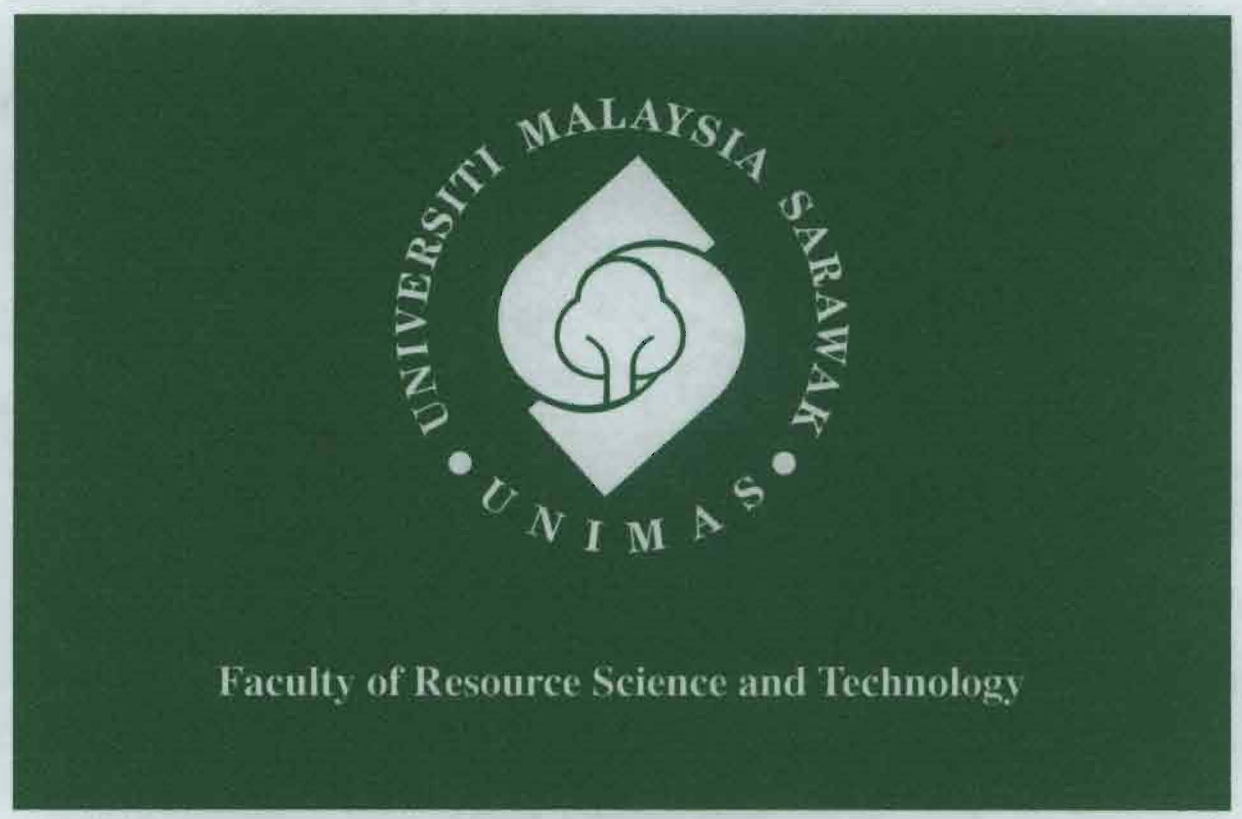

DEVELOPMENT OF PURIFICATION AND CRYSTALLIZATION OF SWEET POTATO SUGAR

Felicia Tan Li Hia

SB
211
S9
T161
2013

Bachelor of Science with Honours
(Resource Biotechnology)
2013

Pusat Khidmat Maklumat Akademik
UNIVERSITI MALAYSIA SARAWAK

DEVELOPMENT OF PURIFICATION AND CRYSTALLIZATION OF SWEET POTATO SUGAR

P.KHIDMAT MAKLUMAT AKADEMIK
UNIMAS
1000246771

Felicia Tan Li Hia
(26309)

A final project report submitted in partial fulfillment of the requirement for the degree of Bachelor of Sciences with Honours.
(Resource Biotechnology)

Supervisor: Professor Dr. Kopli bin Bujang

Department of Molecular Biology
Faculty of Resource Science and Technology
Universiti Malaysia Sarawak
2013

DECLARATION

I hereby declare that no portion of work referred in this project has been submitted in support of an application for another degree qualification of this or any other university or institution of higher learning.

