ENZYMATIC PRODUCTION OF CASSAVA FLOUR USING PECTINASE

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ENZYMATIC PRODUCTION OF CASSAVA FLOUR USING PECTINASE

PRAKASH NAGASVARA RAVO

Thesis submitted in partial fulfilment of the requirements
for the award of the degree of
Bachelor of Chemical Engineering

Faculty of Chemical & Natural Resources Engineering
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JULY 2014

SUPERVISOR'S DECLARATION

We hereby declare that we have checked this thesis and in our opinion, this thesis is adequate in terms of scope and quality for the award of the degree of Bachelor of Chemical Engineering

Signature	:
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STUDENT'S DECLARATION

I hereby declare that the work in this thesis is my own except for quotations and summaries which have been duly acknowledged. The thesis has not been accepted for any degree and is not concurrently submitted for award of other degree.

Signature :
Name : PRAKASH NAGASVARA RAVO
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Dedication

This work is dedicated to my beloved parents and family, not forgetting all my supportive friends

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Thanking the lord his help and guidance which enable me to complete this research project successfully.

Firstly, I would like to extend my sincerest appreciation to Dr. Jolius Gimbun, my Undergraduate Research Project supervisor, for his willingness in supervising the progress of my research proposal from its initial phases till the completion of it. Without his guidance, I would not be able complete this proposal successfully. Next, I would like to extend my appreciation to the encouraging lecturers, and also my research members and seniors, for the roles they had played in giving me guideline and valuable advices during the progress of my proposal.

It was a challenging work with lots of obstacles but in the end some was overcome and the result is this thesis. The experiences and knowledge I have gained throughout the process of completing this proposal will be invaluable experience for me. My sincere thanks also dedicated to my colleagues and lecturers who were there whenever I need their help and assistance. Their views and tips were useful indeed.

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ABSTRACT

This paper presents the studies of enzymatic activity of pectinase enzyme in liberation of starch from cassava (*Manihot esculenta*), especially in cassava flour. The starch granules in cassava is hold up by pectin molecules, in which its cell wall can be broken by pectinase enzyme activity. Hence, the assumption is made where the amount of starch released is inversely proportional to amount of pectin molecules left after the enzyme activity. The experiment was designed to determine the optimum condition for the pectinase activity, where parameter of incubation time, temperature, pH of the solution and concentration of enzyme is taken into consideration. The final product, which is the dried cassava powder (flour) is analyzed for equivalent pectin weight by titration method. The flour produced based on optimum conditions was then analyzed for its nutritional content. Analysis of total protein content, moisture content, ash content, crude fat content, crude fiber content were carried out. Based on the experiment, it is found that the optimum condition for pectinase activity is at between 45-50°C temperature, 7 hours of incubation time, pH value of 5 and concentration of 20 mg/ml. At these conditions, the increase in starch release is about 6%, where this value could be higher when more detailed starch assay is carried out. The nutritional profile is also satisfactory, in some cases, very healthy profile, which means the cassava flour fermented with pectinase enzyme have high potential to replace wheat flour.

Key words: Cassava, starch, pectinase, nutritional profile, enzyme, optimum

ABSTRAK

Kertas kerja ini membentangkan kajian aktiviti enzim pectinase enzim dalam pembebasan kanji daripada ubi kayu (*Manihot esculenta*) terutamanya di dalam tepung ubi kayu. Granul kanji dalam ubi kayu adalah memegang oleh molekul pektin, di mana dinding sel boleh dipecahkan dengan aktiviti enzim pectinase. Oleh itu, andaian dibuat di mana jumlah kanji yang dikeluarkan adalah berkadar songsang dengan jumlah molekul pektin tinggal selepas aktiviti enzim. Eksperimen telah direka untuk menentukan keadaan optimum untuk aktiviti pectinase, di mana parameter masa pengeraman, suhu, pH dan kepekatan enzim diambil kira. Produk akhir, yang merupakan serbuk ubi kayu yang kering (tepung) dianalisis untuk berat pektin bersamaan dengan kaedah pentitratan. Tepung yang dihasilkan berdasarkan keadaan optimum dianalisis untuk kandungan nutrisinya. Analisis daripada kandungan protein, kandungan lembapan, kandungan abu, kandungan lemak mentah, kandungan serat mentah telah dijalankan. Berdasarkan eksperimen, didapati bahawa keadaan optimum untuk aktiviti pectinase adalah di antara 45-50°C, 7 jam masa pengeraman, nilai pH 5 dan kepekatan 20 mg/ml. Pada keadaan ini, peningkatan dalam siaran kanji adalah kira-kira 6%, di mana nilai ini mungkin lebih tinggi apabila kanji asai lebih terperinci dijalankan. Profil pemakanan juga memuaskan, dalam beberapa kes, profil yang sangat sihat, yang bermaksud tepung ubi kayu diperam dengan pectinase enzim mempunyai potensi yang tinggi untuk menggantikan tepung gandum.

Kata Kunci: Ubi kayu, kanji, pectinase, kandungan nutrisi, enzim, optimum

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LIST OF ABBREVIATIONS

A.Niger	Aspergillus Niger
AOAC	Association of Analytical Communities
BSA	Bovine Serum Albumin
CAS	Chemical Abstracts Service
FIIRO	Federal Institute of Industrial Research Oshodi
FOA	The Food and Agriculture Organization of the United Nations
HCL	Hydrochloric Acid
HCN	Hydrogen Cyanide
IITA	International Institute of Tropical Agriculture
KOH	Potassium Hydroxide
MSG	Monosodium Glutamate
NaOH	Sodium Hydroxide
ppm	Parts per million
RPM	Revolutions per minute
TPPIA	Thai Pulp and Paper Industries Association
TTSA	Thai Tapioca Starch Association
UV-VIS	Ultraviolet-Visible

LIST OF SYMBOLS

%	Percentage
°C	Degree Celsius
µm	micrometre
Ca	Calcium
Cal/tan/ha	Calories per tonne per Hectare
g	Grams
K	Potassium
mg/ml	Milligrams per Millilitres
mm	millimetre
Mt/ha	Metric Tonnes per Hectare
nm	nanometre
pH	Power of Hydrogen
Sp.	Species
Tons/ha	Tonnes per Hectare
w/w	Weight by Weight

1 INTRODUCTION

1.1 Background

Increasing population of human being have also increased the demand for staple food with rice, maize and wheat providing two third of them currently. Cassava, meanwhile, have the ability to become the substitute for all of them based on its low production cost, drought-tolerance, high-productivity per unit land, and high nutrients content. The low-in-protein but carbohydrate rich crop, represent an important energy source and staple food source for more than 500 million people throughout tropical Africa, Latin America and certain parts of Asia (Hock *et al.,* 1998). Being the large carbohydrate source, cassava is produced largely in Brazil industrial purpose and as export crop in Thailand. In Africa however, cassava is produced primarily for food consumption making it the most important crop in the continent (Fauquet *et al.,* 1990).

Cassava has emerged has food security for most of the developing country due to its ability to tolerate adverse environmental conditions to grow in diverse ecosystem. It grows best in high rain-fall area (annual rain-fall of 1000-2000 mm) but tolerates drought in low rain-fall area mainly in Africa. Soil type is not a concern for cassava growth as it can grow in soil regardless of pH ranging from alkaline to acidic and marginal or high-productive soil.

The principal parts of cassava plant are roots (50%), stem (44%) and leaves (6%) with the roots and leaves being nutritionally valuable parts which have potential as a feed source. The major carbohydrate source, the roots, consists of 60-65% moisture, 20-31% carbohydrates, 1-2% crude proteins, 0.2-0.6% ether extracts and very low content of minerals and vitamins. However, calcium and vitamin C is rich in cassava roots. Though the quantity is low, the protein quality is high given that the proportion of essential amino acid of total nitrogen is high (Olumide, 2004).

Although the processing of cassava roots yields stable products with the removal of most of the cyanogens, there is still retention of 12-33% of cyanide in cassava flour produced in eastern, southern and central Africa, whereas a relatively low magnitude (1.8-2.4%) of cyanide found in flour produced in West Africa and southern America (Cardoso *et al.*, 2005). Konzo, an epidemic paralytic disease, associated with

consumption of cassava flour of high cyanide content (Ministry of Health Mozambique, 1984). Konzo recorded high in Mozambique due to the low-rainfall in the country, as the cyanide content found to be doubled than to an average content (Cumbana *et al.*, 2007). The degree of gelatinization in cassava starch affects its physical properties such as bulk density and volume, and these changes in the properties can affect the primary use of cassava starch as flour.

1.2 Motivation& Statement of the problems

Pectinase is common enzyme used in food industries. It has been widely used in juice clarification as juice mainly contains polysaccharides such as pectins, cellulose and starch (Wong *et al.*, 2009). During the enzymatic treatment, 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 (Lee *et al.*, 2006). Using of pectinase in starch extraction said to give better release of starch without giving any significant changes to the physical properties of the starch. Espino *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.

However, cassava has some minus points as well. Raw cassava flour, which is actually the powder form of cassava roots, have only about 25% of starch readily available (TTSA., n.d). Relatively lower amount of starch reduces the economic value of cassava flour and at the same time affects the primary use of flour as the gelatinization factor is lesser. Hence, the focus is now on releasing maximum amount of starch from cassava. Enzymatic starch liberation using pectinase is chosen as the pectinase's primary function is to break the pectin molecules which hold up starch granules (Lee *et al.*, 2006). The objectives of the studies is to find out the optimum condition for enzyme activity in breaking the pectin molecules.

Considering climate in Malaysia, the high average annual rain-fall ranging from 1781 mm - 4159 mm (Malaysian Meteorological Department., 2010) would provide the best condition for growth and for production of starch with minimal cyanide content. Pectinase meanwhile can be used to reduce the gelatinization in the starch. Bearing these two factors, Malaysia can optimize the climate condition to become ideal producer of cassava starch worldwide.

1.3 Objectives

The work aims to produce flour from cassava by using pectinase enzyme that meets standards of flour and compare it to that of wheat flour.

1.4 Scope of this research

The study will be focusing on conducting test for each properties mentioned. The sample (roots/tubers) will be collected manually using traditional way of peeling, washing, drying, grinding and adding pectinase. The similar sample will be used for all tests with slight modification (if needed). The area of study narrows to the following:

a) To study the enzymatic starch liberation from cassava by pectinase, whereby the study will focus on identifying the optimum condition for the pectinase activity.
b) To test for nutritional profile of starch (moisture, protein, fat, fiber and ash) where the tests will be carried out on flour produced based on optimum condition.
c) To compare the test results to the specification of standard wheat flour

1.5 Main contribution of this work

The main contribution of the work will be determining the possible potential of pectinase to be used as enzyme to liberate starch which eventually will 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 slight introduction of the cassava plant and cassava flour. The issues related to the cassava flour are also studied where it leads to our motivation and problem statement. This chapter also provides the objectives of the research and the scope of the research to achieve the objective.

Chapter 2 gives a review of previous studies related to the topic. The studies covers applications of cassava, production of cassava plant, the nutritional profile of cassava flour, the degree of gelatinization and the toxicity of cassava flour. This chapter also provides studies about pectinase enzyme.

Chapter 3 is a detailed description of the methodology for the research. It provides explanation on the sample preparation for different parameter studies and the method for analysis.

Chapter 4 provides the overall finding of the study with detailed description for each parameters studied and each analysis made.

Chapter 5 draws together a summary of the thesis and outlines the future work which might be derived from the model developed in this work.

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

Cassava, with the scientific name of *Manihot esculenta*, also called as tapioca when it is dried to a starchy and powdery extract. Cassava is also known as '*yuca*' in Philippines, '*tabolchu*' in North East India and as '*mogo*' in Africa. The leaves of can be consumed, but it is the tubers, or actually its swollen root, that being harvested the most for consumption. The adult cassava plant can grow up to 3 metres tall. The cassava plant is mostly propagated through stem cutting. (Tonukari., 2004)

Figure 2-1: A cassava (*Manihot esculenta*) tuber or root, the main part of the plant.

