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Kinetics Of Lycopene Biosynthesis In Tomato Fruits

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ABSTRACT

The rate at which tomato fruits ripe was investigated via the biosynthesis of lycopene in two tomato varieties under the conditions of field and ambient temperature ripening. Results showed that the biosynthesis of lycopene follows a first-order kinetics in both varieties with calculated rate constants of 1.2×10^{-2} and 1.4×10^{-2} d^{-1} for Ibadan local and Roma types respectively under the field ripening; and 2.1×10^{-2} and 5.3×10^{-2} d^{-1} for Ibadan local and Roma types respectively at the ambient temperature ripening. The formation of β-carotene follows zero-order kinetics in both varieties under the field ripening after 8 days (Roma type) and 10 days (Ibadan-local type), with respective calculated rate constants of 43.81 and 54.7 ppm d^{-1}. No simple order was obtained for the formation of β-carotene at the ambient temperature ripening. The empirical rate law for the lycopene biosynthesis under the field ripening suggests the expression: $\frac{d[\text{Lycopene}]}{dt} = k_1[\text{GGPP}]e^{-k_1 t} - k_0$ where GGPP is geranyl geranyl diphosphate.

KEYWORDS

Kinetics;
Lycopene;
β-Carotene;
Biosynthesis and tomato fruits.

INTRODUCTION

Tomato is any fruit of the numerous cultivated varieties of *Lycopersicon esculentum*, a plant of the nightshade family(Solanaceae) and also the fruit of *Lycopersicon pimpinelli*, the tiny currant tomato[1]. The crop has increased in popularity in the tropics and subtropics until it is the second most important vegetable in these regions since the end of the nineteenth century[2]. The fruits are also grown in all parts of Nigeria.

Lycopene, a member of carotenoid family, which accumulates in human blood(~0.5μ mol/litre plasma) and the tissue(from 1nmol/g wet wt. in adi-

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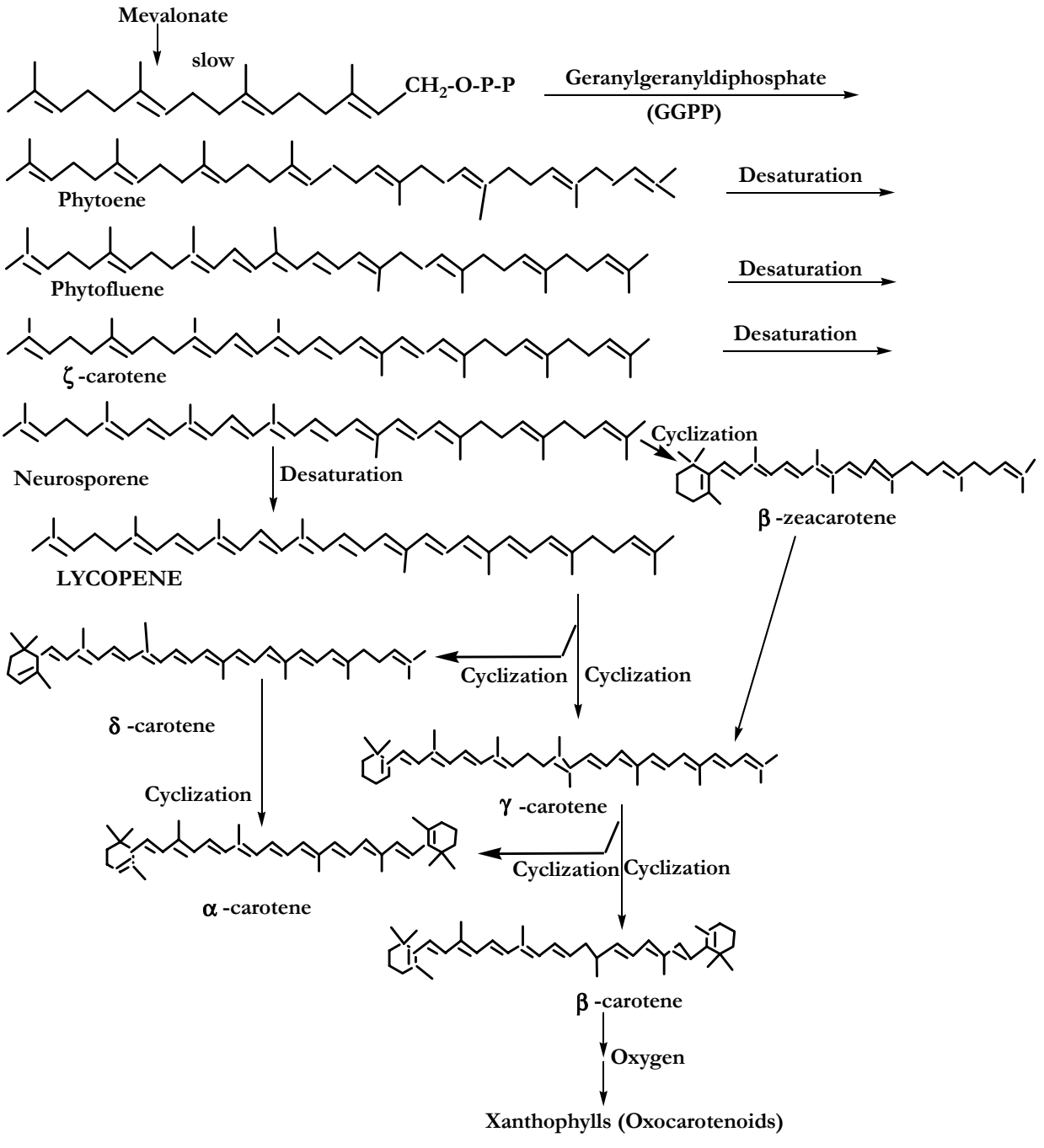

