Microencapsulation of microbial canthaxanthin with alginate and high methoxyl pectin and evaluation the release properties in neutral and acidic condition

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Abstract
Canthaxanthin (CX) is an orange-red keto-carotenoid with high antioxidant activity. This functional pigment is sensitive to oxygen, light, pH and heat. In this study, CX was produced by the Dietzia natronolimnaea HS-1 and was encapsulated in Alginate (Alg) and Alg-high methoxyl pectin (HMP) through O/W/O multiple emulsion/external gelation method to developed resistant microparticles among acidic and neutral pHs. Results showed that initial CX concentration had a significant influence on total CX (TCX), surface CX (SCX), microencapsulation efficiency (EE) and particles size. The highest EE% for Alg (60.21 ± 0.18) and Alg-HMP (70.60 ± 0.68) were obtained with CX initial concentration of 11 and 18 μg/mg, respectively. Alg microparticles showed smaller size compared to Alg-HMP microparticles. Presence of CX in microparticles and good antioxidant activity was confirmed by FT-IR spectroscopy and DPPH assay, respectively. CX in vitro release was 66% and 49% in acidic condition and 76% and 50% in neutral condition for Alg and Alg-HMP, respectively. Thus, Alg-HMP-CX18 microparticles were selected to be used in both neutral and acidic foods such as milk and fermented milks products as an antioxidant and a colorant agent.

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1. Introduction

Carotenoids are a group of yellow to red pigments with highly unsaturated isoprene units. More than 600 carotenoids were discovered in nature because of simple production pathway of them in photosynthetic and non-photosynthetic organisms. Canthaxanthin (CX) (4,4′-diketo-β-carotene) is an orange-red and lipid-soluble keto-carotenoid with high antioxidant activity. Although this pigment is not a vitamin A precursor, but it was categorized as a bioactive compound because of its biological activities such as preventing cancer and cardiovascular disease. The Dietzia natronolimnaea HS-1 is the best. However, CX is a sensitive compound to oxygen, light, pH and heat due to the presence of highly unsaturated bond in its structure. In addition, the lipophilic CX has poor solubility and bioavailability in aqueous systems. Most food products have acidic or neutral pHs with moderate soluble oxygen such as milk and fermented milk products. So methods must be developed to protect CX among these environmental conditions. Microencapsulation is a suitable technique that protects CX from unfavorable conditions and enhances its solubility and bioavailability.

Alginate (Alg) is an ionic biopolymer, consist of β-1, 4-mannuronic acid and α-1, 4-glucoronic acid. Alg is one of the major encapsulating materials because of its easily swollen, cheapness and biodegradability. Alg microparticles are usually produced through emulsification, followed by crosslinking using calcium chloride as an ionic gelation agent. Although these microparticles have good characterist for use in acidic food matrix, but they have shown low resistance to neutral pHs. In this regard, a co-polymer was used for increasing stability of CX microparticles and their applicability in food products.
High methoxyl pectin (HMP) is a kind of plants’ heteropolysaccharide rich of galacturonic acids and consist of ~60% esterified carboxylic groups. Complex of HMP and Alg led to stronger mechanical structure [21]. Recently, using multiple emulsion became interesting for food scientists and several studies have applied water/oil/water (W/O/W) and oil/ water/oil (O/W/O) emulsion [22–24]. Multiple emulsion has advantages such as prolongs release of core materials, protects susceptible materials from hydrolysis and oxidation, entraps both hydrophilic and hydrophobic materials as well as enhancing bioavailability [25,26].

Commercial CX is usually produced through chemical synthesis, but this synthetic CX is not permitted for using in food products. *Dietzia natronolimnaea* HS-1 can produce natural CX by agriculture by-product and industrial wastewater that approach economic advantages. Alg and HMP which are used as encapsulating materials are produced by natural sources and are categorized as biodegradable and inexpensive coating materials. Also, oil phases for production of multiple emulsion are vegetable oils and categorized as nontoxic and biodegradable substances [25]. In microencapsulation of CX by an O/W/O multiple emulsion external/gelation method, nontoxic solvents were applied that caused no environmental pollution considering the green chemistry.

