Utilization of endogenous amylases and Pectinex enzymes for efficient extraction of sweetpotato juice

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Declaration

This thesis is my original work and has not been presented for a degree in any other University

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Abstract

Sweetpotato (*Ipomoea batatas (L.) Lam*) is one of the most important, versatile and underexploited crops in Kenya. Lack of organized marketing, limited consumer interest and low level of value addition are major factor that contribute to this unfortunate scenario. Processing of non-alcoholic beverages and fuel ethanol are feasible options of commercializing the roots, but lack of an efficient and low cost method of extracting juice has been a major constraint especially in third world countries. The most efficient method of extracting sweetpotato juice available currently involves the use of expensive commercial amylases and starch debranching enzymes.

The overall objective of this study was therefore to develop an efficient and low cost method of extracting sweetpotato juice using a combination of endogenous amylolytic enzymes and pectinex 3XL enzyme. The specific objectives of the study were; to determine the effect of sweetpotato variety, temperature and curing duration on endogenous α-amylase and β-amylase activities in three sweetpotato varieties; to study the effect of endogenous α-amylase activity on starch hydrolysis in sweetpotato purees incubated at 60, 65, 70, 75°C for 180 minutes; to determine the effect of pectinex 3XL enzyme on viscosity and yield of juice from saccharified sweetpotato purees and to characterize three local sweetpotato root varieties in terms of yield and quality of juice extracted.

To study the effect of temperature, curing duration and variety on amylolytic activities in sweetpotato roots, a 3x3x3 factorial experiment in a completely randomized design was used. The effects of temperature and curing duration on endogenous α-amylase activity were found to be highly significant while varietal effect was not. The first order interactions between; temperature and time, variety and temperature and second level interactions between the three factors were also found significant. Endogenous β-amylase activity in sweetpotato roots did not change in a consistent fashion; decreasing in Muibai and Gikanda yellow sweetpotato while remaining the same in Gikanda white.

A 4x7 factorial experiment in a randomized complete block design was used to investigate the effect of endogenous α-amylase activity on starch hydrolysis in Muibai sweetpotato purees from roots cured for between 1-6 days at 35°C. ANOVA of the results showed that temperature and α-amylase enzyme activities significantly influenced maltose formation in the purees. The endogenous amylolytic enzymes were able to convert more than 75% of

starch in the sweetpotato purees to sugar, which demonstrates that the enzymes could be used for improving the efficiency and reduce the cost of extracting sweetpotato juice.

A randomized complete block design was used to study the effect of pectinex 3XL enzyme on the viscosity and yield of juice from saccharified sweetpotato purees. ANOVA of the results showed that the effect of pectinex enzyme on puree viscosity and juice yields was highly significant. The yield of juice increased from 30-50% when no enzyme was used to about 80% as little as 200mg/Kg pectinex enzyme was added to the puree. Results of proximate composition analysis of sweetpotato root and juice showed that most of the solids present in the roots were dissolved in the juices and that the sugars contents of juices (14%) was sufficient for fermentation to alcohol.

Therefore, the objectives of the study were achieved which means that commercial exploitation of sweetpotato roots for production of low cost non-alcoholic beverages could be viable options even in less technologically advanced countries such as Kenya.

CHAPTER ONE

1.0 Introduction

Agriculture is the dominant sector of Kenya's economy representing more than 30% of GDP, 45% of government revenue and providing employment for 75% the workforce. It contributes 70% of industrial raw materials and 65% of export earnings. However, the sector is dominated by small-scale farmers, 3.5 million of them with land size averaging 2.5 acres. Small-scale farmers account for 75% of the total agricultural production and over 50% of marketed output. Many of these farmers however concentrate on low risk, low return activities because they cannot access start-up capital and cannot transfer system risks. As a result, there is low agricultural productivity which leads to perennial food shortages, widespread unemployment and poverty. Kenya is a low-income, food deficit country with overall national incidence of poverty at 56% in the rural areas and 49% of urban population (GOK, 2007).

The country's demographic trends indicate that by 2030, the urban population will have tripled while rural populations will have risen by 50%. Therefore, to ensure food security for all, farm productivity will have to increase in such a way that 10 farming households are able to feed 5 non-farming households unlike the present situation whereby 10 farming households feed only 2.5 non farming ones. To achieve this dramatic transformation, marketed food per rural household will have to grow by nearly 3% per year which is a major challenge even under the best of circumstances (Tschirley and Ayieko, 2008).

Root crops offer the best opportunity of meeting these challenges because they are easy and inexpensive to grow, are relatively drought tolerant and give relatively high yields even with minimal inputs. Sweetpotato is the second most important root crop in Kenya after Irish potatoes, but is also one of the most underexploited. Millions of poor, small-scale rural farmers, mostly women, produce sweetpotatoes for home consumption and as a source of cash income (Low et al, 1997). But despite its numerous advantages; high productivity even in poor soils, short maturing season (most varieties being harvested within four to six months) and good nutritional profile, sweetpotato remains one of the most neglected crops in this country.

1.1 Background

Sweetpotatoes are highly nutritious vegetables and are especially rich in calories and biologically active phytochemicals such as β-carotene, polyphenols, ascorbic acid, and dietary fiber (Wolfe 1992). However, consumption has lagged behind production in many countries (FAOSTAT, 2005). Limited choices of sweetpotato products for consumers beyond the raw root, and difficulties in availability, storage, and handling for food processors contribute to this negative trend in consumption (Tsou, 1982; Kays, 1985; Drake et al., 2004). The development of commercially viable products from sweetpotatoes has been recognized as the most viable strategies of enhancing its market value. Processing will add value for farmers and processors, raise rural incomes, create additional employment opportunities while providing consumers with a range of low cost, highly nutritious products.

The possibility of processing non-alcoholic beverages from sweetpotatoes, similar in composition and organoleptic quality to fruit products has been demonstrated to be technically feasible (Truong and Fermentira, 1991; Wireko-Manu et al., 2010). In addition, the high cost of grains inevitably shifts focus to root crops such as Sweetpotatoes as low cost substitutes for producing potable alcohol and fuel ethanol. It has been reported that sweetpotatoes produced 40% more alcohol per unit area than Jerusalem artichoke and sugar beet and about 50% more than Irish potatoes (Mays et al., 1990). However, lack of an efficient process of extracting juice from the root has been a serious technical impediment to widespread use of these technologies in third world countries. Utilization of endogenous sweetpotato enzymes combined with commercial pectinases enzyme treatments is likely to make the extraction of sweetpotato juice more economical and widely adopted in less technologically advanced countries.

Therefore, the main purpose of this project was to investigate the factors that influence the activity of amylolytic enzymes in sweetpotato roots, the heat treatment regimes that would optimize conversion of sweetpotato starch to sugars and the effect of pectinex 3XL enzyme on yields of juice from saccharified sweetpotato purees. It was envisaged that the process would find useful application in the commercial extraction of sweetpotato juice for the formulation of non-alcoholic beverages and in production of alcoholic products such as fuel ethanol.

1.2 Problem statement

Currently available methods of extracting sweetpotato juice for manufacturing non-alcoholic beverages and ethanol utilize a combination of commercial amylases and starch debranching enzymes (glyco-amylase and pullulanase) to hydrolyze starch in the root to sugars, followed by addition of water to facilitate release of juice from the saccharified sweetpotato purees (Sojanovic and Silva, 2005). However, commercial enzymes are expensive and difficult to obtain in less developed countries while use of water dilutes the sweetpotato juice and increases production and transport costs. In a recent study, Wireko-Manu et al., (2010) used maize malt instead of commercial enzymes, but obtained very low saccharification rates. Commercial amylases have also been used in combination with cereal malts to saccharify sweetpotato starch in an industrial process of making alcohol (Mathewson, 1980). Widespread use of cereal malts could expose beverage consumers to afflatoxin poisoning due to unhygienic practices and could also aggravate grain shortage situation.

There is little published information on possible enhancement of endogenous amylolytic activity in sweetpotato roots and their utilization in conjunction with pectinex 3XL enzyme to facilitate efficient juice extraction from sweetpotato roots hence this current study.

1.3 Project Justification

The increasing importance of sweetpotatoes in sub-Saharan Africa is largely attributable to its relatively high productivity across a range of environments, short cropping season and flexible planting and harvesting schedules. Millions of poor, small-scale farmers, mostly women, produce sweetpotatoes primarily for home consumption and as a source of cash income. One of the most remarkable features of the crop is that it gives fairly good yields under relatively stressful climatic and soil conditions (Mutuura et al., 1991). This makes the crop the first choice for majority of poor households with limited ability to purchase agricultural inputs. The rising cost of farm inputs such as fertilizers and pesticides, high population growth, land scarcity and increased frequency of droughts (as a consequence of climate change) is forcing many farmers in medium to high potential areas in Kenya to switch to drought tolerant and high yielding crops such as sweetpotatoes to mitigate against food shortages (Jayne et al., 1998; Rees et al., 1999). The ministry of Agriculture is also promoting sweetpotato production as a food security and commercial crop under the 'orphan' crop program (MOA, 2009).

In general, populations in Eastern and Southern African countries have low availability of vitamin A in their diets and there are indications that the overall supply of vitamin A in East Africa could be decreasing (Low et al., 1997). Vitamin A intake is often inadequate because of the seasonality of food sources such as fruits and vegetables, the early abandonment of breast feeding and the tradition of not giving vitamin A rich foods such as vegetables and eggs to young children. In an effort to reduce incidences of Vitamin A malnutrition in Kenya, new varieties of sweetpotatoes which had high levels of β-carotene were introduced. Some of these varieties are Kakamega or SPK 004 (β-carotene content 5300-7500µg/100g), Jonathan 440014 (β -carotene content 7500-10,300µg/100g), and Japanese 420009 (β -carotene content 5000µg/100g). The productivity of these varieties was reported to be much higher than varieties traditionally produced by farmers (Low et al., 1997). However, some of these newly introduced sweetpotato varieties are bland in taste and watery in texture which limits their acceptability in the local market. Consequently, many farmers now shun these new sweetpotato varieties because of low market demand (Chirchir et al., 2010). To alleviate this problem, value addition options need to be promoted. Processing and consumption of beverages from these orange fleshed varieties could make it possible to address vitamin A deficiency on a mass scale because compared to the fresh sweetpotato roots, processed products are more stable and more convenient to distribute over long distances.

Processing of sweetpotatoes to acceptable beverages is therefore likely to diversify markets for farmers and provide consumers with a range of low cost, nutritious and healthy foods. Compared to fruits, sweetpotatoes offer several advantages; first fruits are seasonal, highly perishable and expensive to produce while sweetpotatoes are available throughout the year, can be left in the ground and harvested piece meal and can be stored for lengthy periods after curing. Secondly, as compared to fruits, production of sweetpotatoes does not require expensive inputs and the crop is relatively drought tolerant. As the world economy continue to suffer from the vagaries of global warming, novel foods such as sweetpotato beverage may become regular every day grocery items, hence the need to develop efficient technologies for extracting the sweetpotato juice. Cottage level processing of non-alcoholic beverages from orange fleshed sweetpotatoes is already taking place in Western and Nyanza provinces.

In addition, the high cost of fossil fuels, the desire of countries to be energy independent and concerns about climate change have stimulated renewed research interest towards the development of green energy (biofuels). Biofuels have been proclaimed as alternative fuels for the future and promise cleaner energy and new market opportunities for farmers in

developing countries (Dong, 2007). Research attention is increasingly focusing on fast growing, high yielding starchy root crops such as sweetpotatoes for fuel ethanol production. However, ethanol producing yeast strains such as Saccharomyces cerevisiae cannot convert starch in sweetpotatoes to ethanol. But unlike other starchy roots such as Irish potatoes and cassava, sweetpotato has the advantage of having a fairly heat stable endogenous amylolytic enzyme system, which is able to convert substantial amounts of the starch in the root to sugars during heat processing. By optimizing this process, production of beverages and fuel ethanol from sweetpotatoes, especially in third world countries, could become viable commercially.

Numerous studies have demonstrated that sweetpotatoes roots are suitable materials for production of alcohol and could substitute cereal grains and potatoes. The conversion rate is about one metric ton of fuel ethanol from eight metric tons of sweetpotatoes (Dong, 2007). Studies also show that sweetpotatoes produce 40% more ethanol per unit area than Jerusalem artichoke and sugar beet and 50% more than Irish potatoes (Mays et al., 1990). But when the short maturing period of sweetpotatoes is considered (3-6months for sweetpotatoes and 9-18 months for cassava), sweetpotato emerges top of cassava in terms of energy conversion efficiency (Liu and Liang, 1983). The other rationale for processing sweet potatoes is to prolong storage life because fresh sweetpotato roots are highly perishable and cannot be stored for any length of time in the tropics.

1.4 Statement of Hypothesis

Subjecting sweetpotato roots to curing temperatures between 25-35℃ is likely to stimulate rapid increase in endogenous amylolytic activity, which together with application of heat-treatment regimes between 60-75℃ could facilitate complete conversion of starch to maltose and that hydrolysis of pectins in the saccharified puree with pectinex 3XL enzyme would optimize extraction of sweetpotato juice.

1.4 Goals and objectives

The overall objective of the study was to develop an efficient process of extracting sweetpotato juice through combined action of endogenous amylolytic activity and pectinex enzymes.

The specific objectives were to:

- determine the effect of sweetpotato variety, temperature and duration of curing on levels of α-amylase and β-amylase activities in three local sweetpotato varieties
- study the effect of α-amylase activity on starch hydrolysis in sweetpotato purees held at 60, 65, 70, 75°C for 180 minutes
- determine the effect of pectinex 3XL enzyme on viscosity and yield of juice from saccharified sweetpotato purees
- Characterize three local sweetpotato root varieties in terms of proximate composition, percent juice yields and composition of extracted juice.

CHAPTER TWO

2.0 Literature review

2.1 Description and agronomic performance of sweetpotatoes

Sweetpotato [*Ipomoea batatas (L.) Lam*], is a dicotyledonous plant belonging to the convolvulacea family. The origin of sweetpotatoes is thought to be central or S. America but is now grown worldwide in tropical and subtropical regions. It is a perennial vine sometimes grown as an annual. The vine produces adventitious roots which grow into fleshy enlargements with varying shapes raging from spindle-shaped to almost spherical. Sweetpotato roots may have a smooth or irregular or ribbed surface, and the skin and flesh may range from almost pure white through cream, yellow-orange or pink, to a very deep purple, depending upon the amount of various pigments present; carotenoids or anthocyanins (Koske, 2004). A single sweetpotato vine may produce 40-50 roots, which also vary greatly in size, shape, color, storage, nutritional and processing characteristics. The roots may range from a few centimeters to over 30 cm in length and may weigh from 100 g to 1 kg, although roots weighing as much as 5 kg have been reported.

