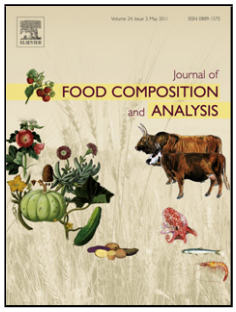

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Original Research Article: Carotenoid stability during storage of yellow gari made from biofortified cassava or with palm oil

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ABSTRACT

The carotenoid composition of gari made from biofortified cassava (BG) was compared to that of existing gari of similar appearance but made from white cassava with added red palm oil (RPG). Storage of both yellow gari products was modelled at ambient temperatures typical of tropical areas (19-40°C) over a 3 month-period at constant relative humidity. Carotenoid content and hence vitamin A activity of the gari products decreased markedly with time and temperature. Trans-β-carotene degradation fitted well the kinetics predicted by the Arrhenius model, in particular for BG. Activation energies for trans-β-carotene were ~~63.1~~60.4 and ~~82.3~~81.0 kJ.mol^{-1} for BG and RPG respectively (R^2 = 0.998 and 0.~~988~~997 respectively): hence the minimum energy to cause degradation of trans-β-carotene in gari was lower with BG. Rates of degradation of 9-cis β-carotene in gari were of the same order as

with trans-β-carotene. Although the initial content of trans-β-carotene was twice as high in the BG compared to RPG, trans-β-carotene in BG degraded much faster. Results showed that the average shelf life at ambient temperature for BG was significantly shorter than for RPG and therefore carotenoids in BG were less stable than in RPG.

Key-words: carotenoid degradation, biofortified cassava, gari, palm oil, storage, temperature, model prediction, vitamin A deficiency, nutritional impact, food composition

Abbreviations: BG: gari from biofortified yellow cassava; RPG: gari from white cassava with added palm oil. FW: fresh weight basis; DW: dry weight basis; R: retention; RAE: Retinol Activity Equivalent; EAR: Estimated average requirement; VAD: vitamin A deficiency; pVACs: provitamin A carotenoids

1. Introduction

Cassava (*Manihot esculenta* Crantz), a tropical root crop, is a starch staple and an important crop for food security for millions of people in sub-Saharan Africa. The short shelf life (2-3 days) of the crop however is a major drawback because it limits its transportation and consumption in its fresh form (Westby, 2002). Hence processed forms of cassava have been developed. Gari is a dried granulated food product with a slight acidic taste is one of the most common processed forms of cassava in West Africa. Gari is produced by grating (to remove cyanide inherent to the root) followed by fermentation (to produce flavour and make the product sour – by lowering pH value, the shelf life is also increased) and drying (to extend the shelf life). Gari presents as a dried form, which makes it stable under ambient conditions, easy to transport and can be stored for many months.

Recently biofortified varieties of cassava that contain significant levels of provitamin A carotenoids (pVACs) have been developed by conventional plant breeding methods and

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released for use by the local populations. These biofortified varieties could be used to help tackle vitamin A deficiency (VAD) (Saltzman et al., 2009) an important public health problem in sub-Saharan Africa and in the world. In some countries higher mortality rates, susceptibility to infections and blindness can clearly be attributed to VAD occurrence. The new varieties have a different visible colour to the traditional varieties because they are yellow compared to traditional cassava that is white and very low in provitamin A. These biofortified varieties produce a gari that is very similar in colour to gari made with added crude palm oil that also contains vitamin A (Abu et al. 2006). However, there are some disadvantages in adding palm oil. Firstly it is not widely consumed, and also, the addition of palm oil adds to the production costs, and finally darkening of gari occurs when added in excess and rancidity can happen during storage (Burri et al. 2012). The use of gari made from biofortified cassava would therefore solve the issue of rancidity and without the additional cost of palm oil help tackle vitamin A deficiency on a wider scale. Nigeria is the most densely populated country in Africa and an emerging country with a fast growing population that could reach 300 million by 2050 (Oshikoya 2008) and the impact of such a product could potentially impact millions. But the challenges are to measure the stability of carotenoids in gari made with biofortified cassava and also to compare it with gari made with crude palm oil that also contains provitamin A.

Understanding how pVACs degrade during storage of vitamin A- containing-gari is critical because it will affect its nutritional impact. Storage of gari at ambient temperatures is a current practice in Nigeria. Gari storage is not only generally practiced at household level but also at commercial level. Periods of storage are on average around six months but some processors can store up to a year. Stability of carotenoids in gari made from white cassava varieties with added palm oil has been studied during processing and storage at ambient

77 temperature (Abu et al., 2006; Gouado et al., 2008; Uzomah et al., 2006). Gouado et al.
78 (2008) showed that during gari processing the product retained a significant amount of the
79 carotenoids from palm oil. In contrast, authors reported a significant loss in carotenoid during
80 storage that followed processing: Abu et al. (2006) measured a loss of 57% in total carotenoid
81 after 4 months at 28°C in Nigeria. However Uzomah et al. (2006) working also at 28°C in
82 Nigeria reported different results: an average loss of 25% after the 2nd week of storage and of
83 50% by the 3rd week. Some gari samples that had lower levels of palm oil lost most of
84 vitamin A activity after only 2 weeks of storage (Uzomah et al., 2006). These dissimilar
85 findings appeal for more research to understand the carotenoid degradation in palm oil gari
86 during storage under controlled conditions.
87
88 The effect of storage on carotenoid in products made from biofortified crops has also been
89 studied. Stability of carotenoids during storage of biofortified maize has been studied by
90 Mugode et al. (2014). It was shown that most of the carotenoid degradation occurred in the
91 first weeks of storage and the degradation rate then lowered. Bechoff et al. (2011a) working
92 on biofortified orange-fleshed sweet potato similarly reported that storage of dried chips had
93 a dramatic effect on carotenoid stability (~80% loss in 4 months). Furthermore, the authors
94 demonstrated that the carotenoid degradation followed a first order degradation (logarithmic
95 curve), which explains why the degradation was higher in the first weeks of storage and then
96 stabilised with time. Temperature and oxygen were the main factors that caused the loss in
97 carotenoids whilst water activity only had a minor effect (Bechoff et al., 2010). A
98 mathematical model was developed to predict the degradation of trans-β-carotene, the main
99 carotenoid in sweet potato, under controlled conditions of temperature, oxygen and humidity
100 and the model was validated by field data (Bechoff et al., 2010).

Little research has been done on biofortified cassava with relation to storage of gari. Ukenye, et al. (2013) observed that the gari made with biofortified cassava was similar in appearance to the gari made with palm oil (traditional gari). Onadipe Olapeju (2011) studied the degradation of total carotenoids in gari from the varieties of biofortified yellow cassava developed in Nigeria (01/1371; 01/1368 and 01/1412). According to the data presented by the author, 50% on average of total carotenoids were lost after 3 month-storage at 30±2°C. However there was minimal information on the degradation rate and the influence of temperature.

