness index value, and glucomannan content, and minimum sulphur dioxide residue. The other physical and structural properties were also determined.

3.2.6 Statistical analysis

All the experimental data were collected in triplicate and the average results were reported. The differences between means were estimated using analysis of variance (ANOVA) and Duncan's multiple range test with a level of significance of $p \leq 0.05$ using the SPSS 16.0 software (IBM SPSS, Chicago, IL, USA). Correlation analysis was applied to investigate for the possible parametric relationships.

CHAPTER 4

RESULTS AND DISCUSSION

4.1 Chemical composition of fresh konjac corms

The results showed that fresh konjac corm consists of 82.83±0.29 % (w.b.) of moisture content and 17.17+0.29 % of solid portion. The chemical composition (AOAC, 2006) of the dried konjac corm is shown in Table 4.1.

Table 4. 1 Chemical composition of fresh konjac corm.

Composition of fresh konjac corm	Result (g/100 g)
Moisture content	82.83 ± 0.29
Total carbohydrate	15.09 ± 0.19
- Dietary fibre	15.05 ± 0.19
- Other carbohydrate	0.04 ± 0.01
Ash	1.02 ± 0.02
Protein	0.94 ± 0.05
Lipid	0.12 ± 0.04
Glucomannan (analysed by 3,5-DNS method)	13.46 ± 0.16
Glucomannan (analysed by HPLC method)	12.82 ± 0.18

Values expressed are means of 3 replicates ± SD

The results show that in solid portion, the main component is carbohydrate (15.09 %) which consists of dietary fibre (15.05 %) and other carbohydrate (0.04 %). The other minor components include ash, protein and lipid (in totally 2.08 %). However, glucomannan content of fresh konjac is 13.46% and 12.82% which analysed by 3,5-DNS and HPLC method, respectively. As for the definition of dietary

fibre, Trowell et al. (1976) published a definition later adopted as consensus by AOAC, defining dietary fibre as plant polysaccharides and lignin which are resistant to hydrolysis by digestive enzymes of man. This definition includes macro constituents of foods which comprise cellulose, hemicellulose, lignin, gums, modified celluloses, mucilages, oligosaccharides, and pectins and associated minor substances such as waxes, cutin, and suberin. However, the definition of dietary fibre that was published by CODEX means carbohydrate polymers including lignin with 10 or more monomeric units, which are not hydrolysed by the endogenous enzymes in the small intestine of humans and belong to the following categories (McCleary et al., 2009):

- Edible carbohydrate polymers naturally occurring in the food consumed.
- Carbohydrate polymers, which have been obtained from food raw material by physical, enzymatic or chemical means and which have been shown to have a physiological benefit to health, as demonstrated by generally accepted scientific evidence to competent authorities.
- Synthetic carbohydrate polymers that have been shown to have a physiological benefit to health, as demonstrated by generally accepted scientific evidence to competent authorities.

From the above information, it can assume that the dietary fibre in konjac corm mainly included glucomannan. This assertion is supported by the findings from several researchers who claimed that the main component of konjac flour is a carbohydrate which is composed of D-mannose and D-glucose in the ratio of 1.6: 1 or 1.4: 1, linked by β-(1→4) glycosidic linkages (Williams et al., 2000). In order to

confirm whether it is glucomannan or not, the experiments described in the next subsequent sections were conducted step. It can be speculated that konjac glucomannan is encapsulated by the other impurities component such as starch, cellulose and also some proteins and lipids in the form of “sacs”. As a result, the konjac solution has a highly turbid appearance (Shimizu and Shimahara (1984); Ohashi et al. (2000); Tatirat and Charoenrein (2011)). Therefore, it is necessary to use the extraction process to improve the purity of konjac glucomannan.

4.2 Effects of anti-browning agents and soaking time for browning control of konjac slices

Browning of fruits and vegetables can occur from both enzymatic and non-enzymatic reaction. Enzymatic browning reaction is the discoloration that results from the oxidation of phenolic compounds by the activity of enzyme such as polyphenol oxidase in the presence of oxygen (Ozdemir, 1997). It is well known that there are several reactions that could result in non-enzymatic browning; four such reactions are (1) the Maillard reaction which results from reaction between reducing sugars such as fructose and glucose and protein or its derivatives such as amino acids and amides, this reaction occurs when the mixtures are heated; (2) the caramelisation or pyrolysis of sugar due to heat treatment above the melting point of the sugar under alkaline or acidic conditions; (3) the decomposition of ascorbic acid; and (4) the lipid oxidation (Ozdemir, 1997).

Based on the possibilities above, it can be seen that the browning of dried konjac slices likely to be mainly due to the enzymatic browning reaction. Since there are some minor compositions of other carbohydrate (e.g. mono- and disaccharide),

protein, and lipid in fresh konjac and the drying condition takes place under low temperature (50 °C) in which the caramelisation, Maillard reaction, or lipid oxidation would be very difficult to occur. Moreover, there are the studies found that browning of konjac caused by activity of polyphenol oxidase (S. Zhang, Zheng, and Zhong (2007a) and (2007b)). Thus, it can be concluded that enzymatic browning reaction is the main factor to the browning problem in dried konjac slices.

In order to inhibit the browning reaction of konjac slices while they were dried for safe storage for the next experiment, the various anti-browning agents were used (see the processing step in Appendix B, Figure B-1). The colour in terms of whiteness index values of treated konjac slices after hot air drying at 50 ºC to remove moisture until 5-8% (d.b.) is shown in Figure 4.1. Generally, the whiteness index value of all samples varied from 50 to 82 (see Appendix C, Table C-1, C-2, C-3). All the three factors (type of antibrowning agents, treatment concentration and soaking time) had significantly effects (p<0.05) on the whiteness index of dried konjac slices. The results in Figure 4.1 is also indicating that sodium metabisulphite was the most effective agent for retarding browning of dried konjac slices. The treatment using 1500 ppm sodium metabisulphite for 30 min resulted in the highest whiteness index value of 82.19, followed by sodium metabisulphite at 1500 ppm concentration for 20 min and 500 ppm for 10 min to produce the whiteness index values of 81.14 and 80.73, respectively. However, these three treatments were not significantly different ($p\leq$0.05) among them (see in Appendix C, Table C-2). Therefore, the treatment consisting of 500 ppm sodium metabisulphite for 10 min was selected as the most effective for preventing enzymatic browning of dried konjac slices while using only small concentration of sodium metabisulphite.

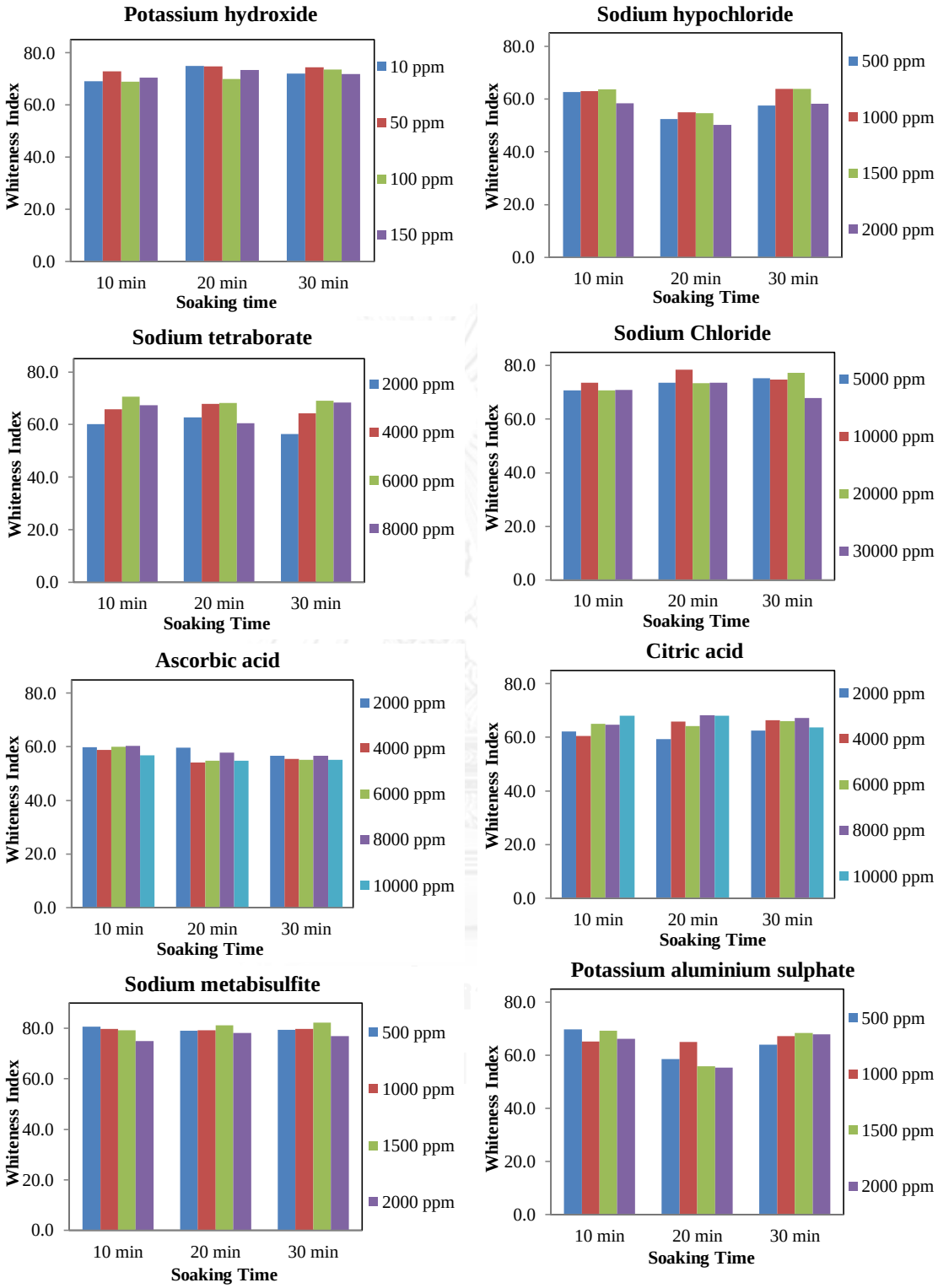


Figure 4. 1 Effect of various anti-browning agents on whiteness index value of konjac *A.muelleri* corm slices.

According to the literature, polyphenol oxidase activity can be regulated by the action of antioxidant agents, enzyme inhibitors, acidulants, chelating agents or complexing agents (Özoglu & Bayindirli, 2002). Sulphur-containing agents such as sodium metabisulphite act on quinines as reducing agents regenerating polyphenols (Queiroz, da Silva, Lopes, Fialho, & Valente-Mesquita, 2011). Many compounds, called sulphiting agents, produce sulphite, among them sulphur dioxide, sodium sulphite, sodium and potassium bisulphites and metabisulphites. Walker (1977) proposed that the primary role of sulphiting agents is to reduce the pigment precursors (*o*-quinone) to colourless, less-reactive diphenols before they can undergo further reaction to form brown pigments. Ferrer et al. (1989) proposed that bisulphate inhibition was due to the reaction of sulphites with intermediate quinones, resulting in the formation of sulphoquinones, which irreversibly inhibited polyphenol oxidase, causing complete inactivation. On the other hand, Madero and Finne (1982) proposed that bisulphite exerted a competitive inhibitory effect on polyphenol oxidase, by binding a sulphydryl group at the active site of the enzyme. The destruction of the disulphide bonds may cause the enzymes to denature or lose their shape because enzymes must have a specific three-dimensional structure to catalyze their biochemical reactions (Fu et al., 2007).

However, Codex General Standard for Food Additives (GSFA) online database limited the maximum level use of sulphiting agent 1,500 mg/kg food (1,500 ppm) or less than 500 mg/kg (500 ppm) as residual SO_2, in dried vegetables and 1,000 mg/kg (1,000 ppm) as residual SO_2, in dried fruits (Codex, 2014b). Thus, the 500 ppm sodium metabisulphite dose applied for 10 min that was used in this study for controlling browning reaction of dried konjac slices was complying with GSFA

regulations. Moreover, sulphite content was decreasing after drying the treated konjac slices at 50 ºC for 6 hours. In addition, dried konjac slices, which were used in this study, were not ready-to-eat products but were destined to be used in further processes for producing konjac glucomannan flour i.e. separation and extraction process including drying process. Thus, the residual sulphite content should be less than 500 ppm which is a safe level for consumers in the final products. The residual sulphite content in konjac glucomannan flour will be reported in the next subsequent sections.

When comparing the ability of each anti-browning agent to inhibit the browning reaction of konjac dried slices, the results show that the average whiteness indexes differed significantly (p<0.05) between treatments (Figure 4.2).

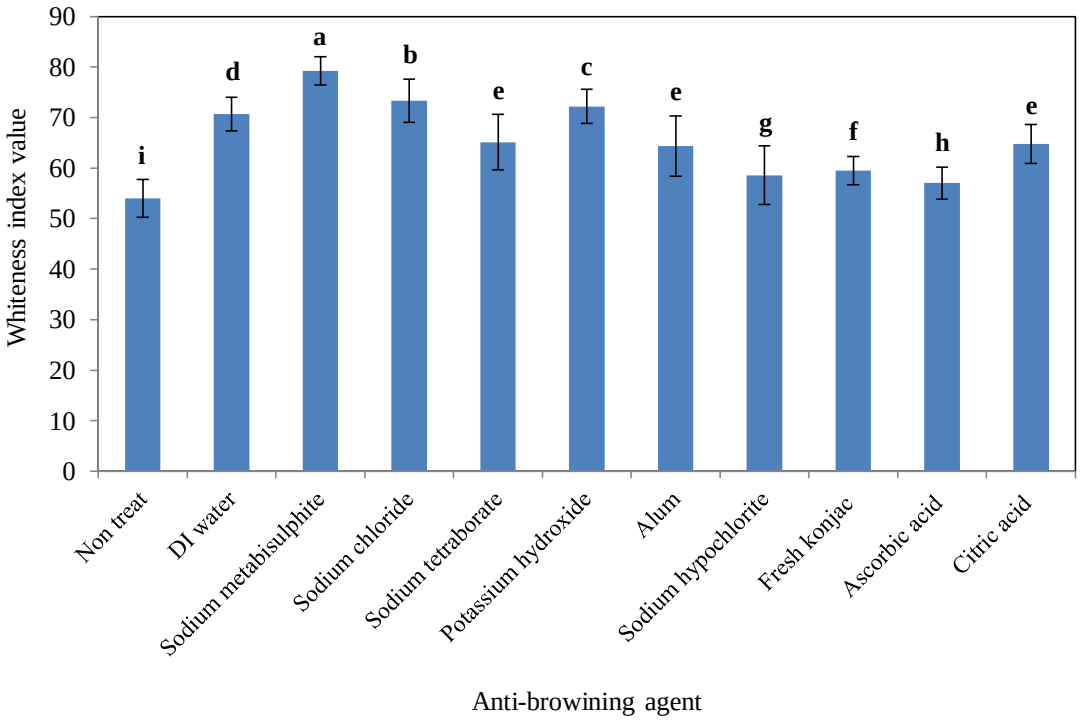


Figure 4. 2 Effect of anti-browning agents on the whiteness index of dried konjac slices. Significantly different treatments are indicated by different letters on the bar graph as per results of Duncan's multiple range tests ($p \leq 0.05$).

Among the anti-browning agents, sodium metabisulphite was the most effective anti-browning agent, while ascorbic acid exhibited the least inhibition activity for protection of browning of dried konjac slices. When comparing within the sulphur-free compounds group, sodium chloride markedly showed the ability to inhibit the browning reaction in dried konjac slices resulting from the use of sodium metabisulphite. Sodium chloride provided a whiteness index value in the range of 68-78. The treated samples were soaked in a 10,000 ppm solution of sodium chloride for 20 min and showed a performance comparable to sodium metabisulphite as shown in Figure 4.3.

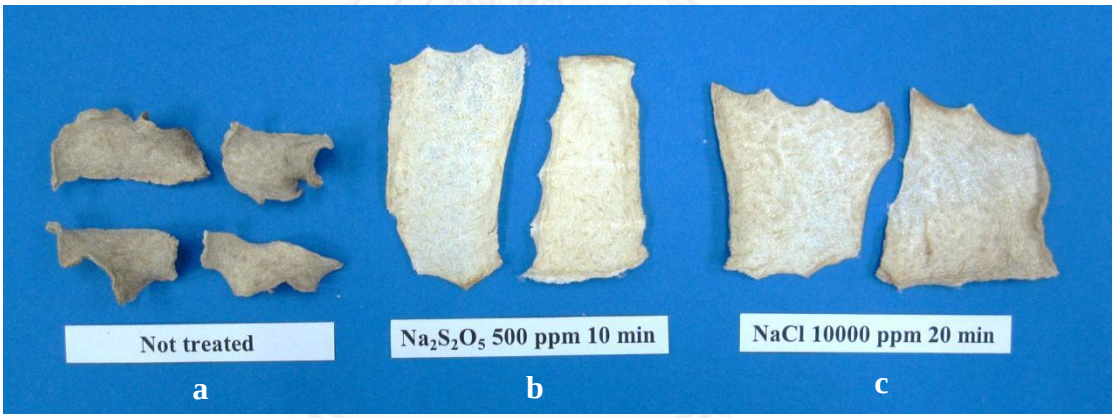


Figure 4. 3 Dried konjac slices after treatment with (b) 500 ppm sodium metabisulphite for 10 min and (c) 10,000 ppm sodium chloride for 20 min, compared to (a) non-treated samples.

Inorganic halides are well-known inhibitors of polyphenol oxidases (Vámos-Vigyázó, 1981). Janovitz-Klapp et al. (1990) determined that sodium fluoride was the most potent inhibitor of apple polyphenol oxidase, followed by sodium chloride, sodium bromide, and sodium iodide. Polyphenol oxidase activity was also observed to decrease with increasing concentrations of sodium chloride for peach (Luh & Phithakpol, 1972) and eggplant and avocado (Knapp, 1965). In addition, Polyphenol

activation by sodium chloride was also described for Fuji apple (Fan, Wang, & Zou, 2005) and Golden Delicious apple (Pizzocaro, Torreggini, & Gilardi, 1993). They found that sodium chloride inhibited polyphenol oxidase by enzyme conformational changes, or altered protein association/dissociation due to modified ionic strength. In practice, sodium chloride and calcium chloride at concentrations ranging between 2 and 4 percent (w/v) are the most commonly used chemicals for the inhibition of browning in many food industries (Steiner & Rieth, 1989).

In conclusion, sodium metabisulphite at 500 ppm and soaking time of 10 min, was the most effective treatment to retard browning reaction. As for sulphur free compounds, sodium chloride at 10,000 ppm and soaking time of 20 min, showed a performance comparable to sodium metabisulphite. Thus, soaking konjac slices in sodium chloride solution before drying can be an alternative way to replace the use of sulphiting agents. However, 500 ppm of sodium metabisulphite for 10 min will be used to prevent browning reaction in this study due to shorter soaking time and higher whiteness index.

4.3 Effects of anti-swelling agents on physic-chemical properties of KGM flour in wet extraction process

After fresh konjac slices were subjected to the most suitable anti-browning treatment as described in section 4.2, they were dried down to 5-8% (d.b.) moisture content and ground into powder. The impurities were eliminate by sifting the powder through a sieve with 125 μm openings (see the processing step in Appendix B, Figure B-2). This separation is based on the difference of size and weight between glucomannan and other impurities such as starch and cellulose. Glucomannan granule

is harder, heavier and larger than starch granules and cellulose (Zhao et al., 2010). Therefore starch and cellulose can be partially separated from glucomannan granules by sifting or air classification. However, there are still have some minor impurities in konjac flour such as proteins, lipids, starch and cellulose. Some of them can adhere tightly to the glucomannan granules and cannot be removed by the sifting or air classification (Shimizu & Shimahara, 1984; Tatirat & Charoenrein, 2011). Hence, further extraction is an important means to remove these impurities. In this study, two anti-swelling agents namely sodium tetraborate (borax) (0.5 - 3.0%w/w) and ethanol (10-95%w/w) were used. The effects of anti-swelling agent on some physicochemical properties of KGM flour are discussed as follows:

4.3.1 Extraction yield

The effects of anti-swelling agents on extraction yield of KGM flour, are shown in Figure 4.7. It appears that the use of ethanol in all concentrations gave a higher extraction yield than the use of sodium tetraborate. The highest percentage of extraction (94.1% extraction yield) was obtained using 70-95% ethanol concentration, see Figure 4.4 (show the raw data in Appendix C, Table C-4).

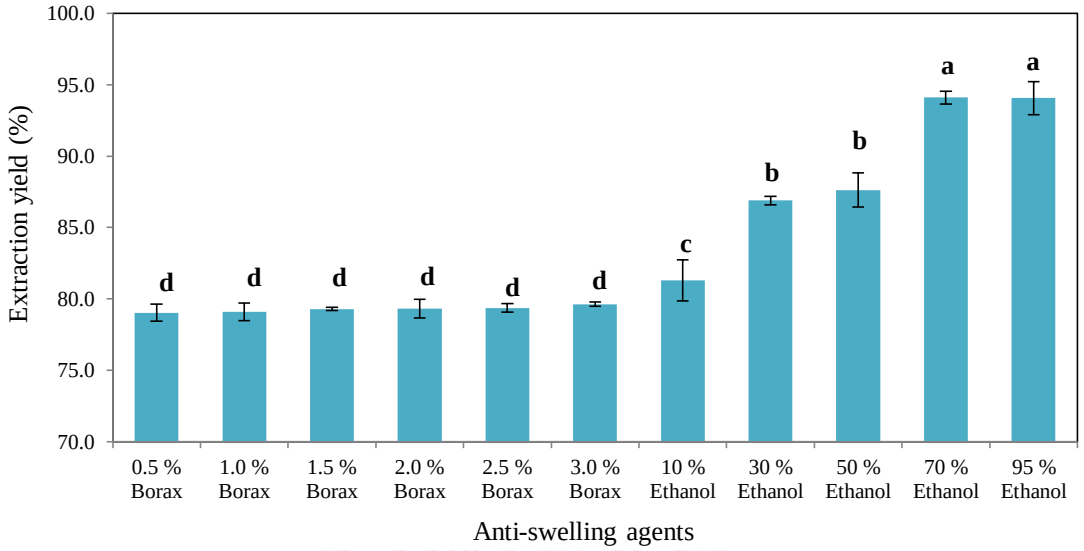


Figure 4. 4 Effect of anti-swelling agents on extraction yield (%) of KGM flour.
Borax = Sodium tetraborate
Different letters indicate significant differences ($p \leq 0.05$) between treatments.

In general, polysaccharide does not dissolve or swell in organic solvents such as methanol, ethanol, propanol, acetone and 5% ethyl acetate-modified ethanol (Shimizu & Shimahara, 1973). Therefore, glucomannan as well as starch and cellulose can be precipitated in organic solvents. Moreover, a higher concentration of organic solvent can precipitate more polysaccharide. When comparing the effects of the extraction using sodium tetraborate without the lower range of concentrations varying between 0.5 and 3.0% (w/w), the extraction yields (79.0 – 79.6%) (Figure 4.4) (show the raw data in Appendix C, Table C-4) were not significantly different ($p>0.05$). However, the extraction yield tends to increase with increasing concentration of sodium tetraborate (borax).

4.3.2 Water uptake

Water uptake follows an opposite direction to the extraction yield. When the concentration of anti-swelling agent is increased, the extraction yield is increased while percentage of water uptake decreases because less water is absorbed on konjac glucomannan granules, especially when using ethanol as anti-swelling agent (Figure 4.5) (show the raw data in Appendix C, Table C-4). When comparing the effect of the extraction by using sodium tetraborate in all concentration varying between 0.5 and 3.0% (w/w), the percentage of water uptake was not significantly different (p>0.05). The concentrations of sodium tetraborate used in this study still not enough to prevent swelling of KGM granules when contact with extraction medium. Using ethanol in the lower concentration (10 % ethanol), showed the same phenomenal as using sodium tetraborate. However, the percentage of water uptake tends to decrease when the ethanol concentration is increased.

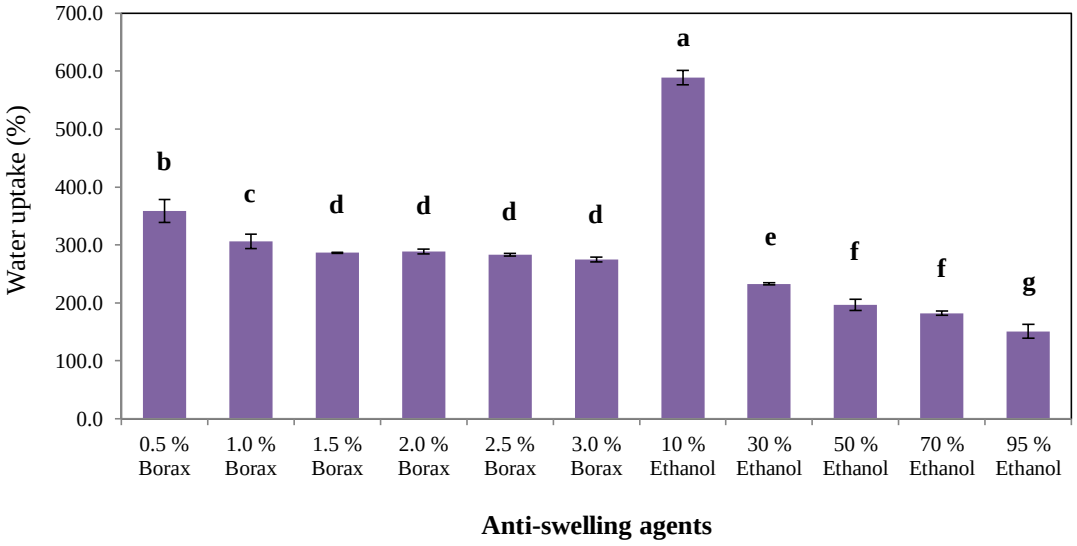


Figure 4. 5 Effect of anti-swelling agents on water uptake (%) of KGM flour.

Borax = Sodium tetraborate

Different letters indicate significant differences (p≤0.05) between treatments.

When comparing the effect of the extraction when using sodium tetraborate within the range of concentrations varying between 0.5 and 3.0% (w/w), the magnitude of water uptake was not markedly different (p>0.05). The concentrations of sodium tetraborate used in this study were not sufficient to prevent swelling of KGM molecules when contact with extraction medium. Using increasing ethanol concentrations showed the same decreasing trend as using increasing concentrations of sodium tetraborate.

4.3.3 Whiteness index

Colour of KGM flour is a very important criterion for consumers, as it should be a white powder. Whiteness index values of KGM flour after wet extraction and freeze drying process are shown in Figure 4.6. The results showed that both anti-swelling agents gave higher whiteness index value than the control sample since more impurities can be removed during wet extraction with anti-swelling agents. Using a higher concentration of ethanol tends to lower the whiteness index value of KGM flour. This may be caused by the fact that a high concentration of organic solvent can damage plant tissue and result in appearance of additional impurities. In contrast, the use of sodium tetraborate within the proposed range of concentrations tends to increase the whiteness index value when its concentration increases but there is no significant difference (p > 0.05) between 2-3%w/w levels. However, this technique is not sufficient to improve colour of KGM flour when compared to commercial sample that has the whiteness index value of 88.8 (show the raw data in Appendix C, Table C-4).

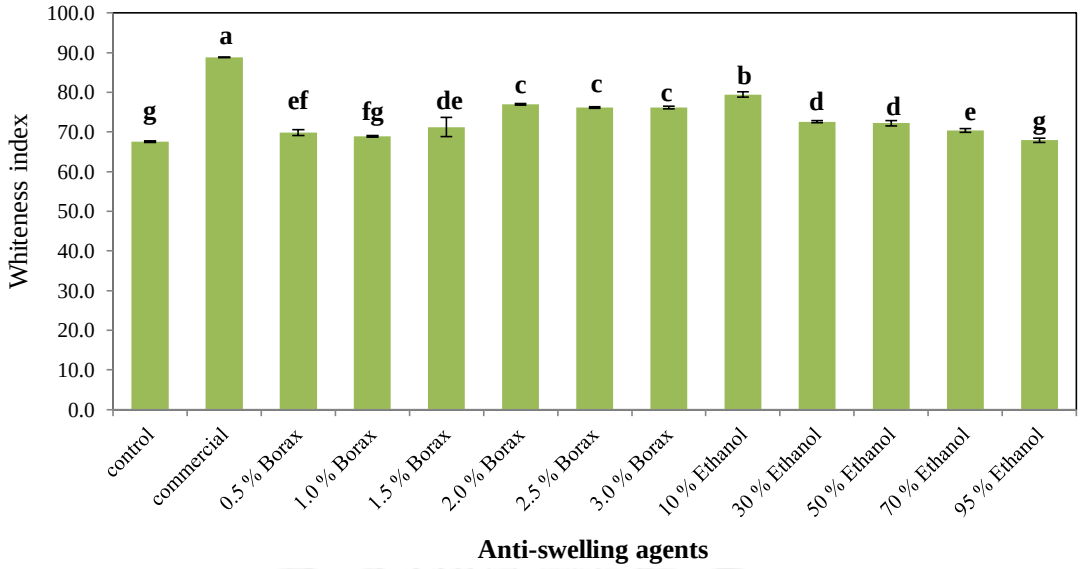


Figure 4. 6 Effect of anti-swelling agents on whiteness index value of KGM flour.
Borax = Sodium tetraborate
Different letters indicate significant differences ($p \leq 0.05$) between treatments.