One of the most remarkable features of the crop is that it gives fairly good yields under relatively stressful climatic and soil conditions. This makes it the first choice crop for the majority of poor households with limited ability to purchase agricultural inputs. It's also cheaper to produce sweetpotatoes than maize, rice or other widely consumed cereal grains (CIP, 2005). Sweetpotatoes can therefore play an important role of alleviating poverty, hunger and malnutrition, which are the most persistent development challenges facing many third world countries including Kenya. Sweetpotatoes are also increasingly being utilized for manufacturing starch, beverages, animal feeds and fuel ethanol

2.2 Production, marketing and utilization of sweetpotato roots

With more than 133 million tons produced annually, sweetpotato ranks as the fifth most important food crop after rice, wheat, maize, and cassava. Among root crops, sweetpotato comes second to Irish potatoes in economic importance (Houghton and Raman, 1998). Asia is the world's leading sweetpotato producing region, with China accounting for 90% of total global production (FAOSTAT, 1998)

Africa has the highest acreage under sweetpotatoes in the world. However, as area planted continues to expand and production moves to more marginal areas, yields have declined. The average yield of sweetpotatoes in Africa currently stands at 5t/ha, which is the lowest yield anywhere in the world (Scott and Maldonado, 1997). Average sweetpotato yields in Kenya stand at 10 ton/Ha (Chirchir et al., 2010), which about a third of the yields realized in Asia. Introduction of high yielding sweetpotato varieties in Kenya and improved crop husbandry practices have significantly increased production yields and volumes. Sweetpotato yields increased steadily from 590,000 metric tons in 2003 to 880,000 metric tons in 2008 while acreage increased from 24,395 to 60,919 ha in the same period. The area under sweetpotatoes this year is estimated as 61,000 Ha. Agronomic trials undertaken on farmer fields in Western and Nyanza provinces demonstrated that under proper management conditions, the yields of some of the exotic orange fleshed sweetpotato varieties could be as high as 30 tons/ha, which indicates that there is still a lot to be done (Gichuki et al., 1997; Low et al., 1997).

Most sweetpotatoes in Kenya are produced in Western and Nyanza provinces. Table 1 shows sweetpotato production statistics for 2008 in selected districts in Nyanza and Western provinces.

Table : Sweetpotato production statistics for year 2008 from 8 selected districts in Western and Nyanza provinces.

	Year 2008		
District	Acreage(Ha)	Yield(t/Ha)	Production(tons)
Bungoma South	1253	10.5	13157
Bungoma West	2959	12	35508
Busia	2368	9.5	22496
Samia	1140	8.5	9690
Rachuonyo	4800	10	48,000
Migori	5035	3.8	19,260
Nyamira	337	15	5055
Kisii Central	6500	15	97,500

(Source: own compilation from data presented by Chirchir et al., 2010)

The highest yield in both provinces was 15t/Ha (Nyamira and Kisii Central) while the lowest was 3.8t/Ha (Migori). Sweetpotato farmers the in the two provinces in western Kenya region produce a variety of sweetpotatoes including SPK004, KEMB 10, Salyboro, Bungoma, Mar

Ooko, Nya-Ugenya, Kampala, Mugenda, Nyawo, Ejumla, Namaswakhe, K117, Polista, Odinga, 292-11-12, Zapallo, Nyathi Odiewo, Javalo, Amina and Kuny kibuonjo. Studies have shown considerable differences in yield and farmer /market preference for these varieties. For example, K117 and Mugande are high yielding, but are disliked by many consumers due to poor eating quality (Kwach et al., 2008). The potential of expanding sweetpotato production in this region is high and is dependent on improved market access and prices for producers. Commercial processing of the sweetpotato roots is likely to play an important role of diversifying markets for farmers while supplying consumers with affordable, nutritious products. The possibility of processing industrial products such as ethanol from sweetpotatoes further expands markets for such farmers.

A major constraint to increased production and marketing of sweetpotatoes in many countries, including Kenya, is the low utilization of the crop (Kays, 1985). In many parts of Asia and Africa, negative consumer attitudes to the crop have long been recognized as serious handicaps to increased utilization of the crop as human food (Wheatley et al., 1995). Many consumers consider sweetpotatoes a poor man's food or survival crop to be consumed during hardships (Watson et al., 1989; Drake, 2004). There is therefore need to enhance efforts to develop good quality and acceptable food and industrial products from the root to expand market opportunities for farmers.

In Kenya, most of the sweetpotato produced is consumed at the household level although recent trends point to increased marketing and cottage level processing (Gakonyo, 1992). At the household level, the main methods of preparing the root are boiling and roasting. However, some communities in Western Kenya have developed varied traditional methods of utilizing sweetpotatoes. In one of the recipes, sweetpotatoes are mashed with maize and beans to produce a local dish known as 'Musenye' (Low et al., 1997). The Teso people use sweetpotato roots for making porridge flour as follows; the roots are washed, peeled, chipped and lemon or tamarind juice added before the chips are sundried. The dried chips are then milled, blended with cereal flours (millet, maize and sorghum) and made into porridge. In Busia district, sweetpotatoes are chipped using knives, sun dried for storage and then eaten piecemeal in form of a paste after boiling with water.

In recent years, the Kenya Government, with assistance from development agencies have promoted cottage processing of sweetpotatoes by rural farmer groups. Sweetpotato farmers club together, register as a common interest group with the department of social services, contribute some money, purchase simple equipment like chippers and after receive training

from agricultural extension officers, they start manufacturing sweetpotato products targeted at the local market. Products such sweetpotato bread, biscuits, cookies and cakes are made either from the flour or boiled roots. Sweetpotato juice is also processed from orange fleshed varieties. The products have however not found widespread acceptance due to poor quality and high cost of production, as compared to commercial variants. In some developing countries such as China, sweetpotato roots are used as a raw material for processing food and industrial products at a commercial scale.

The principal value added products made from sweetpotato roots in China is starch which is in turn used for the manufacture of noodles, candies, composite flour, crisps and alcoholic drinks (Tang et al., 1990; Jiang et al., 2004).In Sichuan province of china, value added processing of sweetpotatoes has catalyzed rapid economic transformation in a region that was for a long time impoverished and that lagged behind the rest of the country in terms of development (Wheatley and Bofu, 2005; CIP, 2005). So important is sweetpotatoes to the economy of the province that inhabitants refer to it as the "the green gold mine". Starch is produced from sweet potato in much the same way as from the other starchy roots except that the solution is kept alkaline (pH 8.6) by using lime, which helps to flocculate impurities and dissolve the pigments. Sweetpotato starch shows properties intermediate between potato starch and corn/ cassava starch in terms of viscosity and other characteristics (Wheatley and Bofu, 2000).

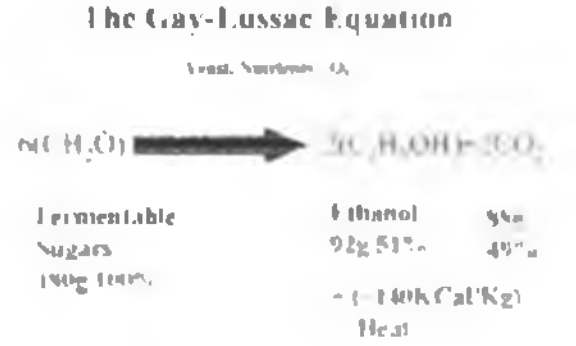
Sweetpotatoes have also been used for formulating weaning foods (Espaniola et al., 1998) and for processing products such as non-alcoholic beverages, jam, and ketchup (Truong and Fermentira, 1991; Fawzia, et al., 1999). Processing of sweetpotato roots to non-alcoholic beverages has been widely studied. The method in common use involves: washing roots, peeling, trimming, chopping, steaming, and blending with water, 12% of sugar, 0.2% citric acid and 232mg/ liter of ascorbic acid. To enhance sensory appeal of sweetpotato beverages, juices/ pulps from mangoes, guavas, lemons and pineapple are commonly added (Truong and Fermentira 1988; Purnamasari et al,. 2003; 2004). The process of manufacturing sweetpotato beverages is already patented in Philippines. Coggins et al (2003) described a process of extracting raw juice from sweetpotato juice using an automatic blender and reported juice yields of 53.6%, but the juice was inedible. Nungo (2005) reported a process of making sweetpotato beverage which involved extraction of juice from boiled sweetpotato roots with hot water then adding citric acid and food flavors. Wireko-Manu et al., (2010) recently described a different approach where he used maize malt to facilitate saccharification of

starch in sweetpotato purees, and then added water to aid juice extraction. He then blended the juice with citric acid and sugar to make a non-alcoholic beverage which was then flavored with either Ginger or lime. Sojanovic and Silva (2005) described a process of extracting sweetpotato juice using a combination of endogenous and commercial amylolytic enzymes. The commercial enzyme preparation included α- amylase and starch debranching enzymes (glycol-amylase and pullulanase). In all these processes, water, malt or commercial enzymes were variously used to facilitate juice extraction from sweetpotato purees, which clearly indicates that a technical problem does exist.

Several methods of processing alcoholic beverages from sweetpotato roots have also been described. An industrial process used in Japan for processing distilled spirits called shochu uses starch from sweetpotatoes (Sakamoto and Bouwkamp, 1985; Kozai et al., 1996). The process is very similar to that of whisky production except that the koji (equivalent to the malt starter in whisky production), is obtained by inoculating steamed rice soaked in water overnight with Aspergillus kawachii for two days at 35 to 37°C. The koji is mixed with starch water and yeast to promote saccharification and fermentation. The filtrate is finally distilled. To begin the fermentation, the activated yeast is mixed in the cooled, pH-adjusted mash in the fermentation tank. The optimum temperature for the fermentation process is between 21-29°C. Fermentation takes 1-4days depending on temperature, pH and type of substrate. The yield is about 800 liters of alcoholic beverage from one ton of sweet potatoes. Sweetpotatoes are also used in Ethiopia for making a traditional beer. The process involves adding two thirds of a kilo of crushed hops to one-third kilo of germinated, ground barley or wheat flour. Water is then added to the mix and fermented for three days, after which twenty kilos of boiled, mashed and dry roasted sweetpotato flour is added. The following day water is added, and the brew left to ferment to a tasty local beer (Kays, 1987).

In yet another industrial process for making sweetpotato alcohol, sweet potatoes are cooked under pressure and subsequently cooled to 71°C. Commercial liquefying amylase enzyme (Taka Therm) is added before addition of malt (1.5-2kg malt to 45kg of sweetpotatoes) at 65°C to facilitate starch saccharification (Mathewson, 1980). The mash is then fermented to alcohol. The conversion of sugar to alcohol follows the equation below.

The Gay-Lussac Equation



2.3 Composition of sweetpotato roots

The chemical composition of sweetpotato roots vary widely according to variety, climatic conditions, degree of maturity and storage duration. On average, sweetpotato roots contain the following: energy 490KJ/100g, moisture 65-81%, protein 0.95-4.0%, carbohydrates 25-32%, fiber 0.9%, ash 0.9-1.4%, calcium 30-34mg/100g, phosphorus 49mg/100g, potassium 373mg/100g, sodium 13mg/100g, β-carotene 0-12mg/100g, thiamin 0.1mg/100g, riboflavin 0.05-0.06mg/100g, niacin 0.6-0.9mg/100g and ascorbic acid 23-25gm/100g (Collins, 1981; Wolfe, 1992). Marked varietal differences have been observed in the levels of carbohydrate components, minerals and vitamins in sweetpotato roots as depicted on Table 2 below.

Sweetpotato starch accounts for over 90% of the total carbohydrates and is highly digestible. This makes sweetpotato suitable as weaning foods, which require carbohydrates that are easily digestible (Palmer 1982). The hydrolysis of some of the starch to sugars during cooking of the root further improves the digestibility and sensory quality of the processed sweetpotato root. It has been reported that majority of East African consumers prefer the white to yellow/ cream colored varieties which are also sweeter. Orange fleshed varieties introduced recently in East-Africa which contain dry matter content above 25% and a dry texture are also liked by consumers (low et al., 1997).

The starch content in sweetpotatoes varies widely. Tewe (2003) reported sweetpotato starch in the range 30.8-41.8%, while Tian et al., (1991) reported values of 6.9-30.7%. These differences are mainly due to the many varieties of sweetpotato available in different parts of the world. Starch naturally exists in form of granules which vary in shape, and size distribution depending on their botanical source and agronomical practices. Native starch granules are semi-crystalline in structure (Blanchard, 1987).

Table : Chemical composition of 49 varieties of sweetpotato roots

Component of Sweetpotato	Range of Values
Starch content	30.80-41.80 g/100g D.M.
Total sugar	3.68-10.40g/100g D.M.
Amylose	21.00-38.40% of starch
Amylopectin	61.60-79.00% of starch
Vitamin A	1.0-8.4 mg/100g D.M.
Vitamin C	4.6-11.4mg/100g D.M.
Tannins	0.02-0.23% of D.M.
Phytin	4.98-14.36 mg/100g D.M.
Oxalate	0.09-1.77%
Crude protein	1.19-9.40%
Ether Extract	0.38-3.03%
Crude Fiber	3.84-5.89%

(Source: Tewe et al., 2003)

Sweetpotatoes starch granules are reported as round, oval and polygonal in shape, sizes ranging between 2-42 μm (Tian et al., 1991; Hoover 2001). Starch granules consist of two major polymers: amylose and amylopectin. Amylose is composed of essentially homogenous linear units of α-(1→4)-D-glucopyranose, which form helicoidal structures in solution (Manners, 1974; Jarvis and Walker, 1993). The interior of the structure is hydrophobic, allowing amylose to from complexes with iodine, fatty acids, butanol (Kennema, 1985). The amylose content and its degree of polymerization (DP) are important determinants of the physical, chemical and technological properties of starch. Tewe et al., (2003) reported that amylose in sweetpotato starch ranged between 21.0-38.4% while Tian et al. (1991) and Takeda et al., (1987) reported a range of 8.5% - 38% and a degree of polymerization (DP) within the range 3025- 4100.