There appears to be little published studies on the prediction of carotenoid degradation during storage although it is a critical issue for gari containing carotenoids and hence gari's potential impact on tackling VAD. More research is needed to understand the stability of gari from biofortified cassava (BG). Traditional gari made with crude "red" palm oil (RPG) is a common product in Southern Nigeria and should also be tested for stability to compare with gari made from biofortified cassava. This information will be useful to understand the potential for the promotion and marketing of gari made from biofortified yellow cassava in Nigeria and its contribution to reducing VAD.

2. Materials and Methods

2.1. Description of samples

Biofortified yellow cassava roots (TMS 01/1371) were harvested from Ikenne (about 2h drive south from IITA, Ibadan, Nigeria). White cassava roots (variety IITA 3303, locally called Oko-Iyawo) were harvested from the Army Barracks field in Ibadan, Nigeria. Cassava roots had a growing period of approximately 12 months after planting. Roots (50kg per variety) were processed into gari by commercial processors based at the Army Barracks, Ibadan:

biofortified yellow cassava variety and white cassava variety with added red palm oil (approximatively 0.45L/32.6kg or 0.328g/32.6kg of grated mash). The amount of red palm oil to add to the mash was selected by the commercial processors. All the processing parameters (time, temperature, pH, quantities etc.) were monitored and the gari produced was of commercial quality. A representative sample of gari was stored in the freezer (-20°C) and maintained frozen during transport and up to start of the storage experiment.

2.2. Storage experiment and sample collection for carotenoid analysis

Gari samples (about 1kg of from either BG or from RPG) were divided into equal portions using a riffle divider. Representative gari sub-samples (50g) were wrapped in a sewed cotton bag and stored in Kilner jars (having metal lever catch and rubber seal) with a saturated salt solution (Sodium Bromide (NaBr) that has a water activity (a_w) of about 0.5). The saturated salt solution was used to maintain the ambient relative humidity constant around the gari product so that only the effect of temperature could be measured. Jars in triplicate were placed in incubators (LMS Cooled Incubator, Sevenoaks, UK) at the Natural Resources Institute (NRI), University of Greenwich, UK and set at four different ambient temperatures (19±1, 26±1, 33±1 and 40±1 ºC). The range of temperatures was comprised between the minimum and maximum ambient temperatures in Nigeria. Hence the degradation of carotenoids during storage of gari could be predicted under similar temperature conditions as those found in Nigeria. Samples were stored in jars for 80 days (20th November 2012- 7th February 2013). The storing system used in the incubators was similar to the one used with dried sweet potato (Bechoff, 2010). Stored gari samples (about 5g) were collected in a representative manner by using a riffle divider and moisture content was checked at the beginning and the end of storage. Sample collections at 19°C were on 24th; 49th; 60th; 80th day; collections at 26°C were on 18th; 24th; 49th; 60th; 80th day; collections at 33°C were on

10^{th}; 18^{th}; 24^{th}; 49^{th}; 60^{th}; 80^{th} day.; collections at 40°C were on 10^{th}; 18^{th}; 24^{th}; 31^{st}; 49^{th}; 60^{th} day. Collected gari samples were immediately stored at -80ºC.

2.3. Carotenoid analysis

The extraction stage was based on Rodriguez-Amaya and Kimura (2004) and described in Bechoff et al. (2011a). Analyses were carried out at NRI, UK. In brief, gari samples (0.6-2.0g depending on the carotenoid content in sample) were rehydrated for 10 minutes in 10 mL tepid deionised water (water was heated at 30ºC to facilitate extraction). The samples were homogenised with 50mL methanol:tetrahydrofuran (THF) (1:1) for 1 minute and filtered. The homogenised extract was rinsed with methanol:THF (1:1) until there was no yellow colour left in the residue. Partition between the aqueous phase and organic phase containing the carotenoids was achieved by the addition of petroleum ether (PE 40-60°C) and sodium chloride (NaCl) solution (10%). The PE phase was further washed with deionised water, dried by addition of anhydrous sodium sulphate, then filtered and made up to volume (50 mL). For the determination of individual carotenoids by HPLC, the carotenoid extracts in PE (~~20ml~~20mL) were dried by flushing with nitrogen in a dry block system at 35º C. Extracts were then dissolved in 500 µLTHF: Methanol (1:1). After vortexing, dissolved samples were collected into a vial with septum for HPLC analysis. A reverse-phase high performance liquid chromatography using an Agilent 1200 system (UK) was used with a polymeric C30 reverse phase column (250 x 4.6 mm i.d. 5µm YMC (EUROP GmbH Germany) having a flow rate of 1 $ml.min^{-1}$, a temperature of 25°C, a running time of 40 minutes and an injection volume of 10µL. The isocratic mix consisted of Methanol: MTBE (80:20). Detection of compounds was performed at 450nm. Concentrations on a fresh weight basis were determined by comparing with standard curve of pure trans-β-carotene (Sigma, UK). Percentages of cis-isomers and other minor compounds such as epoxides were also determined (Bechoff et al. 2011b). Minor

compounds (epoxides of β-carotene) were tentatively identified when these were in very small amounts. Trans-α-carotene was identified by injection of a mixture of carotenoids from carrot extract (Sigma, UK).

2.4. Retention

Retention of trans-β-carotene (TR) was calculated using a simplified equation of the true retention assuming that the dry matter content was constant and therefore the weight of gari did not vary in the samples stored (at constant humidity in incubators) (Equation 1).

$$R(\%) = 100x\frac{\text{trans-}\beta\text{-carotene content per kg of stored gari}}{\text{trans-}\beta\text{-carotene per kg of unstored gari}} \quad \text{(Equation 1)}$$

2.5. Kinetics modelling and statistical analysis

Carotenoid content was determined on a fresh weight basis (FW) at different storage times and temperatures in triplicate. Carotenoid degradation followed a first order kinetics. Hence logarithms of carotenoid content were linear as a function of storage time (Excel, Windows 2007) (Equation 2):

$$\ln C = \ln C_0 - kt \quad \text{(Equation 2)}$$

Where: C: Carotenoid content of gari ($\mu g.g^{-1}$) at storage time t; C_0: Carotenoid content of food ($\mu g.g^{-1}$) at initial time (before storage); t: storage time (day); k: degradation constant rate (day^{-1}). k was determined graphically and using linear regression (XLStat 2014 software. http://www.xlstat.com) for the three replicate data pooled together.

Carotenoid degradation kinetics can be evaluated using different models as in Bechoff et al. (2010). The most common model is Arrhenius model. The Arrhenius model (Equation 3) is

an empirical collision model that describes the relationship between reaction constant rates and temperature using activation energy (E_a) and a pre-exponential factor (k_∞).

$$k = k_\infty e^{-\frac{E_a}{RT}} \quad \text{(Equation 3)}$$

Where: T: temperature (K)

k_∞: value of k at infinite time t = ∞ (day^{-1})

Ea: Activation energy ($kJ.mol^{-1}$)

R: gas constant = 8.314 $J \cdot K^{-1}.mol^{-1}$

The prediction model (Equation 4) is calculated by the equation based on the Arrhenius model and using temperature (T) expressed in Kelvin:

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

Determination of Ea and k_∞ parameters helps predict carotenoid degradation for known storage temperatures and times. Ea and k_∞ were determined using linear regression (XLStat 2014 software).