4.3.4 KGM content

The KGM content of all samples is shown in Figure 4.7 (show the raw data in Appendix C, Table C-4). The results indicate that using sodium tetraborate tends to provide higher KGM content in the extracted samples than using ethanol in the extraction process. This is a result from the formation of network structure of konjac molecular chain and borate anion. These effects can prevent the loss of glucomannan during the extraction process. However, using ethanol at concentrations of 30 and 50% (79.9 $\pm$ 2.4 and 79.3 $\pm$ 2.0 % KGM) was found to be as effective as using 3% sodium tetraborate (81.7 $\pm$ 1.1 % KGM) in extraction process, since they show no significant difference ($p > 0.05$) between them. In addition, KGM content of all samples is still lower than the commercial sample (94.9 $\pm$ 3.2 % KGM). This indicates that the use of anti-swelling agent improves the efficiency of KGM

purification but it is still not powerful enough and thus a further purification step is required. This result agrees with Fang and Wu (2004); Shimizu and Shimahara (1973); Zhao et al. (2010); and Chua et al. (2012) who reported that using 85, 80, 65, and 50 % ethanol, respectively are the most suitable condition to remove the small impurities such as ash and calcium oxalate which adhered on the surface of KGM granules. Therefore, the glucomannan granules could be better purified resulting in a higher glucomannan content.

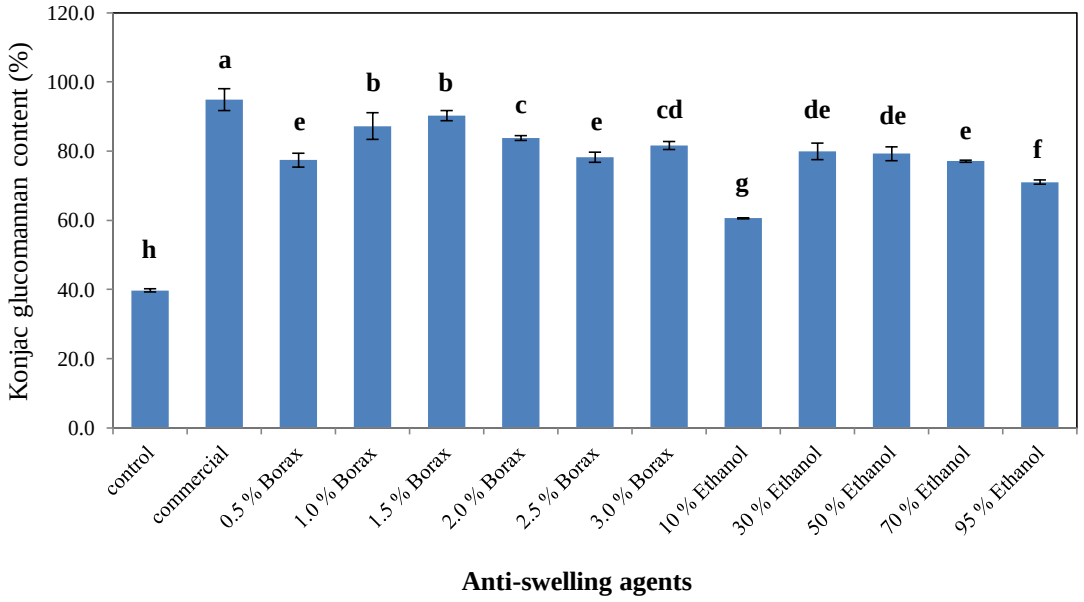


Figure 4. 7 Effect of anti-swelling agents on KGM content in KGM flour.
Borax = Sodium tetraborate
Different letters indicate significant differences ($p \leq 0.05$) between treatments.

4.3.5 Apparent viscosity

The viscosity of 1% (w/w) KGM solution from all samples of KGM flour after wet extraction and freeze drying is shown in Figure 4.8. The result indicated that sodium tetraborate tended to increase the viscosity of KGM solution

when increasing KGM content (show the raw data in Appendix C, Table C-4). It also gives higher viscosity than the commercial sample when using at least 1% sodium tetraborate. This is because of the tetrahedron conformation of borate anion could coordinate with KGM molecular chain easily to form a network structure (Gao, Guo, Wu, & Wang, 2008; Jian, Zeng, Xiong, & Pang, 2011; Li, Pang, & Liao, 2004; Li, Wang, & Sun, 2003). This configuration can obstruct the flow of KGM solution and increases the viscosity. However, this phenomenon is not occurred in samples extracted by ethanol. The viscosity of KGM solution obtained from samples extracted by ethanol showed the lower value in comparison with samples extracted by sodium tetraborate. In addition, it is seen that the viscosity of KGM solution tended to be related with the amount of glucomannan in KGM flour. If the amount of glucomannan in KGM flour is higher than 80%, the viscosity will be increase which caused by more glucomannan content and less residual impurities. However, the commercial sample showed the highest glucomannan content but not the highest viscosity. This result indicated that not only glucomamnan content but also the other factor such as extraction method, drying method, and even species of konjac raw material that affect to the viscosity of KGM solution.

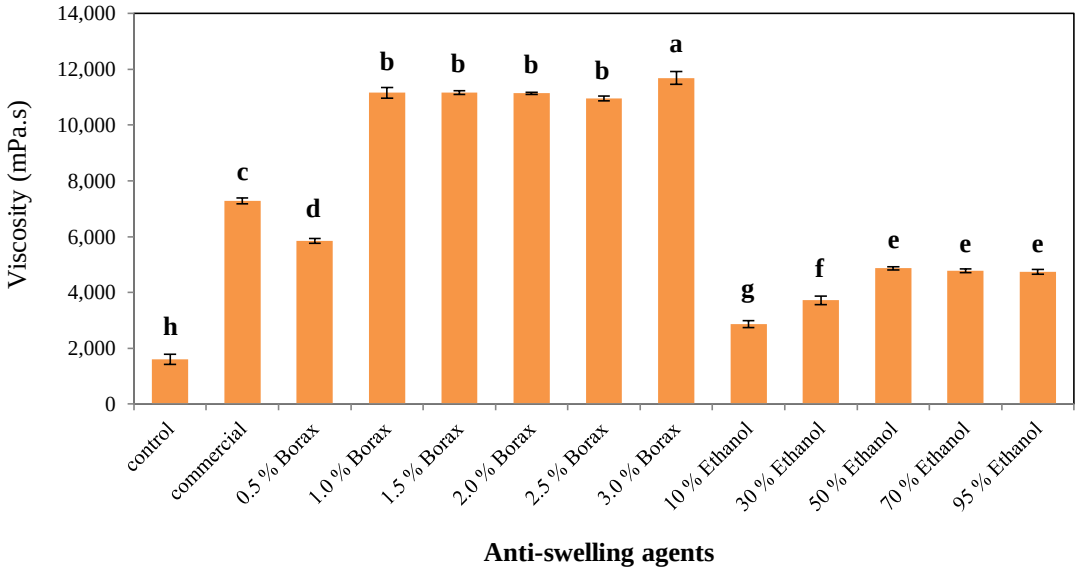


Figure 4. 8 Effect of anti-swelling agents on apparent viscosity (mPa·s) of 1% KGM solution. Borax = Sodium tetraborate.

Different letters indicate significant differences ($p \leq 0.05$) between treatments.

4.3.6 Structural characterisation

Morphology of KGM flour was studied using an image analyzer (Nikon SMZ1000, Japan). Figure 4.9 shows that KGM flour prepared by drying and grinding into powder without the extraction process, is brown and has rough surfaces due to the impurities outside (see Figure 4.9 (a)). Meanwhile the samples that were extracted using 50% ethanol as anti-swelling agents have a better appearance with the oval shape and translucent surfaces. It can also be noticed that the contamination is negligible as shown in Figure 4.9 (b). When considering the samples that were extracted by using 1% sodium tetraborate as anti-swelling agents, it can be seen that the samples have a better appearance than the samples that were extracted with 50% ethanol, with oval shape, smooth and transparent surfaces and less contamination as shown in Figure 4.9 (c). The samples that were extracted using sodium tetraborate

show a better performance of this treatment in eliminating the impurities than using ethanol in extraction process. This result also agrees with the results from the whiteness index value, KGM content and viscosity which show that using sodium tetraborate as anti-swelling agent can provide better results than when using ethanol. However, the results also show that the commercial sample (see Figure 4.9) has the best appearance with transparent surfaces, smaller particle size even at the same magnification and almost no impurities but they show irregular shapes due to the type of grinder used in the industry.

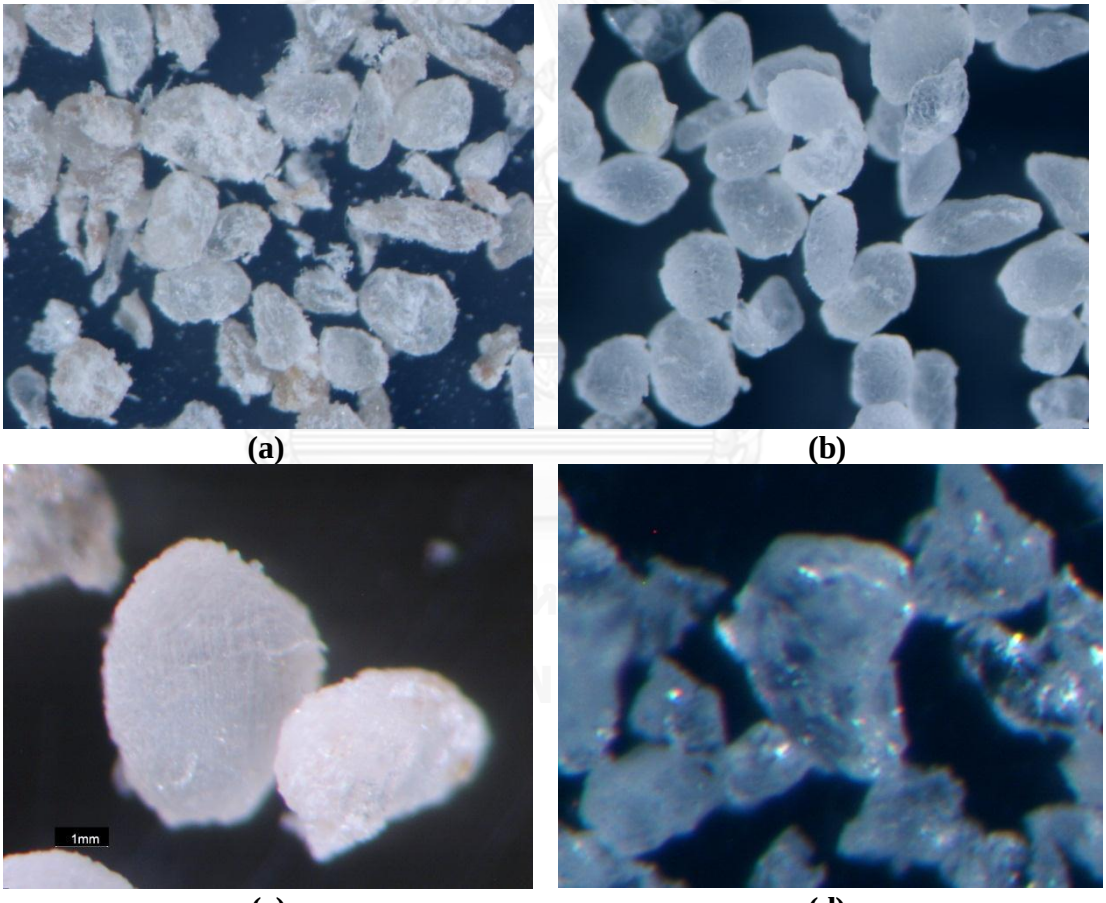


Figure 4. 9 Image analyzer photographs showing morphology of KGM flour. (a) before extraction at 45× magnification; (b) after extraction using 50% ethanol at 45× magnification (c) after extraction using 1% sodium tetraborate at 120× magnification and (d) commercial KGM flour at 120× magnification.

The ability of wet extraction process using ethanol to remove impurities and calcium oxalate crystals from KGM flour is shown in Figure 4.10 and 4.11. These results show the morphology of KGM flour before and after extraction process with ethanol from Scanning Electron Microscope. It can be clearly seen the impurities (Figure 4.10) and the needle-like of calcium oxalate crystals on the surface of KGM granule (Figure 4.11) are reduced after extraction process. This means that extraction process can remove some small impurities such as starch and calcium oxalate which adhered on the surface of KGM granules.

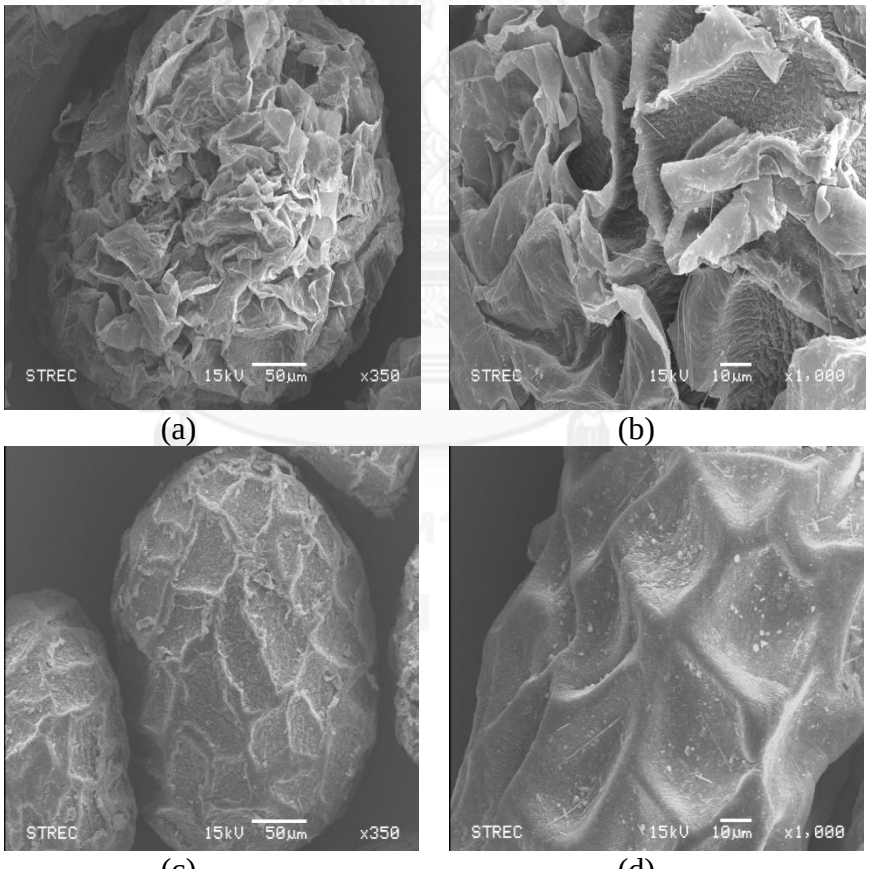
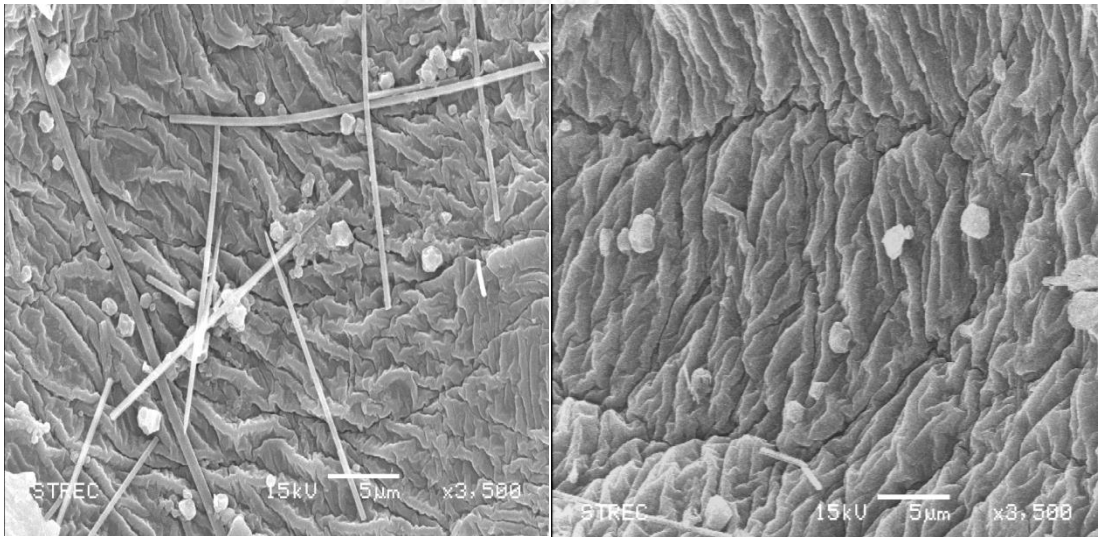


Figure 4. 10 Scanning electron micrographs of KGM flour.
(a) before extraction at 350× magnification (b) before extraction at 1000× magnification (c) after extraction with 50% ethanol at 350× magnification (d) after extraction with 50% ethanol at 1000× magnification.

The results agree with Takigami et al. (1997) who also observed the needle-like crystals in the parenchyma around the konjac cells and confirms the results by elemental analysis using an energy-dispersive X-ray spectrometer (EDX). Shimizu and Shimahara (1973) reported that using 80% ethanol can remove some small impurities such as ash and calcium oxalate which adhered on the surface of KGM granules. Therefore, the glucomannan granules are more purified and present higher glucomannan content. This fact agree with Takigami (2000) who also claimed that the ash content, total protein and total lipid were significantly lower in the purified konjac flour which had been extracted with ethanol than in crude konjac flour.



(a) **(b)**

Figure 4. 11 Scanning electron micrographs of KGM flour.
(a) before extraction (b) after extraction with 50% ethanol at 3500× magnification.

In conclusion, the kind of anti-swelling agents are the main factor that affect to the qualities of KGM flour such as yield of extraction, water uptake, whiteness index value, glucomannan content, and also viscosity of KGM solution. While different concentrations of ethanol affect to all of the properties of KGM flour, the different concentration of sodium tetraborate between 0.5 – 3.0 % did not significantly affect to the yield of extraction, water uptake, and viscosity of KGM solution ($p \leq 0.05$) but showed the significantly difference for the whiteness index value and glucomannan content. Yet, the overall of whiteness index value, glucomannan content, viscosity and morphology of KGM flour samples obtained when using sodium tetraborate were higher and better appearance than in ethanol extracted samples. Since using sodium tetraborate is prohibited in food products but KGM flour that is extracted using sodium tetraborate can still be used in other industrial applications such as paints and waste water treatment. In contrast, using ethanol can provide higher percentage of extraction yield and lower percentage of water uptake than using sodium tetraborate. The result showed that using ethanol at a concentration greater than 50% can reduce the water uptake and improve the yield of extraction. In generally, ethanol shows the ability to precipitate of polysaccharides. Especially, at the higher concentrations of ethanol, the more polysaccharides are precipitated. However, not only polysaccharides were precipitated, but also other impurities that may precipitate simultaneously. As a result lower in glucomannan content and viscosity of KGM solution when using 70-95% of ethanol. Thus, 50% Ethanol was selected to use as anti-swelling agent for further experiment.

4.4 Drying experiment

4.4.1 Hot air drying

The drying behavior of samples subjected to hot air drying treatments (50–80 °C) is shown in Figure 4.12 (a) and (b) which exhibits the change in the moisture content (% d.b.) and moisture ratio of KGM flour, respectively with time under different drying conditions.

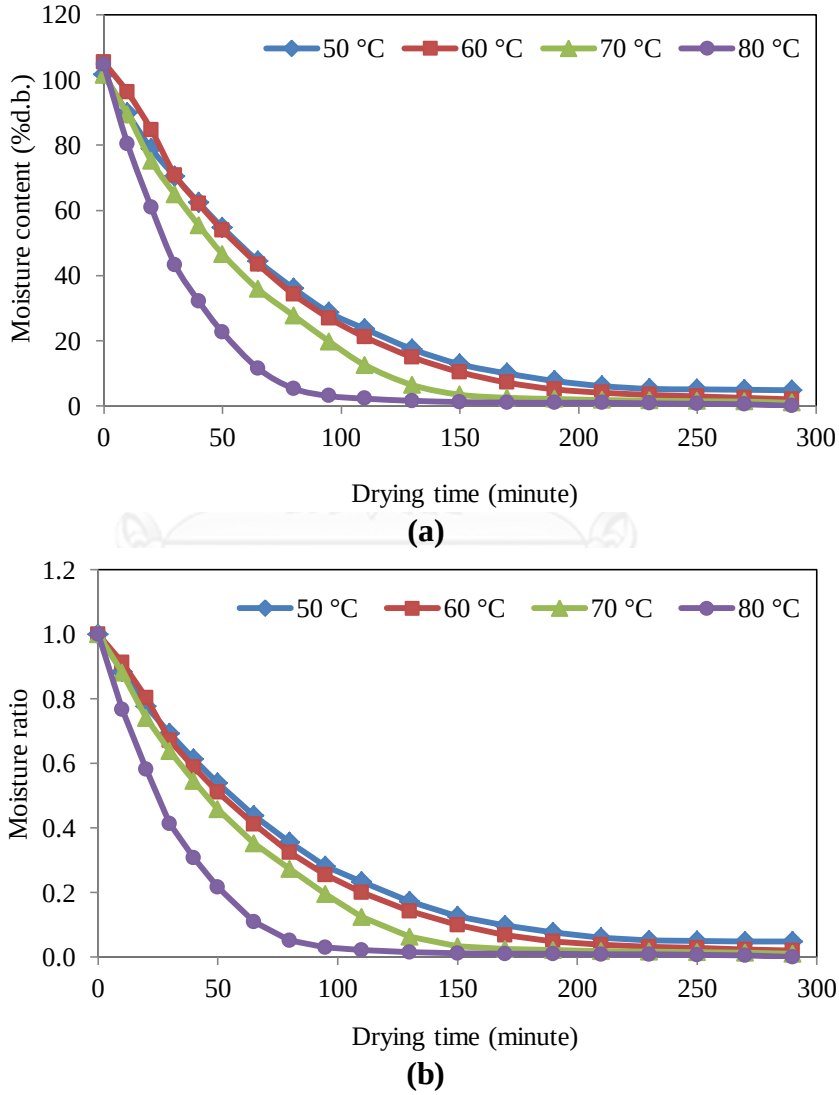


Figure 4. 12 The change of (a) the moisture content (% d.b.) and (b) the moisture ratio of KGM flour versus drying time during hot air drying at different temperatures.

Figure 4.12 indicates the time required to reduce moisture content of semi-dried KGM flour from the initial moisture content 103.34±2.05% (d.b., moisture ratio=1) to target moisture content 5-6% (d.b., moisture ratio=0.05) when drying using hot air at 50, 60, 70, and 80 ºC. The drying behavior for hot air drying process of semi-dried KGM flour was determined from the mass loss in samples of known initial moisture content during drying time. The product temperature and whiteness index value of KGM flour were monitored during drying process. The results in Figure 4.12 (a) and (b) (see the detail in appendix D) indicates that drying KGM flour to the same moisture content level of 5-6 % (d.b.) or moisture ratio at 0.05 using hot air drying at 50, 60, 70, and 80 °C needed 250, 190, 135, and 80 minutes, respectively. Table 4.2 summarises the effects of the drying treatments using hot air dryer on the product temperature of the KGM flour.

Table 4. 2 Effects of drying conditions on moisture content, drying time, and temperature of KGM flour.

Drying air temperature (°C)	Moisture content (% d.b.)		Moisture ratio (M_t/M_i)* after drying	Time to reduce moisture content to 5-6% d.b. (min)	Temperature of KGM flour (°C)
	Before drying	After drying			
50	101.60	5.09	0.05	250	48
60	105.44	5.09	0.05	190	57
70	101.56	5.67	0.05	135	68
80	104.76	5.32	0.05	80	77

*M_t is the moisture on a percentage of dry basis (% d.b.) at any time *t* during drying
M_i is the initial moisture content (% d.b.)

4.4.2 Multistage hot air drying

Using data shown in the hot air drying curves in the previous section (4.4.1) helps developing a strategy for multistage drying process aiming at reducing drying time and improving quality of KGM flour. Since it can be clearly noticed that the colour of KGM flour changed from yellowish to white when increasing the drying time until *t* minutes as shown in Table 4.3 indicates that the colour of KGM flour will change once the moisture content was reduced from about 100% (d.b.) to about 40% (d.b.) or the moisture ratio was reduced from 1.00 to 0.41, respectively and temperature of KGM flour reached in average of 38.25 ºC. After this period, the colour of KGM flour changed only slightly during drying process.

Table 4. 3 Time to change the colour of konjac flour from yellowish to white during hot air drying.

Drying temperature (ºC)	Drying time *(t)* for colour change of KGM flour (min)	Moisture content of KGM flour at time *t* (% d.b.)	Moisture ratio (M_t/M_i)* of KGM flour at time *t*	Temperature of KGM flour at time *t* (ºC)
50	70	41.64	0.41	32.0
60	65	43.43	0.41	37.5
70	55	42.98	0.42	39.0
80	30	43.27	0.41	44.5

*M_t is the moisture on a percentage of dry basis (% d.b.) at any time *t* during drying
M_i is the initial moisture content (% d.b.)

Based on this observation, the multistage can be developed by choosing a two stage drying process. Drying process can be divided into two periods including before and after the change of colour. The possible drying conditions are presented in Table 4.4.

Table 4. 4 The possible drying conditions for developing the multistage hot air drying strategy

Condition	Drying temperature (°C)	Reducing moisture ratio	Drying time (min)	Total drying time to reduce moisture ratio to 0.05 (min)
1	50	1.00 --->0.05	250	250
2	60	1.00 --->0.05	190	190
3	70	1.00 --->0.05	135	135
4	80	1.00 --->0.05	80	80
5	50 + 60	(50°C) 1.00 --->0.41	70	195
		(60°C) 0.41 --->0.05	125	
6	50 + 70	(50°C) 1.00 --->0.41	70	150
		(70°C) 0.42 --->0.05	80	
7	50 + 80	(50°C) 1.00 --->0.41	70	120
		(80°C) 0.41 --->0.05	50	
8	60 + 70	(60°C) 1.00 --->0.41	65	145
		(70°C) 0.42 --->0.05	80	
9	60 + 80	(60°C) 1.00 --->0.41	65	115
		(80°C) 0.41 --->0.05	50	
10	70 + 80	(70°C) 1.00 --->0.42	55	105
		(80°C) 0.41 --->0.05	50	

* Reduction of moisture ratio (please refer to the drying characteristics in Table C-6 in Appendix C)

The comparison of drying time for hot air drying and multistage drying is shown in Table 4.4 show that using multistage drying process under most conditions can shorten drying time versus conventional hot air drying at constant drying temperature. Drying at 50 ºC air temperature requires the longest time (250 min) while when drying at 80 ºC, the process takes the shortest time (80 min) to reduce moisture content of semi-dried KGM flour to target moisture content of 5-6% (d.b., moisture ratio = 0.05). The results also indicate that increasing the drying

temperature results in reduction of drying time. The multistage drying at lower drying temperature and then increasing drying temperature in the second period of drying can significantly shorten the drying time. However, only three conditions: (50 ºC+80 ºC), (60 ºC+80 ºC), and (70 ºC+80 ºC) were selected for the experiments on the effects of drying method on physicochemical properties of KGM flour due to a significant reduction in drying time.

4.4.3 Freeze drying

The drying times required for freeze drying are considerably longer in comparison with conventional hot air drying. The results (data not shown) indicate that the total drying time for drying KGM flour by using freeze dryer was about 9 hours to reduce moisture content to the final moisture content of 5-6 % (d.b.) or moisture ratio of 0.05.

4.4.4 Microwave-vacuum drying

4.4.4.1 Pre-drying step

The semi-dried KGM flour obtained from the extraction process still contains residual ethanol and may cause damage to the microwave-vacuum dryer. Thus, pre-drying step was required to eliminate the ethanol residue from the samples before drying by using microwave-vacuum dryer. Shade dry at room temperature (30 °C), air drying at 40 °C and 50 °C were trialled to find the most suitable condition for the removal of ethanol from the KGM sample. The residual ethanol was checked by using an electronic nose (e-nose) instrument (see more detail in Appendix A, A.4) with chemical sensor array technique (NANOTEC, Thailand). The result showed that

shade dry at room temperature (30 °C), air drying at 40 °C and 50 °C needed 180, 50, and 40 minutes, respectively to eliminate the ethanol residue (Table A-7, Appendix A). Therefore, the most suitable pre-drying condition is drying at 40 °C for 50 min because this condition is mild and does not take too long time. While ethanol was eliminated from the KGM sample, some moisture in the sample was removed as well. The results show that during the ethanol removal process, this process will cause the moisture of the KGM sample to decrease by about 20% (e.g. from 120% (d.b.) to 100% (d.b.)).

4.4.4.2 Microwave-vacuum drying

The drying curves of samples subjected to microwave-vacuum drying are shown in Figure 4.13 (a) and (b). They exhibit the change in the moisture content (% d.b.) and the moisture ratio of KGM flour, respectively with time under different drying conditions. As expected, the higher percentage of weight loss of moisture content occurs in the early stages of drying and the moisture content decreased considerably with increasing drying time. The results in Figure 4.18 also indicate that drying KGM flour using microwave vacuum at 960, 1200, and 1440 W power level needed 10, 8.5, and 7.5 minutes, respectively for reducing moisture content of KGM flour from 100.94$\pm$0.23% (d.b., moisture ratio=1) to 5-6% (d.b., MR=0.05). The target final moisture content was obtained by recording weight of the KGM flour during drying run.