Encapsulated natural CX could be applied in several food products such as dairy foods including cheese, fermented milks and ice cream as colorant and antioxidant agent. In this way, sensory evaluation should be performed [27–29] to find the acceptance of this bioactive component in food products.

To the best of our knowledge, no studies were available on microencapsulation of microbial CX with calcium-Alg and the complex of HMP and Alg by an O/W/O multiple emulsion gelation method and evaluation the release properties of CX microparticles in neutral and acidic condition. Thus, the focus of this study was to develop CX microparticles with Alg and Alg-HMP complex by O/W/O multiple emulsification and external gelation technique for applying in neutral and acidic food matrix.

2. Materials and methods

2.1. Materials

_Bacteria Dietzia natronolimnaea_ HS-1 (DSM 44860) was supplied by Bioprocess Engineering Laboratory (BPEL, University of Tehran, Tehran, Iran). d-Glucose, peptone, malt extract, yeast extract, agar that used for growth of _Dietzia natronolimnaea_ HS-1 and producing CX pigment were purchased from Sigma-Aldrich (St. Louis, MO, USA). Also, beet molasses were supplied by Qazvin Sugar Industry (Qazvin, Iran), CX standard was supplied by Dr. Ehrenstorfer GmbH (Augsburg, Germany). HMP and Alg (from Laminaria hyperborean, molecular mass of 1.97 × 105 Da, mannuronate/guluronate ratio = 0.6) were purchased from Danisco (Copenhagen, Denmark) and BDH (Poole, UK), respectively. 2, 2 Diphenyl-1-picrylhydrazyl (DPPH) and emulsifiers consist of Tween 80 and Span 80 were purchased from Sigma-Aldrich (St. Louis, MO, USA). Soy and Sunflower oil with no added antioxidants were provided by Oilitalia (Forli, Italy) and Nina (Tehran, Iran), respectively. High purity ethanol (99.9%, v/v) was supplied by Kimia Alcohol Company (Zanjan, Iran).

2.2. CX production and extraction

Production and extraction of CX was performed according to the methods that described by Hojati et al. [30]. Media for maintaining _Dietzia natronolimnaea_ HS-1 consist of 10 g/L glucose, 5 g/L peptone, 5 g/L yeast extract, 3 g/L malt extract and 15 g/L agar. For producing the pigment, two step were performed. In first step, liquid pre-culture medium containing glucose, peptone, yeast extract and malt extract (with quantity mentioned above) was prepared and the bacteria cells were transferred to this medium. In second step, the inoculum was transferred to medium containing 10 g/L yeast extract and 40 g/L beet root molasses and incubated at 28 ± 1 °C for 5 days while stirring at 180 rpm for producing CX. After this time, 30 mL of culture was centrifuged at 8000 × g for 5 min and was washed twice by 30 mL physiological serum (9 g NaCl in 1 L distilled water). Then, 15 mL ethanol mixed with the sediment, centrifuged at 8000 × g for 5 min and CX extracted. This procedure performed until all pigment extract from the sediment. Finally, this ethanolic extract was filtered by a 0.2 μm hydrophobic fluorophore membrane (Sigma-Aldrich, St. Louis, MO, USA).

2.3. Preparation of microparticles containing CX using O/W/O multiple emulsion method

Small amount of sunflower oil (Nina, Co., Tehran, Iran) was added to ethanolic extract and ethanol was removed by using a Heidolph Laboratory Digital 4010 rotary evaporator (Heidolph Instruments GmbH & Co., Schwabach, Germany) under vacuum condition at 30 ± 1 °C. Solution of Alg and HMP (1% w/v) were obtained separately in deionized water and maintaining overnight while stirring (200 rpm) at ambient temperature. Homogenization (IKATZ-25-Digital Ultra Turrax, Staufen, Germany) of CX (3, 5 and 7 mL) and Alg solution (100 mL) (for producing calcium-Alg microparticles) or Alg-HMP (1:2 w/v/v) solution (100 mL) (for producing the mix of Alg and HMP microcapsules) containing 1% Tween 80 (w/v) was performed at a speed of 12,000 rpm for 5 min to produce the primary O/W emulsion with final concentrations of 11, 18 and 25 μg/mg of CX. Then, the primary O/W emulsion (100 mL) was mixed with 110 mL soybean oil (containing 1% v/v span 80) using ultra-Turrax at 8000 rpm for 5 min for production of O/W/O-multiple emulsion. This step was performed in a lower shear rate to avoid fracturing of multiple emulsion. Control treatment produced with pure sunflower oil as oil phase of primary emulsion.

