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# Analysis and Estimation of Lycopene Extracted from Tomatoes

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## Abstract

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Lycopene is a carotenoid without provitamin-A activity which functions as an important antioxidant. It is a carotenoid present in human blood and other tissues. The intake of dietary lycopene reduces the risk of many diseases such as eye diseases, diabetes, and cardiovascular diseases. Tomatoes are a major source of lycopene. This study aims at finding a suitable method of extraction and estimation of lycopene from tomatoes. Pectinase, pectinase and ultrasound waves, solid state extraction and spectrometric measurements were used for the said purpose. It was deduced that by using these methods a good quantity of active lycopene can be extracted from red and raw (green) tomatoes which can be used further for therapeutic purposes. These are fast methods to extract lycopene in which pectinase is also extracted simultaneously, thus reducing the cost of buying commercial pectinase. These methods can be used on a small and/or large scale for the preparation of therapeutic medicine as well as for waste management, since vegetable waste can also be used for lycopene extraction. Further alterations can also be made to increase the efficiency of lycopene extraction.

**Keywords:** antioxidant, carotenoids, lycopene extraction, pectinase, phyto-pigments

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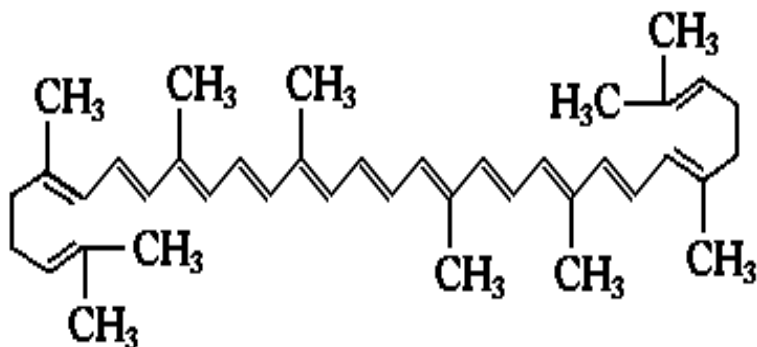
## 1. Introduction

Carotenoids (CRT) are naturally occurring pigments which give a bright colour to various fruits and vegetables. They play an important role in different systems such as in photosynthesis, where they are important in light reaction. CRTs are also important due to their antioxidant (AO) activity. Red, orange and yellow colours of most fruits and flowers are also essential for pollination [1].

Lycopene (LYP) is an important naturally occurring bright red carotenoid pigment and phytochemical which is present in tomatoes and other red fruits and vegetables. It accounts for 80-90% of total pigment content of tomatoes [2].

Tomato products such as paste, sauce, soup and ketchup are all dietary sources of LYP. Recent studies showed that the daily average intake of LYP is 25mg. In processed tomatoes, the bioavailability of LYP is higher as compared to raw tomatoes [3]. It was also found that the availability of LYP is affected by other CRTs. Studies showed that the amount of LYP present with  $\beta$ -CRT is much higher as compared to its sole presence [4].

Scientists have discovered the reason behind LYP presence in different fruits and vegetables. They discovered two genes which are present in citrus fruits and in those fruits which contain LYP. These genes are encoded by lycopene cyclase (LYC) known as Beta-LYC and Eta-LYC [1].



**Figure 1.** Structure of Lycopene Showing Unsaturation [5]

Lycopene consists of eight isoprene (IP) units arranged in a tetraprene (TP). This structure is purely made of carbon and hydrogen. LYP consists of C40 polyisoprenoid compounds. It contains 13 double bonds and unsaturated compounds. Out of the 13 double bonds, two are non-conjugated and the remaining 11 bonds are conjugated.

LYP is a more important antioxidant as compared to  $\alpha$ -tocopherol,  $\alpha$ -CRT and  $\beta$ -CRT [4]. LYP is a fat soluble non-polar CRT. It does not have the  $\beta$ -ionone ring. It also has the ability to do isomerization as the Cis-trans isomer (IM). The induction of IM could be done via chemical reaction, heat exposure and /through light absorption. LYP is present up to 80-97% in all-trans (E), as well as in fruits and vegetables. LYP is present in a combined form as 50% of Trans (E) and 50% of Cis (Z) IM in body tissue and blood plasma [6].