Biosynthesis of Carotenoids showing the central position of lycopene

SCHEME 1

pose tissue to up to 20nmol/g wet wt. in adrenals and testes)[3], is an acyclic, strong unsaturated hydrocarbon formed from isoprene units linked end to end[4]. Carotenoids are biosynthesized by a special branch of the terpenoid pathway(SCHEME 1)[5,6]. Lycopene(11 conjugated and two non-conjugated double bonds) is one of the most potent antioxidants[7–11] with a singlet-oxygen quenching ability twice as high as that of β-carotene and 10 times higher than that of α-tocopherol[10]. The mechanism of the enhanced reactivity of lycopene compared to other C-40 carotenoids remains to be elucidated. It has been speculated that the increased reactivity of lycopene is related to the presence of the two non-conjugated double bonds[10, 11].

This study was undertaken to elucidate overall

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kinetics and mechanisms of lycopene biosynthesis by carrying out (i) the rate at which lycopene accumulates in tomato fruits under different stages of ripening, (ii) the rate of formation of β-carotene in tomato fruits, (iii) the dependence of hydrogen ion concentration on the rate of formation of lycopene. To our knowledge, it appears that no such kinetic study has previously been reported on lycopene biosynthesis in the literature.

EXPERIMENTAL

Sampling

For field ripening, the tomato fruits were plucked at random with respect to the ripening classes every morning of each period. The tomato fruits were plucked at once in the mature green stage and were left to 'self-ripe' in a well-ventilated room of the research laboratory for ripening at the ambient temperature. The description of the tomato varieties used with their ripening classes are given in TABLE 1 and 2. About 3-5 whole fruits were chopped into smaller pieces in a mortar and samples were kept in the refrigerator prior to analysis.

Extraction

Lycopene from tomato fruits was extracted with hexane, methanol, acetone(2:1:1), containing 2.5% 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.

Physical measurements

Optical density of the hexane extract was scanned spectrophotometrically in the wavelength range 400-750nm against a hexane blank using UV-visible spectrophotometer, Unicam Heλ10Sα V 2.05. Concentrations of lycopene and β-carotene were calculated at $\lambda_{max.}$ of 505 and 487nm respectively. The pH of the raw fruit juice at each ripening stage was measured using pH meter (Mettler Toledo) MP 120.

