Abstract: The effects of storage temperature (10; 20; 30; 40 °C), water activity (0.13; 0.30; 0.51; 0.76) and oxygen level (0%; 2.5%; 10%; 21%) on the degradation of carotenoids and formation of volatile compounds during storage of dried sweet potato chips were evaluated. A kinetic model was developed for degradation of trans-B-carotene and it showed that breakdown followed first order kinetics with an activation energy of 64.2 kJ.mol⁻¹. The difference between experimental data under laboratory or field conditions fitted and data predicted by the model was less than 10% for trans-B-carotene, or for total carotenoids. The formation of the volatile compounds, B-ionone; 5,6-epoxy-B-ionone; dihydroactinidiolide; B-cyclocitrinal, was measured by SPME-GC-MS and was clearly related to the degradation of trans-B-carotene. It is also suggested that carotenoid degradation in dried sweet potato was by autoxidation because of the trend in B-carotene degradation rate in relation to water activity or oxygen level.
To: The Editor  
Food Chemistry

Dear Editor,

Please find attached our revised publication entitled "Relationship between the Kinetics of β-Carotene Degradation and Formation of Norisoprenoids in the Storage of Dried Sweet Potato Chips". Please find our revised manuscript in attached document in two versions:

- a revised version (clean: without track changes) (Paper food chem revised.doc);
- a copy of this version with track changes from the previous submitted version (28/07/09) to facilitate the Editor's correction work (Paper food chem. track changes.doc).

The corrections of the manuscript following point by point the reviewers’comments are in the attached file (Reviewers comments food chem.doc).

For your information please find as well our previous publication “Effect of drying and storage on the degradation of carotenoids in orange-fleshed sweetpotato cultivars” submitted to the Journal of the Science of Food and Agriculture (June 09) as supplementary documentation.

Please find also a list of the complete address details of the potential reviewers.

Best regards,

Claudie Dhuique-Mayer

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**Reviewers' comments and correction of the manuscript**

Responses to the reviewers’ comments are in bold. Numbered lines are from the current revised manuscript (see version with track changes).

**Reviewer #1:**
The manuscript mainly dealt with relationship between the kinetics of carotenoid degradation and volatile compounds formation in the storage of dried sweet potato chips. Although the novelty of study is not obvious and there aren't enough new findings, the study is useful for directing the practical storage of potato. As the authors cited, the degradation of carotenoids has been widely studied in food products. Most of the studies in this manuscript were only the validation of previous research. Also as the authors pointed out, further research is required to understand nature of the intermediate compounds between B-carotene and norisoprenoids formed during storage and the kinetics of their formation and degradation. In addition, some questions in the manuscript should be addressed.

1. What is the purpose of undertaking HPLC analysis? Was it used for detection of B-carotene? How was the HPLC analysis undertaken? What were the control compound, analytical column, mobile phases etc.?

HPLC was used for the detection of B-carotene and other minor carotenoids such as 5,6 epoxy-B-carotene (identified in Bechoff et al. 2009a) and also tested for other degradation compounds such as apocarotenoids (Rodriguez and Rodriguez Food Chemistry 2007-“Formation of apocarotenals and epoxycarotenoids from b-carotene by chemical reactions and by autoxidation in model systems and processed foods”). Because degradation compounds could not be identified using HPLC, another technique, SPME-GC-MS, was tested and degradation compounds were successfully found using this technique.

More details about HPLC conditions are given in Materials and Methods lines 139-150 (standard, column details and mobile phases…).

2. Which analytical method was used for quantification of 5,6-epoxy-B-ionone, B-cyclocitral and DHA, SPME-GC method or SPME-GC-MS method?

The SPME-GC-MS was used for the detection and quantification of the norisoprenoids. Line 153.

Although the chemical structures of the three compounds was close to that of B-ionone, I don't think it is acceptable to calculate the concentrations of the three compounds based on the B-ionone standard curve. Especially when the extraction technique SPME was applied, the difference of signal response caused by same concentration between different compounds may be further amplified. In fact, the authors did not obtain the real concentrations of the volatile compounds in samples. It would be more reasonable if
concentrations of the three compounds were expressed as relative abundance ratio of the target compound to internal compound B-ionone.

We agree with these comments. The ratios (also called relative contents) of peak area at initial time divided by peak area at time t were calculated for each volatile. Because we had the B-ionone standard, the concentrations of this volatile could be calculated as well the ratios (double y axis in Figures 3&4. Explanations are given in Materials and Methods (lines 177-184). Real B-ionone contents are given instead of total volatiles contents given in the previous version that were only estimations (lines 389-391; 416-419; 431-441).

Some minor points:
Line 160: is the capillary column 160 m long?
Line 255-256: "a a"?
Line 258: "in it predictions"?
Line 299: "than that"?
Line 346: "enclosed"?
Line 348: "(3):" and "an with"?
Line 432: "lower that"?

Corrected.

Reviewer #2:
Authors treat the rekatuibusguro between the degradation of two carotenoids and the formation of volatile compounds in chips. Introduction and Experimental parts are good designed, but paper fail in the optimization strategy and in the application parts. For this reason, this reviewer suggests more work before publication in Food Chemistry.

In order to give more explanation about the optimization strategy of the study and give some applications, we added Figure 1 that gives the prediction curves of B-carotene, 5,6-epoxy- B-carotene storage times at different levels of carotenoid loss. A corresponding paragraph has been added in Results and Discussion (lines 260-271). Predictions of carotenoid losses in dried sweet potato chips using the kinetic models can be used for practical applications, such as the determination of product shelf life. This supplementary work is a real added value in agreement with the objective of the paper.

In the Introduction a few words about the application have also been added (line 47). One sentence in conclusion (lines 459-462) about a possible application for B-carotene degradation using SPME-GC-MS has been added.

Minor comments: Some typos can be observed in all the text and must be corrected.
* Please change the title, including the name of the two carotenoids.

The title has been altered. For more precision, the word “carotenoids” has been replaced by “B-carotene” in the title and running head and “volatile compounds” by “norisoprenoids” in the title.
Furthermore “5,6 epoxy-B-carotene” has been removed from the abstract in order to put the focus on B-carotene, the main carotenoid in sweet potato. This gives more clarity on the main outcomes of the work.

* In abstract please introduce more details.

A sentence has been added in the abstract about the model prediction: “The difference between experimental data under laboratory or field conditions fitted and data predicted by the model was less than 10% for trans-β-carotene, or for total carotenoids.”

* Mathematical treatment of page 10 is obvious and can be deleted.

OK. Moved under Table 2.

* The number of figures must be reduced to a maximum of four.

This has been achieved as follows:
- Replaced Fig. 1 B-carotene kinetics by Fig 1. A. B.C Prediction curves
- Figure 2 – no change
- Former figures 3,4,5 – deleted
- Former figure 6 now called Figure 3
- Former figure 7 now called Figure 4
- Table 1 – added Kinetics of degradation under various water activities (B) and at different oxygen levels (C)
- Table 2 – no change
- Table 3 – no change

The other changes in the document are the following:
- Replaced “constant rates” by “rate constants” throughout the text
- Line 74 “at constant temperature of 40 °C”
- Line 87 “standard deviation”
- Line 93 “in accordance with Lavelli et al. (2007)”
- Removed paragraph-line 311. (formerly being lines 202-313 in the submitted manuscript). These explanations about U-shape trend between water activity and rate constants were found un-necessary for the understanding of the work.
- Line 473 “We thank David R. Hall for the revision of the manuscript.”
- Figures 3 and 4 Titles “Degradation of trans-β-carotene and production of identified norisoprenoids during storage of dried sweet potato chips at 40 °C under air at different water activities (β-carotene and β-ionone expressed as µg.g⁻¹ fresh weight; volatile degradation products, as ratio: peak area at time t divided by peak area at initial time); Error bars refer to standard deviation (n=3).”
- Other minor changes are indicated by track changes
Relationship between the Kinetics of β-Carotene Degradation and Formation of Norisoprenoids in the Storage of Dried Sweet Potato Chips

Running head: β-Carotene Degradation & Norisoprenoid Formation in Dried Sweet Potato

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Abstract:
The effects of storage temperature (10; 20; 30; 40 °C), water activity (0.13; 0.30; 0.51; 0.76) and oxygen level (0%; 2.5%; 10%; 21%) on the degradation of carotenoids and formation of volatile compounds during storage of dried sweet potato chips were evaluated. A kinetic model was developed for degradation of trans-β-carotene and it showed that breakdown followed first order kinetics with an activation energy of 64.2 kJ.mol⁻¹. The difference between experimental data under laboratory or field conditions fitted and data predicted by the model was less than 10% for trans-β-carotene, or for total carotenoids. The formation of the volatile compounds, β-ionone; 5,6-epoxy-β-ionone; dihydroactinidiolide; β-cyclocitrinal, was measured by SPME-GC-MS and was clearly related to the degradation of trans-β-carotene. It is also suggested that carotenoid degradation in dried sweet potato was by autoxidation because of the trend in β-carotene degradation rate in relation to water activity or oxygen level.

KEYWORDS: carotenoids; dried sweet potato; Ipomoea batatas L; kinetics; norisoprenoids; oxygen; storage; temperature; volatile compounds; water activity
Introduction

Carotenoids are organic pigments found in plants that play an important role as vitamin A precursors in the human diet. In contrast to most plant foods, about 90% of the carotenoid content of orange-fleshed sweet potato (OFSP) is trans-β-carotene (Bechoff et al., 2009a; Bengsston, Namutebi, Larsson Alminger & Svanberg, 2008). Hence OFSP provides a straightforward “model system” for understanding the degradation of β-carotene. The Ejumula variety, for example, cultivated in Uganda, has been reported to contain up to 325μg·g⁻¹ β-carotene on a dry basis (Bengsston et al., 2008), making it a very good source of provitamin A. Such varieties could contribute to tackling vitamin A deficiency, a main public health issue in the developing world (Bechoff et al., 2009a; Bengsston et al., 2008). Degradation of pro-vitamin A during the storage of dried sweet potato chips at ambient temperature has been demonstrated to be a significant problem (Emenhiser et al., 1999; Bechoff et al. 2009b).

The degradation of carotenoids has been widely studied in food products. The major factors influencing carotenoid oxidation, leading to their degradation, are temperature, light, oxygen and acidity (Gayathri, Platel, Prakash, & Srinivasan, 2004). In addition, water activity is a very important parameter to evaluate the quality of dried foods (Lavelli, Zanoni, & Zaniboni, 2007). Previous research on freeze-dried sweet potato cubes has shown higher levels of β carotene degradation at lower water activities (Haralampu & Karel, 1983). High oxygen concentrations have been associated with high levels of carotenoid degradation in dried sweet potato flakes (Emenhiser et al., 1999; Walter, Purcell & Cobb 1970; Walter & Purcell 1974). Storage temperature (4 °C; 25 °C; 40 °C) has also been showed to influence the stability of carotenoid pigments of freeze-dried sweet potatoes with greater losses at high temperatures (Cinar, 2004). In general degradation of carotenoids in a dried food system, under the influence of one or more factors has been demonstrated to be a first order kinetics reaction (Haralampu &
of aroma compounds from carotenoids has also been widely studied because of the application in the flavour industry. Authors have described volatile products resulting from degradation of pure β-carotene; (Handelman, van Kujk, Chatterjee, & Krinsky; Mordi et al., 1993; Waché, Bosser-Deratulld, Lhuguenot & Belin 2003) or naturally present in, for instance, oak wood (Nonier, Vivas, De Gaulejac, & Vitry, 2004); black tea (Ravichandran, 2002); wine (Mendes-Pinto, 2009) and paprika powder (Cremer & Eicher 2000). The highly unsaturated chain of the β-carotene molecule makes it react easily with any radical species present (Krinsky & Kyung-Jin, 2003); carotenoid fragmentation can be caused by autoxidation (air) (Mordi et al., 1993); heating (Cremer & Eicher 2000) and enzymatic activity (Audridge, McCarty & Klee, 2006). The chain reaction is typical of a free-radical reaction where a product is oxidised into secondary products that are themselves oxidised into other products (Goldman, Horev & Saguy, 1983; Krinsky & Kyung-Jin, 2003; Mordi et al., 1993). In all cases β-carotene submitted to oxidation is degraded into epoxides, apocarotenals and apocarotenones. In the latter stages of oxidation these are themselves oxidised into lighter carbonyl compounds which are volatile; these include norisoprenoids. 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. These asymmetric cleavages were achieved using xanthin oxidase (Waché et al., 2003). However the same cleavages can arise from autoxidation (Mordi et al., 1993). Norisoprenoids from β-carotene degradation from either type of cleavage include β-ionone, 5,6-epoxy-β-ionone, dihydroactinidiolide (DHA) and β-cyclocitral.
The first objective of this study was to measure the degradation of β-carotene in sweet potato chips during storage influenced by temperature taking into account oxygen and water activity and be able to predict by modelling thermal degradation in a temperature range (10-40 ºC) close to what is found in many developing countries. The second objective of the study was to relate the degradation of β-carotene to the formation of volatile compounds at constant temperature of 40 ºC. Degradation of pure 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 β-carotene and formation of volatiles (Mordi et al., 1993). However, 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.
**Materials & Methods**

**Raw materials and storage conditions**

Sweet potato chips from Ejumula variety were harvested in Luwero, Uganda in March 2008 after a growing season of 5-6 months. Roots were chipped and dried using open air sun dryer at the National Agricultural Research Laboratories (NARL), Kawanda, Uganda with an average drying temperature±standard deviation of 28.8±2.8 ºC and 53.3±13.6% relative humidity. Dried chips were stored at -20 ºC, where the carotenoid content did not vary significantly during storage in freeze samples over a six month period. A storage study was undertaken at Natural Resources Institute, UK. In order to control relative humidity during storage, dried sweet potato chips (90g) in a sewed cotton bag were placed into a 1.5L-clip top jar containing a salt (150g) saturated with deionised water at 40 ºC to give different water activities in accordance with Lavelli et al. (2007): $a_w = 0.126$ for LiCl, 0.304 for MgCl₂, 0.507 for NaBr and 0.765 for NaCl. Equilibrium between chips and air was achieved after 9 days of storage. Jars in triplicate were placed in incubators (LMS Cooled Incubator, Sevenoak, UK) set at 10±0.5 ºC; 20±1 ºC; 30±0.5 ºC and 40±1 ºC. Such temperatures are similar to those encountered in tropical countries where sweet potato is grown. 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 (Bechoff et al. 2009a). Samples were stored at 10 ºC and 20 ºC for 105 days; at 30 ºC for 95 days and 40 ºC for 54 days. Samples at different water activities were stored at 40 ºC for 54 days. Samples stored at 40 ºC for 21 days at different oxygen levels were flushed with a continuous flow of oxygen:nitrogen mix (containing 0%; 2.5%; 10% or 21% oxygen).

