

## Session IX

### Root and tuber crops for feed and industry

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# Root and tuber crops for feed and industry

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## Abstract

Root and tuber crops (RTC) have had a strong impact on food security of tropical regions of the world. Their bulkiness and logistic problems associated with the storage of RTC have limited their impact from ancient times to the present. In spite of these problems, however, there are increasing opportunities for the industrial uses of RTC in the tropics. The globalization of the economies has made less competitive the tropical production of maize, therefore creating an opportunity for RTC to fill the gap. There are different strategies to adapt these crops for the needs of the industry. **1)** The raw material has to have a competitive price. Therefore varieties and cultural practices should guarantee high and stable yields as well as low production costs. In most cases a key trait that has strong bearing in the value of roots or tubers for the industry is their dry matter content, which typically needs to be maximized; **2)** Costs of processing should be minimized. Certain characteristics of roots and tubers have a strong impact on processing cost. For example roots and tubers whose starch is easier to hydrolyze would be desirable for the bio-ethanol industry; **3)** The quality of the product offers additional opportunities which only recently began to be properly addressed. For the feed industry enhanced nutritional quality (i.e. higher proteins, higher vitamins or lower anti-nutritional compounds) would be a key factor. For the starch industry variation in starch chemistry and functional properties provide huge opportunities already exploited in the cereals. Amylose-free starches have a wide range of applications in the industry. High-amylose (or resistant) starches also have a positive impact for people affected with diabetes; phosphates associated to potato starch define many of its unique properties; **4)** Developing new, high-value products from RTC is also an interesting approach. For example high-quality flour could replace starches for certain uses, which offers economic as well as environmental advantages. The exploitation of foliage for animal feeding has been extensive in the case of sweet potato in China and could be extended to other RTC. To deploy these strategies RTC can now combine different technologies that gradually have been adapted such as the use of marker-assisted selection and genetic transformation. In some instances, breeding and agronomy research needs to be combined. The production of herbicide-tolerant RTC (by genetic transformation or conventional breeding) combined with direct planting practices could have huge beneficial effects by reducing costs of production, increasing yields, and reducing the environmental impact of their cultivation (i.e. reduced soil erosion, improved water use efficiency, conservation of soil fertility). The case of cassava will be used to illustrate many of the strategies highlighted above.

## Introduction

Root and tuber crops (RTC) have had and still have a particular importance in tropical and subtropical regions of the world. Agriculture was invented independently in many sites of the world. One of these sites was in Polynesia where agriculture development was based on RTC. Some of the key traits of these RTC (for example, their bulkiness and short shelf life), have been used to explain why these early societies did not evolve into agriculture civilizations such as those in the Fertile Crescent, where agriculture was mainly based on grains. In spite of the typical problems of RTC, there are increasing opportunities for the industrial uses of RTC in the tropics. The globalization of the economies has made less competitive tropical production of maize, therefore creating an opportunity for RTC to fill the gap. There are different strategies to adapt these crops for the needs of the industry which are briefly discussed, using mostly cassava to illustrate these strategies.

### ***Competitive price of the raw material***

Raw material is a critical component of the production costs of any agro-industry. In the case of starch industry of sweetpotato and cassava in Asia, for example, raw material represent between 70-80% of the cost of production (Fuglie, 2004; 2005 Fuglie et al. 2005; Howeler, 2005; Nelson, 1984; Titapiwatanakun, 1994). Therefore any research that results in a reduction of the costs of production and/or increased yields of RTC would have a positive impact on the competitiveness of agro-industries based on them. Most breeding projects aim at

producing cultivars with high and stable productivity. Countless articles in the literature have reported progress in this regard. There are still new alternatives for reducing costs of production that can and are currently explored. The potential of herbicide tolerance will be used as an example because of its relevance in costs of production and because it highlights an interesting case of complementation between genetic, agronomic and mechanization of field operations research.

Herbicide tolerance in crops offers several advantages. The handling of herbicides can be made in a much more efficient way, applying them at the optimal timing when weeds are most vulnerable. This implies that there is a reduction in the amount of herbicides used, reducing costs of production on one hand, and having a positive impact on the environment, on the other. Perhaps more important is the possibility that herbicide tolerance allows direct planting, which without proper technologies to handle the problem of weeds, is often unviable. Direct planting also offers several advantages: it allows the maintenance of a mulch of crop residues on the soil, therefore reducing soil erosion and maximizing the capture and conservation of water and soil nutrients. Direct planting reduces the operations of soil preparation at planting time, which offer the dual advantages of reducing costs and the negative impact on the environment. There are few alternative approaches to develop RTC tolerant to herbicides, which will be described below.

## **Genetic transformation**

In the case of cassava the first reports on the production of transgenic somatic embryos and plants date back to 1993-1995. Since then several events ranging from herbicide tolerance, reduction of cyanogenic potential to the increase or quality of starch produced by the plant have been reported (Ihemere et al. 2006; Jørgensen et al., 2005; Sarria et al. 1993; Taylor et al., 2004). The development and exploitation of genetically modified crops faces some problems ranging from intellectual property rights, regulatory issues on human health, regulatory issues to prevent gene flow to technical issues related to the process of genetic transformation itself.

### ***Natural occurrence of tolerance to herbicides***

There are many examples reported in the literature where tolerance to different herbicides has been found in different crops (canola, cotton, lentil, lettuce, maize, rice, sugar beet, sunflower, tobacco, tomato, and wheat), which lead to the release of trade marks such as Clearfield, RoundUp Ready and Liberty Link (Sherman et al., 1996; Tan et al., 2005; 2006; Tan and Bowe, 2008). In most of these cases, tolerance to herbicides was based on recessive or partially dominant genes and self-pollinations have facilitated their identification.

### ***Induction of mutations***

Tolerance to herbicides has also been obtained through the induction of mutations using chemicals such as ethyl methanesulfonate (EMS) or radiations like gamma rays (Tan et al., 2005; 2006). It is important to highlight that the use of these natural or induced mutations can overcome some of the regulatory issues that hinder the exploitation of transgenic crops, but nonetheless would have to be used with certain caution to avoid the flow of genes from the herbicide tolerant crop to related weeds. Eco-TILLING (Guang-Xi et al., 2007; Till et al., 2003) is an interesting approach for the identification of natural or induced mutations where the actual site for the mutation is well known. For instance tolerance to imidazolinones relies on a mutation at the AHAS (acetohydroxyacid synthase) gene. CIAT is currently growing a set of about 800 S1 genotypes in five different blocks. Each genotype is represented by two plants in each block. Each of these blocks will be sprayed with a different herbicide, using commercial doses. The aim is to identify partially inbred genotypes showing resistance or tolerance to any of these herbicides.

## **Minimized costs of processing**

The example of bio-ethanol and cassava will be used as example for this section because of its current relevance and the significance of processing costs on the competitiveness of this industry. Bio-fuels are currently based on the production of ethanol from sugars or starch derived from vegetative biomass and grain, or bio-diesel from the more direct use of edible and non-edible plant oils and animal fats (Ortiz et al., 2006). Brazil is the shining example for carburant ethanol production and use (from sugarcane juice), either in pure form or as a blend with petrol. Billions of gallons of bio-ethanol are produced annually in Brazil from sugarcane. Recently the production of ethanol from starch-producing crops has received considerable attention. Key factors for this new development are the emerging technologies and the cost to hydrolyze the starch (originally based on two

stages: liquefaction and saccharification) prior the beginning of the fermentation process (Shetty et al. 2007). Research in microbiology has led to the development of new enzymatic processes that make the hydrolysis of starch more efficient and less expensive. However, using the traditional liquefaction and saccharification process or the new enzymatic approaches, there are expenses involved in the process of conversion of roots or grain into ethanol.

One objective for the RTC research community in this regard would be the development of raw material that reduces the cost of processing. Several alternatives can be mentioned to illustrate the potential of these interventions (Reddy et al., 2008).

### ***Modification of the roots or tubers to produce molecules simpler than starch***

Carvalho and co-workers reported in 2004 a group of interesting “sugary” mutations in cassava that result in storage roots with high free sugars (mostly glucose) and a glycogen-like molecule. The roots from these genotypes have reduced levels of amylose and low dry matter content (DMC). The costs of processing this type of root into ethanol should be considerably lower compared with normal cassava roots. This mutation is widely distributed scattered in the Amazon basin. Although the cost of processing roots from this type of cassava into ethanol would be considerably reduced, a key factor for its ultimate usefulness would be its production of energy per area.

### ***Modification of the roots or tubers to produce starches which are easier to degrade***

Sharma et al., reported in 2007 studies on the enzymatic requirements for the conversion of maize into ethanol, in response to varying proportion of amylose: amylopectin in the starch. They concluded that amylose-free maize (*waxy*) would be better and more efficient for the production of ethanol. There are several reports of amylose-free RTC (described below in this article). The production of ethanol using these amylose-free RTC would be more efficient and competitive. There is an ongoing project to release commercial cassava varieties with “*waxy*” starch.

### ***Maximized dry matter content versus production of energy per hectare***

For most industries the need to reduce costs of production implies that roots or tubers from different crops and cultivars are required to have a maximum amount of DMC. A raw material with low DMC would imply higher costs of starch extraction (i.e. production of higher amount of effluents) or longer periods for drying the roots in the drying yards. Breeders frequently find that genotypes with a maximum productivity of dry matter per hectare are unacceptable because that productivity is based on high production of fresh roots, but at low levels of DMC. These genotypes have been routinely eliminated in spite of their excellent yield potential. In response to these requirements, there has been considerable progress in improving DMC of modern cassava varieties (Kawano, 2003). The bioethanol industry, however, has created a new opportunity for these genotypes with maximum productivity of dry matter per hectare but based on low dry matter contents. In the case of ethanol production it is feasible to envision that, at least in certain periods, fresh roots could be grinded directly to initiate the process of transformation into ethanol. In this case, it may be attractive for the whole process to rely on cultivars with maximum production of energy per hectare independently of the levels of DMC of the root.

## **Qualitative characteristics of roots and tubers**

The quality of roots and tuber offer additional opportunities which only recently began to be properly addressed. For the feed industry enhanced nutritional quality would be a key factor. For the starch industry variation in starch chemistry and functional properties provide huge opportunities already exploited in the cereals.

### ***Enhancement of the nutritional value of roots and tubers***

Recently, an international initiative seeking a reduction in micronutrient malnutrition using plant breeding to develop staple food crops rich in micronutrients, including provitamin A carotenoids, was initiated. The program, known as **HarvestPlus**, involves a global alliance among research institutions and implementing agencies in developed and developing countries. The first six focal crops that comprise staple food for the majority of people in the world, who have or are at high-risk of micronutrient deficiencies, are cassava, sweet potato, maize, rice, wheat, and beans.

In the case of cassava the first step in the identification or production of cassava clones with enhanced nutritional value has been a systematic and massive screening of landraces from CIAT germplasm collection and key improved clones. The screening involved evaluations for many micronutrients but currently has been narrowed to pro-vitamin A carotenoids, minerals (Fe and Zn) and proteins. Germplasm evaluated and quantification methods were described by Chávez et al. (2005). These authors also reported wide genetic variation for levels of pro-vitamin A carotenoids. After five years of rapid-cycling recurrent selection, maximum levels of total carotenoids content has been increased almost three-fold (CIAT, 2008).  $\beta$ -carotene has been found to be the most important component in these measurements (typically 50-80% of total carotenoids is  $\beta$ -carotene). High carotene roots have a tendency for reduced or delayed post-harvest physiological deterioration (Sánchez et al., 2005, CIAT, 2009).

For Fe and Zn, in spite of the earlier promising variation (Chávez et al., 2005), it has become gradually evident that high contents for these two minerals are often the result of contamination from soil or tools used to process the roots. pH of the soil has proven to be more important than content of these elements in the soil or the genotype. Negligible effects of different types and dosages of fertilization to the cassava plant had been found on the contents of Zn and Fe (CIAT, unpublished data).

Variation in protein content of cassava roots has also been reported (Ceballos et al., 2006a). CIAT has also carried out a long-term project for higher protein content in the roots in crosses with wild relatives such as *M. esculenta* subsp. *fabellifolia* and *M. tristis* (Ceballos et al., 2006b). A total of 49 inter-specific crosses, ranging from 6.39 to 10.46% in protein content, have been selected and back-crossed into an elite *M. esculenta* clone. More than 6,000 back-crosses have been made. However, data still being processed suggest that in the case of cassava roots, the N-to-protein conversion factor is considerably lower than the standard 6.25 constant. Genetic variation is still present in these quantifications but the range of variation is drastically reduced from 1-8% (when the measurements are based on the indirect method of N quantification) to 0.5-1.8% (when soluble proteins were estimated through the Bradford method).

High-amylose starches, commonly known as resistant starches, have led to interesting outcomes for human health in recent years. They are described in more detail in the following section but, because their advantages in human nutrition, they are also mentioned in this section.

### **Generation and identification of clones with novel starch types**

Starch is a simple polymer of glucose units that are linked together in two forms (amylose or amylopectin) yet, as stated by BeMiller (1997), after thousand of studies, it remains a beautifully mysterious substance. Amylose is essentially a linear chain, whereas amylopectin (which is typically the major component of starch) is a much larger molecule and is highly branched. The relative proportion of amylose and amylopectin, their degree of branching, and the length of the different branches greatly affect the properties of the starch. One of the most intriguing challenges in starch research is to explain the simultaneous synthesis of these two polymers and to understand the regulation of the several enzymes (and genes) involved (Denyer et al., 2001). RTC offer a great diversity of starches in relation to granule morphology, chemical composition, crystallinity, structure of amylose and amylopectin, swelling power and solubility, gelatinization, rheology, digestibility and retrogradation (Arachchige et al. 2009; Degbeu et al., 2008; Gunaratne and Hoover, 2002; Hoover, 2001; Peroni et al., 2006; Srichuwong and Jane, 2007). Table 1 provides a summary of the main features of starches from several RTC. Starch granule sizes vary from the very small granules of *Disocorea esculenta* (1-5  $\mu$ g) to the large granules of potato (15-110  $\mu$ g). Amylose content ranges from 15.9 in *Nelumbo nucifera* to 38.0% in *Canna edulis*. Cassava has very low levels of lipids (0.1%), whereas *Cucurbita foetidissima* has much higher values (around 1%). Potato starch is recognized because of its levels of phosphates associated to its starch. These phosphates confer enhanced paste clarity, high peak consistency, significant shear thinning and slow rate and extent of retrogradation. Starches belonging to the Diocorea species exhibit a higher pasting temperature and thermal stability than other RTC starches (Hoover, 2001). A characterization of starches from more than 4000 cassava genotypes has recently been published (Sánchez et al., 2009).

Cassava (*Manihot esculenta* Crantz), sweet potato and potato are among the most important sources of commercial production of starch along with maize and wheat. Cassava and sweet potato starches are particularly important in Asia. Starches from cassava and potato share many similarities: they produce relatively bland pastes, with higher viscosity, better clarity and lower retrogradation rates than starches from cereals. They also possess lower levels of proteins and lipids (Davis et al, 2003; Ellis et al., 1998). Many comparisons of the physicochemical and functional properties of starches from different crops have been published, as well as the

effect of the absence of amylose in waxy starches (Peroni et al., 2006; Praznik et al., 1999; Li and Yeh, 2001; McPherson and Jane, 1999; Visser et al., 1997; Hoover and Manuel, 1996; Tetchi et al., 2007).

In spite of the wide variation in starch functional properties among different RTC, it is of utmost interest for the starch industries of each crop to have access to starches varying in their own characteristics within. The potential for new commercial starches can be even greater when biological and chemical modifications are combined (BeMiller, 1997). A key trait defining the functional properties (and therefore, potential uses for the industry) is the relative proportion of amylose and amylopectin in the starch. As a result of efforts to produce modification of starch *in planta* (via conventional breeding or genetic transformation) amylose-free as well as high-amylose starches are now available in potato (McPherson and Jane, 1999; Visser et al. 1997; Hoover, 2001), cassava (Ceballos et al., 2007; Raemakers et al., 2005), and sweet potato (Kitahara et al., 2007). These modifications in the starch main components widen the potential uses of the respective starches. Moreover, since modification is achieved *in planta* rather than *in vitro*, there are undeniable economic and environmental advantages (Davis et al, 2003; Ellis et al., 1998). High-amylose starches are frequently associated with small starch granule size. Small granule morphology has been reported in potato (Noda et al., 2005) and cassava (Ceballos et al., 2008).

The industrial applications of amylose-free starches from different crops are widely reported (Hannah, 2000; Preiss, 2004; Watson 1988). Advantages and commercial applications of high-amylose starches, on the other hand, are more recent. Increased amylose levels leads to slowly digestible and resistant starches (Jobling, 2004; Lehman and Robin, 2007), which have distinctive advantage in health, particularly in diabetes management. Slowly digestible starches may influence satiety and help control overweight problems and have also been linked to improved mental performances (Lehman and Robin, 2007). In addition, high-amylose starches in different crops offer advantages in the production of sweets, adhesives, corrugated boards and in the paper industry, and reduces the uptake of fat in certain fried products (Jobling, 2004). Very high levels of amylose result in “resistant” starches. Maize starches with more than 70% can be produced commercially. Resistant starches cannot be digested but they are rather fermented in the large intestine resulting in the production of butyrate that has been found to be beneficial to colon health (Jobling, 2004).

There are several strategies that lead to the identification of genotypes (including RTC) that modify starch properties *in planta*. The recessive nature of mutations that leads to the production of such genotypes implies certain limitations for polyploid crops because of the difficulties of recessive mutations to express themselves in polyploid genotypes.

**Screening self-pollinated progenies.** For many years the most common approach has been based on careful screening of self-pollinated progenies. Since most of these characteristics are due to recessive mutations, inbreeding is a key step to facilitate their expression and, therefore, identification. One of the earliest genes characterized in any organism is the waxy (*wx*) locus of maize (Hanna, 2000). It is the peculiar waxy texture of the endosperm of amylose-free maize that lead to the naming of the locus. Self-pollinations may possess important limitations in several RTC which have auto-incompatibility such as potato and sweet potato. Systematic self-pollinations, followed by careful screening (including special tests that will allow the identification of genotypes whose starch have special characteristics), allowed the identification of an amylose-free mutation in cassava (Ceballos et al., 2007).

**Induction of mutations.** Breeders have used chemical products or irradiation (i.e. gamma rays) to induce mutations and generate genetic variability with relative success, particularly in the decades of the 1950s and 1960s (Maluszynski et al., 2001; Ahloowalia et al., 2004). That was the approach taken for the induction and identification of a small granule, high-amylose mutation in cassava (Ceballos et al., 2008). In spite of this success, mutation breeding has a few drawbacks. Events are totally random, recessive in nature, and usually appear as chimeras. Therefore, thousands of genotypes need to be evaluated before a useful mutation in the desired gene can be found. With the advent of molecular biology tools, an interesting system was developed to overcome some of the limitations of mutation breeding. DNA TILLING (for *Targeted Induced Local Lesions in Genome*) has been successfully used in different plant species (McCallum et al., 2000; Perry et al. 2003; Till et al. 2003). Sexual seeds are mutagenized and, to avoid ambiguities caused by chimeras in the first generation plants ( $M_1$ ), they are self-pollinated. The resulting plants ( $M_2$ ) are then evaluated while DNA is extracted from them. For screening purposes, DNAs are pooled eightfold to maximize the efficiency of mutation detection (Till et al., 2003).

**Interspecific crosses.** Wild relatives are a common source of genetic variability, particularly in traits that are not commonly found in the cultivated gene pool. CIAT is actively searching for several valuable traits in *Manihot* species other than *M. esculenta*. The project for the introgression of the high-protein trait has already been mentioned. Tolerance to post-harvest physiological deterioration (PPD) in cassava roots has been identified in an inter-specific hybrid between cassava and *M. walkerae*. Accessions of *M. crassisejala* and *M. chlorosticta* are the only genotypes from the primary and secondary gene pool of the crop discovered to possess the waxy starch phenotype (Ceballos et al. 2006). For several years now molecular marker tools and a modified advanced back-cross QTL scheme have been tested for cost-effective pyramiding of useful genes from cultivated and wild gene pools through the elimination of phenotypic evaluations in each breeding cycle.

**Recurrent selection.** The power of recurrent selection to gradually but consistently changing the quality of crops has been well established in the now classic study on maize for altered protein and oils content. (Dudley, 1974). It is feasible, therefore, to implement recurrent selection programs to produce genotypes with altered starch quality (Ceballos et al. 2006b). However, such programs would be expensive and time consuming. Breeders, therefore, find the possibility of a discovery or induction of single mutations that have a drastic effect on starch quality traits more attractive. Recurrent selection has been used to complement the effect of high-amylose mutations in maize (*amylose extender*) for further increasing the proportion of amylose (Jobling 2004; Richardson et al., 2000).

**Genetic transformation.** Several of the starches with modified characteristics described above have been obtained through genetic transformation (Visser 1997, Raemakers 2005, Kitahara et al. 2007, by antisense inhibition or RNA interference. The genetic engineering of cassava varieties to produce waxy starch via antisense, down-regulation of the GBSSI gene has been reported (Salehuzzaman et al., 1993; Munyikwa et al., 1997). The technology of genetic transformation has become a generalized tool in germplasm enhancement. It offers unique advantages, it is very target-specific and commercial varieties with proven adaptability and high yields can quickly be converted to produce specialty starches. However the regulatory issues related to the release of genetically modified organisms, particularly for direct human consumption, has limited the economic impact of this technology. An important contribution of genetic transformation has been the possibility of silencing different genes, thus greatly facilitating the understanding of starch biosynthesis (Denyer et al., 2001)

## Developing high-value products from RTC

Considerable opportunities are also available in the area of processing RTC to develop new products that may offer competitive advantages in the market. The possibility of producing refined flours will be used as an example of the kind of products that can be developed. In many cases, RTC are characterized by low protein and/or fat contents. This has had a negative impact on the value of these crops for animal feeding. However this disadvantage can be turned into an opportunity.

The low levels of fat and protein in many RTC imply that the flour is a product that is close to pure starch, from the chemical point of view. It has been demonstrated that flours can be processed by physical methods to produce refined flour that resembles starch in many properties. The physical treatment implies separation of a considerable amount of fiber present in the flour and a further reduction in the granule size. Refined flours are very white and soft to the tact. Very much like starch, but at a considerable lower price. There are cases where these refined flours can be used to replace the more expensive starch from the respective crop. In the case of cassava, for example, the refined flours have been used successfully by the baking industry to replace up to 10% of wheat flour.. The potential of refined flours offers economic advantage because it is a product that can successfully compete with the more expensive starches from the respective crop. They also offer the advantage of a reduced impact of processing RTC on the environment. Many starch processing facilities fail to properly treat effluent water, therefore, affecting negatively the surrounding environment. Production of refined flour does not require water and does not produce effluents. Therefore the potential impact of production of refined flours from RTC on the environment is very small.

## Concluding remarks

This article aimed at highlighting the diversity of approaches (mostly based on genetic enhancement) that can and are being taken to improve different TRC to better fit the needs of the industry. Below a list of key requirements for research to have a successful impact on different industries that rely on processing RTC.



- For a success in this area a fundamental requirement is a close and intimate interaction between the agricultural and industrial components of this continuum. A clear understanding of the needs of the industry is a key requisite for agriculture research to satisfy them.
- Aggressive and persistent research, frequently requiring several years to yield results, is more often than not an important requirement. A common problem faced by many research teams is the anxiety on the part of donor agencies (public or private) to produce results in an unrealistically short period of time.
- Researches must combine a clear understanding of the needs by the industry with suitable breeding and laboratory tests. For example, it took more than 40 years to identify a naturally occurring waxy-starch genotype in cassava. It was only after breeding techniques (self-pollinations) and special tests (in this case the simple iodine test) were systematically combined that such a mutation could be identified and started to be exploited.
- Researchers must also be very aggressive incorporating new technologies as they become available. Molecular markers (particularly as TILLING) and genetic transformation are becoming available technologies to many RTC. Their incorporation into the research agendas must be judicious and sensible.
- A major advantage for the industrial uses of RTC is that many technologies are crop neutral. Therefore, technologies developed for one crop can frequently be applied to other crops almost immediately. That is the case for example, of the starch hydrolyzing enzymes originally developed for maize, but equally effective on other starch crops.
- The exponential research capacity in the area of molecular markers in the past few years is astonishing. Cost and time wise the sequencing of a crop genome have become almost irrelevant issues. The availability of sequenced genomes and the increasingly efficient application of molecular tools would facilitate enormously the work to develop RTC cultivars that better fit the needs of different industries.
- A fundamental rationale behind the interest of adapting RTC to better serve the needs of the industry is that by doing it, research ultimately promotes rural development (processing of RTC because of their bulkiness is made close to production areas), strengthen and stabilize markets (one of the major drawbacks for producers or RTC) and ultimately alleviate poverty in communities that depend on RTC.

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**Table 1. Descriptors of starches from different crops (adapted from Hoover, 2001)**

Crop species	Starch yield (%)	Size (µg)	Amylose (%)	Lipids (%)	Org P (% dsb)	Inorg P (% dsb)
<i>Amorphophallus paeonifolius</i>	35	3.0-30.0	-	-	-	-
<i>Canna edulis</i>	60.3	13.0-57.6	38.0	0.30	-	-
<i>Colocassia esculenta</i>	55.1	3.0-3.5	21.4	0.39	0.021	-
<i>Cucurbita foetidissima</i>	-	2-24	23.2	0.92-1.14	0.01-0.06	-
<i>Dioscorea abyssinica</i>	-	29.2	29.7	0.05	-	-
<i>Dioscorea alata</i>	84.6	6-100	22.8-30.0	0.03	-	-
<i>Dioscorea cayenensis</i>	87.5	28.5-30.6	21.6-27.0	0.02	-	-
<i>Disocorea dumetorum</i>	88.0	28.5-30.6	10.0-24.6	0.04	-	-
<i>Disocorea rotundata</i>	86.1	10.0-70.0	22.4	0.04	-	-
<i>Dioscorea esculenta</i>	-	1-5	30.0	-	-	-
<i>Ipomoea batatas</i>	-	14.6	26	-	-	-
<i>Lilium maximoroiczii</i>	-	30.0	26.8	-	60 ppm	33ppm
<i>Manihot esculenta</i>	84.5 <sup>a</sup>	16.3 <sup>a</sup>	18.6-23.6	0.1	0.008	0.001
<i>Maranta arundaceae</i>	-	10.0-16.0	19.4	0.32	-	-
<i>Nelumbo nucifera</i>	-	15.0-40.0	15.9	-	48 ppm	-
<i>Pueraria tuberosa</i>	34.2	3.0-23.0	15.1-21.0	0.46	0.005	-
<i>Rhizoma dioscorea</i>	-	19.8-28.4	35	-	-	-
<i>Solanum tuberosum</i>	32	15-110	25.4	0.19	0.089	0.001
<i>Xanthosoma sagitifolium</i>	43.8	10.0-50.0	23.7	-	-	-

<sup>a</sup> Based on new data published by Ceballos et al., in 2008. Also see Peroni et al., 2007.

# Searching for feeding strategies based on sweet potato silage to improve smallholder crop-livestock production systems in Vietnam

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## Abstract

Selected data from sweet potato (SP) clones tested during summer and winter was evaluated for their Root/Total biomass (R/B) ratio to identify potential dual-purpose clones. A native clone and three introduced clones with adequate R/B ratio were selected for further evaluation under two different vine cutting regimes (one and two cuts before root harvest). Clones classified as dual purpose produced 46.6% significantly more total dry matter biomass than the native clone. Two cuts produced 31.6% more total dry matter ( $p < 0.05$ ) than a single cut and the difference was accounted for by the significantly higher dry matter yield of vines developed in the warm and humid weather of the spring. The harvested vines and roots were used to make silage by combining them at different proportions. Four feeding strategies based on the use of SP silage and locally available feed resources were compared with two feeding strategies based on commercial feed. No significant differences in daily weight gain were found among the treatments including silage feeding ( $609 \pm 58$  and  $600 \pm 86$ g/day) but the response to the commercial feed pellets ( $716 \pm 70$ g/day) was significantly higher. However, silage utilization reduced feeding costs by 15.3 and 17.3% and increased the farm benefit by 43 and 50.8% in comparison to the use of commercial feed pellets.

## Introduction

Sweet potato (*Ipomoea batatas*) is a tropical and sub-tropical plant that can be harvested twice per year in the summer and spring-winter seasons in North Vietnam. Roots are mainly used as food and the whole plant is also a good feedstuff because the roots are a source of energy and the leaves provide part of the protein requirement of livestock. As feed, SP can be used as fresh and dried matter or fermented into silage (Woolfe, 1992). In summer time in Viet Nam, sweet potato roots and vines are usually cut into small pieces and dried in the sun but this traditional farmer's way of preserving sweet potato is very demanding in labor. It is then necessary to look for more practical and economic ways of storing SP for their timely inclusion into feeding strategies. It has been shown that in the wet season in North Vietnam, SP roots and vines can be preserved as silage for later use as pig feed (Nguyen, *et al.*, 2006).

The largest component of the total production cost in most crop-livestock production systems is the feed cost and the use of local feed resources is an effective way of reducing feed expenses. In the case of pig feeding the problem is more complex than with ruminants as pigs require high protein levels in their diets, which make the feed more costly compared to ruminant diets. However, the sweet potato-pig fattening operation has a combination of advantages for improving the income of rural households by reducing the feed cost and providing other benefits. To begin with, SP produces roots for market sales and high quality feed for pigs, which convert low-value sweet potato residues into highly desired foods or marketable commodities and provide manure for maintaining and improving soil fertility (Peters, 1998).

The aim of the present research was to evaluate sweet potato varieties for dual-purpose utilization, utilize its vines and roots in different combinations to produce silage and test different feeding strategies based on silage and locally available feedstuff for crossbred fattening pigs. Also, the economic efficiency of those strategies in the context of smallholding farming conditions in North Vietnam was evaluated.

## Experimental procedure

### *Sweet potato varieties and silage*

Three introduced, improved SP varieties (Blesbok, CIP26 and KL25), selected by the ratio of roots biomass to total biomass, and a local variety were compared in two consecutive crop seasons (winter and spring) under two different cuttings regimes (one and two cuts of vines before root harvest). The test was carried out on plots of five smallholders. The two cuts treatment consisted of a harvest of vines at the middle of the crop cycle, followed by the final harvest of vines and roots at the end of the crop cycle (77 and 100 days on winter, respectively). The single cut treatment consisted simply of the total harvest of root and vines at the end of the crop cycle (100 and 112 days on winter and summer, respectively). The total measured fresh production was converted into dry matter. Protein and fiber were analyzed following standard procedures. Energy content was estimated from the literature (NRC, 2008)



**Photo 1. Sequence of silage preparation; A) cutting of vines and roots; B) Mixing of additives (sugar or salt); C) compressing the silage material, and D) Plastic bag without barrel frame**

(6%), fish meal (10%), soybean (10%) and stylo leaf meal (6%); B) rice bran (30%) and corn meal (70%); and C) Fish meal (35%), soybean (34%), cassava leaf meal (9%), Stylo meal (22%). Table 2 shows the chemical composition of each ration component.

Vines and roots from both seasons were used for making silage in big plastic bags (800kg), as shown in Photo 1. Table 1 shows the composition of each silo. Silage was used after 45 days.

**Table 1. Composition (%) of silage prepared with vines and roots of sweet potato from the first and second cuts**

Silage	Vines	Roots	Sugar	Corn meal	Salt
I*	97	--	2.5	--	0.5
II*	93.5	--	--	6	0.5
III**	49.5	50	--	--	0.5
IV**	24.5	75	--	--	0.5
V	74.5	25	--	--	0.5
VI	89.5	10	--	--	0.5

\*Vines from first cut

\*\*Vines from total harvest (first and second cuts)

Farmers use local resources to make different composite feed, which were used as basic feed supplemented by silage in each feeding strategy tested, Table 2. Different average composite feed included the following ingredients: A) corn meal (38%), rice bran (15%), cassava root meal (15%), cassava leaf meal (6%), fish meal (10%), soybean (10%) and stylo leaf meal (6%); B) rice bran (30%) and corn meal (70%); and C) Fish meal (35%), soybean (34%), cassava leaf meal (9%), Stylo meal (22%). Table 2 shows the chemical composition of each ration component.

