Bionatura
Latin American Journal of Biotechnology and Life Sciences

Bionatura Issue 4 Vol 8 No 1 2023

Article

Some characteristics of tomato lycopene and the possibility of its use in fresh milk fortification

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Available from: http://dx.doi.org/10.21931/RB/CSS/2023.08.04.101

ABSTRACT

The results showed that lycopene dye was highly effective in reducing the peroxide number value, as adding 1 mg of the dye to fresh milk led to the lowest peroxide number (0.497 - 2.483) compared to (0.543 - 4.943) of milk with artificial antioxidant BHT and milk without addition, which scored (0.567 - 11.997) for eleven days of storage at 4 °C. Lycopene dye reduced the values of thiobarbituric acid (TBA) in milk stored at 4°C for seven days compared to regular milk without addition. The results also proved that the dry lycopene dye extracted from the fruits of the tomato plant, when dissolved in buffer solutions with different pHs ranging from (3-8), did not have a significant effect on the dyes, as the pH factor on the measured wavelengths ranged from 370.0-370.5 As for the effect of the number of days factor, it ranged from 369.7-370.6 nanometers. It was found that low temperatures did not affect the lycopene pigment extracted from tomato fruits. The results of the sensory evaluation indicate that the total sum of sensory evaluation scores (taste, texture, and appearance) was higher (23.8) in the treatment of lycopene-free milk compared to the milk fortified with lycopene tomato, which scored 22.6.

Keywords: plant pigments, lycopene, fresh milk

INTRODUCTION

Lycopene is the primary source of all animal carotenoids, responsible for the bright colors of most fruits such as pineapple, orange, lemon, grapes, tomatoes, paprika, carrots, beetroot and many flowers—marine fish such as shrimp and salmon [1]. Lycopene is one of the most abundant carotenoids in ripe tomatoes. It contains approximately 80-90% pigments in the other carotenoids (a-carotene, beta-carotene, lutein, and beta-cryptoxanthin (b-cryptoxanthin) [2]. Tomatoes contain about 3-5 mg of lycopene per 100 g of raw material [3].

Among other carotenoids, lycopene has an antioxidant property. It melts at room temperature as vegetable oil. It tends to shrink and precipitate in aquatic systems and become crystalline, a property that makes it less accessible to the body. Lycopene is present as long, needle-shaped crystals in ripe tomatoes. Lycopene is stable in plant tissues or its solid form but becomes completely unstable after tissue extraction and dissolves in non-polar organic solvents [4]. Natural

antioxidants such as lycopene have gained great importance in reducing the oxidation of fats, oils and foods [5].

Several factors, including mechanical processing, heat treatment, and lipid addition [6], influence the stability and biological accessibility of lycopene. Lycopene is also affected by nutritional content as a fat-soluble substance; its consumption with high-fat food sources increases the bioavailability of lycopene [7]. Studies have shown that the bioavailability of lycopene in heat-treated tomatoes is increased compared to fresh tomatoes, and field-grown tomatoes contain higher levels of lycopene compared to greenhouse tomatoes [8].

MATERIALS AND METHODS

The study included the extraction of lycopene from grapefruit according to the method mentioned by [9] to obtain lycopene dye from dried grapefruit at a temperature of 70 °C. 1 g of dried grapefruit powder was taken and mixed with 10 ml of solvent mixture (acetone:hexane: ethyl alcohol) in a ratio of 1:2:1 and mixed in a vortex shaker (Vortex) for ten minutes. Then, 1.5 ml of distilled water was added to the mixture to separate the hexane layer from the acetone and ethyl alcohol layer and mixed for five minutes. Then, the top layer containing the dye lycopene was pulled out and kept in opaque containers.

The resulting pigment concentration in the samples was evaluated using HPLC as per the Macherey-Nagel (mn) Reversed Phase HPLC Application Guide. 0.01 g of all standard materials and samples were weighed. Test Standard was dissolved in a volumetric vial of 20 ml capacity using the mobile phase solution (Acetonitrile + H2O) (5: 95) %. 1 ml of the resulting volume was withdrawn and diluted to 20 ml in a volumetric vial using the same Mobile phase solution to achieve a final concentration of 0.025 g/mL (25ppm). DPPH was tested (Pellegrini et al., 1999)[10] for evaluating the efficiency of lycopene pigments in removing free radicals. It was used at 3 concentrations (0.25, 0.5, 1) mg, and the best concentration in inhibiting free radicals was determined.

Determination of the peroxide value and Thiobarbituric acid (TBA)

The value of peroxide in g was determined for samples of milk fortified with lycopene dye and for milk free of additives and kept cryopreserved at a temperature of 4 C according to the method used by [11]. The value of thiobarbituric acid (TBA) was also determined in 10 ml of a milk sample treated with lycopene dye extracted from tomatoes [12]. The absorbance was measured by a spectrophotometer at a wavelength (538 nm).

