ENZYMATIC CONVERSION OF CASSAVA STARCH TO FLOUR

KONG ZI LING

BACHELOR OF CHEMICAL ENGINEERING (PURE)
UNIVERSITI MALAYSIA PAHANG

ABSTRACT

Cassava (Manihot esculenta Crantz) plays important role as the food source rich with carbohydrate content consumed by human in the world. It is the fourth vital basis of food after the rice, wheat and maize. Cassava starch has been widely used in industrial field including food processing, paper, wood, textile, pharmaceutical and chemical processing. This work aims to produce flour from cassava starch using microbial fungi. The pectinase enzyme derived from *Aspergillus niger* is used in the starch extraction due to its ability to enhance the starch yield without compromise the physical properties of the starch. At first, sample preparation from peeling, washing, drying to grinding was carried out using traditionally method. *A. niger* was cultured for 3 days to produce pectinase as a crude enzyme extract. Then, pectinase precipitate was fermented with cassava solution at different duration (3, 5, 7, 9 hrs) and temperature (30, 40, 50, 60°C) to obtain the optimum condition for cassava starch to reach its maximum extractability. Once the optimum condition was obtained, the starch recovery of cassava flour with pectinase enzyme treatment was evaluated. The nutrition analysis (i.e., moisture, ash, crude fat, crude fiber and protein content) of cassava starch was performed using standard American Association of Cereal Chemists (AACC) and Lowry method. The finding showed that the temperature range of 45-50°C and the duration of 6-7 hrs is the optimum condition for the cassava starch extraction. The cassava flour with and without pectinase enzyme contain 10-12% of moisture, 1-3% of ash, 1-4% of fat, 1-3.5% of crude fiber and 191-364 μg/ml of protein. Besides that, this work also indicated that the pectinase enzyme treatment from *A. niger* had increased about 9% of the starch recovery of cassava flour.

Keywords: cassava, starch, pectinase, Aspergillus niger

ABSTRAK

Ubi kayu (Manihot esculenta Crantz) memainkan peranan yang penting sebagai sumber makanan yang kaya dengan kandungan karbohidrat di seluruh dunia. Ia merupakan sumber makanan yang keempat penting selepas beras, gandum dan jagung. Kanji ubi kayu telah digunakan secara meluas dalam bidang industri termasuk pemprosesan makanan, kertas, kayu, tekstil, farmaseutikal dan industri kimia. Tujuan utama kerja ini adalah untuk menghasilkan tepung daripada kanji ubi kayu dengan menggunakan kulat mikrob. Enzim pectinase yang dihasilkan daripada *Aspergillus niger* digunakan dalam pengekstrakan kanji kerana keupayaannya untuk meningkatkan hasil kanji tanpa mengubah sifat fizikal kanji. Projek ini dimulakan dengan penyediaan sampel seperti mengelupas, membasuh, mengering dan mengisar secara tradisional. *A. Niger* ditapai selama 3 hari untuk menghasilkan pectinase sebagai ekstrak enzim mentah. Kemudian, mendakan pectinase ditapai dengan larutan ubi kayu pada jangka masa (3, 5, 7, 9 jam) dan suhu (30, 40, 50, 60°C) yang berbeza untuk mencapai keadaan operasi yang optima supaya pengeluaran kanji yang maksima. Setelah keadaan operasi yang optima dicapai, pemulihan kanji daripada tepung ubi kayu dengan menggunakan enzim pectinase akan dinilaikan. Analisis nutrisi tepung ubi kayu (kelembapan, kandungan abu, protein, lemak mentah dan serat mentah) akan diuji dengan menggunakan standard American Association of Cereal Chemists (AACC) dan kaedah Lowry. Keputusan dalam kerja ini menunjukkan bahawa julat suhu pada 45-50°C dan jangka masa selama 6-7 jam adalah keadaan operasi yang optima untuk pengekstrakan kanji ubi kayu. Tepung ubi kayu dengan dan tanpa enzim pectinase mengandungi 10-12% kelembapan, 1-3% kandungan abu, 1-4% lemak mentah, 1-3.5% serat mentah dan 191-364μg/ml protein. Selain itu, kerja ini juga menunjukkan bahawa rawatan enzim pectinase dari *A. niger* telah meningkat sebanyak 9% untuk pemulihan kanji tepung ubi kayu.

