



Optimization of extraction of lycopene from carrot and determination of its antioxidant activity

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Abstract

Background: Lycopene is a carotenoid which is abundant in mature red plant fruits, especially in tomato, carrot and watermelon. This study investigated lycopene extraction from carrots and its antioxidant properties. **Methods and results:** Through an orthogonal experiment ($L_9(3)^3$), lycopene extraction was optimized and its antioxidant capacity was assessed by DPPH, ABTS, and hydroxyl radical scavenging assay. The results showed that the maximum yield of lycopene was obtained when the extraction temperature, extraction time and solid-liquid ratio were 40°C, 125 min and 1:2 g/mL, and the influence on the extraction yield of lycopene decreased in the order: solid-liquid ratio > extraction time > extraction temperature. The antioxidant activity test showed that lycopene had strong scavenging ability on DPPH and ABTS free radicals, but poor scavenging ability on hydroxyl radicals. **Conclusion:** The maximum extraction yield of lycopene is 50.82 ± 0.18 µg/g. Lycopene has good antioxidant capacity. This study provides a theoretical framework and statistical support for the continued development of the lycopene from carrots, which is projected to be exploited as a natural antioxidant or health supplement in the food and cosmetics industries.

Keywords Lycopene · Organic solvent extraction · Orthogonal test design · Antioxidant activity · Carrot

Introduction

Vegetables provide essential nutrients in addition to a wide variety of bioactive phytochemicals, which may be necessary for optimum health and defense against chronic diseases [1, 2]. Carrot is a common vegetable in daily life and one of the top ten vegetable crops in the world. It is an annual or biennial herb that can be consumed both cooked and raw. China is the world's biggest producer of carrots, with a domestic carrot planting area of 40×10^4 hm² and

an output of 4000×10^4 t, mainly distributed in Fujian, Shandong, Inner Mongolia, Hebei, Anhui, Henan and other provinces. In recent years, vegetable juice has been widely accepted and consumed by different social classes around the world. In addition to refreshing taste and friendly color, they are rich in nutrition and energy, and also have antioxidant properties [3, 4]. Carrot is rich in carotenoids, polyphenols and flavonoids antioxidant components, which have a good performance in antioxidant capacity [5, 6].

Lycopene, a lipophilic carotenoid hydrocarbon pigment, can be found in pink, red and orange vegetables and fruits such as tomatoes, watermelons, carrots, grapes, peaches, and cranberries [7–9]. It is a non-pro-vitamin A carotenoid which has a straight-chain hydrocarbon with the chemical formula of $C_{40}H_{56}$ and a molecular weight of 536.85. It has 11 conjugated double bonds and 2 nonconjugated double bonds. Lycopene is regarded as an efficient anti-inflammatory and anti-proliferative compound along with its antioxidant, anti-inflammatory, and anti-proliferative capabilities [10, 11]. It has a strong singlet oxygen-quenching ability that is twice as powerful as beta-carotene and 100 times stronger than alpha-tocopherol in terms of physical quenching rate [12]. Several epidemiological and clinical studies have

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shown that lycopene exerts beneficial effects in preventing many diseases, such as cardiovascular disease [13, 14], cancer [15, 16], coronavirus disease 19 [17], nonalcoholic fatty liver disease [18] and diseases of the central nervous system like Parkinson's disease, Alzheimer's disease, epilepsy, depression, etc. [19–21]. Because of its strong antioxidant properties, lycopene is considered as a potential bioactive agent in the treatment of various disorders [22].

The extensive biological activity of lycopene has led more scholars to try to extract it from natural plants. Lycopene production has been the subject of research. Lycopene extraction techniques primarily include organic solvent extraction [23], ultrasound-assisted extraction [24], microwave-assisted extraction [25], supercritical fluid extraction [26], and pectinase assisted extraction [27]. It can also be a mixture of the above two or three methods to improve the extraction rate. Each extraction method has its own advantages and disadvantages. For example, the most common and commercial natural colorant extraction method is organic solvent extraction, but the need for a large number of organic solvents, the toxicity of solvents and the treatment of solvents have always been problems to be solved [28, 29]. In all extraction technologies currently available, supercritical fluid extraction produces the purest, cleanest, and safest product. However, its high cost and availability of instruments limits the application of this method [30]. In a word, only when considering a variety of factors such as environmental protection, economy and laboratory enforceability that the choice of method is the most appropriate method.