Lycopene extraction, if carried out by recombinant DNA technology, can be a long and expensive option for medical industries for the production of such a potent antioxidant. The methodology discussed in the current paper aims at finding a faster and cheaper way of extracting lycopene from natural sources

(fruits and vegetables) and it can prove to be useful in the industrial sector for the production of lycopene [7].

## 2. Methodology

For the extraction of lycopene different procedures were used which are as follows: extraction using pectinase, extraction using pectinase and ultrasound waves, solid state extraction and spectrometric measurements.

### 2.1. Extraction Using Pectinase

**2.1.1. Microorganism.** For extraction, we must use pectinase. However, due to the unavailability of pectinase, it was produced using fungus *A. niger* and the substrate banana peel powder. Fungus was bought from the Department of Agriculture, University of the Punjab and then incubated in at yeast extract agar medium at 30°C for 7 days. Afterwards, the culture was maintained on potato dextrose agar (PDA) for 30 days and was then subcultured.

**2.1.2. Substrate for pectinase production.** Banana peel was washed with tap water and dried at 45°C for 24h. The peel was grinded on 200 mesh sieves until it was converted into powder. For complete drying, the banana peel powder was incubated at 105°C for 48h.

**2.1.3. Pectinase production.** The experiment for pectinase production was carried out in a 250ml flask containing  $MgSO_4$  (0.001% w/v),  $(NH_4)_2SO_4$  (0.001% w/v), 0.01% w/v of  $MnSO_4$ , and  $KH_4PO_4$  (0.04% w/v) in 100ml distilled water. The substrate (banana peel powder) was added to the solution and pH was set at 5.8. Then, the solution was transferred into a sterilized flask. Fungal spores were added in the flask and incubated at 150rpm for 24h at 25-30°C. The culture filtrate was centrifuged at 10000 rpm for 10 min in the centrifuge machine and the supernatant was collected for further testing. For partial purification, 50ml of supernatant was treated with 150 ml of chilled ethanol. The solution was kept at 4°C for 15 minutes until pellet formation. The pellet was treated with 15ml of distilled water. The enzyme was ready for further use [8].

## 2.2. Extraction Using Pectinase

Fresh tomatoes were taken from a close by market and stored at -15°C in the dark for 24h. Their skin was removed before treating them with acetate buffer. The tomatoes were mixed vigorously in the blender. 100g of residue sample was taken and treated with acetate buffer with pH 4.7. It was mixed with various concentrations of pectinase enzyme as 30, 35 and 40 mg/kg samples and incubated at 55°C for 20, 40 and 60 min, respectively. After incubation, it was placed at 95°C for 40 minutes to denature the enzyme. A sample of 5g was picked and mixed with solvent acetone or petroleum at 1:1 ratio. It was placed in a separating funnel and stirred for 10 min and then placed in the dark for 20 min. The upper phase contained LYP which was separated by filter paper coated with 1g of dehydrated sodium sulphate. The researcher took 2ml filtrate

in a cuvette and then wavelength was checked at 503nm.

### 2.2.1. Solid state extraction of LYP.

Tomato paste was extracted 3 times with 10ml acetone and afterwards, it was also extracted 3 times with dichloromethane. Then, combined filtration was done by mixing the extracted product with water and NaCl for breaking emulsions. The filtered product was placed in fume hood for removing acetone and other water soluble components. The petri plate or test tube contained the coloured product (CP). This product was separated and dried over anhydrous calcium chloride. CP dissolved in a few drops of dichloromethane and was used for further investigation such as TLC and column chromatography.

