REVIEW ON EXTRACTION, STANDARDIZATION & ANTIOXIDANT SCREENING OF LYCOPENE

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Abstract

Lycopene, belonging to the carotenoids, is a tetraterpene compound abundantly found in tomato and tomato-based products. It is fundamentally recognized as a potent antioxidant and a non-pro-vitamin A carotenoid. Lycopene has been found to be efficient in ameliorating cancer insurgences, diabetes mellitus, cardiac complications, oxidative stress-mediated malfunctions, inflammatory events, skin and bone diseases, hepatic, neural and reproductive disorders. This review summarizes information regarding its sources and uses amongst different societies, its biochemistry aspects, and the potential utilization of lycopene and possible mechanisms involved in alleviating the abovementioned disorders. Furthermore, future directions with the possible use of this nutraceutical against lifestyle-related disorders are emphasized. Its protective effects against recommended doses of toxic agents and toxicity and safety are also discussed.

Keywords: *lycopene ,antioxidants ,oxidative stress, cancer, diabetes, cardiovascular diseases, skin disorders*

Introduction :

Extraction:

Lycopene is a phytochemical mainly found in tomato and tomato-based products. It is a tetraterpene compound consisting of eight isoprene units and 11 double linear bonds. Lycopene is a non-pro-vitamin A carotenoid [1,2]. However, it is mentioned as an intermediate of carotenoid synthesis in plants [3]. Some fruits' and vegetables' red and orange coloration is attrib [2] free radicals are produced continuously by the body. Oxygen is an element indispensable for life. When cells use oxygen to generate energy, free radicals are produced by the mitochondria. These by-products are generally ROS as well as reactive nitrogen species (RNS) that result from the cellular redox process. The free radicals have a special affinity for lipids, proteins, carbohydrates, and nucleic acids

Standardization:

Natural Sources of Lycopene and Its Use Amongst Different Societies Tomato and tomato-based products are the major dietary sources of lycopene and account for approximately 80% of the consumption of lycopene in western countries. It is also present in a high amount in watermelon, guava, pink grapefruit, rosehips, papaya, and apricot [22]. uted to this liposoluble pigment [2].

Antioxidant screening:

Biochemistry of Lycopene In nature, there are more than 600 carotenoids which are mostly colored, produced by plants, fungi and bacteria. Carotenoids have two main groups: (i) the highly unsaturated hydrocarbons (α, β-, and γ-carotene, lycopene), (ii) xanthophylls (lutein, β-cryptoxanthin, and zeaxanthin). Xanthophylls possess a minimum of an oxygenated group on their end rings, while unsaturated hydrocarbon carotenoids contain just carbon and hydrogen atoms with no oxygen [31].

Health benefits of lycopene :

- Improves vision
- Lowers hypertension
- Goods source of antioxidants
- Prevent urinary tract infection
- Reduce cholesterol level and protects hearts
- Reduces oxidative stress of Type II diabetes

Biological Effect	**Mechanisms of Action**	**References**
Anticancer	Induced cell cycle arrest and modified the potential of mitochondrial membrane, DNA fragmentation	[2,5]
	Treatment of HGC-27 cells with lycopene exhibited significant improvement in LC3-I, Phosphorylated Extracellular Signal-Regulated Kinase (p-ERK) proteins expressions	[6]
	Reduced NADPH oxidase (NOX) 4 activity Lowered invasion, migration, and adhesion, NOX activity, matrix metalloproteinase (MMP)-9, MMP-2 activities and NOX4 protein expression	[4]
Cardioprotective	Decreased low-density lipoprotein-cholesterol (LDL), total cholesterol (TC), and thiobarbituric acid-reacting substances	[1]
	Lowered very-low-density lipoprotein-cholesterol (VLDL), triglycerides (TG) and increased high-density lipoprotein-cholesterol (HDL) level	[2]
	Decreased inflammatory factors (CRP, interleukin (IL)-6), pulse wave velocity, adhesion molecules and endothelial function	[3]

Neuroprotective	Suppressing 4 aminopyridines (4-AP) evoked glutamate release and elevated intrasynaptosomal Ca2+ level Suppressing release of 4-AP-evoked glutamate	[7]
	Improved mitochondrial enzymatic activities, kindling score, oxidative stress	[8]
	Decreased impairment in biochemical, behavioral, neuroinflammatory and neurochemical markers	[1]

Extraction of lycopene:

a) What does lycopene occurs ?