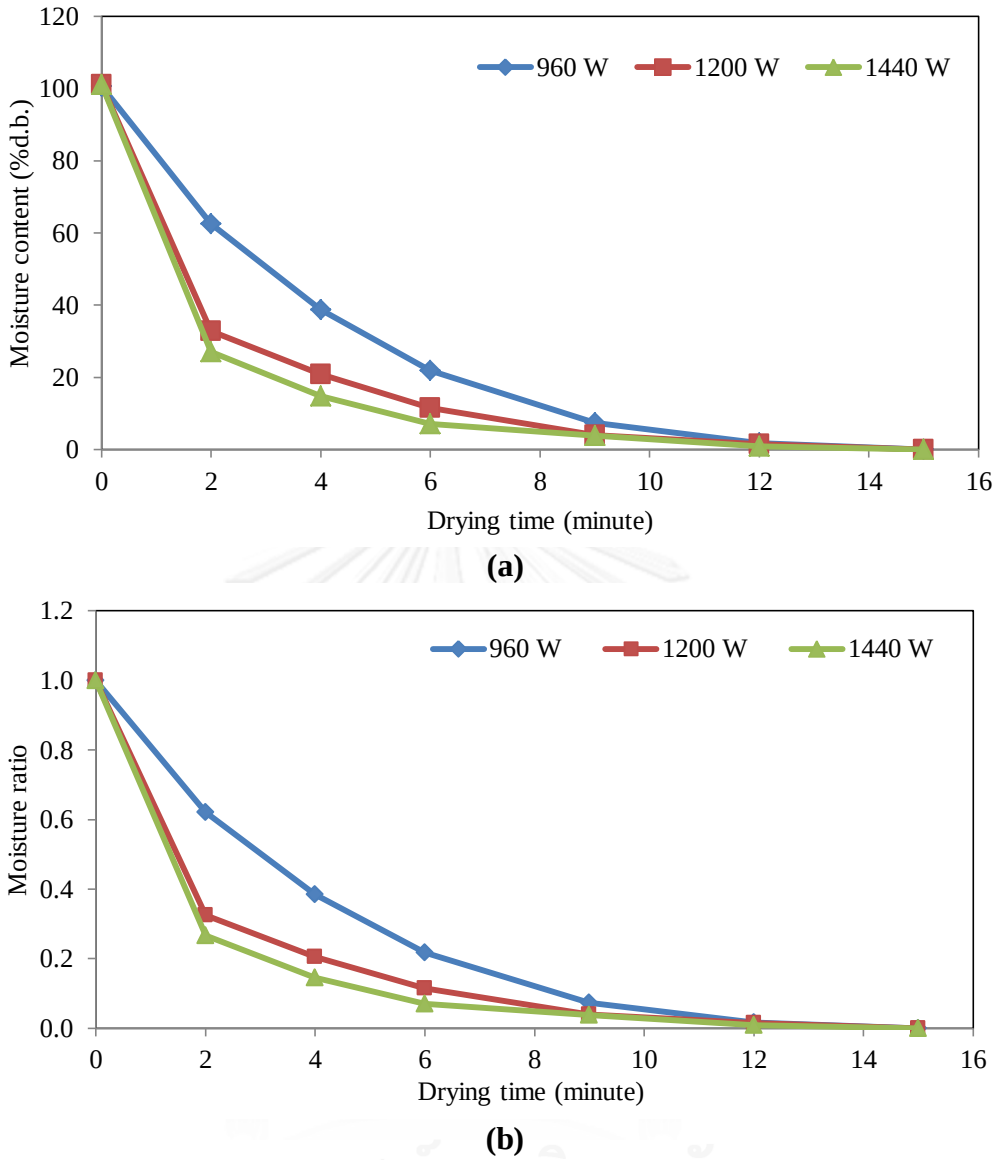


Figure 4. 13 The change of (a) the moisture content (% d.b.) and (b) the moisture ratio of KGM flour versus drying time during microwave-vacuum drying at different microwave power level.

The results indicate that using microwave-vacuum drying needed a much shorter drying time in comparison with hot air drying and multistage hot air drying in every treatment in order to reduce moisture content to the same target moisture level. This is because of microwave spectrum which was absorbed by water

molecules, caused polarized molecules in the KGM flour to rotate, vibrate and build up enormous thermal energy in a process known as dielectric heating. As a result, the product temperature was rapidly reaching the point of evaporation and the evaporating effect was enhanced under vacuum. Thus, water molecules dispersed in KGM matrix became vapour in all parts of the matrix and evaporated to outside in a very short time due to microwave vacuum system. Therefore, the advantage of this method consisted in significantly decreasing the drying time.

4.5 Effects of different drying methods on physical and chemical properties of purified KGM flour

This study compared the effects of hot air drying, multistage hot air drying, freeze drying, and microwave-vacuum drying on various physical quality attributes of KGM flour in term of water activity, Hunter colour value, whiteness index, bulk density, particle density, porosity, apparent viscosity, KGM content, and sulphur dioxide residues. Moreover, the structure characterization of KGM flour was also carried out using SEM, Image analyser, and FTIR. Once the drying curves were obtained from previous steps, the drying experiment was repeated and the required drying time to reach the final desired moisture content of about 5-6% (d.b.) determined. The final moisture content and product temperature are listed in Table 4.5. The residence time during drying of KGM flour from the initial moisture content of about 100% (d.b.) to about 5-6% (d.b.) was 10, 8.5, and 7.5 minutes for microwave vacuum at 960, 1200, and 1440 W power level; 250, 190, 135, and 80 minutes for hot air drying at 50, 60, 70, and 80 ºC; 120, 115, and 105 minutes for multistage hot air drying at 50+80 ºC, 60+80 ºC, and 70+80 ºC; and more than 540 minutes for freeze

drying at -47 ºC. It also can be seen from Table 4.5 that the final product temperature of KGM flour after drying by microwave vacuum drying are varied between 58-62 ºC while hot air drying and multistage hot air drying are varied between 48-79 ºC. The results show that water can be evaporated from the samples at low temperatures and very short time, less than 10 minutes, when using microwaves vacuum drying. Meanwhile, the product temperature of about 60 ºC is obtained when using hot air drying at 60 ºC which takes about 190 minutes to evaporate the same amount of water. However, the final product temperature of freeze dried sample showed below 0 ºC.

Table 4. 5 Effects of drying conditions on drying time, and temperature of KGM flour at the same final moisture content 5-6% (d.b.).

Drying method	Drying condition	Drying time (min)	Final product temperature (°C)
Hot air drying	50 °C	250	48.50 ± 0.71
	60 °C	190	57.75 ± 0.35
	70 °C	135	68.25 ± 0.35
	80 °C	80	78.25 ± 0.35
Multistage hot drying	50+80 °C	120 (70, 50)	78.25 ± 0.35
	60+80 °C	115 (65, 50)	78.75 ± 0.35
	70+80 °C	105 (55, 50)	79.00 ± 0.71
Microwave-vacuum drying	960W	10	57.75 ± 0.35
	1200W	8.5	60.00 ± 0.71
	1440W	7.5	62.25 ± 1.06
Freeze drying	-47 °C, 4.6 Pa	540	Below 0 °C

Results are Mean ± SD (n = 3)

4.5.1 Water activity

Table 4.6 summarises the effects of the different drying techniques on moisture content and water activity of the KGM flour. Table 4.6 shows that since the final moisture content of KGM flour was determined to the same level of 5-6%, thus the moisture content of each samples were slightly different. Water activity of dried samples, has been considered as one of the most important quality factors for dried products especially for long term storage. Water activity is related to moisture content and responsible for biochemical reactions.

Table 4. 6 Moisture content and water activity of KGM flour after drying using different drying techniques under different conditions to the same final moisture content 5-6% (d.b.).

Drying method	Drying condition	Moisture content (% d.b.)	Water activity
Hot air drying	50 °C	$5.59^{ab} \pm 0.08$	$0.249^{b} + 0.008$
	60 °C	$5.62^{a} \pm 0.13$	$0.254^{b} + 0.009$
	70 °C	$5.35^{bc} \pm 0.15$	$0.220^{c} + 0.001$
	80 °C	$5.17^{c} \pm 0.16$	$0.193^{e} + 0.001$
Multistage hot air drying	50+80 °C	$5.31^{c} \pm 0.23$	$0.217^{c} + 0.005$
	60+80 °C	$5.27^{c} \pm 0.08$	$0.207^{d} + 0.002$
	70+80 °C	$5.22^{c} \pm 0.15$	$0.204^{d} + 0.001$
Microwave-vacuum drying	960 W	$5.15^{c} \pm 0.03$	$0.151^{fg} + 0.004$
	1200 W	$5.20^{c} \pm 0.04$	$0.155^{f} + 0.003$
	1440 W	$5.09^{c} \pm 0.05$	$0.144^{g} + 0.001$
Freeze drying	-47 °C, 4.6 Pa	$4.01^{d} \pm 0.07$	$0.129^{h} + 0.001$
Commercial	-	$5.81^{a} + 0.08$	$0.308^{a} + 0.005$

Results are Mean ± SD (n = 3). Letters a, b, c, … in the same column indicate a statistically significant difference at $p \leq 0.05$).

Available water can support the growth of bacteria, yeast and mould, which can affect the safety and quality of food. The values of water activity between 0.20 and 0.40 ensure the stability of the product against browning and hydrolytical reactions, lipid oxidation, auto-oxidation and enzymatic activity (Dirim & Çalışkan, 2012). The results in this experiment showed that water activity value of all samples was ranged from 0.129 to 0.308 which can be made safe to store and retard biochemical reaction. However, KGM flour obtained from hot air drying tend to show higher water activity than other samples since this drying technique may cause case-hardening and prevent water vapour moving from inside the sample and evaporate. During drying, after free water had been removed, the sample still contains some bound water. The microwave energy and also vacuum system can remove bound water more efficiently than in the conventional hot air drying process due to the absorbed energy of water molecules. In contrast, hot air drying may cause case-hardening and prevent water vapour from moving from inside the sample and evaporate. Therefore, lower water activity values can be obtained in microwave vacuum dried samples than in hot air dried ones even at the same final moisture level. This phenomenon was not found in the hot air dried samples. Similar effect of microwave power was found when drying mint leaves (Therdthai & Zhou, 2009) and green peas (Zielinska et al., 2013).

4.5.2 Hunter and whiteness index value measurement

Figure 4.14 and 4.15 show the Hunter values and whiteness index of the KGM flour before and after drying under different condition to the same final moisture content 5-6% (d.b., MR=0.05-0.06).

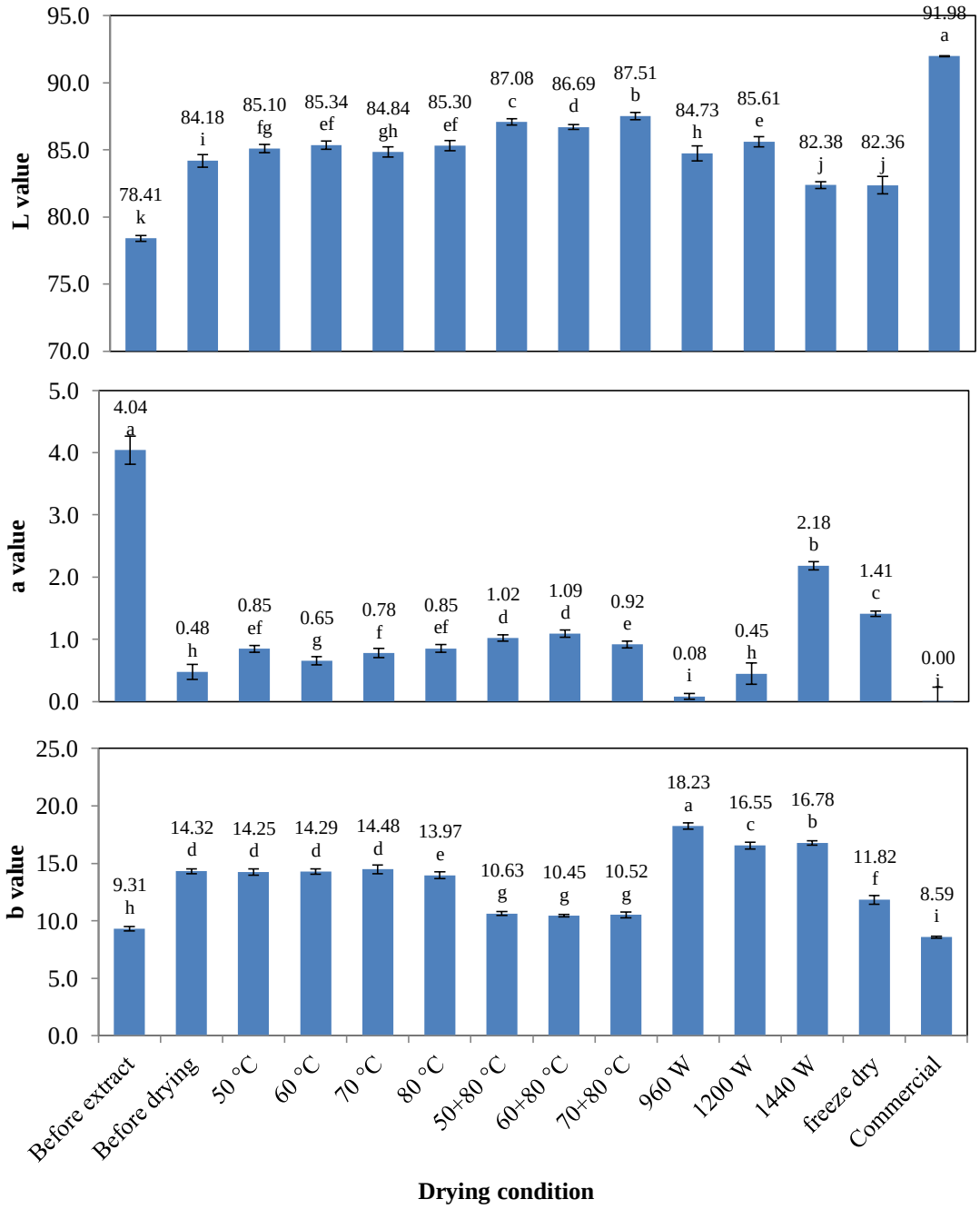


Figure 4. 14 Hunter values (L, a, b) of KGM flour after drying to the same final moisture content 5-6% (d.b.) using different drying techniques under different conditions.

Different letters indicate significant differences ($p \leq 0.05$).

The results indicate that the lightness (L-value), redness (positive a-value), yellowness (positive b-value), and whiteness index of the KGM flour before extraction were 78.41±0.23, 4.04±0.22, 9.31±0.19, 76.14±0.21, respectively while those results of the KGM flour before drying were 84.18±0.45, 0.48±0.12, 14.32±0.21, and 78.65±0.39, respectively (see in Table C-10 and C-11, Appendix C). This means that the extraction process can improve the whiteness of KGM flour since the impurities are removed. However, the overall lightness, redness, and yellowness of the KGM flour tended to increase after thermal drying process (e.g. hot air drying, multistage hot air drying and microwave vacuum drying) as a result of enzymatic browning reaction.

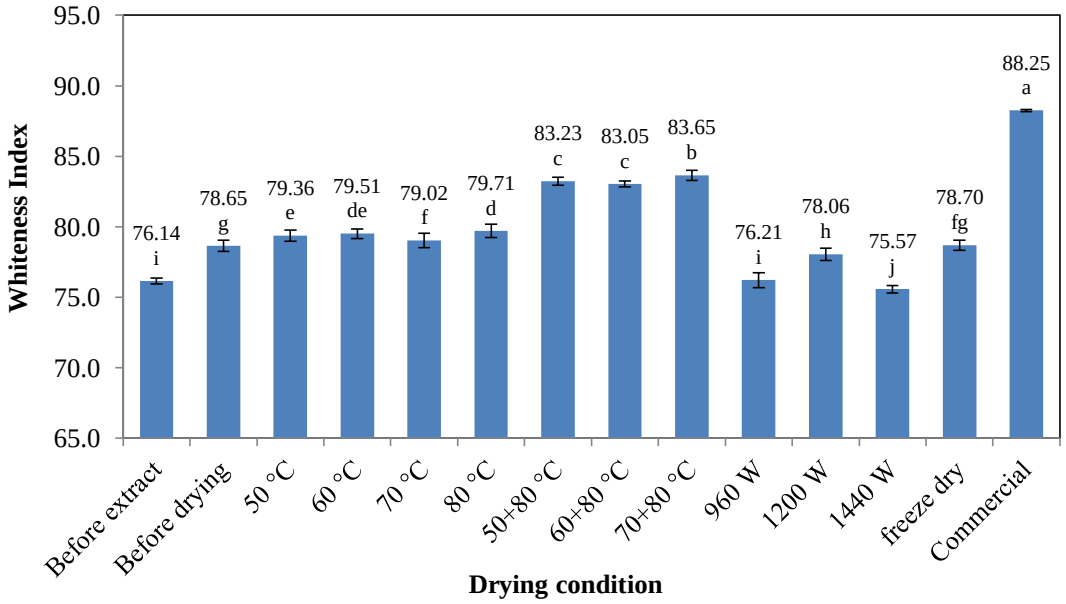


Figure 4. 15 Whiteness index value of KGM flour after drying to the same final moisture content 5-6% (d.b.) using different drying techniques under different conditions.

Different letters indicate significant differences ($p \leq 0.05$).

Enzymatic browning reaction is the discolouration that results when phenol compounds in food reacting with polyphenol oxidase (PPO, EC 1.10.3.1), in the presence of atmospheric oxygen and heat, to produce dark brown, black or red pigments (Walker & Ferrar, 1998). Non-enzymatic browning reaction is a chemical process that produces a brown colour in foods without the activity of enzymes. There are two main reactions of non-enzymatic browning, caramelisation (browning due to sugar-sugar reactions when heated at high temperatures) and the Maillard reaction, which results from reactions between carbonyl groups in reducing sugar and amino group in protein and its derivatives in the presence of water (Quayson & Ayernor, 2007).

For this research, enzymatic browning seems to be the problem in the early step of preparation which was controlled by sodium metabisulphite in extraction and purification steps. In contrast, non-enzymatic browning was not the cause of browning problem in this drying step. Since there are some minor compositions of monosaccharide, disaccharide, protein and lipid were found in KGM flour and the drying condition takes place under low temperature (not exceeding 80 °C) in which the caramelisation, Maillard reaction, or lipid oxidation would be very difficult to occur. Hence, it can be seen that the browning of KGM flour likely to be mainly due to the enzymatic browning reaction. Figure 4.15 and 4.16 show that the whiteness index value of KGM flour was varies according to the product temperature.

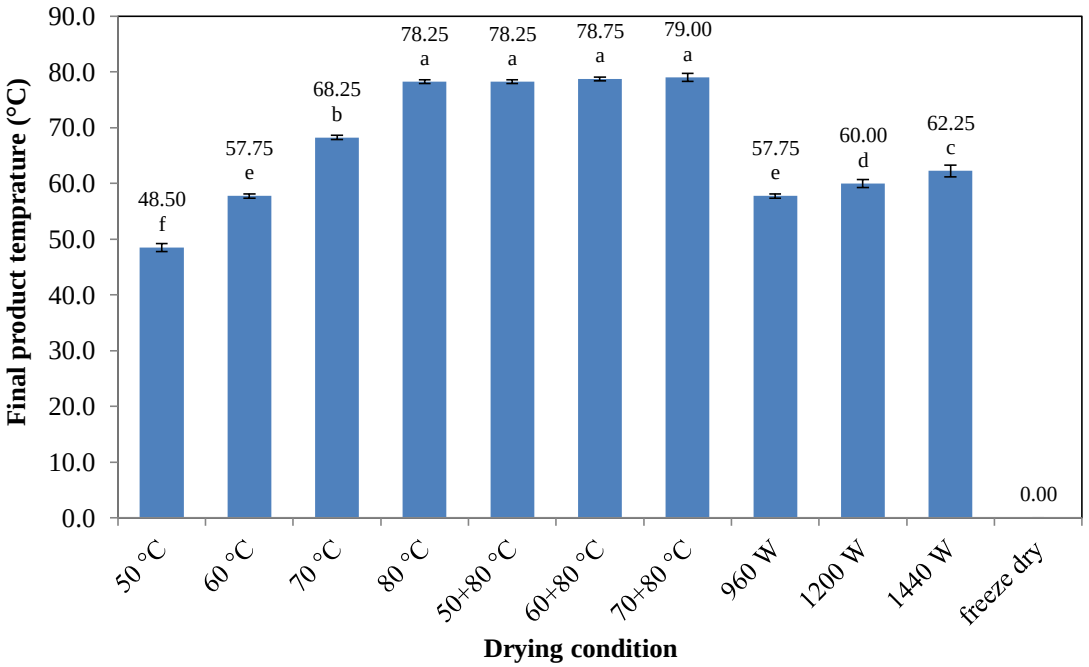


Figure 4. 16 Temperature of KGM flour after drying to the same final moisture content 5-6% (d.b.) using different drying techniques under different conditions. Different letters indicate significant differences ($p \leq 0.05$).

The result show that increasing the product temperature tends to increase the whiteness index of KGM flour. When comparing between drying methods, multistage drying, conventional hot air drying at constant drying temperature, microwave-vacuum drying, and freeze drying, the results show that multistage drying has significantly improved the whiteness index value of KGM flour. In contrast, the whiteness index value of freeze dried sample was lower than that of the other treatments.

In theory, the temperature higher than 50 ºC inactivate PPO activity in enzymatic browning reaction (Martinez & Whitaker, 1995) while the temperatures of 70–90 ºC destroyed their catalytic activity (Vámos-Vigyázó, 1981). The results in this experiment showed that when the final product temperature of KGM flour samples

reached 70-80 ºC, the whiteness index of KGM flour tended to higher than 78.50. Since the final product temperature of KGM flour were reached the level which destroyed the catalytic activity of PPO and inhibited the browning reaction. In contrast to that, the drying temperature which led to the product temperature below 60 ºC can only inactivate PPO activity in enzymatic browning reactions (Martinez & Whitaker, 1995), but PPO still remains intact. Thus, the browning is still visible in microwave-vacuum dried sample and freeze dried sample.

However it appears that the microwave-vacuum drying resulted in samples of KGM flour being slightly darker, less red, more yellow, and lower in whiteness index value. This indicates that there were other causes that affect the browning of microwave-vacuum dried sample besides the enzymatic browning. Such browning may be due to some overheated or burnt spots on the KGM particles of the microwave vacuum dried samples. This browning was more severe near the end of the drying period when the moisture level was low and less evaporative cooling took place (Okos, Narsimhan, Singh, & Weitnauer, 1992).

Although the colour of microwave-vacuum dried KGM flour was significantly different, the samples looked similar when observed with the naked eye. The colour of KGM flour is shown in Figure 4.17. This was also the case when the glucomannan flour was used as a food additive in subsequent work. Although drying using microwave vacuum dryer results in darker KGM flour than drying using other drying techniques, the advantage of microwave vacuum drying is a shorter drying time. From the above information, it can be concluded that drying time is not the main factors that affect the browning of the KGM flour but drying method and especially drying temperature are the most important factor that influence the colour change in

KGM flour. The higher whiteness index of KGM flour obtained when the product temperature is above 60 °C in order to control activity of PPO. Thus, if the high whiteness index value is needed in production of KGM flour, the drying temperature should be controlled in order to obtain the product temperature at above 60 °C.

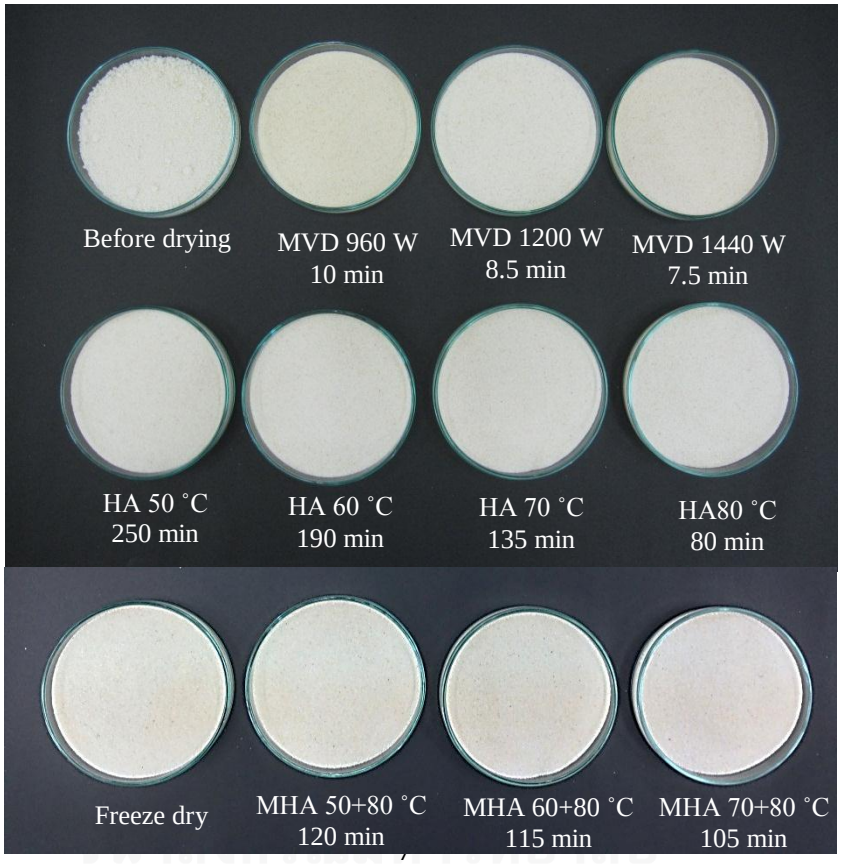


Figure 4. 17 The colour of the KGM flour before and after drying to the same final moisture content 5-6% (d.b.) using microwave vacuum dryer (MVD), hot air dryer (HA), multistage hot air drying (MHA), and freeze-drying under different conditions.

4.5.3 Bulk density, particle density, porosity determination

The bulk density, particle density and porosity of KGM flour after drying by using different drying techniques and condition are shown in Figure 4.18 (see Table C-12 and C-13, Appendix C).

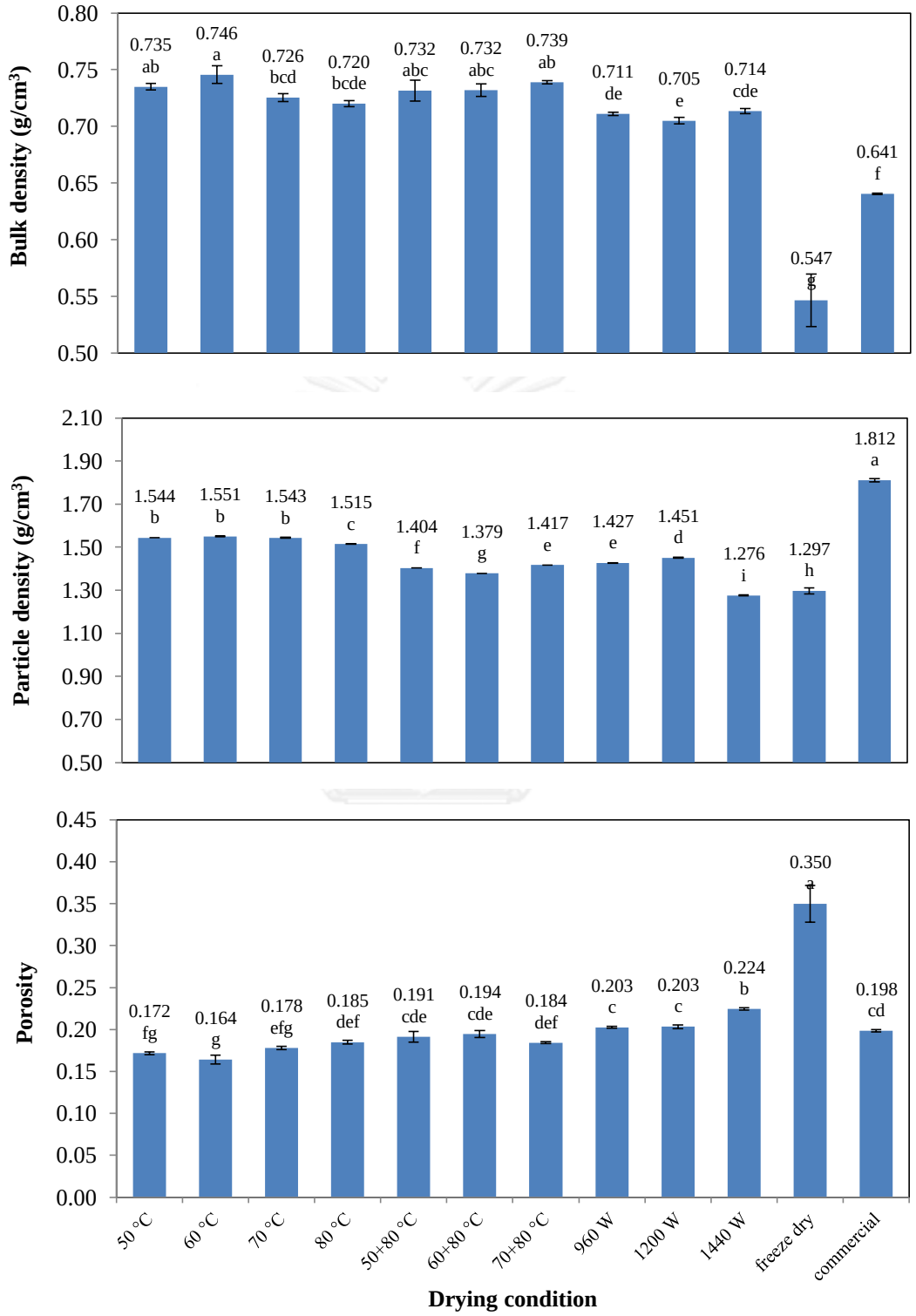


Figure 4. 18 Bulk density, particle density, and porosity of KGM flour after drying to the same final moisture content 5-6% (d.b.) using different drying techniques under different conditions.