Kata kunci: ubi kayu, kanji, pectinase, Aspergillus niger

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LIST OF ABBREVIATIONS

cm	Centimetre
g	Gram
ha	Hectare
hrs	Hours
ml	Milliliter
%	Percentage
°C	Degree Celsius
µ	Micro

LIST OF ABBREVIATIONS

AACC	American Association of Cereal Chemists
BSA	Bovine Serum Albumin
Ca	Calcium
Cl	Chlorine
FOA	Food and Agriculture Organization of the United Nations
H_2SO_4	Sulphuric Acid
HCN	Hydrogen Cyanide
K	Potassium
Na	Sodium
NaOH	Sodium Hydroxide

1 INTRODUCTION

1.1 Background

Nowadays, the cultivation of cassava keep growing serve as a staple crop in the most tropical countries located in the equatorial belt which indicates to its suitability to an extensive of ecosystems. It is the world's fourth most important main food source after rice, wheat and maize and is a major element in the diet of over one billion population. Cassava is widely used in tropical Africa and South America, and in parts of Asia, particularly Indonesia and Thailand (FAO, 2000).The cassava crops have some of the hinge features which are its efficiency in producing carbohydrate, its resistance to drought and to destitute soils, even though it thrives on fertile, sandy-clay soils, and its high flexibility with respect to the timing of planting and harvesting. Hence, cassava plays a vital role for food security, especially in dry areas and marginal soils.

Cassava is grown for its enlarged starch- filled roots while the fresh root contains less protein and ash. Typical composition of cassava root is moisture (70%), starch (24%), fiber (2%), protein (1%) and other substances including low content minerals and vitamins (3%) (Tonukari, 2004). The starch content in the cassava root has many industrial applications, including food processing and in the paper, wood, textile, pharmaceutical, chemical and feed industries. In the traditional settings of North and South America, the tubers are grated and the sap is extracted through squeezing or pressing. The cassava is further dried over a fire to make a meal, or it is fermented and cooked. The meal can then be rehydrated with water or added to soups or stews. In Africa, the tubers are processed into some different ways. Firstly, they may be fermented in water. Then they are either sun-dried for storage or grated and made into dough that is cooked. Alcoholic beverages can be made from the roots (O'Hair, 1995).

Cassava contains two cyanogenic glucosides, linamarin and a small amount of lotaustralin, which are catalytically hydrolysed to release toxic hydrogen cyanide (HCN) when the plant tissue is crushed (Conn, 1981; Balagopalan *et al.*, 1988; McMahon *et al.*, 1995). The intake of large amounts of cyanogens from consumption of high cyanide cassava roots, high cyanide cassava flour and poorly processed cassava leaves can lead to cyanide poisoning with symptoms of headache, nausea, dizziness,

diarrhoea, vomiting and sometimes death (Nhassico *et al.*, 2008). However, cassava starch is mostly used to produce cassava flour which is an important food source in the world. Production of flour with high nutrient content and minimal amount of toxic factor by the extraction of starch from cassava becomes the important issues. Thus, the aim of this study is to produce flour from cassava starch through enzymatic treatment by *A. niger*.

1.2 Motivation and statement of problem

Flour has a big demand in global since several decades and it is expected has a very steady growth in future especially wheat flour. This is because flour is one of the major food supplies for human. The products available in market such as bread and noodle are the products made from flour. According to Global Agricultural Information Network, total production of wheat flour in Malaysia was 75,000 tonnes in 2012 increased by 0.56% in 2011 which totalled at 74,500 tonnes. However, the wheat flour is not enough to consumer in Malaysia due to the growth of the baking industries. Moreover, consumers nowadays are opting for high-fibre whole meal bread. All these factors are boosting wheat imports in Malaysia. This situation is not good in Malaysia may cause economic loss due to the importation of wheat. Thus, using cassava as the raw material to produce flour will be studied.

Cassava is the well-known traditional crop that contributes to the Malaysia food sector. Cassava roots rich in starch content to produce cassava flour. Starch is the most abundant carbohydrate that can be found from various natural sources such as potato, rice, corn, wheat and cassava. Kallabinski and Balagopalan (1991) stated that to obtain high recovery of cassava starch, attempts have been made to replace the traditional mechanical method by the use of commercial cell wall degrading enzymes to release starch from cassava roots. So, using the pectinase enzyme derived from *A. niger* to produce cassava starch to flour will be studied.

