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 ºC), 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-cyclocitral, 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 ºC; 25 ºC; 40 ºC) 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 &

Karel, 1983; Hidalgo & Brandolini, 2008; Koca et al, 2007*;* Lavelli et al., 2007; Walter & Purcell 1974).

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 Kujk, Chatterjee, & Krinsky; Mordi et al., 1993; Waché, Bosser-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-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 $MgCl_2$, 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:

$$\frac{a_w}{(1-a_w)M} = \frac{1}{M_0 C} + \frac{(C-1)}{M_0 C} a_w \quad \text{[Eq. 1]}$$

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 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 H_2O 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 C_{30} 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).

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 ºC 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, 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 (R^2) was 0.9993. The standards for the other norisoprenoids were not available and the selectivities for these compounds by the SPME fibre will vary from that for β-ionone. Therefore the real concentrations of these compounds could not be determined. The peak area response of the detector, however, indicates a relative concentration with storage time and this was sufficient for the follow up of these compounds. For 5,6-epoxy-β-ionone, β-cyclocitral and DHA, identified based on their mass spectra, the ratios (peak area at time t divided by peak area at initial time), also called relative contents, were calculated.

188 **Statistical analysis and kinetics modelling**

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

200

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

203 $$C = C_0 \, e^{-k_\infty \int_0^t e^{\frac{-Ea}{RT}} dt} \quad \text{[Eq. 2]}$$

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

212

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 (R^2) suggest that a first-order equation fitted well the carotenoid degradation. An exception nonetheless was at 10 ºC where correlations observed were lower (R^2~0.8). Lower correlations at low temperatures where there is minimum carotenoid degradation could be explained by experimental errors (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 ($R^2 > 0.990$ 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 ($R^2 > 0.997$ 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.

239

240 Description of total carotenoids by spectrophotometer for Arrhenius and Eyring models
241 fitted well the degradation (R^2 > 0.997 and 0.997 respectively). Activation energy for
242 total carotenoids content (by spectrophotometric reading) being 46.3 $kJ.mol^{-1}$ was
243 similar to the value of 44.3 $kJ.mol^{-1}$ for freeze-dried sweet potato at 60-80 ºC
244 (Stephanovitch and Karel, 1982). Similar activation energies of 45.3 and 48.7 $kJ.mol^{-1}$
245 were measured for total carotenoids on Serio and Monlins wholemeal wheat flour
246 respectively, stored between -20 ºC and 38 ºC (Hidalgo & Brandolini, 2008).

247

248 To test the robustness of the model, it was used to predict the carotenoid content of
249 dried sweet potato sample that had been stored at ambient temperature in a jar and in the
250 dark for 88 days in a laboratory in the UK. For the total carotenoids and trans
251 β-carotene under anisothermic conditions, the difference between the experimental
252 value and value predicted by the model was 4.3% and 3.5% respectively (Table 3). The
253 robustness of the model was further tested by using it to predict the carotenoid content
254 of a dried sweet potato sample (Ejumula) that had been stored in Uganda at ambient
255 temperature in LPDE bags (permeable to oxygen) for 125 days in Uganda (Bechoff et
256 al., 2009b). Similarly, the model was also accurate in its predictions where for total
257 carotenoids under anisothermic conditions with a difference between the experimental
258 value and model of 9.3% (Table 3). Therefore it can be concluded that the model
259 developed with samples stored under controlled laboratory conditions was robust
260 enough to apply to samples stored under field conditions in Uganda and elsewhere.

261

262 Predictions of carotenoid losses in dried sweet potato chips using the kinetic models
263 developed are represented in Fig. 1. These can be used for practical applications, such
264 as the determination of product shelf life. For instance, for a 20% loss in β-carotene

(Fig. 1A), the predicted storage duration in tropical conditions (average 30 ºC) is about 10 days. On the other hand if the dried sweet potato is stored at ambient temperature (average 20 ºC) the predicted storage time is one month. If it is stored in fridge conditions (average 10 ºC) 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 ºC, 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 ºC 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 ºC) 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) 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 rate constants 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 (10-40ºC) with 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 40 ºC, 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). (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). 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 (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 ºC, 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 40º 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

