Investigating carotenoid loss after drying and storage of orange-fleshed sweet potato

Aurélie Bechoff

A thesis submitted in partial fulfilment of the requirements of the University of Greenwich for the Degree of Doctor of Philosophy

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To the memory of
Constance Owori,

who was unable to finish her own thesis
now up in heaven smiling,

for her work
on sweet potato in Uganda

DECLARATION

I certify that this work has not been accepted in substance for any degree, and is not currently submitted for any degree other than that of Doctor of Philosophy (PhD) of the University of Greenwich. I also declare that this work is the result of my own investigations except where otherwise stated.

name ----------
(Student)

name ----------
(First Supervisor)

name----------
(Second Supervisor)

ABSTRACT

Biofortified orange-fleshed sweetpotato (OFSP) is being promoted to tackle vitamin A deficiency, a serious public health problem affecting children and pregnant/lactating women in sub-Saharan Africa. The aim of the study was to quantify and understand the factors influencing carotenoid losses in dried OFSP. Losses were determined in chips after drying and storage. A preliminary study demonstrated that carotenoid levels were not significantly different following either solar or sun drying. Carotenoid loss after drying was generally correlated with high initial moisture content and high carotenoid content in fresh sweetpotato roots. Losses of pro-vitamin A were less than 35% in all cases. Flour made from OFSP could therefore be a significant source of provitamin A. In contrast, storage of chips at room temperature in Uganda and Mozambique for four months resulted in high losses of pro-vitamin A (*ca*. 70-80% loss from the initial dried product). Low-cost pre-treatments, such as blanching, antioxidants and salting, did not reduce carotenoid losses during storage. Enzymatic catabolism of β-carotene in dried OFSP was considered unlikely because of low peroxidase activities at low water activities and the loss of peroxidase activity during storage. To understand further the factors causing the losses, dried sweet potato chips were stored under controlled conditions of temperature (10; 20; 30; or 40ºC), water activity (0.13; 0.30; 0.51; 0.76) or oxygen (0 [under nitrogen]; 2.5; 10 or 21% [air]). Oxygen was the main cause of degradation followed by temperature. An Arrhenius kinetic model was used to show that carotenoid breakdown followed first order kinetics with an activation energy of 68.3kJ.mol^{-1} that was in accordance with the literature. Experimental observations fitted well with data predicted by the kinetic model. The formation of the volatile compounds, β-ionone; 5,6-epoxy-β-ionone; dihydroactinidiolide; β-cyclocitral that were clearly related to the degradation of β-carotene, helped further understand breakdown patterns of β-carotene.

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ABBREVIATIONS

ABTS	2,2'-azino-bis 3-ethylbenzthiazoline-6-sulphonic acid	LA	linoleic acid
a_w	Water activity	LOX	lipoxygenase
BET	Brunauer-Emmett-Teller	LPDE	Low density polythene
CIAT	International Center for Tropical Agriculture, Colombia	MTBE	Methyl-ter-butyl-ether
CIELAB	CIE 1976 (L*, a*, b*) color space	NARL	National Agricultural Research Laboratories, Uganda
CIP	International Center for Potato	OFSP	Orange fleshed-sweet potato
CIRAD	Agricultural Research Institute for Developing Countries, France	POD:	Peroxidase
DCP	Dichlorophenol	R	gas constant = 8.314 J · K^{-1} · mol^{-1}
DHA	Dihydroactinidiolide	RDA	Recommended Dietary Allowance
Ea	Activation energy ($kJ.mol^{-1}$)	RE	Retinol Equivalent
EDTA	Ethylene diamine tetra acetic acid	SO_2	Sulphur gas
FAO	Food Agriculture Organization	SPME/GC/MS	Solid-phase microextraction/Gas Chromatography/Mass Spectra
HIV	Human Immunodeficiency Virus	TDRI	Thailand Development Research Institute
HPDE	High density polythene	Tukey HSD	Tukey's honest significant difference
HPLC	High Liquid Performance Chromatography	VAD	Vitamin A deficiency
ITDG	Intermediate Technology Development Group	VITAA	Vitamin A Partnership for Africa
k	degradation constant rate (day^{-1})	WFSP	White fleshed-sweet potato
		WHO	World Health Organization

CONTENTS

CHAPTER 1.
LITERATURE REVIEW

1.1 BACKGROUND

1.1.1 Introduction

Vitamin A deficiency is a major public health issue in developing countries; with children and pregnant/lactating women the most vulnerable (FAO/WHO 2002). Sub-Saharan Africa is one of the most affected areas with 33 million pre-school children who are deficient, which accounts for a third of the world cases (West 2002).

There are different strategies to tackle vitamin A deficiency. Traditional interventions consist of administration of vitamin A capsules. Although a single dose can be given every six months or every year, these medical interventions are costly (Nestel *et al.* 2006). Food fortification is another approach used to reduce vitamin A deficiency that works by adding vitamin A to food commodities (*e.g.* sugar). An alternative approach is biofortification, which consists of breeding staple crops to increase their content of vitamins and/or minerals. Compared to the two other strategies, biofortification is considered a more sustainable approach because it has the potential to provide vitamins or minerals throughout the year with a one-off intervention and in the longer term at lower cost (Kósambo *et al.* 1998). For the same level of impact on public health, the cost of biofortification is estimated to be half that of vitamin A supplementation (Nestel *et al.* 2006). Moreover, rural and low-income communities, which have been shown to be more at risk, can be reached by this approach and it also creates opportunities for income generation from production and marketing of these crops.

Sweet potato is a very important crop that is widely consumed in sub-Saharan Africa and Uganda one of the major producers (Woolfe 1992). In sub-Saharan Africa the majority of sweet potato that is consumed is white-fleshed and has low levels of provitamin A (β-carotene) (Ameny and Wilson 1997). There are efforts through the Vitamin A Partnership for Africa (VITAA) Initiative and also through a Bill and Melinda Gates

Foundation Project led by the HarvestPlus Challenge Program (Reaching End Users) to promote the use of orange-fleshed varieties that have a high β-carotene content. HarvestPlus is a global alliance of research institutions seeking to improve human nutrition in developing countries by tackling micronutrient deficiencies in iron, zinc and vitamin A. HarvestPlus focuses on biofortification of staple food crops (cassava, sweet potato, maize, rice, bean, millet) that are consumed by the poor. Currently the biofortified sweet potato varieties have shown to be capable of reducing vitamin A deficiency in studies on children in sub-Saharan Africa (Jalal *et al.* 1998; van Jaarsveld *et al.* 2005; Low *et al.* 2007).

The Natural Resources Institute of the University of Greenwich is leading the product development, processing and marketing component of Reaching End Users Project of HarvestPlus. Dried sweet potato can be used in the formulation of a variety of food products such as composite flours, bakery products etc. The work reported in this thesis contributed to this initiative in Uganda and Mozambique with the overall objective of quantifying and understanding the mechanism of losses of carotenoids during the drying and subsequent storage of sweet potato. The outcome of this research should help low-income farmers and food processors use dried orange-fleshed sweet potato (OFSP) that could potentially contribute to tackling vitamin A deficiency.

1.1.2 Vitamin A deficiency

Vitamin A or retinol is essential for human metabolism. Its main function is in the visual cycle in the retina of the eye, but it also plays an important role in growth and development and reproduction, and in the immune system (FAO/WHO 2002). Other functions of vitamin A are not yet fully understood, for example, regarding its role in regulation of gene expression (FAO/WHO 2002).

Vitamin A is found in the diet in two forms. In animal foods, preformed-vitamin A is found as retinyl esters of fatty acids linked to membrane bound-cellular lipids and fat containing storage cells. In plants, precursors of vitamin A called carotenoids are associated with cellular lipids and implanted in cellular structures (FAO/WHO 2002). Preformed vitamin A from meat is more bio-available than carotenoids from plants. However, in developing countries, meat is generally not affordable for most poor people.

It is estimated that 82% of vitamin A consumption is provided by plants in developing countries (Rodriguez-Amaya 1996). Green leafy vegetables (spinach, amaranth, young leaves from other sources), yellow vegetables (pumpkins, squash and carrots) and yellow and orange non-citrus fruits (mangoes, papayas, apricots) are good sources of provitamin A, as are red palm oil, and some indigenous plants and tropical fruits (Rodriguez Amaya 1996; Sommer 1998).

An insufficiency of vitamin A in the diet results in vitamin A deficiency (VAD). Vitamin A deficiency is responsible for night blindness, increased susceptibility to infections and impaired growth and development. Xerophtalmia includes all manifestations of visual deficiency caused by vitamin A from the mild and reversible form of night blindness, conjonctival xerosis, Bitot spots to irreversible form of cornea ulceration where the eye can irreversibly be damaged or lost (Sommer 1998). A large proportion (20-24%) of child mortality from measles, diarrhoea and malaria can be attributed to vitamin A deficiency (WHO 2004). Children are at risk but so are pregnant and lactating women and imunodeficient persons, such as those suffering from HIV and AIDS (Sommer 1998). South Asia and Africa are the parts of the world most affected by vitamin A deficiency (WHO 2004) (Figure 1-1).

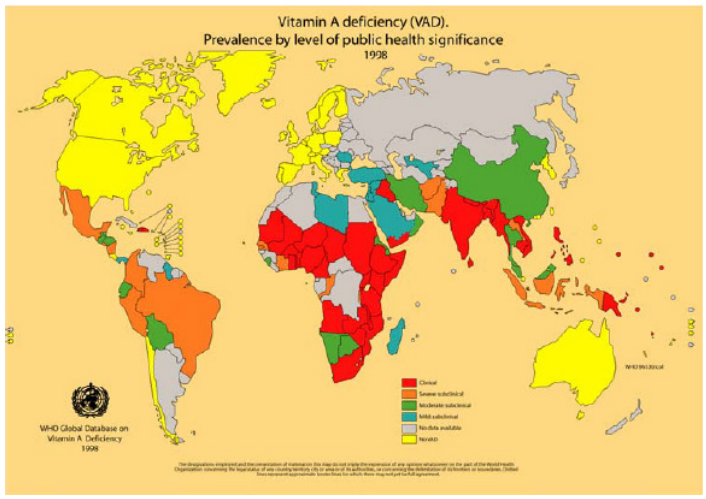


Figure 1-1: VAD prevalence in the world (1998). Red=clinical; Orange=severely sub-clinical; Green=moderate sub-clinical; Blue=mild sub-clinical; Grey=no data available; Yellow=no VAD. FAO/ WHO (2002).

Vitamin A deficiency affects over 127 million pre-school children (West 2002) and is reported to affect 38% of the children under five in Uganda (Ugandan Bureau of Statistics 2001) and 71% of the children in Mozambique (Aguayo and Baker 2005). This corresponds to an estimated 2.3 million children under five in each of Uganda and Mozambique.

In order to tackle vitamin A deficiency, daily nutritional needs in vitamin A for different class-ages were evaluated by FAO/WHO. The mean requirement intake is the minimum intake to prevent xerophtalmia in the absence of clinical or sub-clinical infection. Recommended safe intake is the average intake of vitamin A to permit adequate growth and other vitamin A dependent functions and maintain an acceptable total body reserve of the vitamin (FAO/WHO 2002). Recommended safe intake is also called Recommended Dietary Allowance (RDA). These requirements in microgram retinol equivalents per day (µg RE/day) are summarised in Table 1-1.

Table 1-1: Estimated mean requirement and safe level of intake for vitamin A. FAO/WHO (2002).

Age group	Mean requirement µg RE/day	Recommended safe intake µg RE/day
Infants and children		
0–6 months	180	375
7–12 months	190	400
1–3 years	200	400
4–6 years	200	450
7–years	250	500
Adolescents, 10–18 years	330–400	600
Adults		
Females,19–65 years	270	500
Males, 19–65 years	300	600
65+	300	600
Pregnant women	370	800
Lactating women	450	850

Source: Adapted from FAO/WHO, Rome 1988 (*69*).

Some earlier studies estimated that 6 µg of β-carotene from plant sources were providing one retinol equivalent (RE). More recent studies have however suggested that the appropriate conversion factor varies by plant species and might be much lower (Sommer 1998). Consequently conversions factors published by National Academy of Sciences/Institute of Medicine (2001) stated that 12 µg of β-carotene (or 24 µg of other carotenoids such as α-carotene, 5,6-epoxy-β-carotene and β-cryptoxanthin) found in

food corresponds to one retinol activity equivalent (RAE). A recent study in mashed sweet potato reported a conversion factor of 13:1 (13 µg of β-carotene in mashed sweet potato equivalent to 1 µg retinol equivalent (RE) (Haskell *et al.* 2004).

Two more recent studies (van Jaarsveld *et al.* 2005; Low *et al.* 2007) in South Africa and Mozambique respectively have demonstrated that regular consumption of orange-fleshed sweet potato (OFSP) significantly increased vitamin A status of children. The South African study (van Jaarsveld *et al.* 2005) measured the impact of the consumption of OFSP on primary-school children. The serum retinol of children (n=90) who consumed OFSP was significantly higher as compared with the serum retinol of children consuming white-fleshed sweet potato (WFSP) after 53 school-days. It proved that the consumption of OFSP significantly increased vitamin A status of children. On the other hand, the study undertaken by Low *et al.* (2007) in Mozambique consisted of a two-year-integrated agricultural and nutrition intervention promoting OFSP consumption involving households and young children (*ca.* n=400) through two agricultural cycles. The outcome of the integrated approach also showed that in a rural setting the serum retinol of young children consuming OFSP significantly improved. Moreover the mean plot size of fields devoted to OFSP was nearly ten times higher in the second year. OFSP also emerged as the least expensive source of vitamin A in local markets. This second study further proved that OFSP, as part of an integrated agricultural and nutrition approach, could potentially play a significant role in tackling vitamin A deficiency in developing countries.

1.1.3 Importance of sweet potato

Sweet potato (*Ipomea batata* (L.) Lam.) is a dicotyledonous plant from the *Convolvulacae* family. It is an important crop in food systems in Eastern and Southern Africa. Cultivated in more than 100 countries, sweet potato ranks third of the world root and tuber crops production after potato and cassava (FAOstat 2008). In developing countries, sweet potato is especially valuable because it is a food security crop for the poor that can provide an important part of the dietary carbohydrate of the population (Hagenimana and Owori 1996). Sweet potato grows well in tropical areas, where the majority of poorest people live, and it yields relatively better than most of the major crops under tropical climates (Woolfe 1992). World production has been estimated at 110 million tonnes per annum (FAOstat 2008). Asia is the world's largest producer with

92.5 millions tonnes. China with a production of 85.2 million tonnes represents 77% of the world sweet potato production (FAOstat 2008). In Asia half of the production is used for animal feed and the remaining half for human consumption (Woolfe 1992). On the other hand in sub-Saharan Africa, sweet potato production is 13.7 million tonnes per annum (FAOstat 2008) representing only 12.4% of the world production, but it is almost exclusively used for human consumption.

Sweet potatoes are grown from 40°N to 32°S of the equator and on the equator it is grown from the sea level to 3000m. It has a short harvesting time, 3-8 months from planting, which is less than most other root and tuber crops (Woolfe 1992). High levels of nutrition, high productivity and low levels of input have made sweet potato a valuable resource in the family garden. In particular, it can be a significant resource for families with low income (Villareal and Griggs 1982). Sweet potato has been playing an important role as a life saver during food shortages and famines in the history of humanity. In spite of its numerous qualities, it is much underexploited and this is probably because of its status as a poor man's food (Woolfe 1992).

The sweet potato plant can be divided in three elements (Figure 1-2):

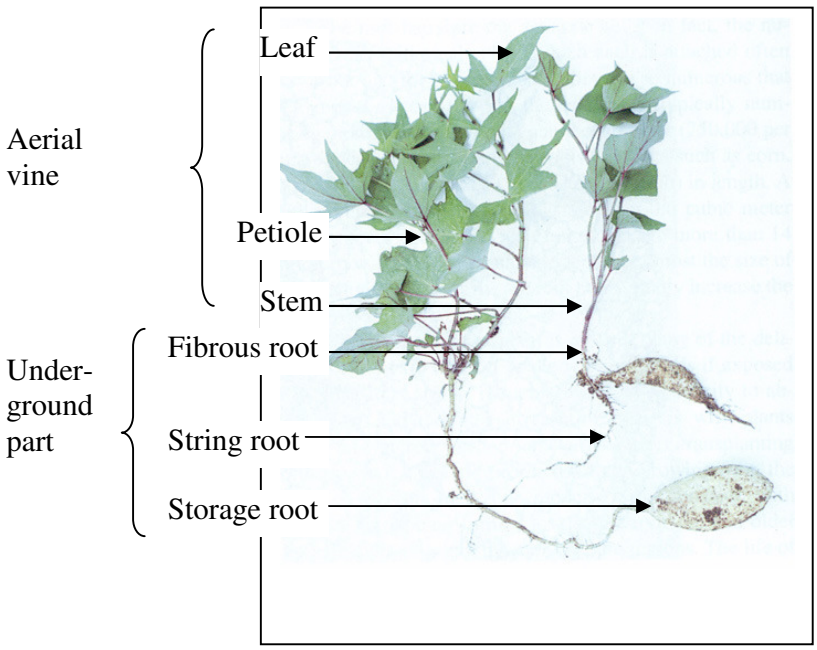


Figure 1-2: Picture showing the morphology of the sweet potato plant. Flowers are not represented in this. University of Illinois (2006).

- the leaves that make energy and sugars from photosynthesis;
- the vines or stems and petioles that transport this energy and nutrients; and
- the reserves produced by the root system within the plant; and the underground storage roots that absorbs water, minerals and nutrients from the ground and store the excess of energy and nutrients (Woolfe 1992).

Human intervention, natural hybridisation and mutation have produced a large number of varieties of sweet potato resulting in wider diversity than in cassava or yam. Varieties vary in the colour of the root skin or flesh (Figure 1-3), in the size and shape of the roots and leaves, depth of rooting, time to maturity and resistance to disease, water content and root texture (Woolfe 1992).

White-fleshed sweet potatoes are commonly cultivated in sub-Saharan Africa, but orange-fleshed varieties, rich in provitamin A, are rarer (Ameny and Wilson 1997). Their promotion is a recent initiative in sub-Saharan Africa. Recent work by Tomlins *et al.* (2007a) with women and children in Tanzania working with OFSP and white-fleshed varieties has shown that there was no difference in acceptability between the specific varieties tested (Figure 1-3).

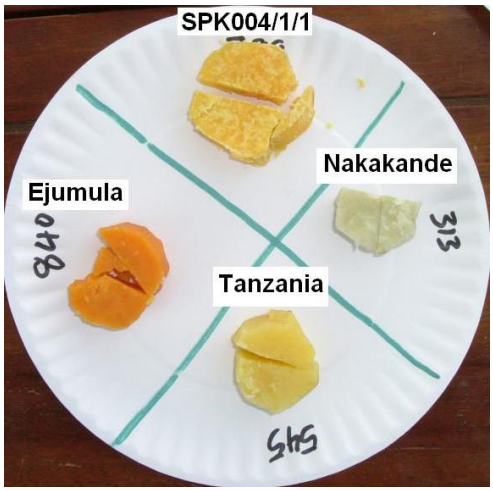


Figure 1-3: Appearance of sweet potato varieties cultivated in Uganda presented for sensory testing. K. I. Tomlins, NRI.

Adoption of OFSP therefore could potentially make a major contribution to overcoming vitamin A deficiency in East and Southern Africa (Tomlins *et al.* 2007a; Low *et al.*

2007). This current Harvest Plus "Reaching End Users" Challenge program project targets Uganda and Mozambique as pilot-countries for the promotion of OFSP.

Uganda is the second highest producer of sweet potato in Africa and the third world highest producer in the world with 2.71 million tonnes (MT) per annum (2% of world production and representing the equivalent of the American production) (FAOstat 2008) close after Nigeria (3.32 MT). The other main sweet potato producers in Africa are, in order of importance: Tanzania (1.32 MT per annum); Kenya (0.89 MT per annum);; Madagascar (0.89 MT per annum); Burundi (0.87 MT per annum) and Rwanda (0.80 MT per annum) (FAOstat 2008). Sweet potato is a staple food in many regions of Uganda, particularly in the North-Eastern and Southern-Western parts. Orange-fleshed sweet potato (OFSP) production is increasing. It was estimated that OFSP occupies 5-10% of production in Central Uganda (Kapinga *et al.* 2003). For a decade, the International Potato Center (CIP) has promoted OFSP in central Uganda, especially in rural communities and, more recently in the Eastern and Western parts of the country, but this was not on a large scale. Promotion has received strong support from policy makers. The HarvestPlus Challenge Program Project on OFSP, which started in 2006, is accelerating the expansion of OFSP by developing and assessing new clones of OFSP with higher provitamin A and better agronomical (yield and resistance to diseases) and sensory characteristics, improving the availability of OFSP vines to farmers, undertaking active promotion to vulnerable groups and promoting, where appropriate, the use of OFSP in new marketable products. Four new biofortified varieties have been released officially in Uganda since 2004; Ejumula, Kakamega (SPK004), Vita (SPK004/6) and Kabode (SPK004/6/6).

Unlike Uganda, sweet potato in Mozambique is a secondary crop representing a production of 0.07 MT per annum, which is a negligible part of world production (0.06%) (FAOstat 2008). CIP has been working for a decade in Mozambique too. The virus constraint is not as great as in Uganda which allows new clones to be more easily developed. At least, nine new varieties have been released since 2000. The markets for OFSP are growing fast in both countries as part of the joint efforts of CIP and HarvestPlus Challenge Program Project.

Uganda and Mozambique were chosen for the HarvestPlus "Reaching End Users with OFSP" Project on basis of the following criteria: high incidence of vitamin A deficiency, progress made in the breeding of suitable OFSP; contrasting nature of their socio-economic and agro-climatic environments and contrasting role of sweet potato crop, as well as the good government support and presence of existing pilot interventions. Promotion of OFSP in these two countries is a first step for HarvestPlus in the promotion of biofortified crops with the vision to expand this to other countries in Africa and other crops in the world.

In order to assess the potential nutritional impact of OFSP, research that goes ahead of the promotion should involve an evaluation of the vitamin A activity in OFSP grown under local conditions and prepared according to local practices. Introduction of OFSP shall lead to new marketing opportunities and the development and sales of processed products. Since OFSP is not consumed raw, losses of carotenoids that occur during different types of processing (including using traditional methods) should be taken into account in the evaluation of vitamin A activity of the product.

1.1.4 Uses of dried sweet potatoes

Traditional consumption

Methods of traditional preparation of sweet potato in sub-Saharan Africa are limited to boiling, steaming, roasting and drying (Hall *et al.* 1998). Using a diagnostic assessment approach based in developing countries, dried products (chips, starch, and flour) were identified as the most promising products from sweet potato compared to the many options available (van Hal 2000). However currently dried chips and flour made from sweet potato are limited in use for household consumption and for sale by small business on local markets in Uganda and other sub-Saharan African countries. No industrial-scale production of sweet potato flour or starch has been reported in Africa, whereas, this does occur for other starch staple crops, such as cassava (Ferris *et al.* 2001). Traditional drying and storage are practiced in northern districts of Uganda (Hall *et al.* 1998). Here, crushed or sliced sweet potatoes are spread on large flat rocks or on cow dung to dry. Sun drying takes about two to three days, but unexpected rains can extend the drying, which often leads to product deterioration, causing the product to turn black or dark grey in colour, resulting in losses in value (Hall *et al.* 1998). Sweet potato dried

slices are called ‘amokeke’ in Soroti and Kumi districts or ‘kaseede’ in Luwero district. Amokeke is reconstituted as a breakfast food during the dry season. Dried crushed roots are known as ‘inginyo’ (Figure 1-4) in Soroti and Kumi districts.

Figure 1-4: Inginyo drying on large rocks in Soroti District, Uganda (December 06). A. Bechoff.

Inginoyo is used to make flour and is mixed with sorghum to produce a stiff porridge called ‘atapa’, a main meal food in some regions (Hagenimana and Owori 1996; Hall *et al.* 1998). Flour or dried sweet potato slices can be re-hydrated in boiling water (Hall *et al.*1998). The dried sweet potato can be also boiled with sauces or tea (Hagenimana and Owori 1996). Inginoyo is stored for longer (6 months) than amokeke (4-6 months). Amokeke has been found to be infested by the lesser grain borer after 4-5 months of storage leading to significant product loss (Hall *et al.* 1998). The two products are generally stored separately in the same granary or in separate granaries. Hall *et al.* (1998) and Owori and Agona (2003) reported that re-drying amokeke was a solution to conserve it longer probably because of the uptake of moisture during storage that favours insect contamination. Some sweet potato varieties, for example, Odepalap and Ateseke are reported to store longer than others. However farmers do not separate dried slices from different varieties (Hall *et al.* 1998; Owori and Agona 2003). Although drying of sweet potato is more scarce than in Uganda, some practices have been reported in Mozambique, in the province of Zambezia (R. Dove; C. Coote, 2008 personal

communications). The sun drying of sweet potato is carried out in a similar way to cassava, which is a crop very commonly dried in Mozambique.

Commercial uses

With regained interest in sweet potato as a cash crop, new processed products are being developed in sub-Saharan Africa. White and coloured varieties can be used indifferently to make chips, flakes and flour (Winarno 1982). In order to tackle vitamin A deficiency in sub-Saharan African by increased consumption of OFSP, CIP and the HarvestPlus Challenge Program have launched trials to incorporate OFSP in various recipes of African foodstuffs (Namanda *et al.* 2005; Owori *et al.,* 2007). A diversity of products has been tested with promising outcomes (Namanda *et al.* 2005). Products from dried OFSP developed for sub-Saharan Africa include porridge, bread, bakery products such as mandazi (traditional doughnut), chapatti, cake etc. (Namanda *et al.* 2005; Owori *et al,* 2007). Provitamin A retention in these products has been determined by Hagenimana *et al.* (1999).

Sweet potato flour has been used to substitute other flours, such as cassava, wheat, and sorghum. In Burundi, Cameroon and Uganda, countries that import 100% of their wheat flour, there is an abundant sweet potato production and there are also considered to be significant sweet potato post-harvest losses (Hall *et al.* 1998). It has therefore been suggested that the imported wheat flour could be replaced with sweet potato. Substitution of wheat flour with fresh, grated roots or sweet potato flour is gaining a foothold in the snack product market in Kenya and Uganda according to Owori and Agona (2003). The profitability is however dependent on the relative cost of sweet potato roots or flour compared to the cost of wheat flour and the degree of substitution (Odaga and Wanzie 1991; Berrios and Beavogui 1992; Owori and Hagenimana 1995; Hall *et al.* 1998). Sweet potato flour (25-50%) could be favourably used for high-value bakery (biscuits, croissants, doughtnuts) (Odaga and Wanzie 1991). Making bread from flour has not always been found to be economically feasible with 16% substitution in the study by Hall *et al.* (1998) and 25% substitution in the study by Odaga and Wanzie (1991). Because of the increased cost of flour compared to fresh roots, Berrios and Beavogui (1992) suggested that raw and grated sweet potato rather than flour should be used for bread making. On the other hand Hagenimana *et al.* (1999) working with Zappalo OFSP variety found that flour was the most effective way of increasing

provitamin A content compared to boiled and mashed roots and grated raw roots in different products. Bread and mandazi from mixed flour (20% OFSP: 80% wheat flour) have been formulated (Kósambo 2004). Porridge prepared with OFSP-sorghum composite (70%/30%) flour (similar to atapa) had significant β-carotene in the final product (Kósambo 2004). These trials therefore indicated that products rich in provitamin A can be produced from OFSP flour.

Substituting cassava flour with sweet potato flour was evaluated in a small-scale processing industry for the making of Kabakagala, a Ugandan traditional deep fried pancake made from a mixture of cassava flour and banana pulp. Considerable interest was shown by low and middle income consumers (Owori *et al.* 2001). Some attempts to prepare Ugali, a kind of stiff porridge widely consumed in Kenya mainly made from white cornmeal or sorghum flour, with sweet potato flour, have been reported (Gakonyo 1992, Oyunga-Ogubi *et al* 2000a*;* 2000b). Gakonyo (1992) however found that Ugali was too sweet with sweet potatoes. Promotion is considered to be needed to help develop the market for these new products (Owori and Agona 2003). Some of processed products from sweet potato are shown in Figure 1-5.

Figure 1-5: Examples of sweet potato processed products from Eastern and Central Africa. Owori *et al.* (2007) Sweet potato Recipe Book. Left to right: Top: boiled fresh roots; bread; mandazi. Bottom: cookies, crisps, juice.

Owori *et al.* (2007) described the processing of OFSP, soya and maize flours by Kasawo Grain millers in Kampala. These products were developed in collaboration with National Agricultural Research Laboratories (NARL), Kawanda. Dehulled roasted maize and

soya grains were mixed with OFSP dried chips and milled together. The composite flour was packed in double polythene plastic bags and sealed. This porridge was destined for children under five years old and their families (Owori *et al.* 2007). This product has attracted some interest and is currently on the market. However in order to reach supermarkets, a higher quality of product is required. Dried OFSP chips supplied by farmers from Luwero and Soroti districts in Eastern Uganda have shown recurrent issue of quality due to provitamin A degradation. However, because of a loss of provitamin A carotenoids (seen as a loss of orange colour) in dried chips, millers from Kampala have been unwilling to buy the dried chips or have bought them at very low price and farmers have not been able to make profit (E. Ekenyu, personal communication, farmer and chip producer in Soroti, 2006). This observation supports the need to investigate carotenoid degradation in the product.

Most of the research on sweet potato flour has focused on developing new products. However there is a need to first improve the quality of the flour such that the provitamin A content is sufficient to have a nutritional benefit (van Hal 2000). Flour is made from milled dried chips. van Hal (2000) argued that the quality of sun-dried roots under non-controlled conditions is not sufficient to make good quality flour that can be used in economically viable processed products. Furthermore intensive production is considered not to be economically viable in tropical areas. Understanding how to improve the quality of dried sweet potato chips produced at the small scale level in developing countries would therefore be a desirable option. If products made with OFSP dried chips are used to tackle vitamin A deficiency, it is necessary to understand and evaluate the stability of provitamin A during the drying and storage of these chips. In order to do so, locally affordable technologies for drying and storage should be assessed on the basis of their effect on provitamin A retention during these process stages. Some of the locally available or adapted technologies for drying and storage are described in the next section.

1.2 DRYING AND STORAGE TECHNOLOGIES ADAPTED TO LOCAL CONDITIONS

1.2.1 Introduction

Sweet potato roots are highly perishable under tropical conditions and have short shelf-life (Fowler and Stabrawa 1992). Preservation of food by drying is one of the most common methods used in developing countries. Drying and storage of sweet potato are traditionally practiced in Asia (*e.g.* China, Philippines, Bangladesh, India), South America (*e.g.* Peru) and Africa (*e.g.* Uganda, Kenya) (Woolfe 1992; van Hal 2000). Other methods of preservation include canning, freezing, pickling and adding sugar (Woolfe 1992), but these methods are not widely practiced in sub-Saharan Africa because of lack of awareness along with lack of resources and infrastructure. Drying has the advantage of being low cost because no extra ingredients are involved and it reduces the weight of the product which facilitates transport and trade and allows storage in granaries. In-ground fresh storage and piece meal harvesting is another method of preservation of sweet potato that is common in sub-Saharan Africa (Hall *et al.* 1998), however, drying permits storage in low season and so enables consumption throughout the year in the regions where crop production is seasonal.

Drying is a mass transfer process that consists of water moisture evaporation from foodstuffs. Moisture (or dry matter) content is defined as the quantity of moisture (or dry matter) contained in the product and water activity describes the amount of water available for hydration of foods and is defined as the vapour pressure of water in the food divided by that of pure water at the same temperature. The initial moisture content of the product influences on the drying rate. During drying, the moisture contained in the product is vaporised under the effect of heat and transferred to the ambient air. Air flow helps heat application through the product and removal of humidity. Relative humidity is defined as the ratio of water vapour in air to water vapor. The lower the relative humidity in the air, the more capacity to remove moisture from the product it would have. There is also a relationship between temperature and relative humidity. The temperature of the air affects the relative humidity (as temperature increases, relative humidity decreases in adiabatic conditions) and this is described on a psychrometric chart (ITDG 1988). At atmospheric pressure, the efficiency of drying therefore depends of the temperature/relative humidity and air flow through the product.

Drying is a critical process, more than the other traditional methods of processing (*i.e.* boiling and steaming). Indeed, the removal of water affects the internal cell structure of the vegetable food leading to higher losses of micronutrients such as provitamin A.

In order to control better provitamin A losses in drying and storage, improved techniques of drying and storage are required. However these technologies should be adapted to the local environment. When working with small-scale farmers, it is necessary to consider issues such as lack of infrastructure, lack of finance, distance from markets, low income and lack of expertise. Technologies for flour processing on a large scale such as drum or spray drying are not appropriate because they require large amounts of energy, high technology and high capital cost (van Hal 2000; Woolfe 1992). Drying technologies suitable for farming areas should have a low initial capital cost; be easy to construct with available natural materials and be easy to operate and maintain (Chua and Chou 2003). There are two types of dryers, artificial or natural (solar or sun) that are suitable in that respect and these are described below.

1.2.2 Artificial drying

Artificial drying can be conducted in a cabinet or tunnel dryer where air is heated by a fuel or electricity (van Hal 2000). Air flow can be perpendicular (cross flow, for example, in a fluidized bed system) or parallel to the product. Other types of dryers that are used with cassava in West Africa include bin and flash dryers (A. Westby, personal communication, NRI, 2009). With artificial dryers, temperature, drying time and air velocity are controlled leading to consistent, high quality products. Cabinet dryers expose sweet potato slices to temperatures between 50 and 80°C for a period of 2.5-24 h. (van Hal 2000). The disadvantage of this system is the high expenditure of energy increasing production costs (van Hal 2000). An alternative to this type of dryer is the biomass dryer cabinet or tunnel dryer using firewood or charcoal. This also requires investment in fuel. These dryers can be expensive to build, but manufacturing costs can be reduced by using materials available in rural places. The disadvantages of these dryers are that the quality of product can be affected by smoke and using firewood contributes to deforestation unless it is produced on a sustainable basis.

1.2.3 Sun drying

Solar and sun dryers are more environmentally friendly systems and are cheaper to operate than artificial dryers because they use natural free energy. Consequently sun drying is one of the lowest-cost preservation methods and is the most commonly used drying method. However traditional open air sun drying involves a number of risks for product quality including dust, insects, mammals, rain and provitamin A damaging UV-sun radiation because of poor control over environmental factors. Improved sun drying was therefore proposed by FAO (1985) in their "Expert consultation on planning the development of sun drying techniques in Africa". Clean smooth raised platforms, blackened surfaces that absorb solar radiation more efficiently or woven mats and mesh trays that facilitate the air movement around the product were recommended. An example of improved sun dryer was a cassava chips dryer (TDRI/CIAT project) consisting of trays with plastic netting (35 holes/cm) stretched on wooden frames and supported by chicken wire (Figure 1-6).

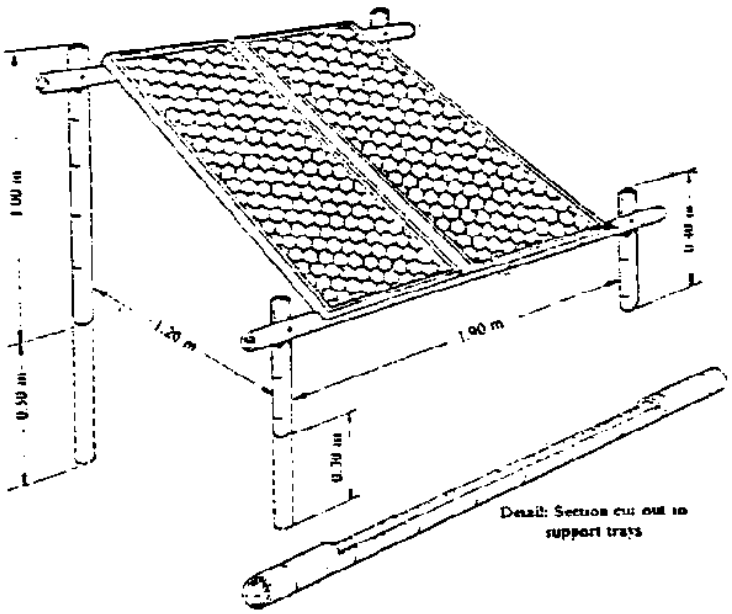


Figure 1-6: Improved sun dryer - Cassava Drying Trays (Colombia). FAO (1985).

Trays were mounted on bamboo poles and sloped at the angle of repose to allow to chips to face the wind (FAO 1985). When the wind was favourable, cassava chips spread in the late afternoon were mostly dried over-night.

Modelling of open air sun drying was undertaken by Garg and Kumar (2000) and Jain and Tiwari (2003) and gave consistent results (in spite of lack of control over environmental conditions). The most important parameters for drying were product thickness and weather conditions. Product thickness should be kept as small as possible for efficient drying (Garg and Kumar 2000).

1.2.4 Shade drying

An alternative to sun drying to limit degradation by sun-light of product nutritional quality is shade drying (Figure 1-7). A critical factor in shade drying is air circulation around the product and the thickness of the layer of product should be limited in order to facilitate drying (FAO 2001). The disadvantage of this dryer is longer drying times that can lead to off-odours (fermentation) if the temperature is low or humidity high.

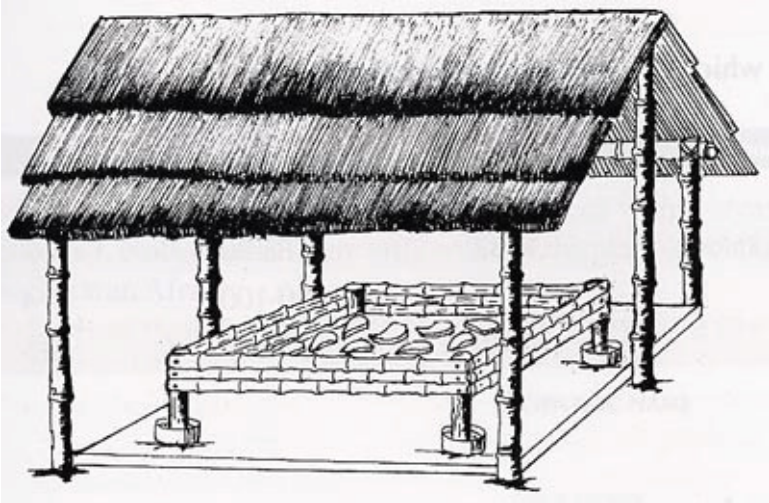


Figure 1-7: Shade dryer. FAO (2001).

Containers of water placed at the four corners of the drying rack prevent crawling insects from reaching the product. The roof structure can be of any material, iron sheet, straw and wood poles, that protects against sun and rain.

1.2.5 Solar drying

Compared to sun and shade dryers, a solar dryer is a more controlled system because the product is placed in an enclosed space (and so not directly exposed to environment). Air heated by solar radiation possesses a higher efficiency of drying food because humidity is reduced. Therefore solar drying is a more appropriate option than sun drying when the

humidity is high (FAO 1985). With optimised ventilation, solar drying is faster than sun drying (Mulokozi and Svanberg 2003; Bengtsson *et al.* 2008). Solar dryers can be active or passive; passive types use natural convection; active types use forced convection by means of a fan for improved ventilation. Passive types can be appropriate for small-scale farmers because there is no need for extra energy and therefore the cost of processing is lowered (Chua and Chou 2003). Solar dryers can also be classified into direct or indirect types. In the direct solar dryer, food is exposed directly to the sun's ray through the clear covering. In the indirect solar dryer, the product is dried by solar heated air only and not in direct exposure to sun radiation. An indirect solar dryer requires generally more investment and technology than a direct solar dryer. Therefore the lowest cost-models of dryers are passive direct solar dryers. The simplest of these is the tent dryer (Figure 1-8).

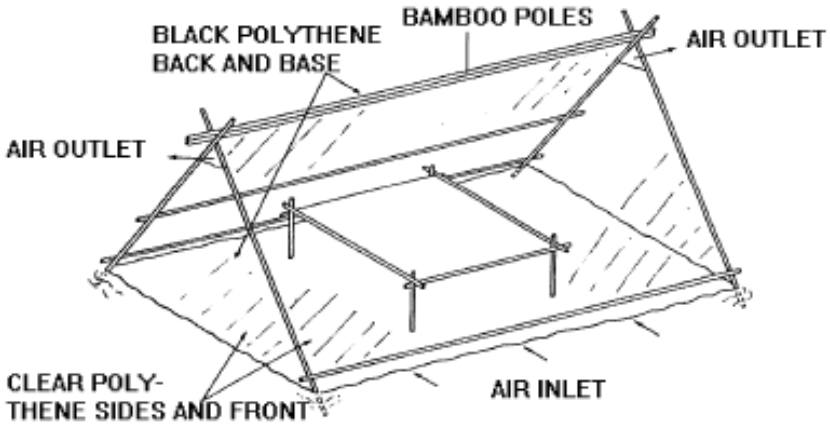


Figure 1-8: Tent dryer. Intermediate Technology Development Group. ITDG (1988).

The tent drier consists of a ridge tent framework, covered in clear plastic on the ends and the side facing the sun, and black plastic on the base and the side in the shade. The clear plastic allows the transmission of the sun light whereas the black plastic absorbs the light and converts it into heat. A drying rack is placed along the full length of the tent. The bottom edge of the clear plastic is rolled around a pole, which can be raised or lowered to control the flow of air into the drier. Moist air rises through outlet at the top corner of the tent. This system is lightweight and fairly fragile in windy conditions (ITDG 1988).

Other types of direct solar dryers are solar cabinet dryer; solar greenhouse dryer and dryers with pre-heating chambers (solar tunnel dryer) (FAO 1985). These models can also work in an active mode (*i.e.* with a fan).

The solar cabinet dryer is an enclosed box covered with plastic. There are holes in the base that allow air to enter and holes in the outlet to evacuate moist air. This dryer can be built from mud, cement or brick (ITDG 1988). An example of cabinet dryer is the model built by NRI for the Fruits of the Nile Company in Uganda (Brett *et al.* 1996).

A type of direct solar dryer is a polyethylene tunnel greenhouse. This dryer was used for drying peppers (Fahrat *et al.* 2004) and apples (Eliçin and Saçilik 2005). The polythene greenhouse is similar to a greenhouse for plant growing (Figure 1-9).

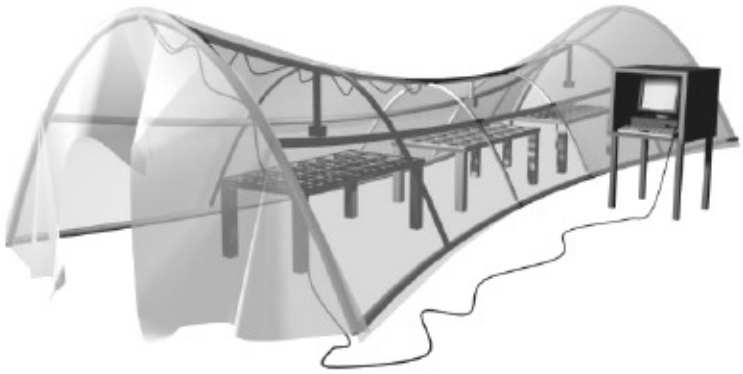


Figure 1-9: A greenhouse solar dryer with temperature and humidity recorded on computer. Elicin and Sacilik (2005).

The polythene coverage protects the samples from insects and rain. Wire mesh trays were used to accommodate apple slices to be dried as thin layer solar drying. The drying time was similar when compared to open air sun dryer in natural convection in Fahrat *et al.* (2004) whereas it was reduced from 2 days to 1.5 drying on the drying reported in Eliçin and Saçilik (2005). If this model of dryer was chosen, a lower cost model should be developed for small scale farmers.

A type of dryer with a preheating chamber is the solar tunnel dryer. The most common tunnel dryer is the Hohenheim type developed 20 years ago for tropical countries. The dryer has three main components: the collector; the dryer and the fan (optional). The

collector is an air heater. It includes an absorber (black - metallic surface) that warms up the air by convection and a clear cover (UV-stabilised polythene) that warms up the air by radiation and reduces heat loss from the absorber. The dryer is where the product is spread on mesh trays (lateral loading); the structure is the same as for the collector without the absorber. An optional fan (forced convection) can be included that forces the air through the collector and dryer. The fans can be driven by mains current, a 12V photovoltaic solar module or a car battery (Gnanaranjan *et al.* 1997). The choice of the materials used for constructing the dryer depends on what is locally available and its cost: for the building of the structure: bricks, cement, wood, plywood, slate, stone and loam; for the heat insulation at the bottom of the collector: polyurethane, cellulose, fibre glass, wood, stone wool, cork, linen. The floor of the drying chamber is covered with sealing foil and mats (bamboo or plastic). A lower cost small scale model was developed for smallholder farmers. The new model worked either with alternate current or solar photovoltaic to activate fans (Mastekbayeva *et al.* 1998) (Figure 1-10).

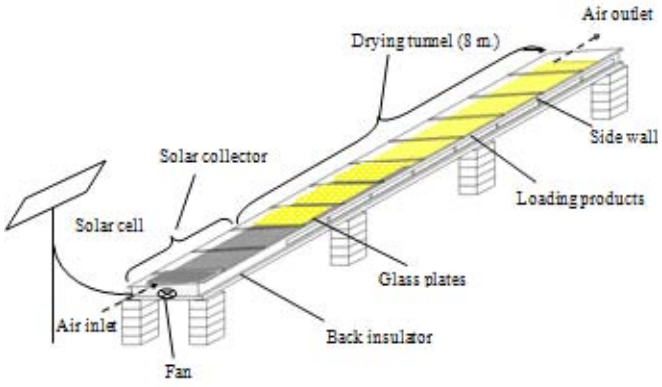


Figure 1-10: A tunnel solar dryer (AIT (Asian Institute of Technology)) with photovoltaic power driven fans. Solar power Thailand (2007); Mastekbayeva *et al.* (1998).

The AIT dryer still represents a high cost for farmers (£5000) (Mastekbayeva *et al.* 1998). Therefore there are however options with natural convection and/or a biomass burner under the dryer (hybrid dryer) to dry when the weather is rainy (see Appendix tunnel dryer at NARL, Uganda- cost: about £200). At the farm level, solar dryers can still represent an unaffordable expense and other options available are direct sun or shade drying (see Appendix tunnel dryer at NARL, Mozambique- cost: about £10-25).

The performance of drying depends on the ventilation system, the model of dryer, and the environmental conditions such as temperature/humidity values . Factors to take into account in the choice of the dryer are efficiency of drying, cost, and effect of drying of product quality such as provitamin A content.

1.2.6 Packaging

In addition to drying, which can affect the quality of dried sweet potato, packaging of the final product is also important. van Hal (2000) reported that "The sweet potato flour was packaged and sealed in different forms of packaging material, which can be porcelain and glass jars, tin cans, paper ''craft'' bags, polypropylene or polyethylene bags, cotton bags, or a combination of two different materials, for example polyethylene and cotton bags together" (Table 1-2).

Table 1-2: Properties of flexible packaging materials. Commonwealth Science Council (1985).

Material	Remarks	Impermeable			Cost
		Water	Oxygen	Light	
Paper	Hygroscopic	no	no	yes	L
Aluminium foil	Excellent	yes	yes	yes	H
Cellulose film	Fragile -sealable	yes	no	no	A
*LDPE (low density)	Thin -sealable	yes	no	no	L
*HDPE (high density)	Thick -Twice less transmission rate of oxygen and water than LDPE- sealable	yes	yes	-	A
Polypropy-lene films	Same thickness as LDPE-sealable	yes	yes	yes	-
Polyester	Very strong film	yes	yes	no	-
Polyamide (nylon)	Used in laminate with LDPE for vacuum packaging	no	yes	yes	-
Coatings	LDPE/Paper for *e.g.*	yes	-	yes	H
Metallization	Thin coating of aluminium with polyester, polypropylene, cellulose	yes	yes	yes	H
Laminates	*E.g.* Paper/foil/LDPE; paper/LDPE/foil/LDPE	yes	yes	yes	H
Jute	Natural material- easily available	no	no	yes	L

L=low; A=average; H=high *Polythene film is of two types: LDPE and HDPE.

The use of the product (*e.g.* household consumption, trading, selling to consumers) will determine the packaging type and presentation. When transporting sweet potato chips for trade exchange, flexible material is more adaptable than rigid one because it allows use of higher volumes at lower cost.

A good packaging material for OFSP chips or flour should protect it against moisture, oxygen and light in order to preserve the provitamin A content. Oxygen can be removed by creating a vacuum, flushing with inert gas such as nitrogen, or using an oxygen absorber. This also requires packaging that limits air exchange during storage such as foil aluminium, laminate, coating, metallization. The best packagings are laminate, coatings, metallization, aluminium foil and pure polypropylene that isolate the product from the three main degrading elements (water, oxygen and light). However their cost and availability does not make them a very easy option for small scale farmers or enterprises (Commonwealth Science Council 1985). The main method of packaging encountered in Uganda by chips producers in Soroti were woven polypropylene bags (*i.e.* the same as for fresh roots). Even though the pure polypropylene is impermeable to water, oxygen and light, the woven polypropylene is not because of spaces between fibres. Other packaging materials available include paper and LPDE (low density polythene resin). Paper is one of the cheapest materials together with LPDE. These types of material are easy to purchase locally but have high permeability to oxygen.

In Mozambique jute sacs were used for the storage of dried chips. The disadvantage of all these packaging materials is their higher permeability in particular to oxygen. Storing sweet potato flour in thick gauge white polythene bag (probably HDPE) was more protective than in enamel cans, plastic cans or calico bags because there was no change in texture and colour and no insects and mites damage after three month-storage (Tewe *et al.* 2003). HDPE (High polythene density resin) might therefore be appropriate.

1.2.7 Pre-treatments to limit provitamin A degradation

Other options that have the potential to limit provitamin A degradation during drying and storage are the use of blanching and the use of additives such as sulphites. Blanching inhibits enzymes that degrade provitamin A such as lipoxygenases and peroxidases (Baloch 1977). The efficiency of additives in retaining provitamin A has been reported in various publications. Sulphite acts as an antimicrobial agent and inhibitor of enzymes and nitrosamine formation (Thane and Reddy 1997). Latapi and Barrett (2006) found that gas sulphured (SO_2) or sodium metabisulphited sun-dried tomatoes had significantly better colour and carotenoid content. Arya *et al.* (1979) confirmed that incorporation of sodium chloride salt, sodium metabisulphite significantly reduced the rate of carotenoid

degradation and non-enzymatic browning in dehydrated carrots. The combination of blanching and sulphiting was found to be the most efficient method in terms of the stability of carotenoids in dehydrated carrots (Baloch *et al.* 1987). However the use of additives implies extra cost of production of dried products that can make them unsuitable for use at the village level. The effect of additives on carotenoids in sweet potato during drying and storage of sweet potato has been scarcely reported as opposed to other roots or vegetables such as carrot. The effectiveness of different additives on reducing provitamin A degradation and the costs and benefits of using them requires investigation.

1.2.8 Storage at low temperatures

Low temperatures slow down enzymatic activities and other chemical reactions in general and therefore help maintain product quality. Carotenoids are best preserved at -20ºC (Rodriguez-Amaya and Kimura 2004). A simple fridge (4ºC) would however help to significantly extend the shelf life of dried sweet potato according to Tang and Chen (2000) and Cinar (2004).

1.3 DEGRADATION OF CAROTENOIDS

1.3.1 Introduction to carotenoids

Locally-adapted techniques of drying and preservation of sweet potato have been described. A further step in improving the process of preserving provitamin A is to understand the properties of provitamin A in plant food and the mechanisms of its degradation. This is presented in this present section.

Provitamin A is found in plant foods as carotenoids. Carotenoids are part of the terpenes family and terpenes are classified as lipids. Structurally carotenoids are usually C_{40} tetraterpenoids that are built from eight C_5 isoprenoid units. Carotenoids are the pigments most widely represented in nature and include more than 600 structures synthesised by plants (Lee *et al.* 1989; Rodriguez-Amaya 1997). Colours vary from yellow to red (Simon 1997). The carotenoids group include carotenes (non-polar) and xanthophylls (polar) (Rodriguez-Amaya 1997). One unsubsidised β-ring with 11-carbone polyethylene chain (β-ionone ring) is the minimum requirement for provitamin

A activity (Rodriguez-Amaya 1997; Tanumihardjo 2002). β-carotene is the carotenoid with the highest provitamin A activity (100%) because the β-carotene molecule can be entirely converted into two molecules of vitamin A or retinol (Figure 1-11; Table 1-3).

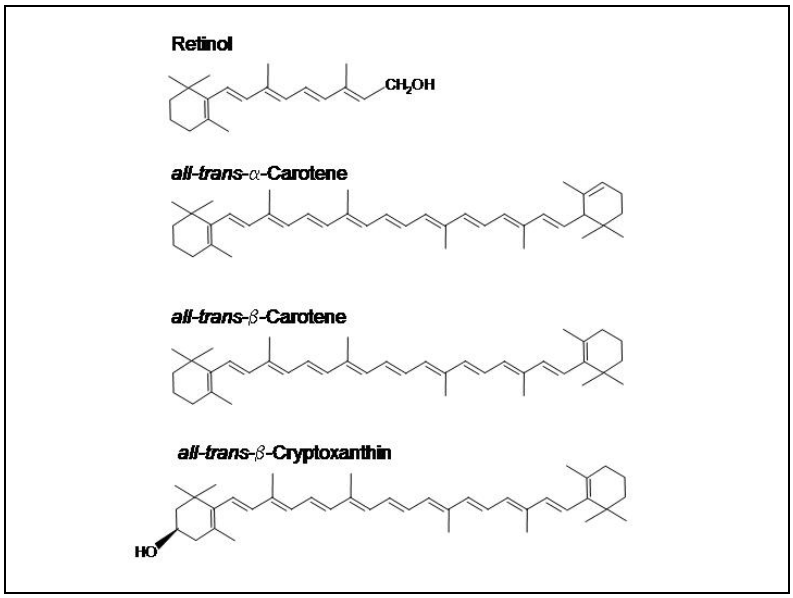


Figure 1-11: Structures of the most common provitamin A carotenoids and retinol. National Academy of Sciences/Institute of Medicine (2001).

Table 1-3: Relatives bioactivities (%) of some provitamins A compounds in comparison with trans-β-carotene as reported by Bauernfeind (1972); Zechmeister (1949) and summarised by Rodriguez-Amaya (1997).

Provitamin A	Bauernfeind (1972)	Zechmeister (1949)
trans-β-carotene	100	100
13-cis-β-carotene	-	53
9-cis-β-carotene	-	38
trans-α-carotene	50-54	53
cis-α-carotene (13-cis?)	-	16
cis-α-carotene (9-cis?)	-	13
trans-5-6-monoepoxy-β-carotene	21	-
trans-γ-carotene	42-50	42
cis-γ-carotene	-	19
trans-β-cryptoxanthin	50-60	57
cis-β-cryptoxanthin (9-cis?)	-	27
cis-β-cryptoxanthin (15-cis?)	-	42
trans-β-apo-8-carotenal	72	-
trans-β-zeacarotene	20-40	-
trans-mutachrome	50	-

About 50 carotenoids are known to have a provitamin A activity (Lee *et al.* 1989). Of these compounds, provitamin A carotenes most commonly encountered include β-

carotene and α-carotene. The provitamin A xanthophyll most commonly encountered is β-cryptoxanthin (Rodriguez-Amaya 1997) Trans-β-carotene represents about 80-90% of the total carotenoids in OFSP (Bengsston *et al.* 2008).

1.3.2 Incorporation of carotenoids in the plant structure and human absorption

The biosynthetic pathway of carotenoid formation in plant starts with the condensation of pyrophosphate molecules, which is common in the formation of many terpenoid compounds (Figure 1-12).

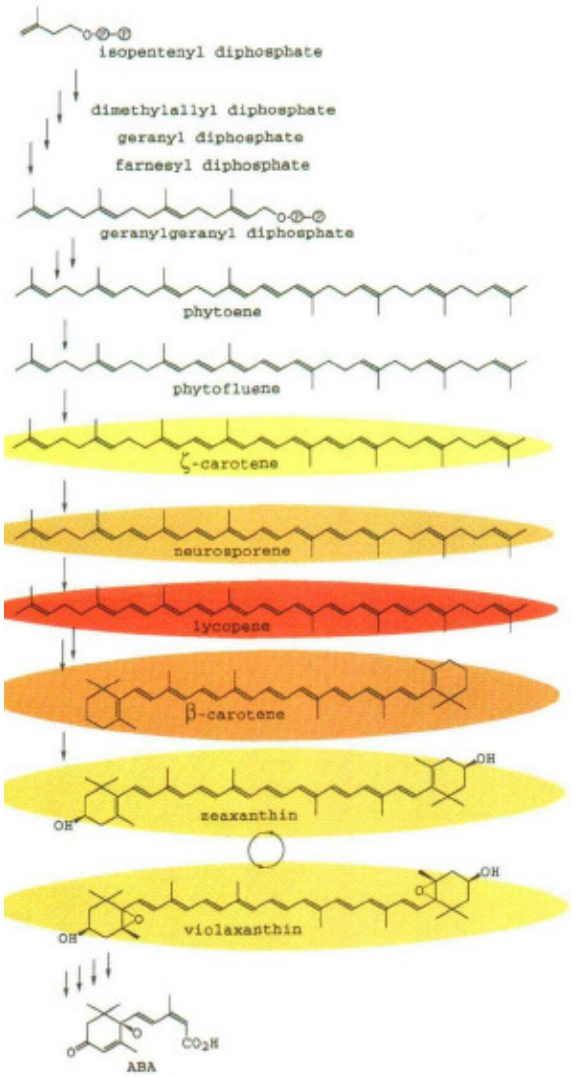


Figure 1-12: Carotenoid biosynthesis in plants. Bartley and Scolnik (1995). ABA: Abscisic acid

β-carotene is formed from lycopene. Xanthophylls can be formed from β-carotene by epoxidation. All these biosynthetic reactions are catalysed by a variety of enzymes (Bartley and Scolnik 1995). Chromatographic analysis of sweet potato (variety

Centenial) has identified carotenoids from the biosynthesis chain being β-carotene (86%), phytoene (3%), phytofluene (2%), ζ-(zeta) carotene (2%), α-carotene (1%), violaxanthin (0.06%) (Purcell and Walter 1968).

Because of their hydrophobic (lipophilic) nature, carotenoids are associated with lipid-protein complexes (Vishnevetsky *et al.* 1999). In plant cells, carotenoids are incorporated in semi-autonomous organelle structures. In leaves, carotenoids are situated in the chloroplast although in fruits or other parts of the plant, roots for example, carotenoids are located in chromoplasts (Figure 1-13).

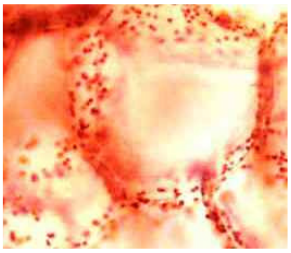


Figure 1-13: A parenchyma cell containing chromoplasts. Each red dot is a chromoplast that contains carotenoids. Webb (2009).

In leaves and raw green vegetables, where carotenoids are present in the chloroplasts, they are linked to protein and chlorophylls that are green in colour and mask the orange colour of the carotenoids (Bartley and Scolnik 1995; Rodriguez-Amaya 1997; Vishnevetsky *et al.* 1999). Chromoplasts usually derive from chloroplasts. During the transformation of chloroplasts into chromoplasts, the photosynthetic apparatus disintegrate and carotenoids accumulate in the novel plastid. This transformation can be observed, for instance, in autumn leaves or during fruit ripening. Microscopic observation of chromoplasts has revealed differential accumulation of carotenoids that led to five different classes: globular, crystalline, membranous, tubular, and fibrillar (Vishnevetsky *et al.* 1999). This classification is on the basis of carotenoid-containing structures: polar lipids, carotenoids, proteins (Vishnevetsky *et al.* 1999). For instance globular chromoplasts are characterised by a very high ratio of apolar to polar components (10:1) (where carotenoids represent about 15-25% of apolar components). Fibrillar structures, however, are characterised by a low ratio of apolar to polar components (1:1) with xanthophylls and equal contents of proteins and lipids (Vishnevetsky *et al.* 1999). In sweet potato, the structure of chromoplasts is not known

(Rees, NRI, unpublished). Fibrillar structures might be more resistant because of association between proteins and carotenoids. It is believed that the structure and association between these three constituents may have an impact on the stability of carotenoids in processing and their body absorption (bioavailability).

Bioavailability is defined as the fraction of carotenoid that is absorbed and available for utilisation in normal physiological functions or for storage (Tanumihardjo 2002). Through the mastication process and through digestion, provitamin A carotenoids are extracted from the food matrix where they are associated with proteins and lipids (Furr and Clark 1997). Enzymatic cleavage of provitamin A from plant origin into retinol (by 15,15'-dioxygenase) occurs in the intestinal mucosal cells (Tanumihardjo 2002). Retinol is incorporated into chylomicra and transported from the lymphatic system, either to the general circulation (Furr and Clark 1997) or to the liver to be stored (Sommer 1998).

Carotenoid absorption and availability for use in the body is therefore dependent on the nature of carotenoids, the structure of chromoplasts and the resistance of food matrix (raw or processed) to mastication and absorption. Although these factors are important in understanding the context of carotenoid degradation "*in vivo*", their influence, however, was not further studied in this thesis. This present research work is restricted to the determination of the level of carotenoid degradation during food processing and the influence of physico-chemical factors on the β-carotene molecule in the sweet potato during processing.

1.3.3 Factors affecting degradation of β-carotene

Disruption of the food's cellular matrix by processing makes carotenoids more vulnerable to oxidative degradation (Kósambo *et al.* 1998). Factors leading to the degradation of β-carotene are illustrated in Figure 1-14.

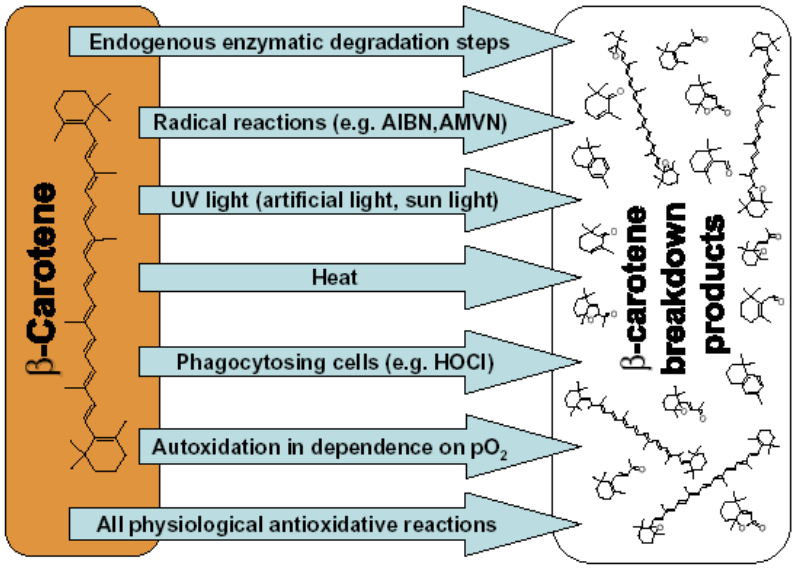


Figure 1-14: Formation of different carotenoid breakdown products from one precursor compound such as β-carotene. Siems *et al.* (2005).

Free radical initiators: 2,2'-azo-bis-isobutyronitril (AIBN);: 2,2'-azo-bis(2,4-dimetylvaleronitril) (AMVN). HOCl: Hypochlorous acid; pO_2: partial pressure in oxygen. Phagocytosing cells and physiological antioxidative reactions are "in vivo" carotenoid degradation mechanisms and are not applicable to this study.

Carotenoids are lipophilic unsaturated structures that are unstable (Gayathri *et al.* 2004). Carotenoid decomposition can result of natural or experimental causes as illustrated in Figure 1-14. The main natural causes are oxygen, heating and light. These could play a role in the breakdown of carotenoid in food products such as sweet potato exposed to environment during drying (heat, air and light). The effects of light, heat, and of autoxidation and enzymatic oxidation on carotenoid degradation will be described in the next sections.

Light

Carotenoids play an essential role in plants as components of photosynthesis in harvesting sunlight energy for chlorophylls because of their conjugated double bounds that enable them to absorb visible light (and produce intense colour from yellow to red in visible spectrum) (Naik *et al.* 2003). However this conjugated system which confers properties to carotenoids also makes them unstable.

Sunlight is the total spectrum of the electromagnetic radiation transmitted by the sun to the earth. The sun light spectrum is composed of a broad range of wavelengths: Infra-

red (>800 nm): heat (long wavelengths); Visible (400-800 nm) (medium wavelengths); Ultraviolet including UVA (315-400 nm) (short wavelengths), UVB (280-315 nm) and UVC (200-280 nm). When white light (sun light or artificial light) passes through or is reflected by a coloured substance, a characteristic portion of the mixed wavelengths is absorbed. The remaining light will then assume the complementary colour to the wavelengths absorbed. Here, complementary colours are diametrically opposed to each other (Figure 1-15).

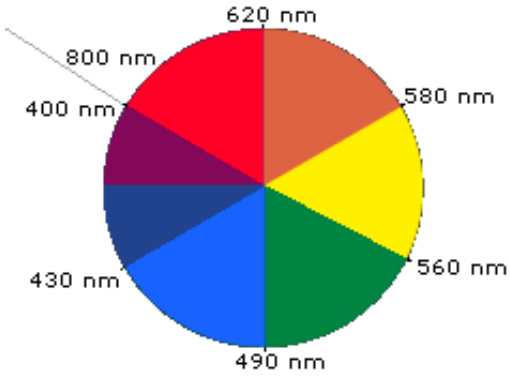


Figure 1-15: Colour wheel representing complementary colours. Michigan State University (2009).

All pigments have the ability to absorb light at different wavelengths. The conjugated double-bond system of carotenoids makes them appear in the visible part (yellow to red part) of the spectrum. Their colour is due to the absorption of complementary colour composed of short wavelengths (400 nm to 500nm: blue-green).

Colour serves as a basis for the identification and quantification of carotenoids by spectrophotometric reading, High Performance Liquid Chromatography (HPLC) and colorimetric methods. The spectrum of β-carotene consists of three peaks which are 425nm, 450nm and 476nm in petroleum ether (PE), and is shown in Figure 1-16.

Loss or change of colour during the analysis can clearly be related to degradation or structural modification (Rodriguez-Amaya 2001). For instance cis-isomerisation causes a slight loss in colour identified by a loss of 2 to 6 nm for mono-cis and accompanied by the appearance of a cis-peak in or near the UV region.

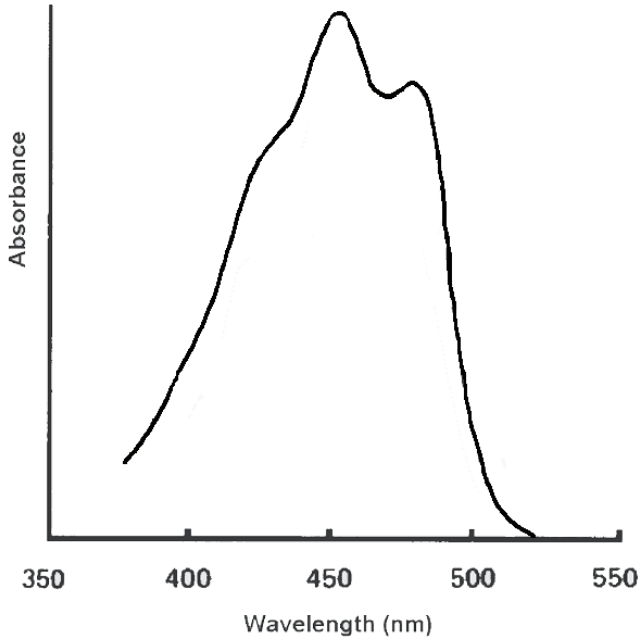


Figure 1-16: Typical absorbance spectrum of β-carotene by spectrophotometry. Rodriquez-Amaya and Kimura (2004).

Because they absorb short wavelengths from the sunlight, carotenoids therefore play a role of protection against UV that are damaging for the skin for instance. But UV and blue light are short wavelengths that can also degrade carotenoids. Lennersten and Lingnert (2000) working on mayonnaise showed that wavelengths between 410 and 450 nm (UV and blue light) had the most degrading effect on β-carotene. On the other hand red light or red-filters protect carotenoids because they filter the UV that can damage the molecule (Lennersten and Lingnert 2000).

1.3.4 Mechanisms of degradation

Chemical degradation occurs during processing by two phenomena: isomerisation and oxidation (Figure 1-17).

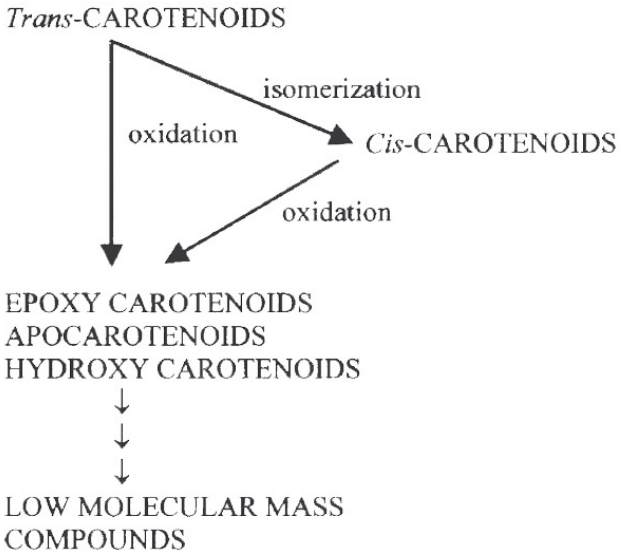


Figure 1-17: Possible scheme for carotenoid degradation. Rodriguez-Amaya and Kimura (2004).

Isomerisation

Carotenoids are found in nature as trans-carotenoids. Under stressful conditions such as heating and UV-light exposure, trans-carotenoids are isomerised into cis-carotenoids (9-cis; 13-cis and 15-cis for β-carotene). Isomerisation could be considered as a negative effect of processing since cis-isomers have less provitamin A activity (about half) than trans-β-carotene. In addition, Deming *et al.* (2002) showed, in gerbils, that cis-β-carotene is less bioavailable than trans-β-carotene. This has been subsequently confirmed in humans (Rodriguez-Amaya and Kimura 2004).

Several researchers have reported that the preferentially formed cis-isomers from all trans-β-carotene are 13-cis and 9-cis (Chandler and Schwartz 1988). In the OFSP variety Jewel (raw: 90µg.g^{-1} on a fresh weight basis), 13-cis was found to be predominant following various processes (blanching, canning, lye peeling, pureeing, dehydrating, microwaving, baking). A small amount of 9-cis and 15-cis were found in canned sweet potato (Chandler and Schwartz 1988; Lessin et al. 1997) (Figure 1-18). Raw roots may contain small amount of the 13-cis isomer if they are stored too long (Chandler and Schwartz 1988). The quantity of isomer formed in processed products is related to the heat and length of treatment (Chandler and Schwartz 1988; Doering *et al.* 1995).

Isomerisation can occur in provitamin A carotenoids at temperatures above 35ºC. 9-cis is predominantly formed above 100ºC whereas 13-cis and 15-cis are formed below 100ºC (Doering *et al.* 1995).

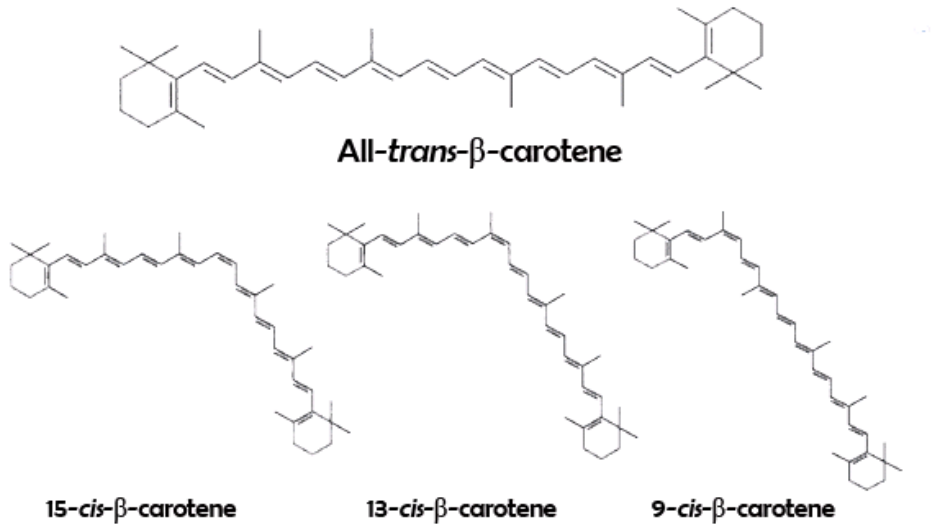


Figure 1-18: Isomers of β-carotene. Rodriguez Amaya and Kimura (2004).

Chandler and Schwartz (1988) reported that processes that most induce cis-isomerisation in OFSP were (in order of less to more damaging): steaming, blanching, pureeing, microwaving, canning, baking and drum drying. Shade and sun drying did not initiate cis-isomerisation in the examples found in literature (Kidmose et al. 2007; Mulokozi and Svanberg 2003) on OFSP or leafy vegetables respectively. The same observation was made by Kidmose *et al.* (2007) working with one variety of OFSP roots called Zappalo and shade dried. These findings should be verified working on different varieties of OFSP and different drying technologies.

Oxidation

Autoxidation

Because of their conjugated double bond system that absorbs free radicals, carotenoids protect the photosynthetic apparatus from the damage of harmful oxygen species (Naik *et al.* 2003). Conjugated double bounds and the electron rich system of carotenoids also induce instability toward oxidation because of attraction for electrophilic molecules (Siems *et al.* 2005). Oxidation (enzymatic or non-enzymatic (autoxidation)) is considered to be the major cause of loss of provitamin A activity during processing and storage. Oxidation occurs through a free radical process. Free radical reactions are

induced by single unpaired electrons that are highly unstable and therefore tend to lead to the destruction of the molecule by a chain reaction. Loss of water during drying has proved to be a risk factor in a free radical process (Chandler and Schwartz 1988). The bleaching process that follows exposure of carotenoids to free-radical species results from the interruption of the conjugated bound (Krinsky and Yeum 2003). The two mechanisms by which β-carotene exerts a protective effect in food are responsible for its autoxidation include:

- quenching singlet oxygen from air; and
- stopping propagation of peroxyl radicals, such as fatty acids in foods (Liebler 1993).