**Table 2. Chemical composition of the components of the ration for each feeding strategy**

Ration component		Dry matter, %	Crude Protein* %	Fiber* %	Energy* Mcal/kg	Cost as fed VND/kg
Silage	I	14.15±1.83	15.43±0.89	18.68±0.92	--	966.47±68.72
	II	17.67±2.34	15.73±2.34	15.43±0.88	--	966.47±68.72
	III	18.69±2.10	9.31±1.27	10.11±0.98	--	1,133.00±69.46
	IV	23.50±1.18	6.43±0.95	6.49±0.86	--	1161±68.45
	V	16.53±2.71	9.59±0.59	16.05±0.81	--	519.01±71.02
	VI	14.52±1.17	11.22±0.50	19.27±1.02	--	4.73±65.41
Composite feed	A	86.14±0.13	14.52±0.35	5.45±0.65	2.91±0.06	6,186.00±354
	B	88.71±0.01	11.19± 0.07	4.70± 0.13	3.46 ±0.01	5,000.00± 0.001
	C	80.37±0.22	36.41±0.97	11.72±0.75	3.01±0.06	10,165.00±425.68
Commercial pellets		86.00±0.01	17.44±1.90	8.58±1.45	3.34±0.11	8,279.20±583.51
Concentrate (CC)		90.08±0.89	47.12±2.08	6.66±0.07	2.885±0.13	15,867±808.29

\* Dry matter base  
VNd 17,500 = 1US\$

### ***Animal response in pig fattening trials***

Six feeding strategies consisting of different combinations of SP silage, local composite feed, commercial pellets and a concentrate were evaluated in a field trial conducted in five smallholders' farms (Table 3). The weight gain of penned crossbred pigs was registered and evaluated. The trial was divided in two stages defined by the seasonal production of sweet potato and the availability of fattening pigs in the farms. Some of the feeding strategies were applied in the first stage and others in the second stage, as shown in Table 3. Each feeding strategy included 20 pigs. Initial weight was 19.80±4.21; 19.93±4.42, 20.03±4.71; 22.00±4.85, 22.47±4.96, 22.50±4.32 kilogram for each feeding strategy, respectively. The total fattening period was ninety days. Table 3

**Table 3. Feeding strategies evaluated for pig fattening in crop-livestock production systems in North Vietnam**

Ingredient within Feeding strategy	Stage 1*			Stage 2*		
	S1	S2	S3	S4	S5	S6
Silage; one type in each month (50% of the ration)	I, III, III	I, II, IV	---	I, VI, VI	II, V,V	I, V,V
Composite feed: A (50% of the ration)	A	A	---	---	---	---
B (32 % of the ration)	---	---	---	B	B	B
C (18% of the ration)	---	---	---	C	C	---
Commercial concentrate (CC) (18% of the ration)	---	---	---	---	---	CC
Commercial pellets (CP) (100 of the ration)	---	---	CP	---	---	

\*Stage 1 and 2 were winter and spring crop season of sweet potato

## **Experimental results**

### ***Sweet potato varieties***

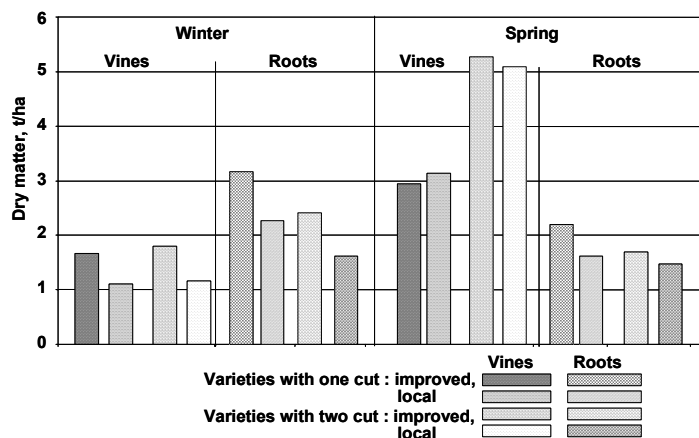
Table 4 shows the dry matter yield (t/ha) of the four varieties by season and management. The yield of vines and roots of the improved varieties was higher ( $p<0.05$ ) than the yield of the local variety (2.92±1.67 and 2.62±1.89, respectively). The varieties KL5 and CIP26 were different to Blesblok on vines and roots yield. Overall, vine production was higher in spring than in winter: 2.99±0.33 and 1.52±0.34 for one and two cuts, respectively and 5.22±0.16 and 1.63±0.38 for two cuts. Vines production increased 41% in spring. However, the production of roots in winter was higher than in summer; 2.94±0.45 and 2.22±0.42 for one and two cuts, respectively; in summer the production was 2.05±0.36 and 1.64±0.20 for one and two cuts respectively. The roots yield increased by 28.4% in winter, in response to an adequate balance of temperature and precipitation. Water excess in spring affected root production but not the growth and yield of vines. The yields of roots and vines



were  $3.43 \pm 0.27$  and  $2.26 \pm 0.34$  for two and one cut, respectively ( $P < 0.05$ ). Root's production was  $2.49 \pm 0.41$  and  $1.93 \pm 0.31$  for one and two cuts, respectively; a significant reduction of 22.5%, in root production occurred with two cuts. However, two cuts tended to produce more total biomass than one cut,  $5.35 \pm 0.51$  and  $4.75 \pm 0.52$  respectively, increasing the total biomass production by 11.2%. Total protein production follows the same pattern, two cuts providing more protein due to the higher vine production, which is a plus for crop-livestock systems in Northern Vietnam. Figure 1 shows the production of vines and roots under the different experimental conditions.

**Table 4. Seasonal dry matter yield of four SP varieties in two cropping season under two different managements (t/ha)**

Crop season	Variety	Vines t/ha	Roots, t/ha	Biomass, t/ha	Vine protein t/ha	Root protein t/ha	Protein yield t/ha	
Winter, A	Blesbok	$1.44 \pm 0.33$	$3.07 \pm 0.73$	$4.51 \pm 0.93$	$0.28 \pm 0.07$	$0.21 \pm 0.05$	$0.49 \pm 0.11$	
	CIP26	$1.92 \pm 0.42$	$3.19 \pm 0.86$	$5.11 \pm 0.99$	$0.35 \pm 0.09$	$0.19 \pm 0.04$	$0.54 \pm 0.10$	
	KL5	$1.62 \pm 0.33$	$3.23 \pm 0.77$	$4.85 \pm 0.90$	$0.30 \pm 0.07$	$0.18 \pm 0.03$	$0.48 \pm 0.09$	
	Local	$1.10 \pm 0.25$	$2.27 \pm 0.48$	$3.37 \pm 0.63$	$0.20 \pm 0.05$	$0.17 \pm 0.04$	$0.37 \pm 0.08$	
	B	Blesbok	$1.59 \pm 0.42$	$2.25 \pm 0.51$	$3.84 \pm 0.80$	$0.36 \pm 0.11$	$0.15 \pm 0.04$	$0.51 \pm 0.13$
		CIP26	$2.07 \pm 0.50$	$2.57 \pm 0.64$	$4.64 \pm 0.84$	$0.44 \pm 0.11$	$0.15 \pm 0.03$	$0.60 \pm 0.12$
		KL5	$1.71 \pm 0.39$	$2.42 \pm 0.70$	$4.13 \pm 0.89$	$0.36 \pm 0.09$	$0.13 \pm 0.03$	$0.49 \pm 0.11$
		Local	$1.16 \pm 0.33$	$1.62 \pm 0.39$	$2.79 \pm 0.63$	$0.24 \pm 0.08$	$0.12 \pm 0.03$	$0.36 \pm 0.09$
Spring, A	Blesbok	$3.09 \pm 1.05$	$1.97 \pm 0.94$	$5.06 \pm 1.32$	$0.39 \pm 0.17$	$0.07 \pm 0.03$	$0.46 \pm 0.17$	
	CIP26	$2.50 \pm 0.87$	$2.46 \pm 1.27$	$4.96 \pm 1.47$	$0.33 \pm 0.13$	$0.10 \pm 0.04$	$0.43 \pm 0.14$	
	KL5	$3.22 \pm 1.38$	$2.17 \pm 1.09$	$5.39 \pm 1.75$	$0.41 \pm 0.20$	$0.06 \pm 0.03$	$0.47 \pm 0.21$	
	Local	$3.14 \pm 1.46$	$1.61 \pm 0.97$	$4.75 \pm 1.57$	$0.44 \pm 0.20$	$0.06 \pm 0.04$	$0.50 \pm 0.19$	
	B	Blesbok	$5.31 \pm 1.49$	$1.49 \pm 0.78$	$6.80 \pm 1.83$	$0.71 \pm 0.20$	$0.05 \pm 0.03$	$0.76 \pm 0.21$
		CIP26	$5.09 \pm 1.47$	$1.90 \pm 1.11$	$6.98 \pm 2.03$	$0.70 \pm 0.17$	$0.06 \pm 0.03$	$0.76 \pm 0.18$
		KL5	$5.40 \pm 1.69$	$1.70 \pm 0.99$	$7.09 \pm 2.18$	$0.70 \pm 0.21$	$0.04 \pm 0.02$	$0.74 \pm 0.21$
		Local	$5.09 \pm 2.40$	$1.47 \pm 1.03$	$6.56 \pm 2.70$	$0.72 \pm 0.36$	$0.03 \pm 0.02$	$0.75 \pm 0.37$



**Figure 1. Sweet potato dry matter production of vines and roots of improved and local varieties under two crop managements and different cropping seasons.**

### Animal response in pig fattening trials

The comparison of the six feeding strategies based on different combinations of traditional feedstuff with SP silage, commercial pellets and concentrate showed (Table 5) that the strategies S3 and S6 (containing commercial pellets and concentrate) were significantly different ( $P < 0.05$ ) from the strategies containing silage and household concentrate (S1, S2, S4, S5). The average daily gain of S3 and S6 were  $0.716 \pm 0.070$  and  $0.620 \pm 0.102$ , respectively whereas for the other strategies daily weight gain ranged from  $0.519 \pm 0.04$  to  $0.609 \pm 0.05$ . The S3 diet included only commercial concentrate and S6 was a combination of commercial concentrate with a high level of protein (48%) and a basic mixture made in the farms (Rice bran, 30% and corn meal 70%) plus silage.

**Table 5. Evaluation of feeding strategies including SP silage and commercial concentrate in Northern Vietnam**

Feeding strategy	Initial weigh, kg	Final weight, kg	Weight gain difference, kg	Daily weight gain, kg/day	Feed cost * VNd/kg	Gross margin VNd
S1	19.80±4.21	74.59±8.24	54.79±5.19	0.609±0.058	17,633±1037	10,025±589
S2	19.93±4.42	73.93±10.59	54.00±7.72	0.600±0.086	17,411±1319	10,493±795
S3	20.03±4.71	84.46±8.39	64.43±6.31	0.716±0.070	21,043±1361	6,450±417
S4	22.00±4.85	68.68±8.35	46.68±4.27	0.519±0.047	19,919±1160	4,868±283
S5	22.47±4.96	71.29±10.92	48.82±6.81	0.542±0.076	20,011±1513	4,766±360
S6	22.50±4.32	78.34±11.37	55.84±9.14	0.620±0.102	21,474±1777	3,140±260

The total production feed cost of each was considered as total cost.

Pig price sale on average was  $30,064 \pm 834$  VNd/kg of live weight

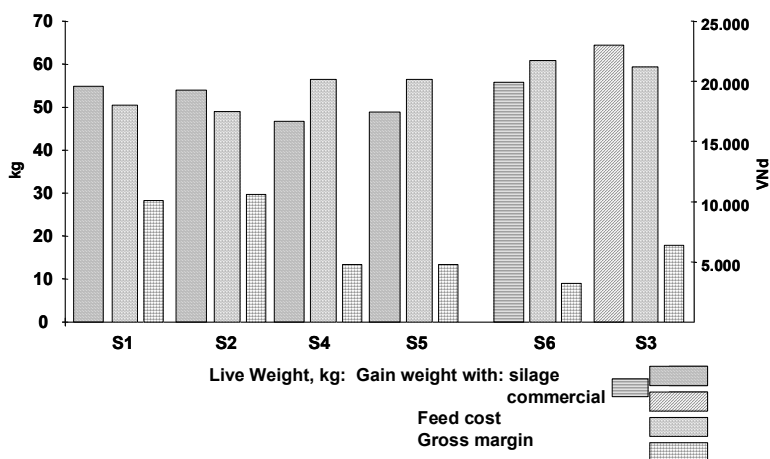
US\$ = 17,500 VND

The total production feed cost of each was considered as total cost.

Pig price sale on average was  $30,064 \pm 834$  VNd/kg of live weight

US\$ = 17,500 VND

Figure 2 shows the gross margins for the feeding strategies. The inclusion of SP silage tends to give a lesser final live weight, but resulted in a more uniform weight gain, which increased the gross margin by 37%. The higher cost of feeding, for strategies 3 and 6, calls for ingredients with higher protein level. The use of SP silage along with the home made concentrate (S4 and S5) resulted in similar gross margins than the strategy with no silage (S3), suggesting that SP silage is a good alternative to supplement the concentrate elaborated with local available resources.



**Figure 2. Comparison of feeding strategies including SP silage, commercial concentrate and home made mixture of local feed resources.**

## Concluding remarks

- Sweet potato can be used in crop-livestock production system as a dual-purpose crop to provide a harvest of vines at seventy days of growth.
- Cutting of vines before the end of the crop period tends to reduce root's yield but increases total biomass production.
- The use of SP silage tends to reduce production cost and increase gross margins of pig fattening production systems when it is combined with local available feed resources.

## Acknowledgement

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# Potential role of sweet potato to improve smallholder crop-livestock production systems

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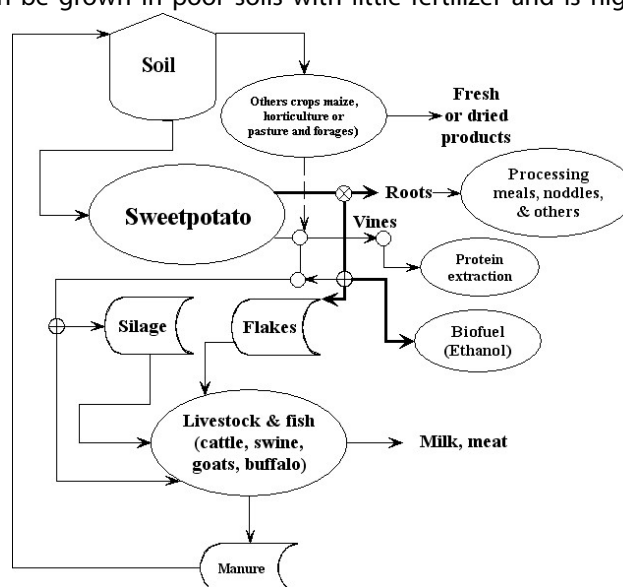
## Abstract

Sweet potato (*Ipomoea batatas*) is grown for different purposes: human consumption, animal feeding, and industrial products (biofuel). Vines, foliage and un-saleable or damaged roots are frequently used to feed animals in Latin America, Africa and East Asia. Demand for feed, fodder, fuel, and traditional supplements are increasing and competing with human needs. Sweet potato (SP) offers a viable alternative to satisfy different demands by using it as a dual-purpose crop. Nineteen clones were selected on the basis of the roots/vines (R/F) ratio for animal feeding and human or industrial use. A modification of traditional crop management consisting in different cutting frequencies was evaluated. Results have shown that cuts at about 75 days of growth reduced root production by 27% while vine production was increased by 25.1%, increasing total biomass by 11-19.5%. This cutting regime provided biomass to be conserved as silage to be used alone or as a supplement for pig feeding as well as roots for different uses. The utilization of SP stands by grazing/foraging pigs caused no significant effect on soil structure or organic matter content, but brought about significant increments in soil nutrients: nitrogen (25%), phosphorus (50%) and potassium (41%). Grazing/foraging or confined swine fed on SP plus silage and a protein supplement showed no significant difference in weight gain between the grazing/foraging treatments but penned pigs gained 17.5% more weight ( $p < 0.01$ ).

Keywords: Sweet potato; cutting regimes; production systems; fattening pigs.

## Introduction

Sweet potato (*Ipomoea batatas*) is cultivated throughout several tropical countries and warm temperate regions wherever there is sufficient rainfall to support their growth. Over 95 percent of the global sweet potato crop is produced in developing countries (CIP, 1984). It can be grown in poor soils with little fertilizer and is highly tolerant to pest and diseases, which makes it a major smallholder's staple crop. Because of its versatility and adaptability, SP ranks as the world's seventh most important food crop—after wheat, rice, maize, potato, barley, and cassava. Sweet potato is high in carbohydrates and vitamin A and produces more edible energy per hectare than wheat, rice or cassava and is also a potential source of biofuel production. The tuberos or storage roots of SP are used both for human and animal consumption while the vines are generally used as animal feed along with the crop residues and unmarketable roots, depending on local preferences and customs (Woolfe, 1992). Sweet potato holds comparative utilization advantages over other crops in semi-subsistence farming systems, Figure 1. The diversity of favorable agronomic characteristics of SP includes general hardiness, low input needs (due at least in part to the presence of vesicular-arbuscular mycorrhiza) and fast vine growth response to fertilizer (Tupus, 1983). Sweet potato has the potential for intercropping, ease of propagation, few crop pests and diseases, and good ground coverage for soil



**Figure 1. Actual and potential use of sweet potato for human consumption, livestock feeding and industrial purposes**

conservation. It has advantageous feeding characteristics in terms of high levels of both energy and protein from the roots and vines, respectively. The vines have high palatability and digestibility for consumption by both ruminants and monogastrics, and due to the low level of enzyme inhibitors in the vines, SP is suitable for its conservation by pre drying or silage (Ruiz, 1982). One major advantage of SP is that besides its use as animal fodder, it may also provide food for human consumption, an optimal integrated crop/livestock management system being able to utilize the sweet potato's good regrowth capacity by continually or sporadically harvesting the vines throughout the growing season before finally harvesting the root (León-Velarde and Gómez, 1996). This management is aimed at enhancing the production of green forage, as harvesting the vines significantly increases the growth rate and yield of the foliage while decreasing root yield.

The aim of the present paper is to summarize the results of the evaluation of SP as a dual-purpose (DP) crop for smallholder's crop-livestock production systems where fresh foliage and roots and the silage of root and vines contributed to the diets for fattening penned or grazing/foraging crossbred pigs. It also presents results of the effects of this crop/livestock system on soil conditions. The results pertain to several experiments conducted by university students under our supervision.

## **Preliminary results**

Preliminary results of SP evaluation suggest that a cutting frequency of 90 days during the crop growing period gave the best balance between forage and root production. A more intensive cutting frequency of 45 days intervals tends to cause a reduced total forage production and nil root harvest. Increasing N fertilization from 0 to 180 kg/ha increased forage production from 6.7 to 9.1 t DM/ha (6 cuts at 45 days interval); 6.1 to 8 t DM/ha (3 cuts at 90 days interval) and from 5.4 to 6.2 t DM/ha for one cut at 135 days of growth (Quispe, 1997, Arteaga, 1997). The use of fertilizer is contingent on the cost and marginal production in relation to other forages. As to forage storage, the combination of SP with maize (75% maize without hear-husk and 25% SP) resulted in good quality silage with no reduction of nutritional value (Guerra, 1998). The digestibility of the obtained silage was around 65%. On the other hand, feeding trials in which silage comprised of 75% of SP foliage and 25 % of roots was fed to milking cows showed that milk production was not affected, but feeding costs were reduced (Sanchez, 1995). As to crop management, studies on direct foraging of SP stands are necessary to better link the root and foliage production with livestock rearing in smallholder's crop-livestock farming systems. As to animal feeding, the trypsin inhibitor activity in the vines and roots of some SP varieties, calls for the definition of a more efficient SP utilization by either ruminants or monogastrics. The large number of clones available allows the selection of dual-purpose varieties with characteristics that favor livestock production.

## **Experimental procedure**

### ***Sweet potato varieties***

The germplasm collection held at the International Potato Center, CIP, includes a large group of SP clones, varieties and accessions. Breeding efforts are focused on root dry matter and starch content. However, in recent years the demand for forage producing varieties has increased. In this context, research on dual-purpose SP (roots and vines) holds a comparative advantage over research on accessions selected only for roots production. Besides providing food for human consumption, dual-purpose varieties will promote the optimal integration of SP based crop/livestock systems by also providing feedstuff. Dual-purpose varieties could be better utilized by continually or sporadically harvesting the vines throughout the growing season before finally harvesting the roots. Based on previous results of trials on plant density, cutting frequency, fertilization, nutritive value and storage (Quispe, 1997, Arteaga, 1997, León Velarde, et al, 1997), nineteen accessions from a data base, which includes data on root and vines production, were initially selected on the basis of the root/foilage (R/F) dry matter production ratio and the characteristic of the leaves (oval or rounded). They were classified in four probabilistically defined groups over a continuous variation of opposing trends of forage and root production as: forage (11); facultative forage DP (2); DP (2); and, facultative root DP (4). Accessions of high root production were not considered. Observations from two evaluations of around 150 days on plots of ten square meters were analyzed by way of a fixed linear model including group and accession (group); only accessions with at least two observations were included in the analysis (SAS, 1996). The variables measured were forage and root dry matter (total and commercial roots), proportion of commercial and no commercial roots (CR/NCR), root/forage (R/F) ratio and weight of commercial roots. Least square means and standard errors are presented in table 1. Selected varieties were evaluated for fresh and dry mater vines and roots yields, in response to two cutting frequencies (in

one case, two cuts at 75 days interval and in the other one single cut at the end of the growing period i.e. 150 days). In both cases, foliage and roots were harvested.

**Table 1. Least means square and standard error for main characteristic of dual-purpose sweet potato evaluation on dry matter production; San Ramón, Perú.<sup>1,2,3</sup>**

Group variety	Relation		Total forage t/ha	Total Roots t/ha	Commercial Roots	Commercial root weight, g	Relation CR/NCR
	RF	RB					
<b>1. Forage</b>	<b>0.32±0.08<sup>a</sup></b>	0.17±0.18	<b>4.66 ± 0.20<sup>a</sup></b>	<b>1.36 ± 0.30<sup>a</sup></b>	<b>0.89 ± 0.23<sup>a</sup></b>	<b>187.77 ± 16.72<sup>a</sup></b>	<b>0.35 ± 0.04<sup>a</sup></b>
ARB-265	0.00	0.00	6.92 ± 0.56	0.00 ± 0.84	0.00 ± 0.66	-	0.00 ± 0.11
DLP-3548	0.00	0.02	5.61 ± 0.56	0.00 ± 0.84	0.00 ± 0.66	-	0.00 ± 0.11
DLP-1308	0.22	0.15	3.94 ± 0.69	0.71 ± 1.03	0.00 ± 0.66	-	0.00 ± 0.13
ARB-UNAP55	0.00	0.00	3.70 ± 0.56	0.00 ± 0.84	0.00 ± 0.66	-	0.00 ± 0.11
DLP-2481	0.14	0.07	5.72 ± 0.56	0.41 ± 0.84	0.34 ± 0.66	216.65 ± 51.18	0.27 ± 0.11
RCBIN-5	0.14	0.14	3.26 ± 0.56	0.46 ± 0.84	0.46 ± 0.66	151.67 ± 41.79	0.50 ± 0.11
DLP-2448	0.35	0.06	3.24 ± 0.69	1.22 ± 1.03	0.53 ± 0.80	141.95 ± 51.18	0.24 ± 0.13
ARB-158	0.30	0.17	5.85 ± 0.79	1.74 ± 1.19	1.60 ± 0.92	160.43 ± 41.79	0.93 ± 0.15
MOCH	0.79	0.41	2.89 ± 0.97	2.18 ± 1.46	2.17 ± 1.13	315.00 ± 51.18	1.00 ± 0.19
ARB-389	0.73	0.46	5.46 ± 0.61	4.09 ± 0.92	1.96 ± 0.72	149.00 ± 36.19	0.41 ± 0.12
DLP-90052	0.95	0.44	4.65 ± 0.56	4.23 ± 0.84	2.67 ± 0.66	179.62 ± 32.37	0.52 ± 0.11
<b>2. Low dual purpose</b>	<b>1.13±0.19<sup>b</sup></b>	0.37±0.16	<b>4.28 ± 0.49<sup>ab</sup></b>	<b>4.3 ± 0.73<sup>b</sup></b>	<b>2.84±0.57<sup>b</sup></b>	<b>236.98 ± 25.59<sup>a</sup></b>	<b>0.68 ± 0.09<sup>b</sup></b>
DLP-2462	1.04	0.26	4.81 ± 0.79	4.77 ± 1.19	2.94 ± 0.93	218.20 ± 41.49	0.60 ± 0.15
DLP-3525	1.23	0.48	3.76 ± 0.56	3.84 ± 0.84	2.74 ± 0.66	255.77 ± 29.55	0.75 ± 0.10
<b>3. High dual purpose</b>	<b>1.68±0.15<sup>c</sup></b>	0.50±0.01	<b>3.33 ± 0.40<sup>b</sup></b>	<b>5.62 ± 0.60<sup>bc</sup></b>	<b>4.45 ± 0.46<sup>c</sup></b>	<b>228.85 ± 20.89<sup>a</sup></b>	<b>0.79 ± 0.08<sup>c</sup></b>
DLP-275A	1.58	0.51	3.36 ± 0.56	5.38 ± 0.84	3.89 ± 0.66	161.78 ± 29.55	0.71 ± 0.11
ARB-394	1.77	0.49	3.28 ± 0.56	5.86 ± 0.84	5.07 ± 0.66	295.55 ± 29.55	0.87 ± 0.11
<b>4. High-Low roots production</b>	<b>2.69±0.13<sup>d</sup></b>	0.65±0.03	<b>3.52 ± 0.35<sup>b</sup></b>	<b>9.26 ± 0.52<sup>d</sup></b>	<b>5.96 ± 0.41<sup>d</sup></b>	<b>198.33 ± 18.39</b>	<b>0.65 ± 0.07<sup>d</sup></b>
ARB-UNAP74	3.13	<b>0.63</b>	4.04±0.56	12.48±0.84	8.13 ± 0.66	267.28 ± 29.55	0.68 ± 0.11
SPV55	2.71	0.67	3.67±0.97	9.80±0.84	6.44 ± 1.13	252.55 ± 51.18	0.67 ± 0.19
SR-90323	2.44	0.68	3.27±0.62	7.97±0.92	5.38 ± 0.71	145.16 ± 32.37	0.68 ± 0.12
CC-89213	2.50	0.63	3.13±0.56	6.79±1.49	3.90 ± 0.66	128.33 ± 29.55	0.56 ± 0.11

<sup>2</sup> Least square means of group with the same letter are not significantly different (P<0.01)

<sup>3</sup> Least square means of variety (group) sorter by total dry matter of forage, roots and commercial roots.

## Silage



**Photo 1. The sequence of sweet potato silage preparation**

It is known that working a traditional silo (bunker, aero) in small farms is difficult, a fact that precludes the adoption of silage making practices. To solve this limitation, a modified small silo, large enough to contain the biomass produced in small plots was designed. As shown in Photo 1, the adapted silo is made of two barrels cut by their side and joined by a door hinge (a). The internal wall and the bottom are covered with plastic joined with plastic glue. A large plastic bag in which the plant material is compressed goes into the container. Adequate drainage is provided by a plastic pipeline located at the bottom of the silo (b). In our trial, vines and roots were chopped and mixed at proportions of 75% and 25% respectively; 3 % of molasses was added as starter (c, d). The vines and roots were compressed in layers (15-20cm) until the silo was full to the top (e). After the process of filling the silo was completed, the barrels were separated by removing the pin, making them available for ensiling another load, if needed (f). The silage was used after 45 days. The characteristics of the silage were adequate, as the animals did not reject it.

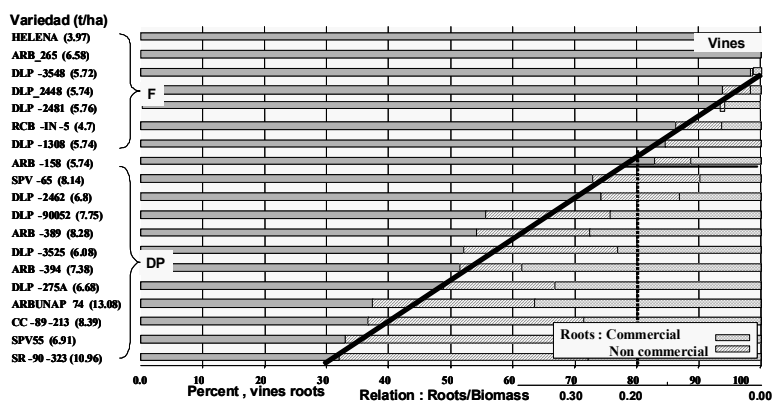
### ***Animal response in pig fattening trials***

The weight gain of pigs in either confinement or grazing/foraging conditions and fed with different combinations of fresh roots and vines, sweet potato silage and a protein supplement was evaluated:

1. Use of vines and roots. Twelve pigs with initial weight of 30 kg were assigned to three treatments: feeding in confinement with vines and roots *ad libitum* plus a fish meal protein supplement at a rate of 10.58 g/kg (A1); foraging on sweet potato plots plus a fish meal protein supplement at a rate of 10.58 g/kg (A2); and, foraging on sweet potato plots plus a fish meal protein supplement at a rate of 3.5 g/kg (A3) Fish meal was fed twice a day (morning and afternoon) by halves. Data were analyzed as repeated (weekly) measurements over 72 days.
2. Use of silage. Sixteen pigs with an initial weight of 20 kg were assigned to four treatments: confinement with fresh vines and roots *at libitum* plus a protein supplement (30% fish meal and 70% soybean meal) (B1); confinement with SP silage plus protein supplement (B2); semi confinement and limited foraging of fresh SP, plus silage and protein supplement (B3); and, foraging plus protein supplement (B4). The protein supplement was supplied at an average rate of 0.5 kg/day as determined by the estimated total dry matter intake. Data were analyzed as repeated (weekly) measurements over 75 days.

## Experimental results

**Sweet potato varieties.** As mentioned before, the initial selection of nineteen clones of sweet potato was based on the root/forage (R/F) dry matter production ratio and the leaf shape (oval or rounded). Based on the R/F ratio the selected clones were classified in five groups: forage (0-1), facultative forage DP (1-1.5), DP (1.5-2.0), facultative root DP (2.0 –3.0) and, high root production (3.0), (León-Velarde, 1997, 2001). Table 1 shows the evaluation of varieties within groups. There was a significant difference among groups ( $P < 0.01$ ) for total forage and root dry matter, total of commercial roots and R/F ratio. Significant differences among accessions within groups on total forage dry matter and commercial roots indicate that there is variability within groups, which gives room for a selection process. The least square means for forage dry matter yield were  $4.66 \pm 0.20$ ,  $4.28 \pm 0.49$ ,  $3.33 \pm 0.40$  and  $3.52 \pm 0.35$  t/ha for groups 1, 2, 3 and 4 respectively. Total root production was  $1.36 \pm 0.32$ ,  $4.31 \pm 0.73$ ,  $5.62 \pm 0.60$  and  $9.26 \pm 0.52$  t/ha for groups [1], [2], [3 and [4] respectively. The total commercial roots produced by groups [2] and [3] were  $2.84 \pm 0.46$  and  $4.45 \pm 0.46$  t/ha, which is 68.7% more root production than group [1] and 25.3% less yield than group [4] opening the opportunity for income generation from root production plus forage availability. The average weights of commercial roots vary from 187 to 228 with no significant difference among groups. The classification of the clones within groups was also analyzed with regard to the total dry matter of vines and roots (Figure 2).



**Figure 2. Clones of dual-purpose sweet potato categorized by root-vines and root-biomass ratio**

Following the above tests, it was noticed that some clones within groups could not be pigeon holed unambiguously as in some cases the classification based on the ratio of roots to vines did not appropriately place some clones. Thus, it was suggested that the ratio of roots dry matter to total biomass  $[R/(R+V)]$  would be more appropriate. When it was done, the number of categories was reduced from five to two: varieties for only forage (F),  $[0 < R/B \leq 0.20]$ , and dual purpose,  $[R/B > 0.20]$ . This classification gives a better definition of dual-purpose sweet potato in the context of crop-livestock production systems where it is necessary to increase or maintain dry matter availability all year round. Based on this classification of forage and dual purpose SP, a set of clones was evaluated under two crop management practices, Table 2.

**Table 2. Vines and roots production at two crop management practices; t/ha of dry matter**

Clones	Relation R/B	Crop management					
		Vines harvested at two cuts (t/ha)				Vines harvested at one cut (150 days)	
		at 75 days	At +75 days	Total vines	Total Roots	Total vines	Total roots
Forage <sup>1</sup> (F)	0.03±0.02	1.70±0.14	3.08±0.21	4.77±0.24	0.07±0.05	4.15±0.56	0.15±0.12
Dual purpose (DP)	0.36±0.12	1.53±0.49	3.34±0.77	4.87±1.01	1.62±0.97 <sup>b</sup>	4.76±1.07	3.00±1.74 <sup>a</sup>

<sup>1</sup> Total dry matter (DM) expressed in t/ha.