### 2.2.2. Thin layer chromatography.

In TLC, the mobile phase consisted of 8ml hexane and 2ml acetone. The stationary phase comprised a plate containing silica gel. The plate was made by dissolving 3gm of silica gel and 1gm of calcium carbonate in 4ml of water and the mixture was stirred vigorously. Then, with the help of a dropper, a small drop of the mixture was put on the clear glass plate and made into a thin layer. After drying the plate two spots were drawn. One spot was made with a high concentration of CRT. The other spot was made with a dilute concentration of CRT. Both points were on the same line position. After drying the spots, the plate was placed in the mobile phase and allowed for elution. When elution was completed, the plate was placed in the iodine chamber because naked eye and UV light couldn't visualize the spots. The concentration spot visualized two points, A and B. The distance covered by the solvent was 5cm and the two points were at a distance of 4.7 and 2.1cm, respectively. However, the dilute

solution spot showed only one point whose movement was 4.7cm. [9].

### 2.3. Extraction of Lycopene by Spectrometric Measurements

Fresh tomato was placed in -15°C in darkness for the night. Then, skin disk was separated and acetone extraction was done by adding 3ml of acetone in the skin disk. It was centrifuged in shaking incubator at 150rpm for 1h. After it was centrifuged, the solution remained at 1000×g for 10 min. Absorbance was checked at 503nm.

**2.3.1. Determination of LYP concentration.** The pulp or diced tomato was dissolved in 4ml acetone and centrifuged in shaking incubator at 200rpm for 3h. Then, centrifugation was done at 1000×g for 10min and absorbance was checked at 503nm. Absorbance was determined by spectrometric measurements. The formula of molar extinction coefficient was used to determine the concentration of lycopene.

**2.3.2. LYP detection test.** LYP was detected by making the dilutions of the solution containing LYP with acetone or dichloromethane. Five milliliter of acetone was used as blank. Four dilutions were prepared from the stock solution keeping a total volume of 5 ml while decreasing the concentration of the dilution by 1 ml each time. Absorbance was checked at 503nm.

## 3. Results

### 3.1. LYP Extraction by Pectinase Enzyme

There were two sample solutions of tomato. One had red colour and the other was of green tomato. Previous studies revealed that green tomatoes lack LYP.

So, the result was positive for the red tomato sample. Absorbance was checked at 503nm and absorbance values are given in the table 1.

For red tomato sample, high absorbance revealed that this sample contained LYP. On the other hand, low absorbance of green tomato sample depicted that it contained a small or negligible amount of LYP carotene.

**Table 1.** The Absorbance of Two Tomato Samples, Red and Green

Sample	Absorbance At 503nm
Red tomato sample	0.382
Green tomato sample	0.149

### 3.2. TLC Test for LYP Extraction

TLC tests showed two distances of the concentrated spot and one of the diluted spot.

Distance covered by the two spots A and B of concentrated drop was 4.7cm and 2.1cm, respectively. The distance covered by the solvent was 5cm. Finally, the distance covered by the spot of diluted drop C was 4.7cm.

So the Rf value of spot

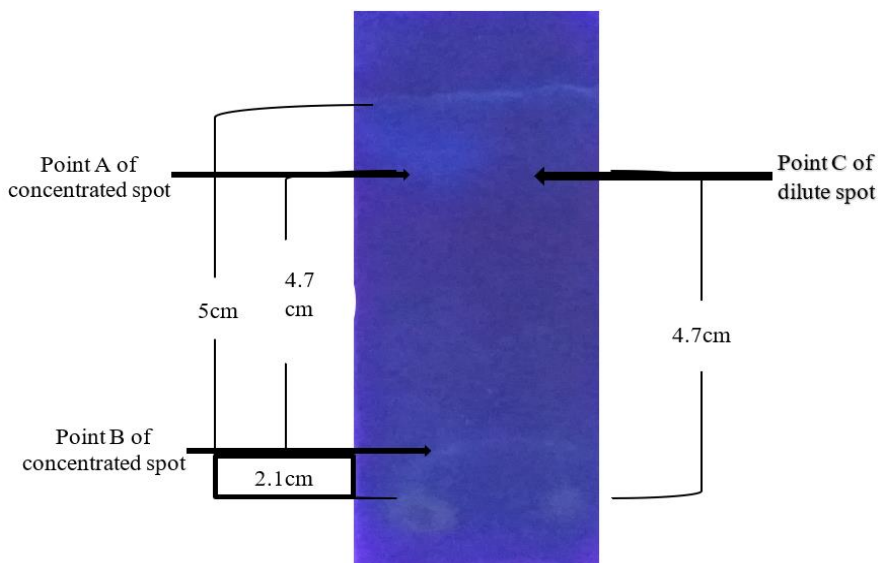
$$A = \frac{\text{distance covered by point A}}{\text{distance covered by solvent}} \\ = \frac{4.7\text{cm}}{5\text{cm}} = 0.94$$

