Aritra Adhya et al /J. Pharm. Sci. & Res. Vol. 12(7), 2020, 920-924

ISSN:0975-1459
Journal of Pharmaceutical Sciences and Research
www.jpsr.pharmainfo.in

A review on antioxidant activity and degradation kinetics study of Lycopene

Aritra Adhya [1] and Dr. Bhaskar Choudhury [2] *
[1,2] *Department of Life Sciences,*
Guru Nanak Institute of Pharmaceutical Science & Technology, Kolkata, West Bengal, India.

Abstract

Among all the dietary carotenoids, lycopene is one of the most potent naturally occurring anti-oxidant and anti-microbial, red coloured plant pigment. Lycopene has unique structural and chemical features which contribute to specific biological properties and pharmacological activities *in-vivo* and *in-vitro*. The kinetic parameters so obtained could prove usefulness in developing countries as a future biotechnological tool in improvement of plant-breeding programs. The anti-microbial and anti-oxidant potential of lycopene may be responsible for its associated health benefits that have attracted an attention of many researchers. Further investigations will be required to evaluate and understand the lycopene-metabolite interactions. This review summarizes the current understandings of lycopene and established its efficacy in prevention of various diseases and future directions of research.

Key Words: - Lycopene, Plant-Breeding, Kinetics, Anti-microbial, Anti-oxidant.

INTRODUCTION

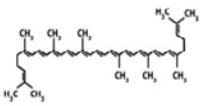
Natural plant pigments have drawn a great attention worldwide. These pigments are nothing but a combination of different phytochemicals in different types of plant cells. Lycopene is a red coloured pigment found in various plants, algae and fungi but not synthesized by any animal. Tomato, papaya, watermelon are considered as major natural source of lycopene. It is a carotenoid olefin and acyclic isomer of beta-carotene [1]. Lycopene possesses a great anti-oxidant property and mild anti-microbial activity due to the presence of conjugated double bonds. High intake of lycopene and its products are associated with prevention chronic diseases like CVD (cardiovascular disease), cancer, diabetes, AIDS & neurological disorders [2]. More specifically it is an acyclic carotene having 11 conjugated carbon-carbon double bonds and 2 unconjugated carbon-carbon double bonds which pre-dominantly exists in 'all-*trans*' configuration.

From the toxicity study, it has been revealed that lycopene doesn't possess any kind acute, chronic and sub-chronic or genotoxicity in animals. So, based on these facts, USFDA has approved it as GRAS (Generally Recognized As Safe) with limited health claim declaration. As a result of it, lycopene is being used in food-industry as a food-additive [3]. Our present mini-review work report the physico-chemical properties, anti-oxidant and anti-microbial properties of lycopene, its degradation kinetics and protective effects from various diseases.

Physico-chemical properties of Lycopene

Molecular Formula:- $C_{40}H_{56}$

Molecular Structure:-



Molecular Weight:- 536.87 Da

Melting Point:- 172-175°C

Density:- 0.889g/cc

Stability:- Sensitive to light, oxygen, high temperature, acids, catalysts and metal ions.

Source:- Tomato (*Lycopersicon esculentum*), Papaya (*Carica papaya*), Watermelon (*Carica lanatus*), Guava(*Psidum guajava*), Micro-algae (*Dunaliella salina*) etc.

Crystal form: - Dark reddish-brown needle like crystals from carbon disulphide and ethanol mixed solvent.

Powder form:- Dark reddish-brown amorphous solid.

Spectral properties of Lycopene

UV-Visible Spectra of Lycopene:-

This spectrum is used to quantify the amount of lycopene present in any solution derived from any natural sources. The amount of lycopene is measured by taking absorbance of solution at 503 nm where $\varepsilon = 17.2 \times 10^4$ mol/cm. In this method, lycopene content is quantitatively detected in "mg/100g" unit. Although the percentage purity is very poor (6-10%) in this method [4].