RESULTS AND DISCUSSION

The description of varieties of tomato fruits used and their ripening classes are given in TABLES 1 and 2 respectively. TABLE 3 shows the concentrations of lycopene in tomato fruits. It could be observed that the lycopene biosynthesis is slow in the first 7-8 days of ripening but reached the maximal of 36.44 and 43.22 ppm for Ibadan-local and Roma types respectively at the light-red stage on the parent plant (field ripening) and maximal of 24.07 and 21.04 ppm for Ibadan-local and Roma types respectively at the pink stage in the detached tomato fruits ripened at the ambient temperature. The highest concentration peaks of lycopene at these ripening stages may be associated to the increasing concentration of geranyl geranyl diphosphate (GGPP) and/or the increasing activation of the enzymes responsible for the conversion. The decrease in concentration of lycopene after 12 days (field ripening) and 10 days(ambient temperature ripening) may be due to conversion of lycopene to β-carotene via cyclization reaction.

The analytical data were subjected to kinetic investigations, and the kinetic data of tomato fruits lycopene at both field and ambient temperature ripening are given in TABLE 5. The variation of lycopene concentration with time is similar to a case of consecutive reaction and can be represented as:

TABLE 1: Description of the tomato varieties used

Type	Ibadan-local	Roma
Shape	Serrated	Gourd
Number of lobes	8-10	2-4
Size	Small-big	Small-big
Color of unripe – fruits	Green	Green
Color of ripe - fruits	Red	Red

TABLE 2: Description of the ripening classes of the tomato fruits

Ripening class	Description#	Time (days)##
Breaker	First appearance of external pink, red or tarnish yellow color not more than 10%	2, 3, 4
Turning	Over 10% but not more than 30% red, pink or tarnish yellow	6, (7or 8)*
Pink	Over 30% but not more than 60% pinkish or red	10
Light-red	Over 60% but not more than 90% red	12
Fully - red	Over 90% red	13

#Percentage (%) refers to both color distribution and intensity; ##days to reach the ripening classes; * 7 days (Ibadan-local) and 8 days (Roma type)

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TABLE 3: Concentration (ppm)[#] of lycopene in tomato fruits

Ripening stage	Field ripening		Ambient temperature ripening	
	Ibadan-local	Roma type	Ibadan-local	Roma type
B_2	0.94 ± 0.02	1.19 ± 0.03	0.97 ± 0.05	0.22 ± 0.02
B_3	1.50 ± 0.10	1.56 ± 0.07	1.21 ± 0.04	1.75 ± 0.05
B_4	1.85 ± 0.05	2.35 ± 0.10	2.09 ± 0.07	3.11 ± 0.10
T_6	2.73 ± 0.10	3.48 ± 0.10	3.05 ± 0.04	4.88 ± 0.06
T_8	3.15 ± 0.08*	4.49 ± 0.01	3.55 ± 0.07*	6.74 ± 0.08
P	9.00 ± 0.01	22.86 ± 0.02	24.07 ± 0.10	21.04 ± 0.10
LR	36.44 ± 0.01	43.22 ± 0.02	18.27 ± 0.05	16.60 ± 0.10
FR	18.59 ± 0.09	19.40 ± 0.06	13.73 ± 0.10	5.22 ± 0.05

[#]Values are means ± SEM, n = 5; *Values at day 7 for Ibadan-local type; B_2, B_3 and B_4 are breaker stage at days 2, 3 and 4 respectively; T_6 and T_8 are turning stage at days 6 and 8 respectively; P is the pink stage; LR is light red stage; FR is the fully-red stage