(Felicia Tan Li Hia)
Resource Biotechnology
Department of Molecular Biology
Faculty of Resource Science and Technology.
Universiti Malaysia Sarawak

ACKNOWLEDGEMENT

First and foremost, I would like to express my utmost gratitude and appreciation to my supervisor, Professor Dr. Kopli bin Bujang for his supervision and encouragement throughout this project. His invaluable help of constructive comment and suggestions throughout the project work have contributed to the success of this study.

Thanks also to postgraduate students of Biochemistry Laboratory, Faculty of Resource Science and Technology, Miss Rubena Malfia Kamal, and Miss Nurjannah for their assistance in this project.

Last but not least, my deepest gratitude to my family for their patience, encouragement, motivation and continued support while the project is on-going and also my sincere thanks to my course mates and friends who willing to share their knowledge and supports.

Pusat Khidmat Maklumat Akademik
UNIVERSITI MALAYSIA SARAWAK

TABLE OF CONTENTS

LIST OF ABBREVIATIONS

AGU	amyloglucosidase units
cm	centimeter
DC	Dry matter
DCW	Dry cell weight
DE	Dextrose equivalent
DNS	3, 5-dinitrosalicyclic acid
FSP	Fresh sweet potato
FSPS	Fresh sweet potato sugar
g	Gram
g/L	Gram per liter
HPLC	High Performances Liquid Chromatography
kJ/ha	kilo joules per hectare
kg	kilogram
KI	Potassium iodide
KNU	kilo novo units
L	Liter
M	molar
MC	Moisture content
mg	microgram
mL	Milliliter
mm	millimeter
NaOH	Sodium hydroxide

nm	nanometer
PAC	Powdered activated charcoal
rpm	Revolution per min
SPF	Sweet potato flour
SPS	Sweet potato syrup
SPFS	Sweet potato flour sugar
t/ha	tonnes per hectare
tons	tonnes
µl	microlitre
wt	weight

LIST OF FIGURES

LIST OF TABLES

Development of Purification and Crystallization of Sweet Potato Sugar

Felicia Tan Li Hia

Programme Resource Biotechnology
Faculty of Resource Science and Technology
Universiti Malaysia Sarawak

Abstract

Sweet potato (*Ipomoea batatas*) sugar can be obtained from purification and crystallization of the sugar syrups. Sweet potato contain 20 to 30 percent starch according to hydrolysis of starch into sugar makes it a potential alternative source of glucose for numerous applications. Liquid sugar was produced from hydrolysis of freshly blended sweet potato tubers. Two basic steps of enzymatic hydrolysis were carried out, namely liquefaction and saccharification involving two enzymes Termamyl-120L and Dextrozyme. Commercial sweet potato flour (SPF) was used as a control to compare with the fresh sweet potato (FSP) starch for their starch and glucose recovery. Glucose produced from enzymatic hydrolysis was purified by PAC filtration using 5 g and 10 g PAC and subsequently crystallized in refrigerator prior for drying in desiccators to obtain sweet potato sugar. Glucose concentration of fresh sweet potato sugar (FSPS) and sweet potato flour sugar (SPFS) obtained after enzymatic hydrolysis were 42.83 g/L and 202.48 g/L, respectively. Starch recovery from FSP and SPF were 38.94% and 46.55%. Recovery of glucose obtained from FSPS and SPFS when using 5 g PAC were 85.62% and 83.82%, respectively. Conversely, there were 79.38% and 79.33%, respectively obtained when using 10 g PAC. Although the glucose recovery of FSPS is slightly higher, it is recommended to use SPFS because it is cheaper and the difference of glucose recovery is only 2%. Hence, this indicated that sugar production from sweet potato starch (commercial SPF) can be produced by the method of purification and crystallization developed from this study.

