



Relationship between the kinetics of β -carotene degradation and formation of norisoprenoids in the storage of dried sweet potato chips

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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 -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 autooxidation because of the trend in β -carotene degradation rate in relation to water activity or oxygen level.

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1. 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; Bengtsson, 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 325 $\mu\text{g g}^{-1}$ β -carotene on a dry basis (Bengtsson et al., 2008), therefore making it a very good source of pro-vitamin A. These varieties could contribute in tackling vitamin A deficiency, a main public health issue in the developing world (Bechoff et al., 2009a; Bengtsson et al., 2008). However, degradation of pro-vitamin A during the storage of dried sweet potato chips at ambient temperature has been demonstrated to be a significant problem (Bechoff et al., 2009b; Emenhiser et al., 1999).

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 & Karel, 1983; Hidalgo & Brandolini, 2008; Koca, Burdurlu, & Karadeniz, 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

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industry. Authors have described volatile products resulting from degradation of pure β -carotene; (Handelman, van Kujk, Chatterjee, & Krinsky, 1991; 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 (Ravi-chandran, 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 °C) 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 °C. 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.

2. Materials and methods

2.1. 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), Kawan-da, Uganda with an average drying temperature $\pm$ standard deviation of 28.8 ± 2.8 °C and $53.3 \pm 13.6\%$ 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 (90 g) in a sewed cotton bag were placed into a 1.5 l-clip top jar containing a salt (150 g) saturated with deionised water at 40 °C 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 °C; 20 ± 1 °C; 30 ± 0.5 °C and 40 ± 1 °C. Such temper-

atures are similar to those encountered in tropical countries where sweet potato is grown. Samples tested at different temperatures were equilibrated with NaBr ($a_w = 0.579 \pm 0.060$) 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 Eq. (1) is expressed as follows:

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

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

2.2. Carotenoid analyses

The samples were extracted using a slightly modified Harvest-Plus method (Rodríguez Amaya & Kimura, 2004). A portion of the homogeneous representative sample (0.5–2.0 g of flour) was homogenised with 50 ml methanol:tetrahydrofuran (THF) (1:1) using an Ultra-turax homogeniser (IKA Janke and Kunkel Labor-technik, Staufen, Germany) at 8000 rpm/min for one minute. 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 50 ml 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 450 nm 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 1 ml 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 Dhui-que-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: 0 min: 40%A/60%B; 0–5 min: 20%A/80%B; 5–10 min: 4%A/81%B/15%C; 10–60 min: 4%A/11%B/85%C; 60–71 min: 100%B; 71–72 min back to the initial condition for re-equilibration. Carotenoids were separated through a C_{30} reverse phase column

(250 × 4.6 mm i.d.) packed with 5 µm YMC (EUROP GmbH, Germany) with a flow rate of 1 ml 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 290–470 nm). Concentrations were determined by comparison to a standard curve using pure β-carotene (Extrasynthese, Genay, France) (Bechoff et al., 2009a).

2.3. 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 (3.0 g) were weighed into a 10 ml glass vial and were capped with an air-tight 20 mm 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 5973 N mass spectrometer. Chromatographic separation was achieved with a DB-Wax (J&W Scientific, Folson, CA) fused silica capillary column (60 m × 0.32 mm 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* 40–300. 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.

2.4. 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*_∞). The Eyring model is based on the transition state theory in which enthalpy of activation ($\Delta H^\ddagger$) and entropy of activation ($\Delta S^\ddagger$) 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 Eq. (2):

$$C = C_0 e^{-k_\infty \int_0^t e^{\frac{E_a}{RT}} dt} \quad (2)$$

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).

3. Results and discussion

3.1. Carotenoid degradation kinetics

The effect of temperature (10; 20; 30; 40 °C) 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 °C 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 °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 (*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 (*E*_a) 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 & 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

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 40 °C in air (B); at various oxygen (flushed) levels at 40 °C (C). Mean of triplicate thermal treatment (standard deviation).

Temperature (°C)		10	20	30	40
(A)					
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)
(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: 0.953 (0.013).