TABLE 4: Concentration (ppm)[#] of β-carotene in tomato fruits

Ripening stage	Field ripening		Ambient temperature ripening	
	Ibadan-local	Roma type	Ibadan-local	Roma type
B_2	29.98 ± 0.05	30.34 ± 0.10	5.92 ± 0.05	6.73 ± 0.15
B_3	31.32 ± 0.20	31.18 ± 0.35	6.81 ± 0.02	7.34 ± 0.04
B_4	30.62 ± 0.10	32.23 ± 0.05	5.96 ± 0.04	8.43 ± 0.05
T_6	30.05 ± 0.10	30.88 ± 0.01	7.22 ± 0.05	7.92 ± 0.09
T_8	29.84 ± 0.09*	37.64 ± 0.10	7.71 ± 0.02*	10.31 ± 0.10
P	48.35 ± 0.05	106.05 ± 0.20	17.79 ± 0.10	17.94 ± 0.05
LR	168.05 ± 0.50	209.21 ± 0.60	11.17 ± 0.05	9.18 ± 0.10
FR	210.04 ± 0.40	252.30 ± 0.92	14.59 ± 0.07	15.42 ± 0.08

*Values at day 7 for Ibadan-local type

The rate of disappearance of GGPP is given as;

$$\text{Rate} = \frac{d[C_{20}H_{33}OPP]}{dt} = K_1[C_{20}H_{33}OPP]^x \quad (1)$$

The rate of formation of lycopene and Beta-carotene are respectively given as;

$$\text{Rate} = \frac{d[C_{40}H_{56}]}{dt} = K_1[C_{20}H_{33}OPP]^x - k_2[C_{40}H_{56}]^y \quad (2)$$

$$\text{Rate} = \frac{d[\beta - C_{40}H_{56}]}{dt} = k_2[C_{40}H_{56}]^y \quad (3)$$

Where x and y are the order of reactions with respect to GGPP and lycopene respectively.

A plot of $In(C\infty - C_t)$ against t gives a pseudo-first order kinetics (Figure 1), where $C\infty$ is the maximum lycopene concentration in each case, C_t is the concentration of lycopene at time t, and $C\infty - C_t$ is the concentration of geranyl geranyl diphosphate, calculable from lycopene concentration. It could be observed that the plots follow a first-order kinetics in both varieties under the field and ambient temperature ripening until after 7 days(Ibadan-local) and 8

TABLE 5: Kinetic data of tomato fruits lycopene

t(days)	Field ripening		Ripening at ambient temperature	
	Ibadan local	Roma type	Ibadan local	Roma type
	$In(C_\infty - C_t)$	$In(C_\infty - C_t)$	$In(C_\infty - C_t)$	$In(C_\infty - C_t)$
0	3.596	3.766	3.181	3.046
2	3.570	3.738	3.140	3.056
3	3.554	3.730	3.129	2.960
4	3.543	3.710	3.090	2.886
6	3.518	3.682	3.045	2.783
8	3.505*	3.657	3.021*	2.660
10	3.312	3.014	∞	∞
12	∞	∞	-	-
13	-	-	-	-

*values at day 7 for Ibadan Local type

$$C_{20}H_{33}OPP \xrightarrow{k_1} C_{40}H_{56} \xrightarrow{k_2} \beta\text{-}C_{40}H_{56}$$
(GGPP) (Lycopene) (Beta -carotene)

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days(Roma type) when the plots deviated from the first-order kinetics; with respective calculated first-order rate constants of 1.2×10^{-2} and 1.4×10^{-2} d^{-1} under the field ripening and 2.1×10^{-2} and 5.3×10^{-2} d^{-1} at the ambient temperature ripening. Since the formation of lycopene follows first-order kinetics (Figure 1), equation (1) can be integrated to give:

$$[C_{20}H_{33}OPP]=[C_{20}H_{33}OPP]_0\, e^{-k_1 t} \quad (4)$$

Where k_1 is the first-order rate constant and $[C_{20}H_{33}OPP]_0$ is the initial concentration of geranyl geranyl diphosphate. Combination of equations (2) and (4) gives equation (5) as follows:

$$\frac{d[C_{40}H_{56}]}{dt}=[C_{20}H_{33}OPP]_0\, e^{-k_1 t} - k_2[C_{40}H_{56}]^y \quad (5)$$

