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Optimizing Analytical Methods to Determine Lycopene Levels in Syrian Tomatoes from Various Regions

Afraa Samir Alnokkari[1]

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ABSTRACT

Background: The particular attention in recent years on high nutritional quality food is increasing because of its health benefits. Tomato, which a common fruit in different regions of Syria, is one of these foods. It contains plentiful antioxidant compounds, including lycopene. Lycopene plays a very significant role in human health and nutrition; it is considered a strong antioxidant due to its ability to trap singlet oxygen and remove the peroxyl radical. This study aims to quantify of lycopene content of fresh tomatoes in Syria and determine the best analytical method (solvent for separation by HPLC) for separation by HPLC.

Methods: Tomatoes from different Syrian regions were quantitatively analyzed for the detection of lycopene, using reversed-phase high performance liquid chromatography through isocratic elution and UV detection. Many mobile phases were under experiments in order to maximum achievement the lycopene separation.

Result: An Analytical method for determination of lycopene in Syrian tomato samples was optimized and the proper solvent for separation and determination of lycopene in Syrian tomato samples was (MeOH/propanol/THF) (15:65:20). The lycopene content in Syrian tomatoes ranged from 55.94 to 103.70 μg/g, this disparity was attributed to many genetic and environmental factors.

This study suggests that Syrian tomato is rich source of lycopene and a major provenance of this powerful antioxidant and can be considered as a possible alternative to antioxidative supplements.

Key words: HPLC, Lycopene, Separation, Solvents, Syrian tomatoes.

INTRODUCTION

Tomato (*Lycopersicon esculentum*) is an abundant constituent in the Mediterranean diet, it is high in antioxidants such as carotenoids. Among the carotenoids, lycopene is a non-provitamin A carotenoid, it has antioxidant properties and it is responsible for the red-colored pigment in both fruits and vegetables. Generally, tomato contains high concentrations of lycopene (Górecka *et al*., 2020). Lycopene concentration increases with the maturity of the tomato, leading to the development of red color (Yoo *et al*., 2017), processing of tomatoes contributes to increasing the lycopene concentration, where the best abundant source of lycopene is processed tomato products such as tinned tomatoes and tomato paste (Beltrán Sanahuja *et al*., 2019). Many epidemiological studies have showed important role of carotenoids in lowering risks for the development of chronic diseases such as cancer and heart disease (Nupur *et al*., Moran *et al*., 2019; Ezzat *et al*., 2020; Sorriento *et al*., 2018). There is particular attention in recent years about lycopene because of the latest studies which confirmed that it is a highly efficient antioxidant and has a high free-radical scavenging ability (Dewantara *et al*., 2022). The 11 conjugated double bonds in lycopene are responsible for the red color and antioxidant activity of it (Sacco *et al*., 2019) and that leads to make it more efficient antioxidant than β-carotene, α-carotene and α-tocopherol (Al-Yafeai *et al*., 2018). Lycopene has many health benefits (Li *et al*., 2021), it serves as a powerful antioxidant, neutralizes free radicals especially those derived from oxygen, present under the lipid membrane and skin cover. Lycopene is transported around the body greatly bound to the low density lipoprotein (LDL) in the plasma and that leads to inhibition its bad effect (Ezzat *et al*., 2020), lycopene can diminish oxidative stress which may help in endothelial function and vascular health (Arnanda and Nurwarda 2019), where lycopene concentrations in serum may serve in the early stage of atherosclerosis. Since the human body is not able to manufacture lycopene, this constitute must be gotten externally in a diet. In addition to its antioxidant properties, lycopene has been shown to stimulate cell to cell communication and modulate hormonal, immune systems which may contribute remarkably in human health (Amer *et al*., 2020). Lycopene may also induce the modulation of cell growth, inflammatory processes, immune function and others. Many studies have shown that lycopene is more efficacious than b-carotene in scavenging singlet oxygen and peroxyl radicals and protecting against oxidation of lipid and protein as the result. Depended on these benefits of

[1]Faculty of Pharmacy, Arab International University, Ghabaghib, Daraa, Syria.