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

*** 90 ml 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).

Carotenoid	Arrhenius model			Eyring model		
	$\ln k_\infty$ (k_∞ days $^{-1}$)	E_a (kJ.mol $^{-1}$)	R^2	$\Delta H^\ddagger$ (kJ mol $^{-1}$)	$\Delta S^\ddagger$ (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:

$k = k_\infty e^{-\frac{E_a}{RT}}$ where: T : temperature (K); k : degradation rate constant at T (day $^{-1}$); k_∞ : value of k at $T = \infty$ (day $^{-1}$); E_a : activation energy (kJ mol $^{-1}$); R : gas constant = 8.314 J K $^{-1}$ mol $^{-1}$.

Eyring model:

$k = \frac{k_B}{h} T e^{-\frac{\Delta H^\ddagger}{RT}} e^{\frac{\Delta S^\ddagger}{R}}$ where: k_B : Boltzmann constant = 1.381×10^{-23} J K $^{-1}$; h : Planck constant = 6.626×10^{-34} Js; $\Delta H^\ddagger$: activation enthalpy (kJ mol $^{-1}$); $\Delta S^\ddagger$: activation entropy (J mol $^{-1}$ K $^{-1}$).

trans-β-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 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 shown 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 β-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 (E_a) 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

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).

Storage time (days)			Initial	Final	Difference (%)	
			($\mu\text{g g}^{-1}$) ^c	Experimental ^c ($\mu\text{g g}^{-1}$)	Predicted by Arrhenius model ($\mu\text{g g}^{-1}$)	
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/100 g).

^b Bechoff et al. (2009b) (a_w from BET model: 0.400 (0.255); range: [0.22–0.58] from chips dry matter 90.5 (3.5) g/100 g; range: [88–92.9 g/100 g]).

^c Mean of triplicate (standard deviation).

be 160, 200 and 110 days for a 40% loss in β -carotene, 5,6-epoxy- β -carotene or total carotenoids respectively.

