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RESEARCH ARTICLE

Supercritical Extraction Of Lycopene From Tomato By Using CO_2 As Solvent: A Potent Antioxidant

Bhoomika Domadia[1*], Dr. Naresh Vaghela[2], Dr. Niral J Modi[3], Dr. Shashi Vivek Ranga[4], Ashish Modi[5], Darshana Dhimmar[6]

[1,*]*Department of Chemical Engineering, Government Engineering College , Valsad, Gujarat Technological University, Gujarat, India Email: bhoomika.domadia@gmail.com*
[2,] *Department of Chemical Engineering, Government Engineering College , Valsad, Gujarat Technological University, Gujarat, India Email: nareshkumar.vaghela@gmail.com*
[3,] *General Department, Government Engineering College , Gandhinagar, Gujarat Technological University, Gujarat, India Email: niralmodi45@gmail.com*
[4,] *Department of Chemical Engineering, Government Engineering College , Valsad, Gujarat Technological University, Gujarat, India Email: shashiranga@gmail.com*
[5,] *Department of Chemical Engineering, Government Engineering College , Valsad, Gujarat Technological University, Gujarat, India Email: admgecv@gmail.com*
[6,] *Department of Chemical Engineering, Government Engineering College , Valsad, Gujarat Technological University, Gujarat, India Email: dhimmardarshana31@gmail.com*

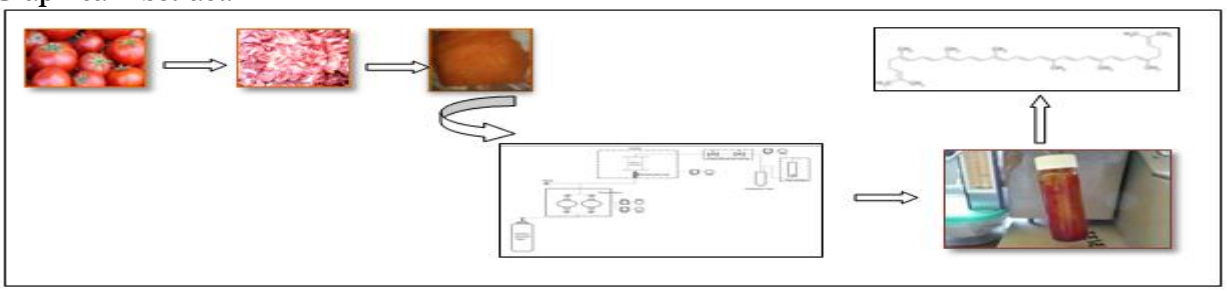
***Corresponding Author:** Bhoomika Domadia*
**Department of Chemical Engineering, Government Engineering College , Valsad, Gujarat Technological University, Gujarat, India Email: bhoomika.domadia@gmail.com*

Abstract

Extraction of Lycopene has received a lot of attention due to reports demonstrating Lycopene's potential to decrease risk of cardiovascular diseases and chronic diseases such as prostate, lung and stomach cancer. Supercritical extraction is an environmentally friendly process to separate the carotenoid by using CO_2 as solvent. This study was investigated for the influence of supercritical extraction parameter such as temperature, pressure, time, and CO_2 flow rate on the extraction yield of lycopene and to find out optimum pressure employed for the isolation of lycopene. The extractions were conducted at pressure ranging from 200 to 550 bar (3000-8000 psi), temperature 60^{0}C and flow rate of SC-CO_2 20 ml/min. During study, feasibility to extract lycopene from tomatoes skin has been developed. The samples of SFT-100 instrument at various pressure has been collected and analyzed. It was able to get yield of lycopene 91.4(mg/100gm) by spectrophotometry analysis from the obtained extract at 400 bar (6000 psi) pressure and 60^{0}C temperature.

