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Analysis of purified lycopene extract obtained from fresh tomatoes

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Abstract

Lycopene is a carotenoid, widely known for its antioxidant properties. In present investigation an attempt was made to observe the effects of purification process on antioxidant activity and isomerisation of lycopene. Antioxidant activity of crude as well as purified lycopene extract was measured as Trolox Equivalent Antioxidant Capacity (TEAC) using ABTS (2,2-azinobis-3-ethylbenzothiazoline-6-sulfonate) method. Crude as well as purified lycopene extract were subjected to spectrophotometric analysis for identification of peaks. HPLC analysis of crude as well as purified lycopene extract was carried out for cis – trans identification of peaks. Purification process significantly ($p < 0.05$) increased the antioxidant activity of crude lycopene extract. Analysis of HPLC chromatograms revealed that isomerisation of lycopene did not take place during purification process and no cis-isomers were observed in purified lycopene.

Keywords: ABTS assay, Lycopene, purification, antioxidant activity, HPLC, tomato

Introduction

Lycopene is carotenoid present in tomatoes and other red colored fruits and vegetables. It contains 13 double bonds, 11 of which are conjugated, which are responsible for its red color and high antioxidant activity. For these reasons, lycopene is among the major components of pharmaceuticals for the treatment of prostate cancer and digestive-tract tumors [1, 3]. Owing to its high solubility in fats and oils, it is widely used in the food and beverages industry as a colorant in foodstuffs. As a consequence of its wide uses, the demand for high-purity and low-cost lycopene is growing continuously. Lycopene is extracted from tomatoes that contain about 30-400 mg/kg of lycopene in pulp and about 20-30 mg/kg in peels [4]. Crude extract so obtained can be purified using column chromatography. But no study has been carried out to study effects of purification process on antioxidant activity and isomerisation of lycopene. Lycopene isomerization from all-trans (most predominant form) to cis-isomer leads to a reduction in its antioxidant activity [5]. The proportions of all-trans forms and cis-forms that directly influence the antioxidant activity of lycopene are also changed during tomato processing and storage [6, 7]. Therefore, in present investigation the effect of purification on antioxidant activity and isomerisation of lycopene was studied because both of these parameters are of immense importance wherever applications of lycopene are concerned.

Materials and Methods

Preparation of raw materials

Fresh red ripe tomatoes were purchased from local market and washed with distilled water and wiped clean. Tomatoes were blanched in boiling water for 5-7 minutes. Blanched tomatoes were separated into three different fractions, viz., skin, pulp and seeds. The seed fraction was discarded. The pulp and skin fractions were collected in separate petriplates and frozen immediately. The fractions were freeze-dried and crushed into powder, and stored at -18°C in amber colored air tight glass bottles until lycopene was extracted and purified. All the organic solvents used were of analytical grade. Lycopene and β -carotene standard was purchased from Sigma-Aldrich at a certified purity grade of 90-95%.

Extraction of lycopene

Crude lycopene was extracted from the mixture of freeze dried pulp and skin fractions (taken in a ratio of 3:2) using petroleum ether and acetone [8]. Five gram of dried powder was taken in a pestle and mortar and extraction with acetone was carried out until the residue was colorless.

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Acetone extract was transferred into a separating funnel having 10-15ml of petroleum ether and mixed gently. Water (20-30ml) was added for phase separation and several washings were carried out to remove acetone. Lower acetone phase was discarded and upper phase of petroleum ether containing dissolved lycopene was collected. Evaporation of solvent was carried out using rotary vacuum evaporator to obtain dry extract. All sample preparations and experiments were performed under dim light conditions to minimize any light-induced degradation and isomerization.

Purification of lycopene

Crude lycopene extract was purified using column chromatography in order to remove impurities and other carotenoids such as β -carotene and xanthophylls. Neutral activated alumina (Grade III) was used as an adsorbent. Dried extract was dissolved in minimum volume of eluting solvent (1 mL of hexane). Lycopene sample was added carefully to the prepared column without disturbing the top surface of the column. Hexane and acetone-hexane mixture was used to elute β -carotene and lycopene from the column, respectively. Yellow β -carotene band was eluted by hexane and 10% acetone-hexane was used to elute red lycopene band from the column.