Lycopene can degrade under various extraction, production, and processing circumstances, since it is unstable to heat, light and oxygen [31]. According to the cultivar, processing technique, storage temperature and time, lycopene degradation changed throughout storage [32]. In addition to changing the color of processed foods, lycopene degradation also has an impact on their nutritional value and biological function. So appropriate extraction, production, and processing techniques should be chosen to increase lycopene's stability.

The objective of this research was to choose an optimal extraction method of lycopene from carrot, and to find out how lycopene yield was affected by extraction temperature, time, solid-liquid ratio, and pH. Lycopene extraction conditions were improved by using orthogonal test design. The antioxidant properties of lycopene were examined using DPPH, ABTS, and hydroxyl radical scavenging assay. This research provides a basis for the further development of lycopene as a natural antioxidant agent or health supplement.

Materials and methods

Carrot sample

From the local market of Hefei, China, premium carrots (*Daucus carota* var. *sativa* Hoffm.) were obtained. The carrots were washed and peeled, and the middle section of the carrots were cut into slices and ground to a paste. The carrot pastes were collected with a beaker, wrapped in tin paper and placed in a cool dark place for the further experiments.

Chemical and reagents

Lycopene standard was purchased from G-CLONE Biotechnology Co., Ltd. (Beijing, China). 2,2-diphenyl-1-picrylhydrazyl (DPPH), 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) were purchased from Sigma Aldrich (St. Louis, MO, USA). All other reagents were analytically pure and purchased from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China).

Selection of lycopene extraction solvent

To each beaker, accurately weighed 10.0 g carrot paste, then added 20 mL of chloroform, n-hexane, glacial acetic acid, ethyl acetate, anhydrous ethanol, and ethyl acetate : acetone (7:1) respectively to extract lycopene. All samples were extracted for 30 min in the dark in a 35°C water bath. The appropriate extraction solvent was used to dilute the filtrate five times. The absorbance at 503 nm was measured using a UV-5100B ultraviolet spectrophotometer (Shanghai Metash Instrument Co., Ltd., Shanghai, China) with the matching extraction agent used as a blank.

Organic solvent extraction

The effects of extraction temperature, extraction time, solid-liquid ratio, and pH on lycopene yield were investigated. Correctly weighed 10.0 g of carrot paste in a beaker, added the best extraction solvent and extracted it in a 35°C water bath without light. A 0.45- μ m nylon filter was used to filter the filtrate after it was diluted five times with ethyl acetate. The absorbance was evaluated at 503 nm using blank extraction reagent as a blank control.

Determination of lycopene

Precisely weighed the lycopene standard 12.5 mg, dissolved in ethyl acetate, constant volume to 250 mL, made of lycopene standard solution. The lycopene standard solution of 0.2, 0.4, 0.6, 0.8, 1.0, 1.2 mL was accurately measured and diluted to 10 mL with ethyl acetate, which was equivalent

to 1.0, 2.0, 3.0, 4.0, 5.0, 6.0 $\mu\text{g/mL}$ standard solution. Ethyl acetate was used as blank control to determine the absorbance and draw the standard curve of lycopene. The lycopene yields were determined with the formula as follows:

$$\text{Lycopene yield}(\mu\text{g/g}) = (C \times V \times N)/m \quad (1)$$

where C is the equivalent lycopene concentration in the sample determined by regression equation, V is the volume of the extraction solvent, N is the dilution factor, and m is the quality of the carrot used for extraction.

Optimization of lycopene extraction

By using an orthogonal experimental design with 3 factors and 3 levels, including extraction temperature ($^{\circ}\text{C}$, A), extraction time (min, B), and solid–liquid ratio (g/mL , C), lycopene extraction conditions were determined. The factors are shown in Table 1 and were determined by using the statistical program SPSS (SPSS for Windows version 20.0; SPSS Inc, Chicago, American.).