Key words: sweet potato, starch content, enzymatic hydrolysis, glucose recovery, sweet potato sugar

Abstrak

Gula ubi keledek (Ipomoea batatas) boleh diekstrak daripada penulenan dan penghabluran sirup gula. Ubi keledek mengandungi 20 hingga 30 peratus kandungan kanji mengikut hidrolisis kanji kepada gula menjadikannya sebagai sumber glukosa alternatif yang berpotensi untuk pelbagai aplikasi. Gula cecair akan dihasilkan melalui hidrolisis daripada campuran ubi keledek. Dua langkah asas hidrolisis enzim telah dijalankan, iaitu pencairan dan pensakarifikasian yang melibatkan dua enzim Termamyl-120L dan Dextrozyme. Kanji komersial telah digunakan sebagai kawalan untuk dibandingkan dengan kanji ubi keledek bagi pemulihan kanji dan glukosa. Hasilan glukosa daripada hidrolisis enzim telah ditapiskan menggunakan 5 g PAC dan 10 g PAC melalui penapisan serbuk arang aktif (PAC) dan kemudiannya dihablur dalam peti sejuk sebelum dikeringkan dalam desikator untuk mendapatkan gula ubi keledek. Kepekatan glukosa ubi keledek dan kanji komersial selepas hidrolisis enzim adalah 42.83 g/L dan 202.48 g/L masing-masing. Pemulihan kanji daripada FSP dan SPF adalah 38.94% dan 46.55%. Pemulihan glukosa daripada ubi keledek dan kanji komersial apabila menggunakan 5 g PAC adalah 85.62% dan 83.82%. Sebaliknya, 79.38% dan 79.33% diperoleh apabila menggunakan 10 g PAC. Walaupun pemulihan glukosa daripada ubi keledek adalah sedikit tinggi, ini disarankan menggunakan kanji komersial kerana ia lebih murah dan perbezaan pemulihan glukosa hanya 2%. Justeru, ini menunjukkan bahawa penghasilan gula daripada kanji ubi keledek (komersial kanji) dapat dihasilkan dengan kaedah pemulihan dan penghabluran yang dimajukan daripada kajian ini.

Kata kunci: ubi keledek, kandungan kanji, pemulihan glukosa, enzim hidrolisis, gula ubi kelede

1.0 INTRODUCTION

1.1 Background of study

Sweet potato (*Ipomoea batatas* L.) known as *ubi keledek* among Malaysians is the seventh most produced food crops in the world in term of annual production (Choi *et al.*, 2007). It is one of the food crops rich in starch and sugar that offers a viable; substitute starchy raw materials besides sago, cassava, corn and other for the conversion of useful sugar feedstock required for the production of ethanol and other added product. Srichuwong *et al.* (2012) also stated that sweet potato roots contain 20 to 30 percent starch based on wet basis makes it an alternative of glucose for numerous applications.

This storage root grows easily to be grown and adapts well in different environments making it suitable to be planted in relatively poor soils where the fertilizer is scarce. Woolfe (1992) stated that the typical composition of the root is starch (60-70%), total sugars (10%), total protein (5%), lipid (1%), ash (3%), total fibre (10%), vitamins, organic acids and other components in low concentrations (less than 1%) in term of dry matter. The high carbohydrate or starch with low fat content in sweet potato makes it acts as a dietary staple and sources of other nutritionally important dietary factors.

Sweet potato provides uses for human consumption, animal feed, industrial products as well as provides great potential to avoid malnutrition and improve food security in developing countries (Lee *et al.*, 2012). Its starch can also produce ethanol to reduce climatic changes which are the current global problem. As renewable energy from biomass is used as biofuel in present or in the future, emission of greenhouse gases and other chemical gases from fossil fuels can be reduced and this increases the energy demand of finding for alternative resources (Zeller and Grass, 2007).