Keywords; Lycopene, Antioxidant Properties, Tomatoes,Supercritical fluid Extraction, SC-CO_2, Optimum Parameters for extraction, Spectrophotometry Analysis, Product yield

Graphical Abstract:



Introduction:

Lycopene, a prominent member of the carotenoid family, is a vital pigment responsible for the characteristic red hue observed in various vegetables and fruits including tomatoes, watermelon, apricots, papaya etc. Lycopene is known for its robust antioxidant properties and potential health benefits[1-3]. A wide range of substances have been identified as antioxidants exerting their oxidation inhibitory effects by different mechanisms. However, the list of lipophilic natural oxidants is limited. Lycopene is an appealing natural compound for lipid scientists As a potent singlet oxygen quencher, it can effectively inhibit lipid oxidation at the initiation stage[3]. Structurally, lycopene is a symmetrical tetraterpene with an extensive system of conjugated double bonds. It has eight isoprene units, with 11 conjugated and 2 non-conjugated double bonds between carbon atoms. The primary dietary source of lycopene are tomatoes and tomato-based products The widespread availability of tomatoes and the growing interest in sustainable and eco-friendly extraction methods further contribute to the appeal of lycopene as a versatile and valuable natural compound for a wide range of applications in the lipid industry and beyond. Extensive research has established a direct correlation between lycopene content in tomatoes and their antioxidant efficacy. Lycopene's exceptional ability to quench singlet oxygen is attributed to its high physical quenching rate, which is much higher than the other lipophilic antioxidants such as β-carotene and α-tocopherol. This efficacy is partly due to the structural transformation which involves the opening of the β-ionone ring into an open chain form. With a concentration of approximately 0.7 mM in human plasma, lycopene demonstrates remarkable potency in quenching O_2.[3]

Beyond its antioxidant prowess, lycopene has been extensively studied for its potential medicinal benefits[4-5]. There are a number of research studies which supports its diverse therapeutic benefits such as in chronic diseases like cardiovascular diseases[6], hepatitis[7] and its antioxidative and antiinflammatory[8] anti viral[9], anti cancer[10], antidiabetic[11], neuroprotective[12], and bone protective[13] properties. These diverse therapeutic attributes underscore lycopene's potential as a nutraceutical agent offering protection against a spectrum of diseases affecting vital organ systems. Lycopene is a deep red solid with a molecular formula of $C_{40}H_{56}$, a molar mass of 536.87 g/mol, density of 0.889 g/ml, and a melting point of 173 °C. Its insolubility in water reflects its inherently lipophilic nature.

Supercritical Fluid Extraction and Extracting Solvent:

The extraction of Lycopene by conventional extraction methods often rely on the use of organic solvents, such as ethanol, ethyl acetate, and hexane[14-15]. However, the use of these solvents can raise environmental and health concerns due to their toxicity and potential residual presence in the final product. In response to these concerns, supercritical fluid extraction (SFE) has emerged as a promising alternative technique. SFE utilizes the unique properties of supercritical fluids, which exhibit enhanced transport characteristics compared to liquids, allowing for faster extraction rates and improved solubility of target compounds. One of the main advantages of SFE is the ability to modulate the solvent strength by adjusting the pressure and/or temperature of the supercritical fluid[16], which directly affects the solubility of the compounds of interest. Additionally solvents used in SFE are generally recognized as safe (Green solvents) , have higher extraction efficiency, reduced extraction times, and the possibility of direct coupling with analytical techniques, such as gas chromatography or supercritical fluid chromatography (SFC).

Among the various supercritical fluids, carbon dioxide (CO_2) is the most commonly used solvent for the extraction of Lycopene from tomato etc. for several reasons[16-18]. CO_2 is an environmentally friendly green solvent. The use of carbon dioxide (CO_2) as a solvent in supercritical extraction offers several advantages, such as its non-toxicity, non-flammability, and low environmental impact. It has low critical temperature (31.2°C) which is crucial for the preservation of thermally sensitive bioactive compounds. Further the quality of extract can be preserved since CO_2 protect it from air contact.

This study aims to optimize the supercritical fluid extraction of lycopene using CO_2. The optimization of the extraction parameters, such as pressure, temperature, and flow rate, can lead to improved yields and reduced extraction times,

Experimental Section:

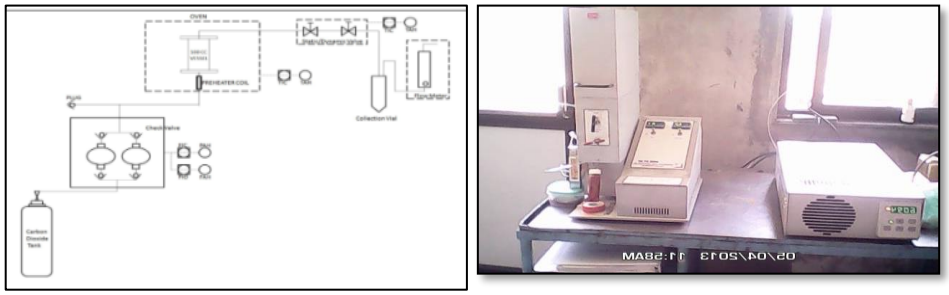
Materials and methods :

In this study, fresh ripe tomatoes were selected and their skin and pulp were separated. The tomatoes used were of a dark reddish color, which is known to contain higher levels of lycopene. Tomato skin powder was obtained as shown below and used as an extractant material.