The Brunauer-Emmett-Teller (BET) equation (Bimbenet, Duquenoy & TRYstram, 2002) that uses the theory for molecular multilayer adsorption was applied to predict data for
water activity ($a_w$) in relation with moisture content in dry basis ($M$). The BET equation was used on the experimental points to calculate water activity from the moisture content. The linearised equation [1] is expressed as follows:

$$\frac{a_w}{(1-a_w)M} = \frac{1}{M_0C} + \frac{(C-1)a_w}{M_0C} \quad \text{[Eq. 1]}$$

$C$ 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.

At least four samples (about 15g of chips per jar) were collected during storage using riffle divider. Samples were milled using a laboratory mill (Model 3600 Perten Instruments, Segeltorp, Sweden).

### Carotenoid analyses

The samples were extracted using a slightly modified HarvestPlus method (Rodriguez-Amaya & Kimura, 2004). A portion of the homogeneous representative sample (0.5-2.0 g of flour) was homogenised with 50mL methanol:tetrahydrofuran (THF) (1:1) using an Ultra-turax homogeniser (IKA Janke and Kunkel Labortechnik, Staufen, Germany) at 8000 rpm/min for one min. Before homogenising the flour, it was re-hydrated for 20 min in 10 ml deionised water. The homogenised extract was filtered through a porosity 2-sintered glass funnel by vacuum and rinsed with methanol:THF (1:1) until there was no yellow colour left in the filtrate. The extracts were combined and poured into a 500 ml separating funnel filled with 40 ml of petroleum ether (PE). After washing once with 50ml 10% NaCl and thrice with 200 ml deionised water, the upper-PE phase containing the carotenoid extract was collected in a 100 ml flask. The PE phase was dried by addition of anhydrous sodium sulphate until some crystals remained loose. This was then filtered into a 50 ml volumetric flask through glass wool and made up to volume with PE. Absorbance at 450nm was read on a diode array Hewlett Packard 8452A
spectrophotometer for determination of total carotenoid content. The carotenoid extracts in PE were dried by flushing with nitrogen in a dry block system at 35°C. The dried extracts were transported to CIRAD, Montpellier, France in an insulated bag containing a freezing gel and stored in the freezer immediately at arrival. The extracts were dissolved in 1ml dichloromethane:MTBE (methyl tert-butyl ether):methanol 50:40:10. Reverse-phase high performance liquid chromatography using an Agilent 1100 system (Massy, France) was used following the method of Dhuique-Mayer et al. (2007). The mobile phases were H2O as eluent A, methanol as eluent B, and MTBE as eluent C. A solvent gradient was programmed in order to enhance compound separation: 0min: 40%A/60%B; 0-5min: 20%A/80%B; 5-10min: 4%A/81%B/15%C; 10-60min: 4%A/11%B/85%C; 60-71min: 100%B; 71-72 min back to the initial condition for re-equilibration. Carotenoids were separated through a C30 reverse phase column (250 x 4.6 mm i.d.) packed with 5μm YMC (EUROP GmbH, Germany) with a flow rate of 1ml.min⁻¹, a column temperature at 25°C and an injection volume of 20 μl. Absorbance was measured with Agilent Chemstation Plus software at 450 nm (Diode array 290-470nm). Concentrations were determined by comparison to a standard curve using pure β-carotene ( Extrasynthese, Genay, France) (Bechhof et al., 2009a).

Norisoprenoid analysis

SPME–GC-MS (solid phase microextraction coupled to gas chromatographic-mass spectra) was used to analyse semi-quantitatively volatile compounds generated during the storage of sweet potato flour. The SPME fibres used were 1 cm long of DVB/Car/PDMS (divinylbenzene/carboxen/polydimethylsiloxane) from Supelco (Bellefonte, PA). 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. Sweet potato flour samples (3.00g) were weighed into a 10ml glass vial.
and were capped with an air-tight 20mm PTFE/silicon septum (Interchim, France). The sample was heated at 50 °C for 15 min to liberate volatile compounds from the flour matrix. All analyses were carried out on Agilent 6980 Gas Chromatographic System (Agilent Technologies, Palo Alto, USA) equipped with an autosampler Combi PAL (CTC Analytics, Zwingen, Switzerland) coupled with an Agilent 5973N mass spectrometer. Chromatographic separation was achieved with a DB-Wax (J&W Scientific, Folsom, CA) fused silica capillary column (60m x 0.32mm i.d.; film thickness= 0.25 μm). Operating conditions 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. Helium was used as carrier gas in constant flow mode 1.5 mL.min\(^{-1}\). 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. 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-cyclocitrinal and DHA, identified based on their mass spectra, the ratios (peak area at time t divided by peak area at initial time), also called relative contents, were calculated.
Statistical analysis and kinetics modelling

Carotenoid contents and norisoprenoid contents were determined on a fresh weight basis. Data were processed on SPSS 15.00 software by one or two way-ANOVA (Analysis of variance; p<0.05) using HSD Tukey test to determine which samples were significantly different from others. The kinetics of carotenoid degradation were modelled using Arrhenius and Eyring models (Cisse, Vaillant, Acosta, Dhuique-Mayer & Dornier 2009). The Arrhenius model is an empirical collision model that describes the relationship between reaction rate constants and temperature using activation energy (E_a) and a pre-exponential factor (k_0). The Eyring model 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.

For the validation of the Arrhenius model at room temperature, the predicted data were calculated using the equation [2]:

\[ C = C_0 e^{-k_0 \int_0^t e^{-\frac{E_a}{RT}} dt} \]  

[Eq. 2]

C is the carotenoid concentration at t = 88 or 125 days of storage and C_0 is the initial concentration. In order to validate the Arrhenius carotenoid degradation model in laboratory conditions, dried samples (Ejumula variety) were stored for 88 days at ambient room temperature (anisothermal or dynamic) conditions in the dark. Temperature and humidity were recorded every h (mean: 21.4 °C/46.8%; min: 13.8 °C/39.3%; max: 25.2 °C/47.6% respectively). The Arrhenius model was also tested with dried samples (Ejumula variety) stored for 125 days at ambient room temperature in Uganda (Bechoff et al., 2009b).
Results & Discussion

Carotenoid degradation kinetics

The effect of temperature (10; 20; 30; 40 ºC) on β-carotene degradation is described in Table 1A. Coefficients of correlation (R²) suggest that a first-order equation fitted well the carotenoid degradation. An exception nonetheless was at 10 ºC where correlations observed were lower (R²~0.8). Lower correlations at low temperatures where there is minimum carotenoid degradation could be explained by experimental errors (Hidalgo & Brandolini, 2008). Degradation rates of trans-β-carotene were significantly different at the four tested temperatures (10; 20; 30; 40 ºC). (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 40 ºC. On the other hand only 35% was lost after 62 days at a lower temperature of 10 ºC. 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 2). For both of the models provided, description of trans-β-carotene fitted degradation (R² > 0.990 and 0.989 respectively). 5,6 epoxy-β-carotene, another carotenoid present in fresh sweet potato (Kosambo, Carey, Misra, Wilkes, & Hagenimana 1998), also followed a first order rate reaction that could also be fitted to the same models (R² > 0.997 and 0.996 respectively). Using the models, the activation energy (Ea) for β-carotene and for 5,6-epoxy-β-carotene was calculated as 64.2 and 78.8 kJ.mol⁻¹ respectively and the enthalpy was 61.7 and 76.3 kJ.mol⁻¹ respectively (Table 2). The energy of activation and enthalpy were both 23% 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 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) being 46.3 kJ.mol\(^{-1}\) was similar to the value of 44.3 kJ.mol\(^{-1}\) for freeze-dried sweet potato at 60-80 °C (Stephanovitch and Karel, 1982). Similar activation energies of 45.3 and 48.7 kJ.mol\(^{-1}\) were measured for total carotenoids on Serio and Monlins wholemeal wheat flour respectively, stored between -20 °C and 38 °C (Hidalgo & Brandolini, 2008).

To test the robustness of the model, it was used to predict the carotenoid content of dried sweet potato sample 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 \(\beta\)-carotene under isothermal conditions, the difference between the experimental value and value predicted by the model was 4.3% and 3.5% respectively (Table 3). 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 (Bechoff et al., 2009b). Similarly, the model was also accurate in its predictions where for total carotenoids under isothermal conditions with a difference between the experimental value and model of 9.3% (Table 3). Therefore it can be concluded that the model developed with samples stored under 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 Fig. 1. These can be used for practical applications, such as the determination of product shelf life. For instance, for a 20% loss in \(\beta\)-carotene
Fig. 1A), the predicted storage duration in tropical conditions (average 30 °C) is about 10 days. On the other hand if the dried sweet potato is stored at ambient temperature (average 20 °C) the predicted storage time is one month. If it is stored in fridge conditions (average 10 °C) the predicted storage time increases up to 70 days. Predicted storage time based on total carotenoids (Fig. 1B) is slightly shorter and when based on 5,6 epoxy-β-carotene, longer (Fig. 1C) 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 °C, the storage duration shall be 160, 200 and 110 days for a 40% loss in β-carotene, 5,6-epoxy-β-carotene or total carotenoids respectively.

**Influence of water activity and oxygen on carotenoid degradation**

The water sorption properties of dried sweet potato chips stored at 40 °C in different saturation salt solutions are described in Fig. 2. The experimental data was 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 usual storage conditions. The sweet potato variety Ejumula stored in Uganda under ambient conditions for four months had a maximum moisture content of 13.6% on a dry weight basis (12% on a wet weight basis) (Bechoff et al., 2009b).

Under different water activities, trans-β-carotene degradation fitted a first order kinetic model with $R^2$ ranging between 0.949 and 0.984 as shown in Table 1B. Under isothermal conditions (40 °C) and in samples stored under air, the lower the water activity the faster the β-carotene degradation. 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 and standard deviations of 0.0493 (0.0029), 0.0460 (0.0032) and 0.0413 (0.0005) \( \text{day}^{-1} \) respectively. On the other hand the degradation rate constant at \( a_w = 0.76 \) was 0.0341 (0.0010) \( \text{day}^{-1} \), 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 \( \beta \)-carotene, it would not be recommended because of the high probability of microbial spoilage. Overall, these results have showed that in the storage of dried sweet potato chips, water activity \( (a_w = 0.13-0.76) \) had a significant impact on carotenoid degradation with rate constants between 0.0341 and 0.0493 \( \text{day}^{-1} \) respectively. However the effect of water activity in \( \beta \)-carotene breakdown was a lot less than the effect of temperature (10-40ºC) with rate constants of 0.0029 to 0.0405 \( \text{day}^{-1} \). Therefore at typical product moisture content of 7-14% on a dry basis (Bechoff et al., 2009b), which corresponds to water activities of 0.2-0.6 at 40 ºC, water activity would have a limited impact compared to temperature.

There was a linear relationship between the \( \beta \)-carotene degradation rate and water activity for the four levels analysed in triplicate \( (R^2 = 0.953) \). (Table 1B). Working with a model system made of microcrystalline cellulose containing 0.5% of \( \beta \)-carotene, Goldman et al. (1983) and Chou & Breene (1972) proved that \( \beta \)-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 that the higher the water activity the lower the \( \beta \)-carotene degradation (Goldman et al., 1983). It has been confirmed in an earlier study on dehydrated sweet potato cubes (Haralampu & Karel, 1983) that lower degradation rates occurred at higher water activities in sweet potato (and \textit{vice versa}). Peroxidase is the main type of enzyme encountered in sweet potatoes (Castillo Leon et al., 2002). At low water activities it has
been demonstrated that peroxidase activity dramatically decreased (Kamiya & Nagamune, 2002). Dioxygenases known for their ability to degrade carotenoids into aroma compounds (Auldridge et al., 2006) also lose activity in non-aqueous environments (Sanakis, Mamma, Christakopoulos, & Stamatis, 2003). For these different reasons, in this study, the possibility of carotenoid degradation due to enzymatic activity seemed unlikely.

Under different levels of oxygen, carotenoid degradation fitted first order with $R^2$ values of 0.944; 0.968 and 0.961 at various levels of oxygen (Table 1C). An exception was under nitrogen where correlation ($R^2$) observed was 0.675. Explication for this poor correlation was a minimum β-carotene breakdown under these conditions as of low temperature (Hidalgo & Brandolini 2008). On samples stored at 40 °C, the degradation rate of β-carotene was highly related to the oxygen level flushed through the sample. Degradation rate constants significantly differed between samples (One way-ANOVA $p<0.05$). Samples flushed with nitrogen had a rate constant of 0.0107 (0.0021) day$^{-1}$, whilst those flushed with 2.5%, 10% and 21% oxygen had respective rate constants of 0.0303 (0.0053), 0.0514 (0.0022) and 0.0853 (0.0038) day$^{-1}$.