β-carotene can react directly with singlet oxygen. Singlet oxygen is an excited state of molecular oxygen (in air) that can initiate free radical reactions with organic compounds such as those found in food. Ground state oxygen (triplet) can be excited into singlet by photosensitisation due to interaction of light and oxygen and photosensitisers (photosensitizers are compounds that can easily absorb light such as chlorophyll and riboflavin in food). Singlet oxygen can generate free radical reactions with a variety of substrates having double-bonds. These oxidation reactions damage components such as fatty acids or other unsaturated compounds found in plant food cells (Bradley and Min 1992). Because of its rich electron system, β-carotene is able to quench singlet oxygen and convert it to triplet state (Bradley and Min 1992; Liebler 1993). This quenching property means that β-carotene can protect cell components from free radical oxidation (Britton *et al.* 2008). β-carotene is actually the most effective singlet oxygen quencher known (Bradley and Min 1992, Liebler 1993). A mole of β-carotene may quench 250-1000 singlet oxygen before being irreversibly oxidised (Bradley and Min 1992).

Alternatively β-carotene can react with peroxides in food. Fatty acids represent only 0.5% of sweet potato composition (Woolfe 1992). However unsaturated fatty acids, such as linoleic acid and linolenic acid, are the main fatty acids in sweet potato and these are very susceptible to singlet oxygen oxidation that transforms them into lipid peroxides (Walter and Purcell 1974). β-carotene is considered a "chain-breaking antioxidant" because it inhibits peroxidation primarily by trapping hydroperoxides (*e.g.* lipid peroxides) that could propagate radical chain reactions. In the course of the reaction β-carotene is oxidised into epoxides (Liebler 1993). The same products can be obtained when β-carotene is oxidised by chemical reagent (Figure 1-19).

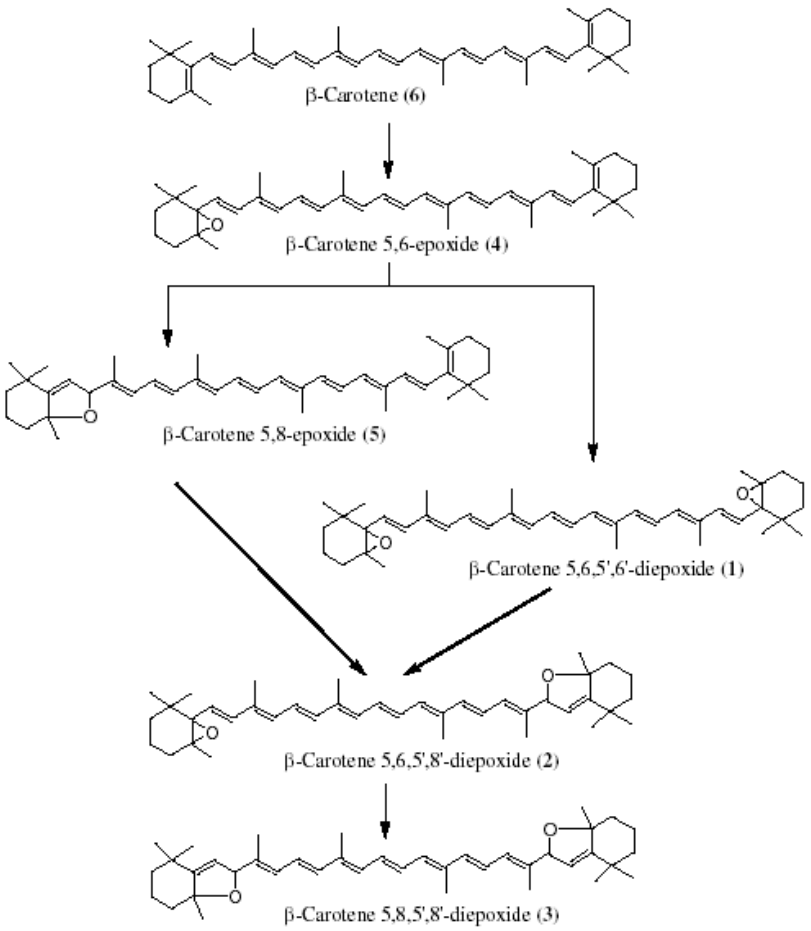


Figure 1-19: Formation of epoxycarotenoids from β-carotene with m-chloroperbenzoic acid. Rodriguez and Rodriguez-Amaya (2007).

The products of these reactions are monoepoxides (5,6 or 5,8 epoxy-β-carotene) or diepoxides (5,6,5'6' ; 5,6,5'8' and 5,8,5',8' diepoxy--β-carotene). Lipid peroxides are also able to degrade highly unsaturated β-carotene molecules. In some cases, the antioxidant reaction can therefore reverse resulting in the complete breakdown of β-carotene by a free radical chain reaction (Bradley and Min 1992).

Enzymatic oxidation

Oxidation of carotenoids can also be a result of enzymatic oxidation. The mechanism is also a free radical reaction. Enzymatic oxidation easily happens during food preparation, such as cutting, peeling or low temperature heating, because tissue disruption frees enzymes that isomerise and oxidise carotenoids (Rodriguez-Amaya and Kimura 2004).

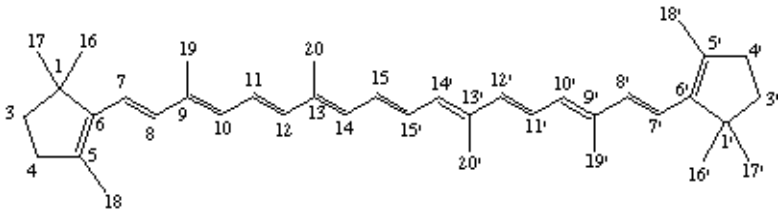
Enzymatic cleavage of carotenoids in plant is catalysed by a family of non-heme carotenoid cleavage dioxygenases (CDDs) that can cleave regiospecifically the 9,10 or 11,12 double bonds of carotenoids leading to apocarotenoid products (Auldridge *et al.* 2006; Huang *et al.* 2009). Alternatively enzymatic cleavage leading to apocarotenoid products can be operated unspecifically by co-oxidation (Bosser and Belin 1994). Co-oxidation of carotenoids and another substrate, for instance fatty acids, such as linoleic acid, using lipoxygenase and xanthine oxidase has been extensively studied by Bosser *et al.* (1995) and Waché (2002; 2003; 2006). It has been reported that carotenoids are oxidised by free radical species generated from another substrate by enzymatic reactions (Waché *et al.* 2002; Zorn *et al.* 2003).

Co-oxidation of β-carotene by lipoxygenase from potato (*Solanum tuberosum*) has been reported (Aziz *et al.* 1999). Presence of lipoxygenase has also been reported in cassava (Isamah 2004), but it has not yet been identified in sweet potato. On the other hand, sweet potato is reported as an excellent source of peroxidase activity, which is mostly situated in the peel (Castillo-Leon *et al.* 2002). Peroxidases can also generate free radical species (Yamazaki *et al.* 1985). Cleavage of β-carotene by peroxidase secreted by the fungus *Lepista irina* was reported by Zorn *et al.* (2003). Cleavage products were the same as with CDDs cleavage or co-oxidation by lipoxygenases or xanthin oxidases (Zorn *et al.* 2003). However in studies on higher plants, catabolism of carotenoids by peroxidase was reported to be dependent on the addition of a phenolic substrate (2,4 dichlorophenol) (Matilde and Martinoia 1982; Kennedy *et al.* 2002; Gandul-Rojas *et al.* 2004) and was enhanced by the further addition of hydrogen peroxide (Matilde and Martinoia 1982). In order to determine the causes (enzymatic or non-enzymatic) of carotenoid degradation one should take into account the presence of these substrates.

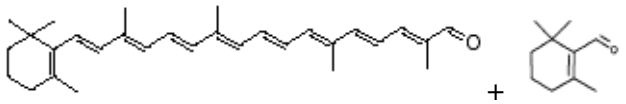
1.3.5 Products of degradation

β-carotene is degraded in a chain radical propagation reaction. The products of oxidation of β-carotene are diverse and include: high molecular weight products, such as, various apo-β-carotenal (8'; 10'; 12'; 14'; 15'), that further degrade; and short chain products, such as, fragrant compounds called norisoprenoids (Siems *et al.* 2005)

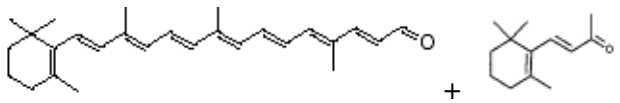
Two types of asymmetric cleavage of trans-β-carotene, 7'-8' and 9'-10', lead to the formation of β-cyclocitral and β-apo-8'carotenal; β-ionone and β-apo-10'carotenal respectively (Figure 1-20).



Trans-β-carotene

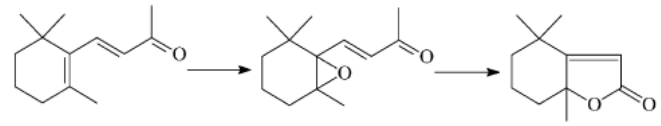


β-apo-8'-carotenal + β-cyclocitral



β-apo-10'-carotenal + β-ionone

-



β-ionone **5,6-epoxy-β-ionone** **dihydro-actinidiolide**

Figure 1-20: 7'-8' and 9'-10'cleavage of β-carotene and formation of 5,6 epoxy-β-ionone and dihydroactinidiolide (DHA) from β-ionone. Mordi *et al.* (1993); Waché *et al.* (2003).

The cleavages can arise from autoxidation (Mordi *et al.* 1993) or enzymatic reactions (Waché *et al.* 2003). Epoxidation of β-ionone into 5,6 epoxy-β-ionone and thermal rearrangement of 5,6 epoxy-β-ionone into dihydroactinidiolide (DHA) has been previously described (Mordi *et al.* 1993; Waché *et al.* 2003) (Figure 1-20).

Norisoprenoids formed after oxidation of β-carotene include β-ionone (fragrance of roses), 5,6-epoxy-β-ionone, β-cyclocitral (flower fragrance with fruit tones), limonene (odour of oranges), DHA (sweet, tea-like odour). These compounds are used in perfumery. The production of aromas from carotenoids has also been widely studied.

Volatile products have been described from the degradation of β-carotene in its chemical form (Handelman *et al.* 1991; Mordi *et al.* 1993; Waché *et al.* 2003); or from carotenoids naturally present in, for instance, oak wood (Nonier *et al.* 2004), black tea (Ravichandran *et al.* 2002), and wine (Mendes-Pinto 2009). Norisoprenoids have also been reported from paprika powder (Cremer and Eicher 2000). But to our knowledge no work has been reported on the degradation pattern of β-carotene from dried root or tuber crop such as sweet potato.

1.4 INFLUENCE OF DRYING AND STORAGE ON CAROTENOID LOSS IN SWEET POTATO ROOTS

1.4.1 Introduction

Following the understanding of the mechanisms of carotenoid degradation, the quantification of carotenoid losses could help define which processes and conditions contribute the most.

In OFSP, initial levels of carotenoids are influenced by variety, root maturation and location (Kósambo *et al.* 1998). Sweet potato varieties can be grouped into four general categories based on their β-carotene content on a dry weight basis (Simonne *et al.* 1993):
- non- detectable <1 $\mu g.g^{-1}$;
- low *β*-carotene 1-39$\mu g.g^{-1}$;
- moderate *β*-carotene 40-129 $\mu g.g^{-1}$; and
- high *β*-carotene >130 $\mu g.g^{-1}$;

The carotenoid content after processing is dependent on the initial content and loss during processing..

1.4.2 Determination of carotenoid degradation

Losses

Quantifying carotenoid losses after processing is a simple means to classify the best processes and optimise the chosen processes. There are two ways of calculating carotenoid loss (Equations 1-1 and 1-2):

- On a dry weight basis:

$$\%loss = 1 - \frac{C}{C_0} \quad \textbf{(Equation 1-1)}$$

- On a fresh weight basis taking into account loss of mass over the process:

$$\%loss = 1 - \frac{mC}{m_0 C_0} \quad \textbf{(Equation 1-2)}$$

C: Carotenoid content of food ($\mu g.g^{-1}$) at time t (*e.g.* after processing or after storage)
C_0: Carotenoid content of food ($\mu g.g^{-1}$) at initial time (*e.g.* before processing or before storage)
m: mass of food at time t (g)
m_0: initial mass of food (g)

The second formula was initially written by Murphy *et al.* (1975). The basis is fresh food weight and not dry matter weight in order to take into account soluble solid losses occurring during cooking (Rodriguez-Amaya 1997; Rodriguez-Amaya and Kimura 2004). Provitamin A true retention was calculated by 1- % loss from Equation 1-2.

In processes where soluble loss is negligible (in the case of drying for instance or in storage of dried foodstuff) both formulas give equivalent results and the first formula is sufficient. However when soluble loss becomes more significant (*e.g.* cooking, blanching, chemical dipping, etc) the second formula that takes into account soluble losses was used.

Degradation kinetics

Another more precise way of measuring the degradation of carotenoids in a food product is the measurement of the kinetics of degradation.

The kinetics of degradation of β-carotene follows either zero-order in an aqueous medium or first-order in an anhydrous medium (Minguez-Mosquera and Jaren-Galan 1995). The degradation rate is described in Equations 1-3 and 1-4:

Zero order: $C = C_0 - kt$ **(Equation 1-3)**

First order: $\ln C = \ln C_0 - kt$ **(Equation 1-4)**

C: Carotenoid content of food ($\mu g.g^{-1}$) at time t (*e.g.* after processing or after storage)
C_0: Carotenoid content of food ($\mu g.g^{-1}$) at initial time (*e.g.* before processing or before storage)
t: time (day)
k: constant rate of degradation (day^{-1})

Carotenoid degradation kinetics can be evaluated using different models. The most well known is Arrhenius model. Other models however exist such as the Eyring model, which is based on the transition state theory in which enthalpy of activation (ΔH*) and entropy of activation (ΔS*) are the model's parameters. The Arrhenius model is an empirical collision model that describes the relationship between reaction constant rates and temperature using activation energy (E_a) and a pre-exponential factor (k_∞). The model's parameters were identified from experimental data measured using linear regressions (Cisse *et al.* 2009).

Carotenoid degradation rate has been described by various authors including Koca *et al.* (2007), Hidalgo and Brandolini (2008) and Cisse *et al.* (2009) using the Arrhenius model (Equation 1-5).

Arrhenius model:

$$k = k_\infty e^{-\frac{Ea}{RT}}$$ **(Equation 1-5)**

k : degradation constant rate (day^{-1})
k_∞ : value of k at T = ∞ (day^{-1})
T : temperature (K)
Ea : Activation energy ($kJ.mol^{-1}$)
R : Molar gas constant = 8.314 J · K−1 · mol^{-1}
Practically the graphical representation gives the following equation:
$\ln k = b - a\frac{1}{T}$ where b and a are constant of linear equation. b= ln k_∞.
Ea was calculated from the linear equation following the formula: $a = \frac{Ea}{R}$

For the validation of the Arrhenius model using temperature (T) expressed in Kelvin, the predicted data can be calculated using Equation 1-6:

$$C = C_0 e^{-k_\infty \int_0^t e^{\frac{-Ea}{RT}} dt}$$ **(Equation 1-6)**

Determination of Ea and k_∞, the parameters helps predict carotenoid degradation for known temperatures and storage times.

1.4.3 Carotenoid losses after drying

Previously reported carotenoid losses (total carotenoid by spectrophotometry or β-carotene by HPLC) following different types of processing as compared with drying are summarised in Tables 1-4 and 1-5.

Table 1-4: Effect of type of process on carotenoid loss in foods other than OFSP

Food Product	Process*	Country	Analyse of carot-enoids	Loss	Reference
Carrot	Steam cooking Water with pressure Water without pressure *Oven drying 65ºC 7.5h* *Water without pressure+ Oven drying*	Brazil	β-carotene	*22%* *23%* *25%* *35%* *32%*	Pinheiro-Santana *et al.* 1988
Leaves: sweet potato, amaranth, cowpea, peanut, pumpkin	*Shade drying 25ºC 24h* *Sun drying*	Tanzania	β-carotene	*74%* *94%*	Mosha *et al.* 1997
12 different fruits	*Oven drying gradual increase (50-65ºC)* *Sun drying*	Croatia	β-carotene	*13%* *11%*	Vedrina-Dragojevic *et al.* 1997
Leaves: savoy beet, amaranth, fenugreek	*Sun drying* *Solar drying* *Shade drying* *Oven drying 65ºC* *Low temperature oven drying 30ºC*	India	β-carotene	*70%* *65%* *68%* *45%* *42%*	Negi & Roy 2000
Leaves: sweet potato, amaranth, cowpea, peanut, pumpkin, ngagani, ngwiba, nsonga, maimbe	*Sun drying* *Cabinet-solar drying*	Tanzania	β-carotene	47% 24%	Mulokozi & Svanberg 2003
OFSP Carrot Collard green	*Cabinet-solar drying*	USA	β-carotene	10% 59% 22%	Mdziniso *et al.* 2006
Cassava	Boiling Gari *Oven drying* *Sun drying* *Shade drying*	Colombia	β-carotene	44% 66% *28%* *62%* *41%*	Chavez *et al.* 2007

*Drying processes are in italics.

Table 1-5: Effect of type of process on carotenoid loss in OFSP

Process*	Variety	Country	Analyse of carotenoids	Loss	Reference
Canning 116ºC 1.5h	Jewel	USA	β-carotene	20%	Chandler & Schwartz 1988
Drum drying 160ºC				*23%*	
Baking 191ºC 1.5h				31%	
Boiling	23 varieties	Kenya	Total	20%	Hagenimana *et al.* 1999
Oven drying 65ºC 12h				*30%*	
Cabinet drying 58ºC 5h	Jonathan	Kenya	β-carotene	*47%*	Kósambo *et al.* 2004
	Kakamega			*28%*	
Sun drying	Jonathan			*72%*	
	Kakamega			*83%*	
Tunnel solar drying	Ejumula	Uganda	β-carotene	*9%*	Stollman *et al.* 2005
	Kakamega			*15%*	
Sun drying	Ejumula			*13%*	
	Kakamega			*29%*	
Boiling	Kakamega, Tainnung, Zappalo, Nyathi Odiewo, 199062.1, Salyboro	Kenya	β-carotene	12%	Kidmose *et al.*2007
Roasting				15%	
Shade drying	Zappalo			*21%*	
Boiling	OFSP (Ejumula, Kakamega, Sowola 6/94/9, SPK004/1, SPK004/6, SPK004/1/1, SPK004/6/6)	Uganda	β-carotene	22%	Bengsston *et al.* 2008
Steamed				23%	
Deep-fat frying				22%	
Oven drying	Ejumula			*12%*	
Tunnel solar drying				*9%*	
Sun drying				*16%*	
Steamed 20min	Yanchu	China	β-carotene	12%	Wu *et al.* 2008
Steamed 20min+oven drying 50C 5h				*35%*	
Steamed 20min+oven drying 50C 11h				*41%*	

*Drying processes are in italics.

Comparison of losses in drying with other processes

The type of processing used influenced on the level of carotenoid loss. Highest retention of carotenoids was obtained with steamed and boiled carrots (under or without pressure) as compared with moist/dry and dehydrated carrots for 7.5 h at 65ºC in a forced air oven, respectively on β-carotene: 22; 23; 25% as opposed to 32 and 35.0% (Pinheiro-Santana *et al.* 1998). Steaming and boiling of 23 sweet potato varieties with various initial carotenoid contents induced an average loss in total carotenoid content of 20% while drying in an oven at 65ºC for 12 h induced a loss of 30% (Hagenimana *et al.* 1999). Using six yellow and orange-fleshed sweet potato cultivars (12.4-108.0 $\mu g.g^{-1}$ fresh basis) from Kenya showed that losses were less during boiling than roasting (respectively 12.1 and 14.9%) while shade drying had higher losses of 21% (Kidmose *et*

al. 2007). Drying generally results in greater losses than steaming, boiling or roasting (Pinheiro-Santana *et al.* 1988; Rodriguez Amaya 1997; Hagenimana *et al.* 1999; Kidmose *et al.* 2007). Processes causing the least to the greatest carotenoid losses were steaming, boiling, roasting, drying, and frying (Rodriguez-Amaya 1997).

There are, however, variations to this classification. Working on three clones of cassava, oven drying, shade drying and boiling caused the lowest *trans*-β-carotene losses with 28; 41 and 44% respectively and, sun drying and gari processing had the highest losses with 62 and 66% respectively (Chavez *et al.* 2007). Long boiling times can explain low retention levels observed here. It has been observed that conditions such as deep frying, prolonged cooking, and combination of several preparations generate large losses (Rodriguez Amaya 1997). Wu *et al.* (2008) showed that steaming followed by drying had a more pronounced effect on carotenoid degradation than steaming only (12% loss). A longer drying (11h against 5h) also increased carotenoid loss (41% against 35% respectively) (Wu *et al.* 2008). Bengsston *et al.* (2008) working on several Ugandan varieties found average losses of respectively 23%, 22% and 22% on steaming, boiling and deep-fat frying. On the other hand, loss in oven drying was only 12% when working with Ejumula OFSP variety.

Generally, comparison between processes demonstrated that there was no clear classification of processes regarding the level of carotenoid degradation. Results vary from one author to another. For a better understanding of the effect of processing on carotenoid degradation, processing conditions need to be recorded precisely. This was not always the case, for example, the temperature and humidity conditions when drying under open air sun conditions are not recorded in most publications (Negi and Roy 2000; Mulokosi and Svanberg 2003; Kósambo 2004; Stollman *et al.* 2005; Kidmose *et al.* 2007; Bengsston *et al.* 2008). On the other hand the evaluation of different processes on the basis of carotenoid loss does not take into account the difference between processes that involve differential biochemical change of the food product (for instance boiling induces OFSP starch gelatinisation while drying induces moisture removal from the cell structures).

Comparison of losses in drying processes

Artificial and natural drying

Working on drying only, there has also been a large variation of losses reported with dryers and food commodities. Trans-β-carotene lost after sun-drying and oven cabinet-drying of OFSP Jonathan variety was 72% and 47% respectively, and after sun-drying and cabinet-drying of Kakamega variety, 83% and 28% respectively (Kósambo 2004). Hence trans-β-carotene content was half in sun-exposed OFSP chips compared to cabinet-dried chips (58°C for 4 hours). Working on other crop than sweet potato, Chavez *et al.* (2007) similarly reported that losses in cassava were lower in oven drying compared to shade and sun drying (28%, 41% and 62% respectively). It has been shown that sun, solar and shade drying generated more β-carotene losses than oven drying on leaves (respectively 70%, 68%, 65% and 45%) (Negi and Roy 2000). In general, in the different food commodities studied, artificial drying (*e.g.* fan-operated oven cabinet) has been reported to retain more provitamin A than natural drying (shade, solar and sun) (Rodriguez-Amaya 1997; Van Hal 2000; Kósambo 2004).

There were however exceptions. In another study on OFSP there were no significant differences between oven (57°C for 10 hours), solar and sun drying (6-10h) (12%, 9% and 16% respectively) (Bengsston *et al.* 2008). A study on total carotenoids in various fruits also showed that losses in an oven dryer at 50-60ºC were similar to losses in the sun (respectively 11% compared to 13%) (Vedrina-Dragojevic *et al.*1997). In both studies levels of carotenoid losses were low and this could explain the lack of differences between artificially and naturally operated dryers.

Solar, shade and sun drying

Few publications have been found on carotenoid retention in natural drying processes (solar, shade and sun drying). Carotenoid losses were higher in sun drying compared to solar drying on OFSP in a study by Stollman *et al.* (2005) (respectively 9% and 13% on Ejumula variety and 15% and 29% on Kakamega variety). Greater retention in solar drying as compared to sun drying can be explained by sun light filtration. It has been shown that exposure to light especially sun-light or UV light may induce trans-cis photomerisation and photodegradation of carotenoids (Rodriguez-Amaya and Kimura 2004). Working on leafy vegetables (sweet potato, pumpkin, amaranth leaves etc), solar

drying retained more provitamin A carotenoids than open-sun drying (Mulokozi and Svanberg 2003) (24% against 47% losses); in another study on leafy vegetables by Negi and Roy (2000) carotenoid losses in solar and sun drying were similar though slightly lower in solar drying (respectively 65% and 70%). When comparing shade and sun drying, which are equivalent in terms of cost and drying technology, it was shown that there was a significant improvement with shade drying of leaves compared to sun drying (74% against 94% loss) (Mosha *et al.* 1994). An improved retention in shade drying than in sun drying of cassava was also observed by Chavez *et al.* (2007). Kidmose *et al.* (2007) corroborated this by demonstrating that shade drying OFSP variety Zappalo for five hours generated a loss of only 21%. Better retention in shade drying in different crops including sweet potato can be explained by minimal exposure to UV sun radiations and lower temperatures than in sun drying (Chavez *et al.* 2007). However there was an exception in the study by Negi *et al.* (2000) working on various tropical plant leaves: the improvement of shade drying as compared to sun drying was not significant. In this study, lower retention in shade drying may be explained by longer drying times in shade drying as compared to sun drying.

From the literature, the levels of carotenoid loss varied greatly between the authors for solar, shade and sun drying. The level of carotenoid loss also varied with the different food products analysed using the same dryer. In a study on carotenoid retention of OFSP, carrot, collard greens in solar drying levels of loss were found to be significantly different being 10%, 59% and 22% respectively (Mdziniso *et al.* 2006). The author suggested that the difference in initial moisture contents of these food products (respectively 75.8%, 90.5% and 89.1%) would have influenced the level of carotenoid lost after drying.

Effect of variety

The effect of variety was also investigated. It was found that carotenoid retention was very variable between varieties. Thirteen Kenyan OFSP varieties, from 1.64µg.g^{-1} to 422µg.g^{-1} *trans-β*-carotene content on a dry basis (db), presented losses on chips varying between 6 and 57% after electric cabinet drying at 58°C for 4 hours (Kósambo 2004). Twenty three varieties with a total carotenoid content of between 2 and 632 µg.g^{-1} db had losses ranging from 0 to 80% (Hagenimana *et al.* 1999). In both studies, reductions

observed were greater in provitamin A carotenoids rich varieties than in ones containing low amounts (Kósambo 2004; Hagenimana *et al.* 1999). More investigation on the effect of variety on carotenoids degradation in drying needs to be undertaken. Inconsistent results in different literature reports clearly illustrate that sun, solar and shade drying processes require more investigation, in particular under different processing and environmental conditions.

1.4.4 Carotenoid losses during storage of dried products

Level of loss

The effect of storage on carotenoid losses in a range of dried food products is reported in Table 1-6.

Table 1-6: Effect of storage of dried food on carotenoid loss

Dried food Product	Storage conditions	Duration (days)	Packaging	Country	Type of carotenoid	Loss (%)	Reference
Freeze-dried carrot	Ambient (16-32ºC)	41	Paper-Al-PE aw=0.23-0.57 aw=0.73	India	Total	45-56% 86%	Arya *et al.* 1979
Freeze-dried papaya	Ambient (16-35ºC)	252	Paper-Al-PE	India	Total	77%	Arya *et al.* 1983
Freeze-dried melon	37ºC	183	Paper-Al-PE	India	Total	50%	Arya *et al.* 1985
Drum dried OFSP flakes	23ºC 55-60% RH	210	PEP air Nylon air Nylon vacuum Nylon O_2 absorber	USA	β-carot.	67% 48% 36% 1%	Emenhiser *et al.* 1999
Oven-dried OFSP slices	Ambient	330	Paper bags	Kenya	Total	11%	Hagenimana *et al.* 1999
Oven-dried Azolla	Ambient	120	Double plastic bag	Belgium	β-carot.	69%	Lejeune *et al.* 2000
Freeze-dried carrot powder-extract	4ºC dark 25ºC dark 45ºC light 45ºC dark	84	Bottles with N_2	Taiwan		18% 29% 40% 47%	Tang & Chen 2000
Drum-dried OFSP flakes	Ambient	91	Foil Paper laminate plastic	Guatemala	β-carot.	43% 46% 54%	Valdez *et al.* 2001
Freeze-dried OFSP powder-extract	4ºC dark 25ºC dark 25ºC light 40ºC dark	45	Bottles	Turkey	Total	11% 60% 64% 88%	Cinar 2004
Oven- dried OFSP chips	Freezer Ambient (25ºC)	120	-	Kenya	β-carot.	0% 50%	Kósambo *et al.* 2004
Oven-dried Gac fruit powder	5ºC	112	PE bag under vacuum	Vietnam	β-carot.	25%	Tran *et al.* 2008

Trans-β-carotene content declined by 50% at room temperature (about 25ºC) in chips from Kakamega and Jonathan varieties in Kenya whereas it remained steady in sweet potato chips stored for 3 months in a freezer (-20ºC) (Kósambo 2004). When analysing the samples stored at ambient temperature without special conditioning there was consistency between losses found by the different authors in relation with storage duration (Arya *et al.* 1979; 1983; 1985; Emenhiser *et al.* 1999; Lejeune *et al.* 2000, Valdez *et al.* 2001; Cinar 2004; Kósambo 2004). On average carotenoid losses after storage were 50% after 3-4 months with these authors. This consistency of results differed from the results from drying processes where no clear trend could be observed (see former paragraph). One study however did not agree with this trend: Hagenimana *et al.* (1999) storing dried slices from 24 sweet potato varieties in opaque paper bags under ambient conditions for 11 months, found that total carotenoids were reduced by only 10%.

Hagenimana *et al.* (1999) further noted that losses during storage of chips/slices were lower than of flour. Consequently it would be therefore more judicious to store chips/slices and make flour as needed. Furthermore these findings agreed with traditional drying practices (Hagenimana *et al.* 1999). It was hypothesised that slices (with a larger surface area) could have contributed to less exposure to oxidation and as a result to carotenoid loss. These observations raised the need to look at the effect of chip size on carotenoid loss in storage. Factors affecting provitamin A carotenoids in storage such as temperature, water activity, light and oxygen have been more clearly identified by the literature than for drying.

Temperature

Freeze-dried carotenoids powder from orange peel; sweet potato and carrot stored at 4; 25; 40 or 45ºC demonstrated a significant difference due to impact of temperature after ten days of storage (Tang and Chen 2000; Cinar 2004).

Loss of carotenoids was found to follow a first order kinetics of degradation in most cases (Baloch *et al.* 1977; Koca *et al.* 2007, Perez-Galvez *et al.* 2005). The effect of temperature can be modelled using the Arrhenius model described earlier. Activation energy (Ea) is given in Table 1-7 for some dried food products.

Different products, butternut squash, OFSP and yellow corn, have also been reported to have various activation energy; being 56.5, 44.4 and 20.1 $kJ.mol^{-1}$ respectively (Stephanovitch and Karel 1982).

Table 1-7: Activation energy of some dried food products

Country	Reference	Dried food Product	Conditions	Dura-tion (days)	Type of carot.	Ea ($kJ.mol^{-1}$)
USA	Stephanovitch & Karel 1982	Butternut squash	60; 70; 80ºC	?	β-carot.	56.5
		OFSP				44.4
		Yellow corn				20.1
Turkey	Koca *et al.* 2007	Sliced &oven-dried carrot	Unblanched 27; 37, 47,57ºC	180	Total (by spectro.)	66.1
			Blanched 27; 37, 47,57ºC			38.9
Italy	Hidalgo & Brandolini 2008	Monlis wholemeal wheat flour	-20; 5; 20;30;38ºC	250	Total (by HPLC)	40.7
		Serio wholemeal				37.7

The initial state of the product before drying (raw; cooked; blanched etc.) also influenced on the carotenoid degradation and therefore the activation energy. Carrots unblanched or blanched, and further dried, had activation energy of 66.1 and 38.9 $kJ.mol^{-1}$ respectively (Koca *et al.* 2007). Activation energy also varied with the variety, for instance on Monlis and Serio wholemeal wheat flours, 40.7 and 37.7 $kJ.mol^{-1}$ respectively (Hidalgo and Brandolini 2008). Hence activation energy should be evaluated for each type of product. Moreover only one reference was found for OFSP (Stephanovitch and Karel 1982) which indicates a need for more research on activation energy in OFSP.

Water activity

Water activity (a_w) has also an impact on storage. Arya *et al.* (1979) demonstrated on a range of 0.0-7.3 a_w in freeze dried carrots at room temperature (16-32ºC) that better stability of carotenoids was obtained with an a_w of 0.43. Lavelli *et al.* (2007) using blanched and unblanched freeze dried carrots stored at 40ºC confirmed that the water activity with best retention ranged between 0.31 and 0.54 (Figure 1-21). Blanched carrots did not exhibit better retention in storage compared to unblanched so enzymatic origin of the degradation was not considered the main cause. On the other hand,

Haralampu and Karel (1983) working on freeze-dried OFSP demonstrated that the lower water activity the higher, the degradation rate (Figure 1-21).

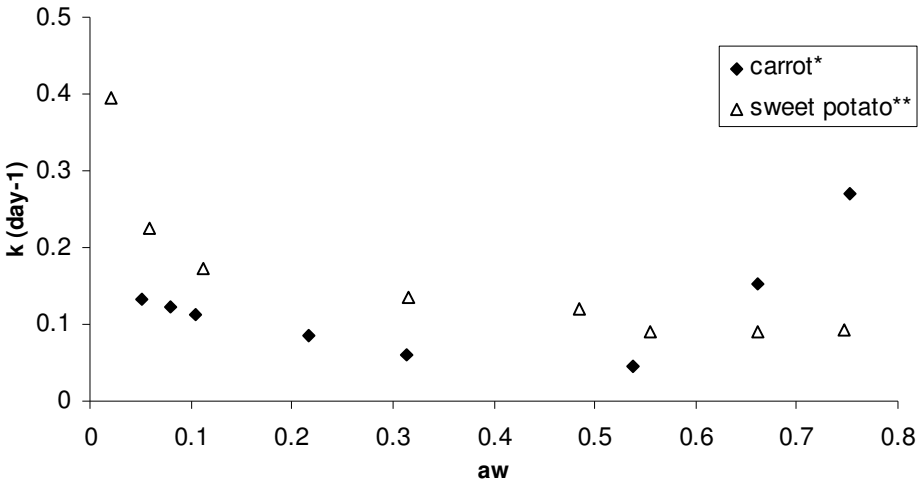


Figure 1-21: Effect of water activity (a_w) on β-carotene rate of degradation at 40°C of freeze dried food powder. * Lavelli *et al.* (2007); ** Haralampu and Karel (1983).

Because earlier results by Haralampu and Karel (1983) showed that water activity had an influence on carotenoid degradation in OFSP and because of scarce data on the effect of water activity on carotenoid content in OFSP or similar food products, there is a need to do more research in this area.

Light

Another factor mentioned earlier that influenced β-carotene degradation was light. Light increased *cis*-isomerisation on *α*-carotene; β-carotene and lutein standards (9-cis and 13-cis) compared to samples stored in the dark (Tang and Chen 2000). There was a relative increase in 9-cis-β-carotene with an associated decline in the 13-cis-β-carotene on mango puree after light exposure reported by Vasquez-Caicedo *et al.* (2007). Chen *et al.* (1996) noticed that 9-cis carotenoids isomer was the major type formed in the light while 13-cis-carotenoids isomers were favoured in the dark. On the other hand, Cinar (2004) did not show an increase of cis-isomerisation on enzyme-extracted pigments from orange peel, sweet potato and carrot. The fact that light generates free-radicals can explain why it promotes oxidation. Moreover the addition of other factors such as moisture and oxygen favour oxidation of lipids generating off-flavours (Commonwealth Science Council 1985).

Oxygen

Oxygen had a greater impact on β-carotene breakdown compared to light (clear or opaque packaging) (Vasquez-Caicedo *et al.* 2007). This was confirmed by Valdez *et al.* (2001). A significant decrease of β-carotene content was observed in sweet potatoes flakes stored at room temperature for 4 months in different packaging. Losses in β-carotene in foil packaging, laminate paper and in plastic (LDPE or HDPE) were 43%, 46% and 54% respectively. Packaging had a minor impact compared to the effect of air oxidation. Emenhiser *et al.* (1999) stored sweet potato flakes at room temperature in a propylene film (high oxygen permeability) with air headspace, a nylon laminate film (low oxygen permeability) with air headspace under vacuum or an Ageless oxygen absorber sachet enclosed had their β-carotene determined at intervals from 0 to 210 days. Results very clearly demonstrated that β-carotene retention was related to apparent availability of oxygen (*i.e.* nylon> polypropylene and oxygen absorber>vacuum>air headspace). Highest losses were obtained with polypropylene headspace (calculated at 66.8%) and lowest with nylon oxygen absorber (calculated from data at 1.0%). The impermeable packaging to oxygen (i.e. laminate) with an oxygen absorber was found the best system to protect provitamin A from the main factor of degradation which is air oxidation (Emenhiser *et al.* 1999). However because of its cost this type of system is not affordable to small-scale farmers in East and Southern Africa. Locally available packaging (*i.e.* LDPE, paper, traditional bags in jute) is permeable to oxygen therefore alternative ways need to be investigated.

The main factors that contribute to the degradation of provitamin A in storage under ambient conditions described in literature were temperature, water activity (moisture), light and oxygen in air. Storage is a critical stage and oxygen was a very significant factor in studies at ambient temperature as demonstrated by study on packaging oxygen permeability (Emenhiser *et al.* 1999; Vasquez-Caicedo *et al.* 2007). More investigations, however, are needed to investigate the relative influence of these factors on the storage of OFSP.

1.4.5 Carotenoid losses during food preparation

Evaluation of finished food products from OFSP to meet nutritional requirements in provitamin A is an important matter. Few published works have been reported on the

carotenoid loss on the finished products made from OFSP. In the few reports or publications found, losses on processed products make from flour were high in all cases. In mandazi (Hagenimana *et al.* 1999; Kósambo 2004; Stollman *et al.* 2005) and bread (Kósambo 2004), losses were greater than 80%. In porridge, losses were greater than 65% (Kósambo 2004). On the basis of these reports solely varieties with high carotenoid content may be able to bring significant contribution to the diet, in particular for mandazi and bread. Further studies are therefore necessary on this aspect.

1.5 SUMMARY OF ISSUES HIGHLIGHTED BY THE LITERATURE REVIEW AND OBJECTIVES OF THE RESEARCH

1.5.1 Summary and outcomes

It has been found that orange-fleshed sweet potato (OFSP) could make a major contribution to tackling vitamin A deficiency in affected countries where sweet potato is commonly consumed. There is a potential to make a variety of products from OFSP, and drying is a viable option for OFSP processing.

Provitamin A compounds are unsaturated unstable carotenoids easily degraded by light (UV), oxygen, and heat (Rodriguez-Amaya 1997) influenced by water activity. It is therefore essential to understand the losses of carotenoids occurring in the drying of orange-fleshed sweet potato (OFSP) in order to achieve products with adequate nutritional quality (Rodriguez-Amaya 1997; Hagenimana *et al.* 1999).

In order to limit the degradation of provitamin A during drying, various types of solar dryers should be compared in terms of carotenoid retention. Their choice depends on the cost; locally available materials and impact of environmental factors in view of humidity and temperature in particular. The methods need to be suited to local conditions with respect to cost and cultural practices (Hagenimana *et al.* 1999).

Inconsistent studies have been reported on the level of carotenoid losses in OFSP during low cost drying techniques, in particular sun and solar drying (Kósambo 2004, DIAS 2006, Mdziniso *et al.* 2006, Kidmose *et al.* 2007, Bengstsson *et al.* 2008) and further investigations are necessary. There is also a need to determine carotenoid losses during

the storage of dried chips which takes place after drying but before the product is traded and consumed. Data found in literature on the level of carotenoid losses during storage of OFSP under ambient conditions needs to be verified under local ambient conditions. It is necessary to identify the conditions or techniques that both reduce carotenoid loss and also are available and also are affordable by farmers and processors.

1.5.2 Hypothesis and objectives of the research

The main hypothesis of this research work is that solar or sun dried OFSP could make a major contribution to the nutritional requirements in vitamin A of children in developing countries.

The main objectives of this research project on dried orange-fleshed sweet potato (OFSP) are the following:

- To choose and assess the methods for analysing carotenoid content and for drying OFSP;
- To quantify the degradation of carotenoids after drying and storage under controlled (in France) and field conditions (in Uganda and Mozambique);
- To understand the conditions that can influence carotenoid degradation including the type of solar dryer, sweet potato variety, chip size, pre-treatments;
- To determine whether enzymes have an impact on carotenoid degradation;
- To measure the impact of temperature, water activity and oxygen under controlled conditions and understand the pathway of carotenoid degradation.

CHAPTER 2.
DESCRIPTION AND ASSESSMENT OF METHODS

2.1 INTRODUCTION

This chapter presents general methods for drying, storing OFSP and analysing the carotenoid content. Some of the methods have been assessed for this specific thesis study. These assessments were necessary to give a reliable basis for the further quantification of carotenoid losses.

2.2 GENERAL DESCRIPTION OF ROOT MATERIAL, DRYING AND STORAGE PROCESSES

2.2.1 Flow chart of sweet potato drying and storing

The different stages of the drying of sweet potato and storage are reported in Figure 2-1.

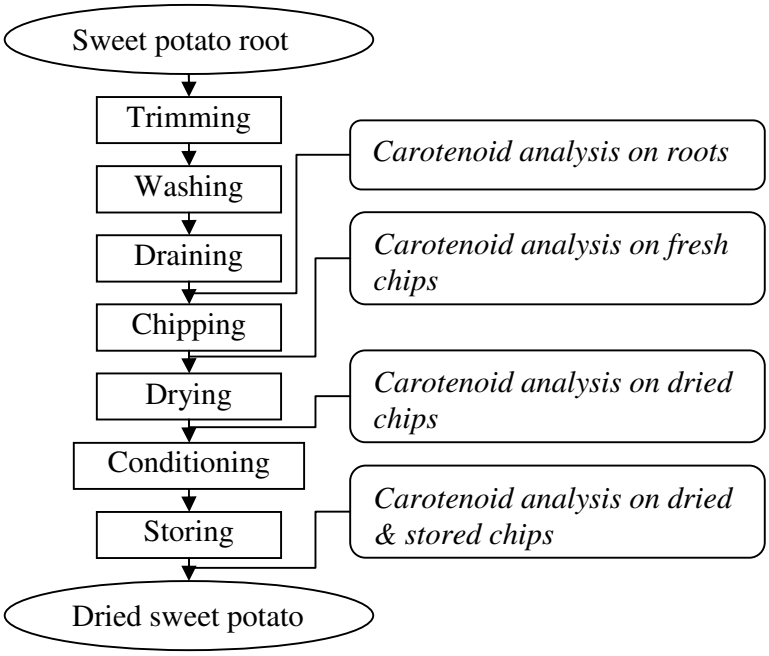


Figure 2-1: Flow chart of dried sweet potato production and carotenoid analysis

2.2.2 Root samples

Roots samples used in this research were collected locally when working in the field (Mozambique or Uganda) or imported when working in France or UK (Table 2-1).

Table 2-1: Sweet potato varieties used in the different experiments (flesh colour, origin country, and planting, drying and storage and laboratory analysis of carotenoid content)

Name	Flesh colour	Origin	Planting	Drying & Storing	Lab. analysis	Thesis Chapter
Rubina ® Agrexo	Red	USA	USA /imported	France	France	2, 3
Beauregard Agrexo	Red	Israel	Israel /imported	France	France	2, 3
Covington	Red	USA	USA /imported	UK	UK	2
Süss-kartoffeln	Red	Israel	Israel /imported	-	UK	7
Resisto	Red	USA	Uganda, Mozambique	Mozam-bique	UK	5
Ejumula	Deep orange	Eastern Uganda	Uganda	UK, Uganda	UK, Uganda	2, 4, 6, 7, 8
MGCL01	Deep orange	-	Mozambique	Mozam-bique	UK	5
SPK004/6	Orange	Bred from SPK004-Uganda	Uganda	Uganda	UK, Uganda	4
SPK004/6/6	Orange		Uganda	Uganda	UK, Uganda	4
Kakamega (SPK004)	Light orange	Western Kenya	Uganda	Uganda	UK, Uganda	4
SPK004/1	Yellow /light orange	Bred from SPK004-Uganda	Uganda	Uganda	UK, Uganda	4
SPK004/1/1	Yellow /light orange		Uganda	Uganda	UK, Uganda	4
Naspot 1	Yellow	Uganda	Uganda	Uganda	UK, Uganda	4

OFSP varieties imported from USA (Rubina®) or Israel (Beauregard) were supplied by the Agrexco platform which was supplied by the Carmel Rungis company in Montpellier, France. Covington variety grown in South Carolina was bought from

Tesco, Chatham. An unknown variety (Süsskartoffeln®) was bought on the Medway campus of the University of Greenwich, Chatham, UK.

Kakamega and Ejumula varieties were officially released in Uganda in April 2004 by the National Agricultural Research Organisation (NARO) as new improved varieties that contain carotenoids to reduce vitamin A deficiency. Some farmers have already adopted them especially in Teso area and Luwero. These sweet potato varieties were selected from a pool of different orange-fleshed varieties based on factors such as agronomic performance, resistance against attack during cultivation, yield, marketability and colour in Namulonge Research Station, Uganda. SPK004 now known as Kakamega is a yellow flesh variety with brown-purple skin that was identified in Western-Kenya and Ejumula is an Ugandan landrace identified by the National Agricultural Research Organisation (NARO); it is an orange flesh variety with light-yellow skin originally from Teso region (North-East) in Uganda. Newly developed varieties Naspot 7 (SPK004/1); Vita (SPK004/6) and most recent varieties Naspot 8 (SPK004/1/1) and Kabode (SPK004/6/6) were bred from Kakamega (SPK004). They differ from SPK004 in their yield, flesh colour and other morphological characteristics (bigger shape and more irregular).Vita and Kabode varieties have been officially released since 2008.

2.2.3 Drying of sweet potato chips

Prior to drying, roots were trimmed, weighed, washed, and drained, then chipped or sliced. Light exposure was minimized as much as possible during handling (samples were covered and not exposed to direct sun-light). Freshly chipped (similar to shredded) or sliced samples were spread equally in each dryer with a determined loading density (quantity of sample per surface). Chips were weighed every 2-4 h to estimate moisture loss during drying. The end of drying was estimated by chips brittleness and sample weight that is related to dry matter content.

2.2.4 Dry matter determination

For roots, fresh chips, dried chips and flour, dry matter content was determined by drying triplicate 5 g samples at 105ºC until constant weight in a cabinet drier (minimum 24 h) (AOAC 1984). Dry matter determination was necessary in order to calculate carotenoid content on a dry weight basis.

2.2.5 Water activity

Water activity (a_w) is defined as the vapour pressure of water in a sample divided by that of pure water at the same temperature. Water activity describes the availability of water in a food system and is related to the susceptibility of food to microbiological spoilage (Decagon Device Services 2009). When vapour and temperature equilibrium are reached, the water activity of the sample is equal to the relative humidity of air surrounding the sample in a sealed measurement chamber (see Chapter 8). Water activity was determined using awmeters in Chapters 3, 7 and 8. Specific conditions of measurements and equipment are given in these chapters. Relationship between water activity and dry matter content for a product at a specified temperature is established using sorption curves (see Chapter 8).

2.2.6 Storage of dried sweet potato samples

Dried samples (representing 1 kg of dried chips or slices minimum) were collected and thoroughly mixed. Dried samples were sub-divided into smaller sample sizes of about 200 g each. Samples for analysis on dried chips were stored with minimum delay (less than a couple of hours) in freezer at about -20°C in zipped bags where air was removed. The remainder of the samples were used for studies at ambient temperature.

2.2.7 Transport of samples

Fresh roots stored at ambient temperature and dried samples that had been stored in a freezer (-20ºC) were transported if possible in a cooler bag with gel packs to the UK. The carotenoid variations were considered negligible between harvest and analysis because of the short interval time (a month maximum). This evidence was based on Ezell *et al.* (1951)'s study in the United States on storage of sweet potato after harvest that showed that there were only small carotenoid variations over 1 month of storage (estimated to be less than 10%) at temperatures ranging between 10 and 21°C.

In the UK, the fresh roots were pureed and stored at -20°C while the dried chips were stored as they were at -20°C for 1-6 month before analysis. All the samples were stored in zip plastic sample bags with surplus air excluded by manual pressure. No significant carotenoid loss was observed on samples in freezer during this interval ($p<0.05$) (see section 2.6).

2.3 DESCRIPTION OF CAROTENOID ANALYIS

Different methods for analysing carotenoid content in OFSP have been used in France (Dhuique-Mayer *et al.* 2005) and, in the UK and Uganda (Rodriguez-Amaya and Kimura 2004). The preparation of samples is similar for both methods.

2.3.1 Preparation of homogeneous samples for carotenoid analysis

Fresh samples

Random sampling is very important because β-carotene content is heterogeneously distributed in sweet potato roots: Resisto OFSP variety β-carotene contents from the same harvest batch and on similar size-sweet potatoes varied between 132-194 $\mu g.g^{-1}$ (Van Jaarsveld *et al.* 2006). Rodriguez Amaya and Kimura (2004) recommended that a minimum of five roots should be quartered longitudinally and opposite sections combined. In our study, five average-sized roots were randomly selected and the whole of the roots were combined.. Roots were peeled and cut into cubes of about 2.5 cm. Alternatively, mixed freshly chipped or sliced sweet potato (500g minimum per sample from a batch of 4 kg) were collected. The cubes, chips, or slices were blended to a fine pulp using a food processor. These operations were carried out under low light to prevent isomerisation or photooxidation of carotenoids. The samples were thoroughly mixed and packed into plastic boxes or zipped plastic bag (protected from light) and stored at -20ºC until analysed (Van Jaarsveld *et al.* 2001, Rodriguez Amaya and Kimura 2004).

Dried samples

Dried chips or slices (minimum 1 kg per sample) were carefully mixed, quartered into 200g samples and stored at -20ºC. In order to undertake the analysis of carotenoid content, dried chips first had to be milled into flour. Different rotary disc laboratory mills have been used in the different experiments (see Chapters 3, 4, 5, 6 and 8).

2.3.2 Colorimetric analysis

Carotenoid content was estimated using colorimetric method. Colorimetric reading is a very rapid and non-destructive technique compared to spectrophotometric and HPLC techniques. A Minolta Chromameter CR 200 was used to determine the colour of flours. The chromameter was calibrated before each measurement using the calibration plate provided with the Yxy values as 93.4, 0.314, 0.319 in C Mode. Reading was taken from

below the Petri dish containing 10g-flour sample. The same Petri dish was used for all the samples. Three measurements were taken on each 10g-flour sample (using different collections from the same sample gave similar results) in the centre and sides. Colour of the flours was measured using CIELAB colour space with 3 variables; L* (luminosity), a* (red), and b* (yellow) (see Chapter 5).

2.3.3 Carotenoid analysis by Dhuique-Mayer's method (France)

Extraction

Carotenoid extraction was carried out according to Dhuique-Mayer *et al.* (2005), which was adapted from Taungbodhitham *et al.* (1998). A portion of the homogeneous representative sample was weighed (1-6 g of fresh tissue or 0.5-2 g of flour following by re-hydratation in 10 ml-deionised water for 20 minutes (see section 2.4). Sample weight varied according to the expected carotenoid content in solution (absorbance readings should be between 0.2-0.8 on the spectrophotometer). The analysis was carried out under low light conditions. The solution was homogenised by magnetic stirring with 35 ml of ethanol/hexane mix (4:3) containing 0.1% 2,6 Di-ter-butyl-p-cresol (as an anti-oxidant) and 0.1 g of magnesium carbonate, $MgCO_3$, (to neutralise plant food acids) for 5 minutes. (Ethanol preferentially extracts polar carotenoids such as xanthophylls whereas hexane preferentially extracts apolar carotenoids such as carotenes; this combination favoured extraction of both apolar and polar carotenoids). The residue was filtered through a porosity 2-sintered glass funnel by vacuum. The residue was re-extracted with a further 35ml of ethanol/hexane (4:3). Ethanol (about 20 ml) and hexane (about 20 ml) were successively added to wash the residue until there was no visible colour left in the filtrate. The extract was transferred to a separating funnel. Fifty millilitres of NaCl (10%) was added in order to increase the aqueous phase density and improve organic/aqueous phase separation. The lower, aqueous phase was discarded. Distilled water (50 ml) was added to remove residual ethanol twice. In the last washing, the lower phase was discarded as completely as possible without discarding the upper phase. The organic phase containing the extract was collected in a 50 ml volumetric flask and dewatered by addition of anhydrous sodium sulphate (Na_2SO_3) until some crystals remain undisolved. The organic phase was filtered through glass cotton to eliminate Na_2SO_3 and concentrated using a rotary vacuum evaporator at 30ºC until dry. The extract was then dissolved in a solution of 500 µl dichloromethane and 500 µl MTBE (Methyl-Ter-Butyl-Ether)/Methanol 80/20. The vortexed extract was transferred

to amber vials before analysis on both spectrophotometric and HPLC methods. For HPLC analysis samples were diluted in the solution ten times for fresh and twenty times for dried samples. For UVIKON 933 UV/Visible double beam spectrophotometer readings, samples were diluted in Petroleum Ether 100 µl/10 ml for fresh samples and 50 µl/10 ml for dried samples respectively.

Spectrophotometric reading

Absorbance at 450 nm was read in PMMA (Poly meta meta acrylate cells, with a range between 300-700 nm). No difference of reading was noticed when compared with quartz cells. Petroleum Ether is a volatile solvent and reading had to be carried out quickly in order to prevent bias and tubes were also sealed with Parafilm. Two readings were taken on the same sample tube. Total carotenoids levels were determined by comparison with a standard curve using pure β-carotene (Purity > 97%; Extrasynthese, Genay, France). Concentration (C in mg.l^{-1}) was calculated by Lambert Beer law from the absorbance (A). The concentration is given by Equation 2-1 (Britton *et al.* 1995):

$$C = 10^3 \frac{MA}{L\xi} \quad \textbf{(Equation 2-1)}$$

Where: the Molar extinction coefficient for β-carotene in Petroleum Ether (ξ) is 138900 L.mol.$^{-1}$.cm.$^{-1}$; and β-carotene molecular weight (M) is 536.88g.mol^{-1}. L is the path length (generally equal to 1 cm^{-1}).

An example of standard curve on the spectrophotometer is given in Figure 2-2.

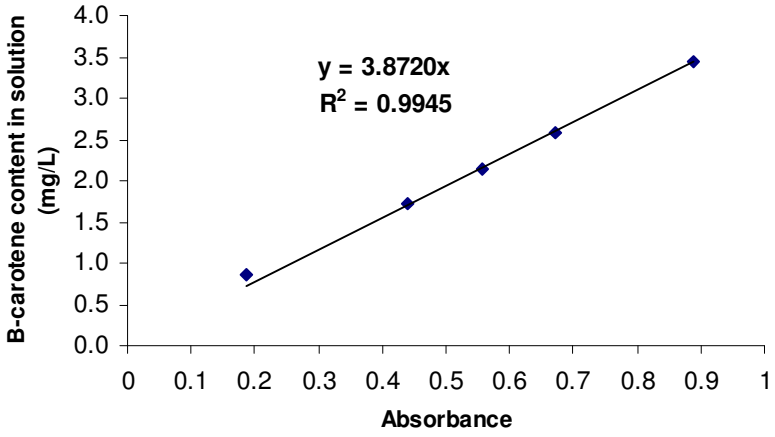


Figure 2-2: β-carotene standard curve at 450nm absorbance using a UVIKON 933 Spectrophotometer, France

β-carotene standard curve was determined by plotting five concentration levels (0.8579, 1.7158, 2.1447, 2.5737, 3.4315 $mg.l^{-1}$). A coefficient of correlation of 0.9945 indicating excellent linearity (y=3.8720x where y is the concentration in $mg.l^{-1}$ and x the absorbance). l^{-1}

HPLC analysis

Carotenoids were analysed by reverse-phase high performance liquid chromatography using an Agilent 1100 system (Massy, France) equipped with autosampler injector, degasser, pump and photodiode array detector set at 450 nm (Dhuique-Mayer *et al.* 2005). The mobile phases were distilled water as eluent A, methanol as eluent B, and MTBE as eluent C. A solvent gradient was programmed in order to enhance compound separation: 0 min.: 40% A /60% B; 0-5 min.: 20% A/ 80% B; 5-10 min.: 4% A /81% B /15% C; 10-60 min.: 4% A /11% B /85% C; 60-71 min.: 100% B; 71-72 min. back to the initial condition for re-equilibration. Carotenoids were separated through a polymeric C_{30} reverse phase column (250 x 4.6 mm inner diameter; 5 µm particle size YMC EUROP GmbH) with a flow rate of 1 $ml.min^{-1}$, a column temperature at 25ºC and an injection volume of 20 µl. The data were stored and processed by Agilent Chemstation Plus software. Concentrations were determined by comparison to a standard curve using pure β-carotene (Extrasynthese, Genay, France). An example of a standard curve is given in Figure 2-3.

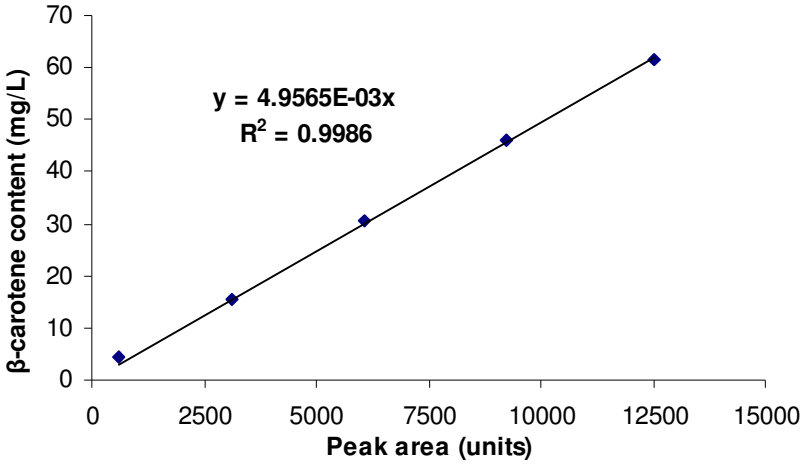


Figure 2-3: β-carotene standard curve at 450nm absorbance. Agilent 1100 system HPLC, France

β-carotene standard curve with five concentration levels (4.38, 15.34, 30.69, 46.08, 61.38 mg.l^{-1}) had a coefficient of correlation of 0.9986 indicating excellent linearity (y=4.9565.10^{-3}x where y is the concentration in mg.l^{-1} and x the area).

2.3.4 Carotenoid analysis by HarvestPlus method (UK and Uganda)

Extraction

Total carotenoids extraction and analysis were based on Rodriguez Amaya (2001) and Rodriguez Amaya and Kimura (2004). A portion from the homogeneous representative sample (fresh or dried) was homogenised with methanol: tetrahydrofuran (THF) (1:1) using a Polytron PT1200E (Kinematica, Switzerland) homogeniser for one minute. The extract was filtered through a porosity 2-sintered glass funnel by vacuum and rinsed with methanol: THF until there was no colour left in the filtrate. The extracts were combined and poured into a 500 ml-separatory funnel where 40 ml of Petroleum ether (PE) had been added. After washing thrice with desionised water (200 ml) the upper-PE phase containing the extract was collected in a 100 ml flask. The PE phase was dewatered by addition of anhydrous sodium sulphate until some crystals remain undissolved, then filtered into a 50 ml volumetric flask through glass wool and made up to volume (see Appendix 3).

Spectrophotometric reading

In Uganda (see Appendix 3), total carotenoid content was determined using a Genesys (Model 10 UV) UV-visible spectrophotometer to measure absorbance at 450 nm in PMMA cuvettes. Concentrations were determined by comparison to a standard curve using pure β-carotene (Purity > 95%; UV synthetic, powder, type I (10g) Sigma-Aldrich, UK) and absorption coefficient of β-carotene in PE of 2592 (Rodriguez-Amaya 2001, Rodriguez-Amaya and Kimura 2004). Carotenoid content was calculated using Equation 2-2 given by Rodriguez Amaya and Kimura (2004):

$$C = 10^4 \frac{A}{A_{1\%1cm}} \quad \textbf{(Equation 2-2)}$$

Concentration (C in mg.l^{-1}) was calculated by Lambert Beer law from the absorbance (A). $A_{1\%1cm}$ is the absorption coefficient of β-carotene in PE (2592) for 1cm^{-1}-path length.

A β-carotene standard curve with seven concentration levels (0.4514, 0.9028, 1.8056, 2.2569, 2.7083, 3.1597, 3.6111 mg.l^{-1}) had a coefficient of correlation of 0.9974

indicating excellent linearity (y=3.8491x where y is the concentration in $mg.l^{-1}$ and x the absorbance).

The same method was carried out at Natural Resources Institute (NRI), University of Greenwich, UK by the same operator. Homogenisation was carried out using Ultra-turax IKA Janke and Kunkel Labortechnik 8000 $rpm.min^{-1}$ and total carotenoid content measured by Diode Array detector spectrophotometer Hewlett Packard HP8452A. The first 300 ml desionised water was replaced by 50 ml NaCl 10% solution to easier phase separation.

A β-carotene standard curve with five concentration levels (0.7948, 1.5895, 1.9869, 2.3843, 3.1790 $mg.l^{-1}$) had a coefficient of correlation of 0.9910 indicating excellent linearity (y=3.7631 x where y is the concentration in $mg.l^{-1}$ and x the area).

HPLC analysis

The HPLC analysis carried out at NRI using a HPLC system consisting of a Waters separation module (Model 600E, Waters Corporation,) auto-sampler (Waters 171 plus) and, photodiode array detector (Waters 996). Twenty millilitres of extract in PE used for spectrophotometric reading was collected in amber glass vial and stored at -20°C (for 2 weeks). The carotenoids solution was then brought to dryness with N_2.

Two different methods were used. In the section 2.5.1, immediately before injection the carotenoids were dissolved in 3 ml HPLC grade acetone and collected in sample vials. 10 µl was injected into the chromatograph. Chromatographic conditions were monomeric C18 reverse phase column ((150 x 4.60 mm inner diameter 3 µm particle size sphereclone ODS(2) Model 00F-4135-E0 Phenomenex, Cheshire, UK) Pump-Waters 600E Autosampler - Waters 717 Detector - Waters 996 PDA**,** flow rate of 1 $ml.min^{-1}$; isocratic elution. Mobile phase was acetonitrile:methanol:ethyl acetate (0.05% triethylamine; 0.01% THF) (80:10:10). Concentrations were determined by comparison to a standard curve using trans-β-carotene (Purity > 95%; UV synthetic, powder, type I (10g) Sigma-Aldrich, UK). The β-carotene standard curve with five concentration levels (7.2, 21.6, 28.8, 36.0, 50.4, 72.0 $mg.l^{-1}$) had a coefficient of correlation of 0.9977 indicating excellent linearity ($y=1.6500.10^{-5}$ x where y is the concentration in $mg.l^{-1}$ and x the area).

In the section 2.6.2, before injection the carotenoids were dissolved in 1 ml THF:Methanol HPLC grade and collected in sample vials. 20 µl was injected into the chromatograph. A polymeric C30 reverse phase column (250 x 4.6 mm i.d. 5µm YMC (EUROP GmbH Germany) with a flow rate of 1 $ml.min^{-1}$, a temperature of 25°C were used to separate the compounds present in an injection volume of 10µl. The isocratic separation was established by using Methanol: MTBE (80:20) and a running time of 40 minutes and detection of compounds at 450nm. The same synthetic β-carotene was used for the preparation of a standard curve for the HPLC. β-carotene standard curve had a coefficient of correlation of 0.9972 and $y=8.311410^{-6}x$.

The identifications of carotenoids including all-trans-β–carotene, 13-cis-β-carotene, 9-cis-β-carotene was achieved by comparison with previous data from CIRAD and from the literature using the retention times and absorbance wavelengths of the compounds.

Calculation of carotenoid content in the sweet potato sample

In order to calculate the carotenoid content in the sweet potato sample (C_{sample}) in $\mu g.g^{-1}$ on a fresh weight basis, the concentration in solution (C in $mg.l^{-1}$) (deducted from the standard curve for total carotenoid or β-carotene (for individual carotenoids)) was multiplied by the volume of solution (V=50 ml) and divided by the sample weight (Equation 2-3) (m in g). Dilution (D) was taken into account.

$$C_{sample} = \frac{CVD}{m_{sample}} \textbf{ (Equation 2-3)}$$

Conversion of carotenoid content on a dry weight basis was achieved by dividing C_{sample} by the dry matter content (%). Percentages of carotenoid loss in storage were calculated from the carotenoid content after drying.

2.3.5 SPME/GC/MS analysis

A SPME (solid phase microextraction) rapid gas chromatographic method was used to analyse semi-quantitatively volatile compounds generated during the storage of sweet potato flour. Analyses were carried out following an adaptation of the method by Laguerre *et al.* (2007) that was developed using rice. Analyses were carried out on a Agilent 6980 Gas Chromatographic System (Agilent Technologies, Palo Alto, Canada) equipped with a autosampler combi PAL (CTC Analytics, Zwingen, Switzerland) coupled with an Agilent 5973N mass spectrometer. Sweet potato flour samples (3.0g)

were weighed into a 10 ml GC glass vial and were capped with an air-tight 20mm PTFE/silicon septum (Interchim, France). Samples were heated at 50ºC for 15 minutes to liberate volatile compounds from the flour matrix. Next SPME analysis was performed by exposing a fibre to the headspace of the glass vial for 45 minutes. The SPME fibres used were 1 cm long of DVB/Car/PDMS (divinylbenzene/ carboxen/polydimethylsiloxane) from Supelco (Bellefonte, PA, USA). (Fibres were conditioned at 270°C under a helium flux for 1 h before use. Prior to each extraction, the fibre was cleaned for 10 min at 250°C to remove contaminants). Then volatiles were thermally desorbed from the fibre to the GC injector operating in splitless mode. Volatiles were then transferred to the mass detector through a DB-Wax fused silica capillary column (60m x 0.32mm i.d. film thickness= 0.25 µm) operating at high temperature (see temperature values in Chapters 5 and 8) with helium as a carrier gas. The oven temperature initial temperature and gradient were set for a helium flow rate of 1.5 $ml.min^{-1}$ (see Chapters 5 and 8). The quantity of volatiles extracted by the fibre is proportional to their concentration in the sample. The MS source temperature and transfer line temperatures were 150 and 250°C, respectively. The mass range scanned was *m/z* 40 to 300. Ionisation was performed under electronic impact (EI) at 70 eV. Volatile compounds were identified using the Wiley Mass Spectral library and a standard curve using β-ionone (purity ≥97%; predominantly trans, Sigma-Aldrich, France) as internal standard (see Chapter 8).

2.3.6 Statistical analyses

Normality of samples was verified by Kolmorogorov Smirnov (sample size > 20) or by Shapiro-Wilk (sample size < 20) normality tests. Analysis of variance (ANOVA) was carried out to determine whether there were significant differences between samples with one up to four factors; a significant difference between samples was determined by a Tukey test. An independent T-test was carried out when comparing two samples solely. Correlations were determined using Pearson tests on average losses ($p<0.05$). All data were processed on SPSS 14.00 or 15.00 for Windows software.

2.4 OPTIMISATION OF EXTRACTION AND REPEATABILITY OF CAROTENOID ANALYSIS

2.4.1 Optimisation of extraction on dried sweet potato

Extraction is a key stage for the accuracy of the carotenoid analysis. Seventeen European laboratories assessed the accuracy of HPLC procedures for the determination of lutein, zeaxanthin, lycopene, α-carotene, and β-carotene in a vegetable blend. Preliminary conclusions were that the chromatographic system was not the main source of variance but the extraction that may have represented about 13% of the overall variance of around 23% (Scott *et al.* 1996 quoted by Rodriguez-Amaya 2001). A good extraction should release all the carotenoids from the matrix (Rodriguez-Amaya 2001, Rodriguez and Kimura 2004).

While carotenoids extraction of homogenised puree made from fresh sweet potato roots was really effective, extraction of flour made from dried sweet potato chips did not give acceptable results: the residue was still coloured after several washings with the extraction mix and carotenoids were visible in the residue after further soaking in the mix during a couple of hours. Hence different pre-extractions treatments were tested on sweet potato flour:

- no treatment;
- soaking in THF:Methanol extraction mix for one hour;
- re-hydration in distilled water for one hour (Table 2-2).

The re-hydrated sample presented significantly higher results than the directly extracted sample. The re-hydrated sample gave the highest total carotenoid content and the precipitate was colourless contrary to other treatments after 3 h in extraction mix at ambient temperature. Re-hydration of dried samples therefore is the best way for achieving a complete extraction of carotenoids from the dried food matrix.

Table 2-2: Effect of pre-treatment on total carotenoid content on Ejumula variety. See method (using HP8452A spectrophotometer) in section 2.3.4.

Extraction pre-treatment	Total carotenoids (ug.g^{-1} dry weight basis)*	Residue colour on filter	Residue colour in beaker after 3 h in 5 ml extraction mix
Dried sample directly extracted	122.5 (3.2)a	Pink	Yellow
Dried sample soaked in extraction mix 1h	140.0 (0.7)ab	Pink	Light yellow
Sample re-hydrated in distilled water for 1h	153.6 (6.8)b	White	White

*Each value corresponds to an extraction in duplicate made on flour from milled dried chips. Values in brackets refer to the standard deviation {mean (standard deviation)}. Values in the same column followed with different letters are significantly different; ANOVA Tukey test ($p \leq 0.05$).

These conclusions are in agreement with authors working with dehydrated food in general (Rodriguez-Amaya 1996, Rodriguez-Amaya and Kimura 2004) and one author (Kosambo 2004) working with dehydrated OFSP, in particular, with a re-hydration time of 1 hour.

The effect of re-hydration time on total carotenoid content was evaluated (Table 2-3).

Table 2-3: Effect of re-hydration time on total carotenoid content on Ejumula variety. See method (using HP8452A spectrophotometer) in section 2.3.4.

Soaking time (min)	Total carotenoids (ug.g^{-1} dry weight basis)*	Residue colour on filter	Residue colour after 3 h in 10 ml** extraction mix
20	162.5(5.2)a	White	White
30	155.9(10.4)a	White	White
60	162.12(1.5)a	White	White

*Each value corresponds to an extraction in triplicate made on flour from milled dried chips. Values in brackets refer to the standard deviation {mean (standard deviation)}. Values in the same column followed with different letters are significantly different; ANOVA Tukey test ($p \leq 0.05$).
**The volume of distilled water was increased to 10 ml in order to cover a 1g flour sample put in a 100 ml-beaker. No significant difference was observed between 5 ml and 10 ml distilled water volume.

There were no significant differences between a sample re-hydrated for 20 minutes; 30 and 60 minutes ($p<0.05$; ANOVA). Our results showed that twenty minutes soaking in distilled water was sufficient time to release all carotenoids from the matrix of dried sweet potato.

2.4.2 Reproducibility of the analysis

Reproducibility of the analysis was assessed on dried sweet potato using two sub-samples of 100 g-flour from a typical sample size of 1.4 kg (Table 2-4).

Table 2-4: Reproducibility in dried sweet potato on HPLC on Rubina® sweet potato. See method in section 2.3.3.

Sample	Moisture content (%)	HPLC *Trans-β*-carotene (ug.g^{-1} dry weight basis)
1	9.93	350.1 (14.8)
2	9.82	§347.07 (7.4)

*Each value corresponds to an average of 3 extractions and 2 extractions § on a 100g-flour from dried slices. Values in brackets refer to the standard deviation {mean (standard deviation)}

No significant difference was determined between the two sub-samples (Independent T Test; $p<0.05$).

Sampling procedure for roots was also evaluated (Table 2-5).

Table 2-5: Reproducibility in fresh roots on spectrophotometer and HPLC for determining carotenoid content (ug.g^{-1} fresh weight basis) on Rubina® sweet potato. See method in section 2.3.3.

Puree of 5 roots-Trial	Dry matter content* (%)	Spectrophotometer Total carotenoids**	HPLC Total carotenoids	HPLC Trans-β-carotene
1	18.3	67.1 (3.0)a	60.1 (4.6)b	51.3 (4.3)b
2	18.0	55.6 (3.2)b	59.3 (7.4)b	50.0 (5.8)b
3	17.7	61.5 (4.6)ab	53.9 (2.9)b	45.0 (2.0)b
4	17.6	§62.6 (2.0)ab	§60.1 (3.8)b	§49.5 (3.4)b
Mean	17.9	61.6(5.3)	58.2 (5.1)	48.9 (4.4)

*Each value corresponds to an average of three extractions. Standard deviation <1%
**Each value corresponds to an average of 3 extractions but exception two extractions§. Average (Standard deviation). Values in the same column followed with different letters are significantly different; ANOVA Tukey test ($p \leq 0.05$).

Intra-lot carotenoids variability is a known fact in OFSP (Van Jaarsveld *et al.* 2006). In order to obtain a representative sample, a minimum number of roots from the same harvest batch must be collected randomly and thoroughly homogenised. The number of roots recommended was three in Kosambo *et al.* (1998) and five in Rodriguez-Amaya and Kimura (2004). Trials were carried out to verify repeatability in fresh peeled roots and compare methods of analysis (Table 2-5) on a puree made of five sweet potato roots.

Total carotenoids and all trans-β-carotene levels were comparable in the four trials in HPLC. There was a significant difference between trials 1 and 2 in spectrophotometric reading. This can be explained by the lower precision on spectrophotometer compared to HPLC. There is, however, globally a good repeatability on both instruments on fresh sweet potatoes.

2.4.3 Comparison of Dhuique-Mayer and Rodriguez-Kimura's methods

Both extraction methods by Dhuique Mayer *et al.* (2005) and Rodriguez Amaya and Kimura (2004) were compared on Agilent HPLC 1100 system. No difference in terms of quantitative results was found ($p<0.05$; T-test). However there were small differences on the spectra; a peak of carotenoids appeared at 29 minutes on the chromatograph of the sample extracted by Dhuique-Mayer's method and was not present on the samples extracted by Rodriguez-Amaya's method (data not shown). The polarity and linkage properties with carotenoids of extracting solvents were suggested to influence the apparition of minor compounds.