Calcium-Alg and calcium-Alg-HMP microcapsules were generated by adding 35 g of calcium chloride solution (25% w/w) dropwise (over about 16 min) to O/W/O multiple-emulsion (210 mL) through magnetic stirring; the mixture was kept stirring for another 30 min. The microparticles were collected by centrifugation (Rotina 35R centrifuge, Hettich, Tuttlingen, Germany) at 6500 × g for 5 min. Then, vacuum filtration was performed by a vacuum pump (DV-42 N 1.5 CFM Platinum Vacuum Pump, USA) and washed several times with deionized water containing 0.1% v/v Tween 80. Finally, the microparticles were frozen at −80 °C for 2 h followed by lyophilization with a freeze drier for 14 h (ALPHA 2–4; Christ, Harz, Germany) [31]. Fig. 1 shows the preparation steps of Alg-CX and Alg-HMP-CX microparticles.

2.4. Evaluation of multiple-emulsion structure by optical microscopy

In order to evaluate the structure of O/W/O multiple-emulsion, an Optical-light microscope (Medline Scientific Limited, Oxfordshire, UK) equipped with a digital camera (Sony, Tokyo, Japan) was used at 8000× magnification.

2.5. Scanning electron microscopy (SEM)

Scanning electron microscopy (SEM) was used to examine the morphology of the microparticles (Oxford Instruments INCA Penta FET × X3). CX microcapsules were sputtered with gold and SEM images were taken at the different magnification and examined using an acceleration voltage of 20.0 kV [32].

2.6. CX encapsulation efficiency (EE)

In order to determine the encapsulation efficiency of CX, UV-visible spectroscopy was used (Shimadzu UV-VIS 1601, Japan). According to previous studies, the maximum absorbance wavelength of CX solution is 474 nm [33] that was used for subsequent measurements of CX. For determination of TCX in microparticles, a 200 mg sample was dispersed in 50 mL pure ethanol and stirred overnight. The solution was filtered,
the absorbance was measured and the concentration was calculated according to the calibration curve. SCX in the powder was determined as the following procedure: 600 mg of powder were weighed in a test tube and extracted with 25 mL hexane for 15 s (shaken at 100 rpm). The tubes were centrifuged at 1000 \( \times g \) for 1 min. Then, the absorbance of the supernatant was measured by spectrophotometer at 474 nm \([30]\). All samples were measured in triplicate. EE were designated using the following equation:

\[
EE\% = \frac{(TCX-SCX)}{Initial\ amount\ of\ CX} \times 100
\]  

2.7. Size distribution of emulsion droplets and freeze dried particles

A laser diffraction particle size mastersizer2000 hydro S (Malvern Ltd., Worcestershire, UK) was used for emulsion droplets and particle size measurements. Diluted solution of microparticles were made and measurements reported as \( d_{0.1}, d_{0.5} \) and \( d_{0.9} \) that is the size of particle below which 10%, 50% and 90% of the samples lies, respectively. Also, polydispersity index (Span) was calculated using following equation:

\[
Span = \frac{D_{0.9} - D_{0.1}}{D_{0.5}}
\]

2.8. Fourier transform-infrared (FT-IR) analyses

FT-IR spectra of sodium Alg powder, HMP powder, Alg-C, optimized Alg-CX, Alg-HMP-C and optimized Alg-HMP-CX microparticles (with the highest EE%), were obtained from wave number in the range of 400–4000 cm\(^{-1}\) using a FT-IR spectrophotometer (Perkin Elmer Spectrum RXI, USA). For preparing samples, they were separately mixed with potassium bromide (KBr) and making pellets under a hydraulic
pressure. For each spectrum, 16 scans at a resolution of 4 cm\(^{-1}\) were obtained.