Corresponding Author: Afraa Samir Alnokkari, Faculty of Pharmacy, Arab International University, Ghabaghib, Daraa, Syria. Email: afraa.alnokkari@gmail.com

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lycopene there should be intake of 5 to 10 mg lycopene per day (Park *et al*., 2020). Because of the anti-oxidant efficacy of lycopene, sundry experiments were achieved to isolate and extract lycopene from different sources but tomato is the best one to yield high amount of lycopene (Park *et al*., 2020). Several analytical methods were used to determine lycopene in food and biological samples, such as UV-Visible spectrophotometry, reverse phase liquid chromatography (RP-LC), High performance liquid chromatography (HPLC) and High performance thin layer chromatography (HPTLC) (Pinela *et al*., 2019; Ropelewska *et al*., 2022). This research aimed to optimize an analytical method for determination of lycopene in tomatoes from different regions of Syria. High Performance liquid chromatography was used to determine lycopene in studied samples, many solvents were experienced to choose the suitable mobile phase for good separation. Lycopene concentrations varied between studied samples and they ranged from 55.94 to 103.70 µg/g.

MATERIALS AND METHODS

The experiment was conducted from October 2021 to April 2022 in the research laboratories at Arab International University. Tomatoes samples were collected from different places in Syria in the same period. Standards of lycopene from tomato (purity 99%) was obtained from Sigma -Aldrich. Ethanol (EtOH), Aceton (ACE), propanol (PrOH), methanol (MeOH), tetrahydrofuran (THF), acetonitrile (ACN), triethylamine (TEA) and hexane (Hex) were purchased from Panreac.

Sample preparation

The fresh tomato was washed with tap water, followed by washing with distilled water (DW), the whole fruit was used and crushed well then 1 g of the resulting mixture was mixed for 15 min with 20 mL of extraction buffer (hexane/acetone/ ethanol 2:1:1) on a magnetic stirring plate, The obtained mixture was filtered and transferred into a separatory funnel. 50 mL of saturated NaCl solution was added and mixed well for 1 min. Once the phases were well-separated, the aqueous phase was thrown away and the hexane phase was recovered and filtered over anhydrous $NaSO_4$ (5 g) which was washed twice with 2.5 mL of extraction buffer. The filtrate was further dried in rotary evaporator (~34°C) and the residue was redissolved in THF with 0.05% TEA to appropriate concentrations before injection.

HPLC/UV analysis of the lycopene

Lycopene was analysed by separation and quantification using reversed-phase high performance liquid chromatography HPLC (KNAUER) with a C18 column (5 ìm, 4.6 × 250 mm), equipped with Smart Line UV Detector 2500, where the detection was carried out at 472 nm through isocratic elution. Separation of lycopene was experienced using various compositions of solvents as mobile phase (MeOH /propanol/THF) (15:65:20), (MeOH /propanol/THF) (20:70:10), (MeOH /ACN/THF) (15:55:30), (MeOH /ACN/ THF) (5:65:30), (MeOH /ACN) (25:75), 0.05% TEA was added to the all-previous mobile phases. The flow rate was 1 mL/min, column temperature was 35°C and the injection volume 20 µL.

Preparation of the standard solutions

1 mg of lycopene standard was transferred to a 25-mL volumetric flask with THF containing 0.05% TEA and before use, the concentration of the lycopene was spectro photometrically determined after dilution in hexane up to solutions giving an absorbance value of below 0.7 units. The concentration of the stock lycopene solution was calculated using the $A^{1\%}$ of 3,450 in hexane at 472 nm (Craft 2001). Furthermore, stock lycopene was diluted with methanol to prepare calibration curves. Lycopene was detected by comparing the retention time with the reference standard and the concentration was measured by external standard calibration based on peak area.

Statistical analysis

All measurements were repeated three times, the values for each sample are represented as the mean ± (SD of the repeated determination). It was noticed from the previous table that there was a significant difference for the Lycopene concentration under the influence of region change, at the level of significance 0.05. Where the highest values appeared in Homs tomato samples, while the values in Tartus tomato samples decreased significantly from the rest of the regions.

To ensure this, the correlation coefficient was calculated between the lycopene concentration and the region, the correlation coefficient was significant as shown Table 1.

RESULTS AND DISCUSSION

lycopene may exist in various geometrical isomers, but in raw foods the all-E-isomer is the most dominate with lower amounts of other Z isomers (Honda *et al*., 2019).

The extraction of lycopene was done using (hexane/ acetone/ ethanol 2:1:1), where the solubility of lycopene in this extraction solvent was optimal and the proposed percentage of each solvent led to efficient extraction. This mixture was used in many researches and achieved efficient extraction of lycopene from tomato samples (Adejo *et al*., 2015). Several analytical methods have been carried out to achieve effective and accurate methodology using HPLC for lycopene analysis in tomato (Amjad *et al*., 2020). For

Table 1: The statistical study of lycopene in syrian tomato samples.