<sup>1</sup> DLP-2448, ARB-158, DLP-2481, DLP-3525, ARB-394

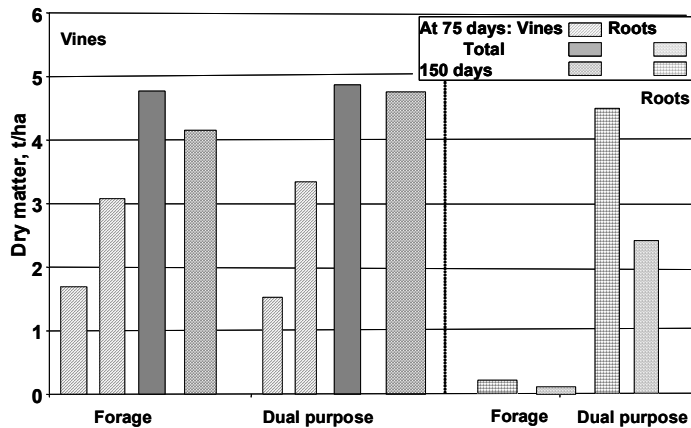
<sup>2</sup> SPV-65, DLP-275A, ARB, 389, ARB-UNAP74, DLP-2462, SR-90323, CC-89213

## Crop management practices

Traditionally SP roots are harvested at the end of the cropping season leaving the vines in the field for any post harvest use. Usually, roots are weighted and marketed for human consumption and the vines are derived for animal feeding purposes. This management is generally applicable to different common varieties but for a dual-purpose variety grown in crop-livestock production systems, a different crop management is required. The novel



approach we propose takes into account the total biomass production, which includes roots and vines. Based on previous results of continuous harvest at 45 days interval, it was determined that cutting intervals of at least 60 days are required for obtaining roots (León-Velarde, et al, 1997; Quispe, 1997). Therefore a modified cutting regime consisting of a harvest of vines at the middle of the growing period was tested. Twelve sweet potato clones classified, as either of the forage or dual-purpose type were included in a test of biomass production as affected by two different managements, table 2. The figure 3 shows the production of vines and roots under the two cutting regimes. On average, the yield of vines was similar on both managements; however, the cut of vines at the middle of the growing period tended to increase the production of vines by 15% while reducing roots yield by 27.1% ( $P<0.05$ ). The total biomass increased by 11% and 19.5% for forage and dual purpose SP, respectively, Table 2.



**Figure 3. Effect of two cutting regimes on the yield of vines and roots of forage only and dual-purpose varieties**

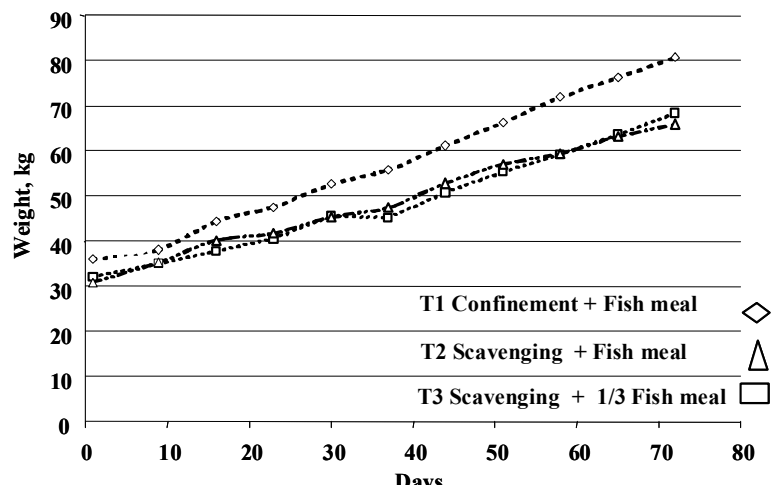
Figure 3 shows the clones evaluated as divided in two groups: forage, and dual purpose. A cut over 75 days tends to reduce by 19.5% the root production of dual-purpose varieties ( $P<0.05$ ). For varieties classified as forage producers the root production is nil but the yield of vines was increased by 15%.

Other aspect of SP management is that vines are traditionally used in a short period of time, due to the problems of conserving them as fresh biomass, which leads to the necessity of preserving them as silage.

### **Animal response in pig fattening trials**

Figure 4 shows the weight gains of penned pigs fed fresh roots and vines, and foraging pigs, both receiving fishmeal as supplement. There was significant difference ( $P<0.05$ ) in weight gain of penned pigs ( $0.620\pm 0.190$  kg/day) compared to foraging pigs fed 1/3 of fish meal ( $0.503\pm 0.132$  kg/day) and foraging pigs receiving the full amount of fish meal ( $0.488\pm 0.021$ ).

The level of fishmeal was enough to cover the protein requirements. Thus, the foraging group with 1/3 of fishmeal allowance showed similar weight increments that the group with full protein supplementation, indicating that the level of protein from sweet potato was enough to obtain adequate weight gain. Incidentally, it happened that fishmeal supplementation was too high as it caused a slight fish odor in the meat.



**Figure 4. Live weight gain of penned and foraging swine fed sweet potato and protein supplementation**

The effect of cut and carry and foraging of SP on the soil was evaluated. Results showed that either management has no significant effect on soil structure and organic matter content (Figure 5). However, foraging caused significant increments in soil nitrogen, phosphorus and potassium, improving residual soil fertility for subsequent crops like maize.

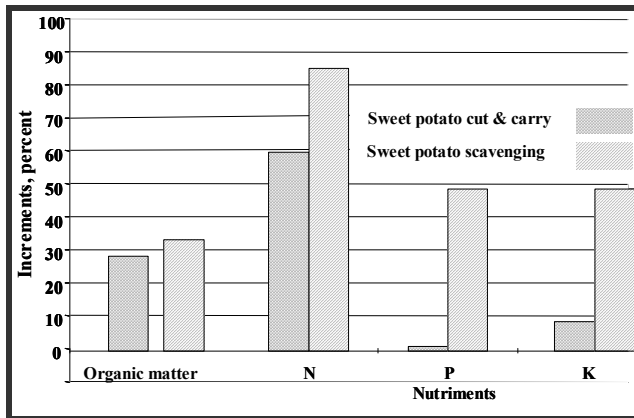


Figure 5. Effect of foraging and cut and carry of SP on soil structure

Figure 6 shows the results of feeding fresh roots and vines to penned and foraging pigs, some of them supplemented with SP silage and protein (fish meal and soybean). The weight gain ( $0.551 \pm 0.150$  kg/day) of the penned pigs fed fresh sweet potato and the protein supplement showed no significant difference with the weight gain ( $0.524 \pm 0.140$  kg/day) of the foraging pigs. On the other hand, the weight gain ( $0.444 \pm 0.125$  kg/day) of penned pigs supplemented with SP silage and protein was significantly higher ( $P < 0.05$ ) than the weight gain ( $0.361 \pm 0.105$  kg/day) of semi confined pigs receiving the same diet. Semi confining management was cumbersome for the continuous back and forth movement between the field and the pen.

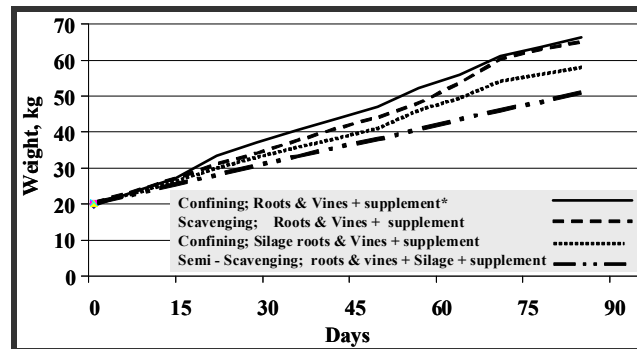


Figure 6. Live weight gain of penned and foraging swine fed on fresh sweet potato, silage and a protein supplement

## Concluding remarks

- Sweet potato varieties can be classified by the ratio of root biomass to total biomass, which provides a useful tool for the identification of dual-purpose varieties suitable for crop/livestock systems.
- Weight gain of penned and foraging pigs fed on the same diet of fresh vines and roots and adequate protein supplementation was similar.
- It was feasible to produce good quality sweet potato silage for the feeding of penned pigs as a supplement or when fresh sweet potato is scarce.
- Foraging of SP fields by pigs improves soil nutrients condition.

## Aknowledgement

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# Kinetics of starch digestion in Australian sweetpotato as affected by particle size

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## Abstract

In root crops, starch is the major component and main energy fraction. Recent trends in human nutrition with respect to health concerns (e.g. obesity and diabetes) have focused on starch digestion to identify digested and resistant starches. Knowledge of digestion kinetics helps to understand the contributions of these components, as well as the factors influencing them. We investigated the kinetics of starch digestion in the *Beauregard* cultivar of sweetpotato as affected by grinding types and conditions. The cultivar was peeled, diced, sulphited, and hot-air dried prior to cryo-milling and hammer milling at 11 different conditions to a range of particle sizes (80 – 380  $\mu\text{m}$ ) in a completely randomized replicated design. Time-course *in-vitro* starch digestion was studied using artificial saliva, pepsin, pancreatin, and amyloglucosidase, and digested starch was measured by glucometry. All the samples essentially exhibited monophasic digestograms, which were described using a modified first-order model. Grinding conditions affected ( $p < 0.05$ ) the particle size, and subsequently the initial or very-rapidly digested starch and rate of digestion ( $K$ ,  $\text{s}^{-1}$ ) because of changes in effective surface area. Significant linear relationships were obtained between the reciprocal of the rate of digestion ( $1/K$ , s) and the square of the average particle size ( $\text{size}^2$ ) for both mills separately and combined. The reciprocal of the slope ( $2.1 - 3.7 \times 10^{-7} \text{ cm}^2 \text{ s}^{-1}$ ) revealed digestion proceeded by diffusion mechanisms. The likelihood of heat generation during hammer milling appeared to influence starch digestion. The digestion parameters of the sweetpotato are discussed with other materials (e.g. cereals), and related to starch properties.

Keywords: *In-vitro* starch digestion, Monophasic digestogram, Diffusion mechanisms, First-order kinetics

## Introduction

Sweetpotato is growing in importance in Australia because of its potential health benefits (vitamins and antioxidants). An estimated 40,000 MT was produced in Australia in 2006, with *Beauregard*, *Northern Star* and *Kestle* as the main commercial cultivars (Maltby *et al.*, 2006). Sweetpotato is a starchy crop (Wolf, 1992), and generally, the modes and patterns of enzymatic digestion of starch define the health and nutritional significance of the food. With the existence of pores or channels in some starch granules, the interlinked stages in enzyme digestion of starch (amylolysis) are thought (Benmoussa *et al.*, 2006; Tester *et al.*, 2006; Sopade *et al.*, 2008) to be (i) random diffusion of enzymes onto the surface of starch granules, (ii) random movement of part of enzyme by capillary action to inside the granules, (iii) amylolysis commences at these points, (iv) amylolysis proceeds radially (centripetal) to create additionally pores and channels to the core of granules, (v) amylolysis proceeds and spreads from the core by enzymes inside the granule (centrifugal). In root crops, enzyme digestion of starch is thought to be mainly by the centrifugal mode (Tester *et al.*, 2006), but it seems the centripetal mode possibly needs to establish the route to the core of starch granules before centrifugal mode can become prominent (Sopade *et al.*, 2008). While these stages appear to mainly apply to extracted pure starches, the situation with starches existing in complete food systems is not as well established particularly in relations to enzyme diffusion.

Using milled sorghum grains, Mahasukhonthachat *et al.* (2009) proposed that starch digestion in sorghum proceeded by diffusion mechanisms by investigating the rate of *in-vitro* starch digestion at different particle sizes. We are not aware of any studies done on root crops, and specifically on sweetpotato. The particle size of food can be reduced (Fellows, 1998) by using various mills (e.g. cryomilling, hammer milling, roller milling, and attrition milling). These mills not only vary in their predominant grinding mechanisms, the extent of frictional heat can differ. The grinding mechanisms (e.g. impact, attrition and shearing) and frictional heat can bring about diverse structural and molecular changes, and affect the functional and digestibility properties of the food. While

impact force (mechanical effects) is common to both cryo- and hammer-milling, cryomilling freezes materials in liquid nitrogen to their glassy state prior to grinding in liquid nitrogen. This effectively eliminates frictional heat, which can be substantial in hammer milling. Hence, we expect both cryo- and hammer-milling to influence functional properties of food differently, as well as the modes and patterns of starch digestion. Therefore, this study investigated how cryo- and hammer-mills affected the mechanisms of starch digestion in non-processed sweetpotato, and examined the dependence of starch digestion on possible frictional heat during hammer milling.

## Materials and methods

### Materials

**Fresh tuber.** The sweetpotato tubers (cultivar *Beauregard*) used were obtained from the Queensland Department of Primary Industries and Fisheries, Gatton QLD 4343. The skin colour was orange, the flesh colour was pale orange, while the cortex was thin, and the tuber shape was obovate (CIP, AVRDC and IBPGR, 1991). The  $L^*$   $a^*$   $b^*$  indices of the flesh were 60.2, 22.6 and 34.9 respectively (white tile,  $L^* = 97.1$ ,  $a^* = 0.1$ ,  $b^* = 1.9$ ), while the moisture content (%) was  $80 \pm 0.3$ , total starch was  $60 \pm 2.8$  g/100g dry solids and the peel was about 6%.

### Method

**Sweetpotato drying.** After washing and peeling, the tubers were diced (3.5 mm cutting disc, 7 mm cutting blade, 1420 rpm; Serial 7-897, Halde RG-7, 26 Kista, SE-164, Sweden) and soaked in a 0.3% sodium metabisulphite solution for 5 min. before air-dried (TD-36T-1-D, Thermoline Scientific Pty Ltd, Smithfield, NSW 2164, Australia) at 40°C and about 0.8 m/s for 4 days. The dried dices were packed in polythene bags and cool-stored before further processing and analysis. The solids yield (total weight of solids in the dried dices) was about 72% of the total weight of solids in the fresh tubers.

**Moisture content.** Moisture content was determined according to AOAC Method 2.2.01 (AOAC, 1995); oven drying at 100°C for 24 hr (constant weight).

**Flour milling.** The dried dices were cryomilled (6850 SPEX Freezer/Mill; SPEX, Metuchen, NJ 08840, USA) and hammer-milled (MFC type DCFH 48, John Morris Scientific Pty. Ltd., Eagle Farm QLD 4009, Australia) using 11 different settings to vary the particle size of the sweetpotato flour. The milling was replicated, and the sweetpotato flours were stored in air-tight plastic bottles until analysed.

**Particle size analysis.** The Malvern Mastersizer Hydro 2000MU (Malvern Instruments Ltd, Malvern WR14 1XZ, UK) was used to analyse the particle size of the flours in water at 2000 rpm. A general purpose analysis model was used with particle refractive and absorption indices of 1.52 and 0.1 respectively, while the refractive index of water as the dispersant was 1.33. Particle size ( $v/v$ ,  $\mu\text{m}$ ) was mainly defined as the volume weighted mean ( $d[4,3]$ ), and particle sizes of the 10<sup>th</sup>, 50<sup>th</sup> (median) and 90<sup>th</sup> percentiles were used in addition to characterize and define the size distribution. However, discussions on the effects of particle size will concentrate on the volume weighted mean, henceforth referred to as the average particle size.

**Total starch analysis.** The total starch content was determined using a method derived from Megazyme (Megazyme International Ireland Ltd., Wicklow, Ireland) based on dimethyl sulphoxide (DMSO),  $\alpha$ -amylase (AA) and amyloglucosidase (AMG) procedure (Mahasukhonthachat *et al.*, 2009).

**In-vitro starch digestion.** The time-course starch digestion was analysed using a rapid *in-vitro* procedure (Sopade and Gidley, 2009; Mahasukhonthachat *et al.*, 2009) that involved digesting about 0.5 g of ground sample with amylase, pectin, pancreatin, and amyloglucosidase in appropriate buffers and pH, and periodically measuring the glucose produced by a glucometer up to 4 hr.

**Statistical analysis.** General linear model analyses (Minitab®, release 15; Minitab Inc.) were conducted, and the significance level for individual tests was 5%. The p-values of tests are reported, as well as the 95%-least significance differences (LSD). Wherever applicable, samples were randomized and, at least, duplicated for all the physicochemical analyses described above.

## Results and discussion

### Particle size analysis

The 11 different settings on the cryo- and hammer-mills yielded sweetpotato flours of different particle size parameters (Table 1), and all the flours exhibited essentially bimodal size distributions (Fig. 1). The mill settings or grinding conditions significantly ( $p < 0.05$ ) affected the average particle size. It can be observed in Table 1 that, generally:

- Finer sweetpotato flour was obtained when the cryomilling cycles were increased. This agrees with the results on sorghum grains (Mahasukhonthachat *et al.*, 2009), and follows from increased milling time and intensity.
- Coarser sweetpotato flour was obtained when the aperture of the retention sieve in the hammer mill was increased. This is expected because of less resistance and reduced residence time in the action zone as the sieve size increased.
- With more passes, finer sweetpotato flour was produced as more particles were further broken down.

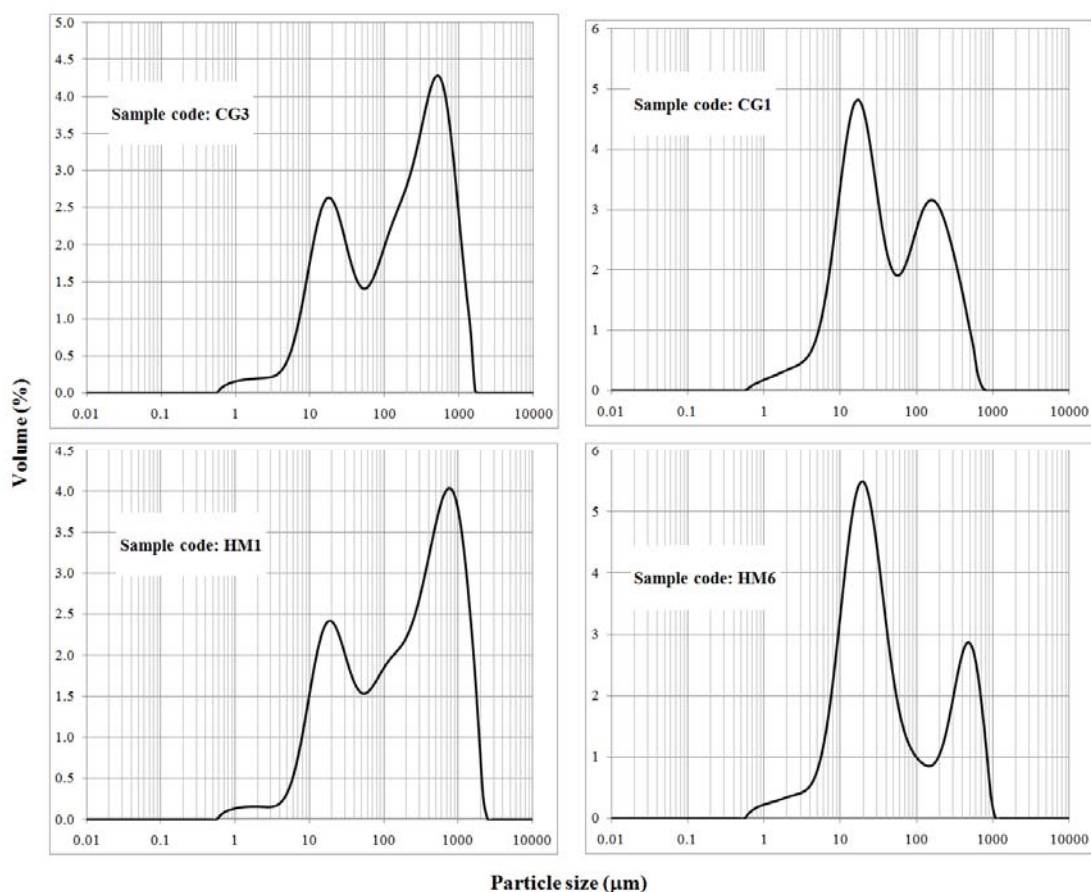
**Table 1. Milling conditions for the sweetpotato flour and the particle size parameters<sup>a</sup>**

Sample code	Milling condition		Particle size parameter (v/v, $\mu\text{m}$ ) <sup>a</sup>							
			d(v,0.1) <sup>b</sup>		d(v,0.5) <sup>b</sup>		d(v,0.9) <sup>b</sup>		Volume weighted mean d(4,3)	
Cryomilling										
	Grinding time (min) per cycle	Number of cycle								
CG1	5 [30]c	6	7•	0.5	28•	2.7	237•	15.3	82	6.1
CG2	5 [10]c	2	8•	0.5	84•	5.8	474•	10.0	171	4.9
CG3	5[5]c	1	11•	0.2	165•	14.5	741•	31.1	283	15.9
CG4	5[15]c	3	7•	0.2	53•	5.3	378•	23.8	132	8.5
CG5	5[20]c	4	7•	0.1	36•	0.5	306•	3.6	105	0.4
Hammer milling										
	Retention sieve (mm)	Number of regrind								
HM1	2	1	13•	0.4	200•	6.7	1016•	62.2	376	20.1
HM2	2	3	11•	0.1	97•	5.1	742•	9.7	256	1.2
HM3	2	6	10•	0.1	77•	2.4	675•	36.2	226	9.7
HM4	1.5	1	10•	0.4	62•	15.8	782•	16.7	256	11.8
HM5	1	1	10•	0.2	60•	0.5	782•	14.2	255	4.2
HM6	0.5	1	8•	0.1	26•	0.8	426•	5.5	119	1.2

<sup>a</sup> Figures are means  $\pm$  standard deviations.

<sup>b</sup> These indicate 10<sup>th</sup>, 50<sup>th</sup> (median) and 90<sup>th</sup> percentiles

<sup>c</sup> Figures in [ ] show the total grinding time. The same pre-cool time (5 min.), intermediate cooling time (2 min.) and impactor speed (10 s<sup>-1</sup>) were used for cryomilling.



**Figure 1. Typical particle size distribution of the cryo-(CG) and hammer-(HM) milled sweetpotato flour**

From published studies (Kerr *et al.*, 2000; Huang *et al.*, 2008; Mahasukhonthachat *et al.*, 2009), in addition to reducing the particle size, milling time and intensity lead to structural and molecular changes, which affect functional and digestibility properties of starch.

**In-vitro starch digestion.** Irrespective of the milling conditions, and consequently the average particle size, starch digestion in the sweetpotato flours exhibited a monophasic digestogram, and significantly ( $p < 0.05$ ) increased with time in a non-linear manner (Fig. 2). The time effect is consistent with an increase in reaction products with time of reaction, and agrees with other studies (Goñi *et al.*, 1997; Sun *et al.*, 2006). Although monophasic digestograms are popular in food systems, the monophasic digestion pattern of the cryo- and hammer-milled sweetpotato flours is contrary to the biphasic pattern obtained with sweetpotato from Papua New Guinea (Liu *et al.*, 2009). We associate this to differences in genotype and environment (G x E), two factors that are well known to affect properties of plant materials. With special reference to digestibility, G x E can affect the rate and extent of digestion, and might influence the dominant digestion mechanisms. The rate and extent of digestion are better quantified by modeling the digestograms to yield parameters and understand the mechanisms of digestion.

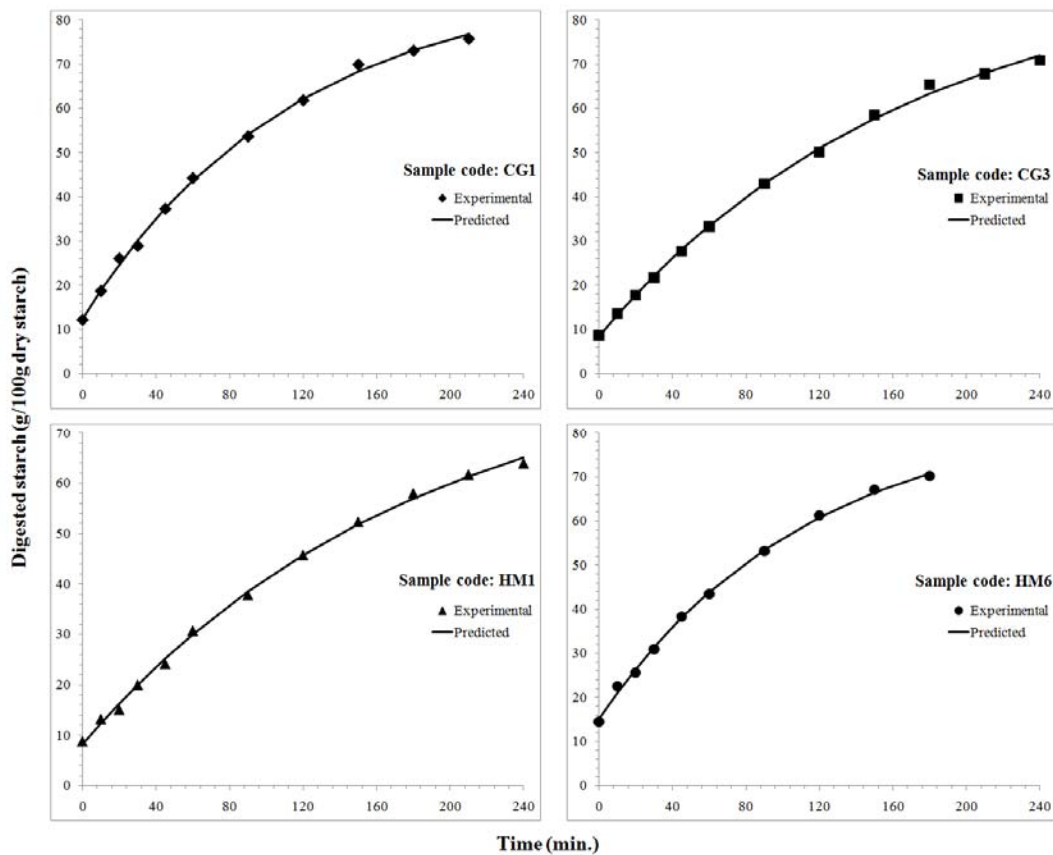
**Modeling in-vitro starch digestion.** Both empirical and theoretical models are widely used in describing digestograms, and of particular importance is the first-order exponential model (Goñi *et al.*, 1997). This model has been modified (Mahasukhonthachat *et al.*, 2009) to include a parameter for the initial digested starch, very rapidly digested starch or a measure of *in-vitro* gastric digestion.

$$D_t = D_o + D_{\infty_o} (1 - \exp [-K t]) \quad (1)$$

$$D_{\infty} = D_o + D_{\infty_o} \quad (2)$$

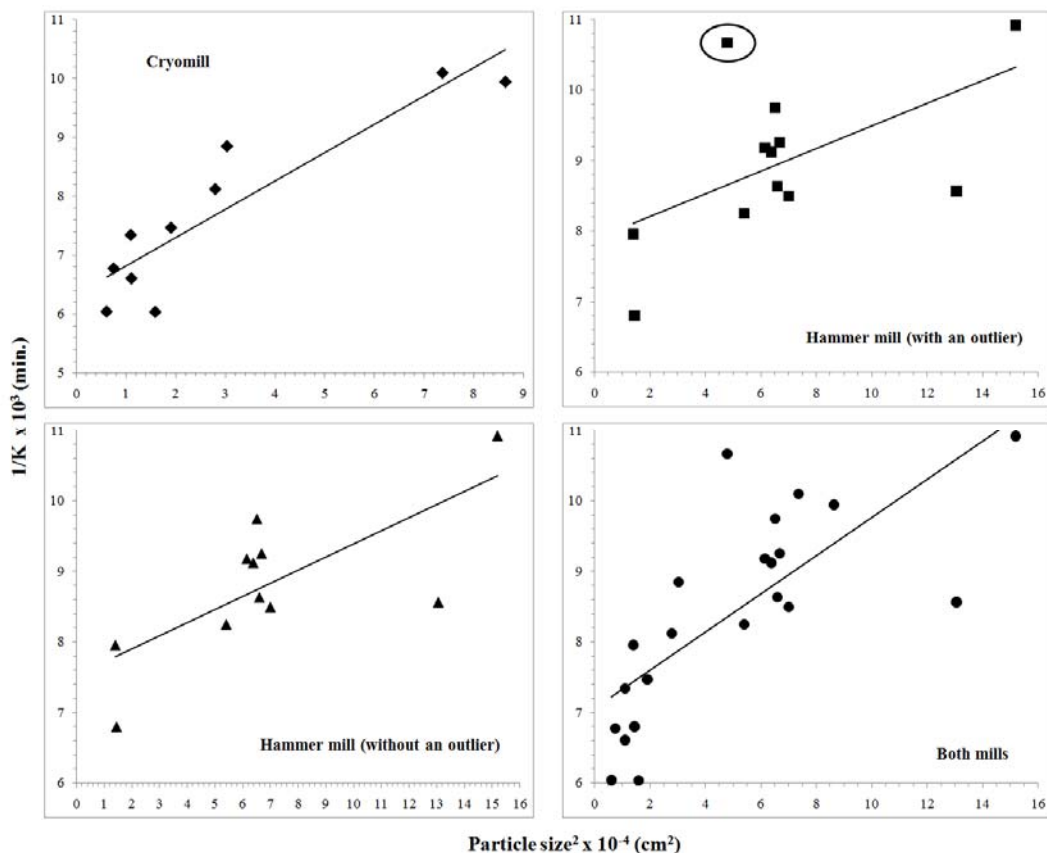
where,  $D_t$  = digested starch at time  $t$  (g/100g dry starch),  $D_0$  = digested starch at time  $t = 0$  (g/100g dry starch),  $D_\infty$  = digested starch at infinite time,  $t \rightarrow \infty$  (g/100g dry starch),  $K$  = rate of digestion ( $\text{min}^{-1}$ ). Using the Microsoft Excel Solver™, and constraining  $D_0 \geq 0$ , and  $D_\infty \leq 100$ , Eqn.[1] adequately described ( $r^2 = 0.981 - 0.999$ ) the digestograms for the cryo- and hammer-milled sweetpotato flours (Fig. 2). Although the parameters of the model are significantly ( $p < 0.05$ ) dependent on the grinding conditions, and hence, the particle size of the flour, the present focus is on the dependence of the rate of digestion on particle size. Generally, the higher the particle size, the lower was the rate of digestion, irrespective of the mill. This is consistent with a reduction in available surface area for enzymatic reactions as size reduces.

The surface area of a spherical particle is directly proportional to the square of the radius or diameter of the particle. If an enzyme diffuses onto the surfaces of spherical particles, and digestion proceeds by first-order kinetics, the rate of digestion is inversely proportional to the square of the particle size ( $1/K = C \times \text{size}^2$ , where  $C$  = constant of proportionality and a measure of coefficient of diffusion). Hence, a plot of the reciprocal of  $K$  against the square of the particle size is linear with a zero intercept, but deviations from the simplified theory are compensated for with an intercept. With or without combining the data from the two mills, starch digestion in the sweetpotato flours followed this simplified diffusion theory (Fig. 3). The reciprocal of the slope equates to the apparent diffusion coefficient ( $D_{app}$ ), and from Smoluchowski analysis, diffusion mechanisms prevail if the apparent diffusion coefficient is of the order of  $10^{-7} \text{ cm}^2 \text{ s}^{-1}$ .



**Figure 2. Typical monophasic digestogram (experimental and predicted) of the cryo-(CG) and hammer-(HM) milled sweetpotato flour**





**Figure 3. The relationship between the reciprocal of the rate constant and square of the particle size for the sweetpotato flour**

Table 2 shows the equations relating the rate of digestion with the square of particle size for the sweetpotato flours, and it can be inferred that starch digestion by amylases in the cryo-milled and hammer-milled sweetpotato flours proceeded by diffusion mechanisms. In an earlier study (Mahasukhonthachat *et al.*, 2009), diffusion mechanisms were also inferred to prevail during starch digestion of sorghum. Although sorghum is a cereal, and sweetpotato is a root crop, the similarity between the prevailing mechanisms during amylolysis could be because both starches have A-crystalline pattern. However, from the apparent diffusion coefficients ( $D_{diff}$ ) in the milled sorghum ( $0.4 - 0.9 \times 10^{-7} \text{ cm}^2 \text{ s}^{-1}$ ) and sweetpotato flours ( $2.1 - 3.7 \times 10^{-7} \text{ cm}^2 \text{ s}^{-1}$ ), digestion was faster in the sweetpotato flours. Generally, sweetpotato starch ( $2 - 72 \mu\text{m}$ ) is bigger than sorghum starch ( $5 - 30 \mu\text{m}$ ) to suggest sorghum would be easier to digest (Sopade *et al.*, 2008). However, unlike sweetpotato, starch-protein interactions are pronounced in sorghum, they limit starch digestibility, and possibly reduce apparent diffusion coefficient of amylases to sorghum starch.