Cassava is a starchy food but contains no gluten. There has benefit for those based on gluten-free diets, people diagnosed with celiac disease and allergies on the gluten can use cassava to make food. Hence, cassava is a good substitute to using wheat, rye or barley, which is the food items that contain gluten. Because of this reason, we use cassava starch to produce the flour. Wheat flour is substituted by the cassava flour

which is more nutrients and no gluten. Even products produced of this flour such as noodles or pasta, are gluten-free. Although baking cakes, bread and other foods requires gluten to enable them to swell in size, it can be substituted with guar and xanthan gum. Due to economic and large resource availability of cassava plant, production of flour from cassava starch is interesting to be studied. Demand of "ready-made" products depends on quality of product itself. Nowadays, educated consumers would prefer to concern on the nutrition of product instead of the price and brand of the product. Hence, nutrition values of cassava starch was analyzed.

1.3 Objectives

The objectives of this research are shown as following:

- To produce flour from cassava by using enzyme derived from *A. niger*
- To perform analysis on characteristics of cassava flour

1.4 Scope of this research

Several scopes have been identified to achieve the objectives of this research:

i) To culture *A. niger* for the production of pectinase enzyme
ii) To study the parameter for the extraction of cassava starch
iii) To obtain the starch recovery after enzymatic conversion
iv) To test the nutritional profile of cassava starch

1.5 Main contribution of this work

The main contribution of this work is to determine the potential of pectinase to be used as enzyme to liberate the starch which could eventually help to produce flour that meets standard specifications.

1.6 Organisation of this thesis

The structure of the reminder of the thesis is outlined as follow:

Chapter 1 provides a brief introduction about the cassava plant. The current issues related to the cassava flour are studied where it leads to the motivation and problem

statement for this work. This chapter also identify the objectives and the scope of the research.

Chapter 2 reviews on the previous researches which is relevant to this topic. This study presents the market survey of cassava in Malaysia and nutritional profile of cassava. Various methods for the derivation of pectinase enzyme from *A. niger* were listed. Besides that, the properties of starch, extraction process of starch and the improved starch recovery by enzymes were discussed.

Chapter 3 is describes the detail methodology for this work. It covers the procedures from sample preparation, product extraction to the nutrition content analysis of cassava starch.

Chapter 4 discussed on the results achieved from the experimental work. The detailed description for each parameter studied in the extraction of cassava starch was presented.

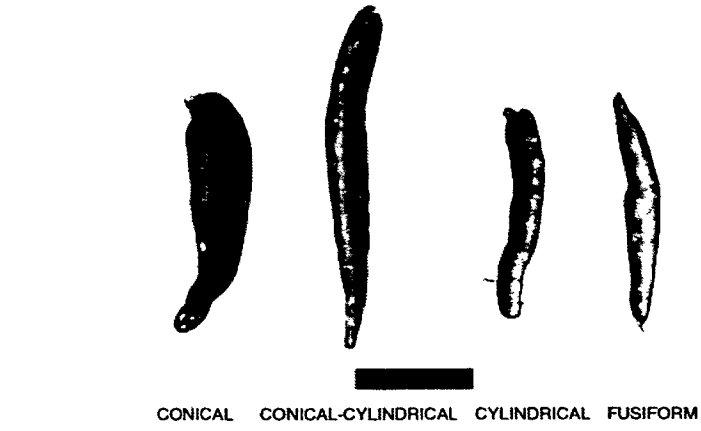
2 LITERATURE REVIEW

2.1 Overview

Studies have been on going to optimize the production of cassava starch with specifications that meets standard requirement and security. Improvement on cassava limitations become our focal point of study with the possible outcome could change the current dependency of the world population towards rice, wheat and maize and staple food.