Sweet potato starch can be hydrolyzed by utilizing the same commercial enzymes applied for other food crops such as cassava, corn, potato, and etc. For instance, thermostable α-amylase from *Bacillus licheniformis*, the mixture of glucoamylase from *Aspergillus niger* and pullulanase from *Bacillus acidopullulyticus* are usually used (Bujang *et al.*, 2000). Hydrolyzed sweet potato starch can then be filtered by multi-filtration to remove impurities prior to crystallize using freeze-drying into solid form of sweet potato sugar.

In addition, sweet potato is believed to have potential value same as other food crops in producing starch, reducing sugar (glucose), and ethanol. Research done by Adeni and Bujang (1998) until recently has proven that sago starch could produce sugars, lactic acid and ethanol. Therefore, the aim in this study is to focus on sugar production from sweet potato developed by purification and crystallization methods.

1.2 Objectives

The main objective of this study is to develop methods of purification and crystallization to produce sugars and its recovery from fresh sweet potato. The particular objectives of this project are to:

- study the purification and crystallization process of sweet potato sugar
- determine the amount of glucose and starch produced from sweet potato
- compare the starch and glucose recovery between fresh sweet potato and sweet potato starch

2.0 LITERATURE REVIEW

2.1 Biology and physiology of sweet potato

Sweet potato (*Ipomoea batatas* L.) known as *ubi keledek* in Malaysia is a type of dicotyledonous plant from the Family *Convolvulaceae* (Taylor, 2007). It is mainly distributed in developing countries such in Southeastern region. According to Loebenstein and Thottappilly (2009), sweet potato in developing countries is placed fifth in economic value production, sixth in dry matter production, seventh in energy production, ninth in protein production and it has wide range of usage and consumption as food, feed and industrial products.

The sweet potato is an herbaceous and perennial plant which cultivated as annual crop. It can be grown in tropical and subtropical region with approximately 10 million ha (Choi *et al.*, 2007). Sweet potato is able to tolerant diverse conditions including drought and typhoons resistance, pests and diseases resistance, and poor soils (Srichuwong *et al.*, 2012). According to Burri (2011), sweet potato is propagated vegetatively by vine cutting as well as grown from seeds. Generally, it takes between 90 to 150 days to harvest sweet potato roots in order to produce maximum yields (Kemble *et al.*, 2006) which shows more early than other roots and tuber crops (Woolfe, 1992).

Studies has reported that environmental factors, including location, year, crop season and length of growing season can influence the percentage raw starch of sweet potato roots (Woolfe, 1992). Significant high starch content harvested at 150 or 180 days after planting as compared to harvest at 120 days after planting in the same season was also studied (Woolfe, 1992).

Pusat Khidmat Maklumat Akademik
UNIVERSITI MALAYSIA SARAWAK

Sweet potato is one of the world's most important food crop producing over 133 million tons globally each year (Abegunde *et al.*, 2012). The largest producing region is occupied by Asia with annual production about 125 million tons, but China accounts for about 90% of worldwide sweet potato production with 117 million tons (Abegunde *et al.*, 2012). According to FAO (2012), Malaysia produced 19,870 tons of sweet potatoes with the yield of 143985.51 Hg/Ha in year 2010.

2.2 Characteristics and compositions of sweet potato

Sweet potato is a growing plant of underground tuberous roots, bearing white or rose violet funnel shaped flowers and leaves (**Figure 1**) that vary in shapes, sizes, and colors depending on the variety grown. The smooth skin color can range between yellow, red, orange, and brown, whereas its flesh can be white, orange (contains carotene), yellow, purple, red, pink and violet (Loebenstein and Thottappilly, 2009). The skin color intensity depends on the environmental conditions where the plant is grown. Typical composition of sweet potato is starch (60-70%), total sugars (10%), total protein (5%), lipid (1%), ash (3%), total fiber (10%), vitamins, organic acids and other components in low concentrations (less than 1%) in term of dry matter (Woolfe, 1992).

