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Interaction of cellulose nanocrystals and amylase: Its influence on enzyme activity and resistant starch content

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ABSTRACT

The aim of this work was to evaluate the effects of cellulose nanocrystals (CNCs) on amylolytic enzyme activity and starch digestibility. For the first time, we investigated the interaction between α-amylase or glucoamylase and CNCs using ultraviolet visible (UV–Vis) absorption spectroscopy, fluorescence quenching method, Fourier transform infrared (FTIR) spectroscopy, and circular dichroism (CD). The results of UV and fluorescence spectra suggested that CNCs interacted with α-amylase and glucoamylase. Increasing the concentration of CNCs caused a reduction of α-amylase and glucoamylase activities. The FTIR and CD results indicated that CNCs induced structural changes in the secondary structure of α-amylase and glucoamylase. By incorporating CNCs into maize, potato and pea starches, the contents of rapid digestible starch and slowly digestible starch of the cooked starches decreased while resistant starch content increased.

1. Introduction

Starch is the most important carbohydrate in the human diet and serves as a major energy source (Hung, Vien, & Phi, 2016). Resistant starch (RS) is a form of dietary fiber and is naturally present in many starchy foods (Raigond, Ezekiel, & Raigond, 2015). The products of RS fermentation help to prevent colorectal cancer, lower the risk of heart disease, and influence metabolic and inflammatory bowel diseases such