~~Other models however exist. The Eyring model (Equation 5) is based on the transition state theory in which enthalpy of activation (ΔH*) and entropy of activation (ΔS*) are the model's parameters. The model's parameters were identified from experimental data measured in triplicate using linear regressions.~~

~~$$k = \frac{k_B}{h} T . e^{-\frac{\Delta H^* - T\Delta S^*}{RT}}$$ (Equation 5)~~

~~Where: k_B: Boltzmann constant = $1.381 \cdot 10^{-23}$ $J.K^{-1}$~~

~~h: Planck constant = $6.626 \cdot 10^{-34}$ J.s~~

~~ΔH*: activation enthalpy ($kJ.mol^{-1}$)~~

~~ΔS*: activation entropy (J.mol^{-1}.K^{-1})~~

Data were processed on SPSS 20.0 software using ~~One Way Analysis of Variance to~~a t-test to determine if there were significant differences ($p<0.05$) between model parameters of the two gari products (BG and RPG).

3. Results and discussion

3.~~2~~1. *Nutritional value of stored gari*

The nutritional value (vitamin A activity) of the gari products at different storage times and ambient temperatures was determined (Table 1). Vitamin A activities per daily "100 g" portion of BG and RPG based on the classical estimate were ~~125.6~~126 and ~~84.8~~85 RAE, respectively. On the other hand unstored BG and RPG had substantial average vitamin A activity based on the new estimate (301~~.4~~ and 203~~.5~~ RAE, respectively). The Estimated Average Requirement (EAR) for a child is 200 RAE based on FAO/WHO (2002) and 275 RAE based on the National Academy of Sciences/Institute of Medicine (2001) s' recommendations. According to the standard estimate for food, 1 µg.g^{-1} Retinol Activity Equivalent (RAE) corresponds to 12 µg.g^{-1} all-*trans*-BC or 24 µg.g^{-1} minor carotenoids (National Academy of Sciences / Institute of Medicine, 2001). Recent studies on the bioconversion of provitamin A from cassava products indicated that the factor might be lower: working with women, Liu et al (2010) showed that the bioefficacy of the BC from porridge made with biofortified cassava was as good as that of a BC supplement (2:1). Later, La Frano et al. (2013) calculated a bioconversion factor of 4.5:1 for biofortified cassava meals and Phorbee et al. (2013), a factor of 6:1. Therefore the conversion factor 5:1 was

suggested here as 'new' estimated bioconversion factor. With either estimate (classical or new), the potential daily contribution of yellow gari to a child's vitamin A intake is close or superior to 50% of EAR.

However, during storage, vitamin A activity of gari products sharply decreased. Our results using the classical estimate showed that after 60 days vitamin A activity was 54~~.1~~ and 64~~.2~~ RAE per 100g at 26°C; 24~~.3~~ and 51~~.4~~ RAE at 33°C, and 24~~.3~~ and ~~8.6~~9 and 33~~.4~~ RAE at 40°C for BG and RPG, respectively. Calculations on estimation of vitamin A activity presented here highlighted that the choice of adequate bioconversion factor (classical or new) is critical because it will be determinant to provide advice on shelf life of the gari product. Using the new estimate, values superior to 50 RAE would only be achieved with BG stored at 33°C for up to 60 days or at 40°C for 35 days. The decrease was temperature dependent. Uzomah et al. (2006) analysed gari products from six different locations in Eastern parts of Nigeria where red palm oil gari is the most common form of gari consumption. Their results similarly reported that vitamin A activity of palm oil-enriched gari significantly decreased when stored at ambient temperature. Initial vitamin A activity in freshly made gari was very variable ranging between 13~~.2~~–723 RAE per 100g (using classical estimate), which shows a wide variation in levels of palm oil added by different communities. Uzomah et al. (2006) reported a loss of 25% in activity after 2 weeks and 50% after 3 weeks of storage at ambient temperature of 28°C. This loss was higher that the results presented here (10% for RPG after 15 days) but other factors under field storage such as light and humidity might have contributed to additional loss (Uzomah et al., 2006).

~~Calculations on estimation of vitamin A activity presented here highlighted that the choice of adequate bioconversion factor (classical or new) is critical because it will be determinant to provide advice on shelf life of the gari product.~~

3.~~1~~2. Carotenoid composition of unstored gari from yellow cassava and white cassava with palm oil

Trans-β-carotene and other carotenoid (9-cis, 13-cis, 5,6 epoxy, 5,8-β-carotene and trans-α-carotene) contents were determined on a fresh weight basis at different storage durations and temperatures (Fig. 2).

Trans-β-carotene content on a fresh basis (FW) in unstored gari from TMS 01/1371 variety was 10.9 $\mu g.g^{-1}$ on average and 13.0 $\mu g.g^{-1}$ as a maximum. Carotenoid content of gari from yellow cassava (BG) obtained by conventional cross breeding techniques has been determined (Chavez et al., 2007; La Frano et al., 2014; Maziya-Dixon et al., 2009; Onadipe Olapeju et al., 2011; Thakkar et al., 2009). Chavez et al. (2007); Frano et al. (2014) reported lower trans-β-carotene content in gari, between 3 and 4 $\mu g.g^{-1}$ on a dry basis (DW) whilst Maziya-Dixon et al. (2009) and Onadipe Olapeju et al. (2011) working on the variety TMS 01/1371 indicated total carotenoid content of 16 and 20 $\mu g.g^{-1}$ FW, respectively that was in accordance with our data: in this study, maximal total carotenoid content determined by spectrophotometer was approximatively 18 $\mu g.g^{-1}$ FW (data not shown). In addition, Thakkar et al. (2009) found a trans-β-carotene content in gari from TMS 01/1371 variety around 15 $\mu g.g^{-1}$ DW, which would be approximately 13 $\mu g.g^{-1}$ FW for a product with approximatively 10% moisture content. Our data on unstored gari from yellow cassava TMS 01/1371 is therefore mostly in agreement with previously published work with the same variety.

299 Carotenoid content of gari from genetically modified cassava has also been measured (Failla
300 et al., 2012). Nonetheless levels of trans-β-carotene reported between 3-8 $\mu g.g^{-1}$ DW were
301 lower than in our study.
302
303 Differences in initial carotenoid content of gari described by different authors may be
304 explained by cassava varietal differences but also by variations in processing steps that
305 influences carotenoid retention from roots into gari.
306
307 Average trans-β-carotene and trans-α-carotene content in unstored RPG were 5.60 $\mu g.g^{-1}$ and
308 3.10 $\mu g.g^{-1}$ respectively, on a fresh basis (FW). Trans-α-carotene content in RPG was about
309 six times more than in BG. The higher concentration of trans-α-carotene in RPG can be
310 explained by the composition of red palm oil (Fig. 1A and B): red palm oil is known to
311 contain both trans-β-carotene and trans-α-carotene (Bonnie Tay & Choo, 2000). The
312 carotenoid content of this gari would depend on the amount of palm added during the
313 process, which can be variable according to practices of gari processors (Gouado et al.,
314 2008). Alpha and β-carotene contents of gari reported by Gouado et al. (2008) were at least
315 100 times greater than ours (trans-α-carotene: 352.6-1572.5 $\mu g.g^{-1}$ and trans-β-carotene:
316 309.7-1624.3 $\mu g.g^{-1}$, for 2 and 8 ml of oil respectively for 210g of gari) and therefore indicate
317 that the analysis was done on the palm oil and not on the gari product itself (2mL for 210g is
318 actually quite close to the amount of oil added in this study). Mortensen (2005) reported that
319 palm oil identified on a C30 HPLC column mainly contained trans-β-carotene and trans-α-
320 carotene and the other compound identified was 13-cis-β-carotene. Although many minor
321 carotenoids (about 15, including lycopene and γ-carotene) are present in palm oil (Bonnie
322 Tay & Choo, 2000; Mortensen, 2005) they were not visible on the present chromatogram
323 (Fig. 1B). Andreu-Sevilla et al. (2009) equally reported that the main carotenoids absorbed in