Different superscripts indicate significantly differences ($p \leq 0.05$).

From the results, drying KGM flour using freeze drying results in lowest bulk density value. In addition, drying using microwave-vacuum results in a lower bulk density value than conventional and multistage hot air drying. Among hot air dried samples, it seems that the bulk and particle density of samples decreased with increasing drying air temperature. This is because when drying at higher temperature, the outer layers of the material become rigid and case hardening is occurring giving the final volume of the dried product (N. Wang & Brennan, 1995). It can be concluded that the drying air temperature had an effect on the density of KGM flour. In the case of microwave vacuum drying, it seems that increasing microwave power slightly affects the bulk density but significantly affects the particle density and viscosity of sample. This applies particularly to microwave power of 1440 W that gave the lowest particle density value of 1.276 g/cm^3 which was strongly related to the highest porosity of 0.224 and the highest viscosity of 17,321 mPa·s as shown in Table C-13 (see Appendix C).

Bulk density is an important characteristic of powder and porous products and it is determined by the mass of the sample and its bulk volume, while the particle density is the density excluding all pores and is determined by the mass of sample and its solid volume (Krokida & Maroulis, 1999). The results show that the bulk density and particle density values of the dried KGM flour differed significantly between drying treatments ($p \leq 0.05$). It appears that the bulk density and particle density of microwave vacuum dried samples tended to be lower than those of hot air dried ones. These results agree with (Krokida & Maroulis, 1999, 2001) who studied structural properties of dehydrated apple, banana, carrot and potato using conventional, vacuum, freeze, microwave and osmotic drying. They found that the

drying method significantly affected the bulk density. Drying fruits using microwave results in a lower bulk density value than conventional hot air drying.

Porosity characterises the overall open structure of a dehydrated material (Vadivambal & Jayas, 2007). This value seems to increase when using microwave vacuum drying compared to hot air drying. This result agreed with Krokida, Zogzas, and Maroulis (1997) who found that air-dried products had low porosity when compared to freeze, microwave and vacuum dried ones. Similar results were also found by Zielinska et al. (2013) who studied drying of green peas using different drying methods including microwave vacuum drying and hot air drying. They found that during microwave vacuum drying, the energy of microwaves is absorbed by water located in the whole volume of the material being dried. Microwave vacuum drying created a large vapor pressure in the centre of the material, allowing rapid transfer of moisture to the surrounding vacuum and preventing structural collapse. Furthermore, the puffing phenomenon, that accompanied microwave vacuum drying, created a porous structure of the green peas and facilitated obtaining a desired product texture, and in this way it reduced the product's density as well as shrinkage. For this experiment, the microwave vacuum drying shows the ability to increase porosity of KGM flour. This phenomenon can be clearly seen in the morphology observations.

From the above information, it can be concluded that drying technique is the main factor that influences the density and porosity in KGM flour. Using conventional hot air drying and multistage hot air drying may cause the case hardening, tightly pack and affect to the density of KGM flour while the porous structure and puffing properties of KGM flour obtained when using freeze or

microwave-vacuum drying. These properties are important to the viscosity of KGM solution.

4.5.4 Apparent viscosity measurement

Viscosity is one of the important criteria for commercialization of KGM flour. This criterion plays an important role in various applications such as gelling agent, thickening agent, emulsifier, stabilizer, film forming agent and coating materials. The high viscosity of KGM solution is required for these applications. The apparent viscosity of 1% KGM solution from KGM flour before and after drying process is shown in Figure 4.19 and Table C-13 (see Appendix C).

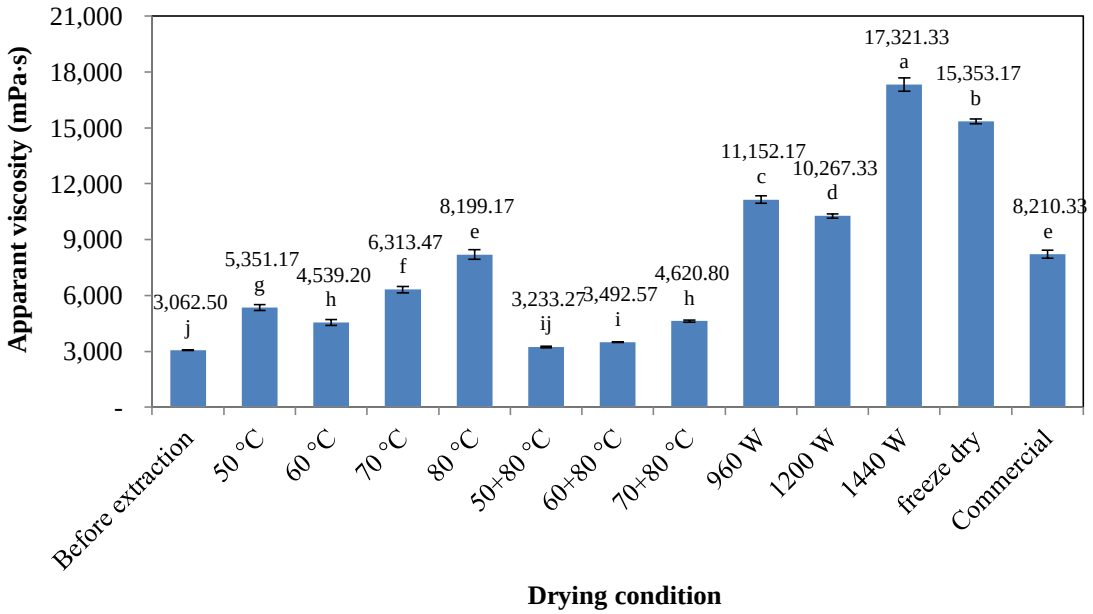


Figure 4. 19 Apparent viscosity (at shear rate of 10 s^{-1}) of 1% KGM solution from KGM flour after drying to the same final moisture content 5-6% (d.b.) using different drying techniques under different conditions.

Different letters indicate significant differences ($p \leq 0.05$).

Figure 4.19 shows that the apparent viscosity of 1% KGM solution from KGM flour was depended on the drying technique. When comparing between drying methods, multistage hot air drying, conventional hot air drying at constant drying temperature, microwave-vacuum drying, and freeze drying, the results showed that using conventional hot air drying and multistage hot air drying lead to a considerable reduction in viscosity, with values differing significantly ($p \leq 0.05$) from values obtained using freeze drying and microwave vacuum drying. This seems to be due to the density and porous characteristic.

The relationship between viscosity and porosity of KGM flour is shown in Table C-13 (see Appendix C). The changes in viscosity and porosity occur in the same direction. From the results, it seems that the changes in viscosity were strongly related to the porosity of the sample. A higher porosity gives KGM particles a higher capacity to absorb water and increase the rehydration rate. A more porous structure enhances water infiltration inside the particle in comparison with less porous structure. As a result, a higher porosity increases water absorption by KGM particles and produces swelling. In addition, since KGM is very hydrophilic, once it absorbs water it can increase its volume more than 100 times and then becomes a thick and viscous solution. When KGM granules are swelling, the volume change of KGM particles due to water absorption may affects the viscosity of the KGM solution. Therefore, more porous granules produce more viscous solution. Similar results of the swelling of biological material have been found in the pharmaceutical field by Ek, Lennholm, Davidson, Nystrom, and Ragnarsson (1995) and Hedenus, Stromme Mattsson, Niklasson, Camber, and Ek (2000). They found that porous cellulose beads consisted of a three-dimensional skeletal fibre system in which the liquid can be taken

up both in the pores between fibres and in the solid fibre matrix itself. Moreover, it was found that the pore size in cellulose beads almost doubled when the beads were soaked in water. For this reason, the hot air dried samples with low porosity will result in a less viscous liquid.

In comparison, hot air drying and multistage hot air drying provided the viscosity in range of 3,233.27 to 8,199.17 mPa·s while freeze drying can lead to a very high viscosity (15,353.17 mPa·s). The highest viscosity was found after using microwave vacuum drying at 1440 W for 7.5 minutes (17,321.33 mPa·s). One of the reason for the high viscosity of microwave-vacuum and freeze dried samples, it may due to the hydrodynamic properties of the porous KGM granule during water uptake process. The high porosity can promote the hydrodynamic force and lead to high viscosity of KGM solution. Thus, using microwave vacuum dryer is an alternative method to improve viscosity properties of KGM flour. In conclusion, drying technique also have significantly affect to the viscosity of KGM sample. Microwave-vacuum drying and freeze drying showed the performance in the porosity production while conventional hot air drying and multistage hot air drying have that limitation. It seems that the changes in viscosity were strongly related to the porous structure of the sample. The high porosity of KGM sample has more ability to absorb water, swell and subsequently produce more viscous solution.

In addition, China's industrial standard for konjac flour (P. Y. Liu et al., 2002) defining the viscosity standards for different grade of konjac flour mentions 14,000, 18,000, and 22,000 mPa·s for second grade, first grade, and top grade of common konjac flour, respectively. From the above information, it was concluded that KGM flour obtained from this experiment, especially when drying by using

microwave vacuum drying method at 1440 W for 7.5 minutes provided a comparable versus with the first grade of common konjac flour in the industrial standard of China (P. Y. Liu et al., 2002).

4.5.5 Microstructure characterisation (Scanning Electron Microscopy)

Figure 4.20 shows the microstructure of KGM flour observed under scanning electron microscope (SEM). The results shows that the morphology of microwave vacuum dried KGM granule was characterised by porous, rough surface and irregular structure whereas the morphology of hot air dried KGM granule was characterized by tightly packed structure, smooth surface and normal shape. The more porous structure may be due to rapid vaporisation of water inside the granule during microwave vacuum drying. For this reason, mass transfer occurred by vaporization (Therdthai & Zhou, 2009). Increasing microwave power tended to increase the evaporation rate and thus enhanced the porosity.

4.5.6 Morphological characterisation (Image analyser)

Further microstructure observations revealed the presence of pores in the granule of microwave vacuum dried KGM samples (Figure 4.21). Some cavities and disruption of the continuity of the cellular structure were observed. These results agree with Zielinska et al. (2013) who also found a porous structure in the green peas after drying under different drying conditions, especially microwave vacuum drying. In case of hot air drying, the structure of KGM granule was compact and tightly packed. This result also strongly related to the high bulk and particle density of KGM flour.

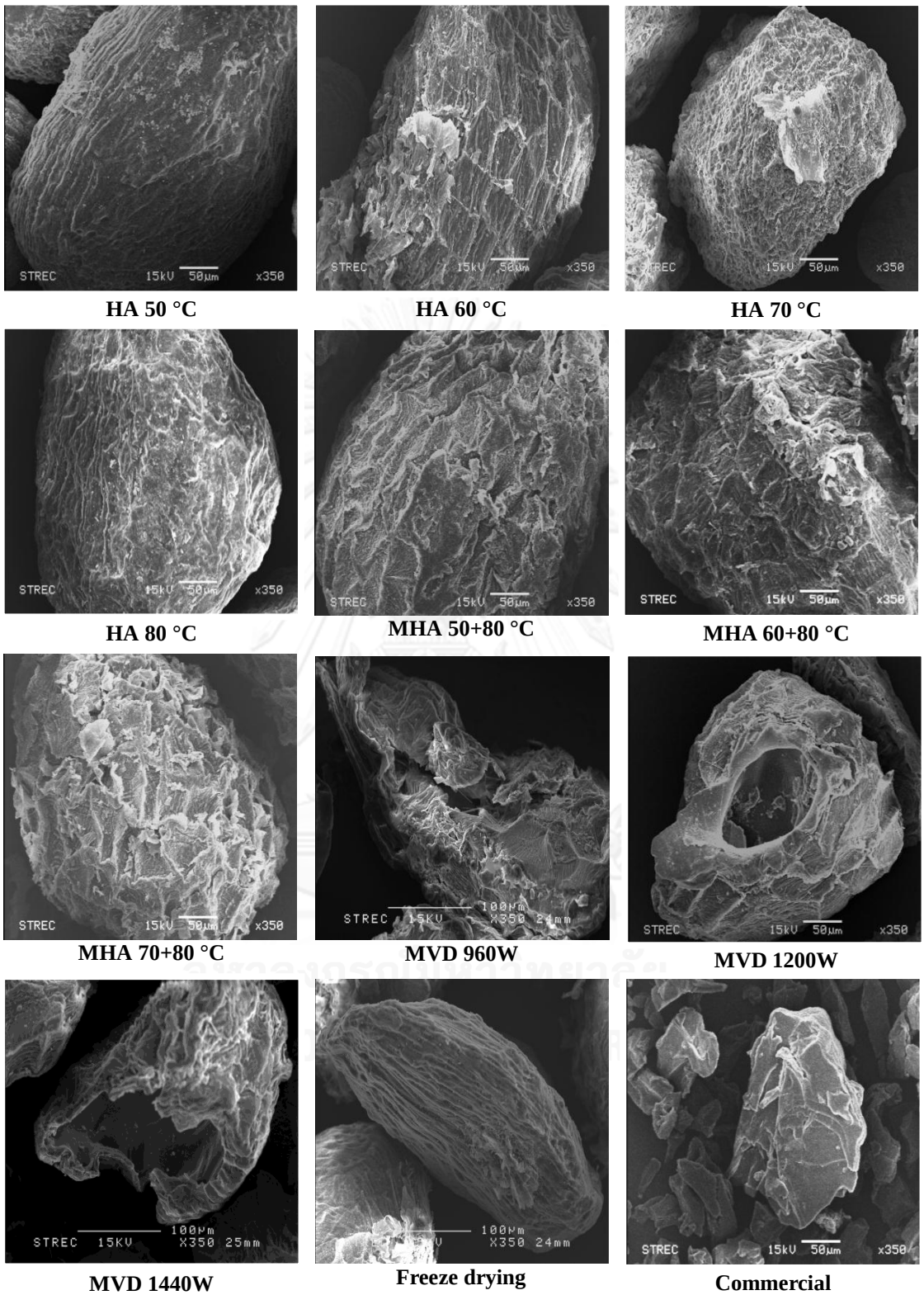


Figure 4. 20 Scanning electron micrographs of the KGM flour after drying to the same final moisture content 5-6% (d.b.) using different drying techniques under different conditions. The KGM flour structure is shown at 350× magnification.

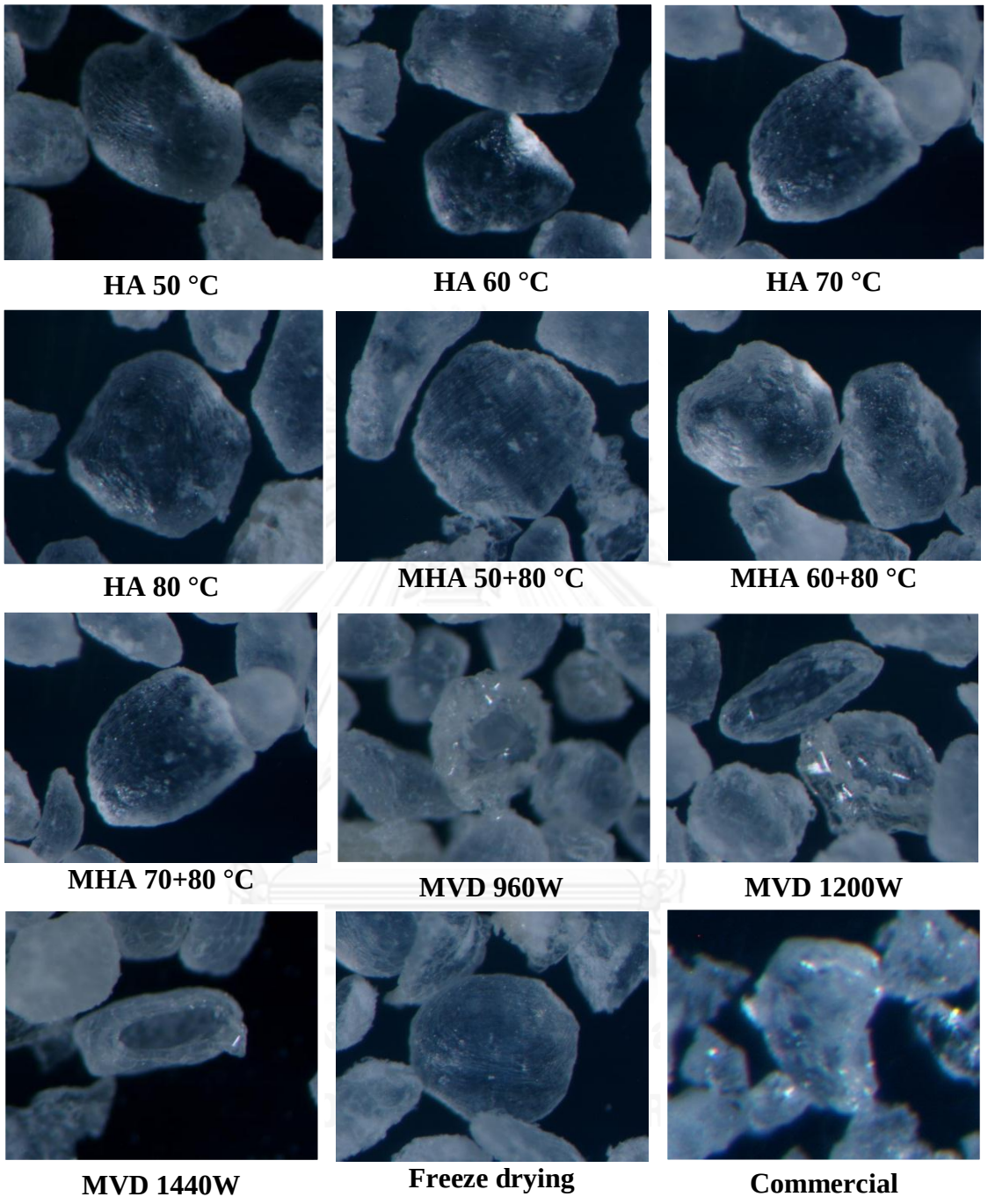


Figure 4. 21 Microstructure pictures obtained by image analyzer of the KGM flour after drying to the same final moisture content 5-6% (d.b.) using different drying techniques under different conditions. The KGM flour is shown at 120× magnification.

Particle shape of KGM flour is also an important factor affecting the viscosity of KGM solution. Figure 4.20 and Figure 4.21 show that the microwave vacuum dried KGM flour samples have an irregular particle shape with rough surface. In contrast, the hot air dried KGM flour sample had a normal oval shape with quite smooth surface. It seems that cavities were generated inside the particles of KGM flour during microwave vacuum drying process. During the microwave vacuum drying, heat was generated and water was evaporated to outside of the KGM granule. Thus, the KGM granules were ruptured and showed a rough shape. Hence, the ratio of the surface area to the volume of the granules has increased and contributed to the increased viscosity of KGM solution. In contrast, a spherical shape possesses a minimum surface area to volume ratio resulting in reduced cohesive forces and improved flow ability of the solution.

4.5.6 KGM characterisation by FTIR

Infrared spectroscopy is a rapid and a non-destructive technique that has been widely used to characterize different polysaccharides (Torringa et al., 1996). When chemical groups interact at the molecular level, changes are seen in FTIR spectra such as shifting of absorption bands. The spectra obtained for dried KGM flour from different drying condition are in agreement with commercial sample (as reference sample) and with those previously reported (Chua et al., 2012; Jacon, Rao, Cooley, & Walter, 1993; Maekaji, 1974; Martins et al., 2012; Nguyen, Do, Nguyen, Pham, & Nguyen, 2011; H. Zhang et al., 2001). The broad band ranging between 3500 and 3100 cm^{-1} were attributed to O-H stretching vibration formed by the hydroxyl group of glucomannan and water (Martins et al., 2012; Nguyen et al., 2011).

The peaks at ~2900 cm^{-1}, ~1370 cm^{-1}, and ~1050 cm^{-1} are assigned to $–CH_2$-stretching vibration, and two C-H bending modes, respectively. The small peak at ~1730 cm^{-1} is due to C=O (carbonyl of acetyl group) stretching vibration (Chua et al., 2012; Jacon et al., 1993; Maekaji, 1974; H. Zhang et al., 2001). The band at 1652 cm^{-1} is due to the in-plane deformation of the water molecule. This water is the strongly bound water of crystallization (H. Zhang et al., 2001). FTIR spectra of KGM samples show a band in the region of 750-1300 cm^{-1} that corresponds to the carbohydrates region. These wavenumbers are within the so-called fingerprint region, where the bands are specific for each polysaccharide (Martins et al., 2012). The peaks at ~1150 cm^{-1} and ~1030 cm^{-1} are usually cited as C-O-C stretching modes from ether groups in the pyranose rings. Peaks attributed to β-glucosidic and β-mannosidic linkages (the characteristic absorption bands of mannose in konjac glucomannan) are observed at ~870 cm^{-1} and ~800 cm^{-1} (Chua et al., 2012; Ye et al., 2006).

FTIR spectra of KGM flour after drying to the same final moisture content 5-6% using different drying techniques and conditions are shown in Figure 4.22. Due to the fact that the spectra of KGM flour obtained from drying by using hot air at 50, 60, 70, 80 °C and multistage hot air drying at 50+80, 60+80, 70+80 °C were similar and also the spectra of microwave-vacuum dried sample at 960, 1200, 1440W microwave power level were not significantly different between them, only the spectra of 50 °C hot air dried, 1440W microwave-vacuum dried, freeze dried, and commercial sample have been presented in Figure 4.22.

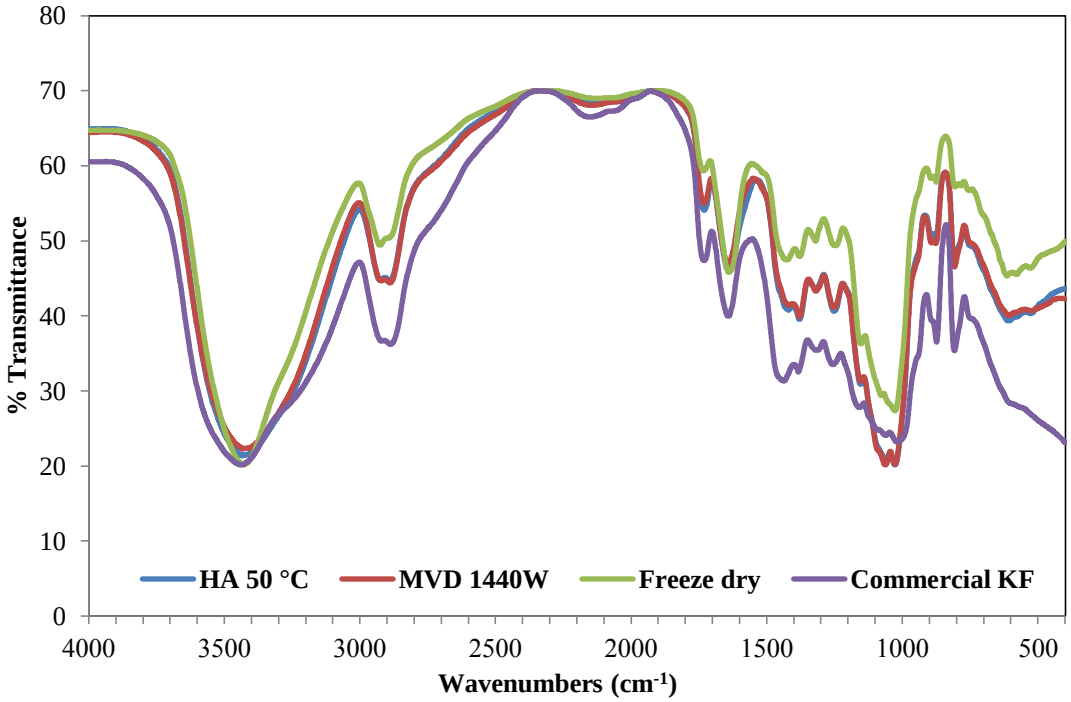


Figure 4. 22 FTIR spectra of KGM flour after drying to the same final moisture content 5-6% (d.b.) using different drying techniques.

The results in Figure 4.22 show that the spectra of all dried samples are almost completely identical. Hence, no new chemical groups were introduced into the structure of KGM during drying. When considering the spectra of dried KGM sample from this experiment and KGM flour from commercial sample, it is obvious that they show the same patterns. This means that the functional groups of KGM molecules are the same and present the glucomannan structure. Since the characteristic peaks of the β-pyranose between mannose and glucose unit in konjac glucomannan were observed at 893 cm^{-1} and 814 cm^{-1}, it can be confirmed that the compound extracted in this experiment is glucomannan. However, FTIR spectra of KGM flour from commercial sample exhibited lower spectra than KGM sample from this experiment. This may be caused by a different species of the raw material.

4.5.7 Comparison of assays to determine glucomannan content

In order to confirm that the extracted matter, obtained from extraction of konjac corms is glucomannan, the quantitative analysis of the glucomannan content was carried out by two methods. The first method using the 3,5-dinitrosalicilic acid (3,5-DNS) was a colorimetric assay (adapted from P. Y. Liu et al. (2002); Zhao et al. (2010); and Chua et al. (2012)) which is widely used in KGM flour industries and is the most reproducible and accurate method for determination of the glucomannan content in konjac samples. This method has been adopted by the Chinese Ministry of Agriculture (CMA) for the classification of konjac flour. As for the second method, it is using high performance liquid chromatography (HPLC) (adapted from Cengiz et al. (2013)). The glucomannan content of KGM samples is shown in Table 4.7.

Table 4. 7 The glucomannan content (%) of KGM flour samples determined by HPLC and 3,5-DNS method.

Sample	% Glucomannan from HPLC	% Glucomannan from 3,5-DNS
Fresh konjac 1	72.34 ± 0.03	72.34 ± 0.15
Fresh konjac 2	71.03 ± 0.86	74.19 ± 0.74
Fresh konjac 3	70.20 ± 0.29	73.52 ± 0.30
KGM after extraction using 50% ethanol	87.40 ± 0.11	88.79 ± 0.89
Commercial KGM flour from China 1	88.19 ± 0.04	89.74 ± 0.74
Commercial KGM flour from China 2	89.38 ± 0.10	90.29 ± 1.19
Commercial KGM flour from China 3	87.29 ± 0.40	90.25 ± 0.00
Commercial KGM flour from China 4	81.93 ± 0.45	82.02 ± 0.45

Results of glucomannan content are shown as Mean±SD (n = 3)

Table 4.7 shows that the glucomannan content of both samples as determined by HPLC and the 3,5-DNS colorimetric assays, ranges from 70.20 to 90.29%. When comparing between the two analytical techniques, the results indicate that both methods show similar values. There were no significant differences between the mean glucomannan values obtained using HPLC and 3,5-DNS colorimetric assays, indicating that both assays are comparable. However, the analysis using the 3,5-DNS colorimetric assay tends to give a higher value of glucomannan content than using HPLC method in every sample. Although the HPLC analysis is more accurate and reliable than the 3,5-DNS method of analysis, it requires an HPLC specialist, involves a higher cost and take longer time than the 3,5-DNS method. Using 3,5-DNS method is more convenient, faster, and cheaper than using HPLC method, especially in industrial konjac flour production. These results agree with Chua et al. (2012) who reported that the 3,5-DNS colorimetric assay was the most suitable method in terms of reproducibility, accuracy and high precision for the determination of the glucomannan content of KGM samples among tested methods including the 3,5-DNS, phenol-sulphuric acid and enzymatic colorimetric assay. From above information, it was confirm that the 3,5-DNS colorimetric assay was the method of choice for use in the later stages of experiments.

4.5.8 Konjac glucomannan content determination

The effects of drying conditions on the glucomannan content in KGM flour are shown in Figure 4.23 and Table C-14 (see Appendix C). The results indicate that air temperature in the hot air drying and multistage hot air drying did not affect the glucomannan content. When comparing the effects of different drying methods, it

appears that drying using hot air drying, multistage hot air drying, or freeze drying did not affect the glucomannan content. The drying temperatures used in this experiment were not high enough to destroy the long chain structure of glucomannan. However, the percentage of glucomannan tended to decrease when using the microwave power level at 960 and 1200 W. This could be the result of the uniformity of the sample. However, the overall glucomannan content of KGM samples were not significantly different and higher than before extraction sample form the result of extraction and purification process.

