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Extraction and Isolation of Lycopene Form Various Natural Sources

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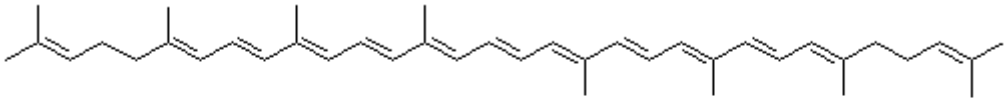
Abstract: *Lycopene is a carotenoid pigment and phytochemical found in tomatoes, water melon and other fruits mostly red coloured. The antioxidant properties of lycopene have attracted attentions due to its biological properties and are thought to be primarily responsible for its benefits in health related problems. Extraction and isolation of lycopene can be done with various methods. Several studies have been undertaken for the extraction of lycopene and for its quantification in various natural sources.*
Keywords: *Lycopene, extraction, hexane, acetone, benzene, ethanol.*

I. Introduction

Fruits and vegetables are main source of natural antioxidant components. Antioxidants give protection against harmful free radicals and reduce rate of cancer and heart disease1. The most efficient carotenoid antioxidant is lycopene. Lycopene is a natural pigment which protects the body by neutralizing the negative effects of oxidants. In the synthesis of vitamin A lycopene plays an important role as an intermediate and carotenoid like β-carotene and β cryptoxent in, influences its development. Lycopene is soluble in fat and synthesized by plants and microorganisms. Regular intake of lycopene containing food reduces the risk of body tumor especially prostate cancer; studies have shown that the antioxidants vitamin E, selenium, and lycopene all reduces risk of prostate cancer. Therefore, it would say that lycopene is very important for cancer prevention; it also reduces LDL cholesterol and cardiovascular diseases. It is a carotenoid and gives red colour to vegetables and fruits.Lycopene in processed foods is mainly in the form of the isomers. Its molecular formula is C40 H56 and 536.88 is its molecular weight. Lycopene is highly unsaturated hydrocarbon with 13 double bonds, it has been reported that 11 unsaturated bonds are conjugated. Conjugated bonds of lycopene molecule gives ability to act as an antioxidant and make it more efficient for the use of human health. Natural food sources of lycopene are tomatoes, watermelon, pink guava, pink grapes, papaya and apricots.Lycopene has been extracted from the several different fruits and berries. It was first isolated from *Tamuscommunis* by Harsten in 1873. When consumption of lycopene from different products up to 150 mg daily shows no side effects.Recent studies have shown that ingested lycopene is metabolized in the body. Several metabolites have now been identified and characterized [1].



Lycopene
Molecular Weight = 536.89
Exact Mass = 536
Molecular Formula = C40H56
Molecular Composition = C 89.49% H 10.51%

Fig. 1: Chemical Structure of Lycopene

II. Material and Methods

1) Benzene Extraction Method-

We made a paste separately of watermelon,tomato and papaya. In the laboratory weigh 100 gm. paste ofeach of the three fruits.100 gm. of sample of watermelon taken in a 250 ml beaker.Then warm the paste and add about 30 ml of warm (400C)benzene to it. Stir well and decant the benzene layer. Again add30 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 papaya and recorded the observations. Identification test of the isolated Lycopene were performed using chemical tests and by microscopic study, identification of chemical structure was done using visible spectrophotometer [1].

2) Methanol Extraction Method-

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 recrystallization 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 [2].

3) Acetone - Ethanol –Hexane Extraction Method-

Three solvents were used for comparison of extraction efficiency: (1) acetone, (2) ethanol, and (3) hexane. Tomato pastes were submitted to extraction with each solvent (1:10 v/v) by means of shaker for 30 minutes. The extraction was performed at room temperature with light protection by covering the vessels with aluminium foil. The lycopene contents were assayed for purity and concentration by UV–Vis spectroscopy at 472 nm (maximum of absorption for lycopene) [3].

4) Acetone-petroleum ether extraction method-

Sample (1.0-1.5 g powder) was extracted with 10mL acetone-petroleum ether (50% v/v). The upper lycopene-containing organic layer was removed by means of a pipette and collected in test tube. Extraction was repeated. The extracts were combined, washed with 15mL saturated aqueous sodium chloride (NaCl) and removed the aqueous wash with a micropipette. The extract was washed with 10mL of 10% aqueous potassium carbonate (K2CO3) and removed the aqueous wash. The lycopene-containing organic layer was dried with a drying agent (calcium chloride). The excess solvent was allowed to evaporate at room temperature for a few minutes in the dark. The tubes containing lycopene extracts were covered with aluminium foil and stored in freezer until further analysis [4].

5) Hexane extraction method-

Sample (0.3 to 0.6 g powder) was weighed in a beaker, 5 mL BHT-acetone solution (0.05%, w/v), 5 mL ethanol and 10 mL hexane was added. The beaker was placed in a bowl of ice on a magnetic stirring plate, stirred for 15 min and added 3 mL distilled water. It was shaken for 5 min on ice and incubated at room temperature for 5 min to allow the separation of both phases. The upper layer containing lycopene was isolated by means of a pipette and collected in a test tube. The tubes containing lycopene extracts were covered with aluminium foil and stored in the freezer until further analysis [4].

III. Discussion

The extraction and isolation of lycopene has been carried out using different methods and the yield of lycopene showed to be high in natural sources of lycopene.

The benzene test initially helps us to identify lycopene in the residue. A simple liquid-liquid extraction method was employed to extract lycopene in minimum organic solvent. Crystals were purified by recrystallization from ether. Obtained crystals were then observed under projection microscope [1]

The extracted lycopene in acetone provided three characteristic peaks at 445, 471 and 500 nm. The use of hexane showed similar characteristic peaks at 445, 471 and 502 nm.It could be stated that acetone with lower toxicity (TLV-TWA 500 ppm) was successfully used as extracting solvent of lycopene in tomato paste [2]

Acetone-petroleum ether extraction resulted in higher crude lycopene yield than hexane extraction. During purification by column chromatography, lycopene had more affinity with alumina due to its high degree of unsaturation and was eluted after yellow-orange carotene pigments. A typical carotenoid such as lycopene displays maximum absorbance at 476nm. Spectrophotometry results revealed that lycopene showed maximum absorbance at 476nm, followed closely by absorbance at 503nm. The purified lycopene content was found to be the maximum in cherry tomato (88.87 mg/kg) as compared to Pakistani tomato (55.84 mg/kg) and watermelon (74.53 mg/kg). Lycopene content in tomato ranges from 55 to 181 mg/kg [41], 4.31 to 5.97 mg/100 g fw [4]

IV. Conclusion

Results of the studies showed that the fruits analyzed having high concentration of Lycopene [1].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 [2]. Hence, lycopene can be extracted using different extraction and isolation methods.

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