3.2. 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

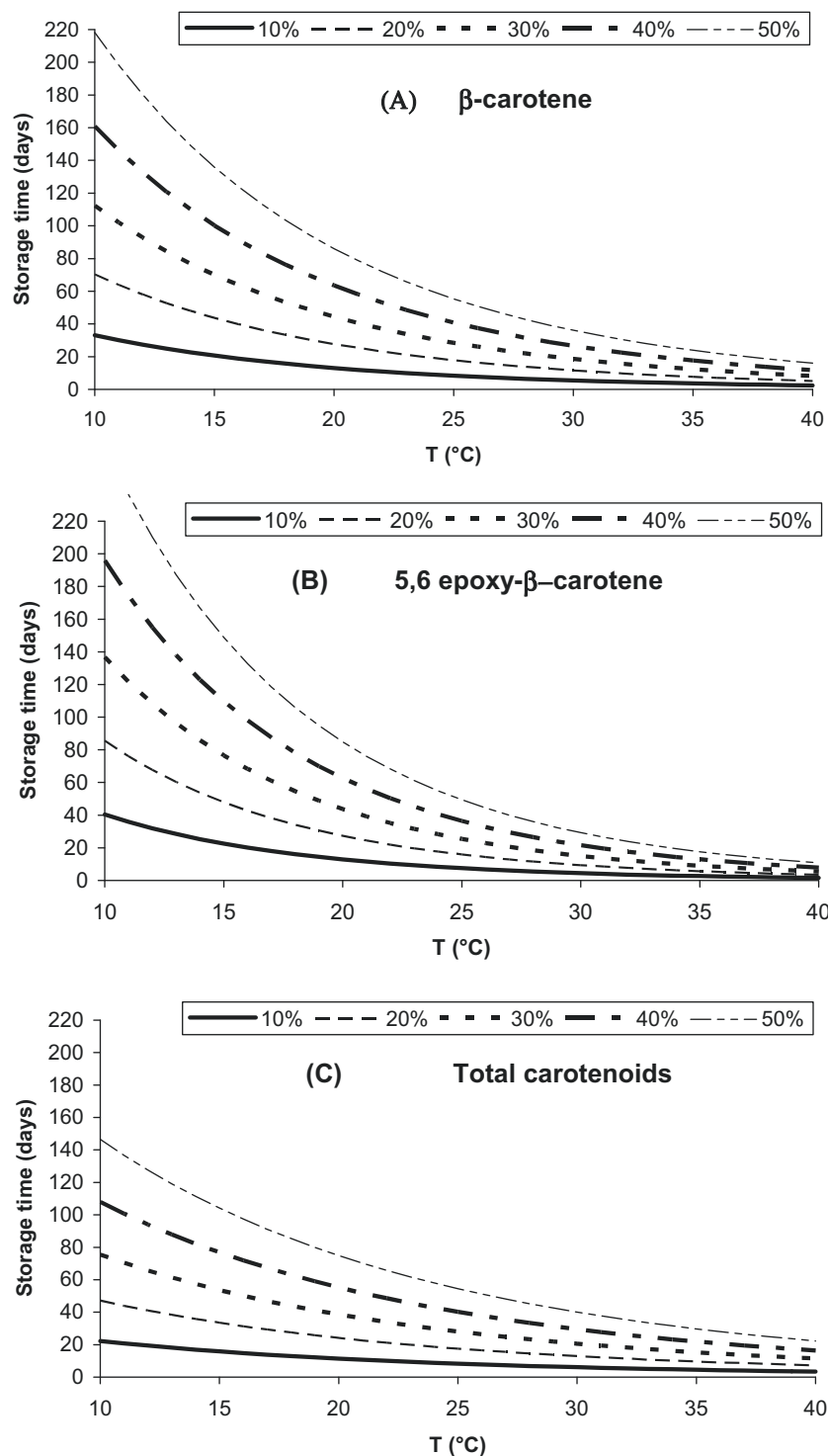


Fig. 1. Prediction curves of carotenoid (β -carotene; 5,6-epoxy- β -carotene; total carotenoids) loss (%) with temperature and with storage time in dried sweet potato chips stored between 10–40 °C in air; a_w 0.52–0.65.

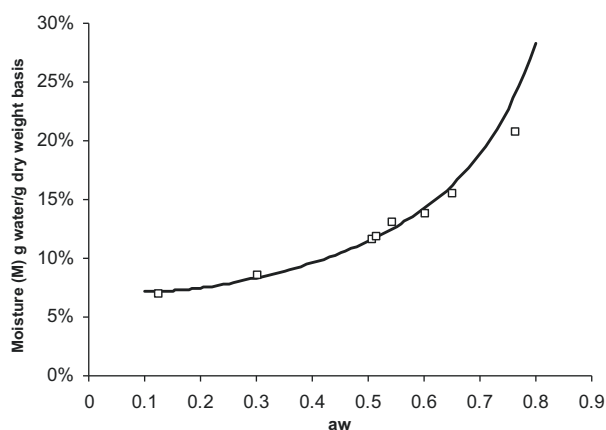


Fig. 2. Water sorption curves of dried sweet potato chips stored at 40 °C for 54 days under air. Experimental data and BET model ($C = -68.88$, $M_0 = 0.0564$).

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) 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 (10–40 °C) with rate constants of 0.0029–0.0405 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 °C, 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 and Breene (1972) proved that β -carotene degradation followed first order kinetics and was accelerated at lower water activities. In particular, when comparing extreme water activities (dry a_w 0.33 and wet a_w 0.84), it was demonstrated 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). Dioxxygenases known for their ability to degrade carotenoids into aroma compounds (Auldrige, McCarty, & Klee, 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 (0.0341–0.0493 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 °C (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 (10–40 °C). 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 °C). In this present study, the β -carotene loss on sweet potato chips stored under nitrogen (16% loss after 21 days) (Fig. 4) 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 °C).

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 autooxidation 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 (Auldrige et al., 2006) emphasized that it had not been proved. This study therefore fills the gap by showing that dehydrated sweet potato