**Table 2: Relationship between the square of the particle size and the reciprocal of the rate constant**

Mill <sup>†</sup>	Equation	$r^2$	$D_{diff} (\text{cm}^2 \text{ s}^{-1}) \times 10^{-7}$
CG	$1/K = 4.8 \times 10^6 (\text{size})^2 + 7.1 \times 10^3$	0.826; $p < 0.001$	2.1
HM	$1/K = 1.6 \times 10^6 (\text{size})^2 + 7.1 \times 10^3$	0.318; $p = 0.035$	6.2
HM (without outlier)	$1/K = 1.9 \times 10^6 (\text{size})^2 + 6.9 \times 10^3$	0.533; $p = 0.005$	5.3
Both mills	$1/K = 2.7 \times 10^6 (\text{size})^2 + 6.9 \times 10^3$	0.562; $p < 0.001$	3.7

<sup>†</sup>CG = Cryo mill, HM = Hammer mill; One HM outlier with standardized residual of 2.5

Although not clearly defined, there were slight but measureable differences in the digestibility of the hammer-milled and cryo-milled sweetpotato flours. This demands further investigations as it appears the hammer-milled flours were easier to digest because the apparent diffusion coefficients of the hammer-milled flours are numerically higher (Table 2) than those of the cryo-milled flours. The slight difference could be because of frictional heat during hammer milling, and/or differences in the mode of size reduction. We measured increases in flour temperatures (5 – 13°C) during the hammer milling to suggest more heat was generated as the diameter of the retention sieve reduced. Hammer milling was suspected to increase the digestibility of sorghum grains in comparison with cryomilling, and differences in the functional properties of hammer- and cryo-milled sorghum flours were reported ((Mahasukhonthachat *et al.*, 2009). Therefore, additional studies on the functional properties (pasting, water absorption, water solubility, gelatinisation, and spectroscopy) of the sweetpotato flours, in progress in our laboratories, might help establish the suspected differences, if any, and if they are measureable in these properties. Moreover, it is worth stressing that even though both the cryo- and hammer-milled sweetpotato flours showed essentially bimodal particle size distributions (Fig. 2), differences could exist between the proportions of small to large particles within and between the mills. In addition to the 10<sup>th</sup>, 50<sup>th</sup> and 90<sup>th</sup> percentiles (Table 1), an approach under investigation in our laboratories is to deconvolute the size distribution curves, and examine the contributions of small and large particles to gastric and pancreatic digestions. This could assist in understanding if, and to what extent, frictional heat during the hammer milling affected starch digestibility in the sweetpotato flours, and the consequences of this to the overall utilisation of sweetpotato as a functional food, and in specialised foods.

## Conclusions

Starch digestion in the *Beauregard* cultivar of Australia sweetpotato proceeded by diffusion mechanisms, and more starch was digested when the particle size was reduced. *In-vitro* pancreatic digestion of the flours was time-dependent in a non-linear manner. The conditions in the cryo- and hammer-mills significantly affected the particle size of the sweetpotato flours, and there were slight but measurable effects of mill type on starch digestibility. Differences in the mechanical action and frictional heat during milling were suspected to be responsible for the differences in the mills, but this demands further investigations. Future studies will investigate the contributions of small and large particles on the diffusion mechanisms, and generally how particle size influences functional properties of the flours. The importance of the particle size-digestibility studies lies in understanding the optimum size and distribution for starch digestibility of specialized products from sweetpotato.

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# Digestibility of starch and potassium in sweetpotato from Papua New Guinea

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## Abstract

Potassium is a major mineral in sweetpotato, and like most root crops, sweetpotato has high starch content. There are many cultivars of sweetpotato with genotype and environmental differences, which influence digestibility and bioavailability. Starch and mineral digestibility in food materials are currently topical because of associated nutritional implications. Time-course *in-vitro* starch and potassium digestibility of 20 samples of sweetpotato from Papua New Guinea were studied using glucometry and electrochemistry/spectroscopy respectively. The samples were made up of six cultivars (*3-mun*, *Carot kaukau*, *Wahgi besta*, *Nillgai*, *Baiyer kaukau*, and *1-mun*) planted in three provinces by three farmers in three different locations. The potassium content of the non-digested samples ranged from 4 – 17 mg/g dry solids, while the starch content was from 47 – 80 g per 100g dry solids and essentially independent of cultivars, farmers and locations. *In-vitro* starch digestibility (2 – 75 g digested starch per 100g dry starch) significantly ( $p < 0.05$ ) varied with time in a non-linear manner, and the starch digestograms exhibited biphasic behaviours irrespective of the sample. We propose that the biphasic behaviour resulted from changes in the digestion kinetics possibly due to barriers to enzyme diffusion by non-starch components. Digestion of potassium was independent of time in *in-vitro* gastric and pancreatic regions, but pancreatic digestion was significantly ( $p < 0.05$ ) higher than gastric digestion. Results are discussed in the light of the importance of resistant starch and bioavailability of (micro)nutrients for increased utilisation of sweetpotato.

**Keywords:** *In-vitro* starch digestion, Potassium release, Glucometry, Resistant starch, Biphasic digestogram, Gastric-pancreatic digestion

## Introduction

Sweetpotato is the most dominant root crop in Papua New Guinea (PNG), where an estimated 520,000 MT were produced in 2007 (FAO, 2009). In PNG, sweetpotato contributes more than 60% of the total food energy derived from staple food crops (Bourke, 1982; 2006). Like other root crops, the starch content of sweetpotato is high and ranges from 30 – 85 g/100g solids, while the major mineral in sweetpotato is potassium (260-380 mg/100g solids), followed by phosphorus, calcium and sodium (Bradbury and Holloway, 1988; Wolf, 1992; Ravindran *et al.*, 1995). Starch is important in human nutrition and health, so also is potassium because of their respective effects on, for example, glycaemic index and acid-base balance (Englyst *et al.*, 1992; McCarron and Reussner, 2001). Generally, the effects of (micro)nutrients in human nutrition and health are dependent on their digestibility and bioavailability, and both are expected to be influenced by genotype, environment, and planting and harvesting conditions because these factors affect the composition of plant materials (Bradbury and Holloway, 1988; Noda *et al.*, 1997; Sopade *et al.*, 2001; Aina *et al.*, 2009). There are more than 1,500 lowland and highland cultivars of sweetpotato in PNG, which although is the 17<sup>th</sup> producer in the world (FAO, 2009), is regarded as the second most important centre of sweetpotato genetic diversity in the world. While the huge number might not be referring to agronomically distinct cultivars, we are not aware of any studies on digestibility of starch and mineral in PNG sweetpotato, just as we have observed limited studies (Ly *et al.*, 1999; Dilworth *et al.*, 2007) generally on digestibility of root crops. We, therefore, report our investigations into the time-course digestion behaviours of starch and potassium in sweetpotato from PNG with a view to identifying differences in cultivars and growing areas.

## Materials and methods

### Materials

Twenty sweetpotato samples (Table 1) were obtained from PNG as part of a study into soil fertility management funded by the Australian Centre for International Agricultural Research (ACIAR). Prior to exporting to Australia, the fresh tubers were peeled, sliced into chips and oven-dried at 40° – 50°C. While in Australia, the dried chips were ground (IKA-Universalmühle M20, Janke & Kunkel GmbH & Co KG, Labortechnik, 79219 Staufen, Germany) to pass through a 1-mm sieve and freeze-stored prior to analysis.

### Method

**Total starch analysis.** The total starch content was determined using a method derived from Megazyme (Megazyme International Ireland Ltd., Wicklow, Ireland) based on dimethyl sulphoxide (DMSO),  $\alpha$ -amylase (AA) and amyloglucosidase (AMG) procedure (Mahasukhonthachat *et al.*, 2009).

**Mineral analysis.** The procedures in Martinie and Schilt (1976) were used. About 0.15 g of sample was accurately (to 4 dp) weighed into a conical flask, to which 15 ml of nitric perchloric acid (nitric:perchloric = 5:1) was added. The flasks were left overnight at room temperature to digest the sample, prior to slow heating to 120°C to minimize foaming. The digest was then heated to 140°C before rapidly heated to and held at 203°C, followed by cooling and dilution (TDI water) to 25 mL. The mixture was transferred to 10 mL plastic vials prior to Inductively Coupled Plasma with Optical Emission Spectroscopy, ICP-OES (Vista Pro; Varian Inc., Palo Alto, CA 94304-1030, USA).

**In-vitro starch digestion.** The time-course starch digestion was analysed using a rapid *in-vitro* procedure (Sopade and Gidley, 2009; Mahasukhonthachat *et al.*, 2009) that involved digesting about 0.5 g of ground sample with amylase, pectin, pancreatin, and amyloglucosidase in appropriate buffers and pH, and periodically measuring the glucose produced by a glucometer up to 4 hr.

**In-vitro mineral digestion.** The procedures for the *in-vitro* starch digestion were adapted for potassium digestion with the assumption that during starch digestion, potassium would be released. About 0.5 g of ground sample was digested with  $\alpha$ -amylase (Sigma A-3176 Type VI-B) in a carbonate buffer (pH 7) to simulate saliva, before pepsin (Sigma P-6887) was added at pH 2 to mimic the stomach. Digestion (gastric) proceeded at 37°C for 60 min, during which analytes were pipetted (10, 30, 60 min.) and neutralized (0.02M NaOH) before potassium analysis. For pancreatic digestion, the digesta from gastric digestion was neutralized, and sodium acetate buffer (pH 6.0) was added before a mixture of pancreatin (Sigma P1750) and amyloglucosidase (Sigma A-7420) in the acetate buffer was added and incubated for a further 120 min, during which analytes were pipetted (10, 30, 60, 120 min.) for potassium analysis. Potassium in digesta was measured with an electrode (Potassium combination ISE electrode, Part N° 27502-39; Extech, Boronia VIC 3155, Australia), and digested potassium was expressed as potassium in digesta relative to the total potassium (2.2.2) in dry sample (solids). Prior to analysis, the electrode was conditioned overnight, and calibrated with different standard values ranging from 1 - 1000 ppm K.

**Statistical analysis.** Analysis of variance (ANOVA) and tests of significance were performed using Minitab® release 15 (Minitab Inc.) with a confidence level of 95%. Wherever applicable, samples were randomised and, at least, duplicated for all the analyses described above.

## Results and discussion

**(Micro)Nutrient content.** Table 1 shows the starch and potassium contents of the samples, and there were no specific effects of province, location, farmer, and cultivar on the (micro)nutrients even though the five cultivars were planted in different provinces and locations by different farmers. The total starch contents varied from 50 – 80 g/100g solids, and samples 8 and 18 were the highest. The potassium contents obtained in the present studies are generally higher (400 – 1680 mg/100 g solids) than the published values on PNG sweetpotato (Bradbury and Holloway, 1988). Based on their potassium contents (mg/100 g solids), the 20 samples can be grouped into low (< 500; samples 5, 14, 17, and 19), medium (500 – 1000; samples 1, 2, 4, 10, 13, 18, and 20), high (1000 – 1500; samples 3, 6, 7, 8, 9, 11, 12, and 15), or very high (> 1500; sample 16).

**Table 1. Sample description, and starch and potassium contents<sup>a</sup>**

Province <sup>b</sup>	Farmer <sup>c</sup>	Location <sup>d</sup>	Cultivar	Code	Total starch (g/100g solids)	Potassium (mg/100g solids)
EHP	F1	S2	3-mun or Carot kaukau	1	51.8 a	880 a
EHP	F2	S2	3-mun	2	50.9 a	660 b
EHP	F3	S2	Carot kaukau	3	55.9 a	1260 c
EHP	F1	S3	3-mun or Carot kaukau	4	55.1 a	610 b
EHP	F2	S3	3-mun	5	59.2 a	470 d
EHP	F3	S3	Carot kaukau	6	55.6 a	1240 c
SP	F1	S1	Wahgi besta	7	51.6 a	1120 e
SP	F2	S1	Nillgai	8	79.5 b	1170 ef
SP	F3	S1	Baiyer kaukau	9	46.9 a	1340 g
SP	F1	S3	Wahgi besta	10	50.9 a	740 h
SP	F2	S3	Nillgai	11	54.3 a	1060 e
SP	F3	S3	Baiyer kaukau	12	52.6 a	1240 c
WHP	F1	S1	Wahgi besta	13	51.4 a	840 a
WHP	F2	S1	Wahgi besta	14	52.2 a	400 i
WHP	F3	S1	1-mun	15	60.8 a	1190 cf
WHP	F1	S2	Wahgi besta	16	49.1 a	1680 j
WHP	F2	S2	Wahgi besta	17	57.3 a	470 d
WHP	F1	S3	Wahgi besta	18	63.1 ab	840 a
WHP	F2	S3	Wahgi besta	19	54.1 a	450 di
WHP	F3	S3	1-mun	20	59.4 a	850 a

<sup>a</sup>For total starch or potassium, figures with the same letters are not significantly ( $p>0.05$ ) different

<sup>b</sup>EHP = Eastern Highland, SP = Simbu, WHP = Western Highland

<sup>c</sup>F1, F2 and F3 are three farmers

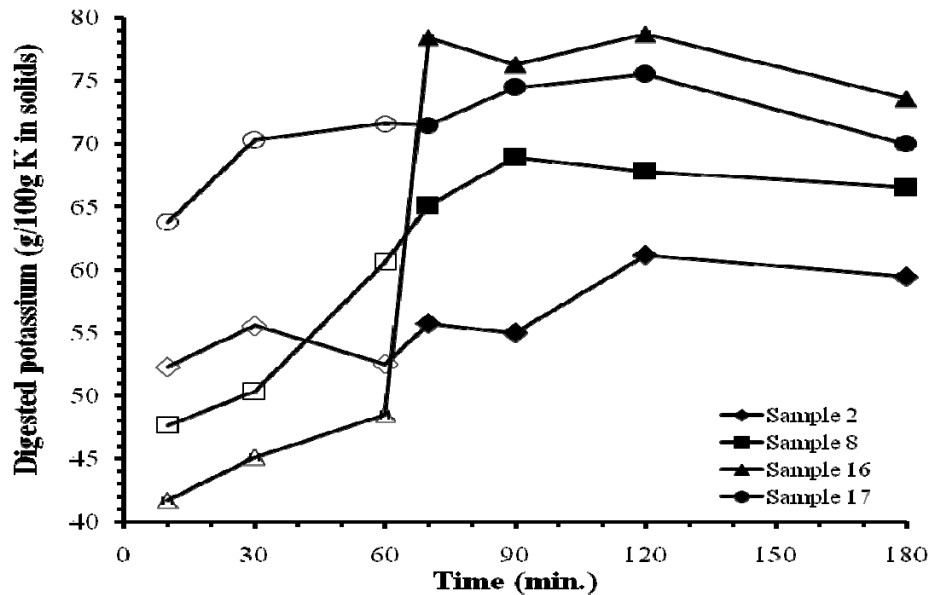
<sup>d</sup>S1, S2, S3 are three locations

*Potassium digestión.* Figure 1 shows typical potassium digestograms of the samples, and it shows changes in potassium digestion between the gastric and pancreatic stages. ANOVA revealed:

- Potassium digestion or release ranged from 40 – 100 (g/100g K in solids) during both phases of digestion, and was significantly ( $p<0.05$ ) affected by the samples. However, there were no specific effects of province, location, farmer, and cultivar. Potassium release was least in samples 2 and 5, while samples 4, 18 and 19 gave the highest release. Remarkably, these five samples are in the low and medium potassium classes as described above. It is noteworthy that although potassium release during gastric digestion was low in sample 16 (the highest potassium content), its potassium release was about 80% in the pancreatic stage (Fig. 1). Generally, potassium release averaged 70 (g/100g K in solids) in the pancreatic stage, and since absorption of (micro)nutrients occurs mainly (Agarwal *et al.*, 1994) during pancreatic digestion (small intestine), it can be inferred that the bulk of potassium in the sweetpotato samples would be released and available to be transported for absorption.
- Gastric or pancreatic digestion time did not significantly ( $p>0.05$ ) affect the release of potassium in the samples. While this is surprising, it shows that potassium release from the sample was almost complete within the first 10 min. Dietary potassium is reported (Demigne *et al.*, 2004) to be very soluble in

gastrointestinal fluids, and the measured rapid release might be connected with its solubility once the cell structures had been enzymatically ruptured. Such a rapid release of (micro)nutrients can trigger off various physiological responses in the host. For example, rapid digestion reduces the feel of fullness (satiety) and increases consumption (or feed intake).

- Although pancreatic digestion was preceded by gastric digestion, more potassium (0 – 40 g/100g K in solids) was significantly ( $p < 0.05$ ) released during the former (Fig. 1). The increase is expected because as more intact sweetpotato cells were penetrated by the (starch-)digestive enzymes, and more starch polymers were digested, more free and possibly bound or physically hindered potassium were released and solubilised.



**Figure 1: Typical potassium digestograms of the sweetpotato (unshaded symbols = gastric digestion; shaded symbols = pancreatic digestion)**

**Starch digestion.** Irrespective of the cultivar, location, province, and farmer, digested starch significantly ( $p < 0.05$ ) increased with time in a non-linear manner (Fig. 2), and two phases are discernible in the starch digestograms (biphasic digestogram). Digestion is dependent on starch properties such as amylose:amylopectin ratio and crystalline structures. Although quarantine requirements hindered detailed characterization of the samples, sweetpotato starch generally exhibits an A-crystalline pattern (Wolf, 1992), which is common in cereals. In our studies of starch digestibility in cereals, we observed monophasic digestograms in milled sorghum grain and waxy maize starch, while regular and high-amylose (50 and 80% amylose) maize starches exhibited biphasic behaviours (Sopade *et al.*, 2008; Mahasukhonthachat *et al.*, 2009; Sopade, 2009). It is doubtful if the crystalline patterns in the sweetpotato samples differed, but amylose content might vary. Although there are genotype and environmental (G x E) variations, amylose content of sweetpotato generally ranges from 20 - 25% (Wolf, 1992). However, since all the 20 samples exhibited the same digestion pattern, the influence of variation in amylose content was probably not pronounced.

Unlike the maize starches that changed in digestion pattern with amylose content (Sopade, 2009), the sweetpotato samples were complete but dried and milled food systems with starch and non-starch components. Sopade *et al.* (2008) proposed that plant cell walls and/or their components possibly hinder (starch-)enzyme diffusion, penetration or transport culminating in a low initial rate of digestion. But once the hindrance had been overcome, the surfaces of the substrates, in this case starch, were then easier to saturate with a concomitant increase in the rate of digestion. Moreover, the possibility of non-starch components

inhibiting or delaying enzyme activity cannot be ruled out. We recognize that even with cell walls and/or their components, complete but milled sorghum grains exhibited monophasic digestogram (Mahasukhonthachat *et al.*, 2009). However, cell wall structures and constituents in cereals and root crops are possibly neither identical nor the same. In addition, it should be stressed that the drying operation, to which the sweetpotato samples were subjected, could have affected their structures and rehydration characteristics. Ly *et al.* (1999) observed changes in digestion as a result of drying.

While there are many theoretical and empirical models to describe monophasic starch digestograms, models to describe biphasic digestograms are non-existent. This is because biphasic digestograms have not been widely reported. We are currently investigating and developing models for biphasic digestograms. However, using digestion at times  $t = 0$  min. as a measure of gastric digestion of starch (or very rapidly digested starch), and  $t = 240$  min. as a measure of pancreatic digestion, ANOVA revealed the samples were significantly ( $p < 0.05$ ) different with no specific location, cultivar, province, or farmer effects. Gastric digestion of the sweetpotato starch ranged from 2 – 9 g/100g dry starch, while pancreatic digestion was substantially higher and ranged from 48 – 75 g/100g dry starch (Fig. 3). Although starch properties determine starch digestion, the amount of starch available for digestion is also important. This is because samples 8 and 18, with the highest starch content (Table 1), were effectively the least digested, and a significant inverse relationship ( $r^2 = 0.566$ ,  $p < 0.001$ ) was obtained between starch content and pancreatic starch digestion.

A remarkable observation from the time-course starch digestion of the 20 samples is the presence of a sample (Sample 8) with a substantial amount of possible resistant starch. Apart from the possibly slight heat-moisture effects of oven drying, the samples were not heat-moisture processed in any way. Therefore, the possible resistant starch in the sample is of the types RS1 (encapsulated starch) and/or RS2 (raw or uncooked starch) according to the Englyst classification (Englyst *et al.*, 1997). Resistant starch and its determining effects on glycaemic index, as well as its associated health and nutritional benefits, are subjects of many studies (e.g. Goñi *et al.*, 1997; Brouns *et al.*, 2002). Samples with a high resistant starch are beneficial to gut health, and can be incorporated into various processed foods to boost the amount of resistant starch. Although detailed characterisation of these sweetpotato samples is required to confirm their suitability as a natural source of resistant starch, the present study suggests samples 8 and 18 are potentially suitable. It is worth stressing that animal studies (Suzuki *et al.*, 2002) revealed sweetpotato helps stabilize blood sugar levels and lowers insulin resistance in diabetic individuals. Therefore, our deductions of a high amount of potential resistant starch in sweetpotato are not untenable.

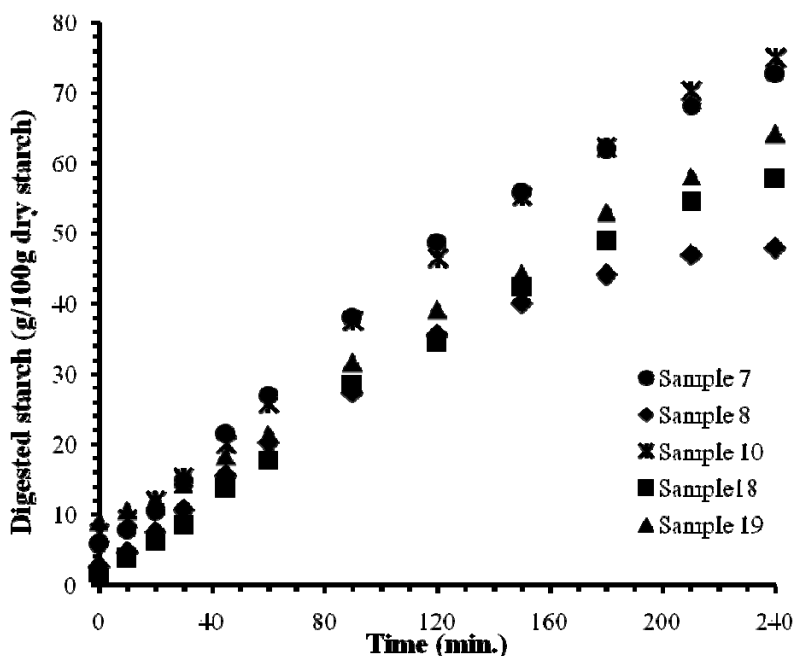
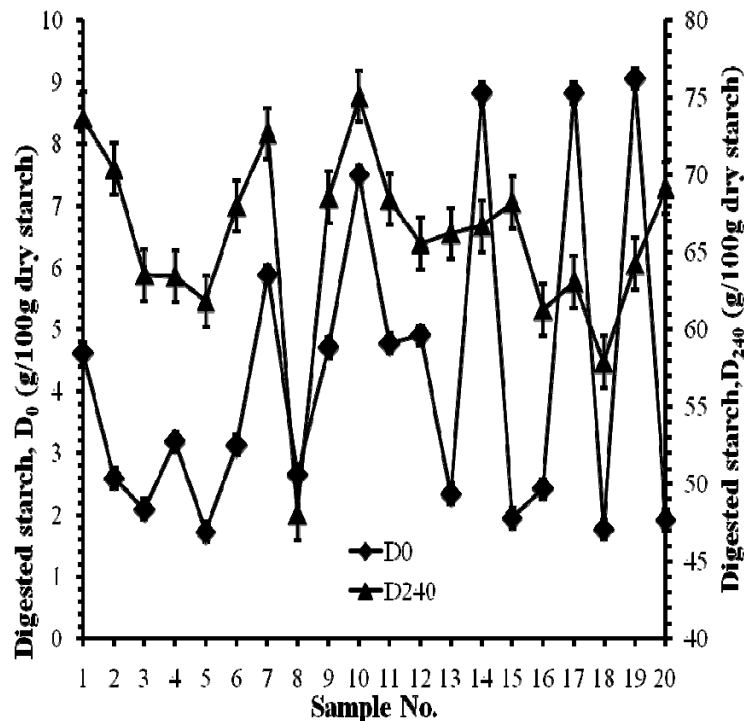


Figure 2. Typical starch digestograms of the sweetpotato





**Figure 3: Line diagrams of digested starch at times  $t = 0$  min. ( $D_0$ , gastric digestion) and  $t = 240$  min. ( $D_{240}$ , pancreatic digestion)**

## Conclusions

Analysis of 20 sweetpotato samples from PNG showed differences in the release of potassium and digestion of starch, but there were no marked genotype and environmental ( $G \times E$ ) effects, even though  $G \times E$  can determine plant properties and functionalities. Irrespective of the sample, potassium was readily released and independent of time of digestion. However, more potassium was released during pancreatic digestion than during gastric digestion. The gastric-pancreatic trend was identical to starch digestion, which was additionally dependent on digestion time in a non-linear manner. Remarkably, some samples potentially exhibited high resistant starch, and being natural food systems, these samples could be used to lower glycaemic index in processed foods, where high glycaemic index is a concern. With many cultivars of sweetpotato important for their vitamin and antioxidant properties (Wallerstein, 2000), identification of some cultivars with potential for high resistant starch, and rapid release of potassium can stimulate further interests in sweetpotato as a valuable food material. This will widen and increase its utilization for health and nutrition benefits, prompting an increase in global production of sweetpotato.

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# Post-harvest deterioration in cassava: from understanding towards control

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The starchy storage roots of cassava (*Manihot esculenta* Crantz) deteriorate within 24 – 72 hours of harvest rendering them unpalatable and unmarketable. With growing urbanization and the entry of cassava into the cash economy this post-harvest physiological deterioration (PPD) has become a major constraint to the development of this important crop, affecting farmers, processors and consumers alike. The physiological changes that occur during PPD are due to the oxidation of phenolic compounds and involve the formation of reactive oxygen species (ROS), alterations in gene expression, protein synthesis and the accumulation and oxidation of a range of secondary metabolites. Biochemical and molecular data confirm that the production and reactions of ROS are central to PPD, and a model suggesting that PPD is a ROS-mediated programmed cell death (PCD) response has been proposed. This model implies that enhancing the anti-oxidant or anti-PCD status of the cassava root at or shortly after harvest may limit the reactions, damage and changes induced by ROS. We are testing this model in transgenic cassava in which we have separately introduced five anti-oxidant genes and three anti-PCD gene driven by a root-specific promoter. The resultant data will be invaluable both for confirming and refining the model, and for moving towards the identification of means by which to usefully modulate the PPD response of cassava roots, as the ultimate goal of this research is to benefit the sustainable livelihoods of resource-poor farmers.

**Keywords:** Cassava, post-harvest physiological deterioration, reactive oxygen species, programmed cell death, transgenic.

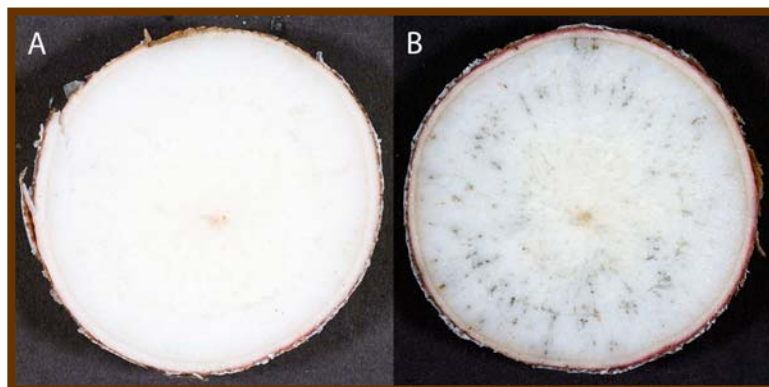
## Introduction

The Green Revolution of the 1960s and 1970s achieved spectacular results in increasing the production of the world's major cereal crops, thereby saving millions from famine and keeping pace with burgeoning population increases; however, the recent recognition of a global food crisis, in addition to the energy and climatic crises, suggest a pressing need to focus again on global crop production in addition to addressing other contributory factors (Schmitz & Kavallari, 2009). Moreover, there is increasing evidence that the gains achievable through classical breeding may have been reached for the major crops and that both new technologies and ignored crops have to become included in improvement programmes (Borlaug, 2000). In addition, Africa, a continent that was bypassed by the Green Revolution and much of whose population suffer from poverty and inadequate diet, desperately needs major research effort focused on improving its important crops, including cassava; crops which may prove more amenable to biotechnological solutions than conventional breeding (Thomson, 2008). It is particularly in Africa that cassava, both as a crop and a resource, has a major contribution to play.

Cassava (*Manihot esculenta* Crantz) is cultivated throughout the humid tropics for its starch-rich storage roots, which yield more energy per hectare than other major crops (Montagnac *et al.*, 2009). It is the world's sixth most important crop in terms of production (Mann, 1997), serves as the staple food for over 500 million, and is particularly important for the sustainable livelihoods of resource-poor farmers in sub-Saharan Africa (Cock, 1985). Increasingly, cassava is also becoming a cash-crop and is being used as input for higher value commodities such as starch or biofuels. Despite its many advantages as a crop, cassava suffers from several constraints that limit its production and its value as a food. In Africa cassava is predominantly cultivated by poor farmers, often on impoverished soils where other crops do not grow well; this severely limits productivity to well below the maximum recorded yield of 90 tonne/ha (El-Sharkawy, 2003). Average productivity for Africa was 8.8 tonne/ha for 2007 - as a point of reference, India's productivity was 32.9 tonne/ha for the same year (FAOSTAT, consulted on 15/07/2009). Therefore, while the distribution of improved germplasm could contribute to improving productivity, it is largely available soil quality and agronomic practices that severely limit cassava productivity in Africa. In addition, cassava suffers from biotic and abiotic constraints. These include: pathogens, particularly viral and bacterial; the possession of cyanogenic glycosides; a short shelf-life of the harvested roots of 24 – 72 hours; and poor nutritional content in terms of quantity and quality of protein, and low abundance of important micronutrients. Due to breeding difficulties with cassava, as a result of high heterozygosity, poor

flowering ability and clonal propagation via stem cuttings, many of these constraints may be more amenable to resolution via biotechnological approaches. Moreover, the production of some novel or high value products from cassava roots could be also facilitated through genetic manipulation.