IR Spectra of Lycopene [5]:-

- 2920.04 cm^{-1} (weak) - The presence of $_{sp3}C$-H bond (stretching).
- 1564.80cm^{-1} (weak) - The presence of $_{sp2}C=C_{sp2}$ bond (stretching).
- 1046.66cm^{-1} (strong) - The presence of $_{sp3}C-C_{sp3}$ bond (stretching).

NMR Spectra of Lycopene:-

NMR spectra of all-*trans* lycopene is determined at 400 MHz (^1H) & 1000MHz (^{13}C) respectively. Nuclear overhouser effect is prominent in lycopene indicating internal steric effect between pi-electron cloud of C=C bonds at particular positions. Tetra methyl silane (TMS) is considered as internal standard and spectrum is observed like $CDCl_3$ & C_6D_6. The result is as follows [6]

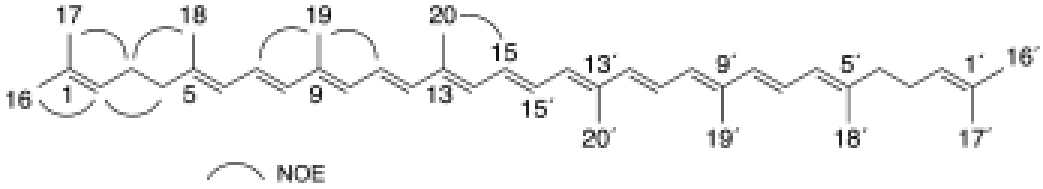


Figure 1: Chemical structure of lycopene. The Nuclear Overhouser effect (NOE) of lycopene molecule

proton	δ in ppm (multiplicity, coupling constant in Hz) in $CDCl_3$ [synthetic lycopene][38]	δ in ppm (multiplicity, coupling constant in Hz) in C_6D_6	carbon	δ in ppm in $CDCl_3$ [synthetic]	δ in ppm in C_6D_6
			C(1), C(1′)	131.72 [131.64]	131.58
H–C(2), H–C(2′)	5.11 (m) [5.11]	5.23 (m)	C(2), C(2′)	123.96 [124.12]	124.50
2H–C(3), 2H–C(3′)	2.11 (m) [ca. 2.11]	2.18 (m)	C(3), C(3′)	26.70 [26.83]	27.10
2H–C(4), 2H–C(4′)	2.11 (m) [ca. 2.11]	2.18 (m)	C(4), C(4′)	40.23 [40.30]	40.62
			C(5), C(5′)	139.47 [139.30]	138.95
H–C(6), H–C(6′)	5.95 (d, *J* = 11.0) [5.95]	6.16 (d, *J* = 11.0)	C(6), C(6′)	125.74 [125.94]	126.77
H–C(7), H–C(7′)	6.49 (dd, *J* = 11.0, 15.0) [6.49]	6.67 (dd, *J* = 11.0, 15.1)	C(7), C(7′)	124.79 [124.87]	125.31
H–C(8), H–C(8′)	6.25 (d, *J* = 15.0) [6.25]	6.44 (d, *J* = 15.1)	C(8), C(8′)	135.40 [135.54]	136.17
			C(9), C(9′)	136.15 [136.15]	136.37
H–C(10), H–C(10′)	6.18 (d, *J* = 11.4) [6.18]	6.36 (d, *J* = 11.6)	C(10), C(10′)	131.55 [131.64]	132.39
H–C(11), H–C(11′)	6.64 (dd, *J* = 11.4, 14.9) [6.64]	6.77 (dd, *J* = 11.6, 15.0)	C(11), C(11′)	125.15 [125.21]	125.66
H–C(12), H–C(12′)	6.35 (d, *J* = 14.9) [6.35]	6.49 (d, *J* = 15.0)	C(12), C(12′)	137.35 [137.46]	138.00
			C(13), C(13′)	136.56 [136.54]	136.83
H–C(14), H–C(14′)	6.24 (m) [6.25]	6.34 (AA′ of AA′BB′ system)	C(14), C(14′)	132.64 [132.71]	133.39
H–C(15), H–C(15′)	6.62 (m) [6.62]	6.70 (BB′ of AA′BB′ system)	C(15), C(15′)	130.07 [130.17]	130.70
3H–C(16), 3H–C(16′)	1.687 (s) [1.688]	1.674 (s)	C(16), C(16′)	25.68 [25.66]	25.84
3H–C(17), 3H–C(17′)	1.614 (s) [1.612]	1.568 (s)	C(17), C(17′)	17.70 [17.70]	17.73
3H–C(18), 3H–C(18′)	1.818 (s) [1.818]	1.749 (s)	C(18), C(18′)	16.99 [16.97]	16.90
3H–C(19), 3H–C(19′)	1.968 (s) [1.968]	1.925 (s)	C(19), C(19′)	12.90 [12.90]	12.98
3H–C(20), 3H–C(20′)	1.968 (s) [1.968]	1.876 (s)	C(20), C(20′)	12.79 [12.81]	12.86

