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Extraction, Purification and Characterization of Lycopene from Tomato (Cv Vijeta) Processing Industry Waste

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Aim: The present study focused on extraction of lycopene from tomato processing industry waste, its purification and characterization by different methods. Lycopene is major carotenoid found in tomato.
Methodology: Different tomato parts like whole tomato, tomato peel and waste generated druing processing were also screened for lycopene content. The extracted lycopene was purified by crystallization method. The purified lycopene was characterized by the different methods live UV-spectroscopy, HPLC, FT-IR and NMR.
Results: Among the different parts of tomato the peel (377.19±1.13 µg/g) contain highest amount of lycopene than industrial waste (175.15±1.09 µg/g) and whole tomato (82.82±0.79). The crystallization method significantly purify the lycopene content which was clearly resulted in UV-spectroscopy, HPLC, FT-IR and NMR results.
Conclusion: This will be beneficial to the industry for purification and characterization of lycopene from tomato processing industry waste.

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Keywords: Lycopene; HPLC; FT-IR; NMR; tomato processing waste.

1. INTRODUCTION

Lycopene, a natural pigment and synthesized by plants and microorganisms however animals are not able to synthesize. It is an acyclic isomer of ß-carotene. Lycopene consists of unsaturated hydrocarbons with 11 conjugated and 2 unconjugated double bonds. Lycopene convert into different *cis-trans* isomers influenced by sun rays, thermal energy, and chemical reactions [1-2].

Lycopene is found mainly in tomatoes in all-*trans* configuration form and the thermally stable form of lycopene is *cis*isomers [1]. In the blood of human beings, lycopene is found as an isomeric assortment, with 60% as *cis*isomers of the total lycopene. The molecular formula of lycopene ($C_{40}H_{56}$) was first determined whereas Willstatter and Escher presented their study showing that lycopene is an isomer of the carotenes [3]. Karrer et al. [4] reported the chemical structure of lycopene, which was subsequently confirmed by Kuhn and Grundmann [5] by characterization of its degraded products following chromic acid oxidation. The molecular weight of lycopene is 536.85 Da, with the general structure being an aliphatic hydrocarbon with 11 conjugated carbon–carbon double bonds, which imparts a red coloration as well as fat- and lipid-soluble characteristics.

Light in the visible range is absorbed by lycopene, and lycopene dissolved in petroleum ether has the highest absorption λ_{max} at 472 nm and a differential emanation wavelength of 3078 [6-7]. As a consequence of the 11 conjugated carbon–carbon double bonds in its backbone, lycopene can tentatively assume 211 or 2048 geometrical configurations [1,8]. All-*trans* i.e. 5-*cis*, 9-*cis*, 13-*cis*, and 15- *cis*are some of common recognized isomeric forms of lycopene, with the stability of different form are as 5-*cis* >all-*trans* >9-*cis* >13-*cis* >15-*cis* >7-*cis* >11-*cis*, so that the 5-*cis* form is thermally more stable than the all-*trans*-isomer [8].

Increasing epidemiological indication proposes that the eating of fresh as well as processed tomato is accompanying with a reduced risk of some types of cancer and a lower incidence of ischemic heart disease [9-11]. Among different antioxidants present in tomatoes, carotenoids, particularly lycopene, is responsible for the brick red color of ripe tomatoes, has got significant attention in recent years for prevention of disease [12-14].

The previous study reviewed the appearance of lycopene in various portions of tomatoes such as skin, the water insoluble portions, and the fibrous portions including the fiber and soluble solids [15-16]. The results showed that 72–92% of lycopene was linked with the water-insoluble fractions and skin. Cell wall degrading enzymes, which can degrade the cell wall components and thus support to liberate intracellular constituents, is an extensively documented method for the extraction of different substances. Different enzymes have been used for the extraction of capsaicinoids and carotenoids from chilli (*Capsicum annuum* L.) in ethanol [17]. In the same cases, it has been projected to increase the extraction of oil from seeds and fruits by enzymatic pretreatment [18]. Plant cell walls comprising with cellulose and pectins, cellulases and pectinases have been used for this purpose.