Figure 1: Sweet potato flower
Source adopted from http://gardeningwithwilson.com/2008/01/04/ever-saw-the-flowers-of-the-sweet-potato-before/

2.3 Sweet potato starch

Starch is a storage polysaccharide of plants that formed from condensation of α-glucose units. It is one of the most important and abundant plant products that act as an energy source in the human diet (Bujang and Ahmad, 2000). Studies have shown that starch productions from cereals contribute approximately 2,050 million tons, while from roots and tubers contribute only 679 million tons annually (Burrell, 2002).

Generally, starch occurs in plants as granules consisting of two major components, namely amylose and amylopectin. Amylose is the simpler form of starch, whereas amylopectin is the more complex one (Campbell and Reece, 2002). According to Majzoobi *et al.* (2003), amylose is a linear unbranched polymer consisting of glycopyranosyl monomers linked together by α-1, 4-glycosidic linkages, while amylopectin is a highly branched polymer consisting of glycopyranosyl monomers linked together by both α-1, 4-glycosidic linkages and α-1, 6-glycosidic linkages.

Insoluble unbranched amylose can be separated from insoluble branched amylopectin fraction through enzymatic hydrolysis. The linear chain of α-1, 4 linked D-glucose residues that compose of amylose is degraded by α-amylase to maltose as mentioned by Aiyer (2005). It forms complex with iodine to produce intense blue color under maximum absorption at 650 nm where this technique is used for the quantitative determination of amylase. On the other hand, the amylopectin which branched with α-1, 6 linkages are degraded by the mixture of glucoamylase and pullulanase. Amylopectin forms purple color when reacts with iodine.

2.4 Utilization of sweet potato starch

Starch is widely used in commercial industrial such as in food industry involving processed foods and products, biofuel industry, textile industry, chemical industry and many other uses. Sweet potato starch is also utilized in many commercial purposes. Starch extracted from sweet potatoes is mostly used as an ingredient in biscuits, breads, cakes, cookies, ice-cream, juices, and noodles by the food industries (Palaniswami and Peter, 2008; Woolfe, 1992). Besides, production of others including sweeteners, sugar syrups, beverages, and citric acids in food industry also contributed by the starch. Animal feed can also obtained from the wastes of starch and alcohol industries (Loebenstein and Thottappilly, 2009).

Apart from that, sweet potato is also used in alcohol industrial for manufacturing of alcohol fuel. With the increased demanding of fossil energy resources as well as the global concern of climate change, the production of biofuel from biomass has becoming the present challenges, especially in developing countries (Zeller and Grass, 2007). By introducing biomass as renewable energy sources, Zeller and Grass (2007) believed that danger of climate change will be reduced such as in emission of carbon dioxide and

greenhouse gases. According to James (2001), sweet potato acts as biomass alternative better than corn for fossil fuels as it yield approximately 1,000 liters of ethanol for half a hectare of roots, whereas only 300 liters for the corn. Moreover, higher production yield of starch from sweet potato per unit of cultivated lands compared to cereal grain making it could be a substitute to cereal grain as a substrate for alcohol production in case of demanding for use as food and feed (Woolfe, 1992).

2.5 Enzymatic starch hydrolysis

Sweet potato starch can be hydrolyzed into reducing sugars by pretreatment of the enzymes. Enzymatic hydrolysis has advantages over acid hydrolysis as less unwanted by-products can be formed and therefore yields more products (Aiyer, 2005). Two basic steps involved are liquefaction and saccharification by utilizing commercial enzymes.

Two commercial enzymes namely Termamyl-120L (thermostable α-amylase) originated from *Bacillus licheniformis* and Dextrozyme 225/75L (a mixture of glucoamylase and pullulanase) originated from *Aspergillus niger* and *Bacillus acidopullulyticus* respectively (Bujang *et al.*, 2000) will be used in this study. The enzymatic activity for Termamyl was 120 KNU/g, while Dextrozyme was 225 AGU/mL as defined by Novo Industries (1990).