2.9. Determination of antioxidant activity

2, 2-Diphenyl-1-picrylhydrazyl (DPPH) was applied for determination of antioxidant activity of microparticles [34]. The method is based on the reduction of DPPH radical at 517 nm in the presence of an antioxidant. Briefly, 25 mg of each sample was added to 50 mL ethanol and stirred overnight. The solution was filtered and 0.1 mL of filtrate was mixed with 3.9 mL of methanolic DPPH solution (0.1 mM). The decrease in absorbance was measured at 517 nm after incubation for 60 min in darkness at room temperature. A DPPH radical solution without sample was used as a control. The DPPH radical-scavenging activity (%) was calculated using following equation:

\[
\text{DPPH scavenging activity (\%)} = \left( \frac{A_{\text{Control}} - A_{\text{Sample}}}{A_{\text{Control}}} \right) \times 100
\]

2.10. In vitro release profile

In vitro release was conducted by acidic and neutral condition with phosphate buffer (pH 7.4) and adjusting pH of phosphate buffer with 1.0 M HCl (pH 4.5). 600 mg of each micro-particle produced with the highest EE% was dispersed in 100 mL acidic and neutral medium at room temperature and kept without stirring in dark. At particular time intervals, 5 mL of sample was withdrawn and was replaced with a same volume of fresh media. The aliquots were centrifuged at 9000 \(\times\) g for 5 min at room temperature and supernatant analyzed using a spectrophotometer (Shimadzu UV-VIS 1601, Japan) at 474 nm. The cumulative percentage of CX released was plotted against time in hours.

2.11. Statistical analysis

SPSS statistical software version 25 (SPSS Inc., Chicago, IL) was used for data analysis. To identify any significant differences between the samples, analysis of variance (ANOVA) followed by the Duncan’s multiple range procedure was used at a 95% confidence level. Each analysis was performed in triplicate.

3. Results and discussion

3.1. Morphology

Previous studies have shown that the most produced carotenoid by Dietzzi natronolimnaea HS-1 is CX [30,35]. In this study, CX was micro-encapsulated in Alg and Alg-HMP microparticles by multiple emulsion and ionic gelation. Small droplets of sunflower oil containing CX molecules dispersed in water droplets (first emulsion) and these phase then dispersed in a continuous soybean oil phase (second emulsion). The structure of these multiple (O/W/O) emulsion and primary O/W emulsion are shown in Fig. 2(a) and (b), respectively. Regular

![Fig. 2. Optical microscopic view of multiple O/W/O emulsion (a), Primary O/W emulsion (b), SEM micrograph of the freeze dried canthaxanthin loaded alginate microparticles (Alg-CX) (c) and alginate-high methoxyl pectin microparticles (Alg-HMP-CX) (d).](image-url)
distribution of droplets is shown in optical micrograph of O/W/O emulsion. **Fig. 2**, also represents the SEM images of the freeze dried Alg-CX microparticles (c) and Alg-HMP-CX microparticles (d). As shown in **Fig. 2**, Alg-CX microparticles had a spherical geometry with uneven surfaces, deep pores and voids in some parts. This finding was reported for microencapsulation of Canola oil in Alg microcapsules [36]. Although Alg-HMP-CX microparticles had unregular geometry, but the surface was even and with low pores in many parts. This finding is in agreement with previous studies about microencapsulation of Ciprofloxacin in Alg-HMP matrix [21]. They suggested that the combination of Alg and HMP had synergist effects and reduced porosity of microparticles as compared to Calcium-Alg microparticles.

