Quantitative Analysis of Lycopene Extract using Pretreated Tomato Samples

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Abstract- *Tomatoes are used extensively in the kitchens worldwide. These are basically used to enhance the flavor and also used as the coloring agent. Tomatoes are rich source of carotenoids, flavonoids and various other antioxidants. The red color imparted by tomatoes is mainly because of a pigment named lycopene. It is believed that lycopene has a role in managing numerous bodily functions like cell proliferation, cell to cell communication etc. Recent studies showed that lycopene can be used as the preventive measure of prostate cancer and also holds therapeutic importance to control various cancers in early stages. This research has been focused to extract lycopene from pretreated tomatoes in different conditions. Spray drier was used to prepare tomato powder which is further used as one of the samples to extract lycopene. Results were obtained from mass spectrometry to deduceconditions best suitable for pretreatment to extract maximum lycopene from tomato samples.*

Keywords- *Lycopene, Drying, Spray Drying, Tomato Paste, Concentration*

I. INTRODUCTION

The ultimate role played in our healthy and nutritious diet by the fruits and vegetables is well accepted. But in recent times, numerous countries have started an initiative to promote these natural products among the consumers.[1] Fruits and vegetables satisfy our body's much needed minerals, vitamins and fibers. These natural products also play a very imperative role in health by preventing heart diseases, cancer [2, 3]and diabetes etc.

Tomato, being such a popular, versatile, easy to grow plant also contains a great taste and high nutritive value.[1] The nutritive value of a tomato can be generally summed up as 18 kcal energy, 1 g protein,0.2 g fat, 3g carbohydrate, 10mg Ca, 0.4mg Vitamin A, and 25mg VitaminC per 100g serving.

Tomatoes as well as tomato products contain ample amount of health-related food components, as they are a rich source of ascorbic acid, Vitamin E, carotenoids, flavonoids, potassium and folate. Carotenoids, ascorbic acid and phenolic components are the main antioxidants.

By processing tomato and changing it into dehydrated products like tomato powder can actually be taken as one of the alternatives of tackling the major issues like rapid ripening, spoilage and senescence in ambient conditions.

Lycopene being a carotenoid pigment is red in color. This phytochemical is generally found in tomatoes and other red fruits and vegetables, the examples being watermelons, red carrots, gac andpapayas. Lycopene is a carotene chemically, despite this fact it has got no Vitamin A activity. Foods that don't appear red may also possess lycopene, like parsley or brown beans. It is one of the most familiar carotenoids in human diet.

Defining structurally, lycopene is a tetrapene and accumulated from eight isoprene units. It is knows as a carotene because it is a member of the family of carotenoids and is composed entirely ofCarbon and Hydrogen. In the natural, all-trans form, lycopene is straight and long, constrained by its eleven conjugated double bonds.[4] Each and everyextension in this conjugated system lowers the energy that is required byelectrons to makethe transition from higher energy states, and thus allows the molecule to absorb visible light having longer wavelengths. Lycopene appears red as it absorbs all but the visible light's longest wavelength.

Though lycopene is not an essential nutrient for regular functioning in humans, but we usually get it from the dishes cooked with tomatoes. After it gets absorbed from the intestine, lycopene gets transported in the blood through different lipoproteins and gets accumulated in the skin, blood, adipose tissue, liver and adrenal glands and even other tissues as well. Lycopene possess an antioxidant activity.[5][6] It leads to quenching of oxygen and scavenging the free radicals. It has few effects on Cross-talk between cells and has the ability to suppress cell proliferation.[3] The overall antioxidant property of tomato can be further increased via thermal treatments and it coincides with the positive effect that increased temperature would have on the lycopene extractability. In comparison, lycopene in tomato is relatively more resistant to thermal degradation than other antioxidants.

