

## A proficient singlet oxygen quenching carotenoid and periodontal disease

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### Abstract

Periodontal disease is one of the most prevalent chronic oral conditions known to affect mankind. Periodontal disease has been linked with oxidative stress either directly or indirectly. An imbalance in the host mechanism to address these oxide free radicals during inflammation forms a major course of chronic diseases. The role of antioxidants associated with decreased risk of chronic diseases has come a long way with applications in general alignments. Lycopene, a highly potent antioxidant, a phytochemical and a carotenoid found in red colored fruits and vegetables are being widely tried for its applicability in chronic diseases. Owing to the sparse data regarding the benefits of lycopene in periodontal diseases, this review highlights the scientific documentation of lycopene as an adjuvant in management of periodontal disease.

**Keywords:** Lycopene, Carotenoid, Phytochemical, Antioxidant, Periodontal disease, Oxidative stress.

### Introduction

Good nutrition will help prevent 95% of all diseases (*Linus Pauling*).<sup>1</sup> Nutrition comprises of macronutrients and micronutrients, Gowland Hopkins<sup>2</sup> recognized “accessory food factors” other than calories, proteins and minerals as organic materials which cannot be synthesized in the body that later came to be known as vitamins and a property most lured about are their antioxidant ability. Antioxidants include vitamins, minerals and phytonutrients. Phytonutrients, also called phytochemicals are chemicals produced by plants and microorganisms. Phytonutrients primarily protect plants from harmful radiation however have shown significant benefits for humans as well. Though they are not considered nutrients that are essential for life, like carbohydrates, proteins, fats, vitamins and minerals, however evidence have shown antioxidant and anti-inflammatory activities that are effective in reducing risks of cancer and heart diseases (USDA).<sup>3</sup>

Till date, more than 600 different carotenoids have been identified among them 20 carotenoids have been found in blood and tissues of humans and as close as 90% of carotenoids formed by beta-carotene, alpha-carotene, lycopene, lutein and cryptoxanthin. Beta-carotene's ability to produce vitamin A and carotenoid's antioxidant properties mark them as an important nutritional supplement.

Naturally occurring potent antioxidant of recent interest is lycopene, the chemical that gives fruits and vegetables its red color. Discovered by Ernst<sup>4</sup> in 1959, several studies have demonstrated the benefits of lycopene as an effective antioxidant, a potent singlet oxygen quencher, reducing risk of cancer and chronic diseases, amongst which is periodontal disease.

Periodontitis is defined<sup>5</sup> as “an inflammatory disease of the supportive tissues of the teeth caused by specific microorganisms or group of specific microorganisms, resulting in progressive destruction of the periodontal ligament and alveolar bone with increased probing depth,

recession or both, when left untreated will progress to tooth loss”.<sup>5</sup> Periodontal diseases is among the most common of chronic diseases known to affect adults,<sup>6</sup> an estimated 40-90% of global population, one of the most prevalent epidemic in the world.<sup>7</sup> The main cause being, inflammatory reaction in periodontium secondary to accumulation of microbial plaque in the dento-gingival complex. The periodontal phenotype that is characterized by hyperinflammation involving excess release of oxygen free radicals by inflammatory cells, especially by polymorphonuclear leucocytes (PMNLs).<sup>8</sup> Neutrophils control bacterial invasion by oxidative and nonoxidative mechanisms, in oxidative mechanism there is formation of reactive oxygen species (ROS).<sup>9</sup> Most ROS formed by PMNLs and phagocytes, have extremely short half-lives and are highly potent they can cause substantial tissue damage by initiating free radical (FR) chain reactions. Normally there exists a dynamic equilibrium between ROS activity and host defense mechanism but a shift in its equilibrium has been considered as one of the major factors responsible for periodontal tissue destruction.<sup>10</sup>

Lycopene has gained popularity over the past few decades as an effective singlet oxygen quencher in management of chronic diseases. This paper compiles the scientific data about lycopene in management of one of the most prevalent chronic oral condition – the periodontal disease.