3.2. Encapsulation efficiency (EE) and particle-size analysis

**Table 1** shows total and surface CX, encapsulation efficiency and particle size of Alg and Alg-HMP microparticles as well as mean diameter and span of primary emulsions. Results showed that increasing the amount of CX content from 11 to 25 μg/mg, influenced TCX, SCX, EE% and particle size. TCX and SCX increased from 6.78 ± 0.12 to 11.86 ± 0.11 μg/mg and 0.28 ± 0.14 to 1.08 ± 0.11 μg/mg for Alg microparticles; and from 7.81 ± 0.21 μg/mg to 16.22 ± 0.04 μg/mg and 0.31 ± 0.17 μg/mg to 1.28 ± 0.16 μg/mg for Alg-HMP microparticles, respectively, as CX increased from 11 to 25 μg/mg. Increasing total core content by increasing initial concentration have reported previously [31,37]. Findings about increasing SCX is in agreement with previous studies [39,40]. Interaction between CX and type of them, the FT-IR analysis were performed. **Fig. 3** presents the FT-IR spectrum of sodium Alg powder, HMP powder, Alg-C, Alg-CX, Alg-HMP-C and Alg-HMP-CX microparticles.

The FT-IR spectrum of sodium Alg powder showed characteristic absorption bands at 3433, 2924, 1621, 1416 and 1031 cm⁻¹ that corresponded to stretching vibration of O−H, C−O−COO− (asymmetric), COO− (symmetric) and C−O−C. These similar findings were reported by other authors [44]. Previous study showed that the spectra of the calcium-Alg was slightly different compared to sodium Alg powder in the wave number of COO− and C−O−C which was related to the crosslinking between carbonyl groups of Alg and Ca²⁺ and covalent bonding, respectively [38]. Alg-C microparticles exhibited characteristic peaks at 3009 cm⁻¹ (C−H stretching vibrations), 2926 and 2855 cm⁻¹ (C−H stretching vibrations), 1746 cm⁻¹ (C=O stretching vibrations), 1643 cm⁻¹ (COO− symmetric stretching vibrations), 1420 cm⁻¹ (COO− asymmetric stretching vibrations) and 1033 cm⁻¹ (C−O−C stretching vibrations) that confirm characteristic wave number for sunflower oil that were reported by other authors [45]. These results indicated that incorporation of sunflower oil in microparticles matrix had no chemical interaction. Previous study represented the characteristic peaks at 3435 cm⁻¹ (OH), 3023–2866 cm⁻¹ (H-Csp3 and H-Csp2), 1654 cm⁻¹ (conjugated CO), 1606 cm⁻¹ and 1557 cm⁻¹ (C=C), 1399 cm⁻¹ and 1364 cm⁻¹ (gem-dimethyl), 1092 cm⁻¹ and 1032 cm⁻¹ (OH) and 975 cm⁻¹ (CH=CH, trans) for pure CX [46]. Alg-CX1 microparticles had little shift in wave numbers (versus Alg-C) from 2926 to 2925 cm⁻¹, 1746 to 1745 cm⁻¹, 1643 to 1633 cm⁻¹ and 1420 to 1418 cm⁻¹ that was indicative of the presence of CX in microparticles. However, CX signals were dominated by sunflower oil and coating materials, because of the small amount of

**3.3. FT-IR analysis**

To detect any interaction between CX and covering biopolymers and type of them, the FT-IR analysis were performed. **Fig. 3** presents the FT-IR spectrum of sodium Alg powder, HMP powder, Alg-C, Alg-CX, Alg-HMP-C and Alg-HMP-CX microparticles.

**Table 1** shows total and surface CX, encapsulation efficiency (EE) and particle size parameters of alginate (Alg) and Alg-high methoxyl pectin (Alg-HMP) microparticles.

