Bioformation of carotenoids in tomatoes (*Lycopersicon esculentum*) under two ripening conditions: A Kinetic study

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Abstract— Lycopene, the main pigment responsible for the typical deep-red color of ripe tomatoes and tomato products, has attracted attention due to its ability to act as a singlet oxygen quencher. Its biosynthesis and cyclization to beta-carotene are responsible for powerful antioxidant functions of tomatoes. In the present study, the rates at which tomato fruits ripe were investigated via the bioaccumulation of lycopene and beta-carotene under field and postharvest ripening conditions in four local cultivars commercially available in Nigeria. Results showed that the bioaccumulation of lycopene in the tomato cultivars studied under both ripening conditions follows first order kinetics with the rate constants of 0.050, 0.079, 0.101 and 0.075 per day in *Ajindi-Kerewa*, *Beske*, *3-Lobes* and b*ig local* cultivars respectively under field temperature ripening. These rate constants are lower for the postharvest ripening except in *big local* cultivar. The bioaccumulation of beta-carotene also follows first order kinetics except in *Ajindi-Kerewa* and *Beske* cultivars. The rate constants obtained were slightly higher for beta-carotene than that of lycopene accumulation for all the four cultivars evaluated and compared under the two ripening conditions. The mechanisms of the bioaccumulation of the carotenoids are discussed. The kinetic parameters so obtained could prove useful in developing countries as a future biotechnology tool in monitoring the pace of improvement in nutritional qualities of tomato fruits during plant breeding programs.

Index Terms— Bioaccumulation; Biotechnology; Carotenoids; Plant breeding; Rate constants; Tomatoes; Kinetics

1 INTRODUCTION

Tomatoes and tomato-based products are considered healthy foods since they are good sources of fiber, cholesterol free and low in fat and calories. They are rich in vitamins A, C and E, potassium, β-carotene and lycopene. The characteristic deep-red color of ripe tomato fruits and tomato-based foods, which serves as a measure of total quality, is mainly due to lycopene. Tomatoes and tomato products are the major sources of lycopene and are considered to be important contributors of carotenoids to the human diet. Other sources of lycopene include watermelon, pink grape fruit, guava and papaya [1]. Carotenoids act as attractants for pollinators to flowers by impacting the red, yellow and orange colors of plant organs [2]. They act as antioxidants which play important biochemical and physiological roles in animals and plants.

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In humans, lycopene exert protective effects against cardiovascular diseases, certain cancers and aging related diseases [3-6]. Carotenoids are essential components of the photosynthetic systems involved in light harvesting in plants and play important roles in preventing photo-oxidative damage [2].

Many of the studies carried out have speculated that the antioxidant activities of carotenoids are a key factor in reducing the incidence of many diseases [7]. It was reported that carotenoids can induce apoptosis in T-lymphocyte cell lines [8] and can protect genome stability [9]. In vitro studies on human lens epithelial cells have shown that addition of lycopene to cell cultures significantly prevents vacuolization [10]. The consumption of processed tomato products has been found to reduce lipoprotein sensitivity to oxidative damage [11] and low serum lycopene has been associated with an increased risk of artherosclerotic vascular events in middle-aged men [12]. High lycopene levels were associated with decreased risk of breast cancer [13], indicating the inhibitory action of lycopene on cell cycle progression at the G1 phase [14]. A high tomato diet can reduce leucocyte oxidative DNA damage and prostate tissue oxidative damage in patient already diagnosed with prostate cancer [15], thus suggesting such food can be used in the treatment of prostate cancer as well as its prevention. In vitro studies with prostate LNCaP cancer cells showed that lycopene in the growth medium reduces their proliferation [16].

The biosynthesis of lycopene and other carotenoids in tomatoes has been studied extensively [17]. Evidences from isotope labeling and functional genomics have demonstrated that the prenyl pyrophosphates utilized in the formation of carotenoids is derived from the plastid localized desoxyxylulose 5-phosphate pathway and not from the mevalonate pathway operating in the cytoplasm [18]. It was reported however, that radioactivity from ^{14}C-mevalonate fed to corn leaves can be recovered in carotenoids and that an in vitro exchange of isopentenyl pyrophosphate (IPP) across the plastid envelope occurs [19]. This indicates the connection between these two pathways and that, prenyl pyrophosphates can be exchanged between both the plastidic and the cytosolic terpenoids pathways [20]. It is therefore suggestive that prenyl phosphates and especially geranylgeranyl pyrophosphate (GGPP) occupy a central position in the synthesis of carotenoids and other terpenoid compounds in plant and that competition for GGPP is responsible for inter pathway regulation [17].

