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Improvement of crocin stability by biodegradeble nanoparticles of chitosan-alginate

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ABSTRACT

This study aimed to improve the stability of crocin, a saffron carotenoid, encapsulating into chitosan (Cs)-sodium alginate (Alg) nanoparticles prepared by a modified ionic gelation method were investigated as a new carrier to improve the stability of a saffron carotenoid, crocin. Response surface methodology was used to optimize the important variables, namely the concentrations of Cs and Alg, and pH influencing the particle size, zeta-potential, and encapsulation efficiency to find the optimum formulation for production of crocin nanoparticles (CNPs). Microscopic analysis and dynamic light scattering examination indicated non-smooth and spherical nanoparticles with the size range of 165–230 nm in weight ratio of Cs:Alg (1:1.25) and pH 4.7. Fourier transform-infrared spectroscopy displayed an extensive hydrogen bonding interaction between the crocin and biopolymers. Encapsulation efficiency, loading capacity and yield of CNPs were 38.16, 30.96 and 48.33%, respectively. The zeta-potential of NPs was about −33.52 MV which resulted in the better stability of NPs during manipulation and storage. Stability studies showed that nanoencapsulation provided enhanced crocin stability with biopolymers compared to the standard crocin under unfavorable environmental conditions.

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1. Introduction

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Saffron, a spice derived from the dried stigmas of *Crocus sativus* L. flowers, has received too much attention in the past two decades because of having various biological properties such as antioxidant, anti-cancer, antispasmodic, anti-inflammatory, and anti-atherosclerotic activities. This plant is primarily grown in some countries including Iran, India, Spain, Italy and Greece as well as several other countries [1–3].

Saffron contains many compounds like crocin ($C_{44}H_{64}O_{24}$), a water-soluble carotenoid, which is the main reason for its common application as a food colorant. As depicted in Fig. 1, it is a glycosyl ester derivative of C_{20}-dicarboxylic acid crocetin (8,8′-diapocarotenedioic acid) [4,5]. Several studies have shown the inhibitory effect/antioxidant activity of saffron on free-radical chain reactions, which is attributed to crocin having stronger antioxidant activity than α-tocopherol [3,5,6]. Akhtari et al. [7] showed that most of the sites in the sugar moiety of crocin have high net charge and they are responsible for the potent antioxidant activity of crocin toward superoxide anions. The sugar moiety in the crocin molecule has a considerable influence on the polarazablity and antioxidant efficiency of crocin [7].

In general, crocin has low stability and after exposure to heat, oxygen, light, acidic environment and the presence of additives during processing and storage of foods, it loses most of its functionality [8,9]. Few studies have shown that orally administered crocin is hydrolyzed to crocetin before or during intestinal absorption, which is partly metabolized to mono- and di-glucoronide conjugates demonstrating the low bioavailability of crocin due to the elimination of glucose moieties [10,11]. Asai et al. [12] investigated the intestinal absorption of orally administered crocetin and crocin in mice. Crocetin and its glucuronide conjugates were also found in crocin-administered mouse plasma, whereas intact crocin

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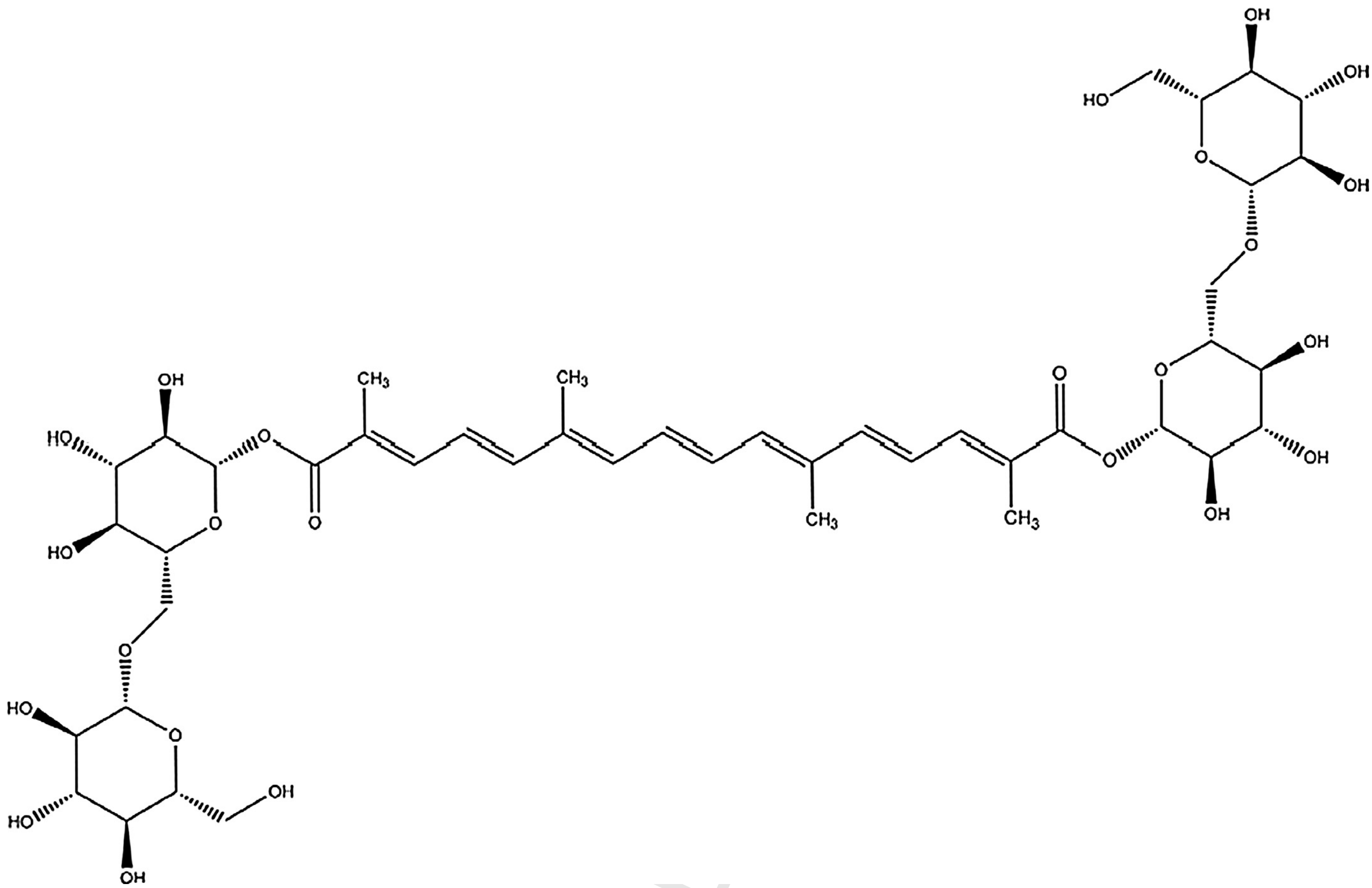

