

SYNERGISTIC ENZYMATIC HYDROLYSIS OF CASSAVA STARCH AND
ANEAROBIC DIGESTION OF CASSAVA WASTE

By

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To the memory of my parents:
Chief Rufus Nyeweiba Aso [Chief Ntoyi]
Mrs. Elizabeth Ndem Aso [Madam Taproot]
Prophetess Mrs. Caroline Ogonda Onyenma

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Cassava is a major root crop and source of food and feed carbohydrate for humans and livestock. Cassava provides economic and subsistence value to more than 800 million to one billion people in Brazil, China, Haiti, India, Indonesia, Nigeria, Thailand and many other countries. However, current processes for conversion of cassava roots to value added products are far from optimum. Glucose is produced by first extracting starch from the roots. The starch is then subjected to two separate operations called liquefaction and saccharification. Each operation incurs many cost elements. Liquefaction for example demands elaborate equipment and energy inputs, while starch extraction is energy, labor and technology intensive. These requirements place glucose and other value added cassava products beyond the reach of many developing nations. It would be efficient and advantageous if liquefaction and saccharification could be carried out simultaneously as one operation and the starch hydrolyzed directly without going through the extraction procedure. Furthermore, over 60 % of global cassava output (which was 252.2×10^9 kg in 2011) is processed annually for human food, generating enormous quantities of organic waste matter with

attendant environmental and disposal issues. Anaerobic digestion of cassava wastes could generate beneficial products and at the same time be environment friendly.

The objectives of this study were to develop and demonstrate a village-scale method of processing fresh cassava root into flour from solar-convection dried cassava chips, produce glucose sweetener from combined liquefaction and saccharification hydrolysis of native starch that was not first extracted, and generate biogas (methane) from anaerobic digestion of cassava waste.

A solar convection dryer requiring no fuel or electricity was used to dry thin slices of cassava root into dry chips that were subsequently ground into cassava flour. The flour as well as freshly ground cassava root (pulp) was used as substrate for conversion into glucose by enzyme hydrolysis. The rates of conversion (kinetics) for both substrates were determined at two different temperatures and compared with those of commercially available refined cassava starch. Cassava waste from peelings and trimmings was used as feedstock for anaerobic digestion into methane fuel.

Results showed that rates of reaction for hydrolysis of all three substrates were similar to each other at both temperatures, but resulted in different extent of reaction. The cassava flour and pulp produced syrup with 3-4% glucose, while the commercial starch produced 10% glucose within 4 hours at 60°C, and 72 hours at 37°C. Anaerobic digestion of cassava waste produced bio-methane yield of 0.25 liter/ gram volatile solids with a production efficiency of 71 %. These findings would suggest the possibility of a cottage industry cassava processing operation that would be self-sufficient in which fresh cassava roots could be solar-convection dried to make flour. The flour and grated root pulp could be hydrolyzed in one step to make glucose syrup sweetener, while the

waste peelings and trimmings could be processed by anaerobic digestion to produce useful energy in the form of biogas for maintaining digester temperature and/or supplementing heat required for hydrolysis and in the solar dryer. In addition, amylolytic (starch hydrolyzing) enzymes could be harvested from the anaerobic digestion liquor, and the post digestion effluent applied to farm land as source of organic fertilizer.

CHAPTER 1 INTRODUCTION

1.1 Background

Cassava is known botanically as *Manihot esculenta* Crantz. It is variously referred to as Mandioca (in Portuguese); Manioc (in French); Yuca (in Spanish); Cassave (in Haitian Creole) and Akpakpuru (in Ikwerre, a Nigerian language). Cassava is a perennial plant widely grown in more than 90 countries in tropical and sub tropical regions of the world (Wilson and Dufour, 2002). It is chiefly cultivated for its starchy roots (FAO, 1990; Chadha, 1961). However, young cassava leaves are consumed by humans as a vegetable (Bokanga, 1994 a; Koch et al., 1994; FAO, 1990; Hahn, 1983; Lancaster and Brooks, 1983), or used as protein supplement in livestock feeds (Borin et al., 2005; Wanapat et al., 2000; Ravindran, 1993, 1992; Hahn et al., 1992; Balagopalan et al., 1988; Moore, 1976). The seeds are used mainly in breeding programs (Ceballos et al., 2012; Nassar and Ortiz, 2010; Rajendran et al., 2005; El-Sharkawy, 2004; Iglesias et al., 1994; Nartey, 1978). Figure 1-1 shows some useful components of the cassava crop.

1.1.1 Importance

Cassava has been reported to be the fourth most important food crop in developing nations after rice, wheat and maize/corn (Johnson et al., 2005; Koch et al., 1994; FAO, 1989). Cassava has also been reported to be world's third largest source of carbohydrates for human food (Nassar and Ortiz, 2010; Claude and Denis, 1990; Fauquet and Fargette, 1990; Phillips, 1982). In Zaire, 60% of the daily calorie intake is provided by cassava roots, while 20% of protein comes from cassava leaves (Koch et al., 1994).



Figure 1-1. Components of Cassava Crop: Roots; Leaves; and Seeds (bing.com, 2013 a).

Globally, cassava provides subsistence for over 800 million to one billion people (Clement et al., 2010; Nassar and Ortiz, 2010; FAO, 2008; Phillips, 1982), serving as food security or hunger alleviator; income provider; employment generator; market developer, including export markets; as well as input for industrial products like chips, flour, starch, sweetener, fuel alcohol and biochemicals (Hakizimana, 2010; Widowati and Hartojo, 2010; Johnson et al., 2009; Eneas, 2006; Srinorakutara et al., 2006; El-Sharkawy, 2004; FAO, 2001; Lynam, 1994; Balagopalan et al., 1988; Berghofer and Sarhaddar, 1988; Srikanta et al., 1987; Matsumoto et al., 1982; Ueda et al., 1981; Okigbo, 1980; Lindeman and Rocchiccioli, 1979).

1.1.2 Advantages

With possible exception of sugarcane, cassava's productivity in terms of calories per unit land area per unit of time is said to be significantly higher than that of other staple food crops (Shetty et al., 2007; Koch et al., 1994; Balagopalan et al., 1988; Okigbo, 1980). As a root crop, cassava has high biological efficiency because the most important edible carbohydrate part (the root) lies underground and does not require support from heavy stems and branches (Coursey and Haynes, 1970). Cassava can be planted most time of the year and is available all year long; is productive on poor soils; tolerant of adverse climatic conditions; adaptable to various farming systems and can be intercropped with beans, maize (corn), yam and several other crops. Cassava is resistant to pests and diseases; is easily propagated by stem cuttings and its production is less labor intensive than yam. Also, cassava has more than two years harvest window. This advantage precludes the need for expensive energy intensive preservation cum storage technique like refrigeration, affords farmers protection against famine, and guarantees flexible labor management schedules. Furthermore, cassava is endowed with good quality starch (Tonukari, 2004; Westby, 2002; International Starch Institute, 1999; O'Hair, 1995; Koch et al., 1994; Hahn, 1992; Balagopalan et al., 1988); that can be transformed to products with huge industrial applications.

1.2 Justifications

1.2.1 Rationale

Despite huge economic and social relevance demonstrated in the enumerated importance and advantages, cassava crop is considered an orphan crop, only suitable for poor subsistence farmers of low economic status. Cassava has traditionally received relatively minor investment and attention for research and development (Ceballos et al.,

2012; FAO, 2008; Cock, 1985; Hahn and Keyer, 1985; Rogers, 1965). The rankings of top 20 agricultural commodities produced in the world in 2011 in terms of quantity and monetary value are presented in Table 1-1 and Table 1-2 for entire planet earth; Table 1-3 and Table 1-4 for Africa; Table 1-5 and Table 1-6 for Asia; and Table 1-7 and Table 1-8 for the Americas.

Table 1-1. Agricultural Commodities in the whole World in 2011 Ranked by Production Quantity (Source: Compiled from FAO online statistical data base; FAO, 2013)

Rank	Commodity	Production Quantity (1 x 10 ⁹ kg)
1	Sugar cane	1794.35
2	Maize	883.46
3	Rice, paddy	722.76
4	Wheat	704.08
5	Cow milk, whole, fresh	606.66
6	Potatoes	374.38
7	Sugar beet	271.64
8	Vegetables fresh	268.37
9	Soybeans	260.91
10	Cassava	252.20
11	Tomatoes	159.02
12	Barley	134.27
13	Indigenous Pig meat	108.64
14	Bananas	106.54
15	Watermelons	104.47
16	Sweet potatoes	104.25
17	Buffalo milk, whole, fresh	93.01
18	Indigenous Chicken Meat	89.55
19	Onions, dry	85.37
20	Apples	75.63

Table 1-2. Agricultural Commodities in the whole World in 2011 Ranked by Monetary Value (Source: Compiled from FAO online statistical data base; FAO, 2013)

Rank	Commodity	Monetary Value (Int \$1 x 10 ⁹)
1	Rice, paddy	187.88
2	Cow milk, whole, fresh	181.27
3	Indigenous Cattle Meat	168.94
4	Indigenous Pig meat	167.00
5	Indigenous Chicken Meat	127.56
6	Wheat	86.27
7	Soybeans	64.14
8	Tomatoes	58.10
9	Maize	57.42
10	Sugar cane	56.63
11	Hen eggs, in shell	53.86
12	Potatoes	50.27
13	Vegetables fresh	46.12
14	Grapes	39.81
15	Cotton lint	37.30
16	Buffalo milk, whole, fresh	36.55
17	Apples	31.84
18	Bananas	29.57
19	Cassava	24.59
20	Mangoes, mangosteens, guavas	23.30

Table 1-3. Agricultural Commodities in Africa in 2011 Ranked by Production Quantity
(Source: Compiled from FAO online statistical data base; FAO, 2013)

Rank	Commodity	Production Quantity (1 x 10 ⁹ kg)
1	Cassava	140.96
2	Sugar cane	83.45
3	Maize	65.05
4	Yams	54.35
5	Plantains	28.81
6	Cow milk, whole, fresh	27.50
7	Rice, paddy	26.53
8	Potatoes	26.32
9	Wheat	22.08
10	Sorghum	20.78
11	Vegetables fresh	17.49
12	Sweet potatoes	17.10
13	Tomatoes	16.55
14	Bananas	15.39
15	Millet	10.78
16	Sugar beet	9.92
17	Groundnuts, with shell	9.43
18	Onions, dry	8.34
19	Oranges	7.13
20	Taro (cocoyam)	7.07

Table 1-4. Agricultural Commodities in Africa in 2011 Ranked by Monetary Value
 (Source: Compiled from FAO online statistical data base; FAO, 2013)

Rank	Commodity	Monetary Value (Int \$1 x 10 ⁹)
1	Cassava	14.63
2	Indigenous Cattle Meat	13.57
3	Yams	11.34
4	Cow milk, whole, fresh	8.33
5	Rice, paddy	7.06
6	Indigenous Chicken Meat	6.51
7	Maize	6.22
8	Tomatoes	6.11
9	Plantains	5.38
10	Bananas	4.33
11	Groundnuts, with shell	3.96
12	Potatoes	3.96
13	Indigenous Sheep Meat	3.53
14	Vegetables fresh	3.29
15	Cocoa beans	3.00
16	Sorghum	2.94
17	Wheat	2.87
18	Indigenous Goat Meat	2.75
19	Sugar cane	2.65
20	Olives	2.59

Table 1-5. Agricultural Commodities in Asia in 2011 Ranked by Production Quantity
(Source: Compiled from FAO online statistical data base; FAO, 2013)

Rank	Commodity	Production Quantity (1 x 10 ⁹ kg)
1	Sugar cane	710.93
2	Rice, paddy	653.24
3	Wheat	317.86
4	Maize	270.86
5	Vegetables fresh	231.29
6	Potatoes	174.63
7	Cow milk, whole, fresh	163.34
8	Tomatoes	96.47
9	Buffalo milk, whole, fresh	90.16
10	Watermelons	87.75
11	Sweet potatoes	83.07
12	Cassava	76.68
13	Indigenous Pig meat	61.88
14	Bananas	61.64
15	Onions, dry	56.73
16	Cucumbers and gherkins	56.72
17	Cabbages and other brassicas	51.85
18	Coconuts	49.19
19	Apples	48.13
20	Eggplants (aubergines)	44.15

Table 1-6. Agricultural Commodities in Asia in 2011 Ranked by Monetary Value
(Source: Compiled from FAO online statistical data base; FAO, 2013)

Rank	Commodity	Monetary Value (Int \$1 x 10 ⁹)
1	Rice, paddy	169.11
2	Indigenous Pig meat	95.13
3	Cow milk, whole, fresh	48.96
4	Wheat	43.40
5	Indigenous Chicken Meat	42.34
6	Vegetables fresh	39.42
7	Indigenous Cattle Meat	36.22
8	Buffalo milk, whole, fresh	35.44
9	Tomatoes	35.04
10	Hen eggs, in shell	31.67
11	Potatoes	26.12
12	Cotton lint	25.54
13	Sugar cane	21.90
14	Apples	20.27
15	Palm oil	18.37
16	Mangoes, mangosteens, guavas	18.10
17	Bananas	17.10
18	Other bird eggs, in shell	15.10
19	Maize	13.56
20	Grapes	12.12

Table 1-7. Agricultural Commodities in the Americas in 2011 Ranked by Production Quantity (Source: Compiled from FAO online statistical data base; FAO, 2013)

Rank	Commodity	Production Quantity (1 x 10 ⁹ kg)
1	Sugar cane	972.68
2	Maize	438.38
3	Soybeans	223.70
4	Cow milk, whole, fresh	179.29
5	Wheat	110.35
6	Potatoes	41.56
7	Indigenous Chicken Meat	39.43
8	Rice, paddy	37.87
9	Oranges	36.15
10	Cassava	34.36
11	Indigenous Cattle Meat	30.29
12	Sugar beet	28.85
13	Bananas	27.86
14	Tomatoes	24.19
15	Sorghum	19.96
16	Indigenous Pig meat	18.03
17	Barley	16.63
18	Rapeseed	15.12
19	Grapes	15.11
20	Hen eggs, in shell	13.20

Table 1-8. Agricultural Commodities in the Americas in 2011 Ranked by Monetary Value
(Source: Compiled from FAO online statistical data base; FAO, 2013)

Rank	Commodity	Monetary Value (Int \$1 x 10 ⁹)
1	Indigenous Cattle Meat	81.83
2	Soybeans	56.80
3	Indigenous Chicken Meat	56.17
4	Cow milk, whole, fresh	54.01
5	Maize	33.67
6	Sugar cane	31.18
7	Indigenous Pig meat	27.72
8	Wheat	15.54
9	Hen eggs, in shell	10.95
10	Rice, paddy	10.32
11	Tomatoes	8.94
12	Grapes	8.63
13	Cotton lint	8.23
14	Bananas	7.70
15	Oranges	6.98
16	Potatoes	6.51
17	Coffee, green	5.10
18	Indigenous Turkey Meat	4.39
19	Rapeseed	4.19
20	Apples	3.88

A careful review of Tables 1-1 through 1-8 reveals that cassava is not receiving commensurate financial value for the quantity produced. Apart from Africa, other regions of the world rewarded large quantities of cassava produced with low monetary

values. Why should a commodity ranked 10th or 12th in terms of quantity produced be ranked 19th or not even within the first 20 in terms of pecuniary value (Table 1-9)? This question begs for reasonable and creative answers. Perhaps a realistic solution might be to diversify cassava into generating industrial-scale value added products such as flour, starch, and chips on the one hand. On the other hand, the potential of cassava in the production of single cell protein, alcohol, enzymes, sweeteners, and biogas could boost its status and thereby enhance cassava's position and value on global agricultural commodities tables.

Table 1-9. Global Rank of Cassava in 2011 among all Agricultural Commodities in terms of Production Quantity and Monetary Value (Source: Compiled from FAO online statistical data base; FAO, 2013)

Region	Production Quantity & Monetary Value			
	Quantity [1 x 10 ⁹ kg]	Rank	Value [Int \$1 x 10 ⁹]	Rank
Africa	140.96	1	14.63	1
Americas	34.36	10	2.05	Below 20
Asia	76.68	12	7.88	Below 20
Oceania	0.19	Below 20	0.01	Below 20
World	252.20	10	24.59	19

1.2.2 Challenges and Opportunities

Challenges that limit utilization of cassava crop include shelf life and mass; two-step hydrolysis procedure; source and format of starch substrate; and enzyme availability.

Cassava roots are bulky and heavy, and therefore expensive to transport over long distances. The roots are also perishable, and must be either consumed or

processed within a few days after harvest. Transforming cassava root into value added product such as glucose sweetener could alleviate this challenge.

A major limitation of conventional method of producing glucose sweetener is the procedure of two separate operations called liquefaction and saccharification.

Liquefaction is the first stage whereby starch is gelatinized by heat treatment and thermostable alpha-amylase enzyme partially hydrolyses the starch into maltodextrins. Saccharification commences in the second stage where the partially hydrolyzed starch is converted to glucose by the action of glucoamylase. This protocol requires more process vessels, piping, and associated equipment; conditioning for enzyme systems; and starch gelatinization is energy intensive. An advantageous alternative approach would be to deploy alpha-amylase and glucoamylase enzymes simultaneously, and carry out both liquefaction and saccharification processes inside the same reactor vessel as a single unit operation. This option should be simpler, and more efficient. It is termed synergism and is the hydrolysis technique investigated in this study.

Maize (corn) is the dominant raw material for global starch production (De Baere, 1999; Gordon, 1999; Swinkels, 1985), accounting for more than 80% of the total starch processed (Johnson et al., 2005; De Braganca and Fowler, 2004; De Baere, 1999). This has relegated other sources of starch such as cassava to the background. In the conventional method of glucose sweetener production, starch is first extracted in the pure form from the native raw material. Subsequently, the extracted starch is subjected to hydrolysis to produce glucose. However, the starch extraction process is time, energy, technology, labor and cost intensive; and therefore not feasible in most developing nations. A more feasible alternative is to use the direct conversion method.

Direct conversion procedure involves hydrolyzing native starch in raw materials with minimal value added history. Two approaches to the direct conversion technique were investigated in this work. The first was to hydrolyze cassava flour. The flour was produced from cassava chips that were dried with a solar-convection dryer developed at the University of Florida (Schiavone et al., 2013). However, the cassava flour accumulated some cost and value adding processes; but not to the extent of extracted starch. The second approach investigated wet ground cassava root as substrate for enzyme hydrolysis. This latter approach would appear to be the best and simplest method over flour and starch because it accumulated the least cost and value adding operations.

Enzymes are expensive. In 2011 a gram of alpha amylase could cost as much as US \$252. Worldwide enzyme sales were over \$1.5 billion in 1998 (Beilen and Li, 2002). This value is projected to rise to \$7 billion in 2013 (ReportLinker, 2009). Because culture medium and substrate are important factors in the synthesis of industrial enzymes, the use of low cost substrates such as cassava peels and other biomaterials or agricultural byproducts can help reduce the cost of sweetener production. Cassava wastes such as peels are generated during the processing and production of numerous cassava based food products such as chikwangue, farinha de mandioca, and gari. With manual peeling, the peel can constitute 20 – 35 % of the total weight of the root (Ekundayo, 1980). Therefore, enormous organic waste is generated from cassava processing. This waste should be good feed stock for anaerobic digestion and fermentation processes that could generate biogas and hydrolytic enzymes. (Adeniran and Abiose, 2011; Silva et al., 2009; Cuzin et al., 1992). It will appear to be a win and

win again opportunity if cassava peel is digested to produce biogas that could be used as a source of energy while extracting hydrolytic enzymes from the digestate at the same time. This study explored both opportunities.

1.3 Objectives

The objectives of the study were to:

1. Demonstrate a cottage industry level process for the production of cassava flour from solar-convection dried cassava chips. The flour could be used for the manufacture of bread and other bakery products.
2. Demonstrate a cottage industry level process for the production of glucose sweetener from synergistic hydrolysis with commercial enzymes on the following substrates:
 - a) Commercially available refined cassava starch.
 - b) Flour produced from solar-convection dried cassava chips.
 - c) Freshly ground cassava root pulp.

The sweeteners would serve as sugar substitutes to consumers; and as industrial ingredient for use in the beverage, confectionery, pharmaceutical and the allied industries.

3. Estimate first-order kinetic parameters (rate constant along with the Arrhenius activation energy) from the enzyme hydrolysis of each substrate at two different temperatures.
4. Demonstrate anaerobic digestion of cassava waste by performing experiments and reporting performance indicators such as: methane yield, sCOD, pH, etc.
5. Isolate and determine the activity of enzymes from post anaerobic digestion broth.

The architecture of the entire scheme of study is presented in Figure 1-2.

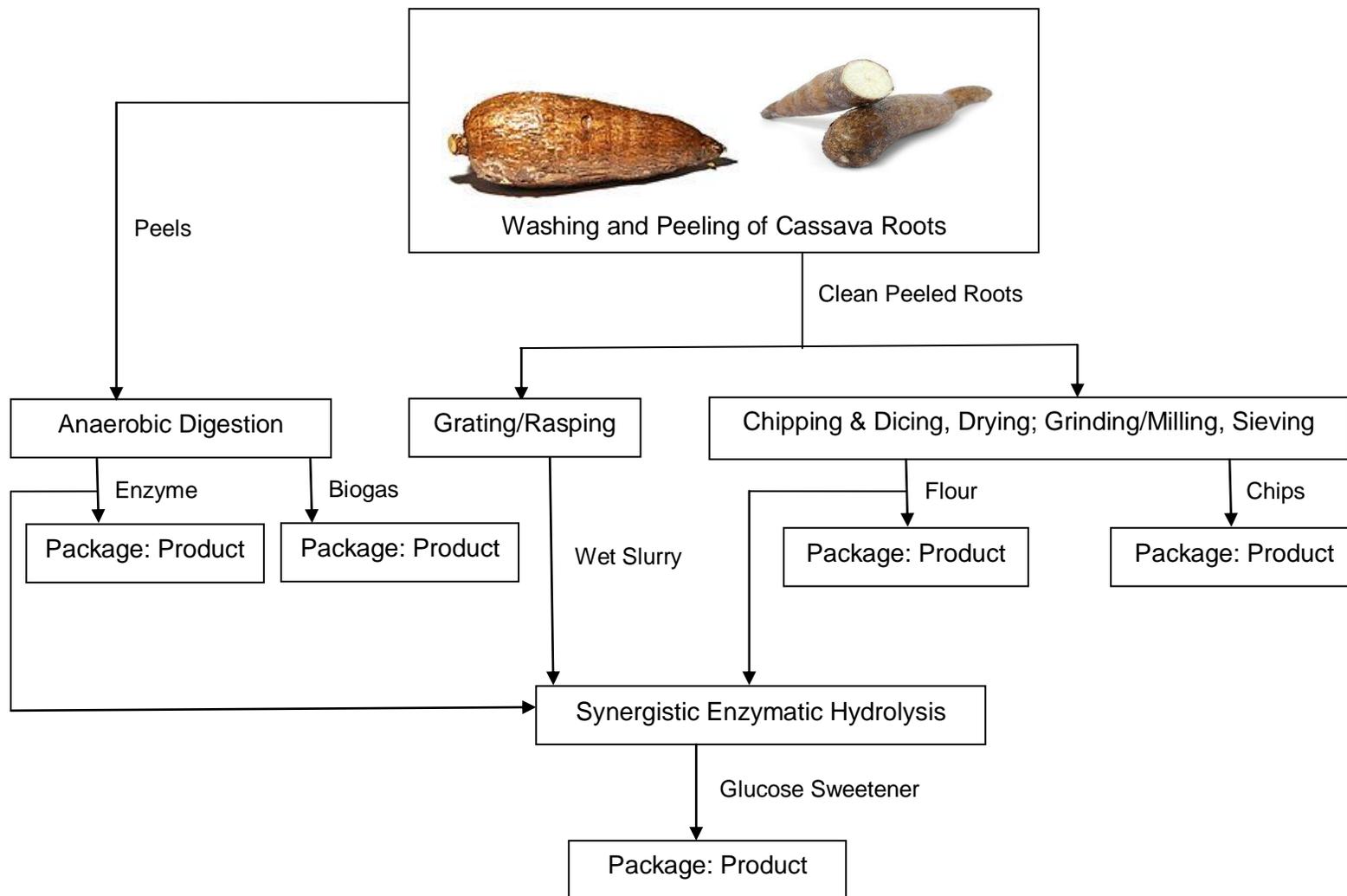


Figure 1-2. Overview/Global Architecture of Scheme of Study

1.4 Organization of Dissertation

The work reported in this dissertation was presented in a series of self-contained chapters as follows. Chapter 1: introduction; Chapter 2: literature review which consisted of two parts. Part 1 dealt with cassava, its products and potential for enzyme hydrolysis. Part 2 discussed the production of enzymes by submerged and solid- state fermentations. Chapter 3 dealt with physical properties of cassava flour made from solar-convection dried cassava chips. Chapter 4 reported on synergistic enzymatic hydrolysis of cassava starch. Chapter 5 reported on experiments done on anaerobic digestion of cassava waste for production of biogas and amylolytic enzymes

Chapters 3, 4, and 5 each began with a concise background that contained an overview of the literature pertinent to that chapter, followed by methodology and results of the work reported in that chapter. Each chapter serves as the basis for preparing a manuscript suitable for submission to be published in a scientific journal.

CHAPTER 2 REVIEW OF LITERATURE

The subject of this dissertation project concerns the enzyme hydrolysis of cassava starch for conversion to glucose. Therefore a review of the literature was undertaken in two parts.

PART 1: Cassava. Its origin, classification, agro-climatic conditions, propagation, production, utilization, packaging, etc.

PART 2: Production of enzymes by submerged and solid-state fermentations.

2.1 Part 1: Cassava

2.1.1 Origin and Distribution

Numerous reports have supported the view that cassava originated in South America (Clement et al., 2010; Nassar and Ortiz, 2010; Léotard et al., 2009; Allem, 2002; Olsen and Schaal, 2001, 1999; FAO, 1990; Fauquet and Fargette, 1990; Balagopalan et al., 1988; Lancaster et al., 1982; Okigbo, 1980; Coursey and Haynes, 1970, Rogers, 1965, Chadha, 1961). Cassava is said to have been domesticated as far back as 4000 BC. By 1500 AD, Portuguese explorers cultivated cassava plantations in South America. The Portuguese introduced cassava into Congo river basins of Africa around 1558 in the 16th century; into Java in 1810; India in 1840; and Singapore and Malaysia in 1850 (FAO, 1990; Fauquet and Fargette, 1990; Balagopalan et al., 1988; Cock, 1985; Lancaster et al., 1982; Hohnholz, 1980; Okigbo, 1980). Today, Cassava is widely distributed and cultivated in most tropical and sub tropical regions of planet earth. In the year 2011, one hundred countries in the world cultivated cassava (FAO, 2013).

2.1.2 Classification

There has been ongoing scientific debate about the botanic classification and domestication of cassava. On the one hand, there is the view that cassava emanated as a hybridization of several species (Ugent et al., 1986, Rogers and Appan, 1973; Rogers, 1965). On the other hand, cassava is postulated to have a sole progenitor (Léotard et al., 2009; Allem, 2002; Olsen and Schaal, 2001; Roa et al., 2000; Olsen and Schaal, 1999; Allem, 1994). Allem (1994) argued the recognition of three subspecies viz: *Manihot esculenta esculenta*; *Manihot esculenta peruviana*; and *Manihot esculenta flabellifolia*. *Manihot esculenta esculenta* comprises the domesticate and includes all cultivars in cultivation. *Manihot esculenta* Crantz belongs to the family Euphorbiaceae and is the flagship of cultivated cassava crop. This family is generally divided into two main groups; the bitter or toxic variety and the sweet or non toxic variety (Elias et al., 2004; Nye, 1991). The bases for classification have ranged from subjective opinions about organoleptic tastes to objective quantifiable cyanide content.

Scientific reports have correlated bitter cassava with high cyanogenic potential (Pereira et al., 1981; Sinha and Nair, 1968). It was reported that the ratio of total cyanide in the peel to that in the parenchyma is low for bitter cassava varieties and high for the sweet varieties. In other words, in “sweet cassava”, the linamarin concentration of the peel is considerably higher than the linamarin concentration of the parenchyma whereas in “bitter cassava”, linamarin concentration is more evenly distributed throughout the entire root (Dufour, 2007, 1988; Gomez et al. 1985). Consequently, sweet cassava with high cyanogenic potential and bitter cassava with low cyanogenic potential have been found (Bokanga, 1994 b; Sinha and Nair, 1968). There is little correlation between the cyanogenic content of the peeled root and the leaves. The latter

tend to be high in cyanogens in both low (sweet) - and high (bitter) -cyanogenic cultivars (Gomez et al. 1985). Furthermore, it was also reported that there is no strong morphological relationship established between cyanogenic potential and other traits (Mahungu, 1994; Nye, 1991; Rogers, 1965). Therefore, using sensory taste to determine cyanogenic potential may be misleading as there is considerable overlap between the cyanide content of bitter and sweet classes. This was demonstrated by Coursey (1973). By graphically representing the results of Sinha and Nair (1968), Coursey (1973), clearly illustrated the overlapping of cyanide content of Sweet, Non-bitter, Bitter, and Very bitter cassava varieties; which were respectively (in ppm HCN [or mg HCN/kg]): 40 to 130, 30 to 180, 80 to 412.5, and 280 to 490 (Okigbo, 1980). Nevertheless, some criteria have been used to distinguish between bitter and sweet cassava varieties.

In one characterization arising from a survey within the framework of the Collaborative Study of Cassava in Africa (COSCA), the sweet non toxic cassava varieties were defined as those which can be eaten raw or after simple boiling; while the bitter toxic varieties were those that must be processed adequately before consumption (Nweke and Bokanga, 1994). This classification has been echoed by others (Clement et al., 2010; Wilson and Dufour, 2002; Mckey and Beckerman, 1993). Bolhuis (1954) noted that Koch (1933) was the author who tried to classify cassava roots according to their toxicity (cyanide content). In that work, three levels of classification were proposed (Tewe, 2004; Balagopalan et al., 1988; Okigbo, 1980; Coursey, 1973; Bolhuis, 1954; Koch, 1933):

- Innocuous: Less than 50 mg HCN per kg of fresh peeled root.
- Moderately poisonous: 50 to 100 mg HCN per kg fresh peeled root.

- Dangerously poisonous: More than 100 mg HCN per kg fresh peeled root.

It should be noted that this classification was based on the premise that 50 to 60 mg HCN is the theoretical lethal dose for an adult weighing 50 kg (Boorsma, 1905). However, citing Rosling (1988), Lynam (1994) noted that up to 100 mg HCN equivalent per day can be detoxified by the body under normal dietary circumstances. Based on the foregoing, some workers now classify sweet cassava varieties as those with low cyanogenic potential defined as having less than 50 mg HCN equivalent per kilogram of fresh peeled root, and bitter cassava varieties as those with more than 50 mg HCN equivalent per kilogram of fresh peeled root (Clement et al., 2010; Elias et al., 2004; Wilson and Dufour, 2002). Other workers have used 100 mg HCN equivalent per kilogram of fresh peeled root to define the limit of low cyanogenic potential (Dufour, 2007; Dixon et al., 1994). On the other hand, World Health Organization (WHO) standard (Codex Alimentarius Standard) for the total hydrocyanic acid content of edible cassava flour is 10 mg/kg maximum (FAO/WHO, 1995).

2.1.3 Agro Climatic Conditions

There is a range of agro climatic conditions conducive for the production of cassava. The crop is suitably cultivated in the areas between 30 ° North and 30 ° South of the equator; at elevations from sea level to 2,500 meters and from areas with as little as 500 mm of rainfall, to tropical rain forest zones with more than 6,000 mm of rain per year. Cassava can adapt to soils pH of 3 to 9.5; temperatures between 8 and 33 ° C; and relative humidity of 15 % to near saturation at 90 % (Wilson and Dufour, 2002; Consultative Group on International Agricultural Research [CGIAR], 1997; Cock, 1985; Hohnholz, 1980; Lozano et al., 1980; Okigbo, 1980; Rogers, 1965). Cassava is noted to perform poorly under flooded soil conditions and at temperatures below 17 ° C or above

37 °C (El-Sharkawy, 2004; Balagopalan et al., 1988; Hohnholz, 1980). Conditions reported for optimum performance of cassava include 1000 to 1500 mm of annual rainfall, pH of 5 to 8, and temperatures between 25 °C and 30 °C (El-Sharkawy, 2004; Cock, 1985).

2.1.4 Propagation

Cassava may be propagated by stem cuttings or seeds. Although seeds have been used for propagation operations (Rajendran et al., 2005; Sambatti et al., 2001; Rajendran et al., 2000; Iglesias et al., 1994), most farmers use stem cuttings for multiplication and planting purposes (Ceballos et al., 2012; Iglesias et al., 1994; Kakes, 1990; Cock, 1985; Nartey, 1978; Chadha, 1961). Stem cuttings or stakes used for propagation may vary between 15 and 30 cm in length (Ceballos et al., 2012; El-Sharkawy, 2004; Hahn and Keyser, 1985). Each planting stake can have 5 to 11 nodes and may be planted horizontally, inclined, or vertically on ridges or mounds formed over shallow pits and troughs. Using different stake lengths of 6.25 cm, 16 cm and 25 cm, it was shown that cassava can be cultivated at densities of 1000 to 4000 stakes per hectare (Keating et al., 1988). However, the number of plants per hectare ranges between 10000 and 15000 (Shetty et al., 2007).

2.1.5 Production

Cassava has emerged as one of the top twenty agricultural commodities in the World. In terms of quantity produced, cassava has consistently ranked among the first ten for the past consecutive twelve years (Table 2-1). Between 1999 and 2011, cassava output worldwide increased by more than 48%, from 169781525 to 252203769 metric tonnes (169.78 to 252.20 x 10⁹ kg) (Table 2-1). The increase in global output may be attributed to improved varieties, better farming systems and increased cultivated land

Table 2-1. Cassava's ranking among World Agricultural Commodities: 1999 to 2011
(Values were achieved, compiled, deduced and/or computed from FAO online statistical data base; FAO, 2013)

Year	Production Quantity [1 x 10 ⁹ kg]	Production Quantity Rank	Monetary Value [Int \$1 x 10 ⁹]	Monetary Value Rank
2011	252.20	10	24.59	19
2010	236.70	9	23.00	19
2009	235.45	8	22.89	19
2008	232.00	8	22.45	20
2007	226.42	9	21.64	20
2006	223.56	9	21.31	20
2005	205.64	10	19.51	20
2004	201.11	10	19.08	20
2003	190.82	9	18.22	20
2002	184.28	9	17.50	20
2001	181.92	9	17.35	20
2000	176.27	9	16.82	20
1999	169.78	9	16.18	20

areas (Phillips et al., 2004; Tewe, 2004; Cock, 1985). Under traditional farming practices, cassava crop yield has been reported at 0.53 to 0.78 kg per plant (Wilson and Dufour, 2002); and 5 to 20 tonnes per hectare (Tewe, 2004; Balagopalan et al., 1988; Keating et al., 1988; Cock, 1985; Hahn and Keyser, 1985; Hahn, 1983; Amsterdam, 1980; FAO, 1977; Chadha, 1961). In 2011, the average yield in India was 36.4 tonnes per hectare (Table 2-2). With modern farming techniques and inputs, yields over 60 tonnes per hectare may be normal (FAO, 1977). Cassava can produce 250 x 10³ calories per hectare per day compared to 200 x 10³ for maize (corn); 176 x 10³ for rice; 114 x 10³ for sorghum; and 110 x 10³ for wheat (Coursey and Haynes, 1970).

The top countries that produce cassava in the world are shown in Table 2-2. Nigeria is the world leader in cassava production. In 2011, Nigeria produced 52403500 metric tonnes (52.40 x 10⁹ kg) of cassava worth about Int \$5.47 x 10⁹. This production

Table 2-2. Global Statistics on Cassava Production in 2011, by Country (Values were achieved, compiled, deduced and/or computed from FAO online statistical data base; FAO, 2013)

Country	Rank of Country in Cassava production	Rank of Cassava Among All the Crops Produced in the Country	Production Quantity and Proportion		Production Value (Int \$1 x 10 ⁹)	Area Harvested [1 x 10 ⁶ Ha]	Yield [1 x 10 ³ kg/Ha]
			Quantity [1 x 10 ⁹ kg]	Proportion [%]			
Nigeria	1	1	52.40	20.7	5.47	3.73	14.0
Brazil	2	5	25.44	10.0	1.32	1.74	14.6
Indonesia	3	2	24.00	9.5	2.45	1.18	20.3
Thailand	4	3	21.91	8.6	2.28	1.13	19.2
Democratic Republic of the Congo	5	1	15.56	6.1	1.61	2.17	7.1
Angola	6	1	14.33	5.6	1.49	1.07	13.3
Ghana	7	1	14.24	5.6	1.48	0.88	16.0
Viet Nam	8	3	9.87	3.9	1.03	0.56	17.6
India	9	20	8.07	3.2	0.84	0.22	36.4
Mozambique	10	1	6.26	2.4	0.65	0.97	6.4
Uganda	11	2	4.75	1.8	0.49	0.42	11.1
United Republic of Tanzania	12	1	4.64	1.8	0.46	0.73	6.2
China	13	Below 20	4.51	1.7	0.42	0.27	16.3
Cambodia	14	2	4.36	1.7	0.45	0.20	21.2
Malawi	15	1	4.25	1.6	0.44	0.19	21.5
Cameroon	16	1	3.90	1.5	0.36	0.28	13.9
Benin	17	1	3.60	1.4	0.37	0.25	13.9
Madagascar	18	2	3.30	1.3	.34	0.36	8.9
Rwanda	19	2	2.57	1.0	0.26	0.20	12.3
Paraguay	20	4	2.45	0.9	0.13	0.18	13.5
Haiti	32	2	0.65	0.2	0.06	0.15	4.1
World		10	252.20	100.0	24.59	19.64	12.8

quantity accounted for 20.7% of the global output. The production quantity was harvested from 3737090 hectares of land at the yield rate of 14.0 tonnes per hectare.

Nigeria was followed by Brazil, second producer in the world; Indonesia, third; Thailand, fourth; Democratic Republic of the Congo, fifth; Angola, sixth; and Ghana, seventh.

These seven nations accounted for more than 60% of global cassava output in 2011 (Table 2-2). Haiti was ranked 32nd, having produced about 0.2% of world output.

However, Haiti is a major cassava producer in the South America and Caribbean region (Henry and Hershey, 2002). In 2011, Haiti was the largest cassava producer in the Caribbean, seconded by Cuba; and cassava was produced in the second largest quantity in Haiti, exceeded only by sugar cane (FAO, 2013).

The 2011 statistics of global cassava output from the different regions of the world is presented in Table 2-3. Africa dominated world production with 55.8% share and 39 countries in active production. Asia followed with 30.4% and 14 countries. The Americas was third with 35 countries producing 13.6% of world cassava output. Oceania produced less than 0.1% of global cassava output in 2011 (Table 2-3).

Table 2-3. Global Statistics on Cassava Production in 2011, by Region (Values were achieved, compiled, deduced and/or computed from FAO online statistical data base; FAO, 2013).

Region	Global Rank of Region in Cassava Production	Rank of Cassava Among All the Crops Produced in the Region	Production Quantity and Proportion		Monetary Value (Int \$1 x 10 ⁹)	Area Harvested [1 x 10 ⁶ Ha]	Total No. of Countries
			Quantity [1 x10 ⁹ kg]	Proportion [%]			
Africa	1	1	140.96	55.8	14.63	13.04	39
Western		1	76.06	30.1	7.94	5.70	15
Middle		1	36.25	14.3	3.72	4.02	9
Eastern		2	28.64	11.3	2.97	3.30	15
Asia	2	12	76.68	30.4	7.88	3.91	14
South-Eastern		3	63.79	25.2	6.59	3.39	10
Eastern		Below 20	4.51	1.7	0.42	0.27	1
Southern		Below 20	8.36	3.3	0.86	0.24	3
Americas	3	10	34.36	13.6	2.05	2.66	35
South		5	32.30	12.8	1.86	2.34	11
Caribbean		5	1.35	0.5	0.12	0.26	16
Central		Below 20	0.70	0.2	0.06	0.05	8
Oceania	4	Below 20	0.19	< 0.1	0.01	0.01	12
Melanesia		13	0.1696	< 0.1	0.0171	0.01	4
Polynesia		6	0.0145	< 0.1	0.0013	0.000929	7
Micronesia		4	0.0089	< 0.1	0.0009	0.000738	1
World		10	252.20	100.0	24.59	19.64	100

2.1.6 Cassava Utilization

Constraints that limit the utilization of cassava crop include shelf life and mass. Cassava roots are bulky and heavy commodities and therefore expensive to transport over long distances. The roots are also extremely perishable, thus must be either consumed or processed within a few days after harvest (Eneas, 2006; Balagopalan et al., 1988; Kuppuswamy, 1961). To overcome these limitations, it may be prudent to process cassava roots near the source of production; and into products that are less bulky and with longer shelf life. Such products include chips, flour, and starch. Other constraints on the utilization of cassava crop are associated with cost and technology as highlighted below.

2.1.6.1 Starch

Cassava root is richly endowed with good quality starch to the extent of 30 – 32 wt% (Westby, 2002; International Starch Institute, 1999; O’hair, 1995; Balagopalan et al., 1988). In addition, Cassava starch exhibits vital benefits and advantages over other sources of starch such as wheat, corn, and waxy maize. The reported advantages and benefits include the following (Central Tuber Crops Research Institute (CTCRI), 2010; Juszczak et al., 2003; Larotonda, et al., 2003; Balagopalan, 2002; Lopez-Ulibarri and Hall, 1997; Balagopalan et al., 1988; Franco et al., 1988; Vijayagopal et al., 1988; Cereda and Wosiacki, 1985; Moorthy, 1985; Swinkels, 1985):

- Unique functional and structural qualities.
- Most bland flavor starch in the world. Does not have any mealy or undesirable starch flavors.
- Gelatinizes and swells rapidly in a narrow temperature range.

- The film has high: clarity, gloss, transparency, smoothness, continuity, flexibility, plasticity, folding endurance, internal strength, tensile strength, solubility, and rewetting capacity.
- High paste viscosity, clarity, and stability.
- High binding force and thickening power.
- Good solubility and dry starch mobility.
- Fair resistance to retrogradation and syneresis (weeping).
- Less corrosion of granules from enzyme attacks.
- Smoother granules structure than potato starch granules.
- No foam building.
- Easy extractability.
- Complete and easier hydrolysis.

The imperfections that have been noted for cassava starch are instability of hot gels and stickiness in cooking, fall in viscosity at elevated temperatures, and poor shear resistance (CTCRI, 2010; Balagopalan et al., 1988; Swinkels, 1985).

Aschengreen et al. (1979) concluded: "... it is possible to obtain isomerization results with syrups from tapioca, wheat, or potato starch, which are as good as those obtained with maize starch based syrups or dextrose." Despite the tremendous advantages and quality of cassava starch, there is no significant application of its use in the manufacture of sweeteners such as glucose and high fructose syrup; unlike the case for corn starch. The limited application appears to be due to the extensive cost and technological requirements associated with the industrial production of glucose and high fructose syrup from corn (Johnson et al., 2009; Hoehn et al., 1983). The devices, equipment, and techniques exploited for cooking/heating; clarification/filtration; concentration/evaporation; refining; enrichment/fractionation; crystallization, dehydration

and other duties include the use of steam, extrusion, centrifugation, vacuum evaporation, carbon/ion exchange chromatographic separation regimes, membrane separation (ultra-filtration) reactors, encapsulation, spray dehydration, and various proprietary polishing, decolorizing and other unit operations methodologies (Cavette, 2010; Wikipedia, 2010; PUROLITE, 2007; Akdogan, 1999; Lopez-Ulibarri and Hall, 1997; Prazeres and Cabral, 1994; Hanover and White, 1993; Darnoko et al., 1989; Hoehn et al., 1983). These levels of input can surpass the financial and technological capabilities of developing nations. Alternative, less cost and technology intensive approaches to the manufacture of sweeteners from cassava starch are therefore necessary; and will appear to be desired options for farmers in developing countries.

2.1.6.2 Flour

Cassava flour is more difficult to preserve in storage (Balagopalan et al., 1988). This may be attributed to the hygroscopic nature of cassava flour (Kuppswamy, 1961), which is more than those of cassava chips and starch (CTCRI, 1986). Cassava flour at 6.7% moisture content that was packed in cloth or gunny bags picked up additional 5% moisture within 6 months of storage (Kuppswamy, 1961). Also, cassava flour is most commonly infested by *Tribolium castaneum* insect species (CTCRI, 1986; McFarlane, 1982; Ingram and Humphries, 1972); *Bacillus* and *Enterococci* species of bacteria, and *Aspergillus* fungi species (Ingram and Humphries, 1972). In one comparative evaluation of packaging materials, polyethylene-lined jute bags were found to be most effective for storage of cassava flour for up to 90 days (Etorma, 1936). Proper initial drying and the maintenance of low moisture content with the use of water-vapor transmission resistant packaging material have been suggested for the packaging of cassava flour in order to prevent fungal or bacterial infection (Balagopalan et al., 1988; McFarlane, 1982). On the

other hand, cassava starch is thought to be neither attacked nor relished by the insects (Nanda and Potty, 1985; MacFarlane, 1982). However, high density polyethylene (HDPE) bags or polyethylene impregnated jute bags were recommended for the storage of cassava starch for up to 180 days in order to determine moisture, viscosity, total microbial count, and viable bacterial and fungal populations (Nanda and Potty, 1985).

2.1.6.2.1 Gluten free flour

Celiac disease is a condition in which the mucous membrane of the small intestine of gluten intolerant people is damaged by gluten, resulting in inflammation and poor absorption of nutrients and consequently, weight loss, diarrhea, anemia, fatigue, flatulence, deficiency of folate and osteopenia (Blades, 1997; Thompson, 1997). The disease was first described as “coeliac disorder” more than 100 years ago by Samuel Gee (St. Bartholomew's Hospital Reports of 1888). Coeliac disorder was defined as a chronic indigestion occurring in people of all ages, particularly in one to- five years old children. Cassava is free from gluten (Rudrappa, 2013). Cassava flour and starch can be used to formulate gluten-free breads (Ahlborn et al., 2005; López et al., 2004; Sanchez et al., 2002; Eggleston et al., 1992). Eating gluten-free diet helps people with celiac disease control the symptoms and prevent complications (Mayo Clinic, 2013).

2.1.6.3 Chips

The requirement to transform cassava tubers to dried chips may be justified on account of the fact that the tubers are very perishable, bulky, and heavy. By transforming into chips, it will be possible to transport cassava economically (by mass and volume) to far distances on the one hand. On the other hand, cassava can then be preserved for a prolonged period because the chips store better and longer than the physiologically active tubers. Consequently, it will be possible to produce flour or starch

from the chips at any time; and from facilities/plants that are distant or remote from the cassava crop production centers.

2.1.6.4 Packaging

A food package is expected to provide protection against humidity, light, oxygen, moisture/condensation, heat/temperature, compression, shock, and vibration exposure that are associated with not only the physical environment, but also the storage, distribution and transportation system (Robertson, 2006; Soroka, 2002). Cassava starch, flour and chips must be protected from unwanted interaction with the environment. Water vapor transmission rate (WVTR) resistance is frequently the prime consideration in protective packaging of cassava products (Balagopalan et al., 1988). This is because to various degrees, starch, flour and chips but especially flour is hygroscopic (CTCRI, 1986; Kuppuswamy, 1961). These products absorb moisture freely and hence suffer from biochemical deterioration, and biological spoilage from insect infestation, microbial growth, etc (Nanda and Potty, 1985; McFarlane, 1982; Parker and Booth, 1979; Balagopal and Nair, 1976).

The relevant unit operations involved, as well as the equipment requirement for the production of cassava products are identified in Figure 2-1 (Aso, 2004; Olomo and Ajibola, 2003; Lopez - Ulibarri and Hall, 1997; Onabolu et al., 1998; Osunsami et al., 1989).

2.1.7 Sweetener Production

Sweeteners are in great industrial demand for applications in the food, confectionary, beverage, pharmaceutical, and allied industries (Kroger et al., 2006; BeMiller, 2002; Coulston and Johnson, 2002; Vuilleumier, 1993; Balagopalan et al., 1988). In these industries, sweeteners may be used to achieve, control, modify, or

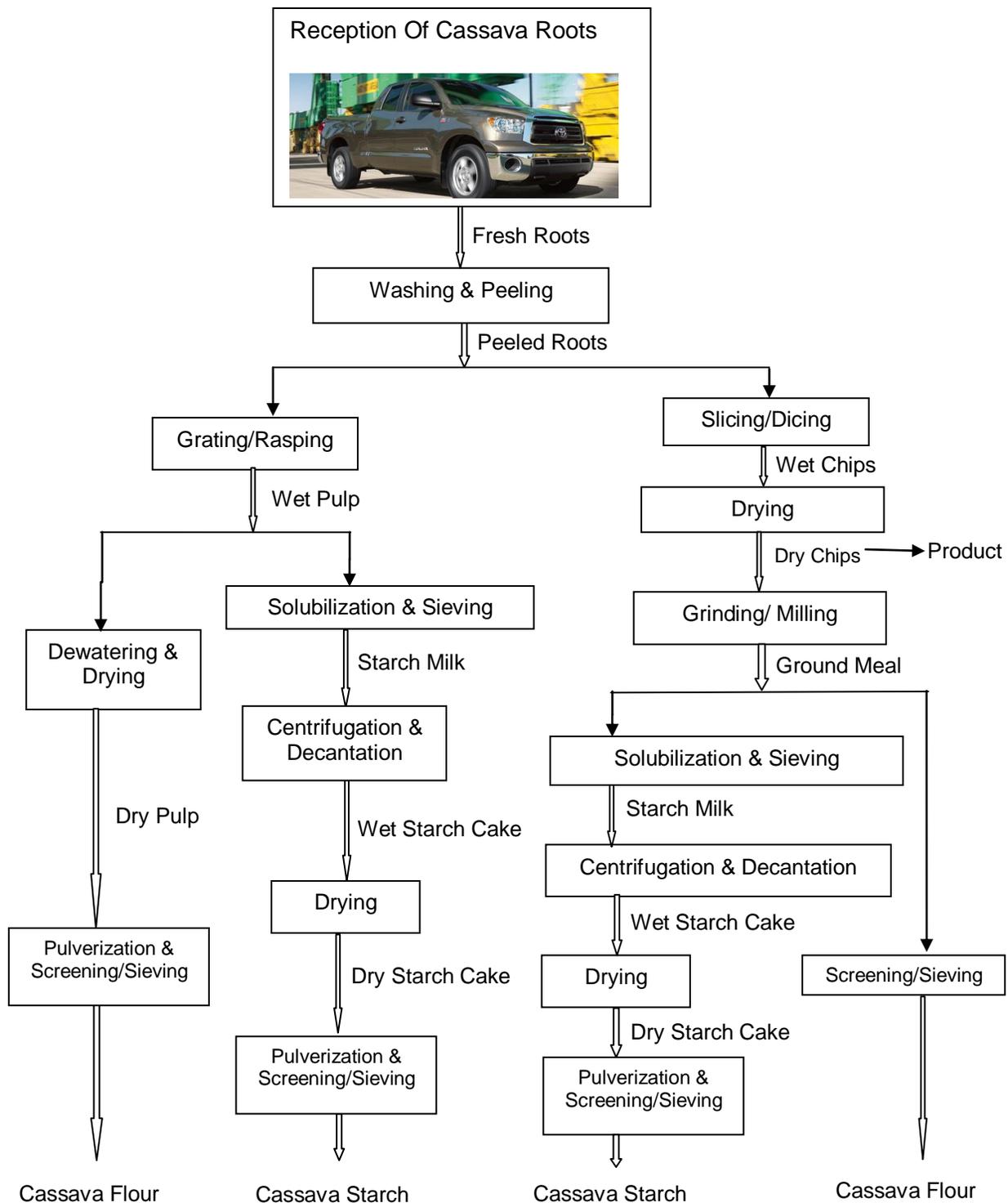


Figure 2-1. Schematic of Unit Operations for the Manufacture of Cassava Chips, Flour, and Starch. Equipment/Machinery Required for Cassava Chips, Flour & Starch Production include: Washers; Peelers; Chipping/Dicing Machines; Wet Grinding/Rasping Machines; Pressing Machines (to dewater); Dryers; Dry Grinding/Milling Machines; Dry Sieving Machines; Wet Mixing Machines (to Solubilize); Wet Sieving Machines; Centrifuges; Packaging Systems.

influence one or more of the following properties: viscosity, hygroscopicity, flavor, size, adhesiveness, appearance, emulsification, etc (Coulston and Johnson, 2002; International Pectin Producers Association (IPPA), 2001; McWilliams, 2001; Nabors, 2001; Hanover and White, 1993; Grenby, 1991; White and Lauer, 1990; Hoehn et al., 1983; Palmer, 1975).

2.1.7.1 Historical overview

The genesis of nutritive sweeteners can be traced to 1811 when Kirchhoff, a Russian chemist discovered that heating a starch-water mixture with dilute acid produced a sugar-like sweet substance (Roberts et al., 1995; Balagopalan et al., 1988; MacAllister, 1980). At the time it was thought that the substance was common sugar (sucrose). However, in 1814 Saussure proved that the substance was indeed glucose (Radley, 1940). In 1866, Dextrose was produced from corn starch; and in 1957, enzymatic conversion of d-glucose to d-fructose was developed (Marshall and Kooi, 1957). Today, a gargantuan quantity of sweeteners is used to produce an astonishing array of products in the bakery, beverage, confectionery, food, pharmaceutical, and other industries. However, the growth in the sweetener arena benefited from and was boosted by advances in other sectors such as starch crop agronomy, enzyme engineering, and process technologies. Following is a brief sketch of the historical background on the production of sweeteners and High Fructose Syrup or HFS (Tomasik, and Horton 2012; Cavette, 2010; Wikipedia, 2010; BeMiller, 2009; Corn Refiners Association, 2007; Ensymm Consulting on Biotechnology, 2005; Hein et al., 2005; Medical Ecology, 2004; Olson, 1995; Roberts et al., 1995; Bagopalan et al., 1988; MacAllister, 1980; Marshall and Kooi, 1957; Dale and Langlios, 1940).

- Early 1700s. Device to shell corn (remove kernels from cob) was patented.

- 1811. GSC Kirchoff (a Russian chemist) produced dextrose and other starch derived sweeteners by heating potato starch in a weak solution of sulfuric acid. [Genesis of acid hydrolysis of starch?!!]
- 1841. The wet milling process (separation of starch from kernels) was patented by Orlando Jones.
- 1842. The first commercial wet milling plant in the USA was established by Thomas Kingsford.
- 1844. The first dedicated corn starch plant in the world operated in Jersey City, New Jersey, USA.
- 1866. Dextrose was produced from corn starch. This was the first corn sweetener produced by adapting the acid conversion of starch method.
- 1882. First manufacture of refined corn sugar (Anhydrous sugar).
- 1900. Introduction of thin boiling starches.
- 1915. Introduction of chlorinated starches.
- 1921. Introduction of crystalline dextrose hydrate.
- 1940. Dale and Langlois introduced enzyme conversion systems for syrups that were preliminarily made by acid hydrolysis. [Genesis of enzyme catalyzed hydrolysis of starch?!!]
- 1940s. Introduction of waxy maize starch.
- Mid 1950s. Introduction of high-amylose corn starch. Development of various corn sweeteners; Introduction of Maltodextrins and low DE (Dextrose Equivalent) syrups.
- 1957. Richard O Marshall and Earl P Kooi developed the enzymatic method of converting d-Glucose to d-Fructose.
- 1967. First commercialization of batch process enzyme catalyzed High Fructose Syrup (HFS) production; at 15% Fructose content.
- 1968. First commercial shipment of enzyme catalyzed HFS production; at 42% Fructose content.
- 1972. A continuous method of enzyme catalyzed starch conversion process was introduced.
- 1975-1985. HFS became the sweetener of choice; and was used extensively by the processed foods and soft drinks industries.

