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Synthesis and characterization of enzymatically cross-linked feruloyl amylopectin for curcumin encapsulation

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ABSTRACT

Feruloyl amylopectin (FAP) was synthesized by the *N*,*N*′-carbonyldiimidazole (CDI) activation method, and the enzymatically cross-linked feruloyl amylopectin (CL-FAP) was prepared via catalysis of horseradish peroxidase (HRP) with the presence of hydrogen peroxide (H_2O_2). RP-HPLC-DAD/ESI-TOF-MS measured ferulic acid and its derivatives in FAP and CL-FAP. FAP was primarily composed of two ferulate monomers, while CL-FAP was composed of two ferulate monomers and two ferulate dehydrodimers. The ester formation in the feruloyl group was confirmed by the presence of carbonyl and aromatic C=C signal near 1725 (1723) and 1510 cm^{-1} in the FT-IR spectra. X-ray diffraction studies showed that the two modified amylopectins lost the ordered A-type crystalline structure, characteristic of maize amylopectin. The encapsulation capacity of curcumin (*ECC*) in 1 mg/mL CL-FAP microemulsion was measured at 88.13 μg/mg by HPLC.

1. Introduction

Natural branched polysaccharides, such as arabinoxylans and arabinans can form highly viscous solutions with gelling capacity by covalent cross-linking the polysaccharide chains through dimerization of ferulic acid substituents under oxidative conditions, e.g., use of enzymatic free radical generating agents such as laccase and peroxidase-H_2O_2 [4]. This process involves some of the arabinose residues that are ester linked on the (O)-5 to ferulic acid (FA) (3-methoxy, 4-hydroxy cinnamic acid). As a natural phenolic acid cross-linker, FA has been investigated for modification of proteins, which can react with some amino acids present at proteins such as tyrosine, lysine, and cysteine to form cross-link bounds. Araghi et al. [1] showed modification of fish gelatin by phenolic acids led to reduced solubility which can be attributed to interaction of polymers by hydroxyl or carbonyl groups that lead to formation of hydrogen or covalent bonds, formation of cross-linking, and so reduction in water solubility of polymer. Studies on synthesis of feruloyl starch have been reported by Ou et al. [21] and Mathew and Abraham [18]. In the activation process of carboxylic acid, some toxic reagent, such as thionyl chloride and phosphorus trichloride have been used. In addition, if the feruloyl starches could form covalently cross-linked polymers has not been investigated. Amylopectin, as a highly branched starch, may have similar characteristics of cross-linking by ferulic acid to that of other natural branched polysaccharides, such as arabinoxylans and arabinans.

Encapsulation of bioactive compounds using emulsions is a common formulation approach used in food, cosmetic and pharmaceutical industries. Particle stabilized emulsions (Pickering emulsions) are stabilized by solid particles instead of surfactants and have been widely investigated in pharmaceutical and cosmetic fields since they present less adverse effects than the classical emulsions [17]. Hydrophobic starch particles, recently being used for Pickering stabilization, have the advantage of the ability to swell during gelatinization [23,24]. Curcumin is often selected as a model bioactive compound because it has significant antioxidant and anti-inflammatory properties and therefore is an attractive target for many food and pharmaceutical applications. Despite these attractive properties, oral delivery of curcumin has been a significant challenge due to its limited stability and poor water solubility. At present there are few studies using the cross-linked feruloyl amylopectin to prepare the water-soluble microencapsulated curcumin.

In this study, we first reported a simple, efficient, and mild homogeneous synthesis of feruloyl amylopection using CDI as the activating agent and DMSO as the solvent. Then, FAP was cross-linked with catalysis of HRP/H_2O_2, got CL-FAP. Furthermore, we studied the physical and chemical characterization of CL-FAP.

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Table 1
Swelling power and solubility of the samples.*

Sample	Swelling power (%)	Solubility (%)
Amylopectin	7.4 ± 0.02[c]	14.6 ± 0.05[a]
FAP	10.81 ± 0.04[b]	8.62 ± 0.03[b]
CL-FAP	19.21 ± 0.06[a]	6.61 ± 0.03[c]

* All values are means of triplicate determinations ± SD. For each characteristic, means followed by different letters (a, b, and c) in each column are statistically different ($p < 0.05$).

Finally, curcumin-in-water (O/W) Pickering emulsions were prepared using CL-FAP as particle stabilizers.

2. Experimental

2.1. Materials

Maize amylopectin was obtained from Sigma Chemical Co., Ltd. (St. Louis, MO, USA). Ferulic acid was from Yuancheng Pharmaceutical Co., Ltd (Hubei, China), and *N*,*N*′-carbonyldiimidazole (CDI) was from Liyang Kaixin Chemical Co., Ltd (Jiangsu, China). The DMSO was obtained from Chengdu Kelong Chemical Reagent Factory (Jiangsu, China). All reagents were of analytical grade. Horseradish peroxidase (EC 1.11.1.7, type VI, 250 U/mg) was from Sigma–Aldrich (Zwijndrecht, The Netherlands).