Spectrophotometric analysis

To obtain UV-VIS spectra of crude as well as purified lycopene extract, the extract was taken in a 50ml volumetric flask and volume was made up with petroleum ether. A 5ml aliquot was taken and further diluted to 50ml using petroleum ether and UV/visible spectra of crude as well as purified lycopene extract was obtained using a spectrophotometer [8].

Measurement of antioxidant activity

Free radical scavenging activity of the crude as well as purified lycopene extract was determined by ABTS method [9] which involved the direct production of ABTS^{•+} chromophore through the reaction between ABTS and potassium persulfate. Trolox equivalent antioxidant capacity (TEAC) measured the relative ability of lycopene to scavenge the ABTS^{•+} (2, 2-azino-bis (3-ethylbenzothiazoline-6-sulfonate) in comparison to the antioxidant capacity of standard amounts of Trolox® (6-hydroxy-2,5,7,8 tetramethylchroman-2-carboxylic acid). In the presence of antioxidants the absorbance at 734nm decreased and the extent of decolorization as percentage inhibition of radical cation was determined as function of concentration and time. The standard curve was prepared by plotting concentration (500 - 5000 μ M) of Trolox (X-axis) v/s% inhibition (Y-axis). One ml of ABTS working solution made with phosphate buffer saline (pH 7.4) was added to microcuvette (1 ml capacity) and absorbance adjusted to 0.70 \pm 0.02 against the buffer. Ten microlitre of sample (extract dissolved in dichloromethane) was added to ABTS working solution as well as in the blank. The contents were mixed for 5 seconds and change in absorbance at 734 nm was recorded over 10 min using SPECORD-200 double beam spectrophotometer (Analytical zena). Based on the % Inhibition of absorbance of sample, trolox equivalent was determined from standard curve using equation as follows:

$$y = 0.014x + 13.23$$

Where;

y: is the% inhibition = [(A 734 nm control - A 734 nm sample)/A 734 nm control]X 100

x is the μ M concentration of trolox

HPLC analysis

HPLC analysis of crude as well as purified extract of lycopene was performed [10] using high performance liquid chromatographic system - HPLC Dionex Ultimate 3000, binary pump. The chromatographic separation was carried out on a Dionex RP C [18] column (250 mm x 4.6 mm I.D. 5 μ m particle size) using tetrahydrofuran, acetonitrile and methanol as isocratic mobile phase (45:45:10 v/v). Mobile phase was filtered through a 0.45 μ m membrane, and degassed ultrasonically prior to use. The mobile phase flow rate was kept 1 ml/min. Identification of peaks was carried out by comparing the retention times of sample with lycopene standard. Cis-trans identification of lycopene isomers was also carried out using RP-HPLC. For data processing and analysis Chromeleon version 6.8 software was used.

Statistical analysis

Data for antioxidant activity was recorded as mean of three replicates. Data was analyzed using MS-Excel 2007. The significance was tested by employing two sample - t-test.

Results and Discussion

Antioxidant activity

Antioxidant activity of purified lycopene extract was measured using ABTS method in order to assess the effect of purification process on antioxidant activity of lycopene. The purified extract possessed significantly higher ($p < 0.05$) antioxidant activity (3.06) in comparison to crude lycopene extract (2.36). This could be attributed to the increase in amount of lycopene in purified extract as other carotenoids possessing less antioxidant activity, e.g., β -carotene and xanthophylls were removed during purification. Therefore, results in present study revealed that purified lycopene can be used as an antioxidant to prevent rancidity and to extend the shelf-life of various food products.

