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ISOLATION AND QUANTIFICATION OF LYCOPENE FROM TOMATO CULTIVATED IN DEZFOUL, IRAN

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Abstract

Lycopene is a pigment principally responsible for the characteristic deep-red color of ripe tomato fruits and products. It, as a natural source of antioxidants, has attracted attentions due to its biological and physicochemical properties. In this study tomato paste prepared from tomato cultivated in Dezfoul (Khozestan) was dehydrated with methanol, then lycopene was extracted with methanol-carbon tetrachloride mixture. Pure lycopene was obtained by twice crystallization of crude product from benzene through addition of boiling methanol. Further purification was achieved using column chromatography with alumina as the adsorbent. Identification of chemical structure of the isolated lycopene was done using UV, IR, NMR and Mass spectroscopy. Average quantity of extracted pure lycopene was calculated as 2.313 mg per 100 g tomato paste.

Keywords:
Carotenoids, Antioxidant, Lycopene, Tomato.

Introduction

Recent epidemiological studies have suggested that the consumption of tomatoes and tomato-based food products reduce the risk of cancer (oral cavity, pharynx, esophagus, stomach, rectum, colon, urinary bladder, prostate and breast) in humans (1-3). This protective effect has been attributed to carotenoids, which are one of the major classes of phytochemicals in this fruit (4). Carotenoids are a family of compounds of over 600 fat-soluble plant pigments that provide much of the color we see in nature. They are important nutritious for the human body owing to their provitamin A and antioxidant activities (5). The most abundant carotenoid in tomato is lycopene, followed by phytoene, phytofluene, ζ-carotene, γ-carotene, ß-carotene, neurosporene, and lutein. Lycopen, a red carotenoid pigment in tomatoes and tomato-based products, is an acyclic form of beta-carotane without pro-vitamin A activity. It has attracted substantial interest during recent times for its beneficial in reducing oxidative stressing coronary heart diseases and other chronic diseases (6-9). Its molecular weight is 536. 89 and molecular formula is $C_{40}H_{56}$ with 89.45 ٪ carbon and 10.51٪

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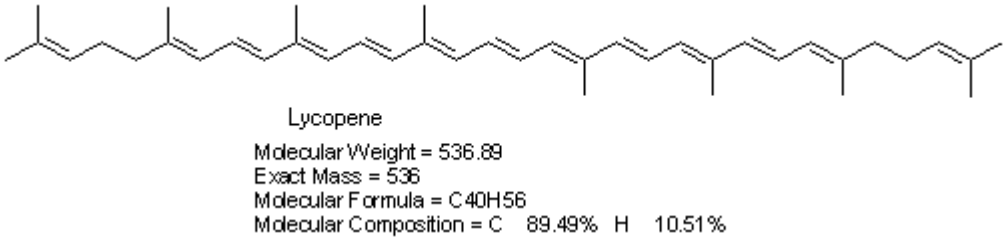

Fig. 1: Chemical Structure of Lycopene.

hydrogen. It is highly unsaturated hydrocarbon containing 11 conjugated and two unconjugated double bonds as it is illustrated in Fig. 1(10, 11).

Different extraction and quantitation methods for lycopene are recorded in the literatures (12-15).

Undesirable degradation of lycopene affects not only the sensory quality of the final products, but also the health benefit of tomato-based foods for the human body. Lycopene in fresh tomato fruits occurs essentially in the all-trans configuration. The main causes of tomato lycopene degradation during processing are isomerization and oxidation. Isomerization converts all-trans isomers to cis-isomers due to additional energy input and results in an unstable, energy-rich station. Determination of the degree of lycopene isomerization during processing would provide a measure of the potential health benefits of tomato-based foods. Thermal processing (bleaching, retorting, and freezing processes) generally cause some loss of lycopene in tomato-based foods. Heat induces isomerization of the all-trans to cis forms. The cis-isomers increase with temperature and processing time. In general, dehydrated and powdered tomatoes have poor lycopene stability unless carefully processed and promptly placed in a hermetically sealed and inert atmosphere for storage. A significant increase in the cis-isomers with a simultaneous decrease in the all-trans isomers can be observed in the dehydrated tomato samples using the different dehydration methods. Frozen foods and heat-sterilized foods exhibit excellent lycopene stability throughout their norma l temperature storage shelf life.