2.5 COMPARISON OF INSTRUMENTS

2.5.1 Correlation between spectrophotometric and HPLC methods

Relationships between carotenoid content measured by spectrophotometric and HPLC methods were depicted for American variety of commercial brand Rubina® submitted to various drying treatments (fresh roots and dried) and for five different varieties of Ugandan sweet potato from two locations (fresh roots) (Figures 2-4 and 2-5).

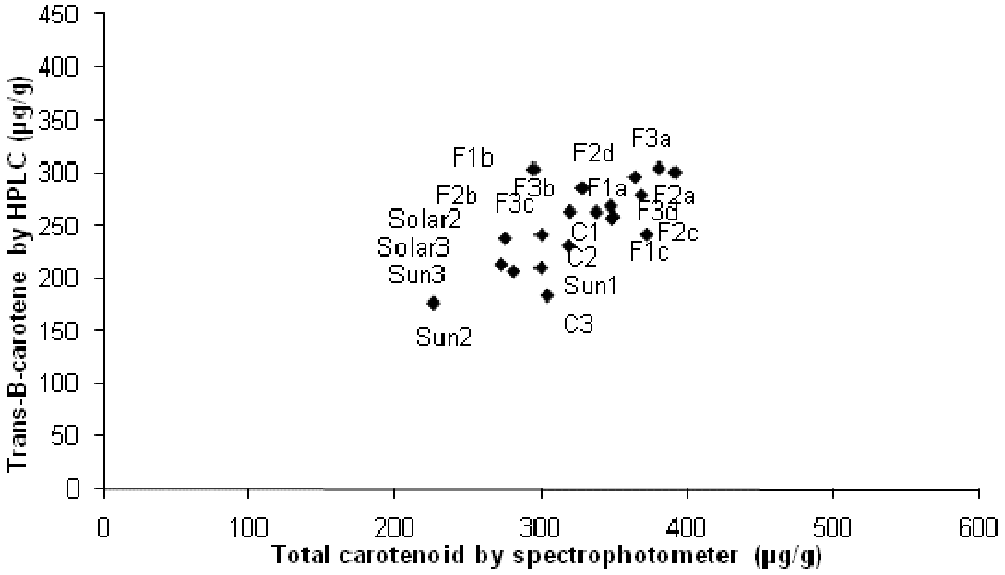


Figure 2-4: Relationship between total carotenoids (µg.g^{-1}) on a dry weight basis measured by spectrophotometric method and Agilent 1100 system HPLC. Each point corresponds to a single extraction measured on both methods. Equation: y = 0.7719x where x=Spectrophotometer total carotenoid content and y=HPLC *trans-β*-carotene content. Pearson correlation R=0.725 p<0.01- 20 extractions – one American variety (See section 2.3.3. Dhuique-Mayer *et al.* (2005)). Solar = solar dried; sun=sun dried; C=cross flow dried; F=fresh root. Different numbers are different extractions and different letters are different lots.

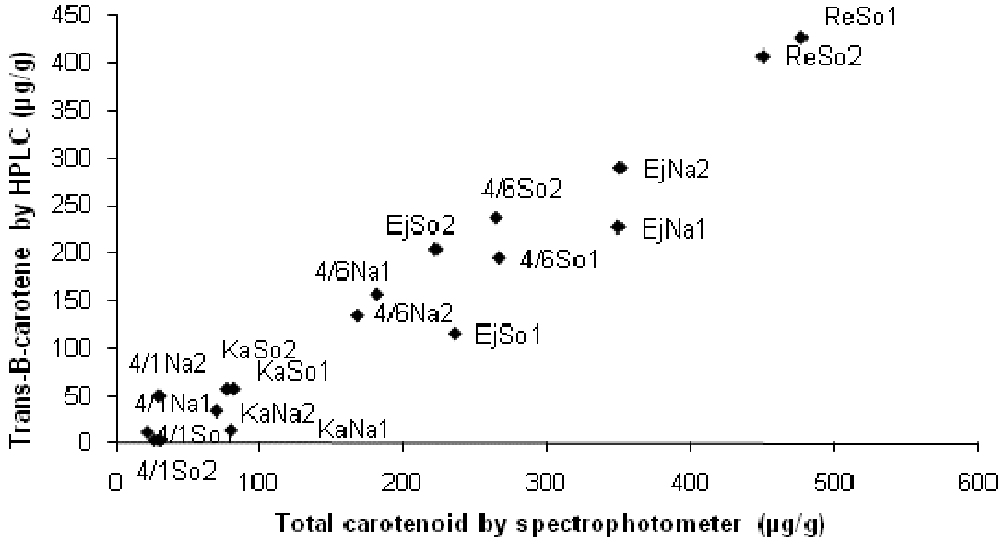


Figure 2-5: Relationship between total carotenoids (µg.g^{-1}) on a dry weight basis measured by spectrophotometric method and Waters 600E HPLC. Each point corresponds to a single extraction measured on both methods. Equation: y = 0.8127x where x=Spectrophotometer total carotenoid content and y=HPLC *trans-β*-carotene content. Pearson correlation R=0.973 p<0.01- 18 extractions – 5 Ugandan varieties from 2 locations (See section 2.3.4. Rodriguez-Amaya and Kimura (2004)). Varieties: Re=Resisto; Ej=Ejumula; Ka=Kakamega; 4/6=SPK004/6; 4/6/6=SPK004/6/6; 4/1=SPK004/1; 4/1/1=SPK004/1/1. Locations: Na= National Crops Resources Research Institute; So=Abuket Soroti District.

Significant correlations were found between total carotenoids measured on spectrophotometer and *trans-β*-carotene measured on HPLC: R=0.725 for a range between 177.3 and 300.8µg.g^{-1} db (Figure 2-4); and R=0.973 for a range between 11.7 and 427.1µg.g^{-1} db (Figure 2-5). Chavez *et al.* (2004) also found a very good correlation (R=0.957) between total carotenoids in spectrophotometer and total carotene in HPLC on yellow-fleshed cassava on 24 samples; measured in four replications on a range of 10-100µg.g^{-1} db. A wide range of carotenoid levels may increase the strength of the correlation as observed in the two Pearson coefficients; correlation on five varieties from two locations was stronger than on one variety.

Other factors can explain these differences such as inter and intra laboratory and method variation. Equations from both figures presented similar coefficients respectively 0.7719 and 0.8127 which suggested that a common trend may exist between spectrophotometer and HPLC measurements in OFSP.

Total carotenoid content by spectrophotometric reading gives a close estimation of the total carotenoid content of a sample determined by HPLC (Table 2-5; Figures 2-4; 2-5): HPLC and spectrophotometer instruments also gave the similar levels of total carotenoids (Independent T-test; $p<0.05$). Total carotenoid content by spectrophotometric reading therefore slightly over-estimates trans-β-carotene content; the percentage of β-carotene was between 86 to 88% of total carotenoids. Kimura *et al.* (2007) also observed that total carotenoid content and all trans-β-carotene content in orange-fleshed Resisto variety were found statistically equivalent because of the predominance of β-carotene but in yellow-salmon-fleshed IAC 60-M-3-Brasilia variety the total carotenoid content was found statistically different because other minor carotenoids represent about 37% of total carotenoids (Kimura *et al.* 2007). Spectrophotometric reading can be used to estimate trans-β-carotene but HPLC would be required for accurate beta-carotene measurement in Rubina® sweet potato. Spectrophotometric method therefore works only for those varieties where it is predominantly trans-β-carotene.

2.5.2 Estimation of total carotenoid content by colorimetric reading

Correlation between β-carotene content and CIELAB colorimetric reading in fresh or dried OFSP have been previously reported (Ameny and Wilson 1997; Hagenimana *et al.*

1999; Bengsston *et al.* 2008). An experiment to correlate colour to total carotenoids (by spectrophotometric reading) was undertaken in 28 samples of dried milled OFSP varieties (see Chapter 5). Significant correlations on Pearson coefficient (one tail; $p<0.01$) were found between L*, a* and b* respectively -0.962; 0.956 and 0.968 suggesting that the method was suitable for total carotenoid content.

2.6 EFFECT OF STORAGE ON ISOMERISATION AND DEGRADATION OF CAROTENOIDS

Unsaturated carotenoids are prone to isomerisation and oxidation and these can cause bias in the storage before the analysis and during the analysis of samples. The effect of storage in the freezer on carotenoid content and the effect of mild or hot temperature on cis-isomerisation were therefore tested.

2.6.1 Effect of freezer storage on total carotenoid content

Samples were stored in the freezer (-20ºC) in zipped-polythene bags where air was manually removed. The effect of storage in the freezer was tested after 21, 26 and 133 days on Covington variety flour (Table 2-6) and after 124, 183 and 217 days on chips from Ejumula variety (Table 2-7).

Table 2-6: Effect of storage in the freezer on carotenoid content on OFSP flour from Covington variety. See method (using HP8452A spectrophotometer) in section 2.3.4.

Storage (days)	Total carotenoid content ($\mu g.g^{-1}$)*	Loss
0	446.9 (8.6) §	-
21	460.4 (6.9) §	-3.0%
26	459.9 (0.7) §	-2.9%
133	465.9 (14.7) §	-4.2%

*On a dry weight basis. Each value corresponds to an average of two extractions§ or three extractions$^{\chi}$ {mean (standard deviation)}. One factor-ANOVA. Tukey Test. $p<0.05$.

Table 2-7: Effect of storage in the freezer on carotenoid content on OFSP chips from Ejumula variety. See method (using HP8452A spectrophotometer) in section 2.3.4.

Storage (days)	Total carotenoid content ($\mu g.g^{-1}$)*	Loss
0	294.5 (13.2) §	-
124	295.1 (2.1) $^{\chi}$	-0.2%
183	283.6 (17.1) $^{\chi}$	3.7%
217	277.7 (13.2) $^{\chi}$	5.7%

*On a dry weight basis. Each value corresponds to an average of two extractions§ or three extractions$^{\chi}$ {mean (standard deviation)}. One factor-ANOVA. Tukey Test. $p<0.05$.

There was no significant loss of total carotenoid content of OFSP flour up to 133 days (4 months) of storage ($p<0.05$; ANOVA). There was no significant difference in the total carotenoid content of sample chips after drying and up to 217 days (7 months) of storage in the freezer. In conclusion storage at -20ºC was proved to maintain the total carotenoid content of OFSP flours up to 133 days and chips up to 217 days.

2.6.2 Effect of elevated temperature storage on β-carotene cis-isomerisation

The effect of mild and high temperature on cis-isomerisation was tested on OFSP flour from Covington variety. Because no changes in the total carotenoid content occurred on samples stored in the freezer, higher temperatures were applied to increase the likelihood that cis-isomerisation could be observed. Hence the temperatures tested are far beyond the range of typical temperatures found during the storage of samples in ambient field conditions.

OFSP flour was heated at either 40 ºC or 100ºC. Triplicate 5g of well mixed OFSP flour were placed in a metal Petri dish (Ø90mm) such that a large surface area was exposed for the temperature treatment. The uncovered Petri dishes that contain the OFSP flour were placed in triplicate (3 x 5g) in an fan-assisted oven (Gallenkamp) at either 40 ºC or 100ºC. At a temperature of 40°C, samples were taken every 17h up to a maximum of 103h. At a temperature of 100°C, samples were collected every 3h up to a maximum of 21h. After the oven treatment, each triplicate set of flour samples were combined and mixed. The carotenoids were evaluated by HPLC (triplicate extraction). The main carotenoids obtained by HPLC were as follows (Table 2-8).

Table 2-8: Identification of carotenoid compounds by Waters 600E HPLC: retention time and main absorption wavelengths

Compound*	5,6 epoxy-β-carotene	5,8 epoxy-β-carotene	13-cis β-carotene	Trans β-carotene	9-cis β-carotene
Retention time (min)	14.4	16.7	18.7	27.8	31.7
Main wavelengths**	447-476	428-457	338-447	452-476	447-476

*Trans β-carotene was identified by co-injection. Other carotenoids were possibly identified from the literature and comparison with results on OFSP samples analysed on Agilent 1100 HPLC

**Carotenoids have three main wavelengths but the first wavelength was not clear. Therefore only two main wavelengths were reported.

The different carotenoid contents at differing temperature and storage time are illustrated in Figure 2-6.

The trend of carotenoid degradation at 100°C and 40°C differed. At 40°C variations in the concentration of carotenoids over a period of 103h were relatively small. Trans-β-carotene and 5,6 epoxy β-carotene slightly decreased. 5,8 epoxy β-carotene 13-cis β-carotene remained steady and 9-cis slightly increased. On the other hand, at 100°C, over a shorter treatment period (21h) trans-β-carotene and 5,6 epoxy β-carotene sharply decreased while 5,8 epoxy β-carotene increased. 13-cis β-carotene sharply increased in the first 3h and successively decreased while 9-cis steadily increased throughout the treatment. Overall there was an increase of isomers (9-cis and 13-cis) and 5,8-epoxide (being an intermediate compound) at 100°C. Hence heating at such a level clearly affects both the trans-β-carotene and its isomers. At 40°C changes in carotenoid levels were small after one week storage (103h).

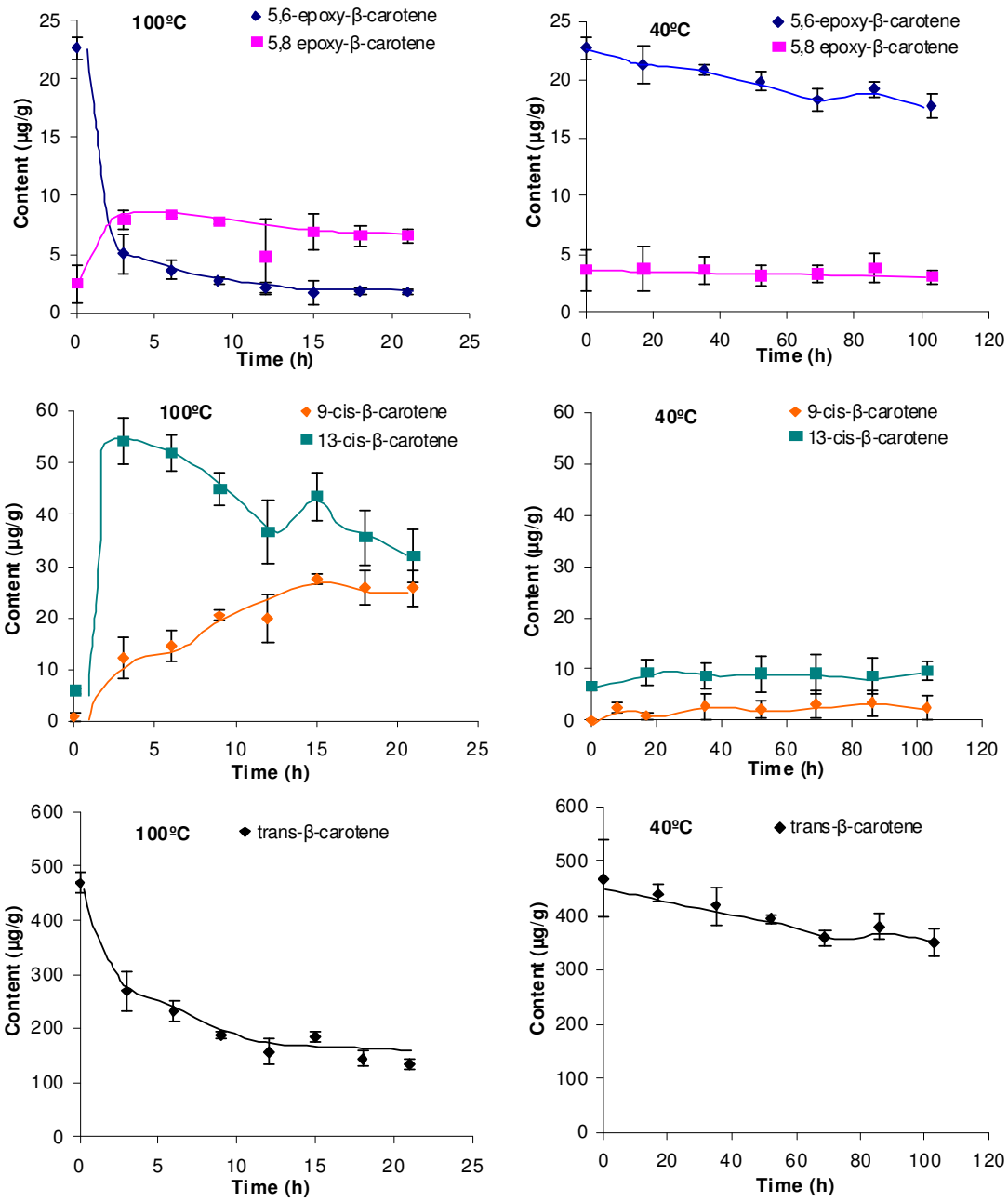


Figure 2-6: Influence of mild (40ºC) or relatively high (100ºC) oven temperature on trans-β-carotene; 9-cis and 13-cis; 5,6 and 5,8 epoxy-β-carotenes. Contents are on a dry weight basis. Mean of $2^2 = 4$ (duplicate extraction on duplication treatment). Error bars refer to standard deviation. Carotenoid extractions were carried out by Marijke School, NRI Msc student for her research project. HPLC analyses using Water 600 E system and interpretation of the results were performed by Aurelie Bechoff (see method in section 2.3.4.).

Doering *et al.* (1995) reported that isomerisation preferentially occur at temperatures higher than 35ºC. Therefore there is little susceptibility for carotenoids to isomer when

Doering *et al.* (1995) reported that isomerisation preferentially occur at temperatures higher than 35ºC. Therefore there is little susceptibility for carotenoids to isomer when stored at lower temperatures than 35ºC. It should be noted that the ambient temperature in Uganda and Mozambique rarely reach the threshold temperature of 35ºC.

2.7 PREPARATION FOR FIELD WORK

2.7.1 Influence of choice of roots or fresh chips as initial point to determine carotenoid losses

Sweet potato roots and freshly chipped sweet potatoes were compared in 11 samples for their total carotenoid levels (extraction in triplicate): 7 varieties from the harvesting lot 1; 2 varieties from lot 2 and 2 varieties from the lot 3. (Figure 2-7).

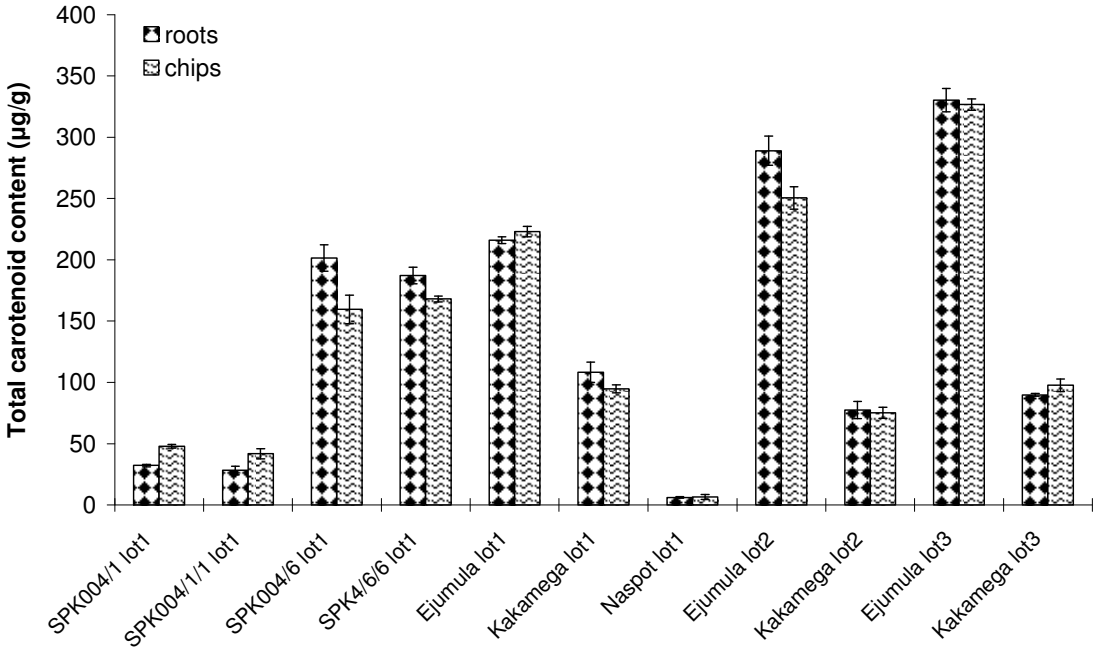


Figure 2-7: Total carotenoid content (µg.g^{-1}) on a dry weight basis in roots and fresh chips from various samples. Variety and date of drying date are for information only. Mean total carotenoid value (standard deviation): roots: 142.4(106.0) µg.g^{-1}; chips: 135.6(96.4) µg.g^{-1}. T-test for equality of means. Sig. (2-tailed) Significant at $p<0.05$. See method in section 2.3.4. (Genesis spectrophotometer).

For the comparison of carotenoid content on roots and freshly cut chips, the number of roots randomly chosen was five and the quantity of fresh chips was 500g (*ca.*1500 chips). There was no significant difference between roots and non-dried fresh chips (One

way-ANOVA; $p<0.05$). In spite of lack of global difference, the trend between roots and freshly non-dried chips total carotenoids level was not always the same as shown in Figure 2-7. This is due to heterogeneity in carotenoid content inside the vegetable material. When analysing each sample separately (one way-ANOVA $p<0.05$), five samples out of eleven presented significant differences between roots and fresh chips. Carotenoid content was significantly higher in fresh chips compared to roots for varieties SPK004/1 and 4/1/1 and significantly lower for varieties (SPK004/6, 4/6/6 and Ejumula from the lot 2. Because the number of particles was five pieces (roots) against 1500 pieces (chips), the reason for the difference may be a non-representative choice of the roots instead of the chips. In addition fresh chips should therefore be preferred because the carotenoid loss would refer to drying exclusively.

2.7.2 Influence of density of OFSP fresh chips on carotenoid losses

Total carotenoid content on dried chips related to three densities on the dryer trays measured before drying (2; 4; 6 kg m^{-2}) is reported in Table 2-9.

Table 2-9: Influence of the density of OFSP chips (Ejumula and Kakamega varieties) on total carotenoid levels of those dried using in open-air sun dryer. See method (Genesis spectrophotometer) in section 2.3.4.

Variety	Fresh chips density ($kg.m^{-2}$)	Dry matter content of dried chips (%)	Total carotenoid content of dried chips ($ug.g^{-1}$)*	Drying time (h)
Ejumula	2	91.3%	182.9(6.6)	5.2
	4	92.5%	194.1(2.7)	6.3
	6	89.2%	155.2(8.1)	9.4
Kakamega	2	92.0%	62.2(2.0)	5.2
	4	92.6%	68.9(0.2)	6.3
	6	88.8%	57.9(3.1)	8.8

*On a dry weight basis. Each value corresponds to an average of three extractions. Values in brackets refer to the standard deviation.

With both varieties, it can be observed that highest carotenoid content was obtained with 4 $kg.m^{-2}$. Drying time increased with chips increasing density. Samples with a density of 6 $kg.m^{-2}$ presented higher moisture content in dried chips (89% dry matter in average compared to 92% for other densities) and longer drying (which can cause higher carotenoid loss). On the other hand a density of 2 $kg.m^{-2}$ limited dryers' capacity.

Therefore an average density of 4 $kg.m^{-2}$ was the optimal density for carotenoid retention when drying.

2.7.3 Influence of freeze-drying

Freeze drying was tested with the purpose to replace fresh roots by freeze dried sweet potato to facilitate air freight transport for analysis in UK or France (Table 2-10).

Table 2-10: Losses of trans- β-carotene after freeze-drying. See HPLC method in section 2.3.3.

Sample	Size reduction	Treatment	Dry matter content (%)	Trans-β-carotene content ($\mu g.g^{-1}$)*	Trans-β-carotene loss (%)
Trial 1: Rubina®	5 mm thick slices	Fresh	18	250.4(8.8)	-
		Freeze-dried	96.1	182.7(12.9)	27.0
Trial 2: Beauregard	pureed	Fresh	15.9	380.2(36.1)	-
		Freeze-dried	90.7	363.9(20.0)	4.3

*On a dry weight basis. Each value corresponds to an average of two extractions. Values in brackets refer to the standard deviation.

Five OSFP roots were peeled, roughly sliced (about 5mm) and freeze-dried (SMH15 Model USIFROID, Maurepas, France). Trans-β-carotene content was measured on fresh and freeze dried samples (Table 2-10).

In the trial 1, freeze-drying took 5 days due to bulkiness of the slices. A significant loss in trans-β-carotene (27.0%) during freeze-drying was found. This result is in agreement with 25% losses observed by Chavez *et al.* (2004) in freeze-drying of yellow cassava that contains β-carotene. The size of the chips was not mentioned however. In the trial 2, freeze-drying took two days. There was a minimal loss of trans-β-carotene (4.3%).

Isomers of β-carotene were present in fresh and freeze-dried samples in similar amounts and no difference was observed between chromatograms of fresh and freeze-dried samples (data not shown). Significant loss observed in the first trial may be due to long time of freeze-drying. Parameters that influence the retention of carotenoids in freeze-drying are the drying time, which related to the particle size. The second trial demonstrated that it was possible to preserve carotenoids with the use of freeze dried

puree. Several authors applied freeze-drying for carotenoid analysis of vegetables (Hedrén *et al.* 2002) and OFSP (Bengsston *et al.* 2008). In accordance with our results, Desorby *et al.* (1998) affirmed that freeze drying of pureed samples was one of the most efficient ways to preserve provitamin A. These results confirmed that freeze-drying could be favourably adopted for the air-freight transport of sweet potato roots when the equipment is available.

2.7.4 Influence of screening plastic coverage on sweet potato flour exposed to artificial sun light

Carotenoids have been found to be mostly photosensitive to wavelengths lower than 470 nm (Sattar *et al.* 1977; Lennersten and Linghert 2000). Plastic coverage used in solar-drying experiment could be wavelength-selective and could therefore bring protection toward destructive sun rays in UV frequency in particular. Transmittance between 200 and 800 nm of different plastic sheeting was measured by UV- visible spectrophotometry. The characteristics of different plastic sheeting are presented in Table 2-11.

Table 2-11: Characteristics of polythene (PE) sheeting

Name (UK Company)	Material (Thickness μm)
Clear super-strength (XLs)	mLLPDE*, PE (150)
Smart Blue (XLs)	mLLPDE, EVA*, PE (150)
Sterilite (XLs)	mLLPDE, EVA, PE (180)
Lumitherm (BPI-Visqueen)	PE (200)
Green (Allpass)	PE (125)
Red (Allpass)	PE (125)
Dark Blue (Allpass)	PE (125)

*mLLPDE: Metallocene Linear Low Density Polyethylene; PE: Polyethylene; EVA:Ethylene Vinyl Acetate

The spectrum of the polythene sheeting was measured between 200-800 nm using a rectangular section of 1 cm thick plastic placed in the centre of the spectrophotometric cuvette so that beam light could perpendicularly cross the plastic sheeting (Krizek *et al.* 2005). Red sheeting that was analysed twice exhibited the same spectrum. Polythene spectra are given in Figure 2-8.

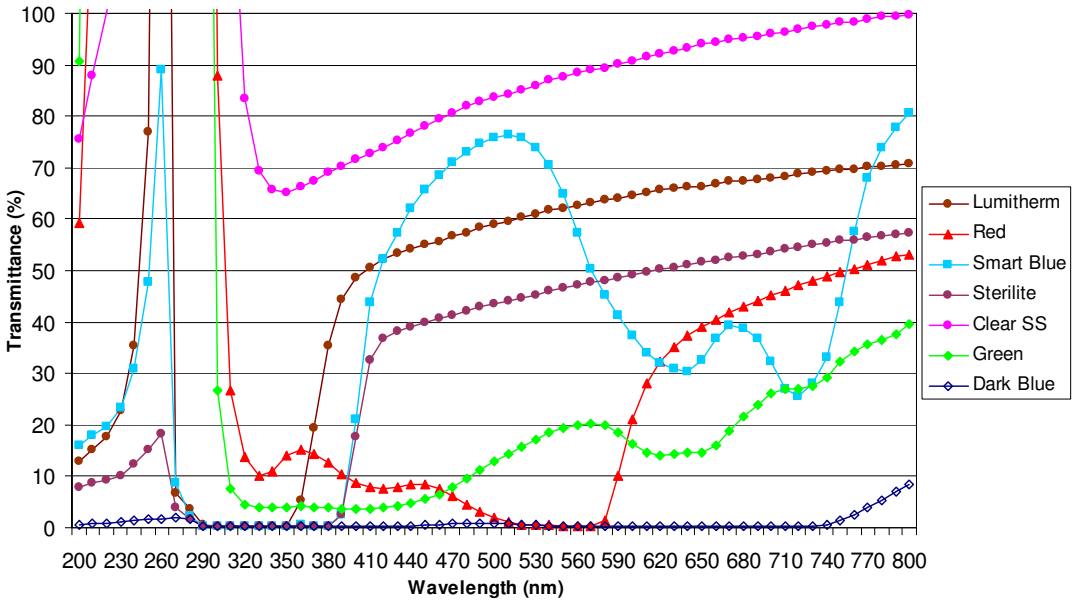


Figure 2-8: Transmittance of various polythene sheeting between 200-800 nm

UV resistant polythenes were Lumitherm, Smart Blue, Sterilite. Red and Green simple polythene stopped also wavelengths lower than 500 nm in visible. Dark Blue did not transmit most of light. On the other hand Clear Super Strength transmitted at all wavelength.

The same plastics were tested using sweet potato flour under artificial sun light. Rubina® OFSP flour (1.5 g) in Petri box was exposed to artificial sun light spectrum radiation (Sun Test CPS Model) for 30 minutes (level energy Sun test: 5 cal.(20.9 J); total irradiance 76.2 W/m^2) with or without polythene coverage. Colour reading on flour was recorded with CR200 chromameter after samples have cooled down. Colour loss was determined by comparison with flour before exposure. L* and b* values did not exhibited a very clear profile. a* values are shown in Figure 2-9.

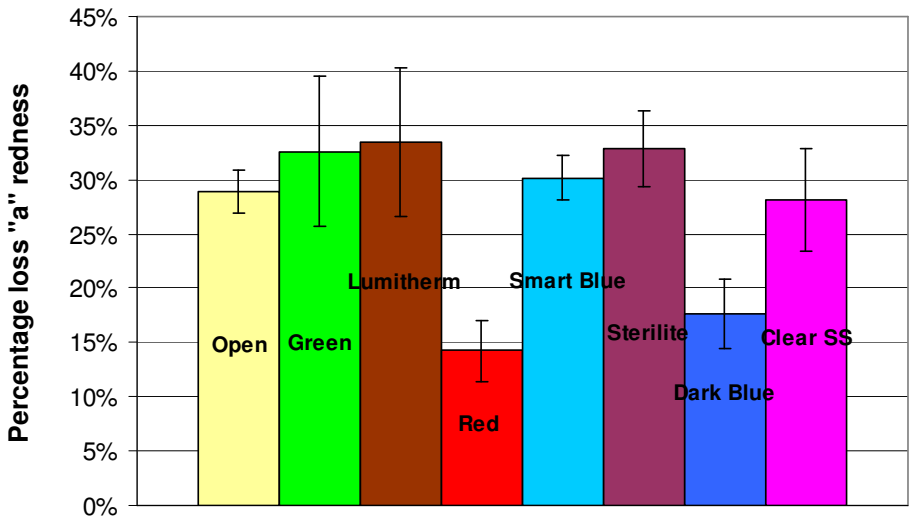


Figure 2-9: Loss of red colour of OFSP flour sample under different polythene coverage. Sun-test exposure was carried out in duplicate.

There were significant differences between non-exposed (control) and exposed flours (one way-ANOVA; $p<0.05$). The mean loss of red colour or "redness" was 27.2%. This hypothetically suggests a significant carotenoid loss. There were significant differences in a* value between exposed flours; red and dark blue had significantly better retention than other plastic coverage (one way-ANOVA; $p<0.05$) respectively 14.2 and 17.7%. The loss of a* value in flour directly exposed (open 28.8%) was similar to loss under Green, Lumitherm, Smart Blue, Sterilite UV resistant and Clear Super Strength polythene. It seems that the type of plastic UV or non-UV resistant would not have influence on the colour under theses conditions. It is risky to conclude too early on carotenoid loss. Notwithstanding a clear conclusion is that the amount of transmitted light influenced the colour of sweet potato flour. More investigation is required to understand loss of carotenoids under polythene sheeting in the field.

2.8 CONCLUSIONS

- The extraction for carotenoid analysis on dried sample was optimized and the repeatability of the analysis assessed. The method of carotenoid extraction used for fresh samples was not effective. For an optimal extraction of carotenoids from dried sweet potato sample, a re-hydration stage (20 minutes at ambient temperature) was necessary. Carotenoid analysis was reproducible on fresh (five roots) and dried sweet

potato (100g of flour) (ANOVA; $p<0.05$). Furthermore, there was no quantitative difference between the two different methods of carotenoid analysis (Rodriguez-Amaya and Kimura 2004; Dhuique-Mayer *et al.* 2005).

- Carotenoid content from OFSP flour or chips could be preserved by storage in the freezer (-20ºC) for several months. The temperature necessary to cause significant cis-somerisation of samples had to be very hot (100ºC in an oven) which suggest that the risk of isomerisation is limited during the analysis of carotenoids and during storage in the freezer or at ambient temperature below 40ºC.
- Spectrophotometric and HPLC methods were highly correlated working with one variety with different drying treatments ($R = 0.725$) and working with a range of varieties from light-yellow to deep-orange fleshed coloured ($R = 0.973$). Moreover total carotenoid contents measured by spectrophotometer or HPLC were similar. Hence the use of spectrophotometer equipment would be suitable to estimate the carotenoid content in conditions where only basic laboratory equipment is available or when rapid methods are necessary.
- The conditions of drying during field work were also tested. The use of freshly chipped sweet potato was suggested instead of the use of five roots earlier recommended by Rodriguez-Amaya and Kimura (2004) because of the more robust and representative sampling with chips. The optimal loading density of sweet potato chips on dryers was 4 $kg.m^{-2}$.
- When choosing the best screening for sun-light in order to preserve carotenoids, the red sheeting was found to retain significantly higher carotenoid levels of sweet potato flours compared to other polythene sheeting.

CHAPTER 3.
EFFECT OF HOT AIR, SOLAR AND SUN-DRYING TREATMENTS ON CAROTENOID RETENTION IN ORANGE-FLESHED SWEET POTATO

3.1 INTRODUCTION

Sun-drying is the most affordable means of drying and is widely practiced in many developing countries. Kósambo (2004) reported trans-β-carotene losses in open air sun-drying and cabinet drying of 83% and 28% respectively working with Kakamega (SPK004) sweet potato variety and of 72% and 47% with Jonathan variety. Lower retention in open air sun-drying was explained by the destructive effect of sunlight and the non-controlled environmental conditions (Kósambo 2004). Since few studies have been reported on open air sun-drying, there was a need to determine β-carotene retention in dried sweet potato on this type of drying and compare β-carotene retention in sun-drying with more advanced drying techniques.

The main objectives of this research work reported in this chapter were to compare the carotenoid losses from OFSP dried in different ways (sun-drying (direct sun-light), solar-drying (greenhouse solar dryer) and hot air drying (using cross flow air dryer)) and to evaluate the vitamin A activity of the flours (from milled chips) after drying. This research work, carried out in France, was a preparative study on the effect of drying on carotenoid loss in preparation for the field studies in Uganda and Mozambique.

3.2 MATERIALS AND METHODS

3.2.1 Raw material

Sweet potato roots having red skin and deep orange flesh imported from the United States of America were purchased locally in Montpellier, France (Rubina® Agrexco Carmel Rungis, France). No information was available on the variety, exact location of

production, harvest batch or transport, but roots were all purchased in a single batch and stored in a conditioning room (14ºC) during the analysis time (1 month).

3.2.2 Sample preparation for drying

Roots were peeled and chipped/sliced using electrical equipment: CL 50 Robocoupe (Vincennes, France) for crimped slices and A200 Hobart (Marne la Vallee, France) for chips. Precautions were taken to protect samples from the light, such as by the use of foil and low light conditions during handling.

3.2.3 Drying of chips

Crimped slices and chips were dried in three dryers described below. Drying times were estimated by weighing the product at regular intervals to an estimated moisture content of 10-11%.

3.2.4 Cross flow dryer

The cross flow dryer made of wood, called SCec-T®, was developed by CIRAD for the drying of granular products such as couscous in West Africa (Méot *et al.* 2007) (Figure 3-1). The air heating system consisted of a butane gas jet and a centrifuge fan (Gomez Eslava 2005). Experiments were carried out indoors. Two temperature probes were positioned between trays and one temperature/humidity probe was placed in the outlet (Gomez Eslava 2005). Warm air entered through a pipe (ϕ200cm) underneath the drying trays with an air temperature between 24 and 45°C (average 42°C). Low drying temperature (mean temperature 42ºC) cross flow drying was used for a comparison to be made with solar-drying (mean temperature 38ºC).

Temperature, humidity, and air velocity through the sample are presented in Figure 3-2. The inlet pipe had three holes (ϕ100 mm) that let air rise and cross flow through the food product placed on three overlaid trays (0.94 m x 0.6 m= 0.564 m² for each tray). The air velocity through the product was 0.28 $m.s^{-1}$. The external ambient temperature ranged between 24 and 30°C and relative humidity between 33 and 59% (Figure 3-2). A 3-mm layer of chips or crimped slices was placed on the trays with an initial loading density of 8 $kg.m^{-2}$ for chips and 15 $kg.m^{-2}$ for crimped slices.

Figure 3-1: SCec-T® cross flow dryer. Views from the front (left) and the back (right).

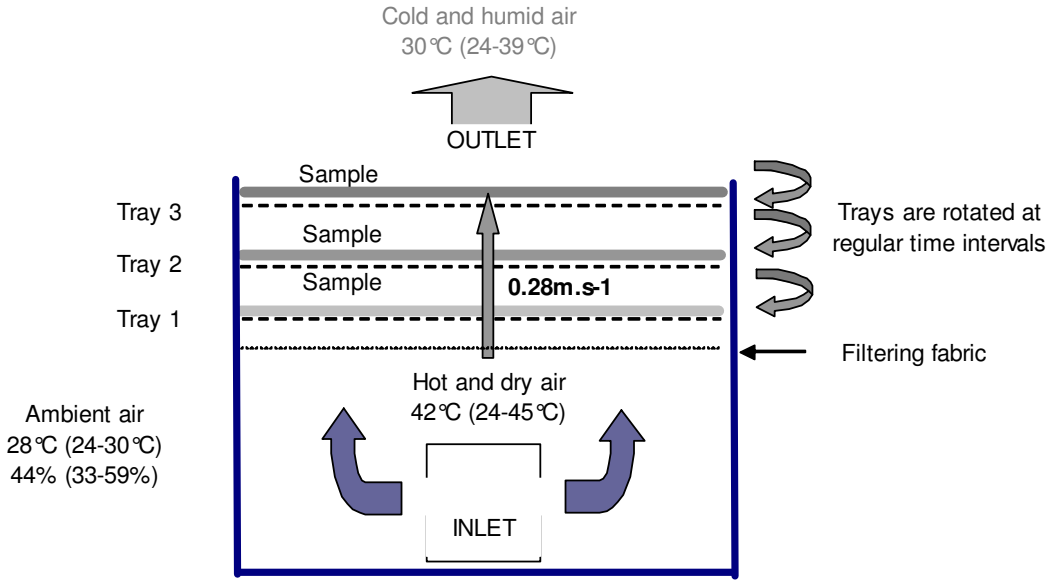


Figure 3-2: SCec-T® cross flow dryer (front view)**.** Temperature/ humidity: mean (min-max).

3.2.5 Greenhouse solar dryer and open air-sun-drying

Solar-drying is achieved by direct sun radiation and the greenhouse effect. A polythene film covered the solar dryer similar to a greenhouse (Gomez Eslava 2005) of 6 m long x 2.5 m wide. A fan was used to force air into the dryer. Five wire mesh trays (2 x 0.94

m) placed 30 cm above the ground, were loaded with a 2 mm layer of crimped sliced or chipped sweet potato placed on terylene tissue (Figure 3-3). Two temperature probes and one temperature/humidity probe were placed between the trays to measure temperature and outlet air humidity. The temperature/humidity within the solar dryer ranged from 27 to 50°C /14 to 52% compared to the external ambient range of 24 to 36°C/24 to 52% (Figure 3-4).

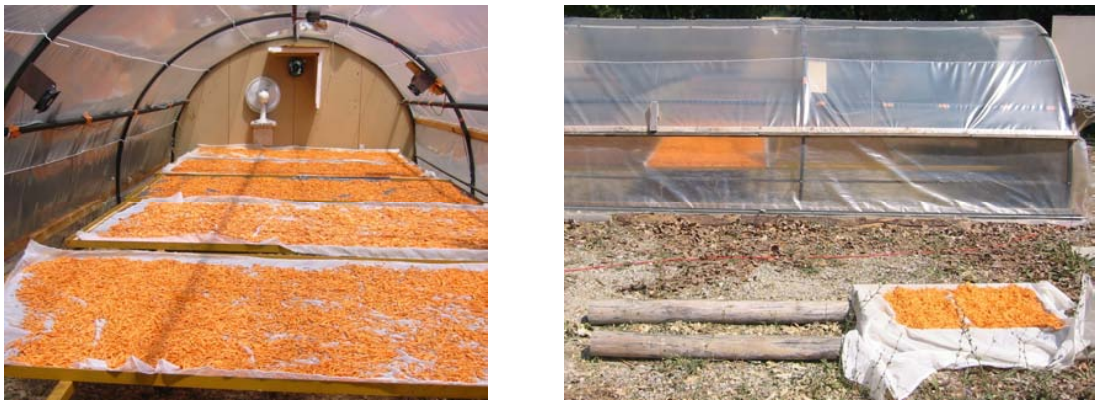


Figure 3-3: Greenhouse solar dryer. Views from the front (left) and the side that includes sun dryer (right).

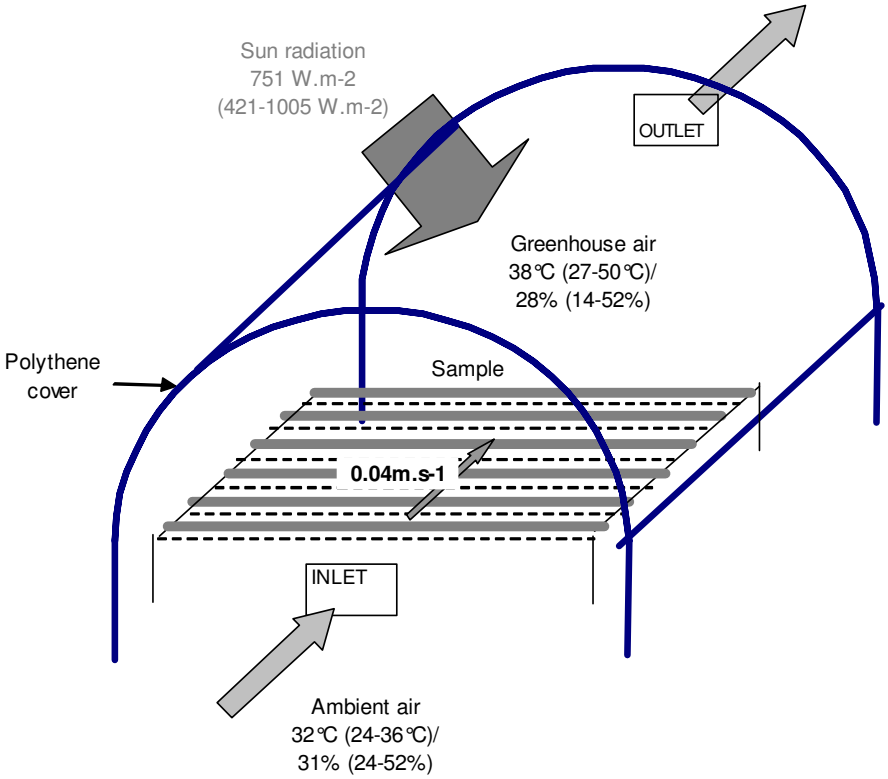


Figure 3-4: SCec-Serre® greenhouse solar dryer (front view). Temperature/ humidity: mean (min-max).

Air velocity was 0.04m.s^{-1}. Solar irradiance (Pyranometer Cimel CE 180 (Paris, France)) ranged between 421 and 1005 W.m^{-2} (9 am to 2 pm) depending on the course of the sun with an average of 751 W.m^{-2}. Temperature and humidity as well as air velocity through sample are presented in Figure 3-4. Tray loading densities were 3.5 kg.m^{-2} for both chips and crimped slices.

Open air sun-drying was carried out concurrently with solar-drying and using the same tray loading density (Table 1). Wire mesh trays (0.43x 0.45 m) were placed in the sun on a stand 10 cm above ground level.

3.2.6 Dimensions of chip and slice samples

Three photographs of samples (chips and slices together) in open air drying at the start of drying; after two hours and at the end of drying were analysed using Image J 1.40g Software (National Institute of Health, USA) (Figure 3-5).

Figure 3-5*:* Crimp and grated chips before/after open-sun-drying

Using the width of the drying tray as known measurement, pixels values were converted into distance units (cm) (11 pixels=1 cm in the three photographs). On each picture thirty chips and slices were selected individually and their visible surface area calculated using ROI (Region of Interest) manager macro in Image J software. Area measured using the Image J software was in agreement with earlier estimation by calliper measurement (0.01 mm precision) done on ten chips/slices at initial time.

3.2.7 Moisture and water activity determination

Dry matter contents were determined by drying triplicate 5 g samples at 105ºC to constant mass (AOAC 1984). Water activity (a_w) was determined in duplicate on finely

blended flour samples using an Aqualab (Decagon, Pullman, WA, USA) controlled with a sodium chloride standard solution (a_w=0.75).

3.2.8 Sample preparation for carotenoid analysis

Fresh samples were prepared according to Rodriguez Amaya and Kimura (2004). Five raw roots were randomly selected, peeled, quartered. Two opposite sections were combined and blended to a fine pulp using a Thermomix multi-purpose household food processor (Vorwerk, Germany). All operations were carried out under dim light. The samples were thoroughly mixed and packed into 100 ml closed plastic boxes wrapped in black plastic and stored at -20ºC before analysis (1 month maximum).

After drying, chips or slices were collected tray by tray and milled into coarse flour using a Thermomix food processor (Vorweck, Germany). Flour was packed into sealed plastic bags under vacuum and stored at -20ºC. Samples were further milled into a fine flour (< 250 µm) on the Laboratory Mill 3100 (Perten Instruments, Roissy, France) before analysis.

3.2.9 Carotenoid analysis

Carotenoid extraction was carried out according to Dhuique-Mayer *et al.* (2005) which was based on Taungbodhitham *et al.* (1998). A sub-sample from the homogeneous representative sample (2 g for fresh and 1 g for dried samples) was extracted. Sub-samples were extracted in triplicate on the same day. Extraction was conducted under low light conditions to limit carotenoid losses.

Carotenoids were analysed by reverse-phase high-performance liquid chromatography using an Agilent 1100 system with photodiode array detection (Massy, France) according to the previously published method of Dhuique-Mayer *et al.* (2005) (Chapter 2. section 2.3.3). Samples from the same extract were analysed on a spectrophotometer UVIKON 933 UV/Visible double beam to measure absorbance at 450 nm. Samples were diluted in Petroleum Ether; 100 µl/10ml for fresh samples and 50 µl/10ml for dried samples. Concentration was calculated by Lambert Beer law from the absorbance (Britton *et al.* 1995).

3.2.10 Statistical analyses

Normality of distribution of sample visible surface area was verified by Kolmogorov-Smirnov test used for small sample size (n=30). Analysis of variance (ANOVA one way - homogeneity of variance test) was carried out to determine whether there were significant differences between means; a significant difference between means was determined by a Tukey test. An independent sample T Test was carried out to determine significant differences between provitamin A compounds before and after drying. All data were analysed using SPSS 14.00 for Windows (Woking, Surrey, UK).

3.3 RESULTS AND DISCUSSION

3.3.1 Quality of flour

Flour from dried sweet potato was evaluated for its moisture content and water activity in order to assess its quality for storage. Tray loading and drying time for each treatment and the moisture contents and water activities (a_w) of flours are shown in Table 3-1. The tray loading rates for the crimp slices were higher than with the chips in the hot air cross flow dryer as a result of operator error.

Table 3-1: Tray loading, drying time, moisture content and water activity of flours made from dried chips and crimped slices

Dryer	Slice type	Tray loading (kg/m^2)	Drying time (h)	Moisture content (%)*	Water activity (a_w)**
Hot air cross flow	Chips	8	2.0	11.0 (0.2)	0.442 (0.001)
	Slices	15	7.5	9.8 (0.1)	0.378 (0.008)
Solar	Chips	3.5	8.5	10.0 (0.4)	0.413 (0.000)
	Slices	3.5	8.5	9.9 (0.3)	0.397 (0.008)
Sun	Chips	3.5	8.0	9.9 (0.3)	0.443 (0.001)
	Slices	3.5	8.0	11.2 (0.1)	0.449 (0.001)

*Mean of three replicates. **Mean of two replicates. Values in brackets refer to the standard deviation.

The flour moisture content was between 9.8 and 11.2%. Flour water activity that ranged between 0.38 and 0.45 should favour carotenoid stability. It was demonstrated on dehydrated carrots in different conditions that best stability of carotenoids was obtained

with water activity of 0.43 (Arya *et al.* 1979) and between 0.31-0.54 (Lavelli *et al.* 2007). Moreover water activity below 0.7 also limits the risk of microbial deterioration and the lowest lipid oxidation is found between 0.2-0.4 (Rahman and Labuza 1999). The water activities of the dried sweet potato flours were therefore considered suitable for storage.

3.3.2 Influence of drying treatment on provitamin retention

Carotenoid losses influenced by drying treatment are reported in the Table 3-2 for chipped sweet potatoes.

Table 3-2: Influence of drying treatment on losses of total carotenoid content and trans-β-carotene content in chips

Dryer	Loss in total carotenoids (%)	Loss in trans-β carotene (%)
Hot air	13a	16a
Solar	21ab	23ab
Sun	33b	34b

Values in the same column followed with different letters are significantly different; ANOVA Tukey ($p \leq 0.05$).

Losses with the different drying techniques ranged from 13 to 33% in total carotenoids content and 16 to 34% in trans-β-carotene content. Losses were low for all treatments including sun-drying. Levels of loss in sun-drying were in contrast to the high levels of loss reported (72 to 83%) previously by Kósambo (2004).

Drying by hot air gave significant higher retention than sun-drying (respectively 13% compared to 33% in total carotenoids content and 16% compared to 34% in trans-β-carotene content) in chips. There was no significant difference between drying by hot air and solar-drying.

Negi and Roy (2000) also reported that solar-drying was equivalent to cabinet drying at 65°C in terms of carotenoid retention in various leafy vegetables (savoy beet, amaranth and fenugreek). However in other studies retention in solar-drying was significantly less in comparison with artificial drying: the same authors found in another study that solar-drying was found to induce more β-carotene losses than cabinet drying at 65°C in savoy beet and amaranth leaves (Negi and Roy 2001). Solar-drying results can be variable

because it depends on the prevailing environmental conditions. In this study, temperature in the solar dryer was similar to the hot air dryer (42ºC). However the hot air cross flow dryer had a better drying performance; higher tray loading and quicker drying (8 $kg.m^{-2}$; 2 h) compared to solar dryer (3.5 $kg.m^{-2}$; 8 h) (Table 3-1). No significant difference between solar and sun-drying was observed for samples dried under the same conditions (3.5 $kg.m^{-2}$; 8 h). Similar results were reported by Mulokozi and Svanberg (2003) working with leafy vegetables, where on the whole solar-drying retained more provitamin A carotenoids than open-sun-drying. However when analysing individual results, it appeared there were no significant differences between solar and sun-drying on five out of seven leafy vegetables.

The low levels of losses obtained in this study with sun-drying as opposed to earlier reports (Kosambo 2004) may be partially explained by environmental factors: the weather was hot and dry during the study with an ambient average of 29°C/39%, which allowed quick drying (8h).; the weather was also windy during the experiment which allowed rapid sun-drying. Traditionally in sub-Saharan Africa, sweet potato pieces are sun-dried for 2-3 days. Chavez *et al.* (2007) reported 62.1% losses in sun dried cassava dried for 2-3 days up to a moisture content of 12%. However a recent study by Bengtsson *et al.* (2008) using Ugandan sweet potato varieties confirmed the results of this study. Losses of trans-β-carotene in oven; solar and open sun-drying on OFSP chips were respectively 12%; 9% and 16% in Ejumula variety from Uganda dried up to 10% moisture. Drying times and temperature were 10 h at 57ºC in oven drying; and between 6-10 h in sun (30-52ºC) and solar-drying (45-63ºC). Bengtsson *et al.* (2008) indicated that there were no significant differences of retention between oven; solar and sun-drying, contrary to previous publications. Bengtsson *et al.* (2008) likewise commented that a quick drying may result in higher retention.

3.3.3 Influence of either chipping or slicing on carotenoid content

The influence of chip size on total carotenoids content under solar and sun-drying carried out under the same conditions (same time and loading density) was investigated The distribution of mean sample visible surface area over 30 chips or crimped slices during drying followed a normal distribution (Figure 3-6).

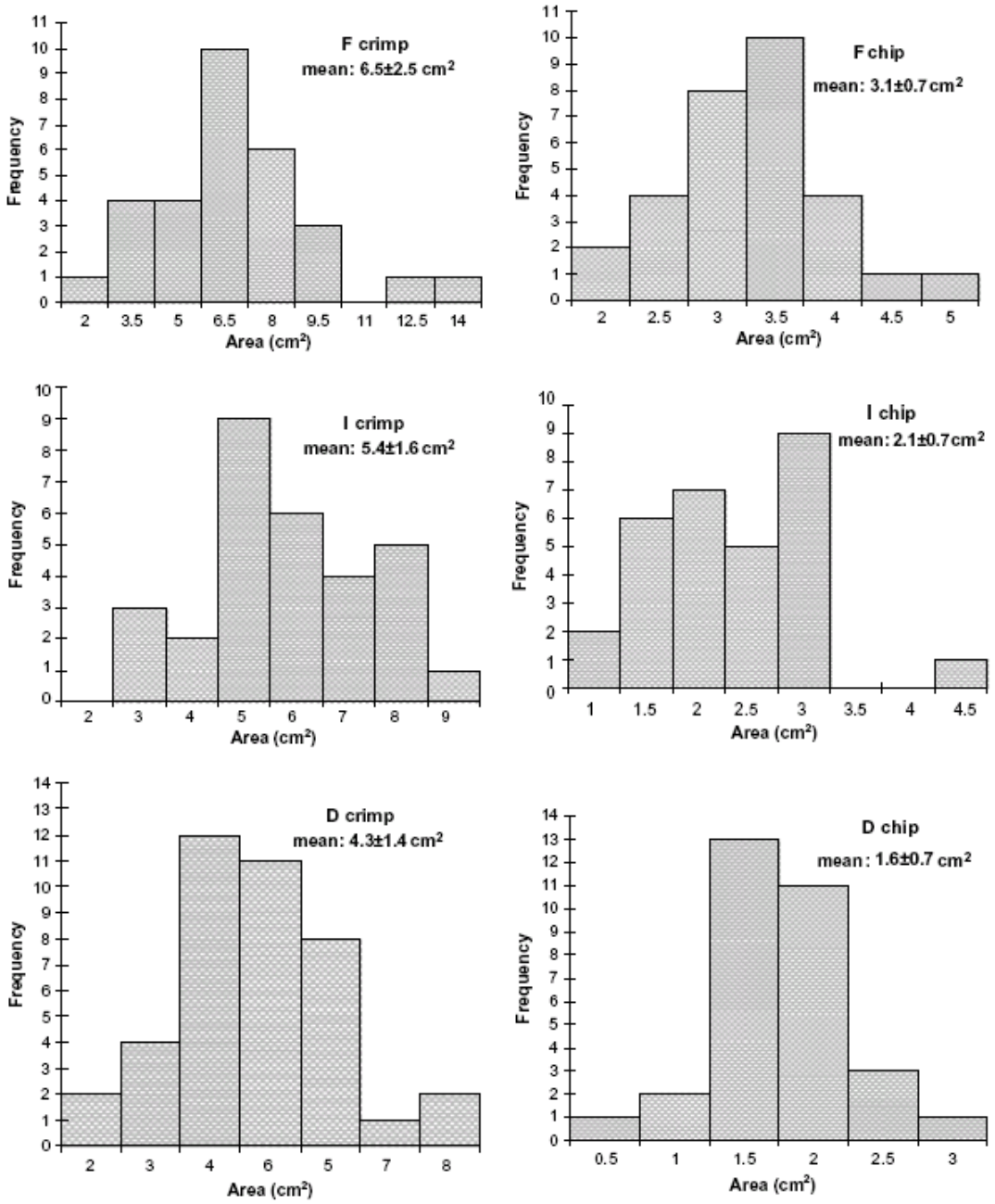


Figure 3-6: Distribution of grated chip and crimp slice visible surface areas during open air-sun-drying. F=fresh; I=after two hours of drying; D=dried. Each histogram represents the area of 30 samples. mean ± standard deviation.

"Shrinkage" of the visible surface area of the samples during drying was more marked for chips (51.2% of the initial area) compared to crimped slices (70.5% of the initial area) (Figure 3-7).

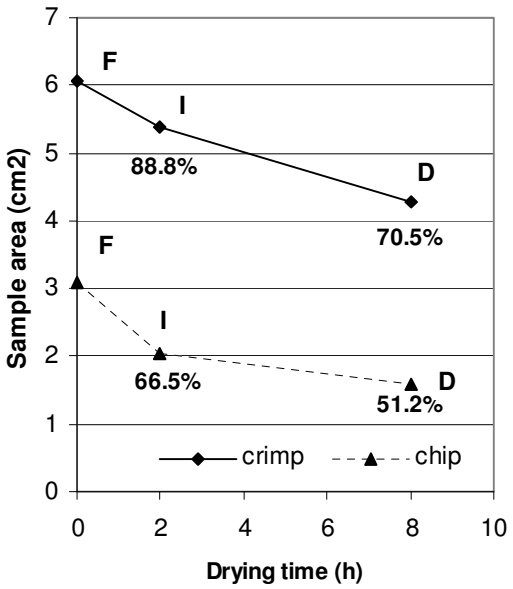


Figure 3-7: Reduction of sample visible surface area during open air-sun-drying. F=fresh; I=after two hours of drying; D=dried. Each value is a mean of 30 samples.

When drying chips, there was a significant difference between sun and solar-drying in terms of carotenoid content under the same conditions (Table 3-3).

Table 3-3: Influence of size reduction and drying treatment on total carotenoid content of dried sweet potato (µg.g^{-1}) on a dry weight basis (db)

	Solar	Sun
Chip	294(17)a	250(8)b
Crimped slice	307(20)a	319(18)a

Each value corresponds to an average of three extractions made on 100g flour from milled dried slices. Values in brackets refer to the standard deviation. Values followed with different letters are significantly different; ANOVA Tukey ($p \leq 0.05$).

The difference was, however, not significant in crimped slices. Although data are only available for sun-dried samples it would appear that chips which had the greatest carotenoid loss had the greatest degree of "shrinkage". It can be therefore hypothesised that there is relationship between degree of "shrinkage" and carotenoid degradation but this would need further investigation.

The role of direct sun radiation in carotenoid degradation in chip samples was not clear; there could be a relationship between cellular collapse caused by "shrinkage" and susceptibility of degradation of provitamin A by sun radiation.

3.3.4 Identification of provitamin A carotenoids

Several carotenoids were observed on the chromatogram of fresh sweet potato (16; 17; 24; 25; 30; 32; 33; 34; 37; 39 minute retention times) (Figure 3-8). Carotenoids were identified by diode array by their peak spectrum at three wavelengths.

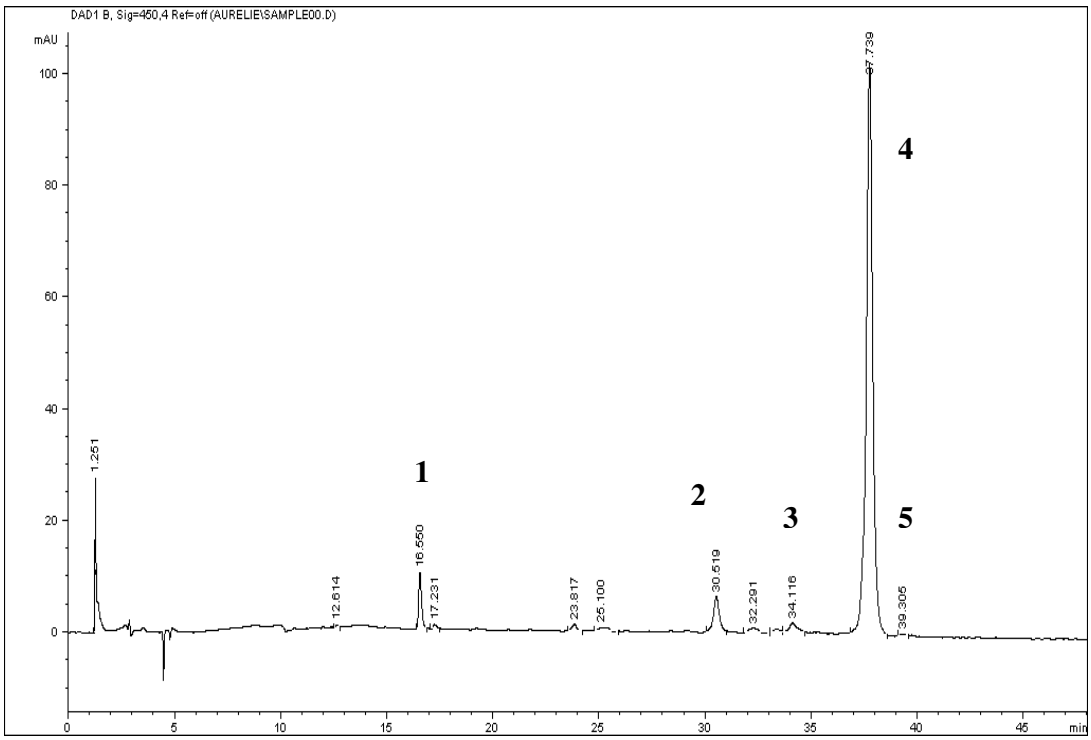


Figure 3-8: Reverse phase HPLC separation of carotenoids in raw sweet potatoes

1. non-identified polar carotenoid; 2. β-carotene 5,6 epoxide; 3. 13-cis- β-carotene, 4. all trans-β-carotene; 5. probably 9-cis - β-carotene

Trans-β-carotene peak appeared at 37 minutes (peak 4). The spectrum of maximum absorption wavelength was 428-452-478 nm in ethanol/hexane, slightly staggered by 2.5 mm compared to literature and % III/II= 13% was in accordance with literature (Rodriguez Amaya and Kimura 2004). (%III/II is an indicator of fine spectral structure calculated as ratio of longest-wavelength absorption peak III and that of the middle absorption peak II) (Figure 3-9).

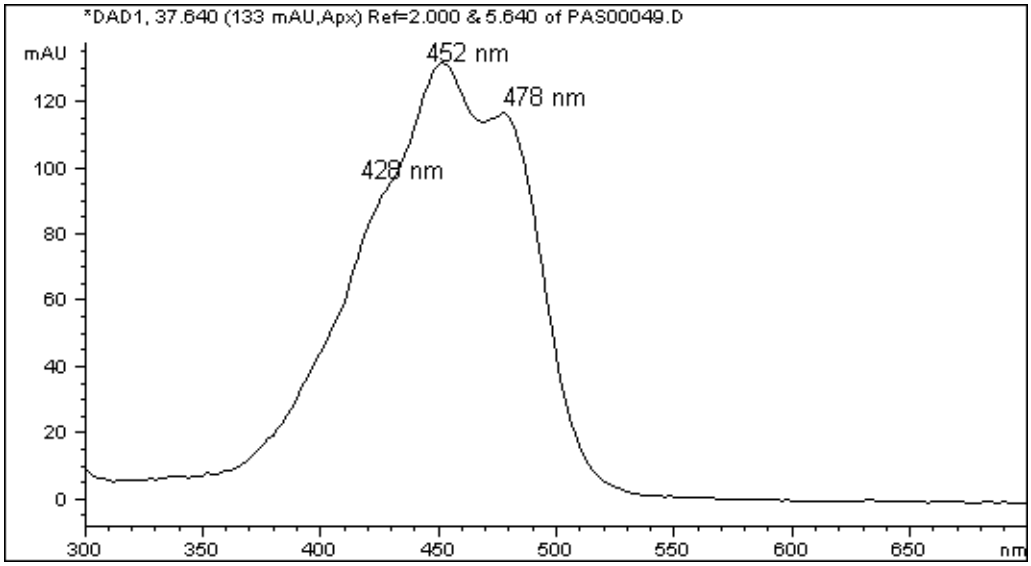


Figure 3-9: UV-Visible spectrum of trans-β-carotene

Apart from trans-β-carotene (peak 4), peaks 1 and 2 were clearly defined (retention time 16 and 30 minutes). Peak 1 did not appear constantly on all samples analysed; the peak 1 was definitely not a carotene: its retention time far from apolar β-carotene indicated a more polar molecular structure such as xanthophylls.

Peak 2 was firstly thought to be β-cryptoxanthin because the retention time was identical to the β-cryptoxanthin standard when co-injected (retention time 30 minutes). However the calculation of the %III/II of peak 2 (%III/II= 46%) was contradictory with β-cryptoxanthin's %III/II equal to 20%. On the other hand it was in agreement with β-carotene 5,6 epoxide's %III/II equal to 57% (Rodriguez and Rodriguez-Amaya 2007). It is to note that the molecular weights of β-cryptoxanthin and β-carotene 5,6 epoxide are the same (552g.mol^{-1}) which make the identification difficult. Furthermore it was found that β-carotene 5,6-epoxide was present in the fresh roots of Kakamega sweet potato variety (Kósambo *et al.* 1998); on the other hand β-cryptoxanthin was not mentioned as part of the carotenoids of sweet potato in literature.

The amounts of both compounds, peaks 1 and 2, were small (less than 10% total carotenoids).

Other compounds were less clearly defined; peak 3 (retention time 34 minute) fitted a typical curved-cis and was identified as 13-cis-β-carotene by co-injection of 13-cis standard. Peak 5 appearing after all-trans-β carotenoids (retention time 39 minutes) was

likely to be 9-cis-β-carotene (Lessin *et al.* 1997; Rodriguez-Amaya and Kimura 2004; Kimura *et al.* 2007). No α-carotene was identified from raw sweet potato.

3.3.5 Quantification of provitamin A carotenoids

The percentage of trans-β-carotenes and minor carotenoids identified are reported in Table 3-4 for fresh and dried sweet potato in the drying treatments jointly analysed.

Table 3-4: Trans-β-carotene and minor carotenoids as percentage of total carotenoids content in fresh and dried sweet potato

Average retention time (min.)	37	34	30	39
Identified compound	Trans-β-carotene (%)	13-cis-β-carotene (%)	β-carotene 5,6 epoxide (%)	9-cis-β carotene (%)
Fresh (%)	86.0 (3.8)a	2.3 (0.7)a	5.6 (1.5)a	1.3 (0.9)a
Dried (%)	88.2 (3.6)a	2.7 (1.0)a	4.5 (0.7)b	1.1 (0.6)a

Each value corresponds to an average of 20 extractions made on a puree from five fresh roots or on a 100g-flour from milled dried chips/slices. Values in brackets refer to the standard deviation. Values in the same column followed with different letters are significantly different; Independent T-test.

The contents of trans-β-carotene and minor compounds: isomers and β-carotene 5,6 epoxide were found to be similar in both fresh and dried samples. These results differ from other previous studies that have indicated that under stressful conditions, such as heating, UV exposure and storage, trans-carotenoids tend to isomerise into cis-carotenoids.

There may be several reasons for these observations: long storage time; heat and length of drying. Raw roots may contain smaller amounts of 13-cis isomers if they were stored too long (Chandler and Schwartz 1988). The presence of small amounts of 9-cis and 13-cis in Rubina sweet potato raw roots could be explained by long root storage time after harvest; these were roots grown in USA and purchased in France. Drying temperatures were not very high (<45°C on average) and drying was quick. The quantity of isomer formed was found to be related to the heat and length of treatment (Chandler and Schwartz 1988; Doering *et al.* 1995). This may explain why carotenoids losses during drying were low (13-40%). In addition, isomerisation in dried samples may need harsher processing conditions to occur. These results were consistent with a study by

Mulokozi and Svanberg (2003) on leafy vegetables submitted to solar and sun-drying in Tanzania where all trans-β carotene 13-cis and 9-cis isomers were similarly affected by sun and solar-drying. 13-cis and 9-cis isomers represented 5% and 15% of β-carotene respectively in Mulokozi and Svanberg (2003) whilst 3% and 6% respectively in this study. Mulokozi and Svanberg (2003) formulated the hypothesis that "the stereo-isomeric forms of β-carotene could be strongly correlated with each other on light exposure and storage"; which means that instead of isomerising, trans-β-carotene could have been converted into oxidative products as well as their isomers. This hypothesis was corroborated by the fact that ratio of trans-β-carotene, 13-cis, β-carotene 5,6 epoxide and 9-cis are the same in fresh and dried samples. This result was confirmed by Kidmose *et al.* (2007) on shade dried OFSP; the same amount of 13-cis-β-carotene was found in root and flour made from dried chips (representing 1% of trans-β-carotene). An interesting and recent work by Hiranvachat *et al.* (2008) showed that a minimum of 5h at constant temperature of 60ºC was necessary to induce formation of 13-cis-β-carotene in oven-dried diced carrot. The absence of isomerisation could therefore be explained since the average temperature in the three dryers was around 40ºC and never went beyond 50ºC. Oxidation occurs through a free radical process and loss of water during drying has proved to be a risk factor (Chandler and Schwartz 1988). Therefore loss of carotenoids (by oxidation) would have occurred rather than isomerisation.

The percentage of β-carotene 5,6 epoxide was significantly lower after drying. This could result from quicker degradation of β-carotene 5,6 epoxide than β-carotene. A combination of factors (light, heat, exposure to oxygen) could have degraded β-carotene 5,6 epoxide slightly more rapidly than trans-β-carotene and stereo-isomers.

3.3.6 Vitamin A activity

Vitamin A activity was calculated using the recent conversion factor of Haskell *et al.* (2004), who demonstrated that bioavailability in fresh sweet potato puree was β-carotene: retinol 13:1. This updated the previous estimation of 6:1 by NAS/NRC (1974). Bioavailability of cis-isomers is estimated as half of trans-β-carotene and that of β-carotene 5,6 epoxide would represent also half of β-carotene activity because it has only one un-substituted β-ionone ring instead of two. Carotenoids contents from minor

provitamin A carotenoids and trans-β-carotene and an estimation of vitamin A activity are summarised in Table 3-5.

Table 3-5: Estimated vitamin A activity of samples of fresh and dried sweet potato

Treatment	Trans-β-carotene (µg.g^{-1} db)	13- cis-β-carotene (µg.g^{-1} db)	β-carotene 5,6 epoxide (µg.g^{-1} db)	9-cis-β-carotene (µg.g^{-1} db)	Estimated vitamin A activity (RE/100g db)*	Contribution to daily vitamin A requirement (% fb)**
Fresh	293.0 (13.3)a	9.1 (0.4)ab	18.4 (1.4)a	6.0 (1.1)a	2,382 (111)a	-
Chipped& cross flow dried	246.9 (22.8)abc	10.2 (0.9)a	14.6 (1.0)ab	4.6 (0.1)abc	2,012 (182)abc	448
Crimped sliced& cross flow dried	232.0 (23.4)bc	10.2 (0.7)a	13.2 (0.5)ab	4.9 (1.5)ab	1,893 (189)bc	427
Chipped& sun dried	198.6 (18.5)c	6.3 (2.7)ab	9.3 (4.9)b	2.4 (1.1)bcd	1,596 (174)c	360
Chipped& greenhouse solar dried	226.0 (16.9)bc	10.4 (1.4)a	12.4 (0.7)ab	5.4 (0.2)a	1,847 (128)bc	416

Each value corresponds to an average of three extractions made on a puree from five fresh roots or on 100g-flour from milled dried chips/slices. Values in brackets refer to the standard deviation. Values in the same column followed with different letters are significantly different. *µg retinol equivalent (RE) = 1/ 13µg trans-β-carotene (Haskell *et al.* 2004) and half of the provitamin A activity for other provitamin A compounds µg retinol equivalent (RE) = 1/ 26µg cis-β-carotene and β-carotene 5,6 epoxide. Calculated on a dry weight basis (db) ** According to FAO/WHO (2002) recommendations are 400 RE per 100 g per day for children (2-6 years old); calculated per 100g of flour on a fresh weight basis (fb).

Estimated vitamin A activity ranged between 1,596 and 2,012 RE per 100g flour and was 2,382 RE per 100g on fresh roots (db). All flours, including sun-dried (1,596 RE), provided a substantial amount of vitamin (about 400% of daily nutritional requirements). These estimations do not take into account further significant losses occurring during the preparation of finished products from the orange-fleshed sweet potato flours. An example of finished product is a traditional doughnut commonly eaten in Uganda called mandazi. Mandazis are usually prepared using wheat flour, but up to 30% of it can be substituted with sweet potato flour (Owori and Hagenimana 2000). These authors reported that dried chips of Zappalo sweet potato variety with a vitamin A activity of 1,170 RE per 100 g (db) resulted in a mandazi with vitamin A activity of 157 RE per 100g (fb) (Hagenimana *et al.* 1999).