The isoprenoid chains of two C_{20}-pyrophosphate units (geranyl pyrophosphate, GPP) condense, tail-to-tail, to form the first C_{40}-carotenoid precursor, phytoene, which is then dehydrogenated sequentially, through the formation of phytofluene, ζ-carotene and neurosporene, to lycopene [1]. Lycopene exists as small globules, that is, in the chromoplasts, which are suspended in the tomato pulp throughout the fruit. Lycopene appears as solid microcrystals and thus the light reflected from them gives the tomato its typical bright red color. This was depicted previously in a schematic way [21]. Phytoene synthase (PSY) catalyzes the first committed and rate-limiting step in carotenoid biosynthesis, and involves the condensation of two molecules of GGPP to form phytoene. This reaction is catalyzed by PSY in higher plants and bacteria [1,22]. The cyclization of lycopene to beta-carotene was also reviewed previously [23]. Two lycopene cyclases, lycopene ε- and β-cyclases, catalyze the conversion of lycopene to either α-carotene or β-carotene [24] and only a single enzyme, lycopene β-cyclase, is required to introduce β-rings at both ends of lycopene to form β-carotene, which is then converted to zeaxanthin via cryptoxanthin. Both ε-CYC and β-CYC are required to introduce one ε- and one β-ring into lycopene to form α-carotene, which is then hydroxylated to lutein. These two competing steps of lycopene cyclization determine the proportion of lycopene channeled to the two branches of the carotenoid pathway [24,25].

As the experimentation with genetically modified crops has become more widespread, the amount of misinformation has increased concomitantly, and the accompanying debate has gotten hotter, while the qualities of the locally bred tomatoes in the developing countries, to the bet of our knowledge, are seemingly unraveled. The aim of the present work is to investigate the rates at which tomatoes ripe via the study of the bioaccumulation of lycopene and beta-carotene in the fruits of the four cultivars (*Ajindi-Kerewa*, *Beske*, *3-Lobes* and *Big Local)* commonly grown in Nigeria under field and ambient temperature conditions. The empirical and kinetic models as well as the mechanisms of the bioaccumulation were discussed. The kinetic parameters so obtained could prove useful as a future breeding tool in monitoring the success or failure in the desired improvement in the plant breeding.

2 MATERIALS AND METHODS

2.1 Sample preparation

The seeds of the four cultivars of the tomatoes were collected from the International Institute of Tropical Agriculture (IITA), Ibadan, Nigeria and were planted on an open farmyard in Ogbomoso, Nigeria between May and September rainy season, 2011. The fruits were identified at National Horticultural Institute (NIHORT), Ibadan, Nigeria. The tomatoes were independently and randomly selected and packed into nylon bags and then taken into the laboratory, where they were rinsed with some doubly distilled water and left to drain for some minutes. Tomatoes subjected to postharvest ripening were harvested at the mature green stages and ripened under ambient temperature. Individual tomatoes were sliced and all parts of the fruits were utilized. The fruit tissue was cut into small pieces and about 500 g of fresh tomato samples was homogenized.

2.2 Extraction and quantification of lycopene and β-carotene

Conventional solvent extraction methods [26] were employed for carotenoid extraction. Lycopene and β-carotene from the tomato fruits were extracted with hexane, methanol and acetone (2:1:1) containing 2.5% butylated hydroxytoluene (BHT). The extract was treated with doubly distilled water, methanol and 20 % KOH/methanol (1:1:1) to saponify any triglyceride present. The extract was then washed with doubly distilled water and re-dissolved in hexane. The absorbance of the hexane extracts were measured at 450 and 502nm nm using Genesys 10S V1.200 spectrophotometer (Buck Scientific, USA). The lycopene and β-carotene concentrations were determined previously reported protocol [27,28] with the molar absorptivities of 1.16×10^5 and 1.72×10^5 L mol^{-1}cm^{-1}for lycopene as well as 1.39×10^5 and 2.63×10^4 L mol^{-1}cm^{-1}for β-carotene at 450 nm and 502 nm respectively [29].

3 RESULTS AND DISCUSSION

3.1 Kinetics of lycopene bioformation in tomatoes

Figures 1A and 1B show the variation of the lycopene contents in *Ajindi-Kerewa*, *Beske*, *3-Lobes* and *Big Local* tomato cultivars with ripening days under vine and ambient temperature conditions. As evident from the Figure 1A, lycopene bioaccumulation in tomatoes ripened on the vine increases with the days of

ripening until maxima of 13.8 and 18.1 µg/g fresh tomato weight were reached at 10 days of ripening in *Ajindi-Kerewa* and *Beske* cultivars respectively. However, the maximum concentrations of 12.9 and 13.9 µg lycopene per gram fresh weight were only reached at the fully-red stage of ripening (12 days) in *3-Lobes* and *Big Local* tomato cultivars respectively. In contrast, the maximum lycopene concentrations in all the tomato cultivars were observed at the twelfth day of ripening when the tomatoes reached a fully red stage under postharvest ripening condition. The maximum lycopene concentrations obtained in tomatoes subjected to ambient temperature ripening were only 50% of those allowed to self-ripe on the vine. This indicates that tomatoes ripened on vine better accumulate lycopene than those subjected to postharvest ripening conditions.