Samples	Lycopene content µg/g
Daraa	91.93b±3.03
Tartus	59.01c±2.39
As suwayda	91.92b±3.17
Homs	99.23a±3.12
P 0.05	0.008***
LSD 0.05	3.945
Spearman correlation	0.68**
CV%	3.44

the selection of stationary phase several columns such as C-8, C-18, C-30 was exercised in previous publications (Dzakovich 2019), among which non-polar C-18 analytical chromatographic column was chosen in this research as the stationary phase for the separation and determination of the non-polar hydrophobic lycopene molecule in tomato samples. This separation was done through isocratic HPLC method which was suitable and easy procedure. The C30 columns are used for the separation of the carotenoids in food samples. However, the acyclic carotenoid lycopene is more highly retained on the C30 column compared to other bicyclic carotenoids such as α and β-carotene (Dzakovich *et al*., 2019). Many compositions of solvents were experienced in order to choose the suitable polarity, the use of binary mixtures of methanol and acetonitrile did not achieve good separation as shown in Fig (1) where a marked overlapping of lycopene peak with another peak when using (MeOH /ACN) (25:75) as mobile phase. For the optimization of the composition of the mobile phase, many different ternary mixtures composed of methanol, propanol and THF with different ratios were examined until the separation of lycopene peak from other peaks was done. THF was chosen because of the solubility of lycopene in it. Fig (2) shows clear overlapping of lycopene peak with other peaks when using (MeOH /propanol/THF) (20:70:10) and the retention time was longer slightly, 20% methanol does not achieve a balance between methanol and other solvents in this mixture which of course may result in great polarity difference. Fig (3) shows overlapping of lycopene peak with another peak when using (MeOH/propanol /THF) (15:65:20) and the retention time was longer where the peaks appeared after 20 minutes. Fig (4) shows HPLC profile of standard Lycopene, Fig (5) shows the HPLC profile of tomato Lycopene as separated peak on C18 column with isocratic elution and using (MeOH /propanol/THF) (15:65:20) and re-dissolving the residue in THF, the retention time was less than 10 min. additional peak was observed near lycopene peak, it was difficult to assign what this peak without proper standard available. The last experimented mobile phase was the more efficient in the separation of lycopene peak without interaction with other constituents in the sample and TEA was added to reduce peak tailing. A flow rate of 1 ml /min

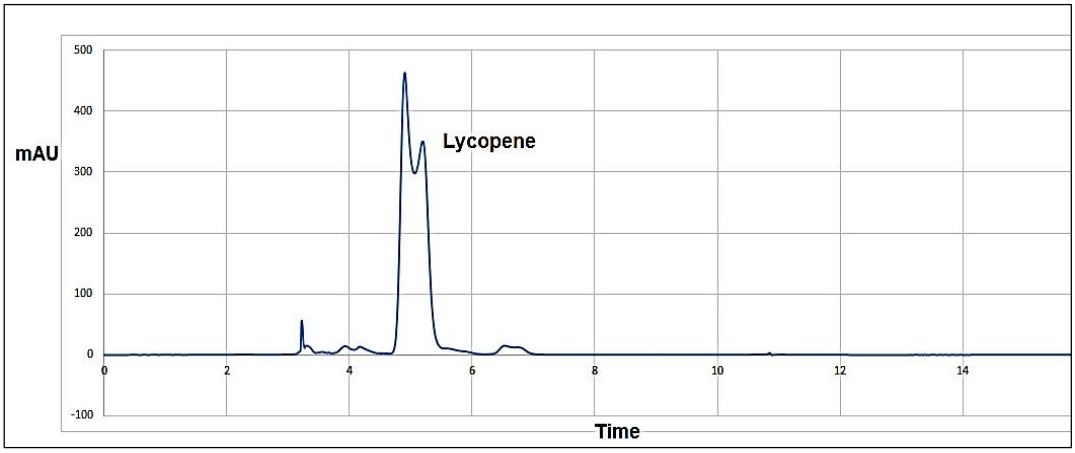

Fig 1: Chromatogram showing the peak of lycopene in tomato sample. The mobile phase: (MeOH /ACN)(25:75). λ=472 nm.