343 showing a direct relationship between oxygen uptake and carotenoid degradation
344 (Walter & Purcell, 1974). The linear relationship for the four levels of oxygen analysed
345 in triplicate (R^2=0.975) between oxygen level and degradation rate signifies that oxygen
346 could be considered as a co-substrate (in excess) of oxidative degradation during
347 storage (Table 1C). A study on sweet potato dried flakes similarly described a linear
348 relationship between the oxygen uptake and carotene destroyed in the first 110 days of
349 storage at 31 ºC (Walter & Purcell 1974).
350
351 Over the range studied (0-21% oxygen) oxygen had a more marked effect on β-carotene
352 than water activity (0.13-0.76) and temperature (10-40ºC). The mean difference
353 between rate constants was 0.0746 day^{-1}, 0.0152 $day^{-1}$and 0.0376 day^{-1} respectively.
354 Studies on other foodstuffs similarly concluded that oxygen was a major factor of
355 degradation during storage. Working on the effect of packaging (polypropylene: high
356 oxygen permeability; nylon laminate film (low oxygen permeability) with air space;
357 under vacuum or using a Ageless oxygen absorber sachet) on sweet potato flakes it was
358 demonstrated that oxygen had a major impact on carotenoid degradation (*3*): sweet
359 potato flakes stored for 210 days in nylon film and with oxygen absorber did not lose
360 significant amounts of β-carotene whilst those stored in polypropylene lost 66% of their
361 initial content at ambient temperature (about 23 ºC). In this present study, the β-carotene
362 loss on sweet potato chips stored under nitrogen (16% loss after 21 days) (Fig. 6) might
363 result of incomplete oxygen exclusion of samples. Alternatively it could also result of
364 the effect of the relatively high temperature used for the storage (40 ºC).
365
366 Studies on a model system made of microcrystalline cellulose and β-carotene similarly
367 demonstrated that the effect of oxygen was important for the degradation of β-carotene
368 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

396 stored at a_w = 0.13 and 0.30; and at 0.51 and 0.76. On the other hand, there was a
397 significant difference in the DHA and 5,6-epoxy-β-ionone ratios for the four water
398 activities (two way-ANOVA; $p<0.05$). This description of differences between volatile
399 relative concentrations agreed with the above description of β-carotene degradation at
400 various water activities.
401
402 Relative contents of β-cyclocitral, β-ionone and 5,6-epoxy-β-ionone were the highest at
403 19 days and subsequently decreased whilst DHA levels reached a plateau after 27 days
404 of storage at 40 ºC (Fig. 3). Epoxidation of β-ionone into 5,6-epoxy-β-ionone and
405 thermal rearrangement of 5,6-epoxy-β-ionone into DHA has been described by several
406 authors (Bosser, Paplorey & Belin, 1995; Mordi et al.,1993; Waché et al., 2003). The
407 similar profile of β-ionone and 5,6-epoxy- β-ionone throughout storage suggested that
408 the two compounds were formed at similar times via the same process whereas the later
409 formation of DHA suggested that the rearrangement into DHA was a slower step.
410
411 Fragmentation of β-carotene in dehydrated sweet potato flakes under oxygen has been
412 reported previously (Walter et al., 1970). Flushing radioactive sweet potato flakes with
413 oxygen in the dark at 22 ºC and storing them for up to 89 days resulted in different
414 radioactive fractions including gaseous products. The degradation was described as an
415 autoxidation reaction (Walter et al., 1970) in accordance with the conclusions in this
416 study. The higher formation of norisoprenoids occurred at the higher oxygen level when
417 the corresponding degradation of β-carotene was also highest. At 40 ºC samples stored
418 flushed with compressed air (21% oxygen) had a β-ionone content of 0.44μg.g^{-1},
419 followed by those flushed with 10% (0.36μg.g^{-1}); 2.5% (0.22μg.g^{-1}) oxygen or nitrogen
420 (0.10μg.g^{-1}), respectively (Fig. 4). Similarly there was a significant increase in the β-