potato fried in palm oil were α and β-carotene, which was in accordance with this present study. In addition, Andreu-Sevilla et al. (2009) reported amounts of lutein, 5,6 epoxy-α-carotene, γ-carotene; δ-carotene; ε-carotene; 15-cis and 9-cis-β-carotene in palm oil that were partially absorbed in fried potato. The minor carotenoids in common for BG and RPG that were identified in our study were all from β-carotene, being 13-cis, 9-cis, 5,6 and 5,8 epoxy-β-carotene. In some cases, minor carotenoids were in very small amount, which made their identification more difficult (Fig. ~~2~~1).

3.3. Kinetics of carotenoid degradation in gari during storage

Globally major and minor carotenoids compounds degraded during storage (Fig. 2). The degradation of the minor compounds was more difficult to model than that of major carotenoids because of the very low concentrations recorded. Degradation kinetics of 13-cis-β-carotene content was globally similar to that of trans-β-carotene and 9-cis-β-carotene but slightly more irregular. According to Achir et al. (2013) 5,6 and 5,8 epoxy-β-carotene were formed from cis-isomers on dried and stored sweet potato; therefore irregular pattern for epoxides may be explained by their formation that precedes their oxidation. Besides, 5,6 and 5,8-epoxy- β-carotene were in very small concentrations, which made mathematical modelling difficult. Overall it appears that all minor carotenoids decreased quite sharply during storage and degradation was increased with temperature and storage duration.

Trans-β-carotene and 9-cis-β-carotene were both the main carotenoids present in BG and RPG (Fig. 2). Degradation of trans-β-carotene followed logarithmic first order kinetics during storage between 19 and 40ºC. Similarly, the degradation of 9-cis-β-carotene also followed a first order kinetics equation.

Degradation rates (k) for major carotenoids are found in Table 2. Rates of carotenoid degradation were determined from the first order kinetics linear curves for BG and RPG stored in incubators. The higher the temperature and the longer the storage time, the greater was the trans-β-carotene degradation and this was greatest for the gari made from yellow cassava (BG) and least for the gari made with palm oil (RPG). Coefficients of determination (R^2) showed that ~~overall~~ globally the first order kinetics equation fitted carotenoid degradation well ($R^2 \geq 0.8$). RPG at lower temperatures (*i.e.* 19ºC) however did not fit the first order degradation as well as BG (R^2 around 0.5-0.7) but the reasons are not clear.

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Degradation rates (k) of trans-β-carotene and 9-cis-β-carotene clearly differ in BG and RPG (Table 2). ~~In addition, trans-α-carotene was identified in significant concentrations in the palm oil gari samples (RPG) (Table 2). Trans-α-carotene followed a first order kinetics and had close rates of degradation as those of trans-β-carotene and 9-cis-β-carotene in RPG.~~ Degradation rates of trans-β-carotene and 9-cis-β-carotene were greater for BG compared to RPG stored under the same conditions. Although the initial trans-β-carotene content and the initial vitamin A activity was about twice higher in BG compared to RPG, trans-β-carotene in BG degraded much faster.

More research would be needed to understand the difference in kinetics of carotenoid degradation in these two types of gari. The more complex matrix of palm oil gari including several different carotenoids having different types of kinetics might explain why the degradation of some minor carotenoids did not fit a first order degradation and why the major carotenoids (trans-β; 9-cis-β-carotene ~~and trans-α-carotene~~) did not fit well a first order degradation at lower temperature. Possibly the lower concentrations of carotenoids in palm

oil gari might also make the linearisation more difficult. Overall, the fitness of the models demonstrates that mathematical modelling for the major carotenoids present is possible when working with yellow cassava in spite of the relatively lower concentrations in cassava compared to orange-fleshed sweet potato (Bechoff et al., 2010).

3.4. Model prediction of β-carotene degradation in gari products

3.4.1. Activation energy of β-carotene degradation based on model prediction

The degradation of trans-β-carotene was further modelled using an Arrhenius ~~and Eyring~~ model~~s~~ (Table 3). The models fitted well trans and 9-cis-β-carotene degradation in incubators between 19 and 40°C for both BG and RPG gari preparations. Degradation kinetics of trans-α-carotene and also more minor carotenoid compounds present in very small amounts in gari (Fig. 2) could not be clearly described by the above models. Concentrations might be too weak to be accurately predicted.

Activation energy for trans-β-carotene in BG (~~63~~60.~~1~~ 4 kJ.mol^{-1}) was of the same order as in dried orange-fleshed sweet potato (64.2 kJ.mol^{-1}) during ambient storage (Bechoff et al., 2010) though the carotenoid content in yellow cassava was much less than in orange-fleshed sweet potato. ~~Interestingly , Eyring parameters- activation enthalpy and entropy being 60.5 kJ.mol^{-1} and 77.1 J. mol^{-1}, respectively in this study and 61.7 kJ.mol^{-1} and 74.3 J. mol^{-1}, respectively in Bechoff et al. (2010) were also similar.~~

Activation energy (Ea) was greater for RPG compared to BG (~~82.3~~81.0 and ~~63~~60.~~1~~ 4 kJ.mol^{-1} for trans-β-carotene, respectively, and ~~75.6~~73.7 and ~~64.1~~61.2 kJ.mol^{-1} for 9-cis-β-carotene, respectively). It is therefore confirmed that the energy needed to degrade trans-β-carotene

was higher in RPG. Palm oil might have coated trans-β-carotene present in gari from white cassava and this might have limited degradation. Oxidation of fatty acids and carotenoids was similarly reported to be a free radical mechanism (Lieber 1993). It is therefore possible that fatty acids present in palm oil might have been oxidised in the place of trans-β-carotene and hence acted as a protection against carotenoid degradation. Achir *et al.* (2010) reported that Ea of trans-β-carotene in pure palm oil was comparable, being 86 $kJ.mol^{-1}$, and this was in accordance with this present study. More research shall be required to understand how different matrices (i.e. different oil types; vegetable cells) can affect activation energy of carotenoids.

