Short communication

Encapsulation of date palm pit extract via particulation of starch nanocrystals in a microemulsion

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Introduction

The participation of bioactive food compounds including phenolics in various metabolisms and their counteractions with disease-developing mechanisms (Rostagno et al., 2010) has caused an increasing attraction to functional foods. Date palm pit extract is a rich source of polyphenols (Ardekani & Kanavi, 2010) that show metal scavengering, antimutagenes, and antimicrobial activities (Bagheri et al., 2013). The utilisation of encapsulated polyphenols in formulation of health-promoting functional foods instead of free counterparts can overcome the drawbacks of their instability, alleviate unpleasant tastes or flavours, as well as improve the bioavailability and half-life of these compounds in blood stream (Fang & Bhandari, 2010).

Starch being a natural, inexpensive, biodegradable, and biocompatible polymer has attracted much attention in encapsulation processes. Starch nanocrystals are amylopectin-originated nanoscalar blocklets obtained through acidic hydrolysis of starch granules amorphous regions at ambient temperatures (Angellier et al., 2004). The acid-resistant crystals are intrinsically rigid, highly crystalline, and low permeable (Wang et al., 2003). These characteristics nominate the nanocrystals as promising candidates for preparation of acid-resistant and mechanically strong carriers for nutraceuticals.

Microemulsions are thermodynamically stable and optically transparent dispersion of two immiscible liquids in which small droplets of one or both liquids are stabilised by surfactant molecules (Destrée & Nagy, 2006). These systems offer the possibility to synthesise nanoscalar supramolecular assemblies from monomers or tiny starting bricks (Lopez-Quintela, 2003). Zhang & Zhong (2009, 2010) implemented a water-in-oil (W/O) microemulsion system to generate whey protein isolate nanoparticles gelled by heat-treatment. There is no report in the literature on preparation of particles from starch nanocrystals. The objective of the present study was therefore to prepare date palm pit extract-loaded spheres from starch nanocrystals using a microemulsion reactor.

Materials and methods

Materials

Native potato starch, hydrochloric acid 37%, sodium hexametaphosphate, sodium hydroxide, sorbitan mono-oleate (span 80), glacial acetic acid, petroleum ether, Folin–Ciocalteu’s phenol reagent, and gallic acid were purchased from Merck (Darmstadt, Germany). Refined sunflower oil was purchased from Frico (Tehran, Iran). Kabkab variety date palm fruit was purchased from a local market in Tehran, Iran. Other chemical and biochemical materials were of analytical grade and used without purification. Bidistilled water was used throughout the work.

Date palm pit extract preparation

Pits were separated from the flesh and sun-dried after soaking in water and washing. Sun-dried seeds were further dried at 50 °C for 4 h in an oven (ShFH 55; Shiraz, Iran) and then were milled with a heavy-duty grinder (Retsch GmbH, Haan, Germany). The milled pit was sieved through 1 mm screens, followed by adding 1 L hot (80 °C) water onto 50 g pit powder. The mixture was incubated (SL1500; Bibby Scientific Limited, Staffordshire, UK) at 30 °C for 7 h while shaken at 100 rpm. The extract was filtered through
Whatman no. 4 filter papers, and sodium azide was added (50 mg L\(^{-1}\)) as antimicrobial agent. The extract was lyophilised (Lyovac GT3; Leybold-Heraeus, Cologne, Germany) and kept at \(-80\) °C until used. Phenolics content of extract was 1582 mg gallic acid equivalent per 100 g dry weight at pH 6.25.

**Preparation of water-dispersive starch nanocrystals**

Starch nanocrystals with mean size of 48 nm were obtained through hydrolysis of starch with 3.7 M hydrochloric acid at 35 °C for 24 days (Jivan et al., 2013). The hydroxyl groups at the reactive surface of nanocrystals become oxygen anions under alkaline conditions enabling to cross-link the crystals with sodium hexametaphosphate through intra- and interester linkages. Nanocrystals were then cross-linked by the method of Ren et al. (2012) with sodium hexametaphosphate to obtain the water-dispersive crystals. The cross-linked starch nanocrystals were stable in aqueous solution for at least 7 days and did not aggregate or settle down spontaneously.