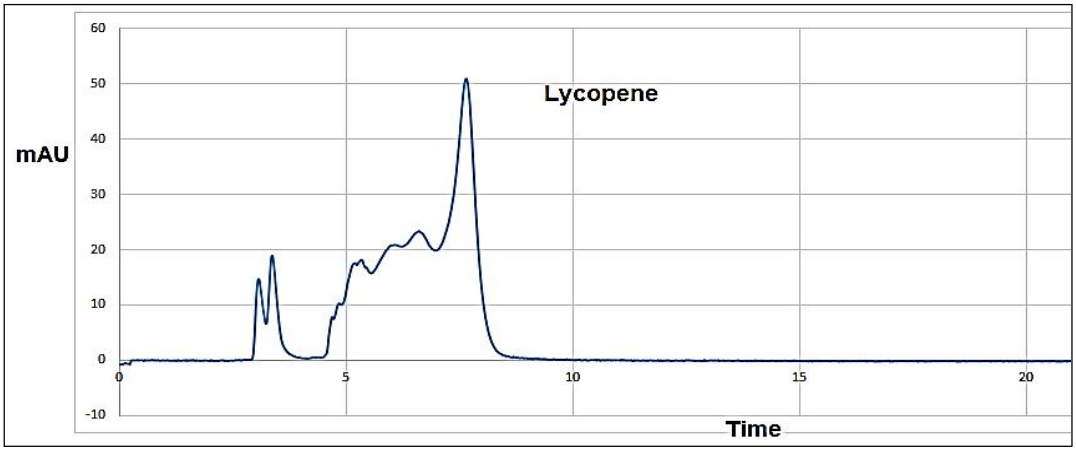

Fig 2: Chromatogram showing the peak of lycopene in tomato sample. The mobile phase: (MeOH /propanol/THF) (20:70:10). λ=472 nm.

was found to be optimum for the achieved retention time, baseline stability and noise. As a result, when mobile phase composition (MeOH /propanol/THF) (15:65:20) + 0.05% TEA was used, chromatogram revealed that the peak of lycopene was well separated without interaction with other peaks in the sample, the retention time was less than 10 min which leaded to fast analysis procedure of lycopene in tomato. The developed HPLC method was applied for quantifying

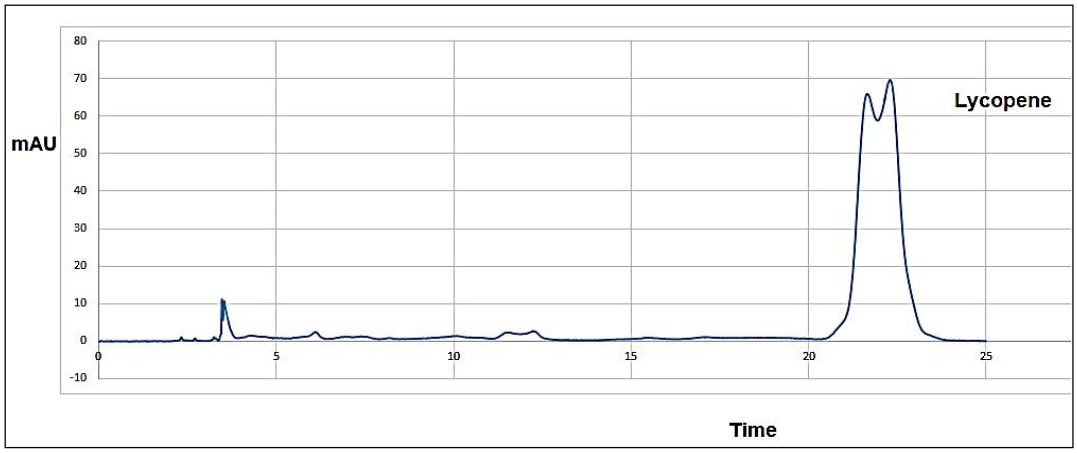

Fig 3: Chromatogram showing the peak of lycopene in tomato sample. The mobile phase: (MeOH /propanol/THF)(15:65:20). λ= 472 nm.

