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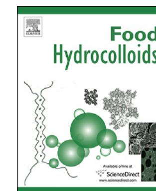

Fabrication of whey protein–pectin conjugate particles through laccase-induced gelation of microemulsified nanodroplets

Behzad Gazme, Ashkan Madadlou*

Department of Food Science and Engineering, College of Agriculture and Natural Resources, University of Tehran, Karaj, Iran

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ABSTRACT

A mixed solution of whey protein isolate, sugar beet pectin and laccase was microemulsified as nanodroplets in a mixture of sunflower oil and span 80. The droplets were transformed into nanoparticles through laccase-induced cross-linking and in situ gelation of biopolymers. Entrapment of caffeine within the conjugate bulk gel of biopolymers postponed the gelation time indexed by dynamic rheometry and decreased the strength of the final gel. The mean size of conjugate particles was 109 nm. Scanning electron microscopy images revealed an almost spherical morphology for uniformly shaped particles. Fourier transform infrared spectroscopy suggested the cross-linking of protein and pectin with participation of ferulic acid groups of pectin. Heat scanning experiments carried out by differential scanning calorimetry indicated the transition of conjugate particles from glassy to rubbery state at lower temperatures than their parent biopolymers.

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1. Introduction

There is an increasing demand for nutraceutical-enriched functional foods because of the augmenting awareness of consumers about the benefits of bioactive substances including antioxidants and vitamins. The bioactives reduce the risk of certain diseases such as cancer and cardiovascular disorders (Lee, Koo, & Min, 2004). Fortification of most food systems with bioactives is however challenging due to the undesirable affects of these compounds on taste, appearance and smell of final products, as well as their sensitivity to ambient and processing conditions encountered during storage and production of most food commodities. Encapsulation is successfully applied to extend the applicability and half-life of nutraceuticals by using both micro and nanoscalar carriers (Abd El-Salam & El-Shibiny, 2012).

Amongst the various food-grade and GRAS (generally recognized as safe)-listed materials, proteins are benefited in encapsulation approaches with enthusiasm because of ease of their preparation (Sundar, Kundu, & Kundu, 2010) and diverse functional characteristics such as gelation, as well as, their potential to fabricate tailor-made structures. Zhang and Zhong (2009) utilized a microemulsion nanoreactor to produce whey protein nanoparticles via enzymatic cross-linking and thermal pretreatments with the aim of heat stability enhancement.

Another category of biopolymers used in fabrication of micro and nano carriers are polysaccharides. Sugar beet pectin is of technological interest for food sector industry and researchers owing to its gelling, surface activity and emulsifying properties (Yapo, Robert, Etienne, Watehelet, & Paquot, 2007). It has been used in encapsulation of bioactive substances including fish oil (Drusch, 2007) as the wall material of capsules (Jung & Wicker, 2012).

Protein–polysaccharide conjugates confer superior characteristics to their parent biopolymers e.g. the resistance of proteins to heat, enzymatic digestion and organic solvents is increased when are conjugated with polysaccharides (Kato, Wada, Kobayashi, Seguro, & Motoki, 1991). Accordingly, conjugates are good candidates for preparation of bioactives-carrying vehicles with reinforced characters. Amongst the various forces to interconnect the proteins and polysaccharides, covalent conjugation offers great advantages such as preserved solubility and integrity of conjugates over a wide range of conditions (Hattori, Okada, & Takahashi, 2000). The enzyme-induced covalent conjugation of proteins with polysaccharides provides a nontoxic, specified function and safe method (Jung & Wicker, 2012). The enzyme laccase has been successfully used to cross-link the proteins and polysaccharides and enhance their functional properties (Chen, Li, Ding, & Suo, 2012; Figueroa-Espinoza et al., 1999; Jung & Wicker, 2012). It is a copper-containing polyphenol oxidase and can oxidize the ferulic

* Corresponding author. Tel.: +98 26 32248804.
E-mail address: a.madadlou@ut.ac.ir (A. Madadlou).

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acid groups in sugar beet pectin as substrate. This may lead to formation of self-supporting pectin gels (Kuuva, Lantto, Reinikainen, Buchert, & Autio, 2003). Laccase can also oxidize some amino acids in proteins such as tyrosine, cysteine and tryptophan (Jung &Wicker, 2012). Mediators such as ferulic acid which is abundantly present in sugar beet pectin enhance the laccase-induced oxidation of proteins (Steffensen, Andersen, Deng, & Nielsen, 2008). Heat treatment of whey proteins and in particular of β-lactoglobulin increase the exposure of tyrosine residues and enhance the laccase-catalyzed protein–sugar beet pectin conjugation (Jung & Wicker, 2012). Ma, Forssell, Partanen, Buchert, and Boer (2011) used the laccase for cross-linking of whey proteins in order to improve their emulsification properties. It was hypothesized by the authors of the present communication that the laccase-induced cross-linking of whey proteins with sugar beet pectin (Jung &Wicker, 2012) may be successfully employed in generation of conjugate particles carrying drugs and/or nutraceuticals.

The objective of the present study was therefore to microemulsify an aqueous phase containing sugar beet pectin, whey proteins and laccase as nanodroplets in an organic phase, followed by generation of conjugate bionanoparticles through the cross-linking action of enzyme. Caffeine was encapsulated as the model core (Gaudin et al., 2012).