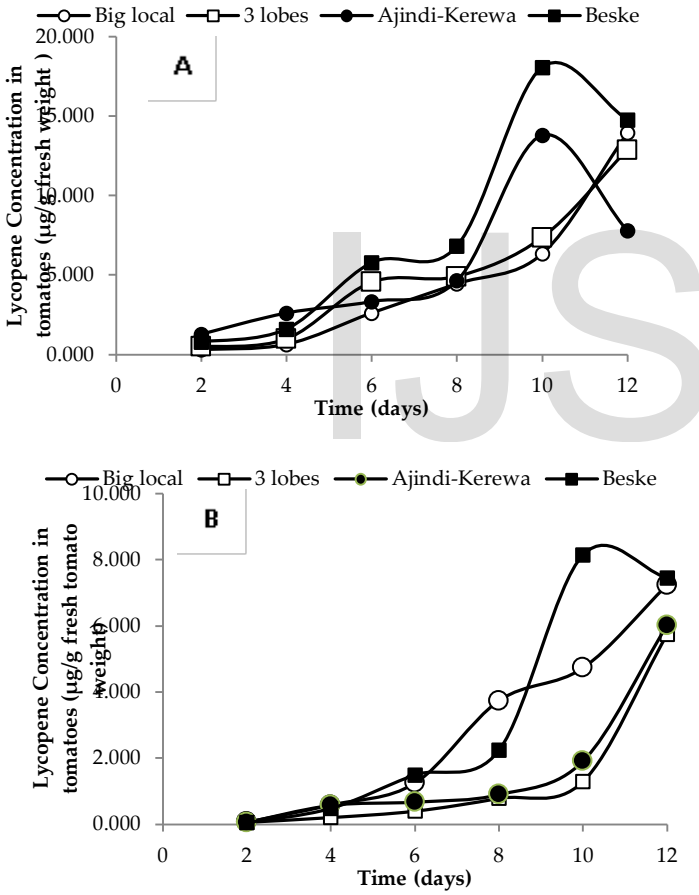

Figure 1: Variation of lycopene concentrations with the days of tomato ripening in *Ajindi-Kerewa*, *Beske*, *3-Lobes* and *Big Local* tomato cultivars under (A) field ripening conditions (B) ambient temperature ripening.

While the kinetics of the lycopene degradation in tomatoes during processing have been studied [30-32] as well as the kinetics of its bioavailability [33,34], the kinetics of the lycopene and beta-carotene (especially in the Nigerian tomato cultivars) seems to be poorly characterized and documented. A study on the Ibadan-local and Roma cultivars suggest that the biosynthesis of lycopene in the tomatoes follows a first order kinetic scheme [35].

