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Effect of Extraction Methods on the Amount of Lycopene Extracted from Tomato Waste

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Abstract. The study aimed to extract lycopene from local tomato and its juice and waste , and study the effect of using different extraction methods on the lycopene yield. mixture of acetone and petroleum ether (T 1) was used in a ratio of (1:1), solvents (hexane: acetone: ethyl alcohol) in a ratio of (2:1:1)respectively (T 2), and a mixture of acetone, ethyl lactate and ethanol was used in a ratio ((1:2:1) (T 3). The results of the study showed that there was a correlation between the type and percentages of solvents used and the yield of lycopene extracted, and the Lycopene percentage extracted (T 1) was 185.6 mg / 100 gm of tomato waste and 25.7 mg / 100 gm of tomato waste, calculated on the basis of dry and wet weight, respectively, and the Lycopene percentage extracted in (T 3) was 165.5 and 11.5 mg / 100 gm of tomato waste on the basis of dry and wet weight, respectively. While the Lycopene percentage extracted in (T 2) was 174.8 mg / 100 gm and 17.3 mg / 100 gm of waste on the basis of color. wet and straight. The study showed the need to achieve the methodology in the balance of the solvent mixture and to choose what is appropriate in extracting the required substance, taking into account the time, temperature and other environmental conditions in order to reach the best degrees of efficiency in the extraction process.

Keywords. Lycopene percentage, Tomato Waste, Extraction methods, Organic solvents.

1. Introduction

In recent decades, there has been an increase in interest and recommendations for the consumption of fruits and vegetables, as they are rich in various phytochemical compounds. They are mostly biologically active substances that play important roles in improving human health. Plants contain primary metabolites, which are the organic compounds that are directly related to the growth and development of the plant. Secondary metabolites that play important roles in plant reproduction (attracting pollinators) and pigmentation of plant parts such as flowers, leaves and fruits. Humans use secondary compounds for specific applications such as perfumes and dyes, for biological and medical purposes, as antioxidants and growth promoters. [1,2]

Carotenoids are one of the secondary compounds and belong to the terpenoid family. The biosynthesis of these quaternary substances begins with the formation of isopentenyl diphosphate and then several reactions (desaturation, isomerization, cyclization, oxygenation) leading to the formation of different types of carotenoids. [3] Carotenoids can be divided into multiple classes, depending on the chemical structure (cyclic and non-cyclic), the presence and absence of oxygen, and the functional groups (hydroxy group and epoxy group) [4]. Of the 600 different types of different group of carotenoids in plants and living organisms, the most important carotenoids are lycopene, lutein, beta-carotene, alpha-carotene, cryptoxanthin, which are mostly of vegetable origin and are soluble in fats and present in human plasma [5].

Lycopene is a natural carotenoid pigment like the rest of the carotenoid pigments, and it is mainly made in plants and is present in microorganisms and some animals[6,7]. Lycopene gives colors ranging from pink to red, contributes to the coloring of fruits and vegetables, plays a role in absorbing light during photosynthesis, and acts as a protection for plants from photosensitization [8]. Lycopene is a hydrophobic carotenoid organic compound with a simple hydrocarbon structure consisting of 8 tetraterpenoids, with the structural formula $C_{40}H_{56}$. Lycopene is characterized by ligands. A long double bond containing 11 conjugate double bonds, which gives the compound important properties at the level of biological and physiological activities.[8;9]Natural lycopene is found predominantly in plants as All-trans-lycopene, and can be Many forms of other engineering isomers, mono- or poly-cis isomers, occur under conditions of elevated temperatures and the presence of oxygen, metals, and other catalysts [8,10].

Lycopene content is affected by several factors such as fruit variety, degree of ripeness, environmental conditions (light, irrigation, temperature, climate, planting site) and storage and processing conditions [8,11] During fruit ripening, total chlorophyll levels decrease to disappear, while Lycopene levels are increased [12,13]. Complementary colors (red and blue) in tomato enhanced lycopene synthesis in the fruit [14]. [15], demonstrated the instability of lycopene under light irradiation by enhancing Lycopene was the most stable bioactive compound even under subsequent evaporation and sterilization, with a loss of only 20% [16,17], and the presence of antioxidants (such as ascorbic acid and phenolic compounds) could be responsible for stability during processing. Moreover, the presence of lycopene in (tomatoes) or in some types of matrix can provide more stability [18,19]. Storage stability of tomato juice was also studied, and it was found that the stability of lycopene Post-processing is closely related to p Efficiency, storage, time and method of processing Lycopene is released due to its hydrophobic nature, and processed lycopene is released more easily than if it was fresh [13,20].