Lycopene can be obtained from natural sources or synthesized chemically. It can be obtained in supplement form, usually as tablets or capsules or in soft gel. Sometimes other nutrients are added to the formulation for additional antioxidant benefits.

Biological sources

Lycopene occurs naturally in plant (vegetable) sources such as tomatoes, and in microbial sources and in cells that have been genetically engineered to accumulate lycopene.

- **Microbial sources**

Microbial sources of lycopene offer an alternative natural product considering that until very recently, the only known source of lycopene was tomatoes. Genetic engineering has made it possible to enhance lycopene content in bacteria, fungi and mammalian cells. Several patents disclose DNA modification and methods for producing carotenoids in biological sources (Olempska-Beer, 2006). It is desirable to obtain edible (suitable for consumption) lycopene from these biological sources while eliminating the need to ingest the biological source.

- **Plant sources**

Tomatoes have one of the highest known concentrations of natural lycopene and most people get much of the lycopene in their diet from tomato products. Other plants known to contain high concentrations of lycopene can also be utilized. They include fruits such as Ruby Red or Red-blush grapefruit, watermelon, and persimmons (Ausich & Sanders, 1999).

- **Tomatoes as a source of lycopene**

The tomato fruit consists of skin, pericarp, and locular contents (Figure 2-2). The epidermal layer has a heavily cutinised outer surface and the skin consists of four or five layers of cells under a thin cuticle. The locular cavities are filled with jellylike parenchyma cells that surround the seeds (Science, n.d.). The chemical composition of the tomato fruit depends on its genetics, ripeness and the conditions under which it was cultivated.

b) Extraction method of lycopene ?

- **Soxhlet extraction**

For this research, the Soxhlet extraction method was used to extract lycopene from dry tomato paste powder. The tomato paste sample was dried, ground into small particles and placed in a porous cellulose thimble for Soxhlet extraction. The flask with the solvent was heated, evaporated into the condenser, and condensed to a liquid, which trickled into the extraction chamber containing the sample.[18]

c) Different solvent used in different methods ?

Soxhlet extraction

(Boiling point of solvent used)

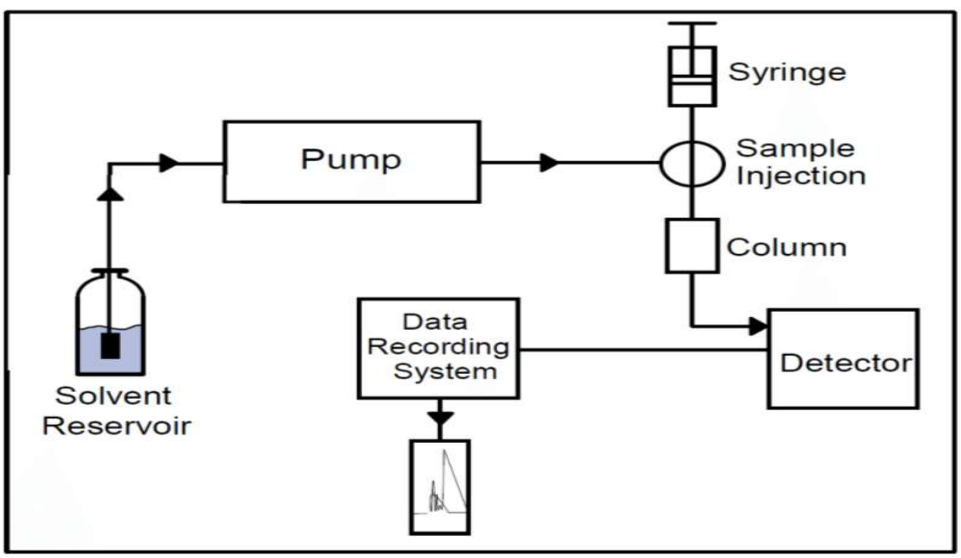
Solvent	Boiling point
n-Hexane	68^0C
Ethyle acetate	77^0C
Ethanol	78^0C
Petroleum ether	40^0C
Dichloromethane	40^0C

d) Different yield of different solvent .