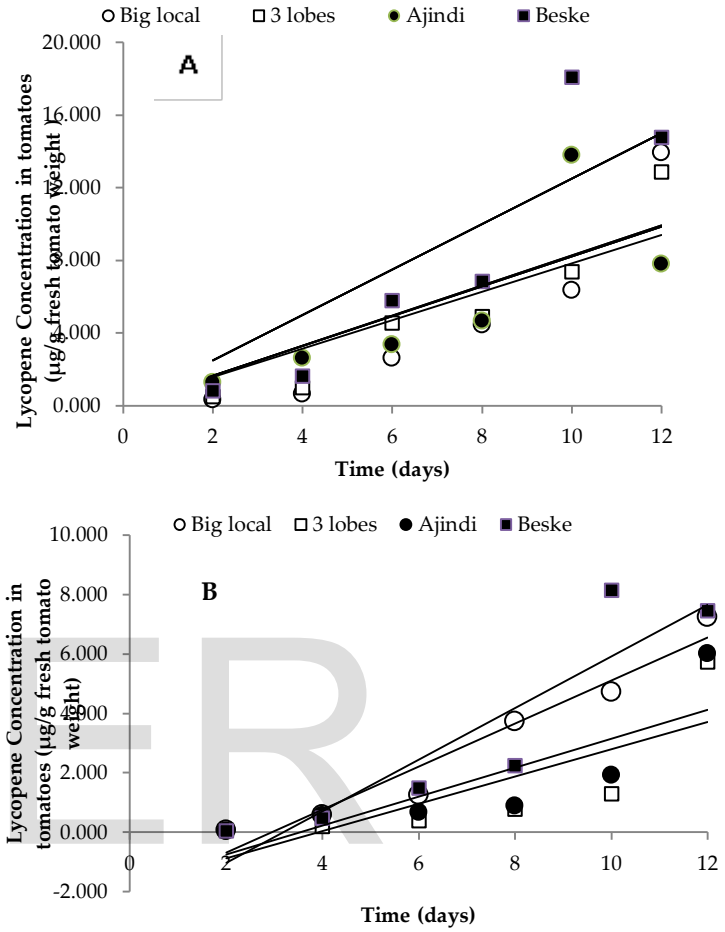

Figure 2: Zero order kinetic plots for lycopene bioaccumulation in *Ajindi-Kerewa*, *Beske*, *3-Lobes* and *Big Local* tomato cultivars under (A) field ripening conditions (B) ambient temperature ripening.

The zero order and first order kinetic plots of the lycopene accumulation in tomatoes under vine ripening were as shown in Figures 2A and 2B while the respective plots for ambient temperature tomato ripening were shown in Figures 3A and 3B. The kinetic parameters so obtained were summarized in Table 1. The coefficients of determination (R^2 values), ranging from 0.843 to 0.985, are all times higher for first order plots than zero order plots with poor correlations (R^2 values ranging between 0.468 and 0.847). This suggests that the bioaccumulation of lycopene in *Ajindi-Kerewa*, *Beske*, *3-Lobes* and *Big Local* tomato cultivars follows a first order kinetic fashion with the respective first order rate constants of 0.050, 0.079, 0.101 and 0.075 per day for tomatoes ripened on the vine. For those ripened at ambient temperature conditions, the first order rate constants of 0.040, 0.054, 0.030 and 0.137 per day respectively were obtained. This implies that the rate of lycopene bioaccu-

mulation in tomato fruits is directly proportional to the concentration of the geranylgeranyl pyrophosphate (GGPP) precursor present in the plant tissue.

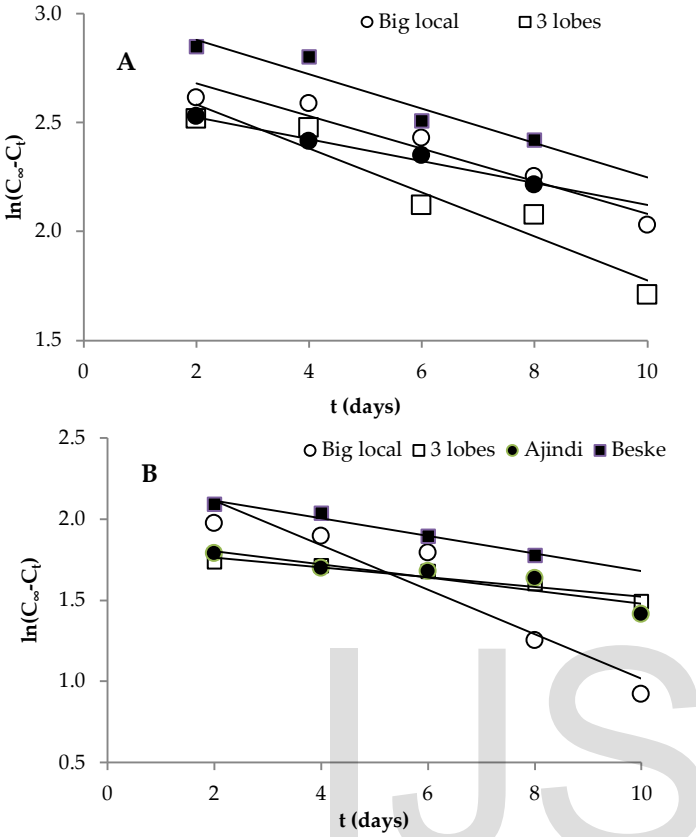

Figure 3: First order kinetic plots for lycopene bioaccumulation in *Ajindi-Kerewa*, *Beske*, *3-Lobes* and *Big Local* tomato cultivars under (A) field ripening conditions (B) ambient temperature ripening.

It was previously reported [17] that during tomato fruit ripening, a massive accumulation of lycopene occurs. Phytoene synthase has been demonstrated to have greater influence over the pathway flux and there was reduction in the cyclisation reaction [41]. The expression of phytoene synthase-1 and desaturase were observed to increase significantly at the onset of ripening, while the cyclases were down regulated [36]. Thus, lycopene accumulation in tomato fruits is as a result of increase of flux through the initial stages of the path way and a restriction on end products that are typically found in vegetative tissues [17]. All these corroborate the suggestion of the present finding that the increase in flux flow is GGPP concentration dependent, which culminate in the accumulation of lycopene in the tomato fruit.

