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Enhancing Carrot Carotenoid Oxidative Stability by Chitosan-TPP Encapsulation

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Short Communication

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Abstract: Carotenoids are an important class of natural products with diverse biological activities, including antioxidant and photoprotective properties, as well as protection against various chronic diseases. However, they are sensitive to oxidation in biological systems and during food processing. Therefore, this study evaluated the impact of protecting carrot carotenoids through chitosan-TPP encapsulation. Results indicated that the chitosan-TPP-carotenoid complex maintained stability at 94.69% and 98.72% after 5 hours against H_2O_2 (8.82 M) and HOCl (15.18 mM), respectively. In contrast, the carotenoid extract degraded to 72.49% and 33.68%, respectively, after only 30 minutes, suggesting that carotenoids are more sensitive to HOCl than H_2O_2, despite the lower concentration of HOCl. Additionally, encapsulation did not affect the antioxidant activity of carotenoids, as both the extract and the complex exhibited similar scavenging activities against hydroxyl radicals. The drug release study demonstrated that carotenoids reached a concentration of 12.985 µM after 12,960 minutes (9 days), corresponding to 88.33% recovery and a half-life of 3,906 minutes (~2.7 days). The release mechanism was found to follow first-order kinetics and was primarily controlled by diffusion, resulting in a decrease in particle size and surface.

1 Introduction

Carotenoids are a class of naturally occurring compounds found abundantly in fruits and vegetables, in addition to occurring in some algae, cyanobacteria and fungi. More than a thousand carotenoids have been identified exhibiting vibrant colours such as yellow, orange and bright red (Hermanns et al 2020), where they provide the characteristic colours to many fruits and vegetables (Saleh et al 2013, Cueto et al 2017). Among vegetable crops, carrots globally rank as one of the top ten; however, 25-35% of the yield is unsuitable for fresh consumption because of the crop deterioration (Klettenhammer et al 2022, Šeregelj et al 2022). Carrots are also recognized as one of the richest plant sources of carotenoids (Meléndez-Martínez et al 2023).

Carotenoids play essential roles in plants, including contributions to photoprotection and photosynthesis. They have also demonstrated protection against some

chronic diseases, such as cancer, cardiovascular diseases, oxidative stress and osteoporosis (Tanumihardjo and Binkley 2013, Šeregelj et al 2022). These health benefits stem from their light-absorbing properties, which offer photoprotection and potent antioxidant capacity. They effectively scavenge free radicals, such as hydroxyl and peroxyl radicals, as well as quench singlet oxygen through energy transfer (Gammone et al 2015).

On the other hand, in the absence of other antioxidants, carotenoids are prone to oxidation, which can occur enzymatically *via* dioxygenases or through exposure to environmental stress factors such as heat, light, oxygen or transition metals, particularly during food processing or cooking. The oxidation of carotenoids produces derivatives such as apo-carotenoids and seco-carotenoids, resulting from the oxidative cleavage of the polyene chain or the ring double bond, respectively, which produces aldehydes and ketones. Additionally, epoxy-carotenoids can result from the epoxidation of any double bond (Mordi et al 2020, Harrison 2022). Therefore, the present study evaluates the potential protection of carrot carotenoids by encapsulation with chitosan-TPP and then examines the effects of encapsulation on carotenoid oxidative stability against naturally occurring oxidants, i.e., hydrogen peroxide and sodium hypochlorite, as well as the impact on the carotenoid ability to scavenge hydroxyl radicals. Drug release kinetics and mechanisms were also examined.

2 Materials and Methods

2.1 Chemicals and Instruments

Chitosan (200-250 KDA) was sourced from Chito-Tech Company. Sodium triopolyphosphate (TPP) was acquired from Advent Chembio Pvt. Ltd. Hydrogen peroxide (30%, 8.820 M) was supplied by Research Lab Fine Chem Industries. Sodium hypochlorite (NaOCl, 15.18 mM) was obtained from Piochem Laboratory Chemicals Company.

The UV-Vis spectrophotometer used was a Thermo Fisher Evolution 300 instrument, and the scan was recorded in the range of 200 to 600 nm. Fourier Transform Infrared-Attenuated Total Reflection (FT-ATR-IR) spectra were obtained using a Bruker Vertex 80/80 V instrument (4000–400 cm^{-1}).