The purpose of liquefaction is to convert a concentrated suspension of insoluble starch granule into a soluble solution of dextrins (Crabb and Mitchinson, 1997) by partial hydrolysis using thermostable α-amylases. Liquefaction is carried out initially by Termamyl-120L (Bujang *et al.*, 2000) which responsible to break down α-1, 4-glycosidic bond of starch. Prior to this, starch undergoes gelatinization that allows easier accessible for the enzymes (Aiyer, 2005).

On the other hand, saccharification is a step where the starch suspension is further saccharified into simple sugar (glucose) by Dextrozyme. It is used to remove β-glucose units of starch by catalyzing the hydrolysis of both α-1, 4- and α-1, 6-glycosidic bond. Hydrolyzed starch of sweet potato will be obtained at the end of the process and conversion yields of starch into reducing sugar will be expressed as dextrose equivalent (DE), that defined as the percentage of reducing sugar present on dry solid basis (Bujang *et al.*, 2000).

2.6 Purification and crystallization of sweet potato sugar

Fine powdered activated charcoal (PAC) is used as absorbent for purification and absorption process (Bujang *et al.*, 2009). PAC packed in columns of 2.5 cm diameter and 50 cm length is used for the hydrolyzed sweet potato starch to be loaded into to remove all the impurities including odor, color, and taste. Sugar decolorization is also occurred in the same time to decolorize sugar to obtain a clear, pure glucose. Bujang *et al.*, (2009) reported that the final recovery of sugar from hydrolysis process can influence by the utilization of PAC for purification and color removal.

On the other hand, oven is used in crystallization of sugar for the drying process at temperature of 60°C based on the lab scale of biochemistry lab. Purified sugar (liquid glucose) obtained is placed in hot oven and allowed to crystallize for 6 days to form solid sugar. However, refrigerator is used in crystallization of sweet potato sugar in this study for the formation of solid at minimum temperature of 4°C. Purified sugar (liquid glucose) obtained is rather put in crucible and placed in the refrigerator allowing to crystallize and form solid sugar of sweet potato. It is then put in desiccators for the completion of drying process.

3.0 MATERIALS AND METHODS

3.1 Materials

3.1.1 Fresh sweet potato (FSP)

Fresh sweet potato tubers (**Figure 2**) were obtained from the local market of 7 Mile Market, Kota Samarahan.

Figure 2: Skinned fresh sweet potato tubers (*Ipomoea batatas*)

Figure 3: Deskinned fresh sweet potato tubers (*Ipomoea batatas*)

3.1.2 Commercial sweet potato flour (SPF)

Commercial sweet potato flour (**Figure 4**) which is to be used as control was obtained from the local supermarket.

Figure 4: Commercial sweet potato flour (SPF)

3.1.3 Hydrolytic enzymes

Two types of commercial enzymes were used. Termamyl-120L, a thermostable α-amylase from *Bacillus licheniformis* (120 KNU/g) was used in the liquefaction step. Dextrozyme, a mixture of glucoamylase from *Aspergillus niger* and pullulanase from *Bacillus acidopullulyticus* (225 AGU/mL) was used in the saccharification step as repeated elsewhere (Bujang *et al.*, 2000).

3.1.4 Starch analysis

Exactly 2.0 g of potassium iodide (KI) was dissolved in 80 mL of distilled water. Then, 0.2 g of solid iodine was added into concentrated KI solution. The mixture was swirled to make sure all the solid iodine is dissolved. Finally, the solution was top-up with distilled water up to 100 mL. The iodine solution was stored in bottle and covered by aluminium foil to avoid direct light penetration (Nakamura, 1981).

3.1.5 Reducing sugar analysis

A solution of 3, 5-dinitrosalicyclic acis (DNS) was prepared by dissolving 10 g DNS, 2 g phenol, 0.5 g sodium sulfite, 10 g sodium hydroxide in 500 mL distilled water. It was stored in a bottle covered by aluminium foil. On the other hand, rochelle salt was prepared by adding 40 g potassium sodium tartarate in distilled water and top-up the volume to 100 mL (Miller, 1959).