3.4.2. Model prediction of retention during storage of gari

Relationships between storage time, storage temperature and predicted retention of trans-β-carotene are described in Fig. 3. Predicted nutritional values for gari (vitamin A activity) were also calculated. According to the Arrhenius model predictions, if BG with an initial nutritional value of 301~~.4~~ RAE (based on the new conversion factor) was stored for 60 days (2 months) at an ambient temperature of 25°C and constant humidity, about ~~32~~44% of the initial trans-β-carotene would be retained (equivalent to ~~135~~ 136 RAE in the product). Periods of storage are 5-6 months on average under tropical temperatures (about 25°C in the daytime) in sub-Saharan Africa (i.e. Nigeria). If BG was stored for 5 months at 25°C, only ~~12~~13% of trans-β-carotene (equivalent to 41 RAE) would be retained and if it was stored for 6 months, about 8% of trans-β-carotene would be retained (equivalent to ~~27~~ 28 RAE) and the nutritional value would be negligible. If the same gari was stored at a lower temperature of 20°C about ~~25~~26% would be preserved after 5 months leading to a nutritional value of about 79 RAE. If the initial carotenoid content were the same as for BG (with further addition of

palm oil), RPG could be stored three times longer, for up to 50 days according to the Arrhenius model predictions. Hence the traditional practices of storage for gari in Nigeria should not be recommended when working with BG. One option may be to lower storage temperature but this would require facilities such as a fridge or freezer.

On the other hand, RPG with an initial nutritional value of 203.5 RAE would retain about 80% of the initial trans-β-carotene after 60 days (2 months) at an ambient temperature of 25°C and constant humidity (corresponding to 142 RAE). About ~~50~~57% would be retained if the same gari was stored for 5 months at 25°C (about 8~~3~~2 RAE). Trans-β-carotene contained in RPG was therefore more stable during storage. ~~If the initial carotenoid content were the same as for BG (with further addition of palm oil), RPG could be stored three times longer, for up to 50 days according to the Arrhenius model predictions.~~

While provitamin A was more stable in RPG, the presence of palm oil created quality issues such as rancidity (Abu *et al.* 2006; Burri et al., 2012): Abu et al. (2006) reported that whilst half of the initial carotenoid content was lost during a 2 month-ambient storage there was a concomitant increase in the peroxide index (six times more than the initial value) of the gari product.

4. Conclusions

The effect of temperature on carotenoid stability was measured in the two types of gari; from yellow cassava (BG) and from white cassava with palm oil (RPG). Carotenoid content was temperature and storage time sensitive. Trans- and 9-cis-β-carotene contents in BG or RPG

447 followed a first order (logarithmic) degradation equation during storage. Although the initial
448 content of trans-β-carotene and the initial vitamin A activity was about twice higher in BG
449 compared to RPG, trans-β-carotene BG degraded much faster. Trans- and 9-cis-β-carotene
450 degradation in BG and RPG was accurately described by the Arrhenius and Eyring models.
451 The mathematical model can therefore be used to predict storage times at various storage
452 temperatures. The addition of red palm oil significantly increased the shelf life of gari in
453 terms of carotenoid retention: fatty acids present in palm may have protected carotenoids
454 against degradation. More research would be needed to understand the role of oil in
455 preserving carotenoids in gari over time. Although gari made with added palm oil could be
456 stored longer, issues of oil rancidity of the latter within the typical market turn-around time
457 for such product will need to be explored. In conclusion, this work has proven that gari made
458 with biofortified cassava has a limited carotenoid stability under ambient temperature
459 conditions and breeders, processors and marketers or supporters should be aware of this
460 constraint.
461
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Table 1. Estimation of vitamin A activity (µg retinol equivalent) for a 100g portion of gari made from yellow biofortified cassava (BG) and from white cassava with palm oil (RPG) (µg RAE[a]) during storage and maintained at constant humidity (a_w = 0.5 for NaBr)

RAE[a] estimate		**5:1[b]**		**12:1[b]**	
T (°C)	Storage days	BG	RPG	BG	RPG
	Initial	301.45±7.36	203.49±5.886	125.61260±3.07	84.7985±2.452
19	24	256.5±11.4	208.9209±10.811	106.9107±4.75	87.0±4.5
	49	205.2±5.45	161.5±24.0	85.5±2.2	67.3±10.0
	60	199.7200±7.3	174.8175±12.713	83.2±3.0	72.873±5.35
	80	155.5156±9.1	139.8140±18.2	64.865±3.84	58.2±7.68
26	18	244.8245±23.523	181.1±8.2	102.0±9.810	75.4±3.4
	24	250.5±25.726	190.3±10.911	104.4±10.711	79.3±4.5
	49	150.3±29.2	135.1±7.0	62.663±12.2	56.3±2.93
	60	129.8130±20.1	154.0±8.79	54.1±8.4	64.2±3.64
	80	99.2±15.1	130.1±12.3	41.3±6.3	54.2±5.1
33	10	200.8201±14.1	147.6148±15.716	83.784±5.96	61.5±6.5
	18	203.9204±11.612	199.6200±5.76	85.0±4.85	83.2±2.4
	24	172.4±14.815	188.7189±8.2	71.972±6.2	78.679±3.4
	49	87. 4±6.77	137.3±9.910	36.4±2.83	57.2±4.1
	60	58.3±1.82	123.3±5.86	24.3±0.81	51.4±2.4
	80	40.841±2.83	92.993±3.84	17.0±1.2	38.739±1.62
40	10	147.8148±25.926	164.0±4.75	61.662±10.811	68.3±2.0
	18	139.6140±7.1	182.7183±5.2	58.2±3.0	76.1±2.2
	24	99.4±3.4	157.0±9.5	41.4±1.4	65.4±3.94
	31	67.668±1.72	143.7144±6.67	28.1±0.71	59.960±2.73
	49	28.629±1.52	99.3±3.5	11.912±0.61	41.4±1.5
	60	20.621±0.91	80.1±6.67	8.69±0.4	33.4±2.83
	80	12.5±0.2	42.3±0.61	5.2±0.1	17.618±0.3

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Mean of triplicate determinations ± standard deviation. [a] Retinol Activity Equivalent. [b] RAE was calculated for a bioconversion factor of 5:1 (La Frano et al., 2013) estimate = {All-trans-β-carotene content /5 + minor β-carotene content/ 10} x unit (g) or for a bioconversion factor of 12:1 (National Academy of Sciences / Institute of Medicine, 2001). Classical estimate = {All-trans-β-carotene content /12 + minor β-carotene content/ 24} x unit (g). Minor compounds are epoxy and cis β-carotene that are estimated to possess half of trans β-carotene activity.