In present study attempts have been made to extraction, purification and characterization of lycopene from tomato processing waste.

2. MATERIALS AND METHODS

2.1 Raw Materials and Chemicals

Fresh tomato processing industry waste (approximate peel to seed ratio of 37:63) was collected from ANS Foods, Sangli, Maharashtra, India. This was dried, in a hot air oven at 50^0C (approximate 10% moisture content) to avoid loss of lycopene during drying, ground and stored in air tight HDPE bags until further use. HPLC grade chemicals were used for experimental work. Wall materials used here included edible gelatin and sucrose.

2.2 Extraction and Purification of Lycopene

The lycopene extraction reported by earlier researchers was followed with slight modifications [16,19]. The dried waste samples were treated with ethanol for 30 min to remove moisture. These were treated with mixture of acetone, ethanol and hexane in the ratio of 1:1:2 (Mass: Solvent was 1:7) for 30 min with continuous shaking. After this deionized water

was added and allowed to stand for 5min for phase separation. The upper solvent layer was collected and concentrated using a rotary vacuum evaporator. This concentrated extract was dissolved in dichloromethane/ethanol (1:4) at 50-60^{0}C temperature, and cooled gradually in an ice bath. This was then refrigerated overnight for crystallization. The crystals were filtered through Whatman No. 1 filter paper, washed with cold ethanol and dried in freeze dryer. The crystallization process was repeated to obtain crystals with higher levels of purity [20].

2.3 Identification of Lycopene

Identification of chemical structure of purified lycopene was done using UV- Spectroscopy, FT-IR and NMR as discussed below.

2.4 UV- Spectroscopy

The purified lycopene was dissolved in hexane and scanned at 350 to 600 nm wavelengths using a UV visible spectrophotometer (Shimadzu Co., Ltd., Japan).

2.5 High-performance Liquid Chromatography (HPLC) Analysis of Lycopene

Separation of analytes were carried out on an isocratic JASCO HPLC-DAD system using KYATECH C_{18} column (250 x 4.6 mm internal diameter, particle size 5µm).The pump used in this HPLC system was PU 2080 pump (Dual piston with gear driven pump). The 20 µl sample solutions of analytes were injected to the chromatographic system using Rheodyne Injector. The PDA detector used in this HPLC system was an UV 2070 detector (Czerny turners mount monochromater) with a deuterium lamp as light source. Chromatographic and the integrated data were recorded using JASCO LC-Net II/ ADC (interface) computer system employing ChromNAV Version 3.2.software for Data processing.

Analysis was carried out by HPLC using acetonitrile: water (60:40 v/v) [21] as a mobile phase at a flow rate of 1.0 mL/min in an isocratic elution mode. Before delivering the mobile phase into the system, it was degassed and filtered through a 0.20 µm syringe filter. Injection volume was 20 µl and the detection was performed at 475nm. The mobile phase is degassed by sonication mobile phase.

2.6 Fourier Transform Infrared Spectroscopy (FT-IR) of Lycopene

Infrared spectra were recorded with a FT-IR spectrophotometer (Cary- 630, Agilent Technology, Mumbai). The purified lycopene crystals were reconstituted with dichloromethane, and the FT-IR was measured in cuvettes with windows of calcium fluoride. The spectrum was recorded in the wavelength region of 4000 to 650 cm^{-1}. Infrared absorption spectrum was recorded and spectrum analysis was done for functional group analysis [22].