2- Materials and Methods

2 -1 Sample preparation

Fresh ripe local tomatoes, a variety (Maikori) cultivated in Karbala Governorate, were used for the summer season 2022-2023. Lycopene was extracted from locally grown tomatoes, its concentration was classified and studied in whole fruits, their juice, and its waste after extracting the juice, as it was the waste of tomato canning factories. The results indicated that lycopene was concentrated in tomato waste. Each sample was dried using an air oven at a temperature of 50 degrees Celsius, then it was ground for the experiment and the dried were used to extract lycopene according to the method described by [21], and packaged in sterile glass bottles and kept in the dark at room temperature and was 2 ± 25 o C..

2-2 Sample Drying

Tomatoes were cleaned and their green and damaged residues were isolated, washed, weighed, the fruits were cut into quarters, then squeezed with an electric mixer. The residues were collected and the juice was isolated aside, and the tomato waste were placed in ovine temperature was set at 50 for the purpose of drying it until the weight stabilized, and after completing the drying process completely, it was grinded automatically and according to the method described by [21], and packaged in sterile glass bottles and kept in the dark at room temperature and was 2 ± 25 o C.

3-2 Lycopene Extraction

- Three methods of extraction for extracting lycopene from tomato waste powder to compare and choose the best method to achieve the greatest lycopene yield, Method that described by [22; 23] to obtain the pigment extract from dried and fresh tomato residues, as a mixture of the two solvents acetone: and petroleum ether was used, with a boiling point of 40-60 °C, as 1 gram of the sample was taken and mixed in a vortex shaker (Vortex) with the addition of 250 ml of acetone in batches at laboratory temperature until the residue became colorless and the extract was collected in a separation funnel containing 10 ml of petroleum ether and a 5% solution of non-aqueous sodium sulfate was added to it and the extract was mixed until it separated into two layers, after which
- The method described by [24] was followed to obtain the dye extract from dried and fresh tomato residues, taking 1 gram of the sample and mixing it with 10 ml of a mixture of solvents (hexane: acetone: ethyl alcohol) in a ratio of (2:1:1) It was mixed in a vortex shaker for 10 minutes, then 1.5 ml of water was added to it to separate the hexane layer from the acetone and ethyl alcohol layer, and it was mixed for another 5 minutes. The top layer containing lycopene was removed and kept in an opaque vial with a lid. I repeated the process by adding another amount of solvents until the residue became colorless The layers containing lycopene dyes were collected together and dried by rotary evaporator at a temperature of 40 o C. The dried dyes were dissolved with petroleum ether and the volume was completed to 50 ml, after diluting it at a ratio of (1:10) with the same solvent an optical absorption reading was taken At a wavelength of 472 nm, the above-mentioned law was applied to obtain the Lycopene percentage estimated in mg per 100 gm of tomato waste.
- The same method mentioned in paragraph (2) was followed to obtain

the bottom layer of acetone was separated from the layer The upper ether layer was then washed again with petroleum ether until the acetone became colorless. The ether extract was concentrated by means of a rotary evaporator at a temperature of 40 °C to a volume of 50 ml. It was diluted with ether in a ratio of (10:1). The optical absorption reading was taken at a wavelength of 472 nm, and the following law was applied to obtain On the Lycopene percentage estimated in milligrams per 100 gm of tomato waste.

Lycopene percentage (mg/100 g waste) =

3.1206 x optical absorption of the sample x volume of the sample x reciprocal of dilution x 100

sample weight in grams x 1000

the dye extract from dried and fresh tomato residues, but by replacing the organic solvent hexane with ethyl lactate.

3.3 Comparison of the Lycopene percentage Extracted from Tomato Waste, Juice and Puree

A quantity of tomatoes was divided into two equal parts, the tomato was cut into quarters in the first part, then squeezed with an electric mixer, and the waste were collected and kept in polyethylene bags, and used to estimate the dye based on dry weight, and the remaining juice was preserved by freezing, while the second part of the tomato was mashed Mixer and the sample was estimated on the basis of dry weight.

3-4 Effect of Solar Drying of Residues on the Lycopene percentage Tomato waste was divided into Petri dishes equal in weight, height and diameter, and the height of the sample inside was approximately 1 cm. The dishes were placed under the sun for 10 hours, and the temperature ranged between (40-50) o, then the remaining moisture was estimated. The drying time for the first model was one day, for the second model two days, and the third for three days. Then the samples were ground with an electric grinder, then extracted using (T 1) and the amount of dye was calculated based on the dry weight and the results were compared.

3-5 The Effect of the Drying Temperature in the Electric Oven on the Lycopene percentage
The electric dryer (AMBIANO. 350 W) of German origin was used to dry the tomato waste, and it was dried using different temperatures (50, 40 and 60) o until the weight stabilized, and the percentage of remaining moisture was estimated for the dried samples. Then, the dried samples were ground using an electric mill and extracted using (T 1).