2.2.1 Production of cassava

Global production of cassava as of 2005 is at the rate of about 160 million tons per year Nigeria is the world largest cassava producer; however Thailand is the number one exporter of dried cassava, where Thailand owns 77% of the total world cassava export in 2005. (FOA., 2008) Thailand is followed far behind by Vietnam, India and Costa Rica in the aspect of exporting country. In Nigeria, almost all the cassava produced is used for human consumption and less than 5% is used in industry. (Ukwuru & Egbonu., 2013)

High number of production in Africa is encouraged by the ability of the cassava plant to tolerate drought, a special character that is rare among all other crops. This means that the cassava plant can be grown in the areas of least annual rainfall and also in the place where the soil quality is said poor with pH ranging 4-9. (Okigbo., 1980)

Okigbo (1980) also identified few other reasons for the rapid growth of cassava plant in Africa besides drought tolerating ability and adaptation to poor soil. Cassava is easily propagated through stem cutting, unlike other major crops. Another major plus point of cassava plant is that is relatively high yielding plant and excellent source of calories. According to DeVries (1967), the potential yield of cassava can be up to 250,000 calories/ha/day and 75 tons/ha.

Figure 2-2: A cassava farm in Nigeria, Africa

2.2.2 Future of Cassava Production

Sub-Saharan Africa is expected to experience the most rapid growth in food demand in root and tubers averaging 2.6 percent per year through 2020 (Scott *et al*., 2000). This growth will account for nearly 122 million metric tons with most of the increase coming largely from cassava, 80 million metric tons (66% of the total). Cassava demand is estimated to grow at 2.0% annually for food and 1.6% per year for feed in developing countries, while total cassava production is projected to reach 168 million tons by 2020 based on the current production rate. However, according to Tonukari (2004) this amount can be far surpassed in developing countries with the right policies and incentives. Current production rate (1993) and estimated production rate in 2020 are shown in table 2.2.

Table 2-1: Cassava Production and Use in 1993, and Projected to 2020

Country/region	Area (million ha)		Yield (mt/ha)		Production (million mt)		Total use (million mt)	
	1993	2020	1993	2020	1993	2020	1993	2020
Sub-Saharan Africa	11.9	15.9	7.4	10.6	87.8	168.6	87.7	168.1
Latin America	2.7	2.7	11.3	15.6	30.3	41.7	30.3	42.9
Southeast Asia	3.5	3.5	12.1	13.7	42.0	48.2	18.9	24.4
India	0.2	0.2	23.6	28.4	5.8	7.0	5.7	7.3
Other South Asia	0.1	0.1	9.4	13.5	0.8	1.3	0.9	1.4
China	0.3	0.3	15.1	20.2	4.8	6.5	5.1	6.4
Other East Asia	na	na	na	na	na	na	1.8	1.9
Developing	18.8	22.9	9.2	12.0	172.4	274.7	152.0	254.6
Developed	...	...	12.1	14.7	0.4	0.4	20.7	20.5
World	18.8	22.9	9.2	12.0	172.7	275.1	172.7	275.1

2.2.3 Cassava as Food Source

Cassava crop provides an important food security to the people in Africa especially for those living in the belt of cassava plant farms. Given the main advantage is drought-tolerance, countries which has low annual rainfall, such as Nigeria and Congo, depend solely on cassava as their main energy source. The term ‘security’ is perfect for cassava plant as the crop or main part of the crop, grows conveniently underground. This means the invaders cannot easily destroy or remove the crop. (IITA., 2009)

Figure 2-3: Cassava is crucial to the food security of millions of people in sub-Saharan Africa

Nearly every person in Africa is consuming about 80 kilograms of cassava per year and it is estimated that 37% of dietary energy comes from cassava. According to International Institute of Tropical Agriculture (IITA), The Democratic Republic of Congo is the largest consumers of cassava in sub-Saharan Africa, followed by Nigeria.

Supplying 38.6% of caloric requirement in Africa in 1970's, which the figure is on the rise to date, cassava crop are now being consumed all over the world where the number of people relying on it reaches 500 million already. (Philips., 1974) In Nigeria especially, cassava roots are used for the preparation of *Gari* (fermented version of tapioca), *fufu* (dough and porridge-like food prepared from cassava), tapioca cakes (starch extracted from cassava) and of course the cassava flour, which possesses normal application of flours. (Etejero & Bhat., 1985)

Figure 2-4: Fufu: A Traditional Cassava Food in Nigeria

Figure 2-5: Gari: Another Traditional Cassava Food in Nigeria

2.2.3.1 Cassava Bread

The use of cassava flour as a raw material for the bakery and pastry industries is fast growing and gaining recognition as reliable partial substitute for wheat. Using high quality cassava flour particularly is suitable since it has no fat content which is important for storage life. Other possible advantages include its bland taste offering no foreign odour or flavour.

Figure 2-6: Cassava Bread or 'Bread of Tropics' in African Countries

Khalil *et al*. (2000), specifically reported that inclusion of cassava flour into wheat flour up to about 30% could still give an acceptable fresh loaf depending on the source of the flour. Federal Institute of Industrial Research Oshodi (FIIRO) Nigeria, has developed cassava bread with 20% high quality cassava flour substituted with 80% wheat flour, which gives similar characteristics of bread produced with 100% wheat flour both in sensory and nutritional properties. The use of composite flour will enable the developing countries, especially in countries where cassava is readily available, to save some scarce foreign exchange expanded on importing flour (Ogunsua., 1989).

2.2.3.2 Cassava Cookies

The need for strategic development and use of inexpensive local resources in the production of popular foods such as cookies has been recognized by organizations such as the Food and Agricultural Organization (FAO), the International Institute for Tropical Agriculture (IITA), Nigeria and the Federal Institute for Industrial Research Oshodi (FIIRO), Nigeria (Falola *et al.,* 2011). Research at IITA has shown that cassava flour (100%) can be used to prepare bakery products such as cookies and doughnuts (Onabolu and Bokanga., 1998). The resulting products are readily available and sold in Nigeria, thus helping to improve food and livelihood security.

Figure 2-7: Cassava Cookies in Brazil

2.2.3.3 Cassava Biscuits

Cassava flour proved effective as a partial substitute for imported wheat flour in biscuits. High quality cassava flour can substitute for up to 30% of wheat flour in sweet dough biscuit and 40% in hard dough biscuit, without consumers being able to detect any adverse change in colour, taste or texture when compared to 100% wheat flour control (Oyewole., 2002). Substitution of more than 40% wheat flour by cassava in biscuits affects the texture and crispiness. Researches are ongoing to find a good solution to finally make cassava a complete wheat substitute in biscuits.

Figure 2-8: Biscuits made from Mixture of Tapioca, Buckwheat and Rice Flour

2.2.4 Secondary Usage of Cassava

Apart from being major consumable energy source, cassava plant also offers various benefits and application to the world. The usages of cassava have been expanded to the industries such as starch, textile and fuel. The usage of cassava have been summarised in Table 2-1

Table 2-2: Cassava Products and Major Uses

Major uses	Products
Human consumption	Raw cassava
	Boiled cassava
	Cooked cassava slices
	Fried cassava slices
	Cassava flakes
	Fermented cassava
	Cassava flour

	Macaroni
	Fufu
	Gaplek
	Composite flour, bread
	Tapioca
	Gari
	Cassaripo or tucupa
	Cassava rice
Livestock feed	Cassava pellets
	Cassava meal
	Cassava chips
	Cassava slices (fresh or boiled)
	Cassava peels
	Cassava-leaf meal
	Broken roots
	Cassava silage
Industrial products	Starch
	Alcohol
	Glucose
	Acetone
	Dextrins
	Glues and pastes
	Binders
	Stabilizer
	Bodying agent (caramel)
	Fillers
	Dusting agent (chewing gum)
	Single-cell protein

Source: Archives of The United Nations University website.

Figure 2-9: Cassava chips for livestock feed

2.2.5 Cassava in Biofuels Production

Cassava is a good feed stock to produce ethanol because it has high starch content. Cassava starch can be converted readily to ethyl alcohol in a two-stage process involving the hydrolysis of starch slurry into glucose by liquefaction so that dextrin and subsequently fermentable sugar can be obtained. The glucose solution is diluted and converted to ethyl alcohol by the anaerobic action of yeast, ethanol of 95.6% w/w comes out through dehydration which is concentrated to 99.5% w/w (Ramasamy and Paramasamy., 2001). Thus cassava-based fuel ethanol is produced and it is usually denatured by small volume of gasoline or other materials added preventing people from drinking it, (Leng *et al*., 2008).

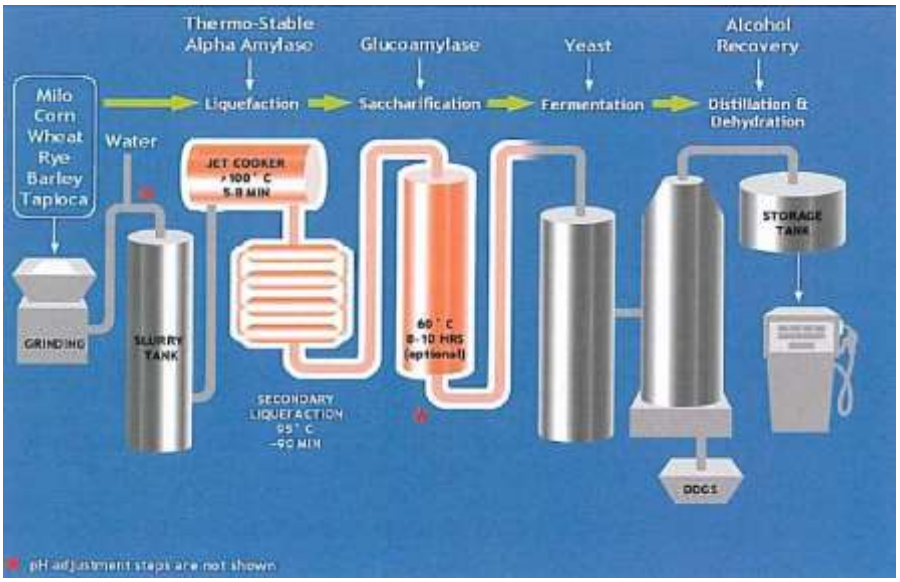

Figure 2-10: Conventional Ethanol Production Process

The ethanol produced is of high quality similar to cereal alcohol. Saccharification can be accomplished either by hydrolytic process or the biological process. The hydrolytic process uses hydrochloric acid or sulphuric acid. Yields are low and continuous use of acid causes equipment corrosion and is dangerous to handlers. The biological process uses amylolytic enzymes, which can be obtained from barley malt and moulds that grow on rice or wheat bran. This process results in higher yields than the acid process.

Figure 2-11: How to make biofuel in five easy steps (left-to-right): chipped cassava stalk; milled cassava stalk; after pre-treatment; after enzyme hydrolysis; post-fermentation – the bioethanol "beer".

Table 2-3: Some advantages of Biofuel Produced from Cassava Starch

It is not poisonous
It does not cause air pollution or any environmental hazard
It does not contribute to the green house effect problem (CO_2 addition to the atmosphere, causing global warming)
It has a higher octane rating than petrol as fuel. That is, ethanol is an octane booster and anti-knocking agent
It is an excellent raw material for synthetic chemicals.
Ethanol provides jobs and economic development to rural areas.
Ethanol reduces country's dependence on petroleum and it is a source of non – oil revenue for any producing country
Ethanol is capable of reducing the adverse foreign balance trade.

2.2.6 Processing of cassava

Cassava plant, before being processed for multi-purposes, especially the tuber part, will need to undergo several simple steps. Processing begins with harvesting of the roots where the woody end of the roots (stem) is cut off. The roots must be processed in 2 days in order to get optimum products.

Figure 2-12: Cassava is carried to Tinh Phong Cassava Starch Processing Factory in Quang Ngai Province, Vietnam

The process is then continued by peeling the skin of the roots and washing. Removal of water from the cassava is done by drying for certain hours. Then the cassava tubers are cut into small pieces before being grinded into powdered form. In the case where fermented starch is preferred (for most of the cases fermented starch is preferred, selected enzyme will be added in the powdered form of the cassava and fermented. The fermentation process is essential here in order to reduce cyanogenic glycosides, which will release hydrogen cyanide, HCN content of the flour produces. According to Tewe (n.d.), fermented cassava products store better and often are low in residual cyanide content.