It was interesting to observe that flushing with air at 40° C dramatically increased the degradation rate (0.0853 day$^{-1}$) compared to samples stored under air but at the same temperature (0.0341 to 0.0493 day$^{-1}$) (Table 1C). 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, Karel, Saguy, & Mizrahi, 1981) that showed that oxygen uptake in a microcrystalline cellulose food model was closely linked to β-carotene degradation and agreed with other study on sweet potato flakes.
showing a direct relationship between oxygen uptake and carotenoid degradation (Walter & 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 (Table 1C). 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).

Over the range studied (0-21% oxygen) oxygen had a more marked effect on β-carotene than water activity (0.13-0.76) and temperature (10-40ºC). The mean difference between rate constants was 0.0746 day$^{-1}$, 0.0152 day$^{-1}$ and 0.0376 day$^{-1}$ respectively. Studies on other foodstuffs similarly concluded that oxygen was a major factor of degradation during storage. Working on the effect of packaging (polypropylene: high oxygen permeability; nylon laminate film (low oxygen permeability) with air space; under vacuum or using a Ageless oxygen absorber sachet) on sweet potato flakes it was demonstrated that oxygen had a major impact on carotenoid degradation (3): sweet potato flakes stored for 210 days in nylon film and with oxygen absorber did not lose significant amounts of β-carotene whilst those stored in polypropylene lost 66% of their initial content at ambient temperature (about 23 ºC). In this present study, the β-carotene loss on sweet potato chips stored under nitrogen (16% loss after 21 days) (Fig. 6) might result of incomplete oxygen exclusion of samples. Alternatively it could also result of the effect of the relatively high temperature used for the storage (40 ºC).

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 and dried media agreed with studies on microcrystalline food model food systems (Chou & Breene, 1972; Goldman et al., 1983; Texeira Neto et al., 1981) where enzymatic activity is excluded and with previous studies on dehydrated sweet potato (Haralampu & Karel, 1983; Walter et al., 1970; 1974). Though autoxidation was mentioned in earlier studies as the cause of β-carotene degradation in dried sweet potato (Haralampu & Karel, 1983; Walter et al., 1970; 1974), a more recent study (Auldridge et al., 2006) emphasized that it had not been proved. This study therefore fills the gap by showing that dehydrated sweet potato and the microcrystalline cellulose food model behaved the same way toward water activity and oxygen which strongly suggests that autoxidation was the mechanism responsible for β-carotene degradation.

**Description of carotenoid degradation in relation with norisoprenoid formation**

Whereas trans-β-carotene degraded during storage, norisoprenoids namely β-cyclocitral, β-ionone, 5,6-epoxy-β-ionone, and dihydroactinidiolide (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). The greatest formation of norisoprenoids occurred at lower water activities (Fig. 3) and this is consistent with the earlier findings that carotenoid degradation is also greater at lower water activities (Table 1B). On average, samples stored at $a_w = 0.13$ had a β-ionone content of 0.47μg.g$^{-1}$, followed by those stored at $a_w = 0.30$ with 0.38μg.g$^{-1}$; at $a_w = 0.51$ had a β-ionone content of 0.30μg.g$^{-1}$ and at $a_w = 0.76$ of 0.31μg.g$^{-1}$ (Fig. 3). 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). No difference in β-cyclocitral ratio was found between samples...
stored at \( a_w = 0.13 \) and 0.30; and at 0.51 and 0.76. On the other hand, there was a significant difference in the DHA and 5,6-epoxy-\( \beta \)-ionone ratios for the four water activities (two way-ANOVA; \( p<0.05 \)). This description of differences between volatile relative concentrations agreed with the above description of \( \beta \)-carotene degradation at various water activities.

Relative contents of \( \beta \)-cyclocitral, \( \beta \)-ionone and 5,6-epoxy-\( \beta \)-ionone were the highest at 19 days and subsequently decreased whilst DHA levels reached a plateau after 27 days of storage at 40 °C (Fig. 3). Epoxidation of \( \beta \)-ionone into 5,6-epoxy-\( \beta \)-ionone and thermal rearrangement of 5,6-epoxy-\( \beta \)-ionone into DHA has been described by several authors (Bosser, Paplorey & Belin, 1995; Mordi et al., 1993; Waché et al., 2003). The similar profile of \( \beta \)-ionone and 5,6-epoxy- \( \beta \)-ionone throughout storage suggested that the two compounds were formed at similar times via the same process whereas the later formation of DHA suggested that the rearrangement into DHA was a slower step.

Fragmentation of \( \beta \)-carotene in dehydrated sweet potato flakes under oxygen has been reported previously (Walter et al., 1970). Flushing radioactive sweet potato flakes with oxygen in the dark at 22 °C 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 \( \beta \)-carotene was also highest. At 40 °C samples stored flushed with compressed air (21% oxygen) had a \( \beta \)-ionone content of 0.44\( \mu g.g^{-1} \), followed by those flushed with 10% (0.36\( \mu g.g^{-1} \)); 2.5% (0.22\( \mu g.g^{-1} \)) oxygen or nitrogen (0.10\( \mu g.g^{-1} \)), respectively (Fig. 4). Similarly there was a significant increase in the \( \beta \)-
cyclocitral, 5,6-epoxy-β-ionone and DHA ratios at the four increasing oxygen levels (two ways-ANOVA; p<0.05).

With no oxygen present in the storage conditions, only a small decrease or no difference in volatile compounds contents was observed. These highlighted how oxygen is important in the degradation scheme of β-carotene into volatile compounds (Fig. 4).

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 (Fig. 6). This is a similar pattern to that observed at different water activities.

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 3&4). 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 μg.g⁻¹, will are much lower than the amounts of carotenoids degraded (Figures 3&4). Waché et al. (2003) 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 clear 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.

**Conclusion**

The Arrhenius and Eyring models correctly described the carotenoid degradation in dried stored sweet potato between 10 and 40 °C; the Arrhenius model was validated using a sample stored at room temperature (non-isotherm conditions). The greater β-carotene degradation rate at lower water activity in particular suggested that the reaction was an autoxidation. 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 relative concentrations of volatiles were recorded. A similar observation was made at lower oxygen levels. One of the applications of these findings could be the development of a rapid and non-destructive method using SPME-GC-MS to measure threshold production of norisoprenoids that would correspond to a critical level of β-carotene breakdown in dried food products and could help in predicting product shelf life. 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. Moreover 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.

**Acknowledgment**
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References


Fig. 1. Prediction curves of carotenoid (β-carotene; 5,6-epoxy-β-carotene; total-carotenoids) loss (%) with temperature and with storage time in dried sweet potato chips stored between 10-40 °C in air; aw 0.52-0.65.
Fig. 2. Water sorption curves of dried sweet potato chips stored at 40 °C for 54 days under air. Experimental data and BET model ($C = -68.88$, $M_0 = 0.0564$).
Fig. 3. Degradation of trans-β-carotene and production of identified norisoprenoids during storage of dried sweet potato chips at 40 °C under air at different water activities (β-carotene and β-ionone expressed as μg.g⁻¹ fresh weight; volatile degradation products, as ratio: peak area at time t divided by peak area at initial time); Error bars refer to standard deviation (n=3).
Fig. 4. Degradation of trans-β-carotene and production of identified norisoprenoids during storage of dried sweet potato chips at 40 °C at different oxygen levels (β-carotene and β-ionone expressed as μg.g⁻¹ fresh weight; volatile degradation products as ratios: peak area at time t divided by peak area at initial time); Error bars refer to standard deviation (n=3).
Table 1. Rate of degradation of carotenoids (k) expressed in day$^{-1}$ in dried sweet potato chips on a fresh weight basis at various temperatures in air; $a_w$ 0.52-0.65 (A); at various water activities at 40ºC in air (B); at various oxygen (flushed) levels at 40ºC (C). Mean of triplicate thermal treatment (standard deviation).

(A)

<table>
<thead>
<tr>
<th>Temperature (ºC)</th>
<th>10</th>
<th>20</th>
<th>30</th>
<th>40</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>β-carotene</strong></td>
<td>k</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.0029 (0.0002)</td>
<td>0.0093 (0.0005)</td>
<td>0.0193 (0.0002)</td>
<td>0.0405 (0.0005)</td>
</tr>
<tr>
<td><strong>5,6 epoxy-β-carotene</strong></td>
<td>k</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.0025 (0.0004)</td>
<td>0.0084 (0.0003)</td>
<td>0.0248 (0.0009)</td>
<td>0.0597 (0.0010)</td>
</tr>
<tr>
<td><strong>Total carotenoids</strong></td>
<td>k</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.0047 (0.0002)</td>
<td>0.0088 (0.0003)</td>
<td>0.0179 (0.0005)</td>
<td>0.0298 (0.0009)</td>
</tr>
</tbody>
</table>

| **R$^2$** | 0.829 (0.071) | 0.922 (0.031) | 0.985 (0.005) | 0.963 (0.007) |
|           | 0.748 (0.167) | 0.902 (0.045) | 0.869 (0.020) | 0.987 (0.009) |
|           | 0.864 (0.091) | 0.982 (0.014) | 0.987 (0.005) | 0.986 (0.006) |

(B)

<table>
<thead>
<tr>
<th>Water activity</th>
<th>0.76*</th>
<th>0.51*</th>
<th>0.30*</th>
<th>0.13*</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>β-carotene</strong></td>
<td>k</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.0341 (0.0010)</td>
<td>0.0413 (0.0005)</td>
<td>0.0460 (0.0032)</td>
<td>0.0493 (0.0029)</td>
</tr>
<tr>
<td><strong>R$^2$</strong></td>
<td>0.984 (0.003)</td>
<td>0.977 (0.001)</td>
<td>0.974 (0.003)</td>
<td>0.949 (0.048)</td>
</tr>
</tbody>
</table>

*Coefficient of correlation between four k levels related to water activity: 0.953 (0.013)

(C)

<table>
<thead>
<tr>
<th>Flushed** oxygen level</th>
<th>0%*</th>
<th>2.5%*</th>
<th>10%*</th>
<th>21%*</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>β-carotene</strong></td>
<td>k</td>
<td>0.0107 (0.0021)</td>
<td>0.0303 (0.0053)</td>
<td>0.0514 (0.0022)</td>
</tr>
<tr>
<td><strong>R$^2$</strong></td>
<td>0.675 (0.290)</td>
<td>0.944 (0.020)</td>
<td>0.968 (0.006)</td>
<td>0.961 (0.013)</td>
</tr>
</tbody>
</table>

*Coefficient of correlation between four k levels related to water activity: 0.975 (0.016)

**90ml.min$^{-1}$
Table 2. Parameters of the Arrhenius and Eyring models for the carotenoids degradation in dried sweet potato chips on a fresh weight basis between 10-40 °C. Oxygen level 21% (air); a_w 0.52-0.65. Mean of triplicate thermal treatment (standard deviation).

<table>
<thead>
<tr>
<th>Carotenoid</th>
<th>Ln k_∞ (k_∞ days⁻¹)</th>
<th>Arrhenius model</th>
<th>Eyring model</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>R²</td>
<td>ΔH* (kJ.mol⁻¹)</td>
</tr>
<tr>
<td>β-carotene</td>
<td>21.5 (0.6)</td>
<td>64.2 (1.6)</td>
<td>0.990 (0.007)</td>
</tr>
<tr>
<td>5,6 epoxy-β-carotene</td>
<td>27.5 (1.5)</td>
<td>78.8 (3.8)</td>
<td>0.997 (0.003)</td>
</tr>
<tr>
<td>Total carotenoids</td>
<td>14.3 (0.6)</td>
<td>46.3 (1.5)</td>
<td>0.997 (0.002)</td>
</tr>
</tbody>
</table>

Arrhenius model

\[ k = k_∞ \cdot e^{-\frac{E_a}{R \cdot T}} \]

Where: T: temperature (K); k: degradation rate constant at T (day⁻¹); k_∞: value of k at T = ∞ (day⁻¹); E_a: Activation energy (kJ.mol⁻¹); R: gas constant = 8.314 J · K⁻¹ · mol⁻¹

Eyring model

\[ k = \frac{k_B \cdot T \cdot e^{-\frac{\Delta H^* - T \cdot \Delta S^*}{R \cdot T}}}{h} \]

Where: k_B: Boltzmann constant = 1.381·10⁻²³ J.K⁻¹; h: Planck constant = 6.626·10⁻³⁴ J.s; ΔH*: activation enthalpy (kJ.mol⁻¹); ΔS*: activation entropy (J.mol⁻¹.K⁻¹)
Table 3. Validation of Arrhenius model for a sample of dried Ejumula sweet potato chips stored under ambient anisotherm conditions during 88 days in the UK\textsuperscript{a} and during 125 days in Uganda\textsuperscript{b} on a fresh weight basis. Oxygen level 21\% (air).