The concentrations of β-carotene in tomato fruits are as shown in TABLE 4. The initial accumulation of β-carotene may be due to the conversion of neurosprene to β-zeacarotene, which in turns forms γ-carotene that isomerizes to β-carotene and the high accumulation of β-carotene after 10 days in both varieties under the field ripening may be attributed to simultaneous conversion reactions of both lycopene and γ-carotene (formed from β-zeacarotene) to β-carotene. Following the biosynthesis of β-carotene (SCHEME 1), there is non-existence of 'induction period' (the period during which no β-carotene is obtained). The concentration of β-carotene is expected to be low at initial stage with high concentration of lycopene if β-carotene is biosynthesized mainly from lycopene; but this is contrary to our observation (TABLES 3 and 4). For example, at the initial stage (i.e. B_2 stage), lycopene concentration was 0.94 ppm (TABLE 3) while β-carotene concentration was 29.98 ppm (TABLE 4). This is an indication that β-carotene is also formed from other intermediate compounds, such as β-zeacarotene, apart from lycopene (SCHEME 1 refers). Our observation suggests that the rate of β-carotene biosynthesis is independent of lycopene concentration at a certain period of ripening.

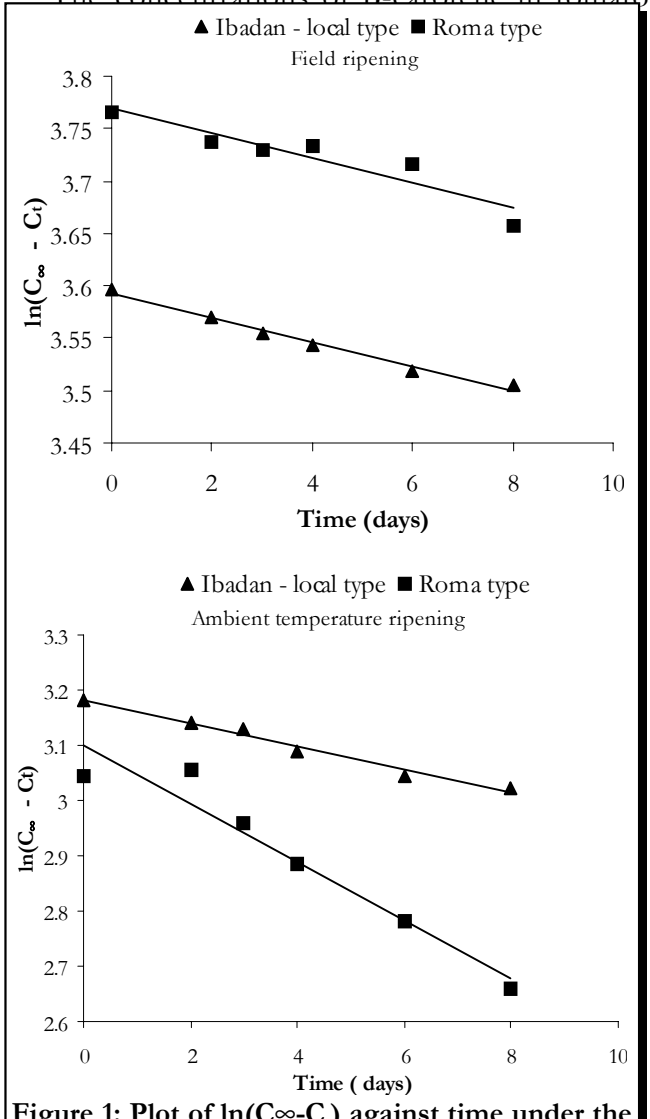

Figure 1: Plot of $\ln(C\infty-C_t)$ against time under the field and ambient temperature ripening

The kinetic plot of β-carotene concentration against the period(days) of ripening of tomato fruits showed zero-order kinetics after 8 days (Roma type) and10 days(Ibadan-local type) (Figure 2), with respective zero-order rate constants of 43.81 and 54.7ppm d^{-1}. Hence, equation(5) can then be re-written as:

$$\frac{d[C_{40}H_{56}]}{dt}=[C_{20}H_{33}OPP]_0\, e^{-k_1 t} - k_0 \quad (6)$$

Where k_0 is the zero-order rate constant. No simple order was obtained for the β-carotene formation in both tomato varieties under ambient temperature ripening (Figure 3). This may be as a result of inhibition of enzymes, resulting from unfavorable condition for the enzymes, thereby suggesting the biosynthetic reaction pathways to be enzymic in nature.