- 1996. About 11.4 billion kilograms of corn were converted to corn syrup and other sweeteners; accounting for over 55% of the nutritive sweetener market in the USA.

2.1.7.2 Acid or enzyme hydrolysis

Depending on the type of sweetener desired (glucose or fructose for example), production may require two or three process steps; excluding downstream processing. Basically starch is hydrolyzed to glucose in two steps (liquefaction and saccharification); and then the glucose is transformed by isomerization into fructose (Aschengreen, 1975; Johnson et al., 2009). Starch hydrolysis may be achieved with the use of acids and or enzymes. Acid hydrolysis is often avoided because of several disadvantages such as low yield, extensive product purification and equipment demands, destruction of starch macromolecules, discoloration and product contamination, charring and dehydration reactions, and the toxic, erosive and hazardous nature of all acids (Aschengreen, 1975; Heitmann and Mersmann, 1997; Johnson et al., 2005; Srinorakutara et al., 2006). For these reasons, the work reported here was conducted with enzyme hydrolysis.

2.1.7.3 Amylolytic enzymes

Enzymes employed in industrial hydrolysis of starch have been noted to be the second largest consumer of the world's enzyme output (Satyanarayana et al., 2004). The glucose producing enzymes alone (the Amylases such as α -amylase, glucoamylase), account for up to 33% of the market share (Nguyen et al., 2002). Generally, the world's industrial enzyme market has steadily increased over the past decades. The market value rose from \$180 million in 1960 to \$817 million in 1985 (Enzymes used in food industry, 2011). In 1998, worldwide enzyme sales were over \$1.5 billion (Beilen and Li, 2002). This value is projected to rise to \$7 billion in 2013 (ReportLinker, 2009). Because culture medium and substrate are important factors in

the synthesis of industrial enzymes, the use of low cost substrates such as cassava peels and other biomaterials/agricultural byproducts can help reduce the cost of sweetener production (Kar et al., 2010; Roses and Guerra, 2009; Silva et al., 2009; Rajagopalan and Krishnan, 2008; Spier et al., 2006; Ayerno et al., 2002; Pandey et al., 2000 a). In addition, the onsite production of such enzymes will obviate transportation and associated logistical costs.

2.1.7.4 Synergistic and direct methods of glucose sweetener production

Perhaps the two major constraints to conventional method of glucose production are the two-step liquefaction cum saccharification procedure, and starch extraction process. The two step procedure requires that liquefaction be carried out first, followed by saccharification. This protocol is inefficient in use of resources: process vessels, piping, and associated equipment, enzyme conditioning, etc. Similarly, the starch extraction is time, energy and labor intensive. It would be advantageous if liquefaction and saccharification processes can be combined as a single unit operation, inside one reactor vessel; and if minimally processed starch source such as fresh cassava root is used as the substrate. The former is called synergism and the latter, direct conversion. Some authors have tried to study these phenomena. Gauss et al., (1976) patented a process for alcohol production by simultaneous saccharification and fermentation of cellulosic materials. Arasaratnam and Balasubramaniam (1993), observed that synergistic hydrolysis of dry milled starch was more efficient than wet milled starch. Linko and Javanainen (1996) claimed 98% yield of lactic acid when 130 g/L starch was simultaneously liquefied, saccharified and fermented. Kearsley and Nketsia-Tabiri (1979) performed direct hydrolysis of native starches in grains, roots and tubers and

reported that unprocessed native starches compared favorably with commercial starches, especially in total starch conversion.

However, little work has been reported in the literature on direct conversion techniques coupled with the synergistic procedure, particularly where glucose sweetener is the desired end product. Most of the reported work was done for the production of alcohol or lactic acid where a reducing sugar, such as glucose, is a transition item to be fermented to the desired end product. The work undertaken in this study was an attempt to combine both direct conversion and synergistic techniques in the production of glucose sweetener, using cassava substrates as the raw materials source. Further objectives included determination of Arrhenius type reaction kinetics: rate constant and activation energy of each substrate at two different temperatures.

2.1.8 Starch Chemistry

Starch, with the chemical or molecular formula $[(C_6H_{10}O_5)_n]$ is a polysaccharide carbohydrate composed of glucose monomer units that are joined by glycosidic bonds. Starch occurs widely in plant tissues in the form of storage granules in fruits (banana, breadfruit), roots (cassava, arrowroot), seeds (corn/maize, rice and wheat), tubers (yam, potato), stem pith (sago) and other parts (Buleon and Colonna, 2007; Eliasson, 2004; Jane et al., 1994; Jenner, 1982). Starch contributes 50–70% of the energy in the human diet (Copeland et al., 2009), and is the principal components of such staple foods like beans, bread, and pasta products (Wang et al., 1998). Pure starch has an auto-ignition temperature of 410°C ; gelatinization temperature of $60^\circ\text{C} - 80^\circ\text{C}$; density of about 1.5 g/cm^3 ; is white, odorless, tasteless, insoluble in cold water or alcohol; and is composed of two major molecules known as amylose and amylopectin (Encyclopedia Britannica: <http://www.britannica.com/EBchecked/topic/563582/starch> [Accessed

10/27/2013]; Water Structure and Science:

<http://www.lsbu.ac.uk/water/hysta.html#r1758> [Accessed 10/27/2013]; Wikipedia:

<http://en.wikipedia.org/wiki/Starch> [Accessed 10/27/2013]; Copeland et al, 2009; Yuryev

et al., 2007) . Figure 2-2 illustrates an overview of the structure of starch granule.

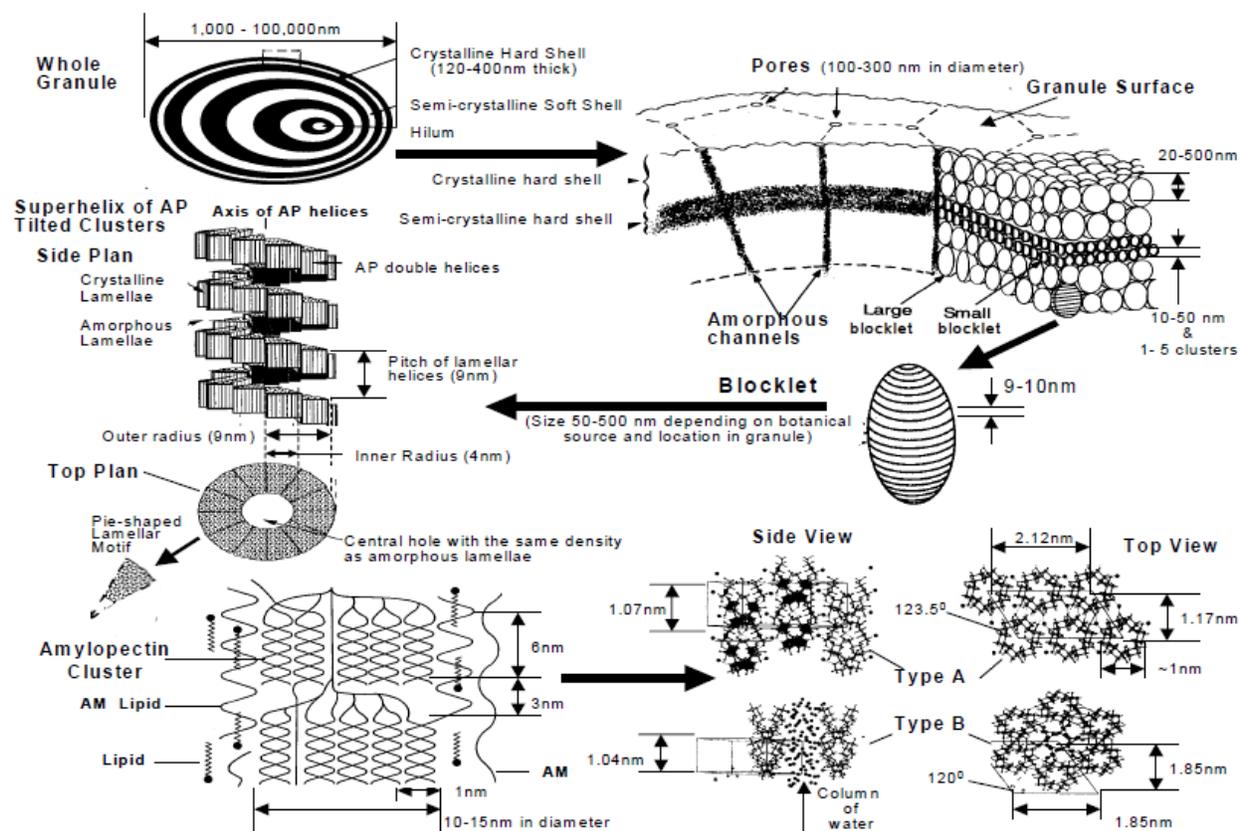


Figure 2-2. An Overview of the Structure of Starch Granule. Source: Purdue University, http://www.cfs.purdue.edu/class/f&n630/pdfs/Starch_yao.pdf [Accessed 10/30/2013]

Depending on botanical source, starch generally may have crystallinity of 15 % to 45 %, granule size of less than 1 μm to more than 100 μm and be composed of 17 % to 25% amylose and 75 % to 83% amylopectin by weight (Perez and Bertoft, 2010; Charles et al., 2004; Lindeboom et al., 2004; Bahnassey and Breene, 1994). However,

waxy starches could have amylopectin contents of over 99% (Bahnassey and Breene, 1994). The structure of starch (Figure 2-2) and the methods of studying it have been reported by many authors (Blazek and Gilbert, 2011; Lopez-Rubio and Gilbert, 2009; Belton, 2007; Limbach and Kremer, 2006; Sanguansri and Augustin, 2006; Ubbink and Mezzenga, 2006; Lindeboom et al., 2004; Molinero et al., 2004; Donald et al., 2001; You et al., 1999; Jenkins and Donald, 1996; Aberle et al., 1994; Jenkins et al., 1993; Hizukuri, 1985; Blanshard et al., 1984; Rosenthal et al., 1974). Starch has varied utilities in the paper, textile, pharmaceutical, bioplastics, and oil drilling and other industries. It is the source of energy and carbon in food and feed; used for texture and functionalities in processed foods; and serves as feed stock for sweetener and ethanol production.

2.1.8.1 Amylose

Amylose is a linear molecule composed of mostly α (1 \rightarrow 4) linked D-glucopyranosyl units. The 1-carbon on one glucose molecule is linked to the 4-carbon on the next glucose molecule giving rise to (α (1 \rightarrow 4)) bonds or linkages. However, recent studies are revealing that amylose contains slight degree of branching with α (1 \rightarrow 6) linkage (Hoover, 2001; Takeda et al., 1992; Hizukuri et al., 1981). Amylose has a packed structure with propensity to retrograde. With its low degree of branching, amylose easily forms insoluble semi-crystalline aggregates of tough gels and strong films that are resistant to digestion. Amylose influences stickiness, texture, water absorption capacity and the digestibility of processed foods (Copeland et al., 2009). It is reported that amylose is positively correlated to hardness and gumminess of starch gels (Sandhu and Singh, 2007). Figure 2-3 represents the structural formula and linear profile of amylose. Some amylose properties of the starches from cereal, legume and root and tuber crops are presented in Table 2-4.

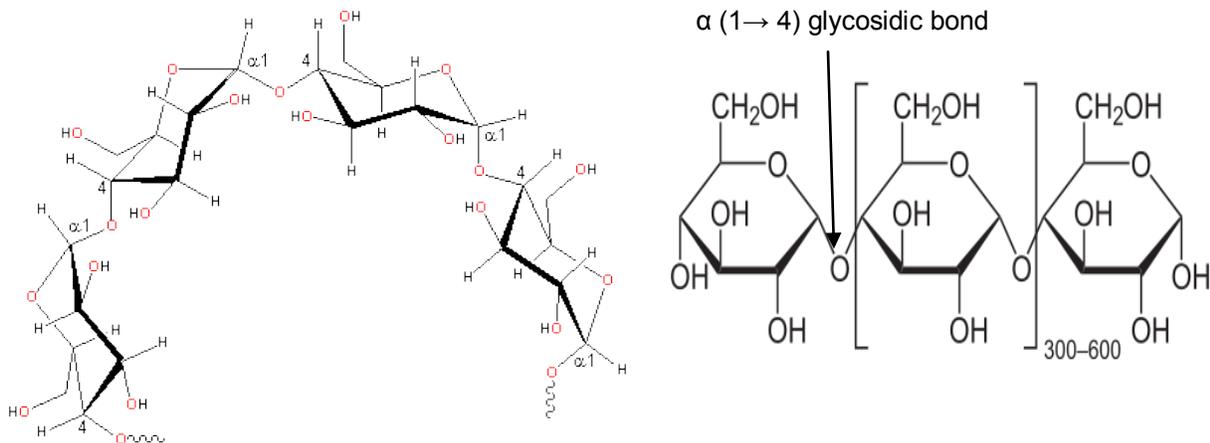


Figure 2-3. Structural Formula and Linear Configuration of Amylose (Sources: Water Structure and Science, <http://www.lsbu.ac.uk/water/hysta.html> [Accessed 11/06/2013]; Wikipedia, <http://en.wikipedia.org/wiki/Amylose> [Accessed 11/06/2013])

Table 2-4. Some Properties of Amyloses from Cereal, Legume and Root and Tuber Starches (Source: Hoover, 2001)

S/N	Property	Starch		
		Cereals	Legumes	Roots and Tubers
1	Blue Value (BV)	1.39 – 1.45	–	1.38 – 1.56
2	Iodine Affinity (I ₂ : g/100g)	9.0 – 20.9	16.0 – 22.0	18.3 – 20.5
3	Organic Phosphorus (ppm)	1.0 – 14.0	–	2.0 – 10.0
4	β-Amylase Limit (%)	61.0 – 95.0	79.0 – 86.9	57.0 – 97.5
5	Number of Branch Linkages (BL)	0.90 – 5.5	–	2.2 – 12.0
6	Number Degree of Polymerization (DP _n)	690 – 1690	1000 – 1900	1273 – 8025
7	Weight Degree of Polymerization (DP _w)	1810 – 5450	–	3320 – 8040
8	Apparent Degree of Polymerization Distribution (DP)	180 – 25200	–	480 – 40000

Specifically for cassava starch, reported amylose values include the following. Blue value: 1.47; Iodine affinity: 16.2 % – 20 %; Organic phosphorus: 7 ppm; β -amylolysis limit: 75 %; Number of chains per molecule: 6 – 8; Chain length: 340; Number degree of polymerization (DP_n): 2600 – 3642; Weight degree of polymerization (DP_w): 6680; Apparent degree of polymerization distribution (DP): 580 – 22400; and Limiting viscosity number: 384 mL/g (Hoover, 2001; Hizukuri and Takeda, 1984; Takeda et al., 1984; Ketiku and Oyenuga, 1972).

2.1.8.2 Amylopectin

Amylopectin is the larger of the two components of starch. It consists of α (1 \rightarrow 4) linked D-glucopyranosyl units, but with more than 5 % α (1 \rightarrow 6) linkages; Figure 2-4.

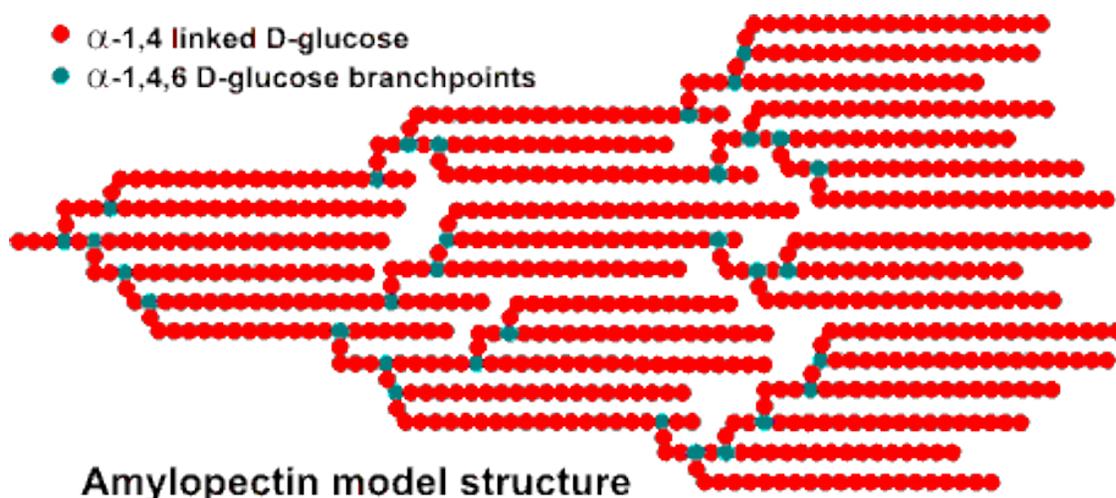


Figure 2-4. Structural Features of Amylopectin Highlighting α (1 \rightarrow 4) Linkages (in red) and α (1 \rightarrow 6) branch points (in blue) (Source: Water Structure and Science, <http://www.lsbu.ac.uk/water/hysta.html> [Accessed 11/06/2013])

This composition gives amylopectin a highly branched tree-like structure, an architecture that led to formation of the cluster model generally used to describe the three dimensional configuration of amylopectin (Manners, 1989; Hizukuri, 1986, 1985).

Figure 2-5 highlights representative structural formula and branching profiles of amylopectin.

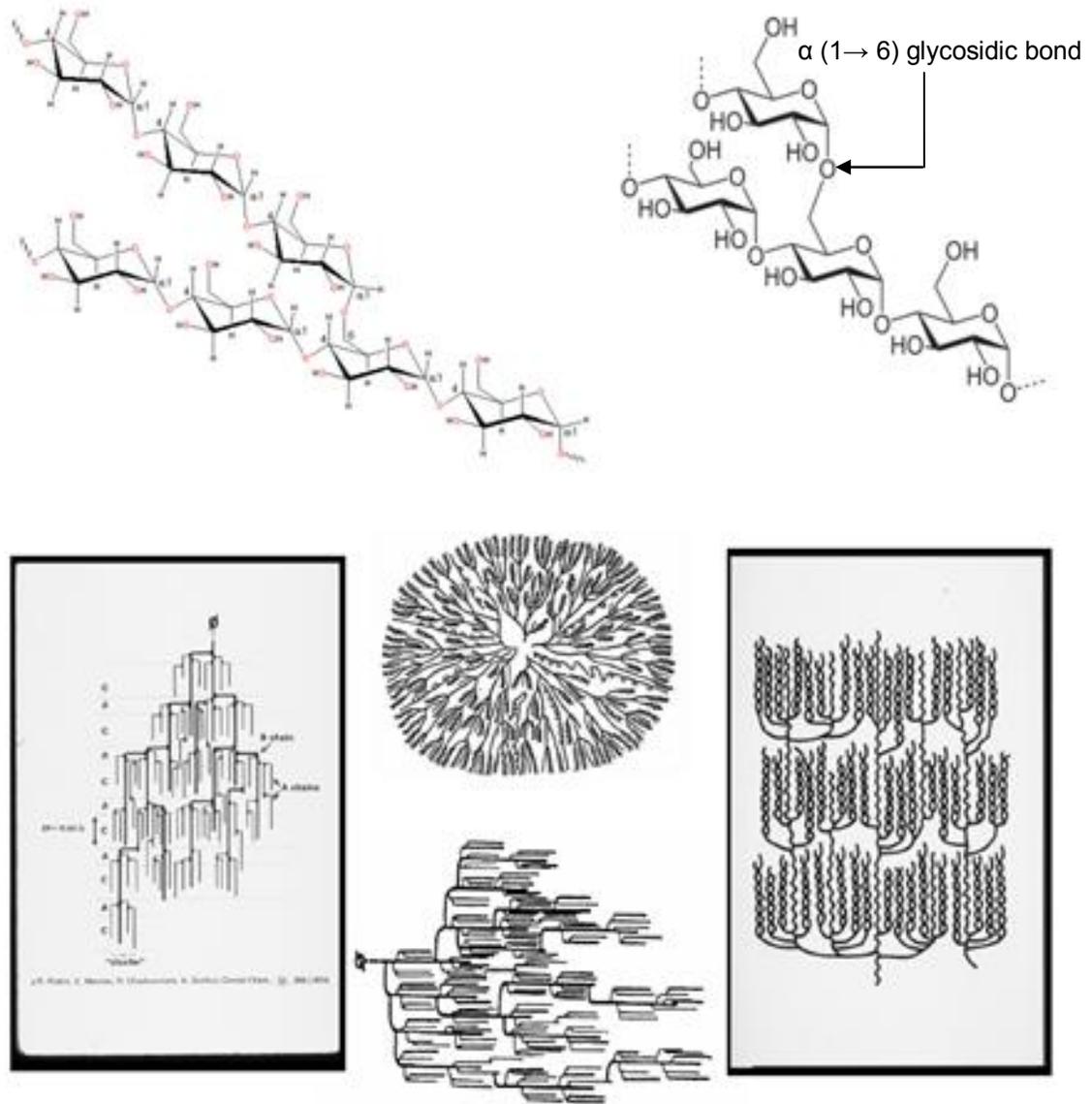


Figure 2-5. Structural Formula and Profiles of Amylopectin Branches and Clusters (Sources: Purdue University, http://www.cfs.purdue.edu/class/f&n630/pdfs/Starch_yao.pdf [Accessed 11/02/2013]; Water Structure and Science, <http://www.lsbu.ac.uk/water/hysta.html> [Accessed 11/06/2013]; Wikipedia, <http://en.wikipedia.org/wiki/Amylopectin> [Accessed 11/06/2013].

The structural design enables amylopectin to impart unique characteristics and functional properties to food systems; influencing crystallinity, gelatinization, retrogradation, cooking and pasting properties (Copeland et al., 2009; Tran et al., 2001; Jane et al., 1999). Amylopectin is reported to play a role in the palatability of rice (Tran et al., 2001), staling of bread and cakes (Copeland et al., 2009), as well as support the crystalline domains and enthalpy of gelatinization of starch granules (Rolland-Sabate et al., 2012; Perez and Bertoft, 2010).

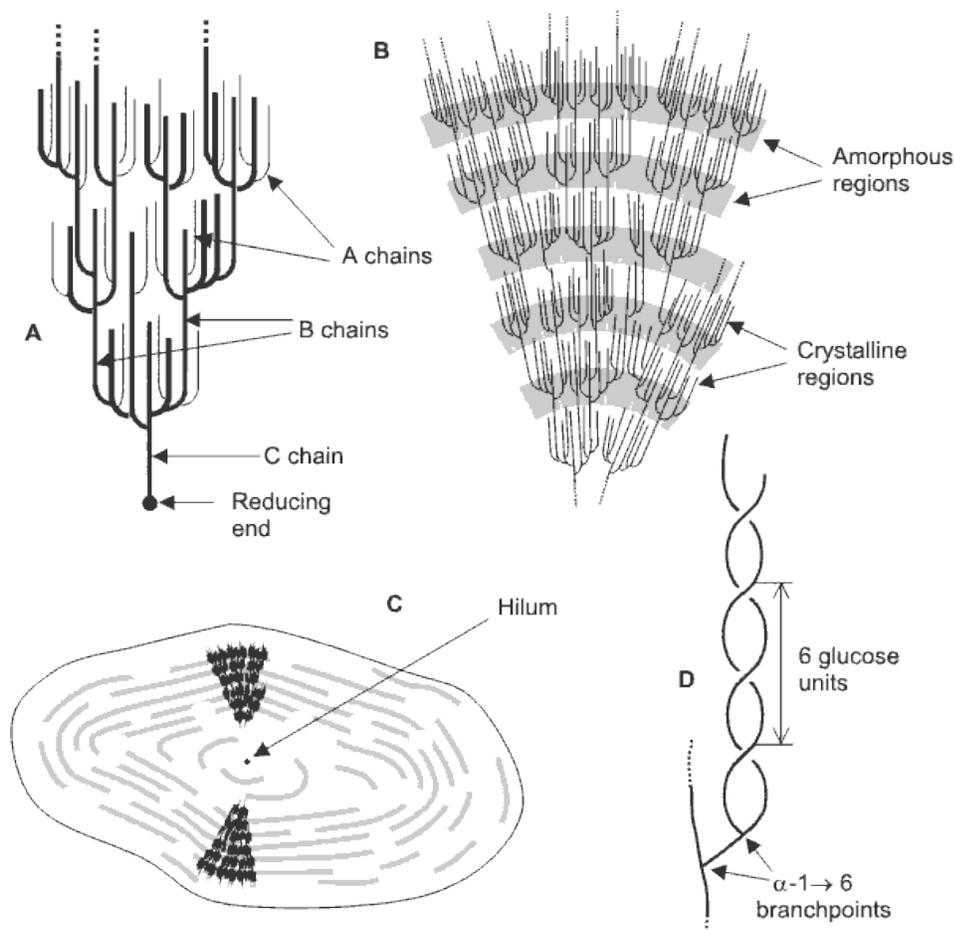


Figure 2-6. Basic labeling of chains in amylopectin. Schematic shows A, B and C chains with the glucose reducing end attached to the C chain; Amorphous and crystalline lamellae; the Hilum or origin of starch formation; as well as α (1→6) linkage points (Source: Water Structure and Science, <http://www.lsbu.ac.uk/water/hysta.html> [Accessed 11/06/2013])

Amylopectin is composed of three classes of glucose chains or branches designated as A, B and C chains (Copeland et al., 2009; Wang et al., 1998; Manners, 1989). As represented in Figure 2-6, the A chains are defined as unsubstituted; they only bind to the B chains. On the other hand, B chains are said to be substituted in that they can bind to other B chains or to the C chains. The C chains feature as the backbone of amylopectin molecules. There is only one C chain per amylopectin molecule, and it is the C chains that carry the reducing glucose (Figure 2-6A). Studies have shown that A chains are the shortest, B chains the longest, while C chains are intermediate in length (Hizukuri, 1986, 1985). Figure 2-7 depicts the building block construction model for amylopectin.

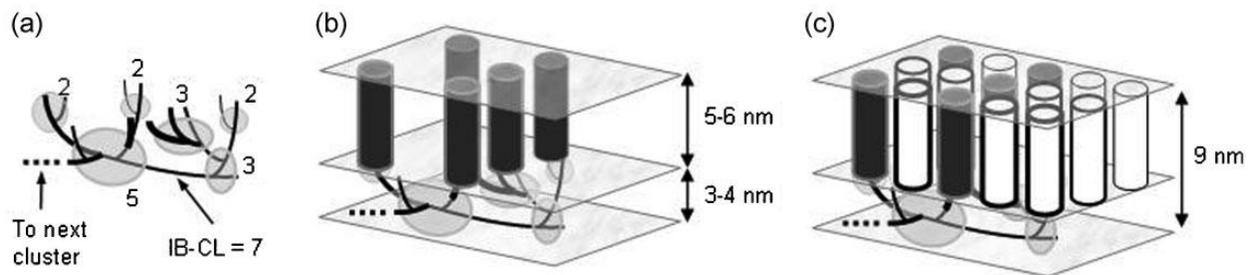


Figure 2-7. A model of how the clusters of amylopectin build up the semi-crystalline granular rings. (a) 3D illustration of the building block structure of the cluster of amaranth amylopectin. The blocks are attached to a backbone forming a network of chains in the amorphous lamella. Numbers indicate types of building blocks with a corresponding number of chains. IB-CL = inter block chain length. (b) The same structure with the external chains added forming five double-helices symbolized as dark cylinders in the crystalline lamella. (c) Several hundred double helices from a large number of clusters interact to build up the crystalline lamella (Source: Perez and Bertoft, 2010).

Some amylopectin properties of the starches from cereal, legume and root and tuber crops are presented in Table 2-5. Specific values for cassava amylopectin include Blue value: 0.104; Iodine affinity: 0 % – 1.39 %; β -amylosis limit: 57 %; and Average

Table 2-5. Some Properties of Amylopectins from Cereal, Legume and Root and Tuber Starches (Source: Hoover, 2001)

S/N	Property	Starch		
		Cereals	Legumes	Roots and Tubers
1	Blue Value (BV)	0.049 – 0.441	–	0.104 – 0.245
2	Iodine Affinity (I ₂ : g/100g)	0.39 – 4.63	1,0 – 5.3	0.06 – 1.1
3	Organic Phosphorus (ppm)	19 – 119	–	21 – 900
4	β-Amyloysis Limit (%)	56 – 61	56 – 66.5	43.8 – 66.7
5	Average Chain length (CL)	19 – 32	20 – 34	19 – 44

chain length (CL): 21 – 27.6 (Hoover, 2001; Jane et al., 1999; Hizukuri, 1986, 1985; Ketiku and Oyenuga, 1972). In Table 2-6 are presented some distinguishing properties between amylose and amylopectin.

Table 2-6. Some Distinguishing Properties between Amylose and Amylopectin

S/N	Property	Unit	Amylose	Amylopectin	Reference(s)
1	Chemical Interaction with Iodine	–	Blue Color	Purple; Reddish-Brown Color	Wang et al., 1998; Whistler and Daniel, 1984
2	Iodine Affinity	%	19 % – 20 %	< 0.2 %	Wang et al., 1998; Rickard et al., 1991
3	General Structure	–	Linear	Branched	Perez and Bertoft, 2010; Jane et al., 1999; Aberle et al., 1994; Rickard et al., 1991
4	Linkages	–	Mainly α[1 → 4]; Less than 0.5 % α[1 → 6]	α[1 → 4] with more than 5 % α[1 → 6]	Copeland et al., 2009; Eliasson, 2004

Table 2-6 Continued. Some Distinguishing Properties between Amylose and Amylopectin

S/N	Property	Unit	Amylose	Amylopectin	Reference(s)
5	Molecular Weight	g/mole	$1 \times 10^5 - 1 \times 10^6$	1×10^8	Copeland et al., 2009; Wang et al., 1998
6	Degree of Polymerization (DP)	Glucose Units	1000 – 10000	Up to 1×10^6 or more	Copeland et al., 2009; Manners, 1989
7	Retrogradation Time	Minutes – Days	Minutes – Hours	Hours – Days	Copeland et al., 2009
8	β -Amyloysis Limit	%	73 – 95	55 – 60	Hoover, 2001; Takeda et al., 1986, 1984
9	Average Chain Length (Glucose Residues)	CL	100 – 10000	20 – 30	Rickard et al., 1991
10	Solubility in Water	%	Variable	Soluble	Rickard et al., 1991
11	Stability in Aqueous Solution	–	Retrogrades	Stable, may retrograde at high conc.	Rickard et al., 1991
12	Conversion to maltose by β -amylase	%	≈ 70	≈ 50	Rickard et al., 1991

2.1.8.3 Cassava Starch

Starch is the principal component of cassava crop, especially the root. Cassava starch is of great value as an important source of nutritional calories in both human food and animal feed. Cassava starch is also finding utilities in the industrial sector. It is used in the production of glucose and high fructose syrup, in the sizing of paper and textiles,

and in the manufacture of adhesives and alcohol (Tonukari, 2004; Rickard et al., 1991; Chadha, 1961). Furthermore, cassava starch has been recommended for the manufacture of extruded snacks, processed baby foods and as filler and bonding agent in the biscuit and confectionery industries. These applications are possible because cassava starch is reported to impart to the products, a fine structure, light color, transparent look, smooth surface, and bland flavor (Seibel and Hu, 1994).

Cassava starch is increasingly being extracted and used in many countries. It is a major source of starch in Brazil, China, India, Indonesia, Philippines and Thailand (Moorthy, 2002; Rickard et al., 1991). Studies have demonstrated that there are variabilities in the physicochemical properties of starch not only between different botanical sources, but also within the same species (Rolland-Sabate et al., 2012; Martinez-Bustos et al., 2007; Charles et al., 2004; Moorthy, 2002; Hoover, 2001; Santisopasri et al., 2001; Defloor et al., 1998; Asaoka et al., 1991; Rickard et al., 1991). However, Perez and Bertoft (2010), stated that the following facts have been established for starch:

- Occurrence of segments of amylopectin with a left-handed, parallel-strand double-helical structure.
- Two types of stable arrangement of double helices can occur, as found in the A and B polymorphs.
- Up to 200 double-helical segments are densely packed in platelet nanocrystals, which are polar and chiral.
- The 1→6 branching does not preclude the formation of double helical arrangements.
- The 'amorphous' amylopectin may contain a significant amount of double helical components.

Some of the important properties that have been reported for cassava starch are presented in Table 2-7.

Table 2-7. Some Properties of Cassava Starch

S/N	Property	Unit	Value	Reference(s)
1	Amylose Content (Normal crop)	%	13.6 – 27	Sanchez et al., 2009; Ceballos et al., 2008; Charles et al., 2005 a, 2004; Hoover, 2001; Jane et al., 1999; Defloor et al., 1998; Asoaka et al., 1991; Olorunda et al., 1981; Ketiku and Oyenuga, 1972
	Amylose Content (Mutated crop)	%	28 – 36	Rolland-Sabate et al., 2012; Ceballos et al., 2008
2	Ash	%	0.02 – 0.49	Nwokocha et al., 2009; Asoaka et al., 1991; Rickard et al., 1991; Rosenthal et al., 1974; Rasper, 1969
3	Crude Fat	%	0.08 – 1.54	Asoaka et al., 1991; Rickard et al., 1991; Soni et al., 1985; Rosenthal et al., 1974
4	Crude Protein	%	0.03 – 0.61	Asoaka et al., 1991; Rickard et al., 1991; Rosenthal et al., 1974
	Gelatinization			
5	Start Tempt. (T_0)	$^{\circ}$ C	54 – 64	Charles et al., 2004; Santisopasri et al., 2001; Bahnassey and Breene, 1994; Defloor et al., 1988
6	Peak Tempt. (T_p)	$^{\circ}$ C	63 – 76	Charles et al., 2004; Santisopasri et al., 2001; Bahnassey and Breene, 1994; Defloor et al., 1988
7	Complete Tempt. (T_c)	$^{\circ}$ C	72 – 83	Charles et al., 2004; Bahnassey and Breene, 1994; Defloor et al., 1988
8	Enthalpy Change (ΔH)	J/g	9 – 16.9	Rolland-Sabate et al., 2012; Charles et al., 2005 a, 2004; Santisopasri et al., 2001; Defloor et al., 1988

Table 2-7 Continued. Some Properties of Cassava Starch

S/N	Property	Unit	Value	Reference(s)
9	Granule Shape	–	Oval, Round, Spherical, Truncated	Ceballos et al., 2008; Soni et al., 1985; Ketiku and Oyenuga, 1970
10	Granule Size	µm	2.4 – 32	Nwokocha et al., 2009; Ceballos et al., 2008; Santisopasri et al., 2001; Defloor et al., 1998; Jane et al., 1994; Asoaka et al., 1991; Ketiku and Oyenuga, 1970
11	Size of Amylopectin Cluster (Repeat Distance)	nm	9.1	Jenkins et al., 1993
12	Nitrogen	%	0.008 – 0.013	Nwokocha et al., 2009; Defloor et al., 1998
13	Phosphorus	%	0.0075 – 0.021	Asoaka et al., 1991; Soni et al., 1985; Rosenthal et al., 1974
14	Starch	%	73.7 – 98.7	Valetudie et al., 1993; Rickard et al., 1991; Rickard and Behn, 1987; This Dissertation (Chapter 4; Table 4-5)
15	Total Pectin	mg GA/kg	314	This Dissertation (Chapter 4; Table 4-5)
16	Crystallinity	%	15.3 – 40	Rolland-Sabate et al., 2012; Hoover, 2001; Asoaka et al., 1991
	Rheological (Pasting) Characteristics			
17	Pasting Temperature (PT)	° C	58.8 – 74.6	Sanchez et al., 2009; Ceballos et al., 2008; Charles et al., 2005 a; Santisopasri et al., 2001; Rasper, 1969
18	Peak Viscosity (PV)	% RVA	24.2.0 – 142.6	Charles et al., 2005 a; Bahnassey and Breene, 1994; Deffenbaugh and Walker, 1989 a, 1989 b

Table 2-7 Continued. Some Properties of Cassava Starch

S/N	Property	Unit	Value	Reference(s)
18 Cont.	Peak Viscosity (PV)	mPa.s	22.0 – 1505.0	Sanchez et al., 2009; Ceballos et al., 2008
19	Minimum or Hot Paste Viscosity at 95 ° C (HPV)	% RVA	11.0 – 30.5	Charles et al., 2005 a; Bahnassey and Breene, 1994; Deffenbaugh and Walker, 1989 a
20	Final or Cold Paste Viscosity at 50 ° C (CPV)	% RVA	12.5 – 127.0	Charles et al., 2005 a; Bahnassey and Breene, 1994; Deffenbaugh and Walker, 1989 a
21	Breakdown (PV – HPV)	% RVA	12.2 – 118.0	Charles et al., 2005 a; Bahnassey and Breene, 1994; Deffenbaugh and Walker, 1989 a
		mPa.s	28.1 – 859.0	Sanchez et al., 2009
22	Setback (CPV – PV)	% RVA	(– 18.8) to (– 9)	Charles et al., 2005 a; Bahnassey and Breene, 1994; Deffenbaugh and Walker, 1989 a
		mPa.s	(– 702.0) to (273.0)	Sanchez et al., 2009
23	Consistency (CPV – HPV)	% RVA	0.5 – 102.4	Charles et al., 2005 a; Bahnassey and Breene, 1994; Deffenbaugh and Walker, 1989 a
		mPa.s	0.0 – 620.0	Sanchez et al., 2009
24	Cooking Ability (Time to PV – Time to PT)	Min.	1.0 – 5.6	Sanchez et al., 2009; Bahnassey and Breene, 1994
25	Solubility at 35 ° C – 95 ° C	%	0.2 – 48	Sanchez et al., 2009; Ceballos et al., 2008; Charles et al., 2005 a; Moorthy, 2002; Hoover, 2001; Rickard et al, 1991

Table 2-7 Continued. Some Properties of Cassava Starch

S/N	Property	Unit	Value	Reference(s)
26	Swelling Power at 35 ° C – 95 ° C	g/g	0.8 – 71.0	Sanchez et al., 2009; Ceballos et al., 2008; Charles et al., 2005 a; Moorthy, 2002; Hoover, 2001; Rickard et al, 1991; Rosenthal et al., 1974
27	Paste Clarity	%	12.5 – 96.6	Sanchez et al., 2009; Ceballos et al., 2008
28	Storage Modulus (G') at 25 ° C and [90 ° C]	Pa	4970 – 19370 [471.7 – 1937]	Charles et al., 2004
29	Loss Shear Modulus (G'') at 25 ° C and [90 ° C]	Pa	453 – 3060 [51.3 – 219]	Charles et al., 2004
30	Loss Tangent at 25 ° C and [90 ° C]	Tan δ	0.12 – 0.19 [0.10 – 0.13]	Charles et al., 2004
31	Iodine Affinity	%	3.07 – 4.7	Jane et al., 1999; Rosenthal et al., 1974
32	Water Holding Capacity	%	71.8	Soni et al., 1991

2.1.8.4 Enzymatic Hydrolysis of Starch

Enzyme technologies are increasingly being used in numerous processes in the pharmaceutical, fine chemical, food, brewing, fermentation, distilling, paper and textile industries, as well as in clinical, medicinal and analytical chemistries (Pandey et al., 2000 b; Solomon, 1978). One of the areas of high application is starch conversion (Satyanarayana et al., 2004; Nguyen et al., 2002). It has been reported that enzyme technology applied to the processing of starch provides higher yields, significant

improvements in product quality, as well as energy savings (Reichelt, 1983; Ueda, 1981). A variety of enzymes may be used for starch conversion. These include alpha amylase, beta amylase, amyloglucosidase, isoamylase, pullulanase, etc. (Tomasik and Horton, 2012; Uhlig, 1998; James and Lee, 1997; Roberts et al., 1995; Whitaker, 1994; Fogarty, 1983; Lyons, 1983; Solomon, 1978; Fogarty and Griffin, 1973).

2.1.8.4.1 Alpha amylase (α -amylase: EC 3.2.1.1)

The α -amylase is alternatively known as α -1,4-D- glucan glucanohydrolase or dextrogenic amylase. It is widely distributed in microorganisms such as bacteria and fungi, and has an optimum pH range of 5 – 7. The structure of α -amylase is shown in Figure 2-8.

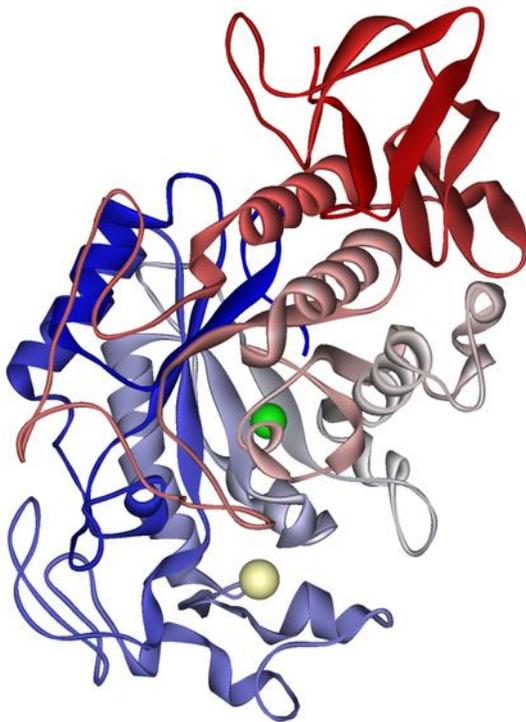


Figure 2-8. Structure of Alpha Amylase with calcium ion (khaki) and chloride ion (green) visible. (Source: Wikipedia, http://en.wikipedia.org/wiki/File:Salivary_alpha-amylase_1SMD.png [Accessed 11/09/2013])

The α -amylase is referred to as endo-acting enzyme in that it cleaves the substrate links in the interior of the molecule, acting from any side of the chain. If starch is treated with α -amylase enzyme, the enzyme will randomly hydrolyze α (1 \rightarrow 4) glucosidic bonds in amylose and amylopectin, whereas α (1 \rightarrow 6) linkages are not attacked. This reaction gives rise to the formation of maltose, maltotriose, glucose, and α -limit dextrans. The dextrans contain all the α (1 \rightarrow 6) linkages (Fogarty, 1983; Solomon, 1978) and polysaccharide chain segments. Figure 2-9 illustrates the operating mechanism of alpha amylase during the hydrolysis of starch.

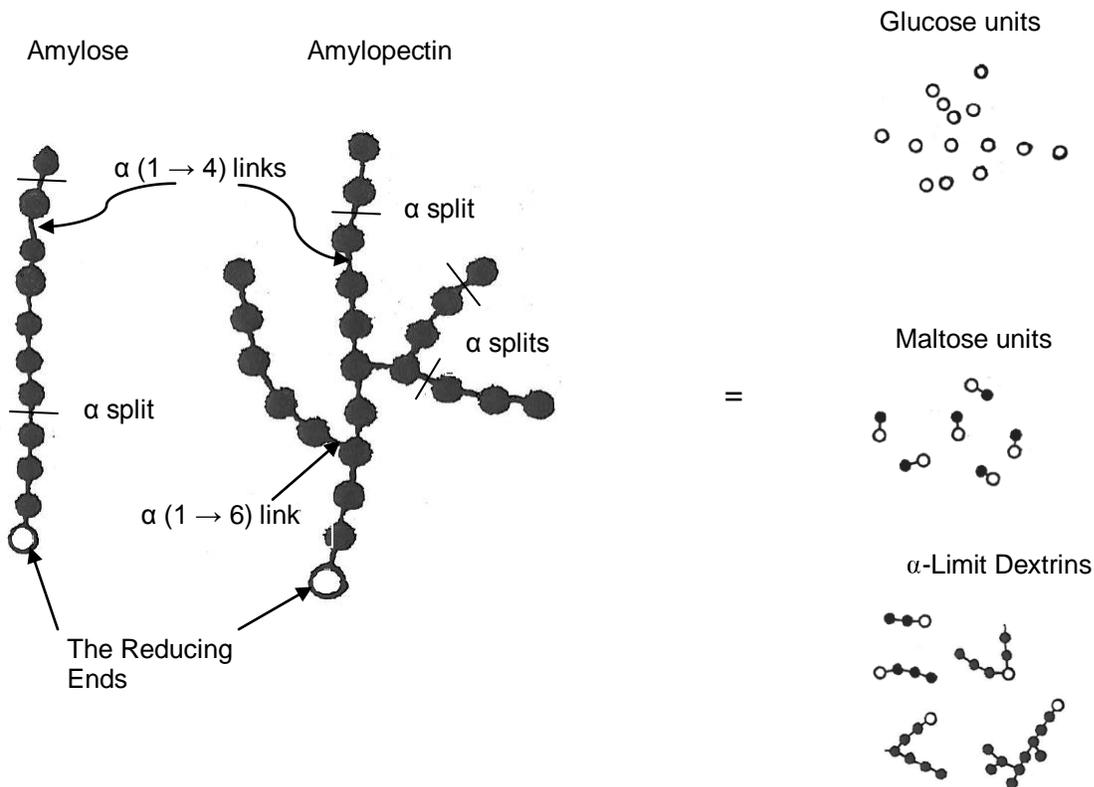


Figure 2-9. Schematics of how α -Amylase randomly splits Starch Molecules to liberate Glucose, Maltose and α -Limit Dextrans.

2.1.8.4.2 Beta amylase (β -amylase: EC 3.2.1.2)

The β -amylase enzyme is also known as α -1,4-D- glucan maltohydrolase or saccharogenic amylase. This enzyme is widely distributed in plants like sweet potatoes and soybeans, but can also occur as extracellular enzyme in microorganisms. It has the optimum pH of about 4 – 5. The structure of β -amylase is represented in Figure 2-10.

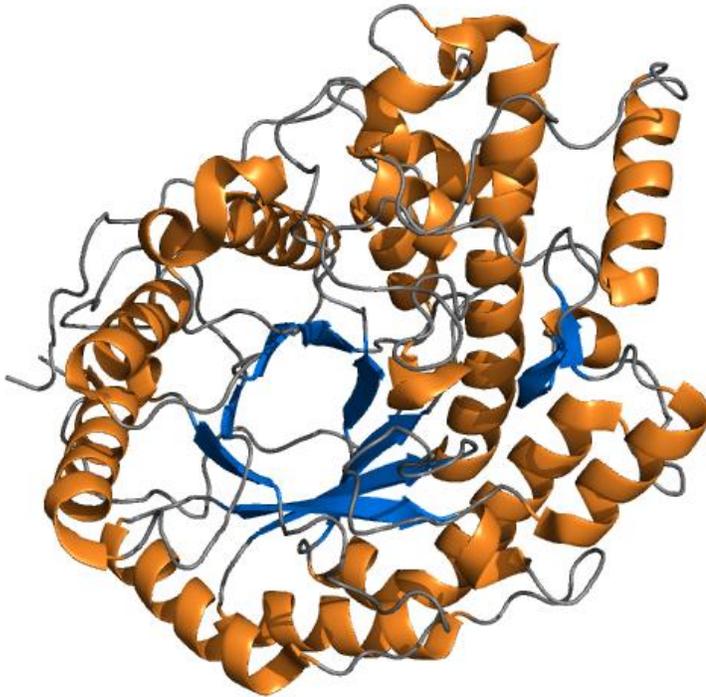


Figure 2-10. Structural Features of Beta Amylase. (Source: Wikipedia, http://en.wikipedia.org/wiki/File:2xfr_b_amylase.png [Accessed 11/09/2013])

The β -amylase is an exo acting enzyme that hydrolyses every second α (1 \rightarrow 4) linkage from the non reducing end, splitting off two glucose units (maltose) at a time. The β -amylase does not bypass or cleave α (1 \rightarrow 6) linkages (James and Lee, 1997), and therefore produces incomplete starch hydrolysis; resulting in 50 % – 60 % conversion to maltose and formation of β -limit dextrans (Perez and Bertoft, 2010; Uhlig, 1998; James and Lee, 1997). The β -limit dextrans contain all unhydrolyzed α (1 \rightarrow 6)

branches and other polysaccharide chain segments. Consequently, if starch is treated with β -amylase, the alternate α (1 \rightarrow 4) linkages of the starch molecules (amylose and amylopectin) are quickly hydrolyzed to maltose (and perhaps some amounts of maltotriose). The α (1 \rightarrow 6) branches are not hydrolyzed, but rather constitute part of the β -limit dextrins; Figure 2-11.

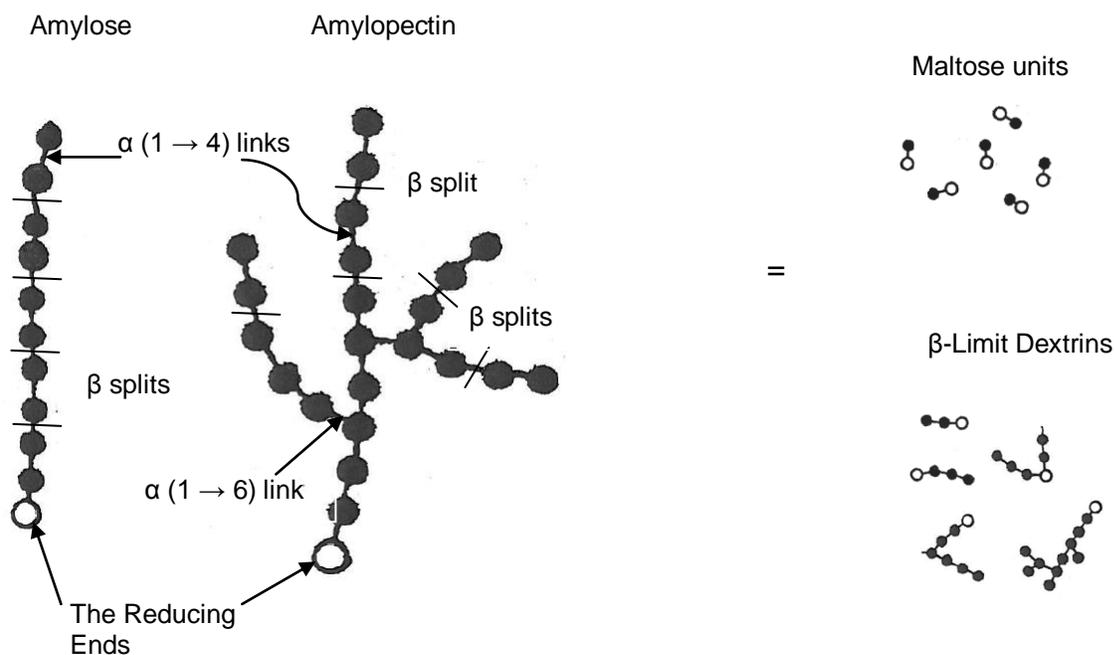


Figure 2-11. Hydrolysis of Starch by β -Amylase to liberate Maltose and β -Limit Dextrins

2.1.8.4.2 Gamma amylase (γ -amylase: EC 3.2.1.3)

The gamma amylase is popularly known as glucoamylase. However, it is also alternatively called α -1,4-D- glucan glucohydrolase, Exo-1,4- α -glucosidase, and amyloglucosidase. The structure of glucoamylase is shown in Figure 2-12. The glucoamylase enzyme is exo-acting and liberates glucose molecules from the non-reducing chain ends of polysaccharides (Whitaker, 1994; Ueda, 1981). It has an optimum pH range of about 3.5 – 5 and is found mostly in fungi.

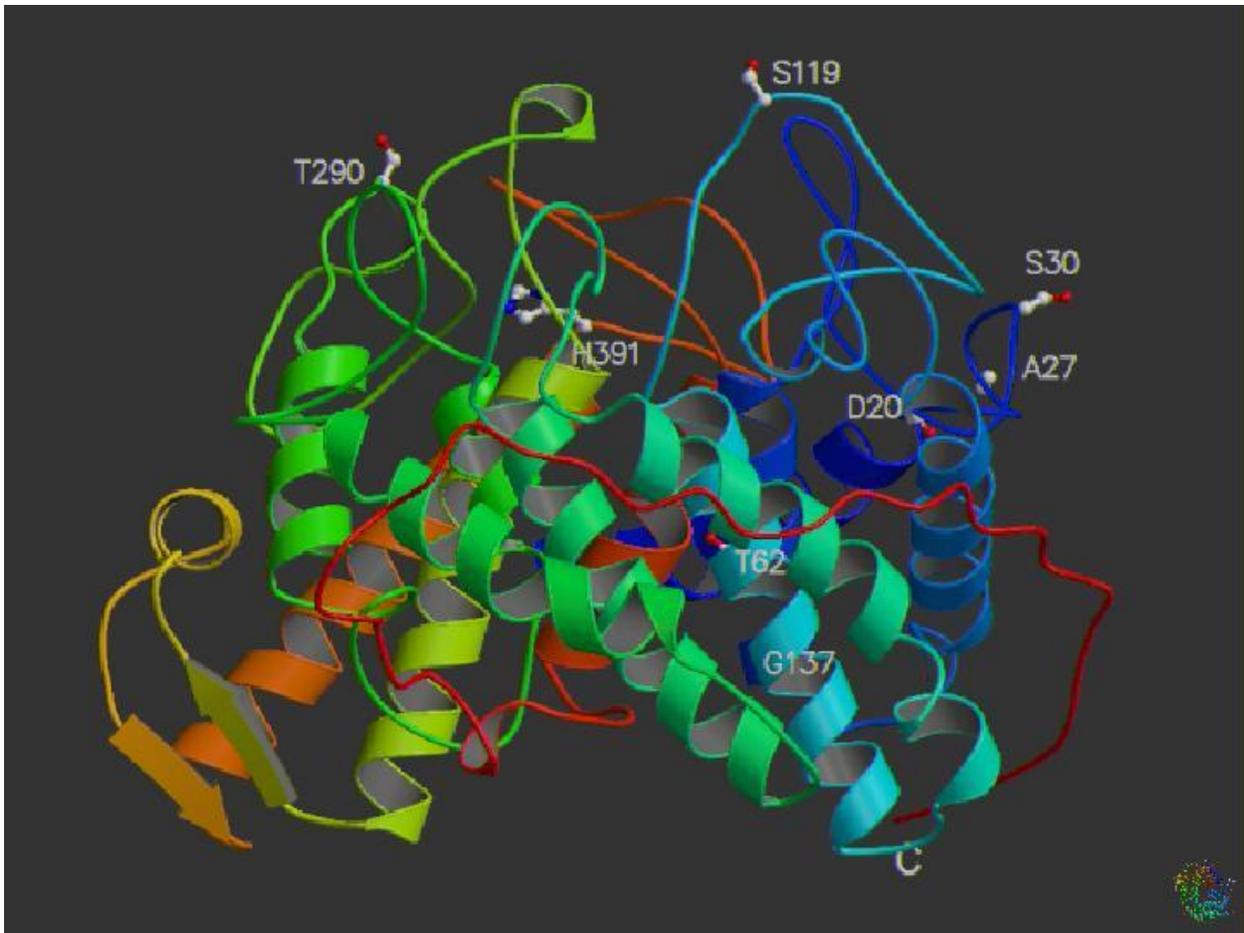


Figure 2-12. Structure of Glucoamylase (Source: bing.com/images, <http://www.public.iastate.edu/~cford/GAstructure.jpg> [Accessed 11/09/2013])

Glucoamylase can cleave both α (1 \rightarrow 4) and α (1 \rightarrow 6) linkages; but the α (1 \rightarrow 6) linkages are hydrolyzed more slowly. When starch is exposed to glucoamylase, the glycosidic bonds (α (1 \rightarrow 4), α (1 \rightarrow 6)) are hydrolyzed consecutively (Fogarty, 1983; Solomon, 1978) to release glucose molecules. Therefore, if incubated for sufficient amount of time, the glucoamylase is able to completely hydrolyze starch (James and Lee, 1997). A model for glucoamylase and its mechanism of hydrolysis of starch has been proposed by the institute of food research in the United Kingdom; Figure 2- 13. In this paradigm, glucoamylase is considered a multi-domain enzyme, consisting of

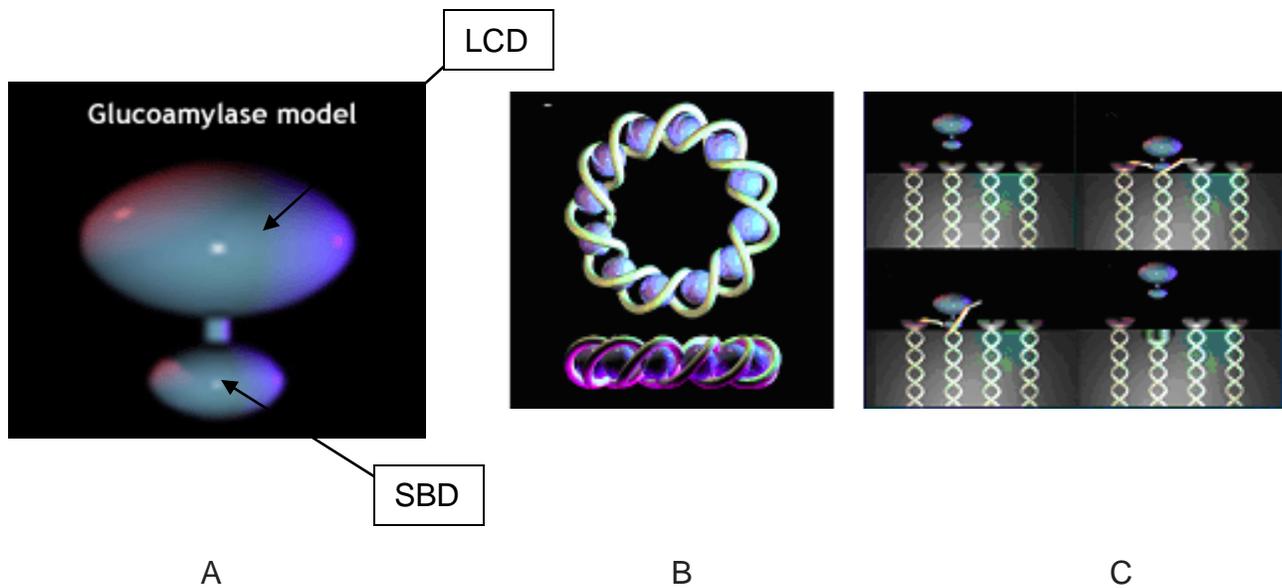


Figure 2-13. Glucoamylase Model and Mechanism of Starch Digestion. A). Proposed model of glucoamylase; B). Coupling of glucoamylase model with starch molecule; C). Locking/binding of glucoamylase model with starch chain ends and subsequent cleavage. (Source: Institute of Food Research, <http://www.ifr.ac.uk/SPM/glucoamylase.html> [Accessed 11/09/2013])

smaller starch-binding domain (SBD) which is attached to the larger catalytic domain (LCD); Figure 2-13A. Complex formation with starch molecule is dominated by the SBD with, the catalytic domain decorating the outside of the starch-SBD ring as illustrated in Figure 2-13B. The nature of starch (amylose) – SBD complex suggests a role for the SBD. The SBD is considered to recognize and dock onto the ends of amylose double helices. This locks the otherwise mobile chain ends at the end of the helix in the vicinity of the LCD (Figure 2-13C), facilitating binding and subsequent cleavage. When this happens to consecutive α (1 \rightarrow 4) bond and α (1 \rightarrow 6) linkages, the result is complete digestion of starch to glucose as represented in Figure 2-14.