<table>
<thead>
<tr>
<th>Microparticles</th>
<th>TCX (μg/mg)</th>
<th>SCX (μg/mg)</th>
<th>EE (%)</th>
<th>Primary emulsion size parameters</th>
<th>Particles size parameters</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Mean diameter (μm)</td>
<td>Span</td>
</tr>
<tr>
<td>Alg-C</td>
<td>0.00⁺⁺⁺</td>
<td>0.00⁺⁺⁺</td>
<td>0.00⁺⁺⁺</td>
<td>2.49⁻⁻⁻</td>
<td>2.03⁻⁻⁻</td>
</tr>
<tr>
<td>Alg-CX₁₈</td>
<td>6.78 ± 0.12⁻⁻⁻</td>
<td>0.28 ± 0.14⁻⁻⁻</td>
<td>60.21 ± 0.18⁻⁻⁻</td>
<td>0.42 ± 0.01⁻⁻⁻</td>
<td>2.49⁻⁻⁻</td>
</tr>
<tr>
<td>Alg-CX₉</td>
<td>9.74 ± 0.01⁻⁻⁻</td>
<td>0.56 ± 0.26⁻⁻⁻</td>
<td>51.04 ± 1.22⁻⁻⁻</td>
<td>0.47 ± 0.11⁻⁻⁻</td>
<td>2.03⁻⁻⁻</td>
</tr>
<tr>
<td>Alg-CX₂₅</td>
<td>11.86 ± 0.11⁻⁺⁺⁻⁺⁺⁺</td>
<td>1.08 ± 0.11⁻⁺⁺⁺⁺⁺⁺</td>
<td>42.81 ± 0.10⁻⁻⁻⁻⁺⁺⁺</td>
<td>0.66 ± 0.03⁻⁻⁻⁻⁺⁺⁺</td>
<td>1.90⁻⁻⁻⁻⁺⁺⁺</td>
</tr>
<tr>
<td>Alg-HMP-C</td>
<td>0.00⁺⁺⁺</td>
<td>0.00⁺⁺⁺</td>
<td>0.00⁺⁺⁺</td>
<td>2.39⁻⁻⁻</td>
<td>1.39⁻⁻⁻</td>
</tr>
<tr>
<td>Alg-HMP-CX₁₈</td>
<td>7.81 ± 0.21⁻⁻⁻⁻⁻⁻⁻</td>
<td>0.31 ± 0.17⁻⁻⁻⁻⁻⁻⁻</td>
<td>69.53 ± 0.32⁻⁻⁻⁻⁻⁻⁻</td>
<td>1.03 ± 0.18⁻⁻⁻⁻⁻⁻⁻</td>
<td>2.13⁻⁻⁻⁻⁻⁻⁻</td>
</tr>
<tr>
<td>Alg-HMP-CX₂₅</td>
<td>13.38 ± 0.01⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻</td>
<td>0.67 ± 0.21⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻</td>
<td>70.60 ± 0.68⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻</td>
<td>1.25 ± 0.02⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻</td>
<td>2.12⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻</td>
</tr>
<tr>
<td>Alg-HMP-CX₂₅</td>
<td>16.22 ± 0.04⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻</td>
<td>1.28 ± 0.16⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻</td>
<td>59.32 ± 1.00⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻</td>
<td>1.39 ± 0.01⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻</td>
<td>1.84⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻</td>
</tr>
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</table>

Data reported are average values ± standard deviations.

Values within each column with different letters are significantly different (p < 0.05).
CX in microparticles. As the result, the differences between FT-IR spectra of Alg-C and Alg-CX₁₁ were not significant.

HMP powder showed characteristic peak at 1634 and 1736 cm⁻¹, with different intensity that correspond to COO⁻ and C=O groups, respectively [47]. Degree of esterification is calculated by this area in the way that stronger intensity at 1736 cm⁻¹ compared to 1634 cm⁻¹ demonstrated higher esterification [20]. Also the FT-IR spectra of HMP showed typical peaks at 3392 cm⁻¹ (O—H), 2913 cm⁻¹ (C—H) and 1105 cm⁻¹ (C—O—C and cyclic C—C) [48,49]. Studies on spectra of Alg-HMP microparticles cleared that more hydrogen bonding occurred between Alg and HMP [37]. As same as Alg microparticles, Alg-HMP-C microparticles exhibited characteristic peaks for sunflower oil that introduced above. Also, as the result of small amount of CX in microparticles, spectra of Alg-HMP-CX₁₈ microparticles had no significant different compare to Alg-HMP-C microparticles.