A major constraint to the development of cassava as a crop is the short shelf-life of its roots, which deteriorate within 24 – 72 hours of harvest (Figure 1; Beeching *et al.*, 1998). This post-harvest physiological deterioration (PPD) is becoming increasingly important due to urbanisation in producing countries and the resultant increase in distances and time between farm and market or processor. A recent *ex ante* impact analysis of the economic benefits to African countries of developing cassava cultivars with delayed PPD indicates that this would have a major impact (Rudi, 2008). The physiological changes that make cassava storage roots unpalatable and unmarketable

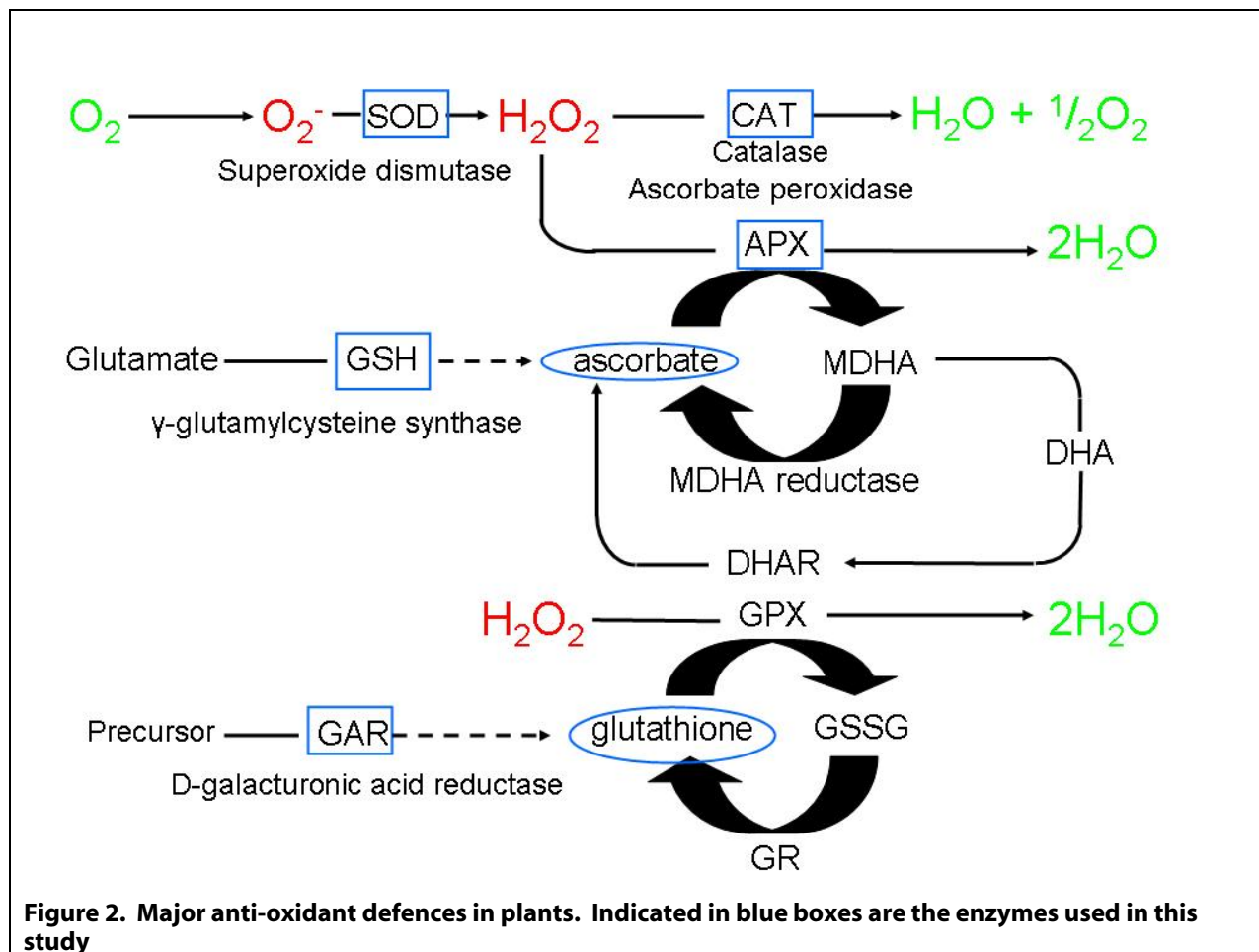


**Figure 1. PPD in cassava. A, section through cassava root at harvesting. B, cassava root at 48 hours after harvesting showing symptoms of PPD.**

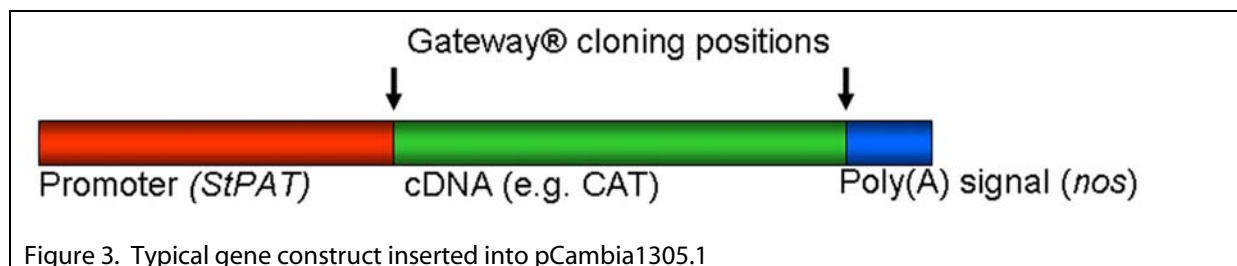
during PPD are due to the oxidation of phenolic compounds and involve the formation of reactive oxygen species (ROS), alterations in gene expression, protein synthesis and the accumulation and oxidation of a range of secondary metabolites (Beeching *et al.*, 1998; Buschmann *et al.*, 2000a; Buschmann *et al.*, 2000b; Han *et al.*, 2001; Reilly *et al.*, 2001). Biochemical and molecular data confirm that the production and reactions of ROS are central to PPD, and a model of PPD as a ROS-mediated programmed cell death (PCD) response has been proposed (Reilly *et al.*, 2003; Reilly *et al.*, 2007). This model predicts that enhancing the anti-oxidant or anti-PCD status of the cassava root at or shortly after harvest may limit the reactions, damage and changes induced by ROS. In this paper we describe experiments to test this hypothesis in transgenic cassava plants. The resultant data from these plants will be invaluable both for confirming and refining the model, and for moving towards the identification of means by which to usefully modulate the PPD response of cassava roots for the benefit of farmers and consumers.

## Experimental

Reactive oxygen species (ROS) play multiple roles in plants: as signalling molecules, in development, and defence, as well as being toxic and damaging. Their role depends largely upon species, concentration and localisation. To protect themselves against the damaging effects of ROS plants produce anti-oxidant enzymes and compounds (Figure 2).

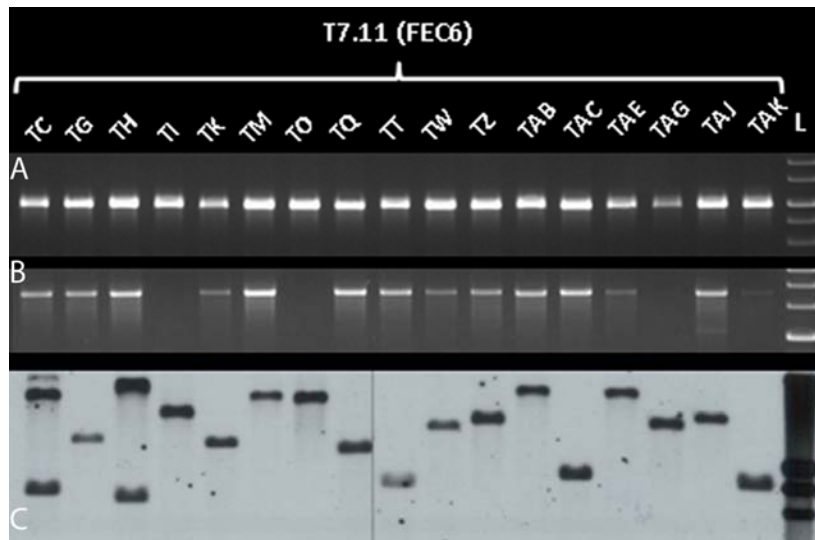


We have isolated full length cDNA clones for Cu/Zn superoxide dismutase, catalase and ascorbate peroxidase from cassava (Gómez-Vásquez *et al.*, 2004; Reilly *et al.*, 2003; Reilly *et al.*, 2007) and cDNAs for  $\gamma$ -glutamylcysteine synthase and D-galacturonic acid reductase from *Arabidopsis* and strawberry, respectively, were obtained (<http://www.brc.riken.go.jp>; Agius *et al.*, 2003). These cDNAs were cloned using Gateway® cloning (<http://www.invitrogen.com>) into pCambia1305.1 ([www.cambia.org](http://www.cambia.org)) that we had modified as follows: the CaMV35S promoter and the GUSPlus reporter gene had been removed and replaced by the *StPAT* patatin promoter, which has been shown to be root-specific in cassava (Ihemere *et al.*, 2006); and the vector had been modified to permit Gateway cloning after the *StPAT* promoter (Figure 3).



A cDNA microarray analysis of PPD had, in addition to ROS modulation, highlighted the expression of pro- and anti-programmed cell death (PCD) genes during the PPD response (Reilly *et al.*, 2007). PCD shares many features with apoptosis, which is much better characterised in animal systems (Delgado *et al.*, 2007). The expression of anti-apoptosis genes in plants has been shown to inhibit oxidative stress induced PCD through

promoting the scavenging of free radicals (Chen *et al.*, 2003; Li & Dickman, 2004). Therefore, we have made gene constructs similar to the anti-ROS constructs above, in which three different anti-apoptotic genes were driven by the StPAT promoter and then transformed them into cassava. These PCD experiments are solely to test the effect of these genes on the plant and root in the laboratory, if the results suggest that this approach might offer a means to control PPD, we would employ plant functional homologues for further work.



**Figure 4. Example of confirmation of transgenesis. A, PCR with hygromycin primers; B, PCR with -glutamylcysteine synthase primers; Southern blot probed with hygromycin gene.**

current understanding of the problem suggests experiments in which the expression of likely candidate genes involve in both anti-oxidant defence and the regulation of PCD pathways can be modulated using an organ-specific promoter. Sufficient lines of cassava plants containing eight candidate genes have been produced and their transgenic nature confirmed. The full analysis of these plants will not only increase our understanding of PPD by should also point to specific genes with the potential to control the deterioration response.

At least 30 transgenic cassava lines for each construct, five anti-ROS, three anti-PCD, plus the necessary controls, have been produced. These have been tested by PCR and Southern blotting to confirm the presence and copy-number of the transgenes (Figure 4). The transgenic plants are currently being grown to maturity so as to enable, phenotypic biochemical and molecular analyses of the developing plants and their storage roots.

## Discussion

Cassava PPD is a signification limitation to the achievement of the full potential of this important crop. While it is a complex response the

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# Extraction and characterization of polisaccharides of *Lepidium meyenii* Walpers root. comparison of a processed product and fresh root after drying and grinding

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## Abstract

Maca (*Lepidium meyenii* Walpers) is a Peruvian plant. The roots are used as food and popular medicine. In this work, we have isolated and characterized the cell-wall polysaccharides. The material was defatted, the enzyme inactivated and treated with  $\alpha$ -amylase and then subjected to aqueous (25 and 93°C) and alkaline extractions sequences (1M, 2M and 4M NaOH at 25 °C). For extractions two materials were used: a commercial product known as maca powder and fresh roots dried and ground. The composition of the neutral monosaccharides was determined by GLC and acidic ones colorimetrically. Although the monosaccharide composition of the fractions was similar, in general, there was a higher content of xylose and less glucose in fractions obtained from maca powder when compared to the roots, suggesting the occurrence of changes during post-harvest processing that affect the solubility of polymers.

**Keywords:** *Lepidium meyenii*, maca, polysaccharides.

## Introduction

Maca (*Lepidium meyenii*) is a plant native of Peru, the roots have economic interest due to their medicinal and nutritional properties. Since the sixteenth century the native people of the Central Andes of Peru believed that the use of maca improves human reproduction and invigorates those who consume it (CHACON, 1961; GONZALES, 2001). This plant is grown from centuries ago and is used by the natives as food and folk medicine, in treating anemia, tuberculosis and sterility (CICERO *et al.*, 2001; ZHENG *et al.*, 2000).

Several investigations attempt to prove the properties attributed to this root, as well as some components of low molar mass were studied (CUI *et al.*, 2003; RONCERO *et al.*, 2005, ZHENG *et al.*, 2000).

The maca root contains a large amount of starch (PAULET; CARRASCO, SÁNCHEZ, 2006), but after an extensive literature review, no data have been found on other polysaccharides. In this study we isolated and characterized the polysaccharides extracted from the cell wall of maca root (*Lepidium meyenii*).

Polysaccharides are important chemical constituents of plants because they are the main components of the cell wall (structural support for plant cells) (REID, 1997). The cell wall is a dynamic mechanical and biological structure, that suffer changes along with life (McCANN *et al.*, 2001) and is involved in the morphology, growth and development of cells and interactions between host plants and their pathogens, and works as a physical barrier against microorganisms and other harmful agents (CARPITA and McCANN, 2000, TALMADGE *et al.*, 1973).

An important part of the cell wall is formed by hemicellulose, which are polysaccharides intimately associated with cellulose, defining structural properties of the cell wall such as regulating the growth and development of plants. The hemicellulose compounds include xyloglucans, glucurono arabinoxylans, mannans, galactomannans, glucomannans and galactoglucomannans. In dicotyledons such as maca, the principal hemicellulose component are xyloglucans.



## Materials and methods

### *Samples*

A commercial processed powder of maca root (*Lepidium meyenii*) was purchased in the market (Maca powder of Santa Natura by Agroindustrias Floris SAC; Lima, Peru). This sample was identified as "M".

A second sample correspondED to a fresh maca root bought in the municipal market (San Camilo) in Arequipa city, Peru. The sample was washed with sodium hypochlorite, sun dried, ground to powder and identified as "R".

### *Extraction of polysaccharides from maca powder*

The maca powder was submitted to enzyme inactivation with methanol–H<sub>2</sub>O (4:1, v/v) under reflux for 20 min, immediately cooled to room temperature, and centrifuged. Insoluble material was sedimented at 15,400g for 20 min, washed with ethanol, dried under vacuum, milled, and defatted with p-toluene– ethanol (2:1, v/v) in a Soxhlet extractor. The solid was dried and used to remove the starch. The maca powder was treated with alpha amylose enzyme TDF-100A A3306 from Sigma Aldrich, at 50 ± 5 ° C. The degradation of starch was monitored with the iodine test. The material was centrifuged and the residue washed with water and ethanol and dried, resulting in maca powder free of starch, which was used for polysaccharide extraction. Successive extractions were performed in a mechanical blender and after each one, centrifugation was carried out and the residue was submitted to the next extraction. Each extract was concentrated and treated with ethanol (2:1, v/v) in order to obtain precipitated polysaccharides, which were then washed three times with ethanol and dried under vacuum.

Maca powder defatted, inactivated and free of starch was subjected to sequential extractions with water at 93 °C and NaOH solutions at concentrations: 1, 2 and 4 mol L<sup>-1</sup>. Alkaline extractions were performed in the presence of sodium borohydride; acidified with 50% acetic acid until pH 5. The polysaccharides were separated by centrifugation, dried under vacuum and labeled as hemicellulose A. The supernatant obtained after precipitation was dialysated, concentrated and precipitated with ethanol 3:1 v/v, yield in formation of the hemicellulose B fraction, which was separated by centrifugation and dried under vacuum.

### *Determination of total sugar, protein and uronic acid content*

Total carbohydrate was measured by the phenol–sulfuric acid method (DUBOIS *et al.*, 1956), using glucose as standard. Uronic acid was estimated by the sulfamate/ 3-phenylphenol colorimetric method (FILISSETTI-COZZI and CARPITA, 1991), using galacturonic acid as standard. Protein was determined according to Peterson (1977), using BSA as standard.

### *Monosaccharide composition*

Polysaccharides were hydrolyzed with 2 M trifluoroacetic acid (5 h, 100 °C), and after concentration to dryness, the residues were reduced with NaBH<sub>4</sub> (WOLFROM and THOMPSON, 1963b) and acetylated with pyridine–acetic anhydride (1:1, v/v, 16 h, at 25 °C) (WOLFROM and THOMPSON, 1963a).

The final residue was solubilized and partially hydrolyzed with 72% (w/w) H<sub>2</sub>SO<sub>4</sub> for 1 h at 0–4 °C, diluted to 8% and kept at 100 °C for 15 h (BIERMANN, 1989). The hydrolysate was neutralized with BaCO<sub>3</sub> and the insoluble material removed by filtration. Monosaccharides were reduced and acetylated as described above.

The resulting alditol acetates were analyzed by gas–liquid chromatography (GLC) using a model 5890 S II Hewlett–Packard gas chromatograph at 220 °C (flame ionization detector and injector temperature, 250 °C) with a DB-210 capillary column (0.25 mm internal diameter x 30 m), film thickness 0.25 µm, with nitrogen as carrier gas (2.0 ml/min).

### *HPSEC–MALLS analysis*

High pressure size exclusion chromatography (HPSEC) was carried out using a multidetection equipment: a Waters 2410 differential refractometer (RI) and a Wyatt Technology Dawn F multi-angle laser light scattering (MALLS) detector. Four Waters Ultrahydrogel 2000/500/250/120 columns were connected in series and coupled to the multidetection equipment. A 0.1 mol L<sup>-1</sup> NaNO<sub>2</sub> solution, containing NaN<sub>3</sub> (0.5 g L<sup>-1</sup>), was used as eluent.

Previously filtered samples (0.22  $\mu\text{m}$ ; Millipore) were analyzed at 1.5  $\text{mg mL}^{-1}$  and the data were collected and processed by a Wyatt Technology ASTRA program.

## Results and discussion

After the defatting, the material was subjected to enzyme inactivation with methanol-water (4:1, v/v). Normally this treatment is also effective in removing low-mass carbohydrates, as saw in this case, where the extract was viscous, indicating the presence of compounds with higher molar mass. The extract was treated with ethanol (3v), promoting the precipitation of a fraction that was called MMe.

According to Paulet, Carrasco and Sanchez (2006) dried maca roots contain 20% of starch. This was characterized (RONDAN-SANABRIA and FINARDI-FILHO, 2008), the maca powder inactivated, and defatted was subjected to hydrolysis with  $\alpha$ -amylase to remove starch and thus facilitate the extraction and study of other present polysaccharides. After the enzymatic digestion, the powder was centrifuged and the residue was washed with water and ethanol and subsequently dried, resulting in maca powder free of starch, which was used for the extractions of the cell wall polysaccharides. The supernatant was boiled for 30 minutes and the denatured enzyme was removed by centrifugation. After removing the enzyme, the supernatant dialysate was concentrated and precipitated with ethanol, resulting in the fraction labeled as MFW.

The maca powder defatted, inactivated and free of starch was subjected to sequential extractions with water at 93 °C and NaOH solutions at concentrations of 1, 2 and 4  $\text{mol L}^{-1}$  at 25 °C.

The extracted fraction after the alkaline treatment was acidified with acetic acid 50% and polysaccharides that precipitated under these conditions were called hemicellulose A. The resulting supernatant dialysate was concentrated and then precipitated with 3 volumes of ethanol, forming the hemicellulose B fraction. For the extract obtained with the 1  $\text{mol L}^{-1}$  NaOH solution, a new precipitate was formed during the dialysis which was called hemicellulose D (dialysis).

The identification codes followed the next criteria: all fractions initially received the letter "M" regarding to maca, followed by "M" that identifies the initial sample as maca powder. The next characters refer to the extraction medium used: HW (hot water or in the case of alkaline extractions, a number corresponding to the molar concentration of the solution was used followed by "A", "B" or "D", according to the type of hemicellulose A, B or D.

The final residue obtained after the extraction was washed with water to neutrality for subsequent hydrolysis.

Table 1 shows yield of polysaccharide, total sugar and protein content in the fractions obtained. All fractions have low yields; among these, the fractions MMe, MMW and MMHW had the highest yield. From the alkaline extraction, the fraction MM4B obtained with 4  $\text{mol L}^{-1}$  NaOH, had the highest yield (1.9%), followed by MM2B (1.3%), obtained with 2  $\text{mol L}^{-1}$  NaOH.

In the alkaline fraction, the yield of hemicellulose B was higher than that of hemicellulose A, suggesting the predominance of high molar mass polysaccharides.

To investigate the differences between polysaccharides present in fresh roots of maca and the commercial product

**Table 1. Yield, total sugar and protein content in polysaccharide extracts from maca powder**

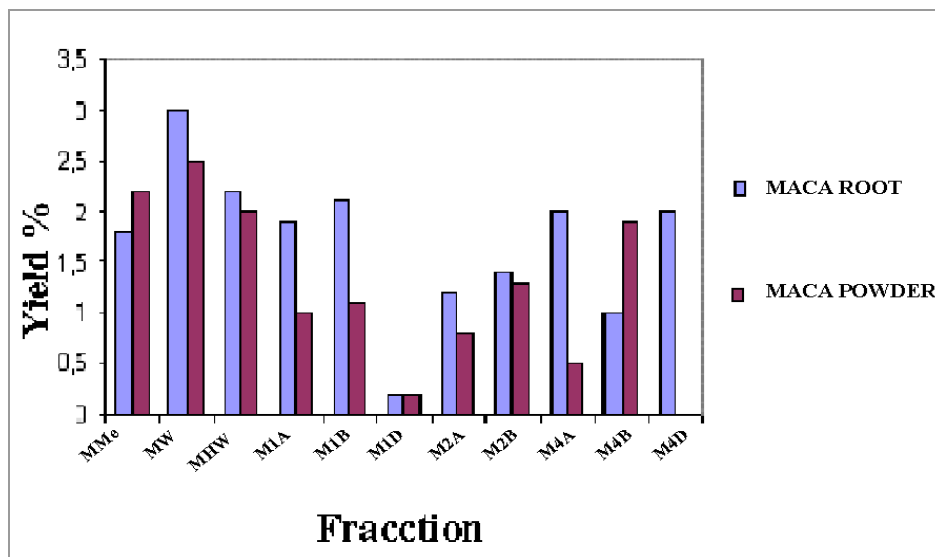
Fractions	Yield <sup>a</sup> % (m/m)	Total sugar <sup>b</sup> (%)	Protein <sup>c</sup> (%)
MMe	2.2	52.2	10.6
MMW	2.5	52.1	18.2
MMHW	2.0	43.0	2.3
MM1A	1.0	29.9	59.1
MM1B	1.1	39.8	33.4
MM1D	0.2	11.6	8.4
MM2A	0.8	9.2	26.7
MM2B	1.3	81.0	14.8
MM4A	0.5	4.3	17.3
MM4B	1.9	34.3	34.2

<sup>a</sup> Of maca powder defatted.

<sup>b</sup> Dubois method (1956).

<sup>c</sup> Peterson method (1977).

known as maca powder, extractions were done using the maca root. This was sun-dried, ground (50g) and subject to the same treatment applied on maca powder. The roots defatted, inactivated and free of starch were extracted in turn with water and alkaline solution using the protocol previously used for extraction of the maca powder. For the identification of fractions, the same criteria used above was followed, except for the substitution of "M" by "R" (roots).



**Figure1. comparison of the polysaccharides yields from roots and maca powder**

The yields of fractions obtained from the roots and maca powder in water had similar values, whereas with the alkaline extractions larger differences were observed. In general, the yield of alkaline fractions from the roots of maca was higher than that obtained from the maca powder. The fractions obtained with 1 mol L<sup>-1</sup> NaOH from the root (MR1A and MR1B) was the double of the fractions obtained from the maca powder (MM1A and MM1B) and the fraction obtained with 4 mol L<sup>-1</sup> NaOH from the root MR4A was four times higher than the fraction from maca powder (MM4A).

The fraction MR4B presented half of the yield from the fraction MM4B. When the total polysaccharides extracted with 4 mol L<sup>-1</sup> NaOH are considered, the yield from the roots was 2.1 times higher than for "maca powder".

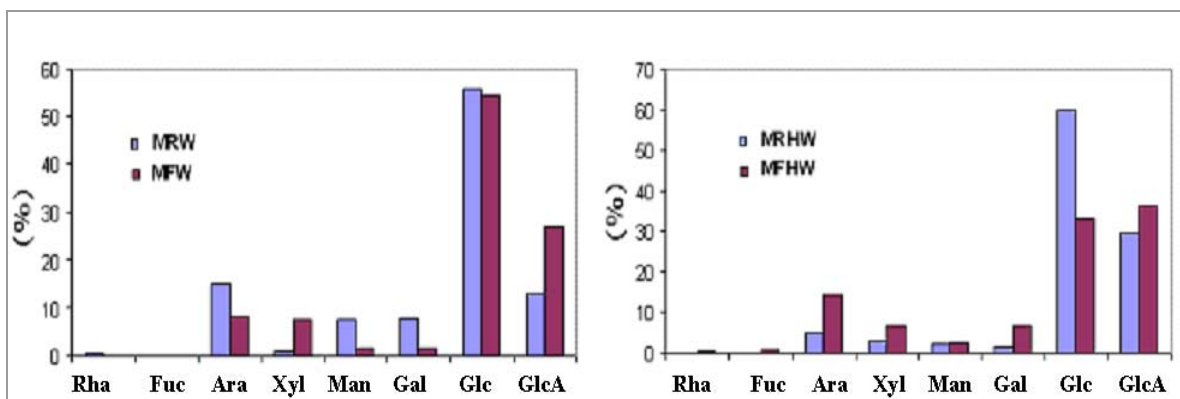
Shiga, Lajolo and Filisetti (2004) studied changes in the cell wall of beans after 24 months storage at room temperature and observed that the amount of soluble material in water practically was not altered. In the meantime, the authors noted that the amount of material extracted with NaOH 4 mol L<sup>-1</sup> presented a small increase after the storage of the beans.

The observed differences may be due to changes that occur during the storage or even may be due to differences during the post-harvest processing, such as time and intensity of exposure to the sun while drying. Graefe *et al.*, (2004) evaluated the effects of post-harvest processing on oligosaccharides of yacon roots (*sonchifolius Smalanthus*) and found that the exposure to the sun for six days decreased the levels of oligosaccharides.

Monosaccharide composition of the fractions obtained from extraction of the maca root is indicated in Table 1.

Comparing the composition of the fractions obtained from roots with those of maca powder, it is noted that the aqueous fractions are similar (Figure 2). The hot water fraction obtained from roots presented a higher content of glucose when compared with maca powder. Moreover, in the same fraction of maca powder there is a higher extraction of polymers containing xylose and arabinose compared to the extract obtained from the root. A much

higher percentage of uronic acid and xylose was observed for MMW fraction when compared to the equivalent fraction obtained from roots (MRW).



**Figure 2. Composition of monosaccharide fractions extracted with water (normal and hot) obtained from root and maca powder**

In Figure 3 the monosaccharide compositions of hemicellulose A and B obtained with NaOH 1, 2 and 4 mol L<sup>-1</sup> are compared.

The alkaline extractions also yielded a higher amount of Xyl in hemicellulose A fractions obtained from maca powder compared to the fractions obtained from roots. Just as observed for aqueous extractions, a greater amount of glucose was detected in the fractions from roots. These results suggest that during drying and storage can occur alterations in the quantity, structure and/or molar mass of polysaccharides, which affect their solubility. Possible changes that may occur in the polysaccharides during post-harvest processing could alter not only the individual polymers, but also could affect the degree of intermolecular association and hence its solubility.

## Conclusion

Based on the aqueous extraction of maca powder (*Lepidium meyenii*) inactivated, defatted and free of starch, the mixtures of polysaccharides were obtained, whose composition suggests the presence of glucans and acid polysaccharides.

Comparing the composition of the fractions obtained by aqueous and alkaline extractions of the commercial product known as maca powder and fresh roots after drying and grinding, some compositional differences were detected, suggesting the occurrence of changes during post-harvest processing that affect the solubility of polymers.

## Acknowledgements

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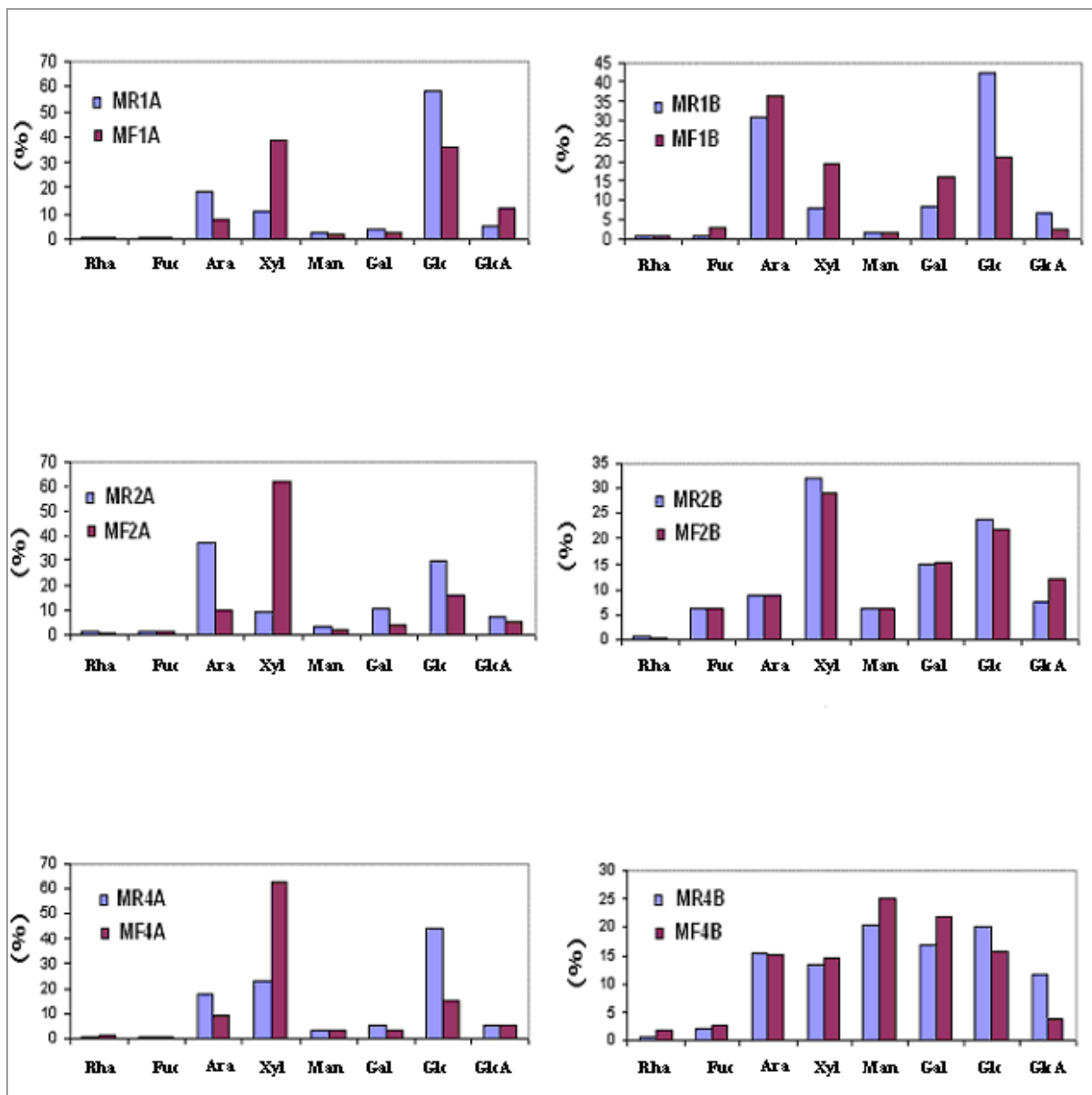


Figure 3. Composition of monosaccharide fractions from alkaline treatment inobtained roots and maca powder".

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# Evaluation of bread made with sweetpotato flour

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## Abstract

Wheat and wheat flour to make bread is expensive and must be imported in most tropical countries. The objective of this study was to determine the nutritional value of bread made from wheat (WF) and sweetpotato flour (SF). Four different treatments were evaluated according to the level of replacement of wheat flour with sweetpotato flour [(1) 100% WF : 0% SF; (2) 90% WF : 10% SF; (3) 80% WF : 20% SF; (4) 70%WF : 30% SF). The parameters determined in treatments were: moisture, protein, fat, fiber, ash, carbohydrates and  $\beta$ -carotene, and the sensorial parameters: color and crust of bread, crumb structure, texture, aroma and flavor. The analysis of variance (ANOVA) showed significant differences between treatments for the variables moisture, protein, fat, fiber, ash, carbohydrates and b-carotene and the DUNCAN test revealed that the bread with the highest substitution of wheat flour (70%WF : 30% SF) has the highest percentage of humidity, fiber, fat, ash and beta carotene. For the qualitative variables, the samples were analyzed by the Kruskal Wallis test. The analysis showed a relationship of intrinsic flavor and aroma to treatment 70%: 30% (WF : SF). However, the best relate to all the sensory attributes was observed for treatment 80%: 20% (WF: SF), which obtained the highest acceptance.

## Introduction

The food supply has been, throughout history, a constant in the fundamental preoccupations of the man (Cheftel and Cheftel, 1992), being well-known the lack of an adapted nutritional contribution in the vulnerable population of our country, like are the pre-schoolers and suckling babies, due to joint of socioeconomic factors that derive in deficiency states. (FAO, 2004).

At the moment, deficiency of micro-nutrients has been recognized in diverse countries, especially in developing countries and it is known that it has seriously effects the health of children, between which they emphasize the undernourishment by protein and deficiency of vitamin A and iron. (Mendez, 2001). Peru is also facing these problems as it reports the rehearse on Nutrition made by the Bottom of the United Nations for the Childhood. The report reveals that the rate of mortality of the minors of five years in Peru is of 58/1000 born alive ones, which is the third highest one of Latin America, after Haiti and Bolivia (UNICEF, 1998).

Many developing countries and several international organizations (OAA/FAO, the WHO, FDA, CIP among others) are establishing strategies to eradicate these nutritional deficiencies. One of them consists on the enrichment and food fortification of massive consumption, to improve the vitamin A ingestion, and another one stimulating the food consumption of vegetal origin, so it is the case of the sweet potato, which is rich in B-carotene, precursor of this vitamin (Mendez, 2001).

Sweet potato has a large potential to be used as a food in developing nations with limited resources because of its short maturity time and ability to grow under diverse climatic condition and on less fertile soil (Collins, 1989). Sweet potato flour can serve as a source of energy and nutrients [carbohydrates, beta-carotene (provitamin A), minerals (Ca, P, K, Fe, and Zn)], and can add natural sweetness, color, flavor and dietary fiber to processed food products (Woolfe, 1992; Ulm, 1988). Addition of various proportion of sweetpotato flour in wheat flour can increase the nutritive values in terms of fibre and carotenoids. This also helps in lowering the gluten level and prevent from coeliac disease (Tilman et al., 2003).

The aim of this study was to determine the effects of adding different levels of SF on the physico-chemical and the sensory properties of bread. The task of improving the acceptance level falls mainly to the consumers, some of the ways to evaluate the quality of a product are: subjective or sensorially and another one using instruments and chemicals to quantify the nutritional composition.

## Materials and methods

The genotype of sweet potato 440442 pertaining to the Bank of Germplasm of CIP-Lima. was seeded in the Experimental Station of La Molina. After harvest, the roots were cured by 2-3 days to room temperature, after that they were washed by immersion and agitation in cold potable water, then with a brush to eliminate the soil adhered to the peel.

