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Synthesis and characterization of a novel potato starch derivative with cationic acetylcholine groups

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ABSTRACT

A novel substance, cationic acetylcholine potato starch (CAPS), was developed for the first time. The synthesis process had three steps: first, carboxymethyl potato starch (CMPS) was synthesized under sodium hydroxide alkaline condition and in isopropyl alcohol organic media; second, bromocholine chloride (BCC) was synthesized with sulphuric acid as a catalytic agent; finally, CAPS was synthesized by the reaction of CMPS with BCC in N,N′-dimethylformamide (DMF). The degree of substitution (DS) of CAPS was determined by ammonia gas-sensing electrode and elemental analysis. CAPS was characterized by Fourier transformed infrared (FTIR) and near infrared (FTNIR) spectroscopy, scanning electron microscopy (SEM), X-ray diffraction (XRD) and differential scanning calorimetry (DSC).

1. Introduction

Starch is one of the most abundant natural polymers and is the major component in many food plants. It is composed of amylase and amylopectin, in a proportion of approximately 20:80 in potato starch [1,2]. The two polysaccharides both have a (1 → 4) linked poly-α-D-glucan backbone. However, amylase is a linear polymer, while amylopectin is highly branched at the C6 positions. Starch and its derivatives have been widely used in food and non-food applications due to their abundance, low cost, and biodegradation [3]. Cationic starch (CS) is a modified starch derivative with positive charge groups, which is commonly prepared by the reaction of native starch with conventional positive charge groups such as amino, imino, and ammonium [4–6]. The most widely used cationic starches are the starch ethers with tertiary amino and quaternary ammonium groups, which are prepared by the reaction of native starch with a cationic reagent 3-chloro-2-hydroxypropyltrimethyl ammonium chloride (CHPTMAC) or 2,3-epoxypropyltrimethyl ammonium chloride (EPTMAC) under various conditions [6–8]. They are usually used as wet-end additives in papermaking industry and as flocculants in wastewater treatment [9–11]. The benefits of their use include better fiber and filler retention, improved mechanical strength and better biodegradability of excess additives [7].

There are many reports about the cationic starch [12–14]. However, few examples of the cationic starch synthesized by native starch with alkaloid as positive charge groups had been reported. In this work, cationic acetylcholine potato starch (CAPS) was synthesized using potato starch as carrier and small molecule acetylcholine (ACh) as functional groups, respectively. Starch is a carbohydrate that could be absorbed, decomposed and changed to several nutritional components, such as glucose, maltose, and dextrin in the presence of α-amylase and β-amylase in human body. ACh has been considered as an important excitatory neurotransmitter [15], and its physiological and pharmacological effects have been well documented [16–18]. Furthermore, ACh also can be absorbed and decomposed in biological body, changed into acetic and choline under acetylcholinesterase (AChe), both of which are useful for biological body [19]. Therefore, it is suggested that the product we synthesized has biology activity and can be used in the areas of food, cosmetics and medicine.

The functional properties of the cationic starches depend strongly on the obtained degree of substitution (DS) and the changes in the granular and molecular structure of the modified starches. Therefore, the reaction conditions including reaction solvent and the mass ratio of bromocholine chloride (BCC) to carboxymethyl potato starch (CMPS) (m_{BCC}/m_{CMPS}), which could affect DS, were optimized. In addition, the information about molecular structural changes was obtained from vibrational spectroscopic

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techniques such as infrared (IR) and near infrared (NIR) spectroscopy. The granular structure of the modified starches was determined by scanning electron microscopy (SEM), X-ray diffraction (XRD) and differential scanning calorimetry (DSC).

2. Materials and methods

2.1. Materials

Native potato starch (food grade, molecular weight of 2.27×10^6, viscosity 1700 mPa s of 2% (w/v) gelatinized solution at 25 °C) was provided by Tengsheng Agricultural Products Group (Gansu, China). Choline chloride (CC) was purchased from Hongxing Chemical Reagent Factory (Shanghai, China). Isopropyl alcohol (IPA), monochloroacetic acid (MCA), sodium hydroxide, sulphuric acid, hydrobromic acid and other chemicals are analytical grade and used as received.

2.2. Synthesis of carboxymethyl potato starch (CMPS)

Carboxymethyl potato starch (CMPS) was synthesized according to a previously reported method [20]. Native potato starch (10 g) and IPA (20 ml) were added into a three necked flask fitted with a mechanical stirrer. NaOH (3 g) was introduced into the flask before the temperature was raised to 35 °C and the mixture was constantly stirred for 45 min. Then, MCA (7.8 g) dissolved in IPA (14 ml) was added, followed by addition of 2.8 g of NaOH. The reaction was allowed to proceed at 45 °C for 100 min. The resulting product was cooled, neutralized, filtrated, and washed with 80% methanol aqueous solution. The final product, CMPS, was then dried in a vacuum oven at 60 °C for 12 h. The degree of substitution was determined by the J acid colorimetric method using a literature procedure [21] and it was determined to be 0.39.