2. Materials and methods

2.1. Material

Whey protein isolate (WPI) was a kind gift from Arla Food Ingredients (Vibyj, Denmark). Sugar beet pectin and caffeine powder were purchased from Herbstreith and Fox KG (Neuenbürg, Germany) and FTZ JC YUJIE International Inc (Qingdao, China), respectively. Laccase (0.5 U mg^{-1}, optimum pH = 6.0) was purchased from Sigma–Aldrich (Munich, Germany). Sunflower oil was commercially supplied by FRICO (Sirjan, Iran). Sorbitan monooleate (Span 80) and other chemicals were supplied by Merck (Darmstadt, Germany).

2.2. Preparation of biopolymers mixture solution

WPI powder was dissolved in distilled water (40 mg mL^{-1}) while stirring at 800 rpm for 30 min at ≈30 °C. A solution of sugar beet pectin was also prepared with identical concentration and procedure. Protein and pectin solutions were charged with sodium azide (0.1 mg mL^{-1}) as antimicrobe and stored for 10 h at 4 and 40 °C, respectively to warrant complete hydration. The protein solution was then denatured through heating at 80 °C for 15 min (Jung & Wicker, 2012) in order to expose the hidden groups to oxidation. The denatured whey protein solution (pH = 6.7 ± 0.1) was mixed with the solution of pectin (pH = 3.0 ± 0.1) on equal volumes at ≈30 °C and pH was adjusted to 6.0 using 5 M NaOH. The already hydrated laccase was added to the biopolymers mixture solution at rate of 2.5 units g^{-1} pectin/protein in order to initiate the gelation. In preparation of caffeine-carrying gels, biopolymers mixture solution was loaded with caffeine at rate of 2.7 mg mL^{-1} before injection of laccase.

2.3. Gelation time determination

Dynamic rheology was applied to determine the gelation point of biopolymers mixture solution using a controlled strain rheometer (Bohlin Gemini II, Malvern Instrument, Worcestershire, UK) with a cone and plate geometry. The diameter and angle of geometry was 40 mm and 4°, respectively. After infusing with laccase, 2 mL of biopolymers mixture solution either loaded with caffeine or not was poured into the geometry and dynamic moduli of storage and loss (G' and G'') were measured over the time within the linear viscoelastic region (frequency = 1 Hz, strain = 0.01, temperature = 40 °C).

2.4. Firmness of gels

The influence of incorporation of caffeine on the firmness of enzymatically-gelled biopolymers mixture solution was studied by measuring the force at the rupture point using a texture analyzer (M350-10CT, Testometric, Lancashire, UK). The biopolymers solution was infused with laccase and incubated for 26 h at 40 °C in order to obtain a mellow gel. Gels with 40 mm diameter and 30 mm height were penetrated with a cylindrical stainless still probe (diameter of 9 mm) at a constant speed of 0.25 mm s^{-1} to a distance of 50% of gels height.

2.5. Synthesis of nanoparticles

A water-in-oil microemulsion was used to fabricate core-free and caffeine-carrying conjugate nanoparticles. Based on our previous study (Sadeghi, Madadlou & Yarmand, 2014) an organic phase composed of sunflower oil and Span 80 on equal masses was infused with the laccase-injected biopolymers mixture solution (with or without caffeine) to the final proportion of 2% (wt/wt). The freshly enzyme-injected aqueous phase was added gradually to the organic phase to obtain a transparent system before the subsequent titration. Cross-linking of whey proteins and sugar beet pectin for 26 h at 40 °C by the enzyme resulted in slow particulation of nanodroplets and finally the microemulsion became unstable. This was followed by centrifugation of the unstable microemulsion at 5000 × *g* for 5 min (MIKRO 220R, Hettich Zentrifugen, Tuttlingen, Germany). After decanting the supernatant, the pellet was washed with chloroform and distilled water and vacuum dried (Croydon, Townson & Mercer Ltd, London, UK) at 180 mm Hg and 60 °C for 5 h. Samples were kept at 4 °C till analysis.

2.6. Size measurement of nanoparticles

The size distribution range and polydispersity of particles were measured based on the dynamic light scattering technique using a Zetasizer Nano range instrument (ZS, Malvern Instrument, Worcestershire, UK). The instrument was equipped with a He/Ne laser operated at 633 nm. The laser intensity was automatically adjusted to a specific range for scattered light detection by an attenuator (Gulzar, Bouhallab, Jeantet, Schuck, & Croguennec, 2011). Experiments were carried out at 25 °C. For this purpose, 5 mg of each core-free and caffeine-carrying sample was dispersed in 1.5 mL distilled water and shaken continuously at room temperature for 1 h before size measurements.

2.7. Morphology of nanoparticles

Core-free and caffeine-carrying particles were imaged with a scanning electron microscope (MV2300, CamScan, Lausanne, Switzerland) at various magnifications. In order to prevent from clumping of specimens, 5 mg of each sample was dispersed in 2 mL distilled water followed by pouring 100 μL of dispersion on an apparatus specific basis. The dispersion was let air dried at room temperature on the stub and then sputter-coated with a 10 nm gold layer before imaging.

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