[a] ^1H and ^{13}C NMR were recorded at 400 and 100 MHz, respectively. s, singlet; d, doublet; dd, doublet of doublets; and m, multiplet.

Figure 2: ^1H and ^{13}P NMR spectral analysis of lycopene molecule from tomato

Anti-oxidant property of Lycopene

The *in-vitro* & *in-vivo* reactivity of lycopene consists of its ability to quench the singlet oxygen atom which is generated in our body through some biochemical reactions [7]. These reactive oxygen species (ROS) are totally scavenged by lycopene or any other carotene. When, lycopene is exposed to free radicals, a visual colour change reaction takes place [8]. Supplement of lycopene inflicts the cellular production of ROSs and slower the rate of tissue destruction from it [11]. As lycopene is lipid-soluble, the anti-oxidant property is more prominent in fatty layer in compared to aqueous layer of our body [9]. The singlet-oxygen-quenching ability of Lycopene is twice of β-carotene and 10 times higher than that of α-tocopherol [1]. Lycopene, under *in-vitro* conditions, undergoes auto-oxidation in presence of light, heat, aerobic oxygen and forms several simpler organic compounds viz. acetone, glyoxal (yields a grass-like odour), methyl heptenone, laevulinic aldehyde [10].

The in-vivo mechanism of action for lycopene degradation towards ROSs can be predicted through 3 possible ways as follows [12, 13]

1. Adduct Formation

In this type of reaction, olefenic lycopene just attacks with alkyl radical and converted to less toxic radical which readily dimerizes and may excretes by kidneys. This type of reaction is reversible and not so feasible kinetically *in-vivo*.

2. Electron Transfer

Under high concentration of ROSs, lycopene scavenges the cellular oxidation through this kind of mechanistic pathway. In this reaction, lycopene lowers the energy of free-radical and converts them into a stable carboanion. This carboanion can take part in conventional s_N2 or E_2 reaction. This reaction is thermodynamically and kinetically spontaneous and experimentally established by using laser flash photolysis.

3. Allylic Hydrogen Substitution

In this type of reaction, allylic hydrogen of lycopene is directly absorbed by free-radical or singlet oxygen molecule. Through this mechanism, ROSs are stabilized through hydrocarbon or alchol formation which can further be oxidized and finally stabilizes as ketones or carboxylic acids respectively.

In a model *ex-vivo* study, Krinsky et. al. confirms that lipid soluble lycopene prevents lipid peroxidation as follows [12]

$$R\text{-}COO^{\cdot} + Ly = R\text{-}COO\text{-}Ly^{\cdot}$$

However, the subsequent reaction mechanisms are not well understood till date. As a polyene, lycopene is expected to be a poor anti-oxidant due to lesser interactions with aqueous phase materials. Besides, it exhibits *synergistic effect* combined with other anti-oxidants (like vitamin C) *in-vitro*. A very advanced approach for detecting anti-oxidant property is DPPH assay test by virtue of which we can measure the extent of free-radical scavenging by lycopene quantitatively through the usage of a single spectrophotometer only [8, 14, 15].