421 cyclocitral, 5,6-epoxy-β-ionone and DHA ratios at the four increasing oxygen levels
422 (two ways-ANOVA; $p<0.05$).
423
424 With no oxygen present in the storage conditions, only a small decrease or no difference
425 in volatile compounds contents was observed. These highlighted how oxygen is
426 important in the degradation scheme of β-carotene into volatile compounds (Fig. 4).
427
428 β-cyclocitral, β-ionone and 5,6-epoxy- β-ionone reached their highest levels after 9 days
429 of storage and tended to subsequently decrease whilst DHA remained almost steady
430 from 9 days (Fig. 6). This is a similar pattern to that observed at different water
431 activities.
432
433 The amounts of β-ionone observed in the SPME-GC-MS analyses corresponded to
434 approximately two orders of magnitude less than the amounts of β-carotene measured
435 by HPLC (Figures 3&4). Calibration curves for the other volatile degradation products
436 were not measured so the accurate amounts present cannot be derived from the SPME
437 analyses. However, these compounds have relatively similar molecular weights and
438 polarities to the β-ionone and so the GC-MS response factors and selectivities of the
439 SPME fibres are expected to be reasonably similar. Accepting these assumptions,
440 amounts of the other degradation products are similar to those of β-ionone with
441 maximum amounts per compounds (β-ionone, 5,6-epoxy-β-ionone, β-cyclocitral and
442 DHA) being approximately 0.4-0.9 $\mu g.g^{-1}$, will are much lower than the amounts of
443 carotenoids degraded (Figures 3&4). Waché et al. (2003) also found that the highest
444 yield obtained from β-carotene catalysed by enzymes in liquid medium was 8.5% in
445 DHA, 2% in β-ionone and 1% in 5,6-epoxide-β-ionone. These results suggested that
446 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. 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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References

Auldridge, M.E., McCarty, D.R & Klee, H.J. (2006). Plant carotenoid cleavage oxygenases and their apocarotenoid products. *Current Opinion in Plant Biology*, *9*, 315-321.

Bechoff, A., Dufour, D., Dhuique-Mayer, C., Marouze, C., Reynes, M,. & Westby, A. (2009a). Effect of hot air, solar and sun drying treatments on provitamin A retention in orange-fleshed sweet potato. *Journal of Food Engineering*, *92*, 164–171.

Bechoff, A., Westby, A., Owori, C., Menya, G., Dhuique-Mayer, C., Dufour D. & Tomlins K. (2009b). Effect of drying and storage on the degradation of carotenoids in orange-fleshed sweet potato varieties. *Journal of the Science of. Food and Agriculture.*, submitted Manuscript ID: JSFA-09-0658.

Bengtsson, A., Namutebi, A., Larsson Alminger, M.& Svanberg, U. (2008). Effects of various traditional processing methods on the all-trans-β-carotene content of orange-fleshed sweet potato. *Journal of Food Composition and Analysis*, *21*, 134-143.

Bimbenet, J.J., Duquenoy, A. & Trystram, G. (2002) Génie des procédés alimentaires: des bases aux applications, edition no. 2, RIA Editions. Lavoisier: Paris, France, 574pp.

Bosser, A., Paplorey, E. & Belin, J.M. (1995). A simple way to dihydroactinidiolide from β-ionone related to the enzymic co-oxidation of β-carotene in aqueous solution. *Biotechnology Progress*, *11*, 689-692.

Castillo Leon, J., Alpeeva, I.S., Chubar, T.A., Galaev, I.Y., Csoregi, E. & Sakharov, IY. (2002). Purification and substrate specificity of peroxidases from sweet potato tubers. *Plant Science*, *163*, 1011-1019.

Chou, H.E. & Breene, W.M. (1972). Oxidative decoloration of β-carotene in low-moisture model systems. *Journal of Food Science*, *37*, 66-68.

Cinar I. (2004). Carotenoid pigment loss of freeze-dried plant samples under different storage conditions. *Lebensmittel Wissenschaft und Technologie*, *37*, 363–367.

Cisse, M., Vaillant, F., Acosta, O., Dhuique-Mayer, C. & Dornier, M. (2009). Thermal degradation kinetics of anthocyanins from blood orange, blackberry, and roselle using the Arrhenius, Eyring and Ball models. *Journal of Agricultural and Food Chemistry*, *57*, 6285-91.

Cremer, D.R. & Eicher, K. (2000). Formation of volatile compounds during heating of spice paprika (*Capsicum annuum*) powder. *Journal of Agricultural and Food Chemistry*., *48*, 2454-2460.

Dhuique-Mayer, C., Tbatou, M., Carail, M., Caris-Veyrat, C., Dornier, M. & Amiot, M.J. (2007). Thermal degradation of antioxidant micronutrient in citrus juice and Newly Formed Compounds. *Journal of Agricultural and Food Chemistry*, *55*, 4209–4216.

Emenhiser, C., Watkins, R.H., Simunovic, N. , Solomons, N., Bulux, J., Barrows, J. & Schwartz, S.J. (1999). Packaging preservation of β-carotene in sweet potato flakes using flexible film and an oxygen absorber. *Journal of Food Quality*, *22*, 63-73.