Spectrophotometric analysis

The UV-VIS spectra of crude lycopene extract is shown in Fig. 1. The shape of the spectrum was characteristic of the lycopene, resulting in a three-peak spectrum at three wavelengths (446, 472 and 503nm). Crude lycopene extract contains other carotenoids also, e.g., β -carotene and xanthophylls. Therefore, in crude lycopene extract β -carotene might have contributed to the height of first peak as it absorbs strongly at that wavelength. In spectra of purified fraction I as shown in Fig. 2, peaks were observed at 446nm and 472nm which were identified as of β -carotene as also reported by others [11]. The peak at 503nm was absent in fraction I indicating absence of lycopene in first fraction. Spectra of fraction II (Fig. 3) exhibited three peaks, first peak at 444nm, second peak at 468nm and third peak at 503nm which are characteristics of lycopene. No peak was identified in Fraction III consisting of xanthophylls as shown in Fig. 4. Therefore, it was inferred that purification process was effective in separating β -carotene and xanthophylls from crude lycopene extract.

HPLC analysis

Crude lycopene extract was subjected to RP-HPLC analysis for cis-trans identification. Chromatograms of all-trans lycopene standard and crude extract were monitored at 471 nm as shown in Fig. 5 and 6, respectively. The overall elution order was found to be β -carotene (peak 1) < lycopene (peak 2) < xanthophylls (peak 3) as shown in Fig. 6. All-trans lycopene standard was used for identification of lycopene peak in chromatogram of crude as well as purified lycopene extract.

The chromatogram of lycopene standard indicated that lycopene eluted in a retention time of 3.5 min. Peak 2 eluted at a retention time of 3.43 min and was identified as trans-lycopene (Fig. 7).

Purified fractions of lycopene were also subjected to RP-HPLC analysis. Chromatograms of all purified fractions, viz., I, II and III were monitored at 471 nm as shown in Fig. 7, 8 and 9, respectively. Fig. 7 depicts that in purified fraction I only β -carotene peak was present which eluted at 2.89 min and other peaks were altogether absent. In purified fraction II (Fig. 8) only lycopene peak was present which eluted at 3.48 min. In purified fraction III (Fig. 9) eluted peak was of xanthophylls. Identification of peaks was based on comparison of retention times with standards. β -carotene standard was used for identification of β -carotene in crude extract and peak 1 with a retention time of 2.74 min was identified as β -carotene.

HPLC analysis of purified lycopene extract did not reveal any peaks that would indicate isomerisation or degradation as single peak was observed. No overlapping of peaks was

observed in all chromatograms. Obtained results are in accordance with other findings [12] who found that mild heating conditions (80 °C) did not cause any apparent influence on isomerization to produce detectable amounts of the unstable cis-isomer from the all-trans form. However, higher temperatures such as 120 and 140 °C greatly influenced the cis-isomer contents, and cis-isomer contents increased with elevated temperatures. In present investigation, the temperature used during extraction was below 50 °C, therefore absence of cis-isomers or presence in undetectable amounts is obvious. The all-trans forms are also more resistant to heat as compared to the cis-isomers under mild treatment conditions [13]. This is also in agreement with the reported 35–96% of all-trans lycopene and <1–7% 13-cis isomer in tomato and other tomato products [14]. Therefore, it can be concluded that lycopene remained stable under the extraction and purification conditions and didn't undergo isomerisation that might have adversely affected its biological properties specially the antioxidant property.