Figure 2-13: Grated cassava and cassava flour

2.2.7 Nutritional Profile of Cassava Flour

Cassava is a starchy staple whose roots are very rich in a major source of energy, carbohydrates. Cassava plant, in fact, is the highest producer of carbohydrates among crop plants with the exception of sugarcane. Cassava reportedly can produce 250 x 10^3 calories/ha/day compared to 176 x 10^3 for rice, 110 x 10^3 for wheat, and 200 x 10^3 for maize (Okigbo., 1980).

Although rich in carbohydrates, cassava often been criticized for its low content of proteins. The uniqueness of cassava protein, meanwhile, is that it is found more in the leaves, the by-product of cassava root harvesting. Cassava roots contain as low as 1% of proteins, but the leaves in-contrast, have about 5% of proteins (Holloway & Bradbury., 1988). However, fermentation of cassava roots results in the enrichment of proteins by a factor of 6-8. Cassava is generally regarded as rich in dietary fiber, calcium and Vitamin C but low in fat.

Moisture content of cassava is between 50% to 70% while the moisture content of dried cassava powder or flour is between 9.2% to 12.3% (Charles *et al.*, 2005). Very high moisture content of cassava is one of the setbacks for cassava. This is because harvested cassava cannot be stored too long unless it is processed into smaller forms (chips or powder) and dried. High moisture content also causes their transportation from rural areas difficult and expensive. Processing the tuber into a dry form reduces the moisture content and converts into a more durable and stable product with less volume which makes it more transportable.

2.2.8 Toxicity of Cassava flour

Cyanide is the most toxic factor restricting the consumption of cassava roots and leaves. There are three different forms of cyanogens present in cassava root and leaves and these include linamarin, acetonehydrin (lotaustralin) and free HCN. The linamarin and lotaustralin undergo a sequential enzymatic breakdown and the final form is toxic free cyanide. Cyanogenic glycosides are a group of chemical compounds that undergo enzymatic hydrolysis to produce linamarin and lotaustralin with a composition of 93% and 7% respectively. The total of these three forms is called cyanogenic potential (Emmanuel *et al.,* 2012). The sweet and bitter taste of cassava is associated with the cyanide content of cassava, where the bitter taste is said to be a result of high cyanide content while sweet taste as a result of low cyanide content. Sweet-cassava products usually contain about 40 to 130 ppm of cyanide, non-bitter ones about 30 to 180 ppm of

cyanide, bitter 80 to 412.5 ppm of cyanide, and very bitter 280 to 490 ppm of cyanide. Consumption of cassava of with high in cyanide can cause acute cyanide poisoning and death. A rough guide to cyanide toxicity has been suggested (Coursey, 1973):-

a) Innocuous: less than 50 mg HCN/kg (fresh, peeled root)
b) Moderately poisonous: 50 - 100 mg HCN/kg (fresh, peeled root)
c) Dangerously poisonous: over 100 mg HCN/kg (fresh, peeled root)

In humans, the clinical signs of acute cyanide intoxication include drop in blood pressure, rapid respiration, rapid pulse, headache, dizziness, stomach pain, diarrhea, vomiting, convulsions, mental confusion, and twitching. When the cyanide level exceeds the limit an individual is able to detoxify, death due to cyanide poisoning can occur. The acute lethal dose of hydrogen cyanide for humans is reported to be 0.5 to 3.5 mg per kilogram of body weight. Children, particularly, are at risk because of their smaller body size (Kwok., 2008). Sweet cassava (low cyanide content) can be made safe for consumption just by peeling and thorough cooking. However, the bitter ones (high cyanide content) requires extensive procedures. After normal peeling and grating of the cassava roots, a prolonged soaking in water to allow fermentation and leaching should be followed, before thorough cooking which will release the volatile hydrogen cyanide gas. These methods are considered traditional ways of cassava cyanide reduction. Wetting method was also found to be effective in reducing the cyanide content of cassava flour in Mozambique (Cumbana *et al.*, 2007)

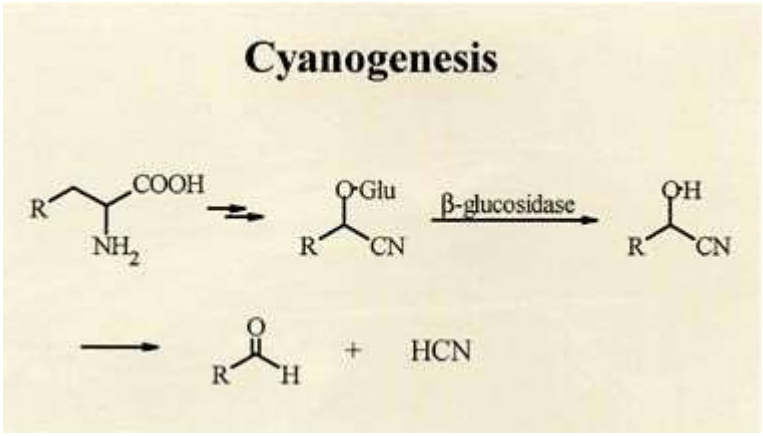

Figure 2-14: Illustration of the cyanogenesis releasing HCN

2.2.9 Degree of Gelatinization of Cassava flour

In plant cells starch is present as granules. Starch polymers (amylose and amylopectin) are tightly packed in granules with a high degree of molecular order and are associated by hydrogen bonding. Raw granules contain highly crystalline regions and are

birefringent in polarized bight. The granules are insoluble in cold water. When exposed to heat in the presence of water, the starch granules undergo an irreversible swelling and destruction of the internal crystalline structure and birefringence is lost. This transformation is termed gelatinization (Holm *et al*., 1988).

2.3 Pectinase

Pectinases were some of the first enzymes to be used in homes. Their commercial application was first observed in 1930 for the preparation of wines and fruit juices. Only in the 1960s did the chemical nature of plant tissues become apparent and with this knowledge, scientists began to use a greater range of enzymes more efficiently. As a result, pectinases are today one of the upcoming enzymes of the commercial sector. These enzymes are responsible for the degradation of the long and complex molecules called pectin that occur as structural polysaccharides in the middle lamella and the primary call walls of young plant cells.

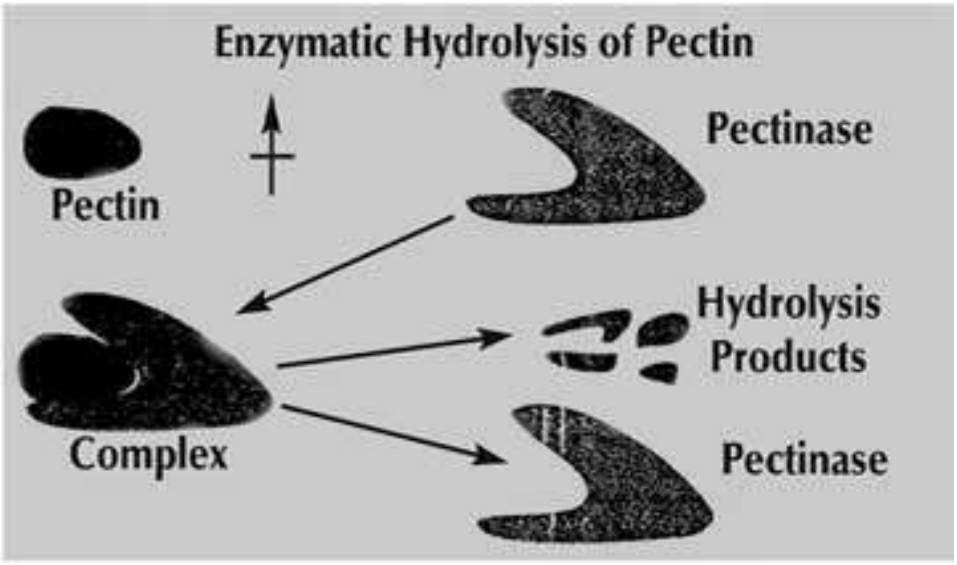

Figure 2-15: Simple Schematic of the Mechanism of Interruption of the Primary Wall by Hydrolysis of Pectin in the Wall by Pectinase

2.3.1 Pectinase in Fruit Juice Extraction

The largest industrial application of pectinases is in fruit juice extraction and clarification. Pectinase contribute to fruit juice viscosity and turbidity. A mixture of pectinases and amylases is used to clarify fruit juices. It decreases filtration time up to 50% (Blanco *et al*., 1999). Treatment of fruit pulps with pectinase also showed an increase in fruit juice volume from banana, grapes and apples (Kaur *et al*., 2004).

Pectinases in combination with other enzymes, viz., cellulases, arabinases and xylanases, have been used to increase the pressing efficiency of the fruits for juice extraction (Gailing *et al*., 2000).

Vacuum infusion of pectinases has a commercial application to soften the peel of citrus fruits for removal. This technique may expand in future to replace hand cutting for the production of canned segments (Baker and Wicker., 1996). Infusion of free stone peaches with pectin methyl esterase and calcium results in four times firmer fruits. This may be applied to pickle processing where excessive softening may occur during fermentation and storage (Baker and Wicker., 1996).

2.3.2 Pectinase in Textile Processing and Bio-Scouring of Cotton Fibers

Pectinases have been used in conjunction with amylases, lipases, cellulases and hemi-cellulases to remove sizing agents from cotton in a safe and eco-friendly manner, replacing toxic caustic soda used for the purpose earlier (Hoondal *et al*., 2000). Bio-scouring is a novel process for removal of non-cellulosic impurities from the fiber with specific enzymes. Pectinases have been used for this purpose without any negative side effect on cellulose degradation.

2.3.3 Pectinase in Waste Water Treatment

The wastewater from the citrus-processing industry contains pectinaceous materials that are barely decomposed by microbes during the activated-sludge treatment (Tanabe *et al*., 1987) have tried to develop a new wastewater treatment process by using an alkalophillic microorganism. Their soil isolate of an alkalophilic Bacillus sp. produces an extracellular endopectate lyase in alkaline media at pH 10.0. Treatment with this strain has proved to be useful in removing pectic substances from the wastewater.

For treatment of wastewater from citrus processing industries various processes have been investigated, which include: physical dewatering, spray irrigation, chemical coagulation, direct activated sludge treatment and chemical hydrolysis followed by methane fermentation (Tanabe *et al*., 1987). These processes have some defects, such as low efficiency due to chemical resistance of the pectic substances, high treatment cost, long treatment periods and complexity of the process 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 (Hoondal *et al*., 2000).

2.3.4 Other Pectinase Applications

Pectinase treatment accelerates tea fermentation and also destroys the foam forming property of instant tea powders by destroying pectins (Carr., 1985). They are also used in coffee fermentation to remove mucilaginous coat from coffee beans.

During papermaking, pectinase can depolymerise pectins and subsequently lower the cationic demand of pectin solutions and the filtrate from peroxide bleaching (Viikari *et al*., 2001).

Citrus oils such as lemon oil can be extracted with pectinases. They destroy the emulsifying properties of pectin, which interferes with the collection of oils from citrus peel extracts (Scott., 1978).

2.3.5 Optimum Conditions for Activity

Pectinase enzyme can be produced by fungus namely Aspergillus Niger (A.Niger) and Bacillus Pumilis.

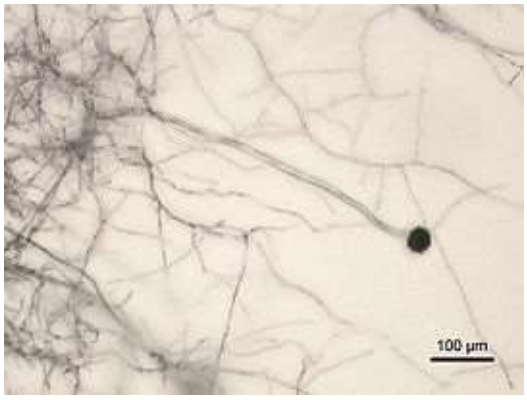

Figure 2-16: Micrograph of A. Niger grown on Sabouraud agar medium (100x magnification)

The optimum temperature and pH value of the pectinase solution differs according to the fungus that produces them. Figure below shows the summary of optimum conditions by Kashyap *et al.* (2001) for pectinase activity.

