Extraction And Isolation Of Lycopene From Solanum Lycopersicum And Citrullus Lanatus For Bioplastic Colouring

Vrinda Nair, Simran Lilwani

Abstract: Lycopene is a carotenoid pigment and phytochemical found in tomatoes, water melon and other fruits mostly red coloured. Due to its structure, lycopene gives its deep red colour owing to its strong colour and non- toxic in nature. Lycopene is non-polar in nature due to which it stains any porous material including plastic. Lycopene diffuses into plastic, making it difficult to remove with hot water or detergent. Since lycopene is insoluble in water but can be dissolved in organic solvent and oil. Extracting lycopene from natural source like tomato and watermelon which contains the highest amount of lycopene leads to a non-toxic colouring agent which can be used in bioplastic colouring. Identification of lycopene was carried out using UV-Visible spectrophotometer and HPLC. The lycopene content was found to be 5.50 and 11.1 (mg/kg fresh wt) in tomato and watermelon respectively. The peaks of lycopene in UV-Visible spectrophotometer were found at 459 nm and 468 nm for tomato and watermelon respectively. HPLC of lycopene was carried out which showed the λMax at 473 nm for tomato and 471 nm for watermelon. The extracted lycopene was added while preparing the bioplastic as the colouring agent. Hence, lycopene proves to be a potent biocolour for the bioplastic colouring.

Index Terms: Lycopene, Carotenoid, UV-Visible spectrophotometer, HPLC, Bioplastic, Tomato (Solanum lycopersicum), watermelon (Citrullus lanatus).

INTRODUCTION

Lycopene is a pigment principally responsible for the characteristic deep-red colour of ripe tomato fruits and products. As a natural source of antioxidants, it has attracted attention due to its biological and physicochemical properties. Lycopene, a red carotenoid pigment in tomatoes and tomato-based products, is an acyclic form of beta-carotene without provitamin A activity. It has attracted substantial interest during recent times for its beneficial in reducing oxidative stressing coronary heart diseases and other chronic diseases. Its molecular weight is 536. 89 and molecular formula is C40H56 with 89.45 ٪ carbon and 10.51٪ hydrogen. It is highly unsaturated hydrocarbon containing 11 conjugated and two unconjugated double bonds. [1]

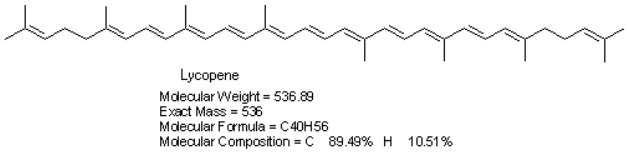

FIGURE 1- *Lycopene structure with molecular weight, exact mass, molecular formula and molecular composition.*

Commercial lycopene is available as standardized tomato extract or from chemical synthesis. Market trends indicate a growing demand for the former product, because of its natural origin and the presence of other phytochemicals, such as β-carotene, phytoene and phytofluene, which are believed to act synergistically with lycopene. Lycopene is in high demand by the pharmaceuticals industry as well as by the food, and cosmetics industries. Natural lycopene is produced today mainly by extraction and concentration from whole tomato fruits, that are grown specifically for this purpose.

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The commercially available product, however, is very expensive and current production from whole tomato fruits is small compared to projections of future demand. This has prompted the search for alternative sources of lycopene and appropriate technologies for its recovery. Lycopene is found predominantly in the chromoplast of plant tissues. In tomatoes, lycopene biosynthesis increases sharply during the ripening process, as the chloroplast undergoes transformation to chromoplast. [2]

MATERIALS AND METHODOLOGY-

Materials- Fresh tomato and watermelon

EXTRACTION OF LYCOPENE-

(A) BENZENE ISOLATION METHOD -

- We made a paste separately of watermelon and tomato. In the laboratory weigh 100 gm paste of each of the fruits.
- 100 gm of sample of watermelon taken in a 200 ml beaker. Then warm the paste and add about 30 ml of warm (40 degree C) benzene to it.
- Stir well and decant the benzene layer.
- Again add 30 ml warm benzene, stir and decant the benzene.
- This has been done about 5 times.
- Then distil off benzene and we got residue of lycopene.
- Recrystallized residue by ether and weighed.
- Repeat the steps with other sample of tomato and recorded the observations. [4]

B) LYCOPENE EXTRACTION WITH HEXANE/ETHANOL/ACETONE-

- Starting with tomato juice, use a 1000 µL micropipette to take the sample.
- Dispense the sample into 15 ml centrifuge tube.
- Add 8.0 ml of hexane: ethanol: acetone (2:1:1) using a micropipette.
- Cap and vortex the tube immediately then incubate out of bright light.

- After at least 10 minutes, or as long as several hours later, add 1.0 ml water to each sample and vortex again.
- Let samples stand 10 minutes to allow phases to separate and all air bubbles to disappear.
- The same procedure was repeated for watermelon sample.

