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Article

Evaluation of some qualitative properties of lycopene extracted from grapefruit

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ABSTRACT

The study was conducted to extract and quantify the amount of lycopene pigment in grapefruit and to evaluate the efficiency of different pigment concentrations as an antioxidant factor in inhibiting free radicals. The study also included evaluating the effect of pH and different temperatures on the dye's effectiveness and the efficiency of the extracted lycopene in inhibiting pathogenic bacteria. The results showed that the percentage of lycopene was 231.02 mg / 100 g, extracted with alcoholic solvents from the fruits of the grapefruit plant. Lycopene dye extracted using HPLC was diagnosed with a concentration of 76.24%. The results of the DPPH test using Elisa showed the possibility of inhibiting free radicals with lycopene, and the highest percentage of inhibition was 65.74% using 1 mg, with a significant difference from the percentage of inhibition 63.21% and 58.54% resulting from the dye at concentrations of 0.5 mg and 0.25 mg, respectively. As for the pH (3-8) of the buffer solutions, they had no clear effect on the concentration of the dye.

Keywords: plant pigments, food spoilage, pathogenic bacteria, citrus.

INTRODUCTION

Lycopene is a red pigment found abundantly in red fruits and vegetables, especially in grapefruits, carrots, tomatoes, and some other plants' fruits. It is one of more than 600 types of carotenoids found in nature (Cadoni et al., 2000)[1]. Lycopene is a hydrocarbon. Polyene contains 40 carbon and 56 hydrogen atoms and is completely soluble in chloroform and ether due to its lipophilic properties (Van and Pajkovic., 2008)[2]. Although lycopene has been used as a natural food coloring for many years, lycopene has recently attracted significant interest as a medicinal ingredient (Ananthanarayan and Choudhari, 2007)[3]. This dye is commonly accepted in the food industry as a food additive for its health benefits as a coloring agent and a natural antioxidant (Rao and Argawal, 1999)[4].

In vitro studies have shown that lycopene is one of the most effective carotenoid antioxidants due to its high antioxidant activity, having a higher free radical scavenging activity than b-carotene and a-tocopherol (Miller et al., 1996)[5]. Lycopene is strongly influenced by nutritional content, and because it is a fat-soluble substance, its consumption of high-fat food sources increases the bioavailability of

lycopene (Ghadage et al., 2019)[6]. The carotenoids found in the fruits of the grapefruit plant, including lycopene, are pigments derived from isoprenoids responsible for the coloring of citrus fruits, which show critical antioxidant activity. They can expel reactive oxygen species generated during stressful conditions as they act primarily as inhibitors of the effect of oxygen, and therefore, carotene contents in plant tissues may affect the system balance as antioxidants (Mascio et al., 1992 Di)[7]. Lycopene is also used as a dietary supplement. Dietary supplements, food additives, pharmaceuticals and cosmetics are considered as bioactive compounds derived from plants Liu et al., 2019[8]. Therefore, this study aimed to i) Extract lycopene dye from grapefruits, ii) Use lycopene dye to inhibit food spoilage microorganisms, and ii) Study the qualitative characteristics of extracted lycopene.

MATERIALS AND METHODS

The study included the extraction of lycopene from grapefruit according to the method mentioned by Thompson et al. (2000)[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 dye concentration in the samples was evaluated using HPLC 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. The study also evaluated the inhibitory activity of lycopene dyes against food-spoilage pathogenic bacteria (Salmonella, E.Coli and Staphylococcus) using the agar well diffusion method. The turbidity was measured at 37°C for 8 hours to obtain a specific number of microorganisms (1.5 x 108) cells/ml Densi Chek. Valgas et al., 2007)[11].

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% (AOAC,1990)[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 (Al-Sheikhly. 2005)[13].

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 231.02 mg/100 gm. This amount of lycopene extracted is good. This may be due to the solvents' efficiency in the extraction process and their penetration deep into plant tissues, reaching the chromoplasts [14].

Diagnosis of lycopene extracted from plants using HPLC.

The results of (Table 1) 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 grapefruit recorded a retention time of Rt of 2.391 and an area peak of 3154987 compared to the standard lycopene dye of grapefruit, which gave a retention time of Rt of 2.390 and a peak of Area 4137693, where the concentration of lycopene dye extracted from grapefruit plant reached 76.24%.