Fig. 1. Chemical structure of crocin.

(glycoside forms) were not detected suggesting the poor absorption and low oral bioavailability of crocin, which is attributed to its lower absorption in the gut [12], hydrolysis by the indigenous β-glucosidase [6] and rapid systematic elimination [13]. Despite the beneficial effects of crocin, its poor stability and high susceptibility to process conditions are major problems for its application.

Nanoscale-drug delivery systems prepared from biocompatible and biodegradable polymers have been considered as a potential approach to drug delivery and their bioavailability increment. Biodegradable carriers are purposely engineered and constructed with nanometer dimensions [14]. Moreover, many of these polymers especially hydrogels are naturally hydrophilic, which is beneficial for the longer *in vivo* circulation times and the encapsulation of water-soluble biomolecules [15].

Alginate (Alg) is a negatively charged molecule and the binary copolymer of (1–4) glycosidically linked β-D-mannuronic acid and α-L-guluronic acid residues. It has been extensively investigated for drug delivery system, especially for proteins due to its biodegradable and biocompatible properties [16–18]. Chitosan (Cs), a natural cationic amino polysaccharide (pK_a 6.5) derived from partial alkaline deacetylation of chitin, is a nontoxic, biodegradable, biocompatible and mucoadhesive biopolymer [19]. Several biopolymers such as Cs have been used in combination with sodium-Alg (Na-Alg) in order to increase the encapsulation efficiency (EE) and drug release profile [16,20,21]. A polyelectrolyte complex is formed when polycations and polyanions in the solution form a dense polymer phase, known as coacervate, which enhances the overall stability of nanoparticles (NPs) [14,22,23]. Due to the influence of different factors such as carrier concentration, carrier to core ratio, mixing time and speed, pH, ultrasonication power and time on the production and characterization of NPs, an optimization approach would be useful for the development of drug delivery systems [23–26]. Tang et al. [27] and Tsai et al. [28] stated that ultrasonication is a common method for preparation and processing of NPs as well as an effective technique for reducing NPs size. Tsai et al. [28] investigated the effect of ultrasonic radiation and mechanical shearing on the particle size of NPs prepared by the ionic gelation method and they find out that the treatment decrease the NPs size. They also indicated that Cs degradation was resulted from the cavitation effect and tearing and/or stretching effect of ultrasonic radiation and mechanical shearing, respectively [28].

Response surface methodology (RSM) can be used to evaluate the relationship between the independent variables and the response as well as their interactions in an effective model [29,30]. To the best of our knowledge, there is no specific study related to the use of nanoparticulate system encapsulating crocin to overcome the deterioration problems. In this research, RSM was applied to optimize the process variables, including Cs content, Alg concentration and pH to obtain the crocin loaded NPs with high EE and small size. The objective of this study was to determine the optimum formulation of NPs with two biopolymers of Cs and Alg using RSM for encapsulating crocin to augment its stability. The findings will have an impact on nutritional and pharmacological applications, where the improved stability of crocin is essential.

2. Materials and methods

2.1. Materials

Low molecular weight Cs (MW = 50–190 kDa, deacetylation degree = 75–85%), Na-Alg (MW = 80–120 kDa, mannuronic

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