One hundred grams of the finished product could therefore meet 40% of the recommended intake of provitamin A for children. Another example is porridge made from sweet potato-sorghum composite flour (70%:30%). Kósambo (2004) reported that dried chips of Jonathan sweet potato variety with a vitamin A activity of 853 RE per 100g (db) resulted in porridge with vitamin A activity of 448 RE (db); considering a moisture content of 75% due to addition of water, one hundred grams of porridge (fb) would meet 30% of the recommended intake of provitamin A for children. In this present study greater vitamin A activities in flour of 1946 RE on average compared to Owori and Hagenimana (2000) and Kósambo (2004) should favourably result in greater vitamin A content in finished products. Products such mandazi and porridge made from orange-fleshed sweet potato could therefore contribute significantly to vitamin A intake in the diet.

3.4 CONCLUSION

The effects of drying treatment and chip size on carotenoid losses in OFSP were investigated. Compared to what was expected, low levels of loss varying between 16 and 34% in trans-β-carotene were obtained for all the treatments. The significant findings are that carotenoid lost through sun-drying were not so different to solar and hot air drying. Another finding was chip shape had an influence on retention: sun-dried samples exhibited significantly greater retention with crimped slices compared to chips. Crimped slice bulkiness or a lesser degree of "shrinkage" may have protected them from damage from the sun's rays and oxidation. These low levels of loss may be attributed by rapid drying (8h) due to the favourable dry, hot and windy climatic conditions. Contrary to expectations, there was not an increase in isomerisation (formation of 9-cis and 13-cis-β-carotenes) due to drying. OFSP flour therefore gave promising results with respect to carotenoid retention. Because of the high β-carotene content of fresh roots (close to 300 $\mu g.g^{-1}$ db) and its high retention even in low cost-sun-drying treatment, orange-fleshed sweet potato demonstrates a potential for a significant contribution to vitamin A in the diet.

CHAPTER 4.
EFFECT OF DRYING AND STORAGE ON THE DEGRADATION OF CAROTENOIDS IN ORANGE-FLESHED SWEET POTATO IN RESEARCH STATION TRIALS IN UGANDA

4.1 INTRODUCTION

In the previous chapter, levels of carotenoid loss from OFSP using different drying technology levels (sun-drying; solar-drying and hot air-drying) were tested in a pilot-scale study. The main findings were that carotenoid losses were low regardless of the level of technology used. The next step was to verify the findings from Chapter 3 under field conditions such as those found in developing countries.

At the time of this study, there was a major concern that losses in carotenoids during sun-drying of OFSP in developing countries were very high (Bouis H, HarvestPlus pers. comm.) and that this would limit the potential of processed OFSP products to contribute to alleviating vitamin A deficiency (Kósambo 2004; Chavez *et al.* 2007). The scarce literature encountered on the extent of provitamin A losses from OFSP under African conditions showed a further need for more extensive research on this aspect. For these reasons, evaluation of carotenoid losses after solar and sun-drying under Ugandan conditions was undertaken. The work is found in this present Chapter.

Exposure to light, especially sun or ultra-violet (UV) light has been reported to induce trans-cis photomerisation and photodestruction of carotenoids (Rodriguez-Amaya and Kimura 2004). It has also been reported that screening from direct sun light had an impact on total carotenoid losses from mango and cowpea leaves; total carotenoid losses were 94% and 63% in sun-drying; 84% and 51% under polythene-covered sheeting (non-UV resistant) and 73% and 44% under visqueen-covered (UV-resistant) sheeting

respectively (Ndawula *et al.* 2004). Therefore the effect of different screening coverage for solar dryers was tested.

Moreover determination of degradation of provitamin A in sweet potato products during the storage period that followed the drying of product needed to be evaluated as this could decrease the nutritional impact of the product for food security. Typically at the household level, dried sweet potato is stored at ambient temperature for 4-6 months.

The objectives of this study were:

- to quantify the losses of total carotenoids from OFSP chips dried in low-cost (see Appendix 2a) solar (using different screening coverage) and sun dryers at a research station in Uganda and subsequently stored and;
- to understand the main factors that influence losses, such as type of dryer, effect of different plastic coverage, sweet potato cultivar and type of packaging of the stored product.

4.2 MATERIALS AND METHODS

4.2.1 Sweet potato root samples

Sweet potato roots were collected from the following locations:

- Orange-fleshed (Ejumula) and yellow-fleshed roots (Kakamega) that had already been released to farmers were harvested from a farm in Luwero District, Uganda.
- Four new cultivars under consideration for release at the time of the study SPK004/1 (Naspot 7), SPK004/1/1 (Naspot 8), SPK004/6 (Vita) and SPK004/6/6 (Kabode), and Ejumula and Kakamega were obtained from the National Crop Resources Research Institute (NaCRRI) at Namulonge and from an experimental field in Bombo, Luwero District.

In all cases, mature roots were harvested after a growing season of six months. Roots were spread on the floor at ambient temperature inside a room to prevent rotting and were processed within 48 hrs of harvest. All cultivars (Table 4-1) have been previously reported to be susceptible to sweet potato weevil and moderately resistant to *Alternaria* blight and Sweet Potato Virus Disease (SPVD).

Table 4-1: Source, flesh colour and yield of selected orange fleshed and yellow fleshed sweet potato cultivars. Source: Mwanga *et al.* (2003); Mwanga *et al.* (2007).

Cultivar	Original source	Flesh colour	Typical reported fresh root yield (tonnes per ha)
Ejumula	Eastern Uganda	Deep orange	36.6
Kakamega (SPK004)	Western Kenya	Yellow/light orange	31
SPK004/1	Bred from SPK004-Uganda	Yellow/light orange	7.4-59.7
SPK004/1/1		Yellow/light orange	7.0-43.2
SPK004/6		Orange	4.6-50.4
SPK004/6/6		Orange	7.9-38.3

4.2.2 Handling and processing prior to drying

Sweet potato roots were trimmed, weighed, washed, and drained in the open air for about 30 minutes. Unpeeled roots were chipped using a rotary disc type chipper (Tonnet Company, Kampala, Uganda). Exposure to light was minimised by using black polythene bags to cover the samples. Chips (thickness 2 mm; width 5 mm; length 69 mm on average) were thoroughly mixed before drying.

4.2.3 Drying of sweet potato chips

Sweet potato chips were dried using dryers that varied in design. Different types of coverage were selected to have different screening effects on UV-radiation for the tent dryers since it was hypothesised that screens, which filter selected UV wavelengths, can reduce losses of carotenoids during drying and that dryer design can influence carotenoid losses during drying. Details of the dryers are given below (Figure 4-1):

- Open air sun dryer
- Tent dryer with UV resistant polythene (Lumitherm BPI-Visqueen®, Herfordshire, UK)
- Tent dryer with non-UV resistant polythene (locally bought in the market in Uganda)
- Tent dryer with red resistant polythene (Allplass®, Hertfordshire, UK)
- Tunnel dryer (UV-resistant polythene sheeting of unknown origin).

The chips were spread over black plastic sheeting that absorbed the sun heat apart from the tunnel dryer where they were spread of cloth netting (mosquito mesh).

Figure 4-1: Solar dryers. From left to right: UV-polythene resistant; non-UV resistant; red-polythene; tunnel dryer and open sun-air dryer

Transmittance of the polythene sheetings of tent-dryers (UV resistant, non-UV resistant and red) were measured by spectrophotometer measurement between 200-800nm (Figure 4-2) (see Chapter 2).

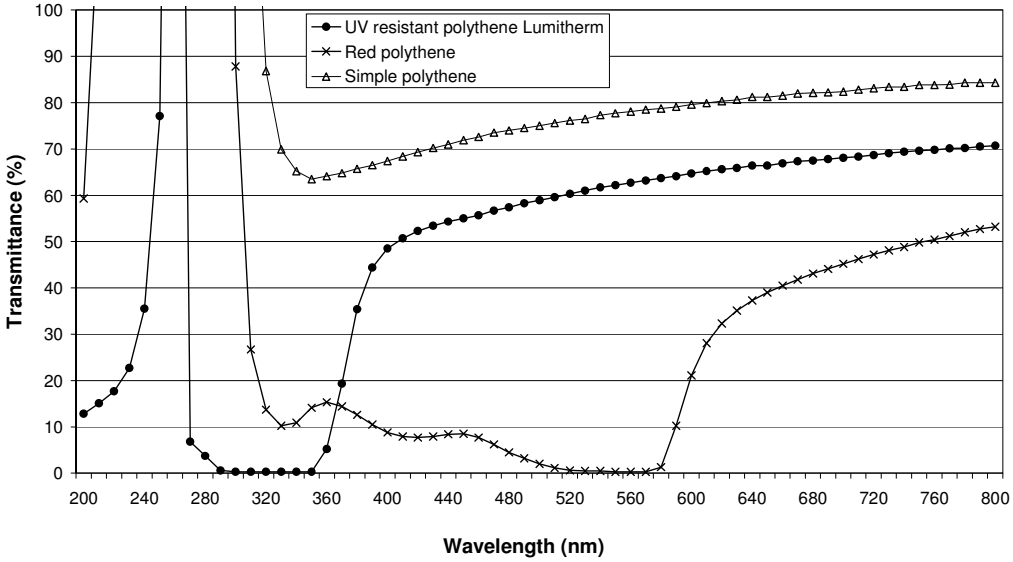


Figure 4-2: UV/visible spectrum of polythene sheeting used in drying studies

Polythene sheeting had different transmittance toward sun light. Red plastic sheeting absorbed at wavelengths between 300-600 nm at which provitamin A is reportedly sensitive (part of UVA and visible). Sattar *et al.* (1977) and Lennersten and Linghert (2000) reported that when exposed to sunlight radiation, provitamin A is more sensitive to ultra-violet (UV) rays, especially at wavelengths close to the maximum absorption of β-carotene of 450 nm and, in general, short wavelengths less than 470 nm cause the most β-carotene degradation. UV-resistant plastic reduced transmission of most UV-wavelengths: between 200-240 nm (UVB and UVC) and between 260-370 nm (UVB, UVA). Simple polythene was not wavelength selective.

All dryers were positioned parallel East-West because of the incidence of the sun's radiation and prevailing wind. For the tent and tunnel dryers, air entered through the inlet placed at the base of the dryers while the moist warm air was evacuated through an outlet in the top corner.

Ambient temperature, humidity, wind speed and irradiance were recorded every 30 min when samples were on the dryers using on a Vantage Pro- meteorological station (Davis Instruments, California, USA). All samples were removed from dryers and placed under a shelter at night or when it rained. Temperature and humidity were recorded inside dryers using Tinytalk temperature/humidity sensors (RS Components Ltd, UK). A loading density of 3.9 kg/m^2 was used when placing the sweet potato chips on the dryers. The mean drying conditions were a temperature of 28.3ºC with a standard deviation of 5.6ºC and a mean humidity of 55.1% with a standard deviation of 23.8%. During drying, chips were weighed every four hours to estimate moisture loss. The end of drying was estimated by chips brittleness and sample weight.

4.2.4 Storage of dried chips for four months

Dried chips (> 1 kg per treatment for Ejumula and Kakamega cultivars) were collected, thoroughly mixed and split into samples of about 200g. Samples were stored under the following conditions: in a LPDE zipped polythene bags (VWR, Leicestershire, UK) protected from light; in knotted black polythene bags; in clear polythene bags placed under a window and so exposed to direct sunlight; or in sealed clear polythene bags stored inside knotted black polythene bags. Black and clear polythene bags were bought from shops in Kawanda, Uganda. No information was available about the supplier.

Samples (200g) per treatment were taken after drying and after storage for four months (125 days) at ambient room temperature. The temperature and humidity was recorded every four hours using Tinytalk temperature/humidity sensors (RS Components Ltd, UK). A four month-storage for dried chips was chosen because it is a typical duration of storage for farmers in Uganda. Samples taken at each storage interval were kept in a freezer (-20°C) until analysed in triplicate.

4.2.5 Total carotenoids extraction and analysis

Carotenoid analysis was undertaken in Uganda (NARL, Kawanda) and in the UK (University of Greenwich).

Carotenoid Analysis in Uganda

Frozen fresh chips (500g) were defrosted at ambient temperature by soaking the plastic bag in tepid water. Fresh chips were then blended to a fine pulp using a Kenwood FP698 Multi Pro Food Processor.

Total carotenoid extraction and analysis were carried out following existing methods (Rodriguez-Amaya 2001; Rodriguez-Amaya and Kimura 2004) but with the following modifications: a portion of the homogeneous representative sample (1-6 g of fresh tissue or 0.5-2 g of flour) was re-hydrated for 20 minutes in 10 ml-desionised water and was homogenised with 50mL methanol: tetrahydrofuran (THF) (1:1) using a Polytron PT1200E (Kinematica, Switzerland) homogeniser for one minute. Total carotenoid content was determined using a Genesys 10UV /UV-visible spectrophotometer at 450 nm. Concentrations were determined by comparison to an external standard curve using pure β-carotene (SIGMA, UK) and an absorption coefficient of β-carotene in PE of 2592 (Rodriguez-Amaya and Kimura 2004).

Carotenoid analysis in the UK

Dried chips were transported to the UK by air flight and were immediately placed in a freezer on arrival. During transport to the UK over a 24 hour period, the dried chips reached ambient temperature during transport, but the carotenoid degradation during transportation was considered negligible (see Chapter 2 section 2.6.2). Samples were immediately placed in a freezer on arrival. Samples were stored in a freezer (-20ºC) for up to 2 months before analysis. Carotenoid content of samples stored in the freezer was checked over a 4 month period and there was no significant decrease in total carotenoid content ($p<0.05$) (see Chapter 2 section 2.6.1).

Prior to analysis, chips were milled into flour and subsequently stored for up to two weeks at -20°C. After careful mixing and sub-sampling using a grain divider, a quarter of the 200g was milled in a Laboratory Mill (Models 3033 or 3600, Perten Instruments, Segeltorp, Sweden). Flour obtained was packed in zipped plastic bags from which excess air was removed manually.

The same extraction method used in Uganda was also used in the UK. The operator was also the same. Homogenisation was carried out using an Ultra-turax IKA Janke and a Kunkel Labortechnik at 322 x g (8000 rpm; diameter 9mm). The total carotenoid content measured by Diode Array detector spectrophotometer (Hewlett Packard HP8452A).

4.2.6 Dry matter determination

Samples were collected for dry matter determination, before and after drying at the same time as carotenoids analysis. Determinations were made by drying triplicate 5g samples at 105ºC to constant weight (minimum 24h).

4.2.7 Statistical analyses

Analysis of variance (ANOVA) was carried out to determine whether there were significant differences between samples with one up to three factors; a significant difference between samples was determined using the Tukey test. Correlations were determined using Pearson tests on average losses. Inter-laboratory difference was tested by one way-ANOVA. All data were processed on SPSS 14.00 for Windows (Woking, Surrey, UK).

4.3 RESULTS AND DISCUSSION

4.3.1 Carotenoid losses in solar and sun drying treatments

The inter-laboratory difference between the carotenoid extraction undertaken in the Uganda and UK laboratories (triplicate extractions of five dried samples of each of Ejumula and Kakamega roots) indicated that there was no significant difference ($p<0.05$) (Table 4-2).

Table 4-2: Comparison between total carotenoid content (µg.g^{-1} db) after drying* determined in the same samples in the UK and Ugandan laboratories

Variety	University of Greenwich, UK	NARL, Uganda
Ejumula	287.1 (5.7)	271.0 (11.8)
Kakamega	92.3 (2.7)	90.3 (5.1)

* n=5[3]; ANOVA. Values in brackets refer to the standard deviation.

The drying of Ejumula and Kakamega (SPK004) sweet potato cultivars was investigated. Total carotenoid losses were on average 7.3% in Ejumula and 10.7% in Kakamega (Table 4-3).

Table 4-3: Total carotenoid loss (dry weight basis) related to processing trial, drying time and drying trial for two cultivars combined (Ejumula and Kakamega) of sweet potato dried on five types of dryers in Uganda

Trial	1	2
Wind Speed* (m/s)	1.8 (1.4)	4.1 (2.1)
T* (ºC)	27.3 (2.6)	31.2 (2.6)
RH* (%)	62.4 (16.4)	32.3 (8.7)
Solar Radiation* (W/m^2)	496 (230.3)	781.9 (183.2)
Drying on dryers time* (h)	11.9 (1.8)	7.2 (1.9)
Total drying time** (h)	59.5 (11.2)	16.9 (10)
Total carotenoid loss(%) Ej.	8.4 (3.3)	6.3 (1.9)
Total carotenoid loss(%) Ka.	13.4 (4.4)	8 (3)
Average- trials	10.9 (4.7)	7.1 (2.7)

Mean (standard deviation); T=Temperature; RH= Relative Humidity.
*These parameters were measured during the total period of drying on dryers (day time). One measurement was taken each half hour. **Including time under shelter at night and when raining. Mean Ejumula total carotenoid loss: 7.3 (2.8)%; Mean Kakamega total carotenoid loss: 10.7 (4.8)%

The mean total carotenoid losses (for the two cultivars analysed jointly) were 10.9% in one replicate study (trial 1) and 7.1% in the other replicate study (trial 2). The two trials had different weather conditions: it was wet for trial 1 and dry, sunny and windy for trial 2. Consequently drying times were reduced for trial 2 (7.2h on dryers in average) compared to trial 1 (11.9h in average). Shorter drying times may have resulted in lower levels of carotenoid loss. Low losses of trans-β-carotene were similarly reported in a recent study involving oven; solar and open sun-drying of OFSP chips in Uganda[23]. Trans-β-carotene losses were respectively 12%; 9% and 16% for the Ejumula cultivar for a drying time and temperature of 10 h at 57ºC in oven drying; between 6-10 h in solar-drying (45-63ºC) and sun (30-52ºC) respectively. In this present study, the low levels of carotenoid losses are in agreement with this more recently published finding.

Losses of total carotenoids from two cultivars of sweet potato dried in dryers fitted with polythene sheeting were determined (Table 4-4). Independent of cultivar and dryer,

losses of carotenoids during drying varied between 2.1% and 18.7% and dry matter contents in dried samples ranged between 88.0% and 92.4%.

Table 4-4: Losses in total carotenoids during solar-drying of Ejumula and Kakamega cultivars of sweet potato dried in various solar and sun dryers under wet and dry weather conditions

Cultivar	Treatment	Dry matter content* (%)	Drying time** (h)	Total carotenoid content*** (µg.g^{-1} db)	Loss (%)
Trial 1: Wet weather					
Ejumula	Fresh	31.4	0.0	250.6(9.1)	-
	Red polythene	90.0	14.0	240.4(2.9)	4.1
	Local polythene	92.4	13.2	227.1(1.7)	9.4
	UV resistant polythene	90.9	12.6	230.1(3.0)	8.2
	Tunnel dryer	90.0	10.4	232.8(4.2)	7.1
	Sun-drying	89.7	9.7	217.8(6.2)	13.1
Kakamega	Fresh	38.1	0.0	75.2(4.4)	-
	Red polythene	88.0	14.1	62.1(0.9)	17.4
	Local polythene	90.0	13.2	65.4(3.3)	13.0
	UV resistant polythene	89.3	12.6	61.1(0.4)	18.7
	Tunnel dryer	91.5	10.4	67.4(4.1)	10.4
	Sun-drying	89.7	9.3	69.8(0.2)	7.2
Trial 2: Dry weather					
Ejumula	Fresh	30.9	0.0	306.3(2.4)	-
	Red polythene	89.7	9.3	291.7(5.8)	4.8
	Local polythene	90.9	7.5	290.9(6.1)	5.0
	UV resistant polythene	89.7	7.3	283.6(5.1)	7.8
	Tunnel dryer	91.0	5.7	279.6(5.0)	8.7
	Sun-drying	91.1	4.8	291.2(6.6)	4.9
Kakamega	Fresh	33.5	0.0	100.3(1.8)	-
	Red polythene	89.6	10.2	91.7(1.7)	8.6
	Local polythene	90.1	8.6	91.2(1.4)	9.1
	UV resistant polythene	90.6	8.0	98.1(3.4)	2.1
	Tunnel dryer	89.5	5.7	91.0(0.3)	10.7
	Sun-drying	91.1	4.9	90.6(6.6)	9.6

*Mean; standard deviation is not given because <1% on triplicate extractions
**Exposure in dryers ***Mean (standard deviation) on triplicate extractions

There was no effect on the loss of total carotenoids from the use of type of dryer (solar or sun) ($p<0.05$). The lack of differences in total carotenoid retention between sun and solar-drying is in contrast with what was previously reported (Mulokozi and Svanberg (2003; Ndawula *et al.* 2004; Chavez *et al.* 2007). Working with leafy vegetables, Mulokozi and Svanberg (2003) has demonstrated that solar-dried products retain significantly more β-carotene than sun-dried products. However, when analysing

individual results from the paper, it appeared there were no significant differences between solar and sun-drying on five out of seven leafy vegetables (Mulokozi and Svanberg 2003). Previous reports (Mulokozi and Svanberg 2003; Bengsston *et al.* 2008) have indicated that solar-drying with natural air convection was faster than sun-drying. An explanation for the lack of differences in provitamin A retention between sun and solar dryers may be that in this study, as opposed to previous reports, sun-drying was faster compared to solar-drying.

In particular there was no effect on the loss of total carotenoids from the different types of polythene sheeting in spite of the various wavelength selectivity ($p<0.05$). Another reason for the lack of differences between dryers may have been because the UV irradiation affected only the surface of OFSP and did not penetrate the inner tissue. The size and shape of chips may also have an impact on carotenoid loss as this was found in Chapter 3. The lack of difference between different screening coverage contrasts with the findings of Ndawula *et al.* (2004) working with mango and cowpea leaves, which are non-starchy foods. Starch being the main component of sweet potato may have also played a protective role in preventing carotenoid losses (Zhao and Chang 1995) and explain the lack of difference in-between the solar dryers and also sun-drying. The lack of direct sun radiation impact is an important findings for sweet potato chips drying and confirmed the results from Chapter 3.

4.3.2 Effect of trial and sweet potato cultivar

On the other hand, the effect of trial and cultivar was significant (two-way ANOVA; $p<0.05$). The effect of trial (which means that not all the dryers behave the same way in the two replicates) can be explained by lack of control over environmental factors in sun and solar-drying. The tunnel dryer had the most consistent results between the two replicates (for both cultivars), probably because it provides more protection from wind and other natural elements compared to the other dryers.

Total carotenoid losses from six OFSP cultivars dried by open sun-drying at NaCRRI (Namulonge) and in Luwero District were on average 14.8% and 7.0% respectively (Table 4). The weather conditions were similar (high wind; low humidity and high solar radiation) for both trials but the drying times differed being half a day at the Namulonge location and half day plus a night at Luwero (Table 4-5).

Table 4-5: Total carotenoid losses related to drying time and weather conditions in open air drying of six OFSP cultivars in Uganda

Trial	Namulonge	Luwero
Wind Speed* (m/s)	5.4 (2.0)	5.1(2.4)
T* (ºC)	32.0(1.8)	32.0(1.6)
RH* (%)	31.7(12.6)	34.1(8.9)
Solar Radiation* (W/m^2)	845.2(137.4)	752.3(277.9)
Drying time on dryers (h)	4.9(0.2)	5.6(0.0)
Total drying time** (h)	4.9 (0.2)	24.5(0.0)
Total carotenoid loss (%)	14.8(4.2)	7.0(2.5)

Mean (standard deviation); T=Temperature; RH= Relative Humidity. *These parameters were measured during the total drying time on dryers. One measurement was taken each half hour.**Including time under shelter at night and when raining. Mean Ejumula total carotenoid loss: 13.6 (4.7)%

Because the samples from the two locations were not dried on the same day a comparison between the factors that influence the carotenoid losses is therefore not possible. Losses in carotenoids per cultivar in both locations are presented in Table 4-6.

Table 4-6: Loss in carotenoids after open air sun-drying of different sweet potato cultivars

Location /Variety	Dry matter content in fresh roots (%)	Total carotenoid content ($\mu g.g^{-1}$ db) before drying	Drying duration (h)	Dry matter content after drying (%)	Total carotenoid content ($\mu g.g^{-1}$ db) after drying	Loss (%)
Namulonge						
Ejumula	31.5	300.5(5.1)	4.7	92.9	236.3(1.7)	21.4
Kakamega	32.1	107.9(1.2)	4.7	92.3	96.5(2.9)	10.6
SPK004/1	32.9	96.2(0.4)	4.7	92.6	84.4(2.2)	12.2
SPK004/1/1	30.3	78.5(6.1)	5.0	92.3	69.4(1.4)	11.6
SPK004/6	28.4	188.5(7.6)	5.2	92.4	160.0(2.3)	15.1
SPK004/6/6	28.8	172.9(1.9)	5.1	92.8	142.0(1.2)	17.9
Luwero						
Ejumula	32.4	223.1(4.3)	5.7	91.3	194.5(5.6)	12.8
Kakamega	32.7	94.7(3.3)	5.7	91.6	101.2(3.5)	-6.8[ʃ]
SPK004/1	31.7	47.9(1.5)	5.6	91.6	41.7(3.4)	13.0
SPK004/1/1	33.1	41.7(4.1)	5.6	91.7	42.2(1.0)	-1.1[ʃ]
SPK004/6	33.8	159.6(11.7)	5.6	91.6	152.7(2.9)	4.3
SPK004/6/6	22.8	168.1(2.3)	5.6	92.0	139.1(5.0)	17.2

*Mean; standard deviation is not given because <1% on triplicate extractions.
**Exposure in dryers ***Mean (standard deviation) on triplicate extractions.
[ʃ]Negative values do not differ significantly from total carotenoid content in fresh chips

Total carotenoid contents of fresh sweet potato varied in the six cultivars between 78.5 and 300.5$\mu g.g^{-1}$ at the NaCRRI and 41.7 and 223.1$\mu g.g^{-1}$ in Luwero Distrct. These

values are in agreement with Bengsston *et al.* (2008) who described trans-β-carotene content varying between 108.1-261.9μg.g^{-1} on the same cultivars including Ejumula, Kakamega (SPK004), SPK004/1; SPK004/1; SPK004/6 and SPK004/6/6 more Sowola 9/94/9 also from the NaCRRI (Namulonge). There were significant differences in fresh chip carotenoid content (before drying) associated with location (two-way ANOVA; $p<0.05$). This has been reported previously on different cultivars (Kósambo *et al.* 1998). These differences were especially marked for the newly developed cultivars, SPK004/1 and SPK004/1/1 (Table 4-6). Sweet potatoes grown on farmer's fields generally had lower levels of total carotenoids than those grown at the research station. This is the first time that the carotenoid contents of these newly developped cultivars have been compared from different locations.

Independent of location, there was a significant influence of cultivar ($p<0.05$) on the levels of total carotenoid loss. Furthermore, a significant correlation between initial dry matter content and carotenoid losses was observed (Pearson coefficient R=-0.518; $p<0.05$) (Figure 4-3A) and between initial total carotenoid content (in fresh chips) and carotenoid losses (Pearson coefficient R=0.589; $p<0.05$) (Figure 4-3B). For an equivalent drying time, cultivars with higher moisture contents and with higher initial carotenoid contents tended to lose more carotenoids during drying.

The observations in this study were confirmed by further analysing the data of Hagenimana *et al.* (1999) who investigated the drying of 24 white, yellow, purple and orange-fleshed sweetpotato cultivars. Initial total carotenoid contents ranged between 2 and 632 μg.g^{-1} dry basis, and dry matter contents in fresh roots between 19 and 34% respectively. Losses of total carotenoids after oven drying ranged between 0 and 80% with an average of 32%. Analysing the data presented (Hagenimana *et al.* 1999), a significant correlation was observed between fresh dry matter and losses during drying and between initial total carotenoid content and losses during drying (Pearson coefficient R=-0.717; $p<0.05$) in cultivars with total carotenoid content greater than 37 μg.g^{-1}. No correlation was observed on 12 other cultivars with carotenoid content lower than 37 μg.g^{-1} (Hagenimana *et al.* 1999). Similar observations regarding the influence of dry matter content and carotenoid losses during drying have been made elsewhere: sweet potatoes (moisture of 75.8 %) had greater β-carotene retention compared to carrots (90.5%) respectively 4.0–5.8%, and 48.9–67.5% (Mdziniso et al. 2006).

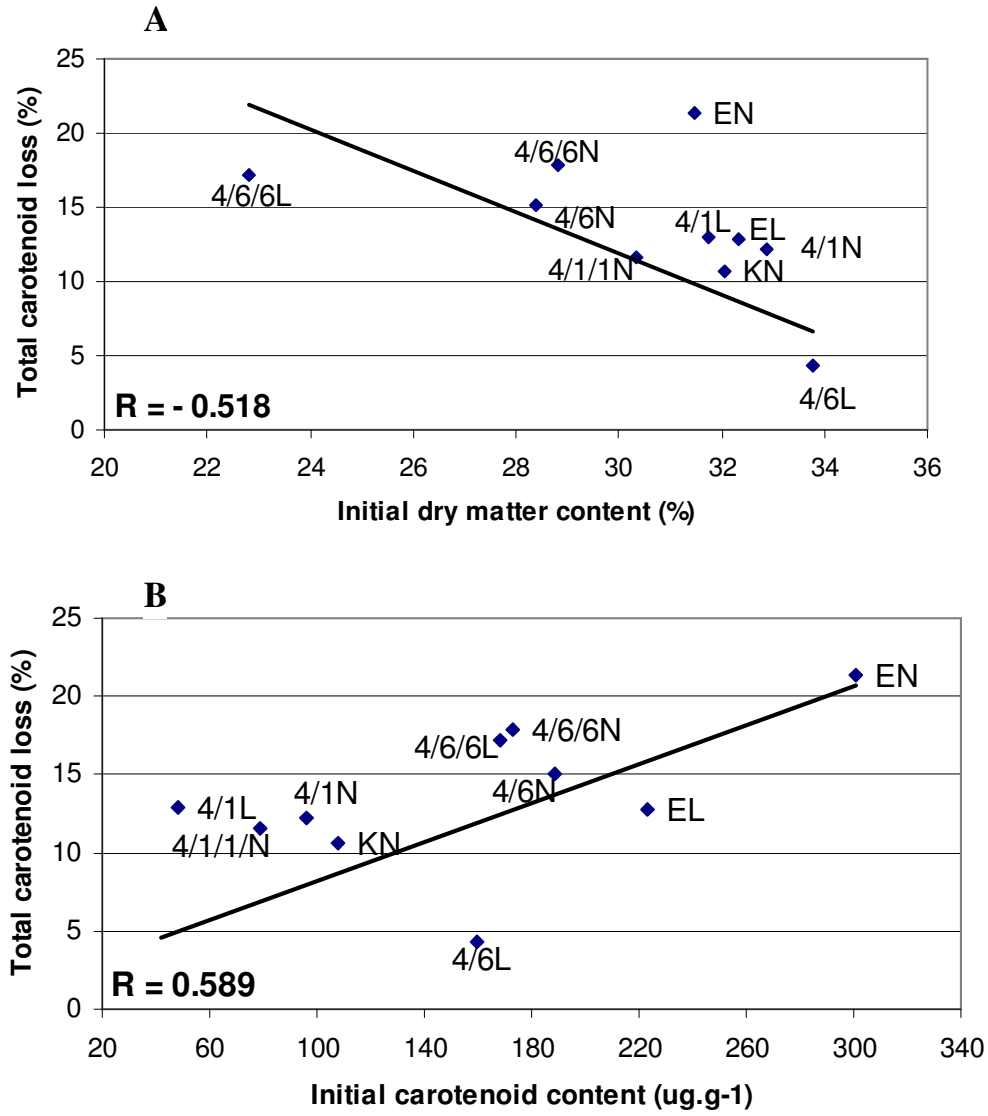


Figure 4-3: Relationship between initial dry matter content (A) or between initial total carotenoid content (dry basis) (B) and total carotenoid loss in drying for six sweetpotato cultivars obtained from Namulonge and Luwero

One point represents the average result per sample (12). Each sample was analysed in triplicate.
The abbreviations are: E, Ejumula; K, Kakamega; 4/1, SPK004/1; 4/1/1, SPK004/1/1; 4/6, SPK004/6; 4/6/6/, SPK004/6/6; N, Namulonge; L, Luwero.
Cultivars KL and 4/1/1L with negative losses are not shown on the figure but taken into account in the calculation of the coefficient of correlation (R).

Our study confirm that there is a linkage between both initial dry matter content and initial carotenoid content and the percentage loss of carotenoid during drying. It is suggested that the relationship between characteristics of the cultivars and losses of

carotenoids during drying should therefore be taken into account in selection of cultivars for promotion.

4.3.3 Effect of storage on provitamin A retention

Because the levels of loss of carotenes after drying were much less than anticipated, changes during storage at ambient temperatures were investigated (Table 4-7).

Table 4-7: Losses of total carotenoids during the storage of OFSP dried chips at ambient temperature in polyethylene (PE) bags for 4 months (125 days)

Cultivar	Treatment	Dry matter content* (%)	Total carotenoid content ** ($\mu g.g^{-1}$ db)	Loss in storage (%)	Overall loss (%)
Ejumula	Before storage	91.3	199.8(5.4)		
	Zipped PE bag	90.4	46.7(4.5)a	76.6	85.4
	Sealed clear PE bag in black PE bag	88.4	64.2(1.0)b	67.9	79.9
	Black PE bag with simple knot	88.4	58.2(4.6)b	70.9	81.8
	Sealed clear PE bag	88.1	69.5(5.7)b	65.2	78.3
Kakamega	Before storage	91.3	52.4(3.6)a		
	Zipped PE bag	90.3	12.0(0.8)b	77.2	84.8
	Sealed clear PE bag in black PE bag	88.8	18.0(0.5)b	65.7	77.2
	Black PE bag with simple knot	88.7	19.0(1.0)b	63.7	75.8
	Sealed clear PE bag	88.0	18.5(1.0)b	64.7	76.5

*Mean; standard deviation is not given because <1% on triplicate extraction
**Mean (standard deviation) on triplicate extractions. Values in the same column (same cultivar) followed with different letters are significantly different; ANOVA two ways Tukey test.

In all cases, the levels of losses of carotenoids during storage were high when compared to losses during drying, averaging 68.2% with a range of 63.7 to 76.6%. The combined levels of loss from drying and storage (overall loss) ranged from 75.8 to 85.4% after four months of storage.

Total carotenoid contents of sweetpotato chips either in sealed or non-sealed (knotted) bags degraded to the same level for both varieties (two-way ANOVA; $p<0.05$). The permeability of packing to oxygen was demonstrated to have a strong influence on level of β-carotene loss in sweetpotatoes stored under ambient conditions (Valdez *et al.* 2001;

Emenhiser *et al.* 1999). Similar levels of loss between sealed and non-sealed bags in this study can be explained by the high air permeability of LDPE.

There were no differences in losses when the dried sweetpotato chips were stored in clear or opaque (black) bags (two-way ANOVA; $p<0.05$). As compared with the effect of oxidation (packaged under nitrogen or air), the effect of photoisomerisation (can or clear bottle) was shown to be minor on mango puree (Vasquez-Caicedo *et al.* 2003). The lack of effect of light exposure during storage has also been reported in work on carotenoid loss from freeze-dried sweetpotato, orange-peel and carrots (Cinar 2004). This is the first time, however, that the lack of effect of light on carotenoid loss during storage of orange fleshed sweetpotato chips has been reported. The lack of effect of light during storage is in agreement with the lack of effect of use of selective polythene sheeting observed during drying.

4.3.4 Effect of sweet potato cultivar on provitamin A retention in storage

The levels of total carotenoids in chips made from Ejumula, Kakamega, SPK004/1, SPK004/1/1, SPK004/6 and SPK004/6/6 cultivars grown at NaCRRI, Namulonge after drying and after storage for four months is illustrated in Table 4-8.

Table 4-8: Levels of total carotenoids (µg/g on a dry weight basis) in dried sweet potato chips of six cultivars grown at Namulonge stored at ambient temperature for four months (125 days) in locally purchased black polythene bags

Cultivar	Dry matter content immediately after drying* (%)	Dry matter content after 4 month-storage* (%)	Total carotenoid content after 4 month-storage** ($\mu g.g^{-1}$ db)	Loss after 4 month-storage (%)
Ejumula	92.9	88.0	58.5(1.7)	75.3
Kakamega	92.3	87.9	29.2(0.7)	69.8
SPK004/1	92.6	88.1	29.1(0.7)	65.5
SPK004/1/1	92.3	88.0	21.5(1.0)	68.9
SPK004/6	92.4	87.8	48.4(1.0)	69.7
SPK004/6/6	92.8	88.1	38.0(0.6)	73.2

*Mean; standard deviation is not given because <1% on triplicate extraction.
**Mean (standard deviation) on triplicate extractions.

Although there was some variability between cultivars, levels of losses were high in all cultivars and averaged 70.4% after four months storage at room temperature (overall losses were 74.7%). Sample moisture contents increased during storage as previously

observed on Ejumula and Kakamega in various packaging types (Table 6). There was no correlation between dry matter and total carotenoid content in fresh sweet potatoes and losses in storage ($p>0.05$).

Levels of loss after 4 months from chips of the six cultivars (70.4%) are consistent with levels of loss observed previously in various packaging after 4 months (68.2%). Considerable losses therefore occur in storage leading to a poor quality product.

4.3.5 Estimation of vitamin A activity in OFSP chips

Vitamin A activities of various OFSP chips were estimated and summarised in Table 4-9.

Table 4-9: Estimation of vitamin A activity in flours made from OFSP cultivars after drying and storage for four months at room temperature (RE. kg^{-1} product on a fresh weight basis)

	Estimated vitamin A activity RE. kg^{-1} product on a fresh weight basis*	
Cultivar	Freshly dried chips	Stored chips (4 month)
Ejumula	15,202	3,561
Kakamega	6,163	1,774
SPK004/1	5,413	1,777
SPK004/1/1	4,430	1,313
SPK004/6	10,235	2,942
SPK004/6/6	9,124	2,317

*1 RE=13 µg of all-trans-β-carotene (Haskell *et al.* 2004). All-trans-β-carotene content is estimated to 90% mean total carotenoids content (Rodriguez-Amaya and Kimura 2004; Bengsston *et al.* 2008; Bechoff *et al.* 2009a). RE= Retinol Equivalents. Recommended daily requirements (RDA) of 2-6 year-olds is 400 RE (FAO/WHO 2002).

Chips made from most of the cultivars had a high vitamin A activity after drying (>4,000 $RE.kg^{-1}$) (FAO/WHO 2002) with an average of about 8,428 RE kg^{-1} (Haskell *et al.* 2004), but after four months of storage none of the chips (regardless of cultivar) had vitamin A activities greater than 4,000 $RE.kg^{-1}$; the average was 2,281 RE kg^{-1}.

In addition to the losses during drying and storage, it is speculated that further losses will occur during the preparation of OFSP flour into finished product (for example, atapa (traditional Ugandan porridge cassava/sweet potato), mandazi (traditional doughnut), or bread) would represent an estimated loss of a further 50% on a dry basis (Hagenimana *et al.* 1999; Kósambo 2004). Nonetheless, most of the cultivars immediately after drying

have the potential to provide a major part of FAO/WHO recommended daily requirements of children (FAO/WHO 2002) assuming that 100 g of finished product was consumed. However, after storage for four months, none of the dried samples would provide a significant source of vitamin A to the diet. In addition to the loss of provitamin A activity, other constraints in the quality of the dried product need to be taken into account, such as, rancidity, browning, and presence of insects. The quality of sweet potato chips during storage is yet to investigate as a new research area. In summary, from a nutritional and quality perspective, it would be recommended to limit the length of storage of dried OFSP as much as possible.

4.4 CONCLUSION

A major conclusion from the work on drying and storage of sweet potato in Eastern Africa was that sun-drying is a relatively less important cause of loss of total carotenoid content than anticipated. An important finding was that the low-cost, controlled direct sun-drying (covering samples at night or in case of rain; and checking carefully the end of drying) was demonstrated to be as efficient as solar-drying in terms of provitamin A retention. Mean losses after drying were as low as 7.3% in Ejumula and 10.7% in Kakamega cultivars.

Sweet potato cultivar had a significant effect on carotenoid losses in drying ($p<0.05$). An interesting fact is that carotenoid loss appeared to be related to the initial carotenoid and moisture content. Cultivars with higher initial moisture and carotenoid contents of cultivars occurred to be related to higher levels of carotene loss in drying. This is the first time that such a finding is clearly shown.

Storage of OFSP chips had a far more significant effect on carotenoid content than drying. Losses over 70% were obtained after room storage for 4 months. Losses over 70% were obtained after 4 months of storage. While the levels of losses in carotenoids were substantial, there was no effect of sun light screening exposure (opaque or clear packaging) after storage as well as after drying. There was furthermore no effect of cultivar tested on losses during storage.

OFSP chips contained a significant amount of provitamin A immediately after drying and this could make a significant contribution to the diet but only if they were consumed soon afterwards. Losses of carotenoids during storage were therefore considered to be more of a constraint to the use of dried sweetpotato than losses during drying.

However, low-cost means of reducing provitamin A losses during storage need to be investigated in order to increase the usefulness of drying as a processing technique in rural areas of Southern Africa. Losses of carotenoids during storage were therefore considered to be more of a constraint to the use of dried sweet potato than losses during drying.

CHAPTER 5.
UNDERSTANDING ON-FARM CAROTENOID LOSSES AFTER DRYING AND STORAGE OF ORANGE-FLESHED SWEET POTATO IN MOZAMBIQUE

5.1. INTRODUCTION

Recent studies (Tomlins *et al.* 2007b) have indicated that experimental results obtained on a research station do not necessarily transfer to the farm situation because of variations in farmer knowledge and the situation on-farm. Therefore, it was important to verify the on-station results obtained in Chapter 4 in a farm situation. In addition, in order to preserve better provitamin A in sweet potato drying, there was a need to determine whether some additional product variables (*i.e.* chip size), which can be relevant for farmers and chips processors, can also influence carotenoid degradation. In laboratory trials, chip size has been reported to influence on carotenoid degradation after sweet potato sun drying in France (Chapter 3) and variety was also reported to influence carotenoid degradation in the study in Uganda (Chapter 4).

Another query was to determine the rate at which carotenoids are degraded during storage. Determination of degradation kinetics of carotenoids on dried sweet potato could help researchers advise farmers about product shelf life with respect to carotenoid content. At this stage of the research, there also was an additional and more fundamental research question about how carotenoids are degraded and into which products. The latter question involved the use of HPLC and GC/MS analysis techniques to identify carotenoids and their degradation products.

The main aim of the study was to verify results obtained in Chapter 4 (studies at a research station in Uganda) using simple and low-cost (see Appendix 2b) drying and storage for orange-fleshed sweet potato in a different setting (on-farm) and a different country (Mozambique). The effect of sun, shaded and solar (tunnel) dryers was tested. In order to draw a more complete picture of carotenoid stability during storage,

carotenoid degradation was measured over four month-period storage using different analytical techniques (spectrophotometer, CIELAB Colorimeter, HPLC and SPME/GC/MS).

5.2. MATERIALS AND METHODS

5.2.1. Sweet potato root samples

Mature sweet potato roots (MGCL01 and Resisto varieties) of about 80 kg per variety were purchased from farmers. The exact root harvest age was not known. All roots were processed in the interval between one and three days after harvest.

5.2.2. Dryers

Three dryers were constructed on a farm belonging to a subsistence farmer at Lualua (105 km from Quelimane, Mozambique). Apart from the greenhouse clear plastic (Strawberry 3 seasons BPI-VISQUEEN®, UK thickness 150 μm), all building materials were obtained locally and constructed by local craftsmen. The on-farm building of dryers involved one carpenter and four men and took four full days of work. Each of the dryers was table-shaped and was fitted with straw mats on which the product was dried.

- **The tunnel dryer** had a total length of 9 m and a width of 1.5 m. The collector (absorber) occupied the first 3.5 m and was formed of an iron metal sheet. The rest of the dryer (5.5 m) was used as the drying area. The floor of the drying area was made of straw mats covered with black plastic sheeting to insulate the structure. Clear greenhouse plastic covered the whole structure apart from the inlet and outlet allowing air flow and protected by mosquito net (0.55m^2 each). The dryer had a 6° slope (see Appendix 2c).
- **The open air dryer** (exposed to direct sun) had a length of 6 m and a width of 1.5m with a height of 0.9m and had a 6° slope (see Appendix 2c).
- **The shade dryer** was identical to the open air dryer and with the addition of a grass lined roof. The roof was about 0.6 m larger and longer than the table in order to protect it from sun light. The shade dryer was flat (*i.e.* without a 6° slope) because of building constraints.
- Each dryer could fit 6 trays of 4 kg fresh sample each and surface area per tray was 1.03m^2 (see Appendix 2c).

The geographical position of the dryers was determined using GPS (GPS 60, GARMIN®). Dryers were positioned facing north; this allowed maximum sun insulation in the southern hemisphere. Temperature/humidity dataloggers (Tinytalk 2 Geminidatalogger, Chichester, UK) were placed in the tunnel dryer and under the shade for ambient temperature.

5.2.3. Drying

Drying trials were carried out in duplicate. Five fresh roots per variety were collected for carotenoid analysis. On the drying day, unpeeled roots were chipped using either a mechanical rotary disc chipper producing either thick and thin chips, or by traditional hand slicing. Size (thickness, width, length) of ten fresh chips or slices was recorded using a digital calliper. Samples (4kg) were weighed (Hanson Electronic; ±1 g) after carefully mixing (using a quartering technique). Sweet potato samples were evenly spread on drying trays (6 trays per dryer) at a density of 3.9 $kg.m^{-2}$ (see Chapter 2 section 2.2.3). Sweet potato thin and thick chips and slices from MCL01 and Resisto after 4 hours of drying are shown in Figure 5-1.

Figure 5-1: OFSP chips of different sizes after 4h of open-air sun drying

Samples were left overnight on dryers because rain was very improbable at the time of the study. A night guard ensured that samples or plastic coverage from the tunnel dryer were secure. The fresh samples of chips/slices per treatment with an initial weight of 4

kg (per sample) reached a final weight of dried chips/slices of 1.5 kg for MGCL01 and 1.0 kg for Resisto after drying.

5.2.4. Collection of dried sweet potato chips for analysis

A carefully mixed portion of dried chips/slices (about 200 g) (see Chapter 2 section 2.2.5) was collected in zip-polythene bags and placed in cooler bag before they were transported (taking a couple of hours) to a freezer in Quelimane . The remainder of the chips/slices were used for the storage study (at ambient temperature). Samples were stored in traditional bags made of jute and hung inside a house constructed from mud in Lualua. In order to measure losses during storage, sub-samples (200 g chips or slices per stored sample) were removed after 1 month (31 days), 2 months (62 days), 4 months (125 days) and placed in polythene bags in a cooler bag and then stored in the freezer. The datalogger recording the ambient temperature during storage was unfortunately lost, but it is estimated from records taken nearby in Quelimane that the temperature in the mud house was on average 25 ºC with minimum/maximum temperatures of 20/31ºC (Weather Underground Quelimane 2007).

5.2.5. Carotenoid analysis

Sample transport was described in Chapter 2 section 2.2.6. Prior to analysis of the dried chips, they were milled using a Laboratory mill 3600 (Perten Instruments, Segeltorp, Sweden) at position three. In cases where slices were not well dried and still soft, liquid nitrogen was added to freeze and harden the samples before milling. Flour samples in zip bags were stored at -20°C and analysed within two weeks of milling in a randomised order. Total carotenoid content by Rodriguez-Amaya and Kimura's (2004) method were determined on fresh roots and dried samples as described in Chapter 2 section 2.3. Duplicate samples of flour were extracted for total carotenoid content. Total carotenoid losses were calculated on a dry weight basis.

5.2.6. HPLC analysis

All extracts of sweet potato carotenoids (20ml) kept at -20ºC were dried under nitrogen using the dry block system at 35ºC and transported to CIRAD, Montpellier, France in an insulated bag with a frozen gel pack and transferred to a freezer immediately at arrival. HPLC analysis was carried out using method by Dhuique Mayer *et al.* (2005) described

in Chapter 2 section 2.3.3.

5.2.7. Dry matter determination and colour measurement

Dry matter determination and colour measurement are described in Chapter 2 section 2.2.4 and 2.3.2 respectively.

5.2.8. SPME/GC/MS analysis

The SPME system is described in Chapter 2 section 2.3.5. Operating conditions used were as follows: splitless injection (4 min); injection temperature, 220°C; initial oven temperature 40°C (held for 5 min), increased by 3°C.min^{-1} to 250°C and held at this temperature for 15 min. Volatile compounds were identified using the Wiley Mass Spectral Library and a standard curve using β-ionone (purity ≥97%; predominantly trans, Sigma-Aldrich, France) as internal standard (see details in Chapter 8). In this chapter, which is a preliminary trial with the measurement of volatile compounds, only peak areas for total volatiles are reported.

5.2.9. Statistical analyses and calculation of carotenoid degradation rates

Statistical analyses (ANOVA and Pearson test) were described in Chapter 2 section 2.3.6.

Carotenoid degradation rate (k) was calculated by linear regression following the formula $C = C_0 - kt$ (zero order kinetics) or $\ln C = \ln C_0 - kt$ (first order kinetics) where C_0 is the total carotenoid content after drying (μg.g^{-1}); C the total carotenoid content at storage time t (μg.g^{-1}); k the rate constant (days^{-1}) and t the storage time (days) (see Chapter 1 section 1.4.2).

5.3. RESULTS AND DISCUSSION

5.3.1. Effect of dryer type on carotenoid retention in OFSP chips

Total carotenoid contents for Resisto and MGCL01 varieties after drying that varied by dryer type and chipping treatment are illustrated in Table 5-1.

Table 5-1: Total carotenoid losses after drying influenced by treatment (dryer, chipping, trial) using MGCL01 and Resisto varieties of OFSP

Variety	Chipping	Dryer	Replica-tion	Dry matter content (%)	Drying time* (h)	Total carotenoid content (µg.g^{-1} db)**	Carotenoid loss (%)
MGCL01	Fresh roots			35.4		235.6(6.5)	
	Thin chips	Tunnel	1st	92.8	26.3	209.7(3.0)	**11.0**
			2nd	95.0	24.8	210.6(7.1)	**10.6**
		Open	1st	91.8	24.0	226.1(9.0)	**4.1**
			2nd	92.4	23.7	222.3(3.1)	**5.7**
		Shade	1st	89.2	26.2	247.7(11.6)	**-5.1*****
			2nd	90.6	26.7	228.4(0.7)	**3.1**
	Slices	Tunnel	1st	91.8	48.1	194.9(4.5)	**17.3**
			2nd	90.9	47.2	214.9(0.8)	**8.8**
		Open	1st	89.3	48.0	211.9(5.5)	**10.1**
			2nd	86.0	47.2	227.0(0.8)	**3.7**
		Shade	1st	86.3	49.9	239.0(7.6)	**-1.4*****
			2nd	87.4	51.5	246.1(4.9)	**-4.4*****
Resisto	Fresh roots			27.0		434.4(0.7)	
	Thin chips	Tunnel	1st	90.7	27.4	384.4(4.1)	**11.5**
			2nd	93.1	24.7	357.8(1.0)	**17.6**
		Open	1st	91.5	26.3	363.1(18.6)	**16.4**
			2nd	91.6	24.5	362.6(7.5)	**16.5**
		Shade	1st	89.2	49.9	408.2(5.8)	**6.0**
			2nd	90.5	51.5	393.8(10.8)	**9.3**
	Slices	Tunnel	1st	88.6	93.1	382.9(4.5)	**11.8**
			2nd	89.0	51.6	369.5(5.2)	**14.9**
		Open	1st	86.1	99.3	366.2(5.0)	**15.7**
			2nd	82.0	51.6	401.0(7.4)	**7.7**
		Shade	1st	83.6	99.4	420.6(1.5)	**3.2**
			2nd	73.0	51.5	415.5(1.6)	**4.4**

* Drying time includes days and nights of samples spent on dryers.
**Each value represents the mean (standard deviation) of two extractions
***Negative value not significantly different from values in fresh sweet potatoes
Average and variation on day/night temperature and humidity respectively were 22°C (12-33°C) and 65% (25-95%) in ambient conditions; 26°C (11-55°C) and 63% (13-100%) inside the tunnel dryer.

Independent of the dryer or variety, average total carotenoid loss during drying was 9.2%. In all cases, levels of loss were less than 17.6%. Because control over drying was limited in these field conditions where final dry matter content varied, dry matter was included as covariate in the analysis of variance. The type of dryer had a significant effect ($p<0.05$) on total carotenoid loss. The three dryers presented significantly different results; on average there were13.0%, 10.0% and 1.9% losses for the tunnel dryer; open air dryer and shade dryer respectively. The ambient weather conditions were as follows. During the day, the relative humidity was low (25% minimum) and temperature was

high (33°C maximum), which favoured quick drying. At night, temperature dramatically dropped (12°C minimum) and relative humidity increased to 95%. Conditions inside the dryers differed. The relative humidity was lower inside the tunnel dryer during the day (13% minimum) but there was condensation (100% relative humidity) during the night. The temperature inside the tunnel dryer was similar to ambient temperature at night (11°C minimum) but was much higher in the day (55°C maximum). Slight but significantly lower retention using tunnel dryer compared to the open air dryer could be explained by higher temperature during drying (up to 55°C whilst the ambient temperature did not go above 33°C).

There was furthermore an issue with moisture evacuation from the product in the tunnel dryer, which is illustrated by the 100% relative humidity reached at night, that may have resulted from disadvantageous wind direction at this time of the year, blowing East-West whereas the tunnel was positioned North-South. As compared with the study on research station that was carried out with an optimally oriented and technically built tunnel dryer, the on-farm tunnel dryer apparently showed technical limitations. Nevertheless, in both studies, the difference between the provitamin A retention in the tunnel and open-air sun dryers was small (13% and 10% respectively) or non-significant (9.0% for all the solar and sun dryers) (see Chapter 4). The tunnel dryer protected against insects and rain but no significant difference in terms of provitamin A was found. This type of dryer was mostly designed for experimental use and, because its cost was estimated to be ten times the cost of an open air dryer and five times that of a shade dryer mainly (see Appendix 2b), it would not be a feasible proposition for Mozambican farmers to adopt. The open air and shade dryer would be the dryers most suited to rural situation in Mozambique because of lower cost and availability of most materials locally (in the farm or the next village).

Generally it was observed that the shade dryer worked well with thin chips but was not well adapted to traditional slices because of longer drying times leading to off-odours. Complete protection from sun light and milder temperatures in shade-drying compared to tunnel and open air sun dryers can explain the improved carotenoid retention from shade drying. Researchers, working with different commodities, have reported conflicting results regarding carotenoid losses during shade-drying. Chavez *et al.* (2007) reported that shaded-dried yellow cassava that contained carotenoids had improved

carotenoid retention compared to sun dried one, while Negi and Roy (2000), working on leafy vegetables, reported that higher carotenoid losses were obtained in shade and sun-drying as opposed to solar (cabinet) drying. Theses inconsistencies in literature could be the result of different environmental conditions (temperature, humidity and wind) and different products characteristics influencing carotenoid retention in drying.

5.3.2. Effect of variety of sweet potato on carotenoid retention in drying

Sweet potato variety had a significant impact on total carotenoid loss ($p<0.05$). Carotenoid loss for the Resisto variety was significantly higher than for MGCL01. Resisto with a dry matter content of 27.0% lost more carotenoids (mean 13.2%) than MGCL01 with a dry matter of 35.4% (mean loss of 5.2%) (Table 5-1). Similarly higher dry matters were associated with lower carotenoid losses during drying when working in a research station in Uganda using six different OFSP varieties (see Chapter 4).

5.3.3. Effect of chipping on carotenoid retention in drying

Traditional slices had an average thickness of *ca.* 5.2 mm whereas thick and thin chips were smaller at 2.9 mm and 0.4 mm thick respectively. When analysing the effect of two size reductions only (thin chipper and traditional slicing) on total carotenoid loss for the two sweet potato varieties (ANOVA two-way-analysis) the effect of chipping was not significant ($p<0.05$) (Table 5-1 seen previously). The effect of chipping treatment using three chipping treatment is reported in open air drying for both MGCL01 and Resisto varieties (Figure 5-2).

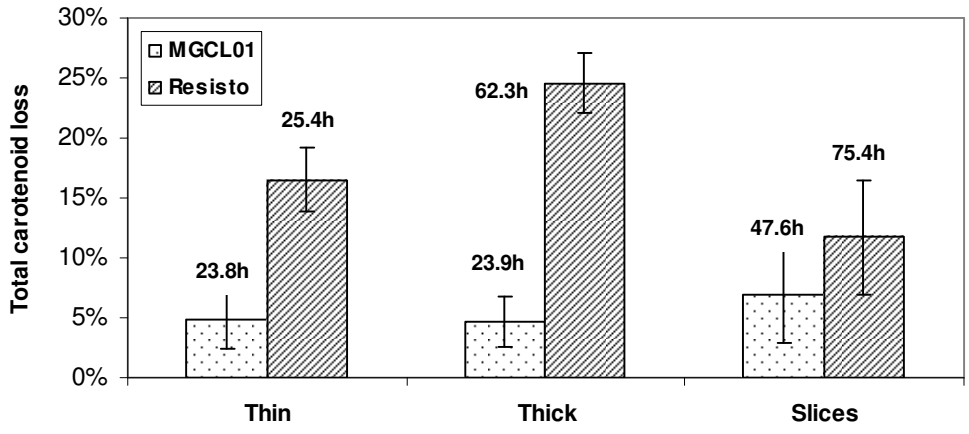


Figure 5-2: Effect of chipping on total carotenoid retention after drying in MGCL01 and Resisto varieties. Drying time (h) is given.

However, when considering the three size reductions and taking into account dry matter content as a covariate, chip size had a significant effect on carotenoid losses ($p<0.05$). There was no difference between the thin chips and slices but drying thick chips resulted in significantly higher total carotenoid losses than the other chipping methods, respectively 14.6% for thick chips and 10.7% for thin chips; 9.3% for traditional slices. Greater losses of total carotenoids in thick chips compared to traditional and thin chips can be explained by inadequate chip size: with a small surface area as compared to the volume, thick chips may have evacuated moisture less efficiently and the core of the chips may have been less protected during drying (as opposed to slices). This shall have resulted in an increased carotenoid degradation.

Results in Chapter 3 also showed that differences in chips surface area resulted in differential carotenoid loss in sun-drying. This is the first time that this has been reported for sweet potato. Working on the drying of carrots, Wang and Xi (2005) reported that β-carotene degradation increased with sample thickness and was also linked to moisture content reached. In this study a new finding was that there was furthermore a major varietal effect associated to chipping ($p<0.05$); total carotenoid loss was on average 17.6% on Resisto and 5.5% on MGCL01. Resisto chips produced using the thick chipper took a long time to dry (62.3h). The influence of chipping on Resisto compared to MGCL01 can be explained by its lower initial dry matter content that requires evacuating more moisture during drying and is in agreement with results from Chapter 4 and Hagenimana *et al.* (1999).

5.3.4. Effect of chipping on carotenoid retention in storage

Overall average losses in carotenoids from stored chips and slices after one month; two and four months of storage were 33.0%, 55.1% and 83.7% respectively (Figure 5-3).

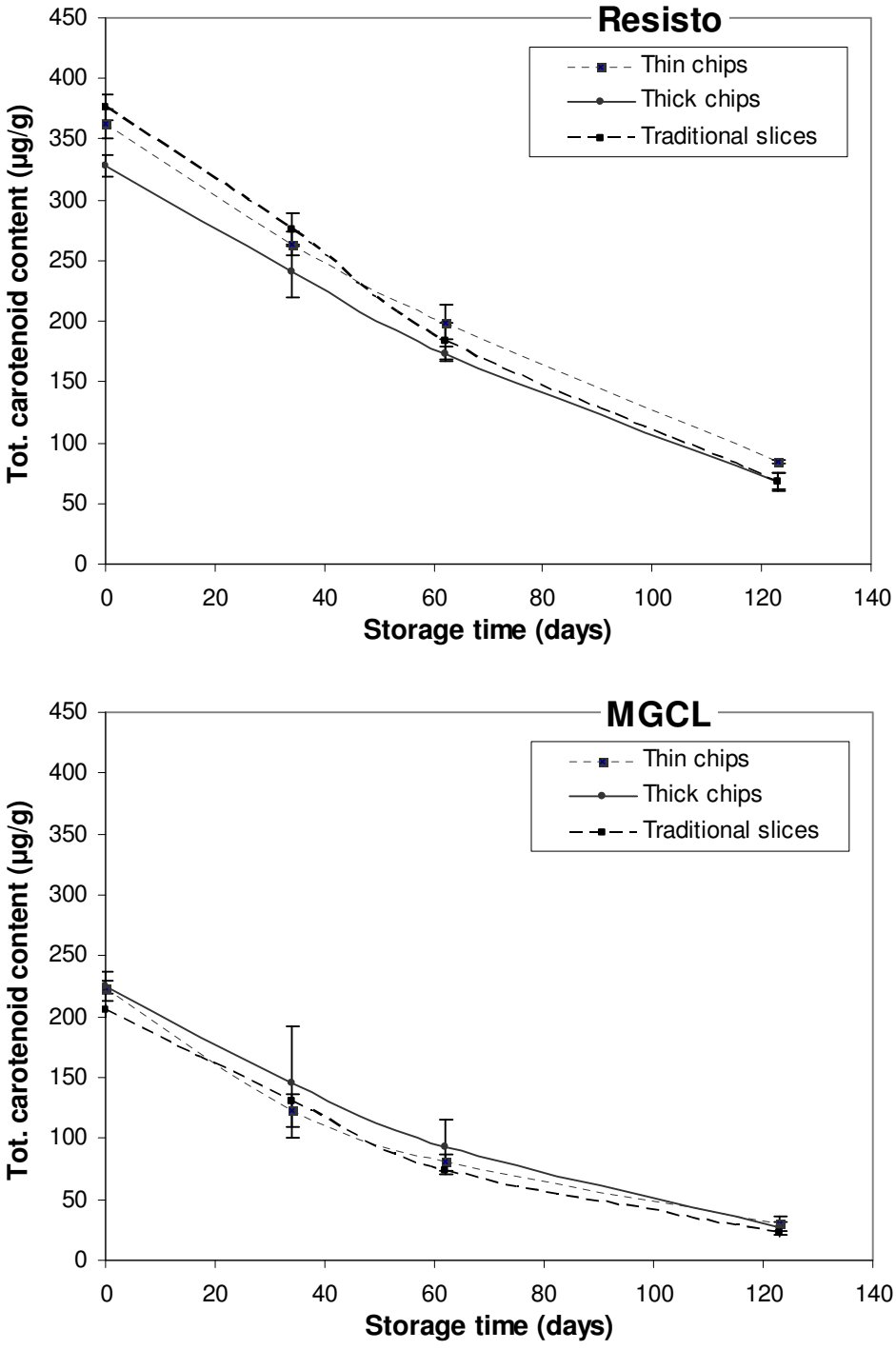


Figure 5-3: Kinetics of total carotenoid degradation (µg.g^{-1}) during storage of Resisto and MGCL01 sweet potato varieties chipped to three different sizes.
Mean of 2^2 replicate; error bars refer to standard deviation.

No effect of chipping was reported when analysing thin or thick chips or slices during storage (ANOVA two-ways; $p<0.05$). There was no interaction of effect of chipping and

storage time that confirmed that there was no effect of chipping consistently throughout the storage period (ANOVA two-ways; $p<0.05$). Working on pure β-carotene encapsulated in dextrose equivalent maltodextrin and comparing spray dried, drum dried and freeze drying, Desorby *et al.* (1997) found that larger particles obtained in drum drying had improved β-carotene stability over storage when compared with the other processes. Mills and Hart (1945) working on dehydrated sweet potato also found that six month-stored sliced material had higher carotene retention than flour at ambient temperature and concluded that sweet potato should be stored in the way they are dehydrated rather than milled into flour.

This present study demonstrated that there was no effect of chip size in stored samples and this for the first time. In this present study, the lack of difference with chipping however did not agree with the results by Desorby *et al.* (1997) and Mills & Hart (1945), but it is believed that the difference of retention observed in these above studies results of the very different sizes of samples tested (*i.e.* flour and slices) that may have resulted in differential porosity to air oxidation. Oxidation (by oxygen) was reported as the main factor responsible for carotenoid degradation in storage (Emenhiser *et al.*1999).The lack of differences in our study is hypothesised to result from similar air diffusion through the different chip/slice sizes (that did not differ as much in size than with flour and slices) of samples stored in jute bags.

5.3.5. Effect of variety on carotenoid retention during storage

There was a significant effect of the trial and variety during storage of dried sweet potato ($p<0.05$). Resisto with 26.8%, 47.8%, 79.3% loss after one, two and four months of storage, had lower total carotenoid losses than the MGCL01 variety with 39.3%, 62.4%, 88.2% respectively (Figure 5-3). This could possibly result from differential composition in other constituents that can enhance or delay carotenoid degradation: for instance, enhancers could be unsaturated fatty acids that are mostly linoleic and linolenic acids in sweet potato (Walter and Purcell, 1974) and were related to lipid peroxidation (Arya *et al.* 1979), and inhibitors of carotenoid oxidation could be phenolic compounds. Phenolic content has been positively correlated to antioxidant activity in various sweet potato varieties (Teow *et al.* 2007). More investigation is required to understand varietal differences with regard to carotenoid retention.

5.3.6. Correlation between spectrophotometer values, HPLC results and colour of samples

Analytical methods were compared for the measurement of carotenoid content. The correlation between the spectrophotometric readings and HPLC determinations was high (R=0.993; n=20; p<0.001) (Figure 5-4).

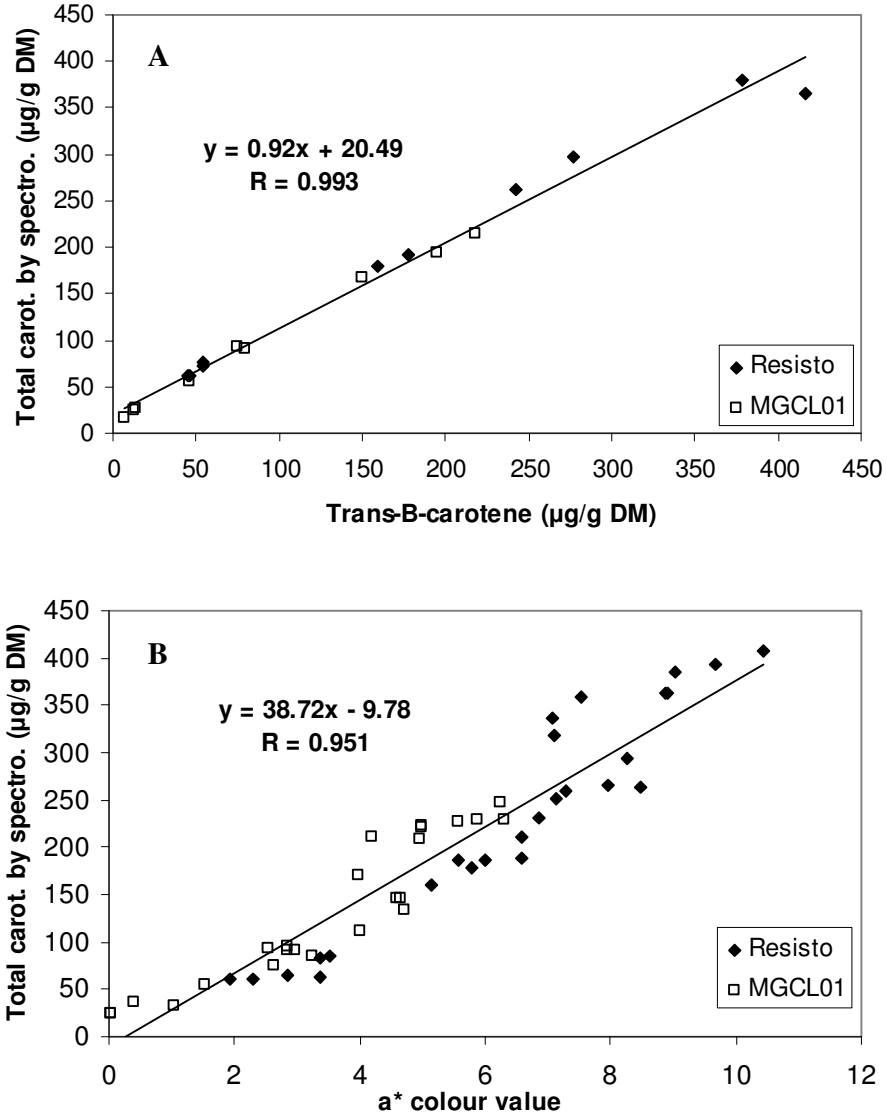


Figure 5-4: Correlation between total carotenoid content measured by spectrophotometer and trans-β-carotene measured by HPLC (n=20 extractions) (A) and correlation between a* colour value and total carotenoid content (n= 51 samples) (B) of sweet potato flour samples from Resisto and MGCL01 varieties measured at different stages of drying and storage.

Spectrophotometric readings can therefore be used for OFSP as an excellent estimate of total carotenoid content as shown earlier by Kimura *et al.* (2007) working with fresh

sweet potato roots of IAC 60-M-3-Brasilia and Resisto varieties. Colour of the flours from MGCL01 and Resisto varieties submitted to various drying treatments (tunnel, open or shade dryers) and three chippings (new, old chipper and traditional slices) was correlated to carotenoid content for each trial (Figure 5-4).

A significant correlation between a* redness and carotenoid content (16-408 $\mu g.g^{-1}$ dry basis) was obtained ($R=0.951$; $n=51$; $p<0.001$). This result was in agreement with Hagenimana *et al.* (1999) who found that a* and b* were the best factors to estimate total carotenoid content on sweet potato flour from yellow and orange cultivars. In closer accordance with our work, Bengtsson *et al.* (2008) showed that a* value was the best estimate of trans-β carotene content on sweet potato flours of seven freeze-dried sweet potato varieties from different batches (50-460 $\mu g.g^{-1}$ dry basis) ($R=0.96$; $n=76$; $p<0.001$). This is however the first time that colorimetric method has been used to estimate carotenoid degradation during storage of dried sweet potato. The very significant correlation between the colour measurement and carotenoid determination confirmed potential for rapid evaluation of carotenoid bleaching of OFSP flour. Rapid colorimetric evaluation could be useful in the process of on-farm development of OFSP drying and for quality monitoring purposes after drying and during storage.

5.3.7. Identification of individual carotenoids before and after storage

The individual carotenoid compounds before and after storage of dried sweet potato were tentatively identified by HPLC (Figure 5-5 A and B).

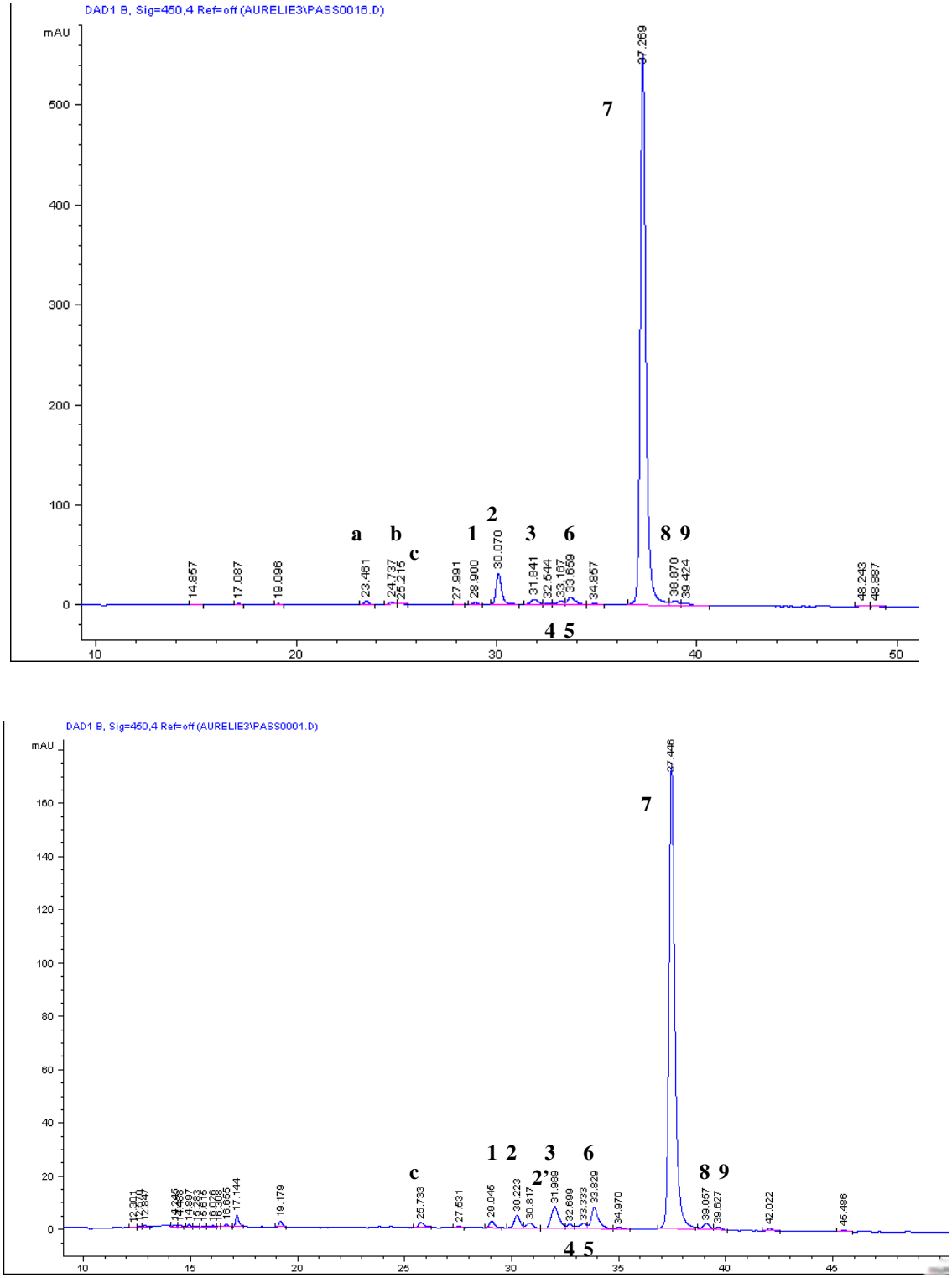


Figure 5-5: HPLC chromatograms at 450nm of the carotenoids of sliced Resisto OFSP after drying (A) and after 120 days storage (B).

a: Possibly -*β*-carotene-5,6,5',6'-diepoxide (23 min.) (414; **440**; 468nm); b: Possibly -*β*-carotene-5,6,5',8'-diepoxide (24 min.) (400; **422**; 450nm); c: unidentified (25 min.) (406; **424**; 450nm); 1: Possibly *13-cis-β*-carotene-5,6 epoxide (29min.) (main wavelengths: 416; **439**; 476nm); 2: possibly *β*-carotene-5,6 epoxide (30min.) (422;**446**; 472nm); 2': non-identified (31min.) (**452**nm); 3: possibly *β*-carotene-5,8 epoxide (32min.) (406;**428**; 452nm); 4&5: unidentified; 6: *13-cisβ*-carotene (34min.) (338;422;**444**; 472nm); 7: all-trans-*β*-carotene (37min.) (**452**; 478nm); 8: possibly 9 cis-*β*-carotene (39min.) (**446**; 472nm); 9: unidentified (39.5min) (**446**; 474nm)

Resisto had the same chromatogram profile as MGCL01. Therefore only the chromatograms for Resisto are shown. The main compound identified was trans-β-carotene (peak 7) resolved at 37 min and representing on average 84% of the total carotenoid concentration, both for dried and stored sliced Resisto or MGCL01 samples. Other peaks were minor compounds mostly degradation products of all-trans-β-carotene and even present in fresh root samples in very small quantities. This has not been reported by other authors. On average (for dried and stored sliced Resisto variety), percentages were the following; β-carotene 5,6-epoxide (4.0%), 5,8-epoxide (3.2%) 9-cis (1.3%) and 13-cis-β-carotene (3.1%). Trans-β-carotene, β-carotene 5,6-epoxide, 9-cis and 13-cis-β-carotene were previously identified using the same HPLC system on a different sweet potato variety (Chapter 3). Whilst the total carotenoid and β-carotene were sharply reduced, the ratio between them did not differ during the storage of dried sweet potato chips. In spite of the degradation of β-carotene, no clear increase of degradation products was clearly observed using the HPLC technique. There are only minor differences between the chromatographic profiles of those samples dried or dried and subsequently stored: peaks a (possibly β-carotene-5,6,5',6'-diepoxide); b (possibly β-carotene-5,6,5',8'-diepoxide) and c (25 min.unidentified) were found in dried chips of both varieties but peak c was only detected after four months of storage. There is also evolution of a peak 2'after four months of storage. This peak might be a degradation product of peak 2 (Dhuique-Mayer, pers. comm.). On the other hand, significant differences in the chromatographic profile of fresh and heated citrus juices (5h; 95ºC) have been described by Dhuique-Mayer *et al.* (2007). This present profile of carotenoids in OFSP flour showed that there were very few qualitative differences in the chromatogram of samples immediately after drying or after storage for 4 months.