### Sources

Some of the good sources of lycopene are found fruits and vegetables that are red colored such as tomatoes, watermelons, pink-grapefruits, apricot, papaya and pink-guavas. About 85% of all dietary sources of lycopene are obtained by tomatoes and their byproducts however; specific variety of tomato and their ripened stage affects the lycopene content. While deep red variety contains 50mg/kg, yellow tomatoes as low as 5mg/kg.<sup>11,12</sup> Lycopene content of different fruits and tomato products are shown in table 1.

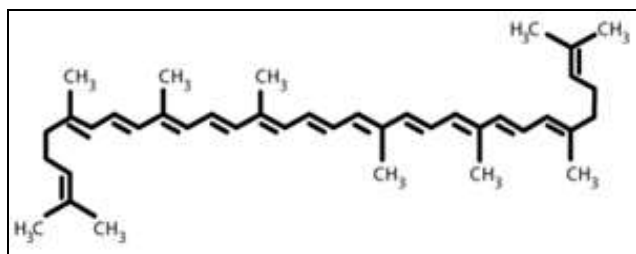
**Table 1: Lycopene content of different fruits and tomato products**

Source	µg/g wet weight
Raw tomato	8.8 – 42
Tomato juice	86 -100
Tomato sauce	63 – 131
Tomato ketchup	124
Watermelon	23 – 72
Pink grapefruit	3.6 -34
Pink guava	54
Papaya	20 -53
Rosehip puree	7.8
Apricot	<0.1

### Chemical Structure and Properties

Lycopene is an acyclic isomer of beta-carotene, which has a highly unsaturated straight chain hydrocarbon of 13 double bonds, 11 of which are conjugated and 2 unconjugated double bonds at each ends which gives the red color. It is the longest carotenoid in the family<sup>13</sup> (Fig. 1).

Unlike alpha and beta carotenoids lycopene is deficient of terminal beta-ionic ring structure and hence the provitamin A activity. Physical properties of lycopene are shown in table 2.

**Fig. 1****Table 2: Physical properties of lycopene**

Molecular Formula	C <sub>40</sub> H <sub>56</sub>
Molecular Weight	536.85Da
Melting Point	172-175 <sup>0</sup> C
Crystal Form	Long red needles separate from a mixture of carbon disulfide and ethanol
Powder Form	Dark reddish brown.
Solubility	Soluble in chloroform, hexane, benzene, carbon disulfide, acetone, petroleum ether and oil; Insoluble in water, ethanol and methanol.
Stability	Sensitive to light, oxygen, high temperature, acids, catalysts and metal ions.

In nature, lycopene is frequently in its thermodynamically stable all trans-isomeric form.<sup>14</sup> However, it can transform to mono or poly isomeric form by light, thermal energy and chemical reactions to cis-isomeric forms, the most common isomer of lycopene being 5-cis, 9-cis, 13-cis and 15-cis. The most stable and the highest

antioxidant property shown by 5-cis isomer followed by the all-trans, 9-cis, 13-cis, 15-cis, 7-cis and 11-cis.<sup>14</sup>

### Bioavailability and Metabolism

In humans only 10 to 30% of consumed lycopene gets absorbed with processed tomato products such as sauce and paste being better absorbed compared to unheated fresh tomatoes.<sup>15,16</sup>

Many factors such as dietary lipid, fiber, and presence of vitamins, minerals and other carotenoids have shown to influence its absorption.<sup>17</sup> Intestinal absorption takes place by formation of spherical molecules, the micelle in which lycopene gets incorporated and are absorbed by epithelial cells via passive diffusion. Following portal circulation lycopene get accumulated in liver where they get packed and released into circulation along with VLDL/LDL particles and transported to various tissue sites such as lung, pancreases, kidneys, adrenals, ovaries, prostate and other tissues.<sup>18,19</sup> The plasma half-life of lycopene ranges from 7 to 14 days.<sup>20</sup>

Very little is known about the in vivo metabolism of lycopene. Few metabolites such as 5,6-dihydroxy-5,6-dihydro lycopene have been detected in human plasma<sup>21</sup> similar to metabolic products obtained in an invitro study using post-mitochondrial fraction of rat intestinal mucosa.