### Flour preparation

The clean roots were taken to the stage of boiling given by water immersion to 100°C by 25 min. The elimination of the peel was realised in manual form, soon the roots were pressed, obtaining a uniform mass, later it was placed in later polythene bags for its freeze-dried, where the product was dried to 37°C ± 2°C with a residual humidity between 5 - 8%, all the process delayed of 19 to 21hr. The milling was realised with an electrical mill allowing addition their sieving (60mesh= 256µm). The final product was packaging in polythene bags and sealed hermetically.

### Bread making

Previously to the elaboration of breads, tests preliminary on small scale were realised with the purpose of establishing the optimal percentage from wheat flour (WF) and sweetpotato flour (SF), obtaining a product of good sensorial characteristics. From these tests we obtained the following treatments expressed in percentage proportion in mass (m/m) of WF and SF: (1) 100% WF: 0% SF; (2) 90% WF: 10% SF; (3) 80% WF: 20% SF; (4) 70%WF: 30% SF (Table 1).

**Table 1. Quantities of wheat flour (WF) and sweet potato flour (SF) as a mixture of ingredients used for making sweet potato bread according to the treatments proposed**

Treatments	WF (g)	SF (g)
100% WF : 0% SF	750	0
90% WF : 10% SF	675	75
80% WF : 20% SF	600	150
70% WF : 30% SF	525	225

## Nutritional evaluation

Determination of the proximal composition of sweet potato breads: for these chemical analyses we applied the recommended methods by A.O.A.C. 1990: (humidity % Part 950.46 pp. 931; Total protein % (N x 6.25) Part 984.13 pp. 74; fat extract %, Part 948.16 pp. 871; Crude fiber %, Part 962.09 pp. 80; Ash %, Part 942.05 pp.70, and carbohydrates by difference).

The determination of the β-carotene content occurred by high performance liquid chromatography (HPLC). Monomeric column C18: with a movil phase: methanol: ter-butyl metil ether, with isocratic elusion: 80:20, with a flux time of 0.8ml/min, both methods proposed by Rodriguez, B.D - Kimura, M. (2004). The method is based on the extraction, in organic phase of carotenoids with acetone, later the carotenes were separated by means of the HPLC.

## Sensory evaluation

A not trained panel was used, integrated by 50 members, to establish what of the treatments has major acceptability (color of the crumb, color of the crust, structures of the crumb, texture, aroma and flavor), evaluated through a preferential test of hedonic scale, where the four treatments were evaluated (Mahecha 1985).

## Physical analysis

We realised a uniaxial compression test, analyzing the texture properties of the sweet potato bread, using a Textometer QTS-25 with software Texture Pro v 2.1, at a speed of 15mm/s with a distance of compression of 50%, calibrated with a cell of load of 5 kg, as shown in figure 1.





**Figure 1. Texture analyzer**

### Statistical experimental design

A complete randomized design was carried out with four treatments ((1) 100% WF: 0% SF; (2) 90% WF: 10% SF; (3) 80% WF: 20% SF; (4) 70% WF: 30% SF). The studied parametric variables (proximal Composition and  $\beta$ -carotene content) were analyzed by an analysis of variance (ANOVA, SAS, 1998). As a multiple comparison procedure the DUNCAN test was used for those results, where the F-test of the ANOVA was significant. The investigated qualitative variables in the sensorial evaluation were analyzed by the nonparametric Kruskal - Wallis test.(Chacín, 2000).

### Results and discussion

The results of the proximal composition of the bread treatments of study are given in table 2. For treatments a similar response was observed regarding to the contents of fat, fiber and ash; the magnitude of these traits increased with the replacement WF by SF.

The ANOVA shows no significant differences between treatments with respect to moisture content. The crude protein content presents a slight diminution when increasing the substitution of WF with SF, as case of the treatment: 70% WF: 30% SF. The results are slightly higher that those reported by Cardenas (1991) who reports 10.6% in dry matter for a bread made from yellow sweet potato. It is important to note that the type of commercial flour contains more protein than the flours to make bread.

**Table 2. Averages for the proximate composition of breads made under the treatments in study**

Treatments	Moisture%	Protein %	Fat %	Fiber %	Ash%	CHOs
90% WF: 10% SF	20.60 <sup>a</sup> ± 0.12	11.55 <sup>a</sup> ± 0.07	10.21 <sup>a</sup> ± 0.01	0.34 <sup>c</sup> ± 0.00	1.05 <sup>c</sup> ± 0.01	55.95 <sup>a</sup> ± 0.07
80% WF: 20% SF	20.82 <sup>a</sup> ± 0.34	11.09 <sup>b</sup> ± 0.05	10.39 <sup>a</sup> ± 0.05	0.67 <sup>b</sup> ± 0.03	1.17 <sup>b</sup> ± 0.01	55.85 <sup>b</sup> ± 0.36
70%WF: 30% SF	20.88 <sup>a</sup> ± 0.01	10.40 <sup>c</sup> ± 0.11	11.38 <sup>b</sup> ± 0.04	0.91 <sup>a</sup> ± 0.01	1.33 <sup>a</sup> ± 0.03	55.09 <sup>c</sup> ± 0.0

Average of three reading

<sup>a,b,c</sup> The values denoted different letters in the same column are significantly different (P < 0.05).

The fat content, measured as fat extract, increases as the proportion of SF increases (70% WF: 30% SF; 90% WF: 10% SF; 80% WF: 20% SF). The observed increase could be attributed to the carotenoid contribution of the sweet potato, since these pigments are soluble in fats and they are quantified with the fat extract. (Béliz and Grosch, 1997).

The crude fiber content, in dry matter increased from 0.34 to 0.91%, due to the rise of the proportion of flour of sweet potato in the treatments. The fiber is an indicative of the cellulose content, hemicellulose and lignin. The ash content increases from 1.05 to 1.33% as sweet potato flour is added to the treatment. This increase can be attributed to the mineral contribution of sweetpotato.

A slight increase of carbohydrates is observed (table 2) with the change from 90% WF: 10% SF to 80% WF: 20% SF, which could be attributed to the humidity diminution in these two treatments. It should be noted that the sweet potato flour causes an increase of the humidity, because the proteins and the starch of the sweet potato retains major amount of water (Bennion, 1970).

## β-carotene content

The β-carotene content of bread from sweet potato in the different treatments appears is given in Table 3. The results obtained by ANOVA and the Duncan test, shows significant differences between treatments ( $P < 0.05$ ).

With increasing percentage of substitution of WF the β-carotene content increases significantly. The treatment 70% WF: 30% SF results in a contribution of 44.43 μg/g of β-carotene in dry matter, which implies an additional contribution to the nutritious value of the wheat: sweetpotato bread, with a value of 372.75μg/100g of (RAE) in dry matter. The need of retinol in infants is of 300 μg per day and the amount of β-carotene to cover this need is of 1800μg per day (Woolfe, 1999). The treatment 80% WF: 20% SF, covers 65% with the daily requirements of this vitamin consuming 100g of wheat: sweet potato bread, which is equivalent to 3 bread units of 34 g.

**Table 3. β-carotene content in the different treatments**

Treatments	β-carotene content (μg/g)	Vitamin A value (μg RAE/100g)
90% WF: 10% SF	5.34 <sup>a</sup>	44.5
80% WF: 20% SF	23.42 <sup>b</sup>	195.16
70%WF: 30% SF	44.43 <sup>c</sup>	372.75

The values denoted different letters in the same column are significantly different ( $P < 0.05$ ).

RAE (Retinol activity equivalents): 12μ β-carotene = 1μg retinol = 1 μg RAE (Trumbo et al., 2001)

## Sensory evaluation

The Kruskal Wallis test shows highly significant differences for the treatments in accordance to the variables color of crust and crumb of bread, crumb structure, texture, flavor and aroma (Table 4).

**Table 4. Average value of sensory variables evaluated for sweet potato bread**

Treatments	CB	CC	CS	T	A	F
100% WF: 0% SF	3.42 <sup>b</sup>	3.10 <sup>b</sup>	3.36 <sup>b</sup>	3.38 <sup>b</sup>	2.92 <sup>c</sup>	2.86 <sup>b</sup>
90% WF: 10% SF	4.08 <sup>a</sup>	3.94 <sup>a</sup>	3.90 <sup>a</sup>	3.94 <sup>a</sup>	3.52 <sup>b</sup>	3.64 <sup>a</sup>
80% WF: 20% SF	4.02 <sup>a</sup>	3.88 <sup>a</sup>	3.72 <sup>ab</sup>	3.98 <sup>a</sup>	3.86 <sup>a</sup>	3.92 <sup>a</sup>
70%WF: 30% SF	4.00 <sup>a</sup>	3.54 <sup>b</sup>	3.74 <sup>a</sup>	3.70 <sup>ab</sup>	3.72 <sup>ab</sup>	3.72 <sup>a</sup>

CB: color of bread crust; CC: crumb color of bread; CS: crumb structure; T: texture; A: aroma; F: flavor.

<sup>a,b,c</sup> The values denoted different letters in the same column are significantly different ( $P < 0.05$ )

The color of bread crumb (CB) is one of the most important visual characteristics of the product bread. The control (100% WF: 0% SF) is significantly less accepted than the other three treatments.



From left to right: (1) 70%WF: 30% SF; (2) 80% WF: 20% SF; (3) 90% WF: 10% SF y (4) 100% WF: 0% SF

**Figure 2. Sweet potato bread with different substitution treatment**

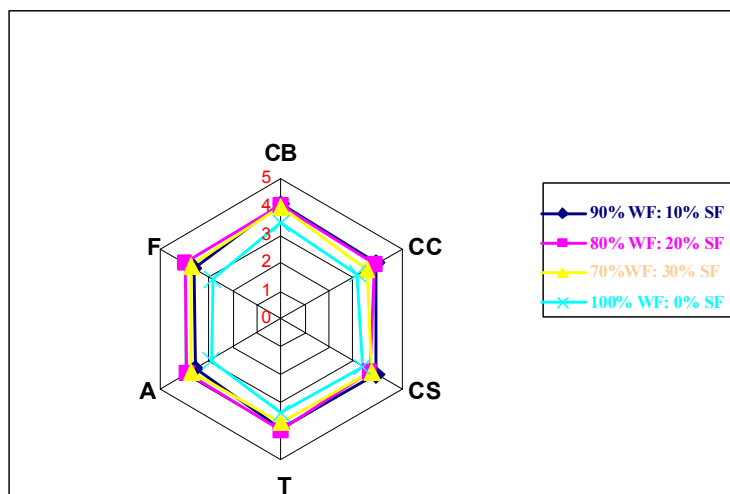
The crust color of bread (CC) - the surface must have a uniform tone -, is directly related to the percentage of substitution. The highest acceptability was observed for 90% WF: 10% SF; however, the difference to 80% WF: 20% SF were not significant.

The crumb structure (CS) is a characteristic that is related to the size of the cells. It must own the same porosity throughout the bread and be free of holes. We observed significant differences ( $p < 0.05$ ) between 100% WF: 0% SF and 80% WF: 20% SF.

The texture (determined by the sense of the tact), indicates the flexibility and smoothness of the crumb. The ideal texture must be smooth and velvety. We observed that treatment 80% WF: 20% SF presents a higher acceptability, which is significant different compared to treatment 100% WF: 0% SF.

The aroma was described as sweet and delicious. The ideal loaf has a pleasant aroma of wheat. On basis of this description we observed that a larger acceptance is obtained with treatment 80%WF: 20%SF, with significant differences in comparison with treatment 100% WF: 0% SF.

The flavor is the most important attribute of good bread. It is the possession of pleasant flavor. We observed that treatment 80% WF: 20% SF presents the highest acceptance, showing significant differences with treatment 100% WF: 0% SF. Figure 3 represents the sensorial profiles of the different treatments obtained of substitution from the averages.



CB: color of bread crust; CC: crumb color of bread; CS: crumb structure; T: texture; A: aroma; F: flavor

**Figure 3. Sensorial profiles of the different treatments obtained of substitution from the averages**

## Principal component analysis

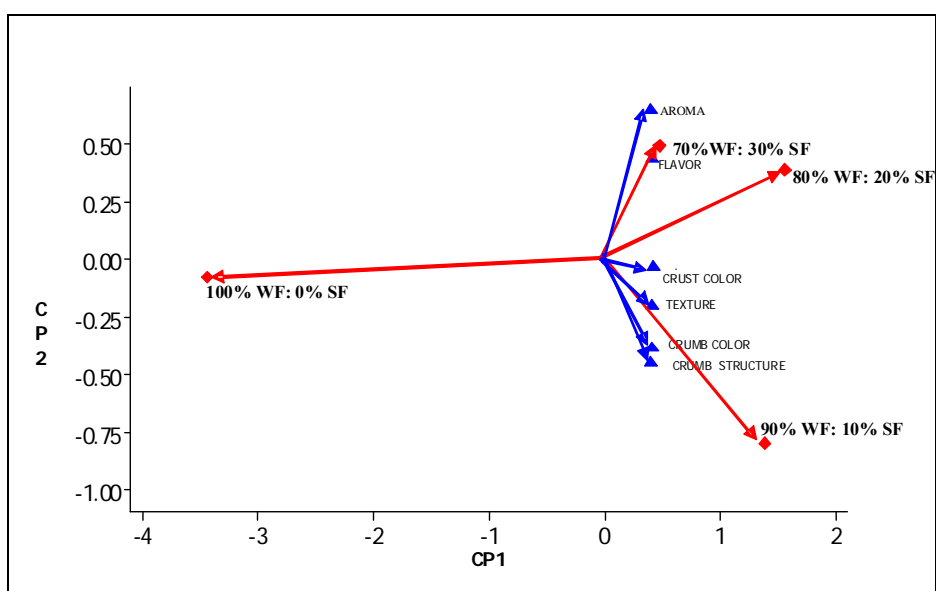
We applied to the ACP taking the average values from the variables of each variety of the total of evaluators (Table 5). Both first components explain the 97.2% of the total variability. The CP1 explains the 91.56% and CP2 the 0.05% of the total variance of the variables. The contribution of the six attributes to CP1 and CP2 is given in table 5 by correlations.

The CP1 is positive for color of bread crust; crumb color of bread, crumb structure, texture, flavor and aroma. The CP2 is positive with flavor and aroma and negative for the color of bread crust; crumb color of bread, crumb structure and texture.

**Table 5. Correlation between attributes and the first two components**

Attributes	CP1	CP2
Color of bread crust	0.42	-0.03
Crumb color of bread	0.41	-0.38
Crumb structure	0.40	-0.45
Texture	0.41	-0.20
Aroma	0.39	0.65
Flavor	0.41	0.43

Projecting the variables to both axes and associating them with the sensorial attributes (figure 4) the following was observed: the treatment 70% WF: 30% SF is associated with the flavor and aroma but do not have a direct relation with color of bread crust; crumb color of bread, crumb structure and texture. On the other hand, treatment 90% WF: 10% SF obtained a direct association with color of bread crust; crumb color of bread, crumb structure and texture but does not have a good relation in accordance to the flavor and aroma. Based on the comparison, it is possible to affirm that the treatment: 90% WF: 10% SF, showed a major acceptability as far as the color of bread crust; crumb color of bread, crumb structure and texture is concerned. On the other hand, the treatment 70%WF: 30% SF, shows to relate with intrinsic flavor and aroma. However, the "best" relates to all sensory attributes, which was observed for treatment 80% WF: 20% SF; this treatment obtained the highest acceptance (Fig. 4).

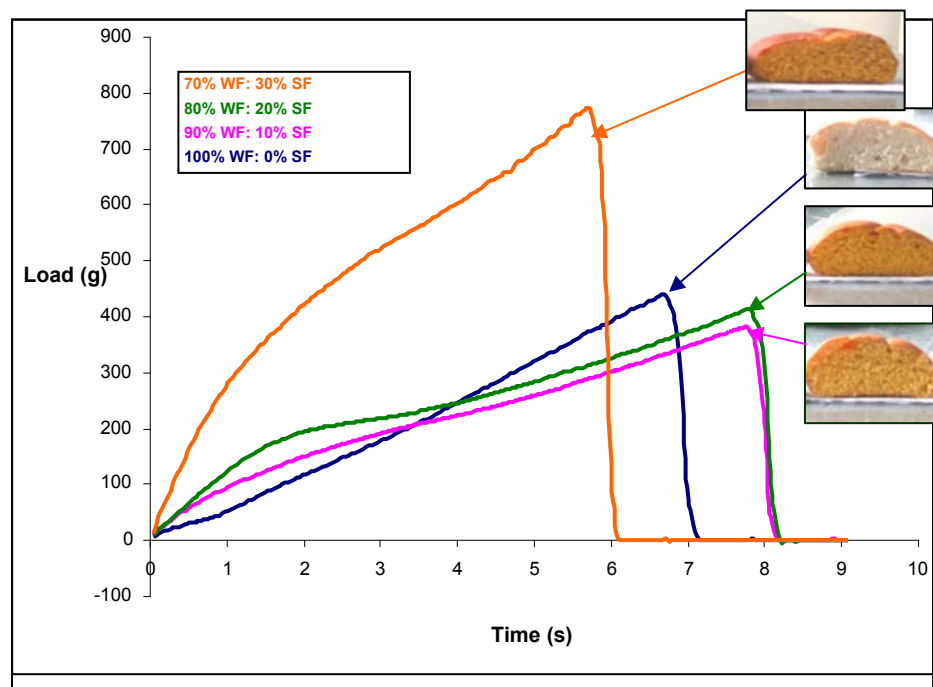


**Figure 4. Plot of the correlations of variables and variables projection to the plane of the first two components**

## Texture profile analysis

The results of analysis of the texture profile presented in figure 5 by a test of uniaxial compression. The treatment 70%WF: 30%SF has a greater hardness due to the nature of the structural characteristics of the bread (for example a smaller volume). This is probably a result of the difficulty that the leavening finds to degrade

alcohol starches and CO<sub>2</sub>. The last one allows the mass to increase the volume. This limitation is possible to be attributed to the deficient formation of the gluten. These evaluations endorse the results of the sensorial evaluation.



**Figure 5. Curves of compression uniaxial to constant speed**

## Conclusions

The results of this study demonstrate that the flour substitution of wheat by flour of sweet potato in the treatment (70%WF: 30% SF) increase the percentage of humidity, fiber, fat, ash and content of  $\beta$ -carotene. The qualitative analyses show an intrinsic relation of the aroma and flavor in the treatment 70%WF: 30%SF. However, the treatment that presents a better relation with all the sensorial attributes is 80%WF: 20%SF that obtained highest acceptability.

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# Potential adoption of the cassava peeling machine in Southwest Nigeria

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## Abstract

Data obtained from three (3) commercial cassava peeling machine fabricators and 90 cassava processors were used to examine the potential adoption of available cassava peeling machines in southwest Nigeria. The commercially available machines are: WAMABCO Peeling Machine (available in three different sizes with capacities of 2 tonnes/day, 4 tonnes/day and 6-7 tonnes/day sold at US\$1500; US\$3000 and US\$3600 respectively); A&H Peeling Machine of 6-7 tonnes/day capacity and FATAROY Peeling Machine of 6-7 tonnes/day capacity sold at US\$2500 each. However, 51.7 percent of the cassava processors were not aware of the machines and 79.3 percent do not have the machine available either at their processing sites or located somewhere close. But majority of the processors (85.6%) were willing to adopt the use of the machine. There were significant relationships ( $p < 0.05$ ) between income ( $r=0.80$ ), number of trainings ( $r=0.92$ ) and the potential adoption of the machine. Yet, majority of the cassava processors (78%) never attended any training in cassava processing. A significant difference ( $p < 0.05$ ) was found between the cost of manual peeling and cost of mechanical peeling ( $t=8.33$ ). The study concluded that processors are willing to adopt the use of the peeling machine if efficient, available and affordable. Commercial fabricators “need” to make the machines more widely available by offering to sell at varying sizes and affordable prices or facilitate arrangements through which processors would have access to use the machine nearby and at reasonable fees.

## Introduction

Failure to adequately develop post-harvest systems for cassava has been a major bottleneck for many years and has limited the contribution of the crop to economic growth and poverty reduction. All processed products of cassava roots in Southwest Nigeria require peeling, yet newly developed processing technologies have been widely adopted for every other stage in the processing of cassava roots to reasonable extents and efficient peeling remains a bottleneck in cassava processing systems. A considerable time is spent in peeling of cassava roots with the indigenous way of using knives. Even though peeling machines believed to be faster and more convenient have been developed locally, however the profitability of these machines has not been tested and the potential adoption has not been ascertained. Against this background, this study examined the potential adoption of commercially available cassava peeling machines in southwest Nigeria. It hypothesized that:

Ho<sub>1</sub>: There is no significant relationship between the socio economic characteristics of cassava processors and the potential adoption of the cassava peeler.

Ho<sub>2</sub>: There are no significant differences in the profitability of the different options for peeling cassava roots.

## Methodology

Three commercially active cassava peeling machine fabricators were purposively selected from the four fabricators identified in southwest Nigeria. These are:

3. 1. Engineer. T.A. Diallo of West African Machine Building Company , Monatan, Ibadan, Oyo State.
4. 2. Alhaji Ahmed of A&H Technical Metal Works, Iwo, Osun State and
5. 3. Mr. Fatai of Fataroy Steel Industry, opposite University College Hospital Mokola Ibadan, Oyo State

A purposive sampling method was also adopted to select cassava processing sites located very close to the commercially producing cassava peeling machine fabricators. Four (4) processing sites were selected from Oyo state, two (2) from Ogun and Osun States. In these locations all the cassava processors on-site made up of forty (40) processors from Oyo State, twenty-two (22) from Ogun State and twenty-eight (28) from Osun State were interviewed for this study.

Questionnaires (containing structured and open-ended questions) and focus group discussions were used to obtain data from selected cassava processors while an interview guide was applied to fabricators. Primary data were obtained from the fabricators on the characteristics of their machines. Primary data were also obtained from cassava processors on their socio-economic characteristics; awareness of the peeling machine; availability of the machine; potential adoption of the machine; the profitability of their peeling operations; opinions on characteristics of a peeling machine that will predispose it for adoption and their willingness to pay for the machine. The reliability of selected questions in the questionnaires were determined by the test-retest method. The coefficients of stability obtained ranged from  $r = 0.75$  to  $r = 0.78$ . Also, content and face validity tests were carried out on the research instruments by known experts.

Chi square analysis was used to test the relationship between the some socio economic characteristics (measured at nominal and ordinal levels) of cassava processors and the potential adoption of the cassava peeling machine. The Pearson Product Moment Correlation was used to test the relationship between the socio economic characteristics of the processors that are measured at interval and ordinal levels. The Students' t- test was used to test the differences in the mean profitability of adopting alternative peeling options.

## Results and discussion

### *Commercially available cassava processing machines*

The commercially available machines are: WAMABCO Peeling Machine (available in three different sizes with capacities of 2 tonnes/day, 4 tonnes/day and 6-7 tonnes /day sold at US\$1500; US\$3000 and US\$3600 respectively); A&H Peeling Machine of 6-7 tonnes/day capacity and FATAROY Peeling Machine of 6-7 tonnes/day capacity sold at US\$2500 each.

### *Description of selected cassava processors*

Table 1 shows that majority (93.1%) of the cassava processors were females and were mostly married (95.4%). Most (89%) belong to household with 3-6 persons; and about two-thirds had formal education (at least completed primary education); but had not attended any traing in cassava processing. More than half (51.7%) were not aware of the existence of cassava peeling machines and 79.3 percent do not have the machine available either at their processing sites or located somewhere close.

**Table 1. Socio-economic characteristics of the cassava processors N= 90**

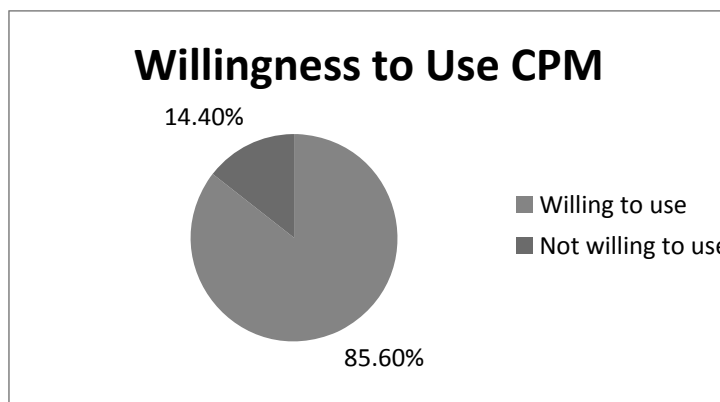
Variables	Mode (Mean)	Standard Deviation
Age	84% 25-50 years old (41 years old)	9.861
Sex	93.1% Female	
Religion	62.1% Christianity	
Marital Status	95.4% Married	
Household size	89% 3-9 persons (6 persons)	2.790
Educational Status	66.7% Formal education	
Number of training	87% None (0.13)	0.389
Number of group affiliated to	65.5% None (0.5)	0.760
Ancestry	53.0% Non-native	

Source: Field survey 2008



Majority of the processors (85.6%) were willing to adopt the use of the machine. Majority (67.1%) of the cassava processors said they were willing to use the machine if is affordable and can completely peel the cassava fed into it without discrimination as claimed by the fabricators, 2.3% wanted the machine in a very comfortable way of operation given that women were the major population in cassava processing, the machines should be fabricated in such a way that it will be easy for the women to operate it as against looking for help from their male counterparts. Washing and offloading channel was another quality the respondents wanted in a cassava peeling machine before deciding to use it, 8.8% of the respondents were under this category.

Figure 1 shows that majority (85.6%) of the cassava processors, were willing to use the machine and (14.4%) were not willing to use the machine. Willingness to use the machine in this study was seen as a proxy for potential adoption. The reasons given for their not wanting to use were social problems and illiteracy on the part of some of the respondents. This is in line with studies that have shown that the factors influencing technology adoption can be social, economic, innovation related, process related or exogenous (Rogers, 2003; Collinson *et al.*, 2001; Agbamu, 1995, Adebayo *et al.*, 2003). In Owe village for example where the IFS funded machine was demonstrated, there was a social problem of who will manage the machine if donated to them, though they claimed that the peeling machine was not needed at the time of the demonstration, that they would have preferred hydraulic press instead, but there was an undertone of social problem in the discussions had with them.



**Figure 1. Cassava processors’ willingness to use cassava peeling machine**

social problem of who will manage the machine if donated to them, though they claimed that the peeling machine was not needed at the time of the demonstration, that they would have preferred hydraulic press instead, but there was an undertone of social problem in the discussions had with them.

### **Test of hypotheses**

Table 2 shows that there are no significant relationships between sex; religion; marital status; educational status; occupation and the potential adoption of a cassava peeling machine. This implies that the potential adoption of a cassava peeling machine depends on other factors apart from all these factors, whether or not, a processor is well educated, and either male or female does not determine his choice of being ready to adopt the use of a cassava peeling machine. There is however a significant relationship between ancestry and potential adoption of cassava peeling machine. This implies that being a native of the study area can affect the choice of adopting the use of a cassava peeling machine. A native can decide to use the machine to improve his livelihood status, he can even take the option of acquiring the machine and use for commercial purposes whereby he serves as an itinerant operator and other cassava processors come for the services at a given fee. There could be a slight difference in the case of a non-native because he could face some social problems which might not enable him to operate successfully as a native would do.

**Table 2. Results of Chi-square analysis (Dependent variable = Potential adoption of cassava peeling machine)**

Variable	Chi-square value	Df	Asymp. Sig. (2-sided)	Decision*
Sex	0.169	1	0.681	NS
Religion	0.328	2	0.849	NS
Marital status	0.752	1	0.386	NS
Educational status	2.534	4	0.639	NS
Major occupation	3.957	7	0.785	NS
Ancestry	8.816	1	0.003	S

\* = Decision criterion is significant when  $p < 0.05$   
Df = Degree of freedom

Table 3 shows significant relationships between household size; number of training; income and the respondents' potential adoption of cassava peeling machine. This implies that the willingness to adopt the use of a cassava peeling machine can be determined by the household number whereby the family labour involved in cassava peeling is not sufficient enough for each production and using a machine for the same purpose will increase production at the end.

The number of training in cassava processing could also positively affect the choice of adopting the use of a machine whereby the processors had attended a couple of trainings in the enterprise and had been enlightened to the advantages of mechanical post harvest techniques in cassava processing especially cassava peeling. Income can also affect the potential adoption of the cassava peeling machine; the higher the income, the higher the tendency of wanting to use a machine for peeling. A processor with a high income can afford to pay for the services of a cassava peeling machine or can even afford to buy one.

Table 3 also revealed that there were no significant relationships between years of experience in cassava processing; number of group affiliated to and the potential adoption of the machine. The potential adoption of a cassava peeling machine is not also determined by the number of group affiliated to; the group a processor belongs to might have nothing to do with cassava processing, it could be a religious group which might not affect the choice using of adopting a cassava peeling machine.

**Table 3. Results of Pearson Product Moment Correlation (Dependent variable = Potential adoption of cassava peeling machine)**

Variable	R value	Approx. value (Sig.)	Decision
Household size	0.86	0.02	S
Number of training	0.92	0.01	S
Income	0.80	0.03	S
Exp. In cassava processing	0.21	0.14	NS
Number of group affiliated to	0.41	0.09	NS

Decision criterion is significant when  $p < 0.05$

The Students' t-test analysis revealed there are differences in the alternative peeling options considered in this study (Table 4). The options were manual peeling; willingness to pay if cassava peeling machine is stationed in their processing sites; willingness to pay if machine is stationed 2km away from their processing sites and the willingness to buy a machine. The respondents were willing to pay less than the amount they were presently paying for manual peeling if the machine is brought to their processing sites and operated by an itinerant operator. The situation is also similar that the respondents were willing to pay less and adopt the use of the machine if stationed two kilometers away from their processing sites.

**Table 4. Results of t-Test analysis (Dependent variable = Manual peeling for Gari production)**

Variable	t-value	df	Sig. (2-tailed)	Decision
Willingness to pay if located at the cassava processing site	8.329	69	0.00	S
Willingness to pay if located 2km away from the processing site	3.629	56	0.00	S
Willingness to buy a cassava peeling machine	4.720	52	0.00	S

Decision criterion is significant when  $p < 0.05$ .

## Conclusion and recommendations

The study concluded that processors are willing to adopt the use of the peeling machine if efficient, available and affordable. The major constraints in the use of cassava peeling machine as found out in this study is the awareness and availability of the machine. The categories of cassava processors who were privileged to have the machine available were processors in research institutes. An average processor was not even aware of the existence of the machine not to talk of having it available for use. Commercial fabricators “need” to make the machines more widely available by offering to sell at varying sizes and affordable prices or facilitate arrangements through which processors would have access to use the machine nearby and at reasonable fees.

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# Fungal and insect contamination of yam and cassava chips in Benin

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## Abstract

In 2003/2004 and 2004/2005 in two agroecological zones fungi, mycotoxins and insects in cassava and yam chips were evaluated. Interaction of season, zone and time with fungal occurrence and incidence (cfu/g) and aflatoxin and fumonsin level. Mean moisture content was 11.9% and 12.4% with levels in the Northern Guinea Savannah (NGS) exceeded the recommended limit of 9-10% (2003-04). In the Sudan Savannah (SS), moisture content ranged from 10.1 to 14.5% and 11.1 to 14.5% in cassava and yam chips, respectively. During the 2004/2005 moisture ranged from 9.5 to 13.6% in the NGS and from 9.2 to 13.8% in the SS. The insects' species identified included *Prostephanus truncates*, *Cathartus quadricollis*, *Carpophilus dimidiatus*, *Tribolium castaneum* and *Sitophilus zeamais*. *P. truncates* and *C. quadricollis* were more prevalent in cassava than yam. After three months, all stored cassava chips were infested by *P. truncates* and *C. quadricollis*, whereas no yam chips in the SS showed insects. *A. flavus* was the most predominant fungal species its prevalence varied with season, during storage period and agroecological zone. Fungal levels in cassava and yam chips reached 8950 cfu/g and 6030 cfu/g, respectively, exceeding the tolerance limit in foodstuffs ( $10^2$ ). Other fungal species included *Penicillium chrysogenum*, *Mucor piriformis*, *Phoma sorghina*, *Fusarium verticillioides*, *Rhizopus oryzae* and *Nigrospora oryzae*. High performance liquid chromatography (HPLC) analysis of both cassava and yam chips showed that they were not contaminated by either aflatoxins or fumonisin B<sub>1</sub>. The limits of detection were 0.1 µg/kg for all the aflatoxins and 0.025 µg/kg for fumonisin B<sub>1</sub>.

**Keywords:** Benin, agroecological zones, cassava and yam chips, fungi, insects.

## Introduction

Cassava and yam are important starchy root crops, eaten and used by millions of people in West Africa, and parts of East and Central Africa. In Benin, cassava and yam are the most important crops with about 2.2 and 1.6 million tons produced in 2007, respectively (FAO, 2007). The production of cassava and yam is hindered by storage problems since they are highly perishable commodities and are easily contaminated by fungi (Ikotun, 1989) and bacteria (Ikotun, 1983) or are subject to sprouting due to increased metabolic activity (Ugochukwu *et al.*, 1974).

