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The Antioxidant Properties of Tomato Lycopene

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Abstract. In this study, the antioxidant activity of tomato lycopene (*Lycopersicon esculentum Mill*.) was investigated. The antioxidant properties were evaluated by using different antioxidant tests, including ferric thiocyanate method, reducing power, metal chelating ability and hydrogen peroxide scavenging. The yield of lycopene from puree was 11.8 mg/100g compared with those in peels and seeds 11.3 and 6.3 mg/100g respectively. Lycopene is a powerful natural antioxidant, 5mg/ml of lycopene exhibited 83.24% inhibition in linoleic acid peroxidation, which was greater at same concentration than the of butylated hydroxy toluene (BHT) 75.68% and α-tocopherol 69.73% after 1 day, whereas, at same concentration the antioxidant activities after 90 days of lycopene, BHT and α-tocopherol were 78.65, 80.09 and 71.91% respectively. By using the reducing power activity assay, it was found that ascorbic acid had the highest reducing power activity (275.49%) followed by lycopene extract (270.83%). The chelating ability on ferrous ions was 84.66% by using at 5mg/ml, and the scavenging effects on H_2O_2 were 50.20–73.74% with 3.5–5mg/ml lycopene extract.

Key words: Tomato Extract , Lycopene, Antioxidant Activity

Introduction

Tomato (*Lycopersicon esculentum* Mill.) is one of the most extensively marketed vegetable food, with a worldwide production of 126 million Tm in 2005. Being a popular fruit, tomatoes find numerous uses in both fresh and processed forms. Processed products include pulp, puree, sauce, juice, paste and peeled whole tomato (17 , 19). Carotenoids belong to the tetraterpenes family and represented by more than 600 known natural structural variants. Carotenoids are synthesized in plants, fungi, bacteria and algae whereas in animals and human they incorporated from their diet. Carotenoids are widespread natural pigments, that confer easily recognizable yellow, oranges, red, or purple colours to some plant and animal organs. The formation of the tetraterpene skeleton, results by losing a proton, and generating a double bond in the center of the molecule. Carotenoids are divided into two classes, carotenes and lycopene, which containing only carbon and hydrogen atoms and oxocarotenoids (Xanthophylls) containing at least one oxygen atom (4 , 15 , 21).

Lycopene ($C_{40}H_{56}$) is an open chain hydrocarbon containing 11conjugated and 2 non-conjugated bonds arranged in a linear array. Lycopene is found predominantly in the chromoplast of plant tissues. In tomatoes the biosynthesis of lycopene increases dramatically during the ripening process, as chloroplast undergo transformation to chromoplast. Globulous chromoplast containing mainly β-carotene is found in the jelly part of the pericarp while chromoplast in the outer part of the pericarp contains voluminous sheets of lycopene.

Many studies reported the occurrence of lycopene in different fractions of tomato fruit such as tomato skin, the water insoluble fraction and the fibrous fraction including the fiber and soluble solids. Their results indicated that 72-92% lycopene was associated with the water insoluble

fraction and skin. Tomato extracts and especially skin extracts contain high amounts of lycopene (2 , 10 , 20 , 21). The protective effect of carotenoids, especially lycopene, is usually ascribed to their ability to act as antioxidants and/or singlet oxygen quenchers.

The quenching constant of lycopene in vitro has been found to be more than double that of β-carotene and ten times higher than that of α-tocopherol (vitamin E). *In vivo* epidemiological studies report a high inverse correlation between the intake of lycopene in the diet and the development of some types of chronic disease such as cancer and coronary heart diseases, as it reduces low.-density lipoprotein (LDL) oxidation and helps reduce cholesterol levels in the blood. The evidence is the strongest for cancers of the lung, stomach, and prostate.

Animals maintained on lycopene diet, consumed on average 142 µg of lycopene per day of which 104 µg was absorbed by the body (which corresponds to 73% net dietary uptake) calculated as the difference between lycopene intake and fecal output. Ingested carotenoids including lycopene are incorporated into dietary lipid micelles, absorbed into the intestinal mucosal lining via passive diffusion. The ingested carotenoids are incorporated into chylomicrons and released into lymphatic system for transport to the liver (13 , 20 , 21 , 22).The objective of this study was to investigate the quantity of the lycopene in puree, peels and seeds and the antioxidant activity of lycopene. Including determination at reducing power and metal chelating activity.

Material and Methods

Samples preparation

Ripe tomatoes (*Lycopersicon esculentum* Mill.) were purchased from a local market in Basrah, the fruit was cut into small parts. Fresh tomato samples were diluted 1:1 (w/v) in distilled water homogenized in blender and were before blending. The homogenized tomato sauce was pressed by clothes cheese to separate peels and seeds. The juice was heated at the boiling point 10% TSS to be more suitable for next steps of extraction. The three samples (peels, seeds and puree) were kept at freezing (-18 ± 2C^{o}) until were assayed.