Amylopectin constitutes of short chains of α –(1-4)- D-glycopyranose branched to α (1-6) - D-glycopyranose to form a highly ramified structure (Blennow et al., 2001). The molecular weight of amylopectin is about 100 times higher than amylose. As compared to amylose, the amylopectin structure is more complex since it is highly branched. Sweetpotato α-amylase and β-amylase are unable to break α – (1-6) -D-glycopyranose bonds in the amylopectin. Therefore, to achieve complete hydrolysis of starch to maltose, a combination of endogenous enzymes and commercial debraching enzyme would perhaps be needed. However, investigations of the possible role of starch debranching enzymes in the process of extraction of sweetpotato juice using endogenous enzymes was beyond the scope of the current study.

When a starch suspension is heated in excess water the granules absorb water, swell, and lose their crystallinity. The term gelatinization is used to describe the swelling and hydration of granular starches. Gelatinization involves the disruption of molecular orders within the starch granules manifested in irreversible changes in properties such as granular swelling, native crystalline melting, loss of birefringence, and starch solubilization. The point of initial gelatinization and the range over which this occurs is governed by starch structure. Both single- and two-stage swelling patterns are exhibited by sweetpotato starches from different varieties (Rasper 1969). The gelatinization behavior of sweetpotato starches show a high peak viscosity, which subsequently becomes thinner rapidly with prolonged cooking, before thickening on cooling (Tian et al., 1991). Gelatinization temperatures of sweet potato starches are reported to be within the range of 58 - 84 °C (Takeda et al., 1986; Tian et al., 1991; Garcia and Walter, 1998). Sweetpotato enzymes α-amylase and β-amylase can only hydrolyze gelatinized starch hence the importance of heat processing to optimize starch hydrolysis in sweetpotato roots.

Starch content of sweetpotato roots also influences the yield of ethanol. Previous studies have demonstrated a positive correlation between starch content in sweetpotato roots and rate of ethanol production. In addition to starch content, alcohol yield is also determined by moisture content, yield of sweetpotatoes per hectare and the growth period (Liu and Liang, 1983). A ton of sweetpotatoes having starch content of 22% and sugar content of 5-6% is expected to yield about 40gallons of alcohol (Mathewson, 1980). Hydrolysis of starch to sugars plays an important role of improving the efficiency of ethanol production from sweetpotato roots.

Apart from starch, sweetpotato roots contain substantial levels of other carbohydrates such as sucrose, cellulose, pectin, and hemicellulose. Pectin, starch, cellulose and hemicelluloses are

collectively termed alcohol insoluble solids, while total carbohydrates will also include soluble sugars such as maltose, glucose, fructose and maltose (Buescher et al., 1976). Softening of sweetpotatoes during cooking has often been associated with the solubilization of pectin (Ahmed and Scott, 1958; Baumgardener and Scott, 1963) by endogenous Pectin methylesterase activity (Buescher et al., 1976). Furthermore, exposure of sweetpotato roots to chilling temperatures is known to induce a disorder called hardcore, in which a hard tissue manifests itself after cooking sweetpotato roots previously exposed to long term chilling. This tissue is softened with pectin lyase but not pectin methylesterase (Buescher et al., 1976). There is sufficient evidence to show that non-starch carbohydrates, notably pectin has an important role to play in the textural quality of sweetpotato roots. Hydrolysis of pectin using enzymes could enhance the extraction of sweetpotato juice from the roots.

Commercial pectic enzymes from fungal sources have been used in fruit juice processing since the 1930's. The main benefits of using pectin degrading enzymes for mash and secondary mash treatment in vegetable processing are: increased yield caused by the enzyme lowering the viscosity of the mash which make liquid/solid separation easier resulting in a higher juice yields. Reduced viscosity of the mash increases processing capacity of horizontal presses, belt presses or decanters. Use of enzymes also increases the extraction of β-carotene content from the puree. The pomace is reduced, soluble solids increase and the press is easily cleaned. Utilization of pectin degrading enzyme could make addition of water, as suggested in most of the currently available methods of extracting sweetpotato juice unnecessary thereby enhancing extraction efficiency.

A number of high-yielding sweetpotato cultivars that are high in both provitamin A and dry matter content have been identified in different agro-ecological zones of East Africa (Carrey et al., 1999; Low et al., 1997). Some of these sweetpotato varieties are reported to have β-carotene contents comparable or even higher than carrots. In some varieties, as much as 300mg/100g of β-carotene (on fresh weight basis) has been found (Wolfe, 1992; Low et al., 1997). Therefore, orange fleshed sweetpotato varieties are considered good sources of pro-vitamin A activity (Hagenimana et al., 1999). Processing non-alcoholic beverages from orange fleshed sweetpotato varieties could be a convenient and cost effective method of supplying β-carotene and combating vitamin A deficiency in vulnerable populations (Hernandez et al., 2005). More than 90% of countries worldwide have a public health problem concerning clinical and subclinical vitamin A deficiency. In general, populations in Eastern and Southern African countries have low availability of vitamin A in their diets and

there are indications that the overall supply of vitamin 'A' in East Africa could be decreasing (Low et al., 1997). Vitamin A intake is often inadequate because of the seasonality of food sources such as fruits and vegetables, the early abandonment of breast feeding and the tradition of not giving vitamin A rich foods such as vegetables and eggs to young children.

Fruit flavored sweetpotato beverages processed from orange fleshed roots were found acceptable to consumers, which suggests a possible market outlet for farmers. Generally, yellow and orange sweetpotato varieties would be preferable for processing non-alcoholic beverages because of the nutritional benefits and the fact that color is an extremely important sensory attribute in determining the acceptability of non-alcoholic beverages (Hely et al., 1994). The stability of β-carotene in some processed foods has been assessed and validated (Hagenimana, 1998; Fawzia et al., 1999). It was envisaged that the process of making sweetpotato beverage would result in significant extraction of β-carotene from sweetpotatoes into the juice and thereby provide an avenue for supplying this vital nutrient to vulnerable populations living far from major sweetpotato producing areas at a low cost. Recently introduced sweetpotato varieties in Kenya have much higher levels of β-carotene than the traditional varieties used in the current study. These sweetpotato varieties were introduced as part of a wider strategy of alleviating Vitamin A deficiency (VAD) in East Africa region (Kapinga et al., 2003). The orange fleshed sweetpotatoes are said to be the cheapest source of dietary Vitamin A that can be produced year round by many small holder farmers and do particularly well in western and Nyanza provinces (Low et al., 1997).

In addition to β-carotene, other types of carotenoids are also found in sweetpotatoes; alpha-, gamma- and zeta-carotenes, phytoene, phytofluene, betacarotene- epoxide, hydroxy-zeta-carotenes and beta-carotene furanoxide. However, these carotenoids normally account for less than 1% of the total carotenoids. Wolfe (1992) found that 86.4-89.0% of the carotenoids in yellow and orange sweetpotatoes were β-carotene. Kays et al. (1993) analyzed β-carotene content of various sweetpotato cultivars and reported that the highest β-carotene content was 190 ug/g dry matter basis. He was however unable to find other provitamin "A" carotenoids such as α-carotene, and β-cryptoxanthin in the analyzed samples.

Sweetpotato roots also contain other functional compounds such as phenolic compounds and anthocyanins which are said to protect the body against many chronic diseases such as diabetes, cardiovascular ailments and cancers (Walter and Schadel, 1981; Gestner, 1993; Hassye et al., 1984; Ames et al., 1993, Scalbert et al., 2005; Panda, 2006). Phenolic

compounds have an aromatic ring with one or more hydroxyl groups and functional derivatives. They are secondary metabolites in plants and are involved in a number of metabolic pathways. They are well known antioxidants that function as singlet and triplet oxygen quenchers and also scavengers of other free radicals (Yoshimoto et al., 2004; Oki et al., 2002; Sahidi and Naczk, 2003). There are two principal phenolic compounds namely; Chlorogenic acid (CHIA) and 3, 5-dicaffeoylquinic acid (3, 5-diCQA) which are present in sweetpotatoes and that are of considerable medicinal importance (Thomson, 1981; Shimozono et al., 1996; Panda, 2006). These compounds have been associated with antioxidant activity, anti-mutagenicity, and free radical scavenging activity reported in sweetpotato roots and leaves (Huang et al., 2004; Rabah et al., 2004). Phenolic compounds have also been reported to suppress various health disorders, including skin cancers in mice, hepatoma invasion (Ames, 1993; Yagasaki et al., 2000), and human immunodeficiency virus (HIV) replication (Pizza, 1993; Zhu et al., 1999). For these reasons, sweetpotato beverages could find a market niche on the health platform.

Wolfe (1992) reported ash content in the range 0.9-1.4%, mainly comprising; calcium 30-34mg/100g, phosphorus 49mg/100g, potassium 373mg/100g, sodium 13mg/100g, elements which are also present in many food materials. The protein content of sweetpotato root is 0.9-4.0% while lipids account for 1.2-2.7% of the total fresh weight of sweetpotato roots. Sweetpotato lipids are composed of neutral lipid, glycolipids, and phospholipids. Sweetpotatoes also contain some organic acids which include malic acid, quinic acid, succinic acid, and citric acid. These acids function as intermediates in metabolism and contribute to the taste of the storage roots.

The levels of tannins, oxalates, phytin and phytin phosphorous showed that apart from the high phytin in some varieties, sweetpotato poses less of a poisoning risk as cassava (Tewe et al., 2003). Trypsin inhibitor activity (TIA) ranging from 20 to 90 percent inhibition has been demonstrated in some sweetpotato varieties. However, heating the roots to 90°C for several minutes inactivates trypsin inhibitors. Cooking sweetpotatoes is also advantageous in that it improves the texture, flavor and digestibility of the root. Cooking sweetpotato roots also improves hygienic quality and destroys toxic compounds such as terpenoid phytoalexins (which accumulate in injured roots) and anti nutritional trypsin inhibitors (Catalano, 1977).

2.4 Characteristics of sweetpotato α-amylase and β-amylase enzymes and role of curing roots

The occurrence of an active diastase enzyme in sweetpotatoes was clearly demonstrated by Gore (1920), who showed that cooking sweetpotatoes slowly through a temperature range of between 60°C to boiling point gave a very high conversion of starch to sugar. Since then, α-amylase has been isolated from sweetpotatoes, crystallized and characterized (Ikemiya and Deobald, 1966). Among the characteristics they reported were its high optimum temperature (70-75°C), heat stability and low activity at ordinary temperatures. The enzyme was observed to show maximum heat stability at pH 6.0, while a much wider pH range (3.6-8.1) was tolerated at lower temperatures (30-40 °C). In their experiments, Ikemiya and Deobald (1966) extracted crude α-amylase enzyme from sweetpotato juice from sweetpotato roots which had been been cured for 5 days at 32.2°C (RH, 85%) and stored for six months at 15.6°C. The roots were shredded and pressed under 5000p.s.i in a hydraulic press. They then filtered the juice through Whatman No.1 paper.

Two methods are commonly used for determining α-amylase activity in sweetpotatoes; amylocrastic method and chromogenic method. The amylocrastic method is based on the rate of hydrolysis of soluble starch substrate. This method depends on the fact that as α-amylase fragments starch substrate, there is progressive decrease in the blue color when iodine solution is added. By matching the color of reactants with serial dilutions of potassium dichromate solutions, the reaction time can be obtained. The α-amylase activity in the sample tested is inversely proportional to the time taken to obtain a match. Results are normally expressed in sweetpotato dextrinizing units/ml. The formula for calculating the α-amylase activity is given by: SDU is 12 · ml x time; where time is in minutes and the volume of juice used to hydrolyze 10mls of 2% soluble starch is expressed given in mls. Where serial dilutions are made from the sweetpotato juice extract, the appropriate dilution factor is multiplied with the volume of juice used.

The Chromogenic method is based on is based on hydrolysis of insoluble derivatives of starch which causes them to release soluble dyes in solution. The amount of dye released being proportional to the enzyme activity in the sample. Amylopectin Azure is one such starch derivative which has been used to measure α-amylase enzyme activity in sweetpotato roots (Hasling et al., 1973; Walter and Purcell, 1973). In the presence of α-amylase enzyme, amylopectin azure releases blue color in the solution which is measured with a

spectrophotometer. The absorbance of the blue solution is determined at 585 and the activity calculated as follows: APA Amylase per ml = As 595-A595 (blank)

As 595 of 0.1M $cuso_4$

The amylocrastic method described by Ikemiya and Deobald (1966) was chosen for the current investigations because it's accurate and easy to use.

Studies show that α-amylase enzyme activity in the fresh sweetpotato roots is initially very low but increases at a uniformly under constant storage conditions (Deobald et al., 1969; Walter et al., 1975). The α-amylase activity in sweetpotato roots increases rapidly even when the roots are held at room temperature. The activity of the enzyme also varies widely between cultivars. The activities of this enzyme in locally grown sweetpotato varieties has not been reported, hence the need to determine them in the current research. The level of α-amylase activity in sweetpotato roots is a good indicator of processing quality of the root (Szyperski et al., 1986). The higher the activity, the more the extent of starch hydrolysis and the sweeter the root becomes after baking or boiling. The level of α-amylase activity also strongly influences the textural quality of cooked sweetpotatoes (Sistrunk, 1971). The enzyme β-amylase has also been isolated from sweetpotatoes and its activity in the roots remains fairly constant throughout. The optimum temperature for β-amylase enzyme is 60-65°c.

If sweet potatoes are to be stored for any length of time, they must be subjected immediately after digging to a suitable combination of temperature and humidity for effective curing of the roots. The primary purpose of curing sweetpotatoes is to heal injuries so that the sweet potatoes remain in good condition for marketing and to preserve "seed" roots for the next crop. Healing takes place rapidly at 29.4°C and 85 to 90 % relative humidity. Curing should start as soon after harvest as possible to heal injuries before disease-producing organisms gain entrance. Healing involves production of cells that are very much like the skin in their ability to prevent infection. These new cells form in a layer just below the surface of the injuries. Because this layer is corky, it is commonly called wound cork. Healing is more rapid under clean cuts and skinned areas than in deep wounds where tissue is crushed. The rate of healing differs a little among varieties. At 29.4-32°C wound cork begins to form in 2 days and is well developed in 5 or 6 days. At lower or higher temperatures than this, wound cork forms less rapidly. Above 35°C, very little, if any, wound cork develops and such temperatures should be avoided. Satisfactory wound-cork formation takes about 4 to 7 days at 29 °C , 8 to 10 days at 26°C , 15 to 20 days at 24°C, and 25 to 30 days at 21°C . At 13°C or

below, wound cork does not form. Slow formation of wound cork increases the opportunity for decay-producing organisms to gain entrance to the root before the layer is sufficiently well formed (Sumner, 2004). The physiology of curing is poorly understood because there is little available information on biochemical transformations that take place. Deobald et al., (1971) report suppression of the activity of the enzyme activity in sweetpotatoes stored after curing. A better understanding of the effect of curing on the endogenous amylolytic activity could potentially be useful in many food processing food applications beyond the scope of the current investigation.