Sample	Pigment type	Ret. Time	Area	Concentration%
Grapefruit	Standard	2.390	4137693	76.24%
	Extract	2.391	3154987	

Table 1. Quantitative and qualitative content of lycopene in grapefruit measured by HPLC technique.

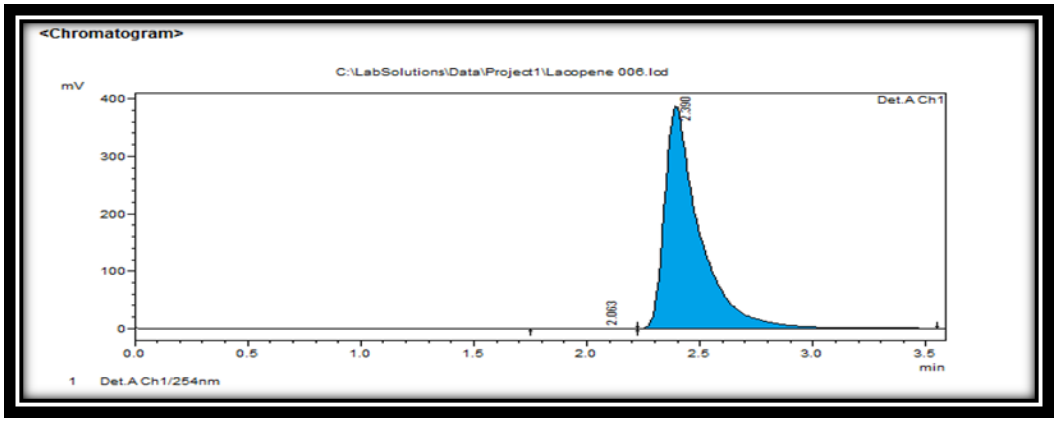

Figure1. HPLC results for Identification of lycopene pigment extracted from grapefruit.

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 (75.47%) compared with concentrations of 0.5 and 0.25 mg, which recorded 63.21% and 58.54%, respectively.

Treatments	Inhibition rate at different conc. (µg)			Average
	0.25	0.5	1	
Grapefruit lycopene	58.54 A	63.21 d	75.47 g	65.74

Table 2. Results of DPPH assay for lycopene pigment extracted from grapefruit.* Means followed by different letters are significantly different, according to Duncan's multiple range test.

Effect of pH on lycopene pigment extracted from grapefruit

The results in Table (4) show a significant effect of pH on the wavelengths of lycopene pigment extracted from grapefruit fruits. At 4 pH, the highest wavelength was 368.1 nm compared to pH 6 and 7, which recorded wavelength values of 367.4 and 367.5 nm, respectively.

Treatments	**wavelengths of lycopene pigment at different storage periods**				
Ph	Day 1	Day 2	Day 3	Day 4	**Average**
3	367.3 bcd	368.3 efg	368.0 def	367.3 bcd	**367.8 ab**
4	368.3 efg	368.3 efg	369.0g	366.7 ab	**368.1 b**
5	367.7 cde	367.3 bcd	368.7 fg	367 abc	**367.7 ab**
6	367.7 cde	367.3 bcd	368.3 efg	366.3 a	**367.4 a**
7	367.7 cde	367.3 bcd	368.3 efg	366.7 ab	**367.5 a**
8	368.7 fg	367.3 bcd	368.3 efg	366.7 ab	**367.8 ab**
Average	**367.9 b**	**367.7 b**	**368.4 c**	**366.8 a**	

Table 3. Effect of pH and storage period on grapefruit lycopene depending on the pigment wavelength. Duncan's multiple range test, authors should* Means followed by different letters are significantly different.

Effect of temperature on lycopene pigment extracted from grapefruit

The results of Table (5) indicate that increasing the temperature decreased the absorbance value of the tested dyes, regardless of the type of solvent. In general, the absorbance of the dry dyes was higher than that of the dyes extracted with petroleum ether when exposed to the same temperature and for the same period. The dry dyes significantly mediated their absorbance at 50°C compared to the rest of the dyes' concentrations measured at other temperatures.