Determination of DPPH free radical scavenging ability

Samples' capacity to scavenge DPPH free radicals was assessed [33]. The carrot sample extract was prepared according to the optimal extraction conditions obtained by the above orthogonal test, and dissolved in acetone in a ratio of 1:1, 1:2, 1:4, 1:6 and 1:8 to obtain the test solution. In a tube, 4 mL of the test solution and 5 mL of 0.1 mM DPPH in ethanol were coupled. After a quick mixing period and 30 min of standing in the dark at room temperature, the absorbance at 517 nm was measured using an ultraviolet visible spectrophotometer. The positive control was ascorbic acid, while the blank was ethanol. The formula below was used to get the radical scavenging rate:

$$\text{Scavenging rate}(\%) = (1 - A_s/A_0) \times 100 \quad (2)$$

where A_s represented the sample's absorption and A_0 the absorbance of the blank control solution devoid of the sample.

Table 1 Orthogonal design factors and levels

Factors	Level		
	1	2	3
Extraction temperature (A , $^{\circ}\text{C}$)	30	35	40
Extraction time (B , min)	100	125	150
solid-liquid ratio (C , g/mL)	1:2	1:3	1:4

Determination of ABTS free radical scavenging ability

The ABTS stock solution was created by combining 10 mL of 7 mM ABTS solution with 5.0 mL of 7.35 mM $\text{K}_2\text{S}_2\text{O}_8$ solution, and letting the mixture remain at room temperature for 12 to 16 h. To achieve the desired absorbance of 0.7 (± 0.02) at 734 nm, the ABTS solution was diluted with ethanol for the study. The carrot sample extract was prepared according to the optimal extraction conditions obtained by the above orthogonal test, and dissolved in acetone in a ratio of 1:1, 1:2, 1:4, 1:6 and 1:8 to obtain the test solution. Appropriate amount of solvent should be taken as blank reading, and ascorbic acid as positive control. After adding 50 μL of the sample solution to 3 mL of the ABTS solution, the mixture was heated to 30°C for 10 min, and the absorbance was then calculated [34]. The formula as follows was used for estimating the proportion of ABTS inhibition:

$$\text{Scavenging rate}(\%) = (1 - A_s/A_0) \times 100 \quad (3)$$

where A_s marked the sample's absorbance and A_0 the absorbance of the blank control solution devoid of the sample.

Hydroxyl radical scavenging assay

The carrot sample extract was prepared according to the optimal extraction conditions obtained by the above orthogonal test, and dissolved in acetone in a ratio of 1:1, 1:2, 1:4, 1:6 and 1:8 to obtain the test solution. The capacity to scavenge hydroxyl radicals was assessed [35]. To begin the reaction, 2.0 mL of H_2O_2 (0.1%) and 4.0 mL of the 1.8 mM FeSO_4 solution were added to 1.0 mL of the sample solution. 3.0 mL of salicylic acid (1.8 mM) was then added after the mixture had been mixed and allowed to stand for 30 min at 37°C . The liquid was then mixed and left to stand for ten minutes at the same temperature. At 510 nm, the absorbance was recorded and contrasted with ascorbic acid. The potential scavenging rate of hydroxyl radicals was calculated using the equation below:

$$\text{Scavenging rate}(\%) = (1 - A_s/A_0) \times 100 \quad (4)$$

where A_s represented the absorbance of the sample, and A_0 represented the absorbance value of the blank control solution without the sample.

Statistical analysis

Data were analyzed with the one-way analysis of variance (ANOVA) using GraphPad Prism 9.0.2 Software (GraphPad Software Inc., San Diego, CA, USA). Three duplicates of