2.5 Mode of action of sweetpotato amylolytic enzymes and effect of temperature on starch hydrolysis

Sweet potatoes contain large amounts of starch which is largely converted during baking into maltose and dextrins. Maltose has been shown to be the sole sugar produced by cooking sweet potatoes. The presence of β-amylase in sweet potatoes and the production of maltose during baking indicate that starch conversion is due in part to this amylolytic enzyme. The other major product of starch conversion, dextrins, are polysaccharides having a broad spectrum of molecular size formed by the action of α-amylase on starch. Hoover (1967) showed that in sweet potato puree, starch was not converted into maltose dextrins until the temperature was above that for starch gelation.

The manner in which these two amylases enzymes depolymerize starch is depicted on Figure 1 below. While α-amylases is an endo-enzyme that randomly split α – (1-4) linkages in starch with retention of anomeric configuration of glucose residues, β-amylases is an exo-enzyme acting from the non-reducing end, releasing β-maltose units from starch, hence the name β-amylase (Kaplan and Guy, 2004). The β-maltose released however undergoes mutarotation into α-maltose (Dicko et al., 2000). Both α-amylase and β-amylase cannot split α-(1-6) linkages in amyl pectin. Therefore, the degradation of starch by the two enzymes is incomplete. Starch liquefying or dextrinizing power is referred to as α-amylase activity while starch saccharifying or saccharolytic power is referred to as β-amylase activity. Plant amylases scarcely hydrolyze raw starch; their action being lower than 5% at room temperature (Dicko et al., 1999). For efficient extraction of sweetpotato juice from sweetpotatoes, high α-amylase and β-amylase activities would be desirable. Heating would also be extremely important for increasing the rate and extent of starch degradation to maltose.

The extent of starch degradation in sweetpotato roots is also influenced by processing temperature and duration of heating (Purcell and Walter, 1988). When the temperature of sweetpotato flesh reaches 77°C, starch is degraded at a rate proportional to the quantity of α-amylase present. Enzyme β-amylase produces maltose from fragments of starch liberated by α-amylase. But as the temperature of the flesh rises, β-amylase is inactivated but α-amylase proceeds to break down starch until it is finally inactivated at 95°C. Without β-amylase to hydrolyze starch fragments to maltose, dextrins accumulate until they inhibit α-amylase (Walter et al., 1975). It has also been reported that more conversion of starch to maltose is achieved during baking of the whole root as compared to boiling (Picha, 1986). Slow boiling of whole sweetpotato roots is also known to result in higher conversion of starch to sugars than normal rates of boiling.

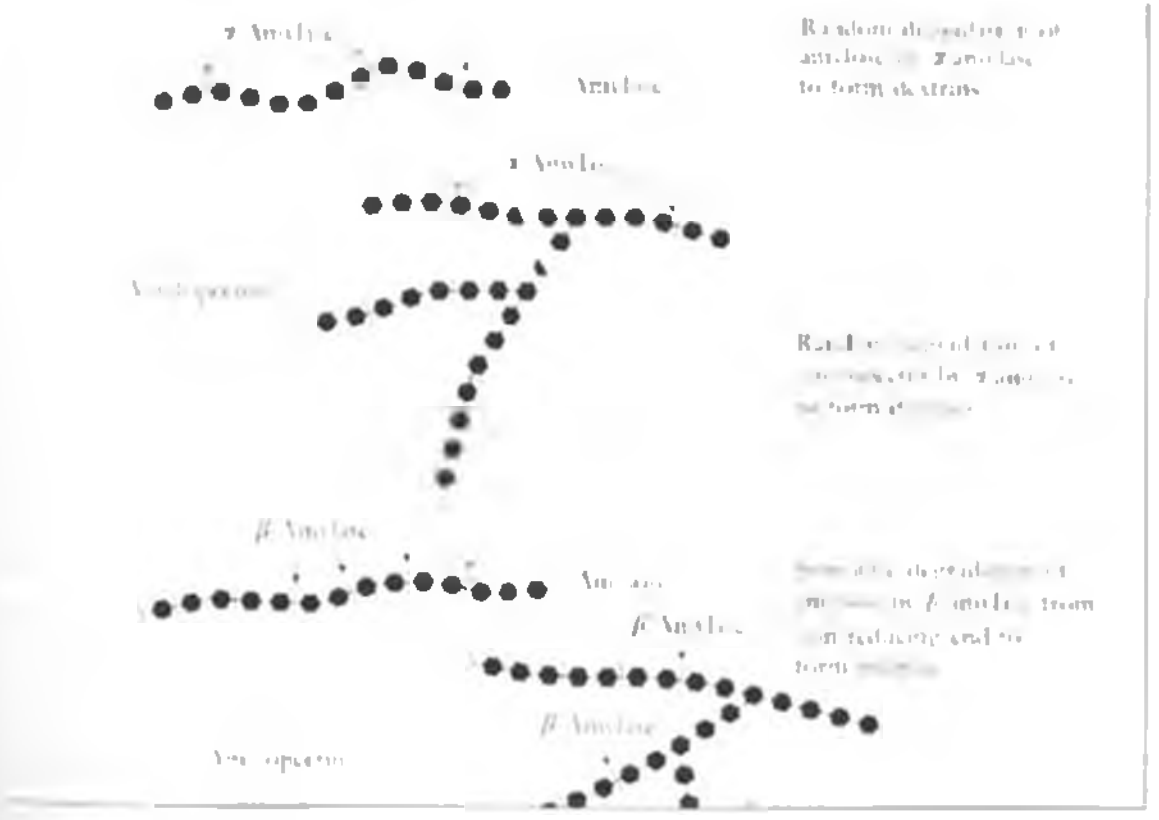


Figure : Starch hydrolysis by amylases enzymes. Source: (Kaplan and Guy, 2004)

Sweetpotato amylolytic enzymes have been used in a variety of applications. Hagenimana et al., (1994) used endogenous sweetpotato enzymes to hydrolyze starch in sweetpotato mashes. Earlier, Etim and Etokikpan (1992) had successfully used sweetpotato flour in place of extraneous enzymes to increase saccharification of starch in sorghum for brewing alcoholic beverages. Fungal amylases have been used to liquefy sweetpotato puree before spray drying (Szyperski et al., 1986; Grabowski, 2006). Grabowski used α-amylase from *Aspergillus oryzae* (Fungamyl 800 L, Novozymes, Bagsvaerd, Denmark) to hydrolyze starch molecules in sweetpotato puree. This alpha-amylase which has optimum activity at a pH around 5 and at temperatures between 50 °C and 60 °C is less heat stable compared to sweetpotato α-amylase. Wireko-Manu (2010) used malt from germinated maize to hydrolyze sweetpotato starch in the process of extracting juice from sweetpotato roots. More effective utilization of endogenous sweetpotato enzymes could make addition of such commercial enzymes and malted cereals unnecessary in the process of manufacturing juice and other products from sweetpotato roots.

2.6 Role of Pectinases enzymes in the extraction of fruit and vegetable juices

Apart from starch, other carbohydrates present in sweetpotatoes such as pectic substances have a very strong influence on the textural quality of sweetpotato roots. Pectins are colloidal carbohydrates which strongly influence the yield of juice from fruit and vegetable pulps. It has been reported that sweetpotato roots have levels of pectins of between 3-5%. The effect of curing treatments on pectin transformations in sweetpotato roots has been studied previously. It was reported that the percentage of soluble pectins in sweet potatoes increased during curing while protopectin showed a corresponding decrease. The rate of this transformation was observed to be greater in roots cured at 40°C' compared to those cured at 30°C (Walter and Purcell, 1975; Buescher, 1976). This is of interest in processing of sweetpotato juice in view of the effect of soluble pectin influencing rheological properties of sweetpotato purees and the fact that curing changes the chemical composition and characteristics of pectic compounds (Truong, et al., 1998). Hydrolysis of native pectins is likely to enhance extraction of juice from sweetpotato roots.

Commercial pectic enzymes from fungal sources have been used in fruit juice processing since the 1930's. The enzymes have found wide applications involving the disintegration of fruit and vegetable pulps and therefore help to extract optimize and clarify juices from carrots, pears, mangoes, apples and bananas. The enzymes disintegrate fruit and vegetable

cells in the pulps and thus enhance juice yields from these fruits and vegetables (Hsu et al., 1990; Asker et al., 1992; Kyamuhangire et al., 2002). The yield of passion juice is normally rather low due to the high pectin content of the fruit pulp surrounding the pips. The use of pectolytic enzymes increases the juice yield by more than 40%. In addition, separation of the pips from the pulp becomes much easier; there is better cloud stability and there is improvement in the natural flavor. Clear passion fruit juice can be obtained by adding pectinase and amylase preparations to the cloudy juice. Alternatively, a clear juice can be produced directly based on a treatment with pectolytic and hemicellulytic enzymes. Kolli et al., (1991) showed that mixtures of pectinases, cellulase and hemicellulase enzymes, when used at recommended rates were more effective than pectinases, cellulase and α-amylase or galactomannase in on their own in reducing viscosity and improving the filterability of puree from both green and ripe bananas

There are several commercial blends of pectinolytic enzymes for different applications. Pectinex 3XL is the trade name of one such enzyme mixture that is widely used for liquefaction of fruit and vegetable mashes to enhance juice yields and also improve filtration and clarification. The mixture contains three different enzymes; pectin lyase, polygalacturonase and pectin esterase. The preparation is especially designed for the treatment of fruit and vegetable mashes and the maceration of plant tissues. Soluble and insoluble pectins as well as haze-provoking polysaccharides are also efficiently degraded. Use of Pectinex 3XL enzyme on fruit and vegetable mashes or pomaces leads to drastically increased capacities in solid/liquid separation (e.g. press, decanter) and higher juice yields.

The enzyme mixture has been used to increase juice yields from mangoes, apples, pears and guavas. It has also been used to facilitate the easy peeling of citrus fruits. As a side activity pectinex also acts on cellulose due to the presence of small amounts of cellulase and hemicelullase. Pectinex enzyme exhibits optimum activity at pH 4.5 and 50°c (Greatvista, 2005). Pectinex enzyme would be most suitable for hydrolyzing sweetpotatoes because pectin, cellulose and hemicellulose all influence the texture of the root. Cellulase and pectinases enzymes are often used in combination in the process of manufacture of clear fruit and vegetable juices (Forgatty, 1981; Ennari et al., 1983). The enzymes are thought to act synergistically to achieve complete liquefaction of fruit purees. Coggins et al (2003) described a different method involving extraction of juice from the raw root using an automatic blender. He reported juice yields of 53.6%. There is little published literature on utilization of pectin degrading enzymes in sweetpotato processing, possibly because the

process of making juice from sweetpotatoes has not been well documented. The effect of pectinex 3XL enzyme on viscosity of sweetpotato puree and extraction yields of sweetpotato juice was the subject of this investigation.

CHAPTER THREE

3.0 Materials and methods

3.1 Materials and equipment

3.1.1 Sweetpotato roots

Sweetpotato roots of Muibai, Gikanda-white and Gikanda-yellow varieties purchased from traders at Wakulima market constituted the main raw materials for this study. The roots were identified on the basis of flesh color, skin color and shape of root which was supplemented with information from market traders. The skin color of Muibai variety was pink, the flesh was white and the shape elongated. Muibai was the most popular with consumers and was always available during the study visits. Gikanda white variety had white skin, white flesh and the root was elongated in shape. Gikanda yellow had white skin but color of fresh ranged from cream to yellow while the shape was almost spherical. The Gikanda-yellow variety was relatively rare in the market during the market visits.

In the market, traders arranged the potatoes in separate heaps on the ground depending on the variety, which made identification convenient. On each visit, 10kgs of medium size roots (each weighing about 100g) from each variety were randomly selected from the relevant heap, packed in labeled polythene bags and transported to the University laboratories at Kabete campus for the study. All the purchases were made in morning, when market traders received fresh supplies from rural suppliers.

3.1.2 Enzymes and chemical reagents

Pectinex 3XL enzyme was obtained from Norvo Fermenti Company (Switzerland) while reagent grade chemicals used were supplied by Kobian (K) Ltd in Nairobi.

3.1.3 Equipment

The electric blender used for milling samples was a Kenwood model. A CONTRAVES co-axial rheometer model 30 with a DIN45 measuring system was used for viscosity measurement.

3.2 Research design and data analysis

To study the effect of temperature, variety and curing duration on α-amylase activity in sweetpotato roots, a 3x3x3 factorial experiment in a completely randomized block design was used. The three factors investigated were: sweetpotato varieties (Muibai, Gikanda white and Gikanda yellow), curing temperatures (25, 30, 35°C) and curing durations (0, 5 and 10 days). The experiment was replicated on five batches of sweetpotatoes purchased over a period of four months. The analysis of β-amylase activity was undertaken on freshly delivered roots and also in roots cured at 35°C following information obtained from a preliminary study on response of the enzyme to curing obtained from 27 sweetpotato varieties. The results between β-amylase enzyme in fresh and cured roots were compared using least difference tests (Lsd).

The effect of α-amylase activity on starch conversion in sweetpotato purees was investigated using a 4x7 factorial experiment in a completely randomized block design. The two factors studied were temperatures at four levels; 60, 65, 70 and 75°C and α-amylase activities at seven levels; 0.46, 0.86, 1.0, 1.09, 1.71, 2.0, and 3.0 sdu/ml. To obtain these levels of enzymes, sweetpotato roots of the muibai variety were held at 35° C in an incubator and samples of the root were removed daily, milled to a paste and a sample of that paste used for α-amylase enzyme activity determination while the rest was portioned and incubated at the four temperatures for 180 minutes. The response variable was reducing sugars concentration (expressed as maltose) in the sweetpotato purees at the completion of hydrolysis.

To determine effect of pectinex enzyme on viscosity and yield of juice from sweetpotatoes, samples of saccharified sweetpotato purees were hydrolyzed at 50°C with incremental levels of the enzymes; 0, 50mg/Kg, 100mg/kg, 200mg/kg, and 400mg/kg. The study was undertaken in a randomized complete block design; the five enzyme treatments (including the control) being replicated in the three varieties of sweetpotato puree (blocks). The relationship between pectinex enzyme concentration on viscosity, soluble solids and juice yield were depicted graphically.

The relationship between the composition of sweetpotato variety and the quality of juice extracted from it was studied using a completely randomized design and the respective means of attributes compared using studentized (t) tests at 0.05 level of significance.