3-6 statistical analysis
The statistical program Statistical Analysis System (2018) – SAS) was used to analyze the data to study the effect of various factors on the studied characteristics, according to a completely randomized design (CRD), and the significant differences between the means were compared with the least significant difference test (L.S.D.) at the level of significance ($P \leq 0.05$).[41]

3 - Results and Discussion
Table No. (1) shows the Lycopene percentage extracted from tomato waste using three extraction methods. The results showed that (T 1) was better than (T 3) and (T 2) in extracting lycopene dye, as the Lycopene percentage extracted in (T 1) 25.7 mg / 100 gm residue and 185.6 mg / 100 gm residues on the basis of wet and dry weights, respectively, while the Lycopene percentage extracted in (T 2) was 17.8 mg / 100 gm residues and 174.8 mg / 100 gm residues on the basis of wet and dry weight, respectively, and reached 11.5 mg / 100 gm waste and 165.5 mg / 100 gm waste in (T 3) on the basis of wet and dry weights respectively.The results of the statistical analysis showed that there were significant differences at a significant level (0.05) between (T1) and T (2) and (3) in the Lycopene percentage extracted from tomato waste on the basis of wet and dry weight.

Table 1. The Lycopene percentage extracted from dried tomato waste using or three different mixtures of solvents.

Sequencing	Solvents	Lycopene percentage extracted mg/100g of waste	
		wet weight	dry weight
1	T (1)	25.7	185.6
2	T (2)	17.3	174.8
3	T (3)	11.5	165.5
4	Values LSD	*3.44	14.92*

Average of Three Replicates
The reason may be due to the efficiency of (T 1) in extracting the dye due to the presence of acetone and ethyl alcohol, which are compounds with high polarity that synergize in the process of penetration into the cellulosic tissues and their ability to mix with water after reaching the chromoplasts. Hexane is also characterized by its low polarity, which helps in dissolving Lycopene dye because it is a low-polar and hydrophobic compound [25]. He showed that the triple system consisting of acetone, ethanol, and hexane, and in the proportions used, led to an increase in the productivity of lycopene extraction by 95% and a high percentage of lycopene. Also, the presence of two The highly polar compounds acetone and ethanol are synergistically better than the presence of one compound with a high polarity, which is acetone, which allows greater penetration of the solvent mixture into the cellulosic tissues of tomato waste, and the dye lycopene is more soluble in hexane than petroleum ether and ethyl lactate.As between [26,27] that the Lycopene percentage extracted from tomato waste and offal depends on the extraction techniques used in addition to the choice of solvent. It is suitable and similar to the polarity of the compound to be extracted. Lycopene is low polar, which is suitable for non-polar solvents such as hexane, petroleum ether and ethyl lactate. It is also necessary to improve a number of conditions from the ratio of the solvent to the matrix, the temperature and the time of extraction with mechanical stirring and to avoid high temperatures due to its sensitivity. Lycopene for a high temperature [25].
In a study conducted by [27] , it was found that the mixture consisting of acetone, ethanol, and

hexane, in ratios (2:1:1), respectively, has high efficiency in extracting carotenoids from tomato waste, as it was able to obtain 14.9 mg / 100 g waste, which is less than the Lycopene percentage extracted. with the same solvents in the current study, which amounted to 185.6 mg / 100 gm waste, and the reason may be due to genetic differences between tomato varieties in their pigment content [28,29] and the product may be subjected to industrial processes such as heating and drying before extraction processes, all of which lead to a difference in the amount of pigments obtained. [30] and in another study, [31], it was found that the mixture of dichloromethane - methyl alcohol at a ratio of (2:1), respectively, is much better than the mixture of acetone - hexane at a ratio of (1:1) on Whereas [32,33] concluded using a mixture of methyl alcohol - carbon tetrachloride at a ratio of (6:2), respectively, to extract carotenoids from tomatoes with high efficiency.In a study conducted by [21], they showed that drying tomatoes Hot air gave the highest Lycopene percentage compared to the lyophilization method, provided that the drying temperature did not exceed 120 o C. The hot air heating process increased the bioavailability of lycopene and the percentage of total phenols and total flavonoids extracted, which increases its antioxidant activity [34]. [35,36] showed the effect of different lycopene extraction methods on the Lycopene percentage extracted from tomatoes, and it ranged from 5 mg to 100 mg / 100 g waste, according to the extraction method.