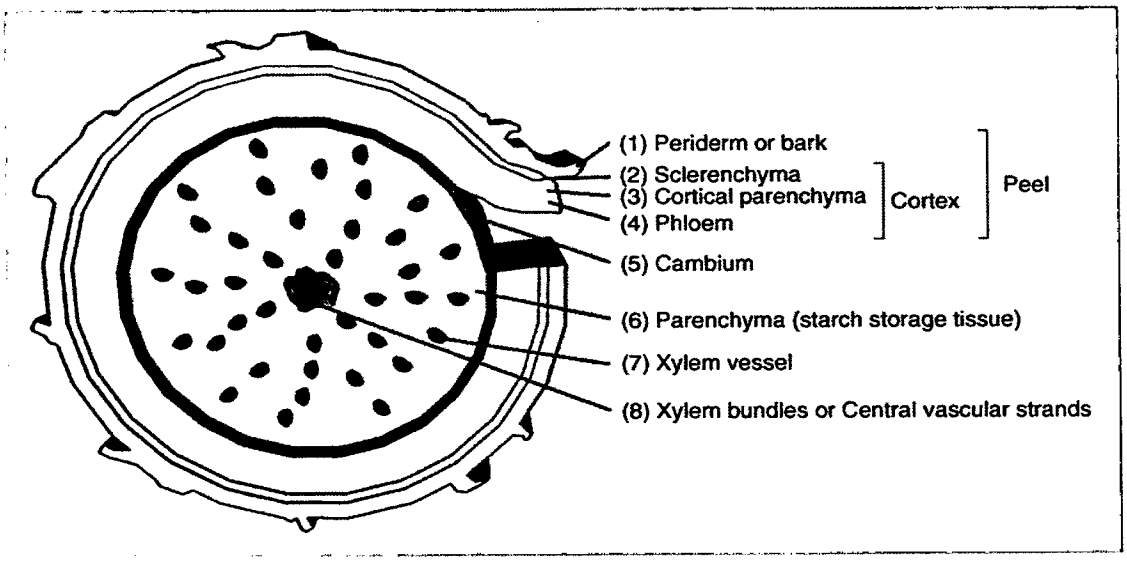
2.2 Cassava

Mature cassava roots have different shapes and sizes depending on the variety, age and growth conditions. The shape can be conical, conical-cylindrical, cylindrical or fusiform while the size ranges from 3 to 15 cm (Figure 2-1 (a)). The outer peel colour varies from white to dark brown. The peel is composed of periderm (outer layer) and cortical region (inner layer) which contains sclerenchyma, cortical parenchyma and phloem tissue (Figure 2-1 (c)). The large central pith of the root is the starch- reserve flesh comprised of cambium, parenchyma tissue and xylem vessels. The peel and the central pith are the two major components of the cross section of cassava roots (Breuninger *et al.*, 2009).



(a)

(b)



(c)

Figure 2-1: Illustration of the cassava structure by Breuninger *et al.* (2009)

(a) Cassava roots with conical, conical-cylindrical, cylindrical and fusiform shapes; (b) Cross-section of cassava roots; and (c) drawing of root cross-section containing different components by Breuninger *et al.* (2009)

2.3 *Production of cassava in Malaysia*

Cassava (*Manihot esculenta*) or Tapioca also called 'Ubi Kayu' in Malaysia is a tropical root or tuber crop under family Euphorbiaceae. Cassava is perennial plant which is the staff of life for millions of people living in tropical countries where cereals will not grow. It is a crop best suited to the lowland tropics which are warm and moist climate where mean the temperature range from 25-29^{0}C (Onwueme and Charles, 1994). Cassava grows on all types of soils, but saline, alkaline and ill-drained soils are not suitable. In Malaysia, there is the potential for cassava production in the peat soils and ultisol soils. Rainfall is heavy in Malaysia which averaging 2000-3000 mm/yr. While the rainy season starts in October but planting and harvesting occur all year round. Most of the cassava in Malaysia is for starch production and some minor production like cassava chips for animal feed (Onwueme, 2002).

Department of Agriculture recorded about 2,396 hectare of tapioca grown in Malaysia in 2007 producing about 38,711 metric tonnes valued at RM 37.5 million. Johor has the largest cassava growing area for about 875 hectare in Malaysia and produced 19,506 metric tonnes which consists of 83% of Malaysia production in 2005. Other states growing cassava are Selangor (173 ha), Perak (89 ha) and Kelantan (23 ha). Malaysia produced 40.998 tons of cassava in year 2012 compared to 37.187 tons in 2009. This increment shows that the government is seriously considering the production of cassava as well as to decrease import of wheat from other countries and competing with other types of flour in the Malaysia food market.