The effect of pH in the extracted lycopene pigments was also studied. Buffer solutions of different pH values 3, 4, 5, 6, 7 and 8 pH were used, each dissolved in 20 Tween at 3% [12], in addition to studying the effect of temperature on the dry extracted lycopene dyes dissolved in petroleum ether. The dye was exposed to temperatures (0,25,50) C for an hour and two hours, and the effect of temperatures (0,25,50,75,100) C for an hour and two hours on dry dyes was studied [13].

Sensory evaluation of milk fortified with lycopene 1 mg/100 ml was conducted by ten specialists in the Department of Food Sciences, Department of Life Sciences and Department of Chemistry. Sensory evaluation forms for milk were used for flavor, appearance and texture [14].

RESULTS

The percentage of lycopene pigment in grapefruit

The results showed an increase in the amount of lycopene extracted from grapefruit fruits using a mixture of solvents (acetone: hexane: ethyl alcohol) at a ratio of 1:2:1. The amount of lycopene extracted was 281.08 mg/100 gm.

Sample	Extract quantity (mg/100g)
Tomato fruits	**281.08**

Table 1. The percentage of pigment extraction from grapefruit

Diagnosis of lycopene extracted from plants using HPLC.

The results of (Table 2) analysis of lycopene pigment extracted using HPLC showed the possibility of determining the concentration of lycopene pigment by comparing the properties of the absorption spectrum and retention time inside the column (Time Retention) Rt with a standard ready-made lycopene dye as shown in Figure (1).

It is also evident from the above Table that lycopene dye extracted with acetone from tomato recorded a retention time of Rt of 2.065 and an area peak of 1436464 compared to the standard lycopene in tomato, which gave a retention time of Rt of 2.078 and a peak of Area 1769466, where the concentration of lycopene dye extracted from tomato plants reached 80.18%.

Sample	Pigment type	Ret. Time	Area	Concentration%
Tomato fruit	Standard	2.078	1769466	80.18%
	Extract	2.065	1436464	

Table 2. Quantitative and qualitative content of lycopene in tomato fruit measured by HPLC technique.

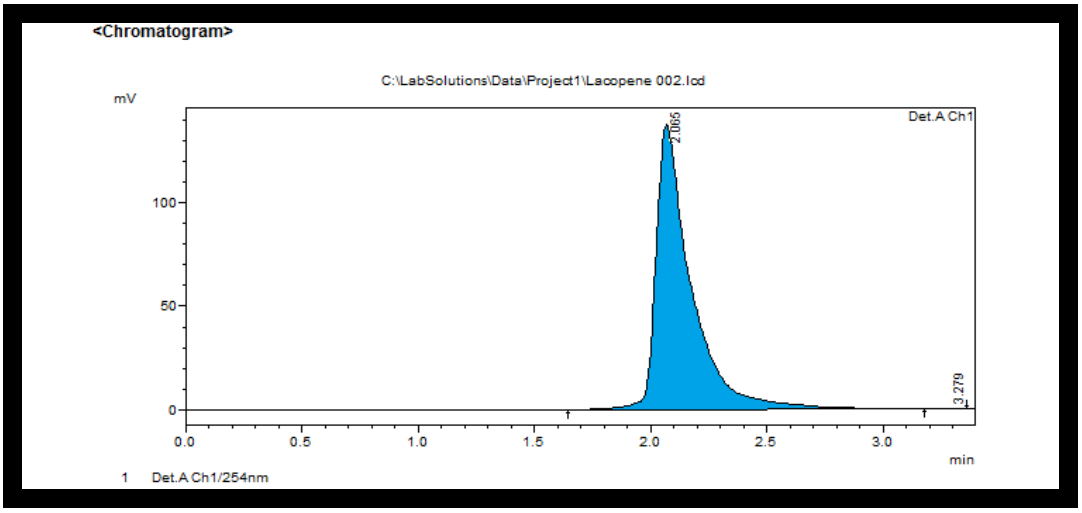

Figure 1 HPLC results for Identification of lycopene pigment extracted from tomato fruit.

The DPPH method was used to determine the antioxidant compounds in grapefruit lycopene, which showed the effectiveness of different concentrations of lycopene in inhibiting free radical DPPH. Lycopene recorded an inhibition or inhibition rate of 67.08%. The highest percentage of free radical inhibition was at the concentration of 1 mg (80.08%) compared with concentrations of 0.5 and 0.25 mg, which recorded 69.43% and 60.94%, respectively. Studies have demonstrated that lycopene stability and biological accessibility are affected by several factors, including mechanical processing, heat treatment, and lipid addition [6]. The study's results agree with [17], who found that the % of antioxidants in lycopene extracted from tomatoes was 80.508%. [18] obtained the antioxidant activity of lycopene extracted from tomato plants with a percentage of 87.7-99.7 % when using a concentration higher than 4 mg.