5.3.8. Kinetics of carotenoid degradation during storage

Kinetics of carotenoid loss per variety are presented in Table 5-2.

Table 5-2: **Kinetic parameters of zero order and first order carotenoid degradation in Resisto and MGCL01 dried slices stored for four months**

	Order	Trans-β-carotene		5,6 epoxide-β-carotene		13-cis-β-carotene		9-cis-β-carotene	
		Rate constant (day-1)	R	Rate constant (day-1)	R	Rate constant (day-1)	R	Rate constant (day-1)	R
Resisto	0	-2.7643 (0.2500)	0.976	-0.1840 (0.0131)	0.985	-0.0376 (0.0054)	0.943	-0.0228 (0.0046)	0.896
	1	-0.0171 (0.0010)	0.990	-0.0249 (0.0025)	0.971	-0.0080 (0.0009)	0.963	-0.0102 (0.0012)	0.963
MGCL01	0	-1.5436 (0.2648)	0.922	-0.0989 (0.0144)	0.942	-0.0261 (0.0081)	0.796	-0.0180 (0.0057)	0.791
	1	-0.0251 (0.0028)	0.966	-0.0315 (0.0035)	0.966	-0.0115 (0.0033)	0.819	-0.0190 (0.0039)	0.893

Each value represents the mean (standard deviation) of two extractions

For trans β-carotene and β-carotene 5,6-epoxide, the coefficients of correlation with storage time were generally higher than R=0.95. MGCL01 variety fitted better first order kinetics whilst Resisto fitted equally zero and first order kinetics and this has not been reported previously. Instead, it has been shown that dried food fitted first order kinetics degradation during storage (Koca *et al.* 2007; Lavelli *et al.* 2007; Hidalgo and Brandolini 2008). Nevertheless, working on pure β-carotene powder, Minguez-Mosquera and Jaren-Galan (1995) demonstrated that degradation followed zero-order kinetics in organic anhydrous medium while in an aqueous medium it followed first-order kinetics. Zero order reactions are found when the substrate is in excess. Because Resisto had twice as much trans-β-carotene as MGCL01 this could possibly explain why the zero order reaction also fitted Resisto. This indicates that the oxidant had no limitation on the substrate which means that oxygen from the air could easily penetrate the product. There are a few discrepancies between the two models because in order zero Resisto degradation was faster than MGCL01 and order one the opposite. Because coefficients of correlation were higher in first order, particularly on MGCL01, the first order was considered. First order rates of degradation were -0.0171 day^{-1} for trans-β-carotene on Resisto and -0.0251 day^{-1} on MGCL01. The rate of degradation of β-carotene 5,6 epoxide was slightly faster than that of trans-β-carotene (0.0249 and -0.0315 day^{-1} on Resisto and MGCL01 respectively). Trans-β-carotene degradation rate has been compared with other carotenoids such as β-cryptoxanthin (Dhuique-Mayer *et al.* 2007) but to our knowledge comparisons between trans-β-carotene and 5,6-epoxy- β-

carotene rates have not been yet reported in the literature.

Although the percent of cis-isomers over total carotenoid increased with storage time (from 1-6% for 13-cis and 0.5-1% for 9-cis), the content of 13-cis-β-carotene and 9-cis-β-carotene, degraded, following first order kinetics. Isomers of β-carotene, 13-cis- and 9-cis-, degraded following first order kinetics however with coefficients of correlation with storage time lower than trans-β-carotene and 5,6 epoxide-β-carotene (*ca.* R~0.80). Rate constants of 13-cis- and 9-cis isomers on Resisto and MGCL01 being 0.0080; 0.0102 and 0.0115; 0.0190 day^{-1} respectively were less than that of trans-β-carotene (0.0171 and 0.0251 day^{-1}). This observation is significant because, to our knowledge, the rate of degradation of cis-isomers has not yet been reported elsewhere when working on storage. A degradation of cis-isomers jointly with trans-β-carotene in solar-drying was however reported in Chapter 3 and also by Mulokozi and Svanberg (2003). Globally the concentration of all the carotenoids was reduced during storage following a first order kinetics; the minor compounds considered as degradation products of β-carotene did not increase with storage. The exception was 5,8-epoxy-β-carotene, whose concentration fluctuated with storage time (Figure 5-6).

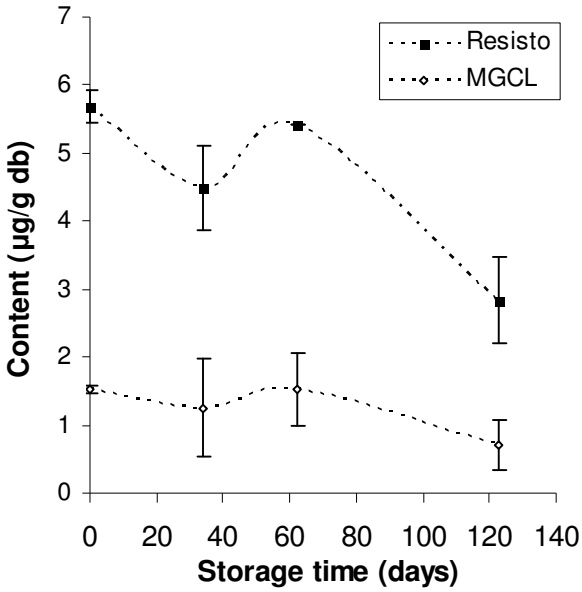


Figure 5-6: 5,8-epoxy-β-carotene content during storage of dried sliced OFSP (Resisto and MGCL varieties) on a dry weight basis (db).

β-carotene 5,8-epoxide could be an intermediate reaction product and could be the result of the degradation of trans-β-carotene or β-carotene 5,6-epoxide (Waché *et al.* 2003).

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Globally the concentration of all the carotenoids was reduced during storage; the minor compounds considered as degradation products of β-carotene did not increase with storage

5.3.9. Volatile formation in dried sweet potato during storage

In spite of the sharp rate of degradation of trans-β-carotene, no clear increase of degradation products was clearly observed in this study. In contrast, a clear increase of apocarotenals and epoxides was observed in other studies when pure trans-β-carotene and submitted to strong heat treatment (Mordi *et al.* 1993) or to chemicals (Rodriguez and Rodriguez-Amaya 2007) and using the HPLC technique.

In order to observe degradation products, another approach was adopted using Gas Chromatography for the analysis of lower-molecular weight compounds. The association of HPLC and Gas Chromatography techniques together with Mass Spectrum analysis has been used to follow the degradation products of pure β-carotene in several studies (Mordi *et al.* 1993). The volatile compounds identified by GC/MS were β-ionone; 5,6 epoxy-β-ionone and dihydroactinidiolide in accordance with the literature (Mordi *et al.* 1993; Sommerburg *et al.* 2003; Waché *et al.* 2003).

Waché *et al.* (2002; 2003; 2006) worked on the degradation of pure chemical β-carotene to produce aroma compounds. β-carotene was cleaved into β-ionone (9'-10' cleavage) that was further epoxidised into β-ionone 5,6 epoxide which was lactonised into DHA (Figure 5-7).

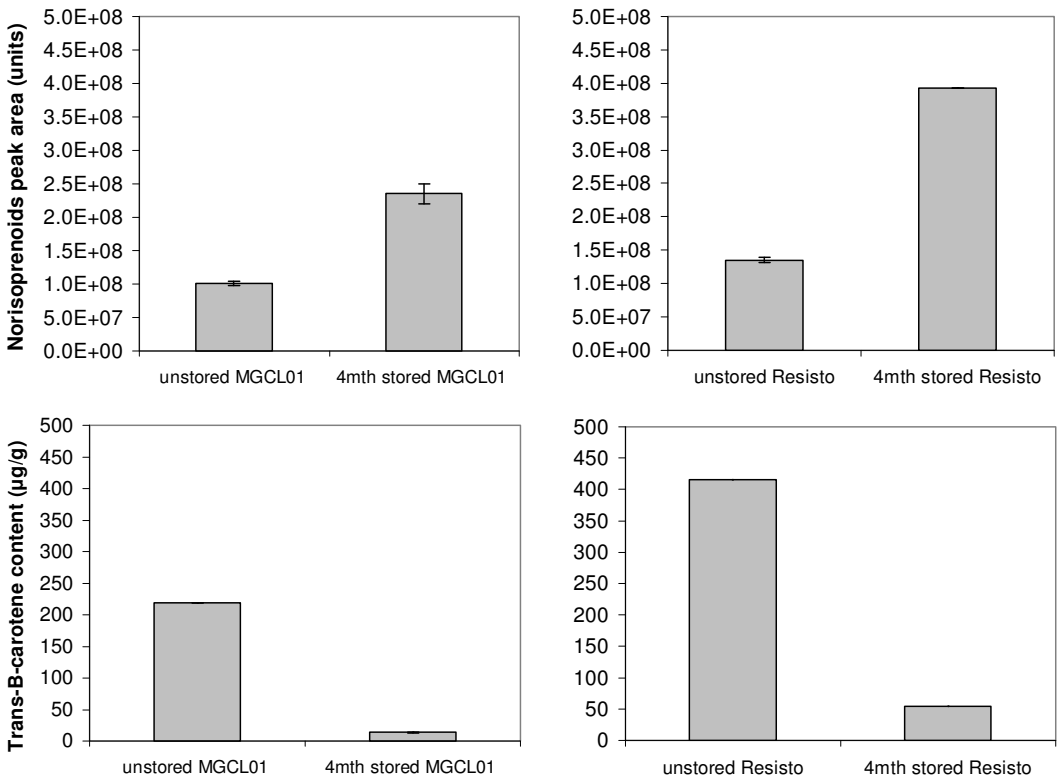


Figure 5-7: Comparison between the norisoprenoid formation and carotenoid degradation in dried traditional slices of Resisto and MGCL01 before storage and after 4 month (120 days) storage. Error bars refer to standard deviation of triplicate determinations.

In this present study, the highest quantities of volatiles (β-ionone; 5,6 epoxy-β-ionone and DHA) were found in 4 month-stored samples of dried slices of Resisto and MGCL01 (peak areas were $3.9.10^8$ and $2.4.10^8$ units respectively respectively) that had the lowest β-carotene content (54.4; 13.7 $\mu g.g^{-1}$ respectively).

The presence of norisoprenoids in unstored sweet potatoes could be attributed to a degradation of carotenoids either during storage of the flour or could be the result of naturally present norisoprenoids in fresh sweet potato. These could be formed by the plant metabolic system during the ripening process (Lewinsohn *et al.* 2005). In the biosynthesis pathway of carotenoid formation (see Chapter 1; Figure 1-12), carotenoid cleavage enzymes that are responsible for the degradation of neoxanthin to abscisic acid can be also responsible for formation of norisoprenoids (Balderman *et al.* 2005).

The lowest quantities of volatiles were found in unstored dried slices of Resisto and MGCL01 (peak areas were $1.4.10^8$ and $1.0.10^8$ units respectively) that had the highest β-

carotene content (416.0 and 218.5 $\mu g.g^{-1}$ respectively). Hence the quantity of volatiles produced seemed to be related to the β-carotene lost in storage: the more β-carotene is lost the more the norisoprenoids is produced However Resisto had more volatile production than MGCL01 in unstored and stored dried sweet potato. Resisto had double quantity of volatiles produced and double initial β-carotene compared to MGCL01. These findings are in agreement with Waché *et al.* publications The novelty of the present work, however, is that the volatile production was measured in a food product (complex matrix) as opposed to Waché's work that used pure β-carotene.

5.4. CONCLUSION

Compared to the earlier study carried out on a research station in Uganda (Chapter 4), retentions of carotenoids after on-farm drying in Mozambique were similar or even higher when working with the same type of dryers (tunnel or sun dryers). It was shown in both studies that a higher level of technology (tunnel dryer) as compared with a lower level of technology (open air sun drying on raised trays) did not necessarily lead to a higher carotenoid retention. This study concluded that drying and storing dried OFSP in a research station or on-farm did make a difference to the level of carotenoid losses.

Some factors influenced the level of carotenoid losses. Open air and shade dryers are better adapted to the rural situation in Mozambique because of the high cost of tunnel dryer for local farmers and processors. There were minimal losses of total carotenoid content when either of the sweet potato varieties tested was dried in the shade in comparison with the tunnel and open-air dryers. Shade dryer was suitable for drying small chips in dry, hot and windy weather but not for slices because of slow drying leading to quality problems. Therefore drying in open air drying (on a raised tray) was considered the most reliable solution for preserving the provitamin A content of OFSP for farmers and processors.

Storage had an major impact on carotenoid losses. This effect of variables such as chipping was limited in storage. It was postulated that was due to oxygen diffusion into the sample and was not restricted by the size of the chip. The lack of difference in carotenoid retention during storage is positive for farmers, because it means that they can limit their management costs because traditional hand slicing of sweet potato was as

good as the use of a mechanical chipper on the retention of carotenoids. On the other hand, the effect of variety was significant in drying and storage. More carotenoid losses were obtained with the variety having the highest moisture content immediately after drying but less carotenoid losses were obtained with this variety during subsequent storage. These observations require investigation on more varieties as this was noted in the research station based study (Chapter 4).

Provitamin A losses during storage were high and those were considered to be slightly greater than the losses determined on-station in Uganda (results after four month-storage). Higher losses in the on farm-study in Mozambique may be explained by higher temperatures and lower relative humidity especially in the day. Moreover the samples in the research station in Uganda were stored in a room and therefore protected from outside elements such as wind, dust whilst the samples stored on-farm were stored in jute bags hung inside a mud house and not protected from these elements. In both cases, however, the conclusions were similar in that the levels of losses were high and some quality issues (smell and insects) were associated with longer storage durations (though their impact was not specifically evaluated). In order to meet a significant part of daily nutritional requirements (see Chapter 4), provitamin A chip samples should not be stored for more than two months for MGCL and three months for Resisto.

For the rapid determination of carotenoid bleaching in sweet potato flour, a colorimetric method was recommended as a non-destructive technique compared to spectrophotometric or HPLC techniques. This could be used without requiring to any equipment other than a colorimeter and therefore adapted for on-farm studies.

Changes in the main carotenoids mainly fitted a first order kinetics degradation during storage in accordance with literature. There were few qualitative differences in carotenoids between un-stored and stored samples by HPLC. An increase in the concentration of products of degradation of β-carotene was not observed by HPLC; but when analysed by SPME/GC/MS production of volatile degradation products (β-ionone; β-ionone 5,6 epoxide and dihydroactinidiolide (DHA)), was observed in relation with the quantity of β-carotene degraded. This helped understand the degradation of provitamin A in dried sweet potato but further investigation is required to measure volatile production with storage time and further understand pathways of degradation.

CHAPTER 6.
EFFECT OF PRE-TREATMENTS ON CAROTENOID LOSSES IN DRYING AND STORAGE OF ORANGE-FLESHED SWEET POTATO

6.1 INTRODUCTION

Research in Chapters 4 and 5 demonstrated that high carotenoid losses occur during storage under ambient conditions. After four months of ambient storage at a research station (Uganda) and on-farm (Mozambique), 70.4% and 83.7% respectively of total carotenoids in average were lost. High losses of carotenoids during storage of dried OFSP chips was considered a critical issue because farmers tend to store dried sweet potato chips over a long periods of time (4-6 months). To decrease carotenoid loss during storage, many techniques could potentially be applied, which include storage at lower temperature (Cinar 2004); in vacuum-sealed or oxygen-free packaging (Emenhiser *et al.*1999); coating with cyclodextrine (Desorby *et al.* 1997) or blanching or chemical pre-treatment (Arya *et al.* 1979; Van Hal 2000). The low level of resources of farmers is a major constraint to find a feasible solution for improving storage. Consequently it was decided to explore the effect of low-cost chemical pre-treatments and blanching.

Pre-treatments of vegetables, fruits or roots have been reported to significantly reduce carotenoid degradation during storage (Dutta *et al.* 2005). Sulphite is being widely used as a food preservative to inhibit oxidation either by oxygen (in air) or enzymes (Isaac *et al.* 2006). Baloch *et al.* (1987) showed that sulphiting using sodium metabisulphite had a significant improvement in the carotenoid retention in diced dried carrots stored at 37ºC for four months. Retention of carotenoids in samples treated with sodium metabisulphite compared to un-treated samples was 76.6% and 51.1% respectively. Moreover, blanching of food using various processes (water, vacuum steam, in-can; microwave) with temperatures varying between 75-98ºC and times between 1 to 10 minutes has been reported to efficiently inactivate enzymes (*e.g.* peroxidases and lipoxygenases) that can

degrade carotenoids (Baloch *et al.* 1977). A combination of sodium metabisulphite (0.2%) plus salt (3%) was also shown to reduce carotenoids destruction in pre-treated and dried carrots (Arya *et al.* 1982). Salting is an ancestral process of food preservation that is still widely practiced in Africa and other parts of the world. Salting reduces osmotic tension of cells increasing the stress on bacteria and enzymes that can degrade food and its constituents such as carotenoids (Wijinker *et al.* 2006). In bread making, sodium chloride (NaCl) has a positive effect on the preservation of carotenoids, because it delays dough oxidation (King Arthur Flour Company 2009). It has been shown in the case of carrot that soaking in 10% NaCl solution for 30 min at 20ºC and blanching for 2.25 min at 96ºC before air drying significantly improved carotenoid stability (Speck *et al.* 1977). An alternative to salting is acidifying. Acidifying by addition of lemon or tamarin juice is used as traditional preservation process in Eastern parts of Uganda (Okwadi J, pers. comm. 2008). Acidity can help reduce enzymatic activity by lowering pH value and in addition some acids have an antioxidant property (*e.g.* ascorbic acid). Addition of ascorbic acid demonstrated a positive effect on the antioxidative activity of dried carrots during storage (Yen *et al.* 2008). Therefore acidifying needed to be further tested as a potential preservation of carotenoids.

The effect of low-cost pre-treatments on carotenoid retention during drying and storage of sweet potato has been reported by very few authors (Singh *et al.* 2006) in comparison with other roots or vegetables, such as, carrot. Therefore, there was a need to prove whether these pre-treatments (single or combined) had a significant effect on carotenoid stability in orange fleshed-sweet potato under conditions encountered in developing countries. This study explored the impact of various pre-treatments to reduce losses after drying and after storage (4-6 months) at ambient temperature in Uganda.

6.2 MATERIALS AND METHODS

6.2.1 Sweet potato root samples

Roots were collected from Wobulenzi, Luwero district, Uganda. Roots were of Ejumula (deep-orange-fleshed) and Kakamega (light-orange-fleshed) varieties. Mature roots (80 kg per variety for two trials) were harvested after a growing season of six months. Within a day after harvest, roots were washed and drained. All drying trials were carried

out in replicate and were undertaken during the same week with a three day interval using the same batch of sweet potatoes. Unpeeled roots were chipped using a rotary disc chipper (mean±standard deviation chip thickness (n=20):1.6±0.6mm;) (see Chapter 4).

6.2.2 Pre-treatments

Freshly chipped sweet potato (4 kg) (wrapped in a stiff mesh cotton net) was immersed either in 5L deionised water mixed with chemical at ambient temperature for 30 minutes or blanched in boiling deionised water. For the initial experiment, the treatment solutions were: sodium metabisulphite (1% w/v) (pH = 4.5); ascorbic acid (1% w/v) (pH = 2.5) and salt (1% w/v) (pH = 6.7). The pH value of the solution was recorded at 25ºC (Thermo Orion pH/conductivity meter). Samples were blanched at a temperature of 96ºC (TEL-TRU Thermometer, Rochester, USA) at NARL, Kawanda, Uganda (altitude 1193m). Temperature in the core of the sample varied between 60-82ºC during the blanching process. In order to determine the optimal blanching time, the peroxidase test was used. The peroxidase test procedure followed an adaptation of FAO peroxidase test for vegetables (Enachescu Dauthy 1995). Deionised water (20ml), 1ml of guaiacol (1% in 96% ethylic alcohol) and hydrogen peroxide (0.3%) were successively added on 10g of crushed, blanched sample. A rapid and intensive brown-reddish tissue and liquid colouring indicated a high peroxidase activity (positive reaction). A gradual appearance of a pink colour indicates an incomplete peroxidase inactivation. However peroxidase was considered inactivated if no colour appeared in the first minute because later appearance of brownish colour could be a bias due to the air oxidation of guaiacol.

For the main experiment, solutions were sodium metabisulphite (0.5% w/v) (pH = 4.9); citric acid (0.5% w/v) (pH = 2.0); ascorbic acid (1% w/v) (pH= 2.5); salt (1% w/v) (pH = 6.7); citric acid: NaCl salt (0.5%:1%); ascorbic salt (1%:1%); sodium metabisulphite:citric acid (0.5%:0.5%) (pH = 2.2) and deionised water (pH = 6.7).

The loss in soluble solid (LS) was defined as the percentage loss between the dry solid mass before drying and after drying (Baloch *et al.* 1977). The following equation (1) was used:

$LS = 1 - \frac{DM\ m}{DM_i\ m_i}$ (1) where DM is the dry matter (%) after drying and DM_i the initial dry matter on fresh chip and m is the mass of sweet potato chips after drying and m_i the initial mass of chips before drying (4kg). Total carotenoid content on an insoluble solid basis was determined by multiplying total carotenoid content on a fresh basis per percentage of insoluble solid: 1-LS.

6.2.3 Drying

Pre-treated chips were evenly spread on drying trays with a density of 3.9 $kg.m^{-2}$ (based on the sample weight before pre-treatment) (see Chapter 2 section 2.2.3). Ambient temperature, humidity, wind speed and irradiance were recorded every 30 min when samples were on the dryers using on a Vantage Pro- meteorological station (Davis Instruments, California, USA) and mean drying time was recorded as described in Chapter 4 (Table 6-1).

Table 6-1: Environmental conditions during open–sun drying of OFSP chips and drying time in Uganda

Experiment	Trial	Ambient Temperature (ºC)	Ambient relative humidity (%)	Wind speed ($m.s^{-1}$)	Irradiance ($W.m^{-2}$)	Time on dryers (h)	Time under shelter* (h)
Initial	1st	26 (18-33)	66 (30-100)	2 (0-6)	392 (64-887)	17	34
	2nd	31 (28-34)	33 (22-49)	4 (0-6)	504 (148-863)	5	0
Main	1st	28 (22-30)	57 (46-77)	9 (6-13)	636 (19-888)	8	18
	2nd	28 (18-34)	49 (28-100)	5 (2-11)	615 (110-958)	13	16

Mean (Minimum-Maximum)
*does not include time on dryers

All samples were removed from dryers and placed under a shelter at night and when it rained. Dry matter content of dried chips ranged between 85.2% and 93.3%.

6.2.4 Storage

The final quantity of dried chips was about 1.4 kg for Ejumula and 1.5 kg for Kakamega. Immediately after drying, two lots of about 200g of chips were collected and placed in the freezer: one sample was for carotenoid analysis on site (NARL); the other sample was for carotenoid analysis in the UK. The rest of the chips were stored in woven polypropylene bags. These were stored in a room at ambient temperature and collected

after 1 month (31 days); 2 months (62 days); 4 months (125 days) and 6 months (187 days). Temperature and humidity were recorded every four hour using Tinytalk temperature/humidity sensors (RS Components Ltd, UK) during storage.

6.2.5 Carotenoid analysis

Carotenoid analysis was undertaken both in the UK (for dried samples only) and in Uganda (for dried samples that have been stored). In all cases, collected samples were stored in the freezer in Uganda (-20°C). Dried sweet potato chips were transported in cooler bag to the UK and stored in the freezer upon arrival. These were milled before analysis using a Laboratory mill 3600 (position 3) (at NRI, UK). Dried and stored sweet potato chips were milled using a Glen Mills, Clifton, model C/11/1 (position 17) (at NARL, Uganda) (Chapter 2 section 2.3.1). In Uganda or in the UK, total carotenoid extraction and analysis were carried out following method by Rodriguez-Amaya and Kimura (2004) (Chapter 2 section 2.3.4). For quicker partitioning 10% NaCl solution (50ml) replaced deionised water. Total carotenoid content was determined using a Genesys 10UV /UV-visible spectrophotometer (Uganda) or a Diode Array detector spectrophotometer (Hewlett Packard HP8452A) (UK) at 450 nm.

6.2.6 Statistical analysis

Normality was assessed for all the samples ($p<0.05$). Analysis of variance (ANOVA) was carried out to determine whether there were significant differences between samples with one up to four factors. A significant difference between samples was determined using the Tukey test ($p<0.05$). Correlations between samples analysed in two different laboratories were determined using Pearson test ($p<0.01$). All data were processed on SPSS 15.00 (SPSS UK Ltd. Woking Surrey) for Windows software.

6.3 RESULTS

6.3.1 Effect of pre-treatments on drying

Initial experiment

The effect of initial experiment with pre-treatments on total carotenoid content after drying are presented in Tables 6-2 and 6-3. Two way-analysis of variance on two varieties and replicate trial were determined both on a dry weight basis and on an

insoluble solid basis. Pre-treatment had a significant impact on carotenoid content using both dry weight basis (Table 6-2) and insoluble solid basis (Table 6-3) ($p<0.05$).

Table 6-2: Total carotenoid content on a dry weight basis (db) for Ejumula and Kakamega varieties of orange-fleshed sweet potato after single pre-treatement and drying

Pre-treatment	Total carotenoid content ($\mu g.g^{-1}$) db		Loss in soluble solid (%)	
	Ejumula	Kakamega	Ejumula	Kakamega
Un-soaked	258.5 (21.1) [a]	123.2 (2.9) [b]	1.67	-0.92
1%Salt	262.6 (44.8) [a]	115.1 (1.4) [a]	8.73	4.94
1% Sodium Metabisulphite	275.3 (21.4) [a]	121.2 (9.0) [ab]	8.21	4.62
1% Ascorbic acid	281.7 (42.0) [a]	125.1 (4.3) [b]	7.54	2.93
Blanched	406.2 (33.0) [b]	164.9 (19.7) [c]	41.56	33.12

Each value represents the mean (standard deviation) of 2^2 analyses (two drying trials; two extractions per trial). Two-way ANOVA (factors: pre-treatement (5 levels); trial (2 levels); Different letters a, b, c, in columns represent significant differences between treatments. Tukey test; $p<0.05$. Pearson correlations ($p<0.05$; $n=10$) between loss in soluble solid and carotenoid content (db): Ejumula: $R= 0.787$; Kakamega $R=0.912$.

Table 6-3: Total carotenoid content on water insoluble basis (insb) for Ejumula and Kakamega varieties of orange-fleshed sweet potato after single pre-treatement and drying

Pre-treatment	Total carotenoid content ($\mu g.g^{-1}$) insb	
	Ejumula	Kakamega
Blanched	206.9 (25.1) [a]	95.0 (1.9) [a]
1%Salt	210.7 (31.2) [ab]	95.5 (2.2) [a]
1% Sodium Metabisulphite	226.9 (27.2) [bc]	101.9 (4.1) [b]
1% Ascorbic acid	230.7 (36.5) [c]	106.1 (4.9) [b]
Un-soaked	233.5 (25.8) [c]	111.9 (5.5) [c]

Each value represents the mean (standard deviation) of 2^2 analyses (two drying trials; two extractions per trial).Two-way ANOVA (factors: pre-treatement (5 levels); trial (2 levels); Different letters a, b, c, in columns represent significant differences between treatments. Tukey test; $p<0.05$.

Determination of total carotenoid content of Ejumula variety on a dry weight basis showed that un-soaked, salt, sodium metabisulphite and ascorbic acid treated samples did not differ significantly. Blanched samples had the highest carotenoid level after drying (Table 6-2). (Using the peroxidase test, the adequate blanching time for peroxidase inactivation was 11 minutes for Ejumula (4kg) and 8 minutes for Kakamega (4kg) varieties respectively). Determination of total carotenoid content of Kakamega variety on a dry weight basis showed that salt-treated samples had the lowest carotenoid

level followed by sodium metabisulphite and ascorbic acid treated samples. Blanched samples had the highest carotenoid content after drying (Table 6-3). On the other hand while total carotenoid content was determined on insoluble solid basis (Table 6-4), blanched samples had the lowest value together with salt-treated samples for both varieties. Sodium metabisulphite treated samples had similar carotenoid level to salt-treated samples for Ejumula but significantly higher for Kakamega. Sodium metabisulphite treated samples were not significantly different from ascorbic acid treated and un-soaked samples for Ejumula variety. Un-soaked samples had significantly higher carotenoid level than the soaked samples for Kakamega variety. Although the general trend was the same for both varieties, varietal effect would need more varieties and more replications would be needed to be assessed.

For all of the samples from the single pre-treatment experiment, very significant correlations were obtained between loss in soluble solid and carotenoid content on a dry weight basis with correlations coefficient R of 0.787 and 0.912 on Ejumula and Kakamega respectively ($n=9$; $p<0.01$) (Table 6-3). A relationship between carotenoid content and soluble solid loss was obtained in the same way by Baloch *et al.* (1977) working on the loss in soluble solids and increase in carotenoid content (basis on leached material) in carrots. This work showed that leaching of soluble solids is a major factor responsible for the apparent increase in carotenoid content (Baloch *et al.* 1977). In order to obtain a fairer value of carotenoid degradation on soaked or blanched samples working on an insoluble solid basis has therefore been recommended. Leaching of solids occurring during blanching can artificially increase the level of retention. Furthermore, it was observed in our trials that drying of blanched chips was difficult because starch gelatinisation rendered the chips sticky. A similar observation was made by Van Hal (2000) who raised the issue that blanching that could "act as a form of incomplete cooking". Gelatinisation could also be a problem at milling because gelatinised samples become very hard (Van Hal 2000). Consequently blanching was not carried out in the second experiment involving combined pre-treatments. In a potential future study, more work should be required to optimise blanching parameters (temperature, time, peroxidase inactivation) in order to limit sweet potato gelatinisation.

Main experiment

Optimal concentrations of pre-treatment solutions have been tested in a pre-trial experiment. In the main experiment pre-treatments concentration of sodium metabisulphite in solution was reduced from 1% to 0.5% because of the risk of allergy. Sodium metabisulphite is a food additive (E223) and FAO/WHO Expert Committee on Food Additives stated that its consumption should not exceed 0-0.07mg/kg body weight/day (Russell and Gould 2003). Results of main experiment on total carotenoid content are presented in Table 6-4 on an insoluble basis.

Table 6-4: Total carotenoid content (µg.g^{-1}) on water insoluble basis (insb) for Ejumula variety of orange-fleshed sweet potato after combined pre-treatement and drying

Pre-treatment	TC insb**
Deionised water (control)	237.7 (24.0) [a]
0.5% Citric acid	232.2 (17.6) [a]
0.5% Sodium Metabisulphite	250.0 (12.8) [b]
1% Salt	251.4 (18.8) [b]
0.5% Citric acid; 1% Salt	254.7 (34.6) [bc]
0.5% Citric acid.; 0.5% Sodium Metabisulphite	259.6 (13.8) [cd]
1% Ascorbic acid.; 1% Salt	260.4 (12.6) [cd]
1% Ascorbic acid	260.8 (11.5) [d]
Un-soaked	269.4 (14.9) [e]

Each value represents the mean (standard deviation) of 2^2 analyses (two drying trials; two extractions per trial).Two way ANOVA (factors: pre-treatement (9 levels); trial (2 levels)); Different letters a, b, c, d, e in columns represent significant differences between treatments. Tukey test; $p<0.05$.

As in the initial experiment, the un-soaked (un-treated) samples had the highest carotenoid content after drying (269.4µg.g^{-1}) ($p<0.05$). Within the soaked samples, samples dipped in deionised water had the lowest carotenoid content (237.7µg.g^{-1}) together with citric acid treated samples (232.2µg.g^{-1}) ($p<0.05$). Compared to samples dipped in deionised water, most pre-treatments (salt (251.4µg.g^{-1}); sodium metabisulphite (250.0µg.g^{-1}); ascorbic acid (260.8µg.g^{-1}); and combined pre-treatments) had a positive impact on carotenoid content. Working with deionised water-treated sample as control therefore proved that pre-treatment had an impact on carotenoid content. Nevertheless this also means that the soaking process had a disadvantageous effect on carotenoid retention after drying compared to non-soaking. Citric acid did not have an influence on the carotenoid retention.

Citric acid only has acidifying properties whilst ascorbic acid has also antioxidant properties, and can therefore reduce oxidation of carotenoids (that is caused, for instance, by exposure to air during drying). Addition of NaCl improved the carotenoid content of citric acid-treated samples (232.2 against 254.7µg.g^{-1} respectively) however the response was not consistent for the two trials (standard deviation of 34.6). Addition of NaCl did not significantly improve the content of those samples treated with ascorbic acid (260.4µg.g^{-1} against 260.8µg.g^{-1} respectively). Addition of citric acid slightly improved the content of sodium metabisulphite-treated samples (259.6 against 250.0 µg.g^{-1} respectively). In summary, the combination of two pre-treatments (citric acid-salt; ascorbic acid-salt; metabisulphite-citric acid) did not make a major impact on carotenoid retention after drying. A lack of consistency between the two trials was in particular observed on the samples treated with salt-citric acid (standard deviation of 34.6). This could have resulted from non-homogeneous application of chemical. Alternatively to non-homogeneous application of chemical, differences between trials might result from the difference of weather observed; rain in the second trial may have washed some of the samples' chemicals; humidity reached 100% in the second trial (Table 6-1).

6.3.2 Effect of pre-treatment on storage

Temperature and relative humidity during storage

The temperature and humidity in the room where the samples were stored was generally very constant and consistent during the periods of storage (Figure 6-1).

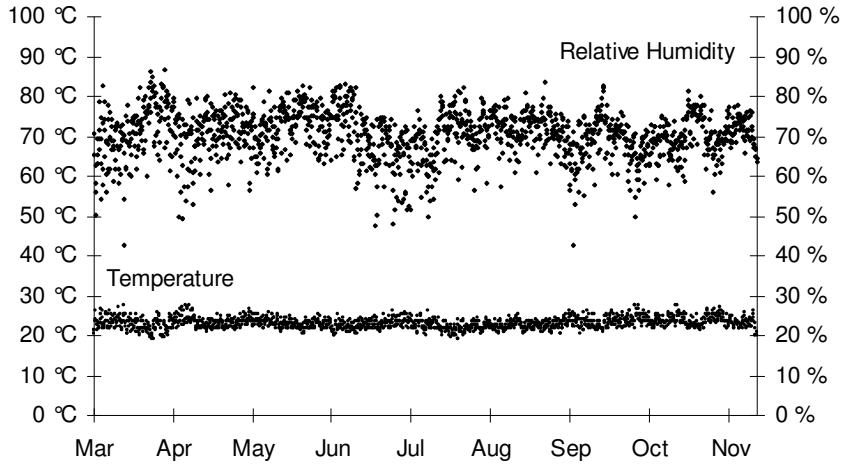


Figure 6-1: Temperature and relative humidity in the storage room for sweet potato chips over 8 months. Record taken every 4h using Tinytalk temperature/humidity sensors.

The mean temperature and humidity were 23.1ºC and 70.5% respectively over an 8 month-period with minimum-maximum variations between 19.1-27.7ºC and 42.8-86.5%.

Carotenoid loss during storage for the main experiment

Total carotenoid loss was determined at different times between one and six months of storage for dried samples of the Ejumula variety from the main experiment (Table 6-5).

Table 6-5: Total carotenoid content (µg.g^{-1}) of Ejumula variety of orange-fleshed sweet potato after one, two, four and six month-storage influenced under combined pre-treatments on an insoluble solid basis

Storage time (mths)	Deio-nised water [a]	0.5% Citric acid [a]	0.5% Citric acid 1% Salt [a]	1% Salt [b]	1% Ascor-bic acid.; 1% Salt [bc]	Un-soaked [c]	0.5% Sod. meta. [cd]	1% Ascor-bic acid [cd]	0.5% Sod. Meta. 0.5% Citric acid [d]
1	123.4 (9.9)^{a}	132.3 (6.1)^{abc}	126.4 (20.8)^{ab}	134.9 (5.5)^{abc}	137.0 (15.6)^{abc}	144.1 (2.8)^{cd}	138.5 (2.8)^{bc}	155.1 (6.0)^{de}	163.0 (3.1)^{e}
2	91.2 (14.4)^{a}	93.4 (12.7)^{a}	98.3 (35.6)^{a}	119.3 (25.4)^{b}	123.5 (12.3)^{b}	126.1 (16.9)^{b}	123.8 (6.7)^{b}	99.4 (31.4)^{a}	126.0 (4.4)^{b}
4	74.5 (12.4)^{b}	54.0 (17.6)^{a}	63.4 (20.3)^{a}	76.2 (20.2)^{b}	84.6 (11.1)^{bc}	104.8 (2.1)^{d}	92.6 (9.5)^{c}	96.1 (7.8)^{c}	81.9 (16.0)^{bc}
6	41.3 (13.1)^{ab}	37.1 (3.9)^{a}	42.1 (29.2)^{ab}	43.0 (22.9)^{ab}	47.7 (16.3)^{ab}	47.4 (14.4)^{ab}	51.2 (2.6)^{b}	51.6 (8.0)^{b}	53.3 (13.5)^{b}

Each value represents the mean (standard deviation) of 2^2 analyses (two drying trials; two extractions per trial). Two way ANOVA (factors: pre-treatement (9 levels); trial (2 levels) per storage month; Different letters a, b, c, d, e in lines represent significant differences between treatments. Tukey test; $p<0.05$.

Globally deionised water, citric acid and citric acid-salt pre-treated samples had significantly lower carotenoid content over storage than the other treatments (Global Two way-ANOVA; $p<0.05$). Salt treated samples had higher retention than deionised water, citric acid and citric acid-salt pre-treated samples but lower retention than un-soaked samples. Salt-ascorbic treated samples had similar retention to un-soaked samples. Ascorbic acid and sodium metabisulphite treated samples also had similar retention to un-soaked samples. Only the combination of sodium metabisulphite-citric acid resulted significantly higher retention than un-soaked samples over storage but similar retention to ascorbic acid and sodium metabisulphite treated samples. Hence pre-

treatments with ascorbic acid and sodium metabisulphite and combined treatments (ascorbic acid-salt; sodium metabisulphite-citric acid) had a significant impact on carotenoid content during storage compared to control in deionised water in a global analysis over storage.

Two way-analysis of variance (factors: pre-treatment (9); drying trial (4)) conducted for each storage time revealed that after one month of storage, sodium metabisulphite-citric acid ascorbic acid, sodium metabisulphite, un-soaked samples had better carotenoid retention than control (deionised water) ($p<0.05$) (respectively 163.0; 155.1; 138.5; 144.1 and 123.4 $\mu g.g^{-1}$) (Two way-ANOVA per storage time; $p<0.05$). Sodium metabisulphite-citric acid treated samples also had significantly greater content than un-soaked (un-treated) samples. On the other hand citric acid and citric acid-salt, salt and ascorbic acid-salt had similar retention to control in deionised water after one month of storage (respectively 132.3; 126.4; 134.9; 137.0 and 123.4 $\mu g.g^{-1}$). Therefore, there was a significant effect of adding sodium metabisulphite or ascorbic acid to the soaking water for samples but there was no effect of adding citric acid or salt. After a two month-storage period there was no difference between un-treated and sodium metabisulphite-citric acid treated samples (respectively 123.8 and 126.0 $\mu g.g^{-1}$). After six months of storage, no difference between the effect of deionised water soaked and un-soaked samples (respectively 41.3 and 47.4$\mu g.g^{-1}$) and between most pre-treated samples though sodium metabisulphite, ascorbic acid and citric-sodium metabisulphite treatment still gave the highest carotenoid values (51.2; 51.6 and 53.3$\mu g.g^{-1}$). Citric acid treated samples had the lowest total carotenoid content ($p<0.05$) (37.1$\mu g.g^{-1}$).

6.4 DISCUSSION

Researchers have investigated the role of a range of pre-treatments, such as sodium chloride, sulphur additives and other anti-oxidants, on the stability of carotenoids in food vegetables, fruits and roots after drying and storage, however, with mixed results (Arya *et al.* 1979). Our work showed that the use of dipping pre-treatment had little effect on carotenoid preservation in OFSP after drying (Table 6-5) and during storage (Table 6-6). In particular, after drying, samples treated with sodium metabisulphite (0.5%) or ascorbic acid (1%) did not have higher carotenoid content than un-soaked samples but had better content than samples soaked in deionised water (Table 6-5). In accordance

with our results, no difference in β-carotene content between sulphurated (by burning element sulphur in an enclosed place) and un-treated (non-sulphurated) apricots after drying in hot air or in sun was shown by Karabulut *et al.* (2007). After drying, however, samples treated with sodium metabisulphite or ascorbic acid had better content than samples soaked in deionised water (Table 6-5). Working on pineapple and papaya, Siahn and Ishak (1990) also reported an improvement of total carotenoid content using sodium metabisulphite (0.2%; 0.4%; 0.6%) after drying with deionised water as a control. However, in another study by Yen *et al.* (2008) working on pre-treatment with a solution of ascorbic acid (0.1%) + glucose (1%) showed that diced carrots treated with this solution and then hot air or freeze dried had significantly better β-carotene content than un-soaked ones, and disagrees with our results. These divergences in results after drying between authors could be the result of the differences in food products composition, type of chemical added and incorporation of the chemical in the food matrix.

After a typical storage period for dried sweet potato (4-6 months), there was no significant effect of sodium metabisulphite (0.5%) as compared with un-treated or deionised water-dipped samples (Table 6-6). Working with diced orange-fleshed sweet potato pre-treated with 0.2% sodium bisulfite for 5 minutes at room temperature, Cinar (2005) also did not show improvement in half life of metabisulphite-treated samples after 120 days at 4ºC or 25ºC. Losses of β-carotene from dried carrots after 12 months at room temperature (22-26ºC) with deionised water as control; 0.05% and 0.2% sulphite treated were 60%; 56% and 59% respectively (Zhao and Chang 1995). This was in accordance with our results that showed that there were few differences between untreated and sodium metabisulphite treated samples after six months. In contrast, Baloch *et al.* (1987) showed that soaking carrots in sodium metabisulphite before dehydration had significant effect on carotenoid retention after drying and after storage at 37ºC for 440 days (14 months). Carotenoid retention was further improved when in addition to sulphiting carrots were also blanched. The differences of results with sodium metabisulphite might be explained by the incorporation of sulphite into the product which may further influence the degradation of sulphite in storage (Baloch *et al.* 1987). Zhao and Chang (1995) described a sharp decrease in residual sulphite content in sulphite-treated carrots over storage (92% loss in 12 month). Sulphite may be lost by reaction with disulfide groups or thiols in proteins and low molecular weight

intermediates (*e.g.* gluthathione). Other reactions such as the formation of complexes between sulphite and aldehydes or other carbonyls compounds (*i.e.* some sugars) can remove its antioxidant activity (Russell and Gould 2003).

Lack of effect of pre-treatments to reduce carotenoid losses during drying can be explained by different reasons. Ascorbic acid is easily oxidised because it is water soluble and temperature sensitive, (Labuza 1973) (Table 6-6). Sodium chloride is an inexpensive traditional food preserver. Salt is known for its impact on osmoregulation and it can denaturize enzymes, such as peroxidases, that could degrade carotenoids in sweet potato. The use of NaCl (1%) did not reduce carotenoid degradation during storage (Table 6-6). Latapi and Barrett (2006) working on tomato, also reported that carotenoid degradation rate was reduced by the use of sodium metabisulphite but not by addition of NaCl (10%). Likewise, Baloch *et al.* 1997 showed that salt (2%) did not have an influence on carotenoid retention in dried tomato powder stored at 40ºC for 90 days. In contrast, Arya *et al.* (1979) reported that NaCl (5%) significantly reduced the degradation of carotenoids in sun-dried carrots stored at room temperature (16-32ºC) for 3 months. It should also be noted that the increase of carotenoid extractability by the use of salt may artificially increase the results (Jaramillo-Flores *et al.* 2005).

Furthermore, soaking samples (30 minutes) at ambient temperature (water temperature ~15ºC) resulted in lower carotenoid content after drying as compared with untreated samples (Table 6-5). This was believed to result from leaching of carotenoids in the water, which had an orange colour after the sweet potato chips had been removed. A similar observation was made by Sian and Ishak (1990) after water blanching (70-100ºC; 1-12 minutes) papaya and pineapple. In most studies, the control sample is soaked in deionised water (Baloch *et al.* 1987; Siahn and Ishak 1990; Jaramillo-Flores *et al.* 2005). It was shown, however, in this study that un-treated samples should also be used as extra control because they gave a different result than deionised water-soaked samples. Moreover the use of un-treated samples is a way to compare the benefit of using no treatment at all (*i.e.* samples simply dried such as farmers would traditionally do) to the benefit of using pre-treatment. In this study, a reduced soaking time could also have been better. A reduced soaking time limited carotenoid loss in the solution (Sian and Ishak 1990). Soaking time of 5 minutes was used by Baloch *et al.* (1987) on carrots. However, in this study, there were no differences in total carotenoid content of soaked or

un-soaked samples after 6 months of storage (Table 6-6). In conclusion the use of soaking as pre-treatment during storage had a limited impact on carotenoid retention.

6.5 CONCLUSION

The tested pre-treatments (chemicals or blanching) were selected because they were affordable and locally available in Uganda.

The main outcomes were the following:

- Soaking the chips in water led to the greatest loss of carotenoids. After drying, un-soaked samples had higher retention than all the soaked samples.
- Most pre-treated and soaked samples (salt-treated; ascorbic acid and sodium metabisulphite-treated) had higher retention than control that had been dipped in deionised water. Therefore the addition of chemicals to the water reduced the loss of carotenoids. Sodium metabisulphite was the most significant in terms of reducing losses. A slight improvement in the carotenoid retention in the first month of storage compared to control (deionised water) was not sufficient for these pre-treatments to be more widely promoted. Combining the chemicals (salt-ascorbic acid; salt-citric acid and citric acid-sodium metasulphite) did not appear to have a synergistic effect. Lack of improvement was believed to result from the degradation of the chemicals in storage as reported by Zhao and Chang (1995). Alternative approaches *(e.g.* coating, improved packaging under vacuum or reduced temperature) to preserving the carotenoid content of dried OFSP chips are therefore required.

It can therefore be concluded that the effect of chemicals did not counteract the negative effect of soaking. The use of pre-treatments successfully reduced the carotenoid degradation in dried products but not in stored ones. Further work should focus on the use of preservers that can resist to chemical degradation during storage.

CHAPTER 7.
INVOLVEMENT OF ENZYMES IN CAROTENOID DEGRADATION DURING STORAGE OF ORANGE-FLESHED SWEET POTATO

7.1. INTRODUCTION

Experiments using pre-drying treatments including blanching and chemical treatment to reduce important carotenoid degradation over storage of OFSP dried chips failed to make a significant improvement (Chapter 6). Another approach to understand the high carotenoid loss during storage was to identify the more fundamental causes of carotenoid degradation. One of the causes of carotenoid degradation during storage can be enzymatic activity. Enzymes that can catalyse a variety of reactions present in plant foods and enzymatic oxidation has been reported to happen when there is a fresh tissue disruption that releases enzymes that isomerise and oxidise carotenoids (Rodriguez Amaya and Kimura, 2004). Enzymatic oxidation of β-carotene by lipoxygenase (Aziz *et al.* 1999; Isamah 2004) and peroxidase (Zorn *et al.* 2003) have been reported (see Chapter 1 section 1.3.3 enzymatic oxidation). Moreover, high peroxidase activity has been found in sweet potato (Castillo-Leon *et al.* 2002). An understanding of the origin (enzymatic or non-enzymatic) of carotenoid catabolism would have implications for the preservation of provitamin A during storage and in the development of new storage approaches. For instance, an optimisation of blanching could be suggested if the degradation should be mainly due to enzymatic activity.

Because enzymatic co-oxidation by peroxidase and non-enzymatic autoxidation both work the same way by producing free radicals that then degrade β-carotene, experiments in the presence or absence of enzymes were carried out in order to differentiate them.

The aim of the study was to find out whether the degradation of β-carotene in dried sweet potato resulted mainly from enzymatic activity or autoxidation. This problem was approached in two directions:

- In the first place, a simplified model system consisting of a β-carotene standard dissolved in buffer medium at pH 6.0 and pH 7.4 at 40ºC was tested for β-carotene catabolism by enzymes including commercial horseradish peroxidase and soybean lipoxygenase and by autoxidation using H_2O_2 and linoleic acid. Influences of 2,4 dichlorophenol (DCP), a free radical generator (that could act as an enhancer of carotenoid oxidation) and ethylenediaminetetraacetic acid (EDTA), a metal chelating agent (that could inhibit oxidation due to metal interaction with carotenoids), were also tested.
- In the second place, peroxidase (and lipoxygenase) activities were determined after drying and after storage of sweet potato and at different water activities in order to understand the involvement of enzymes, and more especially peroxidases that could be responsible for the high carotenoid degradation in dried sweet potato during storage.

7.2. MATERIALS AND METHODS

7.2.1. Sweet potato root samples

Sweet potato flour from Ejumula variety dried chips came from unpeeled roots that had been chipped using a rotary disc chipper and sun-dried for two days in Uganda, conserved at -20ºC and milled just before the analysis using a laboratory mill (Chapter 6). In order to study the effect of storage on enzymatic activity, a portion of the dried chips was stored at 40ºC at controlled humidity (50%) (see conditions in Chapter 8) and analysed after 19 and 54 days. Fresh orange-fleshed sweet potato (OFSP) roots (Susskartoffeln® from Israel) bought from a fruit and vegetable supplier in Chatham, UK was used for comparison with sweet potato flour assays. Roots were washed under tap water, mashed (including peel) and stored at -20ºC before being thawed at ambient temperature and analysed.

7.2.2. pH measurement

The pH value was measured on flour samples that were homogenised with 10ml deionised water using ultra-turax for 5s and topped up to a total volume of 30ml with deionised water. Determination was done in triplicate using Hanna Instruments pH210 Microprocessor pHmeter.

7.2.3. Enzyme assay on β-carotene

β-carotene enzymatic oxidation was evaluated spectrophotometrically following an adaptation of the method of Fleischmann *et al.* (2003) and Baldermann *et al.* (2005).

β-carotene stock solution was prepared : 25mg of β-carotene (Type I approx. 95% UV Sigma, UK) was dissolved in a small quantity of chloroform. Tween 20 (2.5ml) and Chloroform (3.5ml) were slowly added to obtain an emulsion made up to a total volume of 50ml with sodium phosphate buffer (pH 7.4). The solution was homogenised using an ultra-turax for about 10s. For assays, the stock solution was diluted 1/40 in phosphate buffer, which pH value was adjusted to 6.0 or 7.4 with HCl. A transparent solution, which absorbance could be read by UV-visible spectrophotometer, was obtained..

For the assays, 0.2ml substrate (H_2O_2 (20mM), linoleic acid (4mM)), and/or enzyme were added to the β-carotene solution (3ml) in a cuvette. The total volume was adjusted with deionised water to be the same in each tube of the assay so each substrate or enzyme represented 5% of total solution. The reaction mix was incubated at 40ºC and reading of absorbance taken every 5 minutes at 450nm. Blank (β-carotene + deionised water) and positive control (β-carotene + commercial soybean lipoxygenase + linoleic acid) were prepared for each assay. All operations were performed under dim light.

7.2.4. Enzymatic extraction

Sweet potato extract for measurement of peroxidase and lipoxygenase activity was prepared according to Isamah *et al.* (2004). Sweet potato flour (2g) or puree (10g) was homogenised with 20ml of sodium phosphate buffer (pH 7.4) using an ultra-turax homogeniser (IKA Janke and Kunkel Labortechnik) at 290 x g. for 1 minute and immediately maintained on an ice bath. The homogenate was strained through one layer of cheese cloth to remove solid particles. 10ml of buffer was added for rinsing. The

filtrate was centrifuged at 4300 x g. for 10 minutes at 4ºC. The supernatant (crude extract) was collected without further purification.

For the enzyme assay on β-carotene, the supernatant was subjected to acetone precipitation (volume equivalent of supernatant) for 2h in ice in the fridge (equivalent to 0ºC). In order to concentrate the proteins, the resulting pellet was first dried to remove all trace of acetone (in a fume cupboard blowing transversal air) and then dissolved in a small quantity of deionised water (3ml) by vortexing and filtered through 0.45 µm filter.

7.2.5. Measurement of peroxidase activity

Determination of peroxidase activity was according to Childs and Bardsley (1975)'s method. The rate of decomposition of H_2O_2 by peroxidase was determined spectrophotometrically (Super Aquarius Cecil CE9200, Cambridge, UK) by measuring the rate of oxidation of ABTS (2,2'-azino-bis 3-ethylbenzthiazoline-6-sulphonic acid) at 405 nm (ε = 36.8 $mM^{-1}.cm^{-1}$) per minute. The reaction mixture consisted of 3ml of sodium phosphate buffer (pH = 7.32-7.40), 50 µl of ABTS (20mM). 50 µl of hydrogen peroxide (H_2O_2) solution (20 mM) initiated the reaction. ABTS and H_2O_2 solutions were prepared afresh for every assay. H_2O_2 solution was prepared from stock solution kept in the fridge (50-100 µl / 50ml deionised water) and the concentration measured at 240 nm (ε = 39.4 $mM^{-1}.cm^{-1}$). Peroxidase activity was expressed either in nmol ABTS oxidised per minute per ml solution for commercial horseradish peroxidase or in µmol ABTS oxidised per minute per gram of sweet potato flour or puree on a fresh weight basis.

Horseradish peroxidase was chosen because it is widely available commercially and because of the closeness between peroxidase isozymes of sweet potato and horseradish (Rompel *et al.* 2007). Dried commercial horseradish peroxidase (P 6782 Type VI-A Sigma- Optimal pH: 6.0-6.5) was dissolved in water at a concentration of 14$mg.L^{-1}$ and its activity measured before trials. Ethylene diamine tetra acetic acid (EDTA) 10mM was dissolved in deionised water and 2,4 Dichlorophenol (DCP) 36mM was dissolved in acetone.

For the measurement of peroxidase activity at lower water activities, solutions of glycerol and phosphate buffer pH 6 and 7.4 were adjusted at 10%; 40%; 60% glycerol taking into account the volume of reagents in the cuvette. Water activity of

glycerol/solutions at 10%; 40%; 60% was determined using a Degacon CX-1 awmeter (Pullman, Washington) in triplicate.

7.2.6. Measurement of lipoxygenase activity

The preparation of linoleic acid (LA) was adapted from Schweiggert *et al.* (2005) and Gokmen *et al.* (2005). Pure linoleic acid (0.5ml) (99% Sigma, UK) and Tween 20 (0.5ml) (Fisher, UK) were dissolved in 10ml deionised water, homogenised and made up to 2mL with NaOH 1N added to clarify the solution before making up to volume (200ml) with sodium phosphate buffer. The concentration of linoleic acid (molecular weight 280g.M^{-1}) obtained was 8 mM. The commercial lipoxygenase solution was prepared by dissolving dried soybean lipoxygenase (L7395 Fluka, Sigma, UK) (3mg) in phosphate buffer (50ml) giving a concentration of 60mg.L^{-1}. The measurement of lipoxygenase (LOX) activity was carried out following Schweiggert *et al.* (2005)'s method but with some modifications. The reaction solution consisted of 3ml phosphate buffer and 0.2ml of crude enzyme extract. The reaction was initiated by adding 0.2ml of 8mM linoleic acid. Production formation per minute was recorded at 234nm by the accumulation of the conjugated diene (ε = 25.0 $mM^{-1}.cm^{-1}$) per minute. After two weeks storage in the fridge 8mM linoleic acid solution had lost half of its lipoxygenase activity.

7.2.7. Statistical analysis

Data were processed on SPSS 16.00 software by one or two way-ANOVA (Analysis of variance; $p<0.005$) using the Tukey test to determine which samples were significantly different from others. Percentage of retention of absorbance at 450nm was related to time (minutes) by the different mathematical fits in accord with a possible zero-order kinetics or first-order kinetics.

7.3. RESULTS AND DISCUSSION

7.3.1. Measurement of pH value

The pH value of the sweet potato puree and sweet potato flour after reconstituting it with water was slightly acidic (5.87-6.21) (Table 7-1).

Table 7-1: pH values of the fresh sweet potato and sweet potato flour*

Storage time at 40ºC (days)	Fresh sweet potato Susskartoffeln®	Sweet potato flour Ejumula variety
0	5.87 (0.02)	6.21 (0.04)
19	-	5.97 (0.04)
54	-	5.92 (0.03)

*in 30ml deionised water. Mean of triplicate (standard deviation)

In order to mimic conditions found in sweet potato, degradation kinetics of β-carotene in liquid medium were carried out at pH 6.0.

7.3.2. Degradation of β-carotene in liquid medium

Autoxidation and enzymatic oxidation of β-carotene were recorded spectrophotometrically at 40ºC in phosphate buffer pH 6.0 or 7.4 (Tables 7-2 & 7-3 respectively).

Table 7-2: Degradation rate* and mean percentage of β-carotene retained at 450nm after 30 min incubation at 40ºC at pH 6 with various enzymes and/or substrates**

					Subset**						
Enzyme	**LA**	**H_2O_2**	**k* (min^{-1})**	**R^2**	1	2	3	4	5	6	7
POD	**x**	**-**	0.037 (0.007)	0.991 (0.004)	61.7						
POD	**x**	**x**	0.030 (0.004)	0.991 (0.005)		66.5					
LOX	**x**	**-**	0.020 (0.002)	0.994 (0.005)			75.9				
-	**x**	**x**	0.016 (0.000)	0.996(0.002)				79.9			
S	**x**	**-**	0.016 (0.001)	0.994 (0.004)				80.3	80.3		
POD	**-**	**-**	0.015 (0.001)	0.975 (0.024)				80.4	80.4	80.4	
-	**x**	**-**	0.015 (0.001)	0.992 (0.006)				81.4	81.4	81.4	
POD	**-**	**x**	0.014 (0.001)	0.995 (0.007)					82.9	82.9	
S	**x**	**x**	0.013 (0.001)	0.990 (0.012)						82.9	
S	**-**	**-**	0.007 (0.001)	0.992 (0.005)							90.3
S	**-**	**x**	0.007 (0.001)	0.989 (0.011)							90.7
Blank	**-**	**-**	0.007 (0.002)	0.984 (0.007)							90.7
Sigma					1.00	1.00	1.00	0.76	0.05	0.07	1.00

*First order kinetic; mean (standard deviation) of triplicate trial; **Two- way ANOVA; Univariate. Tukey HSD; $p< 0.05$. Factors: enzyme and/or substrate; time of incubation. n= 21 (7 collections (every five minute) x 3 replication trials). x indicates addition of 5% substrate. Substrates: LA: Linoleic acid 0.2mM; H_2O_2 1mM. Enzymes (5% of solution): POD: Horseradish peroxidase; LOX: Soybean lipoxygenase; S: filtered extract from sweet potato flour. At pH = 6, POD activity =2.155±0.044 mmol ABTS.min^{-1}.ml^{-1}; LOX activity = 0.013±0.000 mmol conjugated diene.min^{-1}.ml^{-1}

Table 7-3: Degradation rate* and mean percentage of β-carotene retained at 450nm after 30 min incubation at 40°C at pH 7.4 with various enzymes and/or substrates**

					Subset**					
Enzyme	**LA**	**H_2O_2**	**k* (min^{-1})**	**R^2**	1	2	3	4	5	6
LOX	**x**	-	0.035 (0.013)	0.997 (0.001)	63.9					
POD	**x**	**x**	0.023 (0.005)	0.990 (0.005)		72.5				
POD	**x**	**-**	0.022 (0.003)	0.994 (0.003)		73.2				
-	**x**	**x**	0.017 (0.003)	0.996 (0.001)			78.7			
-	**x**	**-**	0.015 (0.002)	0.992 (0.007)			81.4			
POD	**-**	**x**	0.009 (0.001)	0.993 (0.004)				86.9		
POD	**-**	**-**	0.008 (0.001)	0.978 (0.020)				88.4	88.4	
S	**x**	**-**	0.007 (0.002)	0.989 (0.006)				90.6	90.6	90.6
S	**x**	**x**	0.006 (0.002)	0.983 (0.009)					91.1	91.1
Blank	**-**	**-**	0.005 (0.000)	0.976 (0.018)						93.8
S	**-**	**-**	0.004 (0.000)	0.991 (0.009)						94.3
S	**-**	**x**	0.004 (0.001)	0.985 (0.006)						94.4
Sigma					1.00	1.00	0.54	0.10	0.50	0.09

*First order kinetic; mean (standard deviation) of triplicate trial; **Two- way ANOVA; Univariate. Tukey HSD; $p< 0.05$. Factors: enzyme and/or substrate; time of incubation. n= 21 (7 collections (every five minute) x 3 replication trials). x indicates addition of 5% substrate. Substrates: LA: Linoleic acid 0.2mM; H2O2 1mM. Enzymes (5% of solution): POD: Horseradish peroxidase; LOX: Soybean lipoxygenase; S: filtered extract from sweet potato flour. At pH= 7.4, POD activity = 0.386±0.013 mmol ABTS $min^{-1}.ml^{-1}$; LOX activity = 0.141±0.005 mmol conjugated diene $min^{-1}.ml^{-1}$

In all cases degradation of β-carotene followed a first order kinetics in accordance with Galan and Minguez-Mosquera (1997) working in a liquid medium. All coefficients of correlation R^2 fitted well the first order reaction with values comprised between 0.975 and 0.997.

Oxidation by commercial enzymes (Horseradish peroxidase and Soybean lipoxygenase) was significant as compared with the control (ANOVA; $p<0.05$). Horseradish peroxidase activity was six times higher at pH values of 6 than 7.4 (2.155 against 0.386 mmol ABTS.$min^{-1}.ml^{-1}$). Peroxidase activity was influenced by pH value in agreement with earlier findings in sweet potato (Dogan *et al.* 2007). Influence of pH on enzyme activity is explained by Dogan *et al.* 2007 in that H^+ and OH^- ions probably cause a change in stereo configuration of enzyme influencing on its activity. Influence of pH value on peroxidase activity was perceived as very important compared to other studies (Harvey P., Pers. Comm.). Such a difference would require verification of these results by optimising concentrations of substrates at both pH (Lineaver-Burk plot) (Harvey P., Pers. Comm.).

Soybean lipoxygenase activity was lower at pH value of 6.0 than 7.4 (0.013 against 0.141 mmol conjugated diene.min^{-1}.mol^{-1}). Hence, in presence of linoleic acid and at pH 6.0, horseradish peroxidase was more efficient to bleach β-carotene than soybean lipoxygenase (0.037 against 0.020 min^{-1} respectively) but it was the opposite at pH value 7.4 (0.022 against 0.035 min^{-1} respectively). To our knowledge this is the first time that horseradish peroxidase was compared to soybean lipoxygenase for β-carotene oxidation rate in a buffer medium. This highlights the strong link existing between enzymatic activity due to pH variation and oxidation rate of β-carotene. Lipoxygenase has been demonstrated to be an efficient oxidative agent of β-carotene (Wache *et al.* 2003). This result further shows that peroxidase could be as equally efficient as β-carotene oxidative agent.

An interesting observation is that addition of H_2O_2, which is peroxidase's most usual substrate, did not significantly increase the cleavage activity compared to the simple enzyme both at pH 6.0 and 7.4 (ANOVA; $p<0.05$). Addition of H_2O_2 was shown to increase carotenoid oxidation in the presence of 2,4 Dichlorophenol (DCP) (Matile and Martinoia 1982) in contrast with our study. In our study, the high concentration of H_2O_2 in solution (1mM) may have been inhibitory to the reaction. In addition, the peroxidase could not be regenerated in the absence of DCP (see next section 7.3.3). This suggestion was further supported by Garner (1984) who showed that peroxidation of fatty acids by peroxidase in the presence of H_2O_2 was decreasing from concentrations of H_2O_2 of 0.1 mM. High concentrations were inhibitory whilst low concentrations gave reduced oxidation rates (Garner 1984).

In contrast, catabolism of β-carotene by horseradish peroxidase was enhanced by addition of linoleic acid: at pH 6.0, β-carotene oxidation rate with linoleic acid was 0.037 min^{-1} against 0.015 min^{-1} without; and at pH 7.4, 0.022 min^{-1} against 0.008 min^{-1} respectively. Preston and Barrett (1987) showed that in addition to the use of H_2O_2, peroxidase was able to use linoleic acid peroxide. Our hypothesis is that small quantities of H_2O_2 could be generated from the autoxidation of linoleic acid and then be used by the peroxidase.

The rate of autoxidation in presence of linoleic acid was significant (ANOVA; $p<0.05$). Jaran-Galan and Minguez-Mosquera (1997) working on kinetics of co-oxidation of β-

carotene by soybean lipoxygenase and linoleic acid, and autoxidation of β-carotene by linoleic acid and soybean lipoxygenase similarly demonstrated that autoxidation was significant and β-carotene degradation was accelerated with addition of lipoxygenase at pH 8 (about five times the rate of autoxidation). In the present study, lipoxygenase degradation rate was 0.020 min^{-1} at pH 6.0 and 0.035 min^{-1} at pH 7.4. A faster β-carotene degradation rate at higher pH compared to lower pH resulted of more optimal lipoxygenase activity at alkaline pH. In contrast, autoxidation (with linoleic acid) was not influenced by pH (at pH 6; in presence or absence of H_2O_2: 0.016 and 0.016 min^{-1} respectively and; at pH 7.4: 0.017 and 0.015 min^{-1} respectively). Addition of peroxidase or with H_2O_2 resulted in slower oxidation of β-carotene compared to autoxidation in presence of linoleic acid at pH 7.4 (0.008; 0.009 and 0.015 min^{-1} respectively). This further highlights the importance of autoxidation rate of β-carotene that can be comparable with enzymatic oxidation under some conditions.

Filtered extract from sweet potato flour was not significantly different from the control in terms of rate constant (k) (ANOVA; $p<0.05$). The crude extract or filtered extract did not provoke any degradation of β-carotene with or without hydrogen peroxide. In particular, at pH 6 filtrated extract behaved like the control; autoxidation in presence of linoleic acid with the extract was the same as without the extract (0.016 and 0.015 min^{-1}). However at pH 7.4 filtrated extract had an inhibiting effect toward autoxidation (0.007 and 0.015 min^{-1}). Some reasons can be raised to explain lack of catalytic activity of extract and inhibition effect: first the extract was not purified sufficiently; in addition to acetone precipitation and filtration, Balderman *et al.* (2005) and Fleishman *et al.* (2003) used isoelectric focusing to purify carotenoid cleavage enzymes and other factors (such as antioxidants or proteins) may have hindered peroxidase activity; secondly the extract was not concentrated sufficiently; peroxidase activity in the filtered extract was more than ten times less than in horseradish peroxidase (0.02-0.03 mmol ABTS.min^{-1}.ml^{-1} (Bechoff, A data not shown) and 0.386 mmol ABTS.min^{-1}.ml^{-1} respectively). Optimised extraction and purification of peroxidase from sweet potato would be necessary to measure the impact of oxidation of β-carotene (Harvey P., Pers. Comm.). However because of the closeness between peroxidase isozymes of sweet potato and horseradish (Rompel *et al.* 2007) β-carotene bleaching ability of sweet potato peroxidase is most probable.

7.3.3. Enhancement of β-carotene oxidation using a free radical generator

Bleaching of β-carotene was tested in presence of horseradish peroxidase, hydrogen peroxide, linoleic acid and 2,4 Dichlorophenol (DCP) free radical generator (Table 7-4).

Table 7-4: Instantaneous* effect of 2,4 Dichlorophenol addition on β-carotene absorbance value at 450nm at ambient temperature (about 22ºC)

Sample	DCP	H_2O_2	LA	POD	Absorbance at 450nm**
1	-	-	-	-	0.698 (0.021)
2	x	-	-	-	0.746 (0.039)
3	x	x	-	-	0.739 (0.033)
4	x	-	x	-	0.723 (0.028)
5	x	-	x	x	0.585 (0.018)
6	x	x	-	x	0.174 (0.014)

*the reading was carried out as quickly as possible after the addition of reagents (maximum time of 5 minutes).
**n=3 – average (standard deviation)
x indicates addition of 5% substrate or enzyme. DCP: 2,4 Dichlorophenol 1.8mM in solution (4 ml); LA: Linoleic acid 0.4mM in solution; H_2O_2 1mM in solution. POD: Horseradish peroxidase; at pH= 7.4, POD activity = 0.746±0.046 (mmol ABTS.min^{-1}.ml^{-1}).

Instantaneous bleaching of β-carotene occurred when DCP was added to peroxidase and hydrogen peroxide (absorbance= 0.174). It can be noted that addition of DCP biased the reading of the blank; absorbance was increased with DCP (0.746; blank 0.698). Instantaneous autoxidation of β-carotene in presence of DCP and or H_2O_2 (0.739) or linoleic acid (0.723) did not occur. Bleaching efficiency was therefore attributed to the ability of horseradish peroxidase to use DCP to generate free radicals using H_2O_2 as a substrate. It was demonstrated that the oxidation of carotenoids in the presence of peroxidase was dependent on the presence of DCP (Matilde and Martinoia 1982) but it was not dependent on the addition of H_2O_2. Indeed DCP plays a crucial role in regenerating peroxidase back to its native state (Harvey P., Pers. Comm.). When linoleic acid replaced hydrogen peroxide as substrate the effect was slower (0.585). Bleaching in presence of linoleic acid might result from linoleic acid autoxidation that could then be used by the peroxidase as a substrate to generate free radicals with DCP. Oxidation of β-

carotene by peroxidase in the presence of linoleic acid can be explained by the use of linoleic acid peroxide as substrate (Preston and Barrett 1987) and the production of small amounts of H_2O_2 from the oxidation of linoleic acid that are used by the horseradish peroxidase.

The instantaneous bleaching of β-carotene by horseradish peroxidase highlighted that peroxidases could potentially degrade carotenoids in sweet potato, hypothetically in using phenols, present in sweet potato (Woolfe 1993), to generate free radicals.

7.3.4. EDTA assay

Prevention of β-carotene autoxidation in presence of linoleic acid using metal ion chelator EDTA was tested under dim light conditions (Figure 7-1).