In order to minimize the above problems, cassava and yams require processing. One way is to process tubers into dried cassava and yams chips. The processing of cassava and yams into chips is a common traditional activity in Benin during the dry period, especially in the Central and Northern regions of the country. Chips are subject to attacks by fungi of which the most important are *Aspergillus*, *Fusarium* and *Penicillium* (Wareing *et al.*, 2001; Bassa *et al.*, 2001). Fungal contamination can lead to the discolouration of the chips, mouldy taste, production of off odours (Gwinner *et al.*, 1996) and possibly the production of toxins that are harmful to animals and humans (Constant *et al.*, 1984; Sajise and Ilag, 1987).

The presence of aflatoxins in yam chips produced in Nigeria (Bankole and Mabekoje, 2004) and Benin (Bassa *et al.*, 2001; Mestre *et al.*, 2004) has been reported. Insect infestation and quality changes brought about by insects in cassava chips have been reported in India (Prem Kumar *et al.*, 1996). Little information exists in this respect on cassava and yams chips. Therefore, there is need to establish the relationship between insects, fungi dissemination and mycotoxins contaminated cassava and yam chips in Benin. The main purpose of the present work is to evaluate the magnitude of cassava and yams chips contamination by mycotoxigenic fungi and to

evaluate the level of aflatoxins and fumonisins contamination of cassava and yam chips in Benin. A survey of fungi, mycotoxins, and insect contaminating stored cassava and yams chips in two different geographical regions of Benin including the Northern Guinea Savannah and the Sudan Savannah was undertaken.

## Objectives

6. Identify and enumerate the fungal species on cassava and yam chips from two geographic zones in Benin.
7. Identify and enumerate the insects present on the cassava and yam chips.
8. Determine the aflatoxin and fumonisin levels in stored cassava and yams chips.

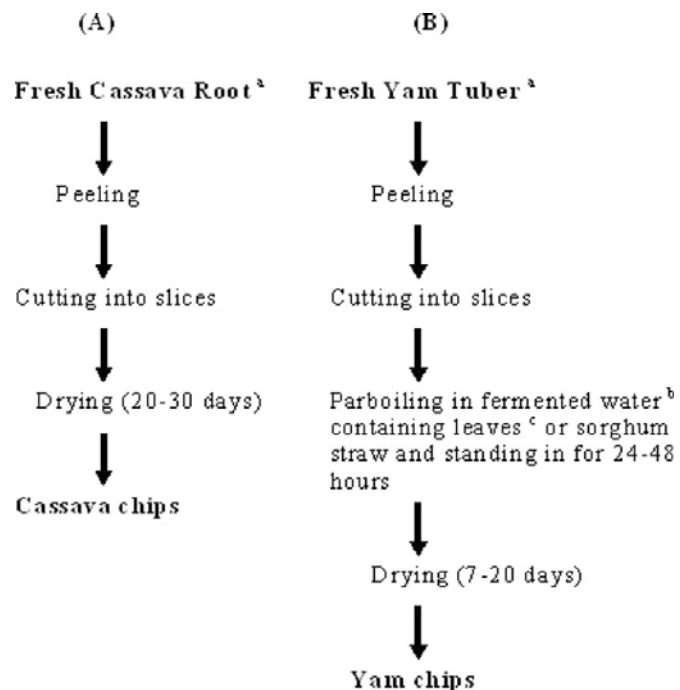
## Materials and methods

### *Agro-ecological zones and villages*

Two countrywide surveys were carried out between 2003 and 2005 in two agro ecological zones [Northern Guinea Savannah (NGS) and Sudan Savannah (SS)] of Benin have been described by Hell et al. (2000) were chosen for the survey to evaluate the natural incidence of insects, fungi and mycotoxins. The fungi of interest were *Aspergillus* spp and *Fusarium* spp whereas the mycotoxins to be studied were aflatoxins (B<sub>1</sub>, B<sub>2</sub>, G<sub>1</sub>, and G<sub>2</sub>) and fumonisin B<sub>1</sub>.

### *Traditional processing of Cassava and Yam chips*

The following flow diagrams show the processing of fresh cassava and yam to chips as described by the processors (Figure 1).



**Figure 1. Flow diagrams of the traditional processing methods of cassava (A) and yam (B) chips in Benin. <sup>a</sup>, fresh harvested cassava root and yam tubers. <sup>b</sup>, supernatant of *ogi* (based on maize or sorghum). The fermented supernatant was obtained from *ogi* that was prepared following the traditional processing method described by Fandohan et al. (2005). <sup>c</sup>, from pawpaw or mango tree.**

### **Survey and sampling procedure**

The surveys were conducted in 20 villages (10 villages per agro ecological zone). Ten farmers were randomly selected in each village (5 processors of cassava chips and 5 processors of yam chips). The same farmers were involved in both surveys. Cassava and yam chips were sampled at the beginning of the storage (after drying for 20-30 days). At least 500g of the cassava and yam chips were randomly selected from each farmer's stock. Samples from each village were pooled together. Part of the 500g sample (200g) was treated as outline in section 2.4 to identify the insect pests. The rest of the sample was ground using a traditional mill stored at 4°C until mycological and mycotoxins analyses. Samples were again collected from the same farmers after three months of storage.

### **Insects collection and identification**

The insects were collected from the unmilled samples, enumerated and the common insects identified in the field. All the insects were taken to the laboratory for confirmation of identity using the keys by Weidner and Rack, (1984) and Dobie *et al.* (1991). They were classified as primary (meaning that is able to attack the raw commodity and causes entrance for other pests) and secondary (that can infest the commodity after the damage of the primary pest) pests. All insect identifications were done at IITA.

### **Determination of moisture content**

Moisture content was determined by heating at 105 °C for 2 h to constant weight (AOAC, 1984).

### **Fungal enumeration and identification**

Fungal genera were enumerated using the dilution plating method. Ground samples (10 g each) were thoroughly mixed with 90 ml of sterile water containing 0.1% peptone water for the 10<sup>-1</sup> dilution. Further serial dilutions to the 10<sup>-4</sup> dilutions were made with 0.1% peptone water. Aliquots (1.0 ml) of each dilution were then transferred to Petri dishes containing potato dextrose agar (PDA). The Petri dishes were incubated at 25 °C in alternating 12-h periods of fluorescence light and dark for 5 days (Singh *et al.*, 1991). Colonies developing on plates were counted at the end of the incubation period and recorded as

Colony Forming Units per gram (CFU/g) (Bankole and Mabekoje, 2004). Isolates from PDA were sub-cultured on malt extract agar (MEA) (Oxoid Ltd, Hampshire, UK) for identification. *Fusarium* species were sub-cultured on carnation leaf agar (CLA) for identification. The MEA and CLA plates were incubated at 25 °C for 7 days under alternating 12-h periods of fluorescence light and darkness. Cultures were identified based on macro and micro-morphology, and on reverse and surface characteristics

of colonies. *Aspergillus flavus* and *A. parasiticus* were distinguished from other *Aspergillus* spp. by the bright orange–yellow reverse coloration on *Aspergillus flavus parasiticus* agar (AFPA). Standard texts such as those of Nelson *et al.* (1983) and Pitt and Hocking (1997) were used in the identification process.

### **Mycotoxins analysis**

**Aflatoxins extraction and detection:** Aflatoxins were extracted and analyzed according to the method described by Bankole and Mabekoje (2004) with some modifications, for thorough description please refer to Gnonlonfin *et al.* (2008).

**Fumonisin B1 extraction and detection:** Fumonisin B1 were extracted and analyzed according to the method described by Shephard and Sewram (2004) with some modifications, for thorough description please refer to Gnonlonfin *et al.* (2008). The method by Doko and Visconti (1994) with modifications was used to determine fumonisin B1 in both cassava and yam chips.

**Mycotoxins recoveries:** Analytical recoveries were determined in triplicate of both cassava and yam chips samples (10 g each) were placed in 250 ml conical flask and spiked with aflatoxin and fumonisin B1 standards at concentration of 1 µg/kg and 2.5 µg/kg, respectively and left to dry overnight. The samples were then extracted according to the above extraction methods and the recoveries calculated. Mean recoveries of added aflatoxins from both cassava and yam chips were 80% and 100% for fumonisin B1.

### 3. Results

#### **Moisture content of stored dried cassava and yams chips in Benin**

Overall, the moisture content ranged from 9.2±0.0% to 15.3±0.0%. In all cases, higher moisture contents were recorded in the samples at the beginning of storage. The moisture content differed significantly from one zone to another and throughout the storage period ( $p<0.01$ ). In general, the mean moisture contents were significantly higher in yam chips than in cassava chips, but decreased significantly after 3 months ( $p<0.01$ ).

#### **Mycological analyses**

Overall, the numbers of isolates varied significantly within and between agroecological zones, from one season to another and throughout the storage period ( $p<0.01$ ) for cassava chips. The number of *A. flavus* isolates in cassava chips during the 2004/2005 NGS sampling season decreased from 8950 cfu/g at the start of storage to 600 cfu/g at the end of storage (Table 1). *M. piriformis* isolates also decreased from 3430 cfu/g at the start to 500 cfu/g at the end of storage. Similar decreases were observed in *A. flavus*, *M. piriformis* and *F. verticillioides* isolates in the SS zone (Table 1).

Regarding yam chips, the number of fungal isolates in samples also varied within and between zones, across the season and throughout the storage period. In all cases, the number of isolates decreased throughout the storage period (Table 2). During the 2003/2004 season, *A. flavus*, *F. verticillioides* and *M. piriformis* isolates decreased from 6290 cfu/g to 770 cfu/g, from 90 cfu/g to 0 cfu/g, and from 4110 cfu/g to 4080 cfu/g in samples collected from NGS, respectively (Table 2). In the samples collected from SS the number *A. flavus* isolates decreased from 2200 cfu/g at the start to 520 cfu/g to the end of storage. As for *F. verticillioides*, the number of isolates decreased from 350 cfu/g at the start to 0 cfu/g at the end of storage. However, there was an increase in the number of isolates of *M. piriformis* from 1890 cfu/g to 4450 cfu/g (Table 2). Significant differences were observed in the number of isolates within zone and throughout the storage period ( $p<0.01$ ), but no significant differences were observed between agroecological zones ( $p>0.05$ ).

Overall, no significant interactive effects of all factors together such as season, agroecological zone and time of sampling were observed on *A. flavus* and *F. verticillioides* occurrence ( $p>0.05$ ). *A. flavus* occurrence was positively and significantly correlated with season in cassava chips ( $r=0.7$ ,  $p<0.05$ ) and in yam chips ( $r=0.8$ ,  $p<0.05$ ).

**Table 1. Major fungal species encountered in stored cassava chips during the 2003/2004 and 2004/2005 seasons. The samples were analyzed at the beginning (start) and after three months of storage (end)**

Fungal species	Cassava chips <sup>a</sup>			
	2003/2004 season [CFU/g (% occurrence)]		2004/2005 season [CFU/g (% occurrence)]	
Northern Guinea Savanna (NGS)	Start	End	Start	End
<i>A. flavus</i>	6030 (47.8)	210 (4.5)	8950 (66.2)	600 (49.6)
<i>Aspergillus</i> . spp	2700 (21.4)	210 (4.5)	160 (1.2)	90 (7.4)
<i>F. verticillioides</i>	0 (0)	0 (0)	60 (0.4)	20 (1.7)
<i>P. chrysogenum</i>	620 (4.9)	0 (0)	170 (1.2)	0 (0)
<i>P. sorghina</i>	780 (6.2)	60 (1.3)	700 (5.2)	0 (0)
<i>M. piriformis</i>	1700 (13.4)	2480 (52.9)	3430 (25.4)	500 (41.3)
<i>R. oryzae</i>	730 (5.8)	1730 (36.8)	50 (0.4)	0 (0)
<i>N. oryzae</i>	60 (0.5)	0 (0)	0 (0)	0 (0)
Sudan Savanna (SS)				
<i>A. flavus</i>	3380 (43.3)	460 (18.1)	4980 (67.9)	440 (86.2)
<i>Aspergillus</i> . spp	2370 (30.3)	360 (14.2)	430 (5.9)	20 (4.0)
<i>F. verticillioides</i>	0 (0)	0 (0)	20 (0.3)	0 (0)
<i>P. chrysogenum</i>	60 (0.7)	0 (0)	30 (0.4)	0 (0)
<i>P. sorghina</i>	0 (0)	120 (4.7)	0 (0)	0 (0)
<i>M. piriformis</i>	1590 (20.4)	1350 (53.2)	1620 (22.1)	50 (9.8)
<i>R. oryzae</i>	410 (5.3)	250 (9.8)	250 (3.4)	0 (0)
<i>N. oryzae</i>	0 (0)	0 (0)	0 (0)	0 (0)

<sup>a</sup>The % occurrence was calculated out of the total CFU/g per season, per zone and per time of storage.

**Table 2. Major fungal species encountered in stored yam chips during the 2003/2004 and 2004/2005 seasons at the beginning (start) and after three months of storage (end)**

Fungal species	Yam chips <sup>b</sup>			
	2003/2004 season [CFU/g (% occurrence)]		2004/2005 season [CFU/g (% occurrence)]	
Northern Guinea Savanna (NGS)	Start	End	Start	End
<i>A. flavus</i>	6290 (24.2)	770 (9.0)	5980 (54.5)	660 (53.6)
<i>Aspergillus</i> . spp	11510 (44.4)	580 (6.8)	620 (5.7)	60 (4.9)
<i>F. verticillioides</i>	90 (0.3)	0 (0)	170 (1.5)	0 (0)
<i>P.chrysogenum</i>	3510 (13.5)	1740 (20.4)	930 (8.5)	140 (11.4)
<i>P. sorghina</i>	110 (0.4)	0 (0)	50 (0.5)	0 (0)
<i>M. piriformis</i>	4110 (16.0)	4080 (47.8)	3010 (27.4)	300 (24.4)
<i>R. oryzae</i>	260 (1.0)	1280 (15.0)	130 (1.2)	50 (4.1)
<i>N. oryzae</i>	60 (0.2)	80 (1.0)	80 (0.7)	20 (1.6)
<b>Sudan Savanna (SS)</b>				
<i>A. flavus</i>	2200 (22.8)	520 (7.6)	2470 (38.6)	410 (21.4)
<i>Aspergillus</i> . spp	2840 (29.4)	110 (1.6)	270 (4.2)	70 (3.6)
<i>F. verticillioides</i>	350 (3.6)	0 (0)	80 (1.3)	0 (0)
<i>P.chrysogenum</i>	2050 (21.2)	350 (5.1)	1530 (23.9)	390 (20.3)
<i>P. sorghina</i>	150 (1.5)	0 (0)	10 (0.2)	0 (0)
<i>M. piriformis</i>	1890 (19.5)	4450 (65.2)	1980 (31.0)	980 (51.0)
<i>R. oryzae</i>	180 (2.0)	600 (8.8)	50 (0.8)	0 (0)
<i>N. oryzae</i>	0 (0)	800 (11.7)	0 (0)	70 (3.6)

<sup>b</sup>The % occurrence was calculated out of the total CFU/g per season per zone and per time of storage.

### ***Mycotoxins analyses: aflatoxins and fumonisin B1***

None of the cassava and yam chips samples collected from the two zones in both seasons i.e. 2003/2004 and 2004/2005 contained detectable amounts of aflatoxin or fumonisin B1.

### ***Insects encountered in stored cassava and yam chips***

Overall, the number of infested chip samples varied from one season to another and across agroecological zones. The percentage of infested cassava chips varied from 10% at the beginning of storage to 100% after 3 month of storage (Table 3).

Although infestation was observed in yam samples, most of the samples did not have insects at the beginning of storage, and very few samples (30%) were infested even after 3 month of storage. The observed insect species included *P. truncatus*, *Carpophilus dimidiatus* (F.) (Coleoptera: Nitidulidae), *C. quadricollis*, *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae), and *S. zeamais*.



**Table 3. Principal insects encountered in stored cassava chips at the beginning and end of storage during the 2003/2004 season in Northern Guinea Savannah (NGS; N= 100) and Sudan Savannah (SS; N= 100)**

NGS zone Insects species	Beginning of storage				End of storage			
	Total insects	% occurrence	# infested samples	% Infested samples	Total insects	% occurrence	# infested samples	% infested samples
<i>Prostephanus truncatus</i>	7	24.1b	2	20	838	48.4a	10	100
<i>Carpophilus dimidiatus</i>	0	0c	0	0	115	6.6c	9	90
<i>Cathartus quadricollis</i>	22	75.9a	3	30	719	41.6b	9	90
<i>Tribolium castaneum</i>	0	0c	0	0	53	3.1c	3	30
<i>Sitophilus zeamais</i>	0	0c	0	0	5	0.3c	1	10
Total	29	100	3	30	1730	100	10	100
% infested samples	30				100			
SS zone								
Insects species								
<i>Prostephanus truncatus</i>	1	6.2b	1	10	650	69.8a	10	100
<i>Carpophilus dimidiatus</i>	0	0c	0	0	80	8.6c	3	30
<i>Cathartus quadricollis</i>	15	93.7a	1	10	190	20.4b	2	20
<i>Tribolium castaneum</i>	0	0c	0	0	11	1.2d	1	10
<i>Sitophilus zeamais</i>	0	0c	0	0	0	0d	0	0
Total	16	100	1	10	931	100	10	100
% infested samples	10				100			

The % occurrence was calculated as the insect population out of the total observed per zone.

Means in a column followed by the same letter are not significantly different from each other (SNK, P = 0.05)

## Discussion

A wide range of fungal species were isolated from the stored cassava and yam chips. The fungal species *A. flavus*, *F. verticillioides*, *P. chrysogenum* and *R. oryzae* that were isolated from yam and cassava chips in this study have been implicated as major causal agents of rots in living, but dormant yam tubers (Amusa et al., 2003), and cassava tubers (Wareing et al., 2001). Being a soil fungus, direct contact between the tubers and soil could be the primary source of contamination by *A. flavus*. It is a common practice in rural Benin not to wash the tubers before peeling. It has been reported that soil adhering to tubers contains many microorganisms that can infect the surface of freshly harvested tubers and root (Osagie, 1992). On the other hand, the fungi may come from bruised and already contaminated tubers that are used to prepare the chips. Fungal pathogens can enter the substrate through natural wounds in the tubers. The wounds can be caused by insects, nematodes and poor handling before, during and after harvest (Amusa et al., 2003).

The most important mycotoxigenic fungal species isolated from both cassava and yam chips were *A. flavus*. The levels of contamination of *A. flavus* were higher than the tolerance limit as given by the International Commission on Microbiological Specification for Food (ICMSF). The level of fungal contamination of cassava and yam chips varied from one agroecological zone to another. Influence of climatic factors on the occurrence of toxigenic fungi such as *A. flavus* and *F. verticillioides* have been reported in Benin (Hell et al., 2000; Fandohan et al., 2005).

No aflatoxins or fumonisin B<sub>1</sub> were detected in the surveyed samples despite the presence of *A. Flavus* and *F. verticillioides*. However, other studies have revealed their presence in yam and cassava chips in Benin (Bassa et al., 2001; Mestres et al., 2004;), in Nigeria (Bankole and Mabekoje, 2004) and in Ghana (Wareing et al., 2001) and in Congo and Tanzania (Manjula et al., 2009).

The absence of mycotoxin in this study could be explained by various factors including climatic conditions, nature of the substrate and processing factors. Indeed, low relative humidity, low moisture content (<13%) and high temperatures have been recorded in the regions where chips were produced during the sampling period.

The diversified mycoflora showed by the isolation of eight distinct fungal genera indicates a competition for available nutrients in both cassava and yam chips. It has been previously pointed out that fungal interaction due to competition could lead to decreased mycotoxin levels (Velluti et al., 2000). The absence of aflatoxins and fumonisin B<sub>1</sub> in both cassava and yam chips produced in Benin may be as a result of interactions between variables which were not fully taken in account in the present study. Anti-microbial and fungitoxic compounds such as scopoletin have been known to accumulate in roots and tubers as a result of postharvest physiological deterioration. These compounds may affect the growth of some of the fungal and inhibit mycotoxin production (Gomez-Vasquez et al., 2004).

The primary pest *P. truncatus* was the most prevalent in both stored cassava and yam chips and has previously been reported as the most common insect problem in these products (Wright, 1993; Birkinshaw et al., 2002; Borgemeister et al., 2003). The secondary pest *C. quadricollis* was also regularly observed. This species can survive on maize powder, such as the one resulting from the attack of *P. truncatus* (Birkinshaw et al., 2002; Borgemeister et al., 2003), we believe that this insect can feed and reproduce on the frass powder resulting from *P. truncatus* damage.

The current study showed that the length of drying time during the processing of cassava chips with a mean of 25 days and a shorter period for yam chips with a mean of 14 days may have an impact on insect infestation longer drying periods increased the risk of infection. Mestre et al. (2004) observed that drying of yam chips in Benin took about 6 days and the moisture content of chips was around 20%. In the present study it was observed that the longer the drying time, the higher the rate of insect infestation. In addition the size of the chips influences the drying time and as a consequence the level of insect infestation (Wareing et al., 2001). Traditional cassava and yam chips in Benin are rather large with lengths measured of a mean of 30 cm (Fandohan, personal observation). Cutting roots and tubers into smaller pieces could help in reducing the drying time and consequently levels of insect infestation (Wareing et al., 2001). Environmental conditions such as relative humidity and temperature that prevailed in both agroecological zones may have been very conducive to infestation. On cassava chips insect infestation appears to start during drying, persist and increase during the storage period. In the current study, all of the cassava chips samples were infested with the primary pest *P. truncatus* after three months of storage, leading to weigh loss and making the product inappropriate for human consumption, but it has been observed that even flour resulting after heavy insect infestation was collected by farmers and used to make a porridge (K.Hell, personal observation).

Chips destined for human consumption in Benin were found to be infested by various insect genera that included *Prostephanus*, *Cathartus*, *Carpophilus*, *Tribolium* and *Sitophilus*. The results of this study showed that the primary pest such as *P. truncatus* population density increased over the storage period as compare to the secondary pest population density which decreased with storage time. This suggested the competition within insects species. Vowotor et al. (2005) in their study showed the interspecific interaction between *P. truncatus* and *S. zeamais* in the stored maize. These two insects species were sparsely aggregated and not associated with each other. The observed poor storage conditions can be the sources of insect infestation as well as a potential source of proliferation of microorganisms that can produce mycotoxins that are dangerous to animal and human health (Marasas, 1995)

Insects were also encountered on stored yam chips, but the observed infestation levels were much lower than those that occurred in cassava chips, this might have been due to the parboiling process (Rajamma et al., 1994). The plants used during parboiling (Vernier et al., 2005; Eze et al., 2006) might have had an insecticidal or repellent effect. It has been reported that parboiling results in partial gelatinisation of starch and the subsequent binding of the gelled starch onto the surface of the yam chips which hardens them (Rajamma et al., 1994) probably making them more storable.

## Conclusion

The absence of mycotoxins in the samples was re-assuring but further investigations are required to carry out a survey of cassava and yam chips offered for sale in Benin markets before definitive statements can be made on the safety of the products. Similarly, the performance of different detection methods for determination of mycotoxins on these products should be reviewed. There is a need to test *A. flavus* isolates for their potential to produce aflatoxins in stored cassava and yam chips. Furthermore, there is a need to investigate on the isolation and inhibitory effect of scopoletin in Beninese cassava and yam varieties.

The results of this study show that cassava and yam chips in Benin were infested by various insect species, mostly Coleopterans. Amongst these insects the specie *P. truncatus* was the more prevalent in both stored cassava and yam chips and also the most destructive. Parboiling the chips, the use of plants during the parboiling process and good storage practices were associated with lower levels of insect infestation. Consequently, any action undertaken to reduce insect infestation during the drying and storage period could help to limit losses to yam and cassava chips, thus increased their storability and marketability.

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# Inhibition of enzymatic browning in *Dioscorea Alata* chips using natural anti-browning agents

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## Abstract

Of all the yam varieties, *Dioscorea alata* is the most susceptible to enzymatic browning. A study was conducted on four natural anti-browning agents (lemon juice, water extract of *Moringa* seed, papaya latex and pectin) to monitor their anti-browning effect on *D. alata* chips. The effects of treatment time and concentration of anti-browning as well as the rate of colour change, were studied. The yam chips were soaked in solutions of anti-browning agents at concentrations of 40% and 50% v/v (for lemon juice) and 1% and 2%w/v (for *Moringa* seed extract, papaya latex and pectin) for 1, 3 and 5 minutes; solar dried for 2 days and their colour differences compared. Sensory evaluation of the treated samples was done using a colour reference sheet. All treatments, except pectin 2% at 3 minutes, significantly different ( $p>0.05$ ) from the control exhibited significant antibrowning effects. Increase in treatment time increased the anti-browning potency of an ABA. Lower concentration had better antibrowning results in DPL, pectin and lemon. The result of the rate of colour change revealed that visual scoring and instrumental measurement produce data sets that differ in quality. Browning in water yam can therefore be controlled with natural anti-browning agents such as papaya latex, water extract of *Moringa* seed, pectin and lemon juice.

## Introduction

The cosmetic quality of food is significantly impacted by colour which is the first attribute used by consumers in evaluating food quality (Guiwen *et al.*, 1995; Calvo, 2004). Colour may be influenced by naturally occurring pigments resulting from both enzymatic and non-enzymatic reactions (Marshall *et al.*, 2000). Maintaining the natural colour in processed and stored food has been a major challenge in food processing due to oxidative processes such as enzymatic browning. (Marshall *et al.*, 2000; Mohammadi *et al.*, 2008; Billaud *et al.*, 2003; Akissoe *et al.*, 2004). The phenomenon is a widespread problem in the food industry, as it leads to undesirable characteristics in food, decrease in food nutrients and quality, shelf life and market value thereby incurring economic losses and food insecurity (Saengnil *et al.*, 2005).

Browning and its control have been extensively studied and reported in many fruits and vegetables such as apples, pears, cabbages, bananas, and potatoes (Soliva-fortuny *et al.*, 2001; Rojas-Grau *et al.*, 2006). Most antibrowning agents used are chemicals of which sulphites are the most prominent. Sulphites are however banned in some countries because they have adverse health effects (Tong *et al.*, 1991; Lee *et al.*, 1995; Son *et al.*, 2000; Hang-ing *et al.*, 2005). According to Jiang *et al.* (2004), consumers are demanding a reduction in the overall use of chemicals on fresh products and alternative methods are being investigated to extend shelf life of fresh-cut fruits.

Yams are second to cassava as the most important tropical root crop due to the absence of cyanogenic compounds. Additionally, they are nutritionally better than cassava due to their higher vitamin C (40-120mg/g edible portion), crude protein (40-140g/kg dry matter) (Opara, 1999). According to Berghofer (2005), minimal processing is one way by which diversified products can be developed. However during processing, yam is prone enzymatic browning which must be controlled (Miyawaki, 2006). *Dioscorea alata* is a common but underutilized yam species in Ghana and browns quickly and intensely than other edible yam varieties (Ozo and Caygill, 2006) hence the motivation for its utilization in this work.

The aim of the study was to assess the potential anti-browning properties of dried papaya latex, water extract of *Moringa* seeds, pectin and lemon juice. The effect of concentration and treatment time of these materials on enzymatic browning in *D. alata* chips was studied. In addition, the rate of browning of *D. alata* was studied for the materials that gave the highest level of browning inhibition from sensory evaluation.

## Methodology

### **Procurement of materials**

*D. alata* (TDa /0099/208) tubers were obtained from Crop Research Institute, Femesua, Kumasi, partially ripened Lemon fruits from the Kumasi European Market, Ghana, powdered citrus pectin from Sigma Aldrich Inc., Germany and dried *Moringa oleifera* seeds from Oda in the Eastern region of Ghana.

### **Preparations of yam chips**

Each tuber was washed, divided into 3 equal parts and a 2/3 fraction from the 'head' region was used for the experiment (Onayemi, 1986). The tubers were peeled, cut and chipped whilst submerged under tap water. Chipping of *D. alata* was done with a hand chipper into average sizes of 4cm x 3cm x 0.1cm.

### **Preparation of anti-browning solutions**

**Dried papaya latex solution.** Incisions were made on four sides of the partially mature papaya fruits, from the stalk end to the tip and the papaya latex that exuded was collected into plastic bowls. Similar incisions were repeated on untapped surface of the same fruit three times at 3-4 days interval. Tapping of papaya latex was done early in the morning before 9.00 a.m. for each day. The liquid latex was solar dried for 2 days into flakes. Powder was prepared from dry flakes and stored in air tight plastic containers prior to use. Concentration of 1% and 2% w/v were prepared and used for the experiment. The solution was allowed to stand for 3 hours while shaken intermittently before being filtered off since the dried papaya latex powder is partially soluble in water.

**Pectin, Lemon and Moringa Seed Extract solution.** The following concentrations were selected and prepared for the study after preliminary test. Both 1% and 2% w/v solutions were prepared from pectin and *Moringa* seed powder respectively. For lemon juice, 40%v/v and 50%v/v were prepared. Warm distilled water (60°C) was used to allow for easy dissolution of the pectin. The *Moringa* seed extract was also agitated intermittently for 2 hours since the solute was partially soluble. The *Moringa* seeds extract had a vitamin C content of 87.93mg/100ml. The raw lemon juice had a pH and titratable acidity of 1.81 and 3.645g/100ml respectively.

### **Treatment of *D.alata* yam chips**

The *D. alata* yam chips (average weight of 10.60g) were soaked in 200ml of the anti-browning agents (ABA) for three time treatments (1, 3 and 5 minutes). Yam chips were strained in a plastic colander to remove residual solution and placed in a solar drier for 48 hours. Treatments were conducted in triplicate. For control experiment distilled water was used.

### **Development of Colour chart**

A colour reference sheet (Plate 1) was developed as a standard for measuring the level of browning of the *D. alata* chips. Yams were prepared as stated earlier. They were grouped into four with each group having three replicates. Yam chips were placed on a white cardboard and solar dried. Pictures of yam chips were taken from 0 min (the time taken out of water) to 3 hours at 30 mins interval. Pictures of the yam samples were taken after 2 days in the solar drier. Eight pictures which showed consecutive differences in browning were selected. The photographs were arranged in ascending order with respect to differences in browning level into a 7 – point ordinal scale where 0 = (no browning) and 7= (heavy browning).

### **Sensory analysis**

The treated samples were presented to 25 trained panellists who scored the degree of browning using the colour reference sheet. The panellists compared the colour of treated yam chips with the colour of *D. alata* chips at different stages of browning on the reference sheet and assigned the corresponding score values from the reference which had the same/nearest browning level as the treated chips.

### **Tristimulus Measurement**

Tristimulus measurement was conducted on fresh *D. alata* samples for the treatment combinations that gave the highest level of browning inhibition (indicated by a lowest mean score) from the sensory evaluation.

The measurement was done with a Minolta Chromameter (Minolta CR-300, Minolta Corp., Ramsey, NJ) at 0min, 20min, 40min, 1 hr, 2hr, 3 hr, 4hrs and 2 days after treatment. The data was recorded using the CIE-L\*, a\*, b\* scale, where L\* represents lightness, a\* represents chromaticity on a green (-) to red (+) axis and b\* represents chromaticity on a blue (-) to yellow (+) axis. Calibration of equipment was done against a standard white tile provided by the manufacturer (L\* = 97.51, a\* = +0.29, and b\* = +1.88). The L\*, a\*, and b\* values recorded are averages of three readings carried out at different point of the sample surfaces. The experiment was carried out at ambient temperature of 22-25°C.



**SCORE: 0**



**SCORE: 1**



**SCORE: 2**



**SCORE: 3**



**SCORE: 4**



**SCORE: 5**



**SCORE: 6**



**SCORE: 7**

**Plate 1. Colour Reference Chart**

### ***Data Analysis***

The Statgraphics Centurion Statistical Software (version XV) was used to perform a multifactor analysis of variance on the means obtained from the sensory evaluation. Fisher's least significant difference (LSD) was used to discriminate among the means. The total colour change of the *D. alata* chips was determined from the L\*, a\* and b\* data using the following equation (Iyudogan and Bayindirli, 2004):

Total colour change ( $\Delta E$ ) was evaluated as:

$$\Delta E = \sqrt{(L^*_t - L^*_{initial})^2 + (a^*_t - a^*_{initial})^2 + (b^*_t - b^*_{initial})^2}$$

"initial" refers to the colour reading of treated *D. alata* at 0 min after treatment.

The rate of browning was determined using Multiple Linear Regression of  $\Delta E$  against time. The equation of the fitted model was of the form:  $y = mx + c$  where the gradient,  $m$  also represents the rate of total colour change. Data analysis was done at a 5% level of significance ( $\alpha = 0.05$ ).