Characterization of microbial pectinases

Producer	Type of pectinase	Opti. pH for activity	Opti. Temp. for activity (°C)	Reference
Acidic pectinases				
Aspergillus niger CH4	Endo-pectinase, Exo-pectinase	4.5–6.0 3.5–5.0	Below 50	Acuna-Arguelles et al., 1995
Penicillium frequentans	Endopolygalacturonase (Endo-PG)	4.5–4.7	50	Borin et al., 1996
Sclerotium rolfsii	Endo-PG	3.5	55	Channe and Shewal, 1995
Rhizoctonia solani	Endo-PG	4.8	50	Marcus et al., 1986
Mucor pusilus	PG	5.0	40	Al-Obaidi et al., 1987
Clostridium thermosaccharolyticum	Polygalacturonate hydrolase	5.5–7.0	30-40	Rijssel et al., 1993
Alkaline pectinases				
Bacillus sp. RK9	PGL	10.0	–	Fogarty and Kelly, 1983
Bacillus sp. NT-33	PG	10.5	75	Cao et al., 1992
Bacillus polymyxa	PG	8.4–9.4	45	Nagel and Vaughn, 1961
Bacillus pumilis	PATE	8.0-8.5	60	Dave and Vaughn, 1971
Amucola sp.	Pectate lyase (PAL)	10.25	70	Bruhlmann et al., 1994
Xanthomonas compestris	PATE	9.5	25-30	Nasuno and Starr, 1967
Bacillus No. P-4-N	PG	10–10.5	65	Horikoshi, 1990
Bacillus stearothermophillus	PATE	9.0	70	Karbassi and Vaughn, 1980
Penicillium italicum CECT 22941	Pectin lyase	8.0	50	Alana et al., 1990
Bacillus sp. DT 7	Pectin lyase	8.0	60	Kashyap et al., 2000
Bacillus subtilis	PAL	8.5	60-65	Chesson and Codner, 1978
Pseudomonas syringae pv. Glycinea	PAL	8.0	30-40	Magro et al., 1994

Figure 2-17: Summary of Optimum Conditions for Pectinase Activity

Collares *et al.* (2012) said that the optimum enzyme loading time for pectinase from A.Niger is 7 hours.

2.4 Cassava Starch

Starch is one of the most abundant substance in nature, renewable and almost unlimited resources (Pandey *et al*., 2000). Starch is produced from grain and root crops. It is mainly used as food but it is also readily converted chemically, physically and biologically into many useful products to date (Matsui *et al*., 2004). Starch is used to produce such diverse product as food, paper textile, adhesives, beverages, confectionary, pharmaceuticals and building materials.

Cassava starch has many remarkable characteristics, including high paste viscosity, high paste clarity, and high freeze – thaw stability, which are advantageous to many industries (Gomes *et al*., 2005). Cassava starch is produced primarily by wet milling of fresh cassava roots but in some countries such as Thailand, it is produced from dry cassava chips (Ceballos *et al*., 2006). Starch is the main constituent of cassava; about 25% starch may be obtained from mature, good quality tuber and about 60% starch may be obtained from dry cassava chips

2.4.1 Native Cassava Starch

Native starches are produced through separation of naturally occurring starch from either grain or root crops such as cassava, maize and sweet potato, and can be used directly in producing certain foods such as noodles (Wang *et al*., 1993). The raw starches produced still retain the original structure and characteristics and are called "native starch". Native starch is the basic starch product that is marketed in the dry powder form under different grades for food, and as pharmaceutical, human and industrial materials. Native starch has different functional properties depending on the crop source and specific types of starch are preferred for certain applications.

Native cassava starches have limited usage, mainly in the food industry, because they lack certain desired functional properties. The native granules hydrate easily when heated in water. They swell and gelatinize, the viscosity increases to a peak value, followed by a rapid decrease, yielding weak boiled, stringy, and cohesive paste of poor stability and poor tolerance to the acidity with low resistance to shear pressure, as commonly employed in modern food processing (Balagopalan *et al*., 1988).

2.4.2 Modified Cassava Starch

Modified starch is native starch that has been changed in its physical and/or chemical properties. Modification may involve altering the form of granule or changing the shape and composition of the constituent amylose and amylopectin molecules. Modification is therefore carried on the native starch to confer it with properties needed for specific uses in many industries such as food, pharmaceutical, textile, petroleum and paper pulp industries. The reasons why native starch is modified include: modifying cooking characteristics (gelatinization); to reduce retrogradation and paste stability when cooled or frozen; to increase transparency and texture of pastes and gels; and to improve adhesiveness between different surfaces such as in paper applications.

2.4.3 Utilization of Cassava Starch in Industry

Although cassava starch is mostly used in food industry and as food source, especially in African countries, it is also plating its role in other industries as well. Paper, adhesive and textile industries are few of them.

2.4.3.1 Paper Industry

Cassava starch has very good properties that are highly desirable for the paper manufacturer. Cassava starch, as a dominant source of starch in Nigeria, possess a

strong film, clear paste, good water holding properties, and stable viscosity (Srirotha., 1999). It should be the most suitable material for the paper industry in West Africa. In the paper and board industries, starch is used in large quantities at three points during the manufacturing process.

When the paper sheet or board has been formed and partially dried, starch generally oxidized (or modified) is usually added to one or both sides of the paper sheet or board to improve the finished product appearance, strength and printing properties. In the coating operation, when a pigment coating is required for paper, starch acts as coating agents and as adhesive.

Figure 2-18: Cassava Starch Consumption in Paper Industry was Estimated at 42000 tonnes/year in 1990 (Thai Pulp and Paper Industries Association-TPPIA)

2.4.3.2 Textile Industry

In the textile industry, the properties of the starch used are abrasion resistance, flexibility, ability to form a bond to the fiber, to penetrate the fiber bundle to some extent and to have enough water holding capacity so that the fiber itself does not rob the size of its hydration. Textile printing or the impression of the design on fabrics requires a carrier for the dyes and pigment and modified starches have found special uses in this application. Printing pastes are high viscosity of media that preferably will not change on ageing and will not resist the effect of added acids or alkalis as required by the colour agent. A sharp image is required and thus a short non stringy paste. Modified starches are frequently mixed with other industrial gums to give the required viscosity and paste characteristics (Balagopalan *et al*., 1988).

Figure 2-19: As of 1990, Cassava Usage in Textile Industry was 1550 tonnes/year in Vietnam

2.4.3.3 Adhesive Industry

Starch is a popular base for adhesives, particularly those designed to bond paper in some form to itself or to other materials such as glass, mineral wool, and clay. Starch can also be used as a binder or adhesive for non-paper substance such as charcoal in charcoal briquettes, minerals wool in ceiling tiles and ceramics before firing. The starches most commonly used for the manufacturing of adhesives are from maize, potato and cassava and according to Graffham *et al*. (1998) cassava starch appears more suitable in several respects.

Cassava starch adhesives are more viscous and smoother for working. They are fluid, stable glues of neutral pH that can be easily prepared and can be combined with many synthetic resin emulsions (Dziedzoave *et al*., 1999). For top quality work, cassava starch is thought to be ideal, because it is slightly stronger than potato starch adhesive while being odourless and tasteless, excellent as an adhesive for postage stamps, envelope flaps, and labels.

Figure 2-20: The exceptional property of cassava starch once frenzied heated or exposed to chemical will developed more adhesive

2.4.3.4 Glucose Production (Monosodium Glutamate)

Monosodium glutamate (MSG), the sodium salt of L-glutamic acid is a popular flavour enhancer and an additive for foods. It was used primarily in Asian foods but its use is now widespread (Jyothi *et al*., 2005). The product is used extremely in many parts of the continent in powder or crystal form as a flavouring agent in food such as meats, vegetables, sauces, and gravies. Cassava starch and molasses are the major raw materials used in the manufacture of monosodium glutamate in the Far East and the Latin American countries (Sanni *et al*., 2005).

Figure 2-21: Cassava Starch Market is expected to Increase to Meet the Demand of The Export Potential for MSG for Fast-Food Industries

The starch is usually hydrolysed into glucose by boiling with hydrochloric or sulphuric acid solution in closed converters under pressure. The glucose is filtered and converted into glutamic acid by bacterial fermentation. The resulting glutamic acid is refined,

filtered and treated with caustic soda to produce monosodium glutamate, which is then centrifuged and dried in drum driers. The finished product is usually at least 99% pure.

2.4.3.5 Animal Feed

Being a cheap carbohydrate source capable of supplying adequate calories, cassava tubers offer great potentials as an animal feed. Cassava is widely used in most tropical areas in feeding pigs, cattle, sheep and poultry. Dried peel of cassava roots are fed to the sheep and goats, and raw or boiled root are mixed into a mash with protein concentrate such as maize, sorghum, groundnut or oil palm kernel meal and mineral salts for livestock feeding (Onuma *et al*., 1983).

Cassava is similar to feed grain as it consist almost entirely of starch and it is easy to digest. The roots are, therefore especially suited to feeding young animals and fattening pigs. Many feeding experiments have shown that cassava provide a good quality carbohydrate, which may be substituted for maize or barley and that cassava rations are especially suitable for swine, dairy cattle and poultry (Nzigamasabo and Ming., 2006).

Figure 2-22: Cassava foliage (leaves and stem), peels and particularly the root; fresh, dried or in silage form; alone or mixed with other feed is used in feeding different species of animals. Dried cassava roots are processed into pellets, chips and meal, mainly for poultry and pig industries

However cassava cannot be used as a sole feedstuff because of its deficiency in protein and vitamins, but must be supplemented by other feeds that are rich in these elements. Proper formulation of the diet is equally important to make the feed nutritionally balanced, since animal performance is highly dependent on it. Cassava leaf meal is a highly nutritious protein-rich ingredient that offers a vast scope for inclusion in root meal diets. However the leaves have to be properly detoxified by drying prior to its

inclusion in compound feeds. Cassava leaves have been found to contribute substantially to the energy requirement of poultry, swine and ruminants (Balagopalan., 2002).

2.4.3.6 Plywood Industry

The cassava starch has freshly been presented to the plywood industry as the manufacturing of the plywood is the supplement of wood using glue. Now, the cassava starch is being used as a component and or ingredient in the production of glue as it has the sticky stuff. The starch is comprised in order to permit the plywood to attach in thick layers and develop strong and durable. Expending starch as an ingredient also benefits to reduce the glue manufacturing cost as well subsequently, it takes up to 50% of the total ingredients. Moreover, cassava starch also has a smooth and charming superficial surface that forms no precipitation in the glue manufacturing process, and a low price.

Figure 2-23: Cassava Starch is an Ingredient to Produce Industrial Glue for Plywood Production

3 MATERIALS AND METHODS

3.1 Overview

This part of the paper presents methodology for starch liberation from cassava using pectinase, which actually is the production of flour from cassava roots or tubers. Temperature, pH value of the solution, time of enzymatic activity of pectinase and the enzyme concentration were the parameters studied. The major works of the study focused on the production of the flour at optimum conditions. As a part justifying the potential of this work, cassava flour produced was then analyzed for its nutritional profile and was compared with commercial wheat flour. The analysis of the flour focused on proximate analysis which includes moisture content, ash content, crude fat, crude fiber and crude protein.

3.2 Materials

Main material needed for the research is, of course, tubers (roots) of cassava plant. For the enzymatic starch liberation, pectinase enzyme (pectinase from Aspergillus Niger) will be used. Common chemicals, such as hydrochloric acid (HCL), sodium hydroxide solution (NaOH) and so will be used for proximate analysis. These chemicals were obtained from UMP Chemical Laboratory store.

