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Gelation characteristics of the sugar beet pectin solution charged with fish oil-loaded zein nanoparticles

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ABSTRACT

It is of interest to fabricate chemical- and sugar-free pectin gels by which nutraceuticals or nutraceutical-loaded particles are delivered to consumers. Fish oil, a well known source of omega-3 fatty acids, was encapsulated in zein via alcohol evaporation. The mean size of the core-free and fish oil-loaded zein particles was 69 and 83 nm, respectively. The oil-loaded zein nanoparticles were then inoculated into the sugar beet pectin solution that gelified subsequently by the action of the oxidative enzyme, laccase. It was found that nanoparticle charging and enzymatic oxidation of pectin solution changed the flow model of the solution from Newtonian to Power law. An initial gelation rate was estimated for laccase-injected pectin solutions by using the data obtained from small amplitude rheometry tests. Nanoparticle charging of pectin solution slowed down the gelation rate ~26 folds and resulted in a much less firm final gel. Fourier transform infrared spectroscopy confirmed that ferulic acid residues of sugar beet pectin were oxidized by laccase, resulting in gel formation.

1. Introduction

Sugar beet pectin (SBP) is a co-processing product of sucrose production from sugar beets. This type of pectin has received an increasing attention by the food industry because of its specific functional properties including oxidative gelability (Norsker, Jensen, & Adler-Nissen, 2000). Some of the arabinose and galactose residues in SBP are esterified with ferulic acid (Takei, Sugihara, Ijima, & Kawakami, 2011) and provide a route for cross-linking of arabinan chains by the oxidative chemicals or enzymes (Zaidel, Chronakis, & Mayer, 2012). The enzyme laccase is a polyphenol oxidase that oxidizes and cross-links the intra- and inter-molecular ferulic acid units (Kuuva, Lantto, Reinikainen, Buchert, & Autio, 2003). Cross-linked arabinan chains in high enough concentrations form a thermo-irreversible gel which can be heated while maintaining integrity (Norsker et al., 2000). Laccase gels pectin through a green mechanism meeting both consumers' and environmental concerns about chemicals. The sugar-free pectin gel may be regarded as a low-calorie healthy product.

Omega-3 polyunsaturated fatty acids such as α-linolenic, eicosapentaenoic and docosahexaenoic acids are health-promoting and physiologically active compounds. These fatty acids play vital roles in the prevention and treatment of cardiovascular diseases, hypertension, arthritis, immune response disorders, and some types of cancers including colon, breast, and prostate (Duan, Jiang, & Zhao, 2011). The ratio of ω-6 to ω-3 fatty acids in the diet is an important determinant of health. The optimal ratio varies from 1/1 to 4/1 depending on the disease under consideration (Simopoulos, 2002). The dietary intake of ω-3 fatty acids of most populations is however considerably low (Mahan, Escott-Stump, & Raymond, 2012). Therefore an increased consumption of ω-3 fatty acids is recommended (Kolanowski, 2006). Fish oil is an excellent source of polyunsaturated fatty acids (Nakanishi, Iitsuka, & Tsukamoto, 2013). Enrichment of foods with fish oil is however, limited due to the insolubility of oil in most food systems and extensive sensitivity of long chain polyunsaturated fatty acids to oxidation. The latter is of particular importance when the enriched food undergoes a complementary heat treatment for microbial inactivation purposes. Encapsulation of the oil may retard or even prevent the thermo-oxidation reactions and widen the range of food commodities intended for enrichment purposes.

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Zein, the prolamin fraction of the corn protein, is insoluble in water but is readily dispersed in binary mixtures of alcohols and water. It has long been recognized for application in food industry (Quispe-Condori, Saldana, & Temelli, 2011). This potential is mostly arisen from the self-assembly property of zein because of its amphiphilic character (Wang & Padua, 2010). The protein is assembled into micro/nanoscaled particles in along with the increasing polarity of solvent, which is achieved through alcohol evaporation or water addition. As the solvent polarity increase, hydrophobic domains of zein molecules are buried inwards to escape from the polar medium (Wang & Padua, 2010). Fat soluble compounds are enclosed successfully within zein particles for encapsulation and controlled release purposes (Parris, Cooke, & Hicks, 2005).

It is of interest to fabricate SBP gels charged with fish-oil loaded zein nanoparticles as the delivery vehicles of ω-3 fatty acids and lipid-soluble drugs to consumers. There was no report in literature on the gelation behavior and gel characteristics of the pectin gels carrying zein nanoparticles. The objective of the present study was therefore to investigate the influence of charging of SBP solution with fish-oil loaded zein nanoparticles on laccase-induced gelation and gel characteristics of pectin.

2. Materials and methods

2.1. Materials

Maize zein, ethanol and laccase obtained from *Trametes versicolor* (optimum pH = 5.0; optimum temperature = 40 °C) were purchased from Sigma–Aldrich (Taufkirchen, Germany). Sugar beet pectin (Betapec Ru 301) was a kind gift by Herbstreith and Fox (Werder/Havel, Germany). Fish oil with the commercial name of "omega 3 fish oil" (Ho 307-Batch VO 10045) was supplied kindly by LYSI (Reykjavik, Iceland).