<table>
<thead>
<tr>
<th>Sample stored in</th>
<th>Storage time (days)</th>
<th>Initial ((\mu g.g^{-1})^c)</th>
<th>Final ((\mu g.g^{-1})^c)</th>
<th>Predicted by Arrhenius model ((\mu g.g^{-1}))</th>
<th>Difference (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>UK\textsuperscript{a}</td>
<td>Trans-β-carotene</td>
<td>88</td>
<td>181.2 (5.9)</td>
<td>74.6 (5.1)</td>
<td>72.0</td>
</tr>
<tr>
<td></td>
<td>Total carotenoids</td>
<td></td>
<td>250.3 (1.1)</td>
<td>94.4 (1.1)</td>
<td>90.3</td>
</tr>
<tr>
<td>Uganda\textsuperscript{b}</td>
<td>Total carotenoids</td>
<td>125</td>
<td>219.6 (1.6)</td>
<td>51.4 (1.5)</td>
<td>46.6</td>
</tr>
</tbody>
</table>

\textsuperscript{a}This present study (Calculated \(a_w\) from BET model: 0.460 (0.012) from chips dry matter (90.4 (0.3) g/100g)

\textsuperscript{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])

\textsuperscript{c}Mean of triplicate (standard deviation).
Relationship between the Kinetics of \( \beta \)-Carotenoid Carotene Degradation and Formation of Volatile Norisoprenoids Compounds Formation in the Storage of Dried Sweet Potato Chips

Running head: \( \beta \)-Carotenoid Degradation & Norisoprenoid Formation in Dried Sweet Potato

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Abstract:
The effects of storage temperature (10; 20; 30; 40 °C), water activity (0.13; 0.30; 0.51; 0.76) and oxygen level (0%; 2.5%; 10%; 21%) on the degradation kinetics of carotenoids and formation of volatile compounds during storage in of dried sweet potato chips were evaluated. Thermal degradation of trans-β-carotene and formation of volatile compounds during storage are described. A kinetic model was developed for degradation of the main carotenoids, trans-β-carotene and 5,6 epoxy-β-carotene, and it showed that breakdown followed first order kinetics with an activation energy of 64.2 and 78.8 kJ mol$^{-1}$ respectively. The difference between experimental data under ambient laboratory or field conditions fitted well with data predicted by the model. The formation of the volatile compounds, β-ionone; 5,6-epoxy-β-ionone; dihydroactinidiolide; β-cyclocitrals, was measured by SPME-GC-MS and was clearly related to the degradation of trans-β-carotene. It is also suggested that carotenoid degradation in dried sweet potato was by autoxidation because of the trend in β-carotene degradation rate in relation to water activity or oxygen level.

KEYWORDS: carotenoids; dried sweet potato; Ipomoea batatas L; kinetics; norisoprenoids; oxygen; storage; temperature; volatile compounds; water activity
Introduction

Carotenoids are organic pigments found in plants that play an important role as vitamin A precursors in the human diet. In contrast to most plant foods, about 80-90% of the carotenoid content of orange-fleshed sweet potato (OFSP) is trans-β-carotene (Bechoff et al., 2009a; Bengston, Namutebi, Larsson Alminger & Svanberg, 2008). Hence OFSP provides a straightforward “model system” for understanding the degradation of β-carotene. The Ejumula variety, for example, cultivated in Uganda, has been reported to contain up to 325μg.g⁻¹ β-carotene on a dry basis (Bengston et al., 2008), making it a very good source of provitamin A. Such varieties could contribute to tackling vitamin A deficiency, a main public health issue in the developing world (Bechoff et al., 2009a; Bengston et al., 2008).

Degradation of pro-vitamin A during the storage of dried sweet potato chips at ambient temperature has been demonstrated to be a significant problem (Emenhiser et al., 1999; Bechoff et al. 2009b). The degradation of carotenoids has been widely studied in food products. The major factors influencing carotenoid oxidation, leading to their degradation, are temperature, light, oxygen and acidity (Gayathri, Platel, Prakash, & Srinivasan, 2004). In addition, water activity is a very important parameter to evaluate the quality of dried foods (Lavelli, Zanoni, & Zaniboni, 2007). Previous research on freeze-dried sweet potato cubes has shown higher levels of β carotene degradation at lower water activities (Haralampu & Karel, 1983). High oxygen concentrations have been associated with high levels of carotenoid degradation in dried sweet potato flakes (Emenhiser et al., 1999; Walter, Purcell & Cobb 1970; Walter & Purcell 1974). Storage temperature (4 °C; 25 °C; 40 °C) has also been showed to influence the stability of carotenoid pigments of freeze-dried sweet potatoes with greater losses at high temperatures (Cinar, 2004). In general-dried sweet potatoes, under the influence of one or more factors has been demonstrated to be a first order kinetics reaction (Haralampu &
The production of aroma compounds from carotenoids has also been widely studied because of the application in the flavour industry. Authors have described volatile products resulting from degradation of pure β-carotene; (Handelman, van Kuijk, Chatterjee, & Krinsky; Mordi et al., 1993; Waché, Bossert-Deratuld, Lhuguenot & Belin 2003) or naturally present in, for instance, oak wood (Nonier, Vivas, De Gaulejac, & Vitry, 2004); black tea (Ravichandran, 2002); wine (Mendes-Pinto, 2009) and paprika powder (Cremer & Eicher 2000). The highly unsaturated chain of the β-carotene molecule makes it react easily with any radical species present (Krinsky & Kyung-Jin, 2003); carotenoid fragmentation can be caused by autoxidation (air) (Mordi et al., 1993); heating (Cremer & Eicher 2000) and enzymatic activity (Audridge, McCarty & Klee, 2006). The chain reaction is typical of a free-radical reaction where a product is oxidised into secondary products that are themselves oxidised into other products (Goldman, Horev & Saguy, 1983; Krinsky & Kyung-Jin, 2003; Mordi et al., 1993). In all cases β-carotene submitted to oxidation is degraded into epoxides, apocarotenals and apocarotenones. In the latter stages of oxidation these are themselves oxidised into lighter carbonyl compounds which are volatile; these include norisoprenoids. 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. These asymmetric cleavages were achieved using xanthin oxidase (Waché et al., 2003). However the same cleavages can arise from autoxidation (Mordi et al., 1993). Norisoprenoids from β-carotene degradation from either type of cleavage include β-ionone, 5,6-epoxide-epoxy-β-ionone, dihydroactinidiolide (DHA) and β-cyclocitral (Mordi et al., 1993).
The first objective of this study was to measure the degradation of β-carotene in sweet potato chips during storage influenced by temperature taking into account oxygen and water activity and be able to predict by modelling thermal degradation in a temperature range (10-40 ºC) close to what is found in many developing countries. The second objective of the study was to relate the degradation of β-carotene to the formation of volatile compounds at constant temperature of 40 ºC. Degradation of pure 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 β-carotene and formation of volatiles (Mordi et al., 1993). However, 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.
Materials & Methods

Raw materials and storage conditions

Sweet potato chips from Ejumula variety were harvested in Luwero, Uganda in March 2008 after a growing season of 5-6 months. Roots were chipped and dried using open air sun dryer at the National Agricultural Research Laboratories (NARL), Kawanda, Uganda with an average drying temperature ± standard deviation of 28.8±2.8 °C and 53.3±13.6% relative humidity. Dried chips were stored at -20 °C, where the carotenoid content did not vary significantly during storage in freeze samples over a six month period. A storage study was undertaken at Natural Resources Institute, UK. In order to control relative humidity during storage, dried sweet potato chips (90g) in a sewed cotton bag were placed into a 1.5L-clip top jar containing a salt (150g) saturated with deionised water at 40 °C to give different water activities in accordance with Lavelli et al. (2007): aw = 0.126 for LiCl, 0.304 for MgCl2, 0.507 for NaBr and 0.765 for NaCl. Equilibrium between chips and air was achieved after 9 days of storage. Jars in triplicate were placed in incubators (LMS Cooled Incubator, Sevenoak, UK) set at 10±0.5 °C; 20±1 °C; 30±0.5 °C and 40±1 °C. Such temperatures are similar to those encountered in tropical countries where sweet potato is grown. Samples tested at different temperatures were equilibrated with NaBr (aw = 0.579±0.060) because it was the closest to the water activity of dried sweet potato chips stored under ambient conditions (Bechoff et al. 2009a). Samples were stored at 10 °C and 20 °C for 105 days; at 30 °C for 95 days and 40 °C for 54 days. Samples at different water activities were stored at 40 °C for 54 days. Samples stored at 40 °C for 21 days at different oxygen levels were flushed with a continuous flow of oxygen:nitrogen mix (containing 0%; 2.5%; 10% or 21% oxygen). The Brunauer-Emmett-Teller (BET) equation (Bimbenet, Duquenoy & Trystram, 2002) that uses the theory for molecular multilayer adsorption was applied to predict data for
water activity \((a_w)\) in relation with moisture content in dry basis \((M)\). The BET equation was used on the experimental points to calculate water activity from the moisture content. The linearised equation \([1]\) is expressed as follows:

\[ \frac{a_w}{(1-a_w)M} = \frac{1}{M_0C} + \frac{(C-1)}{M_0C}a_w \quad [\text{Eq. 1}] \]

\(C\) 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.

At least four samples (about 15g of chips per jar) were collected during storage using riffle divider. Samples were milled using a laboratory mill (Model 3600 Perten Instruments, Segeltorp, Sweden).

Carotenoid analyses

The samples were extracted using a slightly modified HarvestPlus method (Rodriguez-Amaya & Kimura, 2004). A portion of the homogeneous representative sample (0.5-2.0 g of flour) was homogenised with 50mL methanol:tetrahydrofuran (THF) (1:1) using an Ultra-turax homogeniser (IKA Janke and Kunkel Labortechnik, Staufen, Germany) at 8000 rpm/min for one min. Before homogenising the flour, it was re-hydrated for 20 min in 10 ml deionised water. The homogenised extract was filtered through a porosity 2-sintered glass funnel by vacuum and rinsed with methanol:THF (1:1) until there was no yellow colour left in the filtrate. The extracts were combined and poured into a 500 ml separating funnel filled with 40 ml of petroleum ether (PE). After washing once with 50ml 10% NaCl and thrice with 200 ml deionised water, the upper-PE phase containing the carotenoid extract was collected in a 100 ml flask. The PE phase was dried by addition of anhydrous sodium sulphate until some crystals remained loose. This was then filtered into a 50 ml volumetric flask through glass wool and made up to volume with PE. Absorbance at 450nm was read on a diode array Hewlett Packard.
8452A spectrophotometer for determination of total carotenoid content. The carotenoid extracts in PE were dried by flushing with nitrogen in a dry block system at 35ºC. The dried extracts were transported to CIRAD, Montpellier, France in an insulated bag containing a freezing gel and stored in the freezer immediately at arrival. The extracts were dissolved in 1ml dichloromethane:MTBE (methyl_–tertiary–butyl_–ether):methanol 50:40:10 and injected in an HPLC system. Reverse-phase high performance liquid chromatography using an Agilent 1100 system (Massy, France) was used following the method of Dhuique-Mayer et al. (2007). The mobile phases were H2O as eluent A, methanol as eluent B, and MTBE as eluent C. A solvent gradient was programmed in order to enhance compound separation: 0min: 40%A/60%B; 0-5min: 20%A/80%B; 5-10min: 4%A/81%B/15%C; 10-60min: 4%A/11%B/85%C; 60-71min: 100%B; 71-72 min back to the initial condition for re-equilibration. Carotenoids were separated through a C30 reverse phase column (250 x 4.6 mm i.d.) packed with 5μm YMC (EUROP GmbH, Germany) with a flow rate of 1ml.min⁻¹, a column temperature at 25ºC and an injection volume of 20 μl. Absorbance was measured with Agilent Chemstation Plus software at 450 nm (Diode array 290-470nm). Concentrations were determined by comparison to a standard curve using pure β-carotene (Extrasynthese, Genay, France) (Bechoff et al., 2009a). A reverse-phase high performance liquid chromatography using an Agilent 1100 system (Massy, France) was used following the method by Dhuique-Mayer et al. (2007).

Volatile compounds analysis

SPME-GC-MS (solid phase microextraction coupled to gas chromatography–mass spectrometry) was used to analyse semi-quantitatively volatile compounds generated during the storage of sweet potato flour. The SPME fibres used were 1 cm long of DVB/Car/PDMS.
(divinylbenzene/carboxen/polydimethylsiloxane) from Supelco (Bellefonte, PA). 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. Sweet potato flour samples (3.00g) were weighed into a 10ml glass vial and were capped with an air-tight 20mm PTFE/silicon septum (Interchim, France). The sample was heated at 50 °C for 15 min to liberate volatile compounds from the flour matrix. All analyses were carried out on a Agilent 6890 Gas Chromatographic System (Agilent Technologies, Palo Alto, Canada USA) equipped with an autosampler Combi PAL (CTC Analytics, Zwingen, Switzerland) coupled with an Agilent 5973N mass spectrometer. Chromatographic separation was achieved with a DB-Wax (J&W Scientific, Folson, CA) fused silica capillary column (60m x 0.32mm i.d.; film thickness= 0.25 µm). Operating conditions 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. Helium was used as carrier gas in constant flow mode 1.5 mL.min⁻¹. 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. 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 (R²) 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-cyclocitrinal and DHA, identified based on their mass spectrum, which chemical structure was close to that of β-ionone, the concentrations ratios (peak area at time t divided by peak area at initial time), also called relative contents, were calculated based on the β-ionone standard curve.