## Results and discussion

### *Potential anti-browning properties of materials*

All treatments, except pectin 2% at 3 minutes, significantly different ( $p > 0.05$ ) from the control exhibited significant antibrowning effects (Table 2). This antibrowning effect could be due to the active agents- papain and malic acid in DPL (Ponting, 1960; Richard-Forget *et al.*, 1998); carboxyl groups in pectin as well as the coating ability of pectin (Marshall *et al.*, 2000; Oms-Oliu *et al.*, 2008); vitamin C, amino acids and antioxidants in *M. oleifera* seeds (Makkar and Becker, 1999; Anhwange *et al.*, 2004; Grubben and Denton, 2004; Andrew, 2006; Price, 2007) and ascorbic acid and citric acid in lemon (Tchone *et al.*, 2005).

**Table 1. Mean of scores of *D. alata* chips treated with ABAs**

ABA		Treatment Time		
		1min	3min	5min
DPL (Papain)	1%	2.87 (0.309) <sup>a+A</sup>	1.39 (0.867) <sup>b+A</sup>	1.71 (0.358) <sup>a,bA</sup>
	2%	1.93 (0.476) <sup>a+A</sup>	3.20 (0.910) <sup>aB</sup>	1.52 (0.457) <sup>a,bA</sup>
Pectin	1%	4.89 (0.387) <sup>Aa</sup>	1.43 (0.293) <sup>b+A</sup>	3.07 (0.105) <sup>b,A</sup>
	2%	3.88 (0.408) <sup>+A</sup>	4.11 (0.425) <sup>+B</sup>	3.48 (0.118) <sup>+A</sup>
Moringa	1%	3.77 (0.885) <sup>a+A</sup>	3.95 (0.508) <sup>a,A</sup>	2.16 (0.694) <sup>b+A</sup>
	2%	4.24 (0.120) <sup>A</sup>	3.63 (0.808) <sup>A</sup>	3.39 (0.122) <sup>A</sup>
Lemon	40%	3.48 (0.560) <sup>+A</sup>	2.59 (0.303) <sup>+A</sup>	2.09 (1.672) <sup>A</sup>
	50%	4.55 (1.086) <sup>a,B</sup>	2.84 (0.538) <sup>b,A</sup>	2.73 (0.340) <sup>b,A</sup>
Control	-	5.17 (0.272) <sup>a</sup>	3.32 (0.223) <sup>b</sup>	3.95 (0.965) <sup>b</sup>

For each ABA (in rows), means with the same alphabet are not significantly different ( $p < 0.05$ ).

All treatments without alphabets are significantly different ( $p > 0.05$ ) with other treatments in the same row.

Treatments tagged '+' are significantly different ( $p > 0.05$ ) with the control in the same column.

Alphabets in lower case denote significant difference ( $p > 0.05$ ) between treatments of the same ABA subjected to different treatment times.

Alphabets in upper case denote significant difference ( $p > 0.05$ ) between different concentrations of the same ABA (at constant time)

### Effect of treatment time

There was no regular increase or decrease of antibrowning effect in samples treated with DPL, pectin, *Moringa* 1% and the control as treatment time increased. However, treatments at 5min exhibited significantly better ( $p > 0.05$ ) browning inhibition than those at 1min for all ABA except DPL. This could mean that increase in treatment time enhances the adsorption of the ABA (Huan Ng *et al.*, 2003) therefore increasing their performance. Nevertheless, DPL showed no significant difference in antibrowning effect as time increased probably because, the enzyme papain, which is the primary active agent in DPL (Richard-Forget *et al.*, 1998), is required in small amount for its activity (Chandrasekhar, 2002), thus increased adsorption had no significant effect on its browning inhibition.

This study shows that increase in treatment time increases the anti-browning potency of an ABA. However, depends on the type of ABA (Jiang *et al.*, 2004). Since time is an important resource in the food processing



industry, a case such as that of the DPL combination would require that the combination which gives effective browning inhibition with shorter treatment time will be preferred.

### Effect of concentration

Lower concentration in papain (3 min), pectin (3 min) and lemon (1 min) were significantly different ( $p > 0.05$ ) from their corresponding higher concentrations of the same ABA (Table 1). Other treatments were not significantly different ( $p < 0.05$ ) with their corresponding concentrations.

The reduced antibrowning effect of higher concentration of pectin can be attributed to the light brown colour of pectin solution which increased with increase in concentration. Thus, its ability to act as a coating agent due to its gelling and thickening properties, results in the exhibition of this brown colour on the yam chips. Likewise, reduced antibrowning effect in lemon juice as concentration increased may be due to ascorbic acid browning (Davies, and Wedzicha, 1992) - a non-enzymatic browning of the ascorbic acid which involves intermediates similar to those found in maillard browning. It could be that an increase in the concentration of lemon juice had a corresponding increase in the ascorbic acid which in turn enhanced the ascorbic acid browning. It may also be due to the slightly yellow colour of lemon which may have been more evident on the chips at high concentration. In the case of DPL, the high effectiveness of papain at low concentration (Chandrasekhar, 2002) makes its use at high concentration wasteful. However, considering that DPL is an unrefined form of papain, there is room for more explanation on the reason for its reduced effect at high concentration.

### Rate of colour change

The rate of colour change depicts the pace at which each treated yam chip changed colour until the 48 hr stoppage time. It does not necessarily correspond to the  $\Delta E$  at the stoppage time. It can be used to assess the effectiveness of an ABA. Ideally, a treated sample ought to have a lower rate of colour change than the untreated due to inhibition effect of the ABA. Notwithstanding, DPL and Pectin 1% at 3min had higher rates than the control as depicted by 'm' (Table 2).

**Table 2. Rate of colour change from *E* values**

ABA	$R^2$ (%)	Equation of curve ( $y = mx + c$ )		
		$m$ ( $\times 10^{-3}$ )	$c$	<i>E</i> at 48 hrs
DPL 1%:3min	97.6	4.85	1.70039	12.12
DPL 2%:5min	98.29	4.77	1.44834	13.03
Pectin 1%:3min	99.9	5.44	0.38843	13.03
Pectin 2%:5min	98.32	3.97	1.37698	11.31
Moringa 1%:5min	99.07	4.37	0.35323	12.99
Moringa 2%:5min	99.51	4.23	0.23429	12.78
Lemon 40%:5min	99.06	3.85	0.11004	10.3
Lemon 50%:5min	97.06	3.5	1.44522	8.96
Control:3min	98.78	4.59	0.93314	11.11

Pectin 1% at 3min and lemon 50% at 5min had the highest and lowest rate respectively. The discrepancy between this results and the result from sensory evaluation may due to the fact that visual scoring and instrumental measurement produce data sets that differ in quality (Nollet, 2004).

## Conclusion

The materials used have antibrowning potential which can be used to prevent browning in *D. alata*. Treatment time increased the anti-browning potency of ABA, however, this depends on the type of ABA. Lower concentration had better antibrowning results in DPL, pectin and lemon. The result of the rate of colour change revealed that visual scoring and instrumental measurement produce data sets that differ in quality.

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# The suitability of water yam (*Dioscorea alata*) for couscous production

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## Abstract

Consumer utilization of water yam (*Dioscorea alata*) could be enhanced if this specie of yam is processed into a more stable, acceptable and convenient food form. A study on the suitability of *D. alata* in the production of couscous was conducted. Yam couscous was prepared from fifteen varieties of *D. alata*. The products were prepared using the blanched-grated-tuber. Couscous from TDa 99/00528, TDa 291 and TDa 98/001168, TDa 99/00199 and TDa 99/00214 were judged as the most preferred based on general sensory qualities and peak viscosity. However, two variety, TDa 297 and TDa 98/01166 were used for storage studies. The moisture content, pH, microbial load, and sensorial qualities of the yam couscous were determined during a 24 week storage studies. The moisture content, pH and the microbiological load of water yam couscous conforms to food standards for couscous. Although semolina couscous was generally preferred to yam couscous, yam couscous had similar sensory ratings as semolina couscous and was acceptable to the consumers over the storage period. It is therefore possible to produce couscous from *Dioscorea alata* which will be safe for human consumption and keep for not less than 24 weeks in a cool dry place.

**Keywords:** Water yam, couscous, sensory evaluation, microbial load, semolina.

## Introduction

*D. alata*, commonly referred to as 'winged yam', 'water yam' or 'greater yam' has high moisture content and high nutritional content (crude protein - 7.4%; starch - 75-84 %; and Vitamin C - 13.0 - 24.7 mg/100 g (Osagie, 1992). The tubers are bulky and highly susceptible to spoilage hence processing them into more stable product will increase shelf life, availability, enhance its usage and provide variety.

The main processed forms in which yam tubers are consumed or preserved is flour (Akoroda, 1995; Iwuoha, 2003) and in Benin product like *wasawasa* or couscous have been exploited. These products are normally made from the White yam (*Dioscorea rotundata* Poir) whilst neglecting other yams species especially Water yam (*Dioscorea alata*). *D. alata* has been the least choice compared to white and yellow yams due to its generally loose watery texture (Opara, 1999). Consumer utilization of water yam could be enhanced if this species of yam can be processed into a more stable, acceptable and convenient product.

Couscous is a kind of pasta traditionally made from gritty wheat flour (*Triticum turgidum*) (Wright, 2006) rolled into tiny granules of uniform size, with the aid of water and smooth flour. The granules are precooked, dried in the sun, and stored. ([www.en.wikipedia.org](http://www.en.wikipedia.org)). Couscous is prepared by steaming, and served with meat or vegetable stew on it. It can also be eaten alone, flavoured or plain, warm or cold, as a dessert or a side dish (Aboubacar and Hamaker, 1999).

The study therefore seeks to investigate suitability of water yam in the production of couscous and the consumer acceptability of the product.

## Materials and methods

### Raw materials

Fifteen *D. alata* varieties, *Matches*, *Red water yam*, TDa 99/00208, TDa 98/01166, TDa 99/00528, TDa 297, TDa 291, TDa 98/01176, TDa 98/001168, TDa 99/00240, TDa 99/000480, TDa 99/00199, TDa 98/01174, TDa 99/00214

and TDa 99/00049, were obtained from the Savanna Agriculture Research Institute (SARI) Nyankpala; and Crop Research Institute (CRI) Fumesua, Ghana.

Several studies, not reported in this paper, were carried out on the 15 *D. alata* varieties to select varieties for further studies. Based on peak viscosity and general sensory performance by untrained panellists, TDa 98/01176, TDa 98/001168, TDa 297, TDa 99/00528 and TDa 99/0048 were the best varieties for yam couscous. The varieties selected were based on the overall acceptability. TDa 297 (the overall best sample) and TDa 98/01166, which had no significant difference with the second best samples, TDa 99/00528 and TDa 98/01176 were used for storage studies.

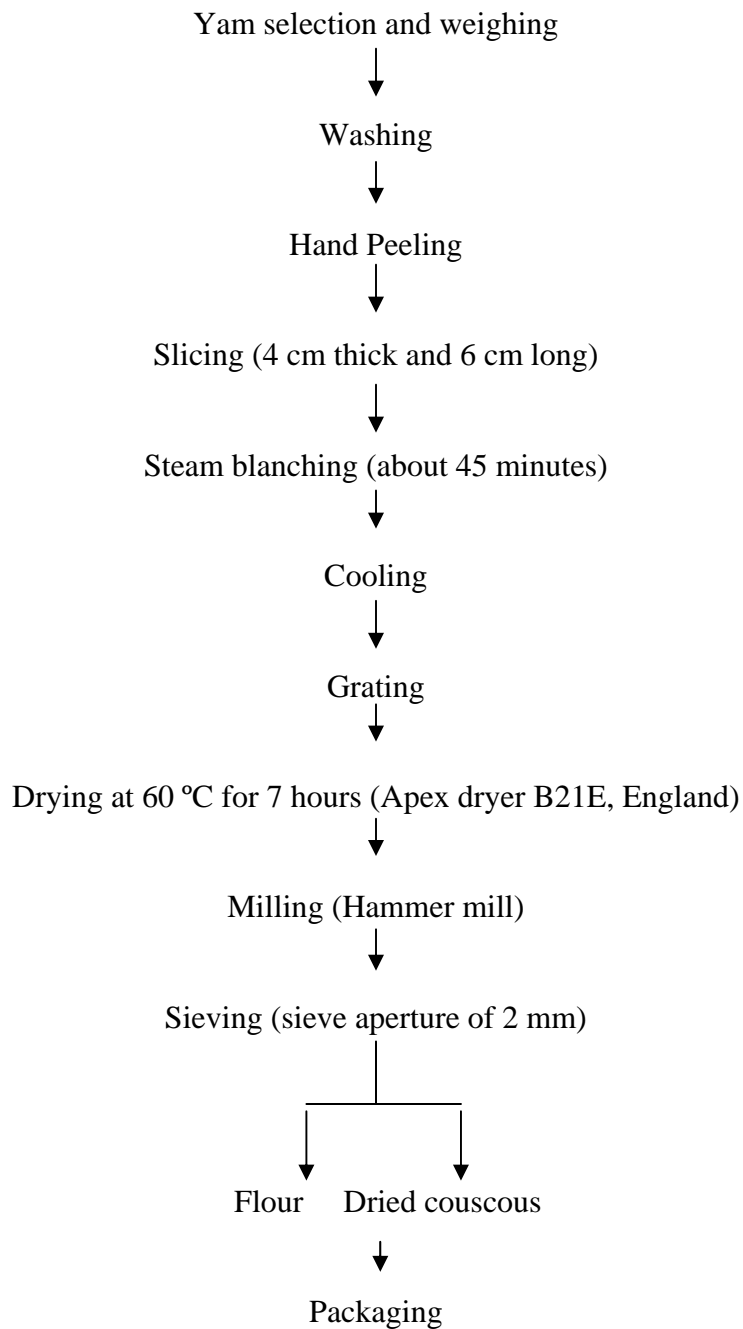
### **Methods**

Couscous was prepared by the blanched-grated-tuber (BGT) method (Figure 1). Storage studies were conducted every 8 weeks, for 24 weeks on samples stored in a cool dry place. This included sensory evaluation by trained panellists, microbiological evaluations, moisture (AOAC, 1990) and pH (AOAC, 2000) of the couscous samples were measured to evaluate the quality of the product over the 24 week period.

All plating for the microbiological evaluations were done by the pour plate method. Aerobic Plate count, yeast, mould and coliform counts were carried out. Confirmatory tests for coliforms and *E. coli* were also carried out.

Sensory evaluation was done by 15 trained panellists from Food Research Institute, Accra, as well as 10 couscous consumers at Paloma Restaurant, Accra, who were made to assess one of the selected yam couscous samples for the storage life studies using the semolina couscous as standard. This group of panellists used a line scale (10cm) of 0 (Dislike very much) to 10 (like very much) to assess the product. Processed couscous were moistened with water and steamed for thirty minutes prior to sensory evaluation. Quality attributes such as colour, texture (hardness), flavour (taste and smell) and overall acceptability were assessed. The assessment of the overall acceptability was done with vegetable stew.

Analysis of variance (ANOVA), were conducted using Statgraphics statistical package (Centurion edition). Significant differences were determined at  $p < 0.05$ . Microsoft Excel was used in the graphical representation.



**Figure 1. Flow diagram for *dried couscous* processed by the blanched-grated-tuber method**

## Results and discussion

### *Effect of moisture on the microbial growth over storage time*

There was a gradual increase in the moisture content of the yam couscous samples over storage time and the variability in the moisture content of the samples were also significant ( $p < 0.05$ ) over the storage time. The moisture content of TDa 98/01166 couscous was 8.1 % when it was freshly prepared. By the 24<sup>th</sup> week of storage, the moisture had increased to 10 %. The moisture content of TDa 297 couscous also increased from 6.6 % on the first day of preparation to 9 % by the 24<sup>th</sup> week of storage. The gradual increase of moisture in the water yam couscous samples may be due to the hygroscopic nature of the inherent starch present. Despite the gradual increase in moisture content of the yam couscous samples over storage time, the values recorded were below the 13.5 % moisture limit specified by Codex standard (Codex, Stan 202-1995). The low moisture content recorded is an indication of the shelf life stability of the product.

### *Effect of pH on the Microbial Growth over Storage Time*

The variability of the pH of the samples and its change over the storage period were statistically significant ( $p < 0.05$ ). There was a slight decrease in pH of TDa 98/01166 couscous sample from 5.58 on the first day of preparation to 5.32 by the 16<sup>th</sup> week, which was followed by a slight increase in pH by the 24<sup>th</sup> week to 5.4. A similar trend was followed by TDa 297 couscous, which had a pH of 5.54 on the first day of preparation, reduced to a pH of 5 after 16 weeks of storage and slightly increased to a pH of 5.1 after 24 weeks of storage. The decrease in pH could have been as a result of fermentation (Adams and Moss, 1995). However, the subsequent increase in pH could be due to the protein mass of the microbes over time. The normal pH range for bacteria growth has been reported to be between 6 and 8 (Adams and Moss, 1995). The pH ranges could have suppressed bacteria growth and could have accounted for the relatively lower Aerobic Plate Count especially in the TDa 297.

### *Aerobic Plate Count*

TDa 98/01166 couscous recorded higher Aerobic Plate Count than TDa 297 couscous. The difference in the Aerobic Plate Count of the sample was significant ( $p < 0.05$ ). TDa 98/01166 had an increase in the Aerobic Plate Count up to the 16<sup>th</sup> week and then decline sharply (Table 1). The increases recorded with the first 16 weeks could be that, there were enough nutrients for the growth of the bacteria. TDa 297 also observed an increase in the Aerobic Plate Count within the first eight weeks and then a subsequent decline. The variations that occurred over the storage period were also significant ( $p > 0.05$ ). On the first day of preparation, the Aerobic Plate Count of TDa 98/01166 averaged  $5.1 \times 10^3$  CFU/g whilst TDa 297 recorded 570 CFU/g. These variations could have been due to differences in levels of exposure of the samples during processing. The bacteria present at this time could be thermophilic organisms since the couscous samples have been dried at 60 °C for 7 hours. At this temperature all the mesophilic and possibly the psychrotrophic organisms which are mostly the food spoilage organisms would have been destroyed (Adams and Moss, 1995). The values recorded for Aerobic Plate Count over the 24 week storage period are all within the Ghana Standards Board acceptable limit of  $1 \times 10^6$  for couscous (Ghana Standards, GS 730/2003).

**Table 1. Mean Aerobic Plate Count (APC) and Yeast and Mould Count (YMC) of dry water yam couscous samples over the 24 weeks storage period**

Sample	Period							
	Week 0		Week 8		Week 16		Week 24	
	APC	YMC	APC	YMC	APC	YMC	APC	YMC
<b>TDa 98/01166</b>	$5.1 \times 10^3$ $\pm 1.4 \times 10^2$	0 <sup>a</sup>	$2.7 \times 10^4$ $\pm 3.2 \times 10^2$	20 <sup>b</sup>	$1.3 \times 10^5$ $\pm 2.8 \times 10^3$	0 <sup>a</sup>	$5.0 \times 10^3$ $\pm 1.4 \times 10^2$	0 <sup>a</sup>
<b>TDa 297</b>	$5.7 \times 10^2$ $\pm 1 \times 10^1$	0 <sup>a</sup>	$5.2 \times 10^3$ $\pm 1.4 \times 10^2$	10 <sup>b</sup>	$7.0 \times 10^2$ $\pm 0.0$	0 <sup>a</sup>	$2.0 \times 10^2$ $\pm 0.0$	0 <sup>a</sup>

Values statistically different at ( $p < 0.05$ ) shares different letters

### **Yeast and Mould Count**

The yeast and mould growth of the samples were however not significant ( $p > 0.05$ ), even though there were significant differences ( $p < 0.05$ ) in their counts over the storage period. There was no mould and yeast growth in the couscous sample on the first day of preparation (week 0), 16<sup>th</sup> week and 24<sup>th</sup> week of storage. Oven drying of the yam couscous samples at 60 °C for 7 hours could have destroyed all the vegetative cells and spores of the yeast and mould that would have been present. According to Adams and Moss (1995), the vegetative cells and spores of yeast and mould are killed below 100 °C in the baking of bread. Yeast and mould growth was observed on the eighth week of storage, which were 10CFU/g and 20CFU/g for TDa 98/01166 and TDa 297 respectively (Table 1). This growth observed may be due to contamination of the samples at the time of analysis. The acceptable limits for yeast and mould levels in couscous are 500 CFU/g according to the US durum couscous specifications (Couscous specifications, 2007) and  $1 \times 10^4$  CFU/g according to Ghana Standards Board specifications for couscous (Ghana Standards, GS 730/2003).

### **Coliforms and *E. coli* count**

There were no Coliforms and *E. coli* growth over the storage period. The Coliforms and *E. coli* which may be present would have been killed during the oven drying of the yam couscous. From the public health stand point, the couscous samples could be recognized as safe due to the absence of Coliforms and *E. coli* (Adams and Moss, 1995).

### **Sensory Evaluation by Trained Panellists**

**Colour (whiteness of colour).** There was a gradual decrease in the whiteness score of the storage period. TDa 297 couscous scored 3.71 on first assessment day. By the 24<sup>th</sup> week of storage, the whiteness score of the panellists have been reduced to 2.4. TDa 98/01166 which was the darker of the two yam couscous also scored whiteness values ranging from 0.97 to 0.93 on the first day and after 24 weeks of storage respectively. The standard couscous which was actually yellow scored an average of 0.83 for whiteness. The relatively low values of whiteness for the yam couscous recorded may be due to the amount of polyphenols that were present prior to processing (Akissoe *et al.*, 2003) and also the ferrous iron present in the tuber oxidizing to ferric iron when the yam tubers are cooked especially in water or with steam (Mornar-Perl and Friedman, 1990). The decrease in the whiteness of the yam couscous as assessed by the panellists was an indication of the darkening of the product over the storage time. The yellow colour of the semolina couscous was relatively stable during the storage period.

**Appearance.** Two parameters were assessed under appearance: the presence of black specks and the uniformity of the couscous grains. Very minimal black specks were recorded. However, TDa 98/01166 had the most of black specks and recorded the highest value of 1.5 on the 16<sup>th</sup> week of storage and the lowest value of 0.8 on the 8<sup>th</sup> week of storage. TDa 297 recorded black speck scores in the range of 0.9 to 0.95 while semolina couscous recorded the lowest black specks values which ranged between 0.2 and 0.24.

The variation in the uniformity of the samples over the storage period was significant ( $p < 0.05$ ) even though yam couscous under the shelf life studies were processed using the same processing procedure. Average values of 9.44, 8.06 and 7.08 were scored for semolina, TDa 98/01166 and TDa respectively. The semolina couscous grains were the most uniform.

**Flavour.** The variability of the flavour acceptability of the couscous samples was not significant ( $p > 0.05$ ). TDa 297 scored the highest flavour value of 7.9 followed by semolina couscous with a value of 7.57 whilst TDa 98/01166 recorded the lowest value of 7.1 on the first day of preparation. There were slight differences in the flavour of the samples on the 8<sup>th</sup> and 16<sup>th</sup> weeks of storage. However, there was a drastic reduction in the flavour of the samples after 24<sup>th</sup> weeks of storage. Semolina couscous, TDa 98/01166 couscous and TDa 297 couscous scored 5.08, 5.55 and 5.26 respectively. The reduction in the flavour score may be attributed to off flavour development in the samples due to absorption of unpleasant flavour components during the storage period (Perera, 2005). It could also be due to breakdown of flavour components (Basha and Young, 1996; Wilkes *et al.*, 2000) and fermentation of the samples (Tiitinen *et al.*, 2006).

**Taste.** Panellists assessed the presence of sour taste and the taste acceptability of the couscous over the storage time. The sour taste of all the couscous samples were virtually negligible on the first day of preparation and were as follows; TDa 297 (0.38), TDa 98/01166 (0.69) and semolina (0.16). According to the judgment of the panellists, there was an increase in the sour taste after 8 weeks of storage to 1.7, 2.2 and 1 for TDa 297, TDa 98/01166 and



semolina couscous respectively. By the 24<sup>th</sup> week of storage, the sour taste had increased to 2, 2.5 and 1.05 for TDa 297, TDa 98/0166 and semolina couscous respectively.

Although the taste acceptability score of all the couscous samples were above average, generally TDa 297 couscous was better than semolina couscous. The taste acceptability score of TDa 98/0166 couscous was the least of the three samples assessed. The variability in the taste acceptability of the sample over the storage period was significant ( $p < 0.05$ ).

**Texture.** The textural attributes of the products evaluated included stickiness, dryness, hardness and mouth feel. The stickiness of the samples on the first day was 3.3 and 2.97 for TDa 297 and TDa 98/0166 couscous respectively. These values were comparable to what was recorded for the semolina couscous (3.36). There was no consistent trend in the stickiness of the products. The 8<sup>th</sup> week recorded the lowest values of stickiness of 3, 2.8, and 3.11 for TDa 297, TDa 98/0166 and semolina couscous respectively. The stickiness of the samples could be attributed to the amount of free starch particles present in the couscous samples. It could also be dependent on the amount of the very small floury particles which may be present in the couscous samples. These free starch particles and smaller floury particles have been reported to contribute to the stickiness of moistened steamed couscous (Aboubacar and Hamaker, 1999).

There was a slight decrease in dryness of the samples with time. The dryness score averaged 3.15, 3.72 and 3.59 for semolina, TDa 98/0166 and TDa 297 couscous respectively. The results imply that the products were relatively moist; nevertheless, hardness and dryness were not significantly variable ( $p > 0.05$ ).

The average score on mouth feel of the samples over the 24 week period were, 7.03, 5.2 and 5.33 for semolina, TDa 98/0166 and TDa 297 respectively. This implies that the mouth feel of semolina couscous is smoother than the yam couscous.

**Overall acceptability.** There was no significant difference in the overall acceptability score of the couscous, which were semolina (7.05), TDa 98/0166 (7.01) and TDa 297 (7). The overall acceptability of the yam couscous products was comparable to that of the wheat couscous. The slight increase in sourness and the slight darkening of the yam couscous did not affect the overall sensory attribute of the product during the 24 weeks of storage.

**Sensory evaluation by couscous consumers at Paloma restaurant.** TDa 297 couscous was used for this category of sensory analysis. The results obtained for the yam couscous was comparable to semolina couscous even though the semolina couscous was the most preferred choice of the panellists. The average score of colour of the semolina couscous and yam couscous were 8 and 5 respectively. This implies that the yellow colour of the semolina couscous was more appealing to the panellists than the off white colour of the yam couscous. The flavour score of 5 recorded by the yam couscous was lower compared to that of semolina couscous which recorded a value of 7. The scores of taste, stickiness, and overall acceptability were all 7 for semolina couscous and 6 for yam couscous.

Stickiness scored 7 for semolina couscous and 5.1 for yam couscous. This was an indication that stickiness of the semolina couscous was preferred to the yam couscous. On the whole, couscous from *D. alata* had comparable and acceptable qualities,

## Conclusion

It can be concluded from this study that the moisture content, pH and the microbiological load of water yam couscous conforms to food standards for couscous. Yam couscous had similar sensory ratings as semolina couscous and was acceptable to the consumers over the storage period. It is therefore possible to produce couscous from *D. alata* which will be safe for human consumption and keep for not less than 24 weeks in a cool dry place.

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# Glucose syrup from yam starch using rice malt as the source of enzymes

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## Abstract

Studies were conducted on the enzymatic hydrolysis of yam starch by the use of enzymes from rice malt of high diastatic activity. Yam starch was isolated from *Dioscorea rotundata* ('Doko bayere') with a percentage starch content of 15.24%. The syrup produced had a dextrose equivalent (D.E.), Brix, total solids, and pH of 38.04%, 70.5, 78.58% and 4.1 respectively. The chemical characteristics of the yam glucose syrup compares favourably with those of commercial syrup. Yam starch can be processed into glucose syrup using enzymes from rice malt.

**Keywords:** Yam, starch, syrup, rice malt, enzymes.

## Introduction

Glucose syrups are essentially industrial sugars used in the manufacture of food products, pharmaceuticals but are mainly consumed in the confectionery industry (Bello-Pérez *et al.*, 2002; Akinola and Ayanlele, 2004). Glucose syrup can be obtained by treating starch with hydrolytic enzymes during which glucosidic bonds are broken (Guzman-Maldonado and Paredes-Lopez, 1995).

According to Raemackers (2001), yam has a starch content of 15-23%. In many tropical countries, yam is produced in large quantities but has a narrow range of utilization and is plagued with high incidence of postharvest losses and limited market expansion (ICRA, 1996; Onwueme, 1987). Product diversification of yam tubers through efficient use will therefore help curtail many of these challenges.

Thus, this study attempts to produce glucose syrup from yam starch using enzymes from rice malt. Enzyme conversion of yam starch to glucose syrup can lead to efficient utilization of yam. The glucose syrup can therefore serve as a complete or partial substitute for sugar in the food and pharmaceutical industries.

## Materials and methods

### *Raw materials*

Paddy rice (*Jasmine 85 variety*) was obtained from the Crop Research Institute (CRI), Fumesua, Kumasi and fresh yam tubers (*Dioscorea rotundata*) were purchased from the Kumasi Central Market, Ghana.

### *Rice malt production*

Five hundred grams of the rice seeds was steeped for 3 days at  $30 \pm 1^\circ\text{C}$  in a micro malting chamber. Grains were air rested for 6 hours. Grains were then germinated for 8 days at a temperature of  $30 \pm 1^\circ\text{C}$  in a micro malting chamber. Kilning of rice seedlings was done in the sun at  $36 \pm 2^\circ\text{C}$  for 2 days. Dried malt was then milled using the plate attrition mill.

### *Glucose syrup production*

Extraction of starch for the glucose syrup production was done using the wet method as described by Barimah and Mantey (2002). The starch obtained was dried with a solar drier tent for 3 days. The final weight of the starch was measured and used to calculate the percentage starch content of the yam used.

One hundred grams (100g) of the dried yam starch was mixed with 500 ml distilled water to form slurry. The slurry was gelatinized at a temperature of 80-85°C for 20 minutes and cooled to 60-65°C. Eight grams (8g) of the rice malt was added to the gelatinized starch and stirred for 10 min in the liquefaction phase. A second batch of 8g rice malt and 100g yam starch was prepared and added to the first batch. The mixture was stirred and allowed to stand for 3 hours. The temperature of the mixture was lowered to 55°C followed by the addition of 16g of the rice malt for the saccharification phase which proceeded for 4 hours. The liquid was then heated for about 20-30 minutes at 80-85°C to inactivate the enzymes from the malt, filtered and heated again to evaporate water and produce syrup. The syrup was packaged in a glass bottle.

### ***Analyses conducted***

Analyses conducted on the syrup included yield, dextrose equivalent, pH, moisture content and total solids and Brix at 25°C. The pH was measured with pH meter (BECKMAN 340, 5894, U.S.A).

The refractometer (Master Refractometer, ATAGO, 781-741-8778, Japan) reading was expressed in degree Brix at 25°C. The moisture content and total solids were determined according to AOAC (1990) methods.

### ***Determination of dextrose equivalent (DE)***

Ten (10) ml of the dextrose solution was taken and diluted to 100ml with distilled water. 10ml of the final solution was taken again and diluted to 50ml with distilled water. 100ml of 0.1M Iodide (I<sub>2</sub>) solution and 10ml of the 0.47M Na<sub>2</sub>CO<sub>3</sub> was added. The solution was then allowed to stand in the dark for 20 minutes. After 20 minutes, 15ml of the 1.02M H<sub>2</sub>SO<sub>4</sub> was added. The excess iodide was then titrated with 0.5M Na<sub>2</sub>S<sub>2</sub>O<sub>3</sub>. The average titre values were then used to calculate the dextrose equivalent.

## **Results and discussion**

The starch yield of yam and moisture content of starch were 15.24% and 21.41% respectively. These values are within the range reported by Raemackers (2001) and Dziedzoave *et al.* (2003). The pH (4.1) of the yam glucose syrup was lower than that reported by Dziedzoave *et al.* (2003) which was between 5.5-5.6. This could be attributed to fermentation that might have occurred during cooling and filtration of the final syrup. The dextrose equivalent of the yam glucose syrup obtained was 38.04%, higher than the minimum recommended standard for glucose syrup (> 20%) (Pancoast and Junk, 1980).

Comparing the yam glucose syrup (YGS) to the commercial glucose syrup (CGG), all the parameters measured were lower in YGS than in CGS (Table 1). This may be attributed to the differences in the production processes (Grace, 1977) as well as the raw material - yam starch and corn starch (www.most.gov.mm) respectively. However, the YGS can be used in products that require low sugar such as canned fruit preserves, ice cream, bakery products, jam, soft drinks, candy and all kinds of confectionery. Large quantities are also used as a booster in the fermentation of alcohol (www.starch.dk).

**Table 1. Comparison of Yam Glucose Syrup and Commercial Glucose Syrup**

Properties	Yam glucose syrup	Commercial glucose syrup (Glaxo)*
pH	4.1	6.7
DE (%)	38.04	48
Total solids (%)	78.58	79
Brix (°)	70.5	78

\*Akinola and Ayanleye (2004)

## **Conclusion**

It can be concluded from this study that potential exists for the preparation of glucose syrup from yam starch using rice malt.

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