3.2 Kinetics of beta-carotene bioformation in tomatoes

The variation of the beta-carotene contents in *Ajindi-Kerewa*, *Beske*, *3-Lobes* and *Big Local* tomato cultivars with ripening days under vine and ambient temperature conditions were as shown in Figures 4A and 4B respectively. As observed from the Figure 4A, beta-carotene concentrations in tomatoes ripened under both conditions increases with the days of ripening with no inflexion point. The highest beta-carotene concentration of 5.3 μg/g fresh tomato weight was reached at 12 days of vine ripening in *Ajindi-Kerewa* while the lowest values were recorded at the second day ripening stage in all the cultivars. On the contrary, the highest beta-carotene concentration in all the tomato cultivars observed at the twelfth day of ripening when the tomatoes reached a fully red stage under postharvest ripening condition were about half the amount recorded for vine ripening technique.

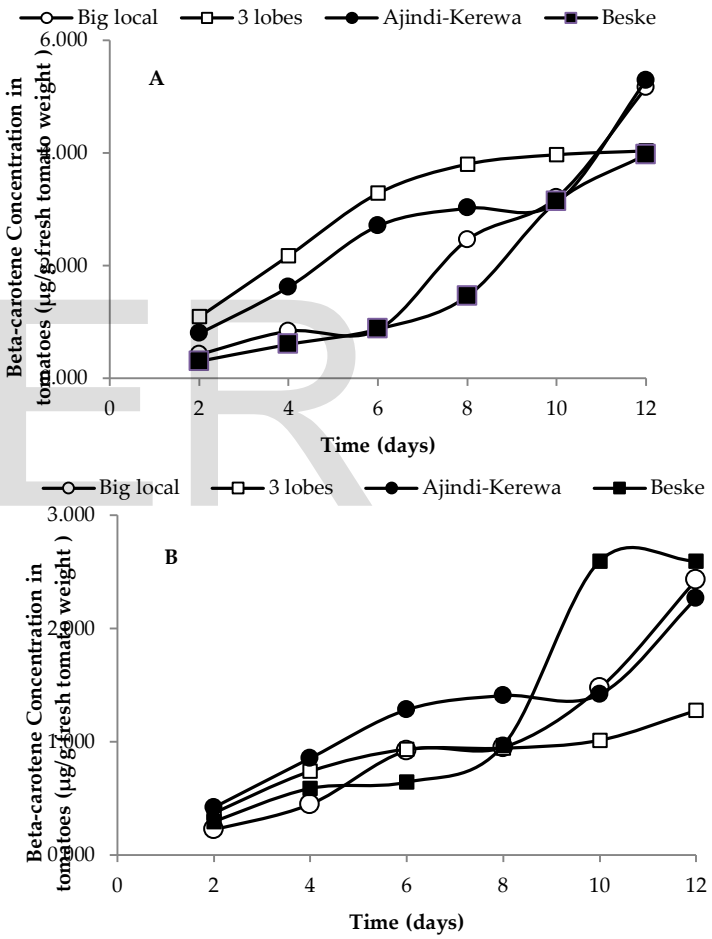

Figure 4: Variation of beta-carotene concentrations with the days of tomato ripening in *Ajindi-Kerewa*, *Beske*, *3-Lobes* and *Big Local* tomato cultivars under (A) field ripening conditions (B) ambient temperature ripening.