Statistical analysis and kinetics modelling

Carotenoid contents and norisoprenoid contents were determined on a fresh weight basis. Data were processed on SPSS 15.00 software by one or two-way ANOVA (Analysis of variance; p<0.05) using HSD Tukey test to determine which samples were significantly different from others. The kinetics of carotenoid degradation was modelled using Arrhenius and Eyring models (Cisse, Vaillant, Acosta, Dhuique-Mayer & Dornier 2009). The Arrhenius model is an empirical collision model that describes the relationship between reaction constant rate constants and temperature using activation energy ($E_a$) and a pre-exponential factor ($k_\infty$). The Eyring model is based on the transition state theory in which enthalpy of activation ($\Delta H^*$) and entropy of activation ($\Delta S^*$) are the model’s parameters. The model’s parameters were identified from experimental data measured in triplicate, using linear regressions.

$$k = k_\infty e^{-\frac{E_a}{RT}} \quad \text{[Eq. 2]}$$

Where:

- $T$: temperature (K)
- $k$: degradation constant rate at $T$ (day$^{-1}$)
- $k_\infty$: value of $k$ at $T = \infty$ (day$^{-1}$)
- $E_a$: Activation energy (kJ mol$^{-1}$)
R: gas constant = 8.314 J · K⁻¹ · mol⁻¹

\[ k = \frac{k_B}{h} T e^{-\frac{\Delta H^* - T \Delta S^*}{RT}} \]  

{[Eq. 3]}

Where:

k_B: Boltzmann constant = 1.381·10⁻²³ J.K⁻¹

h: Planck constant = 6.626·10⁻³⁴ J.s

\( \Delta H^* \): activation enthalpy (kJ.mol⁻¹)

\( \Delta S^* \): activation entropy (J.mol⁻¹.K⁻¹)

For the validation of the Arrhenius model using at room temperature, the predicted data were calculated using the equation \([42]\):

\[ C = C_0 e^{-\frac{E_{a}}{RT} dt} \]  

{[Eq. 42]}

C is the carotenoid concentration at \( t = 88 \) or 125 days of storage and \( C_0 \) is the initial concentration. In order to validate the Arrhenius carotenoid degradation model in laboratory conditions, dried samples (Ejumula variety) were stored for 88 days at ambient room temperature (anisothermal or dynamic) conditions in the dark.

Temperature and humidity were recorded every h (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 (Ejumula variety) stored for 125 days at ambient room temperature in Uganda (Bechoff et al., 2009b).
Results & Discussion

Carotenoid degradation kinetics

The effect of temperature (10; 20; 30; 40 °C) on β-carotene degradation is described in Table 1. Coefficients of correlation (R²) in Table 1 suggest that a first-order equation fitted well the carotenoid degradation. An exception nonetheless was at 10 °C where correlations observed were lower (R²~0.8). Lower correlations at low temperatures where there is minimum carotenoid degradation could be explained by experimental errors (Hidalgo & Brandolini, 2008). Degradation rates of trans-β-carotene were significantly different at the four tested temperatures (10; 20; 30; 40 °C). (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 40 °C. On the other hand only 35% was lost after 62 days at a lower temperature of 10 °C. 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 2). For both of the models provided, description of trans-β-carotene fitted degradation (R² > 0.990 and 0.989 respectively). 5,6 epoxy-β-carotene, another carotenoid present in fresh sweet potato (Kosambo, Carey, Misra, Wilkes, & Hagenimana 1998), also followed a first order rate reaction that could also be fitted to the same models (R² > 0.997 and 0.996 respectively). Using the models, the activation energy (Ea) for β-carotene and for 5,6-epoxy-β-carotene was calculated as 64.2 and 78.8 kJ.mol⁻¹ respectively and the enthalpy was 61.7 and 76.3 kJ.mol⁻¹ respectively (Table 2). The energy of activation and enthalpy were both 23% 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 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) being 46.3 kJ.mol$^{-1}$ was similar to the value of 44.3 kJ.mol$^{-1}$ for freeze-dried sweet potato at 60-80 °C (Stephanovitch and Karel, 1982). Similar activation energies of 45.3 and 48.7 kJ.mol$^{-1}$ were measured for total carotenoids on Serio and Monlins wholemeal wheat flour respectively, stored between -20 °C and 38 °C (Hidalgo & Brandolini, 2008).

To test the robustness of the model, it was used to predict the carotenoid content of a dried sweet potato sample 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 $\beta$-carotene under anisothermic conditions, the difference between the experimental value and value predicted by the model was 4.3% and 3.5% respectively (Table 3). 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 (Bechoff et al., 2009b). Similarly, the model was also accurate in its predictions where for total carotenoids under anisothermic conditions with a difference between the experimental value and model of 9.3% (Table 3). Therefore it can be concluded that the model developed under-with samples stored under 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 Fig. 1. These can be used for practical applications, such as the determination of product shelf life. For instance, for a 20% loss in $\beta$-carotene...
(Fig. 1A), the predicted storage duration in tropical conditions (average 30 °C) is about 10 days. On the other hand if the dried sweet potato is stored at ambient temperature (average 20 °C) the predicted storage time is one month. If it is stored in fridge conditions (average 10 °C) the predicted storage time increases up to 70 days. Predicted storage time based on total carotenoids (Fig. 1B) is slightly shorter and when based on 5,6 epoxy-β-carotene, longer (Fig. 1C) 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 °C, the storage duration shall be 160, 200 and 110 days for a 40% loss in β-carotene, 5,6-epoxy-β-carotene or total carotenoids respectively.

Influence of water activity and oxygen on carotenoid degradation

The water sorption properties of dried sweet potato chips stored at 40 °C in different saturation salt solutions are described in Fig. 2. The experimental data was 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 beyond outside usual storage conditions. The sweet potato of variety Ejumula stored in Uganda under ambient conditions for four months had a maximum moisture content of 13.6% on a dry weight basis (12% on a wet weight basis) (Bechoff et al., 2009b).

Under different water activities, trans-β-carotene degradation fitted a first order kinetic model with $R^2$ ranging between 0.949 and 0.984 as shown in Fig. 3A Table 1B. Under isothermic-isothermal conditions (40 °C) and in samples stored under air, the lower the water activity the faster the β-carotene degradation (Fig. 4A). 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 and standard deviations of 0.0493 (0.0029), 0.0460 (0.0032) and 0.0413 (0.0005) day$^{-1}$ respectively. On the other hand the degradation rate constant at $a_w = 0.76$ was 0.0341 (0.0010) 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 in the storage of dried sweet potato chips, water activity ($a_w = 0.13$-0.76) had a significant impact on carotenoid degradation with constant rate constants comprised between 0.0341 and 0.0493 day$^{-1}$ respectively. However the effect of water activity in β-carotene breakdown was a lot less than the effect of temperature (10-40°C) with constant rate constants of 0.0029 to 0.0405 day$^{-1}$. Therefore at typical product moisture content of 7-14% on a dry basis (Bechoff et al., 2009b), 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 for the four levels analysed in triplicate ($R^2=0.953$) (Fig. 4A) (Table 1B). Working with a model system made of microcrystalline cellulose containing 0.5% of β-carotene, Goldman et al. (1983) and Chou & 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 that the higher the water activity the lower the β-carotene degradation (Goldman et al., 1983).

In contrast to the linear trend depicted in this publication, 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 \( a_w 0.34 \) and 0.54 on freeze-dried carrot flour stored for 30 days at 40 \(^\circ\)C 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). Other authors working on blanched and freeze-dried carrots (Arya, Natesan, Parihar, & Vijayaraghavan, 1979) argued that increased carotenoid degradation rate at higher water activities might have resulted from solubilization of naturally present metal catalysts that accelerate carotenoids antioxidation rate rather than from enzymatic breakdown of carotenoids.

It has been confirmed in an earlier study on dehydrated sweet potato cubes (Haralampu & 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). At low water activities it has been demonstrated that peroxidase activity dramatically decreased (Kamiya & Nagamune, 2002). Dioxygenases known for their ability to degrade carotenoids into aroma compounds (Auldridge et al., 2006) also lose activity in non-aqueous environments (Sanakis, Mamma, Christakopoulos, & Stamatis, 2003). For these different reasons, in this study, the possibility of carotenoid degradation due to enzymatic activity seemed unlikely.

Under different levels of oxygen, carotenoid degradation fitted first order with \( R^2 \) values of 0.944; 0.968 and 0.961 at various levels of oxygen (Fig. 3B, Table 1C). An exception was under nitrogen where correlation \( (R^2) \) observed was 0.675. Explication for this poor correlation was a minimum \( \beta \)-carotene breakdown under these conditions as of low temperature (Hidalgo & Brandolini, 2008). On samples stored at 40 \(^\circ\)C, the degradation rate of \( \beta \)-carotene was highly related to the oxygen level flushed through
Degradation rate constants significantly differed between samples (One way-ANOVA p<0.05). Samples flushed with nitrogen had a rate constant of 0.0107 (0.0021) day\(^{-1}\), whilst those flushed with 2.5%, 10% and 21% oxygen had respective rate constants of 0.0303 (0.0053), 0.0514 (0.0022) and 0.0853 (0.0038) day\(^{-1}\). It was interesting to observe that flushing with air at 40°C dramatically increased the degradation rate (0.0853 day\(^{-1}\)) compared to samples stored under air but at the same temperature (0.0341 to 0.0493 day\(^{-1}\)) (Table 1C). 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 \(\beta\)-carotene flushed with oxygen are in accordance with studies (Texeira Neto, Karel, Saguy, & Mizrahi, 1981) that showed that oxygen uptake in a microcrystalline cellulose food model was closely linked to \(\beta\)-carotene degradation and agreed with other study on sweet potato flakes showing a direct relationship between oxygen uptake and carotenoid degradation (Walter & Purcell, 1974). 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-zero order for the four levels of oxygen analysed in triplicate \(R^2=0.975\)) \(\dot{a}\) between oxygen level and degradation rate signifies that oxygen could be considered as a co-substrate (in excess) of oxidative degradation during storage (Fig. 4B, Table 1C). 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°C (Walter & Purcell 1974). Over the range studied (0-21% oxygen) oxygen had a more marked effect on \(\beta\)-carotene than water activity (0.13-0.76) and temperature (10-40°C). The mean difference
between constant rate constants was 0.0746 day$^{-1}$, 0.0152 day$^{-1}$ and 0.0376 day$^{-1}$ respectively. Studies on other foodstuffs similarly concluded that oxygen was a major factor of degradation during storage. Working on the effect of packaging (polypropylene: high oxygen permeability; nylon laminate film (low oxygen permeability) with air space; under vacuum or using a Ageless oxygen absorber sachet enclosed) on sweet potato flakes it was demonstrated that oxygen had a major impact on carotenoid degradation (3): sweet potato flakes stored for 210 days in nylon film and with oxygen absorber did not lose significant amounts of β-carotene whilst those stored in polypropylene lost 66% of their initial content at ambient temperature (about 23 ºC).

In this present study, the β-carotene loss on sweet potato chips stored under nitrogen (16% loss after 21 days) (Fig. 6) might result of incomplete oxygen exclusion of samples. Alternatively it could also result of the effect of the relatively high temperature used for the storage (40 ºC).

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). It was interesting to observe that flushing with air dramatically increased the degradation rate (0.0853 day$^{-1}$) compared to samples stored under air with various salt but at the same temperature (0.0341 to 0.0403 day$^{-1}$). The increased degradation rate of β-carotene flushed with oxygen agreed with studies (Texeira Neto, Karel, Saguy, & Mizrahi, 1981) that showed that oxygen uptake in a microcrystalline cellulose food model was closely linked to β-carotene degradation and agreed with other study on sweet potato flakes showing a direct relationship between oxygen uptake and carotenoid degradation (Walter & Purcell, 1974).
Both trends of the degradation rate related to water activity and oxygen and dried media agreed with studies on microcrystalline food model food systems (Chou & Breene, 1972; Goldman et al., 1983; Texeira Neto et al., 1981) where enzymatic activity is excluded and with previous studies on dehydrated sweet potato (Haralampu & Karel, 1983; Walter et al., 1970; 1974). Though autoxidation was mentioned in earlier studies as the cause of β-carotene degradation in dried sweet potato (Haralampu & Karel, 1983; Walter et al., 1970; 1974), a more recent study (Auldridge et al., 2006) emphasized that it had not been proved. This study therefore fills the gap by showing that dehydrated sweet potato and the microcrystalline cellulose food model behaved the same way toward water activity and oxygen which strongly suggests that autoxidation was the mechanism responsible for β-carotene degradation.

**Description of carotenoid degradation in relation with norisoprenoid formation**

Whereas trans-β-carotene degraded during storage, norisoprenoids namely β-cyclocitral, β-ionone, 5,6-epoxy-β-ionone, and dihydroactinidiolide (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). The greatest formation of norisoprenoids occurred at lower water activities (Fig. S3) and this is consistent with the earlier findings that carotenoid degradation is also greater at lower water activities (Fig. 4A and Table 1B). The volatile norisoprenoids content was defined as the sum of the four identified major norisoprenoids contents known to be associated with carotenoids degradation. On average, samples stored at \( w_a = 0.13 \) had a β-ionone volatile content of \( 1.230 \pm 0.47 \mu g.g^{-1} \), followed by those stored at \( w_a = 0.30 \) with \( 1.200 \pm 0.38 \mu g.g^{-1} \); at \( w_a = 0.51 \) had a β-ionone volatile content of \( 0.96 \pm 0.30 \mu g.g^{-1} \) and at \( w_a = 0.76 \) of \( 0.920 \pm 0.31 \mu g.g^{-1} \) (Fig. S3). There was no difference between samples stored at \( w_a = 0.13 \) and 0.30 for β-ionone
while the other samples stored at $a_w = 0.51$ and 0.76 differed (two way ANOVA; $p<0.05$). No difference in β-cyclocitril content ratio was found between samples stored at $a_w = 0.13$ and 0.30; and at 0.51 and 0.76. On the other hand, there was a significant difference in the DHA and 5,6-epoxy-β-ionone concentrations ratios for the four water activities (two way ANOVA; $p<0.05$). This description of differences between volatile relative concentrations agreed with the above description of β-carotene degradation at various water activities.

Relative contents of β-cyclocitril, β-ionone and 5,6-epoxy-β-ionone contents were the highest at 19 days and subsequently decreased whilst DHA levels reached a plateau after 27 days of storage at 40 ºC (Fig. 53). 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 (Bosser, Paplorey & Belin, 1995; Mordi et al., 1993; 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 the same process whereas the later formation of DHA suggested that the rearrangement into DHA was a slower step.