	Pigment %			Average
	Temp.	**1 h**	**2 h**	
Ether-dissolved Pigments	**0 C**	1.03 a	1.00 a	**1.01 a**
	25 C	0.93 ab	0.87 bc	**0.90 b**
	50 C	0.83 bc	0.77 c	**0.80 c**

	Average	**0.93 a**	**0.88 a**	
Dry Pigments	**0C**	1.00 a	1.00 a	**1.00 a**
	25C	0.97 ab	0.87 bc	**0.92 a**
	50C	0.83 cd	0.77 cd	**0.80 b**
	75C	0.73 d	0.67 e	**0.70 c**
	100C	0.47 e	0.33 f	**0.40 d**
	Average	**0.80 a**	**0.73 a**	

Table 4. Effect of temperature and exposure period on soluble and dry lycopene extracted from grapefruit.

Bioassay

The results showed that the lycopene dye extracted from the fruits of the grapefruit plant showed a clear ability to inhibit food-destroying pathogenic bacteria. Lycopene dye inhibited Salmonella bacteria with an inhibitory area of 24.17 mm diameter and Escherichia coli with a diameter of 22.83 mm, while the inhibitory corona of Staphylococcus aureus was 22.33 mm in diameter. The results of [22] indicated that the lycopene pigment extracted from tomato had an inhibitory ability against Bacillus cereus with an inhibitory area of 15.3 mm and E.coli with a penetration area with a diameter of (9 to -9.6 mm and against S.typhi with an inhibition diameter of 8.6 mm). Lycopene is an inhibitor of microorganisms, and this is because the hydroxyl group produced by antibiotics leads to a significant degradation of cell components such as DNA, lipids, and proteins.

Food spoilage pathogenic bacteria	**Inhibition zone dia. (mm)**
Salmonella	**24.17cd**
Escherichia coli	**22.83ab**
Staphylococcus aureus	**22.33a**

Table 5. Effect of 1 mg of grapefruit lycopene on pathogenic bacteria based on Inhibition zone diameter

DISCUSSION

[15] obtained lycopene at a concentration of 98.6% from tomato paste and 96.3% from grapefruit. Also, lycopene was obtained at 87-99% from red watermelon and 82-87% for tomatoes when applying UV-vis-HPLC technology to detect lycopene and carotene in vegetables [16].

Studies have demonstrated that lycopene stability and biological accessibility are affected by several factors, including mechanical processing, heat treatment, and lipid addition [8].

In vitro studies have also shown that lycopene is one of the most effective carotenoid antioxidants, with a higher free radical scavenging activity than b-carotene and a-tocopherol (Miller et al., 1996)[5]. The study's results agree with [17], who found that the percentage of antioxidants in lycopene extracted from tomatoes was 80.508%.

The highest wavelengths were recorded in lycopene after three days compared to the higher and lower number of days. This is consistent with a previous study [18] that confirmed the stability of beta-carotene at pH 2.3-8.0 in solutions kept in glass containers. [13] also showed that the stability of lycopene pigment extracted from tomato residues was at a pH ranging from pH3 to 8.

The results indicate that the optimum temperature for preserving lycopene pigment is 0°C. When the lycopene dye is exposed to high temperatures, it leads to anagrams in the dye [19]. [20] indicated that the lycopene concentration does not change when exposed to temperatures less than 70 °C in the absence of adding oil during the manufacturing processes and that the lycopene manufactured in emulsions is destroyed by 25% when exposed to a temperature of less than 70 °C. Another study indicated that dissolving lycopene in canola oil and heating the samples to (100-180) C or increasing the duration of exposure to heat treatment led to an increase in the decomposition of lycopene compared to exposing them to a temperature of 25 C [21].

Cell death occurs mostly by certain oxidation of Guanine (nucleotide nucleotide), where double-stranded DNA forms, then fragmentation and cells die, [23] and [24]. It has been shown that lycopene causes DNA damage in Escherichia coli cells [25].

CONCLUSIONS

The study's results recorded that low temperatures did not affect the lycopene pigment extracted from grapefruit. Lycopene dye extracted from grapefruit fruits effectively inhibited food-damaging pathogenic bacteria salmonella, Escherichia coli and Staphylococcus aureus. The pigment produced an inhibitory zone with a 22.33-24.17 mm diameter against the tested bacteria.

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