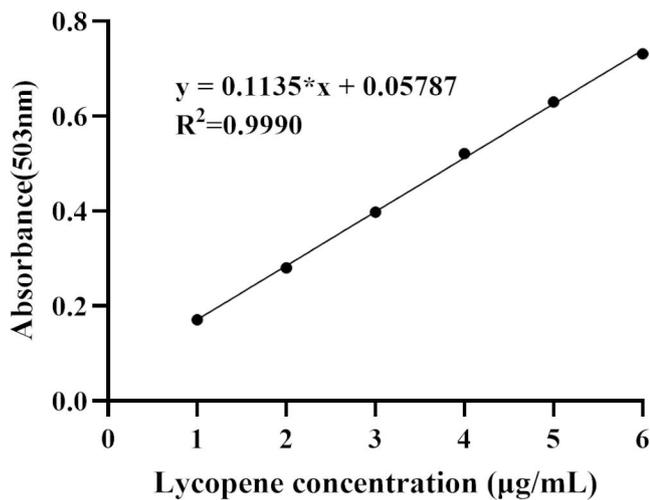


Fig. 1 Standard curve of lycopene

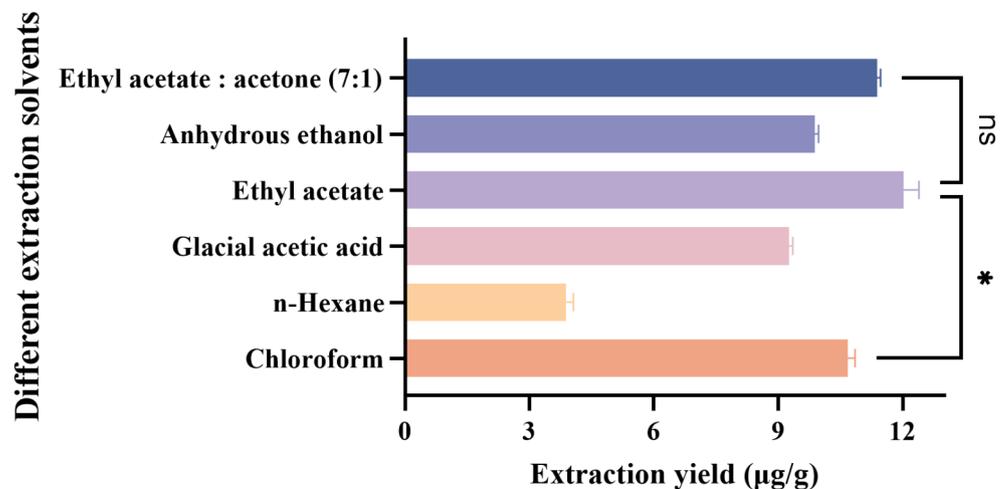
each experiment were carried out. The findings were presented as the mean \pm SD. The $p < 0.05$ level was used to determine whether differences between groups were statistically significant.

Results and discussion

Standard curve of lycopene

Figure 1 depicted the lycopene standard curve. The ordinate was the absorbance at 503 nm, and the abscissa was the quantity of lycopene ($\mu\text{g/mL}$). The linear regression equation is $y = 0.1135x + 0.05787$ ($R^2 = 0.9990$). According to the standard curve, the absorbance of lycopene was linearly positively correlated with the concentration in the range of 1.0–6.0 $\mu\text{g/mL}$.

Fig. 2 Effects of different extraction solvents on the extraction yield of lycopene (ns: not significant, *: $p < 0.05$)



Effects of different solvents on extraction

Different extraction solvents are known to influence the extraction yield. As a result, choosing the right extraction solvent is a crucial stage in optimizing the extraction procedure and achieving the highest extraction yield. Chloroform, n-hexane, glacial acetic acid, ethyl acetate, anhydrous ethanol, and ethyl acetate : acetone (7:1) were chosen as extraction solvents in this investigation based on the solubility properties of lycopene and the properties of organic solvents. Figure 2 illustrated how various extraction solvents affected lycopene yields. Chloroform, ethyl acetate and ethyl acetate : acetone (7:1) had higher extraction yields, with the highest yields found when used ethyl acetate. Chloroform is a volatile organic compound which is sensitive to light [36]. It would react with oxygen in the air and gradually decompose to produce highly toxic carbonyl chloride and hydrogen chloride when exposed to light. So it is not an ideal extraction solvent for laboratory research and industrial production. Ethyl acetate solution without acetone was chosen as the best extraction solvent based on economic considerations, even though the results showed no significant difference between the other two extraction solvents. This solvent would be utilized to extract lycopene from carrot in later trials. Other researchers also have the same extraction solvent selection. Luxsuwong [37] selected a simple ethyl acetate impregnation method to extract lycopene from tomatoes. According to the research by Szabo [38], ethyl lactate followed by ethyl acetate, had the highest extraction rate for lycopene. But ethyl acetate was ultimately chosen because it was a viable, long-lasting, and eco-friendly solvent for carotenoid extraction.