Gayathri, G.N., Platel, K., Prakash, J. & Srinivasan, K. (2004). Influence of antioxidant spices on the retention of *β*-carotene in vegetables during domestic cooking processes. *Food Chemistry*, *84*, 35-43.

Goldman, M., Horev, B. & Saguy, I. (1983). Decolorization of β-carotene in model systems simulating dehydrated foods. Mechanism and kinetic principles. *Journal of Food Science*, *48*, 751-754.

Handelman, G.J., van Kujk, F.J., Chatterjee, A. & Krinsky, N.I. (1991).Characterization of products formed during the autoxidation of β-carotene. *Free Radical Biology and Medicine*, *10*, 427-437.

Haralampu, S.G. & Karel, M. (1983). Kinetic models for moisture dependence of ascorbic acid and β-carotene degradation in dehydrated sweet potato. *Journal of Food Science*, *48*, 1872-1873.

Hidalgo, A.H.& Brandolini, A (2008.) Kinetics of carotenoid degradation during the storage of einkorn (*Triticum monococcum L. ssp. monococcum*) and bread wheat (*Triticum aestivum L. ssp. aestivum*) flours. *Journal of Agricultural and Food Chemistry, 56*, 11300-11305.

Kamiya, N. & Nagamune, T. (2002). Effect of water activity control on the catalytic performance of surfactant – Arthromyces ramosus peroxidase complex in toluene. *Biochemical Engineering Journal*, *10*, 55-59.

Koca, N., Burdurlu, H.S. & Karadeniz, F. (2007). Kinetics of color changes in dehydrated carrots. *Journal of Food Engineering*, *78*, 449-455.

Kosambo, L., Carey E.E., Misra, A.K., Wilkes, J. & Hagenimana, V. (1998). Influence of age, farming site, and boiling on pro-vitamin A content in sweet potato (*Ipomoea batatas* (L.) Lam.). *Journal of Food Composition and Analysis*, *11*, 305-321.

Krinsky, N. I., Kyung-Jin, Y. (2003). Carotenoid-radical interactions. *Biochemical and Biophysical Research Communications*, *305*, 754-760.

Lavelli, V., Zanoni, B. & Zaniboni, A. Effect of water activity on carotenoid degradation in dehydrated carrots (2007). *Food Chemistry*, *104*, 1705-1711.

Mendes-Pinto, M.M. (2009). Carotenoid breakdown products the - norisoprenoids - in wine aroma. *Archives of Biochemistry and Biophysics*, *483*, 236-245.

Mordi, R.C, Walton, J.C., Burton, G.W., Ingold, K.U., Lindsay, D.A. & Moffatt, D.J. (1993). Oxidative degradation of β-carotene and β-apo-8'-carotenal. *Tetrahedron*, *49*, 911-928.

Nonier, M.F., Vivas, De Gaulejac, N. & Vitry, C. (2004). Characterization of carotenoids and their degradation products in oak wood. Incidence on the flavour of wood. *Comptes Rendus Chimie*, *6*, 689-698.

Ravichandran, R. (2002). Carotenoid composition, distribution and degradation to flavour volatiles during black tea manufacture and the effect of carotenoid supplementation on tea quality and aroma. *Food Chemistry*, *78*, 23-28.

Rodriguez Amaya, D.B. & Kimura, M. (2004) HarvestPlus handbook for carotenoid analysis. HarvestPlus Technical monograph series 2.. HarvestPlus handbook for carotenoid analysis. (pp. 1-58) Washington DC, USA: HarvestPlus c/o International Food Policy Research Institute. [Online] http://www.harvestplus.org/pdfs/tech02.pdf

Sanakis, Y., Mamma, D., Christakopoulos, P. & Stamatis, H. (2003). Catechol 1,2 dioxygenase from *Pseudomonas putida* in organic media- an electron paramagnetic resonance study. *International Journal of Biological Macromolecules*, *33*, 101-106.

Stephanovitch, A.F. & Karel, M. (1982). Kinetics of β-carotene degradation at temperatures typical of air drying of foods. *Journal of Food Processing & Preservation*, *6*, 227-242.

Texeira Neto, R.O., Karel, M., Saguy, I. & Mizrahi, S. (1981). Oxygen uptake and β-carotene decoloration in a dehydrated food model. *Journal of Food Science*, *46*, 665-676.

Waché, Y., Bosser-Deratuld, A., Lhuguenot, J.C & Belin, J.M. (2003). Effect of cis/trans isomerism of β-carotene on the ratios of volatile compounds produced during oxidative degradation. *Journal of Agricultural and Food Chemistry*, *51*, 1984-1987.