Lycopene has been prepared from a variety of fruits and berries (13, 14). It was first isolated from *Tamus communis* by Harsten in 1873 (12). The first modern preparation was that of Willstätter and Escher who processed 75 kg tomato concentrate and obtained 11 g once-recrystallized lycopene (15). All extracted lycopene was impure and needs to be purified and stabilized. So, different methods of purification were introduced. Among them solid phase extraction and preparative TLC and HPLC was considered more (16-18). Although pure lycopene can simply be obtained by chromatographic techniques, liquid liquid extractions are methods of choice especially where sophisticated chromatographic instruments are not easily available.

As it is clear, natural products are good sources of antioxidants and evaluation of economic costs and simplicity and availability of extraction methods are very necessary. So in the present work**,** economic extraction and quantification method of lycopene from tomato cultivated in Dezfoul, Iran has been investigated.

Material and Methods

Plant material

Tomato fruits were collected from Dezfoul farms (Khozestan Province, south west of Iran) during summer 2006. It was identified as Lycopersicum esculentum Mill (Solanaceae).

Isolation Procedure

Fifty grams tomato paste was dehydrated by adding 65 ml methanol. This mixture was immediately shaken vigorously to prevent the formation of hard lumps. After 2 hr, the thick suspension was filtered; the dark red cake was shaken for another 15 min with 75 ml mixture of equal volume of methanol and carbon tetrachloride and separated by filtration. The carbon tetrachloride phase was transferred to a separatory funnel; added one volume of water and shaked well. After phase separation, the carbon tetrachloride phase was evaporated and the residue was diluted with about 2ml of benzene. Using a dropper, 1 ml of boiling methanol was added in portion, then crystals of crude lycopene were appeared immediately and the crystallization was completed by keeping the liquid at room temperature and ice bath, respectively. The crystals were washed 10 times using benzene and boiling methanol.

Long, red lycopene prisms were observed under the microscope with some colorless impurity substances. For more purification, column chromatography on active acidic alumina using toluene as eluent was done. The deep red zone was collected. After complete evaporation of solvent, the residue was dissolved in 2 ml benzene. After recrystalization using boiling methanol, no colorless substances observed. Crystalline lycopene is not isomerized but has a tendency to autoxidation (or air oxidation), especially in light, so it was kept in dark evacuated glass tubes prior to use.

Primary identification test were performed using color chemical reactions. Identification of chemical structure of the isolated lycopene was done using UV, IR, NMR and Mass spectroscopy.

Results

The yield of lycopene crystals after column chromatography was 2.313 mg per 100 g tomato paste. In order to identify the lycopene, a few crystal of extracted Lycopene was dissolved in concentrated sulfuric acid, imparting an indigo blue color to the solution. In another test, by adding a solution of antimony trichloride in chloroform to a solution of lycopene in chloroform, an intense unstable blue color appeared. These tests primarily proved the presence of lycopene in the extract.

To further test the purity, structural analysis of the extract was performed. UV Spectrum is shown in Fig. 2, the maximum wavelengths are 447.2, 473.2 and 504.2 nm, which is the maximum wavelengths of pure lycopene reported in the literatures. Vibrational wavelengths of IR Spectrum of extracted lycopene in KBr (FTIR – Bruker, Germany, Fig. 3) are as follow:

(cm^{-1})	
3100:	$CH_{str}(SP^2)$
2918.92, 2851.05:	$CH_{str}(SP^3)$
1670, 1640:	$C= C_{str}$(Trans)
1446.92, 1400:	CH_2(Bending)
1101.07, 1000, 957.33:	CH(Trans OOP)
612.84:	$R_2C=CR$

^{1}HNMR Spectrum was recorded using ^{1}HNMR Spectrophotometer, Bruker 300 and 400 MHz Advanced Ultra Shield, Germany(Fig. 4). The results were as follow:

δ (ppm)	
5.12:	$2H\rightarrow C_2$
2.13:	$4H \rightarrow C_3$
2.23:	$4H\rightarrow C_4$
5.96:	$2H\rightarrow C_6$
6.63:	$2H\rightarrow C_7$
6.26:	$2H\rightarrow C_8$
6.11:	$2H\rightarrow C_{10}$