Effect of extraction temperature on lycopene yield

The yield of the extraction was significantly impacted by the extraction temperature. Figure 3 illustrated how raising

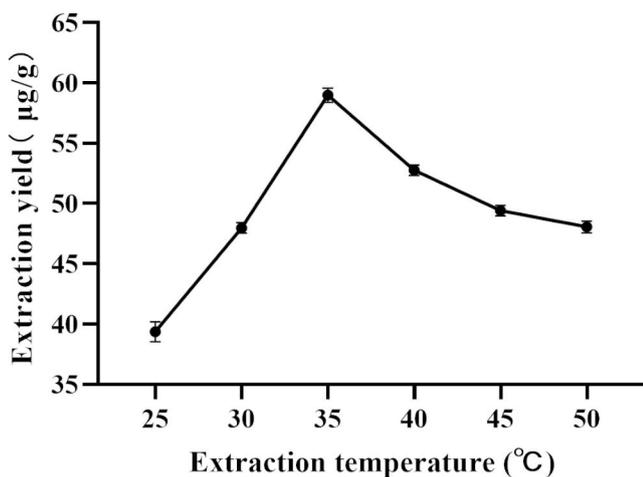


Fig. 3 Effect of extraction temperature (25, 30, 35, 40, 45, 50°C) on lycopene yield

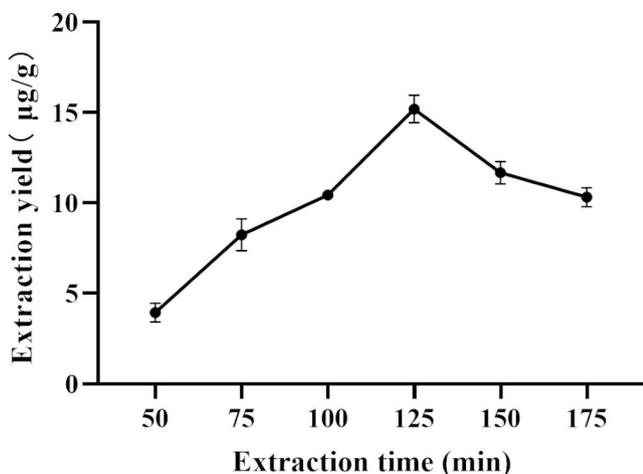


Fig. 4 Effect of extraction time (50, 75, 100, 125, 150, 175 min) on lycopene yield

the extraction temperature from 25°C to 35°C increased the lycopene yield from 39.37 ± 0.83 to 58.98 ± 0.59 µg/g. This suggested that a rise in temperature made it easier for active substances to diffuse, improving the yield of the extraction process [39]. However, continuously rising temperature could have a negative influence on the yield [40]. When the extraction temperature was higher than 35°C, the extraction yield fell. This may be because heating increased the volatilization of ethyl acetate, which in turn impacted the extraction effect.

Effect of extraction time on lycopene yield

Figure 4 illustrated how the length of the extraction process affected the lycopene production. The lycopene yield increased from 3.94 ± 0.52 to 15.20 ± 0.76 µg/g as the extraction period was increased from 50 to 125 min. The

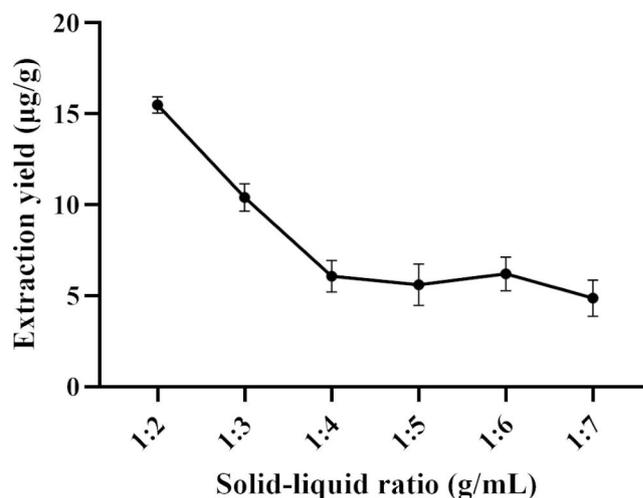


Fig. 5 Effect of solid-liquid ratio (1:2, 1:3, 1:4, 1:5, 1:6, 1:7 g/mL) on lycopene yield

extraction rate peaked at 125 min into the extraction process, as the extraction time rose, the rate gradually decreased. This indicated that the extraction duration was crucial for the extraction of lycopene since prolonging the solid-liquid contact time could promote the compound's diffusion [41]. However, the extraction yield decreased slightly with the extension of time. It is probably due to the fact that ethyl acetate volatilizes slowly at a certain speed in the extraction environment over time, which affects the final extraction effect [42].