As reported by Ribaya-Mercado and associates lycopene has shown to have the fastest degradation rate when compared with other carotenoids.<sup>22</sup>

### Mechanism of Action

Essential effect of lycopene have been attributed to their anti-carcinogenic and anti-atherogenic activities i.e, protecting critical cellular biomolecules that includes lipid, lipoproteins, protein and DNA from getting oxidized. (Fig. 2). Factors that govern their action depend on the molecular and physical structure, location or site of action within the cell, their ability to interact with other antioxidants, concentration and the partial pressure of oxygen.<sup>23</sup>

Anti-carcinogenic property involves inhibition of basic mechanisms of cancer cell proliferation, growth factor signaling and gap junctional intercellular communication.<sup>24</sup>

Anti-atherogenic property of lycopene is expressed by its hypocholesterolemic and antioxidant activity. Hypocholesterolemic activity involves the inhibition of HMG-CoA reductase enzyme on macrophage cholesterol metabolism that suppresses macrophage LDL receptor activity.<sup>24</sup>

Antioxidants may be called as “those substances which when present at low concentrations compared to those of an oxidizable substrate, will significantly delay or inhibit oxidation of that substrate and to protect against FR.”<sup>25</sup>

Antioxidant properties of lycopene as understood till date mainly operates by two mechanisms- physical and chemical quenching, the ability of physical quenching is much higher than that of chemical.

Physical quenching involves the transfer of FR from excitation energy to lycopene that results in isomerized/ excited lycopene. The energy gets dissipated via rotational

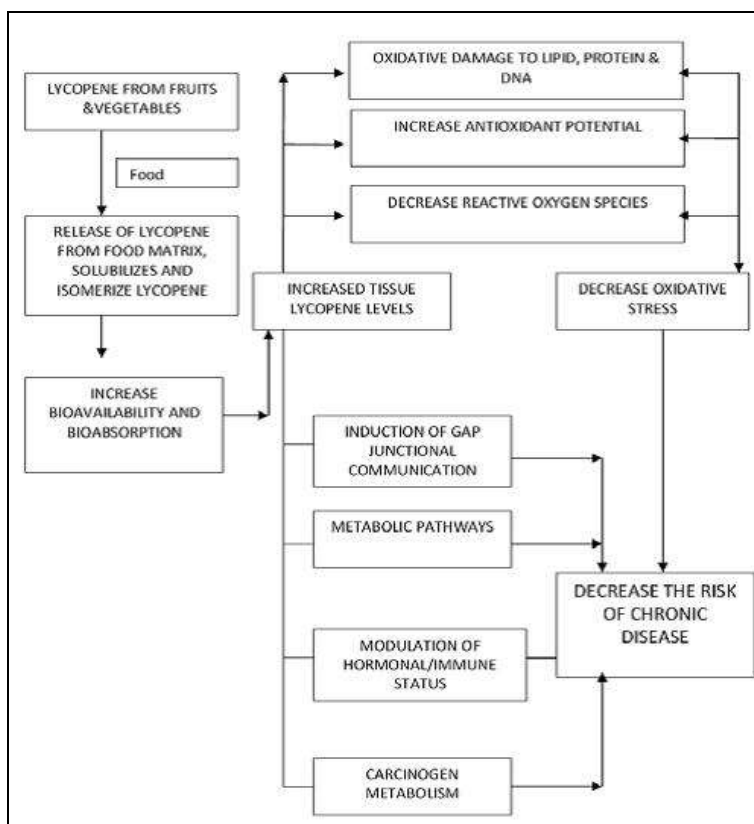


Fig. 2

and vibrational interaction of excited carotenoid with surrounding solvent to yield ground state carotenoid and thermal energy during this stage lycopene remains intact which acts as a catalyst further utilized for quenching. Chemical quenching, adds less than 0.05% of total quenching that results in final decomposition of lycopene.<sup>26</sup>