2.2. Zein nanoparticles preparation

Fish oil-loaded zein nanoparticles were produced by solvent evaporation (Wang & Padua, 2010). Zein (0.2 gr 100 mL^{-1}) and fish oil (0.04 gr 100 mL^{-1}) were dissolved in 80% ethanol (w/w) while stirring at 700 rpm for 15 min at 25 °C. The beaker containing 100 mL zein solution was placed under the hood at 50 °C for 80 min to allow evaporation until the solution volume diminished to 20 mL. Removal of ethanol through evaporation resulted in formation of zein nanoparticles enveloping the fish oil.

2.3. Entrapment of nanoparticles in pectin gel

Sugar beet pectin solution was prepared via dispersing pectin powder in distilled water (7.5 g 100 mL^{-1}), stirring at 500 rpm for 60 min and then storing at 25 °C for 10 h in order to fully hydrate the polysaccharide. Sodium azide (50 mg L^{-1}) was added to the freshly prepared pectin solution to suppress the microbial activities. Authors emphasize that sodium azide is highly toxic for human being and very dangerous for environment and would never be added to a consumer product. The zein nanoparticles aqueous dispersion was added into the pectin solution at ratio of 2:1 so that the final concentration of pectin became 2.5 g 100 mL^{-1}. Subsequently, pH value of the mixed solution was adjusted to 5.0 and then inoculated with laccase (5 unit g^{-1} pectin). The solution was incubated at 40 °C for 5 h to warrant gelation. This resulted in a fully developed pectin gel containing fish oil-loaded zein nanoparticles. The fish oil content of the pectin gel was estimated to be 0.88 mg mL^{-1}. Current recommended intake of ω-3 long chain fatty acids is at the range of 130–260 mg day^{-1} (Hibbeln, Nieminen, Blasbalg, Riggs, & Lands, 2006). Hence, an approximate volume of 147–295 mL of the pectin gel carrying fish oil-loaded zein nanoparticles would be required to meet the daily recommended intake. A control nanoparticle-free counterpart gel was also fabricated.

2.4. Size measurement of zein nanoparticles

Aqueous fresh dispersion of zein nanoparticles was employed for particle size measurement by using a dynamic light scattering particle size analyzer (ZetaPlus, Brookhaven Instruments Co., NY, USA). The number-averaged diameter was selected to express the particle size of nanoparticles. Sample was read three times.

2.5. Atomic force microscopy

The topographic images of pectin gel specimens were acquired by an atomic force microscope (Nanowizard II instrument, JPK, Germany) in the intermittent contact mode using ACTA cantilever with resonance frequency of 1 Hz and force constant of 13–77 N m^{-1}. For this purpose, a thin slice was cut from the produced pectin gel carrying fish oil-loaded zein nanoparticles with a surgery blade, placed on a lamella and let air dried before microscopic imaging.

2.6. Fourier transform infrared (FTIR) spectroscopy

FTIR spectra of zein nanoparticles and pectin gels were characterized by means of a Perkin Elmer FT-IR spectrometer (Perkin Elmer Co., MA, USA) using the potassium bromide disk method in 4000–500 cm^{-1} range with resolution of 4 cm^{-1}.

2.7. Rheological analysis

2.7.1. Viscosity of pectin solutions

The viscosity of the pectin solution impregnated with fish oil-loaded zein nanoparticles and its nanoparticle-free counterpart either immediately after laccase injection or before the injection was measured by using a Brookfield viscometer (LVDV-II Pro, Brookfield Engineering Inc., USA) equipped with the LV spindle at 25 °C. In each test, about 20 mL sample was poured into the measuring cylinder and shear rate was adjusted to increase from 12 to 110 s^{-1} within 12 s intervals. The Newtonian and power law models were used to analyze the rheological behavior of samples.

2.7.2. Gelation rate determination

Gelation rate of the pectin solution charged with fish oil-loaded zein nanoparticles and its nanoparticle-free counterpart was determined through measuring the complex modulus (G^*) immediately after being injected with laccase. A controlled rate Bohlin Gemini 2 rheometer (Malvern Instruments Ltd., Worcestershire, UK) fitted with a cup and vane geometry was employed in the experiment. Specimens were placed within the cup and measurements were carried out at frequency of 1 Hz and strain of 0.5% at 40 °C.

A frequency sweep (0.01–100 Hz) test at 2% strain was also performed on the formed gels 110 min after laccase injection to determine and compare the storage and loss moduli (G' and G'', respectively) of the nanoparticle-loaded and nanoparticle-free pectin gels.

2.8. Statistical analysis

The data were subjected to one-way analysis of variance (ANOVA) by SPSS (ver. 16, IBM software, NY, USA) software. Any