Effect of solid-liquid ratio on lycopene yield

The extraction yield was significantly impacted by the solid-liquid ratio. Figure 5 demonstrated that as the solid-liquid ratio grew, the extraction yield declined. The extraction yield did not considerably alter when the solid-liquid ratio was 1:4 g/mL. This was most likely caused by a decrease in the surface area of plant tissues for solvent penetration and target component solubilization, which was detrimental to the extraction process [39].

Effect of pH on lycopene yield

The pH of the extraction fluid was altered using sodium hydroxide and hydrochloric acid. The lycopene production was unaffected by altering the pH of the extraction solution, as seen in Fig. 6. Thus, in the next orthogonal test, the impact of pH on the extraction yield won't be taken into account. This result was different from those of other researchers on the stability of lycopene. They found that lycopene was less stable in acidic environment and more stable in weakly acid and alkaline solutions [43, 44]. These

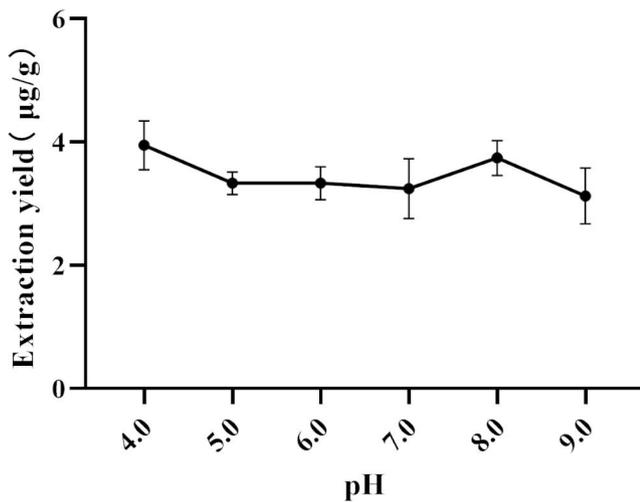


Fig. 6 Effect of pH (4.0, 5.0, 6.0, 7.0, 8.0, 9.0) on lycopene yield

Table 2 Analysis of $L_9(3)^3$ test results

No.	Factors			Extraction yield (µg/g)
	1	2	3	
1	1	1	1	30.64 ± 0.13
2	1	2	2	29.00 ± 0.15
3	1	3	3	21.64 ± 0.27
4	2	1	2	19.66 ± 0.28
5	2	2	3	34.15 ± 0.20
6	2	3	1	41.01 ± 0.43
7	3	1	3	27.10 ± 0.37
8	3	2	1	50.82 ± 0.18
9	3	3	2	36.27 ± 0.42
K1	81.29 ± 0.18	77.41 ± 0.46	122.47 ± 0.28	
K2	94.82 ± 0.14	113.97 ± 0.23	84.94 ± 0.28	
K3	114.19 ± 0.96	98.92 ± 0.78	82.89 ± 0.70	
R	32.90	36.56	39.58	

Table 3 Variance analysis results

Variation sources	Sum of Squares	DF	Mean Square	F-Value	P-Value
A	182.40	2	91.20	13.63	>0.05
B	225.22	2	112.61	16.83	>0.05
C	331.11	2	165.56	24.75	<0.05
Error	13.38	2	6.69		

Note: DF: degree of freedom

variations in experimental outcomes can be brought on by the various sample sources and lycopene detection tools.

Optimization of lycopene extraction parameters

According to the single-factor studies stated above, the extraction temperature, extraction time, and solid-liquid ratio appeared to be the most important factors that affected the yield of lycopene from carrots. In this study, these factors were evaluated using an orthogonal $L_9(3)^3$ test design.