Carrots were purchased from a local market. Carotenoid concentration was quantified at 450 nm (molar absorptivity of 135,800 $M^{-1}cm^{-1}$) as previously reported (Karkar and Şahin 2022).

2.2 Carotenoid Extraction

Carotenoids were extracted by homogenizing 50 g of fresh carrots in 25 mL of 80% acetone. The homogenate was centrifuged and filtered through a cloth sheet. The solvent was evaporated and the residue was dissolved in dichloromethane (CH_2Cl_2). This solution was eluted through a Sep-Pak C18 cartridge using ethanol. The yellow fraction was evaporated and stored under refrigeration for future use.

2.3 Complex preparation and characterization

In 50 mL of 1% acetic acid (0.4%), 200 mg of chitosan was dissolved and then added to 13.14 mg of carotenoid extract dissolved in 190 mL of ethanol (0.07%). A 1% sodium tripolyphosphate (TPP) solution (4.0 mL) was added dropwise, in a CS: TPP mass ratio of 5:1, with continuous stirring for two hours. The resulting mixture was centrifuged, filtered, dried, and stored at -4°C. Loading capacity and encapsulation efficiency were subsequently calculated.

2.4 Oxidative stabilization against H_2O_2 and HOCl

The stability of the carotenoid extract and the chitosan-TPP complex was evaluated against H_2O_2 and HOCl. A mixture containing 300 µL of H_2O_2 (8.82 M) or 300 µL of NaOCl (15.18 mM, adjusted to pH 6.2) and 20 mg of carotenoid extract or its equivalent in the complex was incubated in the dark in stoppered tubes. At each interval, three tubes (replicates) were processed by centrifugation with 5 mL of ethanol. The complex was ground and vortexed for carotenoid extraction, and the absorbance was measured at 450 nm.

2.5 Antioxidant activity

Hydroxyl radical scavenging activity of the carotenoid extract was evaluated based on the method by Smirnoff and Cumbes (1989), modified by Ge et al (2023). The following reagents were added sequentially: $FeSO_4$ (600 µL, 30 mM), H_2O_2 (17 µL, 30%), ethanol (3.0 mL) and carotenoid ethanolic extract (15 µL, 3.31 mM). The tubes were stoppered and incubated for 2 hours and then salicylic acid (750 µL, 18.0 mM) was added. After a further 40 minutes of incubation, the mixture was centrifuged and the absorbance was measured at 510 nm. The same method was used for the complex, employing 1.0 g of the complex with an

equivalent carotenoid content. All experiments were conducted in triplicate.

The reducing power of carotenes against potassium ferricyanide was determined according to the method of Oyaizu (1986).

2.6 Drug Release

A drug release study was performed by adding 10 mL of ethanol to 3 mg of the complex. The solution was shaken, and at specific intervals, 3 mL of clear solution was withdrawn for absorbance measurement at 450 nm. The withdrawn solution was returned to maintain a constant volume. Various drug release models, including first-order, Higuchi, and Hixson-Crowell models, were applied for analysis (Costa and Lobo 2001, Ramteke et al 2014).

2.7 Statistical analysis

Analysis of variance (One-way ANOVA) and Duncan's test ($p \leq 0.05$) were executed using the SPSS 25 package.

3 Results and Discussion

3.1 Complex Preparation and Characterization

The encapsulation efficiency, which reflects the effectiveness of carotenoid entrapment within chitosan-TPP capsules, was determined to be 92.70%. The loading capacity, expressing the proportion of carotenoids incorporated into the complex, was 2.631%.

The IR spectra of the carotenoid extract and the prepared complex are shown in **Fig 1.** The spectrum of the carotenoid extract exhibited a medium-strength ν(C=C) stretching vibration at 1625 cm^{-1} and a strong δ(=C-H) bending peak at 991 cm^{-1}, characteristic of trans-double bonds. In the complex spectrum, these peaks were attenuated due to the reduced concentration of carotenoids. Additionally, a prominent peak at 1535 cm^{-1}, corresponding to δs (N-H) scissoring of chitosan, and a weak peak at 887 cm^{-1}, indicative of N-H wagging and twisting, were observed. Similar chitosan absorption peaks were previously reported (Soleymanfallah et al 2022). A strong peak at 1059 cm^{-1}, attributed to P=O stretching, was also detected (Lustriane et al 2018).