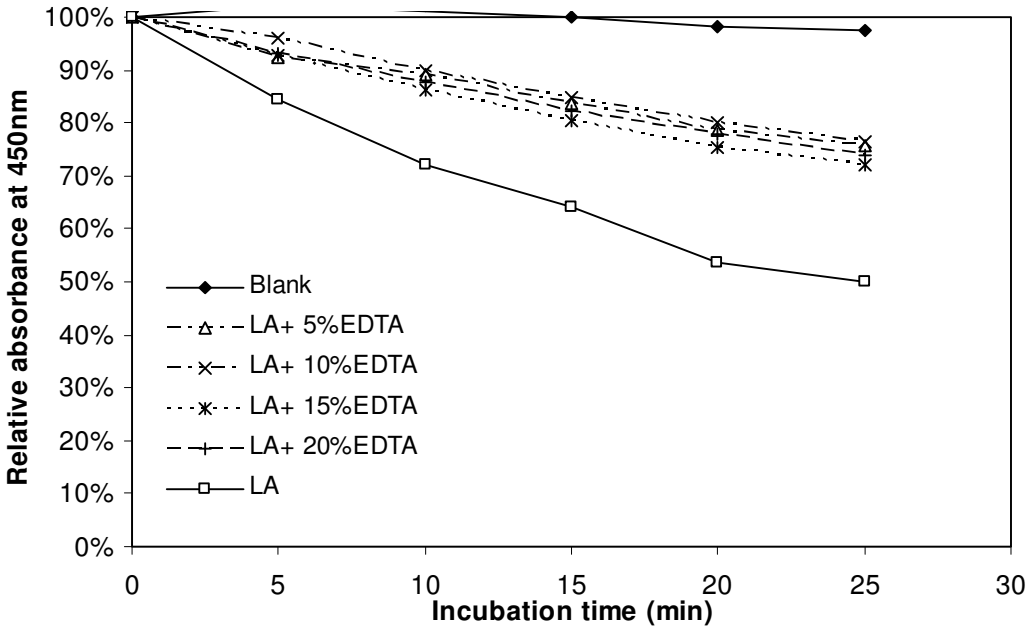


Figure 7-1: Effect of EDTA concentration on β-carotene autoxidation by linoleic acid (LA) in solution at 40°C and at pH 7.4 during 25 minutes
Substrates: Blank: deionised water; Linoleic acid (LA) 0.4mM (representing 5% of solution); EDTA (ethylene diamine tetra acetic acid) (variable concentration 0-20% equivalent to 0-1.5mM in solution)

Fifty percent of β-carotene lost after 25 minutes in presence of linoleic acid by autoxidation confirmed that autoxidation was an important phenomena. EDTA had

partial influence on autoxidation since 75% of β-carotene was lost after 25 minutes in the presence of linoleic acid and EDTA. With increased concentrations (5; 10; 15; 20%) of EDTA there was no dose dependent effect (respectively 75.7%; 76.6%; 72.0% and 74.1%): The partial effect of EDTA is attributed at its role as a metal ion chelator which could have chelated metal ions present in deionised water used in the preparation of the buffer (Haber Weiss reaction). This indicates that other factors than ions such as air oxygen, temperature influence on the autoxidation of β-carotene in presence of linoleic acid. Singlet oxygen is very effective on the peroxidation of unsaturated fatty acids (Bradley and Min 1992). Interaction of light and oxygen is the basis for the formation of singlet oxygen (Bradley and Min 1992) that could then initiate free radical reactions on unsaturated fatty acids and β-carotene. Although the assay was carried out under dim light conditions, formation of singlet oxygen may also have been initiated by a very small amount of light that was in the room.

7.3.5. Effect of storage on peroxidase activity

No lipoxygenase activity either at pH 6.0 or 7.4 was detected in the sweet potato extract.

Peroxidase activity was measured in sweet potato in order to determine the importance of β-carotene oxidation by peroxidase (Table 7-5).

Table 7-5: Peroxidase activity on fresh sweet potato and dried sweet potato before and after storage (µmol ABTS.min^{-1}.g^{-1}) on a fresh weight basis (fb) .

Storage time at 40ºC (days)	Fresh sweet potato Susskartoffeln®	Sweet potato flour Ejumula variety
pH 7.4		
0	377.0(48.3)	380.8 (24.9)
19	-	<5
54	-	<5
pH 6		
0	2321.4 (227.0)	3817.2 (515.2)
19	-	8.9 (4.0)
54	-	<5

*mean of triplicate measurement on two different extracts (standard deviation). n=6

Peroxidase activity in fresh sweet potato was of same order as in dried sweet potato for the same quantity of material (fresh weight basis) (respectively 2321.4 and 3817.2 at pH 6; 377.0 and 380.8 at pH 7.4) indicating that peroxidase activity was still high after drying. Because the fresh and dried sweet potato were not from the same batch and variety a further comparison between peroxidase activity of fresh and dried sweet potato, however, cannot be undertaken.

After 19 and 54 day-storage at 40ºC, peroxidase activity was extremely weak or not detectable. Peroxidase activity disappearance during storage was also found by other authors. Working on Pine needles (*Picea abies*), Has-Schon *et al.* (2005) reported that needles stored at 37ºC retained negligible amounts of peroxidase activity after 30 day-storage. The authors also demonstrated that temperature had a crucial impact on peroxidase activity; samples stored at 24ºC had 40% of the activity remaining after 30 day-storage. Peroxidase activity was demonstrated to diminish with storage time.

7.3.6. Effect of water activity (a_w) on the peroxidase activity

The effect of water activity on peroxidase activity from unstored OFSP flour was tested using glycerol at various solution concentrations (Table 7-6).

Table 7-6: Peroxidase activity from Ejumula dried OFSP (µmol ABTS.g^{-1}.g^{-1}.min^{-1}) on a fresh weight basis (fb), related to water activity adjusted with glycerol concentration

Glycerol concentration	0%	10%	40%	60%
a_w*	Liquid >1.0	0.995 (0.014)	0.906 (0.006)	0.689 (0.005)
pH 7.4	512.5 (4.3)	247.7 (17.9)	<5	<5
pH 6	3678.6 (105.6)	1198.9 (70.6)	48.8 (5.1)	<5

Water activity of unstored sweet potato flour = 0.511 (0.002)
*mean of 3 measurements (standard deviation).

Peroxidase activity was reduced from more than half when working at 10% glycerol compared to 0% (respectively 1198.9 compared to 3678.6 mmol ABTS.min^{-1}.g^{-1} on a fresh basis). At 40% glycerol, only at pH 6.0 did a little activity remain (48.8 mmol ABTS.min^{-1}.g^{-1}). At 60% glycerol, corresponding to a water activity of 0.689, at both pH (6.0 and 7.4) no peroxidase activity remained. Water activity in unstored dried sweet

potato was 0.51 on average. At this water activity therefore peroxidase activity was considered negligible. Other studies similarly reported that peroxidase activity decreased with lower water activity (Lee and Kim 1995; Kamiya 2002). *Arthromyces ramosus* fungal peroxidase complexed in surfactant and toluene sharply decreased its activity for water activities between 0.12-0.84. An explanation for loss of catalytic activity in dried food was that substrate mobility was limited under this condition and exchanges between enzyme and substrate were therefore limited (Arya *et al.* 1979). These results suggest that peroxidase role on carotenoid degradation at low water activities is limited.

7.4. CONCLUSION

When working with a β-carotene liquid medium, commercial horseradish peroxidase was able to oxidise β-carotene. Oxidation was enhanced by the presence of linoleic acid, which in an oxidised form can be an alternative substrate to H_2O_2 for the peroxidase. Since linoleic acid is present in sweet potato this phenomena may play an important role in the oxidation of carotenoids. Moreover β-carotene bleaching by horseradish peroxidase was instantaneous in the presence of DCP and H_2O_2 and showed that phenolic compounds present in sweet potato could favour carotenoid oxidation. Therefore the "ingredients" to initiate enzymatic oxidation of carotenoids are potentially present in sweet potato.

Nevertheless, peroxidase activity was not detected when the flour was stored and at low water activities such as those encountered in dried sweet potato. In addition, there was a strong effect of autoxidation in the presence of linoleic acid. Since oxidation of β-carotene can happen at low water activities in presence of unsaturated fatty acids on sweet potato flakes as reported by Walter and Purcell (1974), autoxidation is the most probable actor of β-carotene catabolism during storage of dried sweet potato.

CHAPTER 8.
CAROTENOID DEGRADATION AND FORMATION OF VOLATILE COMPOUNDS IN DRIED SWEET POTATO CHIPS STORED UNDER CONTROLLED CONDITIONS OF TEMPERATURE, HUMIDITY AND OXYGEN

8.1 INTRODUCTION

Both field studies in Uganda and Mozambique clearly indicated that storage, rather than drying, was a critical step for preservation of provitamin A in dried sweet potato (Chapters 4 and 5). Later pre-treatment studies failed to significantly reduce carotenoid loss in storage (Chapter 6). It was also demonstrated that carotenoid degradation due to enzymatic activity was unlikely (Chapter 7). Following these research studies, there was a further need to measure the separate impact of the main factors, which are temperature; oxygen concentration and humidity, on carotenoid breakdown in sweet potato flour during storage. The conditions in the field did not allow evaluation of these factors separately. In this current chapter, experiments under controlled conditions were carried out in order to measure more precisely the separate impact of the various factors on carotenoid degradation. The kinetics of carotenoid degradation were determined and the production of volatile compounds was tentatively related to the carotenoid breakdown.

The first hypothesis was that the degradation of carotenoid by temperature can be predicted by a mathematical model. The main objective of this study was therefore to measure the degradation of β-carotene in sweet potato chips during storage influenced by temperature taking into account oxygen and water activity and then predict, by modelling, thermal degradation under a range of conditions found in tropical countries.

The second hypothesis was that the degradation of carotenoids could be observed from the degradation products formed. A secondary objective of the study was then to relate the degradation of β-carotene to the formation of volatile compounds. Degradation of standard β-carotene has already been studied using both High Performance Liquid

Chromatography (HPLC) and Gas Chromatography/Mass Spectrometry (GC/MS) for the determination of loss of pure β-carotene and formation of volatiles (Mordi *et al.* 1993). But to our knowledge, a kinetic study including the comparison of β-carotene degradation together with the formation of volatile degradation products has not been reported in a dried food product matrix such as OFSP.

This study therefore seeks to relate β-carotene to its degradation products in order to understand the pathway of β-carotene degradation in a dried food product.

8.2 MATERIALS & METHODS

8.2.1 Raw materials

Sweet potato chips from the Ejumula variety were harvested in Luwero, Uganda in March 2008 after a growing season of 5-6 months. Roots were chipped and dried using an open air sun dryer at the National Agricultural Research Laboratories (NARL), Kawanda, Uganda with an average drying temperature of 28.8±2.8 ºC and 53.3±13.6% relative humidity. Dried chips were stored in a freezer (about -20ºC). Carotenoid content did not change significantly during storage in a freezer over a six month period (Chapter 2).

8.2.2 Storage conditions

Storage system

A storage study was undertaken at Natural Resources Institute, UK. The storing system used is shown in Figure 8-1.

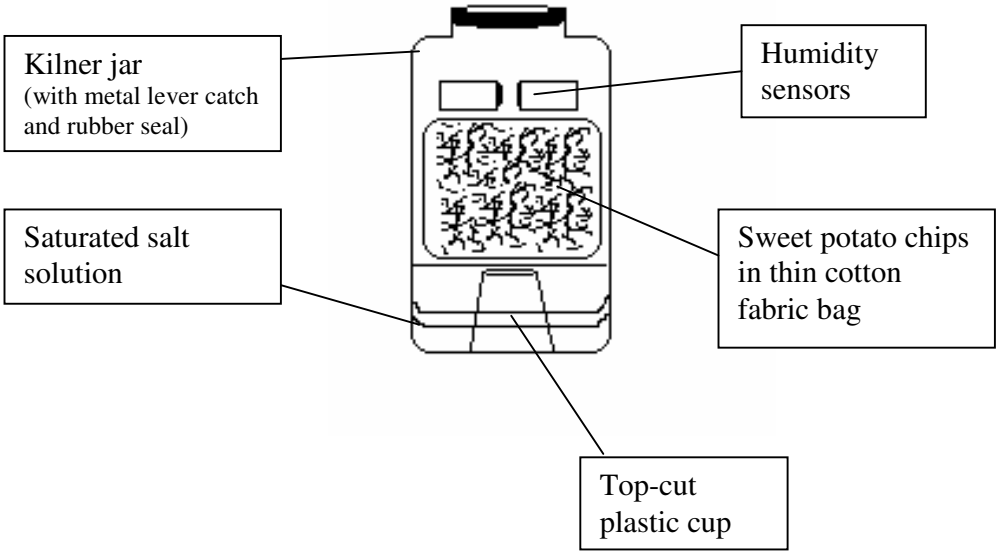


Figure 8-1: Storing system for sweet potato storage trial under controlled conditions. E. Rayment (BSc student).

In order to control the relative humidity during storage, dried sweet potato chips (90g) in a sewed cotton bag were placed into a 1.5L-clip top jar containing deionised water saturated with salt (150g).

Preparation of saturated salt solutions

Saturated salt solutions were prepared according to OIML (1996). 150g of salt was slowly added to deionised water maintained at 60ºC with a hot plate (VWR 600 Hot plate/stirrer, USA) in the flask jar and continuously stirred. The volumes of deionised water for sodium bromide, sodium chloride, lithium chloride, and hydrate of magnesium chloride respectively were 100ml; 150ml; 150ml and 25ml. Equilibrium between chips and air was achieved after 9 days of storage at 40ºC. Water activity (a_w) of chips stored in the different salts was determined in triplicate using an a_w meter CX1 (Decagon, Pullman, Washington, USA). Dry matter was determined by drying a sample (5g) for 24h at 105ºC. The water activities for the chips in the salts were the following: a_w = 0.126 for LiCl, 0.304 for $MgCl_2$, 0.507 for NaBr and 0.765 for NaCl. Samples tested at different temperatures were equilibrated with NaBr (a_w= 0.579±0.060) because it was the closest to the water activity of dried sweet potato chips stored under ambient conditions (Chapter 3).

Sorption curve

The Brunauer-Emmett-Teller (BET) equation by Bimbenet *et al.* (2002) that uses the theory for molecular multilayer adsorption was applied to predict data for a_w in relation with moisture content on a dry weight basis (*M*). The BET equation was used on the experimental points to calculate water activity from the moisture content. The linearised Equation 8-1 is expressed as follows:

$$\frac{a_w}{(1-a_w)M} = \frac{1}{M_0 C} + \frac{(C-1)}{M_0 C} a_w \quad \textbf{(Equation 8-1)}$$

C is the BET constant and M_0 is the monolayer adsorbed gas quantity (in volume units). *C* and M_0 are constant parameters of the model at a given temperature**.** *M* is the moisture content on a dry weight basis.

Incubators

Jars in triplicate were placed in incubators (LMS Cooled Incubator, Sevenoaks, UK) set at 10±0.5 ºC; 20±1 ºC; 30±0.5 ºC and 40±1 ºC. In total five incubators were used (10ºC; 20ºC; 30ºC and 40ºC x 2) and were situated in the same room. Such temperatures are similar to those encountered in tropical countries where sweet potato is grown (*e.g.* Chapter 5- Mozambique: average/min./max.: 22/12/33°C; Chapter 6- Uganda: 28/18/34°C). Samples were stored at 10ºC and 20ºC for 105 days; at 30ºC for 95 days and 40ºC for 54 days. Samples at different water activities were stored at 40ºC for 54 days (Figure 8-2).

For the experiment at different oxygen levels, samples were stored at 40ºC for 21 days. 3L-Kilner jars containing the samples were flushed with a continuous flow of oxygen:nitrogen mix (containing 0%; 2.5%; 10% or 21% oxygen) (Figure 8-3). Inlet tubing was placed at the bottom of the jar below the chips in order to allow gas come up through the chips. A manifold was used to split the gas flow from one gas cylinder to three jars (triplicate). In order to regulate the consumption of the gas in the cylinder the gas flow rate coming out of the cylinder was adjusted between 90-100 $ml.min^{-1}$ using an air flow meter (Model GTF1CHD CT-Platon, Domont, France). In this experiment, it was not possible to have saturated salt solutions in the jar because the continuous air flow dried out the saturated salt solution and also splashed some of the salt outside the jar. The dry matter was however determined throughout the storage.

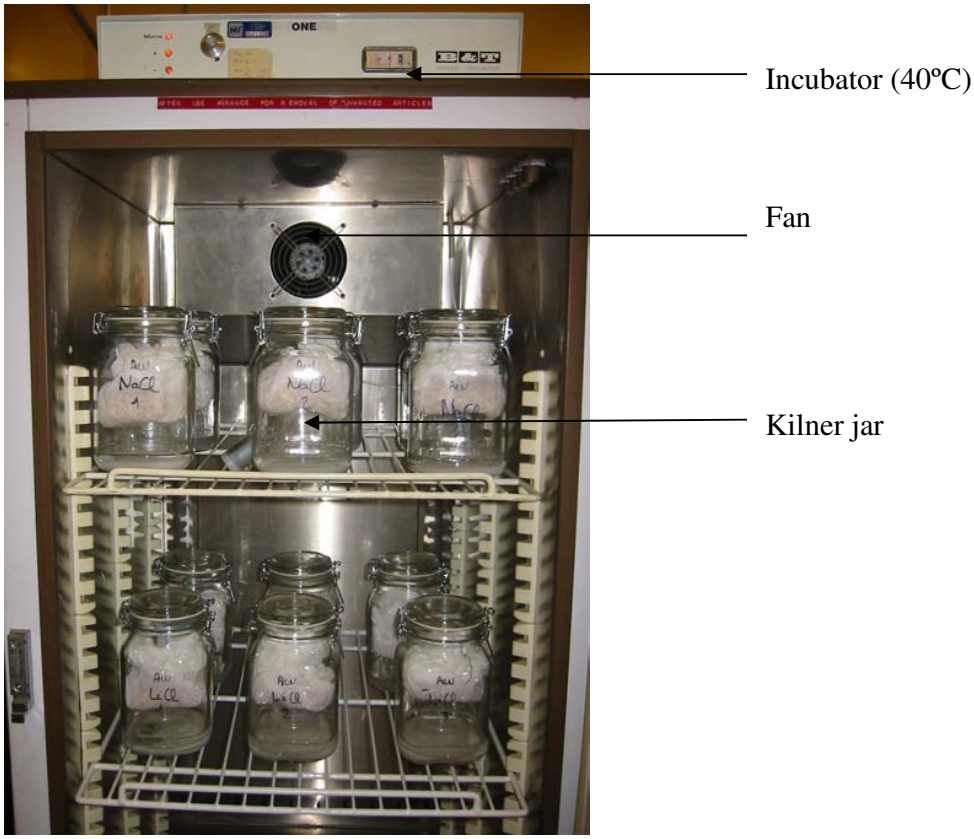


Figure 8-2: Storage system for water activity trial (12 jars).

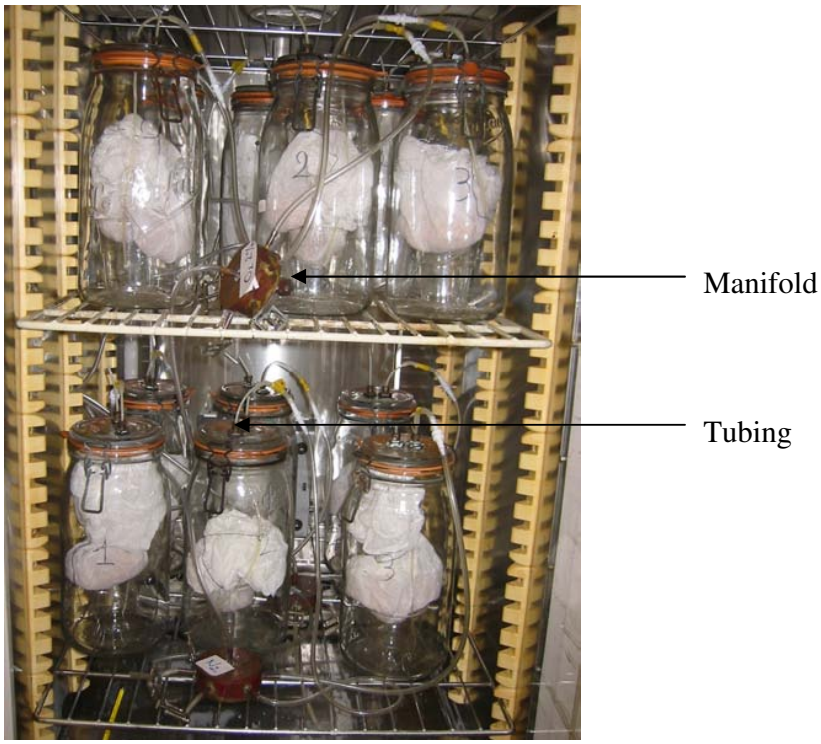


Figure 8-3: Storage system for oxygen trial (12 jars).

The water activity and oxygen experiments were carried out at 40ºC because it is the highest possible temperature in the field and therefore this would accelerate degradation and should highlight differences between treatments.

8.2.3 Experimental design

For a kinetics experiment, samples have to be collected over time. For the temperature, water activity, or oxygen, the experimental design consisted of four levels (10; 20; 30; 40°C), (0.13; 0.30; 0.51; 0.76) or (0; 2.5; 10; 21%) respectively and four or five collections (A, B, C, D, E) over time. The collection times after the start of the trial are given in days (Tables 8-1; 8-2; 8-3).

Table 8-1: Collection times (days) for the temperature* trial in NaBr solution.

Collection	10°C	20°C	30°C	40°C
A	31	31	15	15
B	62	46	28	19
C	94	62	53	27
D	105	94	95	40
E	-	105	-	54

*4 temperatures (°C) / 4-5 collections. Jars are in triplicate.

Table 8-2: Collection times (days) for the a_w* trial at 40°C.

Collection	LiCl	$MgCl_2$	NaBr	NaCl
a_w	0.13	0.30	0.51	0.76
A	15	15	15	15
B	19	19	19	19
C	27	27	27	27
D	40	40	40	40
E	54	54	54	54

*4 a_w / 5 collections. Jars are in triplicate.

Table 8-3: Collection times (days) for the oxygen level* trial at 40°C.

Collection	100% N_2	2.5% O_2; 97.5% N_2	10% O_2; 90% N_2	Simulated air: 21% O_2; 79% N_2
Oxygen level (%)	0	2.5	10	21
A	9	9	9	9
B	11	11	11	11
C	14	14	14	14
D	16	16	16	16
E	21	21	21	21

*4 O_2/ 5 collections. Jars are in triplicate.

8.2.4 Carotenoid analyses

Samples were extracted using the slightly modified HarvestPlus method (Rodriguez-Amaya and Kimura 2004) developed in Chapter 2. Total carotenoid content was determined using the diode array Hewlett Packard 8452A spectrophotometer (see Chapter 2 section 2.3.4) and individual carotenoids were quantified using the HPLC method described by Dhuique-Mayer *et al.* (2005) (see Chapter 2 section 2.3.3).

8.2.5 Volatile compounds analysis

The SPME system is described in Chapter 2 section 2.3.5. The operating conditions for this experiment were as follows: splitless injection (4 min); injection temperature, 250°C; initial oven temperature 60°C (held for 5 min), increased by 4°C.min^{-1} to 240°C and held at this temperature for 10 min. A standard curve using β-ionone (purity ≥ 97%; predominantly trans, Sigma-Aldrich, France) as internal standard for unstored sweet potato flour was performed in triplicate for five concentration levels 0.19; 0.29; 0.39; 0.58 μg.g^{-1} on a fresh weight basis. Coefficient of variation for the triplicate injections was less than 11% and coefficient of correlation (R^2) was 0.9993. The standards for the other norisoprenoids were not available and the selectivities for these compounds by the SPME fibre will vary from that for β-ionone. Therefore the real concentrations of these compounds could not be determined. The peak area response of the detector, however, indicates a relative concentration with storage time and this was sufficient for the follow up of these compounds. For 5,6-epoxy-β-ionone, β-cyclocitral and DHA, identified based on their mass spectra, the ratios (peak area at time t divided by peak area at initial time) were calculated.

8.2.6 Statistical analysis and kinetics modelling

Carotenoid contents were determined on a fresh and dry weight basis and norisoprenoid contents were determined on a dry weight basis. Data were processed using SPSS 15.00 software by one or two way-ANOVA (Analysis of variance; $p<0.05$) using Tukey test to determine which samples were significantly different from others. Carotenoid kinetics degradation was modelled using Arrhenius and Eyring models (Cisse *et al.* 2009). The Arrhenius model (Equation 8-2) is an empirical collision model that describes the relationship between reaction constant rates and temperature using activation energy (E_a) and a pre-exponential factor (k_∞). The Eyring model (Equation 8-3) is based on the

transition state theory in which enthalpy of activation (ΔH*) and entropy of activation (ΔS*) are the model's parameters. The model's parameters were identified from experimental data measured in triplicate using linear regressions.

$$k = k_{\infty} e^{-\frac{Ea}{RT}} \quad \textbf{(Equation 8-2)}$$

Where:

T : temperature (K)
k: degradation constant rate at T (day^{-1})
k_{∞}: value of k at T = ∞ (day^{-1})
Ea: Activation energy ($kJ.mol^{-1}$)
R: gas constant = 8.314 $J \cdot K^{-1} \cdot mol^{-1}$

$$k = \frac{k_B}{h} T . e^{-\frac{\Delta H^* - T\Delta S^*}{RT}} \quad \textbf{(Equation 8-3)}$$

Where:
k_B: Boltzmann constant = $1.381 \cdot 10^{-23}$ $J.K^{-1}$
h: Planck constant = $6.626 \cdot 10^{-34}$ J.s
ΔH*: activation enthalpy ($kJ.mol^{-1}$)
ΔS*: activation entropy ($J.mol^{-1}.K^{-1}$)

For the validation of the Arrhenius model using room temperature, the predicted data were calculated using Equation 8-4:

$$C = C_0 e^{-k_{\infty} \int_0^t e^{\frac{-Ea}{RT}} dt} \quad \textbf{(Equation 8-4)}$$

C is the carotenoid concentration at t days of storage and C_0 is the initial concentration. In order to validate the Arrhenius carotenoid degradation model under laboratory conditions, dried samples (Ejumula variety) were stored for t = 88 days at ambient room temperature (anisothermal or dynamic) conditions in the dark. Temperature and humidity were recorded every hour (mean: 21.4 ºC/46.8%; min: 13.8 ºC/39.3%; max: 25.2 ºC/47.6% respectively). The Arrhenius model was also tested with dried samples from Ejumula variety stored for t = 125 days at ambient room temperature in Uganda (see Chapter 4).

8.3 RESULTS & DISCUSSION

8.3.1 Sampling

In order to get a representative sample, there should be an equal division of the whole sample into sub-samples that should be a representative portion of the total sample.

Sub-sample

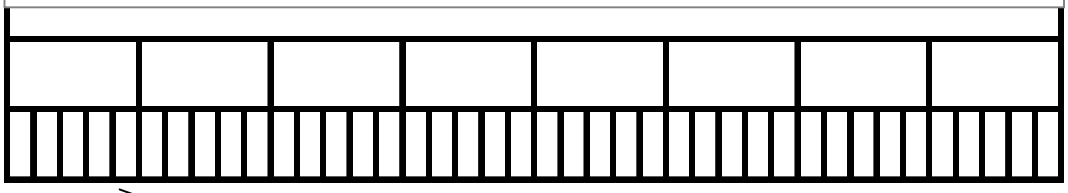
The minimum sub-sample size was estimated by the square root of the total sample size (Walker and Farrell 2003). The total sample weight of chips of the Ejumula variety dried in Uganda and used for this experiment was 3.613.6g. The weight of 10 chips was about 1g. Therefore the minimum sub-sample size should be the square root of the total sample size (36136 chips), that is 190.0947 and is equal to 19g. In practice, the whole sample was equally divided into 2; then 4; then 8 lots using the riffle divider. Then each of the lot was divided into five using the division by hand method used previously in Uganda (Walker and Farrell 2003). Using this method, 40 sub-samples of the total sample were obtained (each weighed about 90g).

Sample collection

On the sub-sample of 90g there should be four collections over time. The maximum collection could be a quarter, that is 22.5g, but reducing too much the sample size during storage could disturb the storage equilibrium. On the other hand the minimum collection (square root) was then 3g. Three grams also represents the minimum sample size for normal distribution (30 particles). Because of the need for analyses to take material losses and miss-analyses into account, collections of samples of about 15g chips were chosen. Practically in order to achieve a representative division of the sub-sample the riffle divider was used. The riffle divider can only split a sample into two at a time so several divisions had to be applied. For instance the initial sub-sample was 90g and was divided into four and a quarter of this was then divided into four. Three quarters of this (3/16) was collected. The rest (13/16) was put back into storage.

The sub-sample reduced with the different collections. Sample division for storage and collection is represented in Figure 8-4.

The whole chips sample (3316.6g) was divided into 8 and then into 5 = 40 sub-samples: 90g each.



Sub-sample: 90g was divided into samples of about 15g					
A	**B**	**C**	**D**	**E**	**Rest of sub-sample**

Figure 8-4: Schematical representation of sub-division of samples for representative collection of sweet potato chip during storage under controlled conditions

1st collection (A): 3/16 of initial sub-sample (90g) ~ 17g
2nd collection (B): 3/16 of sub-sample ~ 14g
3rd collection (C): 1/4 of sub-sample ~ 15g
4th collection (D): 3/8 of sub-sample ~ 17g
5th collection (E) possibly: 1/2 of sub-sample ~ 14g

8.3.2 Assessment of the storage system

Suitable storage conditions (*i.e.* sample containers; controlled temperature/humidity) had to be designed for this experiment. Saturated salts are a suitable way of maintaining constant humidity. Kilner jars of 1.5L were used because they were small enough to fit in the incubators (in comparison with desiccators). Height of the jar was no more than 1.5 times the width of saturation salt solution occupying the bottom of the jar. The greatest ratio width/height is recommended to offer the largest free surface of the saturated salt for reaching humidity equilibrium (OIML 1996).

An adapted device had also to be found to separate the saturated solution, which is liquid, from the dried chip samples. In the first place, the dried sweet potato sample was held above the salt in the jar by a plastic cup, copper gauze and cotton wool. Unfortunately the cotton wool layer held some of the water and was not 100% efficient at stopping chips from falling into the saturated salt solution. Another alternative to the cotton wool was to use a cotton fabric bag containing the chips, which allowed easier removal of the chips. Some modifications were made to the system because it was

realised that copper is a catalyst that could react with highly unsaturated β-carotene and therefore cause a bias in the experiment (Karl Franzman; pers. comm.). The copper gauze was therefore removed and the dried sweet potato sample was rested directly on the plastic cup, whose height was sufficient to maintain the sample away from the salt solution. A cotton fabric bag was used because of its inert properties toward β-carotene (Karl Franzman chemist; pers. comm.). Prior to its use cotton fabric was washed without detergent in a washing machine in order to remove possible additives from the fabric. Cotton bags were then cut and sewed by hand.

The saturation of the salt was tested without and with chips. When using a jar without chips, humidity equilibrium (between the salt water activity and atmosphere inside the jar) was reached after 19h. When using a jar containing dried sweet potato chips at 40ºC, equilibrium with the saturated salt was reached after 6 days (Figure 8-5).

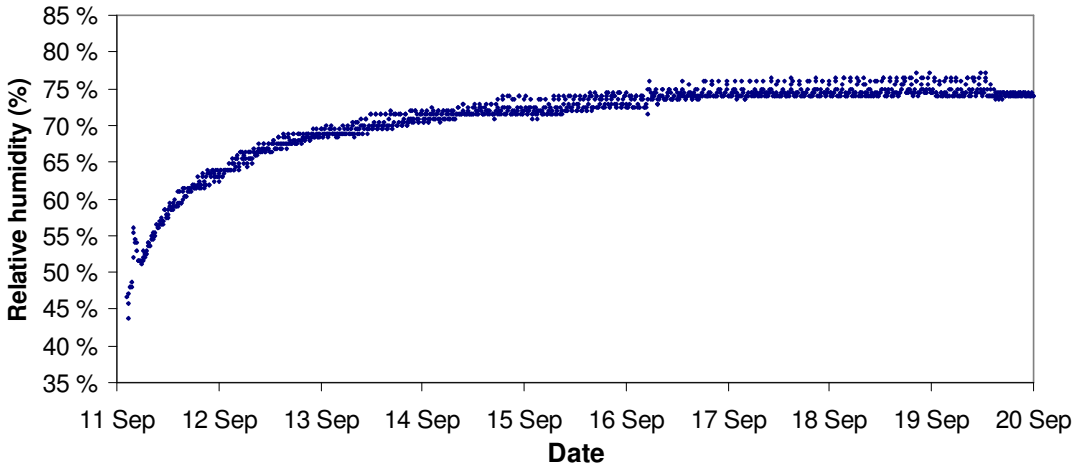


Figure 8-5: Achievement of equilibrium in a jar containing dried chips of orange-fleshed sweet potato (Ejumula variety) and NaCl saturated solution stored in incubator at 40ºC

Therefore the collection time of samples for analysis should be no more than 6 days but preferably 9 days (Figure 8-5). Following these trials, the storage system was considered acceptable for the storage of sweet potato chips under controlled conditions of temperature and humidity.

8.3.3 Carotenoid degradation kinetics

Temperature degradation kinetics

The effect of temperature (10; 20; 30; 40ºC) on β-carotene is shown in Figure 8-6.

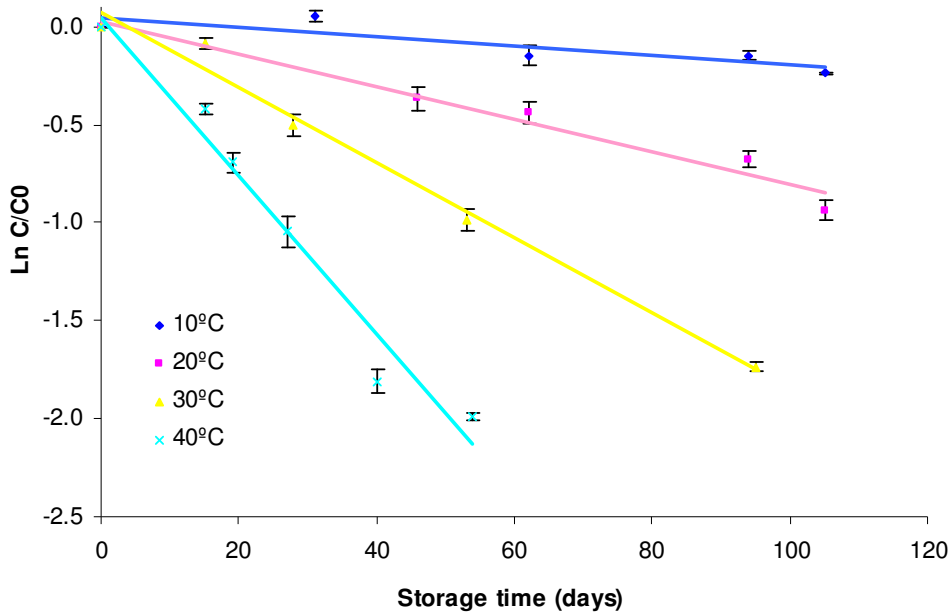


Figure 8-6: Trans-β-carotene degradation kinetics in dried sweet potato chips influenced by temperature between 10 and 40ºC on a dry weight basis (mean of triplicate determination; error bars show standard deviation). Oxygen level 21% (air); a_w 0.52-0.65.

Coefficients of correlation (R^2) in Table 8-4 suggest that a first-order equation fitted well the carotenoid degradation.

Table 8-4: Rate of degradation of carotenoids (k) expressed in day^{-1} at various temperatures in dried sweet potato chips on a dry weight basis. Oxygen level 21% (air) ; a_w 0.52-0.65. Mean of triplicate determinations (standard deviation).

Temperature (ºC)		10	20	30	40
Trans-β-carotene*	k	0.0024 (0.0002)	0.0090 (0.0005)	0.0191 (0.0002)	0.0403 (0.0005)
	R^2	0.757 (0.058)	0.914 (0.031)	0.986 (0.005)	0.965 (0.007)
5,6 epoxy-β-carotene*	k	0.0021 (0.0002)	0.108 (0.0002)	0.0248 (0.0007)	0.0597 (0.0005)
	R^2	0.583 (0.092)	0.889 (0.039)	0.869 (0.017)	0.987 (0.009)
Total carotenoids**	k	0.0040 (0.0003)	0.0083 (0.0002)	0.0174 (0.0005)	0.0295 (0.0010)
	R^2	0.825 (0.116)	0.978 (0.018)	0.987 (0.005)	0.987 (0.005)

* by HPLC; ** by Spectrophotometry

An exception nonetheless was at 10ºC where correlations observed were lower (R^2~0.8). Lower correlations at low temperatures where there is minimum carotenoid degradation could be explained by experimental errors (Hildago and Brandolini 2008). Degradation rates of trans-β-carotene were significantly different at the four tested temperatures (10;

20; 30; 40ºC). (ANOVA one way $p<0.05$; Tukey test SPSS 15.00). Ninety percent of the initial β-carotene was lost after 54 days of storage at 40ºC. On the other hand only 35% was lost after 62 days at a lower temperature of 10ºC. Hence temperature had a significant influence on the degradation of carotenoids. This result is important when storing sweet potatoes under field conditions. Temperature influence on the carotenoid degradation was then modelled using the Arrhenius and Eyring models (Table 8-5) (see calculations in Appendix 1).

Table 8-5: Parameters of the Arrhenius and Eyring models for the carotenoids degradation in dried sweet potato chips on a fresh and dry weight basis between 10-40ºC. Oxygen level 21% (air); a_w 0.52-0.65. Mean of triplicate thermal treatment (standard deviation).

	Arrhenius model			Eyring model		
Carotenoid	Ln k_∞ (k_∞ $days^{-1}$)	E_a ($kJ.mol^{-1}$)	R^2	ΔH^* ($kJ.mol^{-1}$)	ΔS^* ($J.mol^{-1}.K^{-1}$)	R^2
Fresh weight basis						
Trans-β-carotene*	21.5 (0.6)	64.2 (1.6)	0.990 (0.007)	61.7 (1.6)	-74.3 (5.1)	0.989 (0.008)
5,6 epoxy-β-carotene*	27.5 (1.5)	78.8 (3.8)	0.997 (0.003)	76.3 (3.8)	-24.5 (12.5)	0.996 (0.003)
Total carotenoids**	14.3 (0.6)	46.3 (1.5)	0.997 (0.002)	43.8 (1.5)	-134.5 (5.0)	0.997 (0.002)
Dry weight basis						
Trans-β-carotene*	23.1 (0.6)	68.3 (1.6)	0.984 (0.010)	65.8 (1.6)	-61.1 (4.0)	0.998 (0.002)
5,6 epoxy-β-carotene*	28.2 (0.7)	80.5 (1.9)	0.978 (0.005)	78.0 (1.9)	-18.4 (6.0)	0.984 (0.010)
Total carotenoids**	15.5 (0.8)	49.5 (1.9)	0.997 (0.002)	47.0 (1.9)	-124.0 (6.2)	0.997 (0.002)

* by HPLC; ** by Spectrophotometry

For both of the models provided, description of trans-β-carotene fitted well degradation (R^2 = 0.984 and 0.998 respectively on a dry weight basis). 5,6 epoxy-β-carotene, another carotenoid present in fresh sweet potato (Kósambo *et al.* 1998), also followed a first order rate reaction that could also be fitted to the same models (R^2 = 0.978 and 0.984 respectively on a dry weight basis). Using the models (see Chapter 2), the activation energy (Ea) for trans-β-carotene and for 5,6 epoxy-β-carotene was calculated as 68.3 (64.2) and 80.5 (78.8) kJ.mol-1 on a dry (and fresh) weight basis respectively and the

enthalpy was 65.8 (61.7) and 78.0 (76.3) kJ.mol-1 respectively on a dry (and fresh) weight basis respectively (Table 8-5).

On average the energy of activation and enthalpy were both 20% higher on 5,6 epoxy-β-carotene compared to trans-β-carotene. This means that the degradation rate of 5,6 epoxy-β-carotene was more sensitive to the variation of temperature than trans β-carotene.

Description of total carotenoids by spectrophotometer for the Arrhenius and Eyring models fitted well the degradation (R^2 > 0.997 and 0.997 respectively). Activation energy for total carotenoids content (by spectrophotometric reading) was 46.3 kJ.mol^{-1} on a fresh weight basis and was similar to the value of 44.3 kJ.mol^{-1} measured on the same basis for freeze-dried sweet potato at 60-80 ºC (Stephanovitch and Karel 1982). Activation energy for total carotenoids content (by spectrophotometric reading) was 49.5 kJ.mol^{-1} on a dry weight basis and was similar to these of Serio and Monlins wholemeal wheat flour stored between -20ºC and 38 ºC being 45.3 and 48.7 kJ.mol^{-1} respectively on a dry weight basis.

Validation of model

To test the robustness of the model, it was used to predict the carotenoid content of dried sweet potato samples that had been stored at ambient temperature in a jar and in the dark for 88 days in a laboratory in the UK. For the total carotenoids and trans-β-carotene under anisothermic conditions, the difference between the experimental value and value predicted by the model was 4.6% (4.3%) and 2.7% (3.5%) respectively on a dry (and fresh) weight basis (Table 8-6) (see calculations in Appendix 1).

For the 5,6 epoxy-β-carotene, the difference between the experimental value and model value was higher, being 19.4% (60.7%) on a dry (and fresh) weight basis respectively but with lower values. Lower values can be observed to be more difficult to predict using mathematical modelling.

Table 8-6: Validation of Arrhenius model for a sample of dried Ejumula sweet potato chips stored under ambient anisotherm conditions during 88 days in the UK[a] and during 125 days in Uganda[b] on a fresh and dry weight basis. Oxygen level 21% (air).

Storage	Carotenoid	Initial	Final		
		($\mu g.g^{-1}$)[c]	Experimental[c] ($\mu g.g^{-1}$)	Predicted by Arrhenius model ($\mu g.g^{-1}$)	Difference (%)
Fresh weight basis					
UK[a]	Trans-β-carotene	181.2 (5.9)	74.6 (5.1)	72.0	3.5
	5,6 epoxy- β-carotene	12.9 (0.5)	5.7 (0.5)	2.2	60.7
	Total carotenoids	250.3 (1.1)	94.4 (1.1)	90.3	4.3
Uganda[b]	Total carotenoids	219.6 (1.6)	51.4 (1.5)	46.6	9.3
Dry weight basis					
UK[a]	Trans-β-carotene	201.0 (6.6)	82.6 (5.6)	84.8	2.7
	5,6 epoxy- β-carotene	14.4 (0.6)	6.3 (0.6)	5.1	19.4
	Total carotenoids	277.7 (1.3)	104.3 (1.7)	99.6	4.6
Uganda[b]	Total carotenoids	236.3 (1.7)	58.5 (1.7)	49.1	16.0

[a] This present study (Calculated a_w from BET model: 0.460 (0.012) from chips dry matter (90.4 (0.3) g/100g) – storage time of 88 days.
[b] Bechoff *et al.* (2009b) (a_w from BET model: a_w: 0.400 (0.255); range: [0.22-0.58] from chips dry matter 90.5 (3.5)g/100g; range ([88-92.9g/100g]) – storage time of 125 days.
[c]Mean of triplicate (standard deviation).

The robustness of the model was further tested by using it to predict the carotenoid content of a dried sweet potato sample (Ejumula) that had been stored in Uganda at ambient temperature in LPDE bags (permeable to oxygen) for 125 days in Uganda (See Chapter 4). Similarly, the model was also accurate in its predictions for total carotenoids under anisothermic conditions with a difference between the experimental value and model of 16.0% (9.3%) on a dry (and fresh) basis (Table 8-6) (see calculations in Appendix 1).

It can be concluded that the model developed under samples stored using controlled laboratory conditions was robust enough to apply to samples stored under field conditions in Uganda and elsewhere. Predictions of carotenoid losses in dried sweet potato chips using the kinetic models developed are represented in Figure 8-7.

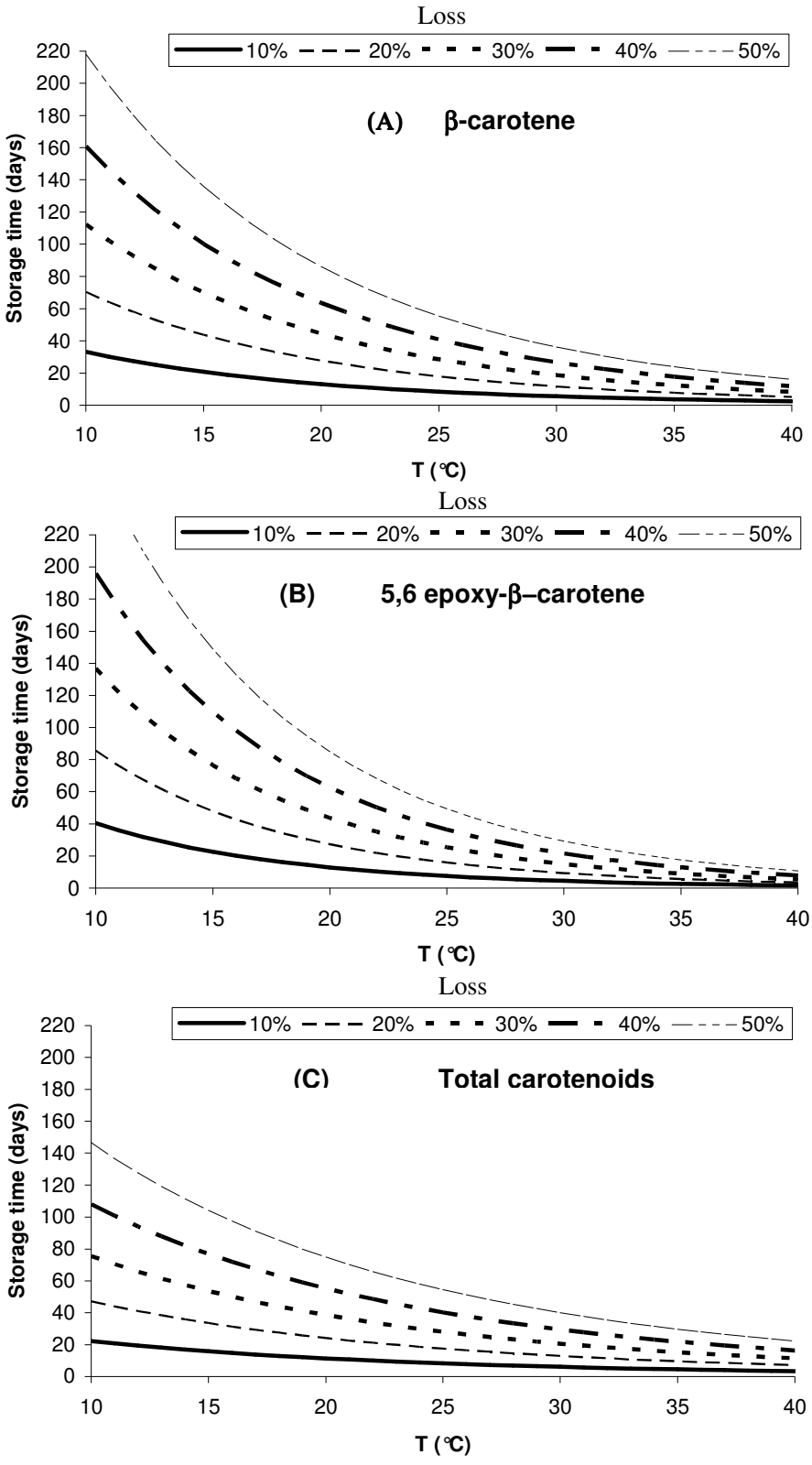


Figure 8-7: Prediction curves of carotenoid (β-carotene (A); 5,6-epoxy-β-carotene (B); total-carotenoids (C)) loss (%) on a fresh weight basis with temperature and with storage time in dried sweet potato chips stored between 10-40 ºC in air; a_w 0.52-0.65.

These curves can be used for practical applications, such as the determination of product shelf life. For instance, for a 20% loss in β-carotene (Figure 8-7A), the predicted storage duration in tropical conditions (average 30 ºC) is about 10 days. On the other hand, if the dried sweet potato is stored at temperate ambient temperature (average 20 ºC), then the predicted storage time is one month. If it is stored in refrigerated conditions (average 10 ºC) the predicted storage time increases up to 70 days. Predicted storage time based on total carotenoids (Figure 8-7B) is slightly shorter and when based on 5,6 epoxy-β-carotene, longer (Figure 8-7C) because the barrier energy to overcome (Ea) is lower for total carotenoids and higher for 5,6 epoxy-β-carotene. For instance, for a sample stored at 10 ºC, the storage duration would be 160, 200 and 110 days for a 40% loss in β-carotene, 5,6-epoxy-β-carotene or total carotenoids respectively.

8.3.4 Influence of water activity and oxygen on carotenoid degradation

Water activity

The water sorption properties of dried sweet potato chips stored at 40ºC in different saturation salt solutions are shown in Figures 8-8 and 8-9.

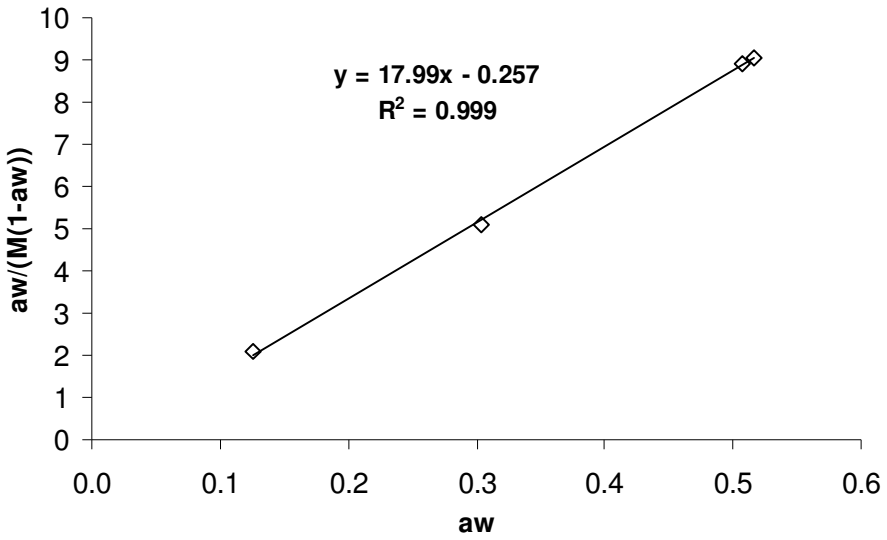


Figure 8-8: Linearised equation of Brunauer-Emmett-Teller (BET) for dried sweet potato chips at 40ºC and stored for 54 days. *C* and M_0 were calculated from the linearised equation. BET model (*C* = -68.88, M_0 = 0.0564).

The experimental data were fitted with the BET equation (R^2 = 0.999) for an a_w interval of 0.13-0.76. At high water activity for a dried flour (a_w = 0.76), the BET model slightly

lost some precision, but this corresponded to a moisture content of 20.8% on a dry weight basis, which is outside the usual range of storage conditions. Sweet potato of variety Ejumula stored in Uganda under ambient conditions for four months had maximum moisture content of 13.6% on a dry weight basis (12% on a wet weight basis) (see Chapter 4).

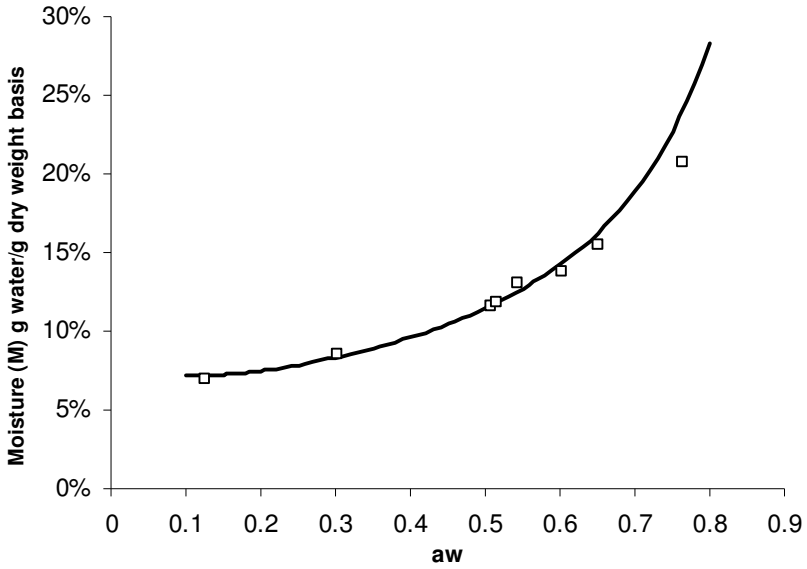


Figure 8-9: Water sorption curves of dried sweet potato chips at 40ºC and stored for 54 days. Experimental data and BET model (C = -68.88, M_0 = 0.0564).

Under different water activities, trans-β-carotene degradation fitted a first order kinetic model as shown in Table 8-7.

Table 8-7: First order rates of carotenoid degradation (k) expressed in day^{-1} at various water activities at 40ºC in dried sweet potato chips on a dry weight basis. Mean of triplicate thermal treatments (standard deviation). Oxygen level 21% (air).

Saturation salt		NaCl	NaBr	$MgCl_2$	LiCl
Water activity		0.76	0.51	0.30	0.13
Trans-β-carotene*	k	0.0332 (0.0009)	0.0413 (0.0004)	0.0462 (0.0033)	0.0496 (0.0032)
	R^2	0.991 (0.001)	0.976 (0.001)	0.970 (0.004)	0.945 (0.048)

Under isothermic conditions (40ºC) and in samples stored under air, the lower the water activity the faster the β-carotene was degraded (Figure 8-10).

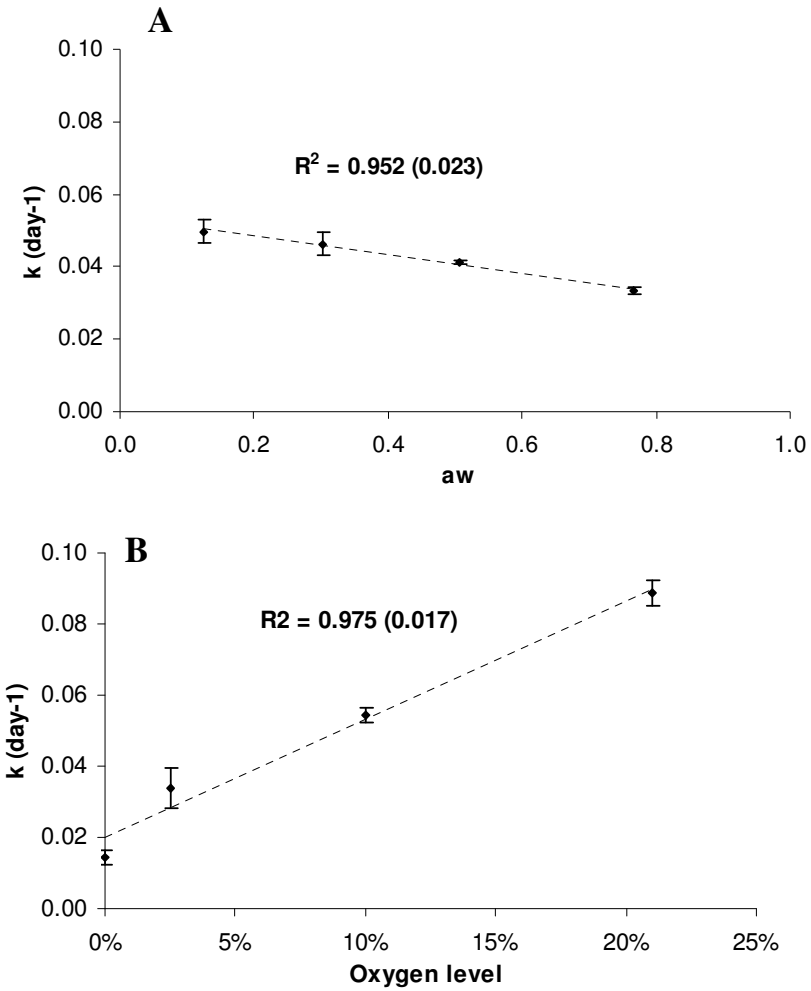


Figure 8-10: Relationship between trans-β-carotene rate constants (k) and water activity of dried sweet potato chips under air (A); and oxygen level (B) for the storage of sweet potato dried chips at 40ºC on a dry weight basis (mean of triplicate determinations; error bars refer to standard deviation).

Samples stored at a_w =0.13 showed greater losses of β-carotene, followed by those stored at a_w = 0.30, 0.51 and 0.76. The degradation rate constant for β-carotene at a_w = 0.13 did not differ significantly from a_w = 0.30, but differed at a_w = 0.51 with means of 0.0496, 0.0462 and 0.0413 day^{-1} respectively. On the other hand, the degradation rate constant at a_w = 0.76 was 0.0332 day^{-1}, which was significantly lower than with the other salts (ANOVA one way $p<0.05$). Although storing the dried sweet potato at high water activity (0.76) improved the retention of β-carotene, it would not be recommended because of the high probability of microbial spoilage. Overall, these results have showed that water activity (a_w = 0.13-0.76) had a significant impact on carotenoid

degradation during the storage of dried sweet potato chips with constant rates comprised between 0.0332 and 0.0496 day^{-1}.

However the effect of water activity was a lot less than the effect of temperature (10-40ºC) with constant rates of 0.0024 to 0.0403 day^{-1}. Therefore at typical product moisture content of 7-14% on a dry basis (see Chapter 4) (which corresponds to water activities of 0.2-0.6 at 40ºC) water activity would have a limited impact compared to temperature. There was a linear relationship between the β-carotene degradation rate and water activity (R^2=0.952) (see Figure 8-10A).

Working with a model system made of microcrystalline cellulose containing 0.5% of β-carotene, Goldman *et al.* (1983) and Chou and Breene (1972) proved that β-carotene degradation followed first order kinetics and was accelerated at lower water activities. In particular, when comparing extreme water activities (dry a_w 0.33 and wet a_w 0.84), it was demonstrated than that the higher the water activity the lower the β-carotene degradation (Goldman *et al*. 1983).

In contrast to the linear trend depicted by Chou and Breene (1972), a U-shaped relationship between the β-carotene degradation rate and water activity (0.05-0.75) has been described in freeze-dried carrots (Lavelli *et al.* 2007). Degradation rate constants were at a minimum between a_w 0.34-0.54 on freeze dried carrot flour stored for 30 days at 40ºC and increased at higher water activities than 0.54. Nevertheless acceleration of degradation at higher water activities was not believed to result from enzymatic oxidation for the reason that blanched and unblanched samples both had U-shaped degradation patterns with water activity (Lavelli *et al.* 2007). Arya *et al.* (1979) working on blanched and freeze-dried carrots argued that increased carotenoid degradation rate at higher water activities might have resulted from solubilisation of naturally present metal catalysts that accelerate carotenoids autoxidation rate rather than from enzymatic breakdown of carotenoids.

It has been confirmed in an earlier study on dehydrated sweet potato cubes (Haralampu and Karel 1983) that lower degradation rates occurred at higher water activities in sweet potato (and *vice versa*). Peroxidase is the main type of enzyme encountered in sweet potatoes (Castillo-Leon *et al.* 2002) (see Chapter 7). At low water activities it has been

demonstrated that peroxidase activity dramatically decreased (Kamiya and Nagamune 2002) and these results have been confirmed in Chapter 7. Dioxygenases known for their ability to degrade carotenoids into aroma compounds (Auldridge *et al.* 2006) also lose activity in non-aqueous environments (Sanakis *et al.* 2003). For these different reasons, in this study, the possibility of carotenoid degradation due to enzymatic activity seemed unlikely.

Oxygen

Degradation of β-carotene was highly related to the oxygen level flushed through the sample as seen in Figure 8-10B. Under different levels of oxygen and at 40ºC, carotenoid degradation fitted first order kinetics (Table 8-8).

Table 8-8: First order rate of carotenoid degradation of (k) expressed in day^{-1} at various water activities at 40ºC in dried sweet potato chips on a dry weight basis. Mean of triplicate thermal treatment (standard deviation). Oxygen level 21% (air).

Flushed gaz		N_2	2.5% O_2: 97.5% N_2	10% O_2: 90% N_2	21% O_2: 79% N_2
Oxygen concentration (%)		0	2.5	10	21
Trans-β-carotene*	k	0.0143 (0.0022)	0.0338 (0.0056)	0.0543 (0.0021)	0.0887 (0.0038)
	R^2	0.754 (0.216)	0.949 (0.020)	0.963 (0.007)	0.961 (0.012)

Degradation rate constants significantly differed between samples (One way-ANOVA $p<0.05$). Samples flushed with nitrogen had a rate constant of 0.0143 day^{-1}, whilst those flushed with 2.5%, 10% and 21% oxygen had respective rate constants of 0.0338, 0.0543 and 0.0887 day^{-1}.

The significant effect of oxygen level on the degradation rate confirmed that oxidative mechanisms are involved in the reaction scheme. The linear relationship (kinetic model of pseudo-order zero) between oxygen level and degradation rate signifies that oxygen could be considered as a co-substrate (in excess) of oxidative degradation during storage (Figure 8-10B). A study on sweet potato dried flakes similarly described a linear relationship between the oxygen uptake and carotene destroyed in the first 110 days of storage at 31 ºC (Walter and Purcell 1974).

Over the range studied (0-21% oxygen), oxygen had a more marked effect on β-carotene than temperature (10-40ºC) and water activity (0.13-0.76). The mean difference between constant rates was 0.887-0.0143 = 0.0744 day^{-1} (21% and 0% oxygen respectively),

0.0403-0.0024 = 0.0379 day^{-1} (40ºC and 10ºC respectively) and 0.0496-0.0332 = 0.0164 day^{-1} (a_w of 0.13 and 0.76 respectively). Studies on other foodstuffs similarly concluded that oxygen was a major factor of carotenoid degradation during storage. It was demonstrated that carotenoid degradation significantly differed in sweet potato flakes kept in packaging with variable permeability to oxygen (Emenhiser *et al.* 1999): Flakes were stored at ambient temperature (about 23ºC) in polypropylene (high oxygen permeability) or in nylon laminate film (low oxygen permeability) 1. with air space; 2. under vacuum or 3. using a Ageless oxygen absorber sachet enclosed. β-carotene content remained steady in sweet potato flakes stored for 210 days in nylon film with an oxygen absorber whilst it was greatly reduced in flakes stored in polypropylene (66% loss) . In this present study, the β-carotene loss on sweet potato chips stored under nitrogen (16% after 21 days) might have been a result of incomplete oxygen exclusion of samples. Alternatively it could also have been the result of the effect of using a relatively high temperature for storage (40ºC).

It was interesting to observe that flushing with air at 40ºC dramatically increased the degradation rate (0.0887 day^{-1}) compared to samples stored under air but at the same temperature (0.0332 to 0.0496 day^{-1}). The significant effect of oxygen level on the degradation rate confirmed that oxidative mechanisms are involved in the reaction scheme. Furthermore the increased degradation rate of β-carotene flushed with oxygen are in accordance with studies (Texeira Neto *et al.,* 1981) that showed that oxygen uptake in a microcrystalline cellulose food model was closely linked to β-carotene degradation and agreed with other studies on sweet potato flakes showing a direct relationship between oxygen uptake and carotenoid degradation (Walter and Purcell, 1974). The linear relationship for the four levels of oxygen analysed in triplicate (R^2=0.975) between oxygen level and degradation rate signifies that oxygen could be considered as a co-substrate (in excess) of oxidative degradation during storage. A study on sweet potato dried flakes similarly described a linear relationship between the oxygen uptake and carotene destroyed in the first 110 days of storage at 31 ºC (Walter & Purcell 1974).

Studies on a model system made of microcrystalline cellulose and β-carotene similarly demonstrated that the effect of oxygen was important for the degradation of β-carotene compared to the effect of water activity (Goldman *et al.* 1983). Both trends of the

degradation rate related to water activity and oxygen in dried media agreed with studies on microcrystalline food model food systems (Chou and Breene 1972; Texeira-Neto *et al.* 1981; Goldman *et al.* 1983) where enzymatic activity is excluded and with previous studies on dehydrated sweet potato (Walter *et al.* 1970; Walter and Purcell 1974; Haralampu and Karel 1983). Though autoxidation was mentioned in earlier studies as the cause of β-carotene degradation in dried sweet potato (Walter *et al.* 1970; Walter and Purcell 1974; Haralampu and Karel 1983), a more recent study (Auldridge *et al.* 2006) pointed that it had not been proved. This study therefore fills the gap by showing that dehydrated sweet potato and microcrystalline cellulose food model behaved the same way towards water activity and oxygen which strongly suggests that autoxidation was the main mechanism responsible for β-carotene degradation.

8.3.5 Relationship between norisoprenoid formation and carotenoid degradation

Water activity

Whereas trans-β-carotene degraded during storage, norisoprenoids, namely β-ionone, 5,6 epoxy-β-ionone, β-cyclocitral and dihydroactiactinidiolide (DHA), mostly formed during storage. These compounds are the main aroma degradation products of β-carotene according to several previous publications (Handelman *et al.* 1991; Mordi *et al.* 1993; Waché *et al.* 2003). In most cases, the greatest formation of norisoprenoids occurred at lower water activities (Figure 8-11) and this is consistent with the earlier findings that carotenoid degradation is also greater at lower water activities (see Figure 8-10A).

On average, samples stored at a_w =0.13 had a β-ionone content of 0.50 (0.47)μg.g^{-1} on a dry (and fresh) weight basis respectively, followed by those stored at a_w = 0.30 with 0.41 (0.38) μg.g^{-1}; at a_w = 0.51 had a β-ionone content of 0.33(0.30)μg.g^{-1} and at a_w = 0.76 of 0.38(0.31)μg.g^{-1}. There was no difference between samples stored at a_w = 0.13 and 0.30 for β-ionone while the other samples stored at a_w = 0.51 and 0.76 differed (two way-ANOVA; $p<0.05$) (Figure 8-11). The amounts of norisoprenoids other than β-ionone were expressed in ratio (peak area at time t/peak area at initial time). There were no differences in β-cyclocitral ratios for samples stored at a_w = 0.13 and 0.30; or at 0.51 and 0.76. On the other hand, there were significant differences in the DHA and 5,6 epoxy-β-ionone ratios for samples stored at these four water activities (two ways-ANOVA; $p<0.05$). Water activity was therefore proved to have an influence on the production of volatile compounds as well as on the degradation of β-carotene.

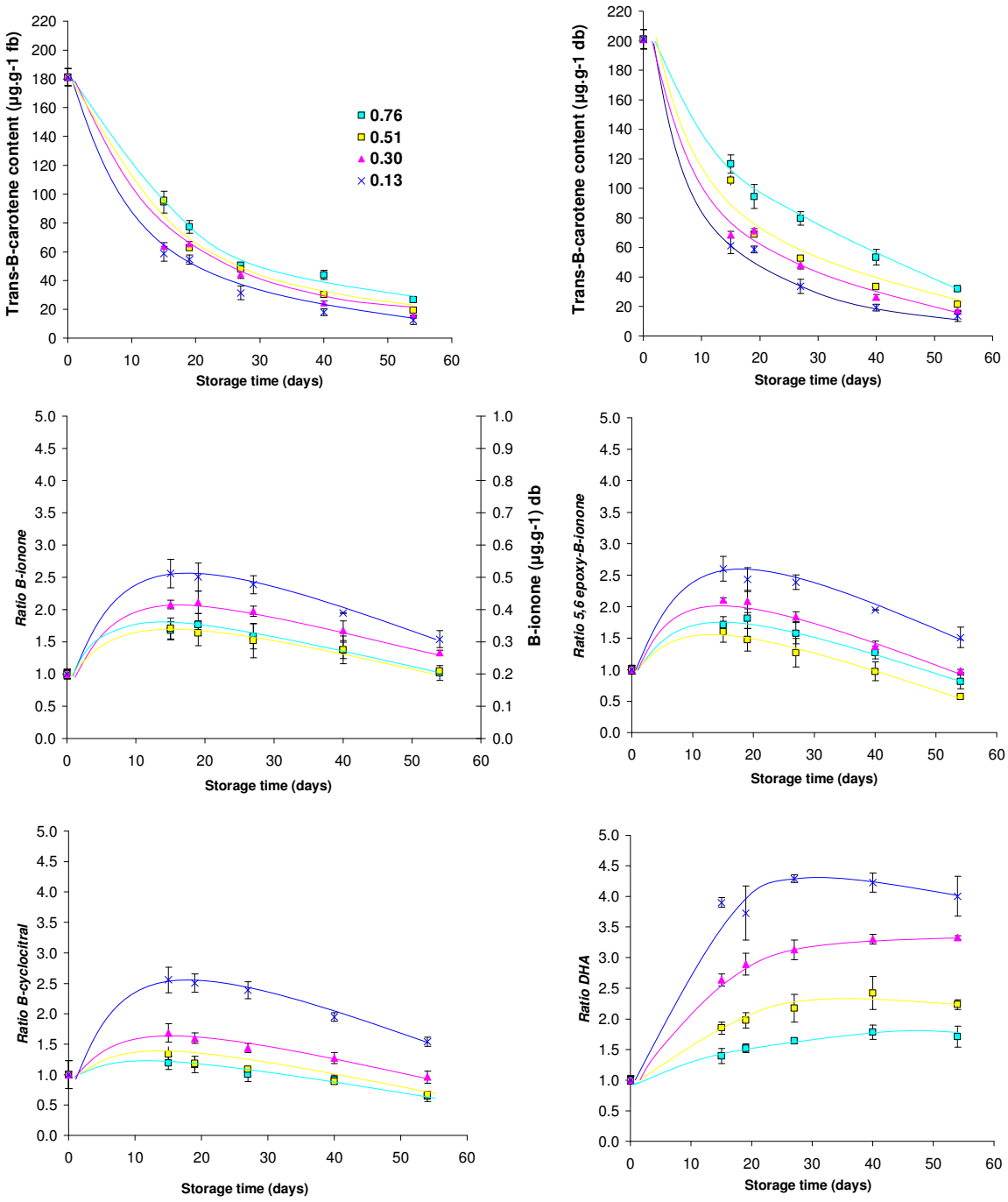


Figure 8-11: Water activity influence on trans-β-carotene on a dry weight (db) or fresh weight basis (fb) and norisoprenoids (β-ionone, 5,6 epoxy-β-ionone, β-cyclocitral, dihydroactinidiolide (DHA)) on a dry weight basis (db) and ratio (peak area at time t divided by peak area at initial time) during 40ºC storage of dried sweet potato chips. Oxygen level 21% (air). Mean of triplicates; error bars refer to standard deviation.

β-cyclocitral, β-ionone and 5,6 epoxy-β-ionone ratios were the highest at 19 days and subsequently decreased whilst DHA levels reached a plateau after 27 days of storage at

40ºC. Epoxidation of β-ionone into 5,6 epoxy-β-ionone and thermal rearrangement of 5,6 epoxy-β-ionone into DHA has been described by several authors (Mordi *et al.* 1993; Bosser *et al.* 1995; Waché *et al.* 2003). The similar profile of β-ionone and 5,6-epoxy- β-ionone throughout storage suggested that the two compounds were formed at similar times via a same process whereas the later formation of DHA suggested that the rearrangement into DHA was a slower step (Dhuique-Mayer pers. comm.).

Oxygen

Fragmentation of β-carotene in dehydrated sweet potato flakes under oxygen has been reported previously by Walter *et al.* (1970). Flushing radioactive sweet potato flakes with oxygen in the dark at 22 ºC and storing them for up to 89 days resulted in different radioactive fractions including gaseous products. The degradation was described as an autoxidation reaction (Walter *et al.* 1970) in accordance with the conclusions in this study. The higher formation of norisoprenoids occurred at the higher oxygen level, when the corresponding degradation of β-carotene was also highest (Figure 8-12). At 40 ºC samples stored flushed with compressed air (21% oxygen) had a β-ionone content of 0.46(0.44)µg.g^{-1} on a dry (and fresh) weight basis respectively, followed by those flushed with 10% (0.37(0.36)µg.g^{-1}); 2.5% (0.24(0.22)µg.g^{-1}) oxygen or nitrogen (0.11(0.10)µg.g^{-1}), respectively. There was a significant difference in the β-cyclocitral, β-ionone 5,6 epoxy-β-ionone and DHA ratios at the four oxygen levels (two ways-ANOVA; $p<0.05$) in agreement with the reported trans-β-carotene degradation at these four oxygen levels.

With no oxygen present in the storage system, only a small decrease or no difference in volatile compounds contents was observed. These observations highlighted the importance of oxygen in the degradation mechanism of β-carotene into volatile compounds (Figure 8-12).

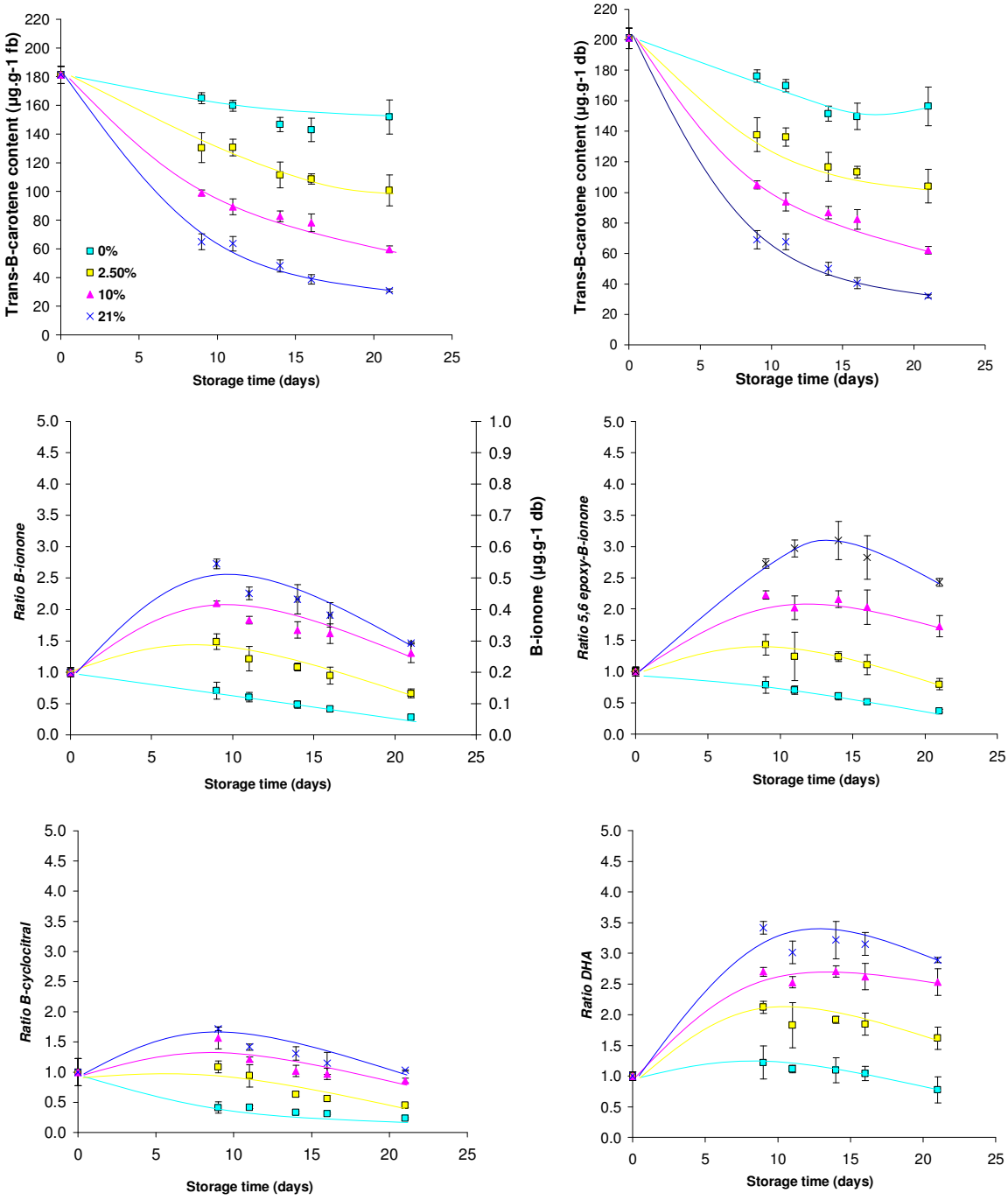


Figure 8-12: Oxygen influence on trans-β-carotene on a dry weight (db) or fresh weight basis (fb) and norisoprenoids (β-ionone, 5,6 epoxy-β-ionone, β-cyclocitral, dihydroactinidiolide (DHA)) on a dry weight basis (db) and ratio (peak area at time t divided by peak area at initial time) during 40°C storage of dried sweet potato chips. Mean of triplicates; error bars refer to standard deviation.