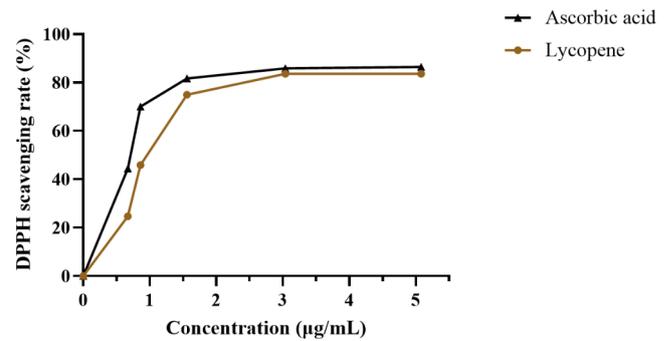


Fig. 7 Lycopene and ascorbic acid on DPPH scavenging rate on lycopene yield

Statistical techniques were used to examine the overall rating index. The findings of the orthogonal test and the investigation of the extremum difference were shown in Tables 2 and 3. The findings from the analysis of variance, which was carried out with the statistical program SPSS 20.0 (SPSS Inc.), were presented in Table 3. Table 2 displayed the outcomes of the orthogonal test. The outcomes showed that the maximal lycopene extraction yield was 50.82 ± 0.18 µg/g. According to the findings, this study was unable to choose the ideal extraction circumstances, therefore, a subsequent orthogonal analysis was necessary. Table 2 listed the calculated values for K1, K2, K3 and R value. It showed that the impact on the lycopene extraction yield was $C > B > A$ based on the observed R value. It was evident that the yield was mostly affected by the solid-liquid ratio. The extraction temperature, time, and solid-liquid ratio that produced the highest yield of lycopene were $A_3B_2C_1$ (40°C, 125 min and 1:2 g/mL), respectively.

DPPH scavenging activity

Figure 7 displayed the findings of the DPPH scavenging rate of ascorbic acid and lycopene in carrot. During the test dose range, the scavenging rate of lycopene was approximately similar to that of the reference standard ascorbic acid, indicating that lycopene had a significant effect of scavenging DPPH free radicals. When the dilution ratio of carrot extract was 1:1 to 1:4 (in the concentration of 0.7, 0.9 and 1.6 µg/mL, respectively), ascorbic acid and lycopene concentrations increased, and this had a considerable impact on the rate of free radical scavenging. As the dilution ratio exceeded 1:4, the rate of free radical scavenging gradually increased. Ascorbic acid and lycopene were found to have scavenging rates of 83.6% and 86.5% when its ratio reached 1:8 (at a concentration of 5.1 µg/mL). The two results above did not differ significantly from one another ($p < 0.05$). It can also be seen from other researchers that lycopene has a high capacity to scavenge DPPH free radicals. Wang [45] observed that lycopene and its inclusion complex

significantly enhanced the ability of DPPH free radical scavenging at the concentration range of 5 to 30 $\mu\text{g/mL}$. Wang [46] found that the Z-isomers in lycopene boosted the antioxidant activity from 5 to 55%, as measured by the DPPH experiment.

ABTS scavenging activity

ABTS assay is the most popular method employed for evaluating antioxidant activity [47]. The findings of the experiment demonstrated a favorable correlation between lycopene content within a specific concentration range and its capacity to scavenge ABTS. The ABTS assay was used to assess the antioxidant strength of lycopene and ascorbic acid, as shown in Fig. 8. Both ascorbic acid and lycopene demonstrated significant ABTS free radical scavenging activity in this investigation, however ascorbic acid was more effective than lycopene. In similar research, Tan [48] found that lycopene in freeze drying and oven drying tomatoes significantly decreased the ABTS radical scavenging activities. Ghellam [49] discovered that the antioxidant activity of autumn olive berries may be brought on by lycopene, carotenoids, vitamins, and other bioactive compounds. Based on the ABTS experiment, which demonstrated a significant connection between them and antioxidant activity, the presence of those compounds had significant antioxidant activity.