In order to confirm whether the lycopene biosynthesis is acid-catalyzed or not, the pH at each ripening stage was measured under the conditions studied and the result is given in TABLE 6. For acid-catalyzed enzymic reaction:

$$Rate=k^*[H^+][Substrate] \quad (7)$$

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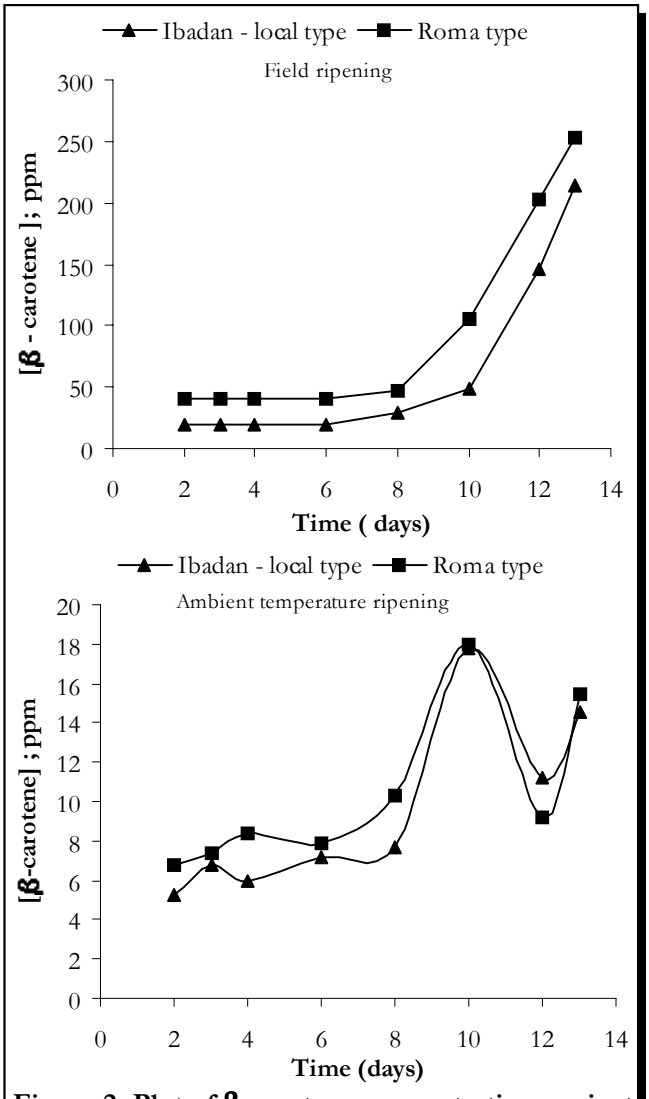

Figure 2: Plot of β-carotene concentration against time under the field and ambient temperature ripening