Lycopene extraction

Lycopene extraction was based on the method of (7). An approximate 1gm of the (puree, seeds and peels) was put in abeaker (50ml) wrapped with aluminum foil, located in an ice bath while that was on ice. Lycopene extraction solution (39ml) consisting of hexane, 0.05% (w/v) butylated hydroxy toluene (BHT) in acetone and ethanol absolute of a ratio 1:1:1 was added to the beaker and shaked for 20 min using magnetic stirrer. 6ml of cold distilled water was added and agitated for an additional 10 min in a separating funnel for better separation of polar and non polar compounds. The funnel was left for 15 min at room temperature to separate into polar and non-polar layers. Supernatant (hexane layer) was evaporated at 40C^{o} by using a rotavapor until 5ml volume. The absorbance of lycopene in the supernatant (hexane layer) was read at 503nm. The hexane was used as blank. The amounts of lycopene in puree, seeds and peels were estimated by the following equation:

$$\text{Lycopene (mg/kg)} = (x/y) \times A_{503} \times 3.12$$

Where x is the amount of hexane (ml), y is the weight of sample (g), A_{503} the absorbance at 503nm and 3.12 is the extinction coefficient.

Chemical analysis:

Determination of antioxidant activity:

Lycopene extract and commercial antioxidants (BHT and α-tocopherol)

were tested for their antioxidant activity by using ferric-thiocyanate method (14).

Extracts or commercial standards (0.5-5mg/ml) in 4ml of 99.5% (v/v) ethanol, 4ml of 2.53% linoleic acid in 99.5% ethanol, 8ml of 0.05 M phosphate buffer (pH 7.0), and 4ml of distilled water were put in a screw test tube and placed at 40Cº in the dark for 3 months. Samples were drawn after 24hr. and after 15 days. To 0.1ml of each sample a solution of 9.7ml of 75% (v/v) ethanol and 0.1ml of 30% (w/v) ammonium thiocyanate were added. Precisely after 3min 0.1ml of 0.02M ferrous chloride in 3.5% HCl was added to the reaction mixture, the absorbance of the developed red color was measured at 500nm. The % inhibition of lipid peroxidation was calculated by following equation:

$$\% \text{ Inhibition} = 100 - [(A_1/A_0) \times 100]$$

Where A_0 is the absorbance of the control reaction and A_1 is the absorbance in the presence of the sample.

The Reducing power

The reducing power was determined according to the method of (16). (2.5ml) of each extract (0.5-5mg/ml) was mixed with 2.5ml of 200mM phosphate buffer (pH 6.6) and 2.5ml of 1% potassium ferricyanide. The mixture after the addition of 2.5 ml of 10% trichloroacetic was incubated at 50 Cº for 20min. The mixture was centrifuged at 1000g for 10min, the upper layer (2.5ml) was mixed with 2.5ml of distilled water and 1ml of 0.1% ferric chloride, and the absorbance was measured at 700nm against a blank. Increased absorbance of the reaction mixture indicated increased reducing power. The control and the standards (BHT, α-tocopherol, Ascorbic acid and citric acid) were subjected to the same procedures of the sample except, the % reducing power was calculated by the following equation:

$$\% \text{ Reducing power} = 100 - [(A_1/A_0) \times 100]$$

Where A_0 is the absorbance of the control and A_1 is the absorbance in the presence of the sample.

Metal chelating activity

The chelating of ferrous ions by the extract and standards was estimated according to (8). 0.4ml of the extract (0.5-5mg/ml) was added to 0.4ml of 2mM $FeCl_2$ and 0.4ml of 5mM 8-hydroxy quinoline in 99% ethanol. The mixture were incubated in the dark at room temperature for 10min.The absorbance of the solution was measured at 562nm. The lower absorbance indicated stronger chelating effect. The control and standards (BHT, α-tocopherol, ethylene diamine tetra acetic acid EDTA, Ascorbic acid, citric acid) were subjected to the same procedures as except.The present chelating effect was calculated as below:

$$\text{Chelating ability } \% = 100 - [(A_1/A_0) \times 100]$$

Where A_0 is the absorbance of the control reaction and A_1 is the absorbance in the presence of the sample.

Scavenging of hydrogen peroxide

The ability of the extract to scavenge hydrogen peroxide was determined according to (8). 1ml of extract (0.5-5mg/ml) was added to 0.6ml of 40mM hydrogen peroxide solution (in 0.1 M phosphate buffer pH 7.4).The absorbance of hydrogen peroxide was determined at 230 nm. After 10 min against a blank solution containing the phosphate buffer without hydrogen peroxide. The scavenging percentages of hydrogen peroxide of both extract and standards (BHT, α-tocopherol, Ascorbic acid and citric acid) were calculated:

$$\% \text{ Scavenged } H_2O_2 = [(A_0/A_1)/A_0] \times 100$$

Where A_0 is the absorbance of the control and A_1 is the absorbance in the presence of the sample.