Standardization of lycopene different methods.

- **High performance liquid chromatography (HPLC)**

The extracted solution of lycopene samples were analysed in an HPLC system consisting of a Waters 515 HPLC pump, Waters 996 photodiode array detector (PDA- UV/VIS detector) and Rheodyne 7725i manual sample injector with a 20- µL sample loop (Figure 3-9). The system was controlled with Waters EmpowerTM 2 Chromatography software



- **Selecting the mobile phase for HPLC**

To identify the optimum mobile phase composition, two different solvents were used: 42:42:16 (v/v/v) acetonitrile: methanol: dichloromethane and 3:1 (v/v) acetonitrile: dichloromethane. The optimum composition was based on the chromatographic response factor. Characterization of the lycopene extract was performed in isocratic mode. The sample injection volume (20 µl in the loop) was injected for 15 minutes into the mobile phase flowing at 1.0 mL/min. The lycopene was detected at a wavelength of 480 nm. The column was controlled at 22˚C (room temperature) and 450-500 psi during analysis. Experiments were done at room temperature in the absence of direct sunlight. [19]

- **High performance liquid chromatography (HPLC) of lycopene**

The structure and composition of the carotenoids influence their separation by HPLC, allowing qualitative and quantitative analysis. A photodiode array detector records the absorption spectrum of each component in the chromatogram. HPLC analyses were done using a C18 column on standard lycopene and samples obtained by extraction from tomato paste with different combinations of solvents. Two different combinations of mobile phases for HPLC were initially used: 42:42:16(v/v/v) acetonitrile, methanol and dichloromethane; and 3:1 (v/v) acetonitrile and dichloromethane. The UV-Vis spectra for lycopene gave a maximum adsorption at 472 and 480 nm.The chromatogram of the standard lycopene shows the standard lycopene peak occurs at 7.5 minutes (Figure 4-11) using a mobile phase of 3:1 acetonitrile: dichloromethane. AU 0.00 0.10 0.20 0.30 Minutes 0.00 2.00 4.00 6.00 8.00 10.00 12.0

Lycopene possesses potent anticancer, antioxidant, antiinflammatory, and antidiabetic potential. In addition, it is a nutraceutical which protects against a wide variety of heart, liver, bone, skin, nervous, and reproductive systems diseases, as evident from numerous studies. However, further investigations are necessary to unveil the underlying mechanisms of actions, with a special emphasis on gene expression studies. Additionally, the recommended and effective doses of this functional food need to be further investigated. Safety concerns about its genotoxicity, maternal toxicity, and teratogenic effects should also be inquired.[20]

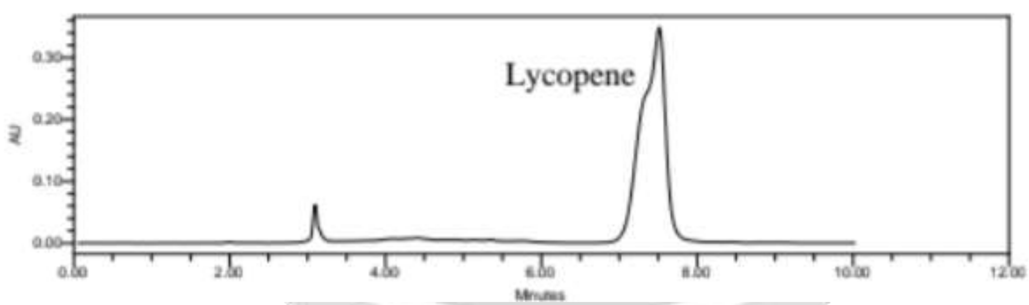


Figure 4-11: Chromatograph of standard lycopene in ethyl acetate, concentration 500 µg/mL with a mobile phase 3:1 acetonitrile: dichloromethane