Table 2. Carotenoid rate of degradation (k) [a] in gari made from biofortified cassava (BG) (A) and from white cassava with palm oil (RPG)

Gari	Temperature (ºC)		19	26	33	40
BG	Trans-β-carotene	k	0.0083±0.0004	0.0158±0.0007	0.0276±0.0010	0.0479±0.0014
		R^2	0.979±0.009	0.948±0.041	0.990±0.013	0.987±0.014
		p	<0.0001*	<0.0001*	<0.0001*	<0.0001*
	9-cis-β-carotene	k	0.0071±0.0005	0.0125±0.0006	0.0234±0.0010	0.0429±0.0016
		R^2	0.968±0.019	0.900±0.054	0.984±0.014	0.991±0.009
		p	<0.0001*	<0.0001*	<0.0001*	<0.0001*
RPG	Trans-β-carotene	k	0.0027±0.0011	0.0039±0.0011	0.0089±0.0013	0.0184±0.0003
		R^2	0.634±0.282	0.869±0.043	0.896±0.079	0.934±0.020
		p	0.016*	<0.0001*	<0.0001*	<0.0001*
	9-cis-β-carotene	k	0.0030±0.0014	0.0040±0.0010	0.0085±0.0010	0.0170±0.0003
		R^2	0.679±0.226	0.837±0.058	0.910±0.061	0.935±0.018
		p	0.029*	0.0001*	<0.0001*	<0.0001*
	~~Trans-α-carotene~~	~~k~~	~~0.0037±0.0010~~	~~0.0049±0.0009~~	~~0.0084±0.0009~~	~~0.0132±0.0006~~
		~~R^2~~	~~0.761±0.357~~	~~0.883±0.120~~	~~0.911±0.103~~	~~0.918±0.001~~

[a] expressed in day^{-1} at storage temperatures on a fresh weight basis and maintained at constant humidity (a_w = 0.5 for NaBr)

R^2: Coefficient of determination. Mean of triplicate determinations ± standard ~~deviation~~error (linear regression; XLSTAT 2014). Standard error is standard deviation divided by the square root of the number of analyses.

k was the slope on the logarithmic carotenoid concentration (Y-axis) vs storage time (X-axis) graph and was obtained by linear regression.

First order equation:

$$\ln C = \ln C_0 - kt$$

Where: C: Carotenoid content of gari (µg.g^{-1}) at storage time t; C_0: Carotenoid content of food (µg.g-1) at initial time (before storage); t: storage time (day); k: degradation constant rate (day-1).

* indicate a significant correlation at p<0.05 (linear regression; XLSTAT 2014).

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Table 3. Parameters of the Arrhenius[a] ~~and Eyring[b]~~ model~~s~~ for the carotenoids degradation in gari on a fresh weight basis between 19-40ºC and maintained at constant humidity (a_w = 0.5 for NaBr) in gari made from yellow cassava (BG) ~~(A)~~ and from white cassava with palm oil (RPG)

Carotenoid	Parameter	Type of gari		p
		BG	RPG	
Trans-β-carotene	Ln k_∞ ($days^{-1}$)	20.1±0.9	27.1±1.2	0.039*
	E_a ($kJ.mol^{-1}$)	60.4±2.1	81.0±3.1	
	R^2	*0.998*	*0.997*	
9-cis-β-carotene	Ln k_∞ ($days^{-1}$)	20.2±0.6	24.2±2.1	0.034*
	E_a ($kJ.mol^{-1}$)	61.2±1.6	73.7±5.2	
	R^2	*0.999*	*0.988*	

[a]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 $J \cdot K^{-1} \cdot mol^{-1}$

~~[b]Eyring model~~

~~$k = \frac{k_B}{h} T . e^{-\frac{\Delta H^* - T\Delta S^*}{RT}}$ Where: k_B: Boltzmann constant = $1.381 \cdot 10^{-23}$ $J.K^{-1}$; h: Planck constant = $6.626 \cdot 10^{-34}$ J.s; ΔH*: activation enthalpy ($kJ.mol^{-1}$); ΔS*: activation entropy ($J.mol^{-1}.K^{-1}$)~~

R^2: Coefficient of determination.

Mean of triplicate determinations ± standard ~~deviation~~error (linear regression; XLSTAT 2014).

Yellow cassava 01/1371 (~~YC~~BC); White cassava IITA 3303 with added palm oil (~~Po-WC~~RPG).

* indicate a significant difference between BG and RPG at p<0.05 (~~One-Way ANOVA~~T-test).

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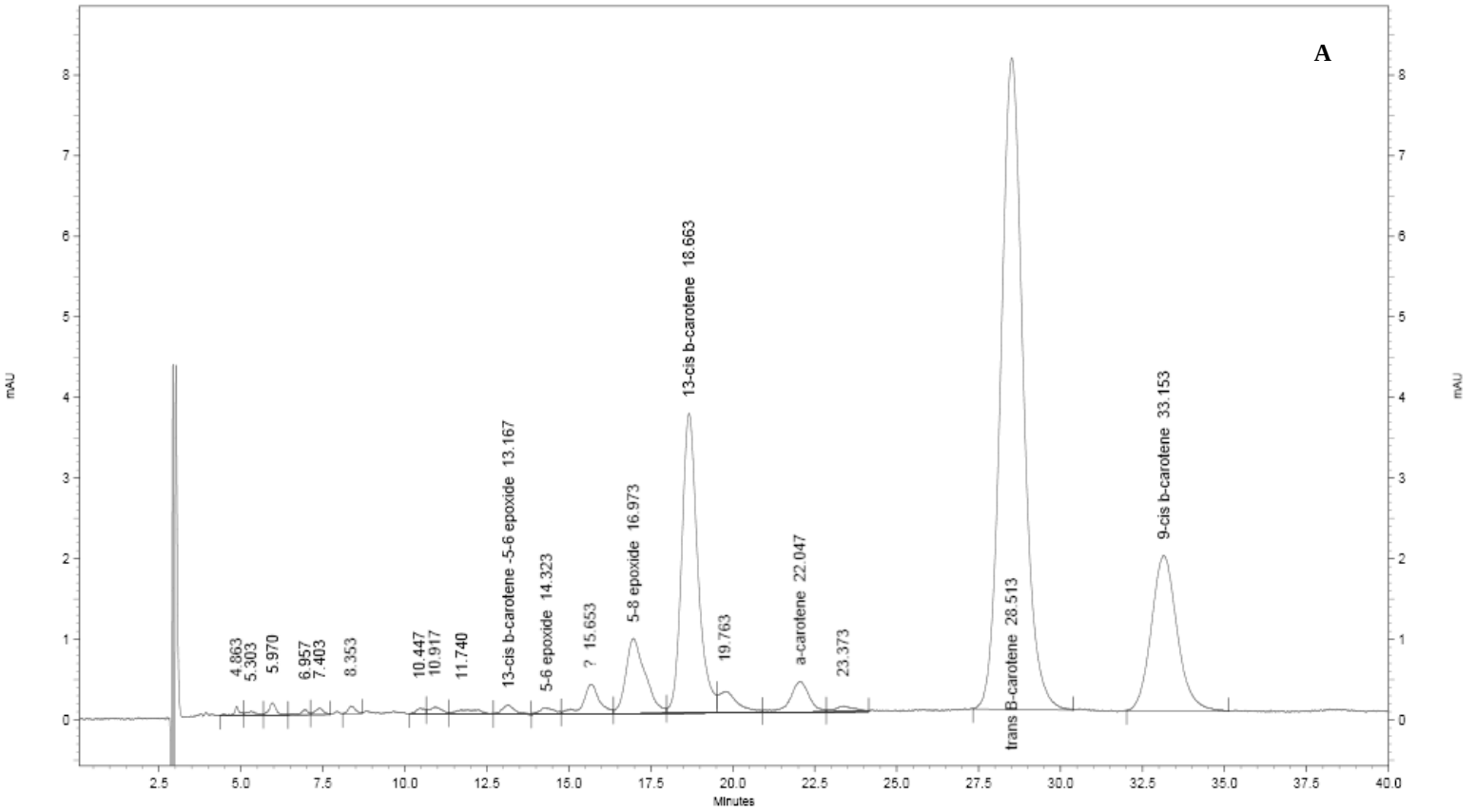