Treatments	Inhibition rate at different conc. (µg)			Average
	0.25	0.5	1	
Tomato fruit lycopene	60.94 a	69.43 D	80.02 g	70.13

Table 3. Results of DPPH assay for lycopene pigment extracted from tomato fruit. * Means followed by different letters are significantly different, according to Duncan's multiple-range test

Effect of lycopene extracted from tomato fruits on the peroxide value (PV)

The results showed that the lycopene extracted from tomato plants added to the milk (T1) led to a higher reduction in peroxide value than the milk sample fortified with the artificial antioxidant BHT (T2) and the additive-free milk (TC). The (T1) recorded the lowest peroxide number (0.497-2.483) mEq/kg fat, while the sample of milk fortified with artificial antioxidant BHT recorded (0.543-4.943) mEq/kg fat compared to (0.567-11.997) mEq/kg fat. In the sample of milk free of additives (TC).

treatments	Day1	Day3	Day5	Day7	Day9	Day11	Average
TC	0.567 ab	1.833 e	3.983 j	6.913 l	9.783 m	11.997 n	5.846 d
T1	0.497 a	0.61 ab	1.09 c	1.13 c	1.88 ef	2.483 h	1.282 a
T2	0.543 ab	0.633 ab	1.977 efg	2.713 i	3.987 J	4.943 k	2.466 c
تأثير الايام	0.525 a	0.88 b	1.903 c	2.677 d	3.946 e	4.949 f	-------

Table4. Effect of lycopene extracted from tomato fruits on the peroxide value (mEq/kg fat) in treated milk * Means followed by different letters are significantly different, according to Duncan's multiple range test. T0, T1, and T2 represent additive-free milk, milk fortified with lycopene, and milk fortified with BHT (artificial antioxidant), respectively.

Regarding the effect of adding lycopene dye on the values of thiobarbituric acid (TBA) for treatments of milk stored in refrigeration at 4 C for periods of 7,5,3.1 days, the results (Table 5) showed that the treatment of the milk sample fortified with lycopene dye in tomato led to a significant reduction in the values of lycopene dye.

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treatments	Day1	Day3	Day5	Day7	Average
TC	0.015 bc	0.033 I	0.041 J	0.049 k	0.0345 C
T1	0.013 a	0.015 Bc	0.018 Ef	0.020 gh	0.0165 A
Average	0.0140	0.024	0.0295	0.0345	---------

Table 5. Effect of adding lycopene to milk on the values of thiobarbituric acid (TBA) mg Malone Aldehyde/kg fat

It is noted from the results (Table 6) that pH 3-8 did not have a significant effect on the lycopene pigment extracted from tomato fruits. In general, the effect of the pH factor on the measured wavelengths of lycopene dye ranged from 370-370.5 nm. The wavelength values increased with increasing days on lycopene extract at different pH. On the second day, pH (6) recorded the highest wavelength as it reached 372 nm, a significant difference from pH (7) on the first day with the lowest wavelength of 369.0 nm. This is consistent with the findings of Soumia et al. (2019)[23] when they extracted lycopene from tomato peels for use as an antioxidant and a natural colorant in Beef Burgers. The effect of pH values (2-10) was determined, and it was shown that the decrease in pH values (7-10) caused a slight change in the content of lycopene pigment, and there was no significant change in lycopene content at 6 pH. In comparison, lycopene pigment was more stable at 7 pH. Also, [24] found that the highest levels of stability and the lowest levels of degradation of carotenoids extracted from tomato peels were in the alkaline medium (7-10) pH. Lycopene extracted from tomato plants is stable in an acidic medium and more stable in an alkaline medium. This is due to the double bonding system linked to carotenoids that cause carotenoids to be tampered with and unstable when exposed to light, high heat, oxygen and acids [25].

Ph	Day1	Day2	Day3	Day4	Average
3	370 abc	370.0 abc	370.7 abcd	369.3 ab	**370 a**
4	372.0 d	370.0 abc	370.0 abc	370.0 abc	**370.5 a**
5	370.7 abcd	371.0 bcd	370.7 abcd	369.3 ab	**370.4 a**
6	370.0 abc	372.0 d	370.0 abc	370.0 abc	**370.5 a**
7	369.0 a	370.0 abc	371.3 cd	369.7 abc	**370 a**
8	370.0 abc	370.0 abc	370.7 abcd	370.0 abc	**370.2 a**
Average	**369.7 a**	**370.3 ab**	**370.5 b**	**370.6 b**	--------

Table 6. Effect of solvent pH on lycopene pigment extracted from tomatoes stored for 4 days *Means followed by different letters are significantly different, according to Duncan's multiple range test.