Amid the naturally occurring carotenoids lycopene has been the most effective quencher of singlet oxygen double that of beta-carotene and potent ROS scavenger than any other antioxidant including vitamin E<sup>27,28</sup> (Table 3). According to data lycopene can deactivate hydrogen peroxide, sulphonyl, nitrogendioxide and thyl types of free radicals.<sup>29,30</sup>

**Table 3: Comparison of antioxidant activities (quenching of singlet oxygen) of different carotenoids:**<sup>27,28,31</sup>

Carotenoid	Rate constant for quenching of singlet oxygen $K_q \times 10^9 (\text{mol}^{-1} \text{s}^{-1})$
Lycopene	31
$\gamma$ - carotene	25
$\alpha$ – carotene	19
$\beta$ – carotene	14
Lutein	8
Astaxanthin	24
Bixin	14
Canthaxanthin	21
Zeaxanthin	10

**Effects on Periodontal Disease**

An inflammatory condition in the periodontium where the homeostatic mechanism between host and oral micro flora gets altered leading to an overwhelming inflammatory reaction that results in host tissue damage manifesting as periodontal disease. Periodontitis produces significant amount of pro-inflammatory cytokines mainly IL-1, IL-6, PGE2, TNF- $\alpha$  reactive oxygen species, proteins, host cells, ions, hormones and markers of oxidative stress and antioxidants. During the process of phagocytosis, non-mitochondrial oxygen consumption may be 10 to 20 times that of resting consumption eventually ending in excessive generation of free radicals and reactive oxygen species such as superoxide & hydroxyl radicals, hydrogen peroxide and hydrochlorous acid. Researches measuring the biomarker generated by ROS reacting with different biomolecules have indicated a positive association between oxidative stress and periodontitis.<sup>32</sup>

Evidence for the role of ROSs in Periodontal Tissue Damage: Halliwell proposed four criteria, similar to that proposed by Robert Koch in 1884, to establish causal relationship between an organism and disease.<sup>33</sup>

1. ROS or the oxidative damage caused must be present at the site of injury.
2. The time course of ROS formation or the oxidative damage caused should occur before or at the same time as tissue injury.
3. Direct application of ROS over a relevant time course to tissues at concentrations found in vivo should

reproduce damage similar to that observed in the diseased tissue.

4. Removing or inhibiting ROS formation should decrease tissue damage to an extent related to their antioxidant action in vivo.

Effects of ROS on Periodontal Tissues and Components: The reactive oxygen species cause periodontal tissue damage by<sup>34</sup>

1. Ground substance degradation
2. Collagenolysis either directly or indirectly or as a result of oxidation of proteases
3. Stimulation of excessive pro-inflammatory cytokine release through NF- $\kappa$ B activation
4. PG-E2 production via lipid peroxidation and superoxide release, both of which have been linked with bone resorption
5. Since IL-1 & TNF- $\alpha$  positively regulate their own production, the additive effects of endotoxin-mediated cytokine production and that arising from respiratory burst of PMNLs in response to the same organisms, lead to periodontal inflammation and subsequent attachment loss.<sup>33</sup>

Beneficial effects of lycopene on periodontal health;

1. Regulate fibroblast migration and proliferation during gingival healing or periodontal repair.
2. By reducing the production of cytokines, chemokines and pro-inflammatory proteins by leukocytes that are responsible for causing cell destruction
3. By neutralizing ROS, they protect fibroblast from toxic substances that release ROS and help in reversing the effect of oxidative damage
4. By promoting the process of wound healing
5. By exhibiting antibacterial and antifungal properties.