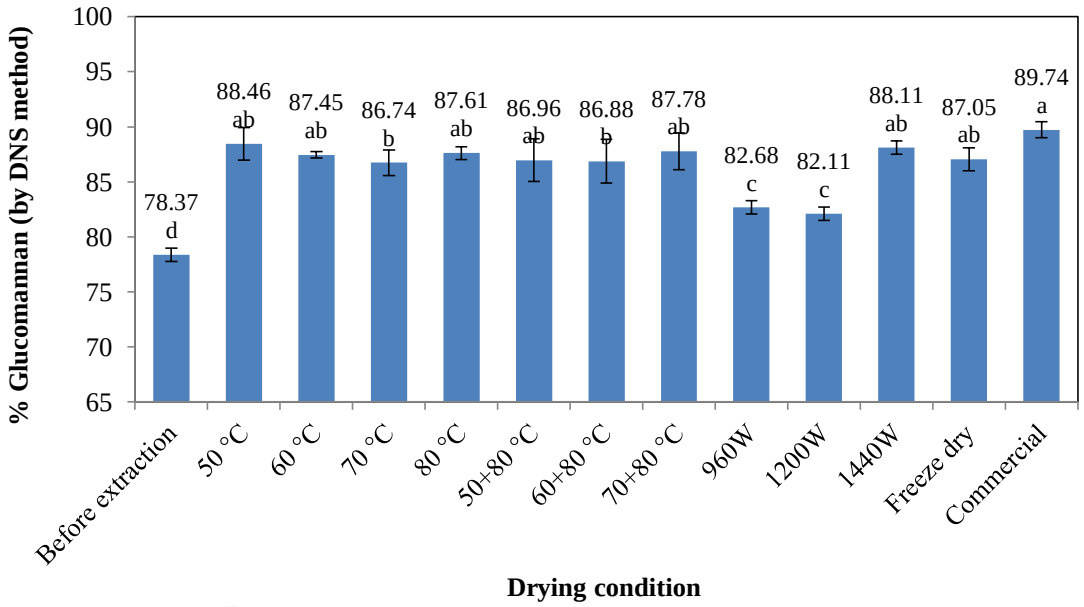


Figure 4. 23 The glucomannan content, determined by 3,5-DNS method, of KGM flour after drying to the same final moisture content 5-6% (d.b.) using different drying techniques under different conditions.

Different letters indicate significant differences ($p \leq 0.05$).

In addition, according to the Chinese Ministry of Agriculture, the acceptance limit of glucomannan content for common konjac flour is between 60 and 70%, and for purified konjac flour it is between 85 and 90% (P. Y. Liu et al., 2002). As shown in Figure 4.28, the glucomannan content of KGM samples as determined

by the 3,5-DNS, ranged from 78.37 to 89.74%. Hence, the glucomannan content from this experiment is higher than the acceptance limits for common konjac flour, and it falls within the acceptance limits for purified konjac flour in some treatments.

4.5.9 Residual sulphur dioxide content determination

The sulphur dioxide residues in KGM flour before and after drying process are shown in Table 4.8. The results indicate that the extraction and heat in drying process can significantly reduce the residual sulphur dioxide content in KGM flour, except in freeze-dried sample which showed the highest sulphur dioxide content due to a low drying temperature. When comparing between drying methods, multistage drying, conventional hot air drying at constant drying temperature, microwave-vacuum drying, and freeze drying, the results showed that microwave-vacuum drying has significantly reduced the sulphur dioxide residues of KGM flour. In particular, the residual sulphur dioxide content in microwave-vacuum dried sample was lower than in other dried samples which were obtained from multistage hot air drying, conventional hot air drying and freeze drying. This finding demonstrates the effect of the higher evaporation rate which was enhanced under microwave vacuum system. Heat from microwave stimulates the evaporation of sulphur dioxide and the vacuum system help to improve the evaporation. The result also indicates that the sulphur dioxide residues of microwave-vacuum dried sample at every microwave power level remained below 300 ppm which comparable to the superior grade of purified konjac flour in the Chinese standard (P. Y. Liu et al., 2002).

Table 4. 8 The sulphur dioxide residues of KGM flour after drying using different drying techniques under different conditions to the same final moisture content 5-6% (d.b., MR=0.05-0.06).

Drying method	Drying condition	Sulphur dioxide residues (ppm)
Before extraction	-	$43.11^{h} \pm 2.52$
Hot air drying	50 °C	$459.04^{c} \pm 1.68$
	60 °C	$434.35^{d} \pm 1.65$
	70 °C	$469.23^{b} \pm 2.50$
	80 °C	$434.63^{d} \pm 0.85$
Multistage drying	50+80 °C	$330.31^{f} \pm 6.62$
	60+80 °C	$361.21^{e} \pm 4.15$
	70+80 °C	$232.69^{g} \pm 0.03$
Microwave-vacuum drying	960W	$45.37^{h} \pm 3.37$
	1200W	$34.56^{i} \pm 0.09$
	1440W	$15.68^{j} \pm 1.71$
Freeze drying	-47 °C, 4.6 Pa	$475.45^{a} \pm 2.42$
Commercial product	-	$11.84^{j} \pm 1.26$

Results are Mean ± SD (n = 3), Letters a, b, c, … in the same column indicate a statistically significant difference at $p \leq 0.05$).

In addition, the results also show that the residual sulphur dioxide content in all samples was as low as safety level while it was also found that there is a small amount of sulphur dioxide that exists in fresh konjac corms (before sample extraction). In addition, the maximum level of sulphur dioxide of 1500 mg/kg (ppm) is approved by Thai Food and Drug Administration for use in dried or preserved fruits and vegetables. Nevertheless, the Japanese specifications and standards for foods and food additives limit the maximum amount of sulphur dioxide for use with KGM flour to 900 mg/kg (Japan External Trade Organization, 2011). The Chinese standard for

the different grades of konjac flour (Table 2.5) defines the sulphur dioxide residues for the superior and first grade of purified konjac flour as 300 and 500 mg/kg, respectively (P. Y. Liu et al., 2002).

4.6 The chemical composition of KGM flour

The results in Table 4.9 showed the chemical composition (AOAC, 2006) of KGM flour obtained from microwave vacuum drying at 1440W compare with commercial sample and konjac flour before extraction. The results indicated that KGM flour from this experiment have more purity when compare with konjac flour before extraction and comparable quality to the commercial sample. After extraction and drying process, KGM flour from this experiment shows higher total carbohydrate, dietary fibre, and glucomannan content and lower impurities content such as protein, lipid, ash and sulphur dioxide content when compare with konjac flour sample before extraction.

Table 4. 9 Chemical composition of KGM flour.

Chemical composition	Konjac flour before extraction (g/100 g)	KGM flour from experiment (g/100 g)	KGM flour from commercial (g/100 g)
Moisture content	8.02 + 0.10	6.62 ± 0.11	8.38 ± 0.08
Total carbohydrate	80.86 + 0.13	86.97 ± 0.57	89.10 ± 0.04
Dietary fibre	80.66 + 0.13	86.10 ± 0.62	88.97 ± 0.10
Ash	5.48 + 0.23	3.31 ± 0.38	1.55 ± 0.04
Protein	5.03 + 0.11	3.00 ± 0.05	0.97 ± 0.03
Lipid	0.61 + 0.10	0.11 ± 0.04	< 0.01
Glucomannan	74.69 ± 0.33	86.74 ± 0.17	88.19 ± 0.04
Sulphur dioxide (ppm)	43.11 ± 2.52	15.68 ± 1.71	11.84 ± 1.26

Values expressed are means of 3 replicates ± SD

CHAPTER 5

CONCLUSIONS

Fresh konjac corm consists of 82.83±0.29 % (w.b.) of moisture content and 17.17+0.29 % of solid portion. In solid portion, the main component is carbohydrate (15.09 %) which consists of dietary fibre (15.05 %) and other carbohydrates (0.04 %). The other minor components include ash, protein, and lipid (in totally 2.08 %). It can be assumed that the dietary fibre in konjac corm mainly includes glucomannan.

The browning of dried konjac slices is likely to be mainly due to the enzymatic browning reaction. Using sodium metabisulphite at 500 ppm, soaking time of 10 min, was the most effective agent to retard browning reaction. In contrast, among sulphur free compounds, sodium chloride at 10,000 ppm, soaking for 20 min, showed a performance comparable to sodium metabisulphite.

The anti-swelling agents are the main factor that affects the quality of KGM flour such as extraction yield, water uptake, whiteness index value, glucomannan content, and also viscosity of KGM solution. Using ethanol at a concentration greater than 50% can reduce the water uptake and improve the yield of extraction. However, 50% ethanol was selected to use as anti-swelling agent for glucomannan extraction.

Drying technique is the main factor that influences several important properties of KGM flour such as colour change, bulk density, particle density, porosity, viscosity, morphology and sulphur dioxide residue. However, drying methods, drying temperature and drying time did not affect the glucomannan content and FTIR spectra of KGM flour. The main factors that affect the glucomannan

content of the KGM flour is the extraction medium and extraction technique. However, using microwave vacuum drying at power level of 1440W for 7.5 minutes resulted in the best quality of KGM flour within the range of experimental conditions studied and provided a comparable result with the first grade of common konjac flour in the industrial standard of China.

It can be confirmed that pre-treatment, wet extraction and drying process used in this experiment can improve the quality of KGM flour in terms of purity, viscosity, and appearance. The results have potential to become useful for the industrial KGM flour production in Thailand. In order to meet the criteria of good quality KGM flour, there are the following suggestions: a higher whiteness index of KGM flour can be obtained when the product temperature is above 60 °C in order to inactivate PPO activity for control the browning of KGM flour; a higher viscosity of KGM solution can be obtained when using a drying method leading to low density and high porosity powder, such as using freeze drying or microwave-vacuum drying; and using thermal and vacuum system in drying process can significantly reduce the residual sulphur dioxide content in KGM flour.

Recommendation for further research

The present study on glucomannan is still limited and thus further research sould focus on:

- The effect of particle size of KGM flour extracted from *A.muelleri* on viscosity within its applications.
- The effect of water content on thermal, rheological and dielectric properties of KGM flour extracted from *A.muelleri*.

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APPENDICES

APPENDIX A

Analysis procedures

A.1 Determination of konjac glucomannan content by 3,5-dinitrosalicylic acid (DNS) colorimetry measurement (adapted from Liu et al., 2002; Zhao et al., 2010; Chua et al., 2012; and Cengiz et al., 2013)

The resultant of reaction is D-glucose and D-mannose which are reductive monosaccharide from KGM extracted by acid hydrolysis. The reducing sugar can be translated into brown amino compounds with 3,5-dinitrosalicylic acid subjected to the alkaline and heating treatment. To a certain extent, there is a relationship between the amount of reducing sugar and the colour intensity of reactive solution, and the KGM content of the konjac flour sample that can be determined by colorimetric measurement.

A.1.1 Preparation of DNS reagent

Solution A: Crystalline phenol 6.9 g is dissolved into 15.2 mL of 10% NaOH, then dilutes to 69 mL with DI water and finally 6.9 g $NaHSO_3$ are added.

Solution B: A mass of 225 g of sodium potassium tartrate ($KNaC_2O_6H_4$) is dissolved into 300 mL of 10% NaOH, then 880 mL of 1% of 3,5-Dinitrosalicylic acid (DNS) are added.

Mix the solutions A and B and store them in a brown reagent bottle at room temperature, then use after 7-10 days and use within 2 weeks.

A.1.2 Construction of standard D-glucose and D-mannose calibration curves

Weigh exactly 0.1000 g of glucose (A.R. grade) (pre-drying in the 105 °C to constant weight), then dissolve in DI water to a constant volume of 100 mL to obtain

a 1.0 mg/mL glucose standard solution. The D-Glucose stock solution (1 mg/mL) is then placed (0.40, 0.80, 1.20, 1.60 and 2.00 mL) into 50 mL volumetric flasks, respectively (using DI water as a blank). DI water was then added to the volume of 2.00 mL, followed by the addition of 3,5-DNS (1.50 mL) to each flask. Each mixture was heated for 5 min in a boiling water bath and cooled to room temperature before being diluted to 50 mL with DI water in a volumetric flask. Absorbance was then measured at 550 nm and a plot of the measured absorbance against the glucose content (mg) constructed. A D-mannose standard curve was constructed using the procedure as described for glucose. The experiment was done in three replicates.

A.1.3 Preparation of KGM sample

Konjac flour (0.2000 g) was added to a magnetically stirred 85% ethanol (50 mL) and mixed for 30 min in a 50 °C water bath. The precipitated KGM was then collected by filtering the mixture through No.1 filter paper to remove soluble sugar. Then the KGM flour was dried at 60 °C or place over a boiling water bath to remove the residual ethanol.

A.1.4 Hydrolysis of KGM sample

Konjac flour (from section 3) was hydrolysed with 10 mL of 3 M H_2SO_4 in a boiling water bath for 90 min and then allowed to cool to room temperature. The KGM solution was then neutralized with NaOH and the volume was adjusted to 50 mL with DI water. This solution can be called the KGM hydrolysate. The KGM hydrolysate was centrifuged at 3000 rpm for 10 min in order to get a supernatant. The experiment was done in three replicates.

A.1.5 Colorimetric reaction assay

A volume of 0.50 mL of the KGM hydrolysate supernatant was then placed in 50 mL volumetric flask and 1.50 mL of 3,5-DNS were added to make the colorimetric reaction. Then the mixture was heated for 5 min in a boiling water bath and cooled to room temperature before being diluted to 50 mL with DI water in the same volumetric flask. Make the same colour reaction by using DI water instead of KGM hydrolysate as the reagent blank. Absorbance was then immediately measured at 550 nm and the glucose content determined from the standard curve. The KGM content was determined by evaluation of equation (A1) and (A2),

$$\% \text{ glucose} = \frac{\text{glucose content}}{\text{net weight of sample}} \times 100 \qquad \text{(A1)}$$

$$\% \text{ KGM} = \% \text{ glucose} \times \boldsymbol{\varepsilon} \qquad \text{(A2)}$$

Where:

ε = Molecular weight ratio of mannose and glucose in the glucomannan residues with in KGM hydrolysate (The molecular weight of mannose or glucose is 180, the molecular weight of residue is 162, giving 162/180 = 0.9)

Table A- 1 The absorbance of standard glucose at different concentrations.

Glucose concentration (mg/mL)	Absorbance at 550 nm			
	Replicate			Average
	1	2	3	
0.0000	0.000	0.000	0.000	0.000
0.0083	0.080	0.084	0.082	0.082
0.0166	0.177	0.179	0.182	0.179
0.0249	0.267	0.272	0.265	0.268
0.0332	0.350	0.349	0.350	0.350
0.0415	0.430	0.415	0.423	0.423

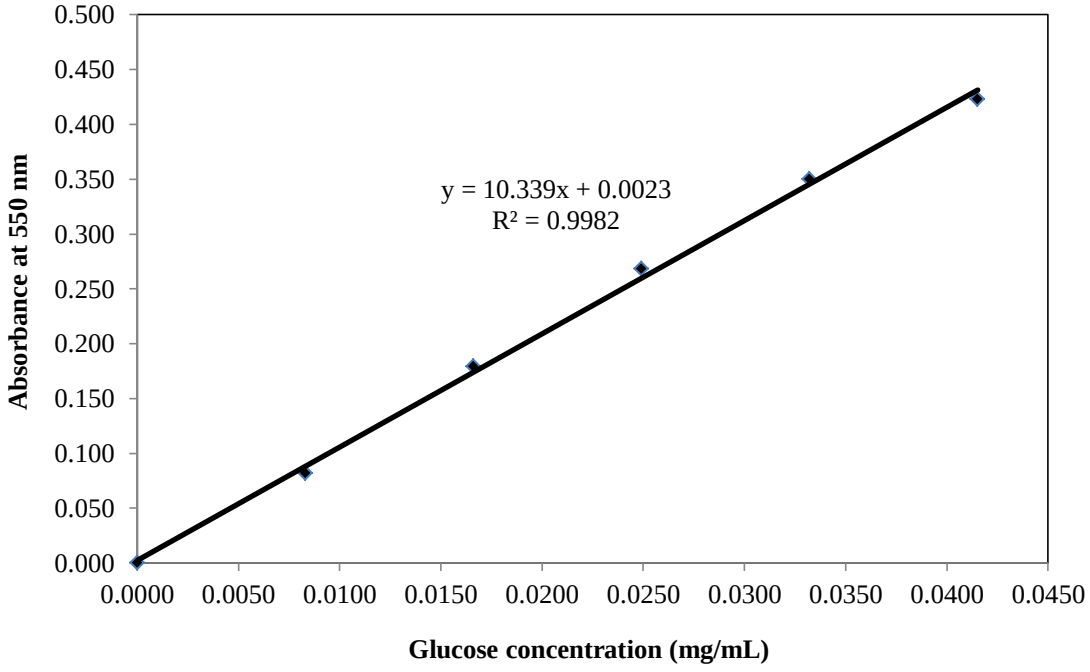


Figure A- 1 Glucose standard curve for glucomannan analysis.

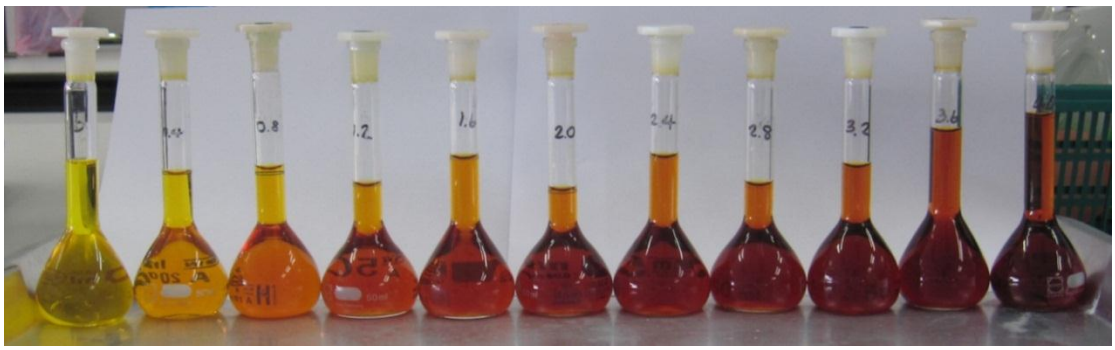


Figure A- 2 The colorimetric reaction of glucose standard at different concentrations.

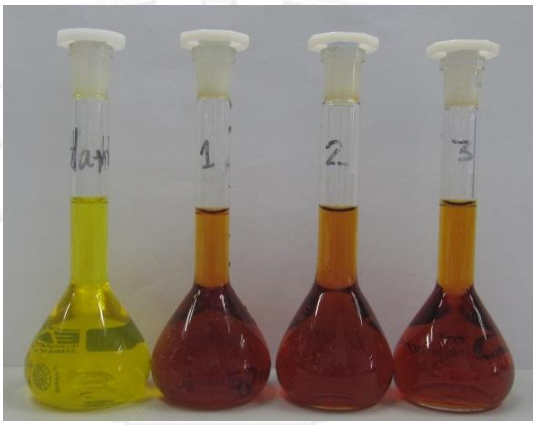


Figure A- 3 The colorimetric reaction of blank and KGM sample.

Table A- 2 The absorbance of standard mannose at different concentration.

Mannose concentration (mg/mL)	Absorbance at 550 nm			
	Replicate			Average
	1	2	3	
0.000	0.000	0.000	0.000	0.000
0.008	0.093	0.092	0.095	0.093
0.016	0.195	0.188	0.193	0.192
0.024	0.287	0.282	0.285	0.285
0.033	0.382	0.366	0.375	0.374
0.041	0.458	0.462	0.465	0.462

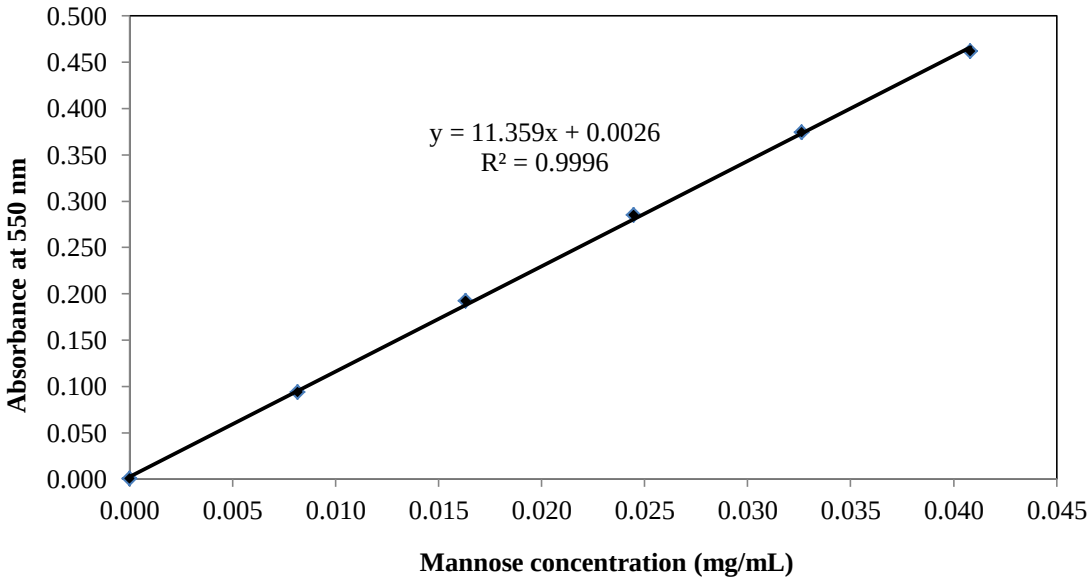


Figure A- 4 Mannose standard curve for glucomannan analysis.

KGM flour 0.2014 g (201.4 mg)

↓

Add 10 mL 3M H_2SO_4

↓

Hydrolyze for 90 min in a boiling water bath

↓

Cool to room temperature

↓

Neutralize by 6M NaOH

↓

Adjust volume to 50 mL

↓

KGM hydrolysates

(201.4 mg/50 mL = 402.8 mg/100 mL = 4.028 mg/mL)

↓

Pipette 0.5 ml into 50 mL volumetric flask

(2.014 mg/0.5mL)

↓

Dilute to 50 ml

(2.014 mg/50 mL = 0.04028 mg/mL)

↓

Colour reaction with DNS reagent

↓

Measure the absorbance at 550 nm

Figure A- 5 Diagram of the determination of konjac glucomannan content by 3,5-DNS method.

sample	Weight of sample (mg)	Net weight of sample (mg/mL)	Absorbance at 550 nm	glucose (mg)	% glucose	% KGM
KGM flour	201.4	0.04028	0.399	0.038369	95.26	85.73

From calibration curve:

$$\mathbf{y = 10.339x + 0.0023}$$

So,
$$x = \frac{y-0.0023}{10.339}$$

y = absorbance at 550 nm (0.399)

x= glucose content (mg)

So,
$$x = \frac{(0.399-0.0023)}{10.339}$$

$$= \mathbf{0.038369\ mg}$$

$$\%\text{glucose} = \frac{\text{glucose content}}{\text{net weight of sample}} \times 100$$

So,
$$\%\text{glucose} = \frac{0.038369}{0.04028} \times 100$$

$$= \mathbf{95.26}$$

$$\mathbf{\%\ KGM = \%\ glucose \times \varepsilon}$$

$$= \mathbf{95.26 \times 0.9}$$

$$= \mathbf{85.73}$$

In the equation:

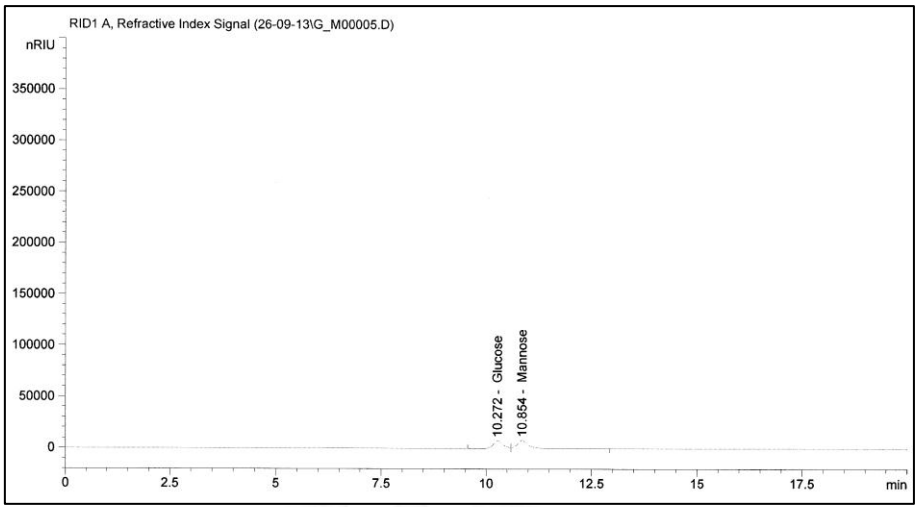
ε = Molecular weight ratio of mannose and glucose in the glucomannan residues with in KGM hydrolysate (The molecular weight of mannose or glucose is 180, the molecular weight of residue is 162, giving 162/180 = 0.9)

A.2 Determination of konjac glucomannan content by HPLC method (adapted from Cengiz *et al*., 2013)

Konjac flour (0.2000 g) was hydrolysed with 10 mL of 3 M H_2SO_4 in a boiling water bath for 90 min and then allowed to cool to room temperature. The KGM solution was then neutralized with NaOH and the volume was adjusted to 50 mL with DI water. This solution can be called the KGM hydrolysate. The KGM hydrolysate was centrifuged at 3000 rpm for 10 min in order to get a supernatant which was then filtered using a 0.45 μm nylon filter (Satorious Stedim Biotech, Germany) and kept in amber glass vial. High Performance Liquid Chromatography with Refractive Index Detector (HPLC-RID, Agilent 1100 Series, USA) operated at 40 °C, equipped with an autosampler and Rezex RHM Monosaccharide column (300 × 7.8 mm) which is operated at 80 °C was used for the determination of glucose and mannose content. A volume of 20 μL of the filtrate was injected to the column. The flow rate of mobile phase (DI water) was set to 0.6 mL/min. Major sugar components (glucose and mannose) were determined by the comparison of retention times with authentic standards and the amounts of sugars were calculated according to the calibration curves for each sugar (0.05, 0.08, 0.10, 0.15, 0.20, 0.40 % D-(+)-glucose and D-(+)-mannose)

A.2.1 The standard calibration curve of glucose and mannose

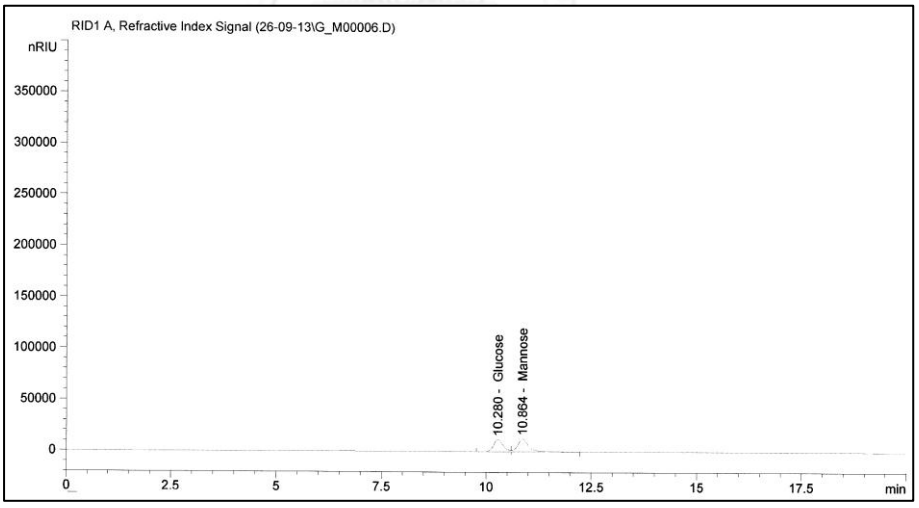
The separation of the standard glucose and mannose on the Rezex RHM Monosaccharide column are shown in Figures A-6 to A-11.



RetTime [min]	Type	Area [nRIU*s]	Amt/Area	Amount [%]	Grp	Name
10.272	VV	1.32168e5	3.87187e-7	5.11737e-2		Glucose
10.854	VV	1.75538e5	3.03621e-7	5.32969e-2		Mannose
Totals :				1.04471e-1		

Results obtained with enhanced integrator!

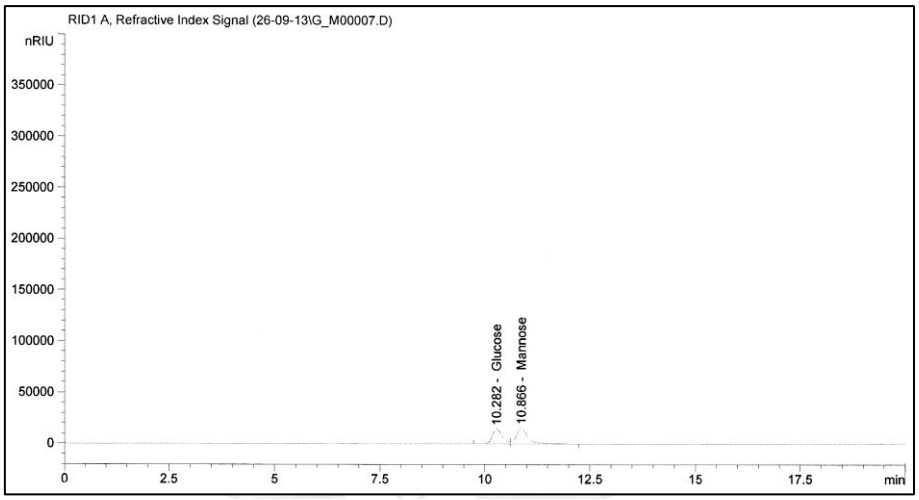
Figure A- 6 Chromatogram, peak area and the amount of glucose and mannose standard at 0.05% concentration.



RetTime [min]	Type	Area [nRIU*s]	Amt/Area	Amount [%]	Grp	Name
10.280	VV	1.97642e5	4.01526e-7	7.93583e-2		Glucose
10.864	VV	2.50632e5	3.12652e-7	7.83605e-2		Mannose
Totals :				1.57719e-1		

Results obtained with enhanced integrator!