Rf value of spot

$$B = \frac{\text{distance covered by spot B}}{\text{distance covered by solvent}} \\ = \frac{2.1\text{cm}}{5\text{cm}} = 0.42$$

Rf value of spot

$$C = \frac{\text{distance covered by point C}}{\text{distance covered by solvent}} \\ = \frac{4.7\text{cm}}{5\text{cm}} = 0.94$$



**Figure 2.** TLC glass plate with LYP on iodine chamber

The results showed that the concentrated spot A contains the carotene pigment LYP. Point B has an  $R_f$  value of 0.42, which shows that the sample may contain more than one carotene pigment. Spot C of the diluted sample showed an  $R_f$  value of 0.94 and it revealed that the diluted drop only contains LYP and not any other carotene pigment (Table 2).

**Table 2.** TLC Tests Result

Spots	Distance Covered by Solute (cm)	Distance Covered by Solvent (cm)	$R_f$ Value
A.	4.7	5	0.94
B.	2.1	5	0.42
C.	4.7	5	0.94

### 3.3. LYP Extraction by Spectrometric Measurements

Absorbance was measured at 503nm on spectrophotometer. The determined absorbance was 0.336. The determined high absorbance showed that the sample contained a high amount of LYP.

### 3.4. LYP Quantification

LYP quantification was determined using the formula of molar extinction coefficient.

Formula:

$$\frac{\text{absorbance}}{\epsilon} = \text{molar concentration}$$

$$\epsilon = 17.2 \times 10^4 \text{ mol cm}^{-1}$$

$$\text{Absorbance} = 0.486$$

By putting the values

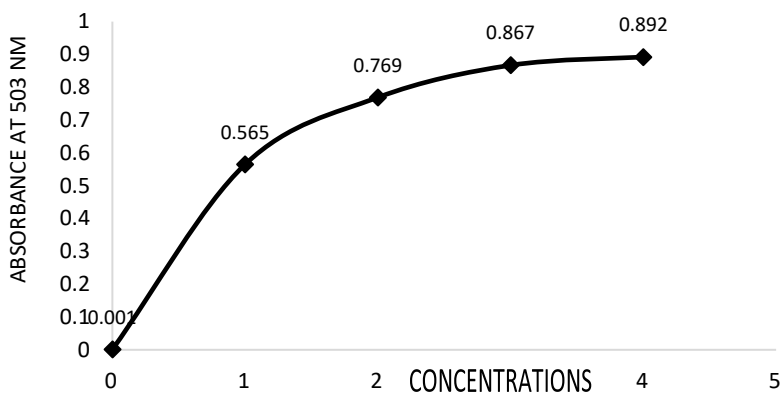
$$= \frac{0.486}{172000} = 2.8 \times 10^{-6}$$

$2.8 \times 10^{-6}$  was the molar concentration of LYP.

### 3.5. LYP Detection Tests

The results of dilutions made is shown in Table 3.

The graph shown in Fig 3 depicts that the dilutions which contained a high amount of sample have a high absorbance. On the contrary, low absorbance showed that the dilutions contained a low amount of sample.