Figure 3-1: Cassava Tubers (Roots) are the Main Material for this Research

3.3 Raw Material Preparation

Cassava roots was obtained from local market in Gambang, were the rainfall averages 2000 mm to 2500 mm annually (Malaysian Meteorological Department., 2010). The plant selected would be an adult one aging 6 months and above. The harvested tuber was peeled and washed before being cut into small pieces. The cut pieces were washed before being dried (either sun-dried or oven-dried) for certain hours, until the moisture content is not significant. The dried pieces were then grinded using grinder and sieved to 600 µm.

Figure 3-2: Cut and Sun-Dried Cassava Roots

50 g of pectinase enzyme from Aspergillus Niger bacteria was bought directly from Sigma Aldrich (CAS number: 9032-75-1) in solid form. The enzyme was kept in chiller at 4°C

Figure 3-3: 50 g of Pectinase from Aspergillus Niger

3.4 Sample Preparation

The 20 g dry weight (dry weight was determined based on moisture content) of raw cassava powder was prepared in 250 ml volumetric flask. The pectinase solution (10 mg/ml), meanwhile, was prepared by adding 5 g of pectinase enzyme (solid phase) into

500 ml ultra-pure water. The pectinase solution was then added into the raw cassava powder.

Figure 3-4: Raw Cassava Powder Added with Pectinase Solution according to Required Concentration (i.e. 5 mg/ml)

The pH value was adjusted to a value of 5.0 using 0.1 M HCL or NaOH. The volumetric flasks were then prepared for incubation (e.g. cover the opening with aluminium foil and cotton wool).

Figure 3-5: The pH value of Solution of Pectinase and Raw Cassava Powder was adjusted using pH meter and either by NaOH or HCL

The flasks were then placed onto incubator platform. The temperature was set at 40°C, RPM at 150 and the process was run for different period of time (e.g. 3, 5,6,7,9 hours).

Figure 3-6: Prepared Sample were Kept in Incubator (i.e. at 150 RPM, 40°C and 2 hours)

Next, the sample was centrifuged at 5000 RPM for 3 minutes and the supernatant liquid was get rid.

Figure 3-7: Incubated Sample was centrifuged and the Supernatant was removed

The wet form of powder was dried in oven at 60°C for at least 24 hours. The dried cassava powder (in solid form) was crushed and pestle into powdered form using mortar before being analysed for equivalent pectin weight using titration method. Best time was chosen and the steps were repeated for different pH value (4, 5, 6 and 7), followed by different temperature (30°C, 40°C, 50°C and 60°C) and finally different enzyme concentration (5 mg/ml, 10 mg/ml 15 mg/ml and 20 mg/ml).

Figure 3-8: The Drying of Wet Cassava Powder (Oven-dried)

3.5 Determination of Equivalent Weight of Pectin

Pectinase enzyme helps to break pectin molecules which holds up starch granules, and subsequently releases starch. So, the identification of equivalent weight of pectin molecules, shows the amount of starch released. The lower the equivalent pectin weight, the higher the amount of starch released. The equivalent weight of pectin was determined using titration method (Aina *et al*., 2012). 5 g of sample was added with 5ml of ethanol. Few drops of universal indicator was added into the solution and the solution was then titrated with 0.01 M NaOH. The equivalent weight of pectin was calculated using the formula as:-

$$\textbf{\textit{Equivalent weight of pectin}}\left(\frac{mg}{mol}\right) = \frac{\textbf{\textit{mass of sample}}}{\textbf{\textit{Volume of Titrate}} \times \textbf{\textit{Molarity of NAOH}}} \times \mathbf{100} \quad \textbf{\textit{eq. (1)}}$$

The equivalent weight of pectin for different sample is plotted into graph to choose the optimum condition.

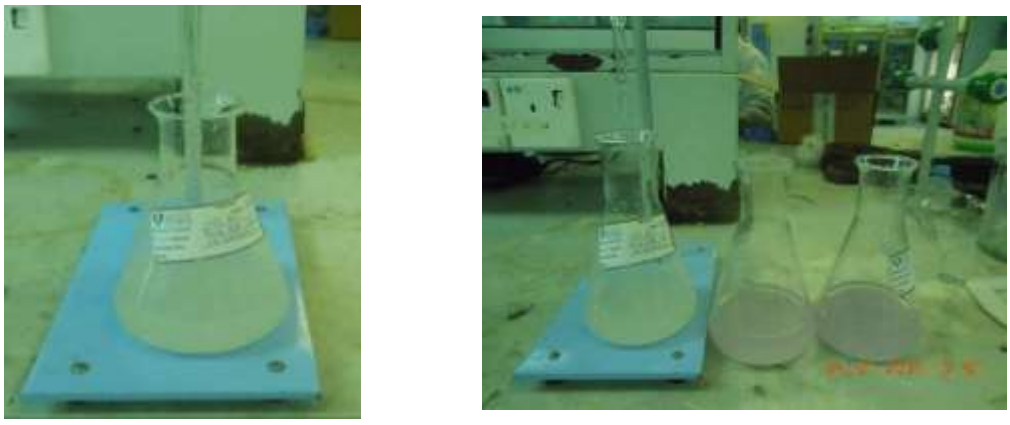

Figure 3-9: Titration Method to Determine Equivalent Pectin Weight

3.6 Proximate Analysis

3.6.1 Moisture Content

The moisture content of cassava flour was determined by gravimetric method as suggested by AOAC method 925.09. One gram (1 g) of sample was pre-weighed (W1) in a beaker and placed in an oven at 105°C for 24 h. The sample was removed from the oven, cooled in a desiccators, and reweighed (W2). Moisture percentage was calculated according to the formula suggested by Numfor (2007).

Moisture (%) = (W1 – W2) / W1 x 100 ***eq. (2)***

3.6.2 Ash Content

Total ash content of cassava flour was determined by ashing method as explained by AOAC method 923.03. Sample (1 g) was weighed into a pre-weighed porcelain crucible and incinerated at least for 24 hours in furnace at a temperature of 600°C. The crucible was removed from the muffle furnace, cooled in desiccators and weighed. Ash content was calculated according to the following formula suggested by Numfor (2007).

Ash (%) = (ash weight / sample weight) x 100 ***eq. (3)***

Figure 3-10: Ashing Method (Furnace at 600°C for 24 hours)

3.6.3 Crude Protein Content

Total protein content was determined through Lowry Method (Lowry *et al*., 1951), where the method of 'protein determination without protein precipitation' was used. Protein content of sample was to be determined from calibration curve based on

standard protein, Bovine serum albumin (BSA) solutions. 400 µg/ml protein standard solution was diluted in water to a volume of 1.0 ml in labelled test tubes. Blank test tube was labelled and added 1.0ml of water. Sample was then added to labelled test tube and diluted to 1.0 ml with water. Then, 1.0 ml of the Lowry Reagent solution was added to standard, blank and sample tubes, allowed to mix well and left for 20 minutes at room temperature. With rapid and immediate mixing, 0.5 ml of Folin & Ciocalteu's Phenol Reagent Working solution was added to each tube, and allowed colour to develop for 30 minutes. The solutions were transferred into cuvets and the absorbance of the standards and sample tubes versus the blank is measured at a wavelength of 700 nm. The absorbance readings are to be completed within 30 minutes. The absorbance values of the standard versus their corresponding protein concentrations was plotted to prepare a calibration curve. The protein content of sample was then determined from the calibration curve.

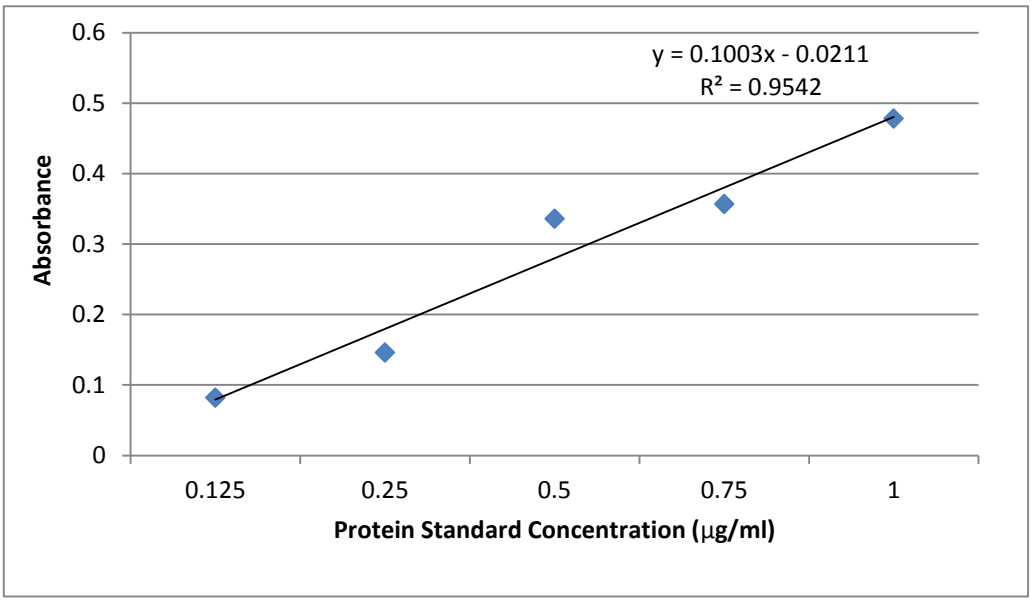

Figure 3-11: Calibration Curve of Standard Protein (BSA)

3.6.4 Crude Fat Content

The crude fat was determined by the method of Soxhlet extraction as explained by AOAC method 920.85. One gram (1 g) sample was weighed into an extraction thimble and covered with absorbent cotton. 50 ml solvent (petroleum ether) was added to a pre-weighed cup. Both thimble and cup were attached to the extraction unit. The sample was subjected to extraction with solvent for 30 min followed by rinsing for 1.5 h. The solvent was evaporated from the cup to the condensing column. Extracted fat in the cup

was placed in an oven at 110°C for 1 h and after cooling the crude fat was calculated using following formula as suggested by Numfor (2007).

Crude fat (%) = (Extracted fat / Sample weight) x 100 ***eq. (4)***

3.6.5 Crude Fiber Content

Crude fiber was determined by extraction as described by AOAC method 920.86. Defatted sample (1 g) was placed in a glass crucible and attached to the extraction unit. 150 ml boiling 1.25% sulphuric acid solutions were added. The sample was digested for 30 min and then the acid was drained out and the sample was washed with boiling distilled water. After this, 1.25% sodium hydroxide solution (150 ml) was added. The sample was digested for 30 min, thereafter, the alkali was drained out and the sample was washed with boiling distilled water. Finally, the crucible was removed from the extraction unit and oven dried at 110°C overnight. The sample was allowed to cool in desiccators and weighed (W1). The sample was then ashed at 550°C in a muffle furnace for 2 h, cooled in desiccators and reweighed (W2). Extracted fiber was expressed as percentage of the original undefatted sample and calculated according to the formula as suggested by Numfor (2007).

Crude fiber (%) = (W1) –(W2)/Weight of sample x 100 ***eq. (5)***

4 RESULTS AND DISCUSSION

4.1 Overview

This chapter presents the entire finding of the topic which includes the experiments results leading to decision on the optimum conditions for pectinase activity, as well as the analysis results which is used to compare the potential of cassava flour from this study to wheat flour.

4.2 Moisture content

Cassava flour production per unit cassava tuber will be very low since the composition of water is more than 60%. However, after drying of the powdered tubers, the moisture content found to be low, in fact lower than the wheat flour. The raw cassava powder is found to contain average of 8% moisture. These moisture content is considered in further processes requiring dry weight sample. The average moisture content after the incubation process is found to be 9%. In both before and after incubation, the moisture content is lower than 10%. This properties of cassava is an important factor contributing to its economic value as its shelf life will be increased.