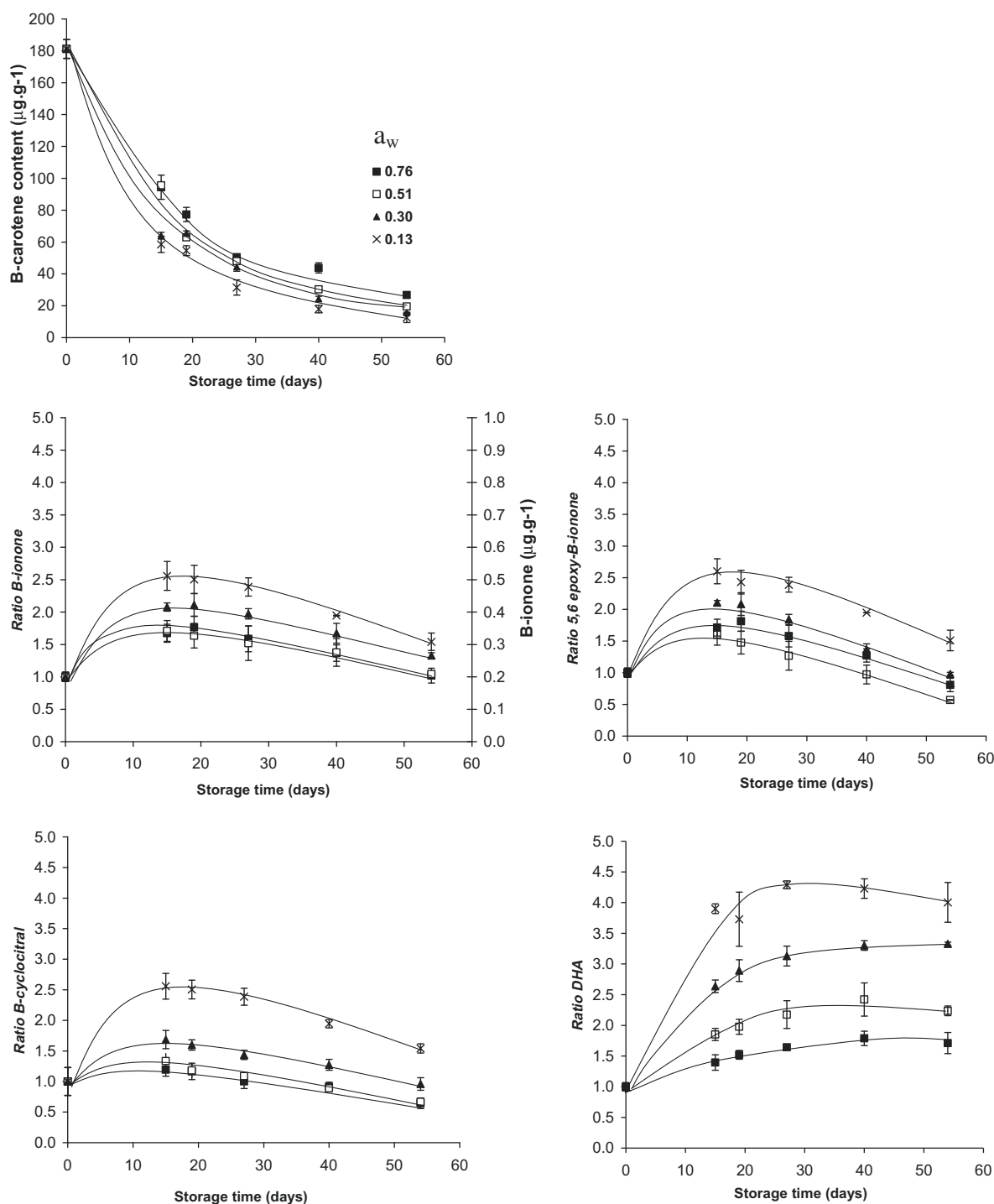


Fig. 3. Degradation of trans- β -carotene and production of identified norisoprenoids during storage of dried sweet potato chips at 40 °C under air at different water activities (β -carotene and β -ionone expressed as $\mu\text{g g}^{-1}$ fresh weight; volatile degradation products, as ratio: peak area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

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.

3.3. 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, van Kujik, Chatterjee, & Krinsky, 1991; Mordí 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 $0.47 \mu\text{g g}^{-1}$, followed by those stored at $a_w = 0.30$ with $0.38 \mu\text{g g}^{-1}$; at $a_w = 0.51$ had a β -ionone