3.4. Antioxidant activity

Antioxidant activity of CX corresponds to a conjugated polyene system in the structure that diminishes at high oxygen concentration [50]. For determination of antioxidant activity, changing the color of methanolic solution of DPPH was measured by a spectrophotometer. Fig. 4 represent the antioxidant activity of unloaded and loaded Alg and Alg-HMP microparticles. Unloaded micro-particles showed low antioxidant activity (maximum 4.9 ± 0.60%). This phenomenon was reported by previous study [51]. Fig. 4 showed that antioxidant activity increased from 15.03 ± 0.13% to 30.41 ± 0.21% for Alg and from 18.03 ± 0.21% to 37.41 ± 0.10% for Alg-HMP microparticles as CX content increased from 11 to 25 µg/mg. Similar results was reported by Gharibzahedi et al. regarding the relation between CX content in the microparticles and antioxidant activity [52].

3.5. In vitro release

For determining the suitability of microparticles to protect CX among destructive condition, in vitro release behavior of CX in acidic and neutral pH of phosphate buffer was studied. Fig. 5 shows release profile of CX from microcapsules in acidic and neutral pHs, Alg-CX₁₁ and Alg-HMP-CX₁₈ microparticles (as represented the optimal EE%), were selected for estimating release in two different media. Results showed that near 66% and 76% of CX were released from Alg-CX₁₁ microparticles in acidic and neutral condition in phosphate buffer after 220 h, respectively. This results confirmed the fact that Alg microparticles were be more swelled in neutral pHs compared to acidic condition. [53]. So the microparticles structures became more disrupted and CX releasing increased. In acidic condition (pH 4.5) near to pkₐ Alg (3.5) [54], shrinkage of Alg microparticles increased [55] and hydrophobic alginic acid with less solubility was formed leading to an increase of viscosity

![Fig. 3. FTIR spectra of sodium Alg powder (a), Alg-C microparticles (b), Alg-CX microparticles (c), HMP powder (d), Alg-HMP-C microparticles (e) and Alg-HMP-CX microparticles (f).](image)

![Fig. 4. DPPH scavenging activities of Alg and Alg-HMP microparticles with and without CX.](image)
and decrease of swelling in Alg microparticles. As a result of more shrinkage structure and higher viscosity, CX release was lower in acidic condition [53]. Release percentages were 49% and 50% for Alg-HMP and decrease of swelling in Alg microparticles. As a result of more porous structure of Alg-HMP-CX microparticles, but low porous structure of Alg-CX microparticles occurred, slow release of CX from microparticles that happen as the result of behavior like Alg-CX microparticles occurred, but low porous structure of Alg-HMP microparticles were performed and different cumulative percent-age were reported [21,53,59]. These differences could be explained by different structure of core material and their specific interaction with coatings, concentration of Alg and HMP in coating initial solutions, release determining medium, temperature and encapsulating procedure.

4. Conclusion

The O/W/O multiple emulsion/external gelation method was suitable for preparation of Alg-CX and Alg-HMP-CX microparticles that protected the biological function of natural CX against environmental condition. SEM images showed spherical shapes with porous surface for Alg microparticles, but irregular shapes and relatively smooth surface for Alg-HMP microparticles. Values of SCX and TCX increased with increasing CX content from 11 to 25 μg/mg while EEs decreased. FT-IR analyses confirmed the presence of CX in microparticles. Also, DPPH assay showed good antioxidant activity for microparticles. In vitro release in acidic and neutral condition demonstrated that Alg microparticles were suitable for protection of CX only in acidic condition, but Alg-HMP were completely suitable for both pHs. Therefore, Alg-HMP micro-particles could protect functional properties as well as color of CX in neutral and acidic condition. Further studies are required for investigation of CX release in different food matrix.

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Declarations of interest

None.

References


Fig. 5. In vitro release profile of CX from Alg and Alg-HMP micro-particles in acidic and neutral condition in phosphate buffer.