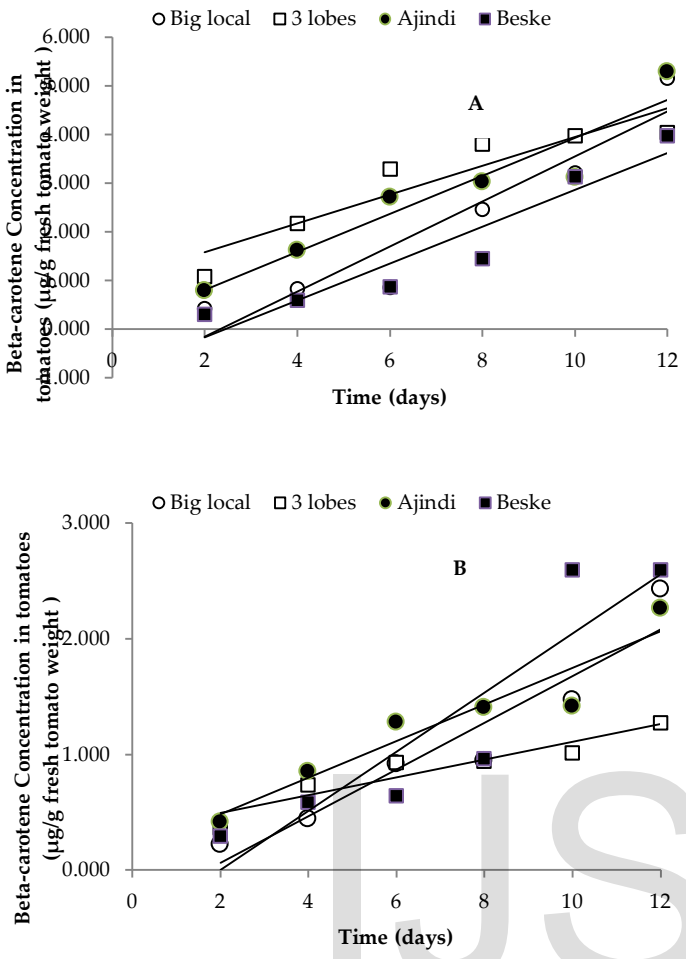

Figure 5: Zero order kinetic plots for beta-carotene bioaccumulation in *Ajindi-Kerewa*, *Beske, 3-Lobes* and *Big Local* tomato cultivars under (A) field ripening conditions (B) ambient temperature ripening.

Previous study on the Ibadan-local and Roma cultivars of Nigerian tomatoes suggest that the biosynthesis of beta-carotene in the tomatoes follows a zero order kinetics for tomatoes ripened on the vine while no simple order for the kinetics of beta-carotene biosynthesis was obtained when the tomatoes were ripened under ambient temperature condition [35]. Figures 5A and 5B show the zero order and first order kinetic plots of the beta-carotene accumulation in tomatoes under vine ripening, while Figures 6A and 6B represent these plots for ambient temperature tomato ripening respectively. The kinetic parameters so obtained were also summarized and included in Table 1. Zero order kinetics favours the bioaccumulation of beta-carotene in *Ajindi-Kerewa and Beske* cultivars under vine ripening as reported earlier for Ibadan-local and Roma cultivars [35] with the rate constants of 0.393 and 0.272 µg/g/day respectively. This is indicating that the bioaccumulation of beta-carotene in these two cultivars of tomatoes may not exclusively dependent on the concentration of the lycopene in the tissue of tomato fruits. However, in *3-Lobes* and b*ig Local* tomato cultivars subjected to vine ripening, it follows a first order kinetic fashion with the respective first order rate constants of 0.480 and 0.112 per day. This deviates from the previous conclusion in other cultivars and may be associated with differences in the genetic background of the cultivars studied.

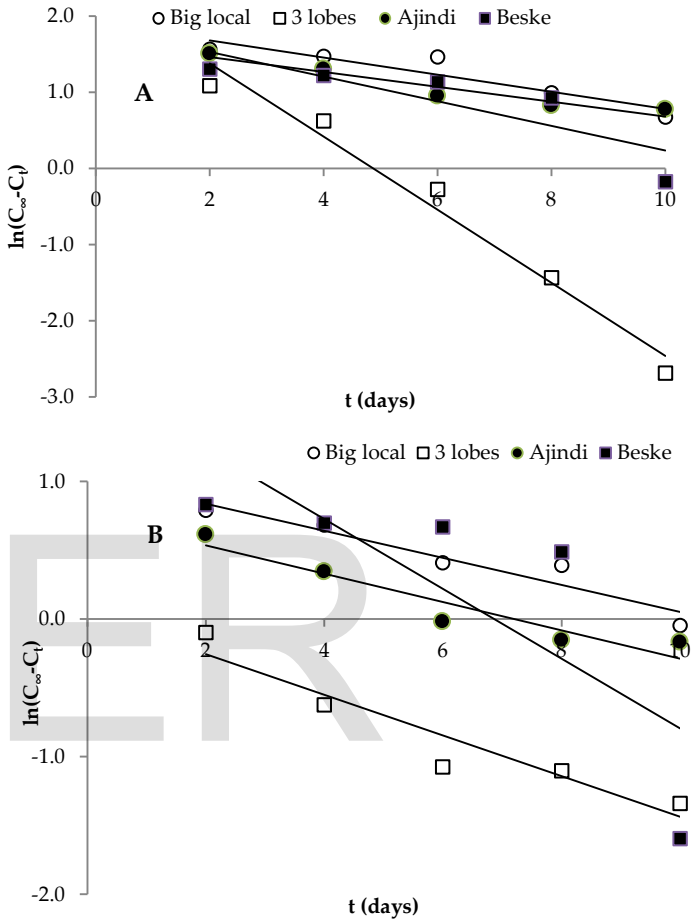

Figure 6: First order kinetic plots for beta-carotene bioaccumulation in *Ajindi-Kerewa*, *Beske, 3-Lobes* and *Big Local* tomato cultivars under (A) field ripening conditions (B) ambient temperature ripening.