ANALYSIS USING THIN LAYER

CHROMATOGRAPHY-

- Analysis were performed in pre-coated silica plates which were cut into 5 * 5 cm. Sample solutions were applied at the bottom of the plate leaving 1 cm with the help of a glass capillary.
- Plates were developed with hexane- chloroform 9:1 as mobile phase in a vapour- equilibrated chamber.
- The development time was approximately 15-20 minutes and was kept in room temperature.
- All operation was performed in a darkened laboratory; solution containers and developing chamber were also kept under darkened condition to protect the sample since lycopene is light sensitive.
- After the development, plates were air dried for 2-3 minutes, and orange-yellow zones of samples were spotted.

UV-VISIBLE SPECTROPHOTOMETRIC DETERMINATION OF LYCOPENE-

- The samples were diluted with methanol in the ratio of 1:1. Since the samples were concentrated after extracting with hexane: ethanol: acetone.
- The UV/Vis spectra were taken in the range from 350 nm – 600 nm. Initially ran a cuvette filled with methanol as the blank (baseline).
- The quartz cuvette was rinsed with the diluted sample. The samples were again diluted in 50 ml methanol and then the readings were taken.

ANALYSIS USING HIGH PERFORMANCE LIQUID (HPLC)-

- The analysis was performed by using Inertsil ODS-3V, C-18, 150 X 4.6mm internal diameter with 5 micron particle size column and PDA detector set at 472 nm, in conjunction with a mobile phase of Methanol, Tetra Hydro Furan and Water in the ratio of 66:30:4 % v/v at a flow rate of 1.5 ml/min.
- The retention time of lycopene was found to be 4.525 minute for tomato and 4.487 minutes for watermelon.
- The injection volume was 10µl
- Methanol, Water and Tetra Hydro Furan, Dimenthylformamide of HPLC grade and double distilled water were used in analysis.
- A mixture of Methanol (HPLC Grade) was prepared, Tetra Hydro Furan (HPLC Grade) and Water (HPLC Grade) in the ratio of 66:30:4 % v/v mixed and sonicated. [5]
- Lycopene was identified by comparing the retention time and the peaks with that of respective standard HPLC chromatogram. Since the detection was maximum at 473 nm and 471 nm for tomato and watermelon respectively of lycopene.

PREPARATION OF BIOPLASTIC-

- Take 1 tablespoon of cornstarch into the 200 ml conical flask.
- Add 4 tablespoons of water.
- Add 1 teaspoon of vinegar.
- Add 1 teaspoon of glycerin.
- Add 1 ml of extracted lycopene
- Mix and turn the heat on medium. Stir continuously. The mixture will turn from a liquid, white mixture to clear, gel-like consistency. Watch carefully and when it begins to boil and bubble, it is done. Turn heat off and remove the conical flask from the heat. [3]

RESULTS-

BENZENE ISOLATION METHOD -

The extracted lycopene and after recrystallization are has shown in the figure.

FIGURE 2- *Extracted lycopene from benzene method*

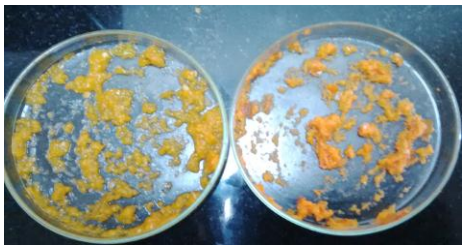

FIGURE 3- *Lycopene after recrystallization with ether*

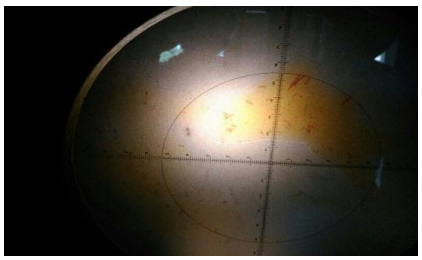

FIGURE 4- *Crystals of extracted lycopene under microscope. [4]*

The extraction of lycopene form benzene method was carried out as a pilot study. This test initially helps us to identify lycopene in the residue. Obtained crystals were then observed under the microscope.

LYCOPENE EXTRACTION WITH HEXANE/ETHANOL/ACETONE-

The samples were extracted with hexane: ethanol: acetone mixture. The procedure develops three different layers from which the top layer with hexane contains lycopene. Approximately 5 ml of sample was collected.

FIGURE 5- *Lycopene forming into top hexane layer and formation of other layer with its pulp*

UV-VISIBLE SPECTROPHOTOMETRIC DETERMINATION OF LYCOPENE-

Calculation of lycopene levels- Lycopene levels in the methanol extracts were calculated according to:

Lycopene – Tomato (mg/kg fresh wt.)