6.86: $2H \rightarrow C_{11}$
6.33: $2H \rightarrow C_{12}$
6.11: $2H \rightarrow C_{14}$
6.20-6.21: $2H \rightarrow C_{15}$
1.63: $12H \rightarrow C_{16}$ and C_{17}
1.83: $6H \rightarrow C_{18}$
1.98: $12H \rightarrow C_{19}$ and C_{20}

Mass Spectrum was obtained using Mass Spectrometer, QP-1000 Shimadzu, Japan. The results are shown below.
Mol. Wt. 536; m/z: 536(37%), 145(38%), 119(25%), 105(27%), 93(30%), 91(30%), 81(40%), 69(77%), 41(60%)
All spectroscopic data state that the extract is pure lycopene.

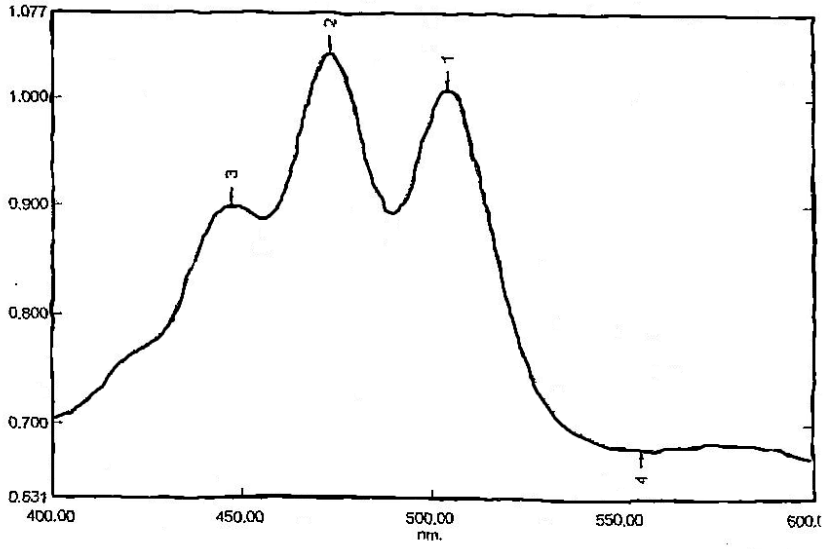

Fig. 2: Visible Spectrum of extracted lycopene crystals in ethanol after three times purification.

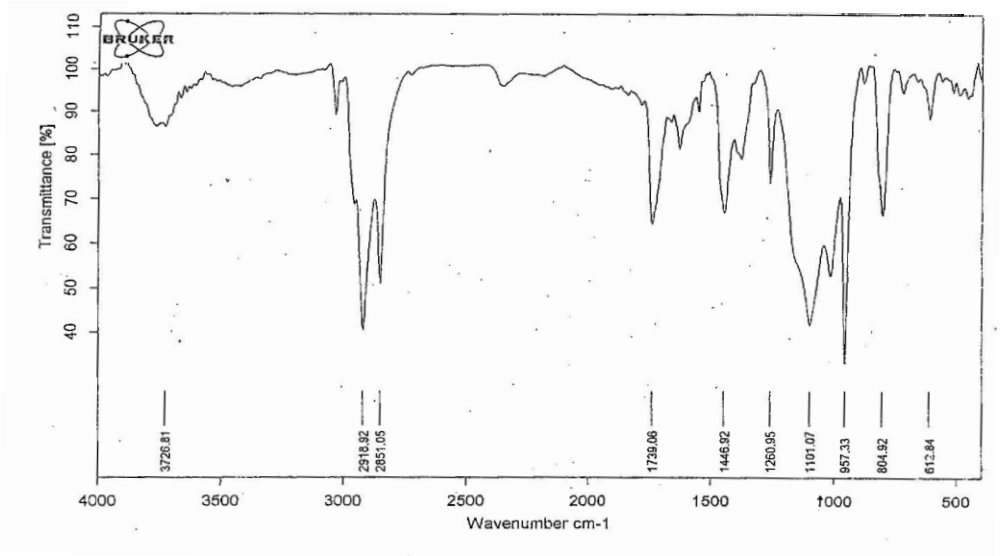

Fig. 3: FT-IR spectra of purified lycopene.