Fragmentation of β-carotene in dehydrated sweet potato flakes under oxygen has been reported previously (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. At 40 ºC samples stored flushed with compressed air (21% oxygen) had a β-ionone mean volatile content of
$1.530.44 \mu g.g^{-1}$, followed by those flushed with 10% (4.220.36\mu g.g^{-1}); 2.5% (0.790.72\mu g.g^{-1}) oxygen or nitrogen (0.40\mu g10\mu g.g^{-1}), respectively (Fig. 64). Similarly there was a significant increase in the $\beta$-cyclocitral, $\beta$-ionone-5,6-epoxy-$\beta$-ionone and DHA concentrations at the four increasing oxygen levels (two ways-ANOVA; p<0.05).

With no oxygen present in the storage conditions, only a small decrease or no difference in volatile compounds contents was observed. These highlighted how much oxygen is important in the degradation scheme of $\beta$-carotene into volatile compounds (Fig. 64).

$\beta$-cyclocitral, $\beta$-ionone and 5,6-epoxy- $\beta$-ionone reached their highest levels after 9 days of storage and tended to subsequently decrease whilst DHA remained almost steady from 9 days (Fig. 6). This is a similar pattern to that observed at different water activities.

The amounts of $\beta$-ionone it was observed in the SPME-GC-MS analyses corresponded to approximately two orders of magnitude less than the amounts of $\beta$-carotene measured by HPLC (Figures 3&4). 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 $\beta$-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 $\beta$-ionone with maximum amounts per compounds ($\beta$-ionone, 5,6-epoxy-$\beta$-ionone, $\beta$-cyclocitral and DHA) being approximately 0.4-0.9 $\mu g.g^{-1}$, will are much lower than the amounts of carotenoids degraded (Figures 3&4). Amounts of volatiles formed were largely lower
that the amount of carotenoids degraded. In other publication (Waché et al., 2003) 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 clear 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.

**Conclusion**

The Arrhenius and Eyring models correctly described the carotenoid degradation in dried stored sweet potato between 10 and 40 ºC; the Arrhenius model was validated using a sample stored at room temperature (non-isotherm conditions). The greater β-carotene degradation rate at lower water activity in particular suggested that the reaction was an autoxidation. 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 relative concentrations of volatiles were recorded. A similar observation was made at lower oxygen levels. One of the applications of these findings could be the development of a rapid and non-destructive method using SPME-GC-MS to measure threshold production of norisoprenoids that would correspond to a critical level of β-carotene breakdown in dried food products and could help in predicting product shelf life. There was a linear relationship between β-carotene degradation rate and water activity, and between β-carotene degradation rate and oxygen concentration. 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. Moreover 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.

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**Fig. 1.** Prediction curves of carotenoid (%-carotene; 5,6-epoxy-%-carotene; total-carotenoids) loss (%) with temperature and with storage time in dried sweet potato chips stored between 10-40 °C in air; $a_w$ 0.52-0.65.
Fig. 1. Degradation kinetics of trans-β-carotenes in sweet potato chips stored between 10-40°C and calculated on a fresh weight basis. Error bars refer to standard deviation (n=3). Oxygen level 21% (air), a_w 0.52-0.65.
Fig. 2. Water sorption curves of dried sweet potato chips stored at 40 °C for 54 days under air. Experimental data and BET model ($C = -68.88$, $M_0 = 0.0564$).
Fig. 3. Degradation kinetics of trans-β-carotenes in sweet potato chips stored at 40°C and calculated on a fresh weight basis. Where (A): influenced by water activity (a_w) in air after 54 days and (B): influenced by oxygen flushing at different concentrations after 21 days. Error bars refer to standard deviation (n=3).
Fig. 4. 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. Error bars refer to standard deviation (n=3).
Fig. 53. Degradation of water activity influence on trans-β-carotene and production of id and identified norisoprenoids during storage of dried sweet potato chips at 40 °C under air at different water activities (β-carotene and β-ionone expressed as μg.g⁻¹ of dried sweet potato chips at 40 °C under air calculated on a fresh weight; volatile degradation products, as ratio: peak area at time t divided by peak area at initial time); basis: Error bars refer to standard deviation (n=3).
Fig. 64. Oxygen influence on trans-β-carotene and identified norisoprenoids in dried sweet potato chips during storage at 40 °C of dried sweet potato chips calculated on a fresh weight basis. Error bars refer to standard deviation (n=3).

Degradation of trans-β-carotene and production of identified norisoprenoids during storage of dried sweet potato chips at 40 °C at different oxygen levels (β-carotene and β-ionone expressed as μg.g⁻¹ fresh weight; volatile degradation products as ratios: peak area at time t divided by peak area at initial time); Error bars refer to standard deviation (n=3).
Table 1. Rate of degradation of carotenoids (k) expressed in day\(^{-1}\) in dried sweet potato chips on a fresh weight basis at various temperatures in dried sweet potato chips on a fresh weight basis. Oxygen level 21% (air); aw 0.52-0.65 (A); at various water activities at 40ºC in air (B)-- at various oxygen (flushed) levels at 40ºC (C). Mean of triplicate thermal treatment (standard deviation).

(A)

<table>
<thead>
<tr>
<th>Temperature (ºC)</th>
<th>10</th>
<th>20</th>
<th>30</th>
<th>40</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>β-carotene</strong> k</td>
<td>0.0029 (0.0002)</td>
<td>0.0093 (0.0005)</td>
<td>0.0193 (0.0002)</td>
<td>0.0405 (0.0005)</td>
</tr>
<tr>
<td>R(^2)</td>
<td>0.829 (0.071)</td>
<td>0.922 (0.031)</td>
<td>0.985 (0.005)</td>
<td>0.963 (0.007)</td>
</tr>
<tr>
<td><strong>5,6 epoxy-β- carotene</strong> k</td>
<td>0.0025 (0.0004)</td>
<td>0.0084 (0.0003)</td>
<td>0.0248 (0.0009)</td>
<td>0.0597 (0.0010)</td>
</tr>
<tr>
<td>R(^2)</td>
<td>0.748 (0.167)</td>
<td>0.902 (0.045)</td>
<td>0.869 (0.020)</td>
<td>0.987 (0.009)</td>
</tr>
<tr>
<td><strong>Total carotenoids</strong> k</td>
<td>0.0047 (0.0002)</td>
<td>0.0088 (0.0003)</td>
<td>0.0179 (0.0005)</td>
<td>0.0298 (0.0009)</td>
</tr>
<tr>
<td>R(^2)</td>
<td>0.864 (0.091)</td>
<td>0.982 (0.014)</td>
<td>0.987 (0.005)</td>
<td>0.986 (0.006)</td>
</tr>
</tbody>
</table>

(B)

<table>
<thead>
<tr>
<th>Water activity</th>
<th>0.76*</th>
<th>0.51*</th>
<th>0.30*</th>
<th>0.13*</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>β-carotene</strong> k</td>
<td>0.0341 (0.0010)</td>
<td>0.0413 (0.0005)</td>
<td>0.0460 (0.0032)</td>
<td>0.0493 (0.0029)</td>
</tr>
<tr>
<td>R(^2)</td>
<td>0.984 (0.003)</td>
<td>0.977 (0.0041)</td>
<td>0.974 (0.003)</td>
<td>0.949 (0.040)</td>
</tr>
</tbody>
</table>

*Coefficient of correlation between four k levels related to water activity: 0.953 (0.013)

(C)

<table>
<thead>
<tr>
<th>Flushed* Oxygen level</th>
<th>0%*</th>
<th>2.5%*</th>
<th>10%*</th>
<th>21%*</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>β-carotene</strong> k</td>
<td>0.0107 (0.0021)</td>
<td>0.0303 (0.0053)</td>
<td>0.0514 (0.0022)</td>
<td>0.0853 (0.0038)</td>
</tr>
<tr>
<td>R(^2)</td>
<td>0.675 (0.290)</td>
<td>0.944 (0.020)</td>
<td>0.968 (0.006)</td>
<td>0.961 (0.013)</td>
</tr>
</tbody>
</table>

*Coefficient of correlation between four k levels related to water activity: 0.975 (0.016)

*90ml min\(^{-1}\)
Table 2. Parameters of the Arrhenius and Eyring models for the carotenoids degradation in dried sweet potato chips on a fresh weight basis between 10-40 °C. Oxygen level 21% (air); aw 0.52-0.65. Mean of triplicate thermal treatment (standard deviation).

<table>
<thead>
<tr>
<th>Carotenoid</th>
<th>Ln k∞ (k∞ days⁻¹)</th>
<th>Ea (kJ.mol⁻¹)</th>
<th>R²</th>
<th>ΔH* (kJ.mol⁻¹)</th>
<th>ΔS* (J.mol⁻¹.K⁻¹)</th>
<th>R²</th>
</tr>
</thead>
<tbody>
<tr>
<td>β-carotene</td>
<td>21.5 (0.6)</td>
<td>64.2 (1.6)</td>
<td>0.990</td>
<td>61.7 (1.6)</td>
<td>-74.3 (5.1)</td>
<td>0.989</td>
</tr>
<tr>
<td>5,6 epoxy-β-carotene</td>
<td>27.5 (1.5)</td>
<td>78.8 (3.8)</td>
<td>0.997</td>
<td>76.3 (3.8)</td>
<td>-24.5 (12.5)</td>
<td>0.996</td>
</tr>
<tr>
<td>Total carotenoids</td>
<td>14.3 (0.6)</td>
<td>46.3 (1.5)</td>
<td>0.997</td>
<td>43.8 (1.5)</td>
<td>-134.5 (5.0)</td>
<td>0.997</td>
</tr>
</tbody>
</table>

Arrhenius model

\[ k = k_{\infty} e^{-\frac{E_a}{RT}} \]

Where: T : temperature (K); k: degradation rate constant at T (day⁻¹); k∞ : value of k at T = ∞ (day⁻¹); Ea: Activation energy (kJ.mol⁻¹); R: gas constant = 8.314 J · K⁻¹ · mol⁻¹.

Eyring model

\[ k = \frac{k_B T e^{-\frac{\Delta H^*}{RT}}}{h} \]

Where: kB: Boltzmann constant = 1.381·10⁻²³ J.K⁻¹; h: Planck constant = 6.626·10⁻³⁴ J.s; ΔH*: activation enthalpy (kJ.mol⁻¹); ΔS*: activation entropy (J.mol⁻¹.K⁻¹).
Table 3. Validation of Arrhenius model for a sample of dried Ejumula sweet potato chips stored under ambient anisotherm conditions during 88 days in the UK and during 125 days in Uganda on a fresh weight basis. Oxygen level 21% (air).

<table>
<thead>
<tr>
<th>Sample stored in UK</th>
<th>Initial</th>
<th>Final</th>
<th>Predicted by Arrhenius model</th>
<th>Difference (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Storage time (days)</td>
<td>Trans-β-carotene (μg.g⁻¹)</td>
<td>Experimental (μg.g⁻¹)</td>
<td>Predicted by Arrhenius model (μg.g⁻¹)</td>
</tr>
<tr>
<td></td>
<td>88</td>
<td>181.2 (5.9)</td>
<td>74.6 (5.1)</td>
<td>72.0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>250.3 (1.1)</td>
<td>94.4 (1.1)</td>
<td>90.3</td>
</tr>
<tr>
<td>Sample stored in Uganda</td>
<td>Initial</td>
<td>Final</td>
<td>Predicted by Arrhenius model</td>
<td>Difference (%)</td>
</tr>
<tr>
<td></td>
<td>125</td>
<td>219.6 (1.6)</td>
<td>51.4 (1.5)</td>
<td>46.6</td>
</tr>
</tbody>
</table>

*This present study (Calculated a_w from BET model: 0.460 (0.012) from chips dry matter (90.4 (0.3) g/100g)

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]))

*Mean of triplicate (standard deviation).
**Effect of drying and storage on the degradation of carotenoids in orange-fleshed sweetpotato cultivars**

<table>
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<th><em>Journal of the Science of Food and Agriculture</em></th>
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| Complete List of Authors: | Bechoff, Aurelie; Natural Resources Institute, University of Greenwich  
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Tomlins, Keith; University of Greenwich, Natural Resources Institute |
| Key Words:        | Ipomoea batatas (L.) Lam, carotenoid, storage, drying, cultivar |

* Supplementary material submitted to JFSA
Effect of drying and storage on the degradation of carotenoids in orange-fleshed sweetpotato cultivars

Running title: Carotenoid degradation in OFSP

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Keywords: Ipomoea batatas (L.) Lam, carotenoid, storage, drying, cultivar.

Abstract: Ugandan orange fleshe sweetpotato cultivars were evaluated for their total carotenoid retention (as an estimate of provitamin A retention) after drying in solar dryers (tunnel dryer, plastic-covered-tents dryers with different light transmission properties) and directly in the sun. Cultivar effect was tested with six cultivars (Ejumula, Kakamega, SPK004/1, SPK004/1/1, SPK004/6 and SPK004/6/6) that differed in carotenoid content: Total carotenoid retention during drying was not dependent on the type of dryer (solar or
sun). Sweetpotato cultivar, however, had a significant effect on retention in drying (p<0.05). Carotenoid loss was generally correlated with high initial moisture content and high carotenoid content in fresh sweetpotato roots. Losses of provitamin A during drying were generally low (15% or less). Dried sweetpotato chips were also evaluated for their carotenoid content after four months of storage at room temperature in Uganda. Losses of provitamin A were high (about 70%) and this was not dependent on the use of opaque or transparent packaging. Losses of carotenoids during storage were therefore considered to be more of a nutritional constraint to the utilisation of dried sweetpotato than losses occurring during drying.