2.7 Nuclear Magnetic Resonance (NMR) of Lycopene

The purified lycopene was subjected to NMR to determine structure. NMR experiments were carried out using a Bruker 800 MHz (BrukerBiospin, Rheinstetten, Germany) spectrometer equipped with a 5 mm TXI cryoprobe. The spectra were recorded at ambient temperature (300 K). Proton spectra for lycopene analysis were referenced to the TMS signal (δ= 0.00 ppm). Chemical shifts of lipid extractions were also compared with commercial standards of lycopene (≈0.5 mg dissolved in 0.6 mL of Dimethyl sulfoxide) purchased from Sigma Aldrich, Mumbai.

3. RESULTS AND DISCUSSION

3.1 Lycopene Content of Different Parts of Tomato

The above results showed that Vijeta cultivar had higher lycopene and carotenoid content than other cultivers, so it was used for further study. Different tomato fruit parts i.e. whole tomato, peel and industrial waste were screened for the lycopene content by solvents (tri-mixture) extraction method and obtained results are expressed in µg/g (Table 1). The data shown that peel of tomato contain highest lycopene (377.19±1.13 µg/g) content followed by industrial waste (175.15±1.09 µg/g) and whole tomato (82.82±0.79 µg/g) on dry wet basis. This indicated that a higher amount of lycopene was accumulated in the peel than other parts. Tomato extracts, especially skin extracts contain high amounts of lycopene [15]. The tomato processing industry waste include seeds and skin residues which may lead to lower down lycopene content than peel [16].

Table 1. Lycopene content of various raw materials of tomato origin

Raw materials	Lycopene content (µg/g)*
Whole tomato	82.82±0.79
Tomato Peel	377.19±1.13
Industrial waste	175.15±1.09

**Values are mean ± SD of three determinations*

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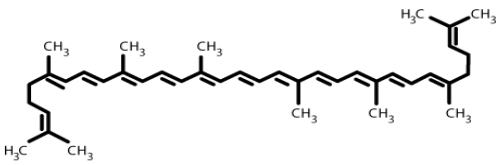

Fig. 1. Structural formula of lycopene

The results obtained for extraction of lycopene (Fig. 1) from tomato peel differ from those obtained using whole tomatoes, on account of the differences in the chemical compositions of the peel and the whole fruit, as well as due to the fact that lycopene is reported to occur in higher concentrations in tomato peel. The peel of tomatoes has the highest total carotenoid concentration, and the locular contents have the highest carotene content. It has been reported that lycopene represents a substantial proportion of the total carotenoid content of tomato products [23]. It is estimated as much as 60–64% of the total carotenoid content consists of lycopene. Considering whole tomatoes, the peel content will be low (5.5–8.1%), which is the reason for lower lycopene content [16, 24].

Lycopene was found predominantly in the chromoplast of plant tissues. In tomatoes, lycopene biosynthesis increases dramatically during the ripening process, as chloroplast undergoes transformation to chromoplast. Globulous chromoplast containing mainly β-carotene is found in the jelly part of the pericarp while chromoplast in the outer part of the pericarp contains voluminous sheets of lycopene [16,23]. Tomato extracts and especially skin extracts contain high amounts of lycopene [15].

The tomato processing industry waste comprises skin and seeds (approximate in the ratio of 37:63), which lower lycopene content as seeds do not contain lycopene [25]. However considering the cost of production of lycopene, it can be concluded that the waste of tomato processing industries, in the form of seeds and skin residues, could provide a useful source of lycopene [16,26].

3.2 Purification and Characterization of Lycopene

The lycopene obtained by solvent extraction method was purified by the crystallization method and subjected to the characterization by TLC, UV - spectroscopy, HPLC, FT-IR and NMR.

3.3 Thin-layer Chromatography (TLC) of Lycopene

The purity of lycopene was confirmed by TLC and it was performed (Data not shown). The data shows that crude extract sample gave 3 coloured spots i.e. red (Rf = 0.16), orange (Rf = 0.58), and yellow (Rf = 0.75), whereas purified lycopene sample showed a single red colored spot, which indicates that the extract is free from other carotenes. The Rf value of the red spot of the purified sample was the same as that of lycopene standard. The orange and yellow spots of the crude extract represent γ-carotene and β- and ζ-carotene, respectively [27-28]. This confirms that the crystallization method used for purification of crude extract of lycopene samples was appropriate.