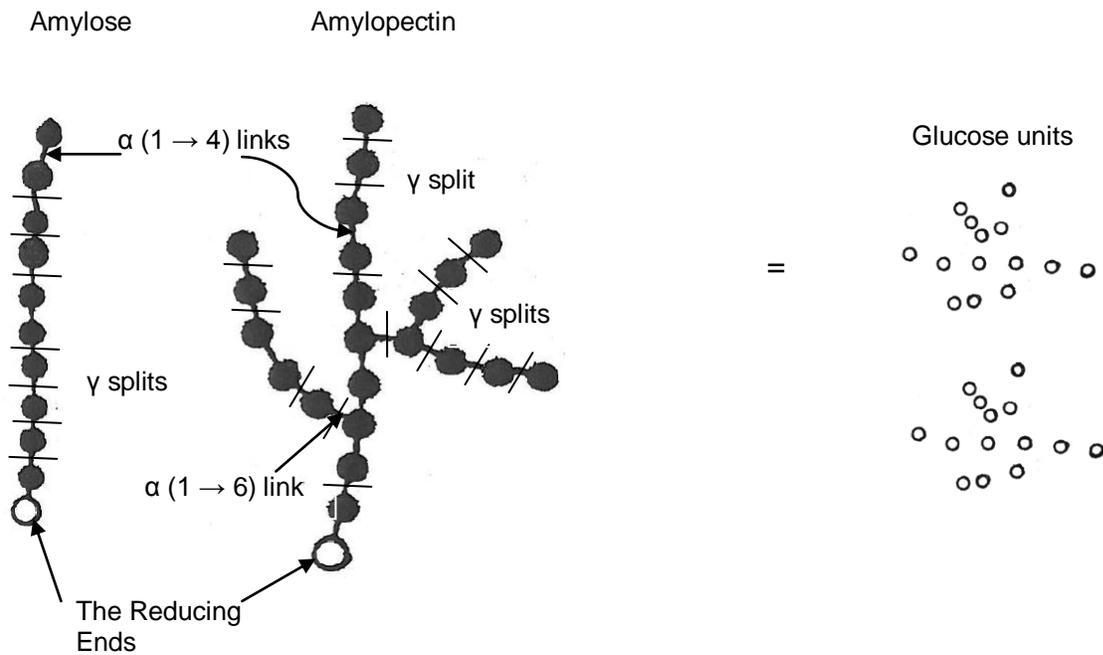


Figure 2-14. Hydrolysis of Starch by γ -Amylase to liberate Glucose Molecules

2.1.8.4.3 Comparison of starch hydrolyzing enzymes

In Figure 2-15 is presented a global view of the major end products achieved by various starch hydrolyzing enzymes. It can be seen how the capabilities of the enzymes vary in the digestion of starch. Apart from γ -amylase (glucoamylase) that could achieve complete digestion of starch to glucose, the other enzymes exhibited incomplete digestion with varying degrees of glucose, maltose, dextrans, etc (Figure 2-15).

However, the digestibility or hydrolysis of starch is a function of numerous factors. These include starch related matters such as botanic source, amylose/amylopectin ratio, extent of molecular association between starch components, degree of crystallinity, gelatinization, retrogradation, germination/sprouting, granule size, available specific surface area, amylose chain length, amylose – lipid complexes,

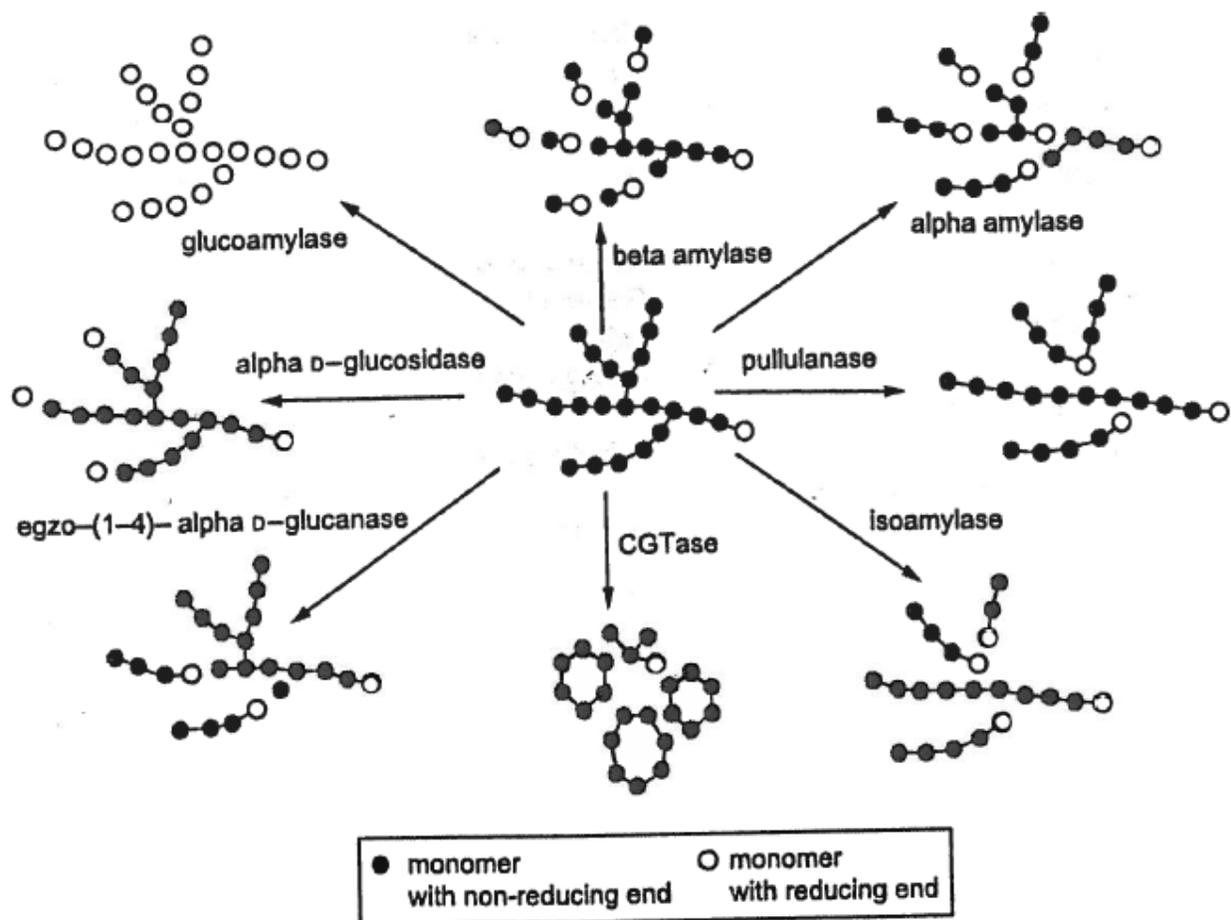


Figure 2-15. Overview of the Pattern of Starch Digestion by major Starch Hydrolyzing Enzymes (Tomasik and Horton, 2012)

porosity, presence of activators/inhibitors, moisture content during heat treatment, structural inhomogeneities and degree of integrity; as well as many other enzyme associated issues (Chen et al., 2011; Copeland et al., 2009; Shariffa et al., 2009; Jayakody and Hoover, 2008; Hoover and Vasanthan, 1994; Jood et al., 1988; Ring et al., 1988; Hoover and Sosulski, 1985; Dreher et al., 1984; Fogarty, 1983; Snow and O'Dea, 1981, Wiseman, 1973).

In general, it has been reported that ungelatinized root and tuber starches are more resistant to enzyme hydrolysis than their cereal counterparts (Valetudie et al., 1993; Rickard et al., 1991; Dreher et al., 1984; Ueda, 1981; Rasper et al., 1974; Leach and Schoch, 1961). On the other hand, cassava starch was found to be the least resistant to enzymatic digestion among the non-cereal starches (Valetudie et al., 1993; Gorinstein and Lii, 1992; Rickard et al., 1991; Rasper et al., 1974), with digestibilities reported to be comparable to those of corn starch (Ueda, 1981; Leach and Schoch, 1961). The activity of an enzyme on the digestibility of native (raw, ungelatinized) starch is often expressed as the percentage of the activity of the enzyme on the native starch as compared to the activity of the enzyme on the gelatinized starch. However, it is difficult to make direct comparisons among the digestibilities of various starches reported in the literature due to a number of reasons. The reasons among others include differences in the type and sources of enzyme used, enzyme purity, enzyme concentration, botanic sources of the starch, varietal differences of the starch; as well as experimental conditions such as time of hydrolysis, pH, temperature, etc. Nevertheless, an attempt has been made here to present some of the reported data on the digestibility of native cassava starch in Table 2-8.

2.1.9 Cassava Toxicity

A major concern for the handling, consumption and use of cassava products is their potential to engender cyanide poisoning. It was reported that diets high in cassava led to increased levels of cyanide in the blood, and elevated concentrations of thiocyanate in the urine (Delange et al., 1973; Osuntokun, 1973). The consumption of cassava has also been associated with such diseases as TAN: tropical ataxic

Table 2-8. Degree of Hydrolysis of Native Cassava Starch Achieved with Various Enzymes and Operating Conditions

S/N	Starch Conc. [%]	Type/Name of Enzyme	Enzyme Activity	Enzyme Conc.	Hydrolysis Tempt. [° C]	Hydrolysis pH	Hydrolysis Time [Hours]	Degree of Hydrolysis Achieved	Reference(s)
1	25	STARGEN 001™	3736 units/g	1 %	35	5 – 6	24	36 %	Shariffa et al., 2009
2	4	<i>Bacillus subtilis</i> α -amylase		2.8 μ kat/g starch	37	6.7	24	44.0 %	Valetudie et al., 1993
3	4	Porcine Pancreatic α -amylase		2.8 μ kat/g starch	37	6.7	24	52.9 %	Valetudie et al., 1993
4	17	Bacterial α -amylase	3490 SKB units		37	5.2	36	57.5 %	Franco et al., 1987
5	80	Glucoamylase from <i>Rhizopus</i> spp		10 mg/mL	55	4.8	2	14 – 16	Rickard and Behn, 1987
6		Glucoamylase from <i>Endomycopsis fibuligera</i>		1.71 units/mL	40	4.5	24	34.9 %	Ueda and Saha, 1983
7	10	Bacterial α -amylase	10 EU/mg		30	6.4	48	4.7 % Maltose	Rasper et al., 1974
8	10	Bacterial α -amylase	10 EU/mg		30	6.4	24	2.5 % Maltose	Rasper et al., 1974
9	10	Fungal Glucoamylase	Grade II		30	5.0	48	4.1 % Maltose	Rasper et al., 1974
10	10	Fungal Glucoamylase	Grade II		30	5.0	24	2 % Maltose	Rasper et al., 1974
11	20	Bacterial α -amylase	16000 SKB	0.5 %	50	6.5	24	55.7 %	Leach and Schoch, 1961
12	10 (Root Pulp)	Bacterial α -amylase (25%) Plus Fungal Glucoamylase (75 %)	5 x 10 ⁶ BAAU/g Plus 1000 AG/g	0.015 %	37	4.3 – 6.6	96	55 %	This Dissertation; Chapter 4

neuropathy (Osuntokun, 1973; Osuntokun et al., 1970), endemic goiter (Bourdoux et al., 1978; Ekpechi, 1973, 1967) and perhaps konzo (Tylleskar et al., 1992; Mlingi et al., 1991). The source of these disorders is believed to be the cyanogenic potential of cassava. The cassava crop is reported to accumulate two aliphatic cyanogenic glucosides known as linamarin: 2(β -D-glucopyranosyloxy) isobutyronitrile, which is the major glucoside in the plant, and to a lesser extent, a higher homologue of linamarin called lotaustralin: 2(β -D-glucopyranosyloxy) 2-methylbutyronitrile (Cooke et al., 1978; Zitnak et al., 1977; Nartey, 1968). The structures of these cyanogens are shown in Figure 2-16. The biosynthesis pathway of linamarin with valine as the precursor is presented in Figure 2-17.

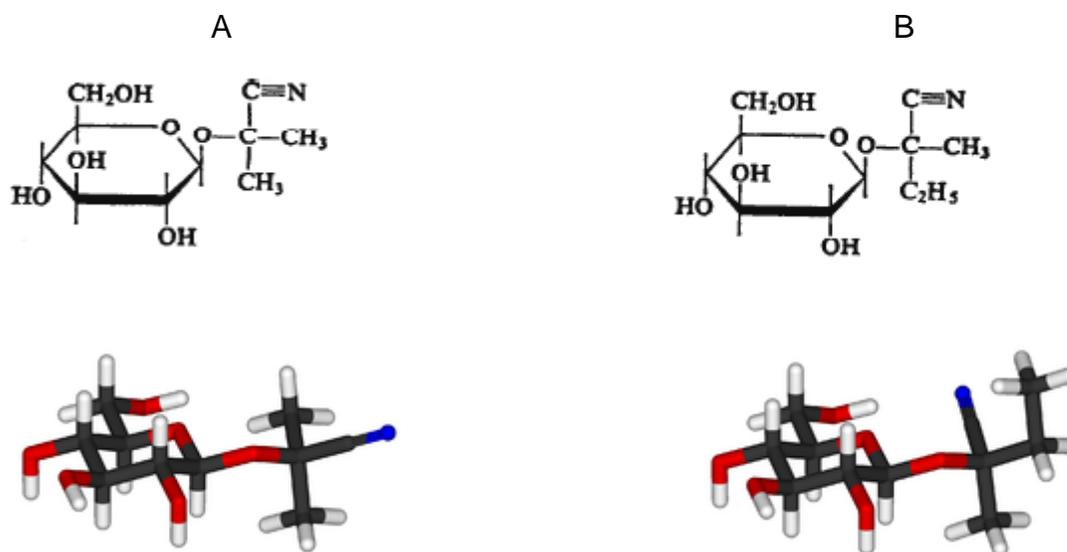


Figure 2-16. Structures of the Aliphatic Cyanogenic Glucosides of Cassava. A: Linamarin and B: Lotaustralin. (Sources: http://en.wikipedia.org/wiki/File:Linamarin_3D_sticks.png [Accessed 11/14/2013]; http://en.wikipedia.org/wiki/File:Lotaustralin_3D_sticks.png [Accessed 11/14/2013]; Nartey, 1968)

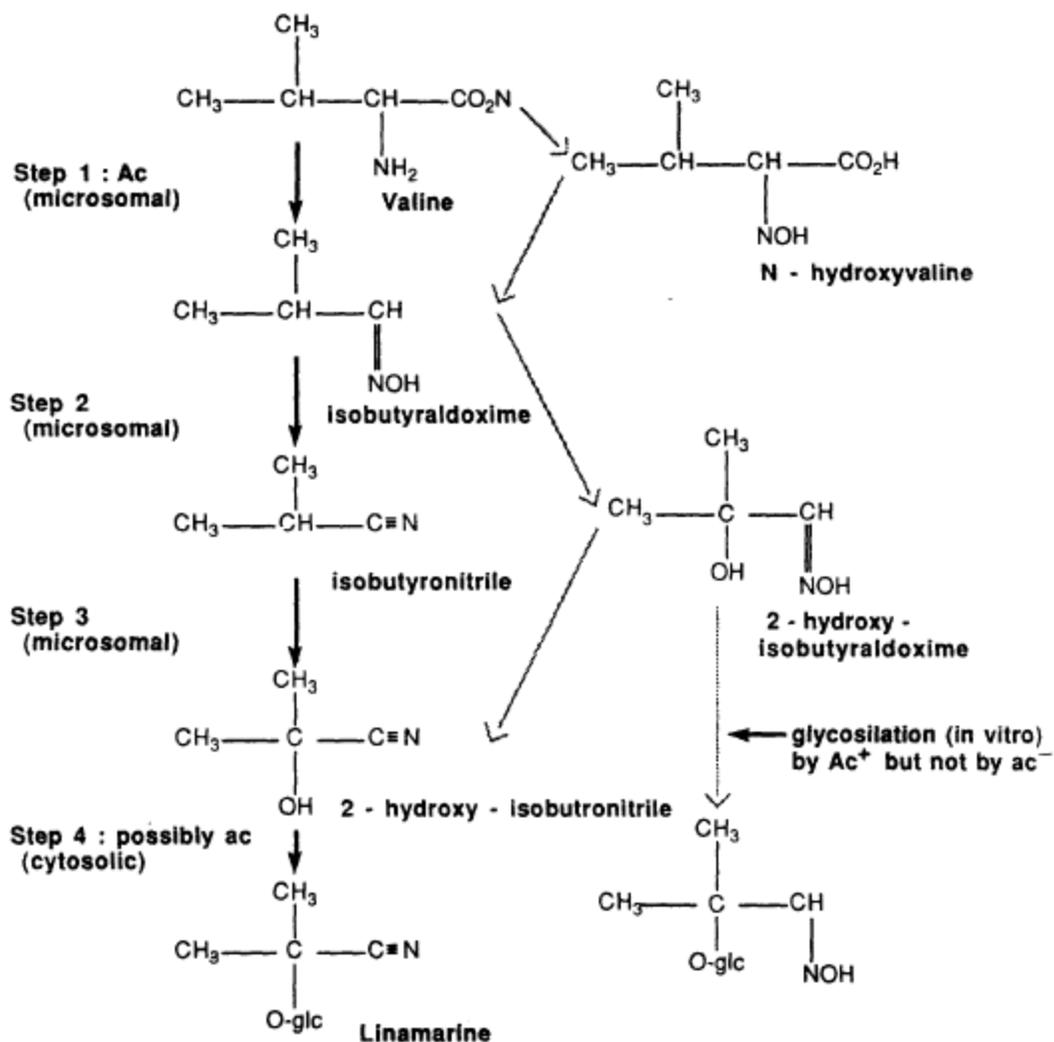


Figure 2-17. The Biosynthesis Pathway of Linamarin showing Valine as the Precursor (Kakes, 1990).

Cassava is also known to harbor a β -glucosidase called linamarase, EC 3.2.1.21 also known as linamarin β -D-glucoside glucohydrolase (Ikediobi et al., 1980; Cooke et al., 1978) and another catabolic enzyme, α -Hydroxynitrile lyase, EC 4.1.2.37 (HNL, acetone cyanohydrin lyase, or oxynitrilase) (Conn, 1994; Hughes et al., 1994; Conn, 1969). Linamarase is an enzyme that catalyzes the hydrolysis of the glucosides to ultimately liberate hydrogen cyanide. The cyanogenic glucoside, linamarin, is thought to

be synthesized in the leaves and petioles and accumulated in the vacuoles (Selmar, 1994; Kakes, 1990), where it is separated from the enzymes; Figure 2-18.

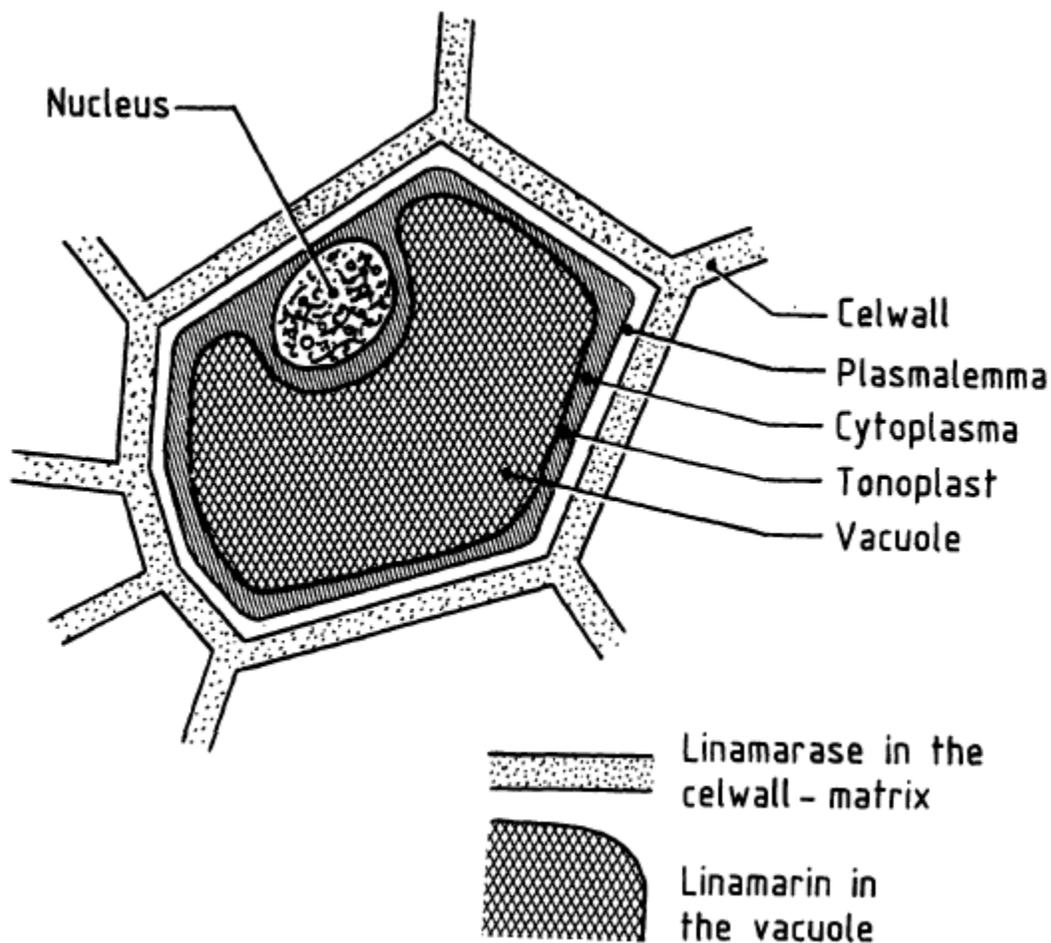


Figure 2-18. The Separation cum Distribution of Linamarin and Linamarase in the Plant System (Kakes, 1990)

The linamarin is thereafter translocated to other parts of the plant via the phloem. The peel of the root and the leaves are the main sinks for the cyanogenic glucosides (Koch et al., 1994; Nambisan and Sundaresan, 1994; Pereira et al., 1981; de Bruijn, 1973; Nartey, 1968). Bokanga (1994 a) noted that the leaves including the petioles

possess the highest cyanogenic potential in a plant. However, it has been argued that synthesis of linamarin does occur in the root also, and at rates comparable to those in the leaves (White et al., 1994). In intact plants, the cyanogenic compounds and the enzymes capable of hydrolyzing them are separated in different compartments, and shielded by the cell walls and other cell components; Figure 2-18. However, if the plant is physically injured or the cell wall becomes ruptured, the enzymes will make contact with the glucosides thereby facilitating hydrolysis of the later and the subsequent production of hydrogen cyanide. The mechanism of hydrolysis is demonstrated in Figure 2-19 and briefly explained here.

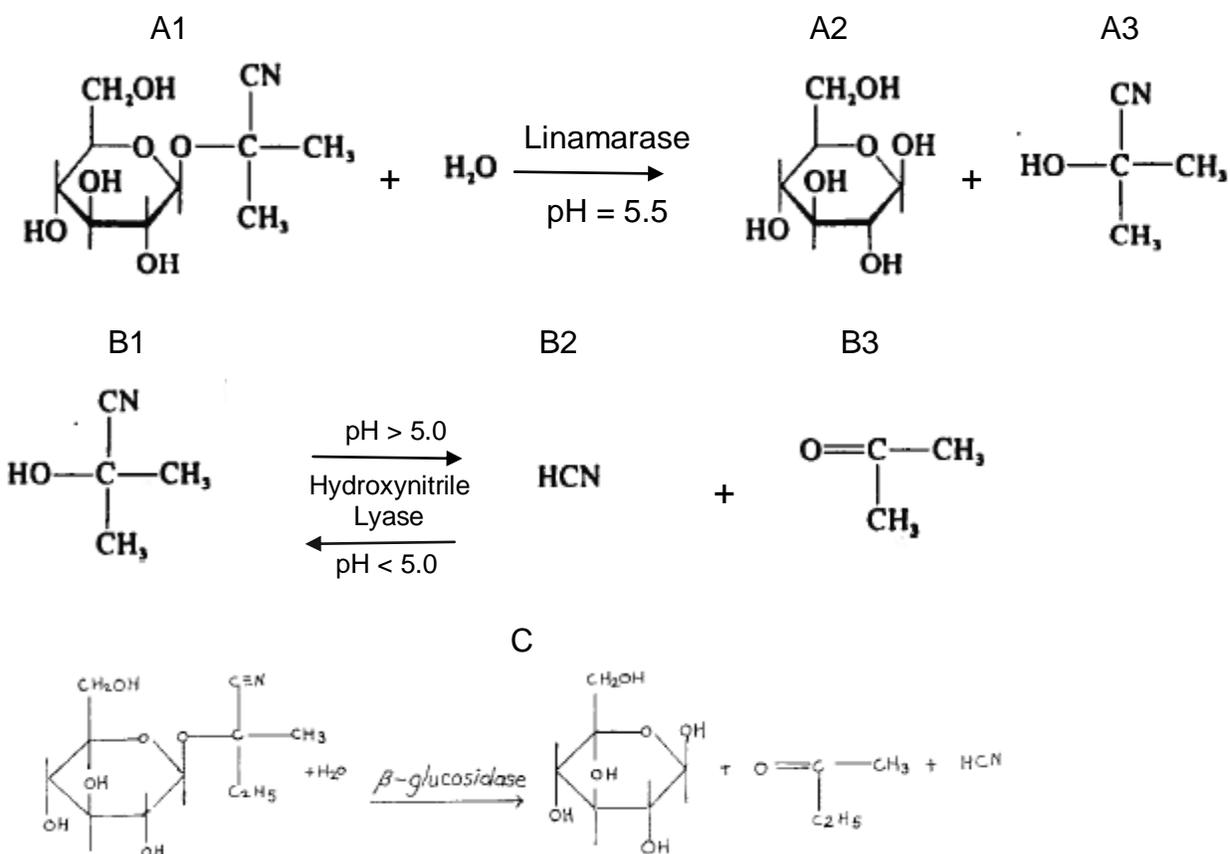


Figure 2-19. Mechanism of Hydrolysis of Cyanogenic Glucosides and Liberation of Hydrogen Cyanide (Conn, 1994; Ikediobi et al., 1980; Meuser and Smolnik, 1980; Cooke, 1978)

The process is a two step reaction. First, linamarin, shown in Figure 2-19 A1 is initially hydrolyzed by the β -glucosidase, linamarase, to form free glucose; Figure 2-19 A2 and the acetone cyanohydrin or 2-hydroxyisobutyronitrile identified by Figure 2-19 A3. This step is favored by pH of about 5.5 (Meuser and Smolnik, 1980). In the second step, if the pH is alkaline or greater than 5, or the temperature is greater than 35 ° C, acetone cyanohydrins will dissociate spontaneously into hydrogen cyanide (HCN); Figure 2-19 B2 (Dufour, 2007; Cooke, 1978) and acetone; Figure 2-19 B3 (Conn, 1994). Otherwise, the reaction can be catalyzed by the second catabolic enzyme, hydroxynitrile lyase to achieve the dissociation (White et al., 1998). Figure 2-19 C illustrates the discussed phenomenon as applied to the hydrolysis of lotaustralin (Ikediobi et al., 1980). Lotaustralin is the higher homologue of linamarin (Nartey, 1968).

Hydrogen cyanide (HCN) has a density of about 0.69 g/cm³; and the boiling and freezing points of about 26 ° C and -14 ° C respectively. These properties enable HCN to rapidly vaporize and dissipate at near room temperature. However, HCN is highly toxic and poisonous. It inhibits cellular oxidative processes and therefore hazardous to human and animal health if consumed. Because cassava roots and leaves are food and feed items, they must be processed to reduce the content of poisonous cyanogens [the cyanogenic glucosides and their hydrolysis products (cyanohydrins and free cyanide ions)] to safe levels in the final products before consumption. The Codex Alimentarius Commission requires that the total hydrocyanic content of edible cassava flour not exceed 10 mg/kg (FAO/WHO, 1995).

2.1.10 Cassava Detoxification

The prime goal of detoxifying cassava is to render it safe for consumption, whether as human food or animal feed. A number of processing techniques have been

used to achieve the detoxification of cassava products. Some of the methods used are dehydration, boiling, baking, steaming, frying, soaking, fermentation, storage, maceration, etc. (Nambisan, 1994; Oke, 1994; Balagopalan et al., 1988). These processing techniques can and have been used in combinations. In Table 2-9 are presented typical detoxification levels achieved with some of the methods. Among the more efficient processes reported are combined grating/pounding, fermentation/dewatering, followed by drying/roasting. These combinations have been reported to detoxify cyanogens in cassava by 80 % to 100 % (Nambisan, 2011; Mahungu et al., 1987).

Table 2-9. Detoxification of Cassava Components and Products by various Processing Methods

S/N	Cassava Component	Process Applied	Detoxification of Total Cyanogens Achieved [%]	Reference(s)
	Leaf and Leaf Products			
1		Pounded; then boiled for 30 minutes	96.3 – 99.6	Ngudi et al., 2003
2		Pounded for 15 minutes	63.0 – 73.0	Bokanga, 1994 a
3		Pounded for 15 minutes, then Boiled for 15 minutes	99.1 – 99.5	Bokanga, 1994 a
4		Chopped and then boiled for 15 minutes	85.5	Nambisan, 1994
5		Crushed or pounded and then boiled	97.0	Nambisan, 1994
6		Wilting at 30 ° C for 5 hours	63	Nambisan, 1994
7		Wilting at 30 ° C for 12 hours	76	Nambisan, 1994
8		Chopped and then dried at 70 ° C	30	Nambisan, 1994

Table 2-9 Continued. Detoxification of Cassava Components and Products by various Processing Methods

S/N	Cassava Component	Process Applied	Detoxification of Total Cyanogens Achieved [%]	Reference(s)
	Leaf and Leaf Products			
9		Kisamvu preparation: Pound fresh young leaves into small pieces	86.0	Gidamis et al., 1993
10		Kisamvu: Pound fresh young leaves into small pieces, boil for 30 to 45 minutes	95.7	Gidamis et al., 1993
11		Apical leaves are grated to a pulp	76.9 – 83.8	Dufour, 1989
12		Apical leaves are grated to a pulp, then cooked for 10 minutes	98.6 – 99.1	Dufour, 1989
13		Apical leaves are grated to a pulp, then cooked for 20 minutes	98.9 – 99.5	Dufour, 1989
14		Apical leaves are grated to a pulp, then cooked for 30 minutes	99.0 – 99.6	Dufour, 1989
15		Apical leaves are grated to a pulp, cooked for 30 minutes, then cooled to ready to eat	99.3 – 99.6	Dufour, 1989
16		Dried at 45 ° C for 24 hours	74.0 – 80.1	Padmaja, 1989
17		Dried at 60 ° C for 24 hours	78.7 – 85.9	Padmaja, 1989
18		Wilted at 30 ° C for 16 hours then Dried at 60 ° C for 24 hours	87.3 – 91.7	Padmaja, 1989
19		Wilted at 30 ° C for 16 hours, Chopped to 1 cm length and then Dried at 60 ° C for 24 hours	77.3 – 87.3	Padmaja, 1989
20		Dried at 75 ° C for 24 hours	64.2 – 84.4	Padmaja, 1989
21		Mpondu: Blanch, pound, cook	95.7	Mahungu et al., 1987
22		Dried leaves	3.7	Bourdoux et al., 1982
23		Mpondu preparation: Wash fresh leaves	6.9	Bourdoux et al., 1982
24		Wash fresh leaves, grind and boil for 15 minutes	94.6	Bourdoux et al., 1982

Table 2-9 Continued. Detoxification of Cassava Components and Products by various Processing Methods

S/N	Cassava Component	Process Applied	Detoxification of Total Cyanogens Achieved [%]	Reference(s)
Leaf and Leaf Products				
25		Mpondu: Wash fresh leaves, grind and boil for 30 minutes	98.3	Bourdoux et al., 1982
26		Mpondu: Blanch for 10 minutes, mash/pound into a pulp	62.0	Maduagwu and Umoh, 1982
27		Mpondu: Blanch for 10 minutes, mash/pound into a pulp, boil for 80 minutes	92.3	Maduagwu and Umoh, 1982
Root and Root Products				
28		Boiling 50 g pieces	25	Nambisan, 1994
29		Boiling 25 g pieces	50	Nambisan, 1994
30		Boiling 5 g pieces	75	Nambisan, 1994
31		Pieces are blanched (boiled for 5 to 10 minutes)	50.0	Nambisan, 1994
32		Peeled and boiled	69.8	Mahungu et al., 1987
33		Crushed and sun dried for 8 hours	96.8 – 98.5	Nambisan and Sundaresan, 1985
34		Sliced into 3 cm x 2 cm x 1 cm pieces and then boiled for 30 minutes	44.5 – 47.2	Nambisan and Sundaresan, 1985
35		Sliced into 3 cm x 2 cm x 1 cm pieces and then oven dried at 70 ° C for 24 hours	25.2 – 29.2	Nambisan and Sundaresan, 1985
36		Sliced into 3 cm x 2 cm x 1 cm pieces and then baked at 110 ° C for 20 minutes	12.9 – 15.0	Nambisan and Sundaresan, 1985
37		Sliced into 3 cm x 2 cm x 1 cm pieces and then steamed in a pressure cooker for 20 minutes	13.5 – 18.3	Nambisan and Sundaresan, 1985

Table 2-9 Continued. Detoxification of Cassava Components and Products by various Processing Methods

S/N	Cassava Component	Process Applied	Detoxification of Total Cyanogens Achieved [%]	Reference(s)
	Root and Root Products			
38		Sliced into 3 cm x 2 cm x 1 cm pieces and then fried in oil for 5 minutes	8.8 – 14.4	Nambisan and Sundaresan, 1985
39		Sun drying 10 mm thick chips	67.4 – 72.2	Nambisan and Sundaresan, 1985
40		Oven drying 10 mm thick chips at 50 ° C	49.5 – 53.6	Nambisan and Sundaresan, 1985
41		Oven drying 10 mm thick chips at 70 ° C	40.0 – 46.2	Nambisan and Sundaresan, 1985
42		Sun drying 3 mm thick chips	41.6 – 48.0	Nambisan and Sundaresan, 1985
43		Oven drying 3 mm thick chips at 50 ° C	35.8 – 40.0	Nambisan and Sundaresan, 1985
44		Oven drying 3 mm thick chips at 70 ° C	20.0 – 25.8	Nambisan and Sundaresan, 1985
45		Oven drying : Peeled chips (5 to 10 x 2 to 4 x 0.3 to 0.6) cm dimensions at 20 kg/m ² loading rate, at 60 ° C for 20 to 22 hours	72.0	Gomez et al., 1984
46		Oven drying : 1 cm thick peeled slices at 20 kg/m ² loading rate, at 60 ° C for 20 to 22 hours	49.0	Gomez et al., 1984
47		Oven drying : Irregular root peel pieces at 14 to 15 kg/m ² loading rate, at 60 ° C for 20 to 22 hours	64.0	Gomez et al., 1984
48		Sweet variety boiled for 20 minutes	87.9	Bourdoux et al., 1982

Table 2-9 Continued. Detoxification of Cassava Components and Products by various Processing Methods

S/N	Cassava Component	Process Applied	Detoxification of Total Cyanogens Achieved [%]	Reference(s)
	Root and Root Products			
49		Pulp: Freeze dried	51.2	Meuser and Smolnik, 1980
50		Pulp: Air dried at 40 ° C	98.5	Meuser and Smolnik, 1980
51		Pulp: Drum dried	99.1	Meuser and Smolnik, 1980
52		Fermented pulp: Drum dried	86.5	Meuser and Smolnik, 1980
53		Fermented pulp: Dried with heated air at 180 ° C	91.4	Meuser and Smolnik, 1980
54		Chips: Dried with heated air at 180 ° C	98.4	Meuser and Smolnik, 1980
55		Chips: Air dried at 40 ° C	98.5	Meuser and Smolnik, 1980
56		Slices: Flash dried	52.0	Meuser and Smolnik, 1980
57		Chips (40 mm x 8.2 mm x 6.8 mm) oven dried at 46.5 ° C for 18 hours	29 % of bound cyanide detoxified; 82.5 % of free cyanide detoxified	Cooke and Maduagwu, 1978
58		Chips (40 mm x 8.2 mm x 6.8 mm) oven dried at 60 ° C for 18 hours	26 % of bound cyanide detoxified; 83 % of free cyanide detoxified	Cooke and Maduagwu, 1978
59		Chips (40 mm x 8.2 mm x 6.8 mm) boiled for 25 minutes	55 % of bound cyanide and > 90 % of free cyanide detoxified	Cooke and Maduagwu, 1978
60		Chips (40 mm x 8.2 mm x 6.8 mm) soaked and stirred in cold (24 ° C) water for 18 hours	50 % of bound cyanide detoxified; 90 % of free cyanide detoxified after 4 hours	Cooke and Maduagwu, 1978
61		Storage: Roots packed in plastic bags, each bag stapled shut, bags then stored in the shade at ambient conditions for 72 hours.	9.93	Dufour, 1988

Table 2-9 Continued. Detoxification of Cassava Components and Products by various Processing Methods

S/N	Cassava Component	Process Applied	Detoxification of Total Cyanogens Achieved [%]	Reference(s)
	Root and Root Products			
62		Whole roots of sweet cassava stored in field clamps for two weeks	50.0	Booth et al., 1976
63		Whole roots of bitter cassava stored in field clamps for two weeks	Toxicity increased by 19.7 %	Booth et al., 1976
64	Attieke	Peel, grate, ferment, add oil and salt, dewater, sun dry	95.9	Mahungu et al., 1987
65	Casabe	Scrape, grate, wash to separate starch, ferment for 48 hours, dewater, cook/toast	92.4 – 98.5	Dufour, 2007; 1989
66		Scrape, grate, wash to separate starch, ferment for 24 hours, dewater, cook/toast	86.8 – 96.8	Dufour, 1989
67		Scrape, grate, cook/toast; obtain fresh casabe	85.4 – 95.2	Dufour, 1989
68	Baton de Manioc	Peel, cut into cubes 20 mm to 40 mm size, remove fibers, soak/ferment for 2 days, dewater for 1 hour, pound/grind to fine paste, wrap in leaves and boil for 20 to 30 minutes	99.3 – 100 (Mean = 99.6)	O'Brien et al., 1992
69	Chikwangue	Peel, ferment (3 days), sieve, steam (45 minutes), knead, boil (1 to 2 hours)	100	Mahungu et al., 1987
70	Chinyanya	Peel, slice and pound to small pieces, sun-dry for 2 to 3 hours, re-pound, sun-dry for 2 to 3 hours, sieve, obtain final flour	82.2 – 86.6	Mlingi et al., 1995; Mlingi and Bainbridge, 1994
71	Eba: Derived from gari	Gari (stored for 4 months), soak in boiling water (2 to 5 minutes), knead into soft paste	99.8	Mahungu et al., 1987
72	Farina	Soak whole roots for 2 to 4 days, peel, grate, ferment for ≥ 3 days, dewater, sift, toast	99.2	Dufour, 1989
73	Farine de Manioc	Peel, cut into 60 mm to 90 mm thick chunks, soak/ferment for 2 days, crush and dewater for 24 hours, sun-dry, pound into flour, boil flour to form thick porridge	91.3 – 99.9 (Mean = 94.4)	O'Brien et al., 1992

Table 2-9 Continued. Detoxification of Cassava Components and Products by various Processing Methods

S/N	Cassava Component	Process Applied	Detoxification of Total Cyanogens Achieved [%]	Reference(s)
	Root and Root Products			
74	Farinha d'agua	Peel, soak, grate, ferment, dewater, toast	99.0	Dufour, 2007
75	Flour preparation	Soak flour 1 : 1.25 ratio (flour : water), spread the mixture out in a thin layer, heat in the oven at 30 ° C for 5 hours	83.3	Cumbana et al., 2007
76		Soak/Wet flour at 30 ° C for 5 hours	55	Bradbury, 2006
77	Flour production	Peel, cut into 4 cm x 4 cm dimensions, sun-dry for 4 days, mill into flour	27.8	Muzondo and Zvauya, 1995
78		Reconstitute flour at 30 % to 35 % moisture content, steam for 20 minutes, cool to room temperature, oven dry for 2 days at 60 ° C; obtain "gelatinized cassava flour"	35.6	Muzondo and Zvauya, 1995
79		Ferment gelatinized cassava flour at 40 ° C with formulated media for 50 hours, obtain "fermented cassava flour"	64.6	Muzondo and Zvauya, 1995
80		Peel; heap, cover and incubate for 3 days; remove mold; crush; sun-dry; pound; obtain flour	82.7 – 98.5	Essers, 1994
81	Fufu	Ghana style: Peel, boil, pound	80.4	Mahungu et al., 1987
82		Nigeria style: Peel, soak (1 day), grate, dehydrate, steam, pound	95.4	Mahungu et al., 1987
83		Zaire style: Peel, ferment (3 days), sun dry, mill, cook	99.8	Mahungu et al., 1987
84		Preparation: Soak whole roots for 3 days	82.6	Bourdoux et al., 1982
85		Peel Soaked roots, break into small fragments and sundry for 3 days	85.9	Bourdoux et al., 1982
86		Grind sun dried fragments into Flour (uncooked fufu)	97.8	Bourdoux et al., 1982

Table 2-9 Continued. Detoxification of Cassava Components and Products by various Processing Methods

S/N	Cassava Component	Process Applied	Detoxification of Total Cyanogens Achieved [%]	Reference(s)
	Root and Root Products			
87	Fufu	Soak whole roots for 3 days, Peel soaked roots, break into small fragments and sundry for 3 days, Grind sun dried fragments into flour, Cook the flour in boiling water to form firm paste, (obtain fufu)	98.7	Bourdoux et al., 1982
88	Fuku	Peel, cut into small pieces, dry (2 days), grind into flour (uncooked fuku)	83.6	Bourdoux et al., 1982
89		Peel, cut into small pieces, dry (2 days), grind into flour, cook flour into a gruel (cooked fuku)	95.3	Bourdoux et al., 1982
90	Gari/gari production	Peel, grate, add water at 75 % (v/w); heat at 50 ° C for 6 hours	> 99 %	Sokari, 1992
91		Peel, grate, dehydrate, ferment, fry	83.2	Mahungu et al., 1987
92		Peel, grate, dehydrate, ferment, fry, store (4 months)	98.1	Mahungu et al., 1987
93		Peel, grate, dewater with screw press for 24 hours, garify (roast)	99.0 – 99.6	Estimated from Maduagwu & Oben, 1981
94		Peel, grate, dewater with traditional press (weights of heavy stones) for 72 hours, garify (roast)	93.5 – 97.9	Estimated from Maduagwu & Oben, 1981
95		Peel, grate, ferment (48 hours), press (48 hours), roast	71.3	Ketiku et al., 1978
96	Ijapu	Peel, boil for 25 minutes, slice, soak slices in cold water for 24 hours, rinse	84.5 – 95.3	Sokari, 1992
97	Konkonte	Cote d'Ivoire style: Dry chips (unfermented), mill, cook	95.4	Mahungu et al., 1987
98	Lafun	Peeling, soaking, fermenting, mashing, drying	88.1	Ketiku et al., 1978
99	Makopa; normal size	Peel, split longitudinally, sun-dry on rock/platform for 8 days, pound, obtain flour	45.7 – 70.7	Mlingi et al., 1995; Mlingi and Bainbridge, 1994

Table 2-9 Continued. Detoxification of Cassava Components and Products by various Processing Methods

S/N	Cassava Component	Process Applied	Detoxification of Total Cyanogens Achieved [%]	Reference(s)
	Root and Root Products			
100	Makopa; normal size	Peel, split longitudinally, sun-dry on rock/platform for 17 days, pound, obtain flour	63.2 – 73.1	Mlingi and Bainbridge, 1994
101	Manicuera	Boiled cassava starch juice beverage	98.6 – 99.6	Dufour, 1989
102	Ntuka	Zaire style: Peel, ferment (3 days), steam (2 hours)	99.8	Mahungu et al., 1987
103	Oyoko	Zaire style: Peel, grate, mix with fermenting pulp (3:1), steam	90.8	Mahungu et al., 1987
104	Plakali	Cote d'Ivoire style: Peel, grate, mix with fermenting pulp (3:1), sun dry, pound, cook	96.6	Mahungu et al., 1987
105	Ugali wa mhogo (from peeled root)	Air Drying Method: Slice peeled roots into 30 mm to 50 mm thick pieces, oven dry at 40 ° C for 3 days	93.7	Gidamis et al., 1993
106		Pound dried pieces and sieve into flour	95.1	Gidamis et al., 1993
107		Prepare Ugali wa mhogo by boiling 1 : 2 (flour : water) ratio and stirring	95.3	Gidamis et al., 1993
108	Ugali wa mhogo (from unpeeled root)	Soaking Method: Soak unpeeled root in water at 30 ° C for 8 days,	62.7	Gidamis et al., 1993
109		Slice soaked roots and oven dry at 40 ° C for 3 days	96.4	Gidamis et al., 1993
110		Pound dried pieces and sieve into flour	96.8	Gidamis et al., 1993
111		Prepare Ugali wa mhogo by boiling 1 : 2 (flour : water) ratio and stirring	98.3	Gidamis et al., 1993
112	Animal Feed	Silage: Unpeeled roots were washed and cut into chips. Chips were packed into wooden silos lined with sheet metal and plastic. Silos were covered with thick plastic sheet and made airtight with wood shavings. Silos were maintained in shaded area and ensiled for 26 weeks	64 – 74.8	Gomez and Valdivieso, 1988

Cardoso et al., (2005), proposed equations that may be used to relate the initial cyanide content of cassava to the percent cyanide retention after processing. Based on their analyses, it may be possible to estimate the initial maximum total cyanide content for a specific processing method to achieve the 10 ppm cyanide safety standard of WHO. Consequently, the more efficient processing methods such as grating/pounding, fermentation/dewatering, that are used to produce farinha and gari should start with cassava having initial maximum total cyanide content of about 222 ppm. On the other hand, to achieve the same safety margin (10 ppm cyanide in the final product), the less efficient process such as frying or the sun drying used for flour production should start with cassava having initial maximum total cyanide content of about 33 ppm (Cardoso et al., 2005). Below is a brief discussion on cassava food systems from around the world.

2.1.11 Cassava Food Products

A wide range of cassava food products have been developed in different parts of the world. Procedures for these food systems may vary from country to country; and even between different regions of the same country. Cassava food systems may be fermented; grated; dried; fried; boiled; or subjected to various other unit operations or combinations thereof. Among food products of cassava prepared and consumed around the world are: akpakpuru, attieke, casabe, chickwangue, farina, fou-fou, fuku, gapek, gari, ijapu, konkonte, kpokpo gari, lafun, landang (cassava rice), mpondu, ntuka, peujeum or tapai, puttu, tapioca, thundam, etc. In many products, grating, fermenting drying etc. minimize hydrocyanic acid concentration (Mahungu et al., 1987; Ketiku et al., 1978). Some of the mentioned cassava food systems are briefly highlighted.

2.1.11.1 Akpakpuru

In the Rivers State of Nigeria and various states in Eastern and Southern Nigeria, a dumpling-type fermented cassava product is produced and consumed both in the rural areas and in the cities. The dumplings have acquired personality of sorts. They are sold as 10 – 12 cm balls wrapped with polyethylene sheets in city streets and touted by different names such as “loiloi” and “packet shirt”. Details of preparation vary from locality to locality. The Ikwerre people of Rivers State of Nigeria call both cassava crop and this product the same name: Akpakpuru. The traditional preparation method is as follows. Whole cassava roots are soaked in 90 – 100 cm diameter 10 – 25 cm deep dug-out pits or fence protected areas at the edges of tidal streams for five or more days. Fermentation occurs during this soaking period, softening the roots parenchyma considerably. Root peels and central fibers are removed; the soft parenchymatous pulp is crumbled and sieved into jute sacks with raffia basket mesh. Mass of sieved pulp is dewatered, wrung dried and stored in the sacks until required for consumption.

Akpakpuru is prepared for consumption by molding the pulp into 10 – 12 cm diameter balls, boiling for 10 – 15 minutes and pounding the balls into dough with pestle and mortar. The boiling and pounding is repeated a couple of times or until the dough is well cooked. Akpakpuru is then eaten as dumpling when served with delicately prepared stews and soups rich in vegetables, game meat, and sea foods such as clams, crabs, oysters, periwinkles and shrimps.

2.1.11.2 Attieke

Attieke is a grated and fermented product in Cote d’Ivoire (Ross, 2012; Balagopalan et al., 1988; Mahungu et al., 1987; Lancaster et al., 1982). It is produced through laborious and time consuming manual unit operations which include peeling,

washing, grating, fermenting, dewatering, sieving, granulating, drying and steaming. Cassava roots are peeled, washed and grated or mashed. A starter culture of 5 – 10 % of the mass of mash is added to facilitate fermentation. About 1 % of palm oil and some salt are also added after which fermentation is allowed to proceed for fifteen hours. The fermented mass is dewatered, sieved and granulated. The granules are sun dried briefly for about an hour and then gelatinized by steaming over a boiling pot of water for thirty minutes. While steaming, the granules are stirred with a spatula to prevent them from sticking together. The fermentation process is reported to impart a slightly sour taste to the finished product (Ross, 2012; Balagopalan et al., 1988; Lancaster et al., 1982). Attieke can be prepared for eating like couscous. Mix equal parts of attieke and water (or broth of choice) in a saucepan and stand for ten minutes. Attieke will absorb the fluid and swell. After the ten minutes wait add salt, butter and desired spices and herbs. Stir and steam cook for ten minutes or until desired tenderness. Like couscous, Attieke may be served and eaten with stewed meats such as beef, fish, pork and poultry; and with vegetables and milk. Attieke can also be used as replacement of grains in various dishes (Ross, 2012).

2.1.11.3 Casabe

Casabe is a flat, cracker-like bread that is made from cassava root. It is a traditional food of the Amazonians, and its preparation can range from simple to elaborate. In the simplest form, fresh cassava roots are peeled and grated into a mash. The mash is subsequently dewatered and placed on hot clay or stone griddle, pressed down into a thin layer and toasted on each side (Figure 2-20). The resulting flat circular cake is the cassava bread known as casabe (Lancaster et al., 1982; Seigler and

Pereira, 1981). A more elaborate method of preparing casabe was described in the literature (Dufour, 2007; 1994; 1989).



Figure 2-20. Production and Toasting of Casabe on a Wood-Fired Griddle (Wikipedia, 2013 B).

2.1.11.4 Chickwangué

Chickwangué is a fermented cassava food product. Variations of it are prepared and consumed in many parts of Africa, including Zaire, Sudan, Gabon, Congo, Central African Republic, Cameroon, and Angola. It has been reported to be the most popular processed food from cassava in Zaire (Hahn, 1992). Basically, the cassava root is fermented in water for 3 – 5 days. Peeling of the root may be effected before or after the fermentation process. Following fermentation, the soft pulp is recovered, washed clean and pounded into a thick paste. The paste is subsequently securely wrapped and tied in leaves of *Megaphrynium macrostachyum*, banana, plantain, or leaves of other plant of *Marantaceae* or *Zingiberaceae* family (Congocookbook.com, 2013; Hahn, 1992). The wrapped paste is then steamed for several hours to near consistency of modeling clay. Viola, chickwangué is done. Sizes of the product vary. In Central Africa,

two sizes of chickwangué wraps are common: 2.54 – 5.08 cm diameter by 30.48 cm long and 10.16 cm diameter by 30.48 cm long (Congocookbook.com, 2013). In Cameroon, “Myondo” and “Bobolo” are cassava products that belong to chickwangué group. Myondo is 1.5 – 2.0 cm in diameter by 15.0 – 20.0 cm long; while Bobolo is 2.0 – 4.0 cm in diameter by 30.0 – 40.0 cm long (Hahn, 1992). Chickwangué and its group of products may be served with soup, stew, or any sauce dish (Congocookbook.com, 2013).

2.1.11.5 Farina

Farina is a popular method of preparing cassava in tropical America. It is prepared in the form of a meal or granules and also called farinha de mandioca in Brazil (Lages and Tannenbaum, 1978). In the preparation of farina, fresh roots are peeled, grated, dewatered and sifted to obtain an even textured mash. The mash is roasted on a griddle with continuous stirring until cooked to less than 10 % moisture content (Oke, 1994). In traditional Amazonia, the grater board, tipiti (a basket work sleeve press), and clay griddle are used respectively for grating/rasping the root, dewatering and roasting the mash (Dufour, 1994). In some preparation styles, the cassava root is soaked or fermented in water for some days before processing (Balagopalan et al., 1988).