Chandra<sup>35</sup> et al. (2007) studied the effects of systemically administered lycopene as single entity and as an additional modality to scaling and root planning (SRP) in patients with gingivitis. Sample comprised of twenty patients with clinical signs of gingivitis were randomly distributed into two treatment groups' i.e, experimental group and control group that were given 8 mg lycopene per day for 2 weeks and placebo for 2 weeks respectively. With an effective result shown by the group that consumed lycopene, the study proposed that lycopene can serve as an adjuvant treatment modality in gingivitis.<sup>18</sup>

In a similar study by Belludi<sup>36</sup> et al (2013) who assessed the efficacy of systemically administered lycopene as an adjunct in scaling and root planning for patients with gingivitis and periodontitis. Statistically significant improvement in clinical attachment level shown in test group compared to control group, while bleeding on probing was statistically non-significant.

Adjunctive use of systemic lycopene along with nonsurgical treatment of chronic periodontitis patients was assessed by clinical parameters and levels of tumor necrosis alpha (TNF- $\alpha$ ), salivary interleukin 1 beta (IL-1 $\beta$ ) and uric acid levels. Improvement in clinical parameters and reduction in salivary IL-1 $\beta$  and uric acid levels were noted

in test group however TNF- $\alpha$  value were not statistically significant.<sup>37</sup>

In a similar study use of systemic lycopene in chronic periodontitis patients with type 2 diabetes mellitus undergoing SRP. 40 diabetes subjects with periodontitis were equally divided into two study groups, group A underwent SRP with 8mg of lycopene administration and group B treated with SRP alone. Clinical parameters like gingival index (GI), probing depth (PD) and clinical attachment levels (CAL) were assessed along with serum markers i.e, malondialdehyde (MDA), C reactive protein (CRP) and glycated hemoglobin (HbA1c) were assessed at baseline, 2 months and 6 months post therapy. Group A subjects showed statistically significant results in decreased mean serum MDA levels at 2 months and 6 months and reduced mean PD along with HbA1c levels at 2months demonstrative of effective role of Lycopene in reducing oxidative stress and altered glycemic levels.<sup>38</sup>

More recently, Linden<sup>39</sup> et al. (2009) investigated, in a representative sample of 1258 men aged 60–70 years drawn from the population of Northern Ireland, the association between periodontal health and the serum levels of various antioxidants including retinol, a-tocopherol, g-tocopherol, a-carotene, b-carotene, b-cryptoxanthin, zeaxanthin, lutein, and lycopene. The population was divided into a group with generalized severe periodontitis, moderate periodontitis and the remaining population. Compared with the remaining population the levels of a- and b-carotene, b-cryptoxanthin, and zeaxanthin were significantly lower both in the moderate and the generalized severe periodontitis group. No significant differences were appreciated in the levels of lutein, lycopene, a- and g-tocopherol or retinol in relation to periodontitis.

### Dose

Till date lycopene has not been recognized nor recommended by medical professionals and government regulatory bodies as an essential nutrient. Evidences have shown daily intake level of 5-7mg in normal healthy human being is considered sufficient to combat oxidative stress and delay onset of chronic diseases. However in condition of cancer and cardiovascular diseases, higher levels of lycopene ranging from 35 to 75mg per day is recommended.<sup>40</sup>

### Commercial Preparations:<sup>41</sup>

Lycored, L-bex forte (lycopene 4000 IU, lutein, beta carotene 10mg), Lyco-first (lycopene, vitamin A palmitate, vitamin E acetate).

### Safety

Lycopene has been considered as a safe product with large amount of dietary intake i.e, 3g/kg/day of formulated lycopene have not shown any adverse effects on health of an individual. High intake of lycopene rich foods or supplements may result in a deep orange discoloration of skin known as lycopenoderma.<sup>41</sup> In a case of excessive lycopene intake a middle aged woman with history of

prolonged consumption of tomato juice, her skin and liver returned orange-yellow and had elevated levels of lycopene in her blood. On discontinuation of lycopene intake following 3 weeks the levels returned to normalcy.

### Conclusion

Lycopene is yet to be considered as an essential nutrient in our balanced diet. However their role in general health in large and periodontal health in particular needs to be scrutinized. Data presented here takes an optimistic stand on the beneficial effect of dietary intake of lycopene in periodontal health. Future scope includes the metabolism, mechanism of action and effects of Lycopene on immunological system in association with periodontal health.

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