Walter, W.M., Purcell, A.E & Cobb, W.Y. (1970). Fragmentation of β-carotene in autoxidizing dehydrated sweet potato flakes. *Journal of Agricultural and Food Chemistry*, *18*, 881-885.

Walter, W.M. & Purcell, A.E. (1974). Lipid autoxidation in precooked dehydrated sweet potato flakes stored in air. *Journal of Agricultural and Food Chemistry*, *22*, 298-302.

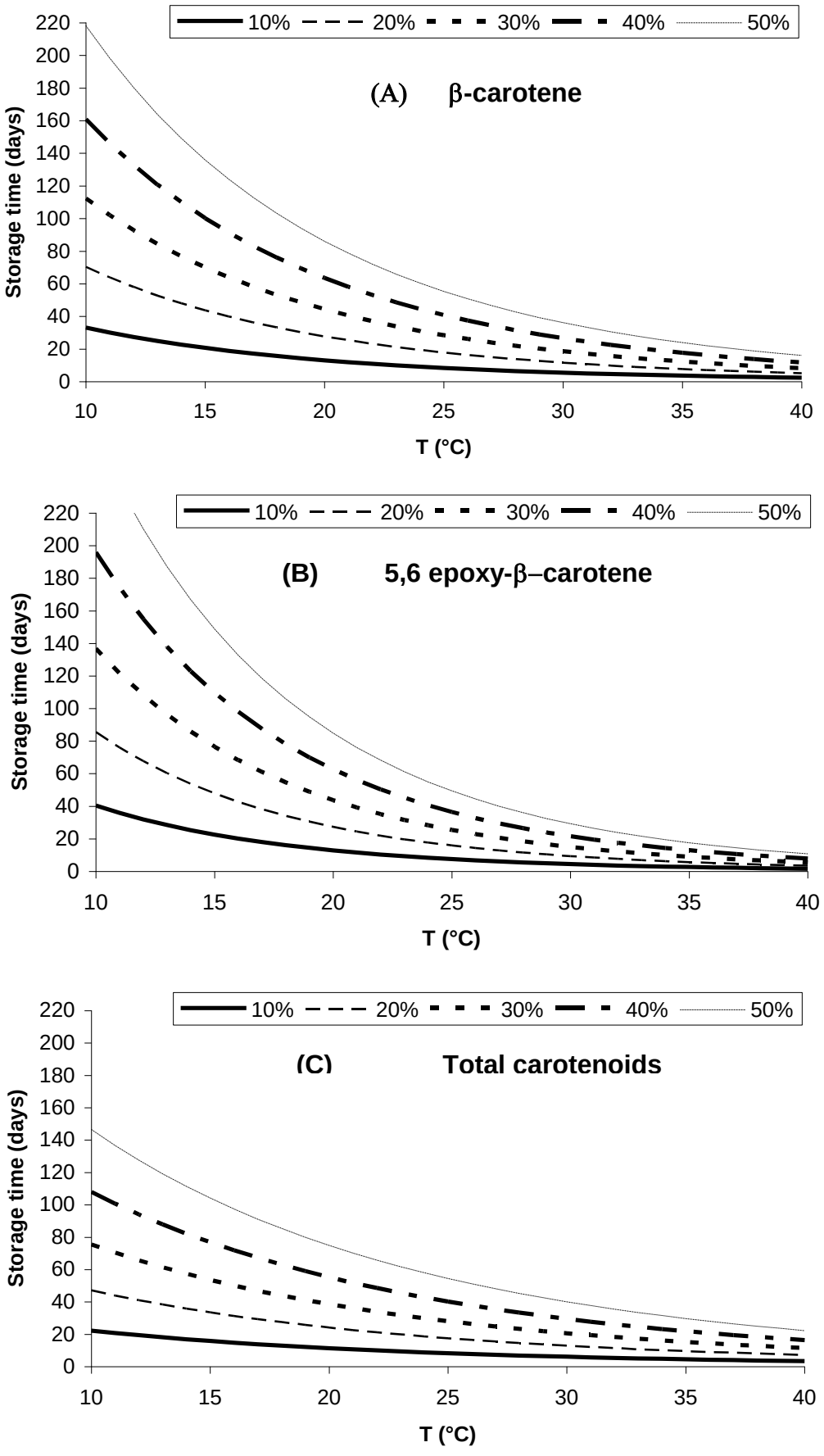

Fig. 1. Prediction curves of carotenoid (β-carotene; 5,6-epoxy-β-carotene; total-carotenoids) loss (%) with temperature and with storage time in dried sweet potato chips stored between 10-40 ºC in air; a_w 0.52-0.65.