2.1.11.6 Fufu

Cassava fufu may be processed in a variety of ways. In one production style, cassava roots are peeled, washed and grated. The grated mash is packed into sacks, weighted down with heavy objects to squeeze out contained juice, and allowed to ferment for a few days. The resulting dough is used to produce fufu (Lancaster et al., 1992). In Ghana, the fufu is said to be prepared by adding quantities of the dough into boiling water and stirring until desired consistency is achieved (Doku, 1969). In another

production method, the peeled cassava roots are first soaked in water and allowed to ferment for several days. The soft root pulp is mashed, sieved in a basin of water, and allowed to sediment or settle. The sediment paste is thinned down in a pot and cooked on low fire with constant stirring until desired consistency is achieved (Ayankunbi et al., 1991)

2.1.11.7 Fuku

Fuku is a cassava root product consumed in Zaire. Cassava roots are peeled, cut into pieces and dried in the sun. Dried chips are pounded into flour and partially fermented with corn. Fermentation is stopped by grilling, and the fuku flour is consumed as a meal gruel prepared in boiling water (Bourdoux et al., 1983).

2.1.11.8 Gaplek

Gaplek is a popular traditional famine protector cassava food product in Java and other parts of Indonesia. Fresh cassava roots are peeled and sliced into 15.24 – 20.32 cm pieces. The pieces are dried in the sun for 1 – 3 or more days depending on weather conditions. The dried pieces are stored in a cool and dry place as vanguard against hunger. When other sources of food become scarce or expensive, gaplek is retrieved from storage, pounded into smaller pieces and cooked and consumed like rice.

2.1.11.9 Gari

Gari is a popular cassava food product consumed extensively in many parts of West Africa including Benin, Ghana, Guinea, Nigeria, and Togo. In these countries, gari may be consumed as a snack in various forms or eaten as the main dish. It is eaten plain just as it is; eaten with groundnuts, coconuts, or palm kernel; eaten soaked in cold water with or without the addition of milk and sugar; or eaten as eba in combination of

soups of fish, beef, game meat, poultry, vegetables, etc. Eba is derived when gari is soaked in hot water and kneaded. The consistency of eba ranges from that of a soft paste to jell to modeling clay. The preparation of gari is similar to that of farina, but requires more elaborate equipment, processes and time. The basic unit operations for gari production are washing, peeling, grating, dewatering/fermenting, sifting, and then garification; which consists of roasting/frying (Aso, 2004; Collard and Levi, 1959). Fresh cassava roots are grated or ground into a mash after washing and peeling operations. The mash is packed into a jute or polypropylene sack and wrung dried/fermented for a few days. The clumped cake is crumbled and sifted through sieves. The sifted mass is then garified (roasted/fried) in shallow iron pots to produce gari. Palm oil may be added during fermentation, and or during garification stage to improve nutritional (Vitamin A) content and minimize sticking to the iron pots. After cooling, the gari may be sieved to obtain homogenous granules.

2.1.11.10 Konkonte

Konkonte is a cassava root product. It is consumed in Cote d'Ivoire, Ghana, and the Caribbean. It is often regarded as poverty food for low income earners. Konkonte is fairly easy to prepare. Fresh cassava roots are peeled, sliced into chips and dried in the sun. Dried chips are milled into flour and consumed as a gruel or runny porridge, being enjoyed with a specially prepared soup.

2.1.11.11 Lafun

For "lafun" production in the Western States of Nigeria, peeled or unpeeled cassava roots are immersed in a stream, in stationary water (near a stream) or in an earthenware vessel, and fermented until the roots become soft. The peel and central fibers of the fermented roots are manually removed and the recovered pulp is hand

mashed or pounded and sun dried. The dried mash is then milled into lafun flour (Oke, 1994; Hahn, 1992). To prepare the lafun, an estimated amount of lafun flour is mixed with an estimated amount of boiling water. The mixture is allowed to cook while stirring continuously to prevent clumps and burning, until the desired consistency is achieved (Ayankunbi et al., 1991). A similar product is reported to be processed in Angola where the dried pulp is called bombo or makessu, and the ground flour is referred to as fuba (Alberto, 1958).

2.1.11.12 Landang (cassava rice)

In the Philippines, a popular dish known as landing or cassava rice is produced by first peeling and grating cassava roots. The grated mass is loaded into jute sacks and the juice squeezed out with applied weight. The mass is then winnowed into small pellets with a basket, dried, steamed, and dried again (Lancaster et al., 1982). Landang can be eaten as is or it may be cooked and eaten like rice or maize.

2.1.11.13 Peujeum or Tapai

Peujeum is a traditional cassava food product in Java. However, it is wide spread in Asia where it is known by various names including tapai ketela in Indonesia; tapai ubi kayu in Malaysia; and binuburang ube or tapay panggi in the Philippines. Peeled fresh cassava roots are steamed to tenderness, cooled to 30 ° C and dusted with a special mixture of flour and spices known as ragi. The dusted root is layered in a basket or wrapped in banana leaves in an earthenware pot and allowed to ferment for a couple of days. The peujeum may be eaten as is or baked before consumption.

2.1.11.14 Puttu

In Sothern India, boiled cassava roots are grated and mixed with shredded coconut. The product is called puttu and is often served with cardamom seed. Grated

roots may be minced, mixed with fried onions, cashew nuts, black gram and coriander leaves. The mixture is then molded into balls, dusted with Bengal gram flour and fried. This product is then called pakkoda (Lancaster et al., 1982). Also, parboiled cassava pieces could be mixed with fat, ginger, coriander and curry leaves, mustard seeds, Bengal dhal and black gram dhal to produce uppuma (Balagopalan et al., 1988).

2.1.11.15 Thundam

In Southern India, thundam is prepared by removing the whole peel from cassava roots, slicing and drying the peeled roots. Dried chips may be boiled or fried for consumption. Alternatively, chips are converted to flour for a variety of uses.

2.1.11.16 Other Cassava food and beverage products

A variety of nonconventional food systems have been produced from cassava. These include cassava rava, porridge, pappads, and wafers (Nanda et al, 2002). Preparations from cassava flour include dumplings, cakes, and fritters. Similarly several beverages have been produced from cassava. Alcoholic drinks include beers, banu or uala, kasili, yaraqui, amoiuare, paya, yakari, tapan, malicha, paiwarri (Balagopalan et al., 1988; Lancaster et al., 1982). Lancaster et al., (1982) reported that the Karinya people produce a non-alcoholic beverage called karato from cassava. Dufour (1989, 1988), noted that Tukanoan Indians in Northwest Amazonia also process bitter cassava into a boiled cassava juice known as “manicuera” and a starch drink called “mingao”.

Mowat (1989), described Chicha as the generic name applied to native beer and other beverages in South America. Chicha may be derived from sweet or bitter cassava, maize, sugar cane, and various fruits. Among the beverages made from cassava by the Amazonian Indians are the non alcoholic drinks like boiled juice that was expressed from grated cassava root with the tipiti; and boiled cassava starch. Beers and other

fermented alcoholic beverages from cassava include bernia, kachiri, kurai, masato, paiwarri and puchokwa (Mowat, 1989).

2.2 Part 2: Production of Amylolytic Enzymes

2.2.1 Background

The development of enzymatic processes and the market for enzymes is growing because the use of enzymes has numerous advantages. These include cheaper production; great specificity and enhanced rate reactions; application to various current and frontier fields like agriculture, chemicals, clean energy, food, pharmaceuticals, clinical, medicinal and analytical chemistries, materials, textile, brewing and distilling industries; the potential to address issues of health, raw materials, sustainability and environment; as well as the possibility to benefit from use of genetics knowledge to produce enzymes specifically tailored for special applications (Beilen and Li, 2002; Pandey et al., 2000 b). The study of amylolytic enzymes is of great importance for the improvement of industrial processes (Gupta et al., 2003), treatment of waste waters and animal nutrition applications (Buendia et al., 2003), among others. There are many sources of amylolytic enzymes. Perhaps the most important source for industrial processes is microbial; especially from some strains of bacteria and fungi (Tomasik and Horton, 2012; Sousa and Magalhães, 2010; Uhlig, 1998; Srivastava and Baruah, 1986; Tani et al., 1986; Pestana and Castillo, 1985; Michelena and Castillo, 1984; Forgarty, 1983).

Glucoamylase and α -amylase are starch converting enzymes used for the production of a wide range of products such as dextrans, modified starches, glucose syrups, organic acids, flavor and aroma compounds, mushrooms and ethanol from

starch degradation (Couto and Sanroman, 2006; Gupta et al., 2003; Stamford et al., 2002; Pandey et al., 2000 a; Labeille et al., 1997). Glucoamylase or amyloglucosidase (EC 3.2.1.3), is an exo-amylase that produces glucose as final product from starch, hydrolyzing glucose units from the non-reducing ends (α -1,4 and α -1,6 linkages) of amylose and amylopectin (Tomasik and Horton, 2012; Anto et al., 2006; Ellaiah et al., 2002; Uhlig, 1998; James and Lee, 1997; Roberts et al., 1995). On the other hand, α -amylase (EC 3.2.1.1) catalyzes the hydrolysis of internal α -1,4-O-glycosidic bonds in polysaccharides. Indeed α -amylase is the most reported endo-amylase that produces oligodextrins as main product (Castro et al., 2011). Figure 2-21 is a photo image of Alpha-Amylase.

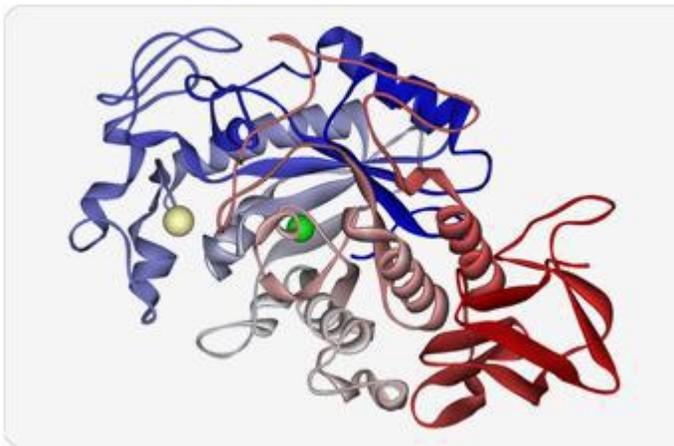


Figure 2-21. Three Dimensional Image of Alpha-Amylase (bing.com, 2013 b; Mobini-Dehkordi and Javan, 2012)

Some of the enzymes that are available for industrial applications do not withstand the extreme processes and conditions in the industries. This has led to the search for extremophile microorganisms that survive non-standard conditions. It is presumed that the enzymes from these microbes can be adapted and optimized for

extreme environmental factors (Atomi, 2005; Van Den Burg, 2003; Demirjian et al., 2001; Hough and Danson, 1999; Niehaus et al., 1999).

The production of exogenous enzymes has been studied using: solid state fermentation (Hashemi, et al., 2011; Murado et al., 1997); submerged fermentation (Aguilar et al., 2008; Amoozegar et al., 2003; Aguilar et al., 2001; Soccol et al., 1994); encapsulated strains (Konsoula and Liakopoulou-Kyriakides, 2006 a; 2006 b); and with the advances in genetics, using recombinant microorganisms (Rasiah and Rehm, 2009; Murakami et al., 2007; Hashim et al., 2005; Lin et al., 2003; Bruins et al., 2001; Lo et al., 2001).

This review highlights fermentation processes and microorganisms used for the production of amylolytic enzymes, as well as the process variables.

2.2.2 Amylolytic Enzymes Production by Solid-State Fermentation

Solid-State Fermentation (SSF) may be defined as a fermentation process that occurs on solid matrix in virtual absence of free water. The solid matrix or substrate provides physical support and may contain sufficient moisture in absorbed form to supply nutrients (Couto and Sanroman, 2006; Pandey, 1992). SSF technology is used for many applications in Asia. Examples include the production of soy sauce, miso and koji. Koji is made from rice with *Aspergillus oryzae* and is used as a starter culture for processing several food products (Hölker and Lenz, 2005). Durand (2003) described SSF as an “ancient art” that is becoming a modern technology. The process is suitable for growth of fungi which are the microorganisms commonly used (Aguilar et al., 2008; Viniegra-González et al., 2003). SSF characteristics are similar to fungi’s natural environment, thus increasing the fungi activity (Barrios-Gonzalez, 2012; Couto and Sanroman, 2006; Hölker and Lenz, 2005; Pandey, 2003; Castilho et al., 2000). Fungi

are also useful in soil bioremediation, enzyme production and as an alternative source of animal feed.

The advantages and limitations of solid state fermentation are presented in Table 2-10 (Aguilar et al., 2008; Couto and Sanroman, 2006; Viniegra-González et al., 2003).

Table 2-10. Advantages and Disadvantages of Solid State Fermentation (SSF)

S/N	Advantages	S/N	Disadvantages
1	Simple Technology: Substrate can serve directly as medium or enriched with nutrients.	1	Process control constraints: humidity, pH, heat, CO ₂ , etc. are difficult to manage.
2	Concentrated product; requiring less downstream processing.	2	Reduced variety of inoculums due to low water activity environments
3	Generated enzymes have low sensitivity to catabolic repression or induction	3	Process Scale-up difficulties
4	Medium is similar to natural habitat of inoculums		
5	Low risk of microbial contamination		
6	Low waste generation		
7	Reduced operational problems		
8	Reduced energy requirements		
9	Reduced water requirements		
10	Low cost media		
11	Low cost operations		
12	Higher productivity (Biomass, Protein retention, Enzyme yield, etc.)		
13	Good circulation		

The process is relatively simple; utilizes marginal inputs; and has low energy requirements and production costs. SSF processes can proceed at water activities of 0.935 – 0.980 (Ruijter et al., 2004; Ishida et al., 2000; Grajek & Gervais, 1987 a, 1987 b) and therefore, produces less waste (Anto et al., 2006; Hölker and Lenz, 2005). In some cases the fermented product is also used as an enzyme source for other hydrolytic processes (Hölker and Lenz, 2005).

Microorganisms used for SSF and the sources from which they are isolated are very diverse. Bacteria may be isolated from hot springs (Sodhi et al., 2005); Cow dung (Swain and Ray, 2009; 2007); or from soils (Kar et al., 2010; Oyeleke and Oduwole, 2009; Kumar and Satyanarayana, 2004; Srivastava and Baruah, 1986). Fungi isolated from soils and seeds are also used (Saxena and Singh, 2011; Varalakshmi et al., 2009; Alva et al., 2007; Anto et al., 2006). In the case of amylolytic enzymes production, the diversity is useful for a variety of applications such as the creation of detergents, bakery products, sugar syrups, brewery products, etc. In SSF, the solid material used can be the source of nutrients for the microorganism and or provide physical support to the system for immobilization. When an inert material such as polyurethane foam, acroleite or a resin is used as SSF support structure, the extraction and purification of fermentation products become less costly and cumbersome than when agro-industrial wastes serve as fermentation matrix (Pandey, 2003; Murado et al., 1997).

Important variables that require control during SSF include physicochemical and biochemical parameters. One-variable-at-a-time approach has often been used to study fermentation processes for enzyme production. However, this kind of approach tends to overlook or cloud interactions between factors and responses that are revealed when

adequate experimental designs and response surface methodologies are adopted (Deepak et al., 2008; Kumar and Satyanarayana, 2004). Some of the SSF control variables are discussed below and include:

- Substrate Support Structure
- Carbon Supplementation
- Nitrogen Supplementation
- Porosity and Aeration
- Moisture Content and Water Activity
- Incubation Temperature
- Inoculums Concentration
- Fermentation Time and pH

2.2.2.1 Substrates support structures

Agricultural, biological and industrial materials, products and wastes have been used as physical support structures in SSF processes with various microorganisms including species of bacteria: *Bacillus*, *Clostridium*, *Streptomyces*; yeast: *Kluyveromyces*, *Saccharomycopsis*, *Zygosaccharomyces*; and many fungi: *Aspergillus*, *Ceratocystis*, *Neurospora*, *Penicillium*, *Rhizopus*, and *Thermomyces*. Products generated include amylases, pectinases, proteases, flavor compounds, etc. The gamut of substrate covers seeds, grains, tubers, leaves, as well as industrial by-products that are used in different formats. For example, cassava has been used and studied in the form of starch, flour, bagasse, peel, gelatinized, and fibrous residue; maize and corn in the format of steep liquor, flour, crushed, and as corn cobs; mustard as seed cake; oat as flour; plantain as peel; potato as flour, starch and solid waste; coffee as husk; rice as bran, flake, flour/powder, grains, hull, and gelatinized; soybean as meal and seeds; sugar beet as pulp; sugarcane as bagasse; sun flour as seeds; while wheat has been utilized in the form of bran, crushed seeds, flakes, flour; and yam as peel. Table 2-11 highlights these substrates, the organisms used as well as the products generated.

Table 2-11. Substrates used in Microbial Fermentations, Organisms Employed and the Products Generated

S/N	Substrate	Organism Employed	Product Generated	Reference(s)
1	Amaranth Grain	<i>Rhizopus oryzae</i>	Flavor (Aroma Compound)	Christen et al., 2000
		<i>Rhizopus oryzae</i> ATCC 34612	Flavor (Aroma Compound)	Bramorski et al., 1998
2	Apple Pomace	<i>Rhizopus oryzae</i>	Flavor (Aroma Compound)	Christen et al., 2000
		<i>Rhizopus oryzae</i> ATCC 34612	Flavor (Aroma Compound)	Bramorski et al., 1998
3	Barley Bran	<i>Thermomyces lanuginosus</i>	Amylase Enzyme	Kunamneni et al., 2005
4	Cassava Bagasse	<i>Aspergillus oryzae</i> TISTR 3605	Alpha Amylase	Pengthamkeerati et al., 2012;
		<i>Kluyveromyces marxianus</i>	Flavor (Aroma Compound)	Medeiros et al., 2001
		<i>Rhizopus oryzae</i>	Flavor (Aroma Compound)	Christen et al., 2000
		<i>A. niger</i> LPB 21; <i>A. niger</i> NRRL 2001; <i>A. niger</i> CFTRI 30; <i>Ceratocystis fimbriata</i> ; <i>K. marxianus</i> ; <i>L. edodes</i> ; <i>P. sajor-caju</i> ; <i>Rhizopus sp.</i> ; <i>R. oryzae</i>	Aroma Compounds; Mushrooms; Organic Acids; Glucose Hydrolysates	Pandey et al., 2000 B
		<i>Rhizopus oryzae</i> ATCC 34612	Flavor (Aroma Compound)	Bramorski et al., 1998
NA	Citric Acid	Soccol, 1996		
5	Cassava (Cooked)	<i>Rhizopus delamer</i> ATCC 34612; <i>Rhizopus oryzae</i> MUCL 28168; <i>Rhizopus oryzae</i> MUCL 28627	Protein enrichment; Alpha Amylase; Glucoamylase	Soccol et al., 1994 A
6	Cassava Fibrous Residue	<i>Streptomyces erumpens</i> MTCC 7317	Alpha Amylase	Kar et al., 2010
		<i>Bacillus subtilis</i> CM3	Alpha Amylase	Swain and Ray, 2007

Table 2-11. Continued. Substrates used in Microbial Fermentations, Organisms Employed and the Products Generated

S/N	Substrate	Organism Employed	Product Generated	Reference(s)
7	Cassava Flour (Gelatinized)	<i>Aspergillus niger</i> ;	Amylases	Soccol, 1996
8	Cassava Flour (Steam cooked)	<i>Rhizopus oryzae</i>	Protein Enrichment	Daubresse et al., 1987
9	Cassava Peel	<i>Aspergillus flavus</i> <i>Aspergillus niger</i>	Amylase Amylase	Sani et al., 1992 Sani et al., 1992
10	Cassava (Raw)	<i>Rhizopus delamer</i> ATCC 34612; <i>Rhizopus oryzae</i> MUCL 28168; <i>Rhizopus oryzae</i> MUCL 28627	Protein enrichment; Alpha Amylase; Glucoamylase	Soccol et al., 1994 A
11	Cassava Root	<i>Rhizopus</i> sp.	Amyloglucosidase; Single-cell protein	Sukara and Doelle, 1989
12	Cassava Starch	<i>Saccharomycopsis fibuligera</i> <i>Rhizopus</i> sp. <i>Aspergillus</i> sp. N-2	Alpha Amylase; Glucoamylase Protein Enrichment Glucoamylase	Gonzalez et al., 2008 Soccol, 1996 B Tani et al., 1986
13	Cassava Waste	<i>Rhizopus stolonifer</i> <i>Aspergillus niger</i> <i>Aspergillus terreus</i>	Cellulases; Beta Glucosidase; Protein Enrichment	Pothiraj et al., 2006
14	Chestnut Flour	<i>Bacillus subtilis</i>	α -amylase; β -galactosidase	Konsoula & Liakopoulou-Kiriakides, 2007
15	Coconut Oil Cake	<i>Aspergillus</i> sp. JGI 12	Amylase	Alva et al., 2007
16	Coffee Husk	<i>Ceratocystis fimbriata</i>	Flavor (Aroma Compound)	Soares et al., 2000
17	Corn cobs	<i>Thermomyces lanuginosus</i>	Amylase	Kunamneni et al., 2005
18	Corn Flour	<i>Bacillus subtilis</i>	α -amylase; β -galactosidase	Konsoula & Liakopoulou-Kiriakides, 2007
19	Corn Starch	<i>Bacillus subtilis</i>	Alpha Amylase	Konsoula & Liakopoulou-Kiriakides, 2006 a; 2006 b

Table 2-11. Continued. Substrates used in Microbial Fermentations, Organisms Employed and the Products Generated

S/N	Substrate	Organism Employed	Product Generated	Reference(s)
20	Corn Steep Liquor	<i>Bacillus subtilis</i> <i>Clostridium thermosulfurogenes</i> SV2	α -amylase; β -galactosidase Beta Amylase	Konsoula & Liakopoulou- Kiriakides, 2007 Rama Mohan Reddy et al., 2003
21	Cowpea Waste	<i>Aspergillus oryzae</i> BS41	Gluco Amylase	Kareem et al., 2009
22	Giant Palm Bran	<i>Kluyveromyces marxianus</i>	Flavor (Aroma Compound)	Medeiros et al., 2001
23	Gram Husk	<i>Bacillus</i> sp.	Amylase	Saxena & Singh, 2011
24	Ground Nut Oil Cake	<i>Aspergillus</i> sp. JGI 12	Amylase	Alva et al., 2007
25	Kumara; a starchy root crop	<i>Aspergillus niger</i>	Citric Acid	Lu et al., 1998
26	Maize (Crushed)	<i>Thermomyces lanuginosus</i>	Amylase	Kunamneni et al., 2005
27	Maize Meal	<i>Thermomyces lanuginosus</i>	Amylase	Kunamneni et al., 2005
28	Millet Cereal	<i>Thermomyces lanuginosus</i>	Amylase	Kunamneni et al., 2005
29	Millet (Pearl Millet) Flour	<i>Clostridium thermosulfurogenes</i> SV2	Beta Amylase	Rama Mohan Reddy et al., 2003
30	Miso	<i>Zygosaccharomyces rouxii</i>	Flavor (Aroma Compound)	Sugawara et al., 1994
31	Molasses Bran	<i>Thermomyces lanuginosus</i>	Amylase	Kunamneni et al., 2005
32	Mustard Oil Seed Cake	<i>Bacillus</i> sp.	Amylase	Saxena & Singh, 2011
33	Oat Flour	<i>Bacillus subtilis</i>	α -amylase; β -galactosidase	Konsoula & Liakopoulou- Kiriakides, 2007
34	Plantain Peel	<i>Aspergillus niger</i>	Beta Amylase	Adeniran et al., 2010
35	Potato Flour	<i>Bacillus subtilis</i>	α -amylase; β -galactosidase	Konsoula & Liakopoulou- Kiriakides, 2007

Table 2-11. Continued. Substrates used in Microbial Fermentations, Organisms Employed and the Products Generated

S/N	Substrate	Organism Employed	Product Generated	Reference(s)
36	Potato Starch	<i>Bacillus subtilis</i>	Alpha Amylase	Konsoula & Liakopoulou-Kiriakides, 2006 a; 2006 b
37	Potato Waste (Solid)	<i>Clostridium thermosulfurogenes</i> SV2	Beta Amylase	Rama Mohan Reddy et al., 2003
		Anaerobic sludge and waste water inocula	Amylase, Carboxymethyl Cellulase, Filter paper Cellulase, Xylanase, Pectinase and Protease	Parawira et al., 2005.
38	Rice Bran	<i>Bacillus</i> sp.	Amylase	Saxena & Singh, 2011
		<i>Aspergillus</i> sp. JGI 12	Amylase	Alva et al., 2007
		<i>Thermomyces lanuginosus</i>	Amylase	Kunamneni et al., 2005
		<i>Aspergillus terreus</i> GTC 826	Glucoamylase	Ali et al., 1989
39	Rice Flake Manufacturing Waste	<i>Aspergillus</i> sp. N-2	Glucoamylase	Tani et al., 1986
		<i>Aspergillus</i> sp. HA-2.	Glucoamylase	Anto et al., 2006
40	Rice Flour	<i>Bacillus subtilis</i>	α -amylase; β -galactosidase	Konsoula & Liakopoulou-Kiriakides, 2007
		<i>Aspergillus awamori</i>	Glucoamylase	Pestana and Castillo, 1985
		<i>Aspergillus foetidus</i>	Alpha Amylase	Michelena and Castillo, 1984.
41	Rice (Gelatinized)	<i>Neurospora</i> sp	Flavor (Aroma Compound)	Pastore et al., 1994
42	Rice Hull	<i>Aspergillus</i> sp. N-2	Glucoamylase	Tani et al., 1986
43	Rice Husks	<i>Penicillium citrinum</i>	Cellulases	Kuhad and Singh, 1993

Table 2-11. Continued. Substrates used in Microbial Fermentations, Organisms Employed and the Products Generated

S/N	Substrate	Organism Employed	Product Generated	Reference(s)
44	Rice Powder	<i>Aspergillus sp.</i> HA-2.	Glucoamylase	Anto et al., 2006
		<i>Aspergillus fumigatus.</i>	Glucoamylase	Cherry et al., 2004
45	Rice Grains	<i>Aspergillus oryzae</i> RIB 128	Volatile Compounds: Alcohols, Aldehydes, Esthers and Ketones	Ito et al., 1990
46	Rice Starch	<i>Bacillus subtilis</i>	Alpha Amylase	Konsoula & Liakopoulou-Kiriakides, 2006 a; 2006 b
47	Soybeans	<i>Rhizopus oryzae</i>	Flavor (Aroma Compound)	Christen et al., 2000
		<i>Bacillus subtilis</i> IFO 3013	Flavor (Aroma Compound)	Besson et al., 1997
48	Soybean (Ground)	<i>Bacillus subtilis</i> IFO 3013	Flavor (Aroma Compound)	Larroche et al., 1999
49	Soybean Meal	<i>Rhizopus oryzae</i> ATCC 34612	Flavor (Aroma Compound)	Bramorski et al., 1998
50	Sugar Beet Pulp	<i>Aspergillus niger</i> I-1472	Feruloyl esterase	Asther et al., 2002
51	Sugar Cane Bagasse	<i>Aspergillus niger</i> UO-01	Amylase	Roses and Guerra, 2009
52	Sugarcane Bagasse Hydrolysate	<i>Bacillus subtilis</i> strain KCC103	Alpha Amylase	Rajagopalan and Krishnan, 2008
53	Sunflower Seeds	<i>Penicillium fellutanum</i>	Protease	Muller dos Santos et al., 2004
54	Tea Waste	<i>Aspergillus niger</i>	Glucoamylase	Selvakumar et al., 1998

Table 2-11. Continued. Substrates used in Microbial Fermentations, Organisms Employed and the Products Generated

S/N	Substrate	Organism Employed	Product Generated	Reference(S)
55	Wheat Bran	<i>Aspergillus oryzae</i> IFO 30103	Alpha Amylase	Dey & Banerjee, 2011
		<i>Bacillus</i> <i>sp.</i> KR-8104	Alpha Amylase	Hashemi et al., 2011
		<i>Bacillus sp.</i>	Amylase	Saxena & Singh, 2011
		<i>Aspergillus niger</i> JGI 24	Alpha Amylase	Varalakshmi et al., 2009
		<i>Aspergillus sp.</i> JGI 12	Amylase	Alva et al., 2007
		<i>Aspergillus sp.</i> HA- 2.	Glucoamylase	Anto et al., 2006
		<i>Thermomyces</i> <i>lanuginosus</i>	Amylase	Kunamneni et al., 2005
		<i>Bacillus sp.</i> PS-7	Alpha Amylase	Sodhi et al. 2005
		<i>Thermomucor</i> <i>indicae-seudaticae</i>	Glucoamylase	Kumar & Satyanarayana, 2004
		<i>Ceratocystis</i> <i>fimbriata</i>	Aroma Compound	Christen et al., 1997
56	Wheat (Crushed)	<i>Thermomyces</i> <i>lanuginosus</i>	Amylase	Kunamneni et al., 2005
57	Wheat Flakes	<i>Thermomyces</i> <i>lanuginosus</i>	Amylase	Kunamneni et al., 2005
58	Wheat Flour	<i>Bacillus subtilis</i>	α -amylase; β -galactosidase	Konsoula & Liakopoulou- Kiriakides, 2007
		<i>Aspergillus oryzae</i>	Alpha Amylase	Rahardjo et al., 2005
59	Wheat Powder	<i>Aspergillus</i> <i>fumigates</i>	Glucoamylase	Cherry et al., 2004
60	Yam Peel	<i>Aspergillus niger</i>	Amyloglucosidase	Adeniran et al., 2010
		<i>Aspergillus niger</i>	Amylase	Uguru et al., 1997

2.2.2.2 Carbon supplementation

For effective performance of microorganisms and optimal secretion of products, substrates are often supplemented with carbon sources. Various sources of carbon such as glucose, lactose, sucrose, and soluble starch, have been studied. These studies revealed that the production rate of enzyme with added sugars could vary widely depending on type of sugar, strain or type of microorganism and composition of substrate. The highest production rates were obtained when sucrose was added to the culture media during fermentation with *Aspergillus* sp. (Ellaiah et al., 2002; Anto et al., 2006) and *Aspergillus niger* (Selvakumar et al., 1998). However, the latter was shown to have a negative impact on enzyme production when fructose and lactose were added (instead of sucrose). For *Aspergillus niger* JG 24, the addition of glucose and sucrose repressed the enzyme production rate when wheat bran was used as the solid material (Varalakshmi et al., 2009). On the other hand, when cassava bagasse was used for enzyme production, the addition of glucose had no significant impact on enzyme yields, but the addition of starch to the culture medium had a negative impact on α -amylase production with *Aspergillus oryzae* (Pengthamkeerati et al., 2012).

During production of β -amylase with *Clostridium thermosulfurogenes*, wheat bran was used as support enriched with potato starch and corn steep liquor. A gradual increase in the yield was observed when concentrations of potato starch and corn steep liquor were increased from 5% to 20% and 0.5% to 2% respectively (Rama Mohan Reddy et al., 2003).

2.2.2.3 Nitrogen supplementation

The effect of organic and inorganic nitrogen has also been studied, revealing an improvement in production when yeast extract (YE) (Anto et al., 2006), malt extract

(Selvakumar et al., 1998), casein, meat extract (Varalakshmi et al., 2009), peptone, tryptone (Kunamneni et al., 2005), and urea (Ellaiah et al., 2002; Kunamneni et al., 2005) were added to the solid media. However, a negative effect on α -amylase production was observed when a mixed substrate of biosludge and cassava bagasse was used with the nitrogen sources (Penghamkeerati et al., 2012). The addition of organic sources enhanced the production of α -amylase when wheat bran and *Aspergillus niger* were used for enzyme production; but, the opposite occurred with urea supplementation (Varalakshmi et al., 2009). In the case of glucoamylase production, supplementation of wheat bran, corn steep liquor and peptone with urea showed varying degrees of improvement in enzyme yields (Ellaiah et al., 2002).

2.2.2.4 Porosity and aeration

Porosity of the bulk solid materials used in SSF plays an important role in the efficacy of solid state fermentations. Porosity can be quantified by the ratio of empty air spaces (pores) to the total bulk volume of the porous material (Durand, 2003). Greater porosity will promote improved aeration, enhancing oxygenation and regulation of humidity and temperature because the combination of air and solid matrix has lower thermal diffusivity compared to water (Pandey, 2003). Porosity also contributes to the distribution of volatile compounds and amount of CO₂ produced during the process (Graminha et al., 2008). Air flow rate affects the production of enzymes such as α -amylase, in which the use of different rates of air flow produce different enzymatic activities during fermentation with *Aspergillus oryzae* IFO 30103 as the biocatalyst and wheat bran as the substrate. One study obtained highest enzyme activity at a flow rate of 0.1 L min⁻¹ g⁻¹ of wheat bran. Beyond that flow rate, the α -amylase activity dropped gradually (Dey & Banerjee, 2012). Anto et al. (2006) used wastes from rice production

of flakes as carbon source for the substrate (coarse waste, medium waste, rice powder), as well as wheat bran. Maximum enzyme production was obtained with wheat bran according to Sodhi et al. (2005) and Ellaiah et al. (2002), followed by coarse and medium waste. These results correlated well with starch content in the samples and characteristics of the substrate used. Coarse wastes contain rice bran particles as substrate and husk as support, increasing the porosity in the media and access to the nutrients, thus allowing germination of spores and growth of mycelia.

Fermentation of wheat dough with *A. oryzae*, Rahardjo et al. (2005) resulted in higher α -amylase and biomass production rates, along with production of aerial mycelia by taking advantage of porosity to increase macroscopic respiration rate. The effect of porosity on microorganism growth was reported by Murado et al. (1997) in which negative signs in terms involving density of the solid support in a mathematical model showed the restriction in intra-particle diffusion when working with high densities using polyurethane foams as solid support during α -amylase production with *A. oryzae*.

In SSF, porosity is a key characteristic because the open pore structure of the solid used as substrate or anchorage benefits the enzyme diffusion and the substrate degradation happens inside. The fragments of substrate that are soluble in water have to diffuse out of the solid matrix where the enzymes action produces metabolizable compounds (Raghavarao et al., 2003).

2.2.2.5 Moisture content and water activity

Moisture content also affects the efficacy of SSF, and should neither be too high nor too low. High moisture content decreases the porosity of the medium and impedes oxygen penetration, while too low a moisture content can impede microbial growth because of poor accessibility to nutrients (Pandey, 2003). Water activity is a measure of

the amount of water for any given moisture content that is freely available to support microbial and biochemical activity. It can determine the type of microorganism that can grow in SSF systems. For example, yeasts and molds can grow at water activities much lower than the higher levels needed for bacteria. The relatively low water activities (a_w) used in SSF influence the physiological aspects of microorganisms, metabolite production and enzyme activity (Graminha et al., 2008). Kareem et al. (2009) tried different levels of initial moisture content for the production of glucoamylase by the cultures *A. oryzae* BS41. Highest enzymatic activity was obtained with 60% initial moisture content. The same moisture content (60%) was also found to be optimum in the production of α -amylase by a strain of *A. oryzae* with cassava bagasse and biosludge as culture medium. Higher moisture contents showed decreasing activity of α -amylase (Pengthamkeerati et al., 2012). Furthermore, initial moisture content of 60% was found to be the optimum for production of glucoamylase from fermentation of tea waste with *Aspergillus niger* NCIM 1248, achieving maximum enzyme production of 198.4 IU/gds (glucoamylase units/gram dry substrate) at 96 hours of fermentation (Selvakumar et al., 1998). The same 60% moisture content was also optimum for fermentation with *Streptomyces erumpens* MTCC 7317 of cassava fibrous residue (Kar et al., 2010).

However, a moisture content of 90% produced the highest enzyme activity (298 U/g) when the thermophilic strain *Thermomyces lanuginosus* was used with wheat bran as the solid material support. Activity of less than 150 U/g was obtained when 60% initial moisture content was used during the same fermentation (Kunamneni et al., 2005). When the fermentation was made with a bacterial strain like *Bacillus* sp. PS-7 for the production of α -amylase, the moisture content that produced the best enzyme yield was

60%; obtained when a ratio of 1:1.5 (wheat bran: tap water) was used (Sodhi et al., 2005). The same ratio of wheat bran and distilled water (60% moisture) was obtained as an optimum during fermentation with *A. oryzae* HS-3 for the production of amyloglucosidase (Singh & Soni, 2001). Figure 2-12 summarizes the optimum moisture contents found for various combinations of microorganism and substrate in enzyme production.

Table 2-12. Optimum moisture content [% W/W] for various combinations of microorganism and substrate in enzyme production

S/N	Microorganism	Substrate	Optimum Moisture Content for Enzyme Produced		Reference
			Gluco-amylase	Alpha-amylase	
1.	<i>Aspergillus oryzae</i> BS41	Not reported	60	–	Kareem et al. (2009)
2.	<i>Aspergillus oryzae</i>	Cassava fibrous residue and biosludge	–	60	Pengthamkeerati et al., 2011
3.	<i>Aspergillus niger</i> NCIM 1248	Tea waste	60	–	Selvakumar et al., 1998
4.	<i>Streptomyces erumpens</i> MTCC 7317	Cassava fibrous residue	60	–	Kar et al., (2010)
5.	<i>Thermomyces lanuginosus</i>	Wheat bran	90	–	Kunamneni et al., (2005)
6.	<i>Bacillus</i> sp. PS –7	Wheat bran	60	–	Sodhi et al., (2005)
7.	<i>Aspergillus oryzae</i> HS–3	Wheat bran	60	–	Singh & Soni, (2001)

2.2.2.6 Incubation temperature

Temperature is a main factor during SSF for enzyme production, not only because it affects the growth of the microorganism, but because during the production of some thermolabile enzymes, the increasing temperature due to microorganism metabolism can cause its denaturation, lowering the yield of the process. Muller dos Santos et al. (2004) developed a mathematical model that showed the behavior of temperature during SSF for the production of protease with *P. fellatanum* in a large scale process with a mixing bioreactor, controlled air flow and water-jacket. This system reached a temperature of 48°C using one volume of air per total bioreactor volume per minute and cooling water temperature at 30°C. However, results showed that only 25% of the enzyme produced retained any activity. On the other hand, using the same air flow rate with water temperature of 14.5°C, approximately 87% activity was retained.

For *A. oryzae* the effect of temperature showed a maximum α -amylase activity when the fermentation process was carried out at 32°C, with a sharp decrease at higher temperatures (Dey & Banerjee, 2012). Similar results were obtained by Ellaiah et al. (2002) at an optimum temperature of 30°C for glucoamylase production with *A. oryzae* SP-3. Francis et al. (2003) also found similar results during optimization of fermentation parameters when producing α -amylase with spent brewers grains and *Bacillus* sp. PS-7. Highest productivity was obtained at 37°C with optimum enzyme activity at 60°C, which remained very thermostable at 50°C during 6 hours (Sodhi et al., 2005). It should be noted that optimum temperatures for the production of the enzyme are not the same as those for enzyme activity. For *A. niger* JGI 24, the maximum α -amylase activity was obtained at 22°C, being negatively affected with higher temperatures (37°C and 40°C). The best activity of the purified enzyme was obtained at 30°C (Varalakshmi et al.,

2009). Enzymes produced by thermophilic microorganisms like *Thermomyces lanuginosus* have better activity during fermentation at higher temperatures. The optimum temperature for amylase production was found to be 50 °C (263 U/g) with very high thermostability at this temperature. Optimal enzyme activity was found at 60°C (Kunamneni et al., 2005), in accordance with the results obtained by Kar et al. (2010) during optimization of fermentation for production of α -amylase using cassava fibrous residue as a culture medium and the actinomycete strain *Streptomyces erumpens* MTCC 7317. Table 2-13 summarizes the optimum temperature for enzyme production and activity with various combinations of microorganism and substrate.

2.2.2.7 Inoculums concentration

The inoculum level is an important factor for the production of amylase. During glucoamylase production with *A. niger* NCIM 1248, different inoculum concentrations were used (1, 2, 3, 4, 5%). An inoculums level of 4% produced maximum glucoamylase (198.4 IU/gds at 96 h of fermentation), with no increase in enzyme production at any higher concentration of inoculum (Selvakumar et al., 1998). In the case of *A. oryzae* TISTR 3605 with cassava bagasse and biosludge as culture medium, an inoculum level of 3×10^7 spores/ml was found to produce the maximum α -amylase activity with 60% initial moisture content and 35°C over 48 hours of incubation. The growth curve for this strain of *A. oryzae* showed an exponential phase of microbial growth during the first 48 hours of incubation followed by a decreasing period after reaching the optimum (Pengthamkeerati et al., 2012). This same behavior was observed by Dey & Banerjee (2012), who obtained a maximum α -amylase activity of 17989 U/gds at 48 hours of incubation followed by reduction of activity beyond the 48 hours.

Table 2-13. Optimum temperature for enzyme production and activity with various combinations of microorganism and substrate

S/N	Microorganism	Substrate	Optimum Temperature for Enzyme Production and Activity (°C)		Reference
			Enzyme Production	Enzyme Activity	
1.	<i>P. fellatanum</i>	Not reported	14.5		Dos Santos et al. (2004)
2.	<i>Aspergillus oryzae</i>	Not reported	32		Dey & Banerjee, (2011)
3.	<i>A. oryzae</i> SP-3	Not reported	30		Ellaiah et al. (2002)
4.	<i>Bacillus</i> sp. PS-7	Spent brewer's grains	37	60	Francis et al. (2003); (Sodhi et al., 2005).
5.	<i>A. niger</i> JGI 24	Not reported	22	30	Varalakshmi et al., (2009)
6.	<i>Thermomyces lanuginosus</i>	Not reported	50	60	Kunamneni et al., (2005),
7.	<i>Streptomyces erumpens</i> MTCC 7317	Cassava fibrous residue	50	60	Kar et al. (2010)

Another strain of *A. oryzae* BS 41 produced optimum glucoamylase production (980 U/ml) with an inoculum concentration of 4%. A lower concentration could be insufficient for initiating the growth and production of the enzyme during fermentation time (72 hours), and a higher concentration would result in diminished enzyme activity due possibly to reduced nutrient availability (Kareem et al., 2009). This inhibitory effect of high levels of inoculum was also seen by Kunamneni et al. (2005) with the highest enzyme production of 267 U/g at an inoculum level of 10% (v/w) and Kumar &

Satyanarayana (2004) during fermentation of wheat bran with the thermophilic mold, *Thermomucor indicae-seudaticae* for glucoamylase production.

On the contrary in the study of Anto et al. (2006), the inoculum size showed little effect on enzyme production during fermentation with *Aspergillus* sp. HA-2, obtaining an activity of 264 ± 0.64 U/gds when wheat bran was used as a culture medium, followed by 211.5 ± 1.44 in rice coarse waste and 192.1 ± 1.15 U/gds in medium waste with an optimum inoculum size of 10^6 spores/ml. Dey & Banerjee (2012) and Sodhi et al. (2005) used an inoculum concentration of 1×10^6 spores/ml for *A. oryzae* IFO 30103 and 1.5×10^6 cells/ml for *Bacillus* sp. PS-7 respectively.

2.2.2.8 Fermentation time and pH

Fermentation time at a given temperature for optimum production of enzyme must also be controlled. In the case of *Streptomyces erumpens*, the optimum time at a given temperature was 60 hours, after which enzyme yield decreased (Kar et al., 2010). In the case of *Thermomyces lanuginosus*, the enzyme activity was observed to increase from 24 to 120 hours, reaching a maximum activity of 262 U/g. Beyond the 120 hours, activity decreased, possibly due to denaturation or loss of moisture during the process (Kunamneni et al., 2005). The pH is also an important factor in fermentation processes, and acts in synergy with other process parameters (Kumar & Satyanarayana, 2004). This became evident in a study Pengthamkeerati et al. (2012) showing lower α -amylase production and activity at pH values other than 7.0 during fermentation with *A. oryzae* TISTR 3605. This pH value of 7.0 was also very close to the optimum value obtained by Varalakshmi et al. (2009) for fermentation with *A. niger* JG24. Some strains of *Aspergillus* sp. have an optimum value of pH in the acidic range of 4 – 6 (Ellaiah et al., 2002; Anto et al., 2006; Dey & Banerjee, 2012).

2.2.3 Bioreactor Designs in Solid-State Fermentation

Important factors that affect the design and scale-up of bioreactors for solid-state fermentation (SSF) are the following:

- Thermal gradients caused by the lack of heat transfer across surfaces and poor thermal conductivity of the substrate, as well as air flow could impede removal of the metabolic heat produced during the growth of the microorganisms (Mitchell *et al.*, 2000).
- Transfer of oxygen, nutrients and enzymes affect the substrate characteristics and moisture content (Durand, 2003; Raghavarao *et al.*, 2003).
- Operation of mixers where microorganisms or substrates are sensitive to shear forces.

Bioreactors for SSF are normally classified by their size or capacity in order to appropriate for laboratory scale, pilot plant, or industrial scale (Durand 2003). Various SSF bioreactor designs commonly used are described briefly below:

2.2.3.1 Packed bed reactor

Packed-bed reactors consist of a column with a perforated base, and use forced aeration, making it possible to control oxygen supply, temperature and moisture content (Pandey *et al.*, 1994; Lu *et al.*, 1998). However, the unidirectional nature of the air flow causes an axial temperature gradient which can cause low yields or cell death (Ashley *et al.*, 1999; Mitchel *et al.*, 2010). Based on this factor, Lu *et al.* (1998) compared the behavior of a glass-packed bed bioreactor using a single layer and a multi-layer configuration. No significant differences in temperature, residual starch concentration or citric acid production could be found among the layers. The single layer configuration showed a temperature gradient up to 2 °C, and the citric acid production was lower than that in the four layer bioreactor when using a strain of *Aspergillus niger* and kumara as substrate. Mitchel *et al.* (1999) observed that the behavior of temperature in packed-bed

reactors using a single layer steadily increased with height. Their study concluded that the bed height must be lower than the height at which the critical temperature is reached during fermentation.

Mitchel et al. (2010) modeled the performance of a multi-layer configuration with 10 levels, using the N-tanks-in-series approach. Sahir et al. (2007) used different operation strategies in order to avoid temperature gradients. Best results were obtained by operating the reactor as a continuous plug-flow, multi-layer packed-bed, which has a lower rate of heat generation than batch operation. Silva & Yang (1998) found that the performance of a packed-bed reactor with forced aeration was superior to that obtained with tray type fermentation reactor.

2.2.3.2 Tray bioreactor

Tray bioreactors are based on a simple design that evolved from ancient technology. It consists of flat trays that can be perforated or not where the substrate is placed in a thin layer (15 cm maximum) and kept under controlled temperature with humidified air. During fermentation of rice for amylase production with *Aspergillus oryzae*, the static solid-state fermentation showed that mass transfer is a problem in this type of reactor, even when the bed depth is only several centimeters (Silva & Yang, 1998), and is used in thermostatic chambers (Couto and Sanroman, 2006; Durand, 2003).

2.2.3.3 Drum bioreactor

In drum bioreactors, mixing can be carried out continuously or discontinuously by gently rotating the entire cylindrical drum, and controlling the temperature by circulating water in a jacket (Durand, 2003; Kalogeris et al., 1999). Other designs use ribbon-shaped baffles for the mixing with rotation of a basket (Raghavarao et al., 2003). In

some cases the continuous agitation can change the characteristics of the medium or cause the damage of the mycelium, and must be avoided (Durand, 2003).

2.2.3.4 Fluidized bed bioreactor

Fluidized bed designs use forced air in order to maintain continuous agitation to avoid adhesion and aggregation of substrate particles, but the shear forces that develop can cause damage to the mycelium and affect the final product yield. Figure 2-22 is a schematic of a fluidized bed bioreactor (Couto & Sanroman, 2006).

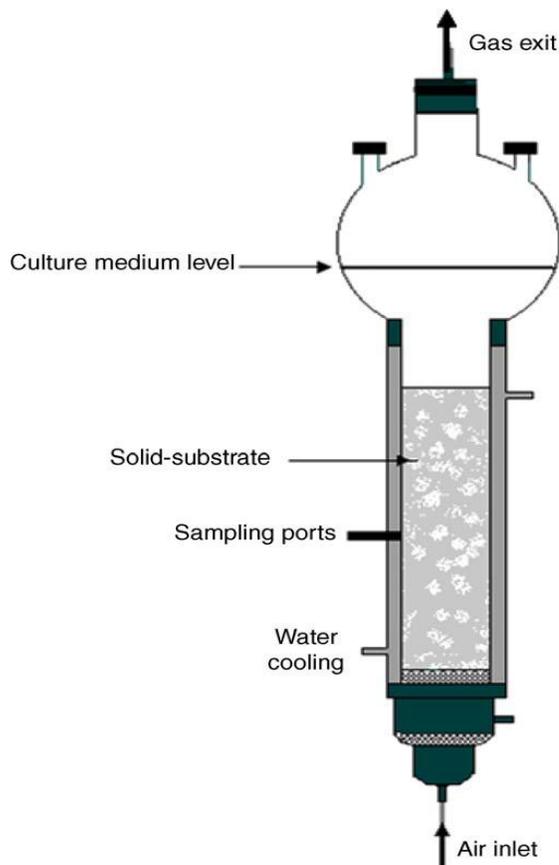


Figure 2-22. Schematic of a Fluidized Bed Bioreactor that uses Humidified Air for Pneumatic Agitation. (Photo from Couto & Sanroman, 2006).

2.2.4 Submerged Fermentation (SmF)

Submerged fermentation (SmF) is carried out by dispersing the substrate ingredients in an excess of water (Singhania et al., 2010). The control of temperature and pH is not as complicated as in SSF. One of the most important variables is oxygen transfer which depends on size and shape of the reactor. The ability to transfer oxygen independently of reactor volume can be quantified by the oxygen transfer coefficient K_{La} (Durand, 2003), which is an important parameter for fermentation process design. Other factors that need to be specified are the medium composition and process conditions that are discussed below.

2.2.4.1 Temperature and pH

Various types and strains of microorganism have been studied under varied temperature and pH conditions to produce amylolytic enzymes. These organisms, each with special characteristics were assessed for enzymes that can be adapted to industrial processes, like starch liquefaction that requires α -amylases with high thermo-stability (Gupta et al., 2003). Some strains such as *Chromohalobacter* sp. TVSP 101, produces thermo-stable, halo-tolerant and alkali-stable α -amylases. Other strains that were studied include the following:

- *Halobacillus* sp. MA-2 (Prakash et al., 2009) ,
- *Micrococcus* sp. NS 211 with the capacity to grow in an acid medium and at high temperatures (Amoozegar et al., 2003),
- *Microbacterium foliorum* GA2, a psychrotolerant amylolytic bacteria (Samie et al., 2012),
- *Bacillus cereus* GA6 (Kuddus et al., 2012),

- *Pseudoalteromonas arctica* (Lu et al., 2010),
- *Bacillus* sp. (Syu & Chen, 1997; Burhan et al., 2003; Goyal et al., 2005; Liu & Xu, 2008), and
- *Streptomyces* sp. ML 12, a marine actinobacterium isolated by Sivakumar et al., (2012).

The strain *Chromohalobacter* sp. produced enzyme over a broad range of pH; from 6.0 to 10 with an optimum of 9.0, at an optimum growth temperature of 37°C. These optima process conditions were also found for *Bacillus* sp. ANT-6 during α -amylase production (Burhan et al., 2003). Since *Chromohalobacter* sp. is quite halo-tolerant, maximum enzyme production was obtained when the culture medium contained 20% NaCl or 15% KCl. Under these conditions, two isozymes were secreted when grown with high-salt contents from 10 to 30% (Prakash et al., 2009). Two α -amylase isoforms were also found during fermentation with *Aspergillus oryzae*, obtaining an increase in concentration of one of these isozymes (AmyB), while the other (AmyA) disappeared through the culture (Sahnoun et al., 2012).

For production of thermo-stable enzymes with *Micrococcus* sp., the highest levels of amylase were found at 85°C. The microbe was capable of growing at temperatures of 110°C and pH from 1.2 to 8.0 with an optimum pH of 3.5 when starch was used as the carbon source in the culture medium (Samie et al., 2012). On the other hand, *Microbacterium foliorum* and *Bacillus cereus* isolated from soil from the Gangotri glacier in western Himalaya-India produced maximum α -amylase at 20°C. Both strains were pH tolerant with a maximum production of amylase at pH 9 and 10 respectively. With the strain of *Bacillus subtilis*, maximum α -amylase yield was obtained at pH 7.0 and 50°C. Supplementation of the medium with calcium stimulated enzyme production and bacterial growth (Asgher et al., 2007). Konsoula & Liakopoulou-Kiriakides (2007)

fermented complex carbohydrates during co-production of α -amylase and β -galactosidase, obtaining high yields of enzymes when the process was carried out under pH 7.0 and 40°C conditions. Neutral values of pH and optimum temperature of 37°C were also observed as the best growth and enzyme production conditions for strains of *Bacillus* sp. (Syu & Chen, 1997; Goyal et al., 2005).

2.2.4.2 Carbon and nitrogen

Aspergillus oryzae was used to produce glucoamylase, α -amylase and α -glucosidase using different sources of carbon, such as glucose, dextrin, maltose and indigestible dextrin. When maltose or dextrin was used, the production of enzymes was higher than that obtained when glucose was used as the carbon source. This diminished production of enzyme was also observed during production of α -amylase with *B. subtilis* when glucose was added to the culture medium along with potato starch (Asgher et al., 2007). Other authors found that for *Hallobacillus* sp., the maximum excretion of amyolytic enzyme was attained with dextrin (3.2 U/ml) followed by starch (2.4 U/ml). In the case of starch, the production of enzyme was found to be constitutive (Amoozegar et al., 2003). For *A. oryzae*, the carbon source that showed highest activities for α -amylase, α -glucosidase and glucoamylase was the indigestible dextrin. The biomass concentration with this carbon source was 35% of that in dextrin culture, which can be useful during industrial processes by improving the viscous fermentation broth rheology with this microorganism (Sujimoto & Shoji, 2012). Other strains of *A. oryzae* used maltodextrins (gruel) as a carbon source with high yields of α -amylase (Kammoun et al., 2008).

Maltose was found to be a good carbon source for fermentation with *M. foliorum*, yielding an amylase production of 4137 units. But, lactose was found to be even better,

producing a maximum yield at 5862 units. However, use of lactose as a carbon source for amylase production with *B. cereus* produced a lesser yield of 4744 units (Kuddus et al., 2012). When starch and glycerol were used with *Bacillus subtilis* IMG 22, a perfect interaction between these variables was found to yield maximum enzyme activity when optimum levels of starch and glycerol were added; the latter with the greater effect on enzyme production (Tanyildizi et al., 2005). The use of starch for enzyme production with *B. subtilis* was also studied by Konsoula & Liakopoulou-Kiriakides (2007). The authors concluded that soluble starch was a good carbon source for enzyme production, while sugars like glucose, maltose or lactose suppressed the production of α -amylase and β -galactosidase.

The alkaline amylase producer *Bacillus* sp. JCM 9141 yielded maximum amylase activity of 45U amylase/ml with starch as carbon source, and 50U amylase/ml with glucose as a carbon source, showing that for this strain, starch is not a great inducer for amylase production (Zhang *et al.*, 2004). Studies on α -amylase production with *Bacillus licheniformis* ATCC 9945a were also reported by Božić et al. (2011). Enzyme production was associated with cell growth for *Bacillus* sp. (Zhang et al., 2004). This same observation was made during fermentation with *Bacillus subtilis* JS-2004 using 1% of waste potato as a carbon source (Asgher et al., 2007), as well as with *Bacillus* sp. (Goyal et al., 2005; Liu & Xu, 2008).

Agroindustrial wastes like yam peel (Uguro et al., 1997), gruel (wheat grinding by-product) (Kammoun et al., 2008) have also been used as carbon sources for submerged fermentation. For industrial fermentation, the design of the medium is very important, as it affects the cost and the product yield. Sivakumar et al. (2012) used

wheat bran, rice bran and tapioca as carbon sources. They showed that wheat and rice bran enhanced the production of α -amylase, resulting in 42.412 IU/ml, while tapioca was not a significant factor during culture media optimization. Production of α -amylase from fermentation of brewery waste water with the strain *Aspergillus niger* UO-1 was found to increase when supplemented with starch and the use of casamino acids, yeast extract and peptone (Hernandez et al., 2006).