In contrast to a previous report [35], stating that no simple order was for beta-carotene biosynthesis under ambient temperature condition, the present work shows that first order kinetics favours beta-carotene bioaccumulation more than zero order for all the tomato cultivars ripened at ambient temperature conditions. The first order rate constants of 0.132, 0.053 0.147, and 0.098 per day were obtained in *Ajindi-Kerewa*, *Beske, 3-Lobes* and *Big Local* tomato cultivars respectively. The expression of zero order alongside first order of reaction for beta-carotene accumulation in tomato points to the possibility that many regulatory mechanisms may be needed to influence beta-carotene bioaccumulation and, combination of genes and promoters may be necessary to manipulate the synthetic

pathway. High beta-carotene formation has been achieved by over-expression of an endogenous lycopene beta-cyclase gene in tomato under a constitutive promoter [39].

Table 1: Kinetic data for lycopene and beta-carotene bioformation in some cultivars of tomatoes

Cultivars	Field Ripening		Ambient Temperature Ripening	
	Zero Order	First Order	Zero Order	First Order
	*Rate Constant ($\mu g\ g^{-1}day^{-1}$)	*Rate Constant (day^{-1})	*Rate Constant ($\mu g\ g^{-1}day^{-1}$)	*Rate Constant (day^{-1})
LYCOPENE BIOFORMATION				
Ajindi-Kerewa	0.827 (0.680)	0.050 (0.985)	0.288 (0.540)	0.040 (0.843)
Beske	1.250 (0.805)	0.079 (0.922)	0.549 (0.690)	0.054 (0.972)
3-lobes	0.823 (0.847)	0.101 (0.928)	0.251 (0.468)	0.030 (0.920)
Big local	0.783 (0.740)	0.075 (0.944)	0.478 (0.809)	0.137 (0.898)
BETA-CAROTENE BIOFORMATION				
Ajindi-Kerewa	0.393 (0.939)	0.096 (0.927)	0.177 (0.934)	0.132 (0.973)
Beske	0.272 (0.849)	0.162 (0.716)	0.197 (0.830)	0.053 (0.934)
3-lobes	0.410 (0.861)	0.480 (0.973)	0.116 (0.834)	0.147 (0.903)
Big local	0.338 (0.854)	0.112 (0.862)	0.163 (0.896)	0.098 (0.916)

* Correlation coefficient, R^2 in the parenthesis

3.3 Mechanism of lycopene and beta-carotene bioaccumulation in tomatoes: A typical consecutive reaction

The immediate precursor of carotenoids is geranylgeranyl pyrophosphate (GGPP). The condensation of two molecules of GGPP to form phytoene is the first committed and rate-limiting step in carotenoid synthesis and is catalyzed by phytoene synthase. Other products are generated from the phytoene stepwise desaturation [reviewed in 23]. These phytoene desaturase genes, identified and isolated from tomato [37], contain a conserved dinucleotide binding site domain at the amino terminus [1].

Thus, this reaction can be illustrated as:

$$2GGPP \xrightarrow{-8[H]} Lycopene + PP_i \quad (1)$$

Since the bioaccumulation of lycopene in the four cultivars of tomato studied has been shown to follow first order kinetics, it follows that:

$$G = G_o e^{-k_1 t} \quad (2)$$

where G_o and G represent the initial concentrations of GGPP and its concentration at tomato ripening day, t with first order rate constant, k_1.

Also reported is that lycopene is cyclized to form β-carotene by only a single enzyme, lycopene β-cyclase, required to introduce β-rings at both ends of, which is then converted to zeaxanthin via cryptoxanthin, whereas both ε-CYC and β-CYC are required to introduce one ε- and one β-ring into lycopene to form α-carotene, which is then hydroxylated to lutein. These two competing steps of lycopene cyclization determine the proportion of lycopene channeled to the two branches of the carotenoid pathway [24]. These and other findings have prompted several attempts to employ lycopene cyclization as a means of manipulating the ratios of the α- and β-carotenes in plants [42].

This can also be illustrated as follows:

$$Lycopene \xrightarrow{k_2} \beta - carotene \quad (3)$$

$$Lycopene \xrightarrow{k_2} \alpha - carotene \quad (4)$$

However, experiments show that the α-carotene is either absent in most tomatoes or its concentration is much lower, below detecting limit [38,39]. This gives rise to negligible value of k_3. Thus, compressing equations 1 and 3, we have:

$$2GGPP \xrightarrow{-8[H],k_1} Lycopene \xrightarrow{k_2} \beta - carotene \quad (5)$$

This follows a consecutive reaction whose reaction scheme has been depicted previously [21]. The rates of bioaccumulation of lycopene and beta-carotene were depicted previously [35] in a single equation (Equation 6).

$$\frac{dL}{dt} = G_o e^{-k_1 t} - k_0 \quad (6)$$

where L is the concentration of lycopene at the tomato ripening day, t; k_0 is the zero order rate constant for beta-carotene accumulation and G_0 and k_1 were as explained previously.