**Figure 4.** The absorbance of LYP sample

**Table 3.** LYP Dilutions Absorbance at 503nm

No. of obs.	Acetone (ml)	Sample (ml)	Absorbance at 503nm
1.	5	0	0.001
2.	4	1	0.565
3.	3	2	0.769
4.	2	3	0.867
6.	1	4	0.892

the current research, LYP was extracted through the use of pectinase enzyme, by solid state extraction and via spectrometric measurements.

Pectinase enzyme was used for the extraction of LYP and due to the less availability of this enzyme, it was extracted from fungus *A. niger* and banana peel [12]. This was an extra step added to the process of lycopene extraction. If industrial pectinase enzyme was available, then the extraction of lycopene would be a direct process [13]. In the pectinase extraction process, tomato paste was treated with acetone and then with pectinase [14]. The solution was treated with dichloromethane for extraction and separating funnel was used to separate the layers. The upper layer was used for further extraction and absorbance at 503nm was 0.324. It showed the presence of CRT and red color showed the presence of LYP. The green sample showed 0.143 absorbance, which confirmed that it lacked LYP [15]. Solid state extraction was then carried out to extract lycopene from the processed sample. Tomato paste was treated with solvents like dichloromethane and

#### 4. Discussion

LYP is the major CRT mostly found in red fruits and vegetables and it is responsible for the red coloring of fruits and vegetables. It is a non-polar, non-soluble compound which has an AO activity [10]. Studies about LYP showed that it is a major carotenoid which can be used for curing different kinds of diseases. The diseases cured by the use of LYP include cardiovascular diseases, kidney diseases, oxidation stress (OS) and many more [11]. Many researches were carried out for the extraction of this CRT antioxidant. Different methods were used for the extraction of LYP and from different sources. LYP may be extracted from tomatoes, watermelons, papaya, peaches and blood plasma. In



acetone and the solution was used for TLC tests. The results showed that the concentration spot had two Rf values, that is, 0.945 and 0.452. The higher value of 0.945 means greater concentration of LYP at this spot. Spectrometric measurements showed the presence of LYP in tomato skin and tomato pulp disk. The absorbance measured was 0.336 at 503nm and it confirmed the presence of LYP in the sample. The detection test of LYP was its dilutions. Absorbance was measured for each dilution. The graph made by these dilutions confirmed the presence of LYP in the tomato sample. The quantity of LYP was measured using the molar extinction coefficient which was  $2.8 \times 10^{-6}$ .

Recently, various other methods were used for lycopene extraction. The antioxidant was extracted from watermelon, pomegranate and industrial waste [16]. Furthermore, methods such as thermal conditioning and ultrasonics were used also to increase the amount of lycopene that can be extracted from different types of fruits and vegetables [17].

Also, recent studies showed that high pressure provided by carbon dioxide can help retain the freshness of the sample for a longer period of time, thus providing an opportunity to extract lycopene in a more favorable environment with less risk of the degradation of the antioxidant [18].

This paper provides the simplest method for the extraction of lycopene which can easily be used for the extraction of the antioxidant at a large scale. However, conditions can be optimized to increase the amount of lycopene extracted from the fruit. Moreover, temperature optimization and pressure settings can be

added to get a higher titer number of the product. For this purpose, further study and research is required in the field to check the feasibility of the said changes to see if they match the designed protocol [19].

Furthermore, the extraction of pectinase can also be optimized by increasing the amount of pectinase extracted from banana peels [20]. Industrial pectinase can also be used which can make lycopene extraction even less time consuming. Moreover, it may be a better option for the production of the antioxidant for therapeutic purposes.

Overall, this is a simple and straightforward method for the extraction of the antioxidant from tomatoes as well as any other fruit. Moreover, this method can be used for small or large scale extraction of lycopene without the use of a lot of chemicals and with less risk of enzyme degradation.

## 5. Conclusion

Lycopene is a potent antioxidant that has vast therapeutic applications. Through the process discussed in this paper, the antioxidant can be easily extracted at a large scale and this process can be used for industrial extraction from common fruits. The extracted lycopene can be used for the treatment of various ailments of heart, eyes etc.

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