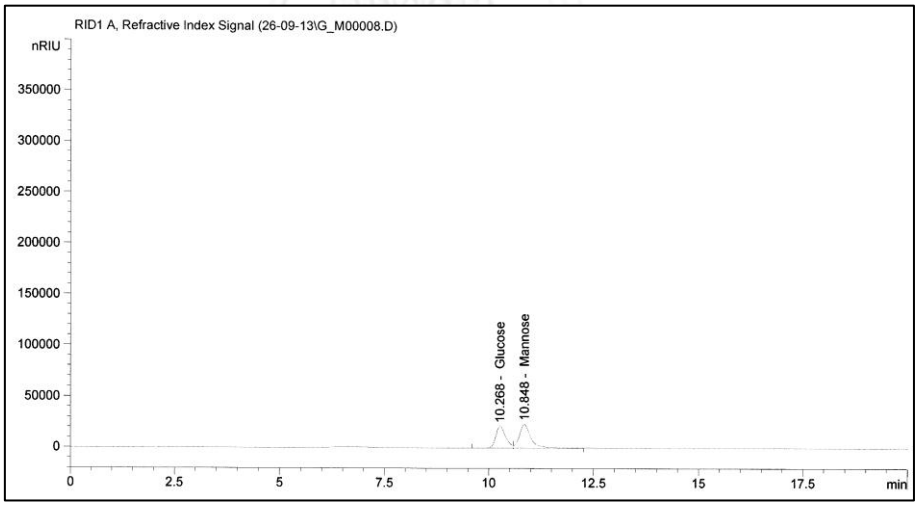
Figure A- 7 Chromatogram, peak area and the amount of glucose and mannose standard at 0.08% concentration.



RetTime [min]	Type	Area [nRIU*s]	Amt/Area	Amount [%]	Grp	Name
10.282	VV	2.47126e5	4.07322e-7	1.00660e-1		Glucose
10.866	VV	3.10121e5	3.16701e-7	9.82155e-2		Mannose
Totals :				1.98875e-1		

Results obtained with enhanced integrator!

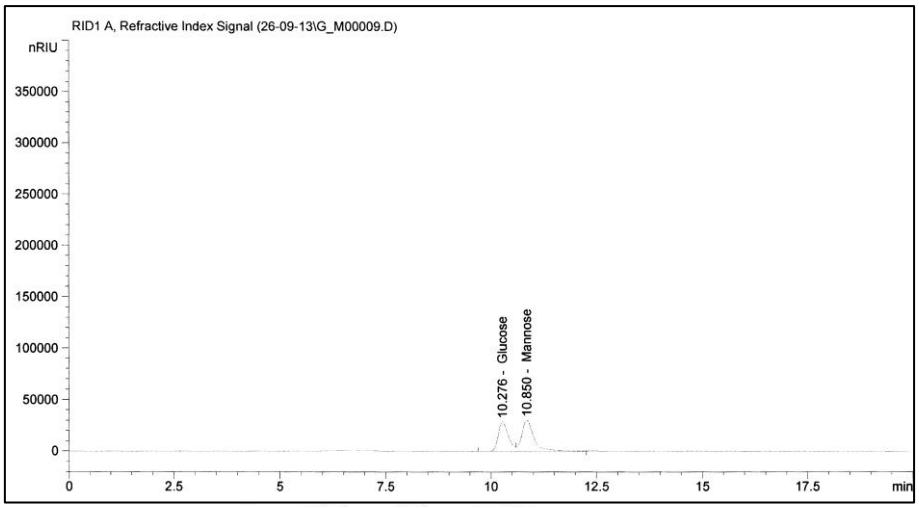
Figure A- 8 Chromatogram, peak area and the amount of glucose and mannose standard at 0.10% concentration.



RetTime [min]	Type	Area [nRIU*s]	Amt/Area	Amount [%]	Grp	Name
10.268	VV	3.52790e5	4.14255e-7	1.46145e-1		Glucose
10.848	VV	4.58487e5	3.22222e-7	1.47734e-1		Mannose
Totals :				2.93879e-1		

Results obtained with enhanced integrator!

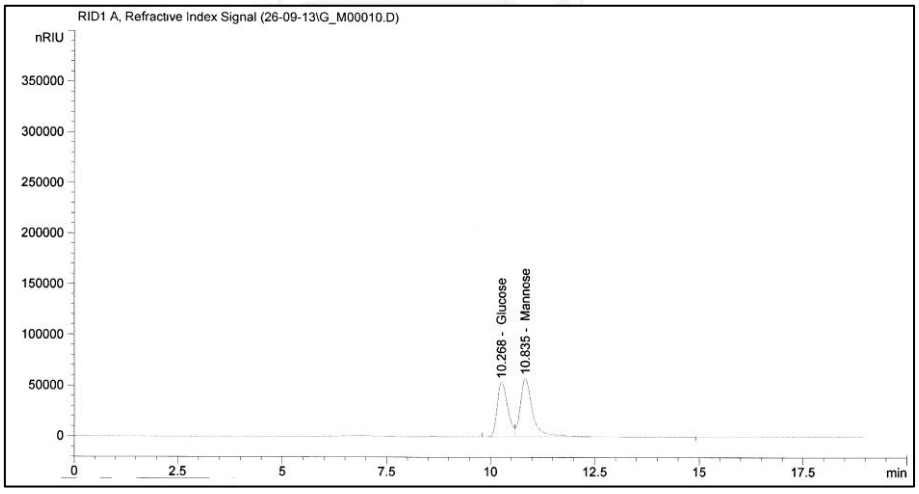
Figure A- 9 Chromatogram, peak area and the amount of glucose and mannose standard at 0.15% concentration.



RetTime [min]	Type	Area [nRIU*s]	Amt/Area	Amount [%]	Grp	Name
10.276	VV	4.84404e5	4.18661e-7	2.02801e-1		Glucose
10.850	VV	6.22161e5	3.25257e-7	2.02362e-1		Mannose
Totals :				4.05164e-1		

Results obtained with enhanced integrator!

Figure A- 10 Chromatogram, peak area and the amount of glucose and mannose standard at 0.20% concentration.



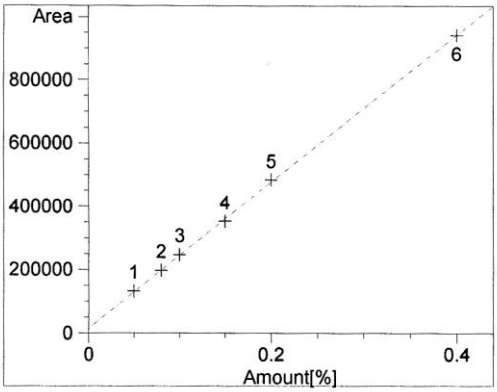
RetTime [min]	Type	Area [nRIU*s]	Amt/Area	Amount [%]	Grp	Name
10.268	VV	9.42182e5	4.24399e-7	3.99862e-1		Glucose
10.835	VB	1.21440e6	3.29404e-7	4.00030e-1		Mannose
Totals :				7.99892e-1		

Results obtained with enhanced integrator!

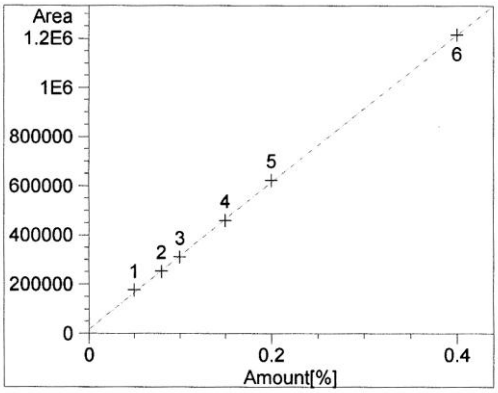
Figure A- 11 Chromatogram, peak area and the amount of glucose and mannose standard at 0.40% concentration.

A linear calibration curve over the range of 0.05–0.40 % standard glucose and mannose were established with the regression equation: y = 2.32304e6x + 13289.51226 (R^2 = 0.99985) and y = 2.99615e6x + 15851.90662 (R^2 = 0.99983), respectively (Figure A-12).

RetTime [min]	Sig	Lvl	Amount [%]	Area	Amt/Area	Ref	Grp	Name
10.268	1	1	5.00000e-2	1.32168e5	3.78307e-7			Glucose
		2	8.00000e-2	1.97642e5	4.04773e-7			
		3	1.00000e-1	2.47126e5	4.04652e-7			
		4	1.50000e-1	3.52790e5	4.25182e-7			
		5	2.00000e-1	4.84404e5	4.12878e-7			
		6	4.00000e-1	9.42182e5	4.24546e-7			
10.835	1	1	5.00000e-2	1.75538e5	2.84839e-7			Mannose
		2	8.00000e-2	2.50632e5	3.19193e-7			
		3	1.00000e-1	3.10121e5	3.22455e-7			
		4	1.50000e-1	4.58487e5	3.27163e-7			
		5	2.00000e-1	6.22161e5	3.21460e-7			
		6	4.00000e-1	1.21440e6	3.29380e-7			



Glucose at exp. RT: 10.268
RID1 A, Refractive Index Signal
Correlation: 0.99985
Residual Std. Dev.: 5802.01415
Formula: y = mx + b
m: 2.32304e6
b: 13289.51226
x: Amount[%]
y: Area

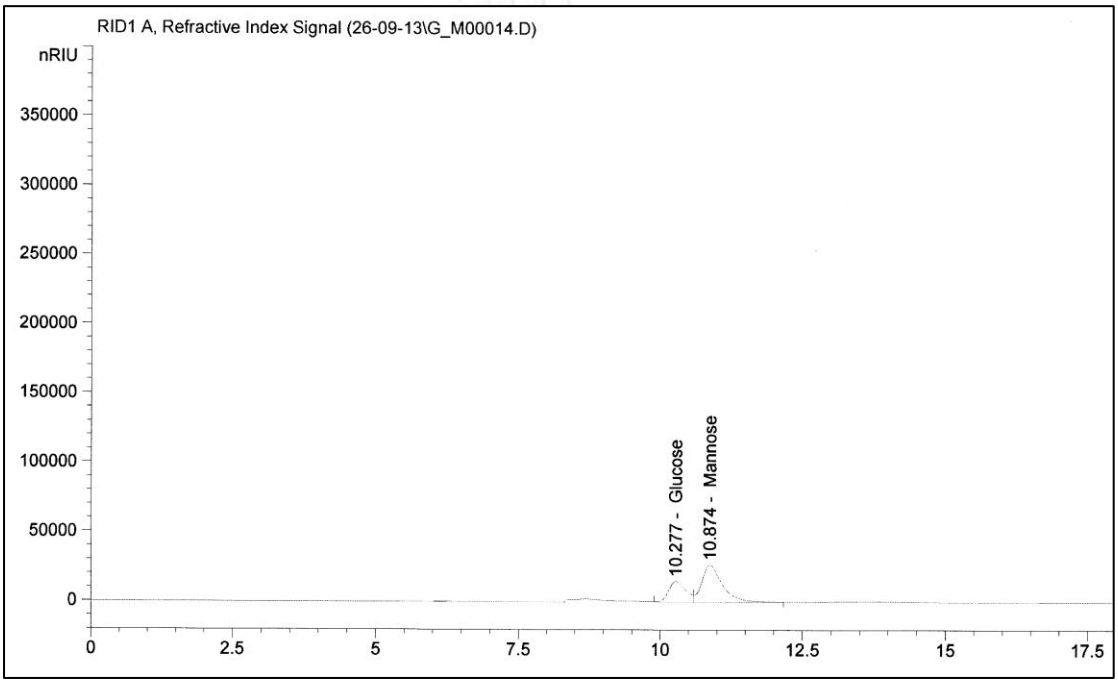


Mannose at exp. RT: 10.835
RID1 A, Refractive Index Signal
Correlation: 0.99983
Residual Std. Dev.: 7849.89790
Formula: y = mx + b
m: 2.99615e6
b: 15851.90662
x: Amount[%]
y: Area

Figure A- 12 Standard calibration curve of glucose and mannose standard at concentration up to 0.40%.

A.2.2 Calculation of the glucomannan content in KGM flour sample

Determination of the glucomannan content was carried out by using the amount obtained by comparing with the peak area of glucose and mannose standard curve under the same condition (see Figure A-13).



RetTime [min]	Type	Area [nRIU*s]	Amt/Area	Amount [%]	Grp	Name
10.277	VV	3.17070e5	4.12429e-7	1.30769e-1		Glucose
10.874	VV	6.97863e5	3.26180e-7	2.27629e-1		Mannose
Totals :				3.58397e-1		

Results obtained with enhanced integrator!

Figure A- 13 Example of chromatogram, peak area and the amount of glucose and mannose from KGM flour.

The experimental procedure was as follows:

KGM flour 0.2014 g (201.4 mg)

↓

Add 10 mL 3M H_2SO_4

↓

Hydrolyzed for 90 min in a boiling water bath

↓

Cool to room temperature

↓

Neutralize by 6M NaOH

↓

Adjust volume to 50 mL

↓

KGM hydrolysates

(201.4 mg / 50 mL = 402.8 mg / 100 mL = 0.4028 g / 100 mL)

↓

Inject 20 μL (0.02 mL) to HPLC

Figure A- 14 Diagram of the determination of konjac glucomannan content by HPLC method.

The calculation was performed as follows:

Glucose 1.30769 x 10^{-1} g in 100 mL solution = 0.130769 g/100mL

Mannose 2.27629 x 10^{-1} g in 100 mL solution = 0.227629 g/100mL

KGM sample (0.4028 g) contains 0.130769 g of glucose

If KGM sample weight 100 g, it contains

(0.130769 × 100)/0.4028 = 32.46 g of glucose (32.46%)

KGM sample (0.4028 g) contains 0.227629 g of mannose

If KGM sample weights 100 g, it contains

(0.227629 × 100)/0.4028 = 56.51 g of mannose (56.51%)

So, the glucomannan content in sample:

% glucose + % mannose = 32.46 + 56.51 = 88.97%

A.3 Determination of sulfurous acid (total) in dried fruit (Colorimetric method) (AOAC Standard method 963.20, 2006)

A.3.1 Preparation of reagent

a) 0.015% formaldehyde solution

Prepare from 40% formaldehyde by diluting in 2 steps: first 10 to 1000, and then 75 of the 10 to 1000 to 2000 mL.

b) Acid-bleached *p*-rosaniline hydrochloride

Place 100 mg *p*-rosaniline HCl and 200 mL H_2O in 1 L volumetric flask. Add 160 mL HCl (1+1) and dilute to volume. Let stand for12 h before use.

c) Sodium tetrachloromercurate

Place 23.4 g NaCl and 54.3 g $HgCl_2$ in 2 L volumetric flask. Dissolve in ca 1900 mL H_2O and dilute to volume.

d) Sulfur dioxide standard solution

Dissolve ca 170 mg $NaHSO_3$ in H_2O and dilute to 1 L. Standardize with 0.01 M I_2 solution before use (ca 100 μg SO_2/mL).

A.3.2 Preparation of standard curve

Add 5 mL mercurate reagent to series of 100 mL volumetric flasks; then add 0, 0.1, 0.2, 0.4, 0.8, 1.0, 2.0, 4.0, 8.0 mL of SO_2 standard solution. Dilute to volume and mix. Transfer 5.0 mL aliquots to 200 mm test tube containing 5 mL rosaniline reagent. Add 10 mL 0.015% formaldehyde solution, mix, and hold 30 min at 22 °C. Read absorbance at 550 nm against 0 standard and plot standard curve.

A.3.3 Determination of Sulfurous acid

Weight 0.50 ± 0.005 g ground sample and transfer to blender with 50 mL H_2O. Cover and blend for 2 min. Withdraw 0.5 mL aliquot from bottom of blender, and transfer to test tube containing 0.08 mL 0.5 M NaOH. Swirl and mix ca 13-30 second. Add 0.08 mL 0.25 M H_2SO_4 and 0.4 mL mercurate reagent, and add DI water to total volume of 2.0 mL. For blank, omit 2 mL sample aliquot.

Transfer 2.0 mL test solution to 200 mm test tube containing 5.0 mL rosaniline reagent. Add 10.0 mL 0.015% formaldehyde solution, mix, and hold for 30 min at 22°C. Read absorbance at 550 nm against blank. Refer to standard curve and convert results to sulphur dioxide (μg/g)

Table A- 3 The absorbance of standard sulphite at different concentration.

Sulphite content (ug/mL)	Absorbance at 550 nm			
	Replicate			Average
	1	2	3	
0.00	0.000	0.000	0.000	0.000
0.10	0.004	0.005	0.005	0.005
0.20	0.009	0.010	0.007	0.009
0.40	0.020	0.019	0.017	0.019
0.80	0.044	0.039	0.038	0.040
1.00	0.059	0.062	0.057	0.059
2.00	0.116	0.116	0.116	0.116
4.00	0.228	0.237	0.223	0.229
8.00	0.439	0.446	0.438	0.441

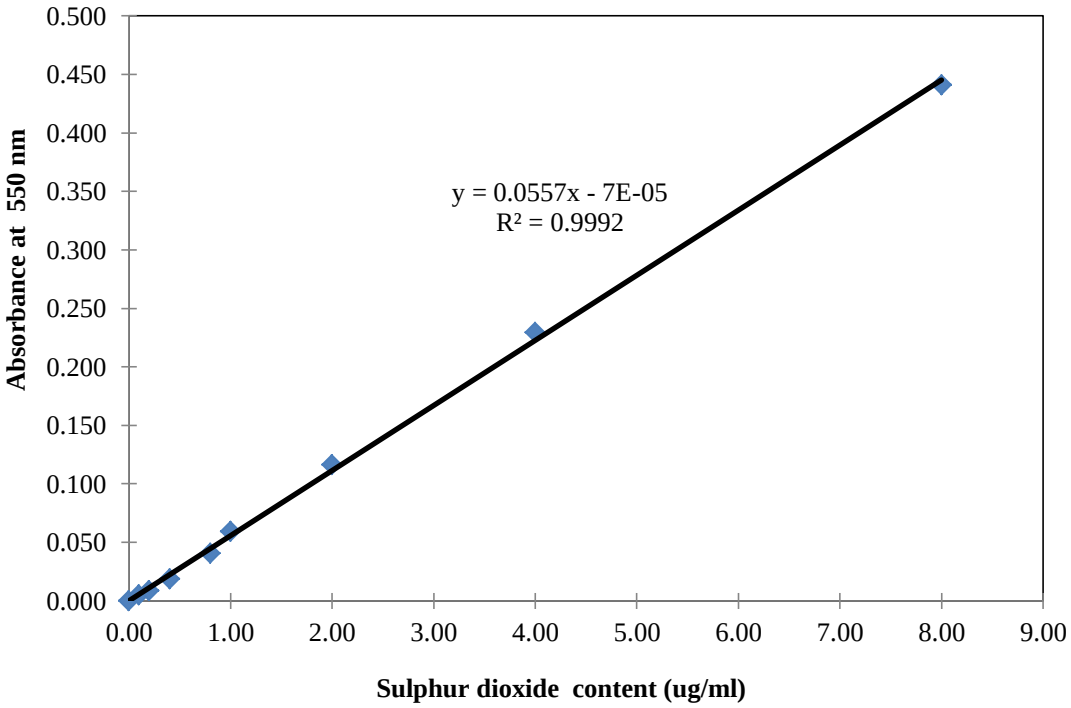


Figure A- 15 Sulphur dioxide standard curve for sulphur dioxide residues analysis.

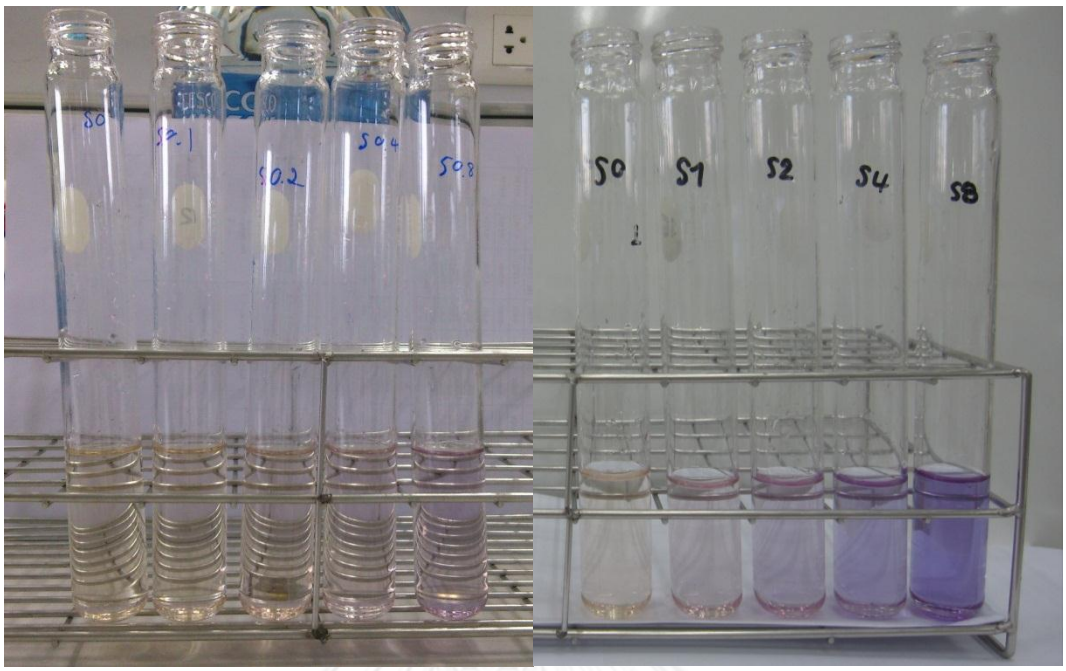


Figure A- 16 The colorimetric reaction of sulphite standard at different concentration.

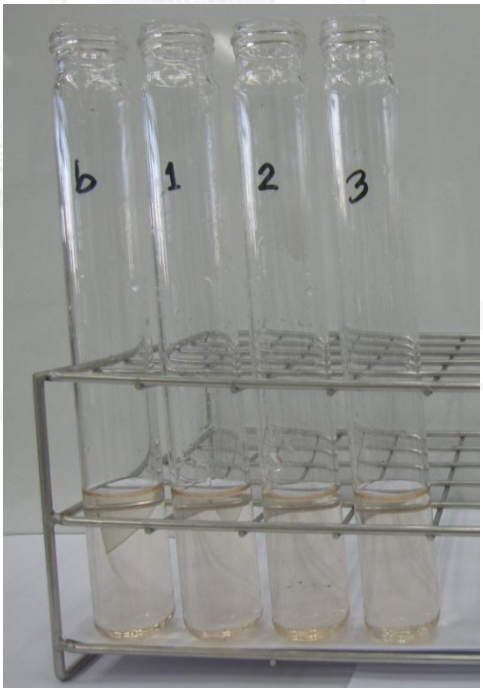


Figure A- 17 The colorimetric reaction of KGM sample and blank.

Sample 0.50 ± 0.005 g

↓

Blend with 50 mL H_2O for 2 min

(0.5 g/50 mL)

↓

Withdraw 0.5 mL aliquot to test tube containing 0.08 mL 0.5 M NaOH

↓

Swirl and mix for 13-30 seconds

↓

Add 0.08 mL 0.25 M H_2SO_4 and 0.4 mL mercurate reagent, and add DI water to total volume of 2.0 mL (0.005 g/2 mL)

↓

Transfer 2.0 mL test solution to test tube containing 5.0 mL rosaniline reagent

↓

Add 10.0 mL 0.015% formaldehyde solution, mix, and hold for 30 min at 22°C

↓

Read absorbance at 550 nm against blank

↓

Refer to standard curve and convert results to sulphur dioxide (μg/g)

Figure A- 18 Diagram of the determination of sulfurous acid residue in KGM flour.

Sample	Weight of sample (g)	Net weight of sample (g)	Absorbance at 550 nm	Sulphite residue (ug)	Sulphite residue (ug/g = ppm)
KGM flour	0.5027	0.0050	0.012	0.2167	43.34

From calibration curve:

$$\mathbf{y = 0.0557x - 0.00007}$$

So,
$$x = \frac{y+0.00007}{0.0557}$$

y = absorbance at 550 nm (0.012)

x = sulphite content (μg)

So,
$$x = \frac{y+0.00007}{0.0557}$$

$$= 0.2167 \ \mu g$$

KGM sample (0.0050 g) has 0.2167 μg of residual sulphite

If KGM sample 1 g, it has (0.2167 / 0.0050) = 43.34 μg/g (ppm) of residual sulphite

A.4 Determination of the residual ethanol by using an electronic nose (e-nose)

The residual ethanol was checked by using an electronic nose (e-nose) instrument (Figure A-19) with chemical sensor array technique (NANOTEC, Thailand). This technique used principles of qualitative analysis to see patterns of each odor attribute to compare the similarities or differences of smell. The e-nose based on a similar principle as human and animal noses was constructed. It comprises an array of chemical sensors that are fabricated from a thin film of polymer-carbon black composite. Since each sensor was made from a different polymer, its response to any volatile chemical is not the same.

Figure A- 19 The e-nose instrument connected to a computer.

When the e-nose 'sniffs' an odour, volatile aroma compound diffuse into the polymer film causing a change in a sensor response, showing numerical values (Tables A-4 to A-6) and producing a set of chromatograms as shown in Figure A-20 and A-21. With appropriate information, the processing algorithm such as principal component analysis or artificial neural network, the e-nose can be trained to recognize an odour by connecting to the database of each odour.

Figure A- 20 Example of chromatogram pattern of odor in KGM sample from five chemicals, temperature and humidity sensors array of e-nose.

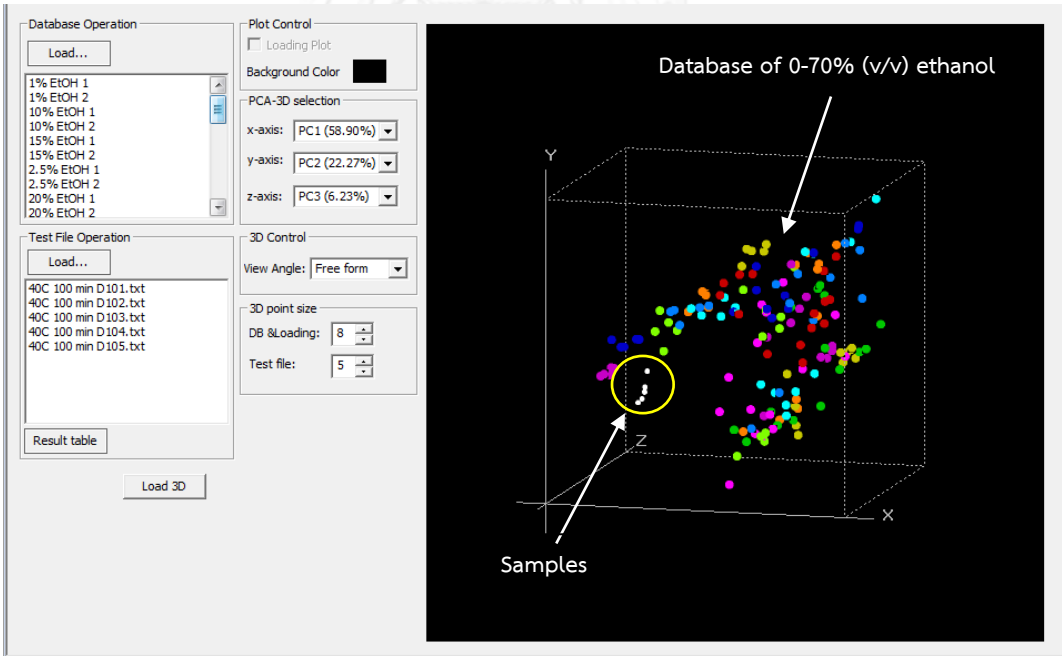


Figure A- 21 Plot pattern of odour from chemical sensors array of e-nose.

Table A- 4 The distance value between the shade dried sample (at increasing drying time) and ethanol database (at different concentrations) from chemical sensors array of e-nose.