INTRODUCTION

Uganda is the world’s third largest producer of sweetpotato (*Ipomoea batatas* (L.) Lam.) with 2.6 million tonnes per annum\(^1\). It is a staple food in many regions of the country, particularly in the North-Eastern and Southern-Western parts. Vitamin A deficiency, a major public health problem in developing countries\(^2\) affecting 38% of the children under the age of five years in Uganda\(^3\). Vulnerable groups susceptible to the effects of vitamin A deficiency include pregnant and lactating women and those who are at risk from immunodeficiency, such as those suffering from HIV and AIDS\(^3\). Two recent studies in South Africa\(^4\) and Mozambique\(^5\) have demonstrated that consumption of orange fleshy sweetpotato (OFSP), rich in provitamin A, significantly increased the vitamin A status of children. White-fleshed sweetpotatoes are the most commonly cultivated in sub-Saharan Africa, while OFSP cultivars are rarer\(^6\) or more recently introduced. Recent work with women and children in Tanzania\(^7\) has however demonstrated that, for the majority of consumers, OFSP cultivars are equally as acceptable as the white fleshed ones.
Substitution of white-fleshed cultivars could make a significant contribution to reducing vitamin A deficiency in East Africa\(^8\).

In addition to consumption of cooked fresh sweetpotato roots, dried sweetpotato products have the potential to increase year round food availability and also provide a source of income for farmers. Drying of sweetpotato is a traditional processing technique practiced in North-Eastern Uganda, and it is also a means of producing a tradable product for use in commercially-available composite flours or as a replacement of wheat flour in bakery products.

In Africa, a concern was that losses in carotenoids during the drying of OFSP 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. Drying studies undertaken in Kenya\(^9\) and Columbia\(^10\) reported high losses of between 72 and 83\% for sweet potato chips and between 41 and 62\% in cassava respectively. Losses as low as 20\% have been reported in Kenya\(^11\) but the drying time was very short and under shade (5 hours). However, in the USA\(^12\) and France\(^13\) much lower losses of carotenoids during the drying of sweet potato of 5-6\%, and 16\% to 23\% respectively were reported which suggests that if drying conditions can be consistently controlled that losses will not be so high. The scarce literature encountered on the extent of provitamin A losses in OFSP under African conditions shows a need for further research. On the other hand there is a need for quantification of provitamin A after drying in order to determine the nutritional value of the dried OFSP product compared to fresh roots; this would inform on whether the dried product can contribute to improving nutrition and health and hence worth being promoted. Moreover, a clearer understanding of the causes of provitamin A losses (type of dryer;
environmental conditions; sweet potato variety etc) during drying of OFSP in developing countries would contribute to the development of improved processing and/or handling techniques, promotion and marketing.

Exposure to light, especially sun or ultra-violet light, has been reported to induce trans-cis photomerisation and photodestruction of carotenoids\textsuperscript{14}. When exposed to sunlight radiation, provitamin A is more sensitive to ultra-violet (UV) rays, especially at wavelengths close to the maximum absorption of $\beta$-carotene of 450 nm; and, in general, short wavelengths less than 470 nm caused the most $\beta$-carotene degradation\textsuperscript{15,16}. It has also been reported that screening from direct sun light had an impact on total carotenoid losses from mango and cowpea leaves\textsuperscript{17}; 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.

Degradation of provitamin A in sweet potato products during storage is important to determine as this would decrease the nutritional impact of the product for food security. Typically at the household level dried sweetpotato is stored at ambient temperature for 4-6 months. A significant decrease was reported in all-trans-$\beta$-carotene content during storage of sweetpotatoes flakes for four months in either plastic or foil packaging. Losses were 43\% in foil packaging, 46\% laminate paper and 54\% in plastic bag at room temperature\textsuperscript{18}. Similar results were reported in Kenya\textsuperscript{9} where trans-$\beta$-carotene content fell by 50\% in sweetpotato chips of Kakamega and Jonathan cultivars stored at room temperature, whereas levels remained steady in chips stored for three months at -20°C. However in another study in Kenya\textsuperscript{19}, storing dried slices from 24 sweetpotato cultivars in opaque paper bags under ambient conditions for 11 months resulted in lesser total-carotenoid
losses of 10%. Differences in room temperature during storage or packaging permeability to oxygen may explain these disparities. This raises a need for further investigation in recorded conditions.

The objectives of this study were (a) to quantify the losses of total carotenoids from OFSP chips dried in low-cost dryers in Uganda and subsequently stored, and (b) understand the main factors that influence losses, such as type of dryer, effect of different plastic coverage, sweetpotato cultivar and type of packaging of the stored product.

MATERIALS AND METHODS

Sweetpotato root samples

Sweetpotato roots were collected from three different 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 (Naspot 9O) and SPK004/6/6 (Naspot 10O), 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 1) have been previously reported to be susceptible to sweetpotato weevil and moderately resistant to Alternaria blight and Sweetpotato Virus Disease (SPVD) resulting of dual infections of Sweetpotato feathery
mottle virus vectored by aphids, and Sweetpotato chlorotic stunt virus vectored by whiteflies \textsuperscript{20,21}.

**Handling and processing**

Sweetpotato 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.

**Drying of sweetpotato chips**

Sweetpotato chips were dried using dryers that varied in degree of exposure to direct sunlight. Different types of coverage were selected to have different screening effects on UV-radiation for the tent dryers since it was hypothesised that UV-radiation would have a significant effect on carotene degradation. Details of the dryers are given below:

- 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).

Transmittance of the polythene sheetings of tent-dryers (UV resistant, non-UV resistant and red) were measured by spectrophotometer measurement\textsuperscript{22} between 200-800nm (Figure
1). A rectangular section of 1 cm width was placed in the centre of the spectrophotometric cuvette so that beam light could perpendicularly cross the plastic sheeting.

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 a Vantage Pro- meteorological station (Davis Instruments, California, USA). All samples were removed from dryers and placed under a shelter at night and 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/m² was used when placing the sweetpotato chips on the dryers. The mean drying conditions were a temperature of 28.3°C with a standard deviation of 5.6°C 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.

Storage of the dried chips

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 4 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.

**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 method\(^\text{14}\), 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 2592.14.

Carotenoid analysis in the UK

Dried chips were transported to the UK by air flight and were immediately placed in a freezer on arrival. Although during transport to the UK over a 24 hour period, the dried chips did reach ambient temperature during transport over a 24 hour period outside the freezer, but the carotenoid degradation was negligible; a maximum of carotenoid loss of 0.88% was calculated for a sample stored at 25°C for 24h (Bechoff A, unpublished). Samples were immediately placed in a freezer on arrival. Samples were stored in a freezer (-20°C) 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).

Prior to analysis, chips were milled into flour. After careful mixing and sub-sampling using a grain divider, a quarter of the 200g was milled 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. Flour samples were stored at -20°C for no more than two weeks before analysis.

The same extraction method was used in the UK by the same operator. Homogenisation was carried out using an Ultra-turax IKA Janke and a Kunkel Labortechnik at 8000 rpm/min. The total carotenoid content measured by Diode Array detector spectrophotometer (Hewlett Packard HP8452A).
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).

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 (SPSS UK Ltd. Woking Surrey) for Windows software.

RESULTS AND DISCUSSION

Provitamin A 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 and there was no significant difference (p<0.05).

The drying of Ejumula and Kakamega (SPK004) sweetpotato cultivars was investigated. Total carotenoid losses were on average 7.3% in Ejumula and 10.7% in Kakamega (Table 2). 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-\(\beta\)-carotene were similarly reported in a recent study involving oven; solar and open sun drying of OFSP chips in Uganda\(^{23}\). Trans-\(\beta\)-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 sweetpotato dried in dryers fitted with polythene sheeting were determined (Table 3). 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%.

Polythene sheeting presented different transmittance toward sun light. Red plastic sheeting absorbed at wavelengths between 300-600 nm which provitamin A is reportedly sensitive\(^{15,16}\) (part of UVA and visible). UV-resistant plastic reduced most UV-wavelengths: between 200-240 nm (UVB and UVC) and between 260-370 nm (UVB, UVA). Simple polythene was not wavelength selective (Figure 1).

There was no effect on the loss of total carotenoids from the use of type of dryer and different types of polythene sheeting in spite of the various wavelength selectivities (\(p<0.05\)). The greater provitamin A degrading effect of sun compared to solar drying has been previously reported by several authors\(^{10,17,24}\) and these are in contrast to the current results.
Working with leafy vegetables\textsuperscript{24}, it has been demonstrated that solar-dried products retain significantly more $\beta$-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\textsuperscript{24}.

Previous reports\textsuperscript{23,24} 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. Furthermore, the lack of differences between dryers may have been because the UV irradiation affected only the surface and did not penetrate the inner tissue. The size and shape of chips may therefore have an impact on carotenoid loss\textsuperscript{13}. Starch being the main component of sweetpotato may have also played a protective role in preventing carotenoid losses\textsuperscript{25} and explain the lack of difference in-between the solar dryers and also sun drying.

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. The effect of cultivar is treated in the next part.

**Effect of sweetpotato cultivar**

Regarding total carotenoid determination in sweet potato cultivar, critics would say that it may not be the best means of measuring provitamin A because it does not estimate the level of cis-isomers of trans-$\beta$-carotene content and these can increase during processing,
especially at temperatures greater to 35ºC. Significant increase of 13- cis-isomer was encountered on drum dried sweet potato. However some authors have found that there is no increase in cis isomerisation of trans-β-carotene after solar and sun drying. Moreover trans-β-carotene was greater than 90% of total carotenoids in these cultivars. Therefore total carotenoid determination could be an acceptable technique to estimate trans-β-carotene quite precisely on these Ugandan cultivars; no significant differences between total carotenoids and trans-β-carotene have been reported in Resisto, an orange fleshed sweetpotato cultivar having around 90% trans-β-carotene.

Total carotenoid losses from six OFSP cultivars dried in 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). Because the samples from the two locations were not dried on the same day a comparison between the provitamin A losses is therefore not possible. Losses in carotenoids per cultivar in both locations are presented in Table 5.

Total carotenoid contents of fresh sweetpotato varied in the six cultivars between 78.5 and 300.5 µg.g⁻¹ at the NaCRRI and 41.7 and 223.1 µg.g⁻¹ in Luwero District. These values are in agreement with the recent study that described trans-β-carotene content varying between 108.1-261.9 µg.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\textsuperscript{29}. These differences were especially marked for the newly developed cultivars, SPK004/1 and SPK004/1/1 (Table 5). Sweetpotatoes grown on farmer’s fields generally had lower levels of total carotenoids than those grown at the research station.

Independent of location, there was a significant influence of cultivar (p<0.05) on the levels of carotenoid loss. While the total carotenoid losses were not very consistent within cultivars, there was a correlation between initial dry matter content and carotenoid losses (Pearson coefficient R=-0.518; p<0.05) (Figure 2). For an equivalent drying time, cultivars with higher moisture contents tended to lose more carotenoids. Similar observations regarding the influence of dry matter content and carotenoid losses during drying have been made elsewhere: sweetpotatoes (dry matter content of 75.8 %) had greater $\beta$-carotene retention compared to carrots (90.5%) respectively 4.0–5.8%, and 48.9–67.5\%\textsuperscript{12}. These results were further confirmed by analysis of data presented by another author\textsuperscript{19}. The effect of oven drying was tested on 24 white, yellow, purple and orange flesht sweetpotato cultivars\textsuperscript{19}. Total carotenoid content ranged between 2 and 632 $\mu$g$\cdot$g$^{-1}$ dry basis and dry matter in fresh roots between 19 and 34\% respectively. Losses of total carotenoids were variable among cultivars ranging between 0-80\% with an average of 32\%. Using the data presented\textsuperscript{19}, a significant correlation was observed between fresh dry matter and losses during drying in nine cultivars with total carotenoid content greater than 37 $\mu$g$\cdot$g$^{-1}$ (Pearson coefficient R=-0.687; p<0.05). No correlation was observed on 12 other cultivars with carotenoid content lower than 37 $\mu$g$\cdot$g$^{-1}$\textsuperscript{19}.  


The cultivars used in this present study demonstrated a positive correlation between initial total carotenoid content (in fresh chips) and carotenoid losses (Pearson coefficient R=0.589; p<0.05) (Figure 3). Cultivars with higher initial carotenoid content tended to lose more carotenoids during drying. The same trend was observed by analysis of other reported data\textsuperscript{19} on 11 cultivars with total carotenoid content greater than 30 μg.g\textsuperscript{-1} (Pearson coefficient R=0.580; p<0.05). As well as for the correlation between fresh dry matter and provitamin A losses, the correlation between initial carotenoid content and loss was stronger on cultivars with carotenoid contents greater than 37 μg.g\textsuperscript{-1} (Pearson coefficient R=0.713; p<0.05); no correlation was observed on cultivars with lower carotenoid content\textsuperscript{19}.

**Effect of storage on provitamin A retention**

Because the levels of loss of carotenes associated with drying were much less than anticipated, changes during storage at ambient temperatures were investigated (Table 6). In all cases the levels of losses of carotenoids during storage were high under these conditions when compared to that lost 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% over four months of storage (Table 6).

Oxygen (in the air), temperature and relative humidity have been reported to be the main causes of carotenoid degradation in low moisture systems during storage and relationships between these factors have been reported\textsuperscript{30}. The temperature and humidity in the room where the samples were stored was generally very constant and consistent during the periods of storage; the mean temperature and humidity were 23.1°C and 70.5% respectively over an 8 month-period with minimum-maximum variations between 19.1-27.7°C and 42.8-86.5% (Figure 4). The chips were stored in low density polythene (LDPE)
bags because this was the most common way of storing them in Uganda and other
countries in Southern Africa. This type of packaging however did not offer a significant
barrier to oxygen. In ambient temperature and relative humidity conditions during the
period storage oxygen was proved to be the main factor of carotenoid degradation. Packaging permeability to oxygen (in air) was demonstrated to have a critical influence on
β-carotene retention in sweet potato flakes during storage. It was reported that
degradation of the carotenoids was significantly reduced during storage at 23°C for 210
days if oxygen was excluded. In our study, while the levels of losses in carotenoids were
substantial, there were no differences in losses when the dried sweetpotato chips were
stored in sealed clear bags, sealed clear bags in black bags or a knotted black bags (two-
way ANOVA; p<0.05). This suggests that the packaging permeability to oxygen might
have been a significant factor in causing these losses. Moreover this suggests that when
stored at room temperature, restricting exposure to sun light did not influence the level of
loss. The lack of effect of storage in the absence of light or dark has also been reported on
the carotenoid loss of freeze-dried orange-peel, carrots and sweetpotato samples. As
compared with the effect of oxidation (packaged under nitrogen or air); the effect of
photoisomerisation (can or clear bottle) was proved to be minor on mango puree.