Equation 6 only works when the bioaccumulations of lycopene and beta-carotene follow first and zero order kinetics respectively as observed with Ibadan-local, Roma, *Ajindi-Kerewa* and *Beske* cultivars of tomato.

In the case that the bioaccumulations of both lycopene and beta-carotene follow first order kinetics, the following deductions can be made:

$$\frac{dG}{dt} = -k_1 G = G_o e^{-k_1 t} \quad (7)$$

$$\frac{dL}{dt} = k_1 G - k_2 L = k_1 G_o e^{-k_1 t} - k_2 L \quad (8)$$

$$\frac{dB}{dt} = k_2 L \quad (9)$$

where G, L and B represent the concentrations of geranylgeranyl pyrophosphate, lycopene and beta-carotene respectively; k_1 and k_2 are the first order rate constants for the bioaccumulation of lycopene and beta-carotene respectively.

Since it is our aim to develop and simplify the analysis of the consecutive reactions involved in the bioaccumulation of lyco-

pene and beta-carotene, using integration method, such that the initial concentrations of lycopene (L_0) and beta-carotene (B_0) are zeros.

By solving the differential equation 8 and applying the limits, it follows that the concentration of lycopene at any tomato ripening day, t is given as:

$$L = \frac{k_1}{k_2 - k_1} G_o (e^{-k_1 t} - e^{-k_2 t}) \quad (10)$$

Consequently by mass balance, the concentration of beta-carotene at any tomato ripening day, t is also given as:

$$B = G_0 - L - G = G_0 \left[1 - \frac{k_1}{k_2 - k_1} (e^{-k_1 t} - e^{-k_2 t}) - e^{-k_1 t} \right] \quad (11)$$

However, in this work it appears that k_2 is slightly higher than k_1 giving rise to the appreciable amounts of lycopene and beta-carotene in the tomato cultivars under study. In the case, where the traits desired in tomato is high lycopene but lower beta-carotene concentrations, the enzymatic cyclization of lycopene to beta-carotene could be inhibited or knocked out appropriately leading a slow or no cyclization reaction. This could be done by down-regulation of the enzyme, lycopene beta-cyclase at the breaker stage of tomato fruit development [37]. If this is achieved, k_2 will be so low that $k_1 >> k_2$ and $k_1 - k_2 \sim k_1$. In this case, equation 10 reduces to:

$$L = G_o (e^{-k_2 t} - e^{-k_1 t}) \quad (12)$$

If $k_1 >> k_2$, the second term in the parentheses rapidly approaches zero while the first term is still near unity. Consequently, the lycopene concentration, L rapidly reaches a value nearly equal to G_0, the initial concentration of GGPP, and then during most of the reaction, lycopene becomes cyclized slowly according to the equation:

$$L = G_o e^{-k_2 t} \quad (13)$$

On the other hand, where the high beta-carotene concentrations traits is desired such as in HighCaro tomato fruits, lycopene beta-cyclase is expressed also at the breaker stage, causing almost complete conversion or cyclization of lycopene to beta-carotene [40]. Under this case, $k_2 >> k_1$ and $k_2 - k_1 \sim k_2$. In this case, equation 10 reduces to:

$$L = \frac{k_1}{k_2} G_o (e^{-k_1 t} - e^{-k_2 t}) \quad (14)$$

Since $k_2 >> k_1$, the second term in the parentheses rapidly approaches zero, while the first term
is still close to unity. Consequently, the concentration of lycopene, L rapidly approaches

$$L = \frac{k_1}{k_2} G_o e^{-k_2 t} \quad (15)$$

Because k_1/k_2 is very small, the maximum concentration of lycopene is much less than G_0, the initial concentration of GGPP.

4 CONCLUSION

The present work studies the kinetics of the carotenoids accumulation in the new breeds of tomatoes and could allow the comparison of the kinetic parameters, thereby enhancing the understanding of the progress being made in plant breeding, most especially in developing countries. Improving the carotenoid contents of tomatoes leads to its increased antioxidant and radical scavenging ability which complement other sources of antioxidants. However, much more dedicated work and further research studies are required to ensure positive progress in improving the shelf life of tomatoes. This will reduce the level of tomato shrinkage, which considerably lower their market value and consumer acceptability.

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