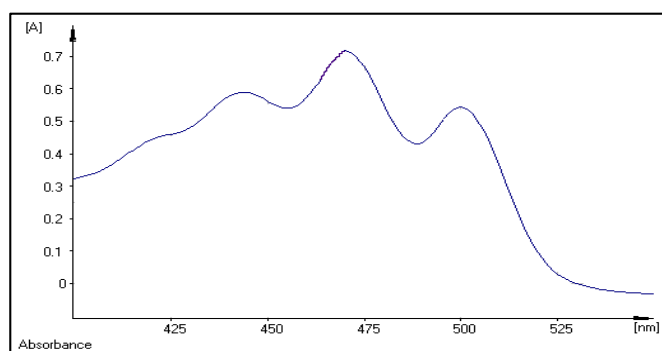


Fig 1: UV/VIS spectra of crude lycopene extract

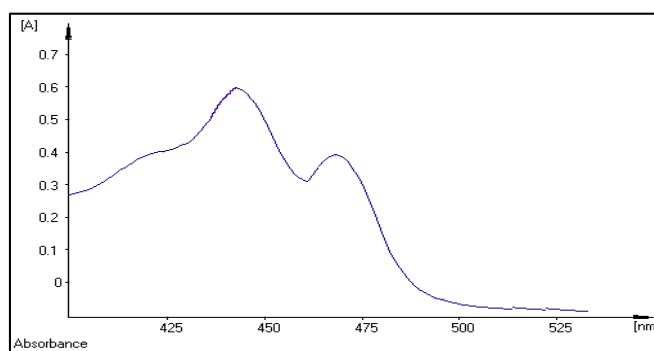


Fig 2: UV/VIS spectra of purified fraction I

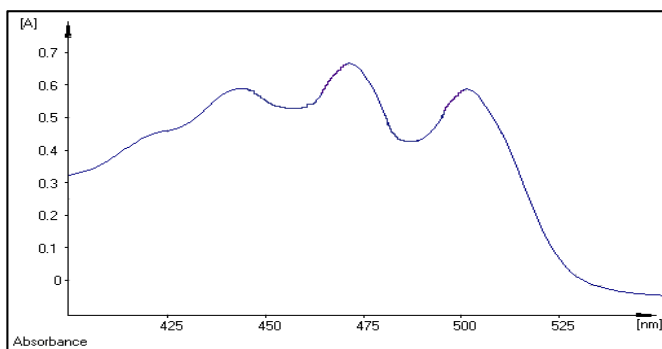


Fig 3: UV/VIS spectra of purified fraction II

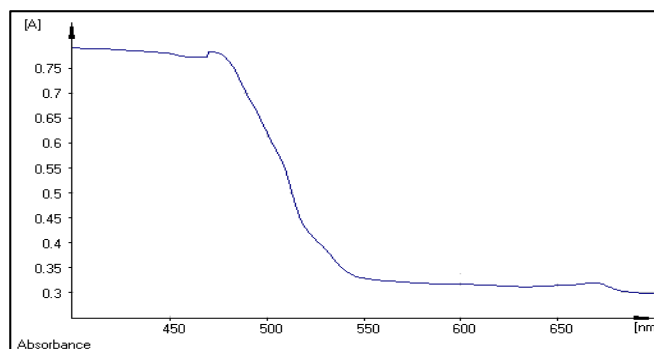


Fig 4: UV/VIS spectra of purified fraction III

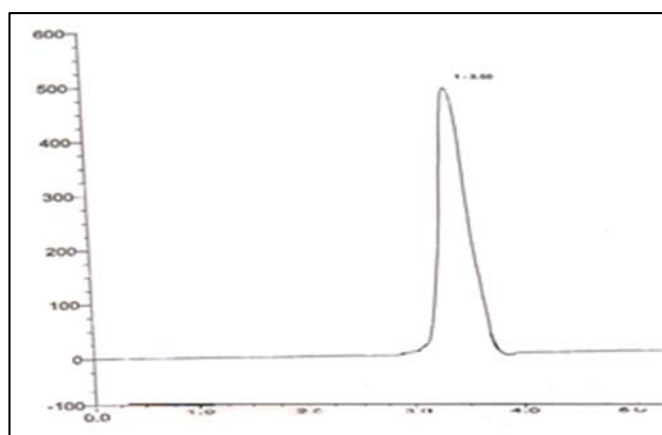


Fig 5: Chromatogram of lycopene standard

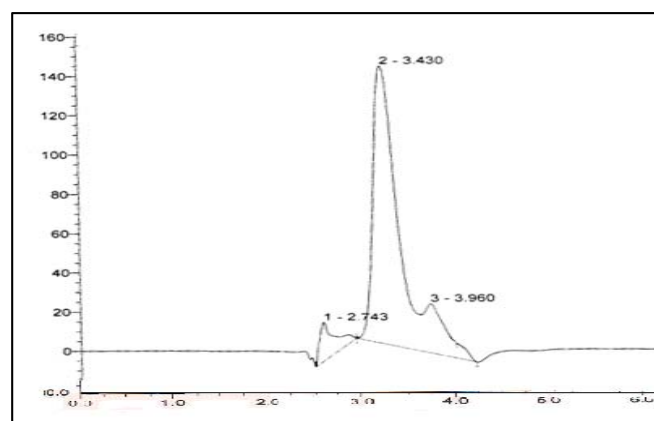


Fig 6: Chromatogram of crude lycopene extract

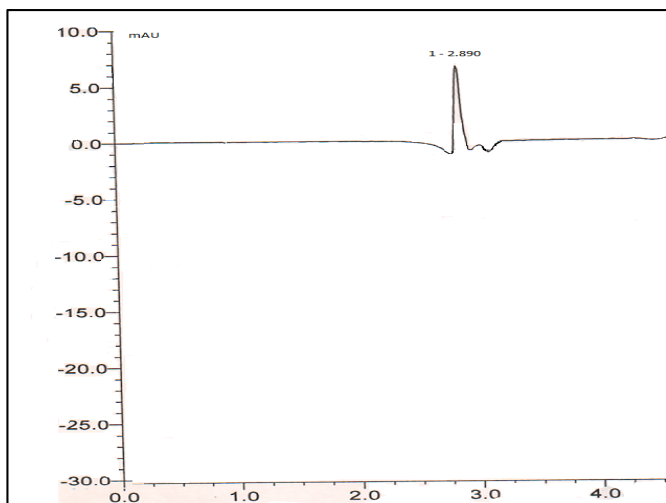


Fig 7: Chromatogram of purified fraction I

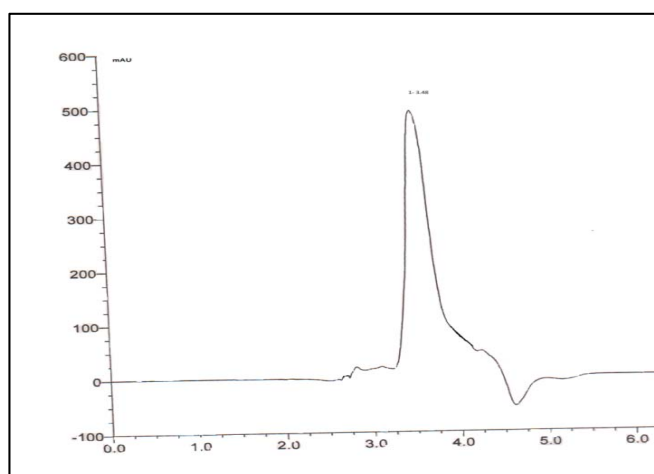


Fig 8: Chromatogram of purified fraction II