2.4 *Nutritional profile of cassava*

Cassava is a drought-tolerant, fundamental food crop grown in tropical where many people are depressed with malnutrition and making it a potentially valuable food source for developing countries. It can maintain their nutritional value for a long time without water (Montagnac *et al.*, 2009). It is rich in carbohydrates, calcium, vitamins B and C, and important minerals. Okigbo (1980) reported that it produces about 250000 calories/hectare/d, which ranks it before maize, rice, sorghum, and wheat. Although rich in carbohydrates, cassava often been criticized for its low content of proteins and fats than in cereals and pulses. However, it is found that more protein in the leaves. Cassava roots contain as low as 1% of proteins, but the leaves in-contrast, have about 5 to 10 times higher than in roots. Cassava is also free from gluten. The roots and leaves, which

constitute 50% and 6% of the mature cassava plant. Cassava roots have calcium, iron, potassium, magnesium, copper, zinc, and manganese contents comparable to those of many legumes, with the exception of soybeans (Montagnac *et al.*, 2009).

2.5 Maturity and yield of cassava

Cassava has no definite lifetime or maturity period as a perennial crop. Maturity period of cassava roots is the point where maximum or near maximum yield is obtained. However, after full development of the canopy, the growth of roots will progressively decrease and finally reach zero. According to Benesi *et al.* (2008), too early to harvest the cassava, it will leads to the lower root yields, while the cassava is too late harvesting leads to development of woody and fibrous tuberous roots, and reduction in starch content. Receptivity reduction in starch content when cassava remains in the soil after maturity appears to be cons for starch production. Most researchers tried to find out the optimum time for harvesting cassava but they have their own suggestions. The time at which cassava matures depends much on the variety and ecological factors (Benesi *et al.*, 2008).

Harvest cassava at full maturity or 6-7 months after planting. The optimal productivity can be indicated by the harvest index, the ratio of root weight to total plant weight, which ranges from 0.5 to 0.7 (Breuninger *et al.*, 2009). Cassava roots for yields various varieties, plant growth conditions like soil, climate, rainfall and agronomic measures such as weed control, irrigation, application of fertilizers and multiple cropping (Breuninger *et al.*, 2009). The crop provides high yields due to the well- managed of the growth conditions. The average composition of mature cassava roots contain 60-70% water, 30-35% carbohydrate, 1-2% fat, 1-2% fiber and 1-2% protein with trace amounts of vitamins and minerals. The starch content of mature cassava roots can range from 15 to 33% depending on the climate and the harvesting time (Breuninger *et al.*, 2009).

However, the optimum maturity stage for maximum starch yield has not been determined. The maximum starch content reaches at the end of the rainy season. Less mature roots will be lower in starch content and higher in water while over mature roots will be lower in recoverable starch content due to the woody texture making starch processing difficult (Breuninger *et al.*, 2009). Baafi and Safo-Kantanka (2007) reported that cassava starch yield and quality is affected by genotype, age, and location.

2.6 *Production of pectinase enzyme from A. niger*

A. niger is an abundant fungi species under *Aspergillus* group and is a very important microorganism in the biology field. It can grow aerobically on organic matter (Schuster *et al.*, 2002) and found in mesophilic environments such as decaying vegetation, dead leaves and compost pile with very little nutrients available. Schuster *et al.* (2002) stated that the abilities and rich production of fungal spores, which are depended by the air, secure the ubiquitous occurrence of the species, with a higher rate of recurrence in warm and humid places. The growth of *Aspergillius* group is affected by several environmental factors such as temperature, water activity and pH. Schuster *et al.* (2002) reported that *A. niger* is able to grow in the wide temperature range of 6-47°C with a relatively high temperature optimum at 35-37°C. The water activity percentage for growth of *A. niger* is 88%, which is relatively high compared to other *Aspergillus* species. *A. niger* is able to grow over an extremely wide pH range: 1.4-9.8.

Many previous studies such as Tjamos *et al.* (2004), Abe *et al.* (1988) and Perrone *et al.* (2006) have been reported the ability of *A. niger* in producing the enzymes. It has been safe to be used for many decades to produce enzymes through fermentation. Since 1960s, *A. niger* has become a source of a variety of enzymes that are well established as technical aids in fruit processing, baking, and in the starch and food industries (Schuster *et al.*, 2002).The potential enzymes produced from *A. niger* are amylase, amyloglucosidase, cellulases, lactase, invertase, pectinases, and acid proteases.