3.4 Characterization by UV- spectroscopy

The characteristic peaks of lycopene were observed at around 445, 472 and 503 (Fig. 2). To minimize interference from other carotenoids, lycopene concentration was determined at 503 nm, using a molar extinction coefficient of 1.585 x 10^5 $M^{-1}cm^{-1}$ [29].

By analyzing the absorption spectra of pure compounds presented in the literature [30], it is found that alpha-carotene and lutein were the most important contribution in the absorption spectrum bands at 420 nm and 444 nm. Lycopene is the most important contribution to the absorption band at 470 nm and 500 nm [30]. Maximum wavelengths i.e. 445, 472 and 503 for lycopene are also reported by various researchers [16].

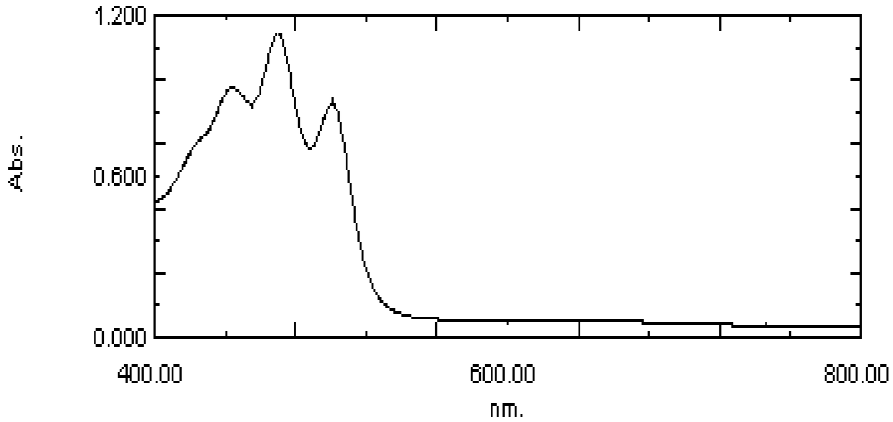

Fig. 2. UV visible spectra of purified lycopene

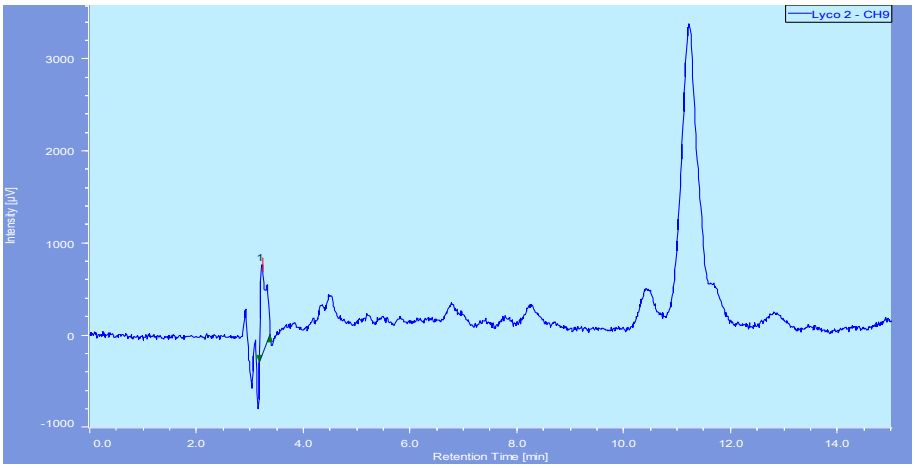

Fig. 3. Chromatogram of lycopene in purified extract

3.5 High-performance Liquid Chromatography (HPLC) of Lycopene Extract

Purified lycopene sample was subjected to the HPLC analysis. Separation of analytes were performed on an isocratic JASCO HPLC-DAD system using KYATECH C18 column (250 x 4.6 mm internal diameter, particle size 5µm).The PU 2080pump was used in this HPLC system (Dual piston with gear driven pump). The sample (20 µl) solutions of analytes were injected to chromatographic system using Rheodyne Injector. PDA detector used in this HPLC system was UV 2070 detector (Czerny turners mount monochromater) with deuterium lamp as light source. Chromatographic and the integrated data were recorded using JASCO LC-Net II/ ADC (interface) computer system employing ChromNAV Version 3.2.software for Data processing. Examination of lycopene was carried out by HPLC using acetonitrile: water (60:40 v/v) [31] as a mobile phase with a flow rate of 1.0 mL/min in an isocratic elution mode. The chromatogram of standard, crude extract and purified lycopene are shown in Fig. 3. The chromatogram of crude extract showed too many picks, which indicated that the extract contain various compounds other than lycopene. Whereas the chromatogram of purified extract showed only one major pick, which conforms that the extract was purified.

3.6 Fourier Transform Infrared Spectroscopy (FT-IR) of lycopene

Functional groups of purified lycopene were recognized by FT-IR. A background-corrected FT-IR spectrum of purified lycopene in dichloromethane is shown in Fig. 4. Carbonyl expanse oscillations were observed at 1744 cm^{-1}, while oscillations owed by the occurrence of