Kuddus et al. (2012) evaluated nitrogen sources, such as casein, glycine, yeast extract, ammonium acetate and ammonium sulfate. Maximum production of α -amylase was obtained with *B. cereus* and ammonium acetate, or with *M. foliorum* and yeast extract. Similar results were found with *Bacillus subtilis* JS-2004 when yeast extract and calcium were added in the medium, increasing bacterial growth and α -amylase production (Asgher et al., 2007). On the contrary, during fermentation with *B. subtilis* IMG 22, the use of yeast extract was not significant for the improvement of α -amylase production (Tanyildizi et al., 2005). It has also been reported that wheat bran when supplemented with metal ions, carbon and nitrogen sources was the best substrate (among those tested) for the production of amylase and protease (Shivakumar, 2012).

2.2.4.3 Salts

The maximum production of enzyme with *Halobacillus* sp. was observed in a medium with 15% (w/v) NaSO₄, followed by 10% NaCl. When sodium nitrate was added to the medium, no extracellular amylase production was observed (Amoozegar et al., 2003). When potassium nitrate, citrate, potassium chloride and calcium chloride were used with *Streptomyces* sp., the salts found to have significant effect on α -amylase activity were sodium chloride and magnesium sulfate (Sivakumar et al., 2012).

The use of calcium to enhance the production of α -amylase with *Bacillus* sp. has been reported by several workers (Asgher et al., 2007; Konsoula and Liakopoulou-Kiriakides, 2007; Kuddus et al., 2012). However, in the case with *A. oryzae* CBS 819.72, the presence of Ca^{2+} had no effect on α -amylase production, while magnesium and phosphate were very important (Kammoun et al., 2008).

2.2.5 Summary on Enzyme Production

Production of amylolytic enzymes suitable for industrial use is very important due to the wide range of applications like detergent industry, bakery products, sugar syrups, brewery etc. Because of the differences between these processes, microorganisms isolated from sources as different as glaciers and hot springs or soil and marine sources have been studied in order to obtain enzymes with special characteristics. Studies in search of optimum combinations of microorganisms, substrate composition and process conditions revealed there was no one-size-fits-all. Optimum conditions for maximum yields varied widely depending on specific strain of microorganism and substrate composition used in the fermentation process. Submerged fermentation continues to be the most widely used method of microbial enzyme production in western countries. However, solid-state fermentation has gained interest in recent years for fermentation of bulk solids. Novel bioreactor designs are of interest for researchers because of the necessity to improve mass and heat transfer during fermentation with large amounts of substrate.

CHAPTER 3
PHYSICAL PROPERTIES OF CASSAVA FLOUR MADE FROM SOLAR
CONVECTION-DRIED CASSAVA CHIPS

3.1 Background

Cassava, *Manihot esculenta* Crantz, sometimes also referred to as Mandioca, Manioc, Tapioca or Yuca is the third largest source of carbohydrate for human food in the world (Nassar and Ortiz, 2010; Claude and Denis, 1990; Phillips, 1982). Most of the world's production comes from developing countries. At present, Nigeria is the number one cassava producer both in Africa and in the world (FAO, 2013; Phillips et al., 2004; Hillocks, 2002). In 2008, Nigeria produced 44.582 million tons of cassava valued at over \$ 3.312 x 10⁹ (Food and Agriculture Organization of the United Nations (FAO), 2010). Haiti is known as a major cassava producer in the South America and Caribbean region (Henry and Hershey, 2002]. The 435,000 tons of cassava produced by Haiti in 2008 ranked her 5th in the region. Haiti's output was exceeded only by Brazil (26.703 million t); Paraguay (2.219 million t); Columbia (1.803 million t) and Peru (1.172 million t) (FAO, 2010). These nations have been increasing their output mainly due to cassava's high productivity; cash and subsistence crop profile; tolerance of poor soils and low rainfall; good resistance to pests and diseases; and more than two years harvest window. The long harvest window affords poor farmers not only some protection against famine, but also guarantees flexible labor management schedules. Because farmers can "store" the starchy roots underground in the field, they do not need to worry about expensive refrigeration or other food preservation techniques. Perhaps more importantly however, is the fact that the cassava crop can be transformed into such products as starch, flour, and ethanol; with huge potential industrial applications (Hakizimana, 2010; Widowati and Hartojo, 2010; Johnson et al., 2009; Eneas, 2006; Srinorakutara et al., 2006;

Balagopalan et al., 1988; Berghofer and Sarhaddar, 1988; Srikanta et al., 1987; Matsumoto et al., 1982; Ueda et al., 1981; Lindeman and Rocchiccioli, 1979).

3.2 Objectives

Constraints that limit the utilization of cassava crop include shelf life and mass. Cassava roots are bulky and heavy, and therefore expensive to transport over long distances. The roots are also extremely perishable, thus must be either consumed or processed within a few days after harvest (Eneas, 2006; Balagopalan et al., 1988; Kuppuswamy, 1961). To overcome these limitations, cassava roots would need to be processed near the source of production using appropriate technology, and into products that are less bulky and with longer shelf life such as chips, flour, starch and sweeteners. The objectives of the work reported in this study were to:

1. Produce dried cassava chips using a prototype solar convection dryer,
2. Produce cassava flour from grinding the chips,
3. Determine and report the following properties of chips and flour:
 - a) Sorption isotherms and drying curves on the chips, and
 - b) Moisture content, particle size distribution, bulk density, solid particle density, porosity, permeability and specific surface area of the flour.

3.3 Methods and Procedures

3.3.1 Scope of Work

The scope of work undertaken in this study consisted of a series of efforts to determine basic physical properties for cassava chips and flour. These included sorption isotherms and drying curves on the fresh cassava pulp to produce the dried chips, and moisture content, particle size distribution, bulk density, solid particle density, porosity, permeability and specific surface area of the flour.

The work was carried out in the physical properties laboratory of the Agricultural and Biological Engineering (ABE) Department at the University of Florida. The purpose

of this work was to obtain estimates for physical properties of cassava chips and flour that could be used for design work.

3.3.2 Fresh Cassava Root

3.3.2.1 Sample preparation

Fresh cassava roots were purchased from Brooks Tropicals, Homestead, Florida. Fresh cassava chips were made by peeling the tubers and slicing them into thin chips with approximate thickness of 2 mm using a Hobart Slicer, Model 1712E (Hobart Corporation, Troy, Ohio). The sliced fresh chips at about 60% (wb) moisture were spread onto screen mesh trays in a single layer, and placed into a prototype solar convection dryer; shown in Figure 3-1, designed and fabricated at the University of Florida (Schiaivone et al., 2013). The chips were dried to approximately 10% (db) moisture, and stored in air-tight containers until ready for use.



Figure 3-1. Prototype Solar Convection Dryer used for Drying Cassava Chips. Photo courtesy of A. A. Teixeira.

3.3.2.2 Moisture content and water activity for sorption isotherms

Moisture content was determined by the oven drying method (AOAC, 2000). Pre-weighed samples of freshly ground cassava root were placed in a drying oven for 72 hours at 105 °C, after which they were weighed once again to determine total moisture removed. Water activity was determined with a water activity meter (AquaLab series 3 TE, Decagon Devices, Inc., Pullman, WA). A 10-gram sample of freshly ground cassava pulp was placed in the sample cup of the meter after the meter had been powered up at least 30 min previously, as per manufacturer's directions. After several minutes, when the meter sensed that equilibrium relative humidity had been reached, the values for water activity and the relevant temperature were displayed and then manually recorded.

In order to establish the sorption isotherm, a series of pre-weighed 10-gram samples of fresh cassava root were placed in sample cups used for water activity readings. Two sample cups were placed in each of five desiccator jars maintained at different equilibrium relative humidity as shown in Table 3-1. At pre-determined time intervals, the cups were removed from jars and weighed. When no further weight change occurred, the samples were removed from their jars for water activity measurements, and placed in the drying oven to determine their moisture content. The isotherm was established by plotting a graph of moisture content verses water activity.

3.3.2.3 Drying curve

The thinly-sliced cassava chips were dried in a prototype solar convection dryer designed and fabricated at the University of Florida (Schiavone et al., 2013). Weigh boats containing approximately 100 grams of pre-weighed sliced fresh chips were placed onto one of the screen mesh trays in the dryer cabinet to dry over time. The

Table 3-1. Saturated Salt Solutions and Associated Equilibrium Relative Humidity when placed in Desiccator Jars.

Saturated Salt Solution	Equilibrium Relative Humidity at 22 ° C (%)
Magnesium Chloride	32.8
Sodium Bromide	57.6
Sodium Chloride	75.3
Potassium Bromide	81.8
Potassium Sulfate	97.3

initial moisture content of these chips was determined using the oven dry method (AOAC, 2000) with sister samples of chips. At pre-determined time intervals, the weigh boats were removed from the dryer for weight measurement, and returned to the dryer. The drying process was terminated after about 28 hours when no further weight loss was observed. The moisture content at each pre-determined time interval was calculated by knowing the initial weight of the sample and the weight reached at that time interval. The drying curve was established by plotting a graph of moisture content (dry basis) verses time. This work was repeated three times to obtain three replicate drying curves.

3.3.3 Cassava Flour

3.3.3.1 Sample preparation

Samples of cassava flour were made by blending dried chips (10% moisture db) with Osterizer® Designer Cycle Blender (Sunbean Corporation, Fort Lauderdale, Florida) until particle sizes less than 0.5 mm could be obtained. Optimum blender

operating conditions were chosen by comparing particle size distribution profiles obtained from different combinations of grinding time and blender speed on chips made from two different varieties of cassava (Algodon and Valencia) and two different chip thicknesses (2mm and 4mm). Particle size distributions were obtained using a vibrating stacked sieving machine (Octagon-2000, Endicotts Ltd. London, England).

3.3.3.2 Physical properties

Bulk density, solid particle density and porosity were determined by use of a multi-pycnometer (Quantachrome®, Boynton Beach, FL). Permeability was measured using an air flow apparatus fabricated in shop facilities of the Agricultural and Biological Engineering Department at the University of Florida. This apparatus is illustrated in Figure 3-2. It provided a means by which a sample of cassava flour contained in a

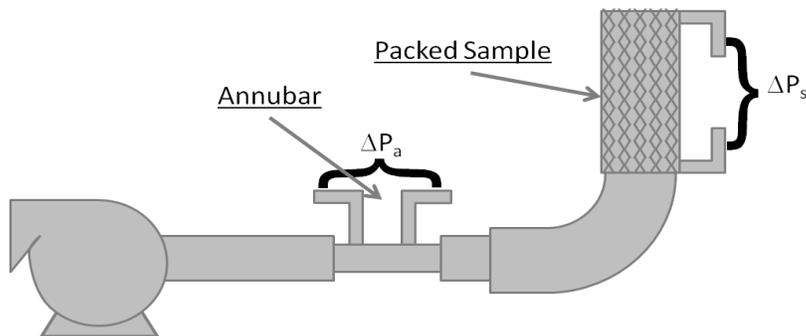


Figure 3-2. Air Flow Apparatus for Determination of Permeability

cylindrical sample holder of known dimensions could be placed in the path of airflow at known flow rate. By measuring the pressure drop across the sample while air was flowing, the permeability of the sample could be determined as a function of this pressure drop and air flow rate. The cylindrical sample holder was 10cm in length and 2cm in diameter. The pressure drop across the sample was measured with an inclined

oil manometer, and flow rate was determined with use of an annubar flow measuring device (F.W. Dwyer MFG. Co., Michigan City, Indiana) for measurement of flow rate. With the airflow on, the pressure drop across the annubar was recorded with the same oil manometer to calculate air flow rate, and the pressure drop across the sample of flour was determined. The permeability (K) was calculated from Darcy's equation (eq. 3-1). Once the permeability was known, the specific surface area of the flour could be estimated as a function of permeability and porosity using the Carmen-Kozeny equation (eq. 3-2).

$$K = (q \times \eta \times L) / (A \times \Delta P_s) \quad (\text{eq. 3-1})$$

$$K = P^3 / [5 \times S^2 \times (1-P)^2] \quad (\text{eq. 3-2})$$

Where K = material permeability (m²), q = air flow rate (m³/sec), η = viscosity of air (1.82E⁻⁵ Pa.s), L = length of cylindrical sample holder (m), A = cross sectional area of cylindrical sample holder (m²), ΔP_s = pressure across sample (Pa), P = porosity (dimensionless), and S = specific surface area (m²/m³).

3.4 Results

3.4.1 Sorption Isotherm and Drying Curves for Fresh Cassava Root Pulp

The sorption isotherm for the fresh cassava root pulp is shown in Figure 3-3. The profile is typical of most sorption isotherms, showing that moisture content decreases rapidly with decreasing water activity at relatively high water activity, but slows down dramatically at water activity less than 0.6. A water activity of 0.6 is well below the water activity at which most yeast and bacterial activity could occur (Isengard et al., 2011). The moisture content at water activity of 0.6 (approximately 10% db) was chosen as target end point for the drying of fresh cassava slices into dried chips.

The three replicate drying curves shown in Figure 3-4 suggest that the target moisture level of 10% db could be reached within 25-28 hours of residence time in the solar convection dryer. This was well within the 48 hours actually used for drying the cassava chips in this study.

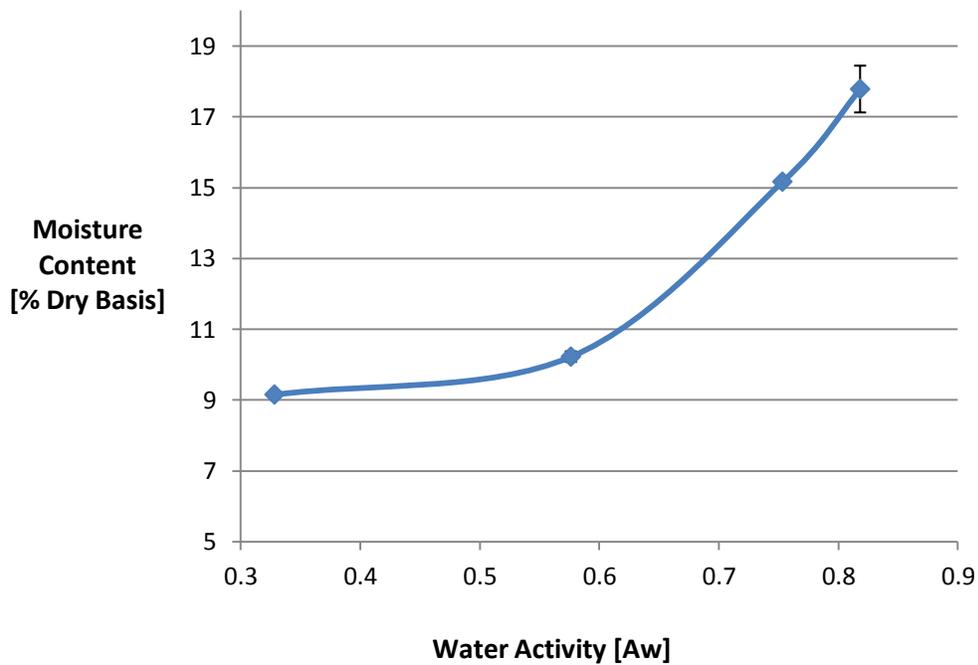


Figure 3-3. Sorption Isotherm of Fresh Cassava Root Pulp Slices for Drying into Chips (Error bars at two standard deviations).

3.4.2 Physical Properties of Cassava Flour

Particle size distributions in samples of cassava flour made from grinding dry cassava chips under various blender operating conditions are shown in Figure 3-5. The particle size distribution profiles shown in the figure were obtained from different

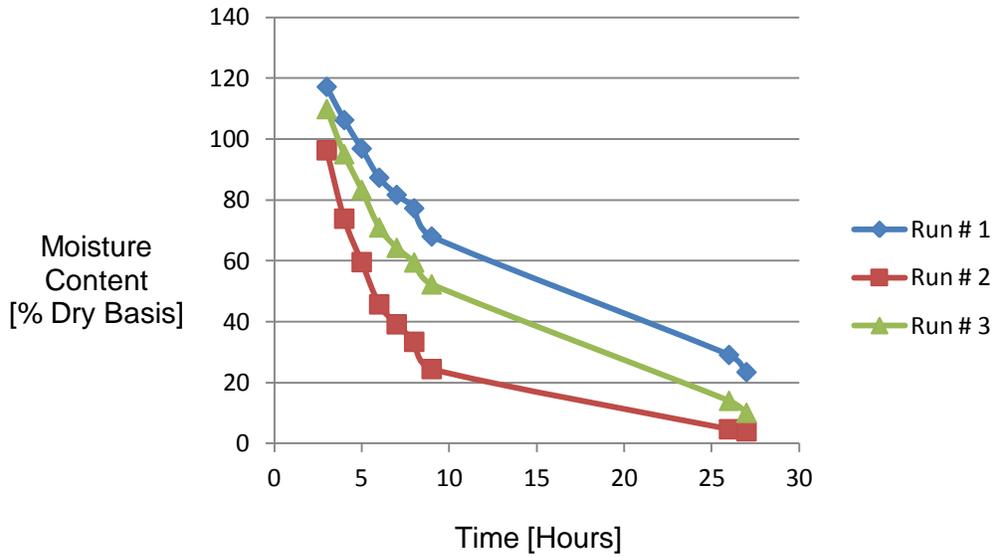


Figure 3-4. Replicate Drying Curves for Drying Cassava Slices into Chips.

overall size distribution

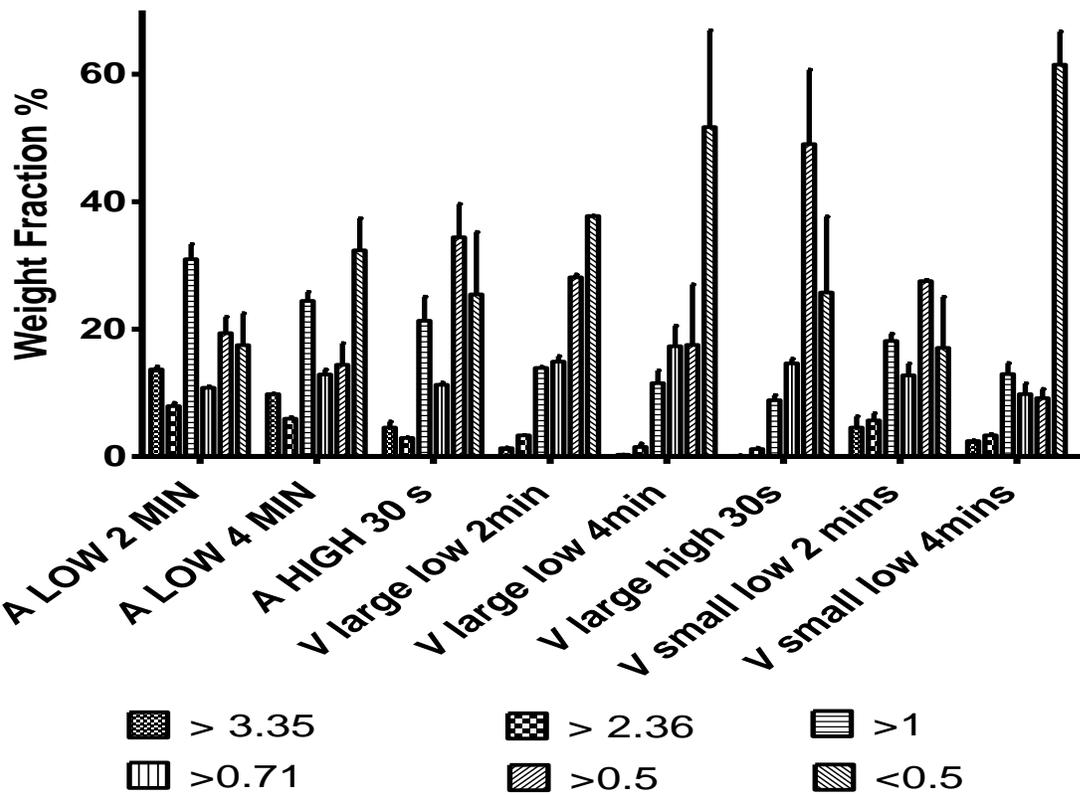


Figure 3-5. Particle Size Distribution in Cassava Flour from Grinding Dry Cassava Chips under various Blender Operating Conditions.

combinations of grinding time and blender speed (high or low) on chips made from two different varieties of cassava, Algodon (A) and Valencia (V) and two different chip thicknesses, small (2mm) and large (4mm). Small chips from Valencia roots were chosen for the study. The profiles for these small chips for two different blending times at slow speed appear at the far right side of the figure. The profile obtained from four minutes at low speed produced a sample in which more than 60% of the sample weight consisted of the desired particle size (less than 0.5 mm). These were the grinding conditions chosen for producing the samples of flour used in this study.

Physical properties of moisture content, bulk density, solid particle density, porosity, permeability and specific surface area are summarized in Table 3-2, along with statistics showing number of replicate measurements and minimum and maximum values found among the replicates.

Table 3-2. Physical properties of cassava flour with particle size ≤ 0.5 mm.

Physical Property	Mean Value	Number of Replicates	Minimum Value	Maximum Value
Moisture Content (%db)	12.35	2	12.34	12.37
Bulk Density (kg/m ³)	550	2	530	570
Solid Particle Density (kg/m ³)	1,480	2	1,442	1,517
Porosity (Dimensionless)	0.636	2	0.630	0.642
Permeability (m ²)	1.0 x 10 ⁻⁶	2	1 x 10 ⁻⁶	1 x 10 ⁻⁶
Specific Surface Area (m ² /m ³)	367	2	367	367

3.5 Summary

The measurements of drying characteristics and physical properties of cassava chips and flour were carried out and reported in this study. These included a sorption isotherm and drying curve on the chips, and moisture content, particle size distribution, bulk density, solid particle density, porosity, permeability and specific surface area of the flour. A prototype solar-convection dryer was used to produce the dried cassava chips which were ground into flour as a means of preservation and adding value to the cassava crop. Data on physical properties reported in this study will be most useful to carry out engineering design of storage, handling, and processing systems for the flour thus produced.

CHAPTER 4 SYNERGISTIC ENZYMATIC HYDROLYSIS OF CASSAVA STARCH

4.1 Background

Sugar generally refers to a class of chemically-related sweet-flavored substances, most of which are used as food. They are carbohydrates, composed of carbon, hydrogen and oxygen. There are different types of sugar which include monosaccharides (fructose, galactose, glucose); disaccharides (lactose, maltose, sucrose); and many others (Wikipedia, 2013 a; Damodaram et al., 2008). Sucrose can be extracted from plants such as sugarcane and sugar beet while fructose and glucose may be derived from starch by hydrolysis reactions. Global sugar production and consumption has been rising in recent years. In 2009/2010, 2011/2012, and 2012/2013, global sugar production (and consumption) were respectively 153.6×10^9 kg (153.7×10^9 kg); 168.2×10^9 kg (160.2×10^9 kg); and 174.0×10^9 kg (163.0×10^9 kg) (USDA, 2013). In 1999, per capita sugar consumption in the world was 24 kg, and this is estimated to reach 25.1 kg by 2015 (FAO, 2002). In the United States, per capita consumption of refined sugar in 1972 was 46.2 kg. This rose to 71.6 kg in 1999; and 48.8% of the total was corn derived sweeteners (Huntrods et al., 2012; Coulston and Johnson, 2002).

Starch is fundamental raw material for production of glucose and fructose sweeteners. These sweeteners are important commercial commodities used extensively in bakery, beverage, confectionery, food, pharmaceutical, and allied industries. However, a great proportion of starch used in the world today comes from corn/maize (Johnson et al., 2005; De Braganca and Fowler, 2004; De Baere, 1999). Similarly, most sweeteners used are produced from corn starch (BeMiller, 2009; Gordon, 1999;

Swinkles, 1985). The conventional method of glucose sweetener production requires first extraction of starch from its raw material, followed by two separate unit operations: Liquefaction and Saccharification (Johnson et al., 2009; Berghofer and Sarhaddar, 1988; Solomon, 1978; Aschengreen, 1975; Pomeranz et al., 1964). This rather round about procedures impose constraints and implications in terms of energy; enzyme; labor; equipment; technology; time and associated costs. It would be more efficient to hydrolyze the starch in its native raw material and to conduct liquefaction and saccharification operations simultaneously in the same reactor if possible.

It has been stated that sweeteners can be made from any starch, and that such sweeteners are essentially identical regardless of which starch is used as the raw material (BeMiller, 2002; Aschengreen et al., 1979). It is also reported that fungal amylases can be used to liquefy starch irrespective of the source of the starch (Witt, 1985). Cassava root is said to be richly endowed with good quality starch to the extent of 30 – 32 wt% (Westby, 2002; International Starch Institute, 1999; O'hair, 1995; Balagopalan et al., 1988). In addition, Cassava starch has been reported to exhibit vital benefits and advantages over other sources of starch such as wheat, corn, and waxy maize (Central Tuber Crops Research Institute, 2010; Juszczak et al., 2003; Larotonda, et al., 2003; Balagopalan, 2002; Lopez-Ulibarri and Hall, 1997; Balagopalan et al., 1988; Franco et al., 1988; Vijayagopal et al., 1988; Cereda and Wosiacki, 1985; Moorthy, 1985; Swinkels, 1985). Therefore cassava crop can be an alternative source of starch for production of sweeteners.

Attempts have been made to obtain reducing or fermentable sugars from starch sources other than corn. Researchers have used cereal grains, seeds, stems and

tubers in various experiments. Nakamura and Park (1975) characterized some physico-chemical properties of cassava starch. Their work demonstrated that fermented cassava starch is more soluble in water, and also less viscous when heated, than unfermented starch. Kearsley and Nketsia-Tabiri (1979) conducted hydrolysis experiments with commercial starches as well as whole grains, tubers and roots of rice, wheat, potato, and cassava. They concluded that it was possible to produce glucose syrups from a wide variety of starch containing crops. Aschengreen et al., (1979) liquefied, saccharified and isomerized starches from sources other than maize, including potato and tapioca. Greenfield and Brooks (1982) investigated the effect of size reduction operations on liquefaction, saccharification and fermentation of cassava roots and stems to ethanol. They discovered that the resulting bimodal particle size distribution peaked around 15 μm ; and represented released starch granules, all of which were readily hydrolysable. Hoehn et al., (1983), produced high fructose syrups from Jerusalem artichoke tubers. Monma et al., (1989) successfully digested starches from potato, rice and sago. Gorinstein and Lii (1992) subjected amaranth, cassava and potato starches to enzymatic hydrolysis. Their report revealed that cassava starch is less resistant to thermostable alpha-amylase than amaranth and potato starches. These cited works clearly demonstrated that reducing and fermentable sugars can be produced from various starch sources other than corn.

However, a major limitation of conventional production of glucose sweetener is the two separate operations of liquefaction and saccharification. Liquefaction is the first stage whereby starch is gelatinized by heat treatment and thermostable alpha-amylase partially hydrolyses the starch to maltodextrins. Saccharification commences in the

second stage where the partially hydrolyzed starch is converted to glucose by the action of glucoamylase. This protocol requires more process vessels, piping, and associated equipment; conditioning for enzyme systems; and gelatinization is energy intensive. It would be advantageous to deploy alpha-amylase and glucoamylase simultaneously and carry out both liquefaction and saccharification processes inside the same reactor vessel as a single unit operation. This option is simple, and more time, energy, equipment and labor efficient. It is termed synergism and has been studied by several authors.

Gauss et al., (1976) patented a process for alcohol production by simultaneous saccharification and fermentation of cellulosic materials with cellulase enzyme and alcohol producing microorganism. Wankhede and Ramteke (1982) studied the synergistic digestibility of native starches by various enzymes. Substrates studied included Ragi (*Eleusine coracana*), Foxtail millet (*Sataria italica*), Jowar (*Sorghum vulgare*) and Rice (*Oryza sativa*). The study revealed that where one enzyme system hydrolyzed native starch by 35.58%, synergistic action hydrolyzed 71.20%. The works of Fujii et al., (1981), Fujii and Kawamura (1985), and Fujii et al., (1988) illuminated synergistic hydrolysis mechanism of alpha-amylase and glucoamylase. According to their model, alpha-amylase catalyzes endo-wise random cleavage of large molecules on the surface of starch granules. The splitting action produces maltodextrins and non reducing sugars that blanket the granule, inhibiting alpha-amylase from further attacks on the substrate. At this juncture, glucoamylase peels off the blanket of maltodextrins, hydrolyzing them to glucose and thereby exposing interior glycosidic bonds of the substrate to new surface attacks by alpha-amylase. After the molecular weight of the

substrate decreases to about 5000, the action of alpha-amylase can be neglected and the rate of formation of glucose is governed by the kinetics of glucoamylase (Fujii and Kawamura, 1985). The cooperative action between alpha-amylase and glucoamylase constitutes synergism and results in enhanced hydrolysis yields and increased enzyme activity, perhaps making the energy intensive gelatinization pretreatment process unnecessary.

Franco et al., (1989) demonstrated increased levels of reducing sugars (glucose and maltose) in hydrolysis systems subjected to synergistic actions of alpha-amylase and glucoamylase. Rattanachomsri et al., (2009) reported increased fermentable sugars (glucose and xylose) and alcohol production from simultaneous multi-enzyme saccharification and yeast fermentation. The process was said to be energy efficient by obviating the pre-gelatinization step. Arasaratnam and Balasubramaniam (1993), observed that synergistic hydrolysis of dry-milled starch was more efficient than wet-milled starch; and that optimum ratio of glucoamylase to alpha-amylase was 1.8 AGU (Amyloglucosidase Unit)/1.0 KNU (Kilo Novo Unit). Shevel'kova and Sinitsyn (1993), reported that the synergistic effect of alpha-amylase and glucoamylase hydrolysis of starch is a function of many variables including substrate composition, concentration, and degree of polymerization, as well as reaction duration. The synergistic technique was applied to production of lactic acid. Linko and Javanainen (1996) claimed 98% yield of lactic acid when 130 g/L starch was simultaneously liquefied, saccharified and fermented. Lactic acid concentration of 162 g/L was reported. Similarly, Anuadha et al. (1999), reported higher yields of 1.21 g/L.hour lactic acid production for simultaneous systems over conventional two step processes. Liakopoulou-Kyriakides et al. (2001),

investigated the synergistic effects of different combinations of alpha-amylase and glucoamylase from various sources. Their report showed that the combination of *Bacillus licheniformis* alpha-amylase and *Rhizopus* mold glucoamylase gave the highest substrate conversion of 76% at 50 ° C; and that combination of *Bacillus licheniformis* alpha-amylase and *Aspergillus niger* glucoamylase enzymes retained initial activity after incubation at 60 ° C.

Another important constraint to conventional glucose sweetener production is the time, energy, and labor intensive starch extraction process. Most of the work reported in literature on glucose production used model systems of soluble or commercial starches. Very few authors have tried the possibility of using direct conversion technique. Direct conversion procedure involves hydrolyzing native starch in raw materials with minimal value adding processing history. Lages and Tannenbaum (1978), conducted laboratory scale batch operations for the production of glucose from tapioca meal (also called farinha de mandioca). Tapioca meal is the flour produced after rasped peeled cassava roots are toasted and dried. The authors reported virtual 100% conversion of the meal after 24 – 36 hours of hydrolysis. This compares to 12 – 24 hours required for 100% conversion of pure cassava starch. Menezes et al., (1978), hydrolyzed cassava root slurry with alpha-amylase and glucoamylase in conventional two stage hydrolysis procedure. However, fungal cellulase was synergistically added at the saccharification stage. The authors reported not only increased rate of sugar formation and starch conversion, but also improved rheological properties. Kearsley and Nketsia-Tabiri (1979), performed direct hydrolysis of native starches in grains, roots and tubers with alpha- and beta amylase enzymes. The results for alpha amylase experiments shown

here in Table 4-1 indicated that unprocessed native starches compared favorably with commercial starches, especially in total starch conversion.

Table 4-1. Some Results of Alpha Amylase Hydrolysis of Starch from Sources other than corn (Kearsley and Nketsia-Tabiri, 1979)

Starch Source	Dextrose Equivalent (DE) of Hydrolysate at 24 Hours	Percent Starch Conversion at 24 Hours
Commercial Starches		
Maize	43	98
Rice	41	96
Wheat	44	94
Potato	40	94
Whole Grain A (Milled Large Particles)		
Maize	22	92
Rice	36	94
Wheat	33	100
Whole Grain B (Milled Small Particles)		
Maize	28	89
Rice	41	93
Wheat	38	99
Whole Tubers and Roots (Minced Particles)		
Potato	30	86
Cassava	38	100

Sreekantiah and Rao (1980), used mixed culture fermentation, enzyme saccharification, and acid liquefaction to produce ethyl alcohol from fresh potatoes, dehydrated tapioca, and dehydrated sweet potatoes. They reported corresponding fermentation efficiencies of 68%, 81%, and 75% for the respective substrates. Maeda et

al. (1982), subjected dried cassava chips slurry to alpha amylase liquefaction. After a clarification and centrifugation step aided by various enzymes, the supernatant fluid was continuously saccharified by immobilized glucoamylase gel. The authors reported glucose content of resulting product to be 94.4%. Srikanta et al., (1987), reported production of ethanol from fresh cassava roots, flour and starch. After liquefaction of 20% solids content substrates with alpha amylase, saccharification was executed with moldy bran or commercial glucoamylase before fermentation with yeast inoculation. Their result indicated that after 4 hours of saccharification at 60 ° C, the hydrolysis scheme yielded 9 – 10% reducing sugars which on fermentation, generated alcohol fermentation efficiencies of 95 to 98% among the different substrates. Berghofer and Sarhaddar (1988), investigated glucose production directly from fresh and dried cassava roots. The authors reported conversion yield of 88 – 89% of the overall starch content of raw material (fresh unpeeled tuber) compared to 82 – 87% conversion through conventional starch extraction processes.

Ghildyal et al., (1989), performed comparative economic analysis of the production of high fructose syrup from cassava chips and extracted cassava starch. They reported that the unit cost for production of 10 t of high fructose syrup per day was US \$ 0.51/kg and US \$ 0.46/kg respectively for cassava chips and extracted cassava starch. They noted that the gain in lower expenses on cassava chips raw material as compared to extracted cassava starch was upset by higher capital investment on plant and machinery; as well as higher operating expenses on process steam, activated carbon, and purification requirements for cassava chips products. The authors concluded that direct utilization of cassava chips does not offer any economic

advantage. Johnson et al., (2009), applied the direct conversion technique to production of glucose and high fructose syrup from cassava roots and sweet potato tubers. They used various enzymes and reported glucose yields of 22 – 25% and 14.0 – 15.7% for cassava roots and sweet potato tubers respectively. Fructose yields were reported to be 8.36 – 9.78% for cassava, and 5.2 – 6.0% for sweet potato. Total starch conversion to glucose was reported to be higher with cassava at 95 – 98%; compared to 88 – 92% with sweet potato. However, the authors used the conventional two-step procedure of liquefaction followed by saccharification, except that one of the enzymes used; Stargen™ 001, had both alpha amylase and glucoamylase activities.

From extensive literature search, it is observed that there is dearth of information on direct conversion technique on the one hand; and on synergistic procedure on the other hand, especially where glucose sweetener is the desired end product. Most of the reported work was done for the production of alcohol or lactic acid, where reducing sugar such as glucose is a transition item to be fermented to the desired end product.

4.2 Objectives

Based on the foregoing, the objectives of the work undertaken in this study were to:

1. Combine both direct conversion and synergistic techniques in the production of glucose sweetener, using cassava crop as the raw materials source.
2. Demonstrate the conversion of starch to glucose sweetener from synergistic hydrolysis with commercial enzymes on the following substrates:
 - a) Commercially available refined cassava starch.
 - b) Flour produced from solar-convection dried cassava chips.
 - c) Freshly ground cassava root pulp.

- Estimate reaction kinetics parameters such as first-order rate constant and Arrhenius activation energy from enzyme hydrolysis of each substrate at two different temperatures.

4.3 Materials and Methods

4.3.1 Enzymes

The enzymes used in this study are presented in Table 4-2.

Table 4-2. Statistics of Enzymes used in the Research Work

Enzyme Manufacturer	Manufacturer's Product Name	Type of Enzyme	Source of Enzyme	Enzyme Activity
Bio-Cat Inc.	Fungal Amylase	Alpha amylase	Fungal: <i>Aspergillus oryzae</i>	100,000 SKB/g. One SKB Unit is the number of grams of soluble starch dextrinized, in the presence of excess beta amylase per hour under the assay conditions
	Glucoamylase	Glucoamylase	Fungal: <i>Aspergillus niger</i>	1000 AG/g. One AG Unit is the amount of glucoamylase that will liberate 0.1 mol of p-nitrophenol per minute from PNPG solution under the assay conditions
BioSun	Alpha Amylase	Alpha Amylase	Bacterial: <i>Bacillus subtilis</i>	5×10^6 BAAU/g. One BAAU Unit is amount of enzyme that breaks down 5.26 mg of Lintner starch per hour under the assay conditions
	Starch Clear-ase	Glucoamylase	Fungal: <i>Aspergillus niger</i>	200 GAU/g One GAU Unit is the amount of enzyme that breaks down 0.1 mole of p-nitrophenol per minute from a substrate of PNPG under the assay conditions.
DSM	Validase BAA 1000L	Alpha Amylase	Bacterial: <i>Bacillus subtilis</i>	1×10^6 MWU/g. One MWU Unit is the amount of enzyme activity that will dextrinize 1 mg of soluble starch to a defined blue color in thirty minutes under the assay conditions
	Validase 400L	GA Glucoamylase	Fungal: <i>Aspergillus niger</i>	400 GAU/mL. One GAU Unit is the amount of enzyme activity that will liberate 1 g of reducing sugar as D-glucose per hour under the assay conditions.

Table 4-2. Continued. Statistics of Enzymes used in the Research Work

Enzyme Manufacturer	Manufacturer's Product Name	Type of Enzyme	Source of Enzyme	Enzyme Activity
Genencor	Spezyme Fred	Alpha amylase	Bacterial: <i>Bacillus licheniformis</i>	17400 LU/g. One LU Unit is the measure of the digestion time required to produce a color change with iodine solution, indicating a definite stage of dextrinization of starch substrate under the assay conditions
	Optidex L-400	Glucoamylase	Fungal: <i>Aspergillus niger</i>	350 GAU/g. One GAU Unit is the amount of enzyme that will liberate 1 g of reducing sugar as glucose from soluble starch substrate per hour under the assay conditions.
	Gensweet SGI	Glucose Isomerase	Bacterial: <i>Streptomyces rubiginosus</i>	3000 GIU/g. One GIU Unit is the amount of enzyme that will convert one micromole of glucose to fructose per minute under the assay conditions.
Novozymes	Liquozyme Supra	Alpha amylase	Bacterial: <i>Bacillus licheniformis</i>	135 KNU/g
	Dextrozyme DX	Glucoamylase & Pullulanase	Fungal: <i>Aspergillus niger</i> and Bacterial: <i>Bacillus subtilis</i>	170 AGU/g & 340 NPUN/g
	Sweetzyme IT	Glucose Isomerase	Bacterial: <i>Streptomyces murinus</i>	400 IGIU-D/g

These enzymes were graciously donated by their respective manufacturers. After initial trials of preliminary studies, Bacterial alpha amylase (Activity: 5×10^6 BAAU/g) and fungal glucoamylase (Activity: 1000 AG/g) were used synergistically in all experiments.

4.3.2 Substrates

Three types of cassava substrates: Root, Flour and Starch were used in all experiments.

4.3.2.1 Roots

Fresh roots were purchased commercially from Brooks Tropicals, Homestead, Florida. The roots were coated with food wax and supplied refrigerated in 18 kg boxes. Each consignment or batch was promptly received and maintained under refrigeration ($< 10^{\circ}\text{C}$) until utilized.

4.3.2.2 Flour

All the flour used in the experiments was of particle size $\leq 45\ \mu\text{m}$. The flour was produced in our laboratories from solar dried chips of the fresh roots.

4.3.2.3 Starch

Cassava starch was purchased from commercial source; My Spice Sage, Yonkers, New York. The starch was supplied in 25 kg packaging and was stored under ambient conditions ($\approx 22^{\circ}\text{C}$, 38% RH).

4.3.3 Sample Preparation

4.3.3.1 Root

Fresh cassava roots were retrieved from refrigeration, placed on a cutting board (Figure 4-1A), and peeled with a kitchen knife (Figure 4-1B). The peeling process involved carefully removing the peel (pellicle (periderm or epidermis) and cortex) from the parenchymatous tissue. The exposed parenchyma was cut into slices of about 10 cm long x 3 cm wide x 3 cm deep dimensions. Subsequently, the slices were rasped into shreds with a kitchen grater (Figure 4-1C). The shreds were pulverized into smooth paste with a blender. Two types of blender were used. Figure 4-1D: Osterizer Designer Cycle Blender (Sunbean Corporation, Fort Lauderdale, Florida), and Figure 4-1E: SmartPower Cuisinart Blender, Model CPB-300 (Intertek Group, London, England). One

blender was used when the other required a period of cooling off. The paste was constituted with distilled water to obtain a 10% (w/w) suspension.

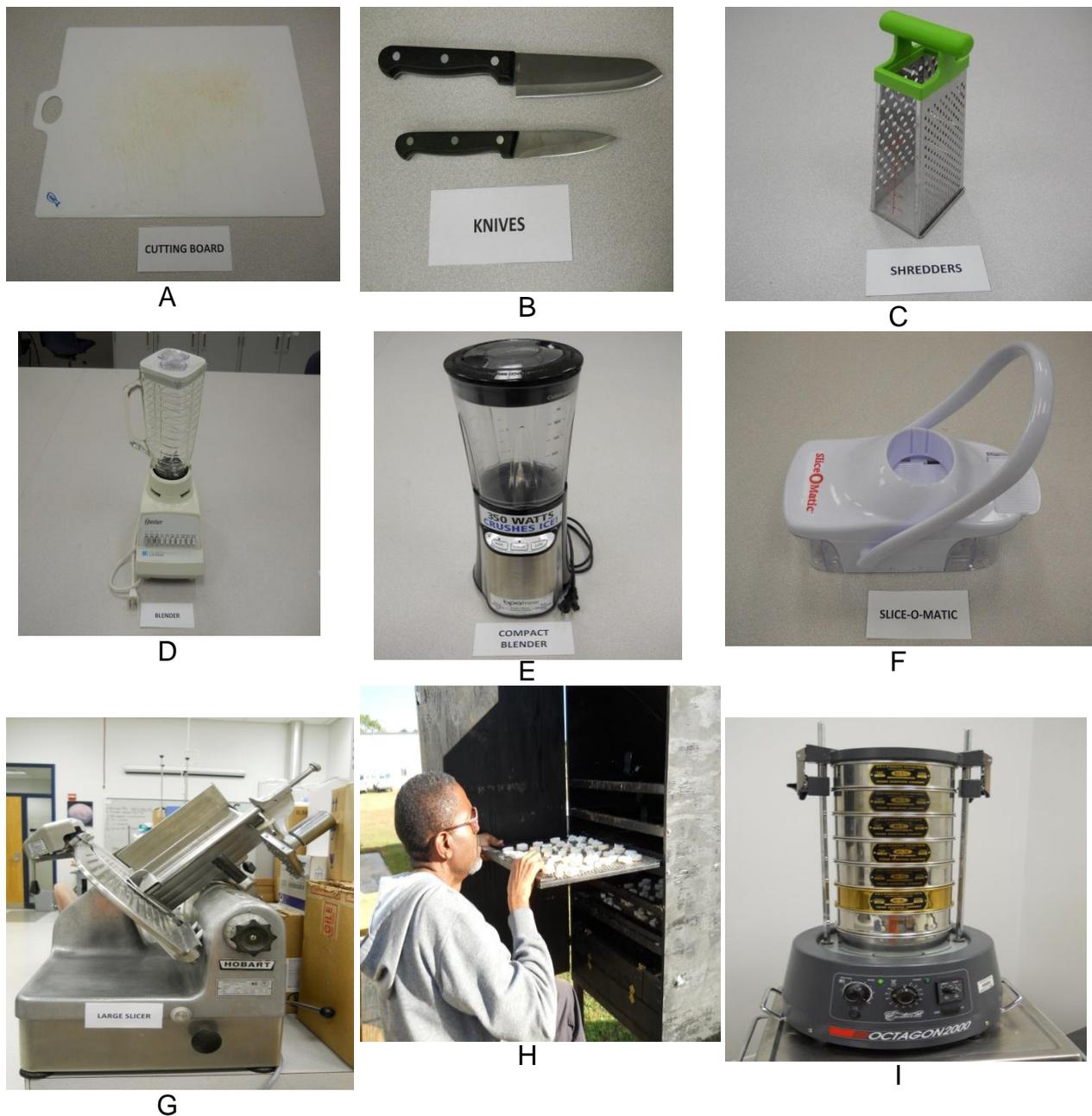


Figure 4-1. Size Reduction Devices used in Sample Preparations. A) Cutting Board B) Knives C) Grater D) Blender 1 E) Blender 2 F) Slice-O-Matic G) Hobart Slicer H) Solar Convection Dryer I) Vibrating Sieving Machine. All photos courtesy of the author.

4.3.3.2 Flour

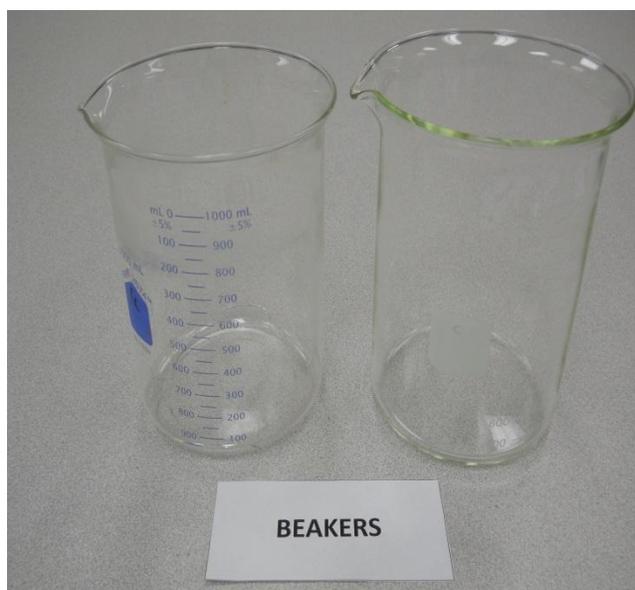
Depending on size of root, either a kitchen knife (Figure 4-1B) or one of two slicers: Slice-O-Matic Slicer (TeleBrands, Fairfield, New Jersey; Figure 4-1F) or Hobart Slicer, Model 1712E (Hobart Corporation, Troy, Ohio; Figure 4-1G) was used for slicing. Peeled roots were sliced into approximately circular chips of 0.5 cm thickness. The sliced chips were subsequently dried to less than 10% moisture content dry basis using a solar natural convection dryer developed at the University of Florida (Schiavone et al., 2013; Figure 4-1H). It took about 1.5 to 3 days to achieve required drying, depending on loading density, solar radiation and other weather and operating conditions. Dried chips were pulverized with blenders and sieved through 45 μm pore sieve (ASTME Standard Sieve No. 325) with the aid of a vibrating stacked sieving machine: Octagon 2000; Serial No. OCT 2000/2 340 96 (Endocotts Limited, London, England; Figure 4-1I). Flour produced was constituted with distilled water to achieve a 10% (w/w) suspension.

4.3.3.3 Starch

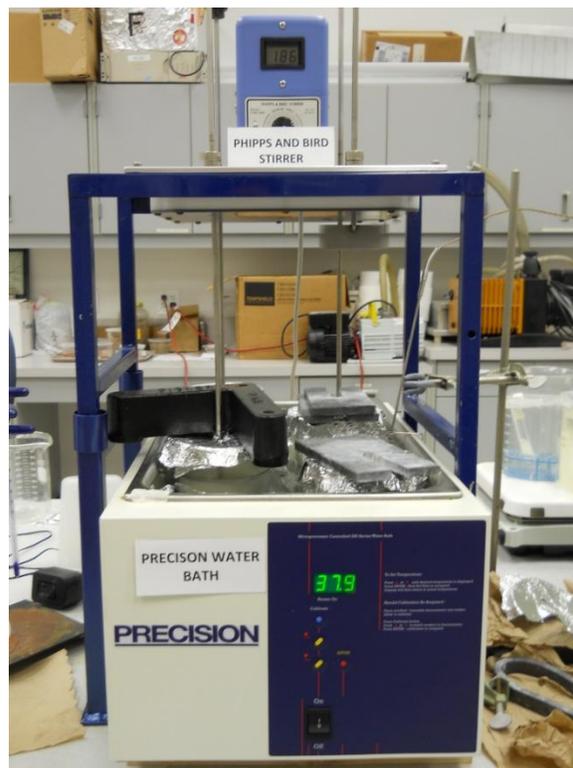
The commercial cassava starch purchased was constituted with distilled water to achieve a 10% (w/w) suspension.

4.3.4 Reactor

The batch reactors used for hydrolysis experiments are shown in Figure 4-2A. They were 1000 mL capacity Pyrex Griffin Beakers (Fisher Scientific Inc., Waltham Massachusetts). The beakers were mounted in a precision microprocessor controlled water bath, Model 285 (Thermo Scientific Inc., Waltham Massachusetts). The system was coupled to a Phipps and Bird Stirrer (Phipps and Bird Inc., Richmond, Virginia). A typical setup of an experimental run of the reactors, water bath and stirrer is presented in Figure 4-2B.



A



B

Figure 4-2. Reactor Devices and Systems for Hydrolysis Experiments A) Reactor Beakers for Hydrolysis Experiments B) Typical Setup of a Test Run System with Reactors, Water Bath and Stirrer. Photos courtesy of the author.

4.3.5 Analytical Methods

4.3.5.1 Proximate composition, starch, and sugar analysis

Proximate composition, starch, and sugar profile analyses were conducted by ABC Research Laboratories, Gainesville, Florida. Samples of cassava root, flour from solar dried chips, and commercial starch were delivered to ABC Research Laboratories sealed in transparent plastic sachets and bowls. Aliquots of selected hydrolysate samples were delivered in sanitary laboratory vials under refrigerated conditions. Reports from ABC Research Laboratories indicated that the following assay methods (Table 4-3) were used, with modifications where appropriate.

Table 4-3. Assay Methods used by ABC Research Laboratories for Analysis of Samples

S/N	Component	Assay Method
1	Ash	AOAC 942.05, 920.153
2	Calories	By Computation
3	Carbohydrate	By Computation
4	Crude Fiber	AOAC 978.10
5	Fat	AOAC 948.15
6	Moisture	AOAC – Moisture
7	Protein	AOAC 981.10
8	Starch	AOAC 996.11
9	Total Pectin <i>Sugars</i>	JBT 6 th Edition # 23; AES Bulletin 570 1
10	Fructose	AOAC 977.20 MOD
11	Glucose	AOAC 977.20 MOD
12	Lactose	AOAC 977.20 MOD
13	Maltose	AOAC 977.20 MOD
14	Sucrose	AOAC 977.20 MOD

4.3.5.2 Glucose Analysis

Glucose is a monosaccharide carbohydrate because it cannot be broken down to any simpler carbohydrate molecule via hydrolysis. Therefore, glucose like any other monosaccharide is called a simple sugar. Glucose is also classified as an aldose. An aldose is a monosaccharide whose carbonyl function is an aldehyde (known as the aldehydo group). A second group of monosaccharides are called ketoses. A ketose is a monosaccharide whose carbonyl function is a ketone (the keto group). Oxidation is the loss of electrons while reduction is the gain of electrons. In a chemical reaction, aldoses can readily give up electrons to an oxidizing agent which accepts electrons from the aldose. In such a reaction, the oxidizing agent is reduced and the aldose is oxidized by acting as reducing agent. Aldoses are therefore referred to as reducing sugars.

However, under alkaline conditions, ketoses are isomerized to aldoses and can thus act as reducing sugars (BeMiller and Huber, 2008). A reducing sugar may therefore be defined as a sugar that contains the free aldehyde carbonyl group or is capable of forming such a free group. Examples of reducing sugars are: arabinose, xylose (pentoses); galactose, glucose, mannose (hexoses). Pentoses have five carbon atoms while hexoses have six carbon atoms (BeMiller, 2010). “The presence of any substituent on the anomeric hydroxyl group of a sugar prevents the sugar from undergoing the equilibrium between ring forms and the open-chain form” (Dr. Jesse F Gregory, 2012; Personal Communication). Consequently, such a sugar does not have a free or reducing end; and so is not reactive. Sucrose is not a reducing sugar because sucrose does not have a free carbonyl group or reducing end. Both anomeric carbons on sucrose are in glycosidic linkage.

4.3.5.3 Determination of Reducing Sugar

One analytical technique used to determine reducing sugars is the 3,5-dinitrosalicylic acid (DNS) method. It was developed by James Sumner and coworker in 1921 to estimate reducing sugars in diabetic patients (Sumner, 1924; Sumner and Graham, 1921). The DNS method tests for the presence of free carbonyl (aldehyde) group in a sample. This is achieved by the oxidation/reduction reaction pathway. When 3,5-dinitrosalicylic acid is reacted with a reducing sugar, aldehyde group is oxidized while 3,5-dinitrosalicylic acid is reduced. The reaction is such that the nitrate group (NO_2) in 3,5-dinitrosalicylic acid is reduced to the amino group (NH_2). The reduction product is called 3-amino-5-nitrosalicylic acid. Figure 4-3 represents classical stoichiometry of the reaction scheme. 3-amino-5-nitrosalicylic acid is a colored aromatic

compound that absorbs light strongly at 540 nm. The absorbance can be read with a spectrophotometer and used to estimate the quantity of reducing sugar in a sample.

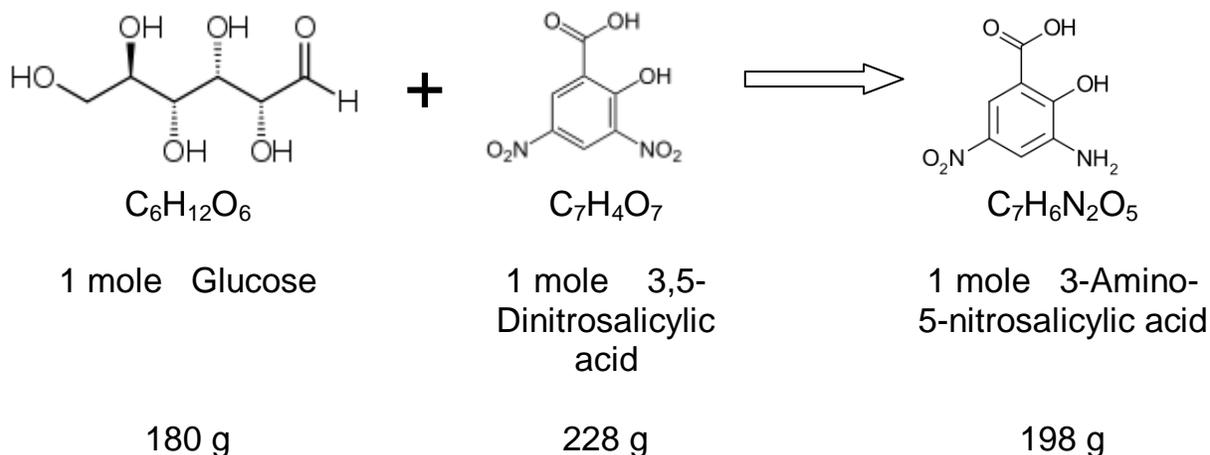


Figure 4-3. Stoichiometry for the Detection of Reducing Sugars as Glucose through Reactions with 3,5-dinitrosalicylic acid (DNS) Reagents

The DNS technique has been adopted, modified, adapted and utilized in various materials, products, and applications. Sumner (1935) applied it to the determination of saccharase activity. Bernfeld (1955) suggested the technique be used to estimate alpha and beta amylases activities. In 1959, Gail Miller modified the reagent to minimize the loss of sugar in the assay (Miller, 1959); and in 1973 Lindsay applied the assay to the measurement of reducing sugars in potatoes (Lindsay, 1973). In the work presented here, the DNS Reagent was composed as follows (Lindsay, 1973; Bernfeld, 1955):

- 1 g of 3,5-Dinitrosalicylic acid
- 20 cm³ of 2M Sodium Hydroxide [2M = Mix 2 g NaOH with 25 cm³ distilled water]
- 50 cm³ of Distilled water
- 30 g of Sodium Potassium Tartrate Tetrahydrate (Rochelle Salt)
- Add distilled water to bring volume to 100 cm³

In the assay for reducing sugars as glucose, 1 mL of relevant hydrolysate or control sample (starch, flour or root) was reacted with 2 mL of DNS reagent in a 10 mL test tube. The test tube containing the mixture was heated for 10 minutes in boiling water and then cooled to room temperature. After addition of 2 mL of distilled water, the absorbance of the reaction product was read at 570 nm with a spectrophotometer (Evolution 60, Model No. 840-189600, Thermo Fisher Scientific Inc., Waltham Massachusetts; Figure 4-4A or Milton Roy Analytical Spectrophotometer, Model No. 335402, Milton Roy Analytical Products, Rochester, New York; Figure 4-4B). A blank sample was prepared in the same manner but without the hydrolysate. The blank was used to zero the spectrophotometers. The percentage of reducing sugar (as glucose) in each sample was estimated by reference to a calibration curve (Figure 4-5); established with reagent grade D-Glucose



A



B

Figure 4-4. Spectrophotometers used for Measurement of Absorbance in Samples Reacted with DNS Reagent in Order to Determine Reducing Sugars as Glucose. A) Evolution 60 Spectrophotometer B) Milton Roy Spectrophotometer. Photos courtesy of the author.