Data analysis: The data was analyzed using ANOVA statistical method described by Steel and Torie (1981) and calculations carried out using MS EXCEL worksheets. Least significance difference method (LSD), t-tests and correlation coefficients were carried out as previously explained.

3.3 Methods

3.3.1 Effect of variety, curing temperature and duration on α-amylase and β-amylase activities in three sweetpotato varieties

Approximately 10 kilos of each of the three sweetpotato varieties; Muibai, Gikanda white and Gikanda yellow were purchased from Wakulima market early in the morning on five different occasions. Two medium sized roots were randomly selected from each delivered lot for α-amylase and β-amylase enzyme activity determination as follows. The roots were washed, trimmed and milled to a fine paste which double folded in a muslin cloth and pressed manually to expel juice weighing approximately a third of the original weight of paste. The juice was clarified in a centrifuge at 3000xg for 10 minutes and analyzed for α-amylase and β-amylase activity as described below.

The rest of the batches of roots were distributed equally in the three temperature and humidity regulated incubators set at 25, 30 and 35°C, while a moist cloth was used to maintain the relative humidity inside the incubator to about 85%. Two sweetpotato roots of each variety were withdrawn from the respective incubator at the 5th and 10th day during the curing period and α-amylase determined. On the 10th day β-amylase activity was also determined in roots cured at 35°C (selected after preliminary studies with 27 sweetpotato varieties showed that β-amylase activity in the root changes erratically during curing)

3.3.1.1 Determination of α-amylase activity in sweetpotato roots (As described by Ikemiya and Deobold, 1966)

A sample of the clarified juice was transferred to a test tube and a few drops of 30% (w/v) calcium chloride solution added before heating the sample to 70°C in temperature regulated baths. The sample was cooled immediately using running tap water. To a series of five test tubes each holding 10ml solutions of pre-heated 2% soluble starch and maintained at 70°C in a temperature controlled water baths was added 1,2,3,4,5 ml samples of the juice extract respectively.

At half minute intervals, 0.1mls of the reactants were withdrawn from the respective tubes using a micropipette and mixed with one ml of the iodine solution in a spotting dish (made according to the AACC method, 1962). The color of the solution mixture was then compared to the Dichromate solutions in comparison tubes prepared earlier according to the method of Olson et al. (1944). (The method involves dissolving 25.0g Cobaltous chloride hexahydrate and 3.84g potassium dichromate in 100 mls of 0.1 Hcl). The time it took to obtain a color match of the iodine solution in the digested sample extract with standard Dichromate solutions was considered as the time the α-amylase in the sweet potato juice extract took to completely hydrolyze the soluble starch in the 2% solution. Therefore, the higher the level of α-amylase in the sweetpotato extract, the shorter it took for the color match to be obtained. To optimize the precision of this method, it is recommended that reaction time should be between 10-30min. If the enzyme activity in the extract was such that the time for hydrolysis is less than 10 minutes, appropriate serial dilutions were prepared. In cases whereby it took more than 30 minutes, α-amylase activity was reported as too low to determine (TLD). Amylase activity was calculated as follows: α-amylase activity in extract (in Sdul/ml) = **12 ÷ volume x time**

3.3.1.2 Determination of β-amylase activity in sweetpotatoes: (As described by Walter et al., 1975).

To 15mls of buffered 2% soluble starch solutions (pH5.0, in 0.1M sodium Acetate buffer) maintained at 70°C in a temperature regulated water bath, 1ml of the clarified sweetpotato juice extracted from the sweetpotato roots was added and at the same time the timer was started. After exactly ten minutes, the reaction was stopped using 2 drops of 0.2N sodium hydroxide solution. Reducing sugar was analyzed in the hydrolyzed sample using the convectional Lane and Eynon Method and β-amylase activity expressed as mg of maltose/ml/min.

3.3.2 Effect of α-amylase activity on starch hydrolysis in sweetpotato purees held at 60, 65, 70, 75°C for 180 minutes

About 10kgs of fresh Muibai sweetpotato roots were purchased from Wakulima market early in the morning on four different occasions and delivered to our laboratories for processing. Three roots were selected randomly from each batch, washed, trimmed and milled to a fine puree from which 100g sample was withdrawn for α-amylase determination as described above. The rest of the puree was divided to four 300g samples, each separately blended with

100g distilled water in 500ml glass flask and distributed in preheated water baths maintained at 60, 65, 70 and 75°C respectively. Changes in soluble solids in the purees were determined at half-hourly intervals using a pocket refractometer until no further change in two subsequent readings was obtained (on average 180 minutes). Whereupon, heating was stopped and reducing sugar analysis carried out for each sample using Lane and Eynon method.

The remainder of the roots was transferred to an incubator maintained at 35°C, RH 85%. Three roots were selected from the batch on a daily basis for the next six days, analyzed for α-amylase activity and then hydrolyzed at 60, 65, 70 and 75°C. The experiment was replicated on four different sweetpotato lots.

3.3.3 Effect of pectinex enzymes on viscosity and extraction yield of juice from saccharified sweetpotato purees

Ten kilos of fresh sweetpotato roots of the three local varieties under investigation Muibai, Gikanda white and Gikanda yellow were purchased from Wakulima market and cured for 5days at 35°C, 85% RH. Two roots were withdrawn for α-amylase and β-amylase enzyme activity determination. The rest of the roots were divided into three batches of 3kgs, peeled and macerated to a fine puree which was held in water baths at 75°C for 180 minutes to facilitate saccharification. The saccharified purees were transferred in five lots each weighing 400g to 500ml glass flasks. The following levels of pectinex 3XL enzymes were separately added to the purees; 50mg/Kg, 100mg/kg, 200mg/kg, and 400mg/kg. The purees were then transferred to temperature regulated water baths preheated to 50°C and continuously stirred with magnetic stirrers. Samples of the puree were then withdrawn at 30 minute intervals for the determination of soluble solids until no further change was noted (average time taken was 90 minutes).

A sample of the puree was removed, cooled to room temperature and viscosity determined using a rotary cylinder type viscometer (CONTRAVES co-axial cylinder rheometer, model Rheomat 30). Shear rate and shear stress were obtained from the speeds and toque readings, according to the tables shown on the rheometer manuals. It has been demonstrated (Rao, et al., 1975) that reliable and consistent readings can be obtained within the mid-range of the viscometer scale when dealing with samples that exhibit pseudo-plastic behavior such as sweetpotato purees). Therefore, determination of viscosity was made at constant rotor speed (III), which was selected after preliminary trials. Apparent viscosity was determined from the

relationship between shear rate and shear stress from the power law model also known as Ostwald-de Waele Model: $\tau = k_{ow} \gamma^n$ where τ is the shear stress (mPa), γ is the shear rate (s^{-1}) and k_{ow} and n are the consistency index ($mPas.^n$) . For pseudo-plastic fluids, it is possible to use the values of apparent viscosity at constant shear rate when $\mu = \eta_a$ and $\mu_0 = \eta_0$ (Savaracos, 1970).

The sweetpotato purees were then weighed and juice manually pressed through a double folded muslin cloth and settled overnight in the cold room. The following day, the clear juice was carefully decanted, weighed and results expressed as % yield based on the original weight of puree. The characterization of sweetpotato juice was undertaken by measuring the following parameters: soluble solids, total solids, specific gravity, total titratable acidity, and relative viscosity, reducing sugars, presence of starch, pH and protein content.

3.3.4 Characterization of sweet potato roots and sweetpotato juice

Ten kilos of fresh sweetpotato roots of the three varieties (Muibai, Gikanda and Gikanda yellow) were purchased from groceries in Nairobi an proximate composition determination carried out.

Preparation of samples from raw root: Three sweetpotato roots were selected from each lot, washed and macerated to a smooth puree in a convectional kitchen type electric blender. Samples of the puree were drawn for the analysis of DM, starch, protein, total sugars, reducing sugars, total carotenoids and ash according to the analytical methods described below.

The rest of the roots were cured for 5days at 35°C and used for extracting clarified sweetpotato juice as previously described. The juice was analyzed for soluble solids, total solids, proteins, total titratable acidity, pH and total sugars. Relative viscosity and relative gravity were also determined on the juice as detailed below. The results of corresponding parameters in the respective roots and juices were compared on each variety.

3.3.4.1 Moisture Content:

Four replicate samples of the puree, each weighing 10g were oven dried in a vacuum oven at 70°C for 24hrs. The difference between the initial and final weight multiplied by ten was expressed as percentage moisture content of samples.

3.3.4.2 Ash Content: (AOAC, 1984)

Three replicate 5g dry samples were transferred to 50ml porcelain crucibles, which had previously been ignited and cooled before weighing. The crucibles and the contents were then ignited to constant weight in a muffle furnace at 500°C. The ash was cooled and weighed and expressed as a percentage of the initial weight of the wet sample.

3.3.4.3 Starch content

Four replicate 5g samples of the dried puree was transferred to a 250ml Erlenmeyer flask and thoroughly extracted with 85% (v/v) Ethanol. The residue was dissolved in 52% perchloric acid and starch was determined. The results were expressed as a percentage on D.M. basis.

3.3.4.4 Protein content (AACC 1983)

Four replicate 5g samples of the dried puree were separately weighed in nitrogen free blotting papers, folded and transferred to dry 800ml Kjeldahl digestion flasks. The catalyst (one selenium tablet) and 20ml of conc. Sulphuric acid were added and the contents heated gently in an inclined position (in a gas chamber). When the initial frothing ceased, reflux condenser was fitted and the mixture heated strongly with frequent shaking until the contents became clear. Gentle heating was then continued for one hour, the contents cooled and washed into a distilling flask with distilled water to a volume of 400ml. Anti-bumping agents were added, the digest made alkaline with 75mls of 50% sodium hydroxide solution, the contents distilled into 50mls of boric acid and drops of methyl blue indicator. After 300mls of the ammonia solution was distilled, the distillate was titrated with 0.1N Sulphuric acid. Factor 6.25 was used for calculating the protein content.

3.3.4.5 Reducing sugar analysis

Four 10g replicate samples of the puree were separately extracted with 85% (v/v) Ethanol. Extracts from the four replicate samples (40g) were then combined and alcohol was removed in a rotary vacuum evaporator at 65°C. The residue was dissolved in 90mls of distilled water, clarified with 10mls of freshly prepared Carez I solution, filtered through Whatman's filter paper #1 and reducing sugars determined according to the Lane and Eynon Method and results expressed on D.M. basis.

3.3.4.6 Determination of sucrose Content

Dried replicate sweetpotato puree samples (10g each) were extracted with 150mls of the 85% ethanol for one hour, centrifuged at 3500rpm for 15 minutes and the residue further extracted

with 50mls of ethanol. The process was repeated and the two extracts combined. The alcohol was removed in the rotary vacuum evaporator and the residue dissolved in 90mls distilled water, followed by clarification and filtration as previously described.

The sample was then transferred to a test tube and maintained at 67°C in a water bath. 5mls of conc. Hcl was added and immediately, the timer was started. After exactly half a minute, 40% (w/v) sodium hydroxide solution was added to stop the reaction. The pH of the solution was maintained at 6.5–7.0. Reducing sugar content of the sample was then determined using Lane-Eynon method as described above. The difference in the two results, Unhydrolyzed and the hydrolyzed sample was multiplied by 0.9 and expressed as the sucrose content.

3.3.4.7 Determination of total carotenoids

Total carotenoid content in raw sweetpotato roots was determined by extracting 10g samples of the puree with 95% ethanol- hexane blend (60:40). After blending, the hexane layer was separated by in a glass separating funnel by adding cold 2% salt solution. The OD of the samples was read at 440nm by use of spectrophotometer and compared to a standard β carotene standard curve to determine total carotenoids (Lanier and Sinstrunk, 1979).

3.3.4.8 Specific gravity:

Samples of 50mls sweetpotato juice were transferred to previously dried and weighed 50ml pycnometer at room temperature. The weight of the juice sample divided by 50 gave the specific gravity (expressed as g/ml) of the juice.

3.3.4.9 Total titratable acidity:

A 10ml sample of sweetpotato juice was titrated against 0.1N Sodium Hydroxide solution in the presence of 1% phenolphthalein indicator solution. The titration was stopped when the color of juice changed to faint pink. Total titratable acidity was calculated as; TA (%) = 0.1 X milliliters of sodium hydroxide used.

3.3.4.10 Relative viscosity of the sweetpotato juice

Relative viscosity of the sweetpotato juice was determined using Ostwald's viscometer at room temperature (25°C). The ratio of time (in seconds) taken by the juice and that taken by distilled water to flow between the two markings on the capillary was expressed as relative viscosity.

3.3.4.11 Total Solids

A 10g sample of the juice was weighed into a tared aluminum filled with dried activated sand, thoroughly mixed and dried in vacuum oven for 24hrs at 70°C. The results expressed as % of the weight of original sample.

CHAPTER FOUR

4.0 Results and Discussion

4.1 Effect of sweetpotato variety, curing temperature and curing duration α-amylase and β-amylase activities in sweetpotato roots

The initial α-amylase activity in the three sweetpotato varieties studied Muibai, Gikanda white and Gikanda were high and spanned a wide range (Table 3). The highest initial α-amylase activity found was 1.54 sdu/ml in Gikanda yellow while the lowest was 0.41 sdu/ml found in Gikanda white. The mean initial α-amylase activity varied widely between batches of sweetpotato roots purchased on different days as indicated by the variances observed. Consequently, it became difficult to predict the level of enzyme activity expected in the fresh roots. In the analysis of the results obtained, sweetpotato roots purchased on the same day constituted the blocks, providing four degrees of freedom in the completely randomized factorial design.