Elementary research has revealed that people consuming tomatoes may carry a lower cancer risk, because lycopene affects few prostate cancer mechanisms. [5, 7]Following are the major three directions where the roles of lycopene in humans are studied: the effects that lycopene has on tumor proliferation, epidemiological study and biochemical/immunological mechanisms of lycopene. Chemo-preventive treatment of cancer can also be strengthened with the help of lycopene. Uncontrollable cell growth inhibition by lycopene has been shown in many experiments. Leukemia is a cancer type, usually quite hard to treat and here lycopene can be used as a boon to treat leukemia in early stages. This project mainly emphasized on the following aims: 1. Production of tomato powder, 2. Extraction of lycopene from pretreated tomato samples and the tomato powder prepared, 3. Preparing standard plot for lycopene from the pure lycopene sample purchased from Sigma Aldrich, 4. Estimation of lycopene content in pretreated samples and the tomato powder by plotting the OD values (502 nm) in the standard graph.

II. MATERIALS AND METHODS

Reagents used during the course of experiment:
Acetone, Methanol, Distilled Water, Gelatin, Malt Extract and Chloroform.

Fresh tomatoes were bought from the local vegetable market of Indian Institute of Technology (BHU), Varanasi.

A. Preparation of tomato powder

Tomato powder was prepared by the technique of spray drying. Tomato pulp weighing 100grams ofwas taken in a beaker and homogenized using the homogenizer. The homogenized sample was then spray dried using a spray drying system. [4]Samples of tomato pulp with a 12% constant total solids concentration were used. Spray drying is the method used for the removal of moisture from slurry by breaking the particles of tomato pulp into small droplets in the presence of hot air to obtain a solid, dry powder. In the spray-drying process, the liquid feed (tomato pulp) is pumped into the drying chamber through an atomizing system. Inside the drying chamber, a stream of heated gas traps the droplets and carries them from the drying chamber to the product recovery system (Fig.1). Evaporation takes place in a few seconds as the relatively cool droplets come in contact with hot gas. Tomatoes have very low solid content, less than 12%. Spray drying must be done by evaporating the pulped tomato to produce a powder containing minimum 30% solids. Tomato pulp is a typical example of a product that is very difficult to spray dried due to the low glass transition temperature of the low molecular weight sugars present. To overcome this problem a constant 12% solid is maintained in the tomato pulp by adding 6g malt dextrin and 6g gelatin to 100ml of the tomato paste formed by the homogenization of tomato in a homogenizer.[8] The physical form of tomato, as a powder, provides a stable,natural, easily dosable ingredient which may be used to impart color and taste for food products.

The atomizer pressure, the feed rate and the inlet temperature were kept at 3 atm, 2.5%(manual), and 240°C. The outlet temperature was observed at regular intervals.

Fig 1Tomato paste spray dried in Spray drier instrument.

The tomato powder produced was transferred to an air tight container in order to prevent moisture contact (Fig.2). The obtained powder is used for the lycopene content analysis and is preserved in freezer to maintain dryness of the powder.

Fig 2Tomato samples in air tight containers to prevent moisture contact.

B. Extraction of Lycopene

Three samples differently pretreated were prepared for the extraction of lycopene. The first sample contained the fresh tomato pulp used for the lycopene extraction. For the preparation of the second sample equal amount of tomato pulp was dried in hot air oven for 1 hour and then used for the lycopene extraction. Tomato powder prepared by spray drying was used as the third sample for lycopene extraction.**Solvent extraction from filtered media** was the method used to extract lycopene from variety of samples. 10 ml tomato pulp was added to 250ml of a solution of 30% potassium hydroxide in methyl alcohol.The mixture was shaken at intervals until well mixed and placed at 5°C overnight.A portion of the saponified paste was mixed with distilled water.A sufficient filter aid was added to disperse the paste conveniently spread on a thinly filter aid percolated filter paper in a large suction funnel.Cake was washed with distilled water until it is approximately free of alkali.The lycopene was extracted from the cake on the filter held tightly at maximum suction, by acetone charges of 50 to 75ml(Fig.3).

Fig 3Positive pressure filtration unit used for the extraction of lycopene from the cake with acetone charges of 50 to 75 ml.