2.2. Esterification and oxidative cross-linking reaction

In a separate flask, 1.2 g (6.2 mmol) ferulic acid was treated with 1.0 g (6.2 mmol) CDI in 10 mL DMSO. The mixture was kept for 16 h at 60 °C by continuous magnetic stirring. Then, the solution of ferulic acid with activated carboxyl groups was prepared. Four grams (24.7 mmol) of amylopectin was dissolved in 72 mL DMSO with a T-18 basic dispenser (IKA) equipped with an S10N 5G dispersion tool at 20,000 rpm/min for 1 min. Then 8 mL of the activated ferulic acid solution was added. The esterification reaction was kept for 5 h at 60 °C by continuous magnetic stirring. Isolation of the feruloyl amylopectin was carried out by precipitation into 80 mL isopropanol followed by filtration and washing with isopropanol (three times with 80 mL). The pellet was dissolved in 80 mL DMSO, and dialyzed against 800 mL DMSO for 24 h (three times in total) and then against 800 mL deionized water that was replaced every 6 h for 48 h. The dialyzed sample was recovered by lyophilization to give the feruloyl amylopectin (FAP) (Fig. 1a).

Oxidative cross-linking of FAP with the peroxidase/hydrogen peroxide system was performed by adding 250 μL of horseradish peroxidase (HRP) (10 mg/mL) and 1.25 mL of hydrogen peroxide (0.5 M) to 50 mL of 0.05 M sodium phosphate, pH 6.0. The mixture was allowed to equilibrate at 25 °C. Subsequently, 50 mL of 1% (w/v) FA solution was added to 10 aliquots of 5 mL each at regular time intervals of 5 min for 1 h. The reaction mixture was maintained at 25 °C for 4 h, and then the reaction was stopped by inactivation of the enzyme (2 min/100 °C). The sample was dialyzed against 1000 mL of deionized water that was replaced every 6 h for 48 h. The dialyzed sample was recovered by lyophilization to give cross-linked feruloyl amylopectin (CL-FAP) (Fig. 1b).

2.3. RP-HPLC-DAD/ESI-TOF-MS analysis of ferulic acid and its derivatives

The sample (0.10 g) was extracted in 20 mL of 2 mol/L NaOH under magnetic stirring at 20 °C for 2 h to free bound ferulic acid. The extracts were then acidified with 6 mol/L HCl to pH 1–2 and extracted three times with two volumes of ethyl acetate. The organic phase was collected and pooled in a 8-mL evaporating flask for rotary evaporator at 60 °C to dryness; 10 mL of 50% (v/v) methanol dissolved the solid.

To identify ferulic acid and its derivatives, an Agilent 1200HPLC (Agilent Technologies, USA) was used, equipped with a binary pump, microvacuum degasser, auto plate-sampler, column compartment, diode array detector, and coupled to an Agilent 6210 TOF-MS (Agilent Technologies).

Samples were dissolved in 1:1 MeOH–water and injected onto a reverse phase Thermo BDS C_{18} column (100 × 2.1 mm, 2.2 μm). These were eluted at 0.4 mL/min with an increasing gradient of MeOH in acidified water as follows: $t = 0$ min, 100% solvent A (0.2% formic acid in water):0% solvent B (0.2% formic acid in MeOH); $t = 8$ min, 10% A:90% B; $t = 8.2$ min, 80% A:20% B; and $t = 10$ min, 80% A:20% B. The column temperature was 30 °C. The FA was detected and quantified at 319 nm using an authentic external standard for retention time and spectral recognition with quantification via area linear regression. The diFAs were also detected at 319 nm but quantified at 280 nm according to response factors as described by Waldron et al. [25]. All solutions were filtered through a 0.45-μm nylon membrane filter (Jinteng Instrument Co., Tianjin, China) before HPLC, and the mobile phase solvents were degassed before use.

The effluent from the RP-HPLC column was split using a T-type phase separator before being introduced into the mass spectrometer (split ratio = 1:3) via negative mode electrospray. The operating parameters were as follows: drying gas (N_2) flow rate, 12.0 L/min; drying gas temperature, 320 °C; nebulizer, 32 psig; capillary, 3600 V; Oct RFV, 220 V; fragmentor voltage, 135 V; and skimmer voltage, 62 V. Mass spectra were recorded from *m*/*z* 100 to 1200. The operation, acquisition, and analysis used the MassHunter workstation software (Version B.02.00, Agilent Technologies). Degree of substitution (DS) of FAP was calculated as: $DS = 162W/[100M - (M - 1)W]$, where 162 is molecular weight of glucose unit, M is weight of feruloyl group (%), and W = molecular weight of feruloyl group (194.19).

2.4. FT-IR spectroscopy

The FT-IR spectra of native amylopectin, ferulic acid and modified amylopectins were recorded in an FT-IR spectrometer (PerkinElmer Spectrum 2000, USA) using potassium bromide (KBr) discs prepared from powdered samples mixed with dry KBr at 1:50.

2.5. X-ray diffraction studies

X-ray diffraction patterns of native amylopectin, ferulic acid and modified amylopectins were analyzed using an X-ray diffraction spectrometer (D8 Advance, Bruker Co., Ltd., Germany) with Nickel filtered Cu Kα radiation (λ = 0.154 nm) at a voltage of 40 kV and current of 30 mA. The diffractometer was equipped with an automatic divergence slit, and the scattered radiation was detected from 5 to 40° (2θ) at 2° (2θ)/min and a step size of 0.06 (2θ).

2.6. Determination of swelling power and solubility

Swelling power (SP) and solubility (S) were determined following the method of Li et al. [15] with a slight modification. 30 mL of 1% sample suspensions (w/v) were heated in a water bath at 95 °C for 30 min. Then each suspension was cooled and centrifuged at 3000 × *g* for 10 min; the decanted was weighed and the supernatant was placed in an air oven at 105 °C for 3 h. SP and S were determined as follows: SP = weight of sediment × 100/(weight of dry sample solids × (100 − solubility)); S = weight of dissolved solids in supernatant/weight of dry sample solids in the original sample × 100.