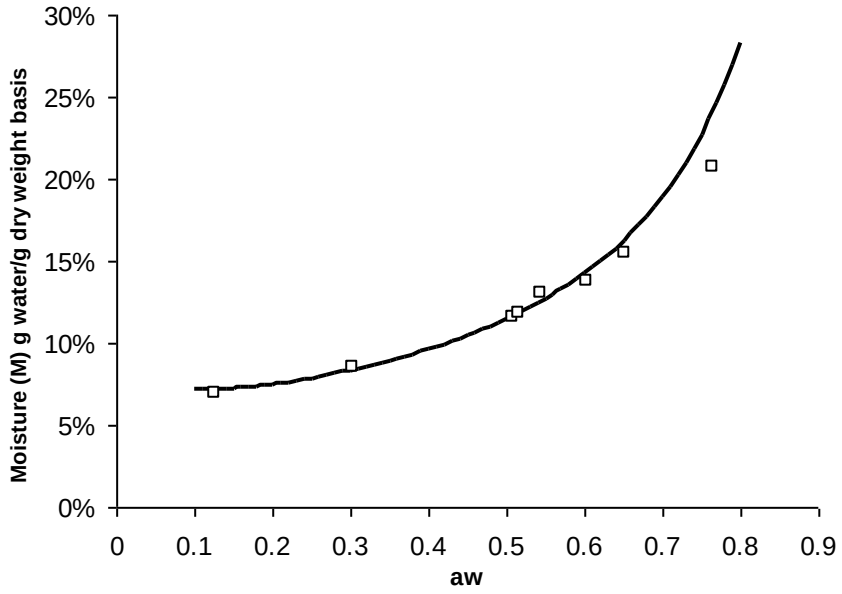

Fig. 2. Water sorption curves of dried sweet potato chips stored at 40 ºC for 54 days under air. Experimental data and BET model (C = -68.88, M_0 = 0.0564).