To counteract the effect of decreasing temperature resulting from rapid evaporation of acetone under reduced pressure, the acetone was heated in a water bath before extraction. The process of washing and extraction was completed in approximately in 25 to 30 min of time.

C. Standardization of lycopene

Standardization of lycopene was done by taking 0.7 mg standard lycopene crystals from the stock of pure lycopene purchased from Sigma Aldrich and then dissolving it in 1ml chloroform. The obtained solution is serially diluted to obtain descending concentrations and the final volume was made up to 3 ml using chloroform. The absorbance (path length=1 cm) was taken at 502 nm using chloroform as a blank.

Finally the absorbance values are plotted on a graph with variable concentration on x-axis and absorbance at 502 nm on Y-axis. [9]The regression coefficient value and the equation for the graph were also derived so as to ease the calculations of concentration of lycopene in tomato samples.

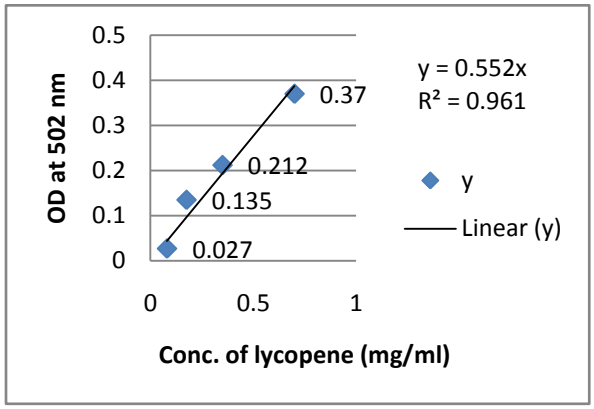

Fig.3. ***Fig 4***Standard graph of lycopene with varying concentration values.

III. RESULTS

To calculate the concentration of lycopene in differently pretreated samples the absorbance was taken at 502nm for all the samples keeping the chloroform as the blank. It was found that the fresh pulp had the absorbance of **0.095**at 502nm. The absorbance at 502 nm for the dried tomato sample was found out to be **0.429**.Finally,the absorbance for the tomato powder produced by spray drying was found out to be **0.186**. After plotting the obtained OD values in the standard plot contrived for pure lycopene from stock sample the concentration of lycopene in the fresh pulp sample was found out to be **0.17mg/ml**. The concentration of lycopene in dried sample was found out to be **0.77mg/ml** by substituting the OD value in the equation obtained from the graph. The amount of lycopene calculated in tomato powder prepared was found out to be **0.34 mg/m**l.

IV. CONCLUSION

Hence from the experimental data we can conclude that the concentrations in the three cases aren't outliers rather comparable. It was observed that the content of lycopene was highest in case of the dried sample i.e. 0.77mg/ml, whereas least lycopene content was found in the fresh tomato pulp sample i.e. 0.17mg/ml. Intermediary lycopene concentration

was observed in case of tomato powder i.e. 0.34 mg/ml.

From the results we can also deduce that the lycopene degradation is resistant to high temperature. The concentration of the lycopene increases with the increase in temperature unlike other carotenoids that are not stable at high temperature.

It can also be seen that the lycopene concentration in the sample in inversely proportional to its moisture content i.e. as the water is removed from the sample the lycopene concentration increases gradually.

V. FUTURE ASPECTS

This research can be used as the basis for extracting lycopene from various tomato sources. As it can be seen from the results and conclusions that different pretreatment conditions lead to change in amount of lycopene content in various samples. As it is known that lycopene has got therapeutic importance hence, the production of lycopene can further be optimized and can be used as a drug for prevention and a remedy to many diseases. The extraction of lycopene from tomato powder can be enhanced by drying the tomato samples first and then converting it to the powder form. It will be really a great achievement if in future we manage to get maximum lycopene from the tomato powder, as powder has greater shelf life, easy storage and availability with further ease in handling for future investigations. The research on therapeutic advantages of lycopene extracted from natural sources can be taken to commercial level if we succeed in enhancement of lycopene content with the help gene engineering and other genetic approaches.

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