Effects of temperature and relative humidity that is related to water activity on
carotenoid degradation in dried sweet potato have been described. At higher temperatures
carotenoids degrade more rapidly. Water activity also had an influence on carotenoid
retention during storage. In our study, for both cultivars analysed collectively, losses
were significantly higher where stored in zipped PE compared to all other packaging even
though the moisture content was slightly lower in the zipped PE bag (90% compared to
88% in other types of packaging) and zipped PE bags were protected from light. A
possible explanation for these observations might be that water has a protective effect on the carotenoids and consequently a drier sample would result in higher carotenoid loss.

Similar results have been reported on low moisture systems of microcrystalline cellulose containing β-carotene; and research on dehydrated sweet potato have also proved that β-carotene degradation rate was faster at lower water activity. Moreover recent but yet unpublished study of temperature related to water activity and oxygen on dried sweet potato was in line with these results (Bechoff A, unpublished).

In general levels of loss during storage in this study agreed with levels of loss encountered in literature which were around 50% or more after 4 months storage at ambient temperature/humidity and air on dried sweet potato chips or flakes.

**Effect of sweetpotato 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 7.

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 sweetpotatoes 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.

**Estimation of vitamin A activity in OFSP chips**

Vitamin A activities of various OFSP chips were estimated and summarised in Table 8. Chips made from most of the cultivars had a high vitamin A activity after drying (>4,000 RE.kg\(^{-1}\))\(^{35}\) with an average of about 8,428 RE.kg\(^{-1}\)\(^{36}\), 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\(^{-1}\).

It is speculated that further losses occurring during the preparation of OFSP flour into finished product (for example, atapa (traditional Ugandan porridge cassava/sweetpotato), mandazi (traditional doughnut), or bread) would represent an estimated loss of a further 50% on a dry basis\(^9,19\). Nonetheless, most of the cultivars immediately after drying have the potential to provide a major part of FAO/WHO recommended daily requirements of children\(^{34}\) 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. From a nutritional and quality perspective, it would not be recommended to store dried OFSP under these conditions for more than two months.

**CONCLUSION**
A major conclusion from the work on drying and storage of sweetpotato 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 were as low as 7.3% in Ejumula and 10.7% in Kakamega cultivars.

Sweetpotato 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.

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. Moreover levels of loss were consistent using various sweetpotato cultivars.

OFSP chips contained a significant amount of provitamin A immediately after drying and this could make a significant contribution to the diet. However, low-cost means of reducing provitamin A losses during storage (eg. pre-treatments such as salting and blanching) or limited shelf life are needed 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 sweetpotato than losses during drying.

ACKNOWLEDGEMENTS
This research was supported by the HarvestPlus Challenge Program “Reaching End Users with orange fleshed sweetpotatoes in Uganda and Mozambique” project. The views expressed are however those of the authors.

REFERENCES


Table 1. Source, flesh colour and yield of selected orange fleshed and yellow fleshed sweetpotato cultivars.

<table>
<thead>
<tr>
<th>Cultivar</th>
<th>Original source</th>
<th>Flesh colour</th>
<th>Typical reported fresh root yield (tonnes per ha)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ejumula</td>
<td>Eastern Uganda</td>
<td>Deep orange(^{20})</td>
<td>36.6(^{20})</td>
</tr>
<tr>
<td>Kakamega (SPK004)</td>
<td>Western Kenya</td>
<td>Yellow/light orange(^ {20})</td>
<td>31(^ {20})</td>
</tr>
<tr>
<td>SPK004/1</td>
<td>Yellow/light orange(^ {21})</td>
<td>7.4-59.7(^ {21})</td>
<td></td>
</tr>
<tr>
<td>SPK004/1/1</td>
<td>Yellow/light orange(^ {21})</td>
<td>7.0-43.2(^ {21})</td>
<td></td>
</tr>
<tr>
<td>Bred from SPK004-Uganda</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SPK004/6</td>
<td>Orange(^ {21})</td>
<td>4.6-50.4(^ {21})</td>
<td></td>
</tr>
<tr>
<td>SPK004/6/6</td>
<td>Orange(^ {21})</td>
<td>7.9-38.3(^ {21})</td>
<td></td>
</tr>
</tbody>
</table>
Table 2. Total carotenoid loss (dry weight basis) related to processing trial, drying time and weather conditions for two cultivars combined (Ejumula and Kakamega) of sweetpotato dried on five types of dryers in Uganda

<table>
<thead>
<tr>
<th>Trial</th>
<th>Wind Speed* (m/s)</th>
<th>T* (ºC)</th>
<th>RH* (%)</th>
<th>Solar Radiation* (W/m²)</th>
<th>Drying on dryers time* (h)</th>
<th>Total drying time** (h)</th>
<th>Total carotenoid loss (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>1.8(1.4)</td>
<td>27.3(2.6)</td>
<td>62.4(16.4)</td>
<td>496.0(230.3)</td>
<td>11.9(1.8)</td>
<td>59.5(11.2)</td>
<td>8.4(3.3)</td>
</tr>
<tr>
<td>2</td>
<td>4.1(2.1)</td>
<td>31.2(2.6)</td>
<td>32.3(8.7)</td>
<td>781.9(183.2)</td>
<td>7.2(1.9)</td>
<td>16.9(10.0)</td>
<td>6.3(1.9)</td>
</tr>
<tr>
<td>Average-varieties</td>
<td>7.3 (2.8)</td>
<td>10.7(4.8)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

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.
Table 3. Losses in total carotenoids during solar-drying of Ejumula and Kakamega cultivars of sweetpotato dried in various solar and sun dryers under wet and dry weather conditions

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

*Mean; standard deviation is not given because <1% on triplicate extractions

**Exposure in dryers ***Mean (standard deviation) on triplicate extractions
### Table 4. Total carotenoid losses related to drying time and weather conditions in open air drying of six OFSP cultivars in Uganda.

<table>
<thead>
<tr>
<th>Trial</th>
<th>Wind Speed* (m/s)</th>
<th>T* (ºC)</th>
<th>RH* (%)</th>
<th>Solar Radiation* (W/m²)</th>
<th>Drying time on dryers (h)</th>
<th>Total drying time** (h)</th>
<th>Total carotenoid loss (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Namulonge</td>
<td>5.4 (2.0)</td>
<td>32.0(1.8)</td>
<td>31.7(12.6)</td>
<td>845.2(137.4)</td>
<td>4.9(0.2)</td>
<td>4.9 (0.2)</td>
<td>14.8(4.2)</td>
</tr>
<tr>
<td>Luwero</td>
<td>5.1(2.4)</td>
<td>32.0(1.6)</td>
<td>34.1(8.9)</td>
<td>752.3(277.9)</td>
<td>5.6(0.0)</td>
<td>24.5(0.0)</td>
<td>7.0(2.5)</td>
</tr>
<tr>
<td>Average</td>
<td>5.3 (4.7)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>13.6 (4.7)</td>
</tr>
</tbody>
</table>

Mean (standard deviation)

*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.
Table 5. Loss in carotenoids after open air sun-drying of different sweetpotato cultivars

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

*Mean; standard deviation is not given because <1% on triplicate extractions

**Exposure in dryers

***Mean (standard deviation) on triplicate extractions

\(\dagger\)Negative values do not differ significantly from total carotenoid content in fresh chips
Table 6. Losses of total carotenoids during the storage of OFSP dried chips at ambient temperature in polyethylene (PE) bags for 4 months (125 days).

<table>
<thead>
<tr>
<th>Cultivar</th>
<th>Treatment</th>
<th>Dry matter content* (%)</th>
<th>Total carotenoid content ** (µg g⁻¹ db)</th>
<th>Loss in storage (%)</th>
<th>Overall loss (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ejumula</td>
<td>Before storage</td>
<td>91.3</td>
<td>199.8(5.4)</td>
<td>76.6</td>
<td>85.4</td>
</tr>
<tr>
<td></td>
<td>Zipped PE bag</td>
<td>90.4</td>
<td>46.7(4.5)a</td>
<td>67.9</td>
<td>79.9</td>
</tr>
<tr>
<td></td>
<td>Sealed clear PE bag in black PE bag</td>
<td>88.4</td>
<td>64.2(1.0)b</td>
<td>67.9</td>
<td>79.9</td>
</tr>
<tr>
<td></td>
<td>Black PE bag with simple knot</td>
<td>88.4</td>
<td>58.2(4.6)b</td>
<td>70.9</td>
<td>81.8</td>
</tr>
<tr>
<td></td>
<td>Sealed clear PE bag</td>
<td>88.1</td>
<td>69.5(5.7)b</td>
<td>65.2</td>
<td>78.3</td>
</tr>
<tr>
<td>Kakamega</td>
<td>Before storage</td>
<td>91.3</td>
<td>52.4(3.6)a</td>
<td>77.2</td>
<td>84.8</td>
</tr>
<tr>
<td></td>
<td>Zipped PE bag</td>
<td>90.3</td>
<td>12.0(0.8)b</td>
<td>65.7</td>
<td>77.2</td>
</tr>
<tr>
<td></td>
<td>Sealed clear PE bag in black PE bag</td>
<td>88.8</td>
<td>18.0(0.5)b</td>
<td>63.7</td>
<td>75.8</td>
</tr>
<tr>
<td></td>
<td>Black PE bag with simple knot</td>
<td>88.7</td>
<td>19.0(1.0)b</td>
<td>63.7</td>
<td>75.8</td>
</tr>
<tr>
<td></td>
<td>Sealed clear PE bag</td>
<td>88.0</td>
<td>18.5(1.0)b</td>
<td>64.7</td>
<td>76.5</td>
</tr>
</tbody>
</table>

*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.
Table 7. Levels of total carotenoids (µg/g on a dry weight basis) in dried sweetpotato chips of six cultivars grown at Namulonge stored at ambient temperature for four months (125 days) in locally purchased black polythene bags.

<table>
<thead>
<tr>
<th>Cultivar</th>
<th>Dry matter content immediately after drying* (%)</th>
<th>Dry matter content after 4 month-storage* (%)</th>
<th>Total carotenoid content after 4 month-storage** (µg.g⁻¹ db)</th>
<th>Loss after 4 month-storage (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ejumula</td>
<td>92.9</td>
<td>88.0</td>
<td>58.5(1.7)</td>
<td>75.3</td>
</tr>
<tr>
<td>Kakamega</td>
<td>92.3</td>
<td>87.9</td>
<td>29.2(0.7)</td>
<td>69.8</td>
</tr>
<tr>
<td>SPK004/1</td>
<td>92.6</td>
<td>88.1</td>
<td>29.1(0.7)</td>
<td>65.5</td>
</tr>
<tr>
<td>SPK004/1/1</td>
<td>92.3</td>
<td>88.0</td>
<td>21.5(1.0)</td>
<td>68.9</td>
</tr>
<tr>
<td>SPK004/6</td>
<td>92.4</td>
<td>87.8</td>
<td>48.4(1.0)</td>
<td>69.7</td>
</tr>
<tr>
<td>SPK004/6/6</td>
<td>92.8</td>
<td>88.1</td>
<td>38.0(0.6)</td>
<td>73.2</td>
</tr>
</tbody>
</table>

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

**Mean (standard deviation) on triplicate extractions.
Table 8. 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).

<table>
<thead>
<tr>
<th>Cultivar</th>
<th>Estimated vitamin A activity RE. kg⁻¹ product on a fresh weight basis*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Freshly dried chips</td>
</tr>
<tr>
<td>Ejumula</td>
<td>15,202</td>
</tr>
<tr>
<td>Kakamega</td>
<td>6,163</td>
</tr>
<tr>
<td>SPK004/1</td>
<td>5,413</td>
</tr>
<tr>
<td>SPK004/1/1</td>
<td>4,430</td>
</tr>
<tr>
<td>SPK004/6</td>
<td>10,235</td>
</tr>
<tr>
<td>SPK004/6/6</td>
<td>9,124</td>
</tr>
</tbody>
</table>

*1 RE=13 µg of all-trans-β-carotene. All-trans-β-carotene content is estimated to 90% mean total carotenoids content. RE= Retinol Equivalents. Recommended daily requirements (RDA) of 2-6 year-olds is 400 RE.
Figure 1. UV/visible spectrum of polythene sheeting used in drying studies.
Figure 2. Relationship between initial dry matter content 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 loss are not represented on the figure but taken into account in the calculation of the coefficient of correlation (R).
Figure 3. Relationship between initial total carotenoid content (dry basis) and total carotenoid loss in drying for the sweetpotato cultivars from harvested from Namulonge and Luwero. One point represents the average result per sample (12). Each sample was analysed in triplicate. One point represents the average result per sample (12). Each sample was analysed in triplicate.

The abbreviations used 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 loss are not represented on the figure but taken into account in the calculation of the coefficient of correlation (R).
Figure 4. Variation in temperature and relative humidity with storage for sweet potato chips stored over 8 months (record was every 4h using Tinytalk temperature/humidity sensors)