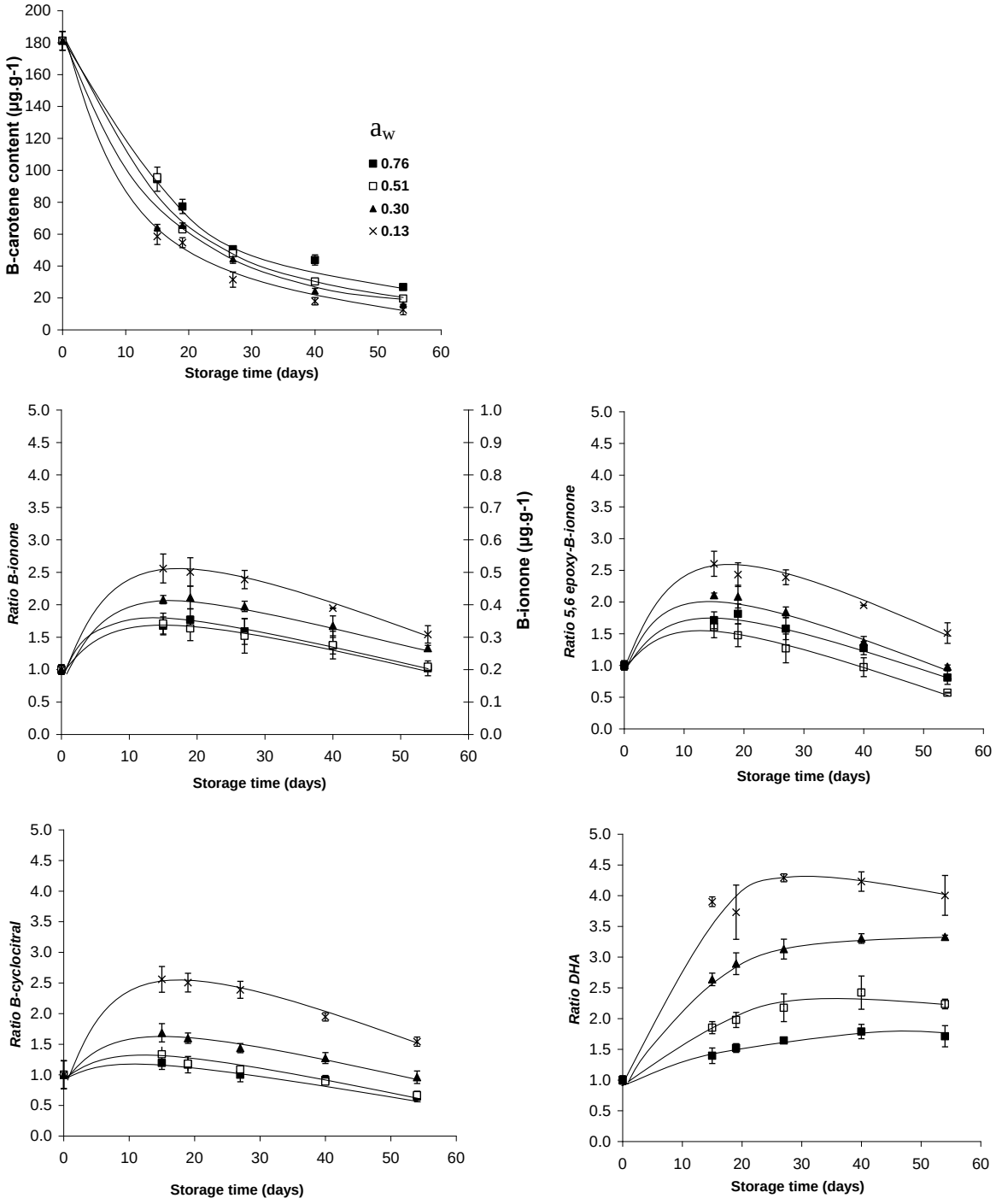

Fig. 3. Degradation of trans-β-carotene and production of identified norisoprenoids during storage of dried sweet potato chips at 40 ºC under air at different water activities (β-carotene and β-ionone expressed as µg.g^{-1} fresh weight; volatile degradation products, as ratio: peak area at time t divided by peak area at initial time); Error bars refer to standard deviation (n=3).

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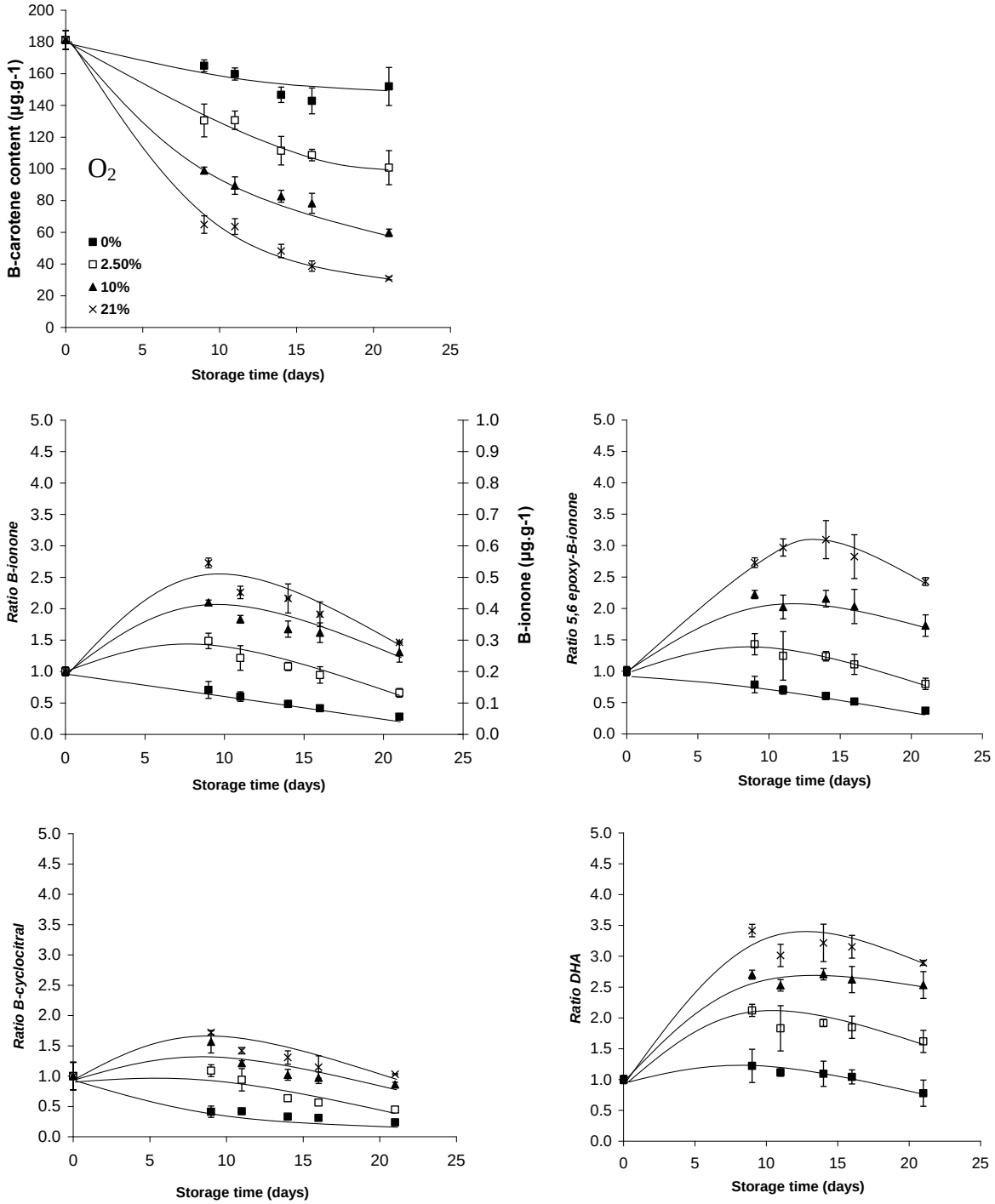