Table : Changes in the levels of α-amylase activity† in three sweetpotato roots during 10 days of curing at 25, 30 and 35°C

Curing temperature	Curing duration (days)	Sweet potato variety		
		Muibai	Gikanda white	Gikanda yellow
25°C	0	0.48±0.36	0.47±0.13	0.48±0.27
	5	2.32±0.34	1.97±0.56	1.34±0.33
	10	6.43±1.28	1.8±0.23	5.04±0.85
30°C	0	0.61±0.32	0.41±0.11	1.54±0.35
	5	1.59±0.66	3.21±0.24	6.38±1.30
	10	2.88±0.61	6.8±1.47	19.54±2.35
35°C	0	0.51±0.12	1.27±0.32	0.42±0.41
	5	7.5±2.46	9.68±1.53	7.83±2.33
	10	19.1±3.25	25.96±1.56	14.52±2.85

† Each value is a mean of five replications expressed and is expressed in sdu/ml

By comparing the calculated and tabulated F values for block effect at $p<0.05$ and $p<0.01$ (Table 4), it was established that the variation between batches of sweetpotato roots was highly significant. Part of the explanation could be accounted by differences in post-harvest handling history of various batches roots purchased. The time lag between harvesting and analysis, storage conditions during transit and in the market are likely to have influenced the results. Sweetpotato roots are produced by different rural smallholder farmers in scattered locations and often pass through a long chain of middlemen before eventually reaching the market. But apart from post-harvest history, harvesting conditions have been reported to influence levels of α-amylase activity in the roots (Deobald et al, 1968). Preliminary investigations carried out on freshly harvested sweetpotato roots from 24 test cultivars also indicated that even at harvesting, there is considerable batch to batch variation in the levels of α-amylase activity (data not shown). High α-amylase activity is an indicator of good processing quality and is desirable in the process of extracting sweetpotato juice from roots.

Curing sweetpotatoes increased α-amylase activity in the three varieties of sweetpotato roots studied. The rate of increase was influenced by temperature, curing duration and sweetpotato root variety. Generally sweetpotato roots cured at 35°C showed the highest level of α-amylase activity at both the fifth and tenth testing dates as compared to roots cured at 30°C and 25 °C respectively. Curing duration also influenced the rate of α-amylase activity in the roots and was highest at the tenth day compared to the initial level of activity and also at the fifth day of curing, irrespective of the curing temperature and the initial α-amylase activity. The highest mean level of α-amylase activity was observed in Gikanda white (25.96 sdu/ml) after curing for ten days at 35°C while the lowest activity was observed in freshly purchased roots of the same variety (0.41sdu/ml). The initial α-amylase activity did not appear to affect the rate of increase in the enzyme activity during curing. ANOVA of the results (Table 4) showed that curing temperature and curing duration significantly affected the level of α-amylases activity in sweetpotato roots. Therefore, to stimulate rapid increase in α-amylase activity in sweetpotato roots and reduce the duration of curing, the higher temperature of 35°C should be used. The results however did not indicate whether this was the highest optimum temperature for curing sweetpotatoes for juice extraction, suggesting need for further investigations in that regard.

Table : Analysis of variance (ANOVA) for process factors on α-amylase activity in sweetpotato roots during curing

Source	df	SS	Mean SS	F
Blocks	4	250.8	62.7	4.2**
Variety	2	34.2	17.1	1.2
Temperature	2	1264.0	632.0	42.8**
Curing Duration	2	2574.9	1287.5	87.8**
Variety x temp	4	638.1	159.5	10.8**
Variety x duration	4	69.3	17.3	1.2
Duration x Temp	4	892.3	223.1	15.8**
Var x dur x temp	8	567.2	70.9	4.8**
Error	104	1536.8	14.8	
Total	134	7827.5		

*significant effect ($p<0.05$) ** highly significant ($p<0.01$)

The results therefore showed that curing sweetpotato is likely to be an important unit operation in the process of extracting sweetpotato juice for beverage and ethanol production. The curing temperatures selected were also within the normal range of ambient temperatures in many parts of Kenya which could imply that no special curing facilities would be required by sweetpotato juice manufacturer. Given that most of the sweetpotatoes purchased from the markets had already undergone partial curing as indicated by the high levels of initial α-amylase activity, it could mean that any deliberate or accidental delay of processing sweetpotato roots is likely to improve processing quality. This would perhaps mean that farmers from distant locations from a sweetpotato juice processing facility would not be disadvantaged as the improved quality occasioned by transport delays could compensate for the high transport cost incurred since extra curing would probably not be needed

The interaction between sweetpotato variety and time was not found significant which means that in the three sweetpotato varieties studied, time of curing stimulated the same response in α-amylase build up. The temperature time interaction was however highly significant, which means that the response of α-amylase activity to different curing durations was dependent on temperature of curing. Within the range of temperature studied, the response was directly related to temperature, with roots cured at 35°C showing the highest increase of α-amylase activity. The upper temperature limit for curing sweetpotatoes of sweetpotatoes was not

determined in this investigation, but based on evidence from previous studies, curing temperatures beyond 35°C promote rapid sprouting and mold attack and are not normally used (Sumner, 2004) . But what effect curing sweetpotato roots temperatures above 35°C might have on the activity of amylolytic enzymes on sweetpotato roots may perhaps merit further study. The second order interaction, between temperature, time and variety was also highly significant. This means that temperature and time interactions differ with the type of sweetpotato variety. This point may require further elaboration in future studies given the wide variety of sweetpotatoes Kenyan farmers are currently producing at different agro-ecological zones. Although some variation was observed in the levels of α-amylase enzyme activities between roots from different varieties, analysis of variance indicated that the observed differences were not statistically significant. These findings are in agreement with those from previous studies (Ikemiya and Deobald, 1966, Deobald et al., 1969 and Walter et al., 1975).

To determine the effect of curing on β-amylase activity in sweetpotato roots, a comparison of the means of β-amylase in three varieties of sweetpotato roots before and after curing for 10 days at 35°C was performed using the least significant difference (lsd) at levels of probability $p<0.05$ and $p<0.01$ (Steel and Torne, 1981) using data presented on table 5, below. The lsd values for Muibai (3.4, 4.4) at $p<0.05$ and $p<0.01$ respectively were less than the absolute difference of means of β-amylase activity in cured and uncured roots which was 8.28. Therefore, the results show that the reduction in β-amylase activity in Muibai sweetpotato roots as a consequence of curing was highly significant. The corresponding values for the other two varieties were lsd (0.05) 6.4 and lsd (0.01) 8.3 for Gikanda white and lsd (0.05) 1.2 and lsd (0.01) 1.6 for Gikanda yellow. The absolute difference in means between β-amylase activity in cured and uncured Gikanda white sweetpotato roots was 0.42 while in Gikanda yellow the difference was 1.2. These results showed that the reduction in β- amylase activity in Gikanda yellow was significant while in Gikanda white, the slight increase in the enzyme activity observed was insignificant. Based on these findings, it was concluded that curing sweetpotato roots reduces the level of β-amylase activity in sweetpotato roots in some sweetpotato varieties while in others it remains the same. Use of a much wider selection of varieties could have been more useful to clarify the position.

Table : Mean β-amylase activities in three sweetpotato root varieties before and after curing at 35°C for 10 days

Sweetpotato variety	β-amylase activity † (Fresh roots)	β-amylase activity (In cured roots)
Muibai	36.64±13.50	28.36±10.33
Gikanda white	39.32±10.32	39.74±2.93
Gikanda yellow	32.89±9.23	38.88±8.84

† β-amylase activity expressed in mg/maltose ml/min. Each value is a mean from 5 replicates.

There is overwhelming evidence in published literature showing that conversion of starch to maltose during heat processing of whole sweetpotato roots and purees is strongly correlated with the level of α-amylase activity in sweetpotato roots. It's also a well established fact that β-amylase is responsible for converting starch and dextrins to maltose and that when the two enzymes act simultaneously, starch degradation proceeds at a faster rate than when either of them acts alone. Therefore, the suppression of β-amylase activity observed as a result of curing some of the sweetpotato varieties was probably more than compensated by increase in α-amylase activity. Evidence suggests that α-amylase in sweetpotatoes hydrolyzes starch in the root randomly to form dextrins and malto-dextrins which are then hydrolyzed to maltose by β-amylase (Bretiler, 1973; Walter et al., 1975). The α-amylase enzyme is responsible for liquefying starch solutions producing starch fragments which are degraded to maltose by β-amylase enzyme. The enzyme β-amylase is an endo-enzyme that hydrolyzes starch, malto-dextrins and dextrins from the non-reducing end of the chain thereby liberating maltose.

In the process of extracting sweetpotato juice using endogenous enzymes, high levels of both α-amylase and β-amylase enzyme are desirable. The optimal levels of β-amylase and α-amylase that would guarantee complete conversion of starch to maltose in sweetpotato roots has not been reported in literature, although it is likely to be a good indicator of optimum curing treatment for sweetpotato roots to be used for extracting the juice. Curing sweetpotatoes therefore is likely to provide an alternative source of amylolytic enzymes instead of using malted cereals for brewing traditional alcoholic beverages whose consumption is likely to increase substantially following their recent legalization. Increased consumption of these beverages could aggravate cereal grain shortages, hence the importance of utilizing sweetpotatoes which are inexpensive to produce and are not popular with many consumers.

Another possible application of cured sweetpotato roots would be for the substituting 'power' flour from malted grains. Flour from malted grains, 'power flour' has been successfully used to reduce the bulk density of cereal porridges and therefore making them more appropriate sources for supplying young children with energy (Naeda, 1978; Ljungqvist et. al., 1981; Mosha et al., 1983). One of the emerging uses for sweetpotatoes is to mix the dried sweetpotato flour with cereal flours to make enriched porridge flour for malnourished children. In such applications, flour from cured sweetpotato flours could provide liquefying enzymes for thinning the porridge and therefore enhance calorific density. Nadutu et al., (2000) used α-amylase for the determination of starch in foods, suggesting another possible commercial use for the enzyme.

4.2 Effect of α amylase activity on starch hydrolysis in sweetpotato purees held at 60, 65, 70, 75°C for 180 minutes

The extent of starch hydrolysis in Muibai sweetpotato purees was significantly influenced by endogenous α-amylase activity in the puree and also on the temperature at which the purees were heated during the conversion. The variation in α-amylase activity in purees was due to the difference in curing durations from 1-6days that the roots were subjected to. Therefore the α-amylase activities indicated are means from four replicates with their respective standard deviations (Table 6). All Muibai sweetpotatoes purees converted at the four constant temperatures investigated had accumulated substantial levels of reducing sugars (maltose) after 180 minutes, the levels being highly dependent on α-amylase present (Table 6). The results of ANOVA of main factors and interactions (presented on Table 7) showed that the effect of temperature on maltose formation in sweetpotato purees was highly significant.

At each level of α-amylase activity present in the puree, the extent of starch conversion to maltose was closely associated with conversion temperature and was lowest at 60°C and highest at 75°C. Temperature of starch conversion in sweetpotato purees significantly influenced maltose formation in the purees. These temperatures were carefully selected and were within the reported optimum range for sweetpotato β-amylase (60-65°C) and sweetpotato α-amylase (70-75°C). The fact that significantly higher conversion of starch to maltose occurred within the optimum temperature for α-amylase provides further evidence that the activity of α-amylase has greater influence on starch conversion in sweetpotatoes than that of β-amylase.

Table : Reducing sugar content in Muibai sweetpotato purees of varying α-amylase activities heated for 180 minutes at 60, 65, 70 and 75°C

Curing duration (days)	α-amylase activity in puree†	Holding temperatures of sweetpotato purees			
		60°C	65°C	70°C	75°C
		% Reducing sugar content (wwb)			
0	0.5±0.2	6.4±1.6	9.3±2.9	11.6±2.3	14.2±0.5
1	0.9±0.3	7.6±1.5	13.5±3.9	13.9±2.7	17.2±0.8
2	1.8±0. 1	10.3±1.9	12.1±2.7	14.7±3.1	16.4±2 5
3	1.1±0.5	13.5±3.4	16.2±1.9	13.65±4.1	17.3±2.2
4	1.7±0.5	14.6±2.8	21.2±3.1	13.4±1.0	22.3±1.8
5	2.0±0.7	18.7±1.4	18.7±1.4	21.2±2.1	24.1±1.2
6	3.8±0.3	20.5±6.2	20.2±1.8	29.3±3.2	26.4±3.5

† α-amylase activity expressed in sdv/ml each value is mean of four replicates. Reducing sugars expressed is a mean from four replicates.

However, maltose formation was expected to be higher within the reported optimum range for β-amylase enzyme and not at temperatures which were much higher. At processing temperatures higher than their optimum, enzymes rapidly lose their biological activity while they exhibit much slower rates of activity below their temperature optimum. The results show that β-amylase converted more starch to maltose at 75°C, a temperature at which it was supposed to have lost all its biological activity since its optimum temperature has been reported in the range 60-65°C. A possible explanation is that in a complex food system as sweetpotato puree, the optimum temperature for enzymes could be influenced by other constituents present. The optimum temperature for α-amylase also appeared to have been above 75°C, which shows the need for studying maltose formation in purees heated above that temperature.

It has been reported that plant amylases scarcely hydrolyze native starch granules, their action being lower than 5% (Dicko et al., 1999). Therefore, gelatinization temperature, which varies widely among different sweetpotato varieties, plays a critical role in the enzymatic degradation of starch by endogenous enzymes. Gelatinization temperatures of sweet potato starches are reported in the range of 58 - 84 °C (Takeda et al., 1986; Tran et al., 1991; Garcia and Walter, 1998). Differences in gelatinization temperatures may also influence starch conversion in sweetpotato purees

The effect of the level of α-amylase activity on the formation of maltose in sweetpotato purees was also found to be highly significant. Although α-amylase is not directly involved in hydrolysis of starch to maltose, it facilitates the saccharification action of β amylase which is simultaneously present in the sweetpotato puree by exposing more non-reducing end from the starch polymer. The formation of maltose in sweetpotato purees is therefore directly related to α-amylase activity within the range tested. In cured sweetpotato roots, there is higher α-amylase activity as compared to fresh roots; hence the differences in levels of maltose formed purees from fresh roots as compared with purees from cured roots. The interaction of temperature and enzyme activity effects was also found to be significant as indicated by ANOVA. This was a clear indication that raising the temperature by the same degree did not cause the same magnitude of response in all purees. For all purees, the highest amount of conversion of starch to maltose which was 75% was achieved at 75°C and α-amylase activity of 2.0sdu/ml. This represents a high starch conversion to soluble sugars and possibly juice extraction efficiency.

Table : ANOVA for process factors on maltose formation in sweetpotato purees

Source	df	SS	Mean SS	F
Blocks	3	29.19	9.73	0.93
Enzyme level	6	2552.68	425.45	40.72**
Temperature	3	846.5	282.17	27.01**
Enzyme x temp	18	387.49	21.53	2.06*
Error	81	846.306	10.45	
Total	111	4662.166		

The calculated linear regression equation relating maltose conversion with α-amylase activity at 75°C was Y =2.328x +13.2. Therefore, at least 9.87sdu/ml of α-amylase enzyme would be required to achieve complete conversion of starch to maltose in sweetpotato purees at 75°C. In the previous investigation, it was demonstrated that this level of enzyme are quite easily achievable through curing sweetpotato roots at 35°C for at least ten days.