β-cyclocitral, β-ionone and 5,6-epoxy- β-ionone reached their highest levels after 9 days of storage and tended to subsequently decrease whilst DHA remained almost steady

from 9 days (Figure 8-12). This is a similar pattern to that observed at different water activities that also suggests that DHA was formed from β-ionone and 5,6-epoxy- β-ionone.

The amounts of β-ionone observed in the SPME-GC-MS analyses corresponded to approximately two orders of magnitude less than the amounts of β-carotene measured by HPLC (Figures 8-11 and 8-12). Calibration curves for the other volatile degradation products were not measured so the accurate amounts present cannot be derived from the SPME analyses. However, these compounds have relatively similar molecular weights and polarities to the β-ionone and so the GC-MS response factors and selectivities of the SPME fibres are expected to be reasonably similar. Accepting these assumptions, amounts of the other degradation products are similar to those of β-ionone with maximum amounts per compounds (β-ionone, 5,6-epoxy-β-ionone, β-cyclocitral and DHA) being approximately 0.4-0.9 $\mu g.g^{-1}$, will are much lower than the amounts of carotenoids degraded (Figures 8-11 and 8-12). Waché *et al.* (2002) also found that the highest yield obtained from β-carotene catalysed by enzymes in liquid medium was 8.5% in DHA, 2% in β-ionone and 1% in 5,6-epoxide-β-ionone. These results suggested that though a relationship between amounts of norisoprenoids formed and carotenoids lost was proved, free radical reaction mechanisms implied further degradation leading to disappearance of norisoprenoids or alternative pathways of degradation involving other reaction intermediates.

Free radical reaction mechanisms implied further degradation leading to complete breakdown of norisoprenoids and alternative pathways of degradation involving various other reaction intermediates (Figure 8-13).

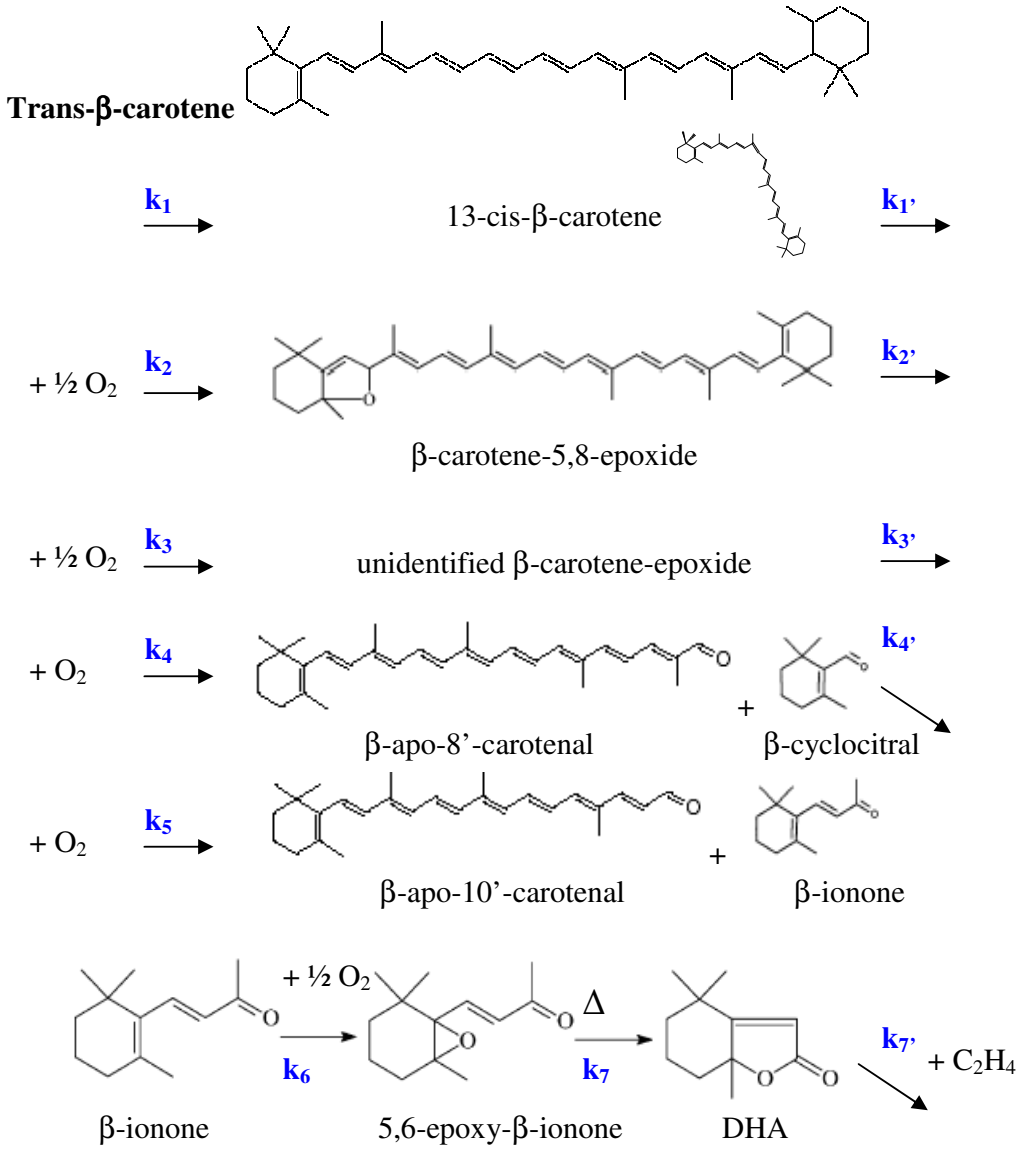


Figure 8-13: Model of degradation pathway of β-carotene in dried sweet potato. k_i: β-carotene degradation constant rate (day^{-1}); $k_{i'}$: intermediate product degradation constant rate (day^{-1}). Based on Waché *et al.* (2003).

The degradation constant rate k_i or $k_{i'}$ can be calculated (Equation 8-5) and the model can be solved using mathematical modelling (for example using MATLAB software).

- d(β-carotene)/dt = - k_1β-carotene
- d(13-cis-β-carotene)/dt = k_1β-carotene - k_1,13-cis β-carotene
- d(β-carotene-5,8-epoxide)/dt = k_2β-carotene - k_2,β-carotene-5.8-epoxide
- d(unidentified-β-carotene-epoxide)/dt = k_3β-carotene – k_3,unidentified-βcarotene-epoxide
- d(β-apo-8'-carotenal)/dt = k_4β-carotene
- d(β-cyclocitral)/dt = k_4β-carotene - k_4,β-cyclocitral
- d(β-apo-10'-carotenal)/dt = k_5β-carotene
- d(β-ionone)/dt = k_5β-carotene – k_6β-ionone
- d(5,6epoxy-β-ionone)/dt = k_6β-ionone – $k_7$5,6epoxy-β-ionone
- d(DHA)/dt = $k_7$5,6epoxy-β-ionone – k_7,DHA

(Equation 8-5)

Molar Balance

5 mol β-carotene + 3 mol O_2 ⟶ 1 mol 13-cis-β-carotene, 1 mol β-carotene-5.8-epoxide, 1 mol unidentified β-carotene, 1 mol β-apo-10'-carotenal, 1 mol β-apo-8'-carotenal, 1 mol β-ionone, 1 mol β-cyclocitral

1 mol β-ionone + $_{1/2}$ mol O_2 ⟶ 1 mol 5,6-epoxy-β-ionone

1 mol 5,6-epoxy-β-ionone ⟶ 1 mol DHA

In order to achieve a full mathematical modelling of the degradation of carotenoids in a food product, these equations should be solved. However this study has not been undertaken as part of this research work.

8.4 CONCLUSION

This study used a storage system to describe the degradation of β-carotene under controlled conditions of temperature, water activity and oxygen.

- The Arrhenius and Eyring models correctly described the carotenoid degradation in dried stored sweet potato between 10 and 40ºC. The Arrhenius model was validated using samples stored at room temperature in the UK and in Uganda (non-isotherm conditions). This model successfully predicted β-carotene degradation using temperature data and could be used in field applications.

- There was also a linear relationship between β-carotene degradation rate and water activity, and between β-carotene degradation rate and oxygen concentration. This relationship requires further research but highlighted the straightforward influence of these factors on carotenoid degradation. Oxygen (0-21%) with degradation constant rates ranging between 0.0143-0.0887 day^{-1} has a far greater influence on carotenoid degradation than water activity (0.13-0.76) with variations between 0.0332-0.0496 day^{-1}. Temperature (10-40ºC) has also an important influence on β-carotene degradation with a range of 0.0024-0.0403 day^{-1}.
- Norisoprenoid formation (β-ionone; 5,6 epoxy-β-ionone; DHA) during storage of dried OFSP chips was clearly related to the corresponding degradation of β-carotene. The higher, the β-carotene degradation, the higher was the norisoprenoid formation. At higher water activities, β-carotene was better preserved and lower concentrations of volatiles were recorded. A similar observation was made at lower oxygen levels.
- With the identification of norisoprenoids products and their variation of concentration during storage, a potential pathway of degradation was proposed. This pathway could be investigated using statistical prediction tools to predict the concentration of norisoprenoid compounds during storage. Further research is required to understand nature of the intermediate compounds between β-carotene and norisoprenoids formed during storage and the kinetics of their formation and degradation.

CHAPTER 9.
GENERAL DISCUSSION AND FUTURE WORK

9.1 OBJECTIVES AND ACHIEVEMENTS

The main objectives of the thesis were to measure the level of carotenoid degradation in orange-fleshed sweet potato (OFSP) after drying and storage. OFSP is currently promoted for tackling vitamin A deficiency in developing countries, and the potential for processed products, in particular dried products, needed to be investigated. Earlier research on food products (such as carrots, cassava, leaves, OFSP) demonstrated that processing, and in particular, solar and sun drying, which can be affordable techniques for small-scale farmers in Africa, had a significant impact on carotenoid stability. However previous research about the losses of carotenoids during solar and sun drying in OFSP in particular (see Chapter 1) was scarce and showed inconsistent results. There was therefore a need to evaluate the impact of solar and sun drying, and subsequent storage on the carotenoid content of OFSP.

- The main achievement of this work has been to quantify carotenoid loss from orange-fleshed sweet potato (OFSP) following drying and storage. Carotenoid loss was related to various process variables (such as type of dryer; chip size; variety; and nature of pre-treatment) both at a pilot scale and field level. Degradation of β-carotene was limited after drying of orange-fleshed sweet potato (less than 35%) but was an important issue during the subsequent storage (70-80% loss).

- A secondary achievement has been to gain a better understanding of how carotenoids degrade during storage of dried OFSP. Some of the observations may be applicable to dried foods containing carotenoids in general. The impact of enzymatic activity and the effects of the main factors on carotenoid degradation (water activity, temperature, oxygen) were measured. Furthermore a quantification of carotenoid losses along with the compounds produced during degradation was achieved.

Further information about the pattern of degradation of carotenoids during storage of dried food products was also obtained.

The main findings are discussed below.

9.2 QUANTIFYING CAROTENOID LOSSES DURING DRYING

9.2.1 Levels of carotenoid loss during drying

The effects of drying treatment and chip size on carotenoid losses in OFSP were investigated. An important finding was that losses after sun drying were relatively low in comparison with what was expected when starting the research project. This was relevant because few literature reports had been found on this subject. A report by the International Center for Potato (CIP) indicated levels of trans-β-carotene loss of 72% from sun-dried OFSP chips of Jonathan variety and 83% of Kakamega variety in Kenya (Kósambo 2004). A presentation at the launch meeting of the HarvestPlus Reaching End Users project (April 2006, Kampala, Uganda) by the program director Howarth Bouis similarly portrayed losses of carotenoid in sun drying of OFSP between 85 to 91% (van Jaarsveld unpublished). Moreover farmers in Soroti who were interviewed in December 2006 reported that OFSP or white fleshed-sweet potato dried in the traditional way (on rocks) could be the same colour after drying, which further indicated that carotenoids could have been entirely degraded during drying. The first results from a pilot-scale study in the South of France showed a different picture. Levels of loss in trans-β-carotene on Rubina® OFSP chips were 34% after sun drying for one day (Chapter 3 or Bechoff *et al.* 2009 in Appendix 4a). In the further field studies at the National Agricultural Research Laboratories, NARO in Uganda, levels of loss in sun-dried chips on Ejumula and Kakamega varieties were only 9.0% and 8.4% respectively (Chapter 4 or Bechoff *et al.* 2010a in Appendix 4b) and in Mozambique losses sun-dried chips on MGCL01 and Resisto were 4.9% and 16.5% respectively (Chapter 5 or Bechoff *et al.* 2010c) after drying for one day up to three days (with coverage at nights). Hence it was shown in all the studies (France, Uganda, and Mozambique) that sun drying could retain most of the carotenoid content. These levels were found consistent with a more recent study resulting from collaboration between the Chalmers University, Sweden and the Makerere University, Kampala, and involving the same varieties. The level of trans-β-

carotene loss after sun drying of Ejumula variety was 16% in this study (Bengsston *et al.* 2008). These results contrasted with the earlier literature that reported high levels of loss after drying. Hypothetical reasons for high levels of loss and inconsistency in these few earlier reports could have been: longer drying times; lack of mixing of sample during drying; reintroduction of moisture (due to rain) to already dried samples; and/or incomplete extraction of carotenoids (from dried chips compared to fresh chips).

9.2.2 Comparison of dryers

Another important finding in this work was that sun drying was equivalent to solar drying in terms of carotenoid retention and this was consistent in the three above studies in France, Uganda, and Mozambique. These findings also agree with the study by Bengsston *et al.* (2008) that compared the retention of trans-β-carotene in oven, solar tunnel and sun drying. However these results contrast with other studies that demonstrated superiority of solar drying compared to sun drying in different foodstuff such as saffron (Raina *et al.* 1996), leaves (Negi and Roy 2000; Mulokozi and Svanberg 2003) or cowpea leaves and mango (Ndawula *et al.* 2004). The lack of difference between solar and sun drying may be explained by the bulkiness of OFSP chips that protects carotenoids from UV-radiation and by faster drying in sun dryer compared to solar dryer with natural convection under these field conditions (Bechoff *et al.* 2009a). Starch, the main component of sweet potato, may have also played a protective role in limiting carotenoid losses, as demonstrated by Zhao and Chan (1995) on starch-coated carrots. It could also explain the lack of difference between the solar dryers and sun drying. Another reason for low carotenoid loss after drying could be the low initial moisture content of the product. OFSP and carrot with respective dry matter contents of 75.8% and 90.5% presented differential β-carotene losses of 10% and 59% respectively. It has been suggested that the removal of more moisture from the product during drying results in increased carotenoid loss (Mdziniso *et al.* 2006). It might be possible that removal of a larger amount of moisture could have increasingly disturbed the integrity of chromoplasts (carotenoids are found within the chromoplast structure within the vegetable cell) and consequently contribute to greater levels of carotenoid degradation.

As compared with sun drying, both hot air drying (Chapter 3) and shade drying (Chapter 5) resulted in slight but significantly higher carotenoid retention in sweet potato chips. The better retention following hot air drying can be explained by quicker drying and lack

of exposure to sun either indirectly (solar) or directly (sun). The better retention of β-carotene following shade drying can be explained by lower temperatures and complete protection against radiation from the sun due to the opaque nature of the coverage. In spite of slower drying, greater retention in shade drying was achieved compared with sun drying and this outcome was in agreement with authors working on leaves of various plants (for example Mosha *et al.* 1997; Negi and Roy 2000) or on cassava (Chavez *et al.* 2007). However due to slow drying and low temperatures, shade drying would not be recommended in humid climates, such as in Uganda, or for samples that have a large surface area such as slices or when there is a limited air circulation (*i.e.* no wind).

9.2.3 Potential for dried products

Because of high retention of β-carotene in OFSP following low cost-sun drying treatment, there is a good potential for varieties such as Rubina® (Chapter 3), Ejumula, Kakamega (Chapter 4), MGCL01 and Resisto (Chapter 5) with a high initial β-carotene content (in roots) to bring a significant contribution to vitamin A in the diet as dried products.

9.3 QUANTIFYING CAROTENOID LOSSES DURING STORAGE

9.3.1 Actual levels of carotenoid loss and their prediction

In contrast with drying, storage of chips at room temperature in Uganda for four months resulted in high carotenoid losses. Total carotenoid contents during storage after four months at ambient temperature were reduced on average by 70.4% on dried chips in Uganda (Chapter 3) and by 83.7% in Mozambique (Chapter 4). The levels of loss were in accordance with the literature on OFSP (Valdez *et al.* 2001, Cinar 2004, Kósambo 2004 (Chapter 1)). Dried chips from Ejumula, Kakamega, SPK004/1; SPK004/6; SPK004/1/1; SPK004/6/6 varieties in Uganda (Chapter 3) were stored in LPDE and those from Resisto and MGCL01 varieties in Mozambique (Chapter 4) in jute bags. These packaging were fully permeable to oxygen. Later a model of kinetic degradation (Arrhenius model) was developed for the storage of dried Ejumula chips between 10 to 40ºC (Chapter 8 or Bechoff *et al.* 2010b in Appendix 4c). The activation energy for trans-β-carotene (calculated on a dry basis) was 68.3kJ.mol^{-1} and for total carotenoids 49.5J.mol^{-1} stored under this range of temperatures. The predicted loss for the model for

total carotenoids (with an initial carotenoid content of 236.3µg.g^{-1}) was 79.2% (49.1µg.g^{-1}) after 125 days (4 months) under recorded temperature (anisothermic conditions) (Chapter 8). The actual loss of total carotenoid content for Ejumula in experimental conditions was 75.3% (58.5µg.g^{-1}) after 4 months storage at ambient temperature (Chapter 4). There was therefore an excellent prediction of the model for Ejumula sweet potato chips stored at ambient temperature in packaging permeable to air.

9.3.2 The issue of carotenoid loss in storage

Typically at household level dried sweet potato is stored at ambient temperature for 4-6 months in traditional storage systems (for example granaries or jute bags). Therefore the high carotenoid losses obtained in storage after four months have major implications for farmers and chip processors. None of the varieties studied in Uganda met the recommended nutritional requirements after a four-month ambient storage period (Chapter 4). A farmer and chip processor pioneer in the project in the Soroti district in Uganda told us that the OFSP chips had lost all their colour (related to provitamin A) after one year of storage and consequently the millers in Kampala were reluctant to buy them. The results of this research study are consistent with this observation.

9.3.3 How to reduce these losses

Because high losses were obtained in the storage of dried sweet potato chips, the factors influencing carotenoid degradation had to be examined and solutions to overcome the loss of quality due to storage were investigated. The size of the chips; influence of variety and use of pre-treatments were tested both in drying and storage. Furthermore storage studies under controlled conditions (temperature, water activity and oxygen) were carried out to gain a more in depth understanding of the degradation of carotenoids.

9.4 QUANTIFYING INFLUENCE OF PROCESS VARIABLES ON CAROTENOID LOSS

9.4.1 Chipping influence

The effect of chipping was tested because when speaking to local farmers they thought that it had an influence on the carotenoid retention. In the pilot-scale study, sun-dried samples exhibited significantly lower trans-β-carotene retention on 1.1 mm-thick chips

(250µg.g^{-1}) than with 1.3mm-thick crimped slices (319µg.g^{-1}) (Chapter 3). Crimped slice bulkiness or lesser degree of "shrinkage" may have protected them from damage from the sun's rays and oxidation. In the field study (Chapter 5), 5.2 mm thick-slices took twice longer time to dry than 0.4 mm-thin chips but there was no difference between them in terms of carotenoid losses (respectively 9.3% and 10.7% on average). On the other hand drying 2.9 mm-thick chips resulted in significantly higher loss (14.6%) than the other chipping methods (Chapter 5). The loss of moisture from the sweet potato slice during drying was earlier demonstrated to be dependent on the slice thickness (Diamante and Munro 1991). Wang and Xi (2005) highlighted that β-carotene degradation increased with sample thickness and moisture content. However chips and crimp slices in Chapter 3 that had similar thicknesses and were from the same sample, but had different retention levels of carotenoid losses. The poorer performance of chips (Chapter 3-Bechoff *et al.* 2009) and thick chips (Chapter 5) during drying in terms of carotenoid retention could therefore be attributed to a small surface area to volume ratio resulting in less efficient moisture loss during drying. More in-depth research is needed to investigate relationships between shape and carotenoid retention during drying of OFSP.

The effect of size reduction on carotenoid losses during storage of dried chips was also investigated. The bulkiness of slices was hypothesized to have a beneficial impact against air oxidation because of the lower level of chip surface exposure during storage. However results obtained showed that thin, thick chips and slices stored in the dark had similar carotenoid degradation levels following four months of storage in Mozambique (Chapter 5). Therefore, there was no effect of chip size on carotenoid losses during storage. The dried starchy structure of sweet potato could limit air diffusion (Diamante and Munro 1991). The effect of oxygen on lipid oxidation is closely related to the product porosity according to Rahman and Perera (2007) (Carotenoids are lipophilic molecules). But the porosity is not dependent of the size or thickness of the product. Therefore dried sweet potato material of different sizes or thicknesses would have similar retention in carotenoid content.

In summary, chip shape was demonstrated to have a small but significant influence on retention in sun-drying but no influence was found during the storage of dried sweet potato pieces.

9.4.2 Varietal influence

In the study (Chapter 5) there was a major varietal effect associated to chipping ($p<0.05$). Total carotenoid loss was on average 17.6% from Resisto and 5.5% from MGCL01. 2.9 mm-thick Resisto chips were sticky and took longer to dry (62.3h) compared to 2.9 mm-thick MGCL01 chips (23.9h). The difference of carotenoid loss between the two varieties can be explained by the difference of moisture content (27.0% for Resisto and 35.4% for MGCL01). A higher initial moisture content in the sweet potato requires more moisture removal during drying and would lead to greater losses of carotenoids. . When working with different varieties that were sun-dried (Chapter 4) it was observed that the carotenoid losses increased with the higher initial moisture content. A significant correlation was obtained between the initial dry matter content and the total carotenoid content lost during drying ($R=-0.518$; $p<0.05$). There was also a cultivar effect and there was a significant correlation between the initial carotenoid content and the total carotenoid content lost during drying ($R=0.589$; $p<0.05$) (losses increased with higher initial carotenoids content in roots). These observations were in agreement with Hagenimana *et al.* (1999). A genetic link between high carotenoid content in fresh roots and low dry matter has been previously suggested that could explain these correlations (Gruneberg *et al.* 2005).

After four months of storage, there was a similar reduction in the carotenoid level in the different varieties analysed in Uganda (Chapter 4). All varieties SPK004/1; 4/1/1; 4/6 and 4/6/6 were bred from Kakamega (SPK004). Ejumula was the only variety from a different genetic pool but losses of carotenoids during storage were similar to those of Kakamega derived varieties. In Mozambique after four-month storage there were significant differences between the two varieties tested (*i.e.* Resisto and MGCL01 with a respective loss of 79.3% and 88.2%) (Chapter 5). These varieties had different genetic origins and these differences in losses might therefore have a genetic basis. The difference between the carotenoid retention in storage of these varieties may result from differential composition in other constituents that can enhance or delay carotenoid degradation (for example unsaturated fatty acids such as linoleic and linolenic acids; polyphenolic compounds (Teow *et al.* 2007) or differential porosity of dried chips between these two varieties (Chapter 3)). These differences in composition were not investigated in this thesis.

9.4.3 Influence of pre-treatment

It was hypothesized that carotenoid retention could be improved by simple means in developing countries where costly packaging materials that exclude oxygen are not affordable. Therefore chemical pre-drying treatments, sodium sulphiting, acidifying (ascorbic acid/citric acid, salting) and blanching were tested. After drying it was shown that most pre-treated samples (salt-treated; ascorbic acid and sodium metabisulphite-treated) had higher true retention than the control that is dipped in deionised water (Chapter 6). Only samples dipped in citric acid did not have a better carotenoid level after drying. Soaking however, had a negative impact on carotenoid retention: un-dipped samples had better retention than samples dipped in deionised water.

In the first month of storage there was a slight improvement in total carotenoid content of ascorbic acid and sodium metabisulphite-pre-treated samples compared to control (deionised water) but the effect of pretreatment did not last more than one to two months. Lack of improvement was believed to result from the degradation of the chemicals in storage as reported by Zhao and Chang (1995). Chemical pre-treatment or blanching therefore did not solve the issue of high losses during storage of dried chips (Chapter 6).

Other ways of reducing carotenoid losses during storage need to be investigated such as control of oxygen for instance using improved packaging or reduced temperature. Hence a better understanding of the fundamental factors that can explain the degradation of carotenoids was needed in order to understand better how to reduce carotenoid losses during storage.

9.5 UNDERSTANDING INFLUENCE OF PHYSICO-CHEMICAL AND BIOCHEMICAL FACTORS ON CAROTENOID LOSS

9.5.1 Light

The characteristic feature of carotenoids is the long conjugated double-bond system that absorbs light in the visible region of the electromagnetic spectrum (400-500nm) and gives them the property to harvest sunlight energy for photosynthesis (Britton *et al.*

2008). The side effect of this structure however is that light can be damaging for the molecule. The degrading effect of UV and short visible wavelengths (blue light) (between 410-450nm) on mayonnaise colour has been described by Lennersten and Lingnert (2000). By comparing the use of different screening filters in solar drying (open sun drying, UV blocking-Visqueen®-covered solar dryer and non UV- blocking polyethylene covered solar dryer) it has been proved that UV-blocking polythene could significantly better preserve carotenoids of cowpea leaves and mango compared to the other coverage (Ndawula *et al.* 2004). Consequently the effect of light screening coverage has been investigated in this study. Preliminary trials on sweet potato flour under light-screening polythene and exposed to artificial sun light demonstrated that colour of samples under the red polythene were significantly less affected than with the other plastic sheeting (Chapter 2). These results were in accordance with Lennersten and Lingnert (2000) and agreed with usual recommendations to work under red light during carotenoid analysis (Rodriguez-Amaya and Kimura 2004). When the same red polythene was tested in the field as solar dryer coverage it did not result in significant improvement in carotenoid retention compared to UV-resistant and non-UV resistant polythene coverage (Chapter 4). This was believed to result from slower drying due to the red coverage that blocked a significant amount of the sun-light wavelengths (between 300-600 nm) and consequently cooled the inside of the dryer (Chapter 4). Moreover there was no significant difference between solar dried (under UV-resistant or non-UV-resistant; or under red-plastic) and sun-dried samples (Chapter 4). Therefore the effect of light in drying was not proved significant on carotenoid catabolism during the drying of sweet potato in accordance with Bengsston *et al.* (2008).

The effect of light on carotenoid retention was also tested in storage. When storing sweet potato chips in transparent or opaque packaging at ambient temperature in Uganda, there was no difference in the total carotenoid content independently of packaging type after four-months. These results were in agreement with Cinar (2004), working on sweet potato extracted pigments exposed in the light or in the dark, where no significant difference between total carotenoid contents exposed or non-exposed after 45 days at 25ºC was found.

Light is also known to be responsible for cis-isomerisation. Cis-isomerisation of β-carotene can decrease the provitamin A activity of dried sweet potato since cis-isomers

(the main isomers formed are 9-cis and 13-cis) have half of the provitamin A activity of the trans- β-carotene molecule (Rodriguez-Amaya and Kimura 2004). Isomerisation under the effect of light has been reported in storage of carrot juice (Chen *et al.*1996) and mango puree (Vasquez-Caicedo *et al.* 2007). The effect of light on isomerisation during storage (in clear or opaque packaging) was not determined; however it was measured during the pilot-scale study during drying. In the pilot-scale study (Chapter 3), individual carotenoids (including cis and trans-β-carotene) were measured after drying in solar (UV-resistant polythene), sun or hot air drying (non-exposed to sun-light). There was no increase in cis-isomerisation (formation of 9-cis and 13-cis-β-carotenes) after drying in these three dryers. A comparable result was found on a study comparing sun and solar dried leafy-vegetables by Mulokosi and Svanberg (2003) and Kidmose *et al.* (2007), who suggested that all stereo-isomers; trans-β-carotene, 9-cis and 13-cis were likely to be oxidised following the same trend (Chapter 3).

9.5.2 Temperature

Cis-isomerisation could also be caused by temperature (Doering *et al.* 1995). Formation of 9-cis and 13-cis isomers due to boiling, canning, cooking and drum drying treatments of sweet potato has been described by Chandler and Schwartz (1988). Hiranvarachat *et al.* (2008) showed that a minimum of 5 h at constant temperature of 60ºC was necessary to induce formation of 13-cis-β-carotene in oven-dried diced carrot. Using dried-sweet potato flour put in the oven (to simulate accelerated and extreme storage conditions) (Chapter 2), it was demonstrated that at 40ºC there was a negligible isomerisation after 103h whilst at 100ºC there was a significant isomerisation after 3h of treatment. Throughout storage at 100ºC 9-cis increased whilst 13-cis decreased. The same observation was made for samples under light at ambient temperature however with lower levels of cis-isomerisation (Chen *et al.*1996; Vasquez-Caicedo *et al.* 2007).

Cis-isomerisation is therefore highly temperature-dependent. The absence of isomerisation in solar and sun drying could be explained since the average drying temperature in all the devices was around 30-40ºC and never went beyond 55ºC (Chapters 3, 4 and 5).

Absence of cis-isomerisation was further observed during the storage of dried sweet potato chips. During the storage of dried samples at ambient temperature in the dark (*ca.* 25ºC) in Mozambique for four months (Chapter 5), trans-β-carotene, 9-cis and 13-cis all degraded following first order kinetics.

As opposed to light, temperature during storage was shown to have a very significant impact on carotenoid catabolism of dried sweet potato in accordance with literature (Cinar 2004; Kósambo 2004; Koca *et al.* 2007; Hidalgo and Brandolini 2008) (Chapter 8). It was also shown that carotenoid content of samples of dried OFSP stored in the freezer (-20ºC) for four months remained constant (Chapter 2).
The effect of temperature (10-40ºC) that can be found in tropical and sub-Saharan climates such as those of developing countries was evaluated during storage. Carotenoids degraded following a first order kinetics of degradation in accordance with many authors including Koca *et al.* (2007); Lavelli *et al.* (2007) working on dehydrated carrots; Hidalgo and Brandolini (2008) working on wheat flour. A predictive model (Arrhenius model) developed in laboratory was further assessed with data from the field in Uganda (Chapter 8). This model was able to successfully predict the carotenoid degradation as affected by temperature and storage time and could be used as a tool to evaluate sweet potato dried chips shelf life.

9.5.3 Water activity

The lowest degradation of carotenoids occurred at the highest water activity level in accordance with the studies on food model and on dehydrated sweet potato (Haralampu and Karel 1983) and was explained by the protective effect of water against air oxidation (Chou and Breene 1972). Water activity is an important parameter for the measurement of food quality and preservation. Moreover water activity was proved to influence carotenoid content in the storage of foodstuff (carrots (Arya *et al.* 1979; Lavelli *et al.* 2007); sweet potato (Haralampu and Karel 1983)) and β-carotene food model systems (Chou and Breene 1972; Goldman *et al.* 1983) (Chapter 8). As compared with water activity, temperature and oxygen had a stronger effect on carotenoid degradation (Chapter 8).

9.5.4 Oxygen

Oxygen (0 [under nitrogen]; 2.5; 10 or 21% [air]) had the greatest effect on carotenoid degradation rate as compared with temperature and water activity (Chapter 8). In the absence of oxygen the carotenoid degradation was strongly reduced, in accordance with earlier studies on sweet potato flakes (Emenhiser *et al.* 1999). The degradation of carotenoids is a free-radical mechanism where oxygen intervenes in the equation. There is also an interaction between singlet oxygen, unsaturated fatty acids such as linoleic acid or linolenic acid and carotenoids present in sweet potato that favours oxidation (Walter and Purcell 1974). In the presence of linoleic acid and β-carotene in a liquid medium (emulsion) spontaneous oxidation of β-carotene (autoxidation) was proved to be significant (Chapter 7).

9.5.5 Enzymatic oxidation

There are two main ways for carotenoid oxidation (using oxygen in air): either autoxidation or enzymatic activity. Though earlier studies on dried sweet potato described carotenoid degradation as an autoxidation, the roles of enzymes had not been resolved. Two approaches were undertaken to understand this issue:

The first approach (indirect) was the observation of carotenoid rate with water activity (Chapter 8). Indeed, enzymatic activity for most enzymes is the highest when substrate and enzyme are most in contact; at water activities close to those of pure water (1.0). Under the conditions encountered in the dried sweet potato the carotenoid degradation was the highest at the lowest water activity where enzymes are less likely to be in contact with their substrate (Chapter 8).

The second approach (direct) was to measure the enzymatic activity (Chapter 7). Peroxidase activity of the sweet potato flour or roots was shown to be high in accordance with literature (Castillo-Leon *et al.* 2002). Pure peroxidase was also proved able to degrade β-carotene in a liquid model system (Chapter 7). However at low water activities and during storage, peroxidase activity in dried sweet potato became negligible. Consequently catabolism of β-carotene by peroxidase activity in the dried sweet potato was unlikely. Lipoxygenase activity was not detected in dried sweet potato. Enzymatic activities other than peroxidases or lipoxygenases were not measured

(Chapter 7). However dioxygenases which are susceptible to catabolise β-carotene, are considered not to be very active at low water activities (Sanakis *et al.* 2003).

Consequently both approaches (direct or indirect) indicate that carotenoid degradation in sweet potato flour during storage is more likely to be caused by autoxidation than by enzymatic oxidation.

Products of β-carotene degradation were also measured. Results indicated the formation of norisoprenoids, including β-ionone, 5.6-epoxy-β-ionone, β-cyclocitral and dihydroactinidiolide, which is in agreement with previous studies. The pathway of degradation of β-carotene was tentatively identified (Chapter 8). The small molecular weight degradation products that include norisoprenoids further proved that the oxidation way is preferred as opposed to cis-isomerisation in accordance with Mulokozi and Svanberg (2003).

Because enzymatic activity at low water activities and after storage of dried sweet potato was found negligible, the origin of oxidation is more likely to be an autoxidation.

9.6 CONCLUSION AND FUTURE WORK

The main hypothesis of this research work was that solar or sun dried OFSP could make a major contribution to the nutritional requirements in vitamin A of children in developing countries.

A quantification of carotenoid losses in drying and storage of OFSP has been achieved. Low carotenoid losses in drying have been obtained independently of dryer type (solar or sun) in the different trials (in France, Uganda or Mozambique). Therefore after drying, OFSP could bring a significant provitamin A intake to the diet. This is an advantageous conclusion for small scale farmers who have limited capacity for investment. On the other hand, high losses during storage of dried chips were determined after a typical storage time duration for the farmers (four month-storage) in ambient conditions. As a result, dried OFSP after storage may not contain sufficient provitamin A to make a significant contribution to the diet. An alternative could be to

reduce the storage time up to two months and favour drying and/or storage of varieties with very high initial carotenoid content such as Resisto variety. In summary therefore it has been demonstrated that drying of OFSP had a limited effect on β-carotene retention but storage had a major impact. If dried OFSP is to make a major contribution to the vitamin A requirements of children then storage needs to be limited in time. The shelf life (typically 1-4 months) will depend on the initial carotenoid content of the dried OFSP (typically 100-400 $\mu g.g^{-1}$).

Factors influencing the carotenoid retention during drying, and storage, were examined. Chip size, variety or pre-treatment (blanching; chemical pre-treatment) showed a limited effect on carotenoid stability. Therefore the fundamental factors influencing carotenoid degradation have been investigated in particular during storage. Light has negligible effect on carotenoid degradation under these conditions. Water activity has an effect but it was limited. Temperature and oxygen are the main parameters that would need control for the reduction of carotenoid degradation in particular during storage. Low temperature storage under nitrogen, under vacuum or with an oxygen absorber should be envisaged but clearly this would pose great problems for small scale farmers.

Further research work is needed in particular to solve the issue of high carotenoid losses during storage considering oxygen and temperature as main constraints, and to develop a deeper understanding of the mechanisms of carotenoid degradation in a dried food product such as dried orange-fleshed sweet potato. The research questions generated from this work deal with various research fields. They are as follows:

- How does one reduce carotenoid loss in storage? Could affordable packaging under vacuum, for instance, be developed and could samples be stored at reduced temperature? These types of storage conditions may be expensive for the local farmers or processors in developing countries. Economic feasibility of these packaging or storage facilities needs to be determined. Storage at low temperatures or under specific packaging may be valuable when the product is sold into higher value marketing chains.

- What is the relationship between sweet potato composition and stability? In particular, what is the relationship between sweet potato genes, dry matter content

of these cultivars and carotenoid loss in drying or storage? A genetic study of the relationship between carotenoid stability and dry matter content in different sweet potato cultivars should be undertaken.

- What is the pathway of carotenoid degradation in a dried food product? In order to achieve a full mathematical modelling of the degradation of carotenoids in a food product, such as dried sweet potato, further work should focus on a kinetic model involving temperature, water activity and oxygen together. Further research is required to understand the nature of the intermediate compounds between β-carotene and norisoprenoids formed during storage and the kinetics of their formation and degradation. An understanding of this process is necessary to determine the precise pathway of degradation of carotenoids and model the degradation/formation rates of each compound related to storage time. This research should help understand how carotenoids in dried food products degrade during storage and could be transposed to other food products in addition to sweet potato. This may lead to novel means of reducing these losses.

- How much carotenoid content is lost in the last stages of preparation of dried sweet potato? In order to complete the missing link between the flour and the finished product, a study is needed to quantify degradation of carotenoids during preparation of product ready for consumption (such as porridges, bread or fried products). A study on the losses of carotenoid in chapattis and porridge made from OFSP flour was carried out as part of a subsequent field study to the PhD and is summarised in Appendix 5. The average percentage loss (and standard deviation) of β-carotene were 30.7% (6.5%) and 16.4% (3.8%) in chapattis and porridges respectively. This supplementary study completed the quantification of carotenoid loss in the processing chain of dried sweet potato products from the drying of the roots to consumption in order to better tackle the issue of vitamin A deficiency in developing countries.

- What is the bioaccessibility and bioavailability of carotenoids in a dried sweet potato matrix? ("Bioaccessibility is the amount of β-carotene that is released from the food matrix and available for absorption. Bioavailability is defined as the fraction of carotenoid that is absorbed and available for utilisation in normal

physiological functions or for storage". Tanumihardjo (2002)). This study doesn't take into account the bioaccessibility of provitamin A in the different forms of sweet potato. It would be important however to determine the bioaccessibility and also bioavailability because it gives information on the fraction of provitamin A that can be used and available for the body. It was shown that the bioaccessibility is linked to the matrix state of the food, for instance boiled, dried and fried. Failla *et al.* (2009) showed that the bioaccessibility of boiled OFSP was poor, being only 0.6-3% and was less than with carrots. Tumuhimbise *et al.* (2009) showed that the bioaccessibility of trans-β-carotene in different Ugandan sweet potato varieties (Ejumula, SPK004, SPK004/1, SPK004/6, SPK004/6/6 (these very varieties have been worked on in the course of this study)) varied with the processing treatments from less bioaccessible (raw<baked<steamed/boiled<deep fried) to more bioaccessible. However the bioaccessibility of dried OFSP and products made from dried OFSP products such as porridge, bread, bakery products has not yet been studied.

Biofortification is gaining increasing interest amongst the international donor community as a means for reducing micronutrient deficiencies. The work in this thesis, contributes to the development of biofortification since it helps in the understanding of whether dried orange-fleshed sweet potato can provide a significant level of provitamin A in the diet of young children. This knowledge will facilitate the effective promotion of the crop to end-users.

References

Aguayo, V.M. and Baker, S.K. (2005) Vitamin A deficiency and child survival in sub-Saharan Africa: A reappraisal of challenges and opportunities. *Food and Nutrition Bulletin* **26**, 348-355.

Ameny, M.A. and Wilson, P.W. (1997) Relationship between Hunter color values and β-carotene contents in white-fleshed African sweetpotatoes (*Ipomea batatas* Lam). *Journal of the Science of Food and Agriculture* **73**, 301-306.

AOAC (1984) Official Methods of Analysis of the Association of Official Analytical Chemists. The Association of Official Analytical Chemists, Inc. Arlington, VA.

Aryra, S.S., Natesan, V., Parihar, D.B and Viayaraghavan, P.K. (1979) Stability of carotenoids in dehydrated carrots. *International Journal of Food Science and Technology* **14**, 579-586.

Arya, S.S., Natesan, V., Premavalli, K.S., Vijayaraghavan, P.K. (1982) Effect of pre-freezing on the stability of carotenoids in unblanched air-dried carrots. *International Journal of Food Science and Technology* **17**, 109-113.

Arya, S.S., Natesan, V. and Vijayaraghavan, P.K. (1983) Stability of carotenoids in freeze-dried papaya. *Journal of Food Technology* **18**, 177-181.

Arya, S.S., Premavalli, K.S., Siddiah, C.H. and Sharma, T.R. (1985) Storage behaviour of freeze dried watermelon juice powder. *International Journal of Food Science and Technology* **20**, 351-357.

Auldridge, M.E., McCarty, D.R. and Klee, H.J. (2006) Plant carotenoid cleavage oxygenases and their apocarotenoid products. *Current Opinion in Plant Biology* **9**, 315-321.

Aziz, S., Wu, Z. and Robinson, D.S. (1999) Potato lipoxygenase catalysed co-oxidation of β-carotene. *Food Chemistry* **64**, 227-230.

Baldermann, S., Naim, M. and Fleischmann, P. (2005) Enzymatic carotenoid degradation and aroma formation in nectarines (*Prunus persica*). *Food Research International* **8**, 833-836.

Baloch, A.K., Buckle, K.A. and Edwards, R.A. (1977) Effect of processing variables on quality of dehydrated carrot. II. Leaching losses and stability of carrot during dehydration and storage. *Journal of Food Technology* **12**, 295-307.

Baloch, A.K., Buckle, K.A. and Edwards, R.A. (1987) Effect of sulphur dioxide and blanching on the stability of carotenoids of dehydrated carrot. *Journal of Food Science and Agriculture* **40**, 179-187.

Baloch, W.A., Khan, S. and Baloch, A.K. (1997) Influence of chemical additives on the stability of dried tomato powder. *International Journal of Food Science and Technology* **32**, 117-120.

Bartley, G.E. and Scolnik, P.A. (1995) Plant carotenoids: pigments for photoprotection, visual attraction, and human health. *The Plant Cell* **7**, 1027-1038.

Bechoff A., Dufour D., Dhuique-Mayer C., Marouzé C., Reynes, M. and Westby A. (2009) Effect of hot air, solar and sun drying treatments on provitamin A retention of orange-fleshed sweet potato. *Journal of Food Engineering* **92**, 164-171.

Bechoff, A., Westby, A., Owori, C., Menya, G., Dhuique-Mayer, C., Dufour D. & Tomlins K. (2010a). Effect of drying and storage on the degradation of total carotenoids in orange-fleshed sweetpotato cultivars. *Journal of the Science of Food and Agriculture*, *90*, 622-629.

Bechoff, A., Dhuique-Mayer, C., Dornier, M., Tomlins, K., Boulanger, R., Dufour, D. & Westby (2010b). Relationship between the kinetics of β-carotene degradation and norisoprenoid formation in the storage of dried sweet potato chips. *Food Chemistry*, *121*, 348–357.

Bechoff, A., Tomlins, K.I., Dhuique-Mayer, C., Dove, R. & Westby, A. (2010c) On-farm evaluation of the impact of drying and subsequent storage on the carotenoid content of orange-fleshed sweet potato. International Journal of Food Science & Technology. *In Press.* (Accepted 17 Aug. 2010).

Bengtsson, A., Namutebi, A., Larsson Alminger, M. and Svanberg, U. (2008) Effects of various traditional processing methods on the all-trans-β-carotene content of orange-fleshed sweet potato. *Journal of Food Composition and Analysis* **21***,* 134-143.

Bernhardt S., Schlich E. (2006) Impact of different cooking methods on food quality: Retention of lipophilic vitamins in fresh and frozen vegetables. *Journal of Food Engineering* **77**, 327-333.

Berrios, D. and Beavogui M. (1992) Trials for the introduction of sweetpotato in bread making in Burundi. pp373-376 in *Product Development for Root and Tuber Crops Vol III-Africa. Proceedings of the Workshop on Processing, Marketing and Utilisation of Root and Tuber Crops in Africa,* 26 October –2 November 1991 at the International Institute for Tropical Agriculture (IITA) Scott, G.J., Freguson, P.I. and Herrera, J.E. editors International Potato Centre.Lima, Peru.

Bimbenet, J.J., Duquenoy, A. and Trystram, G. (2002) Génie des procédés alimentaires: des bases aux applications, edition no. 2, RIA Editions. Lavoisier: Paris, France, 574pp.

Bosser, A. and Belin, J.M. (1994) Synthesis of β-ionone in an aldehyde/xanthine oxidase/ β-carotene system involving free radical formation. *Biotechnlogy Progress* **10***,* 129-133.

Bosser, A., Paplorey, E. and Belin, J.M. (1995) A simple way to (±)-dihydroactinidiolide from β-ionone related to the enzymic co-oxidation of β-carotene in aqueous solution. *Biotechnlogy Progress* **11***,* 689-692.

Bradley, D.G. and Min, D.B. (1992) Singlet oxygen oxidation of foods. *Critical Reviews in Food Science and Nutrition* **31**, 211-236.

Brett, A., Cox, D.R.S., Trim, D.S., Simmons, R. and Anstee, G. (1996) Producting solar dried fruit and vegetables for micro and small scale rural enterprise development. Natural Resources Institute, Chatham, United Kingdom.

Britton, G., Liaanen-Jensen, S. and Pfander, H. (1995) Carotenoids. Volume 1B. *In* Spectroscopy; Birkhaüser: Basel, Switzerland.

Britton, G., Liaanen-Jensen, S. and Pfander, H. (2008) Carotenoids. Volume 4: Natural Functions. Birkhaüser: Basel, Switzerland.

Castillon Leon, J., Alpeeva, I.S., Chubar, T.A., Galaev, I.Y., Csoregi, E. and Sakharov, I.Y. (2002) Purification and substrate specificity of peroxidase from sweet potato tubers. *Plant Science* **163**, 1011-1019.

Chandler, L.A. and Schwartz, S.J. (1988) Isomerization and losses of trans-β-carotene in sweet potatoes as affected by processing treatments. *Journal of Agricultural and Food Chemistry* **36,** 29–133.

Chavez, A.L., Sanchez, T., Tohme, J., Ishitani, M. and Ceballos, H. (2004) Effect of processing on β-carotene content of cassava roots. Poster. CIAT-HarvestPlus. www.ciat.cgiar.org/yuca/pdf/poster_cbn_2004_effect.pdf [accessed 10 September 2009]

Chavez, A.L., Sanchez, T., Ceballos, H., Rodriguez-Amaya, D.B., Nestel, P., Tohme, J. and Ishitani, M. (2007) Retention of carotenoids in cassava roots submitted to different processing methods. *Journal of the Science of Food and Agriculture* **87**, 388-393.

Chen, H.E., Peng, H.Y. and Chen, B.H. (1996) Stability of carotenoids and vitamin A during storage of carrot juice. *Food Chemistry* **57**, 497-503.

Chen, J.P., Tai, C.Y. and Chen, B.H. (2007) Effects of different drying treatments on the stability of carotenoids in Taiwanese mango (*Mangifera indica* L.). *Food Chemistry* **100**, 1005-1010.

Childs, R.E. and Bardsley, W. G. (1975) The steady-state kinetics of peroxidase with 2,2'-Azino-di-(3-ethylbenzthiazoline-6-sulphonic acid) as Chromogen. *Biochemical Journal* **145,** 93-103.

Chou, H.E. and Breene, W.M. (1972) Oxidative decoloration of β-carotene in low-moisture model systems. *Journal of Food Science* **37**, 66-68.

Chua, K.J. and Chou, S.K. (2003) Low-cost drying methods for developing countries. *Trends in Food Science and Technology* **14**, 519-528.

Cinar, I. (2004) Carotenoid pigment loss of freeze-dried plant samples under different storage conditions. *Lebensmittel.-Wissenschaft und Technologia* **37**, 363–367.

Cinar, I. (2005) Stability studies on the enzyme extracted sweet potato carotenoproteins. *Food Chemistry* **89**, 397-401.

Cisse, M., Vaillant, F., Acosta, O., Dhuique-Mayer, C. and Dornier, M. (2009) Thermal degradation kinetics of anthocyanins from blood orange, blackberry, and roselle using the Arrhenius, Eyring and Ball models. *Journal of Agricultural and Food Chemistry* **57**, 6285-91.

Commonwealth Science Council (1985) Solar dryers their role in post-harvest processing. Commonwealth Science Council, London, UK. 337pp.

Cremer, D.R. and Eicher, K. (2000) Formation of volatile compounds during heating of spice paprika (*Capsicum annuum*) powder. *Journal of Agricultural and Food Chemistry* **48**, 2454-2460.

de Araujo, B.S., de Oliveira, J.O., Salgueiro Machacho, S. and Pletsch, M. (2004) Comparative studies of the peroxidases from hairy roots of *Daucus carota*, *Ipomoea batatas* and *Solanum ariculare*. *Plant Science*, 1151-1157.

Decagon Device Services (2009) http://wateractivity.org [accessed 9 September 2009].

Deming, D.M., Baker, D.H. and Erdman, J.W. (2002) The relative vitamin A value of 9-cis-β-carotene is less and that of 13-cis- β-carotene may be greater than the accepted 50% that of *all trans- β*-carotene in gerbils. *Journal of Nutrition* **132**, 2709–2712.

De Pee, S., West, C.E., Permaesih, D., Martuti, S. and Hautvast, J.G. (1998) Orange fruit is more effective than are dark-green, leafy vegetables in increasing serum concentrations of retinol and β-carotene in schoolchildren in Indonesia. *American Journal of Clinical Nutrition* **68,** 1058-1067.

Desorby, S.A., Netto, F.M. and Labuza, T.P. (1997) Comparison of spray-drying, drum-drying and freeze-drying for β-carotene encapsulation and preservation. *Journal of Food Science* **62,**1158-1162.

Desobry, S. A., Netto, F. M. and Labuza, T. P. (1998). Preservation of β-carotene from carrots. *Critical Reviews in Food Science and Nutrition* **38**, 381–396.

Dhuique-Mayer, C., Caris-Veyrat, C., Ollitrault P., Curk, F. and Amiot M.J. (2005) Varietal and interspecific influence on micronutrient content in citrus from the Mediterranean area. *Journal of Agricultural and Food Chemistry* **53**, 2140-2145.

Dhuique-Mayer, C., Tbatou, M., Carail, M., Caris-Veyrat, C., Dornier, M. and Amiot, M.J. (2007) Thermal degradation of antioxidant micronutrient in citrus juice and newly formed compounds. *Journal of Agricultural and Food Chemistry* **55**, 4209–4216.

Diamante, L.M. and Munro, P.A. (1991) Mathematical modelling of hot air drying of sweet potato slices. *International Journal of Food Science and Technology* **26**, 99-109.

DIAS (2006) Semi-Annual and Annual Progress Report HarvestPlus. Kenya. 21pp.

Doering W. von E., Sotiriou-Leventis, C. and Roth, W.R (1995) Thermal interconversions among *15-cis*, *13-cis*, and all-trans-β-carotene: kinetics, Arrhenius parameters, thermochemistry, and potential relevance to anticarcinogenicity of all-trans-β-carotene. *Journal of American Chemical Society* **117**, 2747-2757.

Dogan, S., Turan, P. Dogan, M., Arlan, O. and Alkan, M. (2007) Variations of peroxidase activity among *Salvia* species. *Journal of Food Engineering* **79***,* 375-382.

Dutta, D., Chaudhuri, U. and Chakraborty, R. (2005) Structure, health benefits, antioxidant property and processing and storage of carotenoids. *African Journal of Biotechnology* **4**, 1510-1520.

Eliçin, A.K.and Saçilik, K. (2005) An experimental study for solar tunnel drying of apple. *Ankara University Faculty of Agriculture Journal of Agricultural Sciences* **11**, 207-211.

Emenhiser, C., Watkins, R.H., Simunovic, N., Solomons, N., Bulux, J., Barrows, J. and Schwartz, S.J. (1999) Packaging preservation of β-carotene in sweet potato flakes using flexible film and an oxygen absorber. *Journal of Food Quality* **22**, 63-73.

Enachescu Dauthy, M. (1995) On-line simplified methods for enzyme activity check In *Fruit and Vegetable Processing. FAO Agricultural Services Bulletin No.119*. Food and Agriculture Organization of the United Nations
Rome, 1995**.** Online http://www.fao.org/docrep/v5030e/v5030e0q.htm

Ezell, B.D. and Wilcox, M.S. (1951) Influence of storage temperature on carotene, total carotenoids and ascorbic acid content of sweetpotatoes. Plant Physiology 81-94.

Failla, M.L., Thakkar, S.K. and Kim, J.Y. (2009) In vitro bioaccessibility of β-carotene in orange-fleshed sweet potato (Ipomoea batatas, Lam). Journal of Agricultural and Food Chemistry 57, 10922-10927.

FAO (1985) Expert consultation on planning the development of sundrying techniques in Africa. Proceedings of the Expert Consultation on Planning the Development of Sundrying Techniques in Africa. Food and Agriculture Organization of the United Nations, Rome.

FAO (2001) Processing, Preservation and Storage. Home Gardening Technology. In Improving Nutrition through Home Gardening. A training package for preparing field workers in Africa. Nutrition Programmes Service Food and Nutrition Division Food and Agriculture Organization of the United Nations, Rome, 2001. http://www.fao.org/documents/show_cdr.asp?url_file=/DOCREP/003/X3996E/x3996e42.htm [accessed 12 March 2010].

FAO/WHO Rome (2002). *Vitamin A in Human Vitamin and Mineral Requirements*. Report of joint FAO/WHO expert consultation Bangkok, Thailand. pp 87-107.
FAOStat (2008) Food Agriculture Organization. Statistical database. http://faostat.fao.org [accessed 12 March 2010].

Farhat, A., Kooli, S., Kerkeni, C., Maalej, M., Fadhel, A. And Belghith, A. (2004) Validation of a pepper drying model in a polyethylene tunnel greenhouse. *International Journal of Thermal Sciences* **43**, 53-58.

Ferris, S., Muganga, A., Matovu, R., Kolijn, S., Hagenimana, V., and Karuni, E. (2001) *Marketing Opportunities for Starch and High Quality Flour Production from Cassava and Sweetpotato in Uganda*. International Institute of Tropical Agriculture. Resource and Crop Management. Research Monograph No.29. 88pp.

Fleischmann, P., Watanabe, N. and Winterhalter, P. (2003) Enzymatic carotenoid cleavage in star fruit (*Averrhoa carambola*). *Phytochemistry* **63**, 131-137.

Fowler, M.H. and Stabrawa, A.M. (1992) A Rapid Appraisal of Sweetpotato Marketing in Uganda. pp253-275 in *Product Development for Root and Tuber Crops Vol III-Africa. Proceedings of the Workshop on Processing, Marketing and Utilisation of Root and Tuber Crops in Africa,* October 26-November 2, 1991 at the International Institute for

Tropical Agriculture (IITA) Scott, G.J., Freguson, P.I. and Herrera, J.E. editors. Ibadan, Nigeria.

Furr, H.C. and Clark R.M. (1997) Intestinal absorption and tissue distribution of carotenoids. *Nutritional Biochemistry* **8,** 364-377.

Gakonyo, N. (1992) Processed sweetpotato: responding to Kenya's urban food needs pp337-357 in *Product Development for Root and Tuber Crops Vol III-Africa.* Proceedings of the *Workshop on Processing, Marketing and Utilisation of Root and Tuber Crops in Africa,* 26 October-2 November 1991 at the International Institute for Tropical Agriculture (IITA) Scott, G.J., Freguson, P.I. and Herrera, J.E. editors. Ibadan, Nigeria.

Gandul-Rojas, B. Roca, M.and Minguez-Mosquera, M.I. (2004) Chlorophyll and carotenoid degradation mediated by thylakoid-associated peroxidative activity in olives (*Olea europaea*) cv. Hojiblanca. *Journal of Plant Physiology* **161**, 499-507.

Garg, H.P. and Kumar, R. (2000) Thermal modelling and performance prediction of drying processes under open air sun drying. Not published. http://www.kenes.com/Ises.Abstracts/Htm/0458.htm [accessed 12 March 2010].

Garner, C.W. (1984) Peroxidation of free and esterified fatty acids by horseradish peroxidase. *Lipids* **19**, 863-868.

Gayathri G.N., Platel K., Prakash J. and Srinivasan K. (2004) Influence of antioxidant spices on the retention of β-carotene in vegetables during domestic cooking processes. *Food Chemistry* **84**, 35-43.

Gnanaranjan, N.P., Rakwchian, W., Donoghue, J.O. and Kumar, S. (1997) Field performance of a solar tunnel drier. Proceedings of ISES (Solar World Congress).

Gokmen, V. Savas-Bahceci, Serpen, A. and Acar, J. (2005) Study of lipoxygenase and peroxidase as blanching indicador enzymes in peas: change of enzyme activity, ascorbic

acid and chlorophylls during frozen storage. *Lebensmittel-Wissenschaft und Technologie* **38**, 903-908.

Goldman, M., Horev, B. and Saguy, I. (1983). Decolorization of β-carotene in model systems simulating dehydrated foods. Mechanism and kinetic principles. *Journal of Food Science* **48**, 751-754.

Gomez Eslava, C. (2005). Séchage de produits alimentaires dans les pays en voie de développement. Validation de principes de séchoir. Rapport de stage. 40Pp. Montpellier, France: INSA Toulouse-CIRAD.

Gruneberg, W.J., Manrique, K., Zhang, D. and Hermann, M. (2005) Genotype x environment interactions for a diverse set of sweetpotato clones evaluated across varying ecogeographic conditions in Peru. *Crop Science* **45**, 2160-2171.

Hagenimana, V. and Owori, C. (1996) Feasibility, acceptability, and production costs of sweetpotato-based products in Uganda. *Program Report 95-96*. International Potato Centre (CIP) http://www.cipotato.org/Market/PgmRprts/pr95-96/program6/prog68.htm# Methods

Hagenimana, V., Carey, E., Gichuki, S.T., Oyunga, M.A. and Imungi, J.K. (1999) Carotenoid contents in fresh, dried and processed sweetpotato products. *Ecology in Food and Nutrition* **37**, 455-473.

Hall, A., Bockett, G. and Nahdy, S. (1998) *Sweetpotato Postharvest Systems in Uganda: Strategies, Constraints, and Potentials*. International Potato Center Social Science Department Working Paper No. 1998-7. 81pp.

Handelman, G.J., van Kujk, F.J., Chatterjee, A. and Krinsky, N.I. (1991).Characterization of products formed during the autoxidation of β-carotene. *Free Radical Biology and Medicine* **10**, 427-437.

Haskell, M.J., Jamil, K.M., Hassan, F., Peerson, J.M., Hossain, M.I., Fuchs, G.J. and Brown, K.H. (2004) Daily consumption of Indian spinach (*Basella alba*) or sweetpotato

has a positive effect on total-body vitamin A stores in Bangladeshi men. *American Journal of Clinical Nutrition* **80**, 705-714.

Has-Schon, E., Lepedus, H. Jerabek, L. and Cesar, V. (2005) Influence of storage temperature on total peroxidase activity in crude extracts from *Picea abies* L. Karst. Needles. *Croatica Chemica Acta* **78**, 349-353.

Haralampu, S.G. and Karel, M. (1983) Kinetic models for moisture dependence of ascorbic acid and β-carotene degradation in dehydrated sweet potato. *Journal of Food Science* **48**, 1872-1873,.

Hedrén, E., Diaz, V. and Svanberg, U. (2002) Estimation of carotenoid accessibility from carrots determined by an in vitro digestion method. *European Journal of Clinical Nutrition* **56**, 425-430.

Hidalgo, A.H. and Brandolini, A. (2008) Kinetics of carotenoid degradation during the storage of einkorn (*Triticum monococcum L. ssp. monococcum*) and bread wheat (*Triticum aestivum L. ssp. aestivum*) flours. *Journal of Agriculture and Food Chemistry* **56**, 11300-11305.

Hiranvarachat, B., Suvarnakuta, P. and Devahastin, S. (2008). Isomerisation kinetics and antioxidant activities of β-carotene in carrots undergoing different drying techniques and conditions. *Food Chemistry* **107**, 1538-1546.

Humphrey, J.,H., West, K.P. and Sommer, A. (1992) Vitamin A deficiency and attributable mortability among under-5-year-olds. *Bulletin of the World Health Organization* **70,** 225-232.

Huang, F.H., Horvath, G., Molnar, P., Turcsi, E., Deli, J., Schrader, J., Sandmann, G., Schmidt, H. and Schwab, W. (2009) Substrate promiscuity of RdCCD1, a carotenoid cleavage oxygenase from *Rosa damascena*. *Phytochemistry* **70**, 457-464.

Isaac, A., Livingstone, C., Wain, A.J., Compton, R.G. and Davis, J. (2006) Electroanalytical methods for the determination of sulfite in food and beverages. *Trends in Analytical Chemistry* **25**, 589-598.

Isamah, G.K. (2004) ATPase, peroxidase and lipoxygenase activity during post-harvest deterioration of cassava (*Manihot esculenta* Crantz) root tubers. *International Biodeterioration and Biodegradation* **54**, 319-323.

ITDG (1988) Intermediate Technology Development Group. Practical answers to poverty. Drying of Foods. NRI. Technical Brief. NRI, UK. http://practicalaction.org/practicalanswers/product_info.php?products_id=88%7B1%7D1andattrib=1. [accessed 9 August 2009]

Jain, D.and Tiwari, G.N. (2003) Thermal aspects of open sun drying of various crops. *Energy* **28**, 37-54.

Jalal, F., Nesheim, M.C., Agus, Z., Sanjur, D. and Habitcht, J.P.(1998) Serum retinol concentrations in children are affected by food sources of β-carotene, fat intake, and anthelmintic drug treatment. *American Journal of Clinical Nutrition* **68,** 623–629.

Janjai S., Lamlert, N., Intawee P, Mahayothee, B. , Bala, B.K., Nagle, M. and Muller J. (2009) Experimental and simulated performance of a PV-ventilated solar greenhouse dryer for drying of peeled longan and banana. *Solar Energy* **83**, 1550–1565.

Jaramillo-Flores, M. E., Lugo-Martinez, J.J., Ramirez-Sanjan, E.R., Montellano-Rosales, H., Dorantes-Alvarez, L. and Hernandez-Sanchez, H. (2005) Effect of sodium chloride, acetic acid and enzymes on carotene extraction in carrots (*Daucus carota* L.) *Journal of Food Science* **70**, S138-S142.

Jaren-Galan, M. and Minguez-Mosquera, M.I. (1997) β-carotene and capsanthin co-oxidation by lipoxygenase. Kinetics and thermodynamic aspects of the reaction. *Journal of Agricultural and Food Chemistry* **45**, 4814-4820.

Kamiya N. and Nagamune, T. (2002) Effect of water activity control on the catalytic performance of surfactant- *Arthromyces ramosus* peroxidase complex in toluene. *Biochemical Engineering Journal* **10**, 55-59.

Kapinga, R., Lemaga, B., Ewell, P., Zhang, D., Tumwegamiire, S., Agili, S., and Nsumba, J. (2003) Increased promotion and utilization of orange-fleshed sweetpotato as part of the food based approaches to combat Vitamin A deficiency in sub-Saharan Africa. FoodAfrica, 5-9 May 2003, Cameroon.

Karabulut, I., Topcu, A., Duran, A., Turan and S. Ozturk, B. (2007) Effect of hot air drying and sun drying on color values and β-carotene content of apricot (*Prunus armenica* L.). *Lebenswissenshaft and Technologie* **40**, 753-758.

Kennedy, K., Alemany, K. and Warith, M. (2002) Optimisation of soybean peroxidase treatment of 2,4-dichlorophenol. *Water SA* **28***,* 149-158.

Kidmose, U., Christensen, L.P., Agili, S.M. and Thilsted, S.H. (2007) Effect of home preparation practices on the content of provitamin A carotenoids in coloured sweet potato varieties (*Ipomoea batatas* Lam.) from Kenya. *Innovative Food Science and Emerging Technologies* **8**, 399-406.

King Arthur Flour Company (2009). Vermont, USA. http://www.kingarthurflour.com/professional/salt.html [accessed 30 July 2009].

Kingsly, R.P., Goyal, R.K., Manikantan M.R. and Ilyas, S.M. (2007) Effects of pretreatments and drying air temperature on drying behaviour of peach slice. *International Journal of Food Science and Technology* **42**, 65–69.

Kimura, M., Kobori, C.N., Rodriguez-Amaya, D.B. and Nestel, P. (2007) Screening and HPLC methods for carotenoids in sweetpotato, cassava and maize for plant breeding trials. *Food Chemistry* **100**, 1734-1746.

Koca, N., Burdurlu, H.S. and Karadeniz, F. (2007) Kinetics of color changes in dehydrated carrots. *Journal of Food Engineering* **78**, 449-455.

Kósambo L., Carey E.E., Misra A.K., Wilkes J. and Hagenimana, V. (1998) Influence of age, farming site, and boiling on pro-vitamin A content in sweet potato (*Ipomoea batatas* (L.) Lam.). *Journal of Food Analysis* **11**,305-321.

Kósambo L. (2004) Effect of storage and processing on all-trans-β-carotene content in fresh sweetpotato (*Ipomoea batatas* (L.) Lam) roots and its products. CIP Funded Research Project: Annual Report (July 2003 – June 2004). Kenya Industrial Research and Development Institute. Nairobi. 11pp.

Krinsky, N. I. and Yeum, K.J. (2003). Carotenoid-radical interactions. *Biochemical and Biophysical Research Communications* **305**, 754-760.

Krizek, D.T., Clark, H.D. and Mirecki, R.M. (2005) Spectral properties of selected UV-blocking and UV-transmitting covering materials with application for production of high-value crops in high tunnels. *Photochemistry and Photobiology* **81**, 1047-1051.
Labuza T.P. (1973) Effect of dehydration and storage on nutrient retention in foods. *Journal of Food Technology* **27**, 20–5.

Laguerre, M., Mestres, C., Davrieux, F., Ringuet, J. and Boulanger, R. (2007) Rapid discrimination of scented rice by solid-phase microextraction, mass spectrometry, and multivariate analysis used as a mass sensor. *Journal of Agricultural and Food Chemistry* **55**, 1077-1083.

Latapi, G. and Barrett, D.M. (2006) Influence of pre-drying treatments on quality and safety of sun-dried tomatoes. Part II. Effect of storage on nutritional and sensoray quality of sun-dried tomatoes pretreated with sulphur, sodium metabisulfite or salt. *Journal of Food Science* **71**, S32-S37.

Lavelli, V., Zanoni, B. and Zaniboni, A. (2007) Effect of water activity on carotenoid degradation in dehydrated carrots. *Food Chemistry* **104**, 1705-1711.

Lee, C.Y., Simpson, K.L. and Gerber, L. (1989) Vegetables as a major vitamin A source in our diet. *New York's Food and Life Sciences Bulletin* **126,** ISSN0362-0069.

Lee, S.B. and Kim, K.J. (1995) Effect of water activity on enzyme hydration and enzyme reaction rate in organic solvents. *Journal of Fermentation and Bioengineering* **79**, 473-478.

Lejeune, A., Peng, J., Le Boulenge, A., Larondelle, Y. and van Hove, C. (2000) Carotene content of Azolla an its variations during drying and storage treatments. *Animal Feed Science and Technology* **84**, 295-301.

Lennersten, M. and Linghert, H. (2000) Influence of wavelength and packaging material on lipid oxidation and colour changes in low-fat mayonnaise. *Lebensmittel-Wissenschaft und Technologie* **33**, 253-260.

Lessin, W.J., Catigani, G.L. and Schwartz, S.J. (1997) Quantification of cis-trans Isomers of Provitamin A Carotenoids in Fresh and Processed Fruits and Vegetables. *Journal of Agricultural and Food Chemistry* **45**, 3728-3732.

Lewinsohn, E. Sitrit, Y., Bar, E., Azulay, Y., Ibdah, M., Meir, A., Yosef, E., Zamir, D. and Tadmor, Y. (2005) Not just colors – carotenoid degradation as a link between pigmentation and aroma in tomato and watermelon fruit. *Trends in Food Science and Technology* **16**, 407-415.

Lieber, D.C. (1993) Antioxidant reactions of carotenoids. *In Carotenoids in Human Health.* Canfield, L.M. and Olson, J.A. The New York Academy of Sciences, New York. pp 20-31.

Lisiewska Z., Kmiecik, W. and Slupski, J. (2004) Contents of chlorophylls and carotenoids in frozen dill: effect of usable part and pre-treatment on the content of chlorophylls and carotenoids in frozen dill (*Anethum graveolens* L.), depending on the time and temperature of storage. *Food Chemistry* **84**, 511–518.

Low J., Walker T. and Hijamans R., The potential impact of orange fleshed sweetpotatoes on vitamin A intake in sub-Saharan Africa. *The VITAA Project, Vitamin A and Orange-fleshed Sweetpotatoes in Sub-Saharan Africa*, Nairobi, May 2001. [Online].

(2001). Available: http://www.cipotato.org/vitaa/Publications/Paper_vitaa.pdf [accessed 27 January 2009].

Low, J.W., Arimond, M., Osman N., Cunguara, B., Zano, F., and Tschirley, D. (2007) A food-based approach introducing orange-fleshed sweet potatoes increased vitamin A intake and serum retinol concentrations in young children in rural Mozambique. *Journal of Nutrition Community and International Nutrition* **137,** 1320-27.

Mastekbayeva, G.A., Leon M.A. and Kumar, S. (1998) Performance evaluation of a solar tunnel dryer for chilli drying. ASEAN Seminar and Workshop on Drying Technology 3-5 June 1998, Phitsanulok, Thailand. http://www.retsasia.ait.ac.th/Publications/PERFORMANCE-SOLAR%20DRYER-pitsanulok.pdf.

Matilde, P. and Martinoia, E. (1982) Catabolism of carotenoids: involvement of peroxidase? *Plant Cell Reports* **1**, 244-246.

Mdziniso, P., Hinds, M.J., Bellmer, D.D., Brown, B. and Payton, M. (2006) Physical Quality and carotene content of colar-dried green leafy and yellow succulent vegetables. *Plant Foods for Human Nutrition* **61**, 13-21.

Mendes-Pinto, M.M. (2009) Carotenoid breakdown products the - norisoprenoids - in wine aroma. *Archives of Biochemistry and Biophysics* **483**, 236-245.

Méot, J.M., Marouzé, C. and Rivier, M. (2007) CSec-T: Un séchoir pour les produits granulés. Principes et méthode de dimensionnement. "Transformation, conservation et Qualité des aliments: une Nouvelle approche de la lutte contre la pauvreté" Séminaire régional organisé du 21 au 23 Novembre 2007 par Agence Universitaire de la Francophonie et Université Cheikh Anta Diop de Dakar. Dakar, Africa : AUF.

Michigan State University (2009) Department of Chemistry. Visible and ultra-violet spectrophotometry. http://www.cem.msu.edu/~reusch/VirtualText/Spectrpy/UV-Vis/spectrum.htm. [accessed 7 August 2009].

Mills, R.C. and Hart, E.B. (1945) Studies of the stabilization of carotene in dehydrated feeds and foods. *Journal of Dairy Science* **28**, 1-13.

Minguez-Mosquera, M.I. and Jaran-Galan, M. (1995) Kinetics of the decolouring of carotenoid pigments. *Journal of Agricultural and Food Chemistry* **67***,* 153-161.

Mordi, R.C, Walton, J.C., Burton, G.W., Ingold, K.U., Lindsay, D.A., Moffatt, D.J. (1993) Oxidative degradation of β-carotene and β-apo-8'-carotenal. *Tetrahedron* **49***,* 911-928.

Mosha, T.C, Pace, R.D., Adeyeye, S. Laswa, H.S. and Mtebe, K. (1997) Effect of traditional processing practices on the content of total carotenoids, β-carotene, α-carotene and vitamin A activity of selected Tanzanian vegetables. *Plant Food for Human Nutrition* **50**, 189-201.

Mulokozi, U. and Svanberg, G. (2003) Effect of traditional open sun-drying and solar cabinet drying on carotene content and vitamin A activity of green leafy vegetables. *Plant Foods for Human Nutrition* **58**, 1–15.

Murphy, E.W., Criner, P.E. and Gray, B.C. (1975) Comparison of methods for calculating retention of nutrients in cooked foods. *Journal of Agricultural Food Chemistry* **23**, 1153-1157.