Fig. 4. Degradation of trans-β-carotene and production of identified norisoprenoids during storage of dried sweet potato chips at 40 ºC at different oxygen levels (β-carotene and β-ionone expressed as $\mu g.g^{-1}$ fresh weight; volatile degradation products as ratios: peak area at time t divided by 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)

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

(B)

Water activity		**0.76***	**0.51***	**0.30***	**0.13***
***β*-carotene**	**k**	**0.0341 (0.0010)**	**0.0413 (0.0005)**	**0.0460 (0.0032)**	**0.0493 (0.0029)**
	R^2	0.984 (0.003)	0.977 (0.001)	0.974 (0.003)	0.949 (0.048)

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

(C)

Flushed oxygen level**		**0%***	**2.5%***	**10%***	**21%***
***β*-carotene**	**k**	**0.0107 (0.0021)**	**0.0303 (0.0053)**	**0.0514 (0.0022)**	**0.0853 (0.0038)**
	R^2	0.675 (0.290)	0.944 (0.020)	0.968 (0.006)	0.961 (0.013)

*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 ºC. Oxygen level 21% (air); a_w 0.52-0.65. Mean of triplicate thermal treatment (standard deviation).

Carotenoid	Arrhenius model			Eyring model		
	Ln k_∞ (k_∞ days^{-1})	E_a (kJ.mol^{-1})	R^2	ΔH* (kJ.mol^{-1})	ΔS* (J.mol^{-1}.K^{-1})	R^2
β-carotene	21.5 (0.6)	64.2 (1.6)	0.990 (0.007)	61.7 (1.6)	-74.3 (5.1)	0.989 (0.008)
5,6 epoxy-β-carotene	27.5 (1.5)	78.8 (3.8)	0.997 (0.003)	76.3 (3.8)	-24.5 (12.5)	0.996 (0.003)
Total carotenoids	14.3 (0.6)	46.3 (1.5)	0.997 (0.002)	43.8 (1.5)	-134.5 (5.0)	0.997 (0.002)

Arrhenius model

$k = k_\infty e^{-\frac{Ea}{RT}}$ 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

$k = \frac{k_B}{h} T.e^{-\frac{\Delta H^* - T\Delta S^*}{RT}}$ 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[a] and during 125 days in Uganda[b] on a fresh weight basis. Oxygen level 21% (air).

			Initial	Final		
		Storage time (days)	($\mu g.g^{-1}$)[c]	Experimental[c] ($\mu g.g^{-1}$)	Predicted by Arrhenius model ($\mu g.g^{-1}$)	Difference (%)
Sample stored in UK[a]	**Trans-β-carotene**	88	181.2 (5.9)	74.6 (5.1)	72.0	3.5
	Total carotenoids		250.3 (1.1)	94.4 (1.1)	90.3	4.3
Sample stored in Uganda[b]	**Total carotenoids**	125	219.6 (1.6)	51.4 (1.5)	46.6	9.3

[a] This present study (Calculated a_w from BET model: 0.460 (0.012) from chips dry matter (90.4 (0.3) g/100g)
[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])
[c]Mean of triplicate (standard deviation).