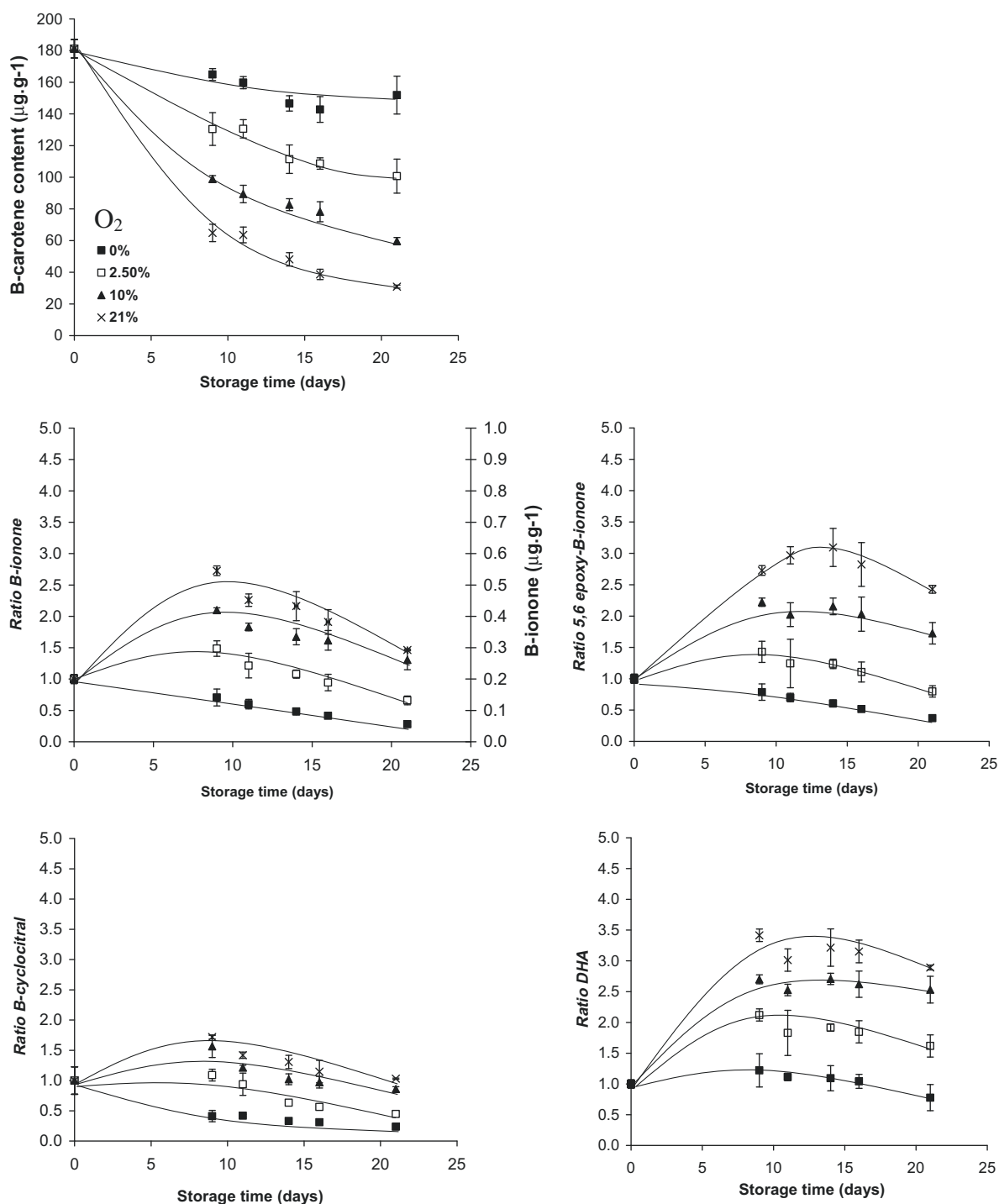


Fig. 4. Degradation of trans-β-carotene and production of identified norisoprenoids during storage of dried sweet potato chips at 40 °C at different oxygen levels (β-carotene and β-ionone expressed as μg g⁻¹ fresh weight; volatile degradation products as ratios: peak area at time *t* divided by peak area at initial time); Error bars refer to standard deviation (*n* = 3).

content of 0.30 μg g⁻¹ and at *a_w* = 0.76 of 0.31 μg 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-β-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 β-carotene degradation at various water activities.

Relative contents of β-cyclocitral, β-ionone and 5,6-epoxy-β-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 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 (Bossier, 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 °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 β -carotene was also highest. At 40 °C samples stored flushed with compressed air (21% oxygen) had a β -ionone content of $0.44 \mu\text{g g}^{-1}$, followed by those flushed with 10% ($0.36 \mu\text{g g}^{-1}$); 2.5% ($0.22 \mu\text{g g}^{-1}$) oxygen or nitrogen ($0.10 \mu\text{g g}^{-1}$), respectively (Fig. 4). Similarly there was a significant increase in the β -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. 4). 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 (Figs. 3 and 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 – $0.9 \mu\text{g g}^{-1}$, will be much lower than the amounts of carotenoids degraded (Figs. 3 and 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.

4. 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.

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