Table (2) shows the Lycopene percentage extracted from tomato waste and its juice, and the whole tomato puree consisting of waste and juice, as the highest amount of dye extracted from tomato waste was 155.7 mg / 100 g waste on the basis of dry weight and 14.6 mg / 100 g waste on the basis of wet weight. The Lycopene percentage extracted from tomato puree was 93.5 mg/100 residue on dry weight basis and 6.2 mg/100g residue on wet weight basis. While the amount of dye in tomato juice was 48.9 mg/100g waste and 3.4 mg/100g waste based on dry and wet weight, respectively. Table (2) showed that there were significant differences at the level of 0.05 between tomato waste, puree and juice, on the basis of dry and wet weight. It was noted that the highest Lycopene percentage was in the tomato waste sample due to the high proportion of solids in it, and this is consistent with what was mentioned by [37]. Where it was found that the tomato waste taken from the wastes of tomato processing plants contained a higher Lycopene percentage than the whole tomato fruits, as it gave 12.3 mg/100g compared to 3.5 mg dye/100g in the whole fruits and on the basis of fresh weight.

Table 2. Comparison of quantities of lycopene extracted from tomato waste, puree and juice.

Sequencing	**Sample type**	**Lycopene percentagebased on dry weight mg / 100g waste**	**Lycopene percentage based on wet weight mg/100g waste**
1	Tomato waste	155.7	14.6
2	Tomato puree	93.5	6.2
3	Tomato juice	48.9	3.4
4	Values LSD	*17,295	*2,106

[29], found that Lycopene percentage in the juice amounted to 15.9 mg / 100 gm on the basis of wet weight. The higher Lycopene percentage in the current study than what was mentioned in those sources was explained by the genetic differences between tomato varieties, the degree of maturity of the fruits, and the difference in Environmental conditions, storage and handling conditions [32].

Table No. (3) shows the Lycopene percentage extracted from tomato waste dried under the sun, as the estimated remaining moisture percentage for samples dried for one, two, and three days was (0.4, 0.6, 0.7), respectively. The Lycopene percentage extracted from tomato waste for samples dried under sunlight for one, two and three days were (73.5, 45.8 and 120.6) mg / 100g residues, respectively. The results of the statistical analysis showed that there were no significant differences in the Lycopene percentage on the basis of weight. Wet at a significant level of 0.05. When following up the results, it was observed that the longer the duration of sun drying, the greater Lycopene percentage extracted, since the content of carotenoids in tomatoes, especially lycopene, increases with the increase in fruit ripening. Carotenoids in tomato and this is confirmed by [38].

Table 3. Shows the Lycopene percentage extracted from tomato waste dried under the sun for several days.

	Sample	Residual Humidity rate %	The Lycopene percentage based on dry weight (mg /100 g of waste)
1	Sun dried for one day	0.7	45.8
2	Sun dried for two days	0.6	73.5
3	Sun dried for three days	0.4	120.6
4	Values LSD	0.3.6 N.S	*16,835

As [39] explained, the ripening enzymes such as Pectinesteras, Cellulase, Polygalacturans continue to be effective even after harvesting the fruits and cause the cell walls to decompose, which helps in the liberation of the dye and its ease of extraction with solvents.

Table No. (4) also showed the effect of different temperatures of drying tomato waste with an electric dryer on the Lycopene percentage. The percentage of moisture remaining in the samples was estimated after drying them at temperatures (60,50 and 70) o, and they were (0.8, 1.2, 1.9). %Straight. The Lycopene percentage extracted from samples of dried tomato waste at temperatures (60.50 and 70) °C was estimated to be (137.8.165.4 and 104.9) mg/100g residue based on dry weight, respectively. The results of the statistical analysis showed a significant superiority at the level of 0.05. For treatment (1) in terms of the amount of lycopene extracted at a temperature of 50°C on each of the (T2) and (3). By comparing the results, it is clear that the temperature of 50°C is the best drying temperature with an electric dryer in terms of the Lycopene percentage and its stability against transformation and structural change [40,41]. The reason may be due to the fact that this temperature is an assisting factor in the extraction of the dye with the presence of ripening enzymes, which play an important role in breaking down the cell walls and facilitating the exit of the dyes. Studies also indicated that these enzymes continue to be effective even after the harvesting process. The fruits can be transferred to the tomato waste, especially the waste, as it works on the decomposition of the cell walls and the release of pigments, as the temperature of 50°C is considered the optimum in the activity of these enzymes [39].

Table 4. Effect of different temperatures of electric drying on the Lycopene percentage.

Sequencing	Sample	Humidity rate %	Lycopene percentage is mg/100 g of waste for 36 hours
1	dryer sample in50g	1.9	165.4
2	dryer sample in60g	1.2	137.8
3	dryer sample in70g	0.8	104.9
4	Values LSD	*0.447	*22,051

Conclusions

It is possible to take advantage of the remnants of tomato factories in the production of lycopene dye, and adopt the method of extracting it with the solvents referred to in (T1) as it was distinguished from the rest of the treatments by the high Lycopene percentage. The study indicated that the solar and mechanical drying process gave a high extraction rate compared to the undried samples.

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