Infrared analysis of lycopene

Extract of lycopene was analyzed by (FTIR-84005) Fouler Transform Infrared Spectrophotometer by the assistance of Chemistry Department, College of sciences

Results and Discussion

The extraction yield obtained with hexane layer of lycopene from peels, seed and puree were 11.3, 6.3 and 11.8mg/100g respectively. Lycopene, according to the finding of (17 , 20) is stable during heating and industrial treatment, except at extreme conditions (for example, very high temperature or very long heating times), and treatments are able to improve lycopene bioavailability, (18) suggested that homogenization and heat treatment disrupt cell membranes and protein-carotenoids complex, making carotenoids more accessible for extraction. However, the increase in lycopene just after processing coincided with depletion of phytoene and neurosporene contents. The tomato cell walls were not completely disrupted during the tomato puree preparation. Therefore, for the cells that were disrupted, lower temperatures (as 80C^o) could be enough to release lycopene. On the other hand, for the cells that were not disrupted during the puree preparation, for example, the cells of tomato skin, a longer heating treatment time or higher temperature may be necessary to disrupt the cell walls sufficiently to release all or most of the lycopene from cells. That is, longer heating time at 100C^o, or higher temperature (120C^o) may increase the extraction yield of lycopene in tomato puree. In food processing, long-time-low-temperature combination and short-time-high-temperature combination can have the same effects on the tomato matrix (20). Lycopene is concentrated in tomatoes its content varies from 0.85mg to 13.6mg/100g according to fruit ripening and to the tomato variety (21). A mixture of hexane with acetone and ethanol or methanol is often used because other components, such as diethyl ether and tetrahydrofuran, may contain peroxides that react with carotenoids, and the stability of lycopene extracts obtained with hexane/acetone or hexane/ethanol is higher than that of extracts obtained with other organic solvents, such as chloroform, methanol or dichloromethane (11).

The effect of lycopene extract on the prevention of linoleic acid peroxidation was investigated by ferric-thiocyanate method. As seen in Fig.1, it was showed that the antioxidant activity increased with the increased concentration of lycopene extract, BHT and α-tocopherd (Vit. E) after 1 day. Lycopene exhibited high antioxidant activity than BHT and α-tocopherol after 45 day 94.07, 91.11 and 88.89% respectively at 5mg/ml. Lycopene extract showed a slight decrease in antioxidant activity, from 82.61% after 60 day to 78.65% at 5mg/ml after 90 day than the antioxidant activity of BHT alone Figs. (2, 3, 4). A high tomato consumption plays a protective role in inhibiting the propagation of radical chain reactions due to the carotenoid constituents, particularly lycopene and B-carotene, and also a variety of natural antioxidants such as ascorbic acid and polyphenols.

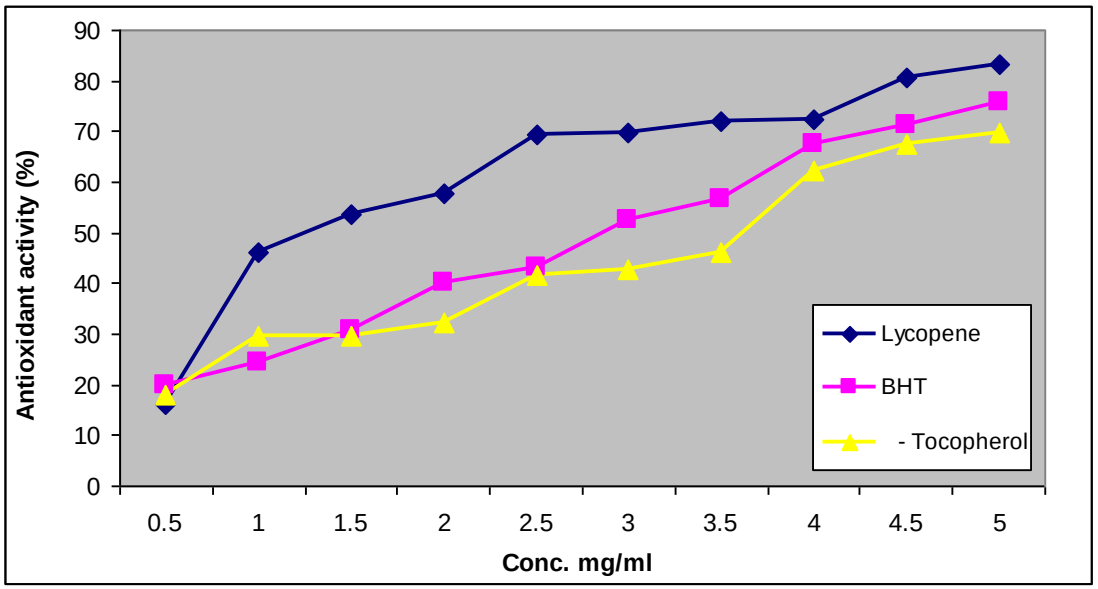

Fig. (1) Antioxidant activity (%) of lycopene extract after 1 day.

During ripening, the total antioxidant activity increases and this increase is mainly due to changes in to the lipophilic antioxidant activity. Hydrophilic antioxidant activity remains practically unchanged (9 , 17). The antioxidant activities of putative antioxidants have been attributed to various mechanisms. Among these are prevention of chain initiation, binding of transition metal ions catalysts, decomposition of peroxides, prevention of continued hydrogen abstraction, and radical scavenging (6). (12) reported the antioxidant activity was based on the electron transfer of antioxidants to stabilize the radical cation. Basically, there are three possible mechanisms for the reaction of carotenoids with radical species which are radical addition, electron transfer and allylic hydrogen abstraction.