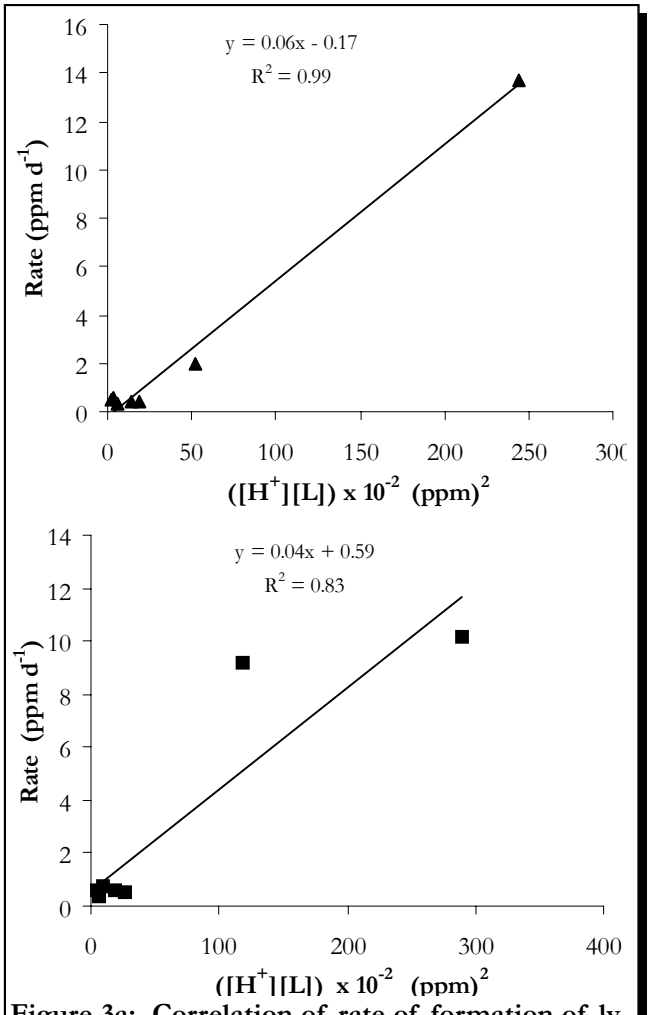

Figure 3a: Correlation of rate of formation of lycopene with the product of concentrations of hydrogen ion and lycopene under the field ripening. The R^2 value and the linear least square fit equations are given in the figures

TABLE 6: Hydrogen ion concentration* of the tomato fruits

t (days)	Field ripening		Ripening at ambient temperature	
	Ibadan local	Roma type	Ibadan local	Roma type
2	2.300 ± 0.071#	3.398 ± 0.096	4.211 ± 0.100	3.902 ± 0.212
3	2.319 ± 0.571	3.708 ± 1.080	4.169 ± 0.095	3.719 ± 0.170
4	3.412 ± 0.619	4.216 ± 0.111	4.192 ± 0.010	3.811 ± 0.067
6	5.188 ± 0.204	5.206 ± 0.166	3.986 ± 0.246	3.912 ± 1.124
8	6.026 ± 0.510	5.888 ± 0.200	4.075 ± 0.250	4.169 ± 0.135
10	5.756 ± 0.133	5.188 ± 0.012	6.918 ± 0.400	6.614 ± 0.304
12	6.719 ± 0.690	6.717 ± 0.657	7.099 ± 0.490	7.765 ± 0.179
13	8.428 ± 0.485	13.581 ±1.560	6.918 ± 0.280	6.317 ± 0.290

* $[H^+]$(10^{-5} mol dm^{-3}); #values are means ± SEM, n = 5

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TABLE 7: Dependence of rate of lycopene biosynthesis on the hydrogen ion concentration

t(days)	Field ripening				Ripening at ambient temperature			
	Ibadan local		Roma type		Ibadan local		Roma type	
	Rate (ppm/day)	*$[H^+]$ $[L]\times 10^{-2}$ $(ppm)^2$	Rate (ppm/day)	*$[H^+][L]\times 10^{2}$ $(ppm)^2$	Rate (ppm/day)	*$[H^+][L]\times 10^{-2}$ $(ppm)^2$	Rate (ppm/day)	*$[H^+][L]\times 10^{-2}$ $(ppm)^2$
2	0.470	2.162	0.595	4.044	0.485	4.085	0.110	0.858
3	0.560	3.479	0.370	5.784	0.240	5.044	1.530	6.508
4	0.350	6.312	0.790	9.908	0.880	8.761	1.360	11.852
6	0.440	14.163	0.565	18.117	0.480	12.157	1.770	19.091
8	0.420	18.982	0.505	26.437	0.500	14.467	0.930	28.099
10	1.950	51.804	9.185	118.598	6.840	166.516	7.150	139.159
12	13.72	244.840	10.180	290.031	-2.900	129.699	-4.440	128.899
13	-17.85	156.677	-23.820	263.471	-4.54	94.984	-11.380	32.975