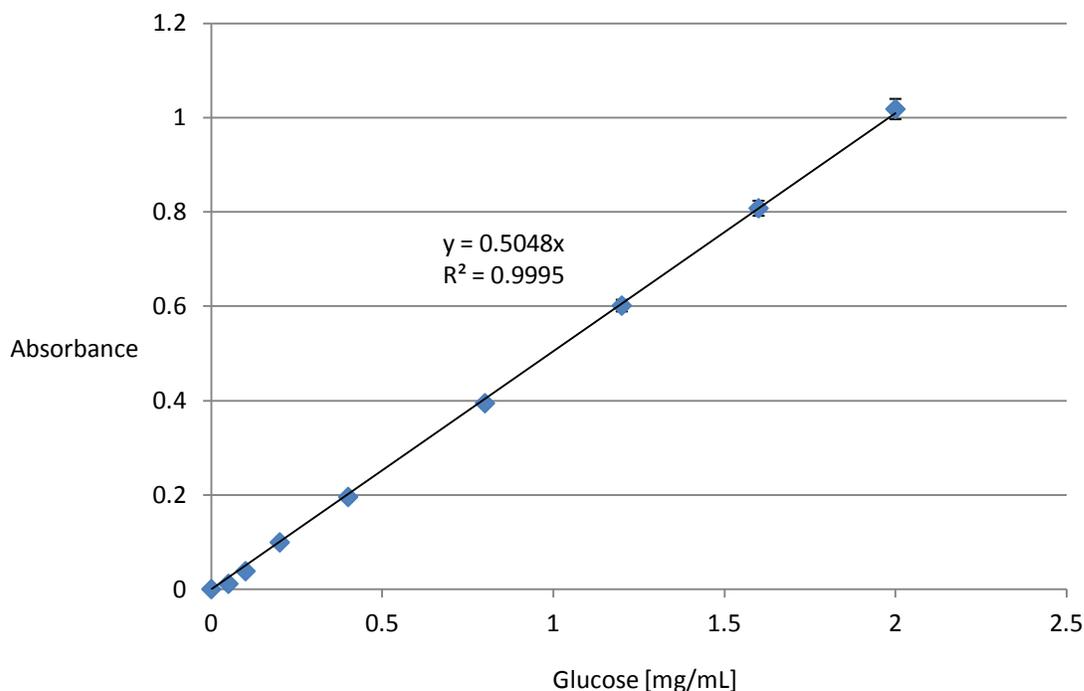


Figure 4-5. Calibration Curve used for Estimation of Reducing Sugars (As Glucose) in the 3, 5-dinitrosalicylic acid (DNS) Analytical Method. The Absorbance was read with Spectrophotometers at 570 nm with 1 cm path length cuvette cells. (Graph is showing the mean of six replications with error bars at two standard deviations).

4.3.6 Experimental Procedure

The experiments were conducted in batches and at two temperatures: 60 ° C and 37 ° C. Total reaction mass for each batch was 800 g and the repartition of components was as follows: Substrate, 10%; Enzyme, 0.015%; Distilled water made up the balance. The two enzymes applied synergistically were bacterial alpha amylase (BAA) and fungal glucoamylase (FGA). The ratio of enzyme application was 3 : 1 (FGA : BAA). A typical sequence of experimental run at 60 ° C required preheating the water bath to the operating temperature. The desired substrate was constituted in the reactor beaker and an aliquot \approx 7 mL was withdrawn into a test tube to be used to characterize the reaction

mass at time zero (T_0). Mass of the beaker with sample was then measured and recorded (Ohaus Adventure Scale, Model AR 5120, Ohaus Corporation, Pine Brook, New Jersey; Figure 4-6A).



Figure 4-6. Scales used for Mass Measurements. A) Ohaus Scale; Precision at 0.1 g
B) Mettler Scale; Precision at 0.001 g. Photos courtesy of the author.

The weighed reactor was carefully positioned in the water bath and covered with aluminum foil sheet that had a slit. The flat blade stirrer was inserted through the slit and installed so as not to touch any side or bottom of the beaker. A mercury-in-glass thermometer was also installed into the reactor beaker for temperature tracking. Finally, a fabricated steel mass was placed over the reactor as a ballast, to insure stability. The stirrer was then started to operate at 180 ± 5 rpm. On attainment of the operating temperature, in this case 60°C , the two enzymes were carefully measured out, introduced into the reactor simultaneously, and hydrolysis proceeded. Measurement of the mass of enzymes to three decimal places was achieved with Mettler Scale, Model PE 160 (Mettler-Toledo, Columbus, Ohio; Figure 4-6B). Samples were withdrawn for analysis at predetermined time intervals (T_1 : 5 minutes (0.083 hours); T_2 : 10 minutes

(0.166 hours); T3: 15 minutes (0.25 hours); T4: 30 minutes (0.5 hours); T5: 45 minutes (0.75 hours); T6: 60 minutes (1.0 hour); T7: 90 minutes (1.5 hours); T8: 120 minutes (2.0 hours); T9: 180 minutes (3.0 hours); T10: 240 minutes (4.0 hours); T11: 480 minutes (8.0 hours); T12: 720 minutes (12.0 hours); and T13: 1440 minutes (24.0 hours)}. At each sampling time, the stirrer was stopped. The stirrer and thermometer were disengaged. The reactor beaker with substrate sample was then withdrawn from the water bath, wiped dry with a clean cloth and reweighed. The water lost by evaporation was replaced with distilled water at 60 ° C. A separate beaker with distilled water was maintained in the water bath for this purpose. The replaced evaporated water was thoroughly stirred into the reaction mass with a metal spatula to achieve a homogeneous hydrolysate. At this juncture, all necessary measurements (pH, soluble solids, etc) were made and ≈ 7 mL aliquot of hydrolysate was withdrawn with a pipette (Eppendorf, Hamburg, Germany; Figure 4-7) into a test tube. The test tube with aliquot



Figure 4-7. Pipettes used in Sample withdrawals and Analysis. Photo courtesy of the author.

was capped and immediately immersed into boiling water for thirty minutes to inactivate enzyme activities. Boiled samples were stored under refrigeration until assayed (within

48 hours) for reducing sugars as glucose using the DNS methodology. A control reactor without enzyme input was incubated and sampled in a similar way.

The mass of the beaker and remaining substrate sample was weighed again and recorded. The beaker was returned to the water bath; the stirrer, thermometer, aluminum foil sheet and steel ballast were reinstalled and timing for the experiment was continued when the reaction mass temperature reached 60 ° C. The time spent in the sampling process was discounted. In other words, only the actual time the substrate sample was maintained at the operating temperature was computed into sampling time requirements. This sampling scheme was applied to all the sampling times; including 24 hours and 96 hours for 60 ° C and 37 ° C experiments respectively. No other control was imposed on the system. No cofactors were added and no buffers were added. The pH of reaction mass was as obtained by mixing substrate sample with distilled water. However, hydrolysate pH was routinely assessed at selected sampling time intervals with Orion 3 Star portable pH meter, serial no. A 11326 (Thermo Fisher Scientific Inc., Waltham, Massachusetts; Figure 4-8A). Soluble solids were occasionally monitored with

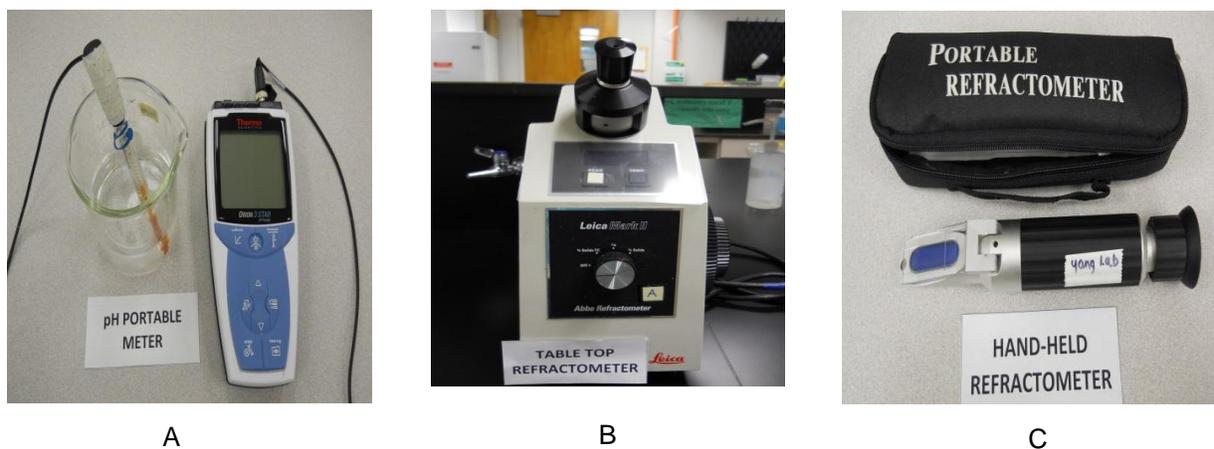


Figure 4-8. Monitoring Devices for pH, and Soluble Solids. A) pH Meter B) Table Top Refractometer C) Portable Refractometer. Photos courtesy of the author.

the aid of refractometers; Abbe Mark II, Model # 10480 (Leica Inc., Buffalo, New York, Figure 4-8B).

The described scheme was replicated three times for each substrate at 60 ° C. The same protocol was adopted at 37 ° C. However, at 37 ° C each experiment was run for 96 hours. Therefore additional hydrolysate samples were withdrawn for glucose assay at T14: 2880 minutes (48 hours); T15: 4320 minutes (72 hours); and T16: 5760 minutes (96 hours). Table 4-4 highlights the experimental design executed.

Table 4-4. Experimental Design for Synergistic Enzymatic Hydrolysis of Different Cassava Substrates

Temperature [° C]	Substrate	Replication	Operating pH	Substrate Concentration	Enzyme Concentration	Enzyme Type and Proportion
60 for 24 Hours	Commercial Cassava Starch	S1 @ 60	As obtained by mixing substrate with distilled water	10 %	0.015 %	Fungal Glucoamylase 75 %
		S2 @ 60				
		S3 @ 60				
	Flour from solar dried cassava chips	F1 @ 60				PLUS
		F2 @ 60				
		F3 @ 60				Bacterial Alpha Amylase 25 %
	Fresh Cassava Root Pulp	R1 @ 60				
		R2 @ 60				
		R3 @ 60				
37 for 96 Hours	Commercial Cassava Starch	S1 @ 37	As obtained by mixing substrate with distilled water	10 %	0.015 %	Fungal Glucoamylase 75 %
		S2 @ 37				
		S3 @ 37				
	Flour from solar dried cassava chips	F1 @ 37				PLUS
		F2 @ 37				
		F3 @ 37				Bacterial Alpha Amylase 25 %
	Fresh Cassava Root Pulp	R1 @ 37				
		R2 @ 37				
		R3 @ 37				

All the hydrolysate remaining in the reactor at the end of each experiment: 24 hours for 60 ° C and 96 hours for 37 ° C were pooled together, clarified by sedimentation, and concentrated to various soluble solids content using Precision vacuum evaporator Model Cat. No. 65486 (Precision Scientific Company, Chicago, Illinois; Figure 4-9).

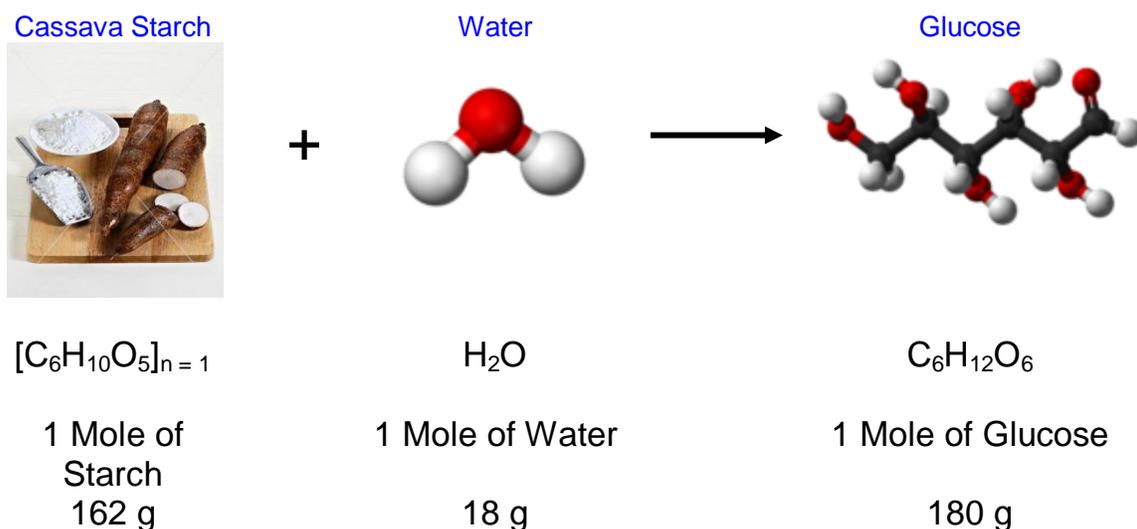


Figure 4-9. Vacuum Evaporator Used for Glucose Syrup Concentration . Photo courtesy of the author.

4.3.7 Mass Balance Stoichiometry

To determine the efficiency of hydrolysis in terms of the rate and absolute conversion of starch to glucose, the law of conservation of matter (Antoine-Laurent de

Lavoisier; 26 August 1743 – 8 May 1794) was applied. Since the enzymes only catalyzed the hydrolysis, they did not go into the hydrolysis equation. Therefore the hydrolysis stoichiometry was derived for cassava starch reaction with water to produce glucose. The stoichiometry as presented in Figure 4-10 revealed that 1 gram of starch yields 1.1111 gram of glucose. This was the basis for all mass balance computations.



Therefore: 1 g Starch \approx 1.1111 g Glucose

Figure 4-10. Mass Balance Stoichiometry for Enzymatic Hydrolysis of Different Cassava Substrates. Image of water and glucose structure courtesy of Wikipedia.

4.3.8 Reaction Kinetics

Mathematical models capable of simulating the process of enzyme hydrolysis are very useful because they are able to predict processing outputs in response to different input variables, such as substrate concentration and hydrolysis time. The kinetic model most widely used to describe enzymatic hydrolysis is the Michaelis-Menten rate

expression (Shuler and Kargi, 2002). Well-designed batch reactors with cell-free enzymes are typically operated as homogeneous systems in which the enzyme and substrate are well mixed to ensure uniform distribution of temperature, pH, and concentration throughout the reactor. Under these conditions, process design and operation rely essentially on the reaction rate or enzyme kinetics. Conventional methods for determining kinetic parameters for the Michaelis-Menten rate expression are used to obtain the reaction rate as a function of substrate concentrations (Zhou et al., 2003) and then perform a graphical method of data analysis or a direct linear plot (Cavaille and Combes, 1995), such as the Lineweaver-Burk plot (Doran, 1995).

However, it is difficult to find a proper model for an enzymatic reaction that obeys inhibition kinetics. In addition, a kinetic model obtained in this way is only valid for an enzyme reaction under predetermined optimal process conditions. Two well-known mathematical models based on the Michaelis-Menten rate expression were used to show the complexity of describing these types of enzymatic reactions. Model 1 is for enzyme hydrolysis in which the reaction rate is a function of substrate concentration only. Most studies of animal and vegetable protein hydrolysis showed inhibition by the substrate (Kristinsson and Rasco, 2000; Van Den Heuvel and Beeftink, 1988), as well as uncompetitive inhibition in the case of vegetable proteins, which was assumed for model 1 (Marquez Moreno and Fernando Cuadrado, 1993). Model 2 reflects the more complex situation in which the reaction rate is a function of both substrate and active enzyme concentrations.

In the work undertaken in this study, the enzyme reaction was carried out under predetermined optimal process conditions of enzyme and substrate concentrations.

Under these conditions only the substrate composition changes with time as the enzyme hydrolysis proceeds. This type of reaction can be described by first order kinetics, which was the type of model chosen for this work.

4.3.9 Statistical Analysis

Data were analyzed by descriptive statistics (Ott and Longnecker 2010; Montgomery, 2009). Means, standard deviations, correlation coefficients, etc. were computed using Microsoft Office Excel Software Package 2007. The excel software was also used to plot graphs, derive and display equations, as well as generate constants.

4.4 Results and Discussion

4.4.1 Compositional analysis of different substrates

Results from laboratory analyses on the composition of each substrate are summarized in Table 4-5. Aside from differences in moisture content which were approximately 11 %, 8 % and 61 % respectively for commercial cassava starch, flour from solar dried cassava chips and ground fresh cassava root pulp, these data show that pure starch accounted for more than 80% total solids in the commercial starch, about 75% of total solids in the cassava flour, and 29% of total solids in the cassava root pulp. These differences dictated the maximum glucose concentration that could be reached when hydrolyzing substrates made with the same weight percent of substrate ingredient.

4.4.2 Enzyme hydrolysis at 60°C

Profiles of glucose concentration over time during enzyme hydrolysis at 60°C of commercial cassava starch, flour from solar dried cassava chips and ground fresh cassava root pulp are shown in Figures 4-11, 4-12 and 4-13, respectively. All three

profiles reveal a classic exponential decay of unaccomplished glucose conversion over time, with more than 90%, 80% and 60% conversion of available starch accomplished within the first four hours of hydrolysis respectively for commercial cassava starch, flour from solar dried cassava chips and ground fresh cassava root pulp. The maximum glucose concentrations reached reflected the quantity of pure starch available in each of the substrates. The substrate made with 10% pure commercial starch converted to virtually 10% glucose, while substrates made with 10% flour or ground root pulp contained only 8% or 7% starch to begin with.

Table 4-5. Compositional Analysis of Different Cassava Substrates *

S/N	Chemical Component [% Mass of Sample; Otherwise stated]	Substrate		
		Commercial Cassava Starch	Flour Produced from Solar-Convection Dried Cassava Chips (Particle size $\leq 45 \mu\text{m}$)	Fresh Cassava Root
1	Ash			0.78
2	Carbohydrate			37.73
3	Fat	NE	NE	0.29
4	Fiber (Crude)			1.04
5	Protein			1.64
6	Moisture	11.20	7.86	60.52
7	Starch	80.16	74.60	29.08
	Sugars			
8	– Fructose		0.644	0.267
9	– Glucose		0.958	0.414
10	– Lactose	ND [DL = 0.1]	ND [DL = 0.1]	ND [DL = 0.1]
11	– Maltose		0.293	ND [DL = 0.1]
12	– Sucrose		5.56	1.46
13	Energy (Calories) [Cal/100 g]	NE	NE	160.09
14	Total Pectin [mg GA/kg]	314	2 478	349

* NE: Not Evaluated; ND: Not Detected; DL: Detection Limit

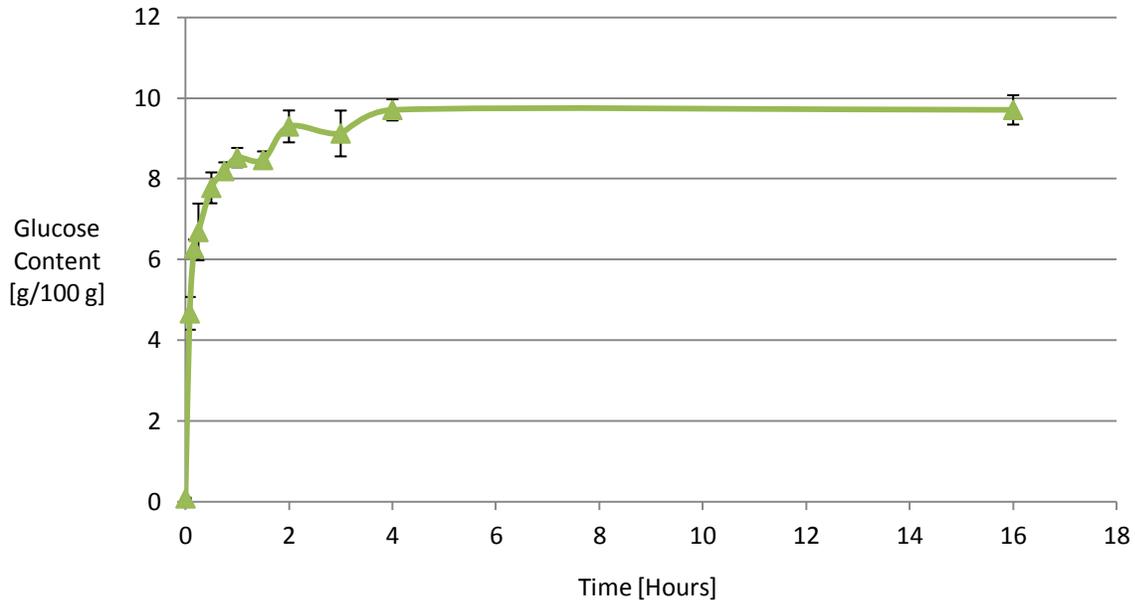


Figure 4-11. Profile of Glucose Content Vs. Time during Enzyme Hydrolysis of Commercial Cassava Starch at 60 ° C (Mean of three replications; and error bars of standard deviations)

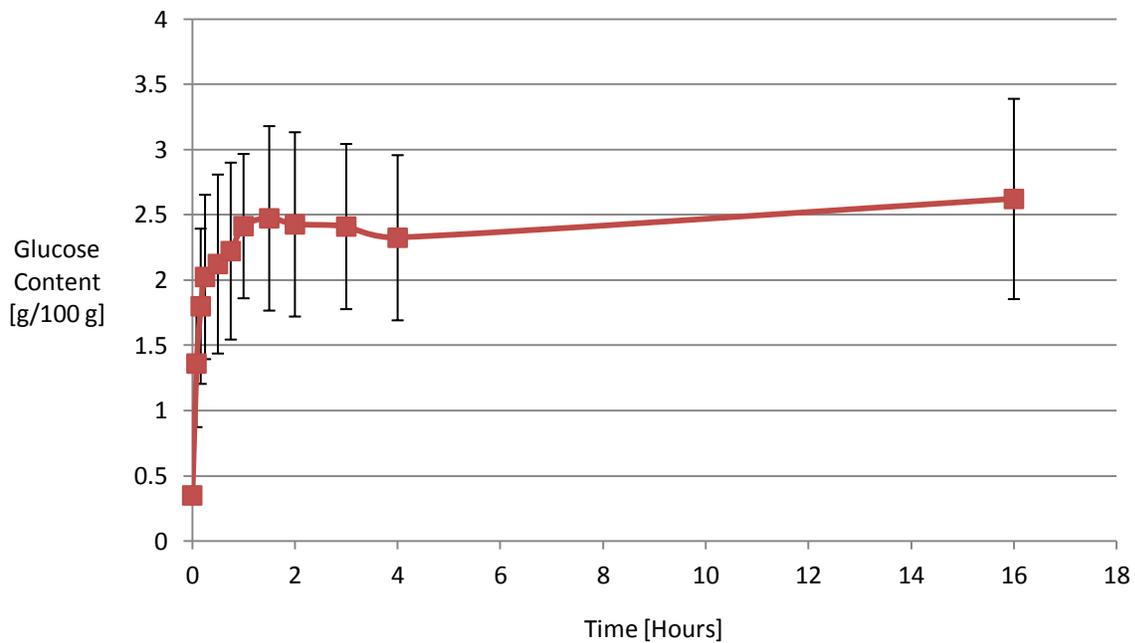


Figure 4-12. Profile of Glucose Content Vs. Time during Enzyme Hydrolysis of Flour Produced from Solar Dried Cassava Chips at 60 ° C (Mean of three replications; and error bars of standard deviations)

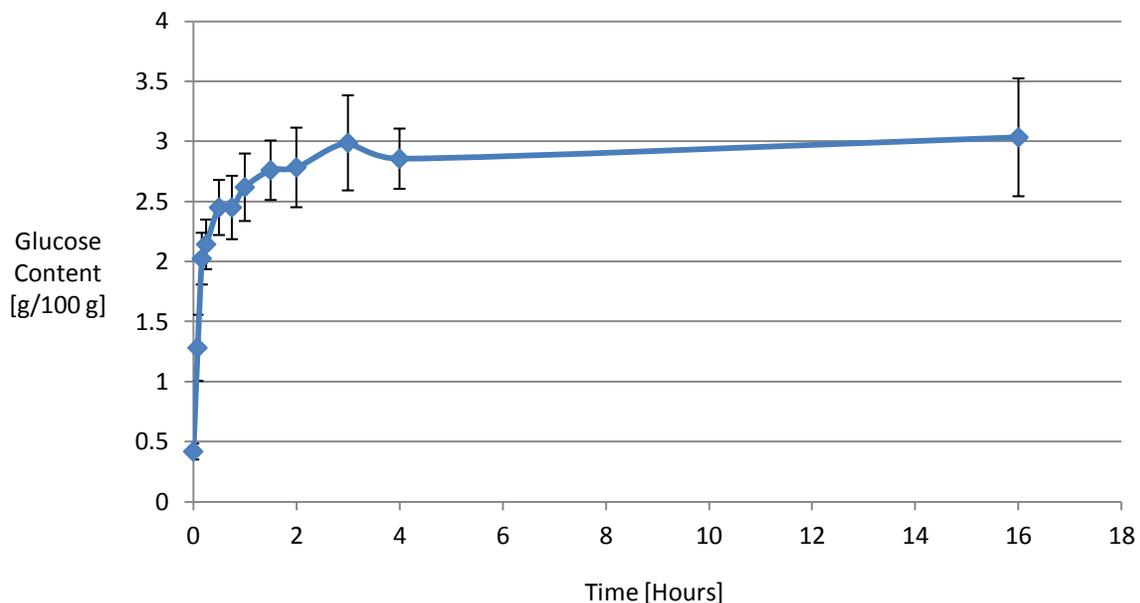


Figure 4-13. Profile of Glucose Content Vs. Time during Enzyme Hydrolysis of Ground Fresh Cassava Root Pulp at 60 ° C (Mean of three replications; and error bars of standard deviations)

Maximum starch conversions reached after 24 hours with each substrate are presented in Table 4-6. A comparison of all three glucose-time profiles is shown as a family of curves on one graph in Figure 4-14. A similar comparison using bar graphs to represent percent starch conversion to glucose with each substrate at various points in time during hydrolysis is shown in Figure 4-15. The results are similar to those of Srikanta et al. (1987) who also reported greater conversion with pulp than with flour.

Table 4-6. Maximum Starch Converted to Glucose in Different Cassava Substrates after 24 hours of Enzyme Hydrolysis at 60 ° C

Substrate	Starch Conversion [%]
Commercial Starch	96
Flour from Solar Dried Chips	27
Ground Fresh Root Pulp	53

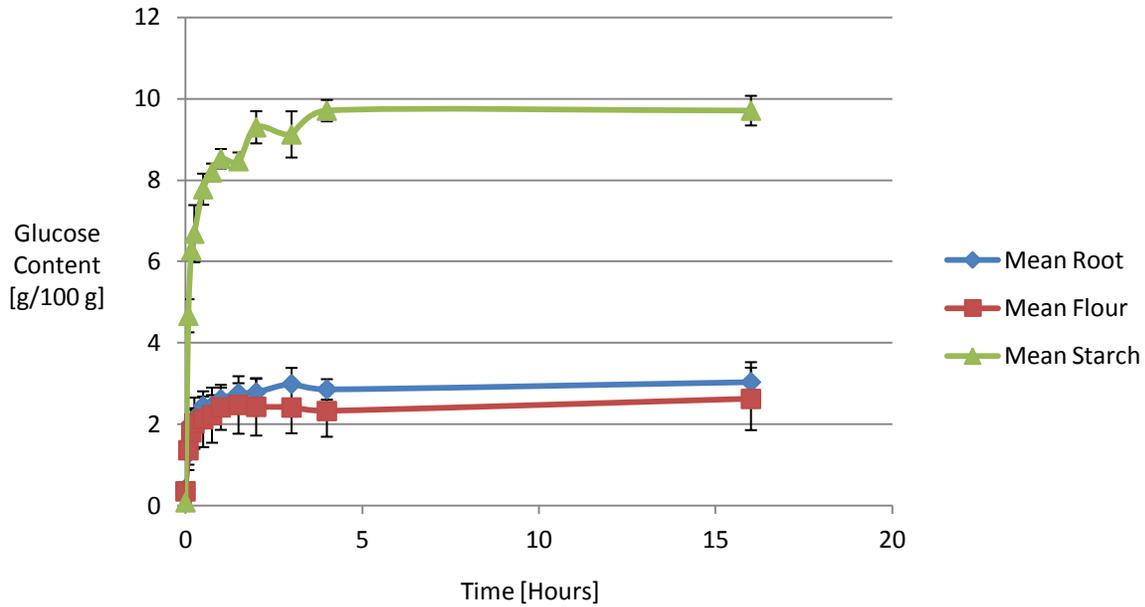


Figure 4-14. Comparison Profile of Glucose Content Vs. Time during Enzyme Hydrolysis of Commercial Cassava Starch, Flour Produced from Solar Dried Cassava Chips and Ground Fresh Cassava Root Pulp at 60 °C (Means of three replications; and error bars of standard deviations)

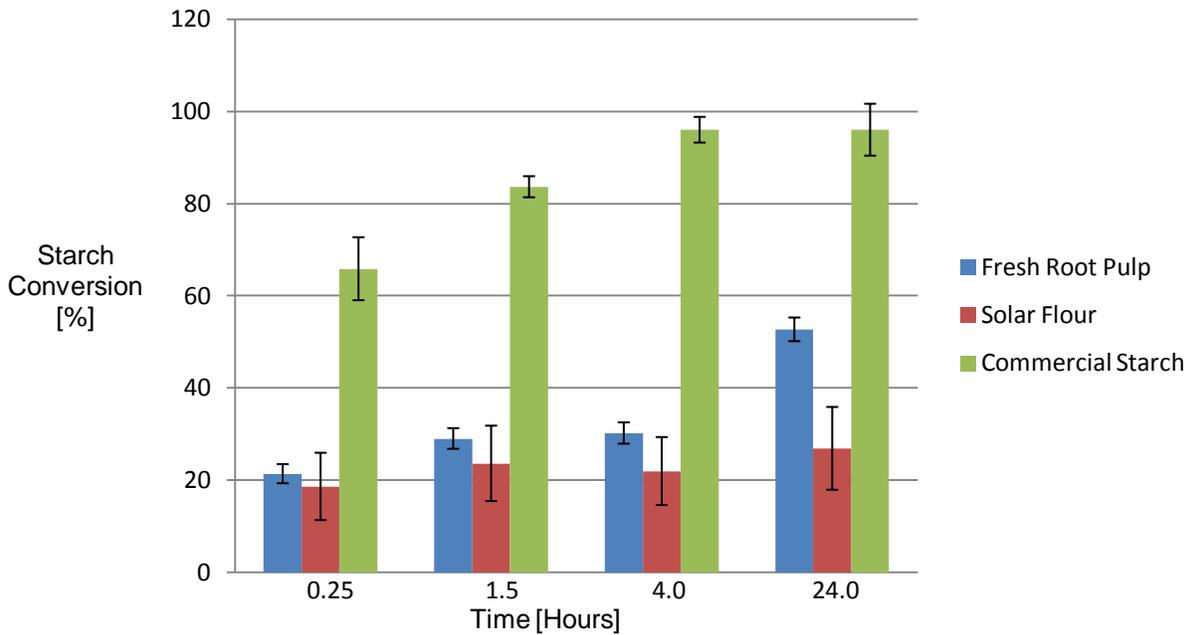


Figure 4.15: Conversion Profile of Enzyme Hydrolysis of Starch to Glucose in Different Cassava Substrates at 60 °C. (Values were estimated from three replications of each substrate and error bars are for standard deviations).

4.4.3 Enzyme hydrolysis at 37°C

Profiles of glucose concentration over time during enzyme hydrolysis at 37°C of commercial cassava starch, flour produced from solar dried cassava chips and ground fresh cassava root pulp are shown in Figures 4-16, 4-17 and 4-18, respectively.

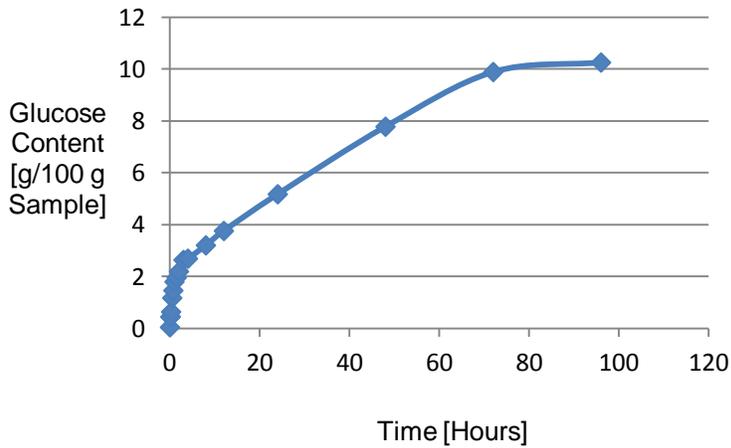


Figure 4-16. Profile of Glucose Content Vs. Time during Enzyme Hydrolysis of Commercial Cassava Starch at 37 ° C

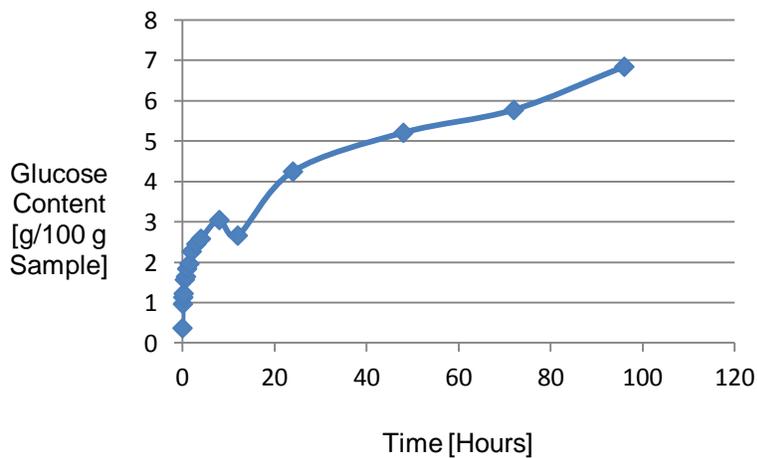


Figure 4-17. Profile of Glucose Content Vs. Time during Enzyme Hydrolysis of Flour Produced from Solar Dried Cassava Chips at 37 ° C

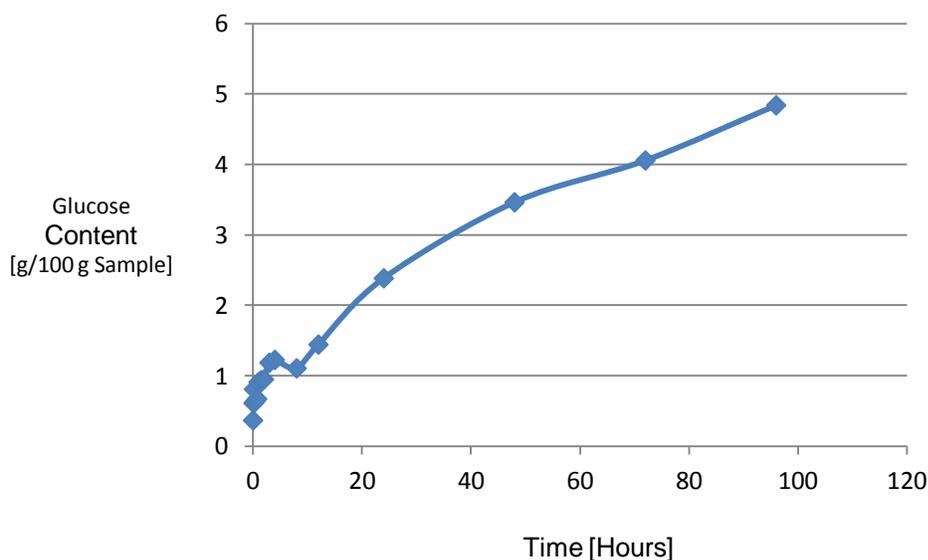


Figure 4-18. Profile of Glucose Content Vs. Time during Enzyme Hydrolysis of Ground Fresh Cassava Root Pulp at 37 ° C

All three profiles also reveal a classic exponential decay of unaccomplished glucose conversion over time, but much more slowly than at 60 ° C. At 37 ° C, hydrolysis must proceed for approximately 72 hours (three days) in order to reach 80 – 90% conversion of available starch to glucose. Again, the maximum glucose concentrations reached after 96 hours of hydrolysis reflected the quantity of pure starch originally present in each of the substrates, and are given in Table 4-7.

Table 4-7. Maximum Starch Converted to Glucose in Different Cassava Substrates after 96 hours of Enzyme Hydrolysis at 37 ° C

Substrate	Starch Conversion [%]
Commercial Starch	100
Flour from Solar Dried Chips	72
Ground Fresh Root Pulp	55

A comparison of all three glucose-time profiles is shown as a family of curves on one graph in Figure 4-19. A similar comparison using bar graphs to represent percent starch conversion to glucose with each substrate at various points in time during hydrolysis is shown in Figure 4-20.

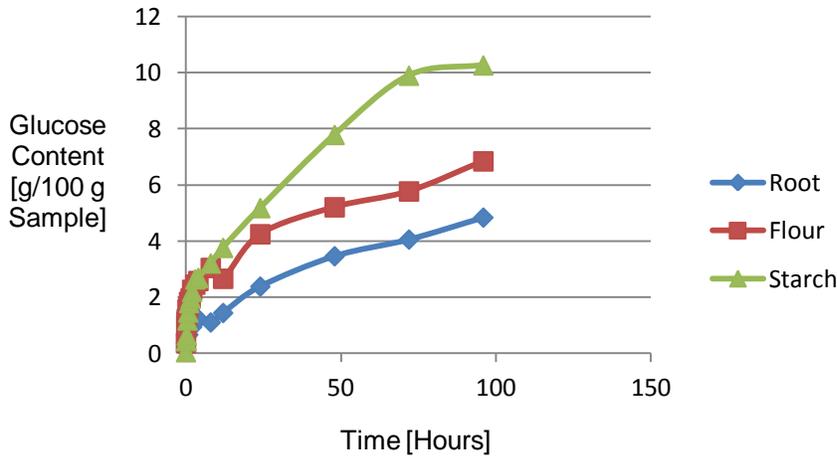


Figure 4-19. Comparison Profile of Glucose Content Vs. Time during Enzyme Hydrolysis of Commercial Cassava Starch, Flour Produced from Solar Dried Cassava Chips and Ground Fresh Cassava Root Pulp at 37 ° C

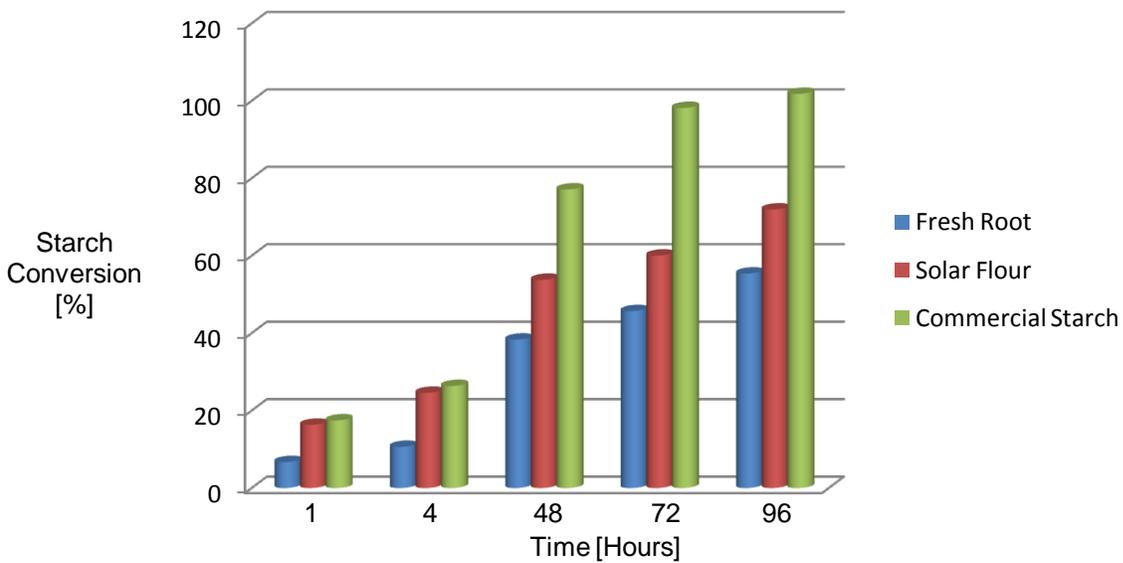


Figure 4-20. Conversion Profile of Enzyme Hydrolysis of Starch to Glucose in Different Cassava Substrates at 37 ° C

4.4.4 Reaction Kinetics of Enzyme Hydrolysis

As noted earlier, all glucose-time profiles revealed a classic exponential decay of unaccomplished glucose conversion over time. All experiments were carried out using the same substrate and enzyme concentrations. Under these conditions, any differences in performance of hydrolysis with different substrates would be noted in the rate of reaction. Therefore, the exponential decay of unaccomplished glucose conversion over time could be treated as a first order biochemical reaction that could be described by first-order reaction kinetics. These kinetics can be quantified by reporting numerical values for first-order rate constants (k) at different temperatures, and the Arrhenius activation energy (E_A) describing the temperature dependency of the rate constants.

4.4.4.1 First-order rate constants

The first-order rate constants were taken from the slopes of straight lines obtained on a semi-log graph of unaccomplished glucose conversion over time. The unaccomplished glucose conversion was expressed mathematically as the difference between maximum glucose concentration reached [C_{max}] and the glucose concentration at any point in time, [C]. Semi-log plots of unaccomplished glucose conversion over time for all three substrates at 60°C are presented in Figure 4-21, and at 37°C in Figure 4-22.

First-order rate constants [k] were taken from the slope of the straight line of best fit by linear regression for each substrate, and are listed in Table 4-8 and Table 4-9 for hydrolysis at 60°C and 37°C respectively.

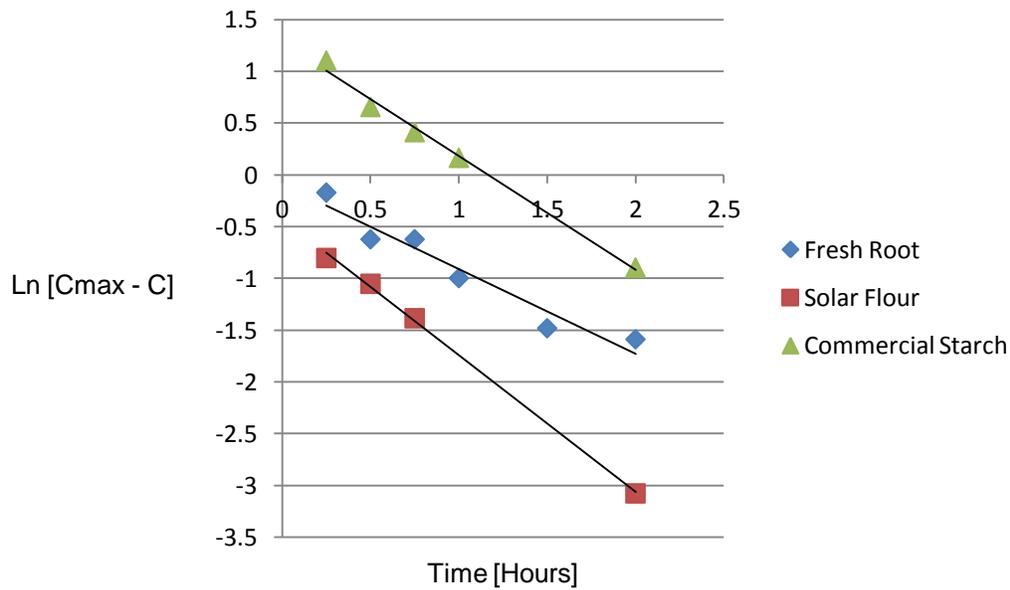


Figure 4-21. Semi-log Plot of Unaccomplished Glucose Conversion over Time for Estimation of First Order Reaction Rate Constants for Enzyme Hydrolysis of Starch with Different Cassava Substrates at 60 ° C

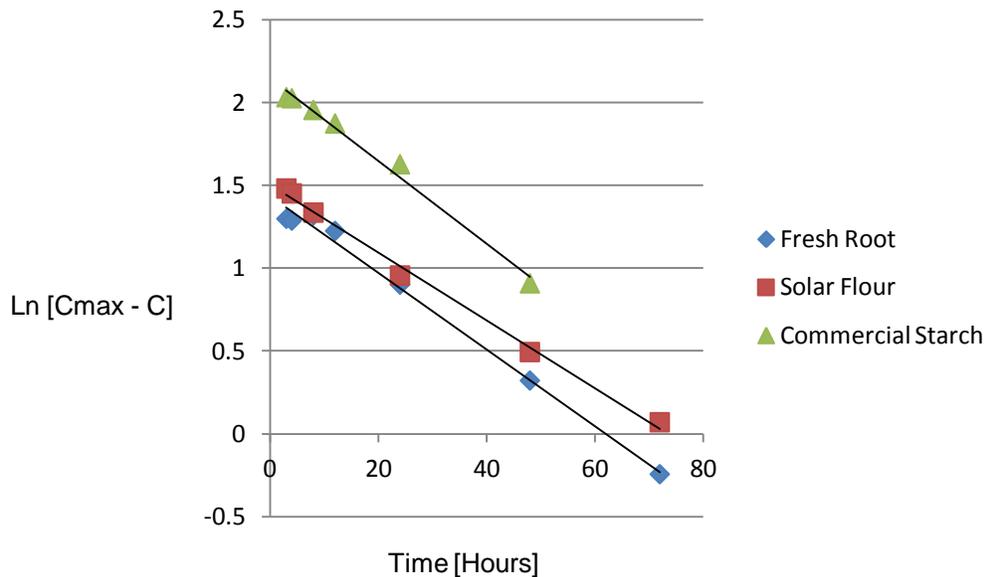


Figure 4-22. Semi-log Plot of Unaccomplished Glucose Conversion over Time for Estimation of First Order Reaction Rate Constants for Enzyme Hydrolysis of Starch with Different Cassava Substrates at 37 ° C

Table 4-8. Reaction Rate Constant (k), Correlation Coefficient (R^2) and Regression Equation of First Order Reaction for Enzyme Hydrolysis of Different Cassava Substrates at 60 ° C.

Substrate	k Value [Hour ⁻¹]	R^2	Equation
Commercial Starch	1.1017	0.9920	$y = -1.1017x + 1.2825$
Solar Flour	1.3198	0.9986	$y = -1.3198x - 0.4222$
Fresh Root	0.8164	0.9397	$y = -0.8164x - 0.0949$

Table 4-9. Reaction Rate Constant (k), Correlation Coefficient (R^2) and Regression Equation of First Order Reaction for Enzyme Hydrolysis of Different Cassava Substrates at 37 ° C.

Substrate	k Value [Hour ⁻¹]	R^2	Equation
Commercial Starch	0.0250	0.9886	$y = -0.025x + 2.1476$
Solar Flour	0.0205	0.9953	$y = -0.0205x + 1.5058$
Fresh Root	0.0232	0.9921	$y = -0.0232x + 1.4363$

4.4.4.2 Activation energy

The Arrhenius activation energy (E_A) describing the temperature dependency of the rate constants was taken from the slope of the straight line obtained on an Arrhenius plot of the natural log of rate constant against reciprocal absolute temperature.

Arrhenius plots for each substrate are shown in Figure 4-23, and the resulting activation

energies for each substrate are listed in Table 4-10. A full report of the reaction kinetics for each of the three substrates is summarized in Table 4-11.

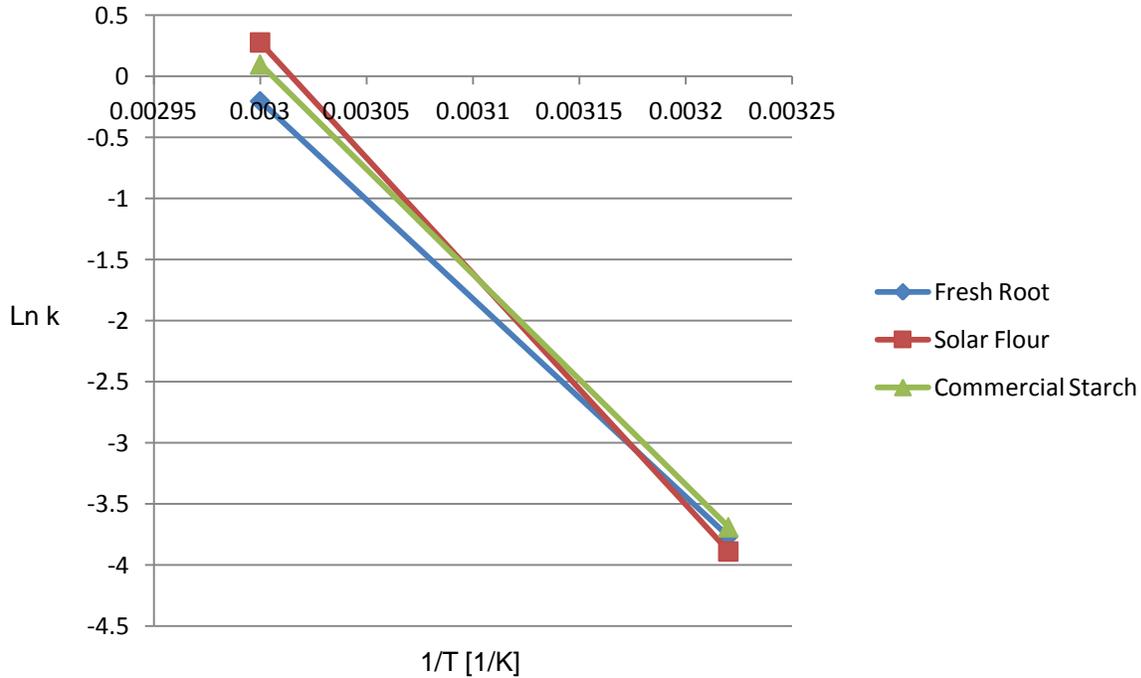


Figure 4-23. Semi-log Plot of First Order Reaction Rate Constants versus Reciprocal Absolute Temperature for Estimation of Arrhenius Activation Energy for Enzyme Hydrolysis of Cassava Starch at 37 ° C and 60 ° C

Table 4-10. Activation Energy (E_A) and Regression Equations for Estimating the Arrhenius Temperature Dependency of Rate Constant for Enzyme Hydrolysis of Different Cassava Substrates

Substrate	E_A [kJ/mol]	Equation
Commercial Starch	143.067	$y = -17208x + 51.720$
Solar Flour	157.392	$y = -18931x + 57.070$
Fresh Root	134.562	$y = -16185x + 48.353$

Table 4-11. Reaction Kinetics Parameters for Enzyme Hydrolysis of Starch in Different Cassava Substrates

Substrate	Reaction Rate Constant; k [Hour ⁻¹]		Activation Energy; E _A [kJ/mol]
	37 ° C	60 ° C	
Commercial Starch	0.0250	1.1017	143.0670
Solar Flour	0.0205	1.3198	157.3920
Fresh Root	0.0232	0.8164	134.5620

CHAPTER 5 ANAEROBIC DIGESTION OF CASSAVA WASTE FOR PRODUCTION OF BIOGAS AND AMYLOLYTIC ENZYMES

5.1 Background

As a result of climate change and other environmental issues, inevitable depletion of fossil fuels supply, sustainability, renewability and biodegradability concerns, energy security, balance of trade and foreign exchange matters, as well as associated socio economic implications, considerable efforts are being deployed globally to the development of renewable energy sources (Nigam and Singh, 2011; Demirbas, 2009, International Energy Agency (IEA), 2009; Demirbas, 2007; Lin and Tanaka, 2006; Wyman et al., 2005; Lynd and Wang, 2004; Wyman, 1999; Lynd et al., 1991; Menezes, 1982). To this effect, biomass in its various forms appears to be a major energy source (Bloomberg New Energy Finance, 2012; Nigam and Singh, 2011; Renewable Energy Policy Network for the 21st Century (REN21), 2011; Ruiz et al., 2011; Demirbas, 2009; Herrera, 2006; Lin and Tanaka, 2006; Ragauskas et al., 2006). However, the use of edible parts of food crops (e.g. sugarcane, corn/maize, soybean, palm oil) for biofuel (e.g. bioethanol, biodiesel) production has raised ethical concerns about diversion of food to fuel production (Pullammanappallil, 2013; Cheng and Timilsina, 2011; Glover et al., 2010; Godfray et al., 2010; Thomson et al., 2010; Fargione et al., 2008; Searchinger et al., 2008; The Royal Society, 2008). Therefore, a reasonable alternative is biofuel production from agricultural residues and wastes, as well as from microalgae, and lignocellulosic biomass originating from non-food sources.

Cassava wastes such as peels are generated during the processing and production of numerous cassava based food products like casabe, chickwangué, farinha de mandioca, fufu, fuku, gapek, gari, konkonte, landang, and peujeum. The

cassava peel is reported to constitute 15 – 19 % fresh weight of the cassava root (Gomez and Valdivieso, 1983; Gomez et al., 1985). With manual peeling, the peel can constitute 20 – 35 % of the total weight of the root (Ekundayo, 1980). More than 60 % of global cassava production is processed as food for human consumption (Tonukari, 2004). In 2011, world cassava output was 252.20×10^9 kg and most of it was produced by developing countries (FAO, 2013). Therefore enormous organic waste is generated from cassava processing. This waste could potentially be a good feed stock for anaerobic digestion and fermentation processes for the production of biogas and hydrolytic enzymes.

Agricultural residues such as cassava peel, cowpea waste, plantain peel, rice bran; yam peel, etc have been reported as suitable fermentation substrates for the production of enzymes (Adeniran and Abiose, 2011; Adeniran et al., 2010; Adeniran and Abiose, 2009; Kareem et al., 2009; Silva et al., 2009; Alva et al., 2007; Swain and Ray, 2007; Uguru et al., 1997; Sani et al., 1992). Also, cassava stem residues, waste water and peel are reported to have been used in biogas production (Zhu et al., 2013, Gao et al., 2012; Cuzin et al., 1992). However, the author did not identify any report that evaluated simultaneous production of biogas and enzymes from cassava waste. It will appear to be a win and win again situation if it is possible to use cassava peel waste in anaerobic digestion to produce biogas that could be used as a source of energy on the one hand; and also to extract a natural cocktail of hydrolytic enzymes from the post digestion broth on the other hand. This study explored these possibilities.