3.2 Carotenoid and Complex Stabilization

The stability of the carotenoid extract and chitosan complex was evaluated against hydrogen peroxide and sodium hypochlorite. Both oxidants, commonly generated in biological systems, can react with the conjugated double bonds of carotenoids. High concentrations of the oxidants were used to assess the enhanced stability conferred by encapsulation. Results in **Table 1** demonstrate that the chitosan complex provided significant stability, retaining 94.69% and 98.72% of carotenoids after 5 hours of exposure to H_2O_2 (8.82 M) and HOCl (15.18 mM), respectively. In contrast, the carotenoid extract degraded to 72.49% and 33.68% under the same conditions within 30 minutes. This also indicates that carotenoids are more sensitive to HOCl than H_2O_2, despite the lower HOCl concentration.

Oxidation reactions were also followed by recording the UV-Vis spectra at various intervals (**Fig 2**). In H_2O_2 oxidation, the carotene characteristic peak at 450 nm decreased continuously, indicating the destruction of the polyene structure, while peaks at 240, 260 and 290 nm of the products were developed. In HOCl oxidation, the 450 nm peak gradually vanishes with an increase in 205 nm absorbance, indicating a decrease in polyene conjugation.

3.3 Antioxidant Activity

Hydroxyl radicals, among the most reactive and harmful radicals in biological systems (Edge and Truscott 2021), can be scavenged by carotenoids through electron transfer, radical adduct formation or hydrogen atom transfer processes (Chen et al 2011). To reveal whether the encapsulation affects the carotenoid antioxidant activity, the abilities of both carotenoid extract and chitosan complex to scavenge the hydroxyl radical generated by the Fenton Reaction were assessed. Results indicated that carotenoid extract could prevent 63.19% of salicylic acid degradation, as shown in **Table 2**. Notably, the chitosan complex exhibited a similar scavenging efficiency (62.16%), indicating that encapsulation did not affect the antioxidant activity of the carotenoids.

Carotene extract and the chitosan complex also exhibited reducing power activity. The complex showed lower activity (0.876±0.019 and 0.629±0.018, respectively), possibly due to the ease with which the hydroxyl radical reaches the encapsulated carotenes compared to ferricyanide.

3.4 Drug Release

A drug release study (**Fig 2**) revealed a continuous increase in carotenoid concentration, reaching 12.985 μM after 12,960 minutes (9 days), equivalent to 88.33% recovery based on the total carotenoid concentration (14.701 μM) derived from the loading capacity (2.631%).