~~**Fig. 1A.** HPLC Chromatogram[a] of gari from TMS 01/1371 biofortified variety (BG)~~ [a]~~Identification of minor carotenoids (*i.e.* α carotene) is tentative.~~

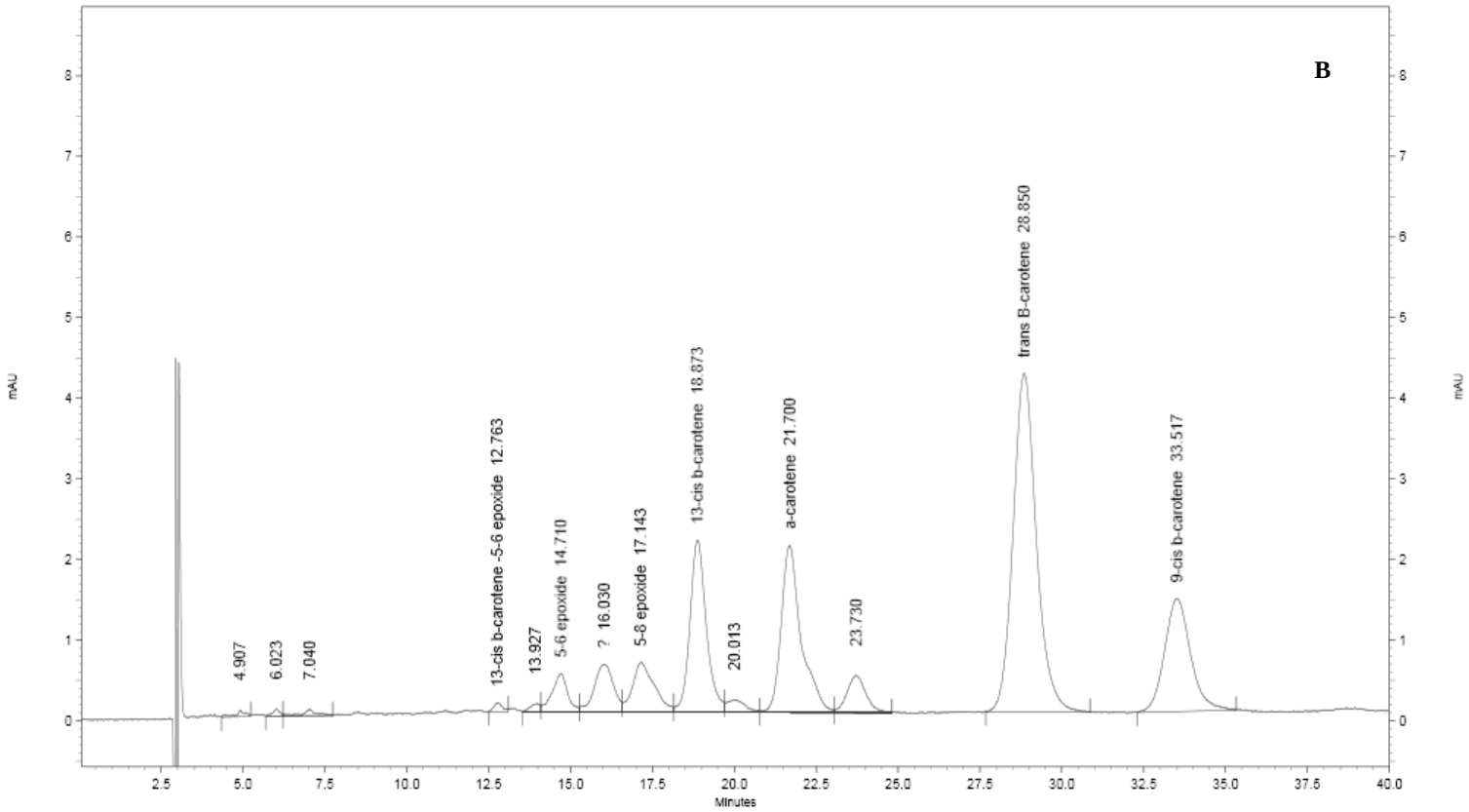

Fig. 1. HPLC Chromatogram[a] of gari from TMS 01/1371 biofortified variety (BG) (A) ~~**Fig. 1B.**~~ and ~~HPLC Chromatogram[a]~~ of ~~Gari~~ gari from IITA 3303 variety with palm oil (RPG) (B) [a]Identification of minor carotenoids (*i.e.* 5,6 and 5,8 epoxy-β-carotene) is tentative. Alpha-carotene was identified by

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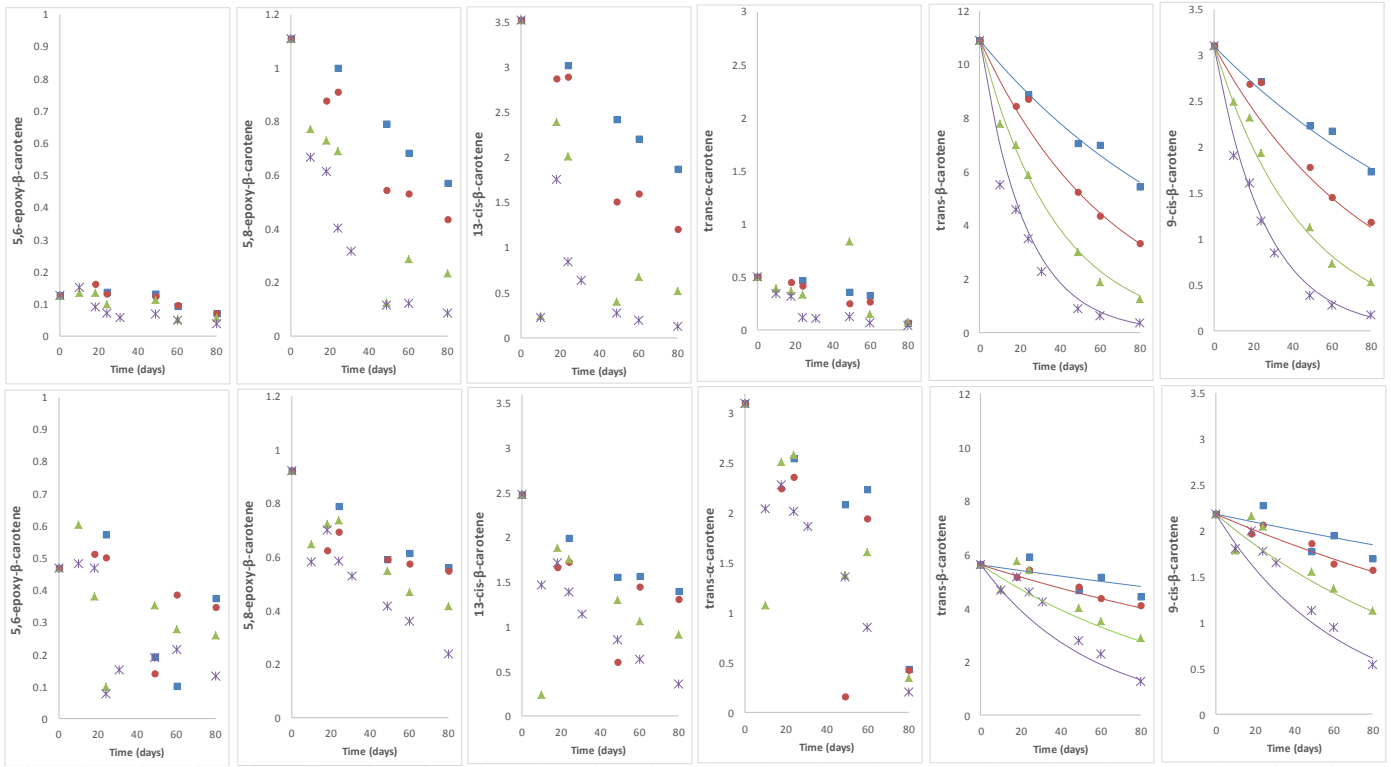