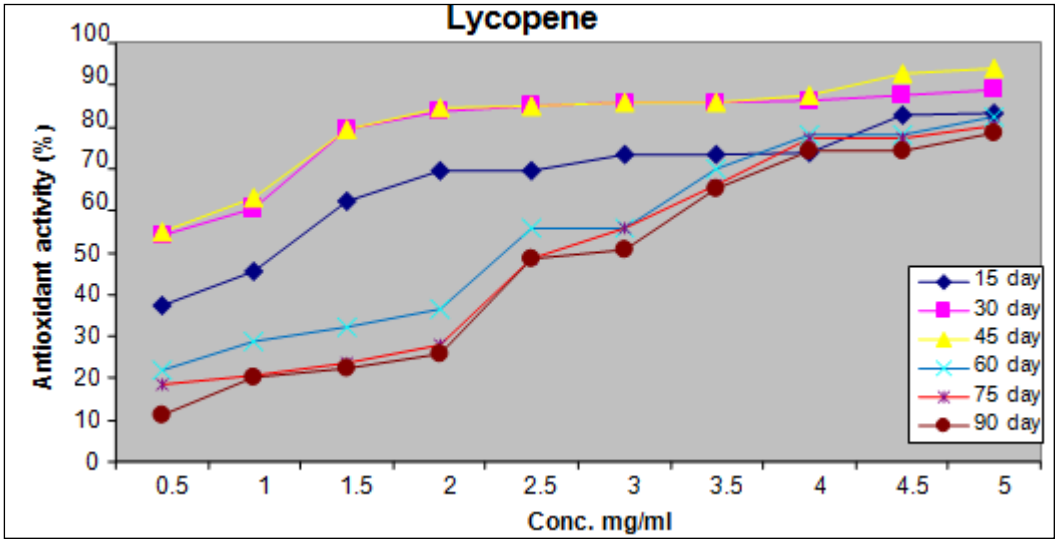

Fig. (2) Antioxidant activity (%) of lycopene extract after 3 months.

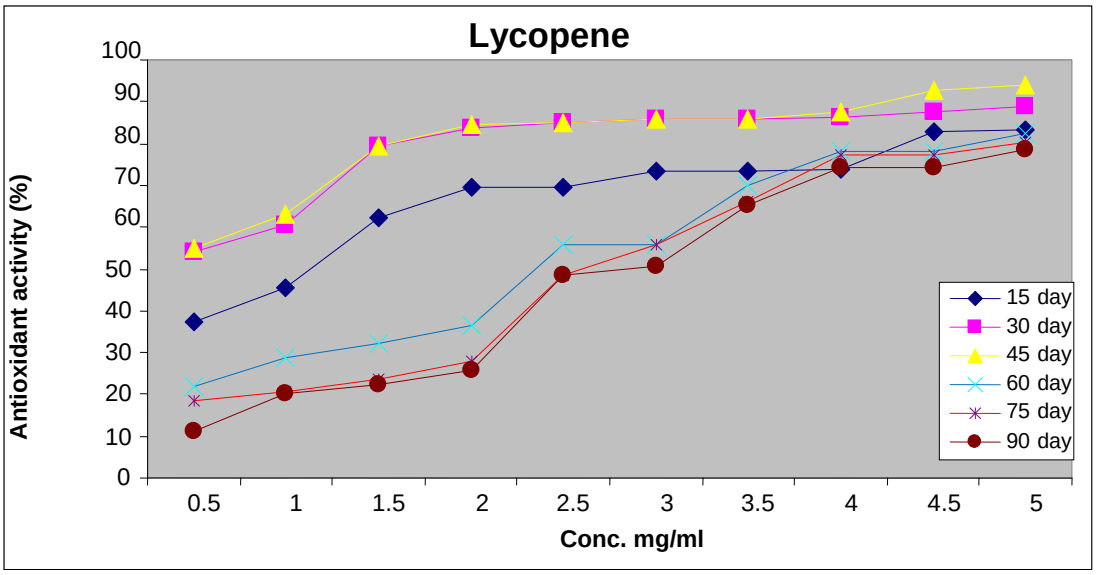

Fig. (2) Antioxidant activity (%) of lycopene extract after 3 months.