- **Reactions and fate in foods**

The chemical structure of lycopene, particularly the long chain of conjugated carbon-carbon double bonds, predisposes lycopene to isomerization and degradation upon exposure to light, heat, and oxygen (Lee and Chen, 2002) and the subsequent loss of its colouring properties (Xianquan et al .; Yang et al. 2006); this would render tomato extract ineffective as a food colour. Page 5 of (9) The Committee received data on lycopene stability in representative foods based on monitoring of the lycopene content in food and the colour of food during 5 days storage under fluorescent light and storage conditions appropriate for each food (room temperature, 4°C, or frozen) . The concentration of lycopene in different food products, to which the commercial product Lyc-O-Mato Oleoresin containing 6% lycopene was added, was in the range of 0.5 to 60 mg/kg (Table 2). Equivalent commercial food products, which were either not coloured or coloured with control colorants such as β-carotene, were used as control samples. Both the test and control samples were analyzed for colour using a Hunter Colorimeter and for lycopene content using HPLC.

Food	**Lycopene level in food (mg/kg)**	**Control colorant level in food (mg/kg)**
Orange gelatin	10-30	Yellow 6/Red (40)
Yellow cake	20-30	Beta carotene (80)
Lemon beverage	3-60	Not coloured
Orange hard candy	5-20	Not coloured
Ice cream	10-20	Not coloured
Salad dressing	20-50	Not coloured
Margarine	0.5-1.0	Beta carotene (2)

Visual inspections and Hunter Colorimetry showed no significant changes in colour after 5 days of storage. The HPLC data showed that ninety-five percent of the added lycopene was recovered at the time of formulation and ninety percent 5 days after formulation. These results demonstrate that tomato extract is stable in a variety of foods under appropriate storage conditions. Lycopene stability was also assessed in a fruit preparation containing apple and Aloe vera formulated with tomato extract. The level of lycopene in the product decreased from approximately 83 mg/kg to 77 mg/kg after four months of storage.

Summary:

Lycopene is a strong antioxidant that can protect your body against oxidative stress and offer some protection from certain environmental toxins and chronic diseases.

Diets rich in the antioxidant lycopene may help prevent the development of prostate cancer. It may also protect against cancers of the lungs, breasts and kidneys, but more human-based research is needed to confirm this.

Lycopene's strong antioxidant properties may help improve cholesterol levels and reduce your likelihood of developing or dying prematurely from heart disease.

Lycopene may help increase your skin's defense against sunburns and damage caused by UV rays. However, it is no replacement for sunscreen.

Refrences:

1] Costa-Rodrigues, J.; Pinho, O.; Monteiro, P. Can lycopene be considered an effective protection against cardiovascular disease? Food Chem. 2018, 245, 1148–1153. [CrossRef]

2] Kumar, R.; Salwe, K.J.; Kumarappan, M. Evaluation of antioxidant, hypolipidemic, and antiatherogenic property of lycopene and astaxanthin in atherosclerosis-induced rats. Pharmacogn. Res. 2017, 9, 161.

3] Cheng, H.M.; Koutsidis, G.; Lodge, J.K.; Ashor, A.; Siervo, M.; Lara, J. Tomato and lycopene supplementation and cardiovascular risk factors: A systematic review and meta-analysis. Atherosclerosis 2017, 257, 100–108

4] Dos Santos, R.C.; Ombredane, A.S.; Souza, J.M.T.; Vasconcelos, A.G.; Plácido, A.; das GN Amorim, A.; Barbosa, E.A.; Lima, F.C.; Ropke, C.D.; Alves,M.M. Lycopene-rich extract from red guava (Psidium guajava L.) displays cytotoxic effect against human breast adenocarcinoma cell line MCF-7 via an apoptotic-like pathway. Food Res. Int. 2018, 105, 184–196. [CrossRef]

5] Morgia, G.; Voce, S.; Palmieri, F.; Gentile, M.; Iapicca, G.; Giannantoni, A.; Blefari, F.; Carini, M.; Vespasiani, G.; Santelli, G. Association between selenium and lycopene supplementation and incidence of prostate cancer: Results from the post-hoc analysis of the procomb trial. Phytomedicine 2017, 34, 1–5. [CrossRef]

6] Zhou, S.; Zhang, R.; Bi, T.; Lu, Y.; Jiang, L. Inhibitory effect of lycopene against the growth of human gastric cancer cells. Afr. J. Tradit. Complement. Altern. Med. 2016, 13, 184–190. [CrossRef