Drying condition	rep	Blank	1% EtOH	2.5% EtOH	5% EtOH	10% EtOH	15% EtOH	20% EtOH	25% EtOH	30% EtOH	35% EtOH	40% EtOH	45% EtOH	50% EtOH	55% EtOH	60% EtOH	65% EtOH	70% EtOH
Room temp 0 min	1	16.54	14.41	14.43	13.15	13.07	12.29	12.04	11.48	10.41	6.39	7.88	5.12	10.86	9.75	11.04	10.23	11.72
Room temp 0 min	2	15.41	12.95	12.94	11.67	11.63	10.96	10.75	10.29	9.37	5.65	6.84	4.24	9.81	8.75	10.00	9.28	10.77
Room temp 0 min	3	15.18	12.89	12.99	11.85	12.10	11.40	11.08	10.25	7.97	5.61	5.81	2.76	10.95	9.36	11.08	10.07	11.76
Room temp 0 min	4	16.74	13.94	13.71	12.34	12.27	11.31	10.93	10.05	8.36	4.71	5.95	3.19	10.17	8.61	10.32	9.21	10.87
Room temp 20 min	1	14.97	12.32	12.26	10.99	10.90	10.31	10.18	9.92	9.51	5.80	6.98	4.78	9.16	8.35	9.36	8.84	10.22
Room temp 20 min	2	12.92	9.88	9.98	8.95	9.43	9.01	8.82	8.20	6.06	5.20	4.08	1.98	9.49	8.04	9.62	8.85	10.48
Room temp 20 min	3	14.60	10.83	10.45	9.21	9.48	8.71	8.32	7.29	4.41	1.25	2.53	0.51	9.13	7.27	9.21	8.13	9.85
Room temp 20 min	4	12.24	9.20	9.44	8.59	9.34	9.07	8.88	8.20	5.34	3.99	2.27	2.71	10.16	8.61	10.26	9.49	11.11
Room temp 40 min	1	12.87	9.06	8.93	7.99	8.69	8.30	8.04	7.18	3.80	5.66	2.96	1.62	9.69	7.99	9.76	8.90	10.51
Room temp 40 min	2	15.17	10.52	9.70	8.35	8.52	7.62	7.14	5.88	2.48	4.18	1.43	0.55	8.49	6.49	8.52	7.39	9.04
Room temp 40 min	3	12.64	7.60	7.02	6.09	6.94	6.74	6.53	5.74	2.42	1.77	3.14	4.90	9.07	7.51	9.10	8.43	9.85
Room temp 40 min	4	14.78	10.00	9.16	7.77	7.92	7.05	6.61	5.46	2.53	3.89	0.82	2.85	7.95	6.04	8.00	6.95	8.60
Room temp 60 min	1	13.48	10.00	9.90	8.87	9.42	8.90	8.60	7.68	4.48	5.20	3.09	0.96	9.80	8.04	9.88	8.93	10.60
Room temp 60 min	2	12.21	9.50	9.88	9.16	10.04	9.80	9.59	8.81	5.42	6.73	2.53	3.36	11.07	9.42	11.16	10.32	11.95
Room temp 60 min	3	14.09	10.70	10.53	9.37	9.72	9.06	8.74	7.84	5.18	4.48	3.21	0.95	9.42	7.69	9.53	8.53	10.24
Room temp 60 min	4	13.96	10.72	10.63	9.52	9.94	9.32	9.00	8.09	5.25	1.27	3.44	1.28	9.77	8.03	9.87	8.88	10.58
Room temp 80 min	1	15.10	11.01	10.42	8.98	8.98	8.07	7.67	6.71	4.82	2.88	2.33	0.91	7.96	6.18	8.06	7.00	8.72
Room temp 80 min	2	13.47	10.13	10.06	8.97	9.44	8.91	8.63	7.81	5.12	1.40	3.30	1.45	9.51	7.85	9.61	8.71	10.38
Room temp 80 min	3	14.16	11.12	11.07	9.94	10.30	9.66	9.35	8.48	5.88	4.78	3.89	1.16	9.86	8.17	9.97	8.98	10.69
Room temp 80 min	4	13.48	10.20	10.15	9.07	9.55	9.01	8.74	7.91	5.21	1.44	3.40	1.46	9.60	7.94	9.71	8.79	10.47

Table A-4 (continue) The distance value between the shade dried sample (at increasing drying time) and ethanol database (at different concentrations) from chemical sensors array of e-nose.

Drying condition	rep	Blank	1% EtOH	2.5% EtOH	5% EtOH	10% EtOH	15% EtOH	20% EtOH	25% EtOH	30% EtOH	35% EtOH	40% EtOH	45% EtOH	50% EtOH	55% EtOH	60% EtOH	65% EtOH	70% EtOH
Room temp 100 min	1	12.18	9.12	9.40	8.72	9.65	9.44	9.23	8.41	4.76	6.87	2.58	3.78	10.99	9.32	11.06	10.23	11.84
Room temp 100 min	2	12.17	7.83	7.60	6.62	7.36	7.14	6.99	6.37	3.83	2.22	2.94	3.51	8.83	7.35	8.90	8.25	9.77
Room temp 100 min	3	12.32	9.53	9.87	9.14	10.02	9.76	9.54	8.73	5.25	2.45	4.41	3.39	11.06	9.39	11.14	10.29	11.93
Room temp 100 min	4	11.94	9.09	9.48	8.82	9.77	9.59	9.40	8.64	5.19	6.92	4.49	2.70	11.06	9.43	11.14	10.34	11.95
Room temp 120 min	1	13.19	9.30	9.07	7.97	8.48	8.00	7.72	6.90	4.09	4.87	2.55	1.26	9.02	7.35	9.10	8.24	9.88
Room temp 120 min	2	14.40	10.74	10.39	9.05	9.16	8.42	8.12	7.37	5.68	3.59	0.94	3.25	8.32	6.74	8.44	7.53	9.20
Room temp 120 min	3	11.71	8.68	9.07	8.50	9.55	9.44	9.27	8.51	4.86	7.30	3.07	4.28	11.20	9.57	11.27	10.49	12.07
Room temp 120 min	4	13.49	10.13	10.07	9.00	9.50	8.96	8.67	7.81	1.19	4.99	3.25	1.61	9.65	7.95	9.75	8.82	10.50
Room temp 140 min	1	13.83	10.01	9.69	8.42	8.68	8.04	7.76	6.99	4.96	3.96	2.73	1.17	8.40	6.80	8.52	7.64	9.30
Room temp 140 min	2	13.86	10.01	9.72	8.60	9.06	8.45	8.11	7.11	3.84	0.55	2.48	2.12	9.37	7.55	9.44	8.45	10.13
Room temp 140 min	3	12.87	9.51	9.55	8.63	9.33	8.94	8.68	7.83	4.55	5.74	3.43	1.54	10.09	8.39	10.17	9.29	10.93
Room temp 140 min	4	12.48	9.50	9.71	8.82	9.50	9.18	8.98	8.28	2.18	5.81	4.00	2.44	10.11	8.55	10.22	9.42	11.05
Room temp 160 min	1	13.21	9.25	8.96	7.77	8.16	7.66	7.42	6.71	4.57	1.84	2.62	1.91	8.43	6.88	8.54	7.75	9.37
Room temp 160 min	2	12.41	9.42	9.65	8.77	9.47	9.16	8.97	8.27	5.47	2.22	4.01	2.51	10.13	8.58	10.24	9.45	11.07
Room temp 160 min	3	11.34	8.15	8.54	7.91	8.93	8.88	8.75	8.13	5.00	3.03	4.36	3.94	10.58	9.07	10.66	9.97	11.52
Room temp 160 min	4	13.95	10.02	9.64	8.39	8.69	8.02	7.70	6.82	1.09	4.03	2.32	1.26	8.56	6.85	8.66	7.72	9.40
Room temp 180 min	1	8.81	3.40	4.15	4.33	6.08	7.12	7.45	7.77	6.89	9.40	6.94	7.78	10.18	9.53	10.25	10.30	11.32
Room temp 180 min	2	10.22	4.14	3.73	3.19	4.59	5.60	5.99	6.51	6.60	8.50	6.39	7.52	8.58	8.13	8.65	8.83	9.75
Room temp 180 min	3	10.07	4.08	3.80	3.33	4.75	5.76	6.15	6.67	6.71	8.58	6.48	7.54	8.70	8.25	8.77	8.96	9.88
Room temp 180 min	4	10.31	4.02	3.45	2.90	4.37	5.44	5.85	6.42	6.62	8.63	6.51	7.73	8.54	8.13	8.61	8.82	9.70

Table A-4 (continue) The distance value between the shade dried sample (at increasing drying time) and ethanol database (at different concentrations) from chemical sensors array of e-nose.

Drying condition	rep	Blank	1% EtOH	2.5% EtOH	5% EtOH	10% EtOH	15% EtOH	20% EtOH	25% EtOH	30% EtOH	35% EtOH	40% EtOH	45% EtOH	50% EtOH	55% EtOH	60% EtOH	65% EtOH	70% EtOH
Room temp 240 min	1	8.54	2.13	3.28	4.43	6.56	7.90	8.27	8.63	7.60	11.26	8.45	9.75	11.65	11.02	11.67	11.77	12.66
Room temp 240 min	2	7.24	2.55	4.67	5.89	8.01	9.30	9.65	9.92	8.40	12.02	9.16	10.10	12.88	12.17	12.92	12.95	13.93
Room temp 240 min	3	7.91	2.13	3.86	5.06	7.19	8.52	8.88	9.21	7.98	11.59	8.76	9.89	12.17	11.52	12.21	12.29	13.21
Room temp 240 min	4	8.33	1.99	3.28	4.19	6.25	7.59	7.99	8.43	7.64	10.75	8.13	9.23	11.10	10.55	11.14	11.29	12.19
Room temp 300 min	1	1.81	6.67	9.66	10.92	12.85	14.19	14.61	15.00	13.37	15.62	13.33	12.97	17.02	16.49	17.12	17.23	18.27
Room temp 300 min	2	2.15	6.85	9.80	11.00	12.93	14.21	14.60	14.93	13.13	15.40	13.09	12.66	17.00	16.40	17.10	17.16	18.24
Room temp 300 min	3	2.63	6.20	9.13	10.30	12.21	13.50	13.89	14.24	12.57	14.80	12.50	12.15	16.28	15.71	16.39	16.47	17.53
Room temp 300 min	4	2.10	6.57	9.56	10.83	12.80	14.11	14.50	14.82	12.99	15.47	13.06	12.76	17.01	16.40	17.11	17.17	18.24
Room temp 360 min	1	1.66	8.02	11.08	12.70	14.76	16.28	16.73	17.17	15.49	18.36	15.83	15.75	19.50	19.01	19.58	19.75	20.71
Room temp 360 min	2	1.60	8.16	11.23	12.84	14.89	16.40	16.85	17.28	15.58	18.42	15.90	15.79	19.59	19.10	19.68	19.84	20.81
Room temp 360 min	3	1.44	6.40	9.45	10.95	12.91	14.43	14.91	15.43	14.11	16.58	14.23	14.14	17.48	17.09	17.57	17.80	18.72
Room temp 360 min	4	1.62	8.26	11.32	12.93	14.98	16.49	16.94	17.38	15.68	18.50	15.99	15.86	19.68	19.19	19.77	19.93	20.89
Room temp 420 min	1	1.43	7.90	10.96	12.59	14.60	16.16	16.64	17.14	15.66	18.37	15.92	15.83	19.33	18.92	19.42	19.64	20.56
Room temp 420 min	2	1.28	7.95	11.01	12.62	14.62	16.17	16.65	17.16	15.69	18.33	15.91	15.79	19.31	18.90	19.39	19.62	20.54
Room temp 420 min	3	1.09	8.04	11.10	12.67	14.66	16.20	16.68	17.19	15.74	18.28	15.90	15.72	19.27	18.88	19.36	19.59	20.52
Room temp 420 min	4	1.11	8.26	11.32	12.88	14.85	16.38	16.87	17.38	15.92	18.40	16.05	15.83	19.42	19.03	19.52	19.75	20.67
Room temp 480 min	1	1.77	8.11	11.17	12.81	14.86	16.40	16.87	17.32	15.69	18.56	16.04	15.97	19.63	19.17	19.72	19.90	20.84
Room temp 480 min	2	1.53	8.05	11.12	12.74	14.78	16.31	16.77	17.23	15.60	18.41	15.91	15.81	19.50	19.04	19.59	19.78	20.72
Room temp 480 min	3	1.49	8.11	11.18	12.80	14.83	16.37	16.83	17.30	15.70	18.47	15.99	15.88	19.55	19.09	19.63	19.83	20.77
Room temp 480 min	4	1.42	8.01	11.08	12.69	14.73	16.26	16.73	17.20	15.61	18.37	15.90	15.79	19.44	18.99	19.52	19.72	20.66

* The highlighted numerical values show the shortest distance between the sample and ethanol database

Table A- 5 The distance value between the sample (drying at 40 °C with increasing drying time) and ethanol database (at different concentrations) from chemical sensors array of e-nose.

Drying condition	rep	Blank	1% EtOH	2.5% EtOH	5% EtOH	10% EtOH	15% EtOH	20% EtOH	25% EtOH	30% EtOH	35% EtOH	40% EtOH	45% EtOH	50% EtOH	55% EtOH	60% EtOH	65% EtOH	70% EtOH
40C 0 min	1	8.13	4.82	4.04	6.78	4.48	5.15	4.52	7.54	5.22	6.05	6.28	3.17	8.25	7.50	8.30	8.27	9.31
40C 0 min	2	9.07	5.87	5.00	3.86	4.78	5.02	3.45	6.55	4.28	5.35	5.82	3.29	7.78	6.79	7.83	7.62	8.79
40C 0 min	3	8.16	6.48	7.26	7.15	8.63	9.01	4.66	8.38	5.50	8.88	9.62	4.35	11.56	10.23	11.62	11.13	12.53
40C 0 min	4	8.55	5.90	5.80	5.34	6.69	7.01	6.05	7.56	4.09	7.07	7.65	3.50	9.86	8.59	9.90	9.48	10.77
40C 20 min	1	9.75	7.58	7.68	6.97	7.97	7.93	2.84	6.26	4.17	7.24	8.22	4.16	9.88	8.40	9.95	9.31	10.81
40C 20 min	2	8.22	7.33	8.57	8.73	10.34	10.78	6.13	9.78	5.53	10.61	11.37	7.27	13.34	11.94	13.40	12.85	14.29
40C 20 min	3	8.27	6.84	7.76	7.64	9.09	9.44	4.90	8.37	4.61	9.21	10.02	5.99	11.81	10.47	11.88	11.37	12.80
40C 20 min	4	9.10	7.21	7.65	7.29	8.59	8.75	3.83	7.62	3.43	8.32	9.17	5.42	11.10	9.64	11.16	10.55	12.00
40C 40 min	1	7.63	6.46	7.64	7.86	9.53	10.07	5.87	5.38	6.50	10.10	10.76	7.40	12.84	11.52	12.89	12.42	13.79
40C 40 min	2	7.92	6.30	7.15	7.20	8.79	9.26	5.08	4.64	5.69	9.25	9.91	6.85	12.02	10.70	12.07	11.60	12.96
40C 40 min	3	7.63	6.38	7.50	7.82	9.54	10.12	5.49	10.13	6.49	10.24	10.82	7.92	13.07	11.75	13.11	12.65	13.98
40C 40 min	4	7.36	5.25	5.91	6.04	7.74	8.37	5.08	9.15	4.83	8.72	9.18	7.33	11.46	10.28	11.50	11.15	12.40
40C 60 min	1	6.31	4.93	6.32	6.74	8.55	9.34	6.18	9.91	6.86	9.81	10.28	7.86	12.34	11.26	12.39	12.12	13.36
40C 60 min	2	5.55	4.43	6.15	6.83	8.76	9.71	6.96	10.70	7.55	10.40	10.76	8.69	12.87	11.88	12.93	12.73	13.91
40C 60 min	3	5.92	4.42	5.86	6.49	8.41	9.32	6.58	10.48	7.07	10.00	10.35	8.55	12.56	11.54	12.61	12.39	13.57
40C 60 min	4	5.61	4.45	6.13	6.78	8.70	9.62	6.85	10.56	7.47	10.29	10.67	8.55	12.76	11.76	12.81	12.61	13.80
40C 80 min	1	2.91	4.57	7.40	8.82	10.92	12.27	10.42	13.97	10.97	13.47	13.62	11.92	15.65	14.94	15.71	15.73	16.76
40C 80 min	2	3.05	4.20	6.96	8.31	10.41	11.73	9.88	13.44	10.44	12.91	13.07	11.42	15.11	14.38	15.16	15.18	16.21
40C 80 min	3	2.97	4.93	7.75	9.26	11.39	12.77	10.97	14.60	11.45	14.02	14.13	12.54	16.23	15.52	16.29	16.32	17.33
40C 80 min	4	3.49	3.81	6.41	7.65	9.73	10.99	9.08	12.66	9.65	12.11	12.29	10.68	14.34	13.58	14.40	14.38	15.44

Table A-5 (continue) The distance value between the sample (drying at 40 °C with increasing drying time) and ethanol database (at different concentrations) from chemical sensors array of e-nose.

Drying condition	rep	Blank	1% EtOH	2.5% EtOH	5% EtOH	10% EtOH	15% EtOH	20% EtOH	25% EtOH	30% EtOH	35% EtOH	40% EtOH	45% EtOH	50% EtOH	55% EtOH	60% EtOH	65% EtOH	70% EtOH
40C 100 min	1	2.79	3.84	6.63	8.07	10.20	11.57	9.98	13.59	10.49	12.86	12.95	11.66	15.04	14.36	15.09	15.14	16.14
40C 100 min	2	3.23	4.62	7.36	8.78	10.90	12.22	10.31	13.97	10.80	13.41	13.55	11.91	15.67	14.91	15.72	15.72	16.75
40C 100 min	3	3.18	4.27	6.98	8.38	10.50	11.83	9.99	13.65	10.48	13.03	13.16	11.65	15.28	14.54	15.34	15.34	16.37
40C 100 min	4	2.98	3.95	6.71	8.11	10.23	11.57	9.86	13.47	10.38	12.81	12.93	11.51	15.02	14.31	15.07	15.10	16.11
40C 120 min	1	2.02	5.64	8.65	10.36	12.45	14.02	12.92	16.29	13.48	15.57	15.55	14.33	17.47	16.99	17.53	17.73	18.62
40C 120 min	2	2.35	5.92	8.93	10.63	12.73	14.28	13.07	16.47	13.63	15.80	15.80	14.47	17.73	17.23	17.80	17.98	18.89
40C 120 min	3	2.94	6.33	9.28	11.00	13.13	14.66	13.32	16.83	13.82	16.15	16.16	14.78	18.17	17.62	18.23	18.38	19.30
40C 120 min	4	1.88	5.33	8.36	10.01	12.11	13.64	12.42	15.80	13.00	15.13	15.15	13.82	17.06	16.56	17.13	17.30	18.22
40C 140 min	1	2.99	7.37	10.35	12.13	14.22	15.83	14.74	18.11	15.29	17.43	17.39	16.07	19.29	18.85	19.36	19.58	20.46
40C 140 min	2	2.69	6.85	9.85	11.60	13.68	15.27	14.16	17.49	14.74	16.84	16.83	15.47	18.70	18.25	18.77	18.99	19.87
40C 140 min	3	2.53	6.46	9.49	11.19	13.28	14.84	13.66	16.98	14.26	16.37	16.38	14.95	18.24	17.77	18.31	18.51	19.41
40C 140 min	4	2.22	6.03	9.06	10.77	12.84	14.43	13.39	16.69	13.99	16.00	15.98	14.72	17.84	17.40	17.90	18.13	19.01
40C 160 min	1	2.26	7.78	10.81	12.52	14.58	16.16	14.94	18.16	15.58	17.69	17.71	16.07	19.49	19.05	19.57	19.79	20.69
40C 160 min	2	2.54	7.16	10.17	11.92	13.98	15.59	14.57	17.83	15.18	17.20	17.17	15.82	18.99	18.58	19.06	19.30	20.17
40C 160 min	3	3.07	7.16	10.12	11.92	14.00	15.63	14.71	18.05	15.26	17.29	17.22	16.07	19.11	18.71	19.17	19.43	20.27
40C 160 min	4	2.79	7.19	10.17	11.95	14.02	15.64	14.69	17.98	15.28	17.28	17.23	15.99	19.07	18.68	19.14	19.40	20.25

* The highlighted numerical values show the shortest distance between the sample and ethanol database

Table A- 6 The distance value between the sample (drying at 50 °C with increasing drying time) and ethanol database (at different concentrations) from chemical sensors array of e-nose.

Drying condition	rep	Blank	1% EtOH	2.5% EtOH	5% EtOH	10% EtOH	15% EtOH	20% EtOH	25% EtOH	30% EtOH	35% EtOH	40% EtOH	45% EtOH	50% EtOH	55% EtOH	60% EtOH	65% EtOH	70% EtOH
50C 0 min	1	17.42	14.36	13.93	6.88	11.92	6.81	9.07	6.05	10.64	5.75	7.99	5.40	9.19	9.06	10.08	7.73	6.52
50C 0 min	2	17.48	13.67	12.92	6.17	10.65	5.02	7.38	4.66	8.81	3.60	6.17	3.31	7.44	7.27	8.42	6.24	7.39
50C 0 min	3	16.82	13.23	12.62	5.70	10.53	5.09	7.40	4.60	8.93	4.08	6.28	3.70	7.50	7.39	8.45	6.22	7.46
50C 0 min	4	15.24	11.27	10.67	4.21	8.95	2.56	4.72	2.20	6.35	2.80	3.72	2.17	4.88	4.77	5.75	3.61	9.96
50C 20 min	1	15.77	11.40	10.55	4.23	8.49	2.46	4.81	1.71	6.29	2.18	3.67	2.88	4.86	4.87	5.95	4.12	9.48
50C 20 min	2	16.68	12.04	10.96	4.71	8.45	3.57	5.93	2.34	7.24	2.47	4.73	4.36	5.88	6.03	7.16	5.56	8.22
50C 20 min	3	16.25	11.33	10.16	4.32	7.70	2.52	4.84	1.78	6.09	1.87	3.63	3.83	4.75	4.96	6.10	4.76	9.32
50C 20 min	4	14.91	10.17	9.27	4.04	7.55	0.46	2.71	2.22	4.24	2.58	1.64	2.29	2.78	2.87	3.90	2.61	11.49
50C 40 min	1	12.88	8.06	7.39	1.35	6.11	3.02	3.93	4.51	5.47	4.82	3.71	4.10	3.98	4.76	5.17	4.40	11.51
50C 40 min	2	13.04	8.12	7.37	1.34	5.97	3.04	4.02	4.47	5.52	4.76	3.75	4.23	4.04	4.84	5.28	4.54	11.37
50C 40 min	3	13.31	8.29	7.45	1.41	5.85	3.08	4.19	4.38	5.64	4.64	3.83	4.38	4.17	4.98	5.49	4.75	11.11
50C 40 min	4	13.48	8.64	7.85	1.50	6.23	3.01	4.33	4.15	5.84	4.44	3.87	4.17	4.34	5.05	5.62	4.68	10.79
50C 60 min	1	1.93	5.52	8.59	10.55	12.08	13.43	12.38	15.39	13.17	15.80	13.37	13.40	12.61	13.35	12.44	12.87	21.03
50C 60 min	2	1.93	5.34	8.41	10.28	11.87	13.12	12.06	15.08	12.86	15.50	13.05	13.09	12.30	13.03	12.11	12.55	20.81
50C 60 min	3	2.17	4.61	7.59	9.02	10.85	11.93	10.95	13.86	11.82	14.28	11.90	11.92	11.18	11.94	11.08	11.43	19.60
50C 60 min	4	2.16	4.46	7.44	8.86	10.67	11.80	10.84	13.73	11.71	14.14	11.78	11.82	11.07	11.83	10.98	11.34	19.44
50C 80 min	1	1.27	7.39	10.46	12.61	14.08	15.47	14.38	17.42	15.13	17.85	15.39	15.33	14.62	15.32	14.35	14.79	22.91
50C 80 min	2	1.18	6.62	9.69	11.50	13.20	14.35	13.30	16.28	14.10	16.72	14.29	14.19	13.55	14.23	13.30	13.67	21.86
50C 80 min	3	1.53	6.23	9.29	11.00	12.75	13.87	12.84	15.80	13.66	16.23	13.82	13.73	13.09	13.78	12.87	13.22	21.38
50C 80 min	4	2.10	5.80	8.84	10.22	12.16	13.13	12.17	15.04	13.05	15.47	13.11	12.99	12.42	13.12	12.24	12.52	20.59

Table A-6 (continue) The distance value between the sample (drying at 50 °C with increasing drying time) and ethanol database (at different concentrations) from chemical sensors array of e-nose.

Drying condition	rep	Blank	1% EtOH	2.5% EtOH	5% EtOH	10% EtOH	15% EtOH	20% EtOH	25% EtOH	30% EtOH	35% EtOH	40% EtOH	45% EtOH	50% EtOH	55% EtOH	60% EtOH	65% EtOH	70% EtOH
50C 100 min	1	1.31	7.79	10.85	12.98	14.49	15.83	14.73	17.77	15.49	18.20	15.75	15.66	14.98	15.66	14.69	15.12	23.24
50C 100 min	2	1.49	7.84	10.90	13.11	14.55	15.95	14.84	17.90	15.58	18.33	15.86	15.79	15.09	15.77	14.80	15.24	23.38
50C 100 min	3	1.67	8.16	11.22	13.42	14.90	16.17	15.02	18.13	15.74	18.57	16.06	15.97	15.28	15.93	14.93	15.39	23.73
50C 100 min	4	1.42	8.23	11.30	13.34	14.93	16.16	15.05	18.09	15.81	18.53	16.07	15.93	15.31	15.97	14.99	15.39	23.57
50C 120 min	1	1.35	8.04	11.11	13.19	14.74	16.02	14.92	17.95	15.67	18.39	15.93	15.81	15.17	15.84	14.86	15.27	23.43
50C 120 min	2	1.30	7.96	11.02	13.10	14.65	15.95	14.86	17.88	15.62	18.32	15.87	15.75	15.11	15.78	14.81	15.22	23.34
50C 120 min	3	1.35	7.63	10.70	12.87	14.34	15.73	14.63	17.67	15.37	18.10	15.65	15.58	14.87	15.56	14.59	15.03	23.15
50C 120 min	4	1.39	7.87	10.93	13.08	14.58	15.91	14.80	17.85	15.54	18.29	15.82	15.73	15.05	15.72	14.74	15.18	23.36

* The highlighted numerical values show the shortest distance between the sample and ethanol database

Table A- 7 Drying conditions and the amount of residual ethanol.

Drying condition	Shade drying at room temperature (30 °C)	Hot air drying at 40 °C	Hot air drying at 50 °C
Drying time (min)	% Ethanol residual		
0	45	45	45
20	43.75	27.50	22.50
40	41.25	25	5
60	41.25	1	0
80	40	0	0
100	38.75	0	0
120	38.75	0	0
140	38.75	0	-
160	33.75	0	-
180	4	-	-
240	1	-	-
300	0	-	-
360	0	-	-
420	0	-	-
480	0	-	-

Results are means from 4 replicates

APPENDIX B

Experimental procedures

B.1 Determination of anti-browning agents and soaking time for browning control of konjac slices

Fresh konjac corms

↓

Peeling and cutting

↓

Immersing in anti-browning solution

Sulphur containing compounds	**Sulphur- free compounds**
- sodium metabisulphite	- potassium hydroxide
- potassium aluminium sulphate	- sodium hypochlorite
	- sodium tetraborate
	- sodium chloride
	- ascorbic acid
	- citric acid

↓

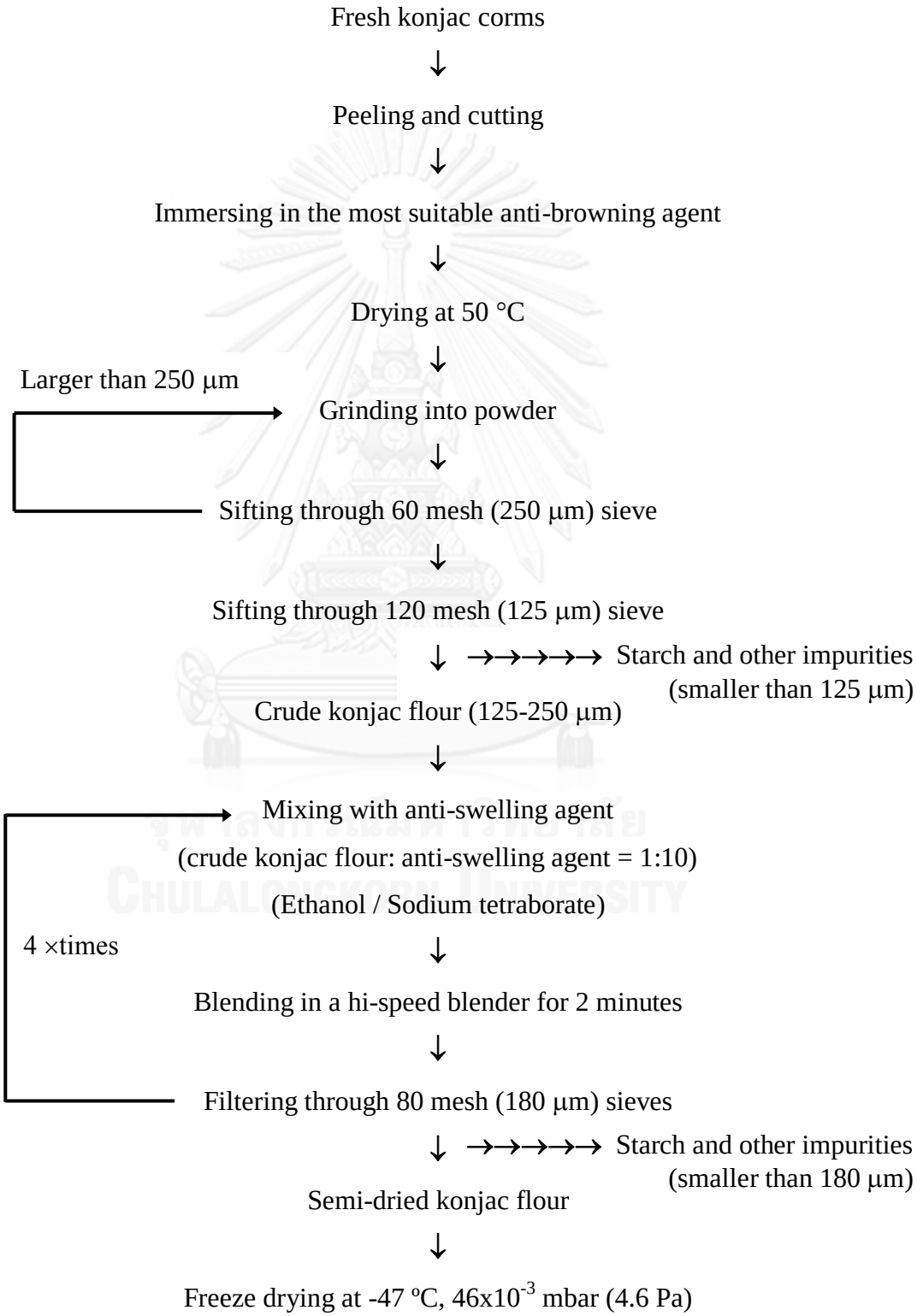
Hot air drying at 50 °C

↓

Whiteness index value measurement

Figure B- 1 Diagram of the determination of anti-browning agents and soaking time for browning control of konjac slices.