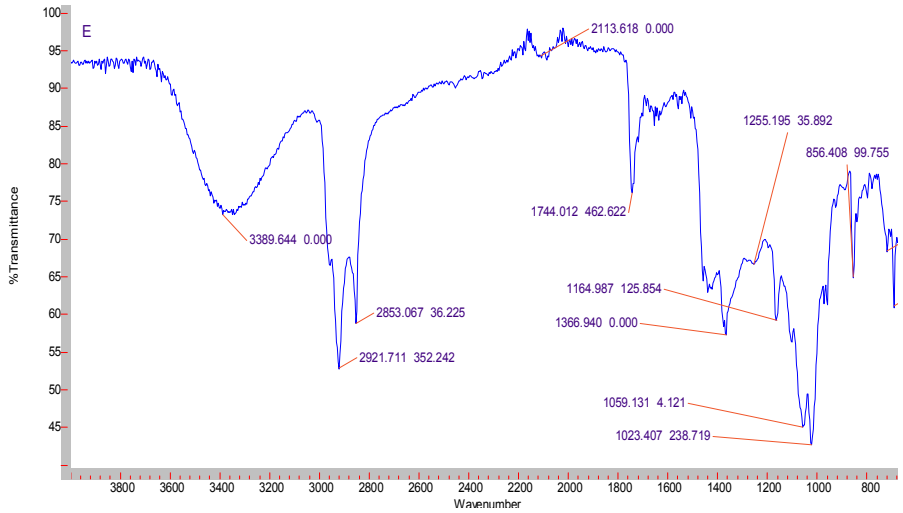

Fig. 4. FT-IR spectra of purified lycopene

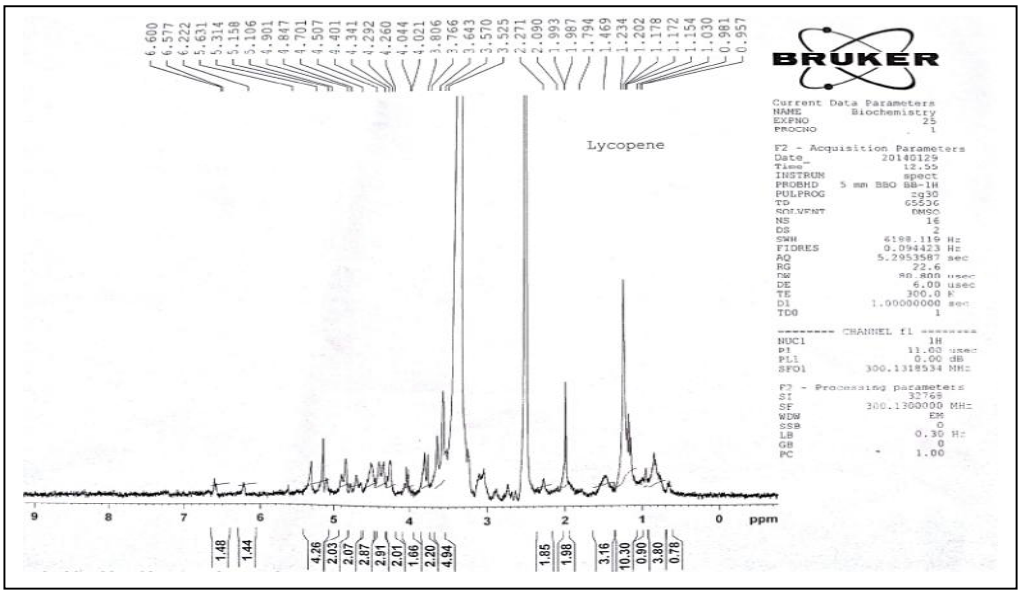

Fig. 5. NMR spectra purified lycopene

hydroxyl groups (predictable in the range of 2400–3400 cm^{-1}) were not noticed. Consequent distortion oscillations to carbon and oxygen double bond stretch oscillations were noticed at 1023, 1059 and 1164 cm^{-1}. Auxiliary indications were recognized as CH3/CH2 stretch oscillations at 2921 cm^{-1} and 2853 cm^{-1} with consistent distortion, CH3 deformation vibrations at 1366 cm^{-1} and C=C stretch oscillations at 1744 cm^{-1}. Major carbonyl oscillations as noticed here are characteristic for α, β-unsaturated carbonyls. Lycopene is one member of the carotenoids family; it contains an extended manacle of conjugated double bonds through two open end rings. Lycopene has the longest structure among all carotenoids. Molecular weight of Lycopene [$C_{40}H_{56}$] is 536.85 and has unsaturated hydrocarbon carotenoid containing 13 C=C, Out of these 11 are conjugated linear arrangements [30].

3.7 Nuclear Magnetic Resonance Spectroscopy (NMR) of Lycopene

The lycopene structure is recognized based on 1 and 2 dimensional NMR spectra, comprising ^{1}H