However, in practice, it is well known that α-amylase cannot completely hydrolyze starch to maltose due to α-[1, 6] glycosidic bonds present in the highly branched amylopectin polymer,

which requires the involvement of a debranching enzyme. However, increasing the level of α-amylase enzyme activity has been demonstrated to cause a reduction in the average sizes of the molecular sizes of β-limit dextrins produced (Walter et al., 1975). But without adding an exogenous debranching enzyme, hydrolysis of amylopectins will not proceed beyond the β-limit dextrins (Fullbrook, 1980). It is likely that addition of a debranching enzyme such as pullulanase, which is able to hydrolyze α [1, 6] glycosidic bonds would be required to achieve complete conversion of starch to maltose. Amylose contents of sweet potato starches vary between 8.5% and 38% (Tian et al., 1991; Takeda et al., 1987) while the balance consists of the branched amylopectin polymer.

Recent studies have shown that sweetpotato roots accumulate substantial levels of α-amylase enzyme inhibitor during wound healing, tuber formation and sprouting (Sakisiran et al.; 2002). The effect of this enzyme inhibitor could have had some influence on starch conversion at the lower temperatures. Studies on starch conversion during boiling and baking in previous investigations report much higher starch conversion in intact roots as compared to heating purees at constant temperature (Hoover, 1967; Picha, 1986; Deobald et al., 1969; Losh et al., 1981; Walter et al., 1984). The enzyme activation technique described by Hoover (1967) was based on heating sweetpotato puree at constant temperature within the range 71-85°C for 2-60 minutes depending on the level of α-amylase present and desired. Deobald et al., (1969) noted that the greatest increase in maltose formation in sweetpotato purees was in the temperature range of 75-78°C. But Walter and Walter et al., (1984) demonstrated that heating sweetpotato strips directly with steam resulted in minimum conversion of starch to maltose. They also observed that raising the mashing temperature above the optimum 75°C inhibited amylolytic activity. Losh et al., (1981) reported that roots baked at 200°C and 230°C scored significantly higher for sweetness, moistness and softness than roots baked at 150°C and 180°C. Slow cooking of within the temperature range 60-100°C has been reported to convert most of the starch to sugars. The results of this study are therefore in agreement with previous studies on the subject.

Starch hydrolysis during cooking of sweetpotato roots significantly influences rheological properties. In sweetpotatoes, starch molecules are degraded during processing, losing the ability to swell and increase viscosity. According to Walter et al., (1975), the degradation of large molecules such as starch lowers viscosity and their water binding ability thus contributing to a moist mouthfeel of cooked sweetpotatoes. Rao et al., (1975) reported that apparent viscosity (moistness) of cooked sweetpotato roots generally decrease with

increasing storage. Degradation of starch by endogenous amylolytic enzymes is therefore likely to enhance the extractability of sweetpotato juice. Therefore, to achieve optimum juice extraction from sweetpotato roots, the breakdown of starch to sugars is vital.

4.3 Effect of pectinex 3XL enzyme on the puree viscosity, soluble solids and yield of extracted sweetpotato juice

The effect of pectinex enzyme concentration on viscosity, soluble solids and yield of juice from saccharified sweetpotato purees from the three sweetpotato varieties, Mubai, Gikanda white and Gikanda yellow are depicted on figures 2, 3 and 4 below. The saccharified sweetpotato purees were hydrolyzed at four pectinex enzyme levels; of 50mg/Kg, 100mg/kg, 200mg/kg, and 400mg/kg and one control was also included in each run to make five treatments for each sweetpotato variety.

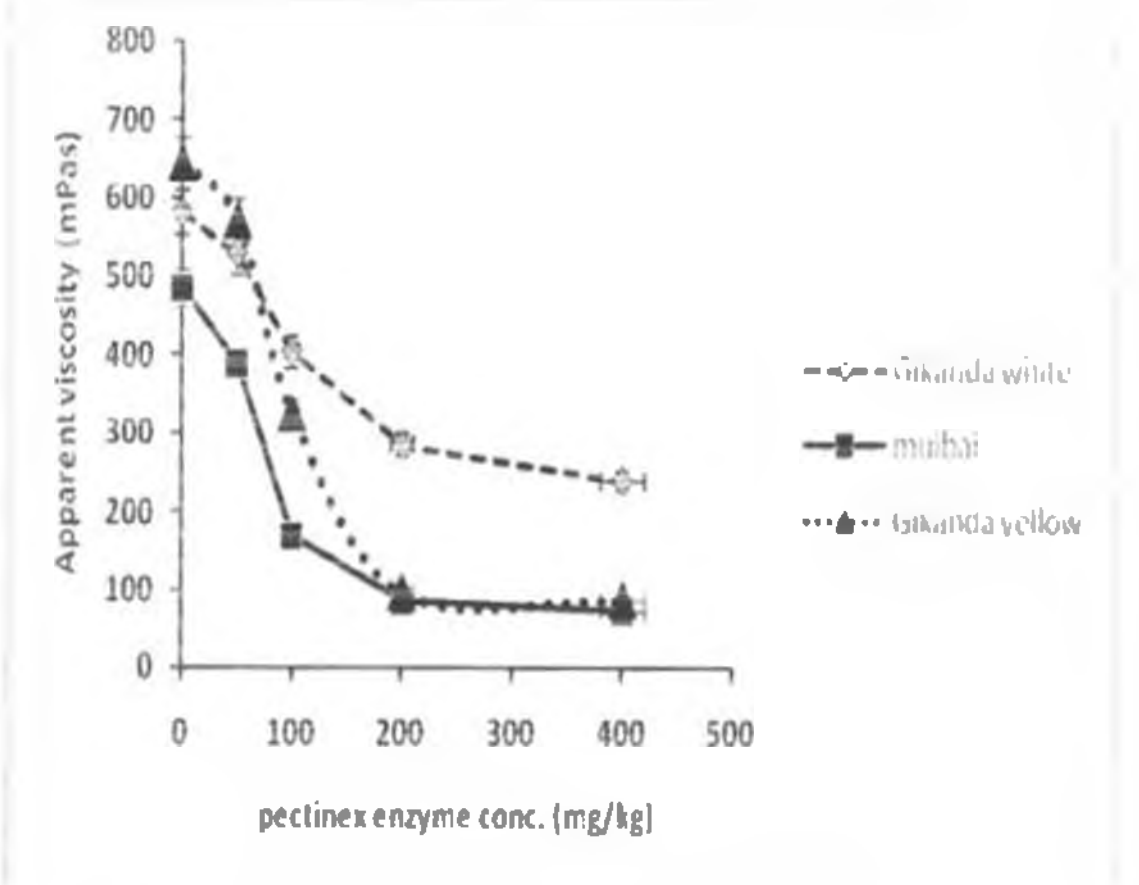


Figure : Effect of Pectinex 3XL enzyme concentration on the viscosity of saccharified sweetpotato purees

The curve relating pectinex concentration and puree viscosity shows an inverse relationship (figure 3) between the two variables. As the level of pectinex enzyme in the three purees is

increased, there is a steep drop in viscosity until a concentration of 200mg/kg of enzyme is reached after which the viscosity decreases marginally. The greatest viscosity reduction was observed in purees prepared from Gikanda white and Muibai and was lowest in purees from Gikanda yellow. Further analysis of data using correlation coefficients (Table 8) showed a significant negative correlation coefficient between pectinex enzyme concentration and viscosity of the puree. Degradation of pectin in the puree is therefore critical to the reduction of sweetpotato puree viscosity. This information provides direct evidence that pectin has an important role to play in rheological properties of sweetpotato purees obtained from the three sweetpotato varieties studied.

Figure 4 shows the relationship between yields of clarified sweetpotato juices extracted from the puree and the level of pectinex enzyme applied on the sweetpotato puree. When the concentration of pectinex enzyme in the sweetpotato puree was increased from 0-200mg/kg, the yield of juice increased from 30-52% to over 75% in the three purees studied. However, when the concentration of the enzyme was increased above 200mg/kg, the increase in yields was quite marginal; suggesting that pectinex concentration of 200mg/Kg was the optimum. When no pectinex enzyme was used, the yield of juice from Gikanda yellow, Muibai and Gikanda white purees were 30, 40 and 52% respectively, a trend which could be explained by viscosity values obtained. Further analysis of data (Table 8) showed a highly significant positive correlation between enzyme concentration and juice yields from the purees.

The study however failed to establish a direct relationship between soluble solids in the extracted juice and the concentration of pectinex enzyme given the low correlation coefficient found. The graphical representation of the information on Figure 5 shows a slight increase in soluble solids as the enzyme concentration in the sweetpotato purees was increased from 0 to 200ppm. The increased yield of sweetpotato juices as a result of addition of pectinex enzyme could be explained by the role of pectin degrading enzymes in food systems. The enzymes open up hydrocolloids polymers in foods and decrease their water holding capacity. If no solids were extracted simultaneously by the action of the enzymes, increased juice yields would have been attributed fully to the release of this water which was held and this would have diluted the extracted juice thereby causing a reduction in soluble solids. Therefore, these results demonstrate that pectinex enzyme enhanced extraction of solids from the sweetpotato purees. So although the correlation coefficient between pectinex enzyme concentration and soluble solids was not significant (Table 8), the increase in juice yield could be attributed to the extraction of soluble solids from the puree.

Pectin degrading enzymes are also commonly used for extracting juice from fruits of high pectin contents such as apples, mangoes and guava. Indeed, commercial pectic enzymes from fungal sources have been used in fruit juice processing since the 1930's. The enzymes have found wide applications involving the disintegration of fruit and vegetable pulps and therefore help to extract optimize and clarify juices from carrots, pears, mangoes, apples and bananas. Pectinex 3XL is a just one of the commercial mixtures used for the purpose and contains several types of pectinases enzymes such as pectin lyase, polygalacturonase and pectinesterase (Forgatty, 1981). As a side activity, Pectinex also contains small amounts of hemicellulases and cellulases Matta et al., (2000) used pectinex enzyme at a concentration of 0.02% (v/v) to extract juice from West Indian cherry. Jansen (1997) reported viscosity reduction of 50% and juice extraction yield of 90% when pectinex 3XL enzyme was used for extracting mango juice from pulp. In addition, Chan and Chiang (1992) have reported the use of the enzyme for clarification of guava nectar.

Table : Correlation coefficients of pectinex concentration (0-200mg/kg) on sweetpotato viscosity, soluble solids and % yield of juice

Sweetpotato variety	Correlation coefficient Enzyme/viscosity	Correlation coefficient Enzyme/(%yield)	Correlation coefficient Enzyme/soluble solid
Gikanda white	-0.92	0.87	-0.081
Gikanda yellow	-0.87	0.84	0.39
Mulasi	-0.83	0.91	0.44

In previous studies sweetpotato juice was extracted with water after cooking whole roots (Nungu, 2005; Wireko-Manu, 2010) or from sweetpotato strips (Truong and Fermentira, 1991). Use of pectinex enzymes to hydrolyze the mass makes use of water unnecessary and is therefore likely to improve the cost of recovering juice from sweetpotato purees.

However, use of pectic enzymes for processing sweetpotato products has not been widely studied.

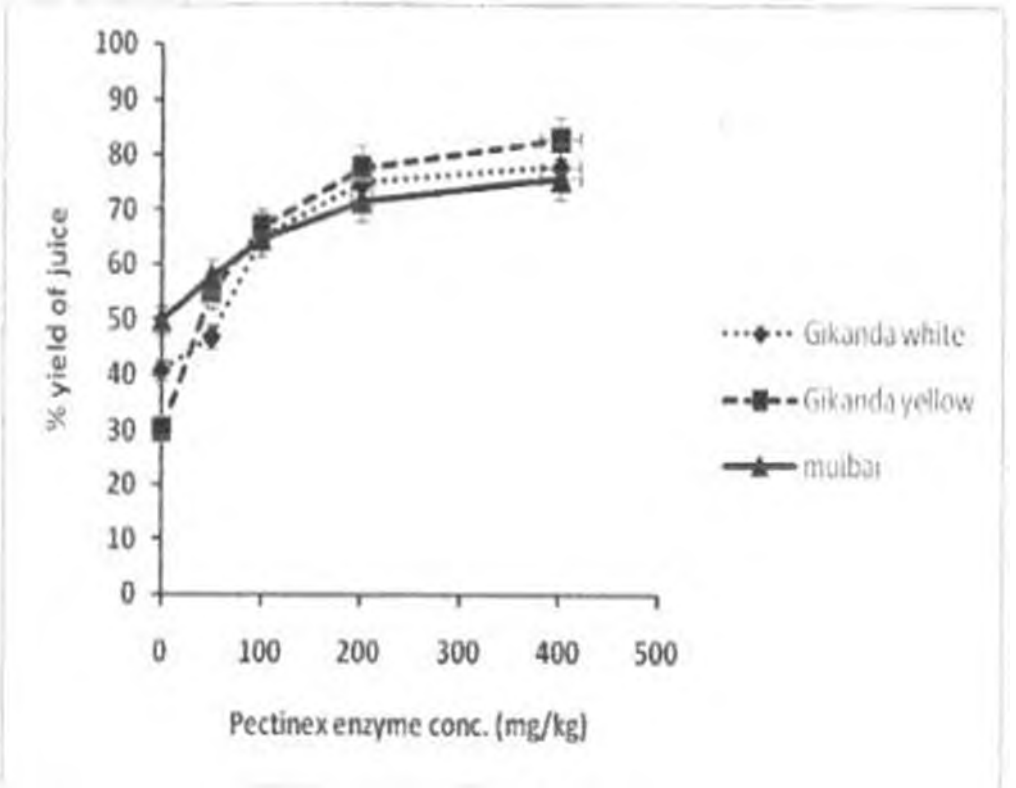


Figure : Effect of Pectinex 3XL enzyme concentration on yield of juice from saccharified sweetpotato purees

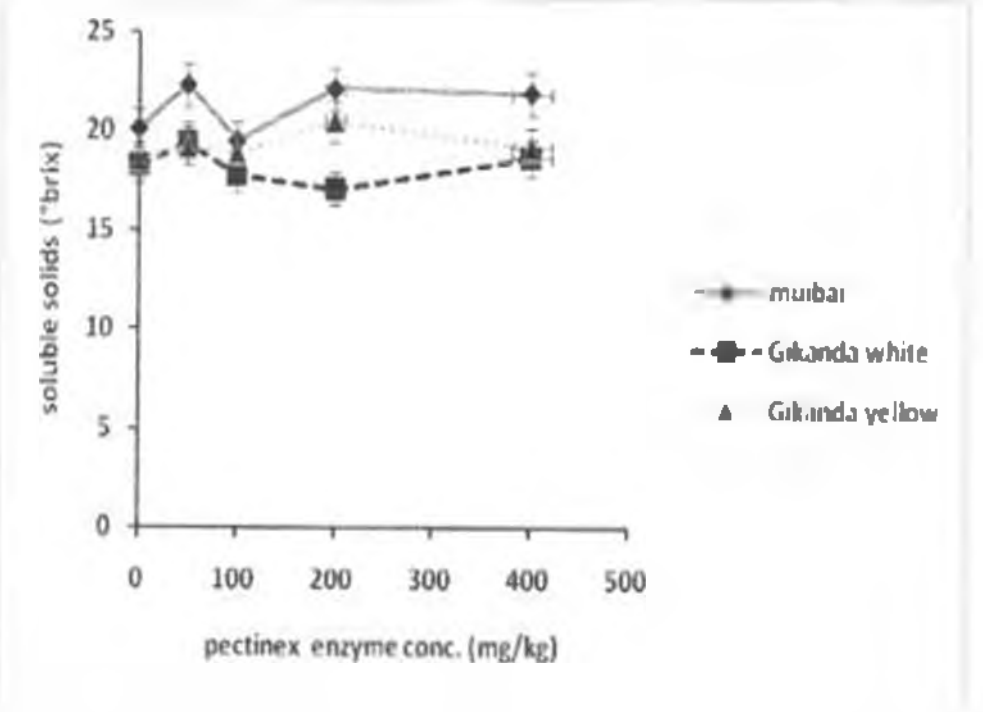


Figure : Effect of pectinex enzyme concentration on soluble solids in saccharified sweetpotato purees

Pectins have been reported to play an important role on the canning quality of sweetpotato roots. Firming of the texture of canned sweetpotatoes is extremely important and pectin degrading enzymes present in the root have been demonstrated to play a critical role in this regard. The firming of sweetpotato roots as a result of low temperature blanching treatment has been attributed to de-esterification of pectic substances by endogenous pectin methyl esterase (PME) which facilitate cross- linking between carboxyl groups in the pectin molecules by calcium bridges (Truong et al., 1998).