Hydroxyl radical scavenging activity

Figure 9 displayed the outcomes of ascorbic acid and lycopene's activity of hydroxyl radical scavenging. Ascorbic acid and lycopene were found to have 83.63% and 21.38% hydroxyl radical scavenging capabilities, respectively, at a concentration of 6.37 $\mu\text{g/mL}$. It was obvious that ascorbic acid had better hydroxyl radical scavenging, while lycopene had much lower free radical scavenging capacity than ascorbic acid. In similar research, some researchers had different results. Ren [33] found that SJP extract and purified SJP had hydroxyl radical scavenging effects of 60.32% and 70.78%, respectively, at 0.6 mg/mL concentration, and ascorbic acid showed 82.72% scavenging effect at this concentration. Nie [50] discovered that Majiayou pomelo Harvest I and II had a greater capacity to scavenge DPPH free radical, hydroxyl radical and superoxide anion radical than Harvest III, and some antioxidant compounds (such as ascorbic acid, lycopene, carotenoids, etc.) had a significant positive correlation with enzyme activity and free radical scavenging capacity ($p < 0.01$). Different treatments and testing equipments are likely required for various occurrences [51].

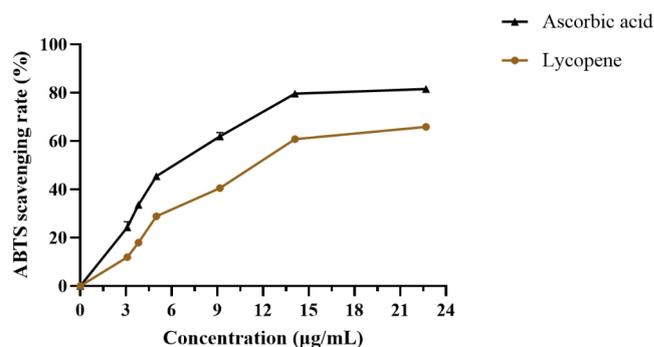


Fig. 8 Lycopene and ascorbic acid on ABTS scavenging rate

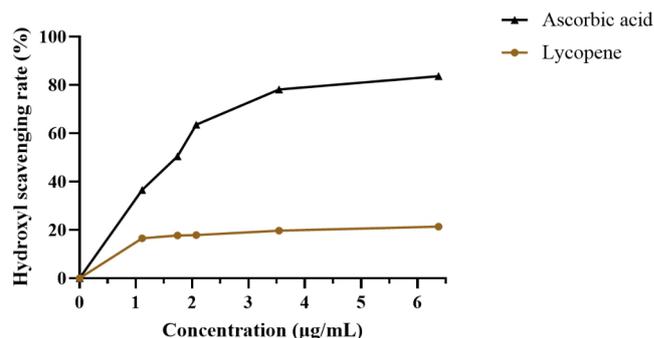


Fig. 9 Lycopene and ascorbic acid on hydroxyl scavenging rate

Conclusions

In this article, a single factor test was used to examine the effects of extraction temperature, extraction time, solid-liquid ratio, and pH on the amount of lycopene extraction from carrots. The extraction process for lycopene was optimized using an orthogonal $L_9(3^3)$ test design in agreement with the aforementioned results. The maximum extraction yield of lycopene was $50.82 \pm 0.18 \mu\text{g/g}$, which was obtained when the extraction temperature, time, and solid-liquid ratio were 40°C , 125 min, and 1:2 g/mL, respectively. Furtherly, the antioxidant capacity of lycopene was studied. Strong DPPH and ABTS radical scavenging were displayed by lycopene, but hydroxyl radical scavenging was only moderately effective. By consulting relevant literatures, there are few studies on lycopene in carrots. Further studies would be carried out to elucidate the stability of lycopene in carrots and other in vitro activities such as antibacterial activity. In general, the lycopene found in carrot is anticipated to be used as a natural antioxidant or health supplement in the food and cosmetics industries, and this research offers a theoretical foundation and data support for its further development.

Authors' contributions Bichen Ge and Wei Wang conceived, designed and performed the experiments; Bichen Ge and Xiaoju Chen analyzed the data; Xiaoju Chen and Yurong Gao contributed reagents and materials; Bichen Ge wrote the paper.

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Availability of data and materials Not applicable.

Code availability Not applicable.

Declarations

Conflict of interest Not applicable.

Ethics approval Not applicable.

Consent to participate Not applicable.

Consent for publication Not applicable.

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