*$[H^+]$, mol dm^{-3} is equivalent to 1000 ppm; [L] = [Lycopene] in ppm

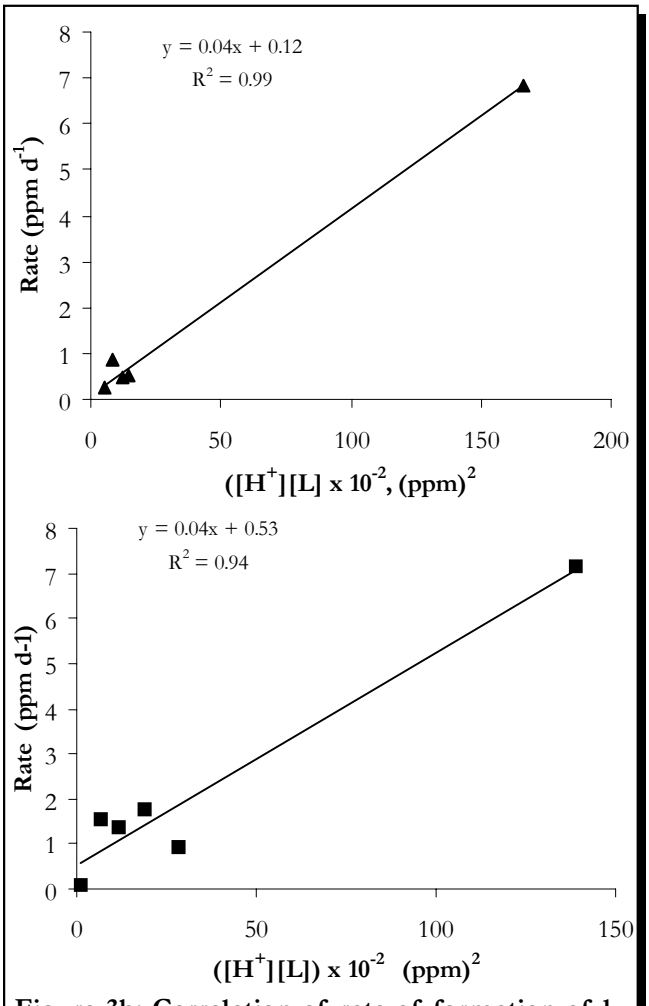

Figure 3b: Correlation of rate of formation of lycopene with the product of concentrations of hydrogen ion and lycopene at the ambient temperature ripening. The R^2 value and the linear least square fit equations are given in the figures

Where lycopene is the substrate and k^* is the acid-catalyzed rate constant. Based on equation (7), we investigated the dependence of $[H^+]$ concentration on the rate of formation of lycopene at both field and ambient temperature ripening as a means of confirming whether the enzymic reaction for lycopene biosynthesis is acid-catalyzed or not. The calculated rates of formation of lycopene were plotted against the product of concentrations of hydrogen ion and lycopene. The rate of formation of lycopene is linearly correlated with $[H^+]$[Lycopene] (Figure 3), the linear least squared fit to the plotted data had an R^2 values of 0.9899 and 0.8266 for Ibadan-local and Roma types respectively under the field ripening (Figure 3a); and 0.9922 and 0.9453 for Ibadan-local and Roma types respectively at the ambient temperature ripening (Figure 3b). Thus, the rate of formation of lycopene is directly proportional to the hydrogen ion concentration for both conditions studied; and the linear least squares fit equation can be used as a predictive equation for the rate of lycopene biosynthesis, by measuring the lycopene content and pH of the tomato fruit juice, and inserting each value into the linear equation.

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