4.4 Characteristics of raw sweetpotato roots and juice

Table 9, below shows the results of proximate composition of the three sweetpotato varieties studied.

Table : Proximate composition of three local sweetpotato varieties

Sweetpotato Variety	%Dry Matter	%Starch (dwb)	%Proteins (dwb)	%Reducing Sugars (wwb)	% sucrose	Total carotenoids (µg/100) (wwb)	%Ash (wwb)
Muihai*	35.9±1.8	33.3±2.8	2.8±0.2	0.6±0.3	1.4±0.4	22.8±3.6	2.0±0.2
Gikanda (yellow)	40.0±1.3	35.8±3.0	2.4±0.5	0.7±0.2	1.5±1.0	218.8±20.6	1.1±0.2
Gikanda (white)	37.5±3.7	34.6±2.5	3.5±0.2	0.7±0.3	1.8±0.4	37.1±4.7	2.3±0.1

Each value is a mean from six replicates *Local names commonly used in central Kenya and parts of Nairobi. Dry matter basis (dwb) and Wet weight basis (wwb).

The dry matter content in the three varieties of sweetpotato roots, Muihai, Gikanda white and Gikanda yellow were in the range 35.9-40.5% (Table 8). Over 80% of the total solid content was starch, which clearly indicated that the sweetpotato varieties selected for this study were suitable for extracting juice. Since conversion of starch to maltose was a major objective of this study, starch content was considered critical selection criteria. It has been reported that East African adult consumers generally prefer sweetpotato roots with dry matter content above 25% (Low et al., 1997) which means that the trend was not accidental. The sucrose content (non reducing) of the root was below 2% in the three sweetpotato varieties studied. Although sucrose content in raw sweetpotatoes was found to be low, previous studies have demonstrated a steep increase during curing and storage of sweetpotato roots. Deobald et al., (1969, 1971) showed that sucrose content peaks in sweetpotatoes stored for 9-17 days after

curing. He therefore concluded that curing and storing sweetpotato roots for at least a fortnight significantly increased sucrose content and improved the processing quality of the root. However, the effect of curing and storage on levels of sucrose content in sweetpotato roots was beyond the scope of the current study.

The protein content in all the fresh sweetpotato roots was low, although the values are within the range reported by others. In non-alcoholic and alcoholic beverages, high molecular weight proteins have been associated with storage instability related problems such as the development of post-bottling haze in clear fruit juices and in filtered wines and beers. This is due to the formation of protein-tannin complexes which tend to form during storage. In fruit juices, the combination of phenolic compounds and proteins has been associated with development of post bottling haze in clear fruit juices such as apple and pear juice (Hsu et al., 1990; Thomson, 1981). In the process of manufacturing alcoholic beverages, some of the proteins could be metabolized by the yeast and reduce the problem. However, not all the nitrogen analyzed as proteins in sweetpotato roots is actually protein, given the high levels of non protein nitrogen present (Walter et al., 1984). Low molecular weight proteins and nitrogen compounds have no role to play in post-bottling haze in alcoholic and non-alcoholic beverages. So, it is unlikely that the protein level in sweetpotatoes is of any technological importance in the stability of such beverages.

The protein content of juice (table 9) was less than 50% of the levels in the root suggesting that some of the protein might have been insoluble in water and was left in the press cake. Some of the protein could also have been sedimented during the cold clarification of juice.

Table : Properties of clarified sweetpotato juices from three local varieties sweetpotato roots

Juice variety	Colour	starch	pH	TTA	Relative Viscosity	Specific Gravity	% Total Solids	% Soluble Solids	% Protein	% Total Sugars
Muthoi	Light yellow	-ve	6.5±0.3	0.3±0.1	1.8±0.3	1.1±0.2	20.1±1.2	20.2±0.8	0.45±0.1	14.0±2.4
Ghanda White	Light yellow	-ve	6.8±0.3	0.3±0.1	1.9±0.4	1.1±0.3	15.5±1.80	15.5±0.4	0.45±0.3	14.0±1.5
Ghanda Yellow	Bright yellow	-ve	6.5±0.3	0.3±0.1	1.8±0.2	1.12±0.4	19.1±0.8	19.1±0.3	0.37±0.1	14.3±2.3

* Total titratable acidity expressed as % citric acid. Each value is a mean from four replicates

The total carotenoids content in the three sweetpotato varieties was generally low but varied considerably between and within varieties. Gikanda had the highest level of total carotenoids content (expressed as β carotene) and the results showed that there was considerable variation in the levels between different batches. The intensity of the yellow color of all the sweetpotato juices closely mirrored the β carotene content of the root from which the juice was extracted. The color intensity varied from light-yellow (for Muibai and Gikanda white) to deep yellow in juice extracted from Gikanda yellow roots (Table 9). The results were also quite surprising because a colorless juice was expected from the white fleshed variety, not the yellow colored juice that was obtained. This could be an indication of the high efficiency of this method in terms of optimal extraction of natural pigments, particularly β-carotene from the root.

The findings are quite significant because optimal extraction of β-carotene would be of interest to manufacturers who intend to use the juice for formulating non alcoholic beverages targeting niche markets such as foods for boosting body immunity. Consuming low cost foods such as orange fleshed sweetpotatoes has been recommended as the most effective strategy of addressing Vitamin A deficiency, which is currently one of the major health challenges facing many developing countries including Kenya (GOK and UNICEF, 1995). The feasibility of using sweetpotatoes for processing sweetpotato juice rich in vitamin β-carotenes (Vitamin A pre-cursor) has been explored in several studies.

Color is extremely influential in the overall acceptability of non-alcoholic drinks (Frances, 1977; Booth, 1987; Schiffman, 1979). Therefore, the level of carotenoids is an important selection criterion for processing non-alcoholic beverages from the juice (Truong et al., 1991). In non-alcoholic sweetpotato beverages, β-carotene enhances sensory appeal and the nutritional image of the product (Truong and Fennentura, 1991). Collins (1981) reported that juice prepared from high β- carotene sweet potato cultivars contained approximately 1 mg β-carotene 100 g-1 and could furnish more than 40% of the adult RDA of vitamin A. However, the method proposed by Wireko-Manu et al., (2010) where they used malt from germinated maize grains for the saccharification of sweetpotato puree does not appear to have succeeded in extracting much of the β-carotenes in the root in view of the low levels of Vitamin A they report in their juices (3.28-10.11µg/100g). This study has therefore established a more efficient process of extracting juice rich in β-carotenes from sweetpotato roots.

Sweetpotato juice is a low acid product with a pH of 6.5 and total titratable acidity of 0.25-0.3% (calculated as citric acid). The acidity has been attributed to three organic acids; malic acid (0.16%), succinic acid (0.05%) and quinic acid (0.05%). Although the exact role of these acids is unknown, they are thought to influence the pH of the root. Acids enhance the sensory quality of alcoholic and nonalcoholic drinks. In the formulation of non-alcoholic beverages from sweetpotato juice, citric acid is added to enhance the sensory quality of the products. Truong and Fermentira (1991) added 0.45%-0.65% acid while the beverages prepared by Wireko-Manu (2010) had total titratable acidity in the range 0.45-1.6% (calculated as citric acid).

Total solids in sweetpotato juices ranged from 15.5-20.1%, most of which was soluble. Total sugars constitute 69, 75 and 90% of soluble solids in extracted juices from Muibai, Gikanda yellow and Gikanda white. This was a clear indication that the extent of starch hydrolysis has an important influence on extraction yield of sweetpotato. In contrast, the methods described by Truong and Fermentira (1991), extracted negligible solids from sweetpotato roots (less i.e. than 2%). The reducing sugar content of the juices was fairly consistent in all juices and averaged 14%. This could possibly be due to inhibition of α-amylase enzyme by β-limit dextrins which accumulate substantially when the β-amylase enzyme has been inactivated. Recent studies have also demonstrated the presence of an endogenous α-amylase inhibitor in sweetpotato roots whose level increases during curing and sprouting (Sakistran et al., 2002).

Normally, for single strength juices, the acceptable level of sucrose is about 12%. However, the sweetness of maltose is only 0.32 relative to sucrose. This means that to achieve the required level of sweetness for a single strength juice, about 7% of sucrose would be required. This represents a saving of 42% in sugar as compared to processing sweetpotato juice using previous methods. Truong and Fermentira (1991) added 12% sugar to sweetpotato juice to achieve the required level of sweetness. Furthermore, the sweetpotato juice made by using our method would have over 20% soluble solids (mostly sugar and dextrins), which makes it suitable as an energy booster due to high energy density.

CHAPTER FIVE

5.0 Conclusions and Recommendations

5.1 Conclusions

Curing of sweetpotato roots at temperatures between 25-35°C was found to significantly increase levels of α-amylase activity while β-amylase activity changed in an erratic manner. It was further established that maltose formation in cured sweetpotato purees incubated at 60, 65, 70 and 75°C was related to the temperature and level of endogenous α-amylase activity present in the puree. The proportion of sugars to total solids in sweetpotato juices varied among the sweetpotato varieties studied and was lowest in Muibai (69%) followed by Gikanda yellow (75%) and highest in Gikanda white (90%).

The level of Pectinex 3XL enzyme was found to significantly influence the viscosity of saccharified sweetpotato purees and the yield of juice extracted from the purees. When no pectinex enzyme was used, the yield of juice varied from 30-52% and increased to over 75% when at least 200mg/kg of pectinex 3XL enzyme was added. The three sweetpotato varieties studied had starch content above 30%, which means that processing of sweetpotato juice for beverage and ethanol production from them would most likely be commercially viable. A comparison of the composition of sweetpotato roots and composition of the corresponding juices extracted showed that the color of juice was closely associated with the β-carotene content of the root. The three juices had total sugar content of at least 14% as compared to 2% in the roots, which showed the effectiveness of using endogenous enzyme to saccharify of starch in sweetpotato purees. The β-limit dextrins generated and the possible presence of an endogenous α-amylase enzyme inhibitor were suspected to have been responsible for the incomplete conversion of starch to maltose, hence the need to investigate higher incubation temperatures and the effect of adding commercial debranching enzymes in future studies.

These results indicate that a process for extracting sweetpotato juice using endogenous amylolytic enzyme and pectinex 3XL enzyme is a practical possibility and could contribute to commercialization of beverages and fuel ethanol from local sweetpotatoes varieties. The specific objectives of the project were therefore achieved and it is expected that this work will make a contribution towards stimulating commercial utilization of sweetpotato roots as a raw material for processing of alcoholic beverages, non-alcoholic beverages and fuel ethanol. The work is likely to benefit Sweetpotato farmers by providing them with reliable markets for

their produce; provide food processors with low cost raw materials and provide consumers with affordable, hygienic and nutritious (high β-carotene content) beverages. This method of extracting sweetpotato juice could also reduce significantly the cost producing biofuels from sweetpotatoes, and therefore reduce the import bill for fossil fuels, reduce environmental pollution and contribute to overall economic growth of the country.

5.2 Recommendations

Following the many positive findings of the study, the following recommendations are made for immediate and future implementation:

1. To reduce Vitamin A malnutrition in the country, high yielding, orange-fleshed sweetpotato varieties such as K117 and Mugande which are unpopular for consumption should be promoted for processing affordable , β-carotene rich non-alcoholic beverages for mass distribution to vulnerable population segments such as young children living in marginal areas.
2. To enhance food security, consumer safety and to satisfy the anticipated explosion in demand of traditional alcoholic beverages following their recent legalization, manufacturers of these brews should be trained and encouraged to substitute malted grains with cured sweetpotatoes and pectinex 3XL enzymes.
3. The Government should provide incentives such as tax breaks to encourage manufacturers to utilize sweetpotatoes for the production of nutritious non-alcoholic beverages, potable alcohol and fuel ethanol.

5.3. Suggestions for future work

1. The optimal ratio for α-amylase and β-amylase activity and incubation temperature necessary to achieve complete conversion of starch to maltose in sweetpotato purees requires further investigations.
2. Further work should also be undertaken to investigate the effect of saccharification of sweetpotato purees by endogenous amylolytic enzymes at temperatures above 75°C, the possible inhibitory role of endogenous α amylase enzyme inhibitor, and possible use of a combination of endogenous amylolytic and commercial debranching enzymes to attain complete conversion of starch to maltose.

3. Possible utilization of endogenous pectin methyl esterase (PME) enzyme in the process of extraction sweetpotato juice should also be investigated with a view to avoiding the use of commercial pectinex enzymes and reducing the cost of extracting the juice.

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