4.3 Relationship between time and pectinase activity

For the first 4 hours, the equivalent weight of pectin does not show significant reduction. However, after 4^{th} hour, the changes begin to rise where the equivalent weight of pectin reduces, which means than more starch was released. The shaded area reflects optimum temperature where the activity of pectinase is very significant after 6 hours of incubation period where the pectin molecules is reduced up to 10%. However, the time factor should be carefully chosen as it will also affects the overall processing time. Hence, the chosen result, more than 6 hours (i.e. 7 hours) is significant with Collares *et al.* (2012) where the enzyme activity time is found to be 7 hours. The relationship between time and equivalent pectin weight is as below:-

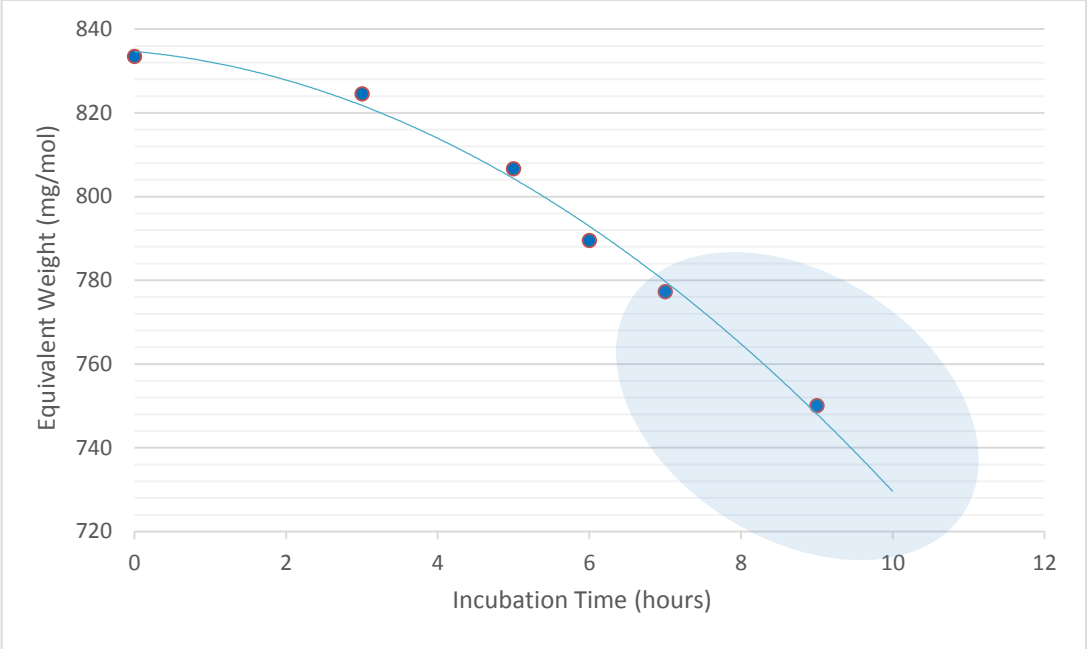

Figure 4-1: Graph of equivalent weight of pectin against time of incubation

4.4 Relationship between pH value of solution and pectinase activity

The activity of pectinase is at maximum at pH value of 5.0, at slightly acidic condition. Kashyap *et al.* (2001) also stated that the optimum pH value for pectinase activity should be between 4.5 to 6. Too high or too low a PH would also result in the enzyme denaturing. The logical explanation of why the enzyme denatures would be that if the pH is decreased too much, there would be too many H+ ions around the protein and thus the H+ ions would be attracted to the places in the enzyme which were more negative than the enzyme, thus forming a hydrogen bond there. Also, if the pH is increased, there would be too many OH- ions and they would interact with the positive regions in the protein. This negative or positive region could possibly be in the active site, and even if it is not it does end up disfiguring the enzyme. Due to the fact that the enzyme-substrate interaction is so specific, even the slightest deformity of the active site directly or indirectly will result in the enzyme involved not working properly. This change, if permanent, will render the enzyme useless. The graph below clearly indicates the maximum pectinase activity at pH 5.

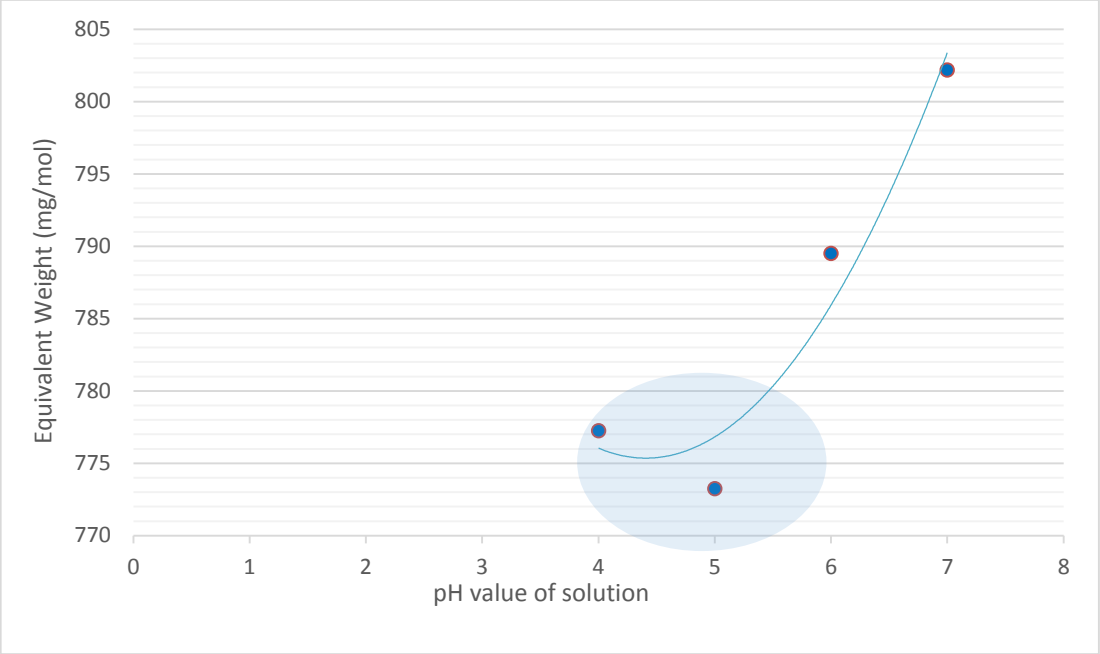

Figure 4-2: Graph of equivalent weight of pectin against pH of solution

4.5 Relationship between concentration and pectinase activity

As estimated, the concentration of pectinase activity is directly proportional to pectinase activity. In our case, the maximum concentration, which is 20 mg/ml pectinase able to break more pectinase.

The logicality is very simple, where the higher concentration of pectinase enzyme would increase the probability of breaking more pectin molecules and thus releasing more starch. This is because there will be more presence of active sides that will bind to cell wall of pectin, thus breaking them.

However, in our study, the using of pectinase is limited to maximum concentration of 20 mg/ml due to its availability. Hence, the possibilities of using aspergillus niger bacteria, which produces pectinase enzyme, should be studied. The relationship between concentration and pectinase activity is illustrated as below:-

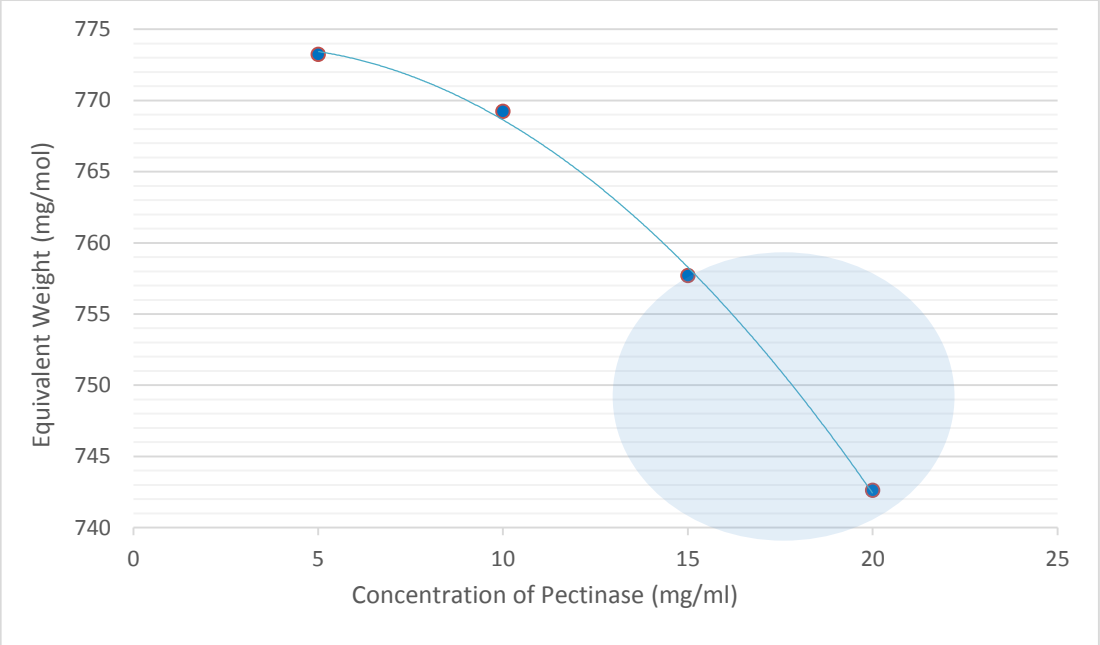

Figure 4-3: Graph of equivalent weight of pectin against concentration of pectinase

4.6 Relationship between temperature and pectinase activity

As for temperature, the pectinase enzyme activity is at optimum between 45°C-50°C. At higher temperature, the higher temperature will make the reaction go faster. However, the higher temperature will eventually denature the enzyme, ultimately destroying the enzymatic action.

The readings comply with previous study for optimum temperature of 50°C (Kashyap *et al*., 2001). The relationship between temperature and pectinase activity is illustrated in figure 4-4 below.

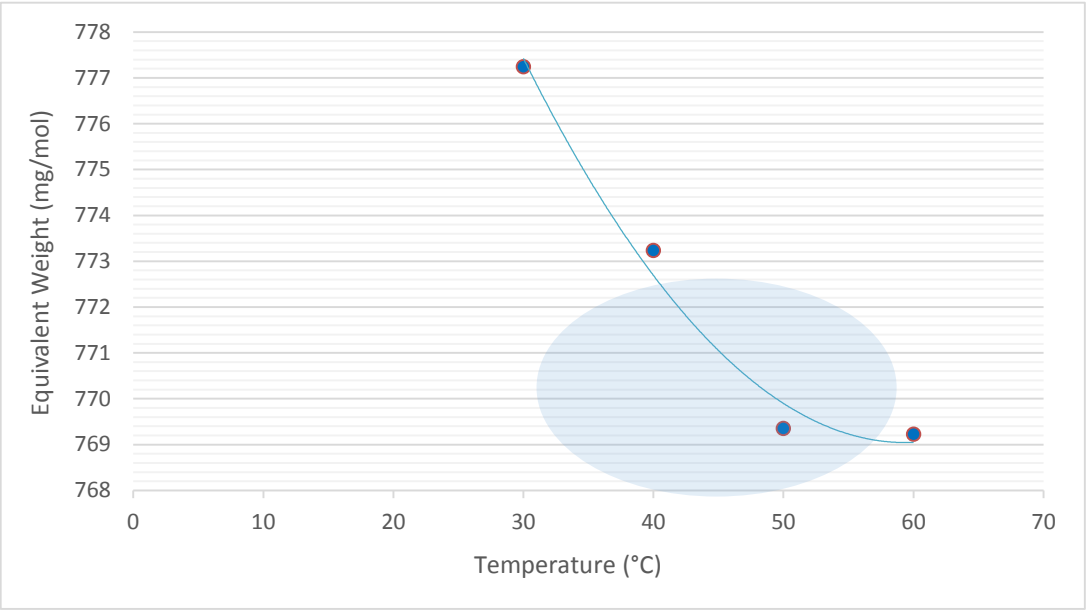

Figure 4-4: Graph of equivalent weight of pectin against temperature of incubation

4.7 Optimum condition for pectinase activity

After conducting experiment on different parameters, the best conditions is identified. For verification purpose, the experiment (from sample preparation until analysis) is repeated to verify the outcome. It is found that the optimum conditions give similar results for each repetition, meaning that the experimental result is acceptable. Figure 5 shows the repetition for optimum condition while Table 4-1 shows the summary of optimum conditions for pectinase activity.