Effect of temperature on lycopene pigment extracted from grapefruit

The results and Table (7) show the studied interaction of dry dyes for lycopene dye extracted from tomatoes. The highest significant effect with absorbance amounted to (1.20 nm) was exposed for one or two hours at a temperature of 0 C compared with the lowest absorbance value recorded in the second hour when the dyes were exposed to a temperature of 0° C. 100°C, which recorded 0.42 nanometers. [26] found that when temperatures (90,70,45) C were applied to tomato lycopene, high temperatures recorded small amounts of lycopene extracted while lower temperatures achieved greater amounts of lycopene.

	Pigment %			Average
	Temp.	1 h	2 h	
Ether-dissolved Pigments	0 C	1.20 a	1.20 a	1.20 a
	25 C	1.10 ab	0.98 bc	1.02 b
	50 C	1.00 bc	0.91 c	0.93 b
	Average	1.10 a	1.00 a	
Dry Pigments	0C	1.20 a	1.20 a	1.20 a
	25C	1.07 b	0.96 c	1.01 b
	50C	1.0 bc	0.90 cd	0.95 b
	75C	0.79 d	0.70 e	0.75 c
	100C	0.56 e	0.42 f	0.49 d
	Average	0.92 a	0.84 a	--------

Table 7. Effect of temperature and exposure period on soluble and dry lycopene extracted from tomato fruit.* Means followed by different letters are significantly different, according to Duncan's multiple range test.

Sensory evaluation

The results of the sensory evaluation (Table 8) showed that the flavor scores of the additive-free milk 7.80 were higher than the milk fortified with lycopene dye extracted from tomatoes 7.30 with a slight difference. Also, the appearance trait points for regular milk were higher and slightly different than those recorded for the lycopene-treated milk of tomato. The gradation of the previous scale was used to indicate the acceptability of the consistency of milk fortified with lycopene extract. Texture values did not differ between milk in both treatments. The results indicate that the total sum of sensory evaluation scores (taste, texture and appearance) was the highest for regular milk without additives (23.8), with a significant difference from the total points of generalized milk with lycopene of tomato (Table 8).

Treatments	Taste / of 8	Appearance /of 8	Texture/of 8	Total/of 24
Plain milk	7.80 b	8.00 b	8.00 a	23.8 C
Milk+lycho pin	7.30 a	7.35 a	7.95 a	22.6 A

Table 8. Sensory evaluation of some characteristics of milk fortified with lycopene extracted from tomatoes.

DISCUSSION

The amount of lycopene extracted was sufficient. This may be due to the efficiency of the solvents used in the extraction process and their penetration deep into plant tissues, reaching the chromoplasts [15].

The ability of a solvent or a mixture of solvents to extract lycopene is affected by several factors, including the nature and type of plant from which the dye is extracted. Also, the raw material under extraction was subjected to various manufacturing treatments before the solvent extraction process, including exposing the material to different temperatures, cooling or drying [16].

The permissible limits for the value of the peroxide number in foods, in general, are 10 mEq/kg fat, as indicated by [19], where fats and oils become unacceptable for human consumption when the PV value exceeds 10 mEq / This was confirmed by the Iraqi Standard for Standardization and Quality Control [20], which indicated the same value.

Thiobarbituric acid (TBA) compared to other treatments. The value of thiobarbituric acid increased significantly with the increase in the number of days. The lowest value of thiobarbituric acid was recorded on the first day and increased to reach the highest value on the seventh day. This confirms previous results [21] that oils are rancid and are not acceptable for human consumption when TBA values exceed 0.2 mg Malone Aldehyde/kg fat. The values of thiobarbituric acid were recorded as 0.021 mg malondehyde/kg fat on the first day and 0.034 mg malondehyde/kg lipid on the seventh when milk was fortified with whey protein concentrate and iron [22].

In a study of the physical and chemical properties of lycopene extracted from tomatoes[25], it was found that lycopene exposure to high temperatures leads to a significant decrease in lycopene content, as 26.1% and 35% of lycopene were lost when heated for a while. Three hours at 65°C and 100°C, respectively, which led to the discoloration of the lycopene pigment.

CONCLUSIONS

The study concludes by extracting lycopene pigment from tomato fruits, 2- Supporting lycopene-free foods, and 3- Studying the qualitative characteristics of lycopene pigment added to raw milk.

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Received: May 15, 2023/ Accepted: June 10, 2023 / Published: June 15, 2023
Citation: Adnan WH Al-Mudhafar and Ahmed A-R J Alikhan**.** Some characteristics of tomato lycopene and the possibility of its use in fresh milk fortification. Revista Bionatura 2023;8 (2) 63. http://dx.doi.org/10.21931/RB/CSS/2023.08.04.101