5.2 Objectives

The objectives of the work reported in this study were to:

- Determine the proportions of cassava root components. These include the peel (periderm/epidermis or outer coat and cortex), the parenchymatous tissue, the head and the tail ends.
- Demonstrate the feasibility of anaerobic digestion of cassava waste by performing experiments with cassava peels and reporting performance indicators such as: methane yield, chemical oxygen demand (COD), pH, etc.
- Explore feasibility of extracting from the post anaerobic digestion broth, active enzymes that are capable of hydrolyzing cassava starch.
- Determine the activity of any extracted enzyme.

5.3 Materials and Methods

5.3.1 Cassava Roots

Fresh cassava roots were purchased from Brooks Tropicals, Homestead, Florida. The roots were coated with food wax and supplied refrigerated in 18 kg boxes. Upon receipt, each consignment or batch was promptly received and maintained under refrigerated storage ($< 10^{\circ}\text{C}$) until utilized.

5.3.2 Reactor

The reactor used in this study constituted mesophilic anaerobic digester. The digester vessel was fabricated from a Pyrex glass jar about 0.2540 m high from base to shoulder and 0.1651 m in diameter (Figure 5-1 b). The neck was approximately 0.0889 m in length and 0.0889 m in diameter. Volume capacity of the reactor was $\approx 0.0054\text{ m}^3$; of which 0.003 m^3 was utilized in each batch of the experiments. The reactor was charged from top through the neck opening. The opening was capped with air and liquid-tight closure system that consisted of Pyrex lid, Teflon O ring and Stainless steel clamp. The vessel was fitted with ports at top of the lid and on the side close to the bottom for biogas transfer and leachate withdrawal respectively. The reactor vessel was incubated at mesophilic temperature ($36 \pm 3^{\circ}\text{C}$) in a converted household refrigerator

chamber, heated with a 65 W Sylvania light bulb (Sylvania lighting Services Corporation, Danvers, Massachusetts). The bulb was controlled by BFG thermostat, model #6910796 (Whirlpool Corporation, Benton Harbor, Michigan). Biogas production was monitored with a positive displacement type gas meter that was connected from outside of the incubator chamber. The meter device consisted of a transparent PVC U-tube that was filled with orange colored anti-freeze solution; float switch (W W Grainger, Lake Forest, Illinois); solenoid valve (Fabco-Air Inc., Gainesville, Florida); Dayton off time delay relay model 6X153E (W W Grainger, Lake Forest, Illinois); and a counter (Redington Counters Inc., Windsor, Connecticut). The design assembly of the anaerobic reactor digester and biogas measurement system is shown in Figure 5-1.

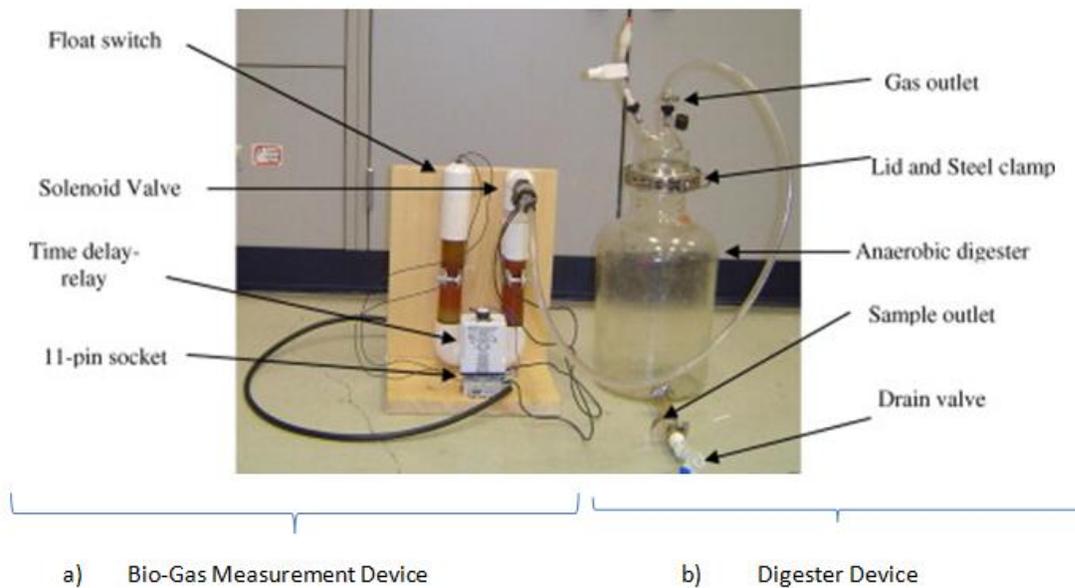


Figure 5-1. Anaerobic Digestion System Design

5.3.3 Inoculum

About 0.003 m³ of mixed liquor from an active municipal solid waste fed mesophilic lab-scale digester was used as inoculum to start up the first experiment. Subsequently, mixed liquor from previous experiment was used as inoculum for next experiment.

5.3.4 Bulking Agent

To prevent compaction of peel waste in the digester, about 1.5 kg of lava rocks was used as bulking agent. The rocks which were irregularly shaped and about 2.5 cm in average dimension were obtained from a landscaping supplier.

5.3.5 Sample Preparation

5.3.5.1 Root components proportion

For each experimental run, between 1780 g to 2828 g of fresh cassava roots was retrieved from refrigeration and used. Roots were weighed with heavy duty shipping scale serial # HD 150 (My Weigh, Phoenix, Arizona, Figure 5-2). Deliberate efforts were



Figure 5-2. My Weigh Shipping Scale used to weigh out Cassava Roots

made to include for each batch, small, medium and large size roots. The measured roots were placed on a cutting board shown in Figure 5-3 A, and then peeled with a kitchen knife (Figure 5-3 B).

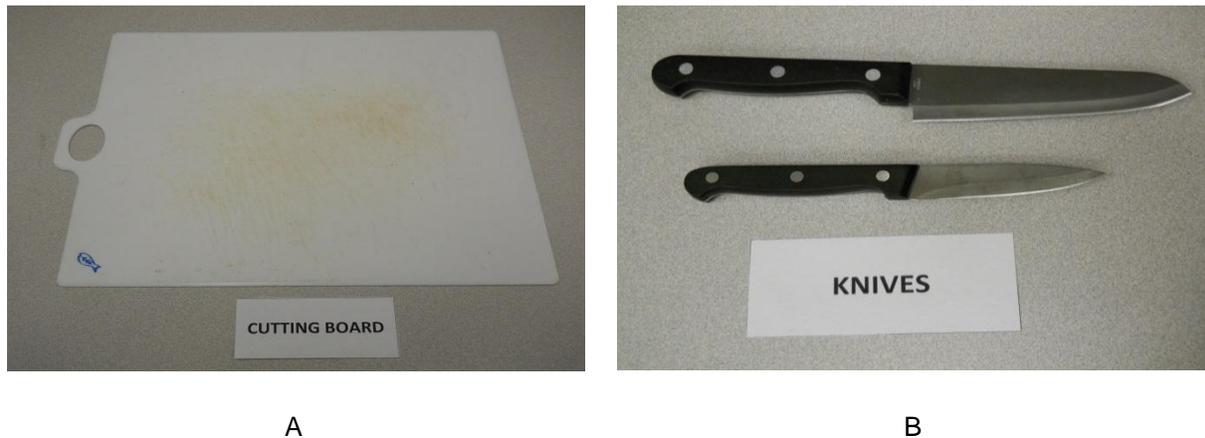


Figure 5-3. Implements used to Peel Cassava Roots and Prepare the Peelings for Experiments. A) Cutting Board B) Peeling Knives. Photos courtesy of author.

The peeling process involved two steps; first, carefully cutting off the head and tail ends of each root such that the main edible portion (the parenchyma) was not affected. Secondly, the peel (periderm/epidermis or bark/outer coat, and cortex) was separated from the parenchymatous tissue, again making sure that the parenchyma was not affected. The root components: Head and Tail ends; Periderm/Epidermis; Cortex; and Parenchyma were subsequently weighed separately to determine root mass proportions. The masses were thereafter added and compared to the initial mass of roots to estimate the lost mass. Figure 5-4 shows the different components and the types, shapes and sizes of roots encountered. The described operations were replicated four times.

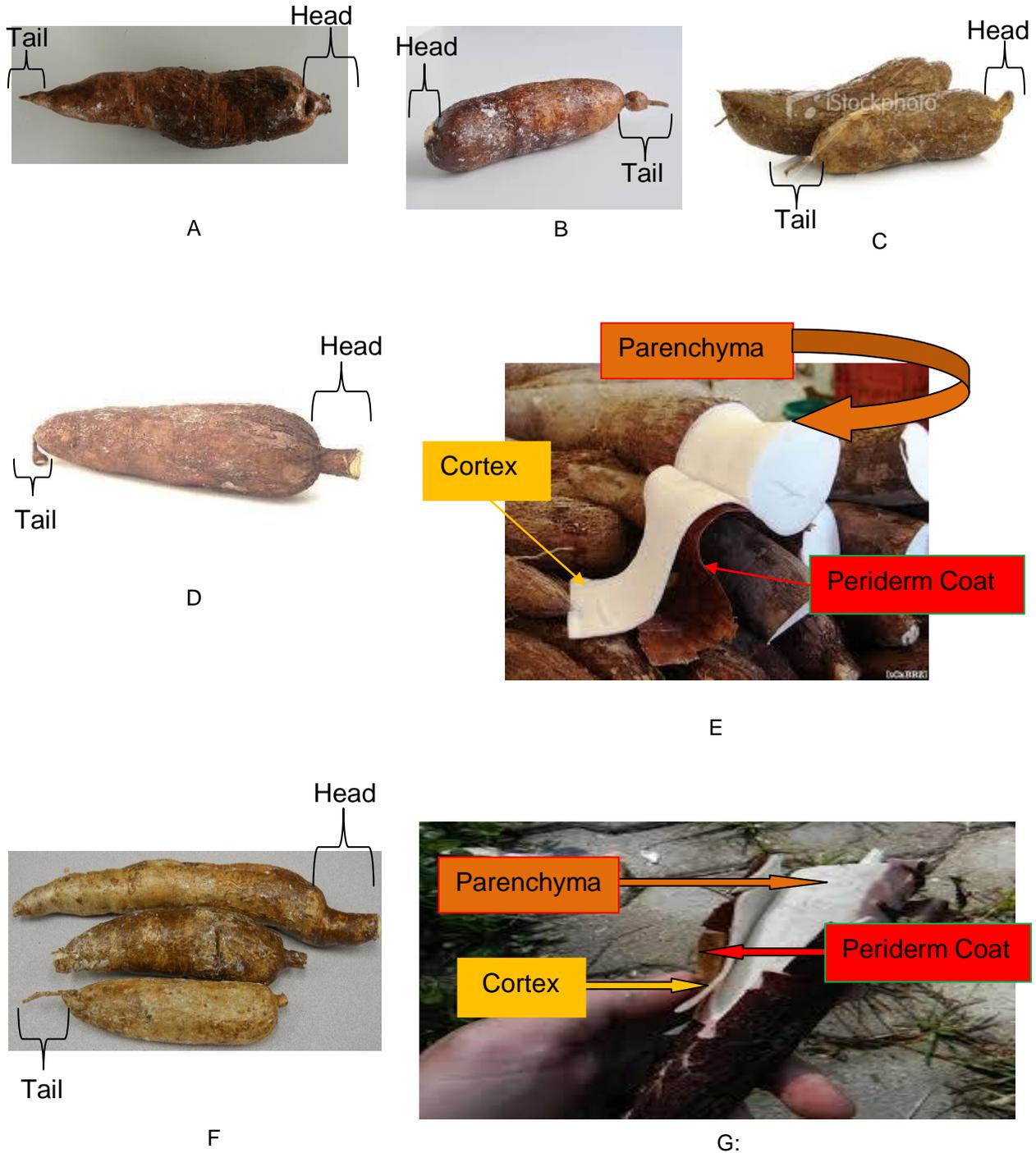


Figure 5-4. Components of Cassava Root. A), B), C), D) and F) Head & Tail Ends Shown E) and G) Periderm/Epidermis, Cortex and Parenchyma Shown. Photos B, C, D and E courtesy of google.com images; Photo G courtesy of bing.com/images. The remainder of the photos courtesy of author.

5.3.5.2 Anaerobic digestion samples

For anaerobic digestion experiments, the cortex which was 0.203 to 0.224 cm thick was cut into pieces of 2.5 cm average dimension. Similarly, the head and tail ends, as well as the periderm or epidermis was reduced to pieces of about 2.5 cm average dimensions. These cut up three root components (Head and Tail ends, Periderm/epidermis, and Cortex) were combined, mixed thoroughly and used as substrates in all the anaerobic digestion experiments. The substrate as prepared was used either immediately or stored under frozen ($\leq -10^{\circ}\text{C}$) refrigeration until required.

5.3.6 Anaerobic Digestion Procedure

Two hundred grams of substrate was charged for each batch of anaerobic digestion experiment. Freshly prepared substrate was used or previously frozen sample was thawed overnight before utilization. To start the loading process, the substrate was divided into two equal portions of one hundred grams each; and the 1.5 kg lava rock bulking agent was divided into three equal portions of five hundred grams each. The reactor vessel cap was then disengaged and one portion of the bulking agent (five hundred grams) layered on the bottom of the vessel. The first one hundred grams portion of substrate was spread on the rocks. Then the second portion of five hundred grams of lava rocks was layered over the substrate and the remaining one hundred grams portion of substrate spread on the rocks. Finally, the remaining five hundred grams of lava rocks was layered over the substrate. The first run was inoculated with 3000 cm^3 of mixed culture inoculums and 35 g of soda ash (sodium bicarbonate) was added to buffer against pH changes. The digester cap was replaced and clamped tight;

digester placed in the incubator chamber; biogas lines were connected; the incubator door was closed; and anaerobic digestion allowed to proceed.

When gas production capacity was depleted (no biogas produced in two or more consecutive days), the process was interrupted and stopped. The cap was opened and the second batch (200 g) of substrate was loaded from the top. Similarly, the third and fourth batches of 200 g substrate each was charged at the end of the second and third runs respectively. Each subsequent run was therefore initiated by the post digestion broth of the preceding experiment; no additional inoculums or buffer were added. At the end of the fourth run, all the contents of the digester were discharged and the digestion broth and substrate digestate (residue) were analyzed for enzyme activity. For the fifth experiment, 200 g of substrate was prepared and layered as described for run number one above. The remaining broth from the first four runs was made up to 3000 cm³ with fresh inoculums and used for inoculation; no additional buffer was added.

Each batch of experiment was operated in a batch mode; as a single-stage, leach-bed, and unmixed system. The digester contents were not agitated.

5.3.7 Analysis

5.3.7.1 Biogas composition

The methane and carbon dioxide composition of biogas produced was analyzed with Gas Chromatograph (GC) Series 580 (Gow-Mac Instrument Co., Bethlehem, Pennsylvania) that was coupled with Gas Partitioner Model 1200 (Fisher Scientific Inc., Waltham, Massachusetts). The GC was equipped with thermal conductivity detector and calibrated with a mixture of nitrogen (N₂), methane (CH₄), and carbon dioxide (CO₂) in the volume ratio of 25:45:30 [N₂:CH₄:CO₂]. The GC analytical system that is being described and was used in the experimental work is shown in Figure 5-5.



Figure 5-5. Gas Chromatograph (GC) System used to Estimate the Methane and Carbon dioxide Contents of Biogas Generated by Anaerobic Digestion of Cassava Peel Waste. Photo courtesy of author.

In operation, the GC system was turned on and allowed thirty minutes of come-up time to attain operating temperature of 50 ° C. Thereafter about 20 mL of biogas sample was drawn with a syringe and injected into the gas partitioner. The GC automatically performs analysis; gas chromatograms were processed and stored with SP 4290 Integrator (Spectra Physics, Santa Clara, California).

5.3.7.2 Soluble chemical oxygen demand (sCOD)

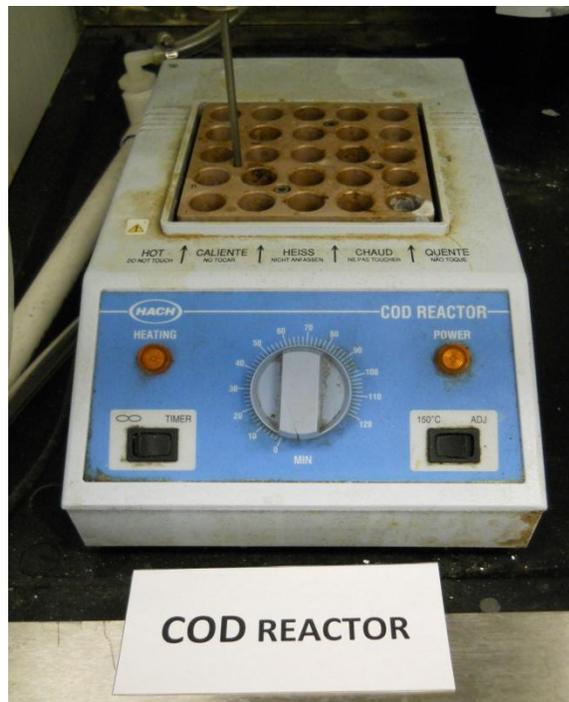
The soluble chemical oxygen demand (sCOD) was analyzed for daily leachate samples drawn from the digester using Hach COD reagent kit (Hach Company, Loveland, Colorado). About 4 mL of leachate was withdrawn from the digester and centrifuged for ten minutes at 2500 rpm with Marathon Micro H centrifuge (Fisher Scientific Inc., Waltham, Massachusetts; Figure 5-6 A). The supernatant was



A



C



B

Figure 5-6. Laboratory Equipment used in sCOD analysis of Leachate from Anaerobic Digestion of Cassava Peel Waste. A) Marathon Micro H Centrifuge B) Hach sCOD Reactor C) Hach sCOD Colorimeter. Photos courtesy of the author.

filtered with 0.45 μm pore size filter paper (Whatman Plc., Maidstone, Kent, UK). The filtrate was diluted twenty times and 2 mL pipetted into Hach COD reagent vials (with

the range: 20 – 1500 mg/L (2 – 150 ppm) COD). The vials were digested (incubated) for two hours at 150 ° C in the COD reactor model # 45600-00 (Hach Company, Loveland, Colorado; Figure 5-6 B). Subsequently, the COD of the leachate was read with DR/890 colorimeter (Hach Company, Loveland, Colorado; Figure 5-6 C), after zeroing with a blank.

5.3.7.3 pH

The pH of digester broth was monitored about the same time daily with Orion 3 Star pH Bench top (Thermo Scientific Inc., Waltham Massachusetts). Assay technique involved withdrawing 20 mL of broth in a plastic vial. The pH meter was turned on and warmed for thirty minutes. Meter probe which had been properly calibrated with neutral and acidic standard media was rinsed with distilled water and wiped dry with lint free kimwipes (Kimberly-Clark Corporation, Irving Texas). The probe was subsequently inserted into the broth and held until the meter gave a stable reading.

5.3.7.4 Total solids (TS), volatile solids (VS), and other properties

Total solids (TS) and volatile solids (VS) constituents of the peel waste (feedstock) were determined gravimetrically. About 100 g of sample was dried for 72 Hrs at 105 ° C using Isotemp Oven Model 350 G (Fisher Scientific Inc., Waltham, Massachusetts). The dried sample was then burnt in Isotemp Muffle Furnace (Fisher Scientific Inc., Waltham, Massachusetts) at 550 ° C for 2 Hrs. From the masses of the sample, dried sample, ash residue, sample pan, etc., TS, VS, and other properties were computed. The formulae used for computations and methane yield analysis are

presented in Table 5-1. The procedure described was executed for each batch or replication of the experiment.

Table 5-1. Formulae for Methane Yield Analysis and for Determination of Properties of Cassava Peel Waste used as Feedstock/Substrate in Anaerobic Digestion for Biogas and Enzyme Production

Parameter Symbol	Parameter ID	Unit	Computational Formula
A	Mass of Dish and Sample Dried for 72 Hrs at 105 °C	g	–
B	Mass of Dish Alone	g	–
C	Mass of Wet Sample and Dish	g	–
D	Mass of Dish and Sample Burnt for 2 Hrs at 550 °C	g	–
E	Cumulative Methane Production	L	–
F	Batch of Feedstock (Peel Waste) Charge in the Digester	g	–
G	Total Solids	%	$\frac{(A - B)}{(C - B)} * 100$
H	Volatile Solids	%	$\frac{(A - D)}{(A - B)} * 100$
I	Volatile Solids Proportion	g VS	$\frac{(F * G * H)}{(100 * 100)}$
J	Fixed Solids	%	$\frac{(D - B)}{(A - B)} * 100$
K	Moisture Content; Wet Basis	%	$\frac{(C - A)}{(C - B)} * 100$
L	Moisture Content; Dry Basis	%	$\frac{(C - A)}{(A - B)} * 100$
M	Methane Yield Per Feedstock Charge	L/g	$\frac{(E)}{(F)}$
N	Methane Yield Per Volatile Solids	L/g VS	$\frac{(E)}{(I)}$
O	Methane Production Efficiency	%	$\frac{(N)}{(0.35 L/gVS)} * 100$

5.3.8 Enzyme Extraction and Enzyme Activity

The work performed included extraction of enzymes as well as testing the activity of extracted enzymes.

5.3.8.1 Enzyme extraction

It has been noted that the location of extracellular enzymes impacts the capacity to degrade substrate (Parawira et al., 2005). Depending on whether the enzyme is released into the surrounding environment or attached to the cell surface, the enzyme could be described as cell free or cell associated (Priest, 1984). In the work carried out in this study, three divisions of enzyme were identified (Zhang et al., 2007). These were cell free enzyme; cell associated enzyme; and biofilm associated enzyme.

5.3.8.1.1 Cell free enzyme

This is the enzyme that has been secreted into the environment and is potentially solubilized in the leachate broth. To extract this class of enzyme, 10 mL of leachate was withdrawn into a plastic vial and centrifuged at 3000 g for ten minutes with International Clinical Centrifuge, Model CL 96327H-3 (Fisher Scientific Inc., Waltham, Massachusetts; Figure 5-7). The supernatant was used as source of cell free enzyme.



Figure 5.7: Clinical Centrifuge used for Enzyme Extraction. Photo courtesy of the author.

5.3.8.1.2 Cell associated enzyme

The cell associated enzyme is presumed to be attached to the surface cells of organisms that are freely suspended in leachate liquor (broth). On centrifugation, suspended cells are separated from the liquor into a pellet; carrying the attached enzymes with them. The pellet is harvested and the cell associated enzyme extracted from it thus:

- Wash the pellet resulting from cell free enzyme extraction two times. For each washing, centrifuge with 10 mL of potassium dihydrogen phosphate buffer (pH 7.0; 0.1 mol L⁻¹) for two minutes at 3000 g.
- Suspend the washed pellet in 2 mL of sodium acetate buffer (pH 6.0; 0.05 mol L⁻¹). Then centrifuge the suspension at 3000 g for ten minutes. The supernatant is collected as the source of cell associated enzyme.

5.3.8.1.3 Biofilm associated enzyme

In the milieu of anaerobic digestion, some microbes are attached to the surface of the substrate as biofilm. Those enzymes that are attached to microbes that themselves are attached to the substrate are called biofilm associated enzymes. A biofilm associated enzyme is neither in solution in the leachate broth (cell free enzyme) nor attached to the surface of organisms that are suspended in the leachate broth (cell associated enzyme). Biofilm associated enzyme was extracted with a procedure similar to that used for cell associated enzyme.

- About 5 g of substrate was taken out of the anaerobic digester (or 5 g of digestate was taken at the end of a batch anaerobic digestion operation).
- The substrate/digestate was washed twice. Each washing process was carried out with 10 mL of potassium dihydrogen phosphate buffer (pH 7.0; 0.1 mol L⁻¹); by centrifuging for two minutes at 3000 g.
- Washed substrate/digestate was suspended in 2 mL of sodium acetate buffer (pH 6.0; 0.05 mol L⁻¹). The suspension was centrifuged at 3000 g for ten minutes. The supernatant was then collected as the source of biofilm associated enzyme.

5.3.8.2 Enzyme activity

The determination of enzymatic activity was carried out for each of the three divisions of enzyme: cell free; cell associated; and biofilm associated. Specifically, amylase activity was estimated by method of Bernfeld (Bernfeld 1955), but with modifications where appropriate. Assay system used to determine activity for each enzyme division contained 0.5 mL of the crude enzyme solution and 0.5 mL of 1 % commercial cassava starch. The 1 % starch may be used as is; not buffered to estimate overall amylolytic activity. The starch may be buffered with disodium hydrogen phosphate buffer (pH 6.9; 0.1 mol L⁻¹) to estimate amylase activity (Adeniran et al., 2010; Zhan et al., 2007). Alternatively, the 1 % starch solution may be buffered with sodium acetate buffer (pH 4; 0.1 mol L⁻¹) to estimate glucoamylase activity (Adeniran et al., 2010; de Moraes et al., 1999; Zanin and De Moraes, 1998). The mixture was then incubated at 37 ° C for thirty minutes and the released reducing sugar (as glucose) estimated with the 3,5-dinitrosalicylic acid (DNS) procedure. The DNS methodology was defined and discussed in chapter four of this dissertation.

One unit of enzyme activity (EU) was defined as the amount of the enzyme that liberated 1µg of reducing sugar (as glucose) per minute, under the assay conditions. Specific activity was expressed as enzyme unit per µg protein (EU/ µg protein). The protein content of each enzyme solution was estimated by the modified Lowry's method (Thermo Fisher Scientific Inc., 2011; Lowry et al., 1951), taking liquid bovine serum albumin or BSA (Thermo Scientific, Rockford Illinois) as the standard.

5.4 Results and Discussion

5.4.1 Cassava Root Components Proportions

Table 5-2 shows the proportion of cassava root components on fresh mass basis. The major human food component, the parenchyma, constituted the largest mass with the average value of 81.16 % and range of 80.72 % to 81.75 %. The cortex was second with the range of 12.76 % to 13.33 % and mean value of 13.11 %. The periderm or epidermis, also known as the bark or outer coat, averaged 3.31%; while head and tail ends had a mean value of 1.71 %. Since the peel consists of cortex and periderm, its mean proportion of cassava fresh root was computed to be 16.42 %. This value is similar to the work of Gomez and Valdivieso (1983) and Gomez et al., (1985) who

Table 5-2. Mass Distribution of Cassava Root Components

S/N	Component	Mass Proportion [% Fresh Mass]				Mean
		Run No. 1	Run No. 2	Run No. 3	Run No. 4	
Edible Portion						
1	Parenchyma	81.30	81.75	80.88	80.72	81.16
Waste Portion						
2	Cortex	13.33	12.76	13.24	13.11	13.11
3	Periderm	3.51	3.19	3.38	3.15	3.31
4	Head & Tail Ends	1.35	1.18	1.79	2.54	1.71
Lost Portion						
5	Lost Mass	0.51	1.12	0.71	0.48	0.71
6	Total Waste = Sum of waste portion = 18.13 %					

reported cassava root peel proportions of 15 % – 18 % and 15 % – 19 % respectively.

Dufour (1988), reported relative contributions of the bark (periderm) and cortex fractions

to root fresh weight of 1.6 % and 14.3 % respectively. Furthermore, it has also been reported in the literature that after 4 months to 10 months of cultivation, the cortex constituted about 15 % of total fresh weight of root (Cooke and de la Cruz, 1982).

An interesting root component presented in Table 5-2 is termed lost mass. It was discovered that the sum of the masses of conventional root components (parenchyma, cortex, periderm, and head and tail ends) was less than the total mass of root that was started out with. The lost mass may be due to evaporative loss of moisture, micro and nano particles that may have drifted away, volatilization and loss of the wax coating, etc. The lost mass concept was therefore introduced to account for these irrecoverable losses. Consequently, the lost mass was discounted, and the total waste associated with the peeling process was reported to be the sum of cortex, periderm, and head and tail ends. The sum of these waste components, and therefore the total waste, averaged 18.13 % of the fresh mass of cassava root.

5.4.2 Composition of Cassava Peel Waste

The characteristics of the cassava peel waste are presented in Table 5-3. The mean moisture content of the peel wastes was 66.79 % wet basis. The values ranged from 63.14 % for run number one to 73.33 % for run number two. Total solids composition averaged 33.19 % and on average, 96.37 % of the solids was volatile. The range for total solids varied from 26.66 % (run number two) to 36.85 % (run number one); while that for volatile solids varied from 95.38 % (run number four) to 96.90 % (run number three). Furthermore, the dry weight mean was 66.36 g, ranging from 53.32 g for run number two to 73.70 g for run number one, while for the fixed solids, the range was from 3.09 % for run number three to 4.61 % for run number four, with the mean value of 3.61 % (Table 5-3).

Table 5-3. Substrate Characteristics and Bio-Methane Potentials of Mesophilic Anaerobic Digestion of Cassava Peel Waste

S/N	Variable Parameter	Run Number One	Run Number Two	Run Number Three	Run Number Four	Mean
1	Mass of Wet Substrate Charge [g]	200	200	200	200	200
2	Moisture Content, Wet Basis [%]	63.14	73.33	63.21	67.50	66.79
3	Moisture Content, Dry Basis [%]	171.26	275.00	171.82	207.69	206.44
4	Total Solids [%]	36.85	26.66	36.78	32.50	33.19
5	Dry Weight of Solids [g]	73.70	53.32	73.56	65.00	66.36
6	Volatile Solids [%]	96.81	96.42	96.90	95.38	96.37
7	Volatile Solids Proportion [gVS]	71.35	51.42	71.30	62.00	64.01
8	Organic Load [gVS/L]	23.78	17.13	23.75	20.66	21.33
9	Fixed Solids [%]	3.18	3.57	3.09	4.61	3.61
10	Cumulative Methane Production [L]	14.21	15.94	12.71	19.11	15.49
11	Methane Yield Per Substrate Charge [L /g]	0.0710	0.0797	0.0635	0.0955	0.0774
12	Methane Yield Per Volatile Solids Proportion [L @ STP /gVS]	0.1991	0.3099	0.1782	0.3082	0.2488
13	Residence Time [Days]	30.37	24.93	19.35	33.28	26.98
14	Biogasification Efficiency [%]	56.89	88.55	50.91	88.06	71.10

5.4.3 Biogasification of Cassava Peel Waste

The profiles of cumulative methane yield versus time for the four runs are presented in Figure 5-8. Run number two had the highest performance, followed by run

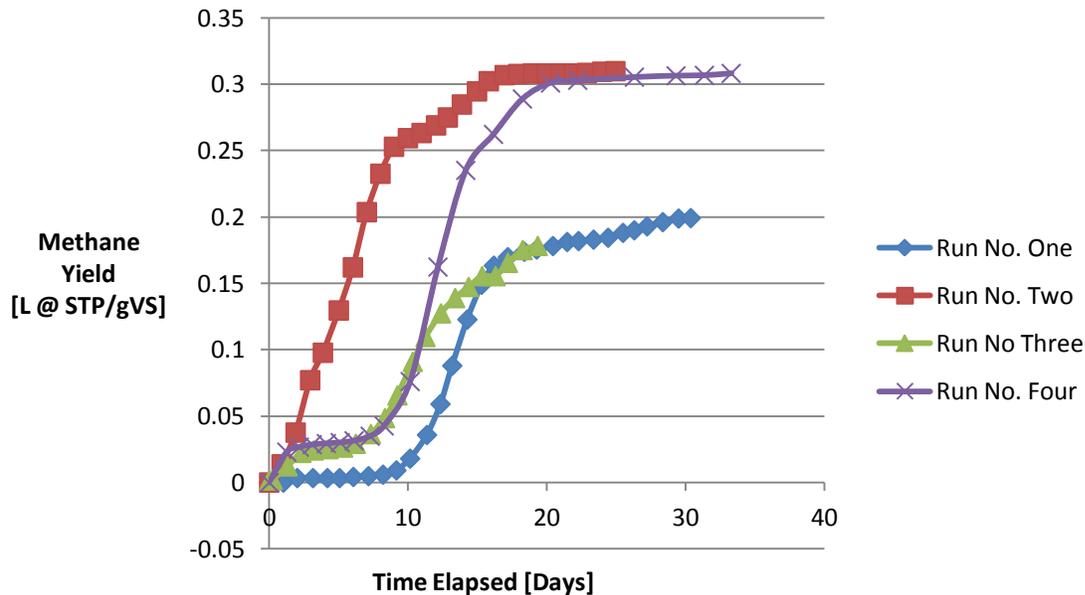


Figure 5-8. Methane Yield of Mesophilic Anaerobic Digestion of Cassava Peel Waste

number four. Run number one Biogasification was initiated with mixed liquor from an anaerobic digester treating municipal solid waste. It is quite possible that the organisms were not adapted to wastes from cassava processing. This may explain the long lag time of six days that it took to commence methanogenesis and biogas production in run number one. However, eight days later, on day fourteen from date of start up, methane production rate peaked at 2.48 L per day. After about nineteen days from commencement of the experiment, methane production dropped to 0.12 L per day. Gas production capacity was eventually exhausted by the thirtieth day when gas production rate dropped to zero. Run number two was initiated with the now active broth from run number one. It can be seen from Figure 5-8 that there was no lag time in commencing

biogas production. Methanogenesis started quickly and gas production rate peaked on day seven at 2.14 L per day. Biogas production thereafter slowly tapered off and was exhausted by day twenty five with the cumulative methane yield of 0.31 L @ STP/gVS.

Residence time for the batch operations (the time from when the digester was charged or loaded to the time when no biogas was produced in two or more consecutive days) ranged from 19.35 days for run number three to 33.28 days for run number four. The mean residence time was 26.98 days. The mean cumulative methane yield for all the runs was 0.25 L @ STP/gVS. The range was from \approx 0.18 L @STP/gVS for run number three to 0.31 L @ STP/gVS for run number two and four; Table 5-3.

The range and mean results of cumulative methane yield for cassava peel waste are in accord with what have been reported in the literature as biochemical methane potential for biomass and waste feedstocks such as sorghum, napiergrass, poplar, sugarcane, willow, municipal solid waste, as well as spent sugar beet processing by-products (Koppar and Pullammanappallil, 2008; Polematidis et al., 2008; Hutnan et al., 2001, 2000; Chynoweth et al., 1993; Stoppok and Buchholz, 1985; Frostell et al., 1984).

5.4.4 Soluble Chemical Oxygen Demand (sCOD)

Profiles of Soluble Chemical Oxygen Demand (sCOD) of Mesophilic Anaerobic Digestion of Cassava Peel waste for run number one and run number three are shown in Figure 5-9. The day one sCOD for run number one was 2440 mg/L. Ten days later, on day eleven of the experiment, sCOD for run number one peaked at 14340 mg/L. On that peak date, the cumulative methane production, cumulative methane production rate, and cumulative methane yield for run number one were respectively 2.56 L, 1.27 L per day, and 0.0359 L @ STP/gVS. The sCOD thereafter tapered down and

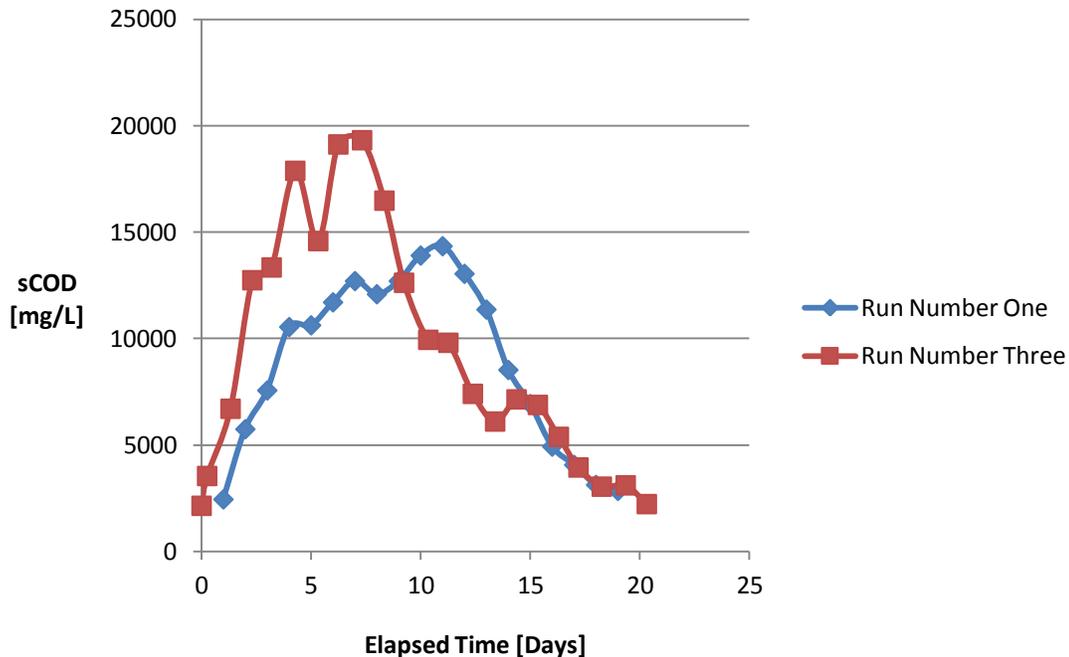


Figure 5-9. Profiles of Soluble Chemical Oxygen Demand (sCOD) for Mesophilic Anaerobic Digestion of Cassava Peel Waste

approached the value of 2840 mg/L by day nineteen of the run.

Run number three started with sCOD of 2140 mg/L on the loading day. Six hours later the value was 3540 mg/L, and by the next day (day one) sCOD for run number three rose to 6700 mg/L. The sCOD value continued the rise and peaked on day seven at 19320 mg/L. On that peak date of sCOD of run number three, the cumulative methane production, cumulative methane production rate, and cumulative methane yield were respectively 2.61 L, 0.528 L per day, and 0.0366 L @ STP/gVS. The sCOD thereafter tapered down and approached the value of 2220 mg/L at the end of the run.

A careful study of Figures 5-8 and 5-9 revealed some interesting phenomena. First, the cumulative methane yield for runs one and three were similar on the days the sCOD peaked. These were 0.0359 L @ STP/gVS (on day eleven) and 0.0366 L @ STP/gVS (on day 7) respectively for run number one and run number three; Figure 5-9.

Secondly, methane production rate peaked on day fourteen for run number one and on day ten for run number three; Figure 5-8. It is interesting to note that these dates were respectively three days after sCOD peaked. It may appear like maximum methane production rate is preceded by three days of sCOD peak. These might be relevant data for optimal design of mesophilic anaerobic digesters/reactors for cassava peel waste and similar biomass.

5.4.5 Total Solids (Dry Matter) and Volatile Solids Reduction

The total dry matter and volatile solids reduction and their computational scheme are presented in Table 5-4. After four runs, the 254.8 g of dry matter loaded had only 76.08 g remaining; thus 70.14 % (178.72 g) was digested. Similarly, out of 245.55 g of volatile solids loaded, 71.06 g remained; translating to 71.06 % volatile solids reduction or the digestion of 174.49 g of volatile solids, Table 5-4. The percent volatile solids reduction corresponded with the Biogasification efficiency of the cassava peel waste presented earlier in Table 5-3.

Table 5-4. Dry Matter and Volatile Solids Reduction during Anaerobic Digestion of Cassava Peel Waste.

S/N	Variable Parameter	Parameter ID and Computing Formula	Parameter Value
1	Dry matter added during the four runs	A	254.8 g
2	Dry matter remaining at the end of four runs	B	76.08 g
3	Total dry matter (solids) reduction	$C = [(A-B)/A]*100$	70.14 %
4	Volatile solids added during the four runs	D	245.55 g
5	Volatile solids remaining at the end of four runs	E	71.06 g
6	Volatile solids reduction	$F = [(D-E)/D]*100$	71.06 %

5.4.6 Enzyme Activity

The activities of enzymes isolated as a consequence of anaerobic digestion of cassava peel waste are presented in Table 5-5. When the experiment was performed with cut up pieces of whole unpeeled cassava roots, higher activities were obtained. The data are shown in Table 5-6. It appears like the production of amylolytic enzymes

Table 5-5. The Activities of Enzymes Produced by Anaerobic Digestion of Cassava Peel Waste (Mean of duplicate measurements).

Time of Enzyme Extraction [Days]	Enzyme Activity [EU = (μ g/mL min)]		
	Cell Free Enzyme	Cell Associated Enzyme	Biofilm Associated Enzyme
1	29.58	–	–
2	29.58	2.50	–
3	29.18	2.64	–
46 (Last Day)	13.93	0.79	9.17

Table 5-6. The Activities of Enzymes Produced by Anaerobic Digestion of Pieces of Whole Unpeeled Cassava Roots (Mean of duplicate measurements).

Time of Enzyme Extraction [Days]	Enzyme Activity [EU = (μ g/mL min)]		
	Cell Free Enzyme	Cell Associated Enzyme	Biofilm Associated Enzyme
2	68.93	2.37	26.08
3	48.79	2.70	13.66
4	46.28	4.55	10.10

will require adding the parenchyma component of the root in the feedstock. Peels alone may not be good enough for production of high activity amylolytic enzymes.

The specific activities (EU/ μ g protein) of the enzymes are summarized in Table 5-7. Despite the wide differences in the total activities of the enzymes presented in Table 5-5 and Table 5-6, the specific activities of the enzymes are similar (Table 5-7). This similarity may be attributed to the protein concentration in each enzyme solution.

Table 5-7. Specific Activities of Enzymes Produced by Anaerobic Digestion of Cassava Root Materials (Means of duplicate measurements)

S/N	Enzyme System	Specific Activity [EU/ μ g protein]
1	Cell Free Enzyme	0.011 – 0.044
2	Cell Associated (Pellet) Enzyme	0.012 – 0.033
3	Biofilm Associated Enzyme	0.016 – 0.065

5.5 Dividends and Applications of Anaerobic Digestion of Cassava Peel Waste

Apart from the enzymes that could be derived or harvested from anaerobic digestion of cassava peel waste, other benefits include the biogas generated and the fertilizer value of the post digestion effluent. In this section, we analyze the biomethane (energy) potential and applications of the biogas as well as the fertilizer value of the effluent.

5.5.1 Biomethane Potential and Applications of Biogas

Mass balance analysis for processing one tonne (1000 kg) of cassava roots and the anaerobic digestion of generated waste are presented in Figure 5-10. At 18.13 % peeling waste capacity, 181.30 kg peel waste was generated and subjected to

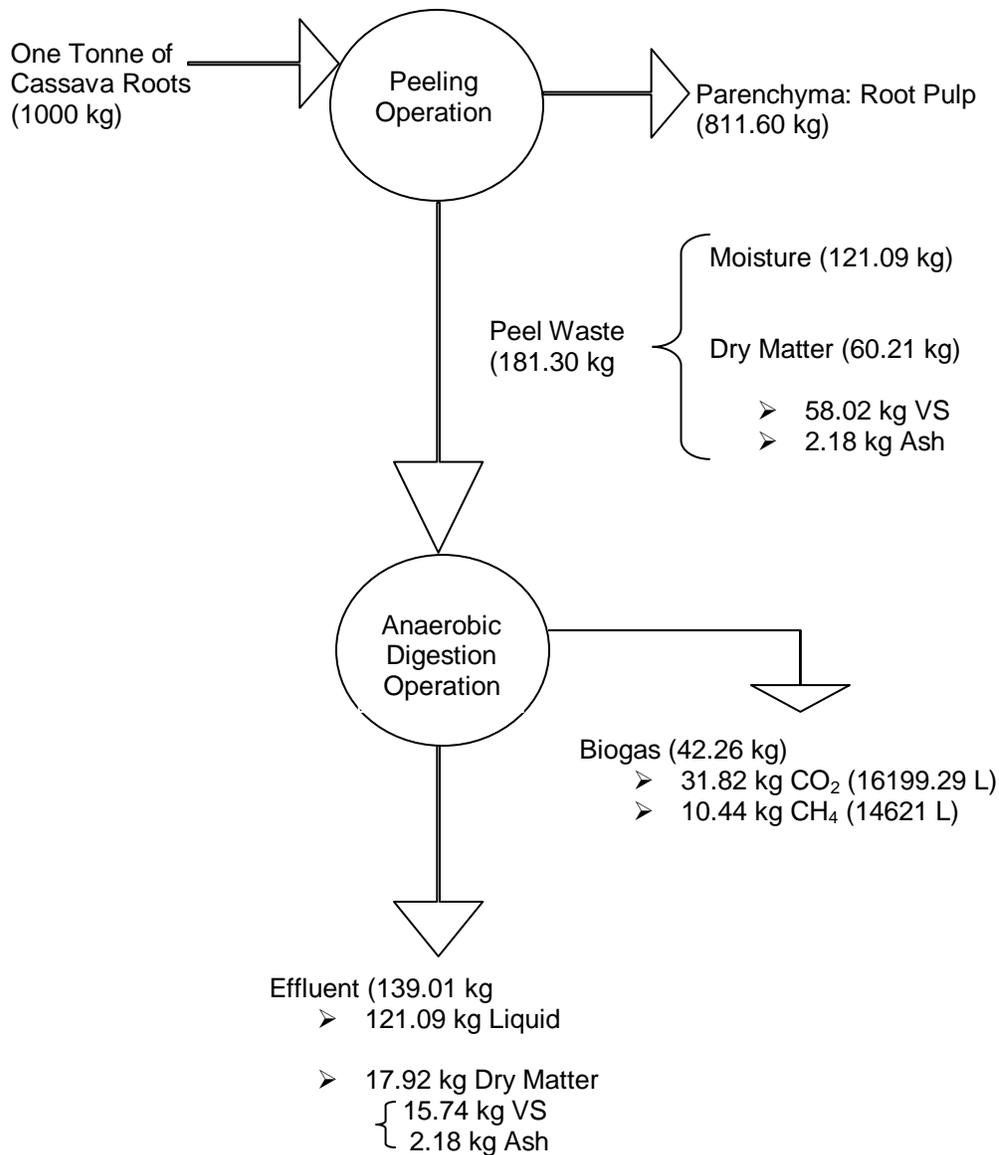


Figure 5-10. Mass Balance for Anaerobic Digestion of Peel Waste from one tonne (1000 kg) of fresh Cassava Root

anaerobic digestion. About 81.16 % (811.6 g) of the fresh root mass was realized as root pulp or edible parenchyma, while the balance of 0.71 % (7.1 g) represented the lost mass; Table 5-2. The parenchyma could be used to produce chips, flour, starch, glucose, etc. However, anaerobic digestion of the peel waste generated 10.44 kg (14621 L) of methane with estimated energy value of 581.25 MJ computed as follows.

The high heating value (heat of combustion) and molecular weight of methane are respectively 890.8 kJ/g mole and ≈ 16 g/g mole (Doran, 1995). Therefore the 10.44 kg of methane will yield: $\{[(890.8 \text{ kJ/g mole})/(16 \text{ g/g mole})] \times [(10.44 \text{ kg CH}_4)(1000 \text{ g/kg CH}_4)]\} = 581247 \text{ kJ}$ or 581.25 MJ of thermal energy. This energy could have at least two possible applications.

a. Boil Water: The energy [Q (kJ)] required to boil a given mass of water [M (kg)] can be estimated with the equation: $Q = MC_p\Delta T + M\Delta h_v$ (eq. 5-1) where C_p is specific heat of water (4.187 kJ/kg ° C); ΔT is temperature difference (° C); and Δh_v is the latent heat of vaporization of water (2256.9 kJ/kg). From equation 5-1, to boil 1 kg of water initially at 25 ° C, with $\Delta T = 100 - 25$ or 75 ° C requires: $\{(1 \text{ kg} \times 4.187 \text{ kJ/kg } ^\circ \text{C} \times 75 ^\circ \text{C}) + (1 \text{ kg} \times 2256.9 \text{ kJ/kg})\} = 2570.925 \text{ kJ}$. Consequently, the water that could be boiled with 581247 kJ from the anaerobic digestion of the cassava peel waste is $(581247 \text{ kJ}/2570.925 \text{ kJ}) \times 1 \text{ kg} = 226.08 \text{ kg}$.

b. Generate Electricity: Alternatively, the 581247 kJ (581.247 MJ) energy value of the biomethane can be transformed to electricity. At the conversion factor of 0.2778 kWh_e/MJ, and conversion efficiency of 40 %, the electricity obtained would be $\{(581.247 \text{ MJ} \times 0.2778 \text{ kWh}_e/\text{MJ}) \times (40/100)\} = 64.588 \text{ kWh}_e$. This electric energy could be used to light homes in a rural village and enable children to do home work and read/study at night.

The Figure 5-11 shows the mass balance for anaerobic digestion of one tonne (1000 kg) of cassava peel waste. The composition of the fresh peel waste as shown in Figure 5-11A consisted of moisture: 66.8 % (668 kg), volatile solids: 32 % (320 kg), and ash: 1.2 % (12 kg). After the peel waste was subjected to anaerobic digestion, the results were carbon dioxide, 55 % (176 kg), methane, 18 % (57.6 kg), and residual organic matter, 27 % (86.4 kg); Figure 5-11B. This amount of methane could boil 1247.34 kg of water or generate 356.35 kWh_e. To achieve this quantity of peel waste would require processing approximately 5515.72 kg of fresh cassava roots.

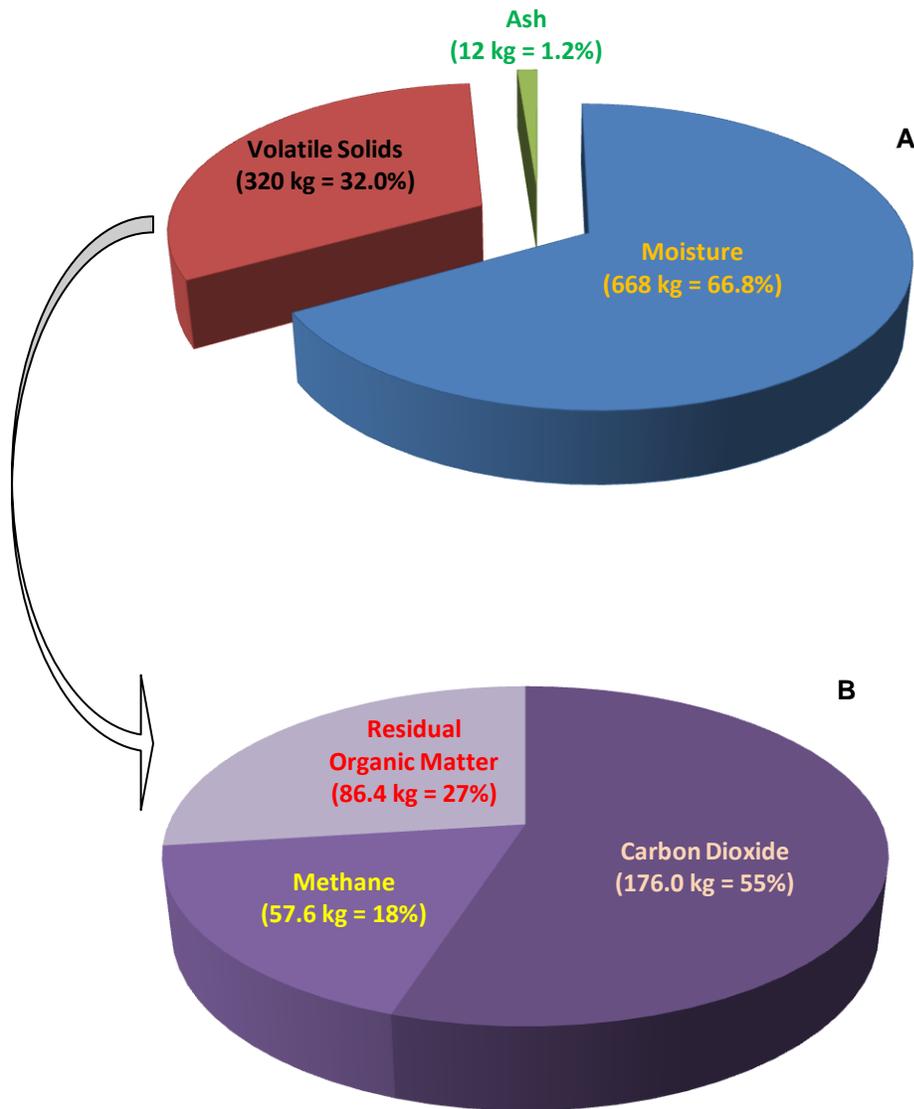


Figure 5-11. Mass Balance for Anaerobic Digestion of 1 tonne (1000 kg) of Cassava Peel Waste. A) Components distribution of the fresh Cassava Peel Waste before anaerobic digestion. B) Repartitioning of Volatile Solids after anaerobic digestion.

5.5.2 Fertilizer Value Potential of Effluent from Mesophilic Anaerobic Digestion of Cassava Peel Waste

The liquid effluent from anaerobic digestion of the cassava peel waste was analyzed to determine its value as a source of organic fertilizer if applied on farm land.

The analyses for Nitrogen (N), Phosphorus (P), and Potassium (K) and other constituents were conducted according to the methodologies presented in Table 5-8.

Table 5-8. Methodologies used for the Analysis of Fertilizer Components of Post Anaerobic Digestion Liquor. (All the fertilizer component analyses were carried out by the University of Florida Institute of Food and Agricultural Sciences Analytical Services Laboratories)

S/N	Fertilizer Components	Analytical Methods and Instruments
1	Ammonia	EPA 350.1 on a Continuous Flow Autoanalyzer. Alpkem Flow IV (O-I Analytical, College Station, TX 77842)
2	Nitrate + Nitrite	EPA 353.2 on a Continuous Flow Autoanalyzer. Alpkem Flow IV (O-I Analytical, College Station, TX 77842)
3	Total Kjeldahl Nitrogen	EPA 351.2 on a Continuous Flow Autoanalyzer. A2 Analyzer (Astoria Pacific International, Clackamas, OR 97015)
4	Metals	EPA 200.7 on an Inductively Coupled Plasma Spectrophotometer (ICP). Spectro Arcos (Spectro Analytical Instruments, Mahwah, NJ 07430)
5	Ortho-Phosphorus	EPA 365.1 on an AQ2 Discrete Analyzer (Seal Analytical, Mequon, WI 53092)
6	Total Phosphorus	EPA 365.1 on a Continuous Flow Autoanalyzer. Alpkem Flow IV (O-I Analytical, College Station, TX 77842)

The results showed that each liter of effluent contained 572.7 mg, 30890 µg, and 1066 mg of (N), (P) and (K) respectively. It has been estimated that 600 kg of NPK (15: 15: 15) fertilizer per hectare is required to produce 25 tonnes of cassava roots (<http://cassavabiz.org/agroenterprise/farming.htm> [Accessed 10/17/2013]). It is also reported that the fertilizer nutrients are available to plants as elemental nitrogen for (N);

as P₂O₅ for (P); and as K₂O for (K) (http://en.wikipedia.org/wiki/NPK_rating [Accessed 10/27/2013]). Based on these data, we compute that 24 kg of NPK (15: 15: 15) fertilizer is required to produce one tonne of cassava roots; with the 15 % of (N) supplied or available as elemental nitrogen; the 15 % of (P) available as P₂O₅; and the 15 % of (K) available as K₂O.

5.5.2.1 Nitrogen

The nitrogen requirement is estimated as:

$$\begin{aligned} \text{Nitrogen} &= 15 \% [24 \text{ kg (elemental N)/t cassava root}] \\ &= 3.6 \text{ kg (N)/t cassava root.} \end{aligned}$$

Nitrogen that can be obtained from digestion is estimated as:

From effluent analysis, (N) concentration = 572.7 mg/L.

Thus for the 3 L of digester volume, (N) = 3 L (572.7 mg/L) = 1718.1 mg.

Nitrogen fertilizer value of the effluent can now be estimated.

Note that 1718.1 mg is mass of (N) in the dry matter of 4 experimental runs.

$$\begin{aligned} \text{Since the dry matter was 254.8 g, the mass of (N) per unit dry matter} \\ = 1718.1 \text{ mg (N)/254.8 g dry Wt.} \times (1 \text{ g}/1000 \text{ mg}) = 0.00674 \text{ g (N)/g dry Wt.} \end{aligned}$$

However, for 1tonne of roots, only 60.21 kg dry matter was generated.