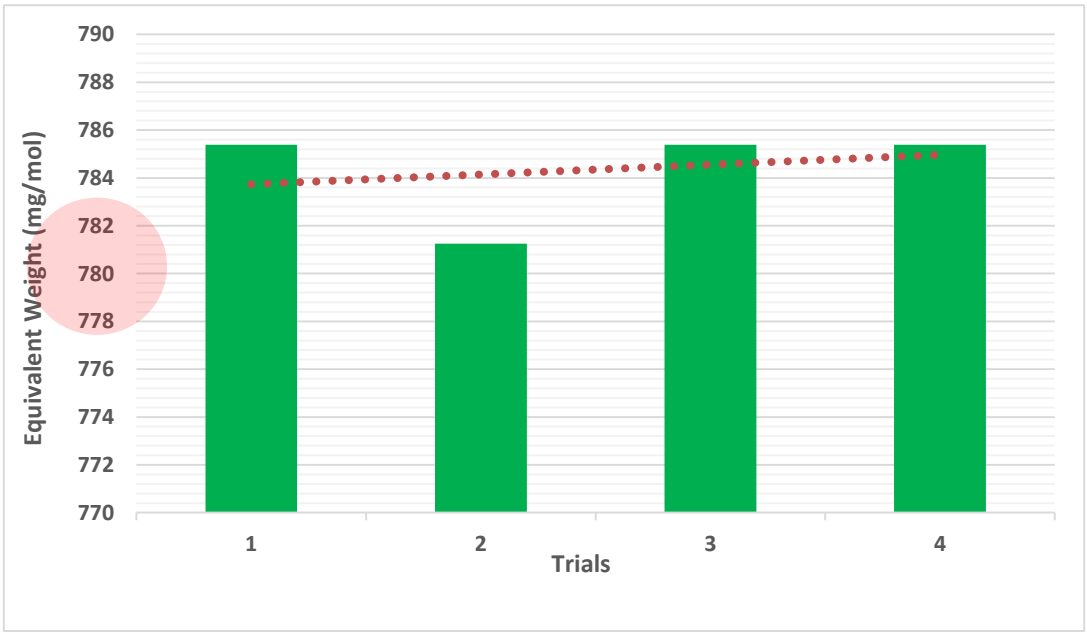

Figure 4-5: Verification of Optimum Conditions

Table 4-1: Optimum Conditions for Pectinase Activity for Each Parameters

Optimum Conditions for Pectinase Activity	
Temperature	45- 50°C
pH of solution	5
Enzyme Concentration	Directly proportional (20mg/ml maximum for this study)
Incubation Hour	>6 hours

4.8 Nutritional Profile of Cassava Flour

4.8.1 Moisture Content

As mentioned in section 4.2, the moisture content of cassava flour both before and after fermented with pectinase enzyme, remains at a lower percentage compared to wheat flour. Raw cassava powder found to have moisture contain of about 8% while processed cassava powder have moisture content of about 9%. Slight changes in the moisture content is actually negligible as these values are still lower than 10%. Wheat flour, meanwhile, has moisture content of 12%, significantly higher than the cassava flour. The lower moisture content gives added economic values to cassava flour as it can last much longer. This is mainly because microorganism prefers high moisture condition to live, and thus infecting the product. Considering this, it can be said that life span of cassava flour will be longer, best said, the expiry date of cassava flour will be longer.

Table 4-2: Moisture Content of Raw Cassava Flour

Moisture Content (%)	
Trial 1	*8.46*
Trial 2	*8.21*
Trial 3	*8.45*
Average	**8.38**

Table 4-3: Moisture Content of Processed Cassava Flour

Moisture Content (%)	
Trial 1	*8.89*
Trial 2	*8.78*
Trial 3	*9.39*
Average	**9.02**

Table 4-4: Moisture Content Analysis Summary

Moisture Content (%)	
Raw Cassava Flour	***8.38***
Processed Cassava Flour	***9.02***
Wheat Flour	***12.00***

4.8.2 Protein Content

The protein content was determined based on the calibration curve in figure 3-1, where BSA was used as standard protein sample. The absorbance values are given in table 4-5, where graph plotted based on these values are used to measure the protein content of cassava sample.

Figure 4-6: UV-VIS Spectrometer Reading of BSA

Table 4-5: Absorbance (nm) versus Protein Concentrations (%)

Sample	Concentration (%)	Absorbance (nm)
BSA	12.5	0.082
BSA	25.0	0.146
BSA	50.0	0.336
BSA	75.0	0.357
BSA	100.0	0.478
Sample 1 (raw)	X	0.001*
Sample 2 (processed)	Y	0.001*
Sample 3 (processed)	Z	0.001*
Blank	0.0	0.000

*Percentage (X,Y,Z) corresponding to these values were estimated as the protein content of the samples.

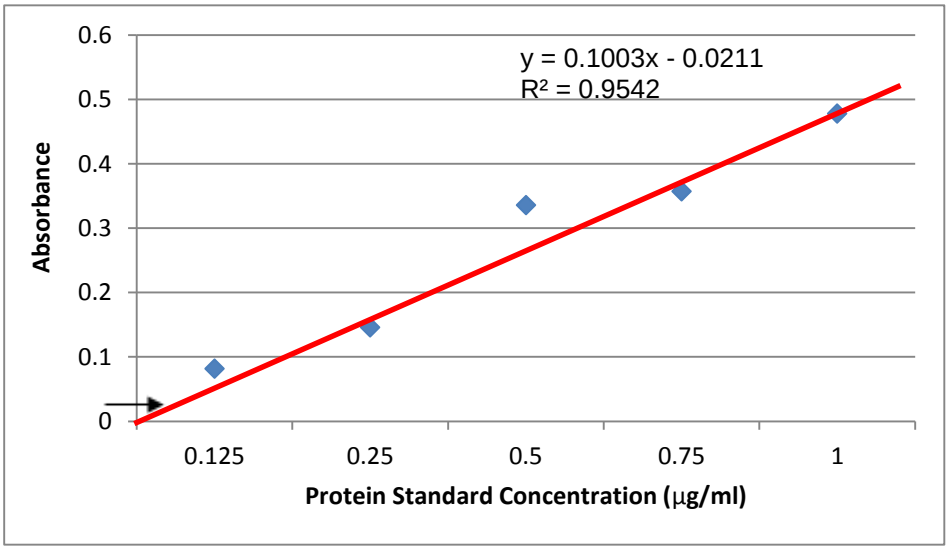

Figure 4-7: Protein Content Determination of Samples

From the standard curve, it was difficult to determine the exact amount of protein in sample since the value of absorbance are too small, in which in some trial it showed no value (absorbance = 0.000 nm). Hence the smallest value is chosen and based on the chosen values, the protein content was found to be at the range of 1% - 2%. These values tally with the values suggested by Olumide (2004).

One of the setbacks of cassava, perhaps the most significant one, would be its very low protein content. Unlike wheat, which has protein content between 8% -13%, cassava based product could not be consumed alone, but need to consumed along with other protein source. However, Olumide (2004) stated that the cassava protein quality is high given that the proportion of essential amino acid of total nitrogen is high.

4.8.3 Ash Content

Ash content is a measure of total amount of minerals present within a food, whereas the mineral content is the specific inorganic component present within a food, such as Calcium (Ca) and Potassium (K). Determination of ash content is important as it determines the quality, microbiological stability and processing of the food. Below are the brief function of ash content:-

- Quality: The quality of many food depends on the concentration and type of the minerals they contain, including their appearance, texture and stability
- Microbiological Stability: High mineral content are sometimes used to retard the growth of certain microorganism.
- Processing: It is essential to know the mineral content of food as it may affect the physiochemical properties of food.

The very low amount of mineral content in cassava flour reflected by the lower amount of ash content. The raw cassava sample and fermented sample contains about 0.7 % ash. The ash content calculation are stated in table 4-6 to table 4-8.

Table 4-6: Ash Content of Raw Cassava Flour

Ash Content (%)	
Weight of Crucible (g)	*92.77*
Weight of Sample + Crucible (g)	*93.77*
Weight of Sample (g)	*1.00*
Weight of Sample + Crucible after ashing (g)	*92.78*
Ash Content	*0.0072*
ASH CONTENT (%)	**0.72**

Table 4-7: Ash Content of Processed Cassava Flour (Sample I)

Ash Content (%)	
Weight of Crucible (g)	*75.50*
Weight of Sample + Crucible (g)	*76.53*
Weight of Sample (g)	*1.02*
Weight of Sample + Crucible after ashing (g)	*75.51*
Ash Content	*0.0074*
ASH CONTENT (%)	**0.72**

Table 4-8: Ash Content of Processed Cassava Flour (Sample II)

Ash Content (%)	
Weight of Crucible (g)	*77.65*
Weight of Sample + Crucible (g)	*78.66*
Weight of Sample (g)	*1.00*
Weight of Sample + Crucible after ashing (g)	*77.66*
Ash Content	*0.0068*
ASH CONTENT (%)	**0.68**

Table 4-9: Ash Content Summary

Ash Content (%)	
Raw Cassava Flour	*0.72*
Processed Cassava Flour (Sample I)	*0.72*
Processed Cassava Flour (Sample II)	*0.68*
Average ash content of fermented cassava flour	*0.70*
Percentage Difference	*0.018**
ASH CONTENT RANGE (%)	**0.6 – 0.75**

*Percentage difference is very low (<1 %), and hence can be ignored

As suggested by Yeshajahu *et al.* (2000), ash is the inorganic residue remaining after the water and organic matter have been removed by heating in the presence of oxidizing agents, which provides a measure of the total amount of mineral within a food. Ash

content is used based on the fact that the minerals are not destroyed by heating, and that they have a low volatility compared to other food components.

Based on the calculation, the ash content of cassava flour is relatively very low, which means the overall mineral content is very low as well as suggested by Olumide (2004). Hence, specific mineral content analysis is not necessary. On the other hand the average ash content of wheat flour is 1.3%.

4.8.4 Crude Fiber Content

The fiber content of cassava flour, unlike ash and protein content, is high compared to wheat flour. This suggest that the cassava flour is healthier as the fiber's main function is to keep digestion system healthy and ensure it functions properly. On the other hand, fiber aids and speeds up the excretion of waste and toxins from the body, preventing them staying in the intestine for too long.

Table 4-10: Crude Fiber Content of Raw Cassava Flour

Crude Fiber Content (%)	
Crucible	*92.66*
Sample+crucible	*93.76*
Sample	*1.10*
Weight after 110°C (W1)	*93.52*
Weight after 550°C (W2)	*93.49*
W1-W2	*0.033*
Crude Fiber Content (%)	***3.02***

Table 4-11: Crude Fiber Content of Processed Cassava Flour (Sample I)

Crude Fiber Content (%)	
Crucible	*92.83*
Sample+crucible	*93.87*
Sample	*1.04*
Weight after 110°C (W1)	*93.62*
Weight after 550°C (W2)	*93.59*
W1-W2	*0.035*
Crude Fiber Content (%)	***3.36***

Table 4-12: Crude Fiber Content of Processed Cassava Flour (Sample II)

Crude Fiber Content (%)	
Crucible	*92.71*
Sample+crucible	*93.76*
Sample	*1.05*
Weight after 110°C (W1)	*93.56*
Weight after 550°C (W2)	*93.52*
W1-W2	*0.036*
Crude Fiber Content (%)	***3.40***

Table 4-13: Crude Fiber Content Summary

Crude Fiber Content (%)	
Raw Cassava Flour	***3.01***
Processed Cassava Flour (Sample I)	***3.36***
Processed Cassava Flour (Sample II)	***3.40***
Average ash content of fermented cassava flour	***3.38***
Percentage Difference	***0.364****
ASH CONTENT RANGE (%)	**3-3.4**

*Difference is so small that it can be ignored

4.8.5 Crude Fat Content

Perhaps the most important advantage of cassava will be its fat content. Cassava could be well replacing wheat among people who looking for healthy food, as its fat content is about 5 times lower than that of wheat.