Fresh Tomatoes Tomatoes Skin(Dry) Skin Powder

The Skins were dried in a controlled environment, avoiding direct sunlight, to prevent degradation of the lycopene. The Drying process took approximately 5-6 days, during which time the skins were dried in the shade or at room temperature. Once the skins were completely dry, they were ground into a fine powder using a suitable grinding method.

Supercritical CO_2 Extraction Apparatus:



In this study, for extracting lycopene from tomato skins, we have used the SFT-100 system. The Supercritical Carbon Dioxide (SC-CO_2) Extraction System (SFT-100) is a state-of-the-art technique, designed to extract bioactive compounds from various plants. The SFT-100 system consists of several key components, including a high-pressure pump for CO_2, a back pressure regulator, a wet gas meter a chiller, a heating chamber and a 10 mL vessel, The system was designed to operate at different pressures. The inlet flow rate of CO_2 was set at 20 ml/min. The temperature was maintained at 60°C to optimize the extraction process. The tomato skin powder as obtained in the previous step, was taken in the 10 mL vessel, and liquid CO_2 was fed into the system from a CO_2 cylinder. The CO_2 was compressed, controlled and cooled using the high-pressure pump and chiller, respectively, to maintain its liquid state. The CO_2 was then heated in the heating chamber to transform it into a supercritical state, which enhances its solubility and extraction capacity. To optimize the extraction process, we varied the pressure conditions and observed the effects on the extracted yield and purity of lycopene. We also investigated the effect of using fresh tomato skin powder versus used powder on the extraction efficiency. The extracted lycopene was collected in sample collection vials, which were wrapped in aluminum foil and transported to the laboratory for analysis.

Spectrophotometry Analysis

0.1 g of tomato powder as obtained in previous step was taken and homogenized in a mixture of 8 ml of 2:1:1, v/v/v mixture of hexane:ethanol:acetone . The homogenate was centrifuged for 10 minutes at a speed of 4000 rpm, to separate the solvent layers. The absorbance of the upper hexane layer was measured at 503 nm against a hexane blank using a double beam UV-VIS spectrophotometer (Elico -Sl 159). A standard solution of lycopene was prepared in hexane at a concentration of 0.04 mg/mL and stored protected from light at 4°C. All the solvents and reagents used were of AR grade. Working standards of appropriate concentrations were prepared by serial dilution with hexane. The absorbance of standard solutions was measured and a calibration curve was constructed by plotting absorbance against concentration. Lycopene content in the tomato samples was calculated by Beer-Lambert law:

$C = (A503 \times MW \times V) / (\varepsilon \times l \times w)$

Where, C = lycopene concentration in mg/kg,

A503 = absorbance at 503 nm,

MW = molecular weight of lycopene (537 g/mol)

V = final volume of hexane extract (8 mL),

ε = extinction coefficient of lycopene in hexane (172 $mM^{-1}cm^{-1}$),
l = cuvette's path length (1 cm), and
w = weight of sample extract (0.1 g).
Lycopene (mg/kg) = (A503 x 537 x 8 x 0.55)/(0.10 x 172)
= A503 x 137.4
[Here 0.55 is the volume ratio of the upper layer to the mixed solvents]

Result and Discussion:
Overall experiments were performed at different pressure range and amount of product extracted was noted after completing the reaction hours. The time duration of one experiment run was 8 hour. The red colour of the skin powder was changed after extraction. The difference in colour of material is as follows:

Skin powder Before Extraction Obtained Product Skin powder After Extraction

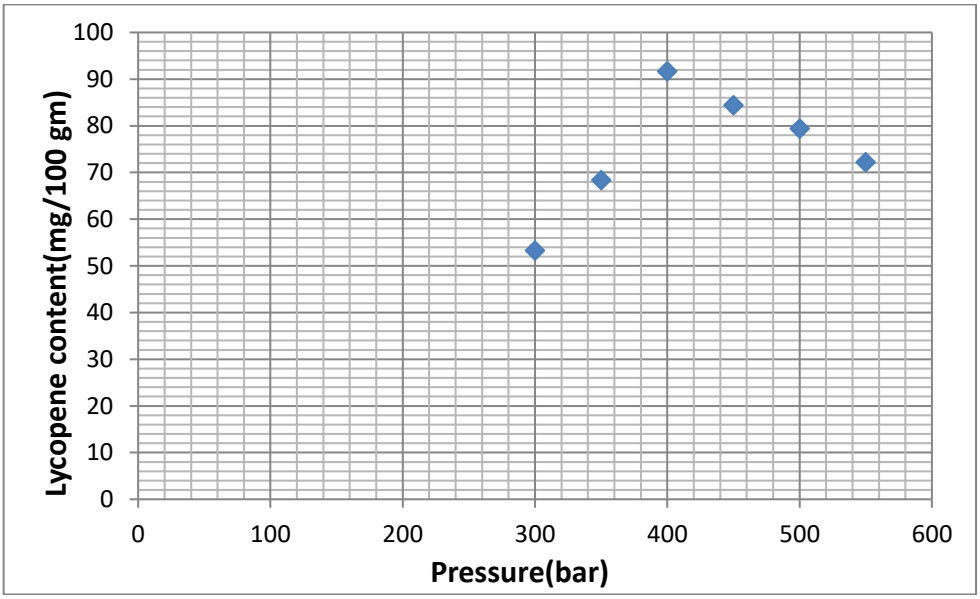
Initially extraction was performed at 200 bar but at this condition the fluid did not extract lycopene except in trace amounts. In order to obtained an extract richer in lycopene, the extraction must be carried out at high pressure. So, Experiments were performed at different pressure more than initial condition. This was done on dried ripe tomato skins powder. Table 1 shows the lycopene content obtained in supercritical CO_2 from ripe tomato skin by varying pressure & keeping temperature 60^0C constant.

Table 1: Lycopene content at different pressure with 20 ml/min flow rate of SC-CO_2 and temperature 60^0C

Sr.no.	Pressure		Lycopene content (mg/100 gm)
	bar	Psi	
1.	200	3000	-
2.	300	4500	53.3
3.	350	5200	68.4
4.	400	6000	91.7
5.	450	6600	84.4
6.	500	7300	79.4
7.	550	8000	72.2

Analysis of table 1 inferred that concentration of lycopene increases with increase the pressure upto 400 bar and decreases with increase pressure condition upto 550 bar. Its solubilization could be more difficult at low temperature and pressure. The reason for this, at low pressure there may possibilities exist a competition between carotenoids and lipids for supercritical solvent and the extraction of lycopene may occurred only after major part of lipids as triglycerides which have higher solubility in CO_2 were extracted. But lycopene concentration decreases with respect to 400 bar though increase in pressure. The reason for that there may be extract other carotenoid with lycopene, present in tomato. The structure of β-carotene and lycopene pigments is very similar, they may present a different solubility in the SC-CO_2.There are different concentration of the two pigments in various parts of vegetable tissues. I tried to optimize the method to obtain a product that should be richer in lycopene and free from impurities. Other reason for this it may be due to solvent properties of CO_2. At very high pressure, it may be not completely penetrate the matrix. At,400 bar (6000 psi) pressure and 60^0C

temperature, the solubility of lycopene may be higher in SC-CO_2 than other condition. It was able to get lycopene content 91.7(mg/100gm) at this pressure. It is not possible to get 100% yield cause of degradation of the product. The graph 1 is plotted by using data obtained by spectrophotometric analysis which is concentration of Lycopene content in mg/100gm of product vs. Pressure in bar.



Graph 1: Lycopene content of the extraction conducted at different pressure and at 60^{0}C.

The concentration of Lycopene in tomatoes is quite variable and depends on both type of tomatoes and maturation temperature. In tomatoes, there are also present other carotenoids, lipids, ascorbic acid and phenols etc.

Conclusion:

SFT-100 was tested for lycopene extraction from tomato skin powder. For the purpose of this study seven values of pressure were selected in the range of 200-550 bar at (3000-8000 psi). Concentration of lycopene vary with pressure varies. This study indicated that,400 bar pressure and 60^{0}C temperature is the optimum pressure-temperature condition to get maximum lycopene content 91.7(mg of extract/100gm of material) from tomato skin. Based on experimental studies, it can be concluded that tomato skin powder can be used at smaller scales for effective lycopene extraction from herbs.

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