This dry matter was fed the digester, see mass balance schematics; Figure 5-10.

$$\begin{aligned} \text{Consequently, potential (N)} &= 0.00674 \text{ g (N)/g dry Wt} \times (60.21 \text{ kg dry Wt.}) \\ &= 0.4058 \text{ kg (N)} \end{aligned}$$

But 3.6 kg (N) is required to produce one tonne of cassava roots.

Therefore proportion of (N) met by anaerobic digestion

$$= 0.4058 \text{ kg (N)/} 3.6 \text{ kg (N)} \times (100) = 11.27 \%$$

5.5.2.2 Phosphorus

The phosphorus requirement is estimated as:

$$\begin{aligned}\text{Phosphorus} &= 15 \% [24 \text{ kg } (\text{P}_2\text{O}_5)/\text{t cassava root}] \\ &= \{[3.6 \text{ kg } (\text{P}_2\text{O}_5)/\text{t cassava root}] \times [62 \text{ kg (elemental P)}/142 \text{ kg } (\text{P}_2\text{O}_5)]\} \\ &= 1.5718 \text{ kg (P)}/\text{t cassava root}.\end{aligned}$$

Phosphorus that can be obtained from digestion is estimated as:

From effluent analysis, (P) concentration = 30890 $\mu\text{g/L}$.

$$\begin{aligned}\text{Thus for the 3 L of digester volume, (P)} &= 3 \text{ L } (30890 \mu\text{g/L}) \\ &= 92670 \mu\text{g or } 92.67 \text{ mg}.\end{aligned}$$

Phosphorus fertilizer value of the effluent can now be estimated.

The 92.67 mg is mass of (P) in the dry matter of 4 experimental runs.

$$\begin{aligned}\text{Since the dry matter was 254.8 g, the mass of (P) per unit dry matter} \\ &= 92.67 \text{ mg (P)}/254.8 \text{ g dry Wt.} \times (1 \text{ g}/1000 \text{ mg}) = 0.000363 \text{ g (P)}/\text{g dry Wt}.\end{aligned}$$

However, for 1tonne of roots, only 60.21 kg dry matter was generated.

This dry matter was fed the digester, see mass balance schematics; Figure 5-10.

$$\begin{aligned}\text{Consequently, potential (P)} &= 0.000363 \text{ g (P)}/\text{g dry Wt} \times (60.21 \text{ kg dry Wt.}) \\ &= 0.021898 \text{ kg (P)}\end{aligned}$$

But 1.5718 kg (P) is required to produce one tonne of cassava roots.

Therefore proportion of (P) met by anaerobic digestion

$$= 0.021898 \text{ kg (P)}/ 1.5718 \text{ kg (P)} \times (100) \approx 1.40 \%$$

5.5.2.3 Potassium

The potassium requirement is estimated as:

$$\begin{aligned}\text{Potassium} &= 15 \% [24 \text{ kg } (\text{K}_2\text{O})/\text{t cassava root}] \\ &= \{[3.6 \text{ kg } (\text{K}_2\text{O})/\text{t cassava root}] \times [78 \text{ kg (elemental K)}/94 \text{ kg } (\text{K}_2\text{O})]\}\end{aligned}$$

= 2.9872 kg (K)/t cassava root.

Potassium that can be obtained from digestion is estimated as:

From effluent analysis, (K) concentration = 1066 mg/L.

Thus for the 3 L of digester volume, (K) = 3 L (1066 mg/L)

= 3198 mg.

Potassium fertilizer value of the effluent can be estimated thus:

We now have 3198 mg of (K) in the dry matter of 4 experimental runs.

Since the dry matter was 254.8 g, the mass of (K) per unit dry matter
= $3198 \text{ mg (K)}/254.8 \text{ g dry Wt.} \times (1 \text{ g}/1000 \text{ mg}) = 0.01255 \text{ g (K)}/\text{g dry Wt.}$

However, for 1tonne of roots, only 60.21 kg dry matter was generated.

This dry matter was fed the digester, see mass balance schematics; Figure 5-10.

Consequently, potential (K) = $0.01255 \text{ g (K)}/\text{g dry Wt} \times (60.21 \text{ kg dry Wt.})$

= 0.755696 kg (K)

But 2.9872 kg (K) is required to produce one tonne of cassava roots.

Therefore proportion of (K) met by anaerobic digestion

= $0.755696 \text{ kg (K)}/2.9872 \text{ kg (K)} \times (100) \approx 25.30 \%$

The organic fertilizer generation capability of anaerobic digestion of cassava peel waste is summarized in Table 5-9. Obviously, anaerobic digestion can comfortably satisfy at least one quarter of the potassium requirement.

Table 5-9. Organic Fertilizer Potential of Anaerobic Digestion of Cassava Peel Waste

S/N	Organic Fertilizer Element	Quantity Required [kg/tonne of Cassava Root Produced]	Quantity Generated by Anaerobic Digestion of Cassava Peel Waste [kg/tonne of Cassava Root Processed]	Proportion of Fertilizer Requirement Met [%]
1	Nitrogen (N)	3.60	0.406	11.30
2	Phosphorus (P)	1.57	0.022	1.40
3	Potassium (P)	2.99	0.756	25.30

CHAPTER 6 CONCLUSIONS AND RECOMMENDATIONS

6.1 Conclusions

The researches conducted for this doctoral dissertation demonstrated several value added potentials for the cassava crop. Namely dehydration of cassava root parenchyma to produce chips using solar-convection dryer; production of cassava flour from the solar-convection dried cassava chips; glucose sweetener production by simultaneous and synergistic enzymatic hydrolysis of native unprocessed cassava starch; as well as anaerobic digestion of cassava waste to produce biomethane and amylolytic enzymes. Based on these demonstrations, the following conclusions can be drawn.

- 1 Fresh cassava roots can be processed into flour from solar-convection dried cassava chips. The drying process does not require any payment for fuel or electricity.
- 2 The characterized physical properties of the chips and flour such as sorption isotherm, drying curve, moisture content, particle size distribution, bulk density, solid particle density, porosity, permeability and specific surface area are beneficial to the execution of engineering design of storage, handling, and processing systems.
- 3 Simultaneous and synergistic enzymatic hydrolysis of cassava starch is possible. In this technique, liquefaction and saccharification processes are executed as a single unit operation.
- 4 The simultaneous and synergistic application of fungal gluco-amylase (FGA) and bacterial alpha-amylase (BAA) at ratio of 3:1 (FGA:BAA), yielded 96 %,

- 27 %, and 53 % conversion of starch to glucose at 60 ° C after 24 hours of hydrolysis respectively for commercial cassava starch, flour produced from solar-convection dried cassava chips , and fresh cassava root pulp.
- 5 At 37 ° C operating temperature and 96 hours incubation time, the synergistic hydrolysis protocol achieved 100 %, 72 %, and 55 % conversion of starch to glucose respectively for commercial cassava starch, flour produced from solar-convection dried cassava chips, and fresh cassava root pulp.
 - 6 Glucose sweetener can be obtained by direct conversion of cassava starch substrate. With this technique cassava root pulp or flour can be hydrolyzed directly to glucose without going through the expensive, time and energy intensive starch extraction process.
 - 7 The simultaneous/synergistic and direct conversion approaches minimize energy, equipment and technical inputs, as well as the overall cost hitherto required for the production of sweeteners by first extracting the starch from cassava root.
 - 8 First-order kinetic parameters (rate constant along with the Arrhenius activation energy) were determined for the enzyme hydrolysis of each substrate at the two different temperatures.
 - 9 Cassava root processing wastes such as peels can be converted to bio-methane via anaerobic digestion. The methane is a clean and renewable energy that could reduce dependence on fossil fuels and thus mitigate associated adverse environmental impacts.

- 10 The biomethane may be used to heat/boil water or generate electricity. The electrical power can help pupils in villages do their homework and study at night.
- 11 Processing one tonne (1000 kg) of fresh cassava root creates 181.30 kg of peel waste. The waste can be digested in anaerobic reactor to generate 10.44 kg (14621 L) of methane with energy value of 581.25 MJ. This thermal energy can boil 226.08 kg of water which was initially at 25 ° C. Alternatively; the thermal energy could be used to generate 64.58 kWh_e for illumination or motive power applications.
- 12 The post digestion effluent can be used as source of organic fertilizer to supplement the nitrogen (at 11 %) and potassium (at 25 %) requirements for cassava cultivation.
- 13 The anaerobic digestion of cassava waste demonstrated viability of digester reactor operation with the following performance indices. Organic load of 20 gVS/m³ (gram volatile solids/cubic meter); residence time of 27 days; bio-methane yield of 0.25 L/gVS (liter/ gram volatile solids); and bio-methane production efficiency of 71 %.
- 14 It is possible to obtain amylolytic (starch hydrolyzing) enzymes from mesophilic anaerobic digestion of cassava waste.
- 15 Extracellular enzymes were located in the three divisions defined as cell free (leachate) enzyme; cell associated (pellet) enzyme; and biofilm associated enzyme.

- 16 Beta amylase and Glucoamylase activities were identified in the cell free, cell associated and biofilm associated portions of the digester substrate.
- 17 Anaerobic digestion of pieces of whole unpeeled cassava root resulted in much higher activities than those obtained with the peel waste alone.
- 18 The highest activity was found in the cell free portion, followed by the biofilm associated portion. The cell associated portion had minimal activity.
- 19 The activity of the cell free enzyme was 1.5 to 3.0 times higher than that of the biofilm associated enzyme at 13.93 EU to 68.93 EU and 9.17 EU to 26.08 EU respectively for the cell free and biofilm associated enzymes.
- 20 The specific activities (EU/ μ g protein) for all the enzyme systems; cell free, cell associated and biofilm associated were found to be similar.
- 21 The processes demonstrated can be accommodated by developing nations to foster appropriate technologies for cottage industries, encourage the use of indigenous raw materials, enable local capacity building, as well as promote economic empowerment.

6.2 Recommendations for Future Work

This is an exciting endeavor in that we are engaged in a new research frontier of simultaneous and synergistic hydrolysis reactions, as well as mesophilic anaerobic digestion of agricultural and biological materials. The results of our research can be applied by people with low income or financial resources and little or no technical skills anywhere in the world for the production of flour, sweeteners, biomethane and enzymes that could be used by the households and industries. However, in order to maximize the value adding potentials of these endeavors, optimization of the research demonstrations are urged. Areas to be considered for further/future work include:

- Development and implementation of purification system for the digester derived enzymes.
- Evaluation of activity and stability of the digester derived enzymes at different temperatures.
- Evaluation of the capability of the digester derived enzymes to hydrolyze native un-extracted starch in cassava flour and root pulp.
- To understand and characterize the relationship, if any, between biomethane production, enzyme production and enzyme activity.
- Development of an economic model with comprehensive plant design and cost benefit analysis for a closed-loop system that simultaneously produces biomethane and amylolytic enzymes. The enzymes would be used for hydrolysis of cassava starch for the production of sweeteners while the methane would be used to heat both the anaerobic digester and the hydrolysis reactors.

APPENDIX A
PHOTOGRAPHS OF SOME MATERIALS AND EQUIPMENT USED AND THE
PRODUCTS CREATED*

A-1. Solar-Convection Dryer



* All the photos courtesy of the author unless otherwise indicated.

A-2. Cassava Chips that were Dried by Solar-Convection Dryer



A-3. Flour from Cassava Chips that were Dried by Solar-Convection Dryer



A-4. Electric-Convection Oven



A-5. Cassava Chips Dried by Electric-Convection Oven



A-6. Flour from Cassava Chips that were Dried by Electric-Convection Oven



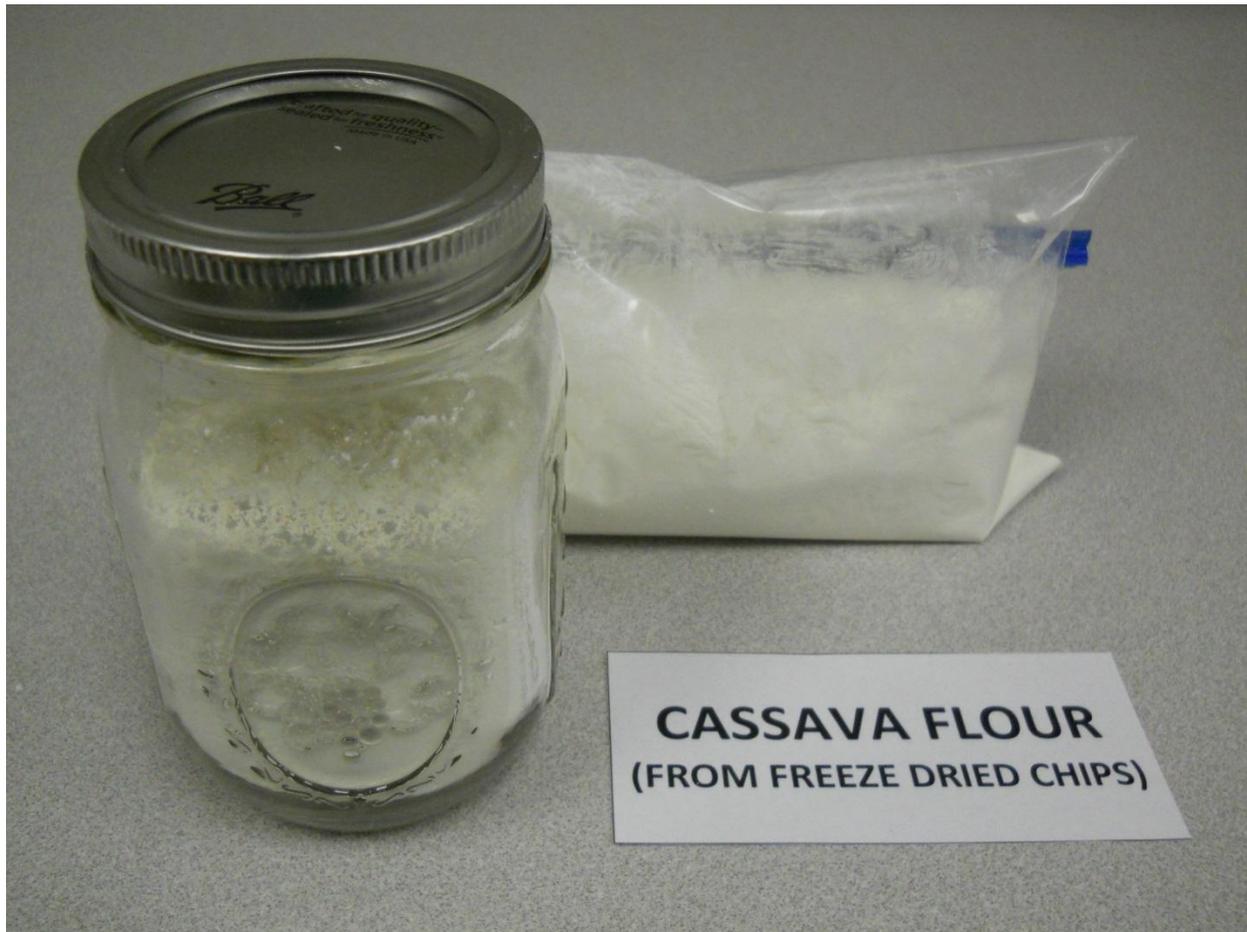
A-7. Freeze Dryer



A-8. Freeze Dried Cassava Chips



A-9. Cassava Flour Produced from Freeze Dried Cassava Chips



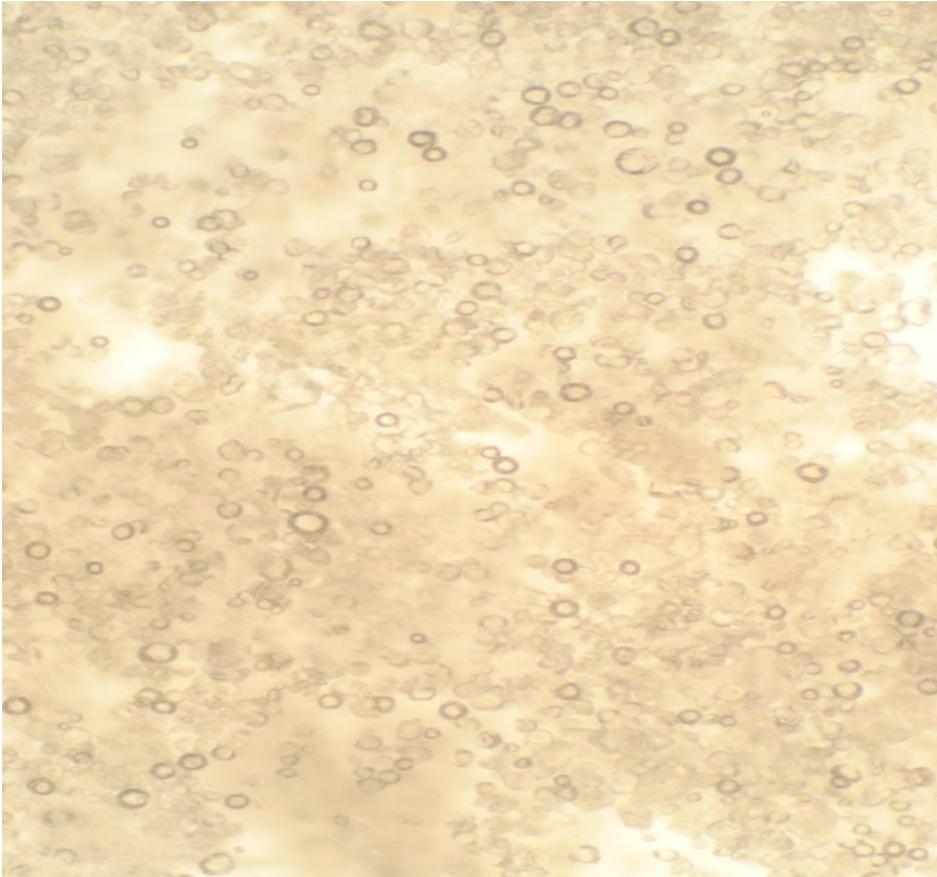
A-10. Cassava Chips that were Dried by Different Drying Methods



A-11. Flour from Cassava Chips that were Dried by Different Drying Methods



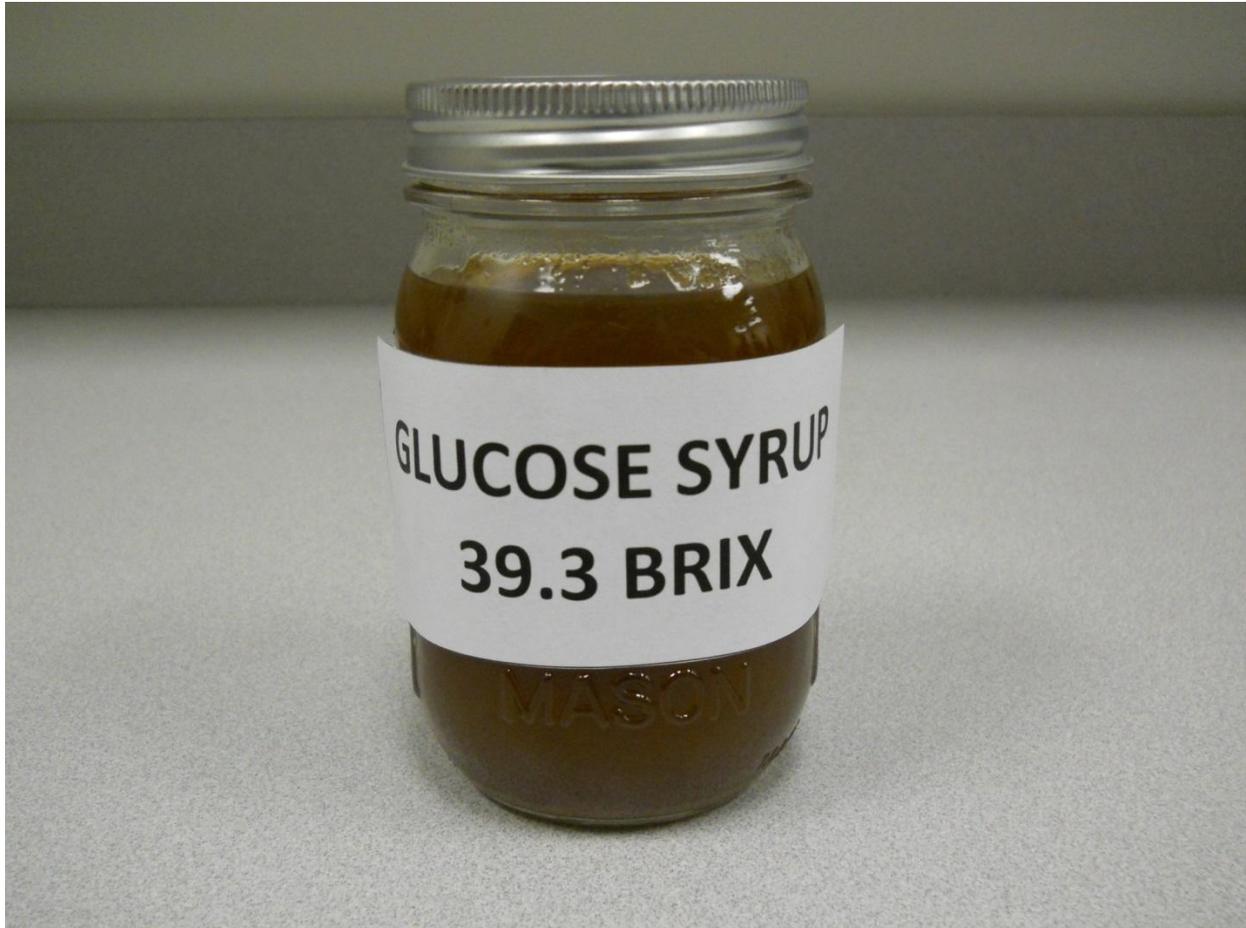
A-12. Microphotograph of Cassava Flour



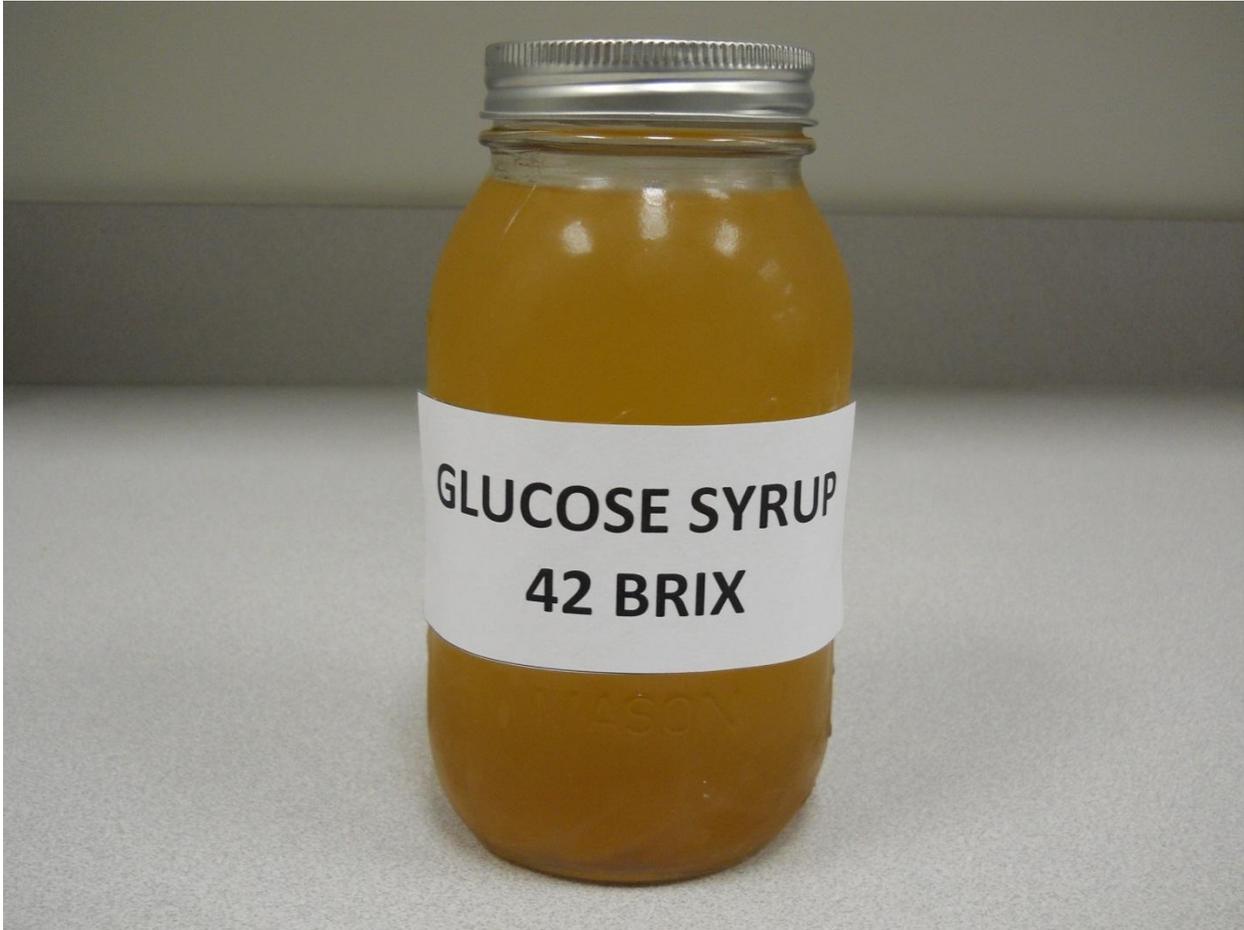
A-13. Glucose Syrup [27.7 ° Brix] Produced by Synergistic Enzymatic Hydrolysis
of Cassava Substrates (Commercial Starch, Flour, and Fresh Roots]



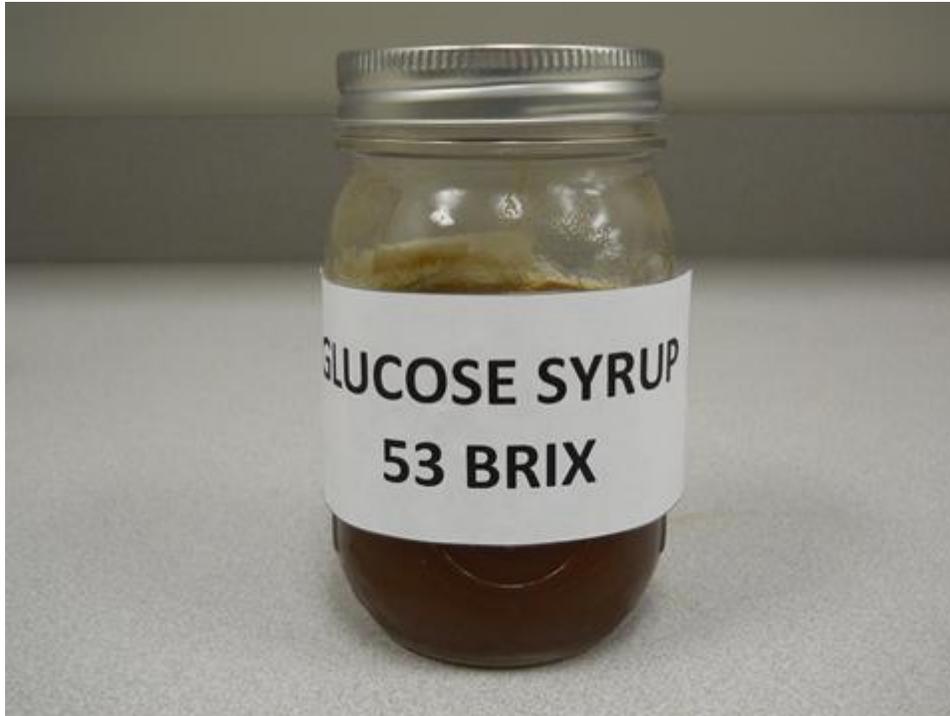
A-14. Glucose Syrup [39.3 ° Brix] Produced by Synergistic Enzymatic Hydrolysis
of Cassava Substrates (Commercial Starch, Flour, and Fresh Roots]



A-15. Glucose Syrup [42 ° Brix] Produced by Synergistic Enzymatic Hydrolysis of Cassava Substrates (Commercial Starch, Flour, and Fresh Roots]



A-16. Glucose Syrup [53° Brix] Produced by Synergistic Enzymatic Hydrolysis of
Cassava Substrates (Commercial Starch, Flour, and Fresh Roots]



A-17. Glucose Syrup of Various Soluble Solids [$^{\circ}$ Brix] Produced by Synergistic Enzymatic Hydrolysis of Cassava Substrates (Commercial Starch, Flour, and Fresh Roots]



A-18. Various Packaging Options for Value Added Cassava Products: Chips; Flour, Glucose Syrup



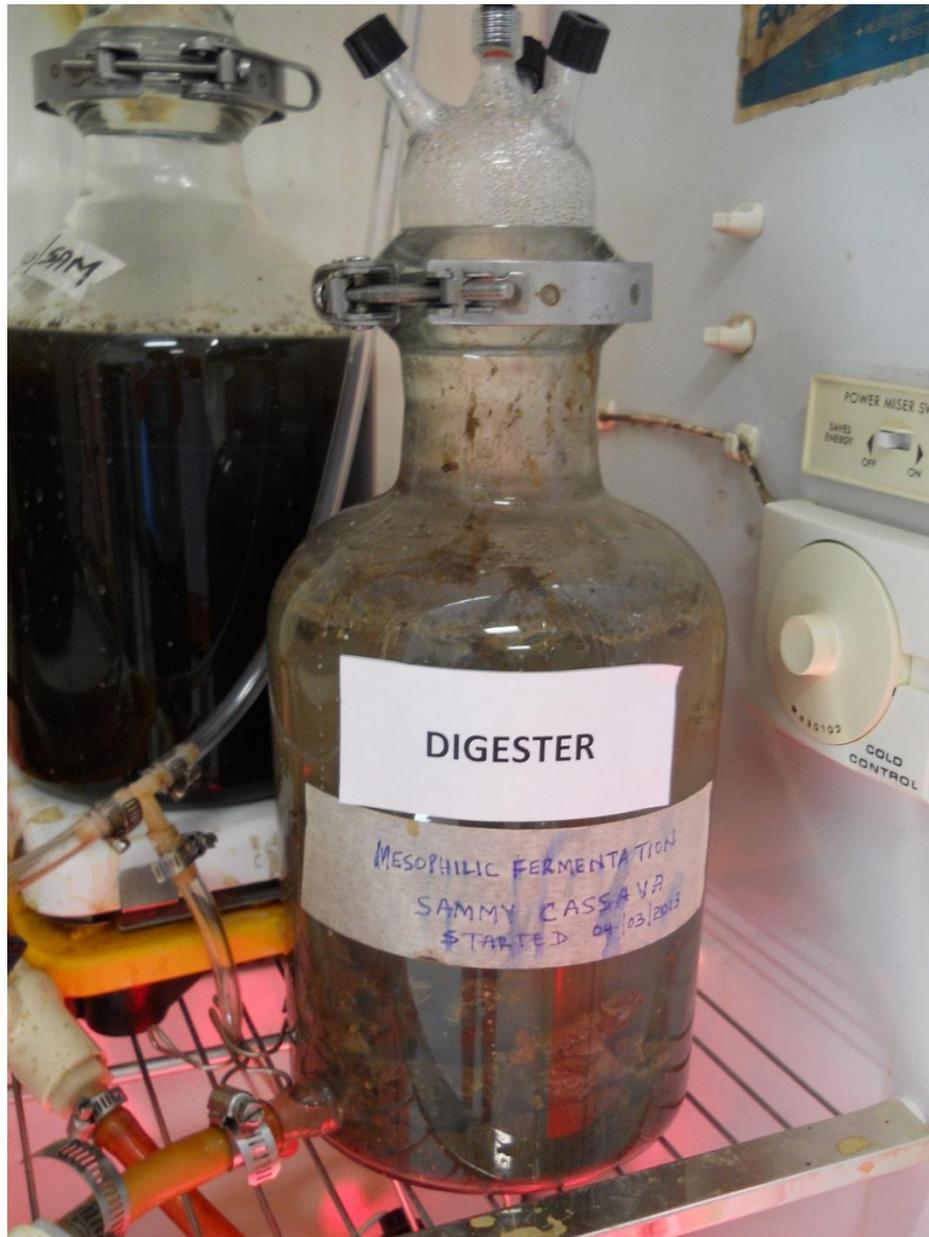
A-19. ASTM Standard Sieves Used for Particle Size Analysis



A-20. Incubators for Anaerobic Digestion Reactors



A-21. Mesophilic Anaerobic Digester Incubating at $26 \pm 3 \text{ }^\circ\text{C}$



A-22. Cylinders for Carrier Gases used with GC Equipment (background) for Biogas Analysis



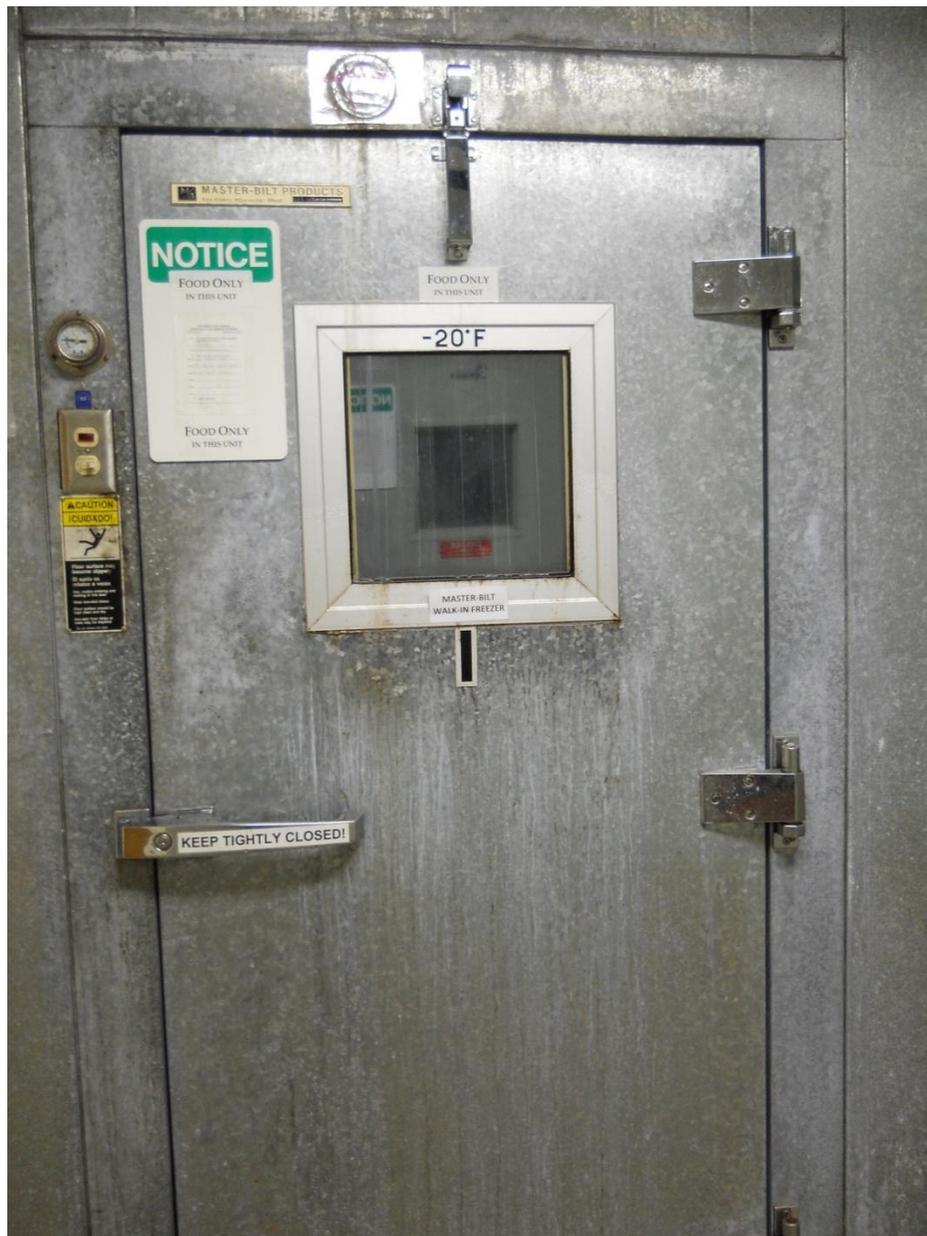
A-23. Electric Range and Pots Used to Concentrate Glucose Syrups



A-24. Mason Jars Used for Packaging and Canning Cassava Products



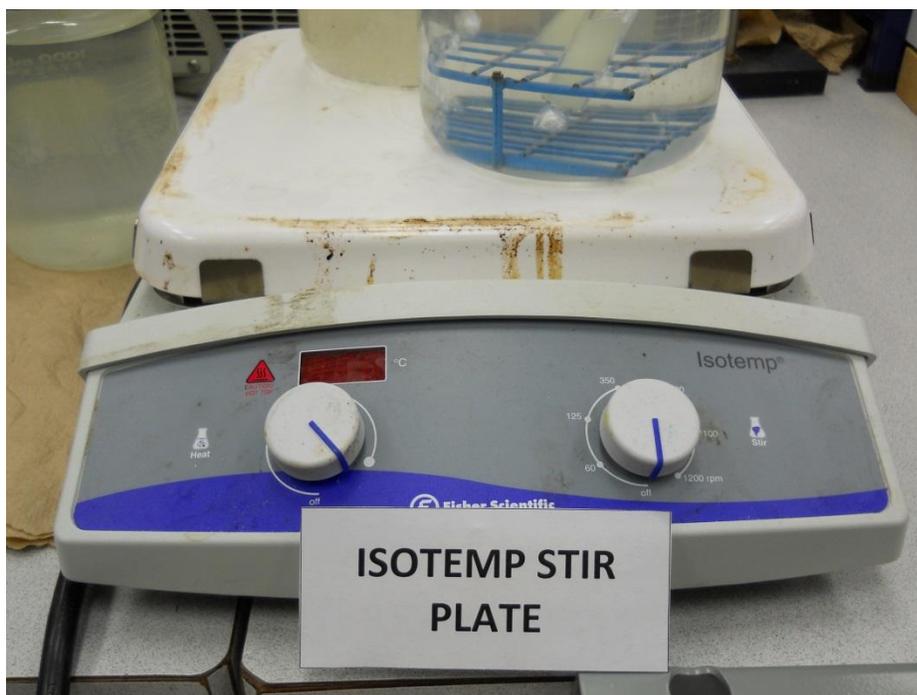
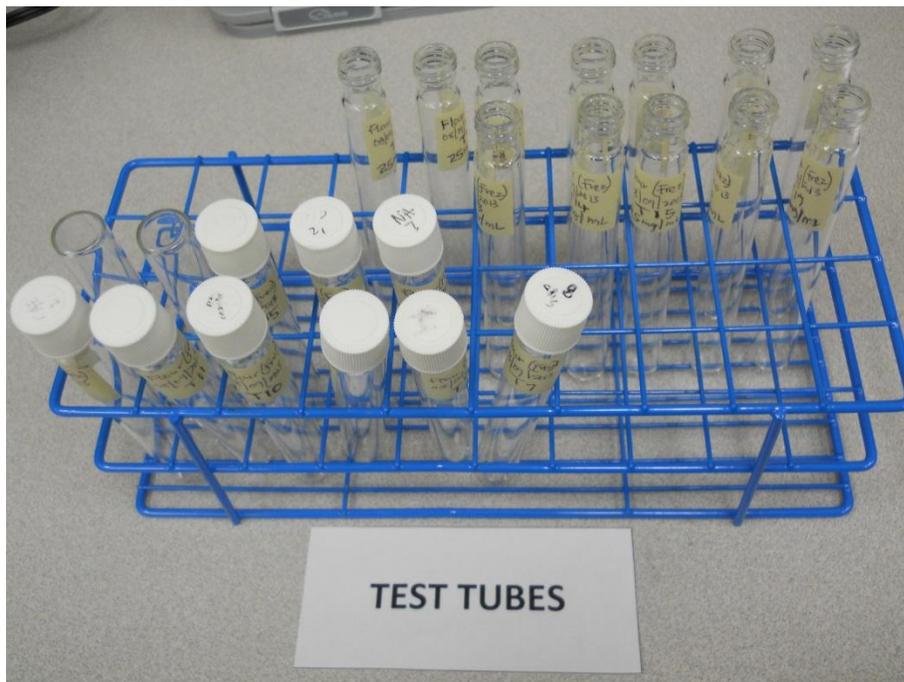
A-25. Walk-in Freezer Used to Freeze Samples at -20°C



A-26. Vortex Machines Used to Homogenize Research Samples for Analysis



A-27. Rack, Test Tubes and Stir-Plate/Heater Used to Hold and Prepare Samples for Analysis



A-28. Desiccator Jars and Cassava Samples Used for Sorption Isotherm Experiments



A-29. Weights Used as Ballast to Stabilize Research Samples under Centrifugal Force Field



A-30. Components of Cassava Root



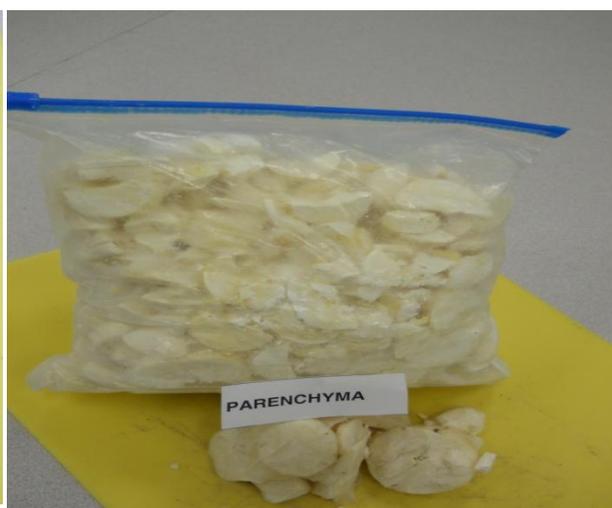
Heads & Tails: 1.71 %



Periderm: 3.31 %



Cortex: 13.11 %



Parenchyma: 81.16 %

Lost Mass: 0.71 %

A-31. Heads & Tails of Cassava Root



A-32. Periderm/Epidermis (Bark or Outer Coat) of Cassava Root



A-33. Cortex of Cassava Root



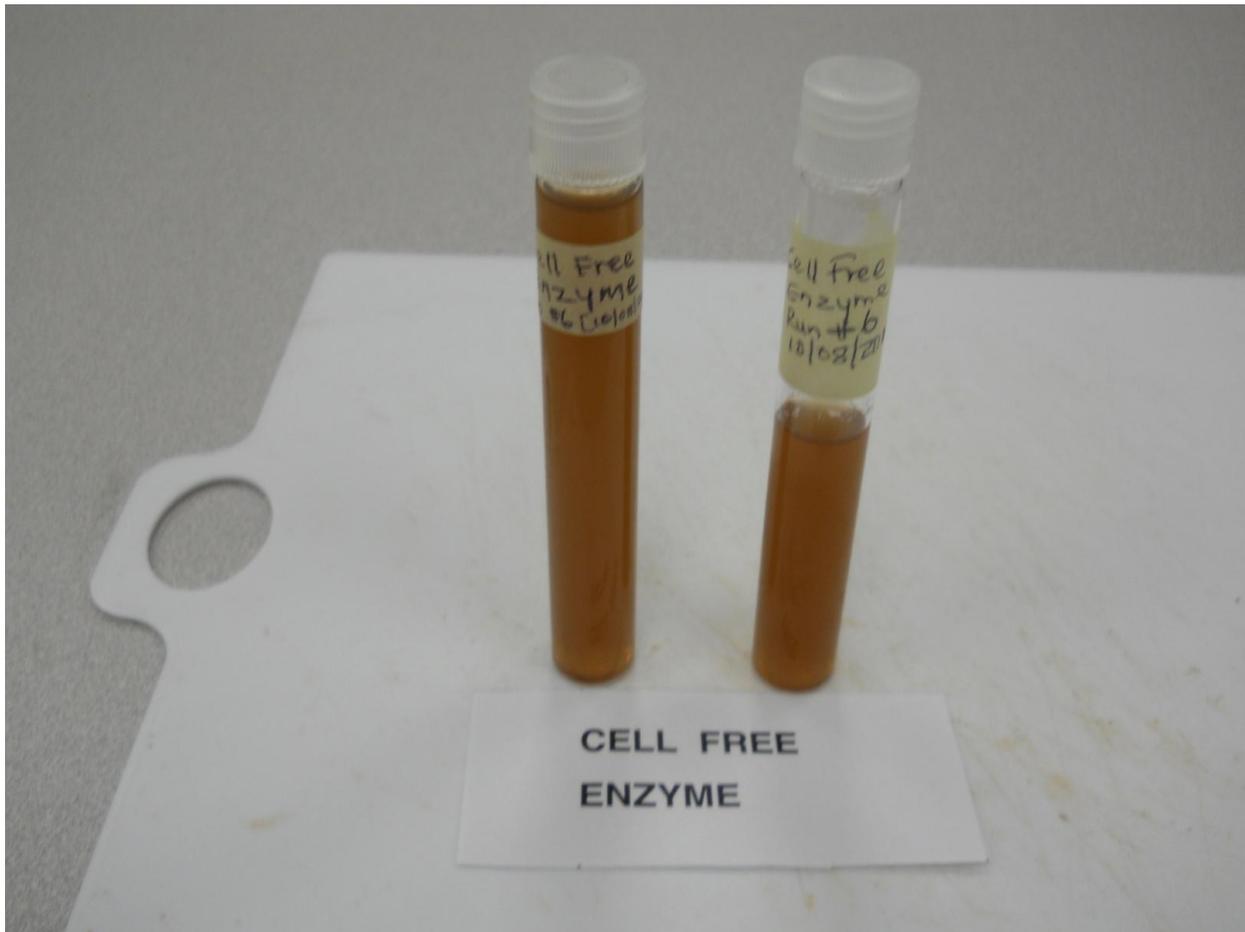
A-34. Parenchyma (Pulp) of Cassava Root; Frozen Slices



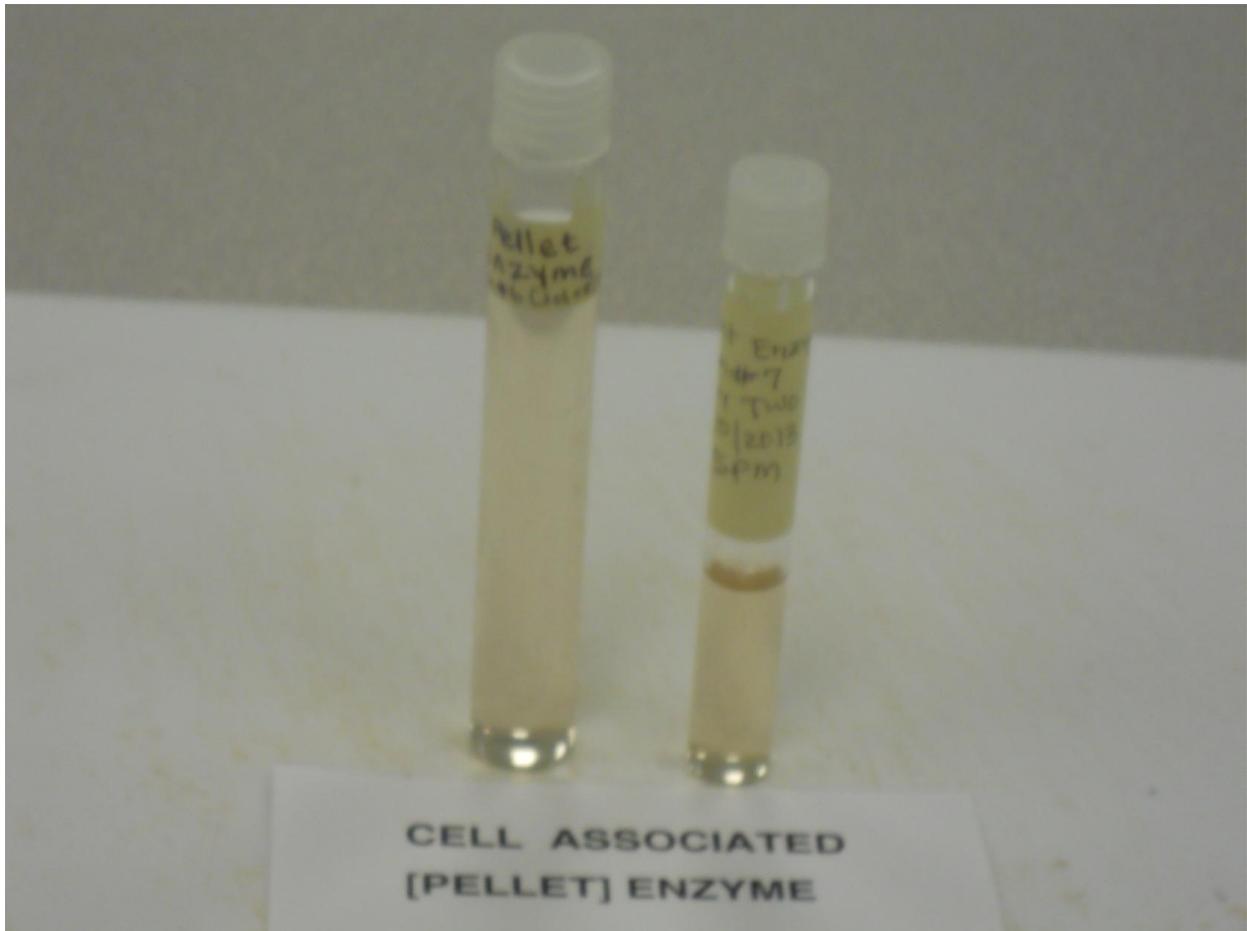
A-35. Cassava Root Peeling Waste (Heads & Tails, Periderm and Cortex) used as Feedstock/Substrate in Anaerobic Digestion Experiments for Biomethane and Enzyme Production



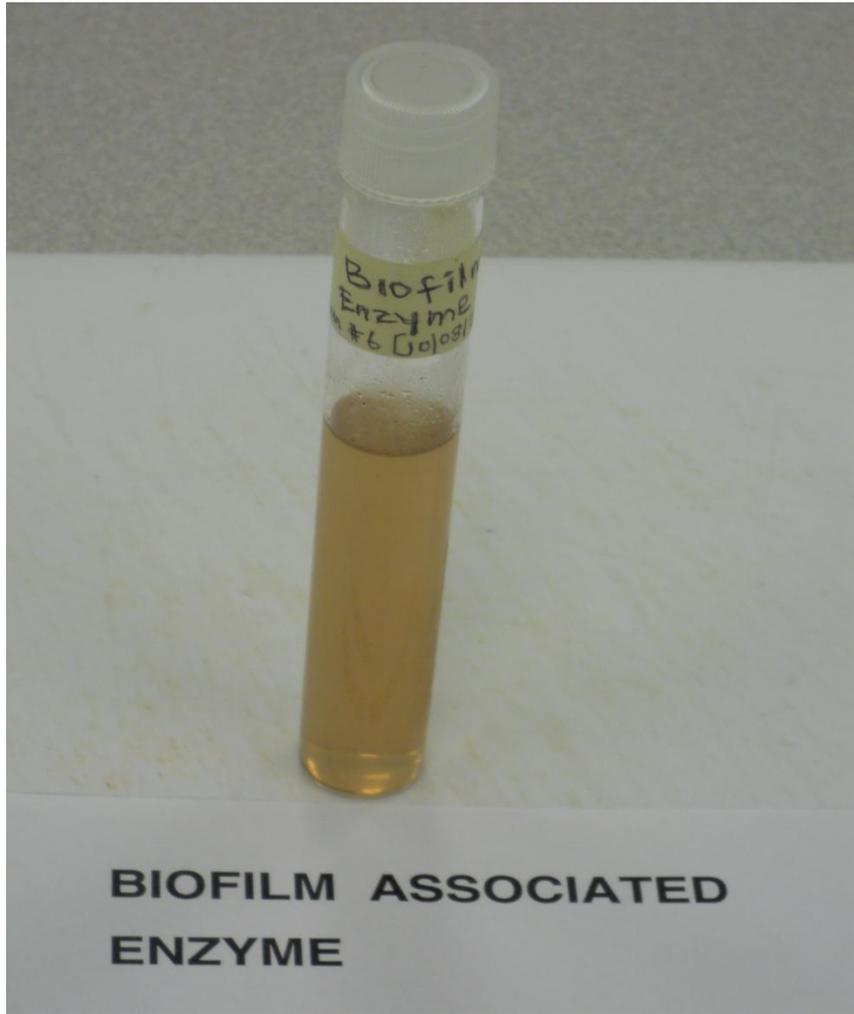
A-36. Cell Free Enzyme Derived from Anaerobic Digestion of Cassava Waste



A-37. Cell Associated (Pellet) Enzyme Derived from Anaerobic Digestion of Cassava Waste



A-38. Biofilm Associated Enzyme Derived from Anaerobic Digestion of Cassava Waste



A-39. PhD Candidate & Graduate Committee Members [Front row from Right to Left: Dr Wade Yang, Member; Dr Arthur A Teixeira, Chairman; Sammy Aso, PhD Candidate. Back row from left to right: Dr Bruce A Welt, Member; Dr Robert P Bates, Member; Dr Spyros A Svoronos, Member, and Dr Pratap C Pullammanappallil, Member



A-40. Graduate Committee Members



A-41. Hydrolysate Samples' Analysis



APPENDIX B RECOMMENDED REFERENCES FOR FUTURE READING

B-1. Catalytic Efficiency

http://chemwiki.ucdavis.edu/Physical_Chemistry/Kinetics/Complex_Reactions/Catalytic_efficiency_of_enzymes [Accessed Friday 27th April 2012]

B-2. Enzyme Kinetics

<http://graphpad.com/curvefit/introduction63.htm> [Accessed Friday 27th April 2012]

<http://users.rcn.com/jkimball.ma.ultranet/BiologyPages/E/EnzymeKinetics.html>
[Accessed Friday 27th April 2012]

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BIOGRAPHICAL SKETCH

Samuel NwaneLe Aso [Sammy] was born in the Village of Elemenwo, Rivers State, Nigeria. He started elementary education in the then St. Marks School Elemenwo. His elementary education was interrupted by the Nigerian civil war from 1966 to 1969. In 1970 he resumed elementary education and received the first school leaving certificate in 1971. Sammy enrolled into County Grammar School Ikwerre/Etche in 1972 for secondary education; graduating in 1976 with the West African Examinations Council (WAEC) certificate for secondary education.

In the fall of 1977 he enrolled into Utah State University, Logan, but transferred to The University of Tennessee, Knoxville, in the summer of 1978. Sammy received his bachelor's degree in agricultural engineering from The University of Tennessee in December 1981 and enrolled into the master's degree program in the spring of 1982 at the same institution. He received the Master of Science degree with concentration in food engineering in August 1983.

Sammy immediately returned to Nigeria and on completion of National Youth Service Corps (NYSC) program in 1984, served as the general manager of Erijoy Nigeria Limited, a multi branch hotel/catering firm situated in Port Harcourt, Nigeria. He joined Rivers State University of Science and Technology (RSUST) in 1991 as assistant lecturer. Sammy rose through the ranks and became a senior lecturer in food engineering at RSUST by 2006. In the spring of 2010, He commenced this Doctor of Philosophy degree program in food engineering at the University of Florida.

The research interests of Sammy include life cycle assessment (LCA) of food engineering systems; food irradiation; ohmic food processing; high pressure food processing; heat pipe technology; and the engineering of consumables (especially food)

for extraterrestrial body-surface and micro-gravity environments such as Space Shuttle Orbiters, International Space Station, Lunar and Martian Outposts, Asteroid Mining Bases, as well as other ETI and Planet-Surface habitats. Sammy is a deep space enthusiast and lover of space mementos.