Table 4-14: Crude Fat Content of Raw Cassava Flour

Crude Fat Content (%)	
Extraction Unit Weight	*134.56*
Extraction Unit + Sample	*135.77*
Sample	*1.20*
Extraction Unit Weight (After Extraction)	*135.76*
Extracted Fat	*0.0028*
Crude Fat (%)	***0.23***

Table 4-15: Crude Fat Content of Processed Cassava Flour (Sample I)

Crude Fat Content (%)	
Extraction Unit Weight	*135.75*
Extraction Unit + Sample	*136.75*
Sample	*1.00*
Extraction Unit Weight (After Extraction)	*136.74*
Extracted Fat	*0.0039*
Crude Fat (%)	***0.39***

Table 4-16: Crude Fat Content of Processed Cassava Flour (Sample II)

Crude Fat Content (%)	
Extraction Unit Weight	*134.65*
Extraction Unit + Sample	*135.76*
Sample	*1.10*
Extraction Unit Weight (After Extraction)	*135.75*
Extracted Fat	*0.0032*
Crude Fat (%)	***0.29***

Table 4-17: Crude Fat Content Summary

Crude Fat Content (%)	
Raw Cassava Flour	***0.23***
Processed Cassava Flour (Sample I)	***0.39***
Processed Cassava Flour (Sample II)	***0.29***
Average ash content of fermented cassava flour	***0.34***
Percentage Difference	***0.11****
ASH CONTENT RANGE (%)	**0.2 – 0.4**

*Very small range of difference which can be neglected.

This low fat content, if can be fully utilized, will surely be demand full to meet the requirement of healthy food globally.

5 CONCLUSION

5.1 Conclusion

The optimum condition for pectinase activity in cassava flour production is at more than 6 hours incubation time, 45°C-50°C of temperature, pH value of 5 of the solution and at high concentration of enzyme. The overall results shows that the cassava flour fermented with pectinase has high potential to replace wheat, where more starch is released compared to raw cassava flour and supported by its healthier nutritional profile. In a nutshell, the objective of this study is achieved.

5.2 Recommendation

For further support the result, more detailed starch assay should be conducted while to improve the sustainability, the pectinase enzyme should be obtained from A.Niger bacteria or other source instead of buying, which proved to be costly. The analysis method for nutritional profile, which all are basic analytical method, will need to be replaced by detailed analysis to obtain more accurate and precise readings. Lastly, other alternatives also should be studied to reduce the setbacks of using cassava flour as wheat replacement, such as cyanide content and low protein content.

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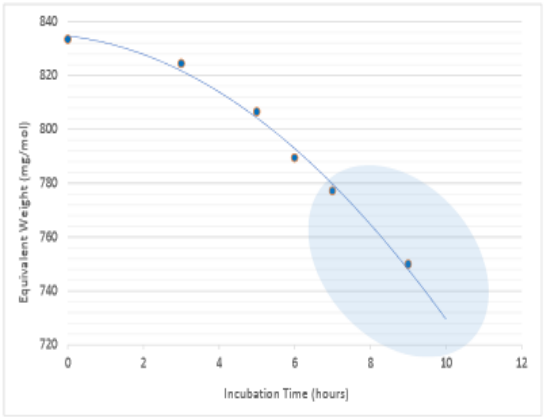
APPENDICES

*pH 5 *40°C *10mg/ml

Incubation Time (hours)	Volume of NaOH (ml)	Equivalent weight (mg/mol)	Average Equivalent Weight (mg/mol)
0	6.0	833	833
	6.1	820	
	5.9	847	
3	6.1	820	825
	5.9	847	
	6.2	806	
5	6.2	806	807
	6.3	794	
	6.1	820	
6	6.3	794	790
	6.3	794	
	6.4	781	
7	6.4	781	777
	6.4	781	
	6.5	769	
9	6.6	758	750
	6.7	746	
	6.7	746	

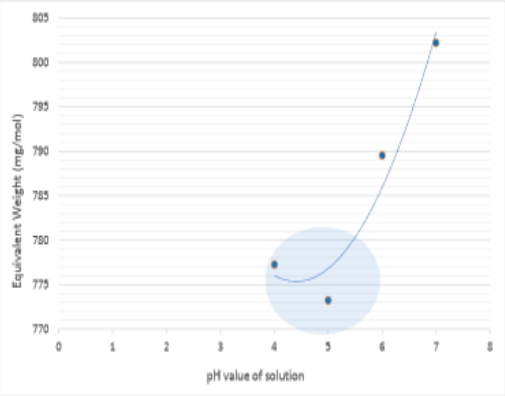
0	833
1	
2	
3	825
4	
5	807
6	790
7	777
8	
9	750
10	



*7 hours incubation time *40°C *10mg/ml

pH	Volume of NaOH (ml)	Equivalent weight (mg/mol)	Average Equivalent Weight (mg/mol)
4	6.4	781.25	777.24
	6.4	781.25	
	6.5	769.23	
5	6.4	781.25	773.24
	6.5	769.23	
	6.5	769.23	
6	6.3	793.65	789.52
	6.4	781.25	
	6.3	793.65	
7	6.3	793.65	802.18
	6.2	806.45	
	6.2	806.45	

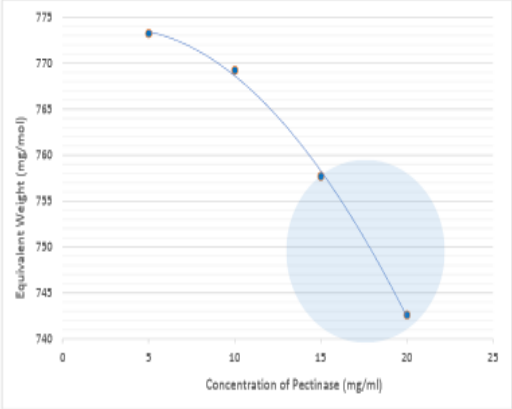
4	777
5	773
6	790
7	802



*7 hours incubation time *40°C *pH 5

Concentration of pectinase (mg/ml)	Volume of NaOH (ml)	Equivalent weight (mg/mol)	Average Equivalent Weight (mg/mol)
5	6.4	781.25	773.24
	6.5	769.23	
	6.5	769.23	
10	6.5	769.23	769.23
	6.5	769.23	
	6.5	769.23	
15	6.6	757.58	757.69
	6.7	746.27	
	6.5	769.23	
20	6.7	746.27	742.61
	6.7	746.27	
	6.8	735.29	

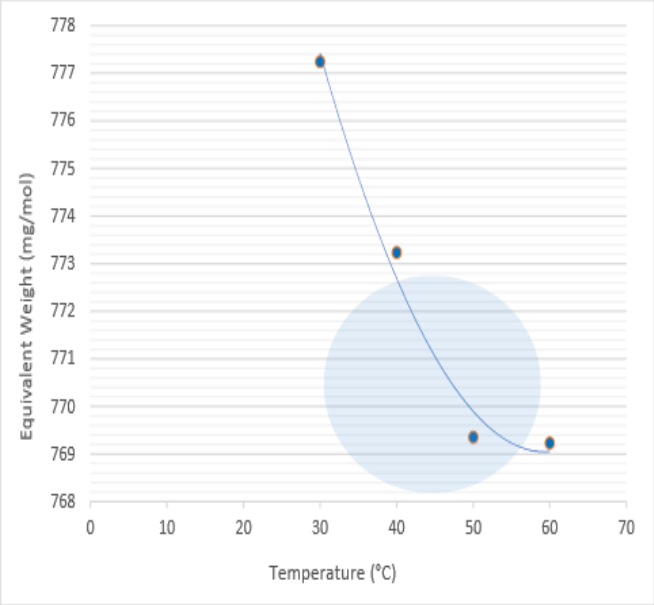
5	773
10	769
15	758
20	743



*7 hours incubation time *20mg/ml *pH 5

Temperature (°C)	lume of NaOH (l	valent weight (mg/	Average Equivalent Weight
30	6.4	781.25	777.24
	6.4	781.25	
	6.5	769.23	
40	6.4	781.25	773.24
	6.5	769.23	
	6.5	769.23	
50	6.4	781.25	769.35
	6.6	757.58	
	6.5	769.23	
60	6.5	769.23	769.23
	6.5	769.23	
	6.5	769.23	

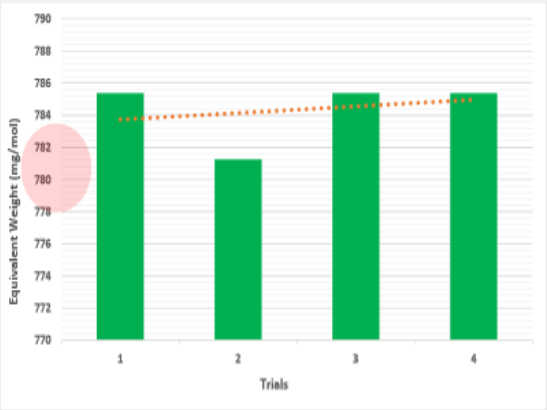
30	777
40	773
50	769
60	769



*7 hours incubation time *20mg/ml *pH 5 40°C

Trial	Volume of NaOH (ml)	Equivalent weight (mg/mol)	Average Equivalent Weight (mg/mol)
1	6.4	781.25	785.38
	6.3	793.65	
	6.4	781.25	
2	6.4	781.25	781.25
	6.4	781.25	
	6.4	781.25	
3	6.3	793.65	785.38
	6.4	781.25	
	6.4	781.25	
4	6.3	793.65	785.38
	6.4	781.25	
	6.4	781.25	

1	785
2	781
3	785
4	785



ASH CONTENT

RAW SAMPLE	
Crucible	92.7695
Sample+crucible	93.7705
Sample	1.001
Sample+crucible (after ashing)	92.7767
Ash Content	0.0072
Percentage	0.719281

FERMENTED SAMPLE I	
Crucible	75.5047
Sample+crucible	76.5258
Sample	1.0211
Sample+crucible (after ashing)	75.5121
Ash Content	0.0074
Percentage	0.724709

FERMENTED SAMPLE II	
Crucible	77.6521
Sample+crucible	78.6567
Sample	1.0046
Sample+crucible (after ashing)	77.6589
Ash Content	0.0068
Percentage	0.676886

CRUDE FIBER CONTENT

RAW SAMPLE	
Crucible	92.6566
Sample+crucible	93.7612
Sample	1.1046
Weight after 110°C (W1)	93.5234
Weight after 550°C (W2)	93.4901
W1-W2	0.0333
Crude Fiber (%)	3.014666

FERMENTED SAMPLE I	
Crucible	92.8321
Sample+crucible	93.8676
Sample	1.0355
Weight after 110°C (W1)	93.6224
Weight after 550°C (W2)	93.5876
W1-W2	0.0348
Crude Fiber (%)	3.360695

FERMENTED SAMPLE II	
Crucible	92.7073
Sample+crucible	93.7612
Sample	1.0539
Weight after 110°C (W1)	93.5601
Weight after 550°C (W2)	93.5243
W1-W2	0.0358
Crude Fiber (%)	3.396907

AVERAGE **3.3788**

CRUDE FAT CONTENT

RAW SAMPLE	
Extraction Unit Weight	134.5645
Extraction Unit + Sample	135.7651
Sample	1.2006
Extraction Unit Weight (After Extraction)	135.7623
Extracted Fat	0.0028
Crude Fat (%)	0.233217

Fermented Sample I	
Extraction Unit Weight	135.7456
Extraction Unit + Sample	136.7487
Sample	1.0031
Extraction Unit Weight (After Extraction)	136.7448
Extracted Fat	0.0039
Crude Fat (%)	0.388795

Fermented Sample II	
Extraction Unit Weight	134.6544
Extraction Unit + Sample	135.7565
Sample	1.1021
Extraction Unit Weight (After Extraction)	135.7533
Extracted Fat	0.0032
Crude Fat (%)	0.290355

AVERAGE **0.33957**

BSA	Absorbance
0.125	0.082
0.25	0.146
0.5	0.336
0.75	0.357
1	0.478