Nowadays, pectinase is one of the essential enzymes widely applied in the industrial sectors especially for the food processing industries, e.g. pulp extraction and clarification of fruits juices, vegetables and wines (Ibrahim *et al.*, 2014). According to Mrudula and Anitharaj (2011), pectinase produced by *A. niger* can be categorized as submerged and solid-state fermentation. Synthesis of pectinase from *A. niger* by these two fermentation approaches have been studied extensively by many researchers such as Palaniyappan *et al.* (2009), Martos *et al.* (2009), Khairnar *et al.* (2009), Castilho *et al.* (2000), Nazneen *et al.* (2011) and Khan *et al.* (2012). Based on these previous studies, the productivity of pectinase is significant influenced by several parameters, i.e. temperature, moisture content, cultivate time and medium. Therefore, selection of optimum growth conditions during fermentation process of pectinase enzyme from *A. niger* is important to obtain the maximum yield and expected quality of enzyme. The summary of some previous researches with various fermentation methods and culture

conditions was listed in Table 2-1 below. Based on the Table 2-1, the solid-state fermentation method is the most popular to be employed due to its simple process, high productivity and concentrated products over the submerged fermentation approach. The previous study by Mrudula and Anitharaj (2011) also agreed with the higher yield production of pectinase could be obtained via solid-state fermentation method. Thus, solid-state fermentation method will be employed for this work.

Table 2-1: Various condition for the production of pectinase from *A. niger*

Microorganism (Enzyme)	Fermentation method	Culture condition	Parameter	Optimum condition	Reference
A. niger (pectinase)	• Solid-state	PDA medium for 96 hrs at 30 °C	• Different substrates (Wheat Bran, Rice Bran, Cassava Starch, Potato Starch, Rice husk) • Temperature (30-50°C) • Moisture content (50, 60, 70 and 80%) • Time (up to 9th days) • Carbon source of pectin (4 - 13%) • Nitrogen source such as urea, peptone yeast extract, $(NH_4)_2SO_4$ • Aeration	• Wheat bran + potato starch • 7 days • 9.68% pectin • 1.69% $(NH_4)_2SO_4$ • 40 °C of temperature • 60% moisture • Optimum aeration was examined having different sizes of conical flasks (750 ml)	Nazneen *et al.* (2011)
	• Solid-state • Medium: Glucose, $NaNO_3$,K_2HPO_4, $MgSO_4.7H_2O$, KCl,$FeSO_4.7H_2O$, agar • wheat and soy brans, $(NH_4)_2SO_4$ and HCl	Culture for 13 to 96 hrs at 30°C	• Time (13-96 hrs) • Moisture content (25-70%) • Temperature (30°C)	• 40% moisture • 22 hrs	Castilho *et al.* (2000)

Table 2-1: Various condition for the production of pectinase from *A. niger* (cont'd)

Microorganism (Enzyme)	Fermentation method	Culture condition	Parameter	Optimum condition	Reference
A. niger (pectinase)	• Solid-state • Medium: $NaNO_3$, KCl, $MgSO_4.7H_2O$, $FeSO_4.7H_2O$, K_2HPO_4, yeast, sucrose, distilled water	Czapek's agar medium for 72 hrs at 30°C	• Wheat bran, sugar cane bagasse, nutrient solution • Time (24-144 hrs) • pH (4-7)	• 40 °C • pH 5 • Substrates (90% of wheat bran and 10% of sugarcane bagasse • 96 hrs.	Suresh and Viruthagiri (2010)
	• Submerged • Medium: $KHPO_43H_2O$, yeast extract, sucrose, Czapek concentrate ($NaNO_3$, KCl, $MgSO_4.7H_2O$,$FeSO_4.7H_2O$)	Culture for 120 hrs at 30°C	• Substrate (wheat flour, pectin, corn flour) • Temperature (20-70°C) • pH (3-8) • Time (24-120 hrs) • (100-200) rpm • carbon sources (glucose, sucrose starch)	• Wheat (1%), • 30°C • pH 5.5 • 72 hrs • 170 rpm • Starch (0.025%)	Palaniyappan *et al.* (2009)
	• Submerged • Medium: $(NH_4)_2SO_4$,$MgSO_4 \cdot 7H_2O$, KH_2PO_4, $FeSO_4 \cdot 7H_2O$, powder of sunflower head	Culture for 120 hrs at 34°C on 300 rpm	• Substrate (glucose, sucrose) • Nitrogen source ($(NH_4)_3PO_4$, $(NH_4)_2SO_4$) • Time (24-120 hrs)	• Glucose (4–6%) • $(NH_4)_2SO_4$ • 72hrs	Patil and Dayanand (2005)