Lycopene as an Anti-microbial Agent

Lycopene is considered as one of the major phytochemical, synthesized by plants and microorganisms but not by animals. Being a carotenoid (due to the presence of C=C bond), it possesses anti-microbial role due to its anti-oxidant property. For this reason, lycopene is being used as food additive which confers health-benefits and gives a red colour to the food product. Conventional microbial plating and Dot-ELISA method shows that lycopene has a weaker anti-microbial activity compared to standard antibiotics [16, 17].

Degradation Kinetics of Lycopene

Lycopene, due to its acyclic nature, undergoes a slow biotransformation at room temperature but at elevated temperature, the rate of biotransformation process is enhanced. In case of lycopene, biotransformation process includes cyclisation and isomerisation by virtue of which lycopene converts to β-carotene, α-carotene & 6-cis isomers (15-cis, 13-cis, 11-cis, 9-cis, 7-cis, 5-cis lycopene) [1,13]. Moreover, it is also oxidized aerobically at elevated temperature (40°C and above) [or at room temperature in presence of catalyst] to yield "long apo-lycopenals/ones" and "short apo-lycopenals/ones" as major product through unstable lycopene monoxide intermediate.[13] The kinetics of lycopene degradation reactions are influenced by reaction medium, physical and environmental conditions.

Now, the change can easily be detected and monitored through visual colour measurement with the help of a Hunter colorimeter. It determines the visual colour in terms of lightness (L), redness & greenness (a) and yellowness & blueness (b). To detect the rate of biotransformation we have to measure the change of L, a, b values under experimental conditions. [22] At first, the colorimeter is calibrated with a white tile (L= 90.55, a= -0.71, b= 0.39) and with respect to this, visual colour change is measured and recorded quantitatively.[22]

The kinetics of degradation of both pigment and visual colour change has been reported to follow first-order reaction adequately [22, 23]. The first order kinetic model can be expressed as follows:-

$$\ln (L/L_0) = -k_1 t \quad \text{.....(1)}$$

Where, L - Amount of lycopene at time "t".

L_0 - Initial amount of lycopene [at time "0"]

$-k_1$ Reaction rate constant [in $hour^{-1}$]

t - Heating time [in hours]

The Arrhenius model can be applied to describe the temperature dependence of this biotransformation reaction. This is done as follows:

$$k = k_0 \exp(-E_a/RT) \quad \text{..... (2)}$$

where, k_0- frequency factor, E_a- activation energy, R- Universal gas constant, T- absolute temperature .

Now, the first order reaction in terms of fractional conversion maybe represented as:-

$$\ln (1-f_1) = -k_2 t \quad \text{.....(3)}$$

Where, f_1 denotes the extent of transformed lycopene.

Now, we cannot predict this value directly but from the different hunter colour values (a, b) as they are proportional to each other [15]. Usually, (a*b) values are analyzed for colour degradation as follows:-

$$\ln [(a*b)/(a_0*b_0)] = -k_3 t \quad \text{.....(4)}$$

Where, a*b- product of the redness-greenness and yellowness-blueness parameter at any time t = t.

a_0*b_0- product of the redness-greenness and yellowness-blueness parameter at time t=0.

Now, correlating these standard kinetic equations with visual colour changing parameters we get:-

$$\ln [(a_0*b_0) - (a*b)/ (a_0*b_0) - (a_\alpha*b_\alpha)] = -k_4 t \quad \text{..... (5)}$$

or, $\ln (1-f_2) = -k_4 t$... (6) [f_2 denotes colour value correlated fraction of chemically converted lycopene]

Where, $a_\alpha*b_\alpha$- product of the redness-greenness & yellowness-blueness parameter at time $t=\alpha$ (infinite); infinite time refers heating time = 10 hours.