= (A459 x 537 x 8 x 0.55)/ (0.10 x 206)
(1)
= A459 x 137.4
(2)
= 0.048*114.6
= 5.50 mg/kg fresh wt

Where 537g/mole is the molecular weight of lycopene, 8 mL is the volume of mixed solvent, 0.55 is the volume ratio of the upper layer to the mixed solvents, 0.10 g is the weight of tomato added, and 206 mM-1 is the extinction coefficient for lycopene in methanol.

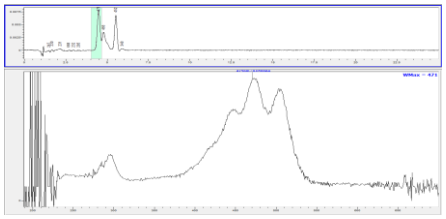

FIGURE 6- *UV-Visible spectrophotometer of tomato showing absorbance of 0.097 at 468 nm*

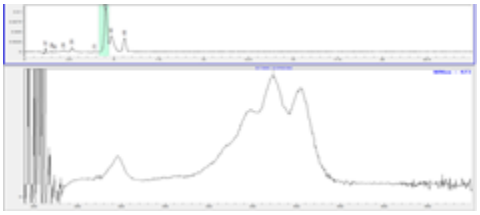

FIGURE 7- *UV-Visible spectrophotometer of watermelon showing absorbance of 0.048 at 459 nm*

Calculation of lycopene levels- Lycopene levels in the methanol extracts were calculated according to:

Lycopene – Watermelon (mg/kg fresh wt.)

= (A468 x 537 x 8 x 0.55)/ (0.10 x 206) (1)

= A468 x 137.4 (2)

= 0.097*114.6
= 11.1 mg/kg fresh wt

Where 537g/mole is the molecular weight of lycopene, 8 mL is the volume of mixed solvent, 0.55 is the volume ratio of the upper layer to the mixed solvents, 0.10 g is the weight of tomato added, and 206 mM-1 is the extinction coefficient for lycopene in methanol.

ANALYSIS USING HIGH PERFORMANCE LIQUID (HPLC)-

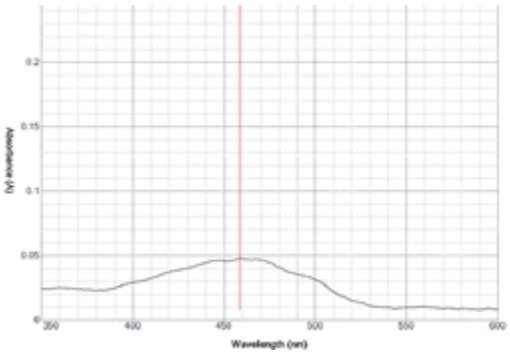

FIGURE 8- *Tomato lycopene spectra in HPLC with λMax at 473 nm*

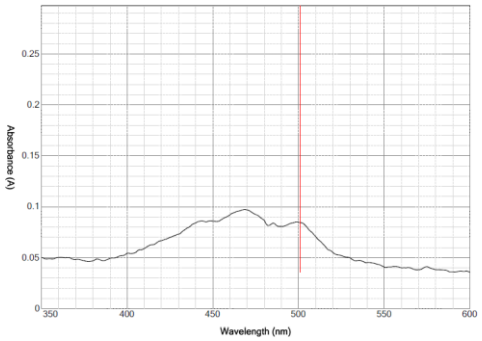

FIGURE 9- *Watermelon lycopene spectra in HPLC with λMax at 471 nm*

BIOPLASTIC-

FIGURE 10- *Lycopene coloured bioplastic*

FIGURE 11- *Centrifuge tube with lycopene layer getting coloured which was kept for 1 whole day*

CONCLUSION-

Results of the studies showed that the fruits analyzed have high concentration of lycopene. Lycopene are naturally occurring substances found in many plants. Studies have proved that carotenoids are beneficial in number of diseases and health conditions owing to their antioxidant potential and anticancer activity. In this study, the highest content of lycopene was observed in Citrullus lanatus (watermelon) and followed by Solanum lycopersicum (tomato). The results of studies can be improved if we use different solvents in extraction process. In present study two methods of extraction were followed viz benzene method and method involving use of mixture of hexane, ethanol and acetone. Since, lycopene is a natural carotenoid pigment found in most of the foods. So, it can be used as potent biocolorant for bioplastics. Use of a natural biocolorant such as lycopene in bioplastics will not have any safety concerns as compared to synthetic colours which might be toxic and difficult to degrade. The points which one needs to be careful about lycopene are its heat and light sensitivity which can lead to photodecomposition. Utmost care should be taken while handling lycopene, one of the solutions to the problem is to add BHT- acetone.Thus, lycopene is a non- toxic and safe colouring agent. Lycopene also provides various benefits and daily uptake is also recommended now-a-days so it's completely a natural colour can also be known as Lycopene- BioColour.

FUTURE STUDIES-

The study can be further improved by concentrating the extracted lycopene and making it intense red colour. Further, studies can be carried out for studying the structure of lycopene in detail.

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