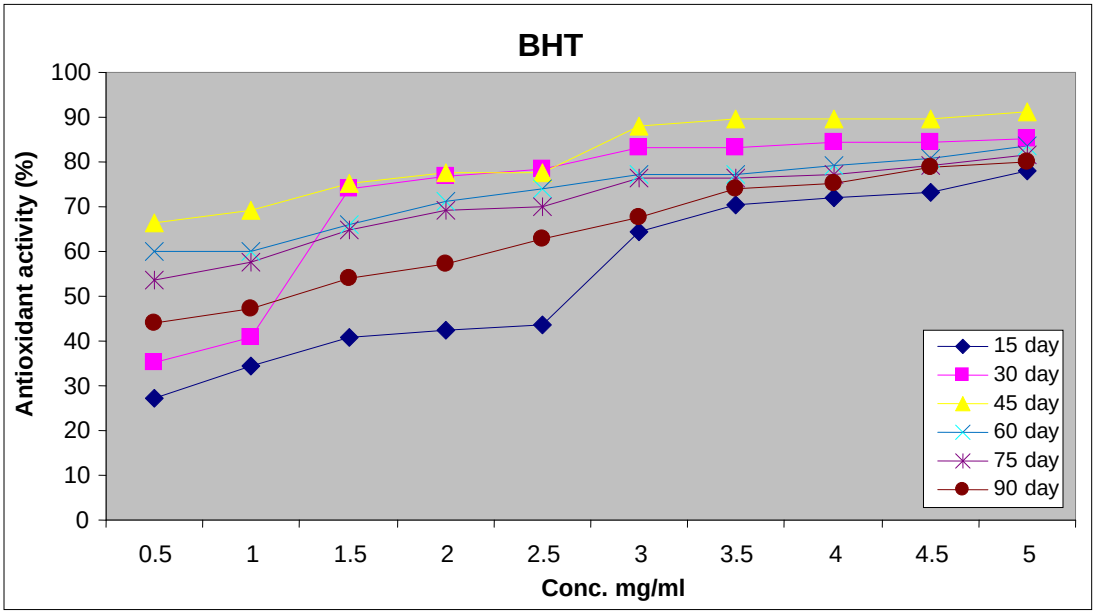

Fig. (3) Antioxidant activity (%) of BHT after 3 months.

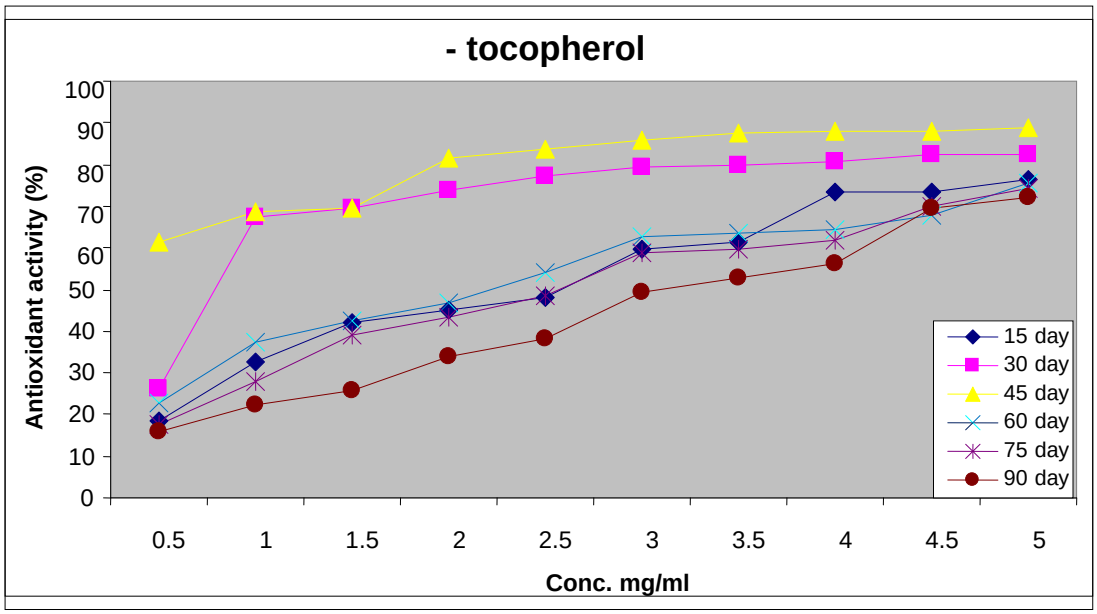

Fig. (4) Antioxidant activity (%) of α- tocopherol after 3 months.

The reducing power of lycopene extract increased with increased concentration and was higher than BHT, α-tocopherd and citric acid 270.83, 160.10, 150.89 and 179.62% respectively, whereas reducing power of ascorbic acid was 275.49% at the same concentration Fig.5. The reducing properties were generally associated with the presence of reductones such as ascorbic acid, which have been shown to exert antioxidant action by breaking the free radical chain by donating a hydrogen atom to convert them into more stable products and to terminate radical chain reactions. Reductones react directly with peroxides and also with certain precursors of peroxides, thus preventing peroxide formation (7, 23).

The chelating effects of lycopene extract on ferrous ions was low at 0.5-4 mg/ml, but notably high at 5mg/ml 84.66% Fig 6. The chelating activity was increased with increased concentration of lycopene extract, but EDTA showed excellent chelating ability at 0.5mg/ml to 5mg/ml 85.01-96.96% BHT and α-tocopherol were not a good chelating agent for ferrous ions and that chelating effect were 25.96 and 23.86% at 5mg/ml respectively, whereas ascorbic acid and citric acid chelated 93.81 and 89.06% respectively of ferrous ions at 5mg/ml. Ferrous ion, commonly found in food systems is well known as an effective pro-oxidant. Polyphenols and carotenoids can chelate pro-oxidant metal ions, such as iron and copper, thus preventing free radical formation from these pro-oxidants (1 , 21). Lycopene was could chelate singlet oxygen, with a chelating constant tow fold higher than that of β-carotene. Furthermore, lycopene can sequester nitrogen dioxide (NO_2^o) and sulfide (RS^o) free radicals and inhibit DNA and cellular membrane damage due to oxidative processes (5).