injection of a mixture of α and β carotenes.

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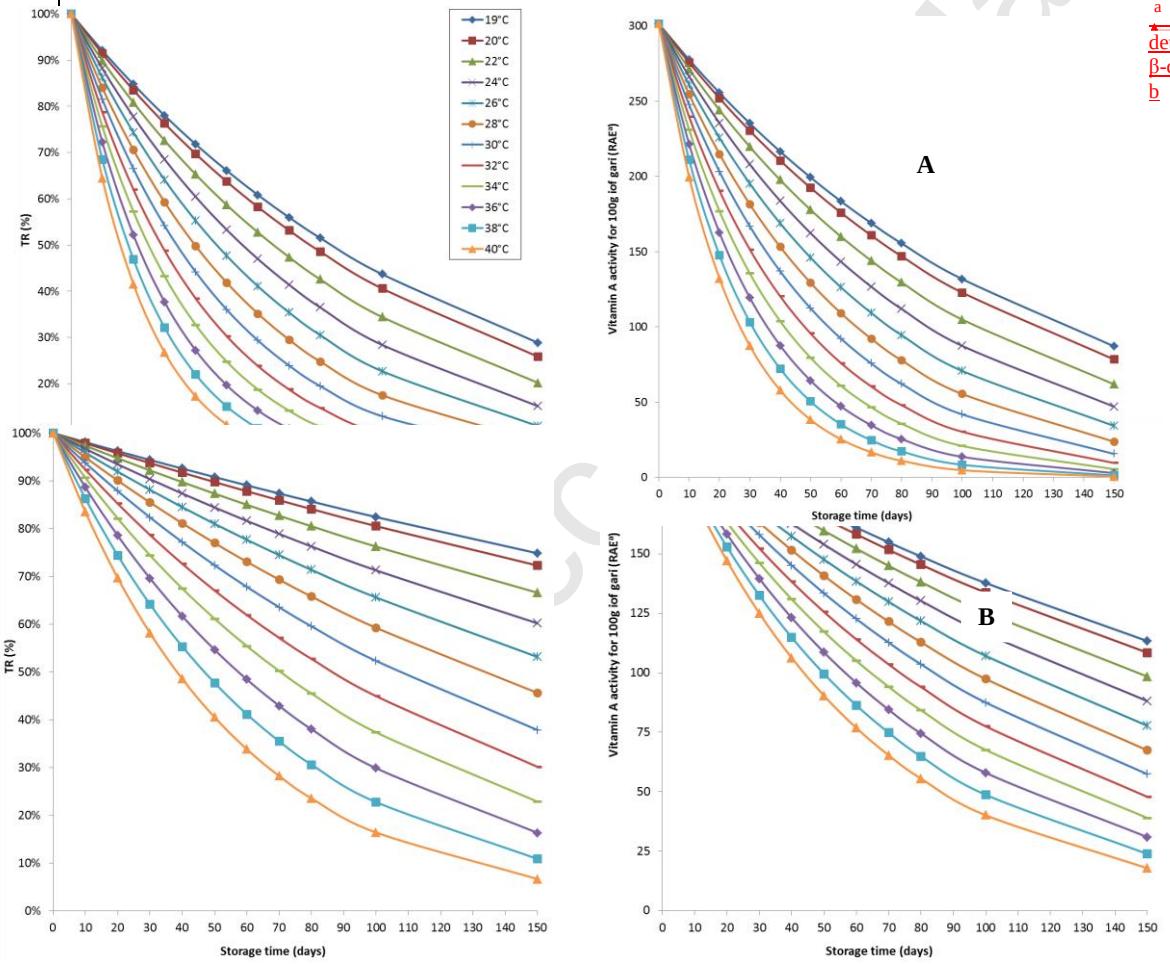
Fig. 2. Carotenoid content (on a fresh weight basis (FW)) of gari made from yellow biofortified cassava (BG) (A) or white cassava with red palm oil (RPG) (B) ($\mu g.g^{-1}$) and stored at four different temperatures ( ) and constant humidity (a_w = 0.5 for NaBr) over 80 days.
Mean of triplicate determination

19°C 26°C 33°C 40°C

Fig. 2. Carotenoid content (on a fresh weight basis (FW)) of gari made from yellow biofortified cassava (BG) (A) or white cassava with red palm oil (RPG) (B) (expressed in $\mu g.g^{-1}$ trans-β-carotene [a]) and stored at four different temperatures [b] (——) and constant humidity (a_w = 0.5 for NaBr) over 80 days. Mean of triplicate determination.



[a] Concentration for all the carotenoids was determined from standard curve of pure trans-β-carotene (Sigma, UK).

[b]

Fig. 3. Relationships between storage time, temperature, trans-β-carotene true retention (TR) and vitamin A activity[a] of gari made from yellow cassava (BG) (A) and from white cassava with palm oil (RPG) (B) based on Arrhenius model predictions.

[a] Retinol Activity Equivalent (RAE) estimate ~~estimate~~ was calculated for a bioconversion factor of 5:1 (La Frano et al., 2013) RAE = {all-trans-β-carotene content

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/5 + minor β-carotene content/ 10} x unit (g).

Highlights

- Trans-β-carotene degradation was modelled during storage of BG and RPG
- Trans and 9-cis β-carotene degradation fitted well the Arrhenius and Eyring models
- Initial trans-β-carotene was twice as high in the BG compared to RPG
- Activation energy was lower (~20%) with BG compared to RPG
- Based on carotenoid stability, shelf life of BG was much shorter than RPG