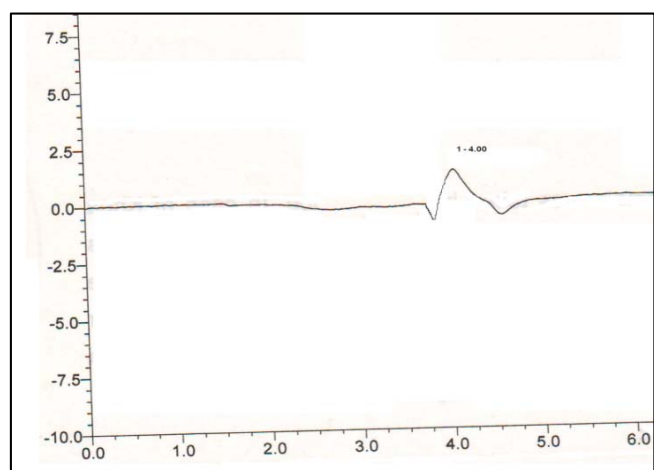


Fig 9: Chromatogram of purified fraction III

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Conclusion

Analysis of purified extract revealed that purification process did not induce any isomerisation from trans-isomers to cis-isomers. Also the purification process was found effective in removing carotenoids such as β -carotene and xanthophylls from crude lycopene extract. No negative impact was observed on the antioxidant activity of lycopene rather a significant increase ($p < 0.05$) was observed in antioxidant activity after purification. Therefore, purified lycopene possesses great potential to be used as a natural antioxidant in foods to extend their shelf-life.