The scavenging ability of lycopene extract on hydrogen peroxide was shown in Fig 7 and compared with that of BHT, α-tocopherol, Ascorbic acid and citric acid. Lycopene extract was exhibited 73.74% scavenging activity on hydrogen peroxide at 5mg/ml. In the other hand, BHT and α-tocopherol exhibited 57.20 and 51.20% respectively hydrogen peroxide scavenging activity at the same

concentration, whereas ascorbic acid was capable of scavenging hydrogen peroxide 83.60% at 5mg/ml. Citric acid had excellent scavenging ability on hydrogen peroxide at 0.5mg/ml to 5mg/ml 82.10-93.42%. Hgrogen peroxide itself is not very reactive, but it can sometimes be toxic to cell because it may give rise to hydroxyl in the cells. Thus, removing H_2O_2 as well as O_2^{o-} is very important for protection of food systems (8).

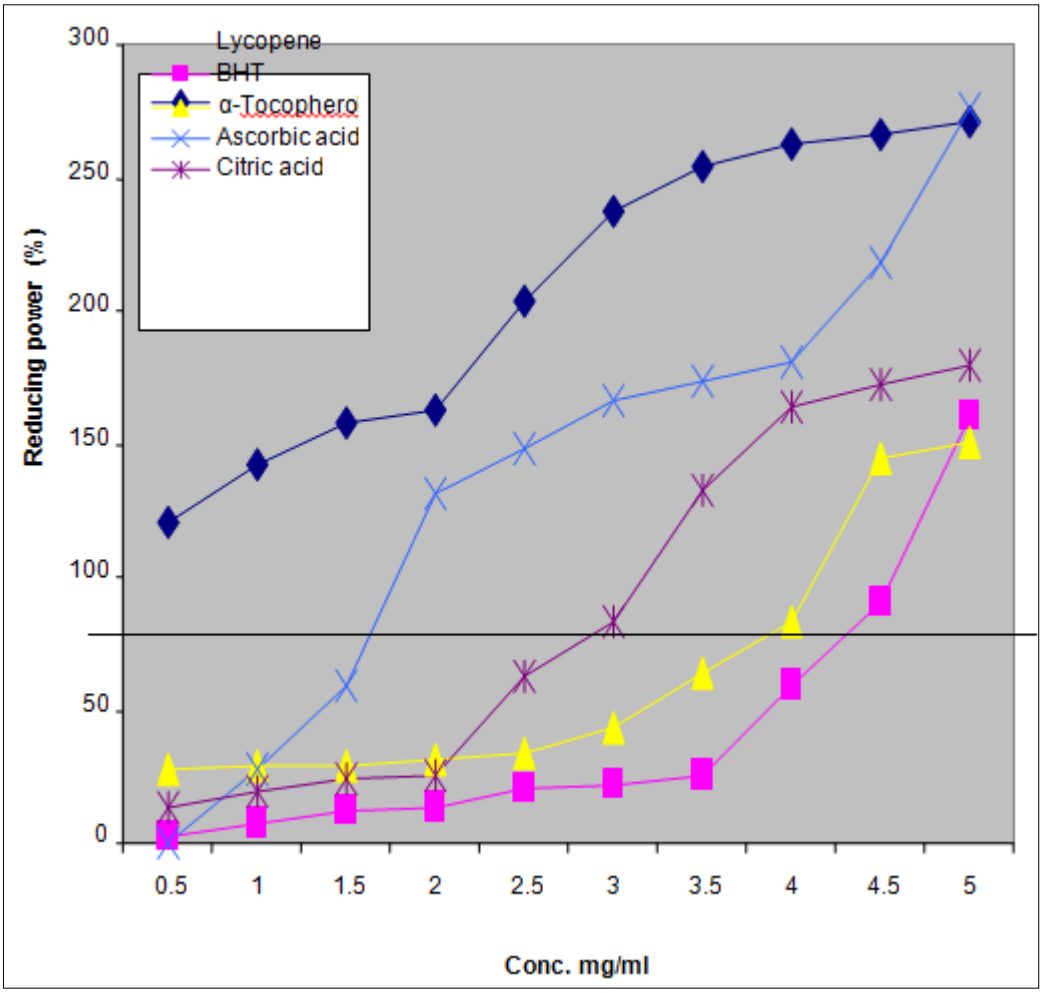

Fig. (5) Reducing power activity (%) of lycopene extract, BHT, α-tocopherol, ascorbic acid and citric acid.