Mwanga ROM *Development of high yielding multiple resistant sweetpotato germplasm.* McKnight Foundation Collaborative Research Program. Research Progress Report**:** April 1, 2002 - March 31, 2003. National Crops Resources Research Institute (NaCRRI), Kampala, Uganda. National Agricultural Research Organization [Online] (2003). Available: http://mcknight.ccrp.cornell.edu/program_docs/project_documents/EHAF_03-1112_spbreeding/03-1112_spbreeding_yr7_02-03_vweb.pdf [accessed 26 January 2009].

Mwanga ROM, Odongo B, Alajo A, Kigozi B, Niringiye C, Kapinga R, Tumwegamire S, Makumbi R, Lugwana E, Namakula J, Lemaga B, Nsumba J and Yencho C, Submission to the cultivar release committee for release of sweetpotato cultivars. National Crops Resources Research Institute (NaCRRI), Kampala, Uganda. National Agricultural Research Organization (2007).

Naik, P.S., Chanemougasoundharam, A., Khurana, S.M.P. and Kalloo, G. (2003) Genetic manipulation of carotenoid pathway in higher plants. *Current Science* **85**, 1423-1430.

Namanda, S. (2005) *Experiences in quality on-farm sweetpotato processing*. Serere Agricultural and Animal Production Research Institute Publisher. NARO-CIP-INIBAP. Uganda.

NAS/NRC (1974). *Recommended Dietary Allowance* (8th Ed.) Washington DC, USA: National Academy of Science.

National Academy of Sciences/Institute of Medicine (2001) Vitamin A. In *Dietary Reference Intakes for Vitamin A, Vitamin K, Arsenic, Boron, Chromium, Copper, Iodine, Iron, Manganese, Molybdenum, Nickel, Silicon, Vanadium, and Zinc*. National Academy Press, Washington, DC.

Ndawula, J., Kabasa, J.D. and Byaruhanga, Y.B. (2004) Alterations in fruit and vegetable β-carotene and vitamin C content caused by open-sun drying, visqueen-covered and polythene-covered solar-driers. *African Health Sciences* **4**, 125-130.

Negi, P.S. and Roy, S.K. (2000) Effect of blanching and drying methods on β-carotene, ascorbic acid and chlorophyll retention of leafy vegetables. *Lebensmittel Wissenschaft und Technologie* **33**, 295-298.

Negi, P.S. and Roy, S.K. (2001) Effect of drying conditions on quality of green leaves during long term storage. *Food Research International* **34**, 283-287.

Nestel, P., Bouis, H.E., Meenakshi, J.V. and Pfeiffer, W. (2006) Symposium: food fortification in developing countries. *Journal of Nutrition* **136**, 1064-1067.

Nonier, M.F., Vivas, De Gaulejac, N. and Vitry, C. (2004) Characterization of carotenoids and their degradation products in oak wood. Incidence on the flavour of wood. *Comptes Rendus Chimie* **6**, 689-698.

Odaga, A. and Wanzie, R. (1991) The use of sweetpotato in bakery products in Cameroon. pp377-384 in *Product Development for Root and Tuber Crops Vol III-Africa. Proceedings of the Workshop on Processing, Marketing and Utilisation of Root and Tuber Crops in Africa,* 26 October –2 November 1991 at the International Institute for Tropical Agriculture (IITA) Scott, G.J., Freguson, P.I. and Herrera, J.E. editors International Potato Centre. Lima, Peru.

OIML (1996) The scale of relative humidity of air certified against saturated salt solutions. OIML R121. Organisation Internationale de Métrologie Légale. Edition 1996 Paris, France 11pp. www.oiml.org/publications/r/r121-e96.pdf

Owori, C. and Hagenimana V. (1995) Assessment of the potential for sweetpotato processed product (doughnuts) for diversified utilisation of sweetpotatoes in Uganda. pp450-453 in *Root Crops and Poverty Alleviation.* Proceedings of the Sixth Triennial Symposium of the International Society for Tropical Root Crops-Africa Branch. Akoroda, M.O. and Ekanayake, I.J. editors. Lilongwe, Malawi.

Owori, C. and Hagenimana, V. (2000) Quality evaluation of sweetpotato flour processed in different agroecological sites using small scale processing technologies. African Potato Association. *African Potato Association Conference Proceedings* **5**, 483-490.

Owori, C., Karuri, E., Mbugua, S., Hagenimana, V. and Ragama P. (2001) Importance of "kabakalaga" processing to the sweetpotato product development needs in Uganda. pp150-156 in *Root Crops: the Small Processor and Development of Local Food Industries for Market Economy. Proceedings of the Eight Triennial Symposium of the International Society for Tropical Root Crops-Africa Branch.* 12-16 November 2001 Akoroda, M.O. editor. Ibadan, Nigeria.

Owori, C. and Agona, A. (2003) Assessment of sweetpotato cultivars for suitability for different forms of processing. In *Sweetpotato Post-Harvest Assessment*. *Experiences from Tanzania*. Edited by Rees, D., van Oirschot, Q. and Kapinga, R. Natural Resources Institute, University of Greenwich.

Owori, C. B., Lemaga, R.O.M., Mwanga, A., Namutebi and Kapinga, R. (2007) Sweetpotato recipe book: sweetpotato processed products from Eastern and Central Africa. African Crop Science Society, Kampala, Uganda. 93pp.

Oyunga, M.A., N'gan'ga, N.M. and Olayo, J.A. (2000a) Sweetpotato processing for improved Vitamin A intake: Gender implications and potential for sustainable technology adoption in Siaya District, Kenya. *Proceedings of the 5th Triennial Congress of the African Potato Association* Kampala, Uganda. May 29–June 2, 2000. pp. 491-495.

Oyunga-Ogubi,M.A., Ng'ang'a N.M. and Oudia, M.I (2000b) Potential market for vitamin A-rich sweet potato products for improved nutrition and food security in rural households of Siaya district, Kenya. In Newsletter Sight And Life. Activity Reports 2000. IVACG Meeting, Hanoi.

Pérez-Gálvez, A., Jarén-Galán, M., Mínguez-Mosquera (2005) Impact of the increased thermal processing on retinol equivalent values of paprika oleoresins. *Journal of Food Engineering* **71**, 379-385.

Pinheiro-Santana, H.M., Stringheta, C.C., Cardoso S.C., Paez, H.H., de Queiroz, VM. (1998) Evaluation of total carotenoids, α- and β-carotene in carrots (*Daucus carota* L.) during home processing. Ciencia e Tecnologia de Alimentos 18 (1). doi: 10.1590/S0101-20611998000100009 *Print version* ISSN0101-2061. http://www.scielo.br/scielo.php?pid=s0101-20611998000100009andscript=sci_arttext

Prakash, S., Jha, S.K. and Datta, N.(2004) Performance evaluation of blanched carrots dried by three different driers. *Journal of Food Engineering* **62**, 305–313.

Preston, C.M. and Barrett, J. (1987) *Heligmosomoides polygyrus*: peroxidase activity. *Experimental Parasitology*, **64**, 24-28.

Purcell, A.E. and Walter, W.M. (1968) Carotenoids of centennial variety sweet potato, *Ipomea batata* L. *Journal of Agricultural and Food Chemistry* **5**, 769-770.

Rahman, M.S. and Labuza, T.P. (1999). Water activity and food preservation. In: *Handbook of Food Preservation* (edited by M. Shafiur Rahman).pp. 339-382. New York, USA: Marcel Dekker, Inc.

Rahman, S. and Perera, R.O. Drying and Food Preservation (2007) pp422 in *Handbook of Food Preservation* CRC Publisher 2nd Edition Oxon, UK.

Raina, B.L., Agarwal, S.G., Bhatia, A.K. and Gaur, G.S. (1996) Changes in pigment and volatiles of saffron (*Crocus sativus* L.) during processing and storage. *Journal of the Science of Food and Agriculture* **71**, 27-32.

Ravichandran, R. (2002) Carotenoid composition, distribution and degradation to flavour volatiles during black tea manufacture and the effect of carotenoid supplementation on tea quality and aroma. *Food Chemistry* **78**, 23-28.

Rodriguez Amaya D.B. (1996) Assessment of the pro-vitamin A contents of foods-the Brazilian experience. *Journal of Food Composition and Analysis* **9,** 196-230.

Rodriguez Amaya D.B. (1997) Carotenoids and food preparation: the retention of pro-vitamin A carotenoids in prepared, processed and stored foods. USAID.OMNI Project.

Rodriguez Amaya D.B. (2001) *A Guide to Carotenoid Analysis in Food*. ILSI Press, Washington DC. 64pp.

Rodriguez Amaya D.B. and Kimura M. (2004) *HarvestPlus Handbook for Carotenoid Analysis*. Copyright HarvestPlus, Washington DC. 63pp.

Rodriguez, E.B. and Rodriguez-Amaya, D.B (2007) Formation of apocarotenals and epoxycarotenoids from β-carotene by chemical reactions and by autoxidation in model systems and processed foods. *Food Chemistry* **101**, 563-572.

Rompel, A. Albers, M. Naseri, J.I., Gerdemann, C., Buldt-Karentzopoulos, K., Jasper, B. and Krebs, B. (2007) Purification, cloning and characterisation of a novel peroxidase isozyme from sweetpotatoes (*Ipomoea batatas*). *Biochimica et Biophysica Acta* **1774**, 1422-1430.

Russell, N.J. and Gould, G. W. (2003) *Food Preservatives* 2nd ed., 2003. Springer, New York, USA. 386 pp.

Sanakis, Y., Mamma, D., Christakopoulos, P. and Stamatis, H. (2003) Catechol 1,2 dioxygenase from *Pseudomonas putida* in organic media- an electron paramagnetic resonance study. *International Journal of Biological Macromolecules* **33**, 101-106.

Sattar, A., DeMan, J.M. and Alexander, J.C. (1977) Wavelength effect on light-induced decomposition of vitamin A and β-carotene in solutions and milk fat. *Journal of the Canadian Institute for Food Science and Technology* **10**, 56-60.

Schweiggert, U., Schieber, A. and Carle, R. (2005) Inactivation of peroxidase, polyphenoloxidase, and lipoxygenase in paprika and chili powder after immediate thermal treatment of the plant material. *Innovative Food Science and Emerging Technologies* **6***,* 403-411.

Sian, N.K. and Ishak, S. (1991) Carotenoid and anthocyanin contents of papaya and pineapple: influence of blanching and predrying treatments. *Food Chemistry* **39**,175-185.

Siems, W. , Wiswedel, I., Salerno, C., Crifo, C., Augustin, W., Schild, L., Langhans, C.D. and Sommerburg, O. (2005) Carotene breakdown products may impair mitochondrial functions_potential side effects of high-dose β-carotene supplementation. *Journal of Nutritional Biochemistry* **16**, 385–397.

Simon, P.W. (1997) Plant pigments for color and nutrition. *HortScience* **32,**12-13.

Simonne, A.H., Kays, S.J., Koehler, P.E. and Eitenmiller, R.R. (1993) Assessment of β-carotene content in sweetpotato breeding lines in relation to dietary requirements. *Journal of Food Composition and Analysis* **6**,336-345.

Singh, S., Raina, C.S., Bawa, A.S. and Saxena, D.C. (2006) Effect of pre-treatments on drying and rehydration kinetics and colour of sweet potato slices. *Drying Technology* **24**, 1487-1494.

Solar power Thailand. Solar Energy Research. Department of Physics. Silpakorn University. http://www.thaisolarpower.com/su/drying/solardry.htm [updated 26 Nov. 2007].

Sommer A. (1998) Xerophtalmia and vitamin A status. *In: Progress in Retinal and Eye Research. Great Britain: Elsevier Science Ltd* **17,** 9-31.

Sommerburg, O., Langhans C.D., Arnhold, J., Leichsenring, M., Salerno, C., Crifo, C., Hoffmann, G.F., Debatin, K.M. and Siems, W.G. (2003) β-carotene cleavage products after oxidation mediated by hypochlorous acid- a model for neutrophil-derived degradation. *Free Radical Biology and Medicine* **35**, 1480-1490.

Speck P., Escher F. and Solms, J. (1977). Effect of salt pre-treatment on quality and storage stability of air dried carrots. *Lebensmittel Wissenschaft und Technologie* **10**, 308-313.

Stephanovitch, A.F. and Karel, M. (1982) Kinetics of β-carotene degradation at temperatures typical of air drying of foods. *Journal of Food Processing and Preservation* **6**, 227-242.

Stollman A. (2005) *Provitamin A in Sweet Potato: Effect of Processing and Assessment of Children's Risk for Vitamin A Deficiency in Rural Uganda.* Chalmers University of Technology, Diploma work in Food Chemistry. 50pp.

Tang, Y.C. and Chen, B.H. (2000) Pigment change of freeze-dried carotenoid powder during storage. *Food Chemistry* **69**, 11-17.

Tanumihardjo, S.A. (2002) Factors influencing the conversion of carotenoids to retinol: bioavailability to bioconversion to bioefficacy. *International Journal for Vitamin and Nutrition Research* **72**, 40-45.

Taungbodhitham, A.K, Jones, G.P., Wahlqvist, M.L and Briggs, D.R. (1998) Evaluation of extraction method for the analysis of carotenoids in fruits and vegetables. *Food Chemistry* **63**, 577-584.

Teow, C.C., Truong, V.D, McFeeters, R. F., Thompson, R.L., Pecota, K.V. and Yencho, G.C. (2007) Antioxidant activities, phenolic and β-carotene contents of sweet potato genotypes with varying flesh colours. *Food Chemistry* **103**, 829-838.

Tewe, O.O. Ojeniyi F.E. and Abu O.A. (2003) Sweetpotato production, utilization, and marketing in Nigeria. Social Sciences Department, International Potato Center (CIP), Lima, Peru, June 2003. 53pp.

Texeira Neto, R.O., Karel, M., Saguy, I. and Mizrahi, S. (1981) Oxygen uptake and β-carotene decoloration in a dehydrated food model. *Journal of Food Science* **46**, 665-676.

Thane and Reddy (1997) Processing of fruit and vegetables: effect on carotenoids. *Nutrition and Food Science* **2**, 58-65.

Tomlins, K.I., Ndunguru, G., Stambul, K., Joshua, N., Ngendello, T., Rwiza, E., Amour R., Ramadhani, B., Kapande, A. and Westby, A. (2007a) Sensory evaluation and consumer acceptability of pale fleshed and orange fleshed sweetpotato by school children and mothers with preschool children. *Journal of the Science of Food and Agriculture* **87**, 2436-2446.

Tomlins, K.I., Ndunguru, G., Kimenya, F., Ngendello, T., Rwiza, E., Amour, R., van Oirschot, Q. and Westby, A. (2007b) On-farm evaluation of methods for storing fresh sweet potato roots in East Africa. *Tropical Science* **47***,* 197-210.

Tran, T.H., Nguyen, M.H., Zabaras, D. and Vu, L.T.T. (2008) Process development of Gac powder by using different enzymes and drying techniques. *Journal of Food Engineering* **85,** 359-365.

Tumuhimbise, G.A., Namutebi, A., Muyonga, J.H. (2009) Microstructure and in vitro β-carotene bioaccessibility of heat processed orange fleshed sweet potato. Plant Foods for Human Nutrition 64, 312-318.

Uganda Bureau of Statistics (UBOS) and ORC Macro (2001), *Uganda Demographic and Healthy Survey 2000-2001.* Calverton, Maryland, USA: UBOS and Opinion Research Corporation (ORC) Macro.

Valdez C., Lopez C.Y., Schwartz S., Bulux J., Solomons N.W. (2001) Sweet potato buds: the origins of a "designer" food to combat hypovitaminosis A in Guatemala. Processing, vitamin A content and preservation characteristics. *Nutrition Research* **21**, 61-70.

van Het Hof, K.H., West, C.E., Westrate, J.A. and Haustvast J G.A.J. (2000) Dietary factors that affect the bioavailability of carotenoids in recent advances in nutritional sciences. *American Society for Nutritional Sciences* **135,** 503-506.

van Hal, M. (2000) Quality of sweetpotato flour during processing and storage. *Food Review International* **16**, 1-37.

van Jaarsveld P., Marais D.W., Harmse E. and Rodriguez Amaya D. (2001) Evaluation of the retention of β-carotene in boiled, mashed orange flesh sweet potato. Copyright VITAA. 23pp.

van Jaarsveld, P.J., Faber, M., Tanumihardjo, S.A., Nestel, P., Lombard, C. J., and Spinnler Benadé, A.J. (2005) β-carotene–rich orange-fleshed sweet potato improves the vitamin A status of primary school children assessed with the modified-relative-dose-response. *American Journal of Clinical Nutrition* **81,** 1080-1087.

van Jaarsveld, P.J., De Wet Marais, Harmse, E., Nestel, P. and Rodriguez-Amaya, D.B. (2006) Retention of β-carotene in boiled, mashed orange-fleshed sweet potato. *Journal of Food Composition and Analysis* **19**, 321–329.

Vasquez-Caicedo, A.L. Schilling, S., Carle, R.and Neidhart, S (2007) Impact of packaging and storage conditions on colour and β-carotene retention of pasteurised mango puree. *European Food Research and Technology* **224**, 581-590.

Vedrina-Dragojevic, I., Sebecic, B. and Horvatic, M. (1997) Effect of blanching, drying, freezing and storage on degradation of β-carotene in different fruits. *Nahrung* **41**, 355-358.

Villareal, R.L. and Griggs, T.D. (1982) Symposium overview. *Sweet Potato*. pp1 in Proceedings of the First International Symposium. Shanhua, Tainan, Taiwan.

Vishnevetsky, M., Ovadis, M. and Vainstein, A. (1999) Carotenoid sequestration in plants: the role of carotenoid-associated proteins. *Trends in Plant Science* **4**, 233-235.

Waché, Y., Bosser-Deratuld, A., Mai Ly, H. and Belin, J.M. (2002) Co-oxidation of β-carotene in biphasic media. *Journal of Molecular Catalysis B: Enzymatic* **19-20**, 197-201.

Waché, Y., Bosser-Deratuld, A., Lhuguenot, J.C. and Belin, J.M. (2003). Effect of cis/trans isomerism of β-carotene on the ratios of volatile compounds produced during oxidative degradation. *Journal of Agricultural and Food Chemistry* **51**, 1984-1987.

Waché, Y., Bosser-Deratuld, A and Belin, J.M. (2006) Dispersion of β-carotene in processes of production of *β*-ionone by cooxidation using enzyme-generated reactive oxygen species. *Process Biochemistry* **41**, 2337-2341.

Walker, D.J. and Farrell, G. (2003) *Food Storage Manual*. Chatham, UK: Natural Resources Institute/Rome: World Food Programme.

Walter, W.M., Purcell, A.E and Cobb, W.Y. (1970) Fragmentation of β-carotene in autoxidizing dehydrated sweet potato flakes. *Journal of Agricultural and Food Chemistry* **18**, 881-885.

Walter, W.M. and Purcell, A.E. (1974) Lipid autoxidation in precooked dehydrated sweet potato flakes stored in air. *Journal of Agricultural and Food Chemistry* **22**, 298-302.

Wang, J.and Xi, Y.S. (2005) Drying characteristics and drying quality of carrot using a two-stage microwave process. *Journal of Food Engineering* **68**, 505-511.

Weather Underground Quelimane (2007). History for Quelimane August-September 2007.
http://www.wunderground.com/history/station/67283/2007/10/13/MonthlyHistory.html [accessed 13 October 2009].

Web, D. A parenchyma cell containing chromoplasts (2009) Photo. University of Hawai at Manoa,
Honlulu.http://www.botany.hawaii.edu/faculty/webb/Bot201/CellTissOrgan/201LabTypVascPlantTheme.htm. [accessed 7 August 2009].

West, K.P. (2002) Extent of Vitamin A Deficiency among Preschool Children and Women of Reproductive Age. *Journal of Nutrition* **132,** 2857–2866.
WHO (2004) Vitamin A deficiency (Chap. 4) *In: Comparative Quantification of Health Risks. Global and Regional Burden of Disease Attributable to Selected Major Risk Factors (Vol. 1)*. World Health Organisation, Geneva.

Wijinker, J.J., Koop, G. and Lipman, L.J.A. (2006) Antimicrobial properties of salt (NaCl) used for the preservation of natural casings. *Food Microbiology* **23**, 657-662.

Winarno, F.G. (1982) Sweetpotato processing and by-product utilisation in the tropics. pp373-383 in *Sweet Potato Proceedings of the First International Symposium* Villareal Ruben L. and Griggs T.D., Editors. Asian Vegetable Research and Development Centre Shanhua Printed by Hong Wen Printing Works, Tainan.Taiwan, China.

Woolfe, J. A. (1992) Post harvest procedures: *Sweet Potato an Untapped Food Source*. Cambridge, UK: Cambridge University Press. 643pp.

Wu, X., Sun, C., Yang, L., Zeng, G., Liu, Z. and Li, Y. (2008) β-carotene content in sweet potato varieties from China and the effect of preparation on β-carotene content in the Yanshu no 5. *Innovative Food Science and Emerging Technologies* **9**, 581-586.

Yamazaki, I., Tamura, M., Nakajima, R. and Nakamura, M. (1985) Physiological aspects of free-radical reactions. *Environment Health Perspectives* **64**, 331-342.

Yen, Y.H., Shih, C.H. and Chang, C.H. (2008) Effect of adding ascorbic acid and glucose on the antioxidative properties during storage of dried carrot. *Food Chemistry* **107**, 265-272.

Zhao, Y.P. and Chang, K.C. (1995) Sulfite and starch affect color and carotenoids of dehydrated carrots (*Daucus carota*) during storage. *Journal of Food Science* **60**, 324–326.

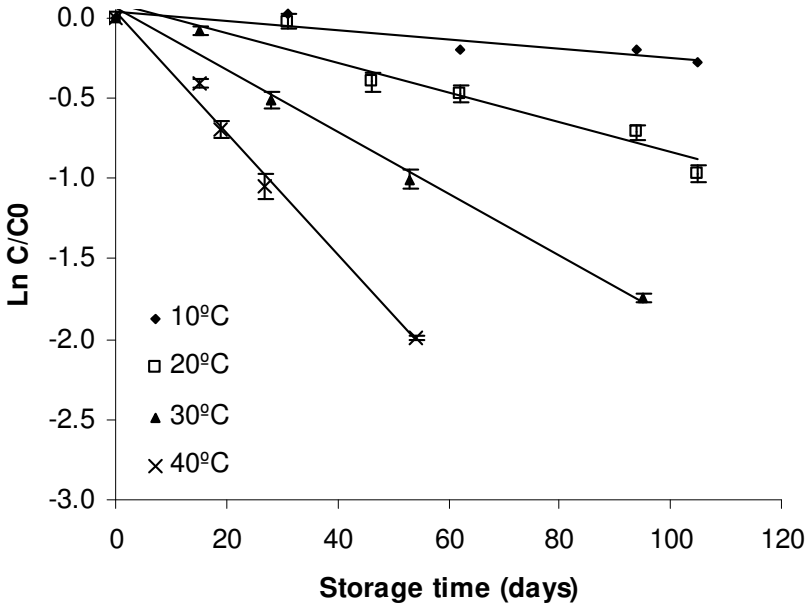
Zorn, H., Langhoff, S., Scheibner, M., Nimtz, M. and Berger, R.G. (2003) A peroxidase from *Lepista irina* cleaves β, β-carotene to flavor compounds. *Biological Chemistry* **384**, 1049-1056.

Appendices

Appendix 1: Kinetic modelling of carotenoid degradation in dried sweet potato

A/Calculation of model parameters of the Arrhenius and Eyring models for the prediction of β-carotene degradation

Temperature degradation kinetics:
The effect of temperature (10; 20; 30; 40ºC) on β-carotene is described in Figure 1 (see Chapter 8).



Temperature (ºC)		10	20	30	40
Trans-β-carotene*	k	0.0024 (0.0002)	0.0090 (0.0005)	0.0191 (0.0002)	0.0403 (0.0005)
	R^2	0.757 (0.058)	0.914 (0.031)	0.986 (0.005)	0.965 (0.007)

Figure 1: Trans-β-carotene degradation kinetics in dried sweet potato chips influenced by temperature between 10-40ºC on a dry weight basis (mean of triplicate (jar); error bars refer to standard deviation). Oxygen level 21% (air); a_w 0.52-0.65.

The degradation is modeled by Arrhenius and Eyring equations.

Arrhenius model:

$$k = k_{\infty} e^{-\frac{Ea}{RT}}$$

k : degradation constant rate (day^{-1})
k_{∞} : value of k at T = ∞ (day^{-1})
T : temperature (K)
Ea : Activation energy ($kJ.mol^{-1}$)
R : Molar gas constant = 8.314 J · K−1 · mol^{-1}

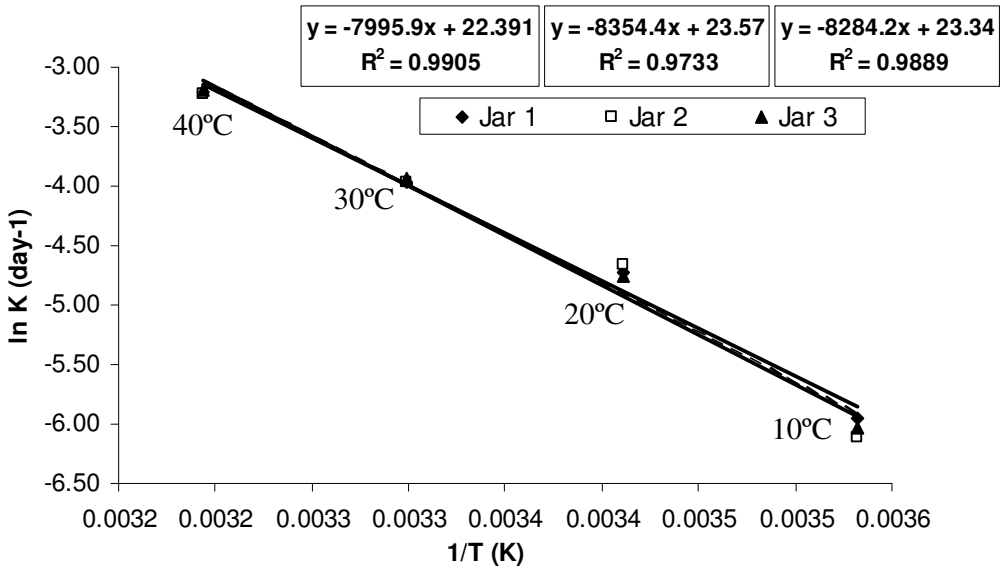


Figure 2: Arrhenius model for temperature between 10-40ºC on a dry weight basis (mean of triplicate (jar); error bars refer to standard deviation). Oxygen level 21% (air); a_w 0.52-0.65.

$\ln k = b - a\frac{1}{T}$ where b and a are constant of linear equation. b= ln k_{∞}.

Ea was calculated from the linear equation following the formula: $a = \frac{Ea}{R}$

In average over the three replicates (jars): Ea = 68.3$kJ.mol^{-1}$; k_{∞} =1.21.10^{10} day^{-1}.

Eyring model:

$$k = \frac{k_B}{h} T . e^{-\frac{\Delta G^*}{RT}} = \frac{k_B}{h} T . e^{-\frac{\Delta H^*}{RT}} . e^{\frac{\Delta S^*}{R}}$$

k_B : Boltzmann constant = $1.381 \cdot 10^{-23}$ J.K^{-1}
h : Planck constant = $6.626 \cdot 10^{-34}$ J.s
ΔG : free activation enthalpy (J.mol^{-1})
ΔH : activation enthalpy (kJ.mol^{-1})
ΔS : activation entropy (J.mol^{-1}.K^{-1})

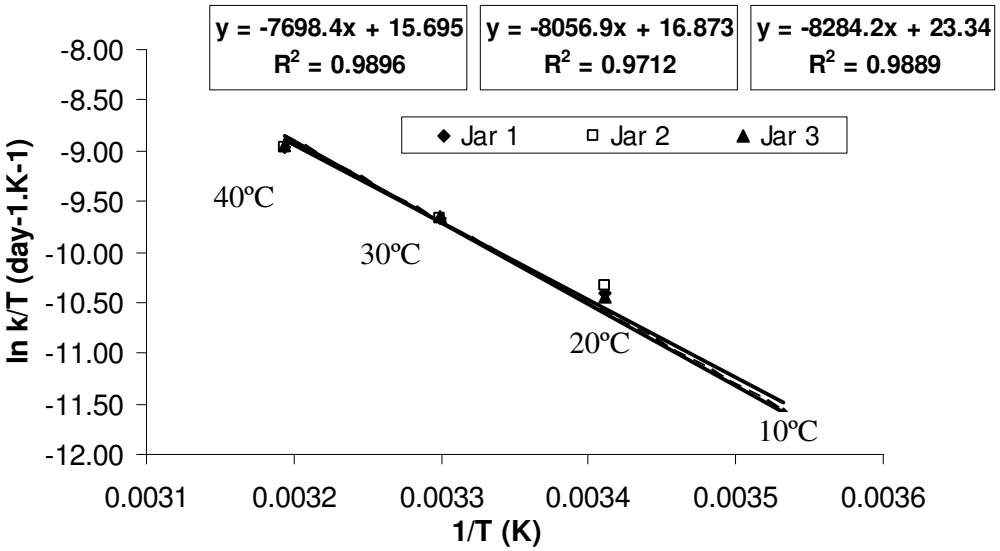


Figure 3: Eyring model for temperature between 10-40ºC on a dry weight basis (mean of triplicate (jar); error bars refer to standard deviation). Oxygen level 21% (air); a_w 0.52-0.65.

$\ln \frac{k}{T} = b - a \frac{1}{T}$ where b and a are constant of linear equation.

ΔH was calculated from the linear equation following the formula: $\Delta H = Ra$ and

$\Delta S = R(b - \ln \frac{k_B}{h})$

In average over the three replicates (jars): ΔH = 65.8kJ.mol^{-1}; ΔH =-61.1 J.mol^{-1}.K^{-1}

B/Calculation of Arrhenius model robustness using experimental points – under variable temperature (anisothermal) conditions in the dark

Solving of the Arrhenius equation for a first order kinetics of degradation

$$\frac{dC}{dt} = -kC \quad \text{(First order equation) (1)}$$

$$k = k_\infty e^{\frac{-Ea}{RT}} \quad \text{(Arrhenius equation) (2)}$$

$$\Rightarrow \quad \frac{dC}{dt} = -k_\infty e^{\frac{-Ea}{RT}} C \quad \text{(1) + (2)}$$

$$\frac{dC}{C} = -k_\infty e^{\frac{-Ea}{RT}} dt \quad \text{(concentrations and times are put separately)}$$

$$\int_{C_0}^{C} \frac{dC}{C} = -k_\infty \int_0^t e^{\frac{-Ea}{RT}} dt \quad \text{(integration)}$$

$$\ln \frac{C}{C_0} = -k_\infty \int_0^t e^{\frac{-Ea}{RT}} dt \quad \text{(integrate of derivate is equal to logarithm of concentration)}$$

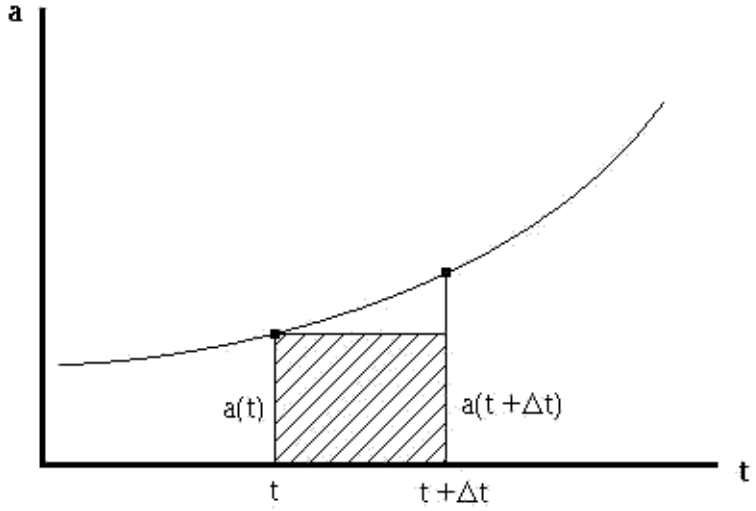
$$\frac{C}{C_0} = e^{-k_\infty \int_0^t e^{\frac{-Ea}{RT}} dt} \quad \text{(exponential)}$$

$$C = C_0 \, e^{-k_\infty \int_0^t e^{\frac{-Ea}{RT}} dt}$$

T=ambient temperature; time= 88 days

$$C = C_0 \, e^{-k_\infty \int_0^t e^{\frac{-Ea}{RT}} dt}$$

Euler estimate: dt = Δt for Δt very small therefore ∫dt = ∑Δt



Time (h)	Temperature				
t_0	T_0	$e\frac{-Ea}{RT_0}$	$e\frac{-Ea}{R\frac{(T_0+T_1)}{2}}(t_1-t_0)$		
t_1	T_1	$e\frac{-Ea}{RT_1}$		$e\frac{-Ea}{R\frac{(T_1+T_2)}{2}}(t_2-t_1)$	
t_2	T_2	$e\frac{-Ea}{RT_2}$			$e\frac{-Ea}{R\frac{(T_2+T_3)}{2}}(t_3-t_2)$
t_3	T_3	$e\frac{-Ea}{RT_3}$			
Etc…	T_x				

Sum: $\sum_0^x e\frac{-Ea}{R\frac{(T_{x-1}+T_x)}{2}}(t_x-t_{x-1})$

$$C = C_0 \, e^{-k_\infty \sum_0^x e\frac{-Ea}{R\frac{(T_{x-1}+T_x)}{2}}(t_x-t_{x-1})}$$

Temperature is integrated for each time interval (Figures 4 and 5).

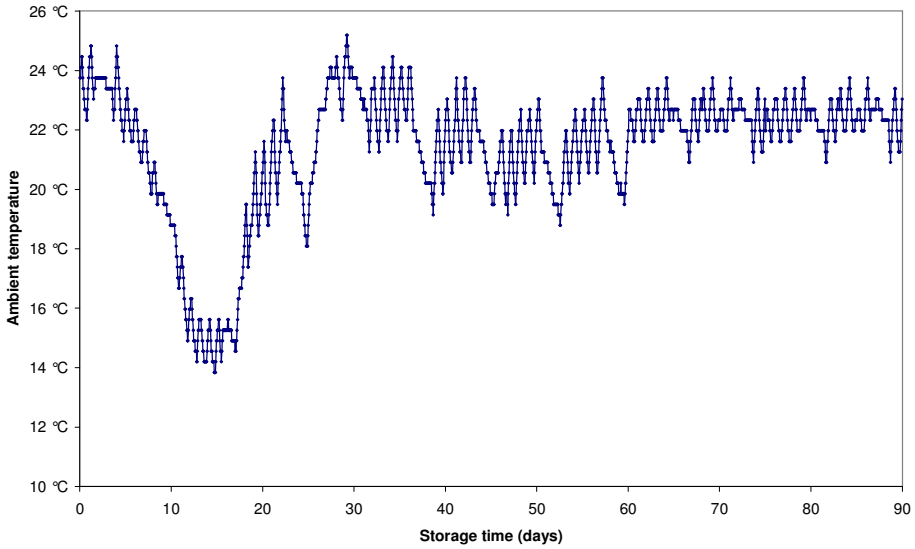


Figure 4: Temperature during storage at ambient room temperature in the UK (88 days) (non-regulated temperature over Christmas).

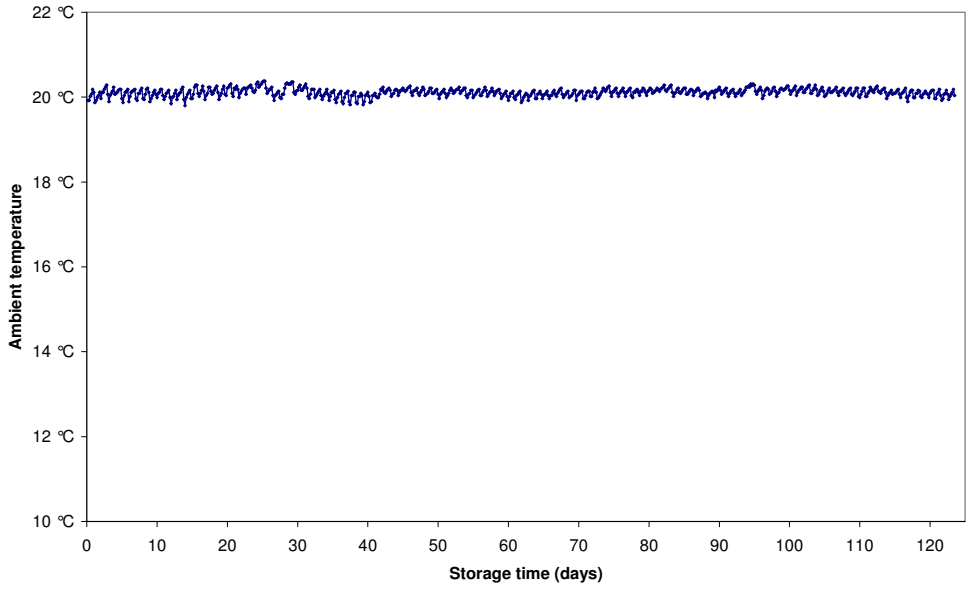


Figure 5: Temperature during storage at ambient room temperature in Uganda (125 days)

1) Integration of temperature in the UK and calculation of the predicted values

a) β-carotene

$t_x - t_{x-1} = 1$h

Co = 201.0 µg.g^{-1}

Ea= 68.3kJ.mol-1

$k_\infty = \frac{1.21.10^{10}}{24}$ h^{-1}

R= 8.314 J. K^{-1}.mol^{-1}

C_{model}= 84.8 µg.g^{-1}

$Cexp$= 82.6 (5.6) µg.g^{-1}
Mean (standard deviation)

Percentage difference:
$|\frac{C - C\exp}{C\exp}| = 2.7\%$

b) total carotenoids (UK)

$t_x - t_{x-1} = 1$h

Co = 277.7 µg.g^{-1}

Ea= 49.5kJ.mol-1

$k_\infty = \frac{1.80.10^{6}}{24}$ h^{-1}

R= 8.314 J. K^{-1}.mol^{-1}

C= 99.6µg.g^{-1}

$Cexp$= 104.3 (1.7) µg.g^{-1}
Mean (standard deviation)

Percentage difference:
$|\frac{C - C\exp}{C\exp}| = 4.6\%$

2) Integration of temperature in Uganda and calculation of the predicted values

total carotenoids

$t_x - t_{x-1} = 1$h

Co = 236.3 µg.g^{-1}

Ea= 49.5kJ.mol-1

$k_\infty = \frac{6.86.10^6}{24}$ h^{-1}

R= 8.314 J. K^{-1}.mol^{-1}

C_{model}= 49.1µg.g^{-1}

$Cexp$= 58.5 (1.7) µg.g^{-1}
Mean (standard deviation)

Percentage difference:
$|\frac{C - C\exp}{C\exp}|$= 16.0%

The model fitted well the experimental value for a non-isotherm trial either with UK or Uganda data working both on the Ejumula sweet potato variety.

Conclusion
The non-isotherm model fitted well with the experimental values (2.7% and 4.6% difference on β-carotene and total carotenoid content respectively). Data from the Uganda (Uganda) also fitted well with the experimental values (16.0% difference).

In conclusion our model was validated for the prediction of total carotenoid and β-carotene degradation in sweet potato dried chips under anisothermal conditions.

Material	**Tunnel dryer**			**Open air dryer**			**Tent dryer**		
	Quantity	Cost /unity (Ush)	Total cost (Ush)	Quantity	Cost /unity (Ush)	Total cost (Ush)	Quantity	Cost /unity (Ush)	Total cost (Ush)
Timber	0.02 x 0.3 x 3.96 15 pieces	15,000	225,000	6 pieces	2,000	12,000	6 pieces	1,500	9,000
Bamboo	-	-	-	6 bundles	15,000	90,000	3 bundles	15,000	45,000
Polythene clear	1.2 x 9.13 30 metres	2,500	75,000	-	-	-	1.2 x 8 metres	2,500	20,000
Polythene black	1.2 x 10 10 metres	2,500	25,000	-	-	-	1.2 x 3 metres	2,500	7,500
Assorted nails	4kg	2,500	10,000	5kg	2,500	12,500	5kg (1/3*)	2,500	4,200 (=12,500/3)
Bricks	600 pieces	80	48,000	300 pieces	80	24,000	200 pieces	80	16,000
Cement	2 bags	20,000	40,000	1 ½ bag	20,000	30,000	1 ½ bag (1/3*)	20,000	10,000 (=30,000/3)
Sand	1 pick-up	20,000	20,000	½ pick-up	20,000	10,000	1/3* pick-up	20,000	7,000 (=20,000/3)
Thumb pins	2 packets	5,000	10,000	-	-	-	1 packet	5,000	5,000
Hook Payson	-	-	-	6 pieces	500	3,000	4 pieces	500	2,000
Papyrus mat	6 pieces	2,000	12,000	-	-	-	-	-	-
Iron sheets	2 pieces	13,000	26,000	-	-	-	-	-	-
Black painting	2 litres	5,000	10,000	-	-	-	-	-	-
Brush for painting	2 pieces	1,500	3,000	-	-	-	-	-	-
Hinges for door	7 pieces	500	3,500	-	-	-	-	-	-
Drum and welding			100,000	-	-	-	-	-	-
Plastic mesh	0.52 x 6 6 metres	15,000	90,000	-	-	-	-	-	-
Cost without labour and transport			**697,500 Ush; about £211****			**181,500 Ush; about £55****			**145,700 Ush; about £44****
1 technician: 15,000sh/day	5 days		150,000	2 days		50,000	2 days		50,000
2 porters: 3000sh /day									
Transport			30,000			30,000			30,000
Total			**877,500 Ush; about £266****			**261,500 Ush; about £79****			**225,700 Ush; about £68****
Capacity fresh chips (kg)			**24**			**16**			**8**
Total per capacity			**About £11/kg**			**About £4/kg**			**About £8/kg**

Material		**Tunnel dryer**		**Open air dryer**		**Shade dryer**		**20 Trays**	
	Cost per unity (MT)	Quantity	Total cost (MT)	Quantity	Total cost (MT)	Quantity	Total cost (MT)	Quantity	Total cost (MT)
Poles	0	ND	0	0	ND	0	0	ND	0
Polythene clear	144 (£3*)	2.5 x 10 m	1440 (£30*)	-	-	-	-	-	-
Polythene black	65	1.5 x 6 x 2 m	780	-	-	-	-	-	-
Nails	40	5 kg	200	3 kg	120	4kg	200	3kg	120
Thumb pins	30	3 boxes	90	-	-	-	-	-	-
Straw mat	40	6 pieces	240	4 pieces	160	4 pieces	160	-	-
Iron sheets+sheers	380	1 piece	380	-	-	-	-		
Black painting+brush	70	2 litres	140	-	-	-	-		
Mosquito mesh	60	1 x 2 m	120	-	-	-	-	1 x 32 m	1920
Cost without labour			**3,390 MT; about £71**		**280 MT; about £6**		**360 MT; about £8**		**2040 MT; about £43**
Labour		3 days= 1 carpenter + 4 farmers workers	1800	0.5 day= 1 carpenter + 4 farmers workers	300	1.5 day= 1 carpenter + 4 farmers workers	900	1 day= 1 carpenter	100
Total			**5,190 MT; about £108**		**580 MT; about £12**		**1260 MT; about £26**		**2140 MT; about £45**
Capacity fresh chips (kg)			**24 (6 trays)**		**32 (8 trays)**		**32 (8 trays)**		
Total per capacity			**About £4.5/kg**		**About £0.4/kg**		**About £0.8/kg**		

*Bought in the UK

Appendix 2c: Low-cost solar dryers

A/Building of low-cost solar dryers

Example: Lualua, Mozambique
Technical information sheet: Aurelie Bechoff;
Building of dryers: Keith Tomlins and Aurelie Bechoff

The building of low cost solar dryers involves the following steps:

1. Preparing the ground
2. Laying foundations
3. Putting coverage
4. Making trays

1. Preparing the ground
The area should be chosen as flat as possible. Clear the ground from all bush shrubs. Orientate your dryers toward the midday sun (South in the Northern hemisphere or North in the Southern hemisphere). Leave sufficient space between dryers (about 2 meters) to limit shade effect of one dryer to another.
Place sticks to mark the pegs of dryers. Use string; spirit level and protractor to measure the appropriate angle for the drying table (the table angle corresponds to the perpendicular angle to the sun at noon; that is the latitude on annual measurement). Mark the height of pegs.

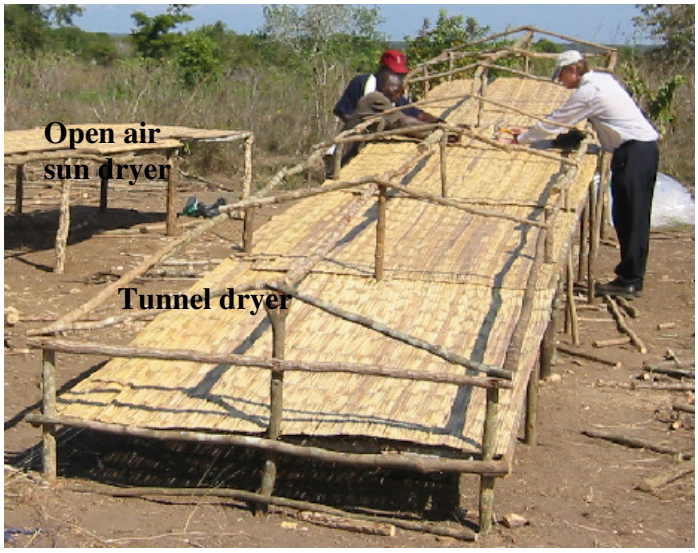
2. Laying foundations

Replace sticks by poles and build the table structure using transversal poles. You would need a hammer and nails preferably (alternatively you could use bush grass to attach poles together).

For the tunnel dryer mount another structure on the top of the table structure.

3. Putting coverage

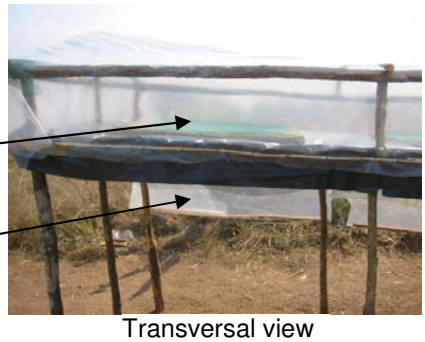
Cover drying tables with mats.



As an option insolate the table of tunnel dryer with black plastic. Put iron sheets (absorber) into place in the first 3 m of the tunnel dryer and paint with black paint.

Cover the structure with clear polythene (10m x 2m) and pin it. Cut both ends for inlet and outlet and cover using mosquito net.

Facial view



Transversal view

Prepare the roof of the shade dryer by securing with poles; two on the sides of the drying table and one in the middle. Mount transversal poles on this structure.

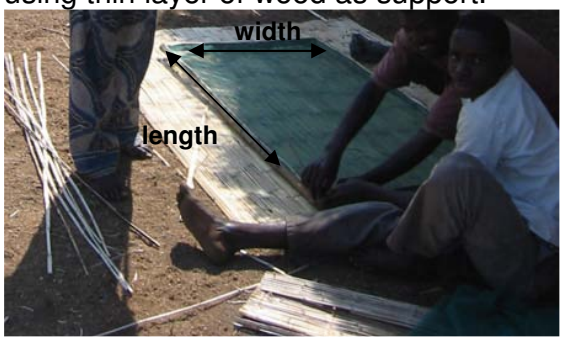
Facial view

Transversal view with transversal poles

Complete the shade dryer by covering with straw and adding poles to hold the straw

4. Making trays

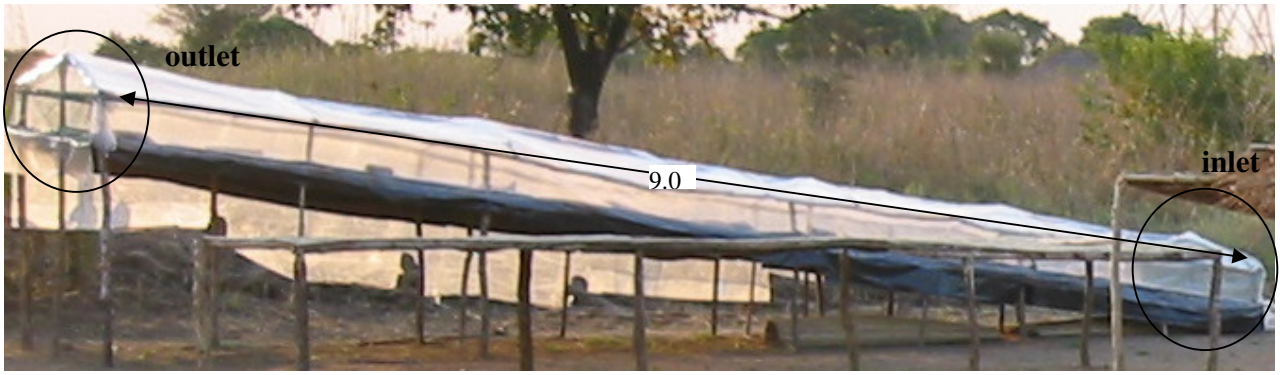
Make mesh trays using 2 pairs of poles. Nail the mosquito net into the pole using thin layer of wood as support.



B/Dimensions of dryers

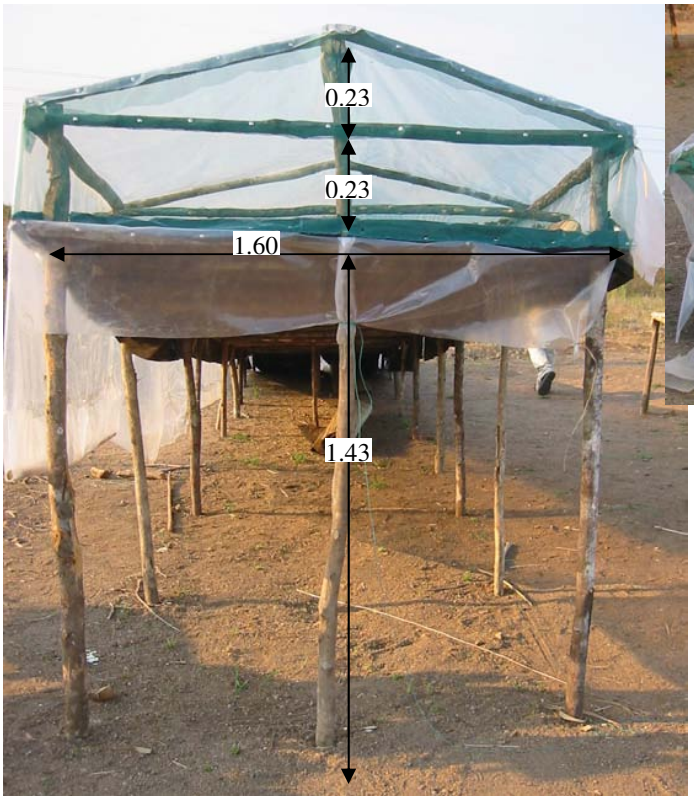
Tunnel dryer

The tunnel dryer was 9 m long: 3.5 m of collector (iron sheet absorber) and 5.5 m of dryer.

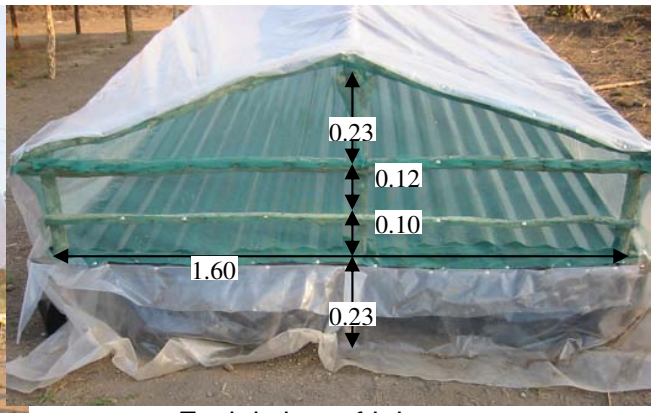


Side view

The width was 1.6 m. There was a 6º angle of drying table. The inlet surface is the same as the outlet surface: 0.23 x 1.60 x 1.5 = 0.552m^2



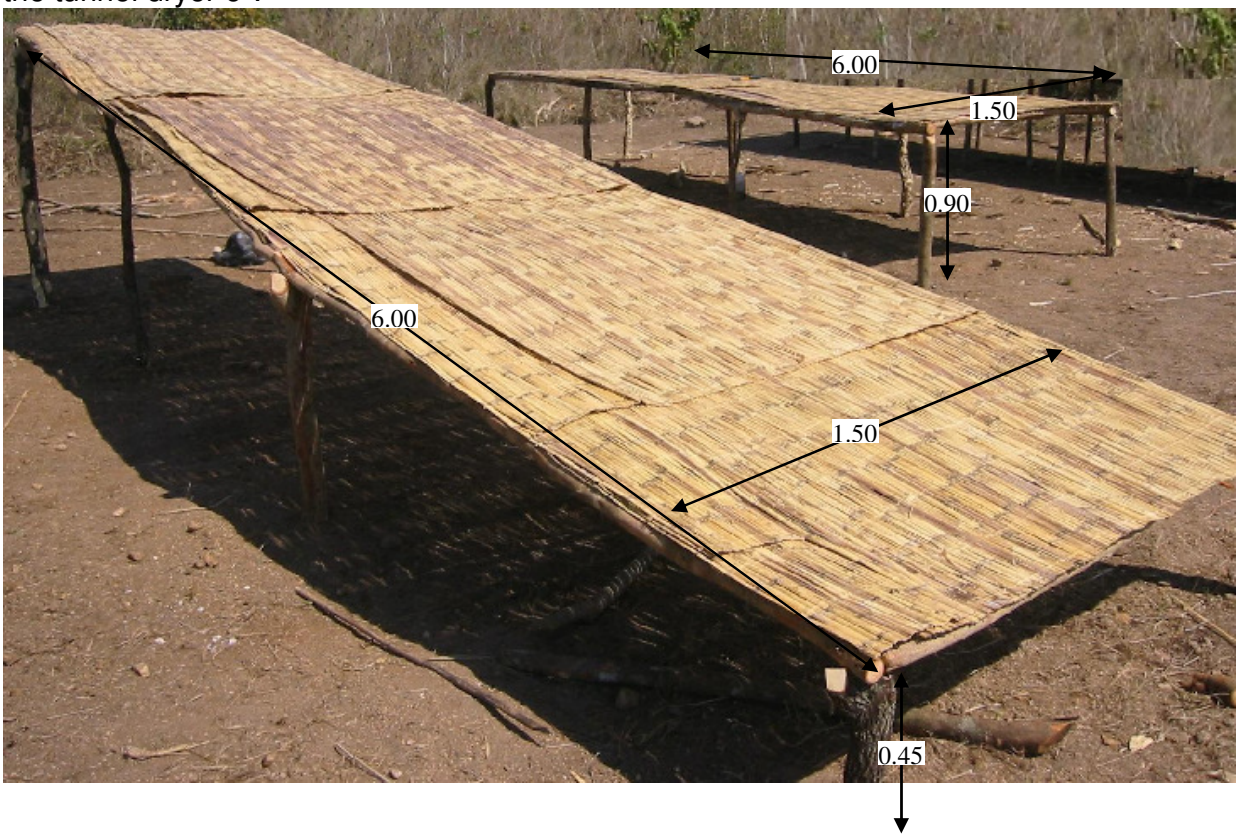
Facial view of outlet



Facial view of inlet

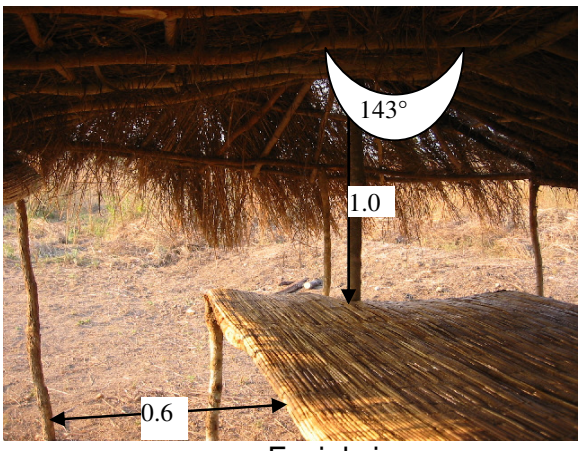
Open air flat and sloped dryers

Open air dryers were 6 m long and 1.5 m wide. The sloped dryer had the same angle as the tunnel dryer 6º.

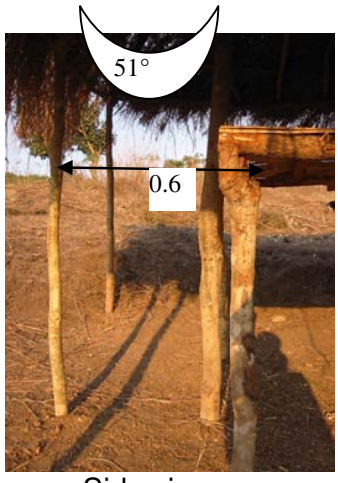


Shade dryer

The shade dryer was similar to the flat open dryer mounted of a roof. The roof was slightly staggered to the table (about 0.6 m on four sides) to protect better against incident sun rays. The height of the roof from the drying table was 1.0 m and the main angle was very wide (143°by calculation) so that persons could stand without bending. The angle on the ends was calculated as 51°.



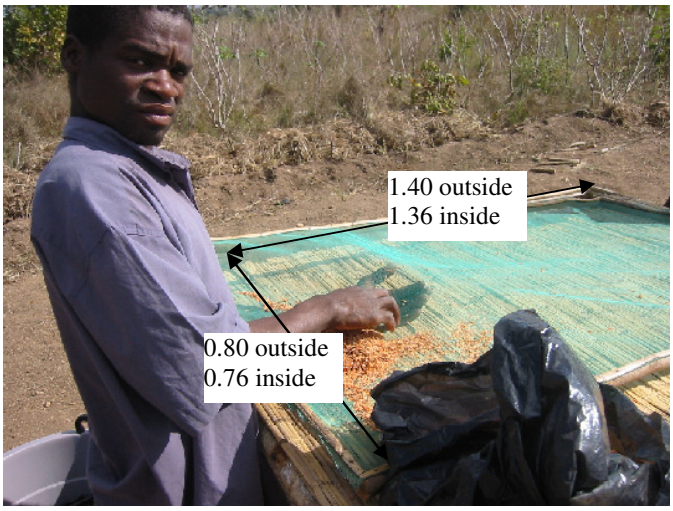
Facial view



Side view

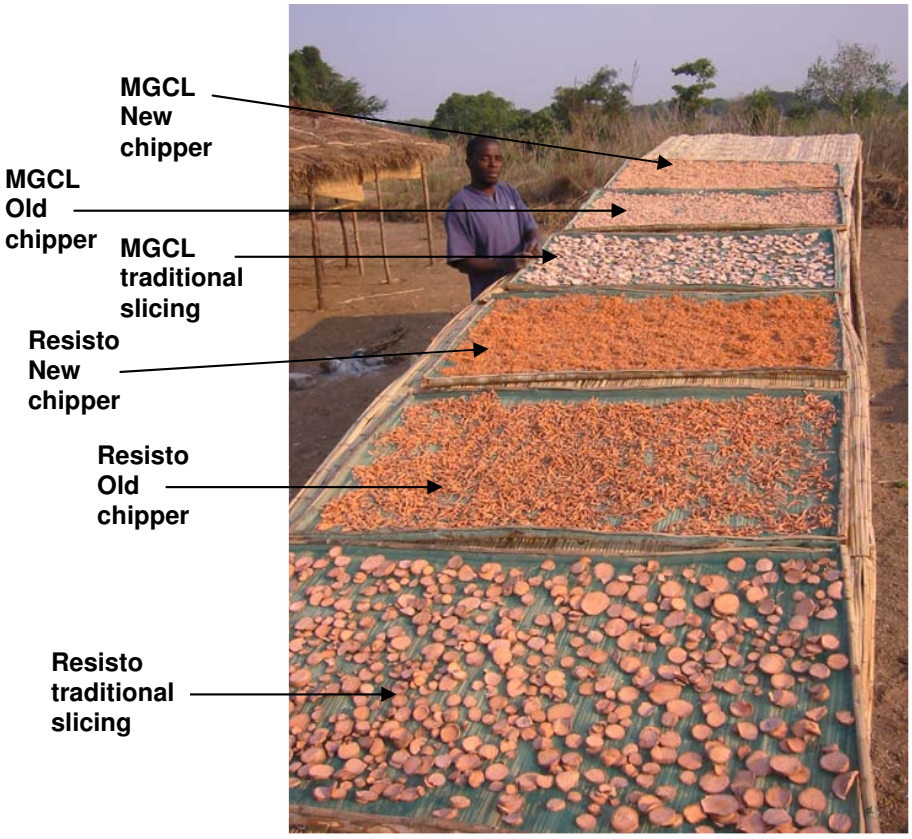
Trays

The internal length was 1.36 m and the width was 0.76 m per tray. Each dryer could fit six trays.



Example of sweetpotato drying

The same weight of sweetpotato chips or slices is loaded on each tray (here 4 kg). Each dryer would therefore have a capacity of 6 x 4 =24 kg.



Appendix 3: Total carotenoids method for Sweetpotato

Based on HarvestPlus Handbook method by Rodriguez Amaya and Kimura, 2004

Setting up -December 2006: Aurelie Bechoff,
PhD student, Natural Resources Institute (UK)
In charge: Constance Owori, researcher Post Harvest Department, NARL, Uganda
Technician: Geoffrey Menya, Post Harvest Department, KARI, NARL, Uganda

REAGENTS

- Methanol: Tetrahydrofuran (THF) (1:1)
- Petroleum Ether 40-60°C
- Sodium Sulphate, anhydrous (Na_2SO_3)

For cleaning:

- Sintered glasses: Sulphuric acid (98%); Hydrogen peroxide solution (100 volumes). *Sulphuric acid is corrosive and should be handled solely under fume cupboard with eye protection; mask and gloves.*
- Volumetric flasks: Acetone

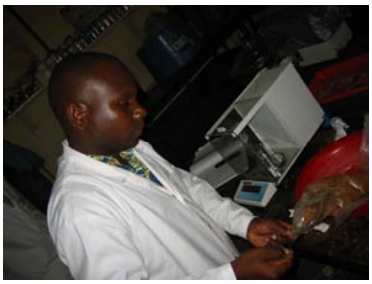
APPARATUS

- Kenwood FP698 Multi Pro Food Processor for chopping fresh sweetpotato
- Polytron Homogeniser suitable for high shear mixing to release carotenoids from vegetable cell membranes
- Stop watch
- Vacuum Pump
- Analytical balance Wagtech with reading 0.0001g
- Spatulas (x 2): 1 long spatula; 1 small spatula
- Beakers, 100 ml (x 3)
- Measuring cylinder, 100 ml (x 1)
- Sintered glass funnels porosity 2 (x 3)
- Vacuum flasks, 250 ml (x 3)
- Separatory funnels 500 ml (x 2)
- Clamp and boss + separatory funnel support (x 2)
- Conical flasks, 100 ml (x 3)
- Glass funnels (x 2)
- Cotton wool
- Volumetric flasks, 50 ml (x 3)
- Cuvettes PMMA reading 280-800nm
- Delicate task wipe for cuvettes
- Genesys 10UV Thermopectonic spectrophotometer to measure absorbance at 450 nm
- Foil to cover samples from light
- Protection equipment: laboratory coats (x 2), gloves, glasses, breathing masks with solvent filter for fume protection to use in case of power shortages (x 2)

1. Sample preparation

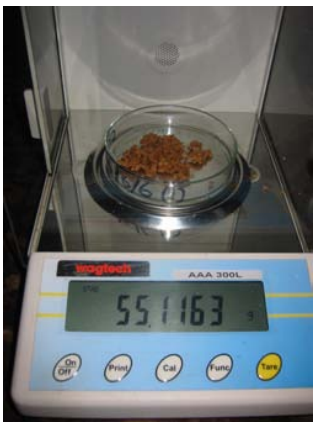
Caution! Carotenoids are subject to photo-oxidation. All operations must be performed under dim light.

- For fresh sweetpotato roots or fresh chips, defreeze by soaking the plastic bag in tepid water (about 30°C). Fresh samples must be blended to a fine pulp using a Kenwood FP698 Multi Pro Food Processor.
- For flour samples milled from dried sweetpotato chips, put samples at ambient temperature for about 10-15 minutes. Add 10ml deionised water to re-hydrate sample; measure 20 minutes before extraction.



2. Sample Weighing

- Homogenise well sample using the long spatula.
- Weigh 5g in triplicate for dry matter determination using glass Petri dishes or ceramic dishes as followed: Tare balance; weigh tare; report tare weight; add 5g of sample; report tare + sample weight. Put samples in oven at 105ºC for 24h minimum. (In case of power shortage, leave for one day more).



- Homogenise again sample using long spatula
- Weigh a portion of the sample for total carotenoids determination in triplicate in beakers using the two spatulas. The weight depends on the carotenoids content of the sample as shown in the table below.

Portion of sample weighed (g) for carotenoids determination

Sweet potato variety	Fresh	Dried
Ejumula	1	0.5
Kakamega	3	1
SPK004/1 or SPK004/1/1	3-6	-
SPK004/6 or SPK004/6/6	2	-
Naspot 1	8-9	-

- Cover with foil and transport to fume cupboard

3. Extraction

- Pour 50 ml mixture of methanol: tetrahydrofuran (THF) (1:1) into the beaker containing the sample; fix on the stand and homogenise for 60 seconds using Polytron homogeniser. Rinse the Polytron rod with deionised water and place it back into the box to protect apparatus from falling.

- Fix vacuum flask, rubber and sintered glass funnel on the stand. Add sample solution. Filter through funnel by vacuum (first filtration). Rinse beaker with 10-15 ml of methanol: THF (1:1) mixture receiving the washing in the vacuum flask through the funnel (second filtration). Use small spatula to homogenise solution in the funnel before filtering for the second time. *The residue or washing must be devoid of colour. If not rinse beaker again and repeat filtration.*

4. Partition

- Pour 40 ml of Petroleum ether (PE) into separatory funnel; add the extract; slowly add 200 ml of desionised water letting flow along the walls of the funnel to avoid formation of an emulsion. Let the two phases separate and discard the lower, aqueous, colourless phase. Wash three times with 300 ml desionised water to remove residual methanol: THF. After the last washing, leave sample for about 15 minutes to allow better separation.

- Discard the remaining lower phase as completely as possible without discarding the upper phase (Use a clear flask to check lower phase; if erroneous separation put lower phase back and repeat the operation).

- Collect the upper organic phase in 100ml-conical flask.
- To remove residual water, add anhydrous sodium sulphate salt until some crystals remain loose. *All glassware should be well dried from that stage.*

- Transfer the solution into a 50 ml-volumetric flask, filtering through glass funnel with cotton wool. Ensure that no sodium sulphate would pass through.

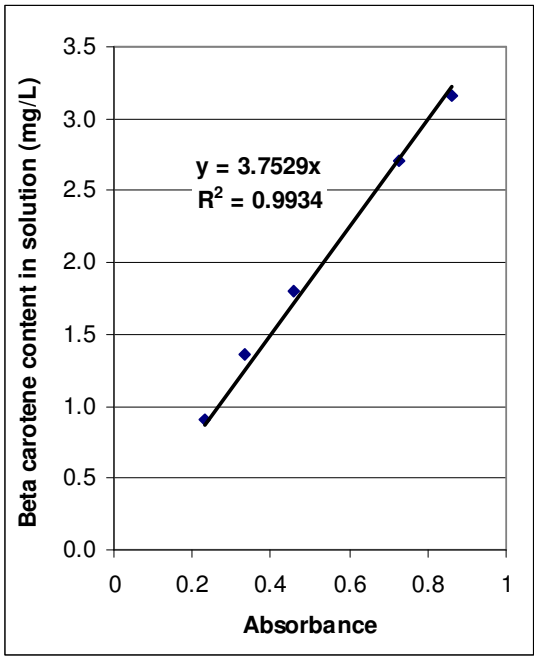
- Make up to volume with petroleum ether.

5. Spectrophotometric reading

- Switch on Spectrometer 5 minutes before reading. Absorbance reading is at 450nm.

- Make blank in Petroleum Ether. (Rinse blank cuvette with Petroleum Ether and ensure that cuvettes are clean and wipe them if necessary using delicate wipe paper).
- Homogenise sample solution and take sample reading three times (changing solution in the same cuvette). Absorbance should be comprised between 0.2-0.8, range measured with standard.
- Solutions are diluted in petroleum ether if the sample absorbance was not included in the range.
  Petroleum Ether is a volatile solvent and reading had to be carried out quickly in order to prevent bias.

- Concentrations are determined by comparison to a standard curve using pure beta carotene, SIGMA, UK. The curve was established prior to analyses in November 2006 and repeated in February 2007.



Beta carotene standard curve
Genesys 10UV Spectro.
KARI, Uganda (Feb. 2007)

Sample concentration in solution (mg/L) is calculated by multiplying the sample absorbance by the trend-line coefficient (here 3.7529)

- Sample concentration per weight deducted by dividing carotenoids content in the 50ml volumetric flask by the initial sweetpotato sample weight (fresh or dried).

Total carotenoids content (µg/g) = $\frac{\text{Absorbance} \times 3.7529 \times 50}{\text{Sample mass (g)}}$

Poster Author: Aurelie Bechoff- April 2007

Publications, award and presentations

Publications:

Bechoff A., Dufour D., Dhuique-Mayer C., Marouzé C., Reynes, M. & Westby A. (2009) Effect of hot air, solar and sun drying treatments on provitamin A retention of orange-fleshed sweet potato. *Journal of Food Engineering, 92* (2), 164-171. http://www.sciencedirect.com/science?_ob=ArticleURL&_udi=B6T8J-4TW10BY-6&_user=5893342&_coverDate=05%2F31%2F2009&_alid=1467419761&_rdoc=2&_fmt=high&_orig=search&_origin=search&_zone=rslt_list_item&_cdi=5088&_sort=d&_st=13&_docanchor=&view=c&_ct=2&_acct=C000027518&_version=1&_urlVersion=0&_userid=5893342&md5=9beac093e0e802ed8879eab2ff6217c6&searchtype=a

Bechoff, A., Westby, A., Owori, C., Menya, G., Dhuique-Mayer, C., Dufour D. & Tomlins K. (2010a). Effect of drying and storage on the degradation of total carotenoids in orange-fleshed sweetpotato cultivars. *Journal of the Science of Food and Agriculture*, *90*, 622-629.
http://onlinelibrary.wiley.com/doi/10.1002/jsfa.3859/abstract

Bechoff, A., Dhuique-Mayer, C., Dornier, M., Tomlins, K., Boulanger, R., Dufour, D. & Westby (2010b). Relationship between the kinetics of β-carotene degradation and norisoprenoid formation in the storage of dried sweet potato chips. *Food Chemistry*, *121,* 348–357.
http://www.sciencedirect.com/science?_ob=ArticleURL&_udi=B6T6R-4Y1NV8H-1&_user=5893342&_coverDate=07%2F15%2F2010&_alid=1467419761&_rdoc=1&_fmt=high&_orig=search&_origin=search&_zone=rslt_list_item&_cdi=5037&_sort=d&_st=13&_docanchor=&view=c&_ct=2&_acct=C000027518&_version=1&_urlVersion=0&_userid=5893342&md5=5ca64dd5bb510fb4708e154be585ddce&searchtype=a

Bechoff, A., Tomlins, K.I., Dhuique-Mayer, C., Dove, R. & Westby, A. (2010c) On-farm evaluation of the impact of drying and subsequent storage on the carotenoid content of orange-fleshed sweet potato. International Journal of Food Science & Technology. *In Press.* (Accepted 17 Aug. 2010).

Bechoff, A., Westby, A., Menya, G. & Tomlins, K.I. (2010d). Effect of pre-treatments for retaining total carotenoids in dried and stored orange fleshed-sweet potato chips. Journal of Food Quality. Currently under review (Submitted 25 February 2010. JFQ-2010-060.)

Award:

Bechoff, A., Natural Resources Institute, University of Greenwich for her research into "Understanding losses of carotenoid during making of porridge from orange fleshed sweetpotato". Natural Resources International Fellowship. Postgraduate Research & Travel Fellowship. Awarded £1,680 for field study in Uganda to use between 1st May 2009 and 30 June 2010.
http://nrinternational.co.uk/_uploads/awards/38.pdf

Presentations:

Bechoff, A., Westby, A., Dufour, D., Dhuique-Mayer, C., Marouze, C., Owori, C., Menya, G., Tomlins, K.I. (2007). Effect of drying and storage on the content of pro-vitamin A in Orange Flesh Sweetpotato: sun radiations do not have significant impact. 10th Triennial Symposium of the International Society for Tropical Root Crops – Africa Branch (ISTRC-AB), "Root and Tuber Crops for Poverty Alleviation through Science and Technology for Sustainable Development", IIAM, Maputo, Mozambique, 8 au 12 octobre. (poster)
http://docs.google.com/viewer?a=v&q=cache:ntbcgvMfyi0J:www.agrosalud.org/index.php%3Foption%3Dcom_docman%26task%3Ddoc_download%26Itemid%3D99999999%26gid%3D37+Effect+of+drying+and+storage+on+the+content+of+pro-vitamin+A+in+Orange+Flesh+Sweetpotato&hl=en&gl=uk&pid=bl&srcid=ADGEESggZm52XExlkeagoEYnC7TXahR0TTXtmMyZw6Rm6DDanCdLgoOv5P4zTJd4sNzSOxyMlDzuPzPqsUy5sSSOLNKvFzyyrizLP6NOEGHDG2iyEBzvs36t64KOjYcb08D3xdgY3Han&sig=AHIEtbRptOxko9wsZehurZSteISINVfGPA

Bechoff, A. Tomlins, K., Dhuique-Mayer, C. and Westby, A. Understanding carotenoid losses after drying and storage of orange-fleshed sweetpotato (Ipomea batata). 15th Triennial Symposium of the International Society for Tropical Root Crops – (ISTRC), "Tropical Roots and Tubers in a Changing Climate: A convenient opportunity for the World", CIP, Lima, Peru, 2-6 november. (oral)