2.7 Pectinase

Pectinase is a commercial and hydrolytic enzyme preparation which breaks down the pectin (a complex polysaccharide substrate), found in the cell wall of plants. It can be produced by submerged or solid-state fermentation. The pectic substances located primarily in the middle lamella between cells in higher plant tissues. The term "pectins" encompasses a group of acidic heteropolysaccharides with distinct structural domains. They are subjected to both biosynthetic and cell wall-based modifications (Palaniyappan *et al.*, 2009). The production of pectolytic enzymes using different substrates and the effect of physical parameters such as temperature, aeration rate, pH, time, carbon source, nitrogen source and type of fermentation were investigated (Palaniyappan *et al.*, 2009; Khairnar *et al.*, 2009; Nazneen *et al.*, 2011; Castilho *et al.*, 2000).

Pectinase is categorized according to their secretion pattern as extracellular and intracellular pectinases. An extracellular enzyme is excreted outside the cell into the medium in which that cell is living. Extracellular enzyme converts large substrate molecules like food for the cell or organism into smaller molecules that can then be more easily transported into the cell. While for an intracellular enzyme generally operates within the confines of the cell membrane. Membrane proteins remain attached in some way to the cell membrane. Both intracellular and extracellular pectinases are categorized on the pattern of their attack on the galacturonan part of pectin molecules (Saranraj and Naidu, 2013).

Pectinase has great biotechnological potential and can be employed in many important industrial processes. In food and related industry, major significance was being attached to the use of enzymes in upgrading quality, increasing yields of extractive processes, product stabilization, and improvement of flavour and by product utilization (Saranraj and Naidu, 2013). It is widely used in fruit juice processing like extraction and clarification, vegetable oil extraction, processing of alcoholic beverages, tea extraction, and a variety of application in food industries (Palaniyappan *et al.*, 2009).

This enzyme splits polygalacturonic acid into monogalacturonic acid by opening glycosidic linkages. Through this process, it softens the cell wall and increase the yield of juice extract from the fruits. Lee *et al.* (2005) reported that pectinase breaks down the

pectin molecules that led to a reduction of water holding capacity and consequently, free water is released to the system and reduces the viscosity during the enzymatic treatment. Vegetable food processing industries release pectin, containing wastewaters as by-product. Pre-treatment of these wastewaters with pectinolytic enzymes facilitates removal of pectinaceous material and renders it suitable for decomposition by activated sludge treatment.

Using of pectinase derived from *A. niger* in starch extraction said to give better release of starch without giving any significant changes to the physical properties of the starch. Tambalo *et al.* (2005) studied that the use of cellulose and pectinase in the extraction of starch from cassava and sweet potato increased the percent yield and subsequent recoveries without compromising the quality of the starch produced. The enzymes break the pectin-cellulosic matrix of cell membranes and thereby facilitate release of starch granules. Different types of pectolytic enzymes show different degree of efficiency in releasing starch from cassava. Continued breakdown of pectic substances from middle lamellar region of cell wall by pectinase, leads to cell separation, loss of tissue coherence and liquefaction of tissue. Pectinase further act on the pectic polymer of the inner cell matrix, resulting in the rupture of plasmolemma of the individual cells leading to release starch (Padmanabhan *et al.*, 1992).

2.8 Starch

Starch has the general chemical formula of $(C_6H_{10}O_5)_n$ with n the number of glucose monomers. Those polysaccharides are produced by all green plants as an energy storage and is a vital energy source for humans (Abbas *et al.*, 2010). Starch is found in large amounts and processed most usually from cereals such as corn or maize, wheat, rye, barley, rice, cassava, and potato.

Starch is deposited in granules in almost all green plants and in various types of plant tissues and organs. For example: leaves, roots, shoots, fruits, grains, and stems. The main site of starch synthesis and accumulation in the cereals is the endosperm, with starch granules that are located within the amyloplasts. The starch content in yam, cassava, potato, maize and sweet potato roots ranges between 65 and 90% of the total dry matter (Preiss, 2000). Yong *et al.* (1995) studied that the starch content of cassava was 70-80% on dry matter basis.