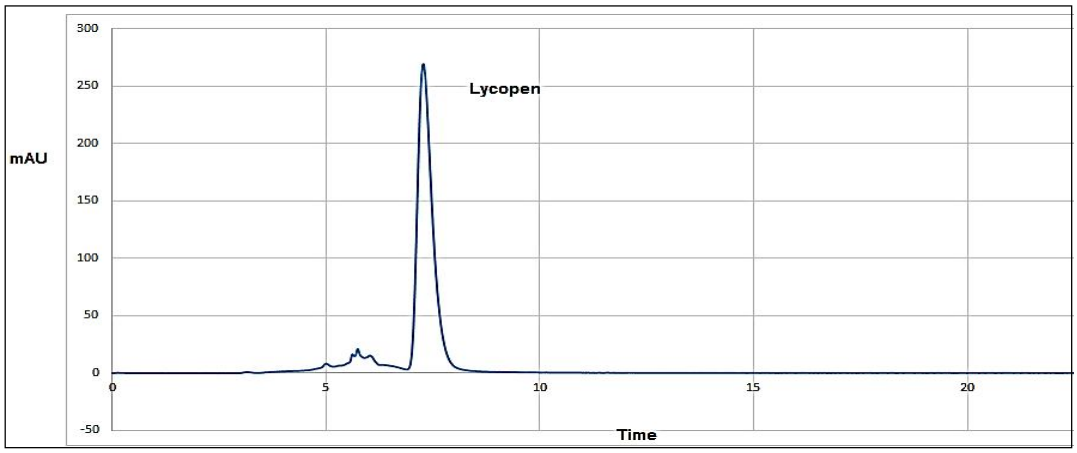

Fig 4: Chromatogram showing the peak of standard Lycopene. The mobile phase: (MeOH /propanol/THF)(15:65:20). λ=472 nm.

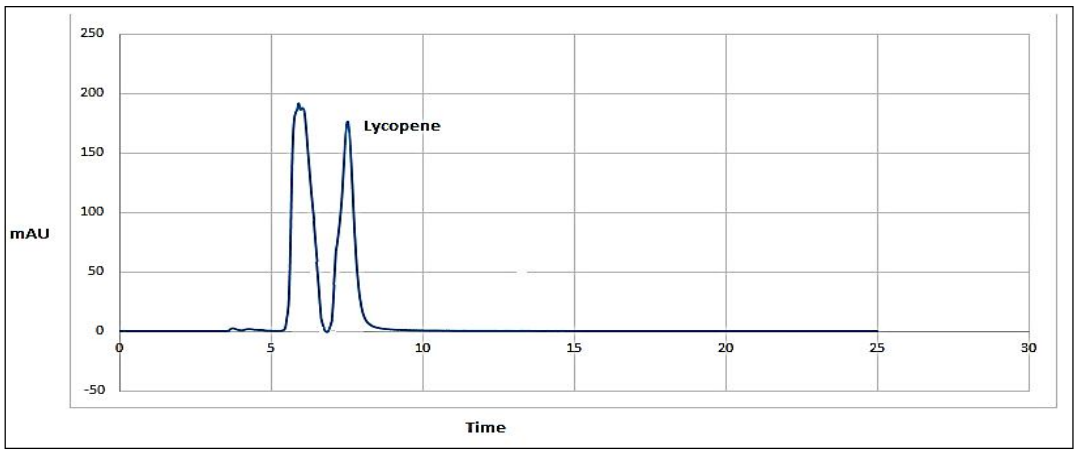

Fig 5: Chromatogram showing the peak of lycopene in tomato sample. The mobile phase: (MeOH /propanol/THF)(15:65:20). λ=472 nm.

all the levels of lycopene in the extracts. An effective, precise, fast isocratic reversed-phase HPLC method for the determination of lycopene in crude sample has been achieved to isolate lycopene from tomatoes; this method is useful for the lycopene content determination. Many methods have been used for lycopene analysis such as spectrophotometric, infrared spectroscopy; however, they consume too much time in addition to the expected interferences. HPLC is the most common method used for separation and determination of Lycopene because it is a rapid and accurate method and this apparatus is available in the most analytical laboratories. Tomatoes were collected from different regions in Syria to detect the differences in lycopene concentration between these regions. The chosen HPLC method was applied in screening of tomato samples for their lycopene amount, since the time between extraction and HPLC analysis was highly minimized, no degradation of lycopene would be happened during analysis. Therefore, comparing retention time with the pure lycopene standard was found to be suitable for identification of lycopene from tomato samples. Table 2 shows the values of lycopene contents in the analyzed samples. Homs tomatoes showed higher concentrations of lycopene, the lycopene concentration in Daraa and Al Suwayda tomatoes were converged because of geographical proximity, the Tartus tomato had the lowest lycopene concentration between the studied samples.

The lycopene content varied between different samples from different regions and in the same region, this may be due to the differences in the cultivar, maturity and cultivation conditions with influence on the lycopene content of tomatoes (Tsouvaltzis *et al*., 2023) Total lycopene content ranged from 55.94 to 103.70 μg/g. Such variability in the lycopene content between different samples is usual and has been formerly reported as well (Górecka *et al*., 2020). The analysis of variance shows that there is significant difference for the (p=0.05) in lycopene content between tomato samples from different regions in Syria.