B.2 Determination of anti-swelling agents for wet extraction process



↓

KGM flour

(final moisture content 5-8% (d.b.))

↓

Grinding and sifting through 120 mesh (125 μm) sieve

↓ →→→→→ Starch and other impurities (smaller than 125 μm)

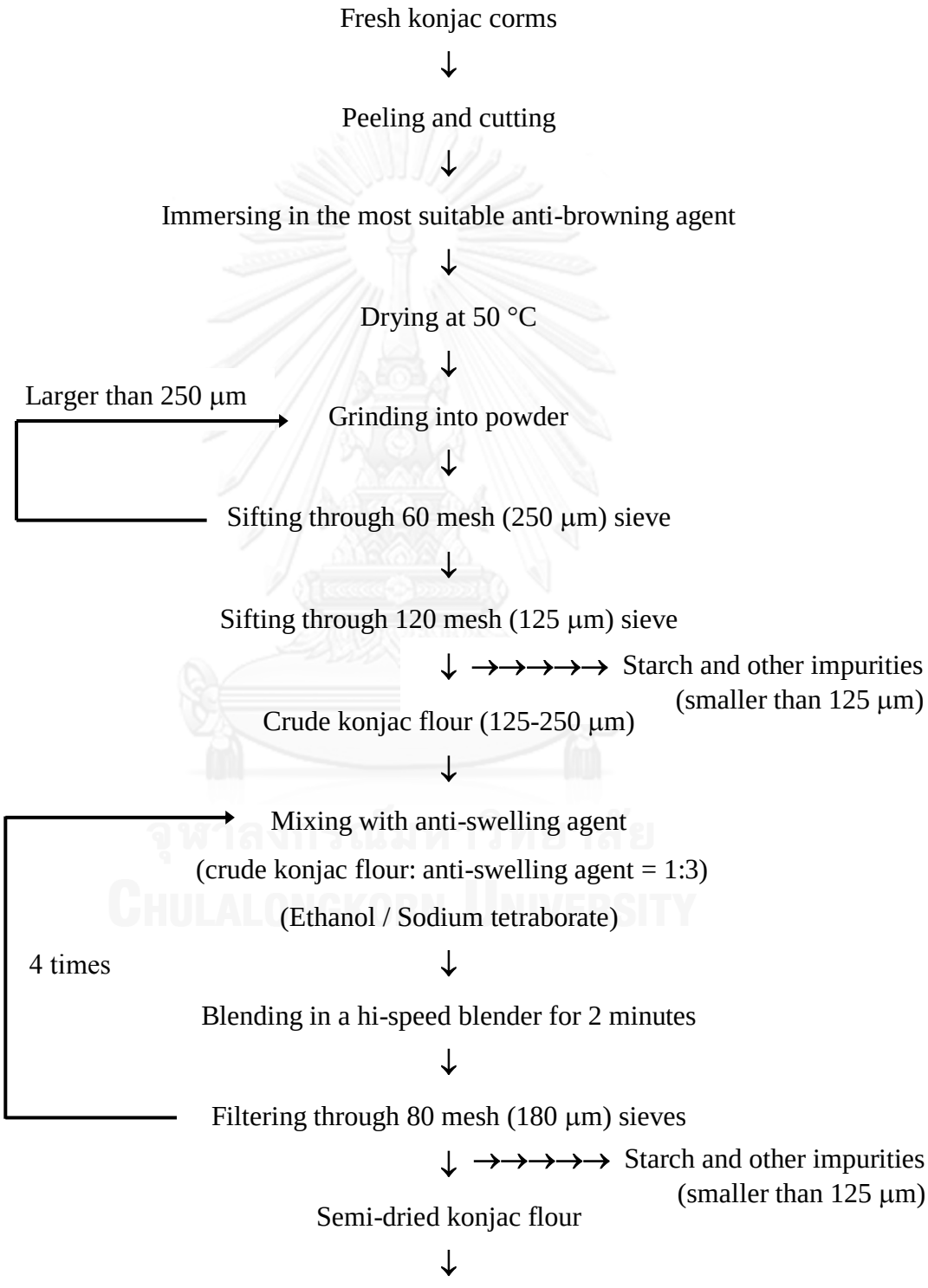
KGM flour (180-250 μm)

↓

Analysis of Physical properties	**Analysis of Chemical properties**
% Extraction yield % Water uptake Whiteness index value Viscosity Morphology	Glucomannan content

Figure B- 2 Diagram of the determination of anti-swelling agents for wet extraction process.

B.3 Study the effects of different drying methods on some physical and chemical properties of purified KGM flour



Hot air drying	Multistage hot air drying	Freeze drying	Microwave-vacuum drying
50, 60, 70, 80 °C	50+80 °C, 60+80 °C, 70+80 °C	-47 ºC, 4.6 Pa	Microwave power: 960 W, 1200 W, 1440 W Controlled pressure: 80 kPa (600 mmHg) Controlled frequency: 2450 MHz

↓

KGM flour

(final moisture content 5-8% (d.b.))

↓

Grinding and sifting through 120 mesh (125 μm) sieve

↓ →→→→→ Starch and other impurities (smaller than 125 μm)

KGM flour (180-250 μm)

↓

Physical properties	Chemical properties	Structure characterization
Moisture content Water activity Whiteness index value Bulk, Particle density Porosity Apparent viscosity	KGM content (3,5-DNS and HPLC) Residual sulphite content	Microstructure characterisation (SEM) Morphological characterisation (Image analyser) KGM characterisation (FTIR)

Figure B- 3 Diagram of the study of the effects of different drying methods on some physical and chemical properties of purified KGM flour.

APPENDIX C

Raw data

Table C- 1 Whiteness index of fresh and dried konjac slices without treatment.

Sample	Whiteness index value
Fresh konjac slices	59.48 ± 2.77
Dried konjac slices (Not treated)	53.98 ± 3.74

Results are Mean ± SD (n = 30)

Table C- 2 Whiteness index of konjac slices after treatment with sulphur containing anti-browning compound and hot air drying at 50 °C.

Concentration of anti-browning agent (ppm)	**Soaking time**		
	10 min	**20 min**	**30 min**
DI water	70.98^{a} ± 2.94	**72.36^{a} ± 1.86**	68.78^{b} ± 3.90
Sodium metabisulphite			
500	**80.73^{bc} ± 1.80**	78.96^{de} ± 1.62	79.38^{d} ± 2.15
1000	79.82^{cd} ± 2.78	79.29^{de} ± 1.72	79.74^{cd} ± 1.86
1500	79.31^{de} ± 1.28	81.14^{ab} ± 1.87	82.19^{a} ± 2.93
2000	74.88^{g} ± 2.46	78.18^{e} ± 2.76	76.93^{f} ± 1.94
Potassium aluminium sulphate			
500	**69.82^{a} ± 3.11**	58.59^{f} ± 3.05	64.00^{e} ± 3.58
1000	65.12^{de} ± 4.55	65.07^{de} ± 2.76	67.14^{bcd} ± 3.82
1500	69.17^{ab} ± 2.70	55.81^{g} ± 4.56	68.43^{abc} ± 2.50
2000	66.18^{cde} ± 3.63	55.28^{g} ± 3.95	67.95^{abc} ± 3.24

Results are Mean ± SD (n = 30)

Letters a, b, c, … in the same row and column for each type of anti-browning agent indicate a statistically significant difference at $p \leq 0.05$)

Table C- 3. Whiteness index of konjac slices after treatment with sulphur-free anti-browning compound and hot air drying at 50 °C.

Concentration of anti-browning agent (ppm)	Soaking time		
	10 min	**20 min**	**30 min**
Sodium hypochlorite			
500	$62.57^{a} \pm 5.08$	$52.35^{d} \pm 2.75$	$57.47^{b} \pm 3.56$
1000	$63.02^{a} \pm 4.14$	$55.04^{c} \pm 3.67$	**$63.86^{a} \pm 3.20$**
1500	$63.69^{a} \pm 2.71$	$54.57^{c} \pm 1.96$	$63.84^{a} \pm 3.68$
2000	$58.35^{b} \pm 3.70$	$50.19^{e} \pm 3.34$	$58.13^{b} \pm 3.51$
Potassium hydroxide			
10	$69.05^{e} \pm 1.80$	**$74.91^{a} \pm 1.00$**	$72.07^{cd} \pm 1.44$
50	$72.91^{bc} \pm 1.60$	$74.82^{a} \pm 3.70$	$74.44^{ab} \pm 1.84$
100	$68.89^{e} \pm 4.36$	$70.05^{e} \pm 4.44$	$73.64^{abc} \pm 2.48$
150	$70.47^{de} \pm 2.30$	$73.37^{abc} \pm 2.23$	$71.90^{cd} \pm 2.63$
Sodium tetraborate			
2000	$60.22^{f} \pm 4.31$	$62.66^{e} \pm 4.24$	$56.40^{g} \pm 3.92$
4000	$65.88^{cd} \pm 2.65$	$67.87^{b} \pm 3.70$	$64.29^{de} \pm 2.57$
6000	**$70.62^{a} \pm 3.04$**	$68.19^{b} \pm 3.17$	$69.09^{ab} \pm 3.87$
8000	$67.33^{bc} \pm 2.48$	$60.42^{f} \pm 3.51$	$68.45^{b} \pm 4.77$
Sodium chloride			
5000	$70.78^{d} \pm 1.82$	$73.57^{bc} \pm 2.43$	$75.32^{b} \pm 4.86$
10000	$73.52^{c} \pm 3.08$	**$78.39^{a} \pm 1.58$**	$74.68^{bc} \pm 1.89$
20000	$70.67^{d} \pm 3.33$	$73.35^{c} \pm 5.19$	$77.25^{a} \pm 1.68$
30000	$70.94^{d} \pm 4.16$	$73.61^{bc} \pm 2.99$	$67.88^{e} \pm 3.65$
Ascorbic acid			
2000	$59.83^{a} \pm 1.10$	$59.60^{a} \pm 2.40$	$56.58^{cd} \pm 1.20$
4000	$58.88^{ab} \pm 1.87$	$54.07^{e} \pm 3.23$	$55.48^{de} \pm 1.40$
6000	$59.93^{a} \pm 2.07$	$54.70^{e} \pm 1.75$	$55.08^{e} \pm 2.38$
8000	**$60.38^{a} \pm 2.14$**	$57.72^{bc} \pm 4.09$	$56.63^{cd} \pm 2.08$
10000	$56.75^{cd} \pm 1.72$	$54.70^{e} \pm 3.20$	$55.03^{e} \pm 2.41$
Citric acid			
2000	$62.09^{hi} \pm 1.66$	$59.35^{j} \pm 3.54$	$62.50^{gh} \pm 2.04$
4000	$60.54^{ij} \pm 2.29$	$65.90^{cde} \pm 3.57$	$66.31^{bcd} \pm 1.80$
6000	$65.06^{def} \pm 4.26$	$64.10^{efg} \pm 2.90$	$65.99^{cde} \pm 1.98$
8000	$64.68^{def} \pm 4.26$	**$68.27^{a} \pm 2.37$**	$67.21^{abc} \pm 2.37$
10000	$68.05^{ab} \pm 2.19$	$68.04^{ab} \pm 1.77$	$63.73^{fgh} \pm 3.99$

Results are Mean ± SD (n = 30)

Letters a, b, c, … in the same row and column for each type of anti-browning agent indicate a statistically significant difference at $p \leq 0.05$).

Table C- 4 Physicochemical properties of KGM flour after using different anti-swelling agents.

Anti-swelling agent	% Yield of extraction	% Water uptake	Whiteness index	Viscosity (mPa.s)	% Glucomannan
control	NT	NT	67.6 ^{g} ± 0.2	1,593.8 ^{h} ± 181.7	39.7 ^{h} ± 0.4
commercial	NT	NT	88.8 ^{a} ± 0.1	7,277.8 ^{c} ± 56.2	94.9 ^{a} ± 3.2
0.5 % Borax	79.0 ^{d} ± 0.6	358.9 ^{b} ± 19.8	69.9 ^{ef} ± 0.8	5,852.5 ^{d} ± 90.7	77.4 ^{e} ± 2.0
1.0 % Borax	79.1 ^{d} ± 0.6	306.4 ^{c} ± 12.5	68.9 ^{fg} ± 0.3	11,159.1 ^{b} ± 187.7	87.3 ^{b} ± 3.8
1.5 % Borax	79.3 ^{d} ± 0.1	288.3 ^{d} ± 4.3	71.2 ^{de} ± 2.4	11,164.3 ^{b} ± 64.2	90.2 ^{b} ± 1.5
2.0 % Borax	79.3 ^{d} ± 0.7	286.3 ^{d} ± 1.2	76.2 ^{c} ± 0.2	11,141.8 ^{b} ± 40.6	83.8 ^{c} ± 0.8
2.5 % Borax	79.4 ^{d} ± 0.3	283.0 ^{d} ± 2.6	76.2 ^{c} ± 0.3	10,957.1 ^{b} ± 88.0	78.3 ^{e} ± 1.4
3.0 % Borax	79.6 ^{d} ± 0.2	274.4 ^{d} ± 4.1	77.0 ^{c} ± 0.2	11,692.1 ^{a} ± 228.3	81.7 ^{cd} ± 1.1
10 % Ethanol	81.3 ^{c} ± 1.4	589.1 ^{a} ± 12.5	79.4 ^{b} ± 0.6	2,865.5 ^{g} ± 129.8	60.6 ^{g} ± 0.2
30 % Ethanol	86.9 ^{b} ± 0.3	232.8 ^{e} ± 1.5	72.6 ^{d} ± 0.3	3,720.3 ^{f} ± 152.7	79.9 ^{de} ± 2.4
50 % Ethanol	87.6 ^{b} ± 1.2	196.8 ^{f} ± 9.7	72.2 ^{d} ± 0.6	4,865.7 ^{e} ± 57.2	79.3 ^{de} ± 2.0
70 % Ethanol	94.1 ^{a} ± 0.4	181.8 ^{f} ± 3.7	70.4 ^{e} ± 0.5	4,776.2 ^{e} ± 66.5	77.1 ^{e} ± 0.3
95 % Ethanol	94.1 ^{a} ± 1.2	150.7 ^{g} ± 12.0	67.9 ^{g} ± 0.6	4,741.9 ^{e} ± 90.5	71.1 ^{f} ± 0.6

NT, not tested. All values are means of 3 replicates, significantly different treatments are indicated by different superscripts letters following mean values within the same column as per results of Duncan's multiple range tests ($p \leq 0.05$).

Table C- 5 Drying characteristics (moisture content % d.b.) of KGM flour during hot air drying at different temperatures.

Drying temperature (°C)	50 °C	60 °C	70 °C	80 °C
Drying time (min)	Moisture content (% dry basis)			
0	101.60	105.44	101.56	104.76
10	89.81	96.22	89.40	80.36
20	78.88	84.67	75.14	60.90
30	70.40	70.77	64.85	43.27
40	62.34	62.01	55.39	32.13
50	54.73	53.93	46.58	22.60
65	44.44	43.43	35.79	11.51
80	36.04	34.21	27.70	5.32
95	28.61	26.92	19.73	3.05
110	23.64	21.12	12.53	2.23
130	17.42	14.95	6.41	1.57
150	12.79	10.38	3.44	1.19
170	9.95	7.16	2.49	1.00
190	7.66	5.09	2.14	0.96
210	6.09	4.05	1.81	0.95
230	5.23	3.39	1.62	0.80
250	5.09	2.93	1.44	0.65
270	4.95	2.47	1.34	0.50
290	4.81	2.01	0.94	0.00

Results are means from triplicate samples

Table C- 6 Drying characteristics (moisture ratio) of KGM flour during hot air drying at different temperatures.

Drying temperature (°C)	50 °C	60 °C	70 °C	80 °C
Drying time (min)	Moisture ratio			
0	1.00	1.00	1.00	1.00
10	0.88	0.91	0.88	0.77
20	0.78	0.80	0.74	0.58
30	0.69	0.67	0.64	0.41
40	0.61	0.59	0.55	0.31
50	0.54	0.51	0.46	0.22
65	0.44	0.41	0.35	0.11
80	0.35	0.32	0.27	0.05
95	0.28	0.26	0.19	0.03
110	0.23	0.20	0.12	0.02
130	0.17	0.14	0.06	0.02
150	0.13	0.10	0.03	0.01
170	0.10	0.07	0.02	0.01
190	0.08	0.05	0.02	0.01
210	0.06	0.04	0.02	0.01
230	0.05	0.03	0.02	0.01
250	0.05	0.03	0.01	0.01
270	0.05	0.02	0.01	0.00
290	0.05	0.02	0.01	0.00

Results are means from triplicate samples

Table C- 7 The change of KGM flour's colour before and after hot air drying.

Drying temperature	Colour of KGM flour before drying	Colour of KGM flour after drying
50 °C		
60 °C		
70 °C		
80 °C		

Table C- 8 Drying characteristics (moisture content % d.b.) of KGM flour during microwave-vacuum drying at different microwave power level.

Microwave power level	960 W	1200 W	1440 W
Drying time (min)	Moisture content (%dry basis)		
0	100.67	101.07	101.07
2	62.50	32.76	27.00
4	38.65	20.78	14.72
6	21.86	11.58	7.11
9	7.36	4.04	3.89
12	1.72	1.52	0.90
15	0.00	0.00	0.00

Results are means from triplicate samples

Table C- 9 Drying characteristics (moisture ratio) of KGM flour during microwave-vacuum drying at different microwave power level.

Microwave power level	960 W	1200 W	1440 W
Drying time (min)	Moisture ratio		
0	1.00	1.00	1.00
2	0.62	0.32	0.27
4	0.38	0.21	0.15
6	0.22	0.11	0.07
9	0.07	0.04	0.04
12	0.02	0.02	0.01
15	0.00	0.00	0.00

Results are means from triplicate samples

Table C- 10 Hunter values of the KGM flour after drying to the same final moisture content 5-6% (d.b., MR=0.05-0.06) using different drying techniques and conditions.

Drying method	Drying condition	L value	a value	b value
Before extraction	-	$78.41^{k} \pm 0.23$	$4.04^{a} \pm 0.22$	$9.31^{h} \pm 0.19$
Before drying	-	$84.18^{i} \pm 0.45$	$0.48^{h} \pm 0.12$	$14.32^{d} \pm 0.21$
Hot air drying	50 °C	$85.10^{fg} \pm 0.30$	$0.85^{ef} \pm 0.06$	$14.25^{d} \pm 0.26$
	60 °C	$85.34^{ef} \pm 0.30$	$0.65^{g} \pm 0.07$	$14.29^{d} \pm 0.23$
	70 °C	$84.84^{gh} \pm 0.37$	$0.78^{f} \pm 0.07$	$14.48^{d} \pm 0.37$
	80 °C	$85.30^{ef} \pm 0.38$	$0.85^{ef} \pm 0.06$	$13.97^{e} \pm 0.31$
Multistage drying	50+80 °C	$87.08^{c} \pm 0.24$	$1.02^{d} \pm 0.05$	$10.63^{g} \pm 0.17$
	60+80 °C	$86.69^{d} \pm 0.19$	$1.10^{d} \pm 0.06$	$10.45^{g} \pm 0.12$
	70+80 °C	$87.51^{b} \pm 0.28$	$0.92^{e} \pm 0.06$	$10.52^{g} \pm 0.24$
Microwave-vacuum drying	960W	$84.73^{h} \pm 0.55$	$0.08^{i} \pm 0.04$	$18.23^{a} \pm 0.28$
	1200W	$85.61^{e} \pm 0.37$	$0.45^{h} \pm 0.17$	$16.55^{c} \pm 0.30$
	1440W	$82.38^{j} \pm 0.25$	$2.18^{b} \pm 0.07$	$16.78^{b} \pm 0.19$
Freeze drying	-47 °C, 4.6 Pa	$82.36^{j} \pm 0.65$	$1.41^{c} \pm 0.04$	$11.82^{f} \pm 0.38$
Commercial product	-	$91.98^{a} \pm 0.04$	$0.01^{i} \pm 0.23$	$8.59^{i} \pm 0.09$

Results are Mean ± SD (n = 3), Letters a, b, c, … in the same column indicate a statistically significant difference at $p \leq 0.05$).

Table C- 11 Whiteness index value of the KGM flour after drying to the same final moisture content 5-6% (d.b., MR=0.05-0.06) using different drying techniques and conditions.

Drying method	Drying condition	Whiteness index value
Before extraction	-	$76.14^{i} \pm 0.21$
Before drying	-	$78.65^{g} \pm 0.39$
Hot air drying	50 °C	$79.36^{e} \pm 0.39$
	60 °C	$79.51^{de} \pm 0.34$
	70 °C	$79.02^{f} \pm 0.51$
	80 °C	$79.71^{d} \pm 0.48$
Multistage drying	50+80 °C	$83.23^{c} \pm 0.29$
	60+80 °C	$83.05^{c} \pm 0.20$
	70+80 °C	$83.65^{b} \pm 0.37$
Microwave-vacuum drying	960W	$76.21^{i} \pm 0.51$
	1200W	$78.06^{h} \pm 0.44$
	1440W	$75.57^{j} \pm 0.28$
Freeze drying	-47 °C, 4.6 Pa	$78.70^{fg} \pm 0.36$
Commercial product	-	$88.25^{a} \pm 0.07$

Results are Mean ± SD (n = 3), Letters a, b, c, … in the same column indicate a statistically significant difference at $p \leq 0.05$).

Table C- 12 Bulk density and particle density of KGM flour after drying to the same final moisture content 5-6% (d.b., MR=0.05-0.06) using different drying techniques and conditions.

Drying method	Drying condition	Bulk density (g/cm^3)	Particle density (g/cm^3)
Hot air drying	50 °C	$0.735^{ab} \pm 0.003$	$1.544^{b} \pm 0.000$
	60 °C	$0.746^{a} \pm 0.008$	$1.551^{b} \pm 0.002$
	70 °C	$0.726^{bcd} \pm 0.004$	$1.543^{b} \pm 0.002$
	80 °C	$0.720^{bcde} \pm 0.003$	$1.515^{c} \pm 0.002$
Multistage drying	50+80 °C	$0.732^{abc} \pm 0.009$	$1.404^{f} \pm 0.000$
	60+80 °C	$0.732^{abc} \pm 0.006$	$1.379^{g} \pm 0.000$
	70+80 °C	$0.739^{ab} \pm 0.001$	$1.417^{e} \pm 0.000$
Microwave-vacuum drying	960W	$0.711^{de} \pm 0.001$	$1.427^{e} \pm 0.002$
	1200W	$0.705^{e} \pm 0.003$	$1.451^{d} \pm 0.002$
	1440W	$0.714^{cde} \pm 0.002$	$1.276^{i} \pm 0.001$
Freeze drying	-47 °C, 4.6 Pa	$0.547^{g} \pm 0.023$	$1.297^{h} \pm 0.014$
Commercial product	-	$0.641^{f} \pm 0.001$	$1.812^{a} \pm 0.008$

Results are Mean ± SD (n = 3), Letters a, b, c, … in the same column indicate a statistically significant difference at $p \leq 0.05$).

Table C- 13 Porosity and apparent viscosity of KGM flour after drying to the same final moisture content 5-6% (d.b., MR=0.05-0.06) using different drying techniques and conditions.

Drying method	Drying condition	Porosity	Apparent Viscosity (mPa·s)
Before extraction	-	-	$3062.50^{j} \pm 8.58$
Hot air drying	50 °C	$0.172^{fg} \pm 0.002$	$5,351.17^{g} \pm 151.08$
	60 °C	$0.164^{g} \pm 0.005$	$4,539.20^{h} \pm 153.07$
	70 °C	$0.178^{efg} \pm 0.002$	$6,313.47^{f} \pm 175.57$
	80 °C	$0.185^{def} \pm 0.002$	$8,199.17^{e} \pm 268.47$
Multistage drying	50+80 °C	$0.191^{cde} \pm 0.007$	$3,233.27^{ij} \pm 47.91$
	60+80 °C	$0.194^{cde} \pm 0.004$	$3,492.57^{i} \pm 20.54$
	70+80 °C	$0.184^{def} \pm 0.001$	$4,620.80^{h} \pm 59.41$
Microwave-vacuum drying	960W	$0.203^{c} \pm 0.001$	$11,152.17^{c} \pm 200.73$
	1200W	$0.203^{c} \pm 0.002$	$10,267.33^{d} \pm 112.38$
	1440W	$0.224^{b} \pm 0.001$	$17,321.33^{a} \pm 354.12$
Freeze drying	-47 °C, 4.6 Pa	$0.350^{a} \pm 0.022$	$15,353.17^{b} \pm 133.38$
Commercial product	-	$0.198^{cd} \pm 0.001$	$8,210.33^{e} \pm 218.46$

Results are Mean ± SD (n = 3), Letters a, b, c, … in the same column indicate a statistically significant difference at $p \leq 0.05$).

Table C- 14 The glucomannan content of KGM flour after drying to the same final moisture content 5-6% (d.b., MR=0.05-0.06) using different drying techniques under different conditions.

Drying method	Drying condition	% Glucomannan from 3,5 DNS colorimetric method
Before extraction	-	$78.37^{d} \pm 0.59$
Hot air drying	50 °C	$88.46^{ab} \pm 1.48$
	60 °C	$87.45^{ab} \pm 0.29$
	70 °C	$86.74^{b} \pm 1.18$
	80 °C	$87.61^{ab} \pm 0.59$
Multistage drying	50+80 °C	$86.96^{ab} \pm 1.94$
	60+80 °C	$86.88^{b} \pm 1.99$
	70+80 °C	$87.78^{ab} \pm 1.68$
Microwave-vacuum drying	960W	$82.68^{c} \pm 0.59$
	1200W	$82.11^{c} \pm 0.59$
	1440W	$88.11^{ab} \pm 0.59$
Freeze drying	-47 °C, 4.6 Pa	$87.05^{ab} \pm 1.03$
Commercial product	-	$89.74^{a} \pm 0.74$

Results are Mean ± SD (n = 3), Letters a, b, c, … in the same column indicate a statistically significant difference at $p \leq 0.05$).

VITA

Miss Rarisara Impaprasert was born on December 29, 1980 in Bangkok, Thailand. She got her B.Sc. degree in Food Science and Technology from King Mongkut's University of Technology Thonburi in 2002 and M.Sc. in Food technology from Chulalongkorn University in 2006. Regarding her work experience, she has worked for Thai Food and Drug Administration (Thai FDA), Ministry of Public Health in the position of Food Scientist from November 2006 - June 2007. Then she was employed by Nestle (Thai) Co., Ltd. in the position of Food Legislation Specialist Corporate Regulatory Affairs from July 2007 to June 2008. In 2008, she received a scholarship from Office of Commission for Higher Education, Ministry of Education, Thailand to study in Food technology Ph.D. program at the Department of Food Technology, Faculty of Science, Chulalongkorn University. Since 2012, she has working as a lecturer for the Department of Microbiology, Faculty of Science, King Mongkut's University of Technology Thonburi.

List of publications

[1] Impaprasert, R., Borompichaichartkul, C., and Srzednicki, G. (2010). Inhibition of enzymatic browning in konjac (Amorphophallus muelleri) tuber slices. Proceeding in Food Innovation Asia Conference 2010: Indigenous Food Research and Development to Global Market, pp. 574-581.

[2] Impaprasert, R., Borompichaichartkul, C., and Srzednicki, G. (2013). Effects of Anti-swelling Agents on Physicochemical Properties of Konjac Glucomannan. Acta Horticulturae, 989, 331-338.

[3] Impaprasert, R., Borompichaichartkul, C., Srzednicki, G., Zhao, J., and Yu, L. (2013). Improving Production of Purified Konjac Glucomannan From Amorphophallus muelleri by Multistage Drying. Acta Horticulturae, 1011, 155-162.

[4] Impaprasert, R., Borompichaichartkul, C., and Srzednicki, G. (2014). A new drying approach to enhance quality of konjac glucomannan extracted from Amorphophallus muelleri. Drying technology: An International Journal, 32(7): 851-860.

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[1] Impaprasert, R., Borompichaichartkul, C., Srzednicki, G. (2010). Inhibition of enzymatic browning in konjac (Amorphophallus muelleri) tuber slices (Poster presentation). Proceedings of Food Innovation Asia Conference 2010: Indigenous Food Research and Development to Global Market, June 17-18, BITEC Bangkok, Thailand, 574-588.

[2] Impaprasert, R., Borompichaichartkul, C., Srzednicki, G. (2012). Effects of Anti-swelling Agents on Physicochemical Properties of Konjac Glucomannan. Proceedings of Asia-Pacific Symposium on Postharvest Quality Management on Root and Tuber Crops, February 21-24, Golden Tulip Hotel, Bangkok, Thailand.

[3] Impaprasert, R., Borompichaichartkul, C., Srzednicki, G. (2012). Improving Production of Purified Konjac Glucomannan From Amorphophallus muelleri by Multistage Drying (Oral presentation). 2nd Asia Pacific Symposium on Postharvest Research, Education and Extension, September 18-20, Grand Quality Hotel, Yogyakarta, Indonesia.

[4] Impaprasert, R., Borompichaichartkul, C., Srzednicki, G. (2012). Improving Production of Purified Konjac Glucomannan From Amorphophallus muelleri by Multistage Drying (Oral presentation). 2012 Malaysia – Thailand Graduate Forum in Life Science, Food Science and Agriculture, December 12-15, Mahidol University, Bangkok, Thailand.