Starch is an important industrial product and has many applications which are not only in the food industry. Starch is being widely used in the paper manufacturing, in glues, such as wallpaper glue, cosmetics and even as a lubricant in oil drilling, wood, textiles, food and others. Dzogbefia *et al.* (2008) and Collares *et al.* (2012) stated that an important source of starch is from cassava which is a widely cultivated starchy root crop in many developing countries, especially in Africa, Latin America and Asia.

2.9 Structure and properties of starch

Starch is a polysaccharide carbohydrate consisting of a large number polymer of glucose units joined together by glycosidic bonds. Even though the glycosidic bonds are stable at high pH, they are hydrolyzed at low pH and at the end of the polymeric chain, a latent aldehyde group known to be the reducing end is present. It constitutes two types of macromolecules: amylose (Figure 2-2) and amylopectin (Figure 2-3), accounting for 20-27% and 73-80% depending on the plant. Amylopectin is a semi-crystalline highly branched polysaccharide with α, 1 – 4 and α, 1 – 6 linked side chains of glucose units while amylose is linear chains of glucose unit with α, 1-4 glycosidic bonds (Blennow, 2000). The relative content of amylose and amylopectin varies with the source of starch which reviewed in Table 2-2.

Amira *et al.* (2012) recommended that the amylopectin internal part is critical to the physical characteristic of granular starch. It is naturally found tightly and radially packed into dehydrated granules with origin-specific shape and size. The diameter of starch granules depend on its source. It ranges from 2 to 100 μm (maize, 2-30 μm; wheat, 1-45 μm; potato, 5-100 μm; cassava, 4-24 μm) The path of the starch chains is considered to be perpendicular to the granule surface. Native starch is partly crystalline. The crystallinity of native starch varies between 15 and 45% depending on the origin and pretreatment (Amira *et al.*, 2012).

According to the currently publicly known notion, amylopectin forms the crystalline component while amylose exists mainly in the amorphous form. Structural studies have shown that native starch has crystalline polymorphism. Cereal starch usually gives A-type modes symmetry of monoclinic whereas tuber starch gives B-type modes symmetry of hexagonal in x-ray diffraction. The crystal lattice of B-type starch contains more water molecules than the A-structures, which is suggested to be the reason for

higher stability of the A-structure. A-type and B-type structures molecular conformations are almost identical. Both of the structures have left-handed double helices with parallel strands. Double helices contain six glucose units per turn in each chain and the glucose units are in a chair conformation. Within the double helix, there are inter-chain but no intra-chain hydrogen bonds. In additional, parallelly packed double helices are connected through a hydrogen bonding network (Amira *et al.*, 2012).

Table 2-2: Ratio of amylose and amylopectin in some starches

Source	Amylose (%)	Amylopectin (%)
Potato	21	79
Maize	28	72
Wheat	26	74
Tapioca (cassava)	17	83
Waxy maize	-	100

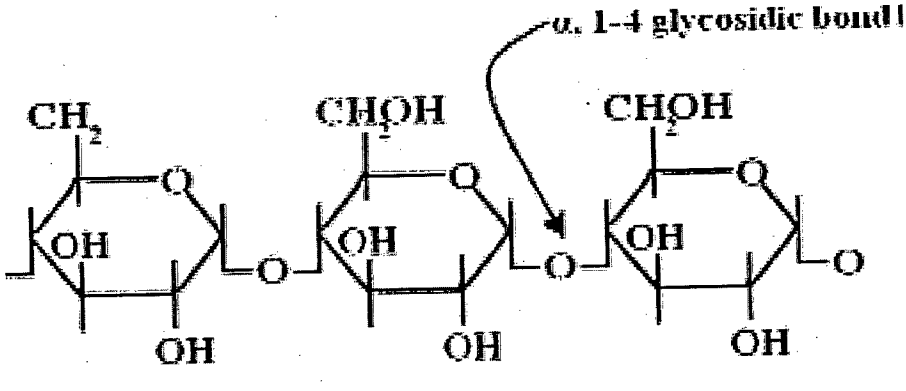


Figure 2-2: Illustration of the structure of Amylose by Amira *et al.* (2012)