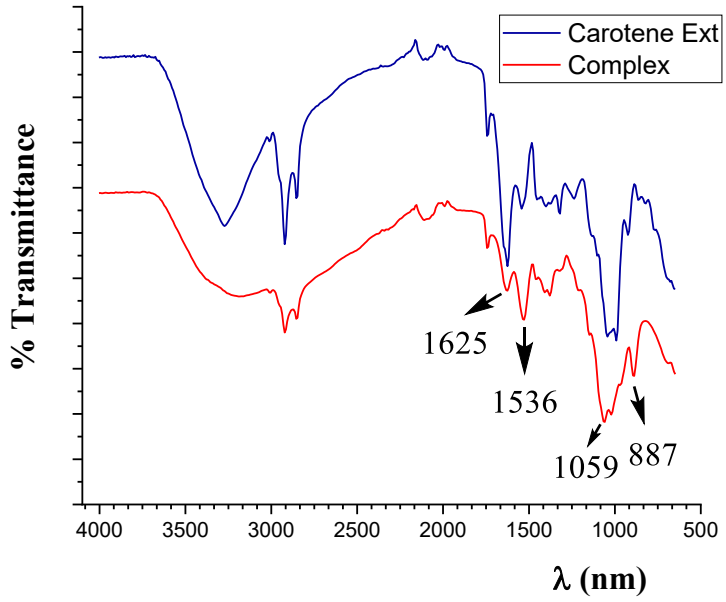

Fig 1. IR spectra of carotenoid extract and chitosan-TPP-carotenoid complex

Table 1. Oxidative stability of extracted carotenoids and chitosan-TPP complex

H_2O_2 (8.82 M) oxidation					
Time (min)	**% Car**	**SD**	**Time (min)**	**% Car**	**SD**
0	101.70	3.16	0	102.74	3.38
1	93.01	3.64	60	100.76	2.17
10	87.90	0.85	300	94.69	2.27
20	78.75	1.08			
30	72.49	0.96			
60	69.56	0.82			
120	62.67	1.83			
HOCl (15.18 mM) oxidation					
Time (min)	**% Car**	**SD**	**Time (min)**	**% Car**	**SD**
0	103.295	3.83959	0	101.18	1.14
2	79.84852	1.79429	60	99.72	4.29
5	66.70594	0.9663	300	98.72	2.50
10	57.79039	0.61046			
15	50.48861	1.7974			
20	43.79796	0.93903			
25	39.972	2.15648			
30	33.68241	2.78176			

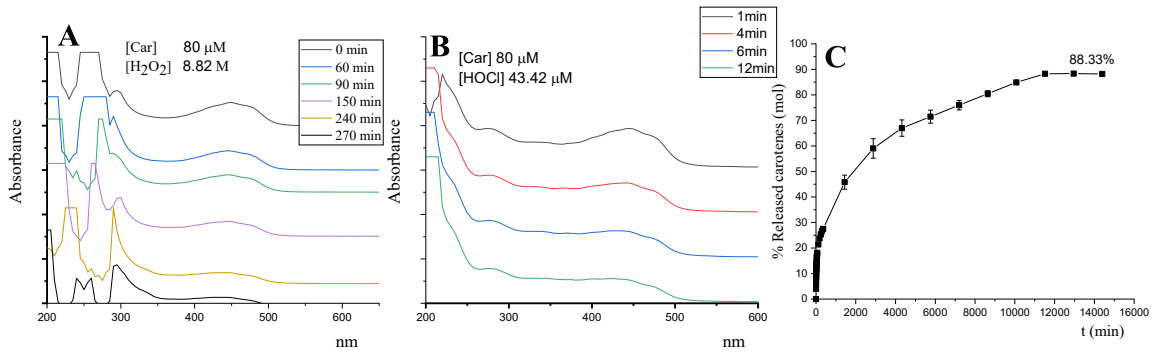

Fig 2. UV-Vis spectra of carotene oxidation by H_2O_2 (A) and HOCl (B) with time, and Drug release (C) of chitosan-TPP-carotenoid complex in ethanol

Table 2. Antioxidant and reduction potential of carotenoid extract and its complex

Material	% Scavenging OH Radicals	Reducing Potential (Absorbance)
Carotenoid extract	63.190 (±0.613)[a]	0.876 (0.019)[a]
Complex	62.175 (0.372)[a]	0.629 (0.022)[b]

Values in the same column with different letters are significantly different

The first-order model indicated that the initial carotenoid concentration was 12.401 µM, whereas the experimental value was 14.701 µM. The rate constant (k) was 1.774×10^{-4} min^{-1}, while the half-life period was 3906 minutes (0.693/k).

Fitting the Higuchi model suggests a homogeneous distribution of carotenoids within the complex, with release primarily influenced by diffusion. Applying the Hixson-Crowell model indicates that carotenoid release is linked to a reduction in particle size and surface area (Costa and Lobo 2001, Ramteke et al 2014).

4 Conclusion

The encapsulation of carotenoids in the chitosan-TPP complex effectively enhanced stability against both H_2O_2 and HOCl while preserving the antioxidant activity of the carotenoids. The drug release study adhered to first-order, Higuchi and Hixon-Crowell models, indicating a diffusion-controlled mechanism associated with a decrease in the particle size and surface area, and suggesting that carotenoids might be present, at least partially, on the particle surface. The half-life was approximately 2 days and 17 hours, with 88.33% recovery achieved within 9 days.

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