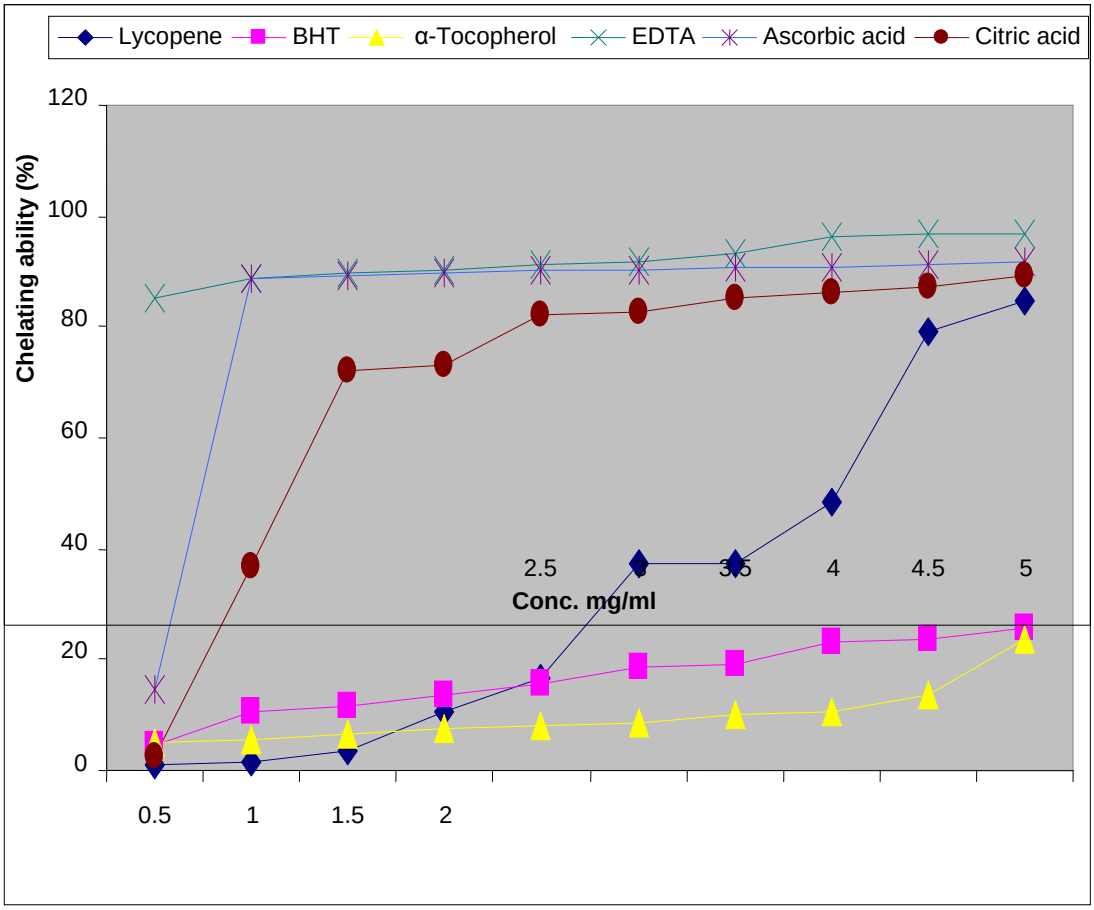

Fig. (6) Metal chelating able of different amount of lycopene extract, BHT, α-tocopherol, EDTA, ascorbic acid and citric acid.

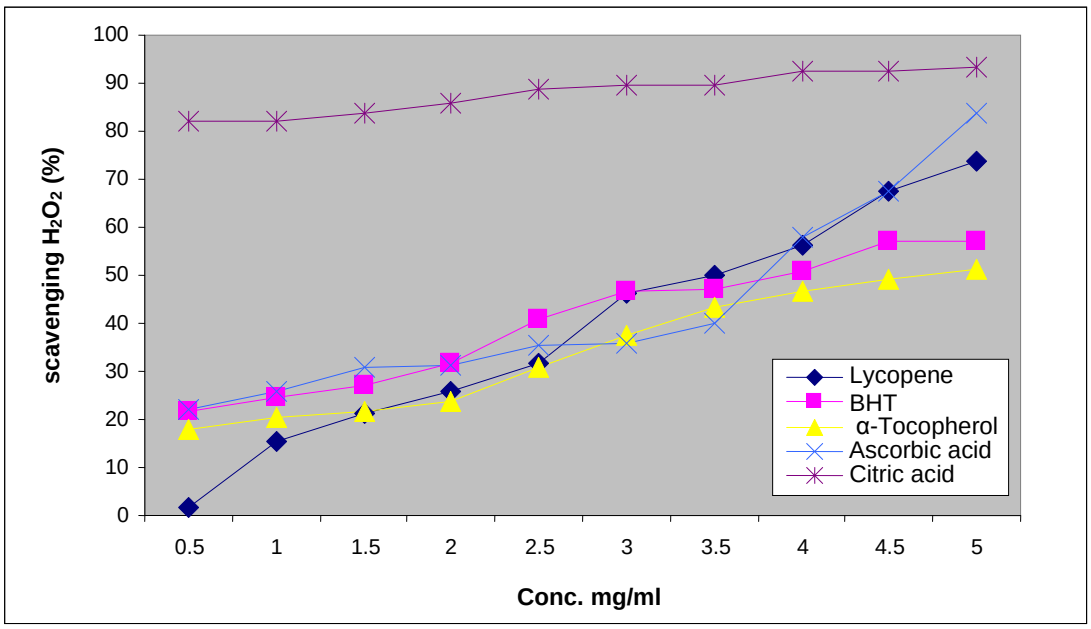

Fig. (7) Hydrogen peroxide scavenging activities of lycopene extract, BHT, α-tocopherol, ascorbic acid and citric acid.