Generally Syrian tomatoes from the different regions showed high concentration of lycopene, but Homs samples had the highest concentration, this value may be attributed to the kind of the tomatoes, where cherry tomato is planted in Homs countryside and this kind of tomato generally has elevated amount of lycopene. Tomatoes with red color with an elevated content of lycopene have better antioxidant activity than tomatoes with higher levels of beta-carotene and lutein, but this antioxidant efficacy is superior in the form of fruit compounds than in the form of lycopene, beta-carotene and lutein pure. The antioxidants in cherry tomatoes have a higher lycopene content than other kinds of tomatoes (Górecka *et al*., 2020). Tartus tomato showed the lowest lycopene concentration between the Syrian samples, this may be attributed to the high percentage of water in Tartus tomato. The lycopene content in tomatoes is highly influenced by a lot of factors, both genetic and environmental factors. The color of the fruit is considered the genetic factor of tomatoes that affects the content in tomatoes (Petrovi´c *et al*., 2022). Lycopene content is generally indicated by color. Several literatures illustrated that the amount of lycopene in tomato varies highly between cultivars, stage of maturity and growing conditions (Gonçalves *et al*., 2020).

The results of the samples analysis have confirmed that concentration of lycopene can be very different also for tomatoes having a similar intensity of red color. Addition to genetic factors, there are other factors that impact lycopene levels in tomatoes like the intensity of sunlight. Temperature and humidity are other environmental factors that can affect the lycopene concentration of tomatoes. The lycopene content in the tomato is affected by the respiration process, in this process lycopene is degraded into terpenes so that leads to the reduction of lycopene content, one of the results of this process is water, it leads to increase the water amount with storage. lycopene content in tomatoes is highly affected by fruit age. Previous studies demonstrated that lycopene content in tomatoes is affected by the time of harvest. Therefore, the levels of lycopene in tomato are not only affected by plant genetics, environmental factors and tomato processing also extremely influence the lycopene in tomatoes (Yakubum *et al*., 2017). Consequently, it is substantial to detect the factors related to pre- and post-harvest conditions to superior understand their effects on the synthesis and accumulation of components such as carotenoids, in addition to antioxidant abilities. All of the previous factors play a role in the determination of tomato quality, especially in terms of the health-related characteristics of tomato. An understanding of the relationship between the factors that affect on lycopene concentration and the content of other components with antioxidant activities is essential in order to potential benefits

Table 2: Lycopene content in Syrian tomato samples μg/g.

Samples	Lycopene content μg/g
Daraa 1	93.16±3
Daraa 2	96.22±8
Daraa 3	88.73±9
Daraa 4	89.37±8
Daraa 5	92.17±5
Tartus 1	55.94±2
Tartus 2	59.10±6
Tartus 3	57.62 ±4
Tartus 4	60.22±3
Tartus 5	62.17±4
As suwayda 1	92.54±4
As suwayda 2	96.16±7
As suwayda 3	89.41±9
As suwayda 4	88.22±3
As suwayda 5	93.26±9
Homs 1	101.25±4
Homs 2	97.12±10
Homs 3	103.70±5
Homs 4	96.47±7
Homs 5	97.59±4

for human health will be taken from tomato consumption. Thus, the use of tomato improves the health of consumer as lycopene is having high nutritional value. Raw tomato seems to be the main contributor to dietary lycopene intake in many countries and associated with intake of lycopene and other antioxidants that have health benefits, it is abundant and cheap fruit in the Syrian kitchen and it is the most valuable source of lycopene in Mediterranean and Syrian diet.

CONCLUSION

There is a great interest on using natural antioxidant bioactive compounds to improve the quality of life such phenolic compounds, due to their antioxidant activity. In this research HPLC method using (MeOH /propanol/THF) (15:65:20) as mobile phase was applied for separation and quantification of lycopene content in Syrian tomato samples, the concentrations ranged from 55.94 to 103.70 ìg/g and the highest content of lycopene was in Homs tomatoes. these results encourage the use of Syrian tomato in diet intensively. Variability in the lycopene content is not unusual and has been reported previously and may be attributed to season, geographical location and cultivation practice which have an effect on the lycopene content of tomato cultivars. Thus, this applied method which is simple, specific, sensitive and accurate can be used in routine analysis of lycopene in tomato.

Conflict of interest: None.

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