and ^{13}C NMR. Chemical modification for proton and carbon indications of the purified lycopene of the study was in good agreement with results reported by Hengartner et al. 1992 [32]. Spectral data on lycopene were similarly autonomously described by various researchers [33-35]. Results of purified lycopene at δ (ppm) was 3.570: $2H \rightarrow C_2$, 2.090: $4H \rightarrow C_3$, 2.271: $4H \rightarrow C_4$, 3.643: $2H \rightarrow C_6$, 4.841: $2H \rightarrow C_7$, 4.292: $2H \rightarrow C_8$, 4.021: $2H \rightarrow C_{10}$, 5.106: $2H \rightarrow C_{11}$, 4.341: $2H \rightarrow C_{12}$, 4.021: $2H \rightarrow C_{14}$, 4.044 – 4.260: $2H \rightarrow C_{15}$, 1.234: $12H \rightarrow C_{16}$ andC_{17} , 1.987: $6H \rightarrow C_{18}$ and 1.993: $12H \rightarrow C_{19}$ andC_{20} (Fig. 5).

4. CONCLUSION

The vijeta cultivar contains a higher amount of lycopene whereas in case of different parts peel had the highest amount of lycopene. The results of UV, HPLC, FT-IR and NMR gives a clear idea about whether the purification method was good enough to purify the lycopene extracted from the tomato processing industry waste. This will be beneficial to the lycopene producing industry for extraction, lycopene from waste generated during processing of tomatoes.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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