IR data exhibited = C-H stretch for SP^2 C-H at (3068 cm^{-1}) as a-medium band, conjugated C=C stretch appeared at lower frequencies (1434.9cm^{-1}) due to highly conjugated, out of plane bend of = C-H appeared at (864 and 769 cm^{-1}). SP^3 C-H stretching appeared as a strong bands at (2960-2867 cm^{-1}) Fig 8 (3).

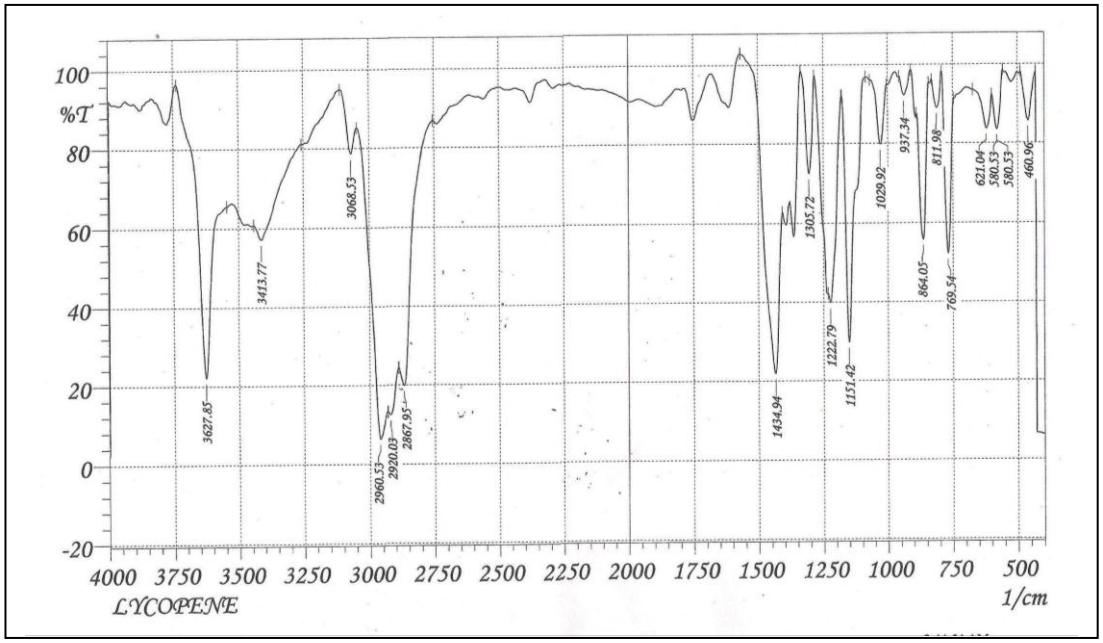

Fig. (8) Infrared spectroscopy of lycopene extract.

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الخواص المضادة للأكسدة لمستخلص لايكوبين الطماطة

روضة محمود العلي

قسم علوم الأغذية والتقانات الاحيائية، كلية الزراعة، جامعة البصرة

المستخلص. هدفت هذه الدراسة الى استخلاص اللايكوبين كمضاد أكسدة من الطماطة (*Lycopersicon esculentum* Mill.). قدرت الخواص المضادة للأكسدة باستعمال اختبارات مختلفة والمتضمنة الفعالية المضادة للأكسدة باستعمال طريقة ثايوسيانيد الحديديك، القوة الاختزالية، قابلية ربط المعادن واقتناص بيروكسيد الهيدروجين. تم الحصول على كمية من اللايكوبين مقدارها 11.8ملغم/100غم لعجينة الطماطة مقارنة بالقشور والبذور 11.3 و 6.3 ملغم/100غم على التوالي. يعد اللايكوبين مضاد أكسدة طبيعي فعال، فعند تركيز 5 ملغم/مل اظهر مستخلص اللايكوبين قابلية تثبيط عالية 83.24% لنظام أكسدة حامض اللينوليك مقارنة بنفس التركيز لكل من BHT و α–tocopherol 75.68 و 69,73% على التوالي بعد يوم واحد من الخزن، بينما كانت الفعالية المضادة للأكسدة بعد 90 يوم لمستخلص اللايكوبين 78.56% مقارنة بكل من BHT و α–tocopherol 80.09 و 71.91% على التوالي عند تركيز 5 ملغم/مل. وجد في اختبار القوة الاختزالية أن حامض الاسكوربيك امتلك أعلى فعالية اختزالية تبعهُ مستخلص اللايكوبين 275.49 و 270.83% على التوالي. كانت قابلية ربط ايونات الحديدوز عالية 84.66% عند تركيز 5 ملغم/مل. أما اقتناص H_2O_2 فكان 50.20–73.74% عند تركيز 3.5–5 ملغم/مل.