7] Lu, C.-W.; Hung, C.-F.; Jean, W.-H.; Lin, T.-Y.; Huang, S.-K.; Wang, S.-J. Lycopene depresses glutamate release through inhibition of voltage-dependent Ca2+ entry and protein kinase C in rat cerebrocortical nerve terminals. Can. J. Physiol. Pharmacol. 2017, 96, 479–484. [CrossRef] [PubMed]

8] Bhardwaj, M.; Kumar, A. Neuroprotective effect of lycopene against PTZ-induced kindling seizures in mice: Possible behavioural, biochemical and mitochondrial dysfunction. Phytother. Res. 2016, 30, 306–313. [CrossRef] [PubMed]

9] Datta, S.; Jamwal, S.; Deshmukh, R.; Kumar, P. Beneficial effects of lycopene against haloperidol induced orofacial dyskinesia in rats: Possible neurotransmitters and neuroinflammation modulation. Eur. J. Pharmacol. 2016, 771, 229–235. [CrossRef] [PubMed]

10] Velavan S. Free radicals in health and diseases. Pharmacol Online 2011;1(1):1062-77.

11] Pennathur, S.; Maitra, D.; Byun, J.; Sliskovic, I.; Abdulhamid, I.; Saed, G.M.; Diamond, M.P.; Abu-Soud, H.M. Potent antioxidative activity of lycopene: A potential role in scavenging hypochlorous acid. Free Radic. Biol. Med. 2010, 49, 205–213. [CrossRef]

12] Yin, Y.; Zheng, Z.; Jiang, Z. Effects of lycopene on metabolism of glycolipid in type 2 diabetic rats. Biomed. Pharmacother. 2019, 109, 2070–2077. [CrossRef] [PubMed]

13]Mozos, I.; Stoian, D.; Caraba, A.; Malainer, C.; Horba ´nczuk, J.O.; Atanasov, A.G. Lycopene and vascular health. Front. Pharmacol. 2018, 9, 521. [CrossRef] [PubMed]

14] Maiani, G.; Periago Castón, M.J.; Catasta, G.; Toti, E.; Cambrodón, I.G.; Bysted, A.; Granado-Lorencio, F.; Olmedilla-Alonso, B.; Knuthsen, P.; Valoti, M. Carotenoids: Actual knowledge on food sources, intakes, stability and bioavailability and their protective role in humans. Mol. Nutr. Food Res. 2009, 53, S194–S218.

15] Aghel N et al (2011). Isolation and Quantification of lycopene from tomato cultivated in dezofoul, IRAN. Jundishapur Journal of Natural Pharmaceutical Products 6(1): 9-15.

16] Amany M. Basuny et al (2009) Tomato lycopene is a natural antioxidant and can alleviate hypercholesterolemia. African Journal of Biotechnology Vol. 8 (23), pp. 6627-6633.

17] Amany M. Basuny et al, (2009) Tomato lycopene is a natural antioxidant and can alleviate hypercholesterolemia. African Journal of Biotechnology Vol. 8 (23), pp. 6627-6633.

18] Xi, J. (2006). Effect of high pressure processing on the extraction of lycopene in tomato paste waste. Chemical Engineering and Technology, 29(6), 736-739

19] Agarwal, S., & Rao, A.V. (2000). Tomato lycopene and its role in human health and chronic diseases. Canadian Medical Association Journal, 163(6), 739-744

20] Barba, A.I.O., Hurtado, M.C., Mata, M.C.S., Ruiz, V.F., & de Tejada, M.L.S. (2006a). Application of a UV-vis detection-HPLC method for a rapid determination of lycopene and beta-carotene in vegetables. Food Chemistry, 95(2), 328-336

21] FAO/WHO, 1965. Specification for the identity and purity of food additives and their toxicological evaluation: food colours and some antimicrobials and antioxidants (Eighth report on the Joint FAO/WHO Expert Committee on Food Additives). Geneva, World Health Organization (WHO Technical Report Series, No. 309).

22] Lee, M.T., and Chen, B.H., 2002. Stability of lycopene during heating and illumination in a model system. Food Chem., 78, 425-432.