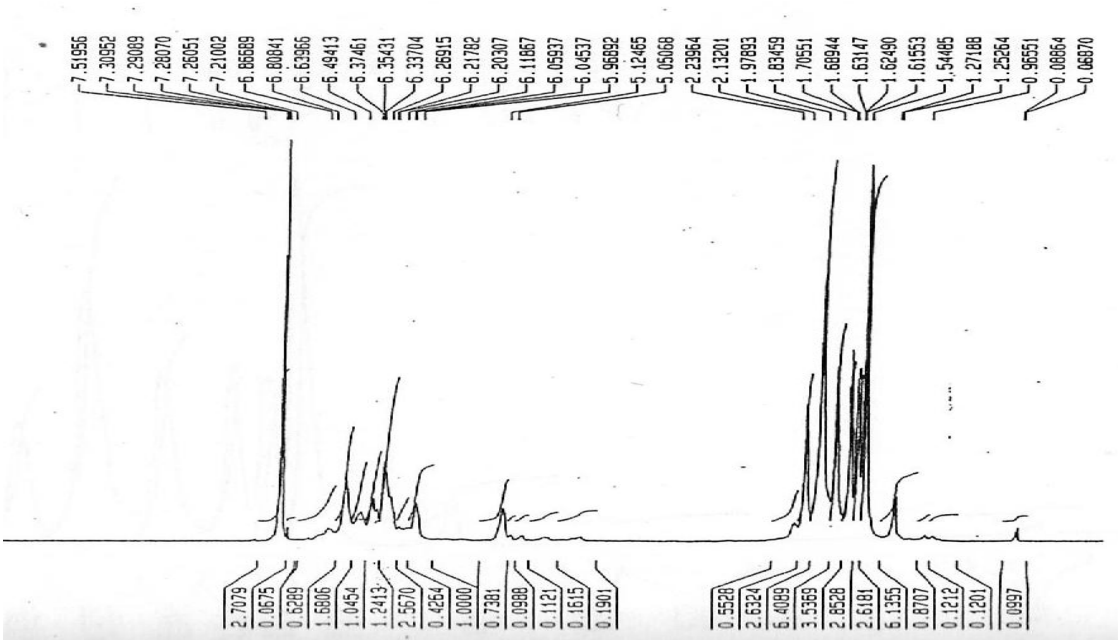

Fig. 4: ^{1}HNMR spectra of lycopene that was purified by column chromatography.

Discussion

Lycopene was extracted from Dezfoul tomato paste by simple liquid-liquid extraction using as minimum organic solvent as possible. The main problem was purification of the extract. Two simple and easy to use purification methods, namely recrystalization and column chromatography using acidic alumina as stationary phase, were used and compared with each other. Crystals obtained by each method was first observed under microscope. Presence of colorless substances indicated the extent of impurity in crystals obtained by three times recrystallization. Combination of recrystalization and column chromatographic method gave completely pure lycopene crystals as no colorless substances was seen. Analysis of NMR and FT-IR spectra also revealed the extent of impurities. Crystals obtained by triple recrystalization showed a few characterized lycopene peaks (19) but those of column chromatography after twice recrystalization was completely the same as lycopene standard without any isomerisation sign. Mass spectra of the extracts also confirmed lycopene (20). However, recrystalization combined with column chromatography using acidic alumina as stationary phase and Tolouene as mobilephase was shown to be the best purification techniques and the crystals produced were pure and all trans. recommended precautions for its storage was considered (exclude air and keep them under nitrogen atmosphere) since it is light sensitive and react easily with oxygen. The amount of pure lycopene was also good (2.313 mg per 100 g tomato paste) compare to those obtained from other studies.

Conclusion

To our knowledge, it was the first report on separation and quantitation of lycopene from Dezfoul tomato. This tomato can be a good natural source of lycopene. Of course, more simple, new, and environment friendly sorbent such as nano and bio materials can be used in the isolation and purification of lycopene from tomato paste.

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