**Preparation of particles and extract-loaded spheres**

The aqueous phase composed of 30 g L\(^{-1}\) water-dispersive starch nanocrystals, and 1.5 g L\(^{-1}\) date palm pit extract powder was microemulsified in the 1:1 mixture of sunflower oil and span 80 at ratio of 4% (w/w). The cosurfactant free microemulsion was destabilised by adding glacial acetic acid gradually resulting in formation of extract-loaded spheres. The generated spheres were precipitated by centrifugation at 18 300 \(g\) for 4 min, followed by neutralising the supernatant with concentrated acid to pH 7.0. The content of phenolic compounds was measured in supernatant according to the method of Singleton & Rossi (1965). Encapsulation efficiency was estimated as follows:

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\text{Phenolics content relased from capsules to supernatant} = \frac{\text{Phenolics content in the microemulsified aqueous phase \times 100}}{\text{Mean size of particulated nanocrystals and extract-loaded spheres are reported in Table 1. Particulation of microemulsified aqueous droplets of water-dispersive starch nanocrystals by acid resulted in generation of nanoscalar particles. Extract-free particulated nanocrystals were smaller but}}
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**Fourier transform infrared spectroscopy**

Particulated nanocrystals and extract-loaded spheres were mixed together with potassium bromide (weight ratio 2:500) and pressed into discs for scanning with a Fourier transform infrared (FTIR) spectrometer (Tensor 27 FT-IR Instructions, Bruker Optik GmbH, Ettlingen, Baden-Württemberg, Germany). Samples were scanned at 4000–500 cm\(^{-1}\) with resolution of 4 cm\(^{-1}\).

**Encapsulation efficiency measurement**

Extract-loaded spheres were disintegrated by suspending 30 g nanosphere in 1 L alkali water (pH 10.5) and shaking for minutes. The discrete nanocrystals were precipitated by centrifugation at 18 300 \(g\) for 4 min, followed by neutralising the supernatant with concentrated acid to pH 7.0. The content of phenolic compounds was measured in supernatant according to the method of Singleton & Rossi (1965). Encapsulation efficiency was estimated as follows:

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\text{Encapsulation efficiency} = \frac{\text{Phenolics content in the microemulsified aqueous phase \times 100}}{\text{Phenolics content relased from capsules to supernatant}}
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The measurement was done in triplicate, and results are reported as means of replication measurements.

**Results and discussion**

Size measurement results of core-free and extract-loaded particulated nanocrystals are reported in Table 1. Particulation of microemulsified aqueous droplets of water-dispersive starch nanocrystals by acid resulted in generation of nanoscalar particles. Extract-free particulated nanocrystals were smaller but...
less monodisperse than the extract-loaded counterparts. The lower polydispersity of extract-loaded particles is attributed to more regular arrangement of nanocrystals around the extract ingredients including phenolic compounds. Polyphenols might interact with starch nanocrystals and acted as stations for arrangement of crystals in microemulsion droplets.

The scanning electron microscopy images of core-free and extract-loaded particulated starch nanocrystals are shown in Fig. 1. It is observed that both core-free and extract-loaded particles were semi-spherical assemblies attached to neighbours and/or overlapped in dry state when examined under microscope (Shi et al., 2011; Bagheri et al., 2013). Starch nanocrystals were not distinguishable in electron microscopy images implying that crystals coalesced by the acid during fabrication of particles.

Figure 2 represents the FTIR spectra of core-free and extract-loaded particulated starch nanocrystals. A broad band due to hydrogen-bonded hydroxyl group (O–H) appeared at 3365 cm\(^{-1}\) and is attributed to the complex vibrational stretching associated with free, inter- and intramolecular-bound hydroxyl groups (Fang et al., 2002). The peak at 1153 cm\(^{-1}\) was due probably to the CO-bond stretching, and the peak at 1014 cm\(^{-1}\) is the characteristic of anhydrous glucose ring O–C stretching (Fang et al., 2004). In preparation of water-dispersive starch nanocrystals, sodium hexametaphosphate reacted with the surface hydroxyl groups of starch nanocrystals, establishing intra- and interester linkages. However, the absorbance peak of P–O–C vibration, located at 1050–850 cm\(^{-1}\), overlapped with the absorbance peak of C6–OH vibration of the starch glucose unit (Ren et al., 2012) making it
difficult to directly characterise the cross-linking occurred. The high similarity between spectra of core-free particles and extract-loaded spheres indicates that date pit extract entrapment within particulated cross-linked nanocrystals was majorly physical, and the probable chemical interactions between core and matrix of spheres were noncovalent Van der Waals and hydrophobic interactions.

The encapsulation efficiency calculated based on the difference of phenolics content in the microemulsified aqueous phase and that released from spheres approximated a value of 62 ± 3%. The remainder of phenolic compounds most probably transferred to inside the surfactant micelles formed in the microemulsion and lost during separation of organic phase. The leaching of phenolic compounds into the oil phase of microemulsion is not expected due to the water solubility of extract used.

Conclusion

The feasibility of microemulsification-particulation technique for fabrication of date pit extract-loaded spheres constructed from starch nanocrystals was successfully established. A more comprehensive study is required to examine the applicability/suitability of fabricated particles in food systems from immunity, sensory, and releasing profiles aspects.

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References