Now, the change in visual colour is a direct manifestation of change in pigment concentration. The hunter values are correlated with lycopene content to get actual kinetic expression or result. The relationship between visual colour and lycopene content [22]

$$(a*b) = (k_a + k_b)* L \quad \text{..... (7)}$$

Where, L denotes lycopene content in µg/gram or mg/100g unit.

k_a- coefficient of 'a'; k_b- coefficient of 'b'; both are f(T) [22]

Now, Kaur et al. [22] studied the kinetics of the reaction and analyzed the experimental data, regression coefficient and correlation coefficient using Statistica (version-5.0).They also considered the activation energy simultaneously and prepared kinetic feasibility of this reaction. The kinetic result is as follows:-

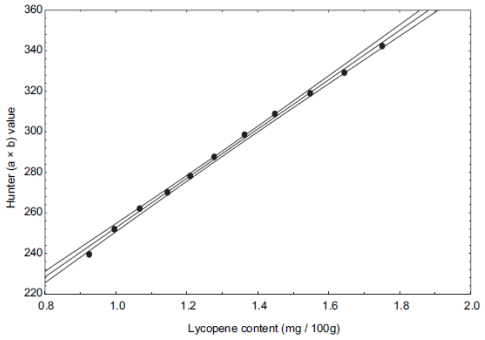


Figure 3: Correlation between lycopene and visual colour of tomato peel at 100° C

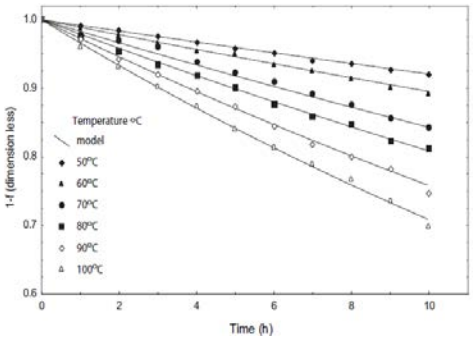


Figure 4: Hunter value (a x b) degradation kinetics of tomato peel at selected temperature

Now, considering Arrhenius equation, we get:-

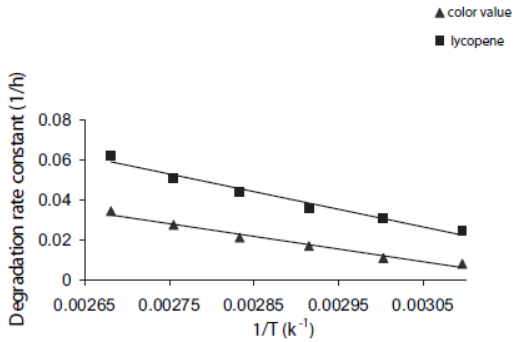


Figure 5 : Dependence of degradation rate constant for lycopene and Hunter colour of tomato peel on temperature using Arrhenius model

Preventive effects of Lycopene

The red pigment lycopene confers preventive health benefits to human beings. Many researchers have already been reviewed the preventive effects of lycopene on several chronic diseases. Here, in this short review, we will discuss about the effects of lycopene on oxidative stress, cancer, cardiovascular diseases (CVD), diabetes and AIDS based on well-established experimental data or facts.

Oxidative Stress

It is one of the major chronic diseases of the world. Cellular free-radicals or ROSs are the potential contributors leading to the oxidative stress which accelerates the aging process by increasing oxidative load. Studies have shown that lipids, proteins, DNAs are subjected to oxidative stress i.e. they can degrade via oxidation [13].

Lycopene-enriched diet and supplementation provides a significant reduction of DNA damage in both of normal and cancerous cell lines [18, 19]. DNA-oxidation scavenging activity of lycopene is more prominent in rats. *In-vivo* toxicity study on rats also suggests that lycopene reduces lipid peroxidation and lymphocytic DNA damage along with least affected kidney fibroblast cells [20]. However, Riso et. al. reported no significant difference in lymphocytic DNA damage and peroxidised markers between a tomato-based drink treated group and control group[13]. In healthy human volunteers, lycopene supplementation reduces lipid oxidation rates compared to lycopene-free diets [1].

Synergistic effect is very clear in reducing oxidative stress i.e. when lycopene is treated with other carotene or anti-oxidant, clinical efficacy has been increased. Lycopene fortified with vitamin C or beta-carotene shows greater anti-oxidant capacity as they reduces protein oxidation by lowering thiobutyric acid reactive substances (TBARS) in urine [13].