2.3. Synthesis of bromocholine chloride (BCC)

Bromocholine chloride (BCC) was synthesized by the following procedure. 7.6 g of hydrobromic acid (40%, v/v) and 2 g of choline chloride (CC) were added into a three necked flask at 88 °C, and then 1.6 g of sulphuric acid was added dropwise. The reaction was carried out for 5 h under a nitrogen atmosphere. The mixture was neutralized with sodium hydroxide. Excess sulphuric acid was removed by adding barium chloride, followed by addition of sodium carbonate. The solid was filtered off and the filtrates were concentrated to dryness. The resulting solid was washed with ethyl ether and dried in a vacuum oven.

2.4. Synthesis of cationic acetylcholine potato starch (CAPS)

CMPS, BCC, and 20 ml of reaction solvent were added in a round-bottom flask. The mixture was thoroughly degassed by bubbling with nitrogen and then heated to 80 °C. The reaction was allowed to proceed for 9 h. The resulting product was washed with ethanol, filtered and then dried in a vacuum oven. The effects of reaction solvent and the mass ratio of BCC to CMPS (m_{BCC}/m_{CMPS}) on degree of substitution (DS) and yield were determined. Values reported for the DS and yield were average of three experiments.

2.5. Determination of degree of substitution (DS)

DS of CAPS was determined by ammonia gas-sensing electrode [22]. 1.0 g of CAPS sample was weighed accurately and added to a 100 ml of Kjeldahl flask with 0.1 g of fine selenide powder and 10 ml of sulphuric acid. The flask was heated until the liquid changed to a colorless and transparent solution. The solution was cooled to room temperature and transferred to a 250 ml volumetric flask. Distilled water was added to scale. 10 ml of the solution was withdrawn and added to a beaker with 37 ml of distilled water, followed by addition of 3 ml of sodium hydroxide (10 M). The mixture was stirred and its electric potential E_1 was determined. Then 0.5 ml of ammonium chloride standard solution (0.1 M) was added dropwise and the electric potential E_2 was recorded. The electric potential E_3 was determined after 55.5 ml of sodium chloride buffer solution (0.2 M) was added. DS was determined according to Eq. (1)–(3).

$$C_X = C_S \frac{V_S}{V_X} (10^{0.301((E_2-E_1)/(E_2-E_3))} - 1)^{-1} \quad (1)$$

where C_X and C_S are molar concentration of overall ammonia of samples and ammonium chloride standard solution (mol L^{-1}), respectively; V_X and V_S are the total volume of the mixed solution and ammonium chloride standard solution volume (ml), respectively.

$$W_N = \frac{C_X \times 14 \times V_X \times f}{m \times 1000} \times 100\% \quad (2)$$

where W_N is the nitrogen mass concentration of the sample (%); m is the weight of the sample (g); f is the dilution times; 14 is the molar mass of nitrogen.

$$DS = \frac{162 W_N}{1400 + W_N(1 - M_r)} \quad (3)$$

where M_r is mole mass of the substituent ($-CH_2COOCH_2CH_2N(CH_3)_3Cl$), (g mol^{-1}); 162 is mole mass of an anhydrous glucose unit of starch.

2.6. Component analysis of CAPS

Contents of carbon, hydrogen, nitrogen in CAPS were determined by an elemental analysis instrument (Germany Elemental Vario EL Corp., model 1106).

2.7. Fourier transformed infrared (FTIR) and near infrared (FTNIR) spectroscopy

Native potato starch, CMPS and CAPS were characterized by Fourier transformed infrared (FTIR) spectrophotometer (170-SX, American) and Fourier transformed near infrared (FTNIR) spectrophotometer (AWTS-AvaSpec-NIR256, American).

2.8. Scanning electron microscopy (SEM)

The granule morphologies of native potato starch, CMPS and CAPS samples were examined using a JSM-6701F SEM instrument (JEOL, Ltd., Japan). The samples were coated with gold prior to observation.

2.9. X-ray diffraction (XRD)

The X-ray diffraction (XRD) measurements for native potato starch, CMPS and CAPS were taken on X-ray powder diffractometer (Shimadzu XRD-6000) with Nickel filtered CuKα radiation (λ = 1.54056 Å) at a voltage of 40 kV and current of 100 mA.

2.10. Differential scanning calorimetry (DSC)

DSC measurements were performed with a Sapphire DSC (Perkin-Elmer, America). About 10 mg of the dried samples were placed in aluminum pans and sealed. The samples were heated from 40 to 100 °C with a heating rate of 10 °C/min under nitrogen atmosphere.