Cancer

Cancer is a major health problem around the world. The disease is caused by several biochemicals, microbiological or genetic factors. This disease can only be cured if it is detected and treated at an early stage. So, prevention of cancer is highly solicited; lycopene can play a key role in near future to fulfil this purpose.

Lycopene is affirmed as a potent anti-cancer agent in various laboratory researches, clinical trials and epidemiological studies [1]. Nkondjock et. al. [23], in a perspective study, revealed that lycopene consumption may decrease the risk of pancreas cancer. Several pre-clinical studies have claimed that it can prevent tumour formation and skin cancer as well. The key role of lycopene to prevent prostate cancer has been widely studied [13]. Further investigations are required to understand the anti-cancer mechanism of lycopene.

Cardio-vascular Diseases

As per the WHO report, cardiovascular diseases (CVD) is the largest killer of the world; particularly in poor, developing countries. To prevent this disease, lycopene could be effective tool because of its cheapness and moderate bioavailability. Cumulative evidences shows that CVD can be prevented for years through consumption of lycopene-rich diet.

Lycopene readily dissolves in fatty (lipid) layer of human body. It suppresses the cholesterol bio-synthesis by inhibiting HMG-CoA reductase. It also reduces LDL & VLDL content in blood serum that also reduces the probability of ischemic strokes [13, 21]. Ahuja et. al. reported that lycopene-rich oil reduces CVD by improvement of lipid content in blood serum in animals [1]. More human trials are necessary to validate this fact strongly.

In addition, lycopene also reduces hypertension, the silent killer. Reduction of hypertension also reduces the chance of heart-attack. Even flavanols, carotenoids and

polyphenols are recommended as DASH (dietary approach for controlling hypertension) material by some dieting associations [1].

Diabetes

Lycopene is closely related to our metabolites interfering their kinetic feasibility. It also affects the blood-sugar governing metabolites. Coyne et. al. suggested serum lycopene content increases along with decrease in plasma sugar level in fasting state. Wang et. al. have found that plasma lycopene reduces the type-2-diabetes in female & middle-aged patients. But, lycopene-metabolite interaction is not well understood and clinical results are often contradictory. Further studies are necessary to fetch a bold conclusion.

AIDS

More recently, several investigations shows that lycopene passively activates T-cells and B-cells by removing strong, harmful oxidizing agents from human body. As T-cells and B-cells become more active they resist the growth of HIV viruses in our body [1].

Effect on other diseases

Lycopene also delivers preventive health benefits over respiratory infections, cognitive behaviour, eye cataract, erythema, neurodegenerative diseases and male infertility. [1,13]

Toxic effects of Lycopene

There is lack of information on the adverse consequences of lycopene in human. Although, Lycopenemia is caused due to high intake of lycopene containing foods.[24] Moreover, it is deposited in liver causing hepatic dysfunction [25]. Acute, sub-chronic, chronic toxicity and genotoxicity studies reveal that lycopene is non-toxic itself but accumulation of this pigment may elongate gestation period causing mild maternal toxicity.

Future Directions of Research

Most of the studies considered tomatoes and tomato originated products as lycopene source. However, it will be the aim of future human intervention studies to include other lycopene-enriched fruits like guava, papaya, watermelon etc. and consider synergistic effects with other food components and their importance *in-vivo*. Much more studies are required to understand the anti-inflammatory and anti-atherogenic mechanism of lycopene and its phenotypic modulation. The effect of lycopene on renal cell and its' carcinoma, renal dysfunction and oxidative stress could be our future interest of research. The clinical effect of lycopene in endothelial functionality is still unknown along with its correlation with endoplasmic reticulum stress; we can make study on it.

CONCLUSION

Our present review supports the importance of lycopene in improving our normal body functionality and primary & secondary prevention of diseases. The possible kinetic study depicts the chemical and thermal stability of the pigment by which we can conclude that it can exhibit its beneficial pharmacological activity within us. It is a safe, non-toxic compound which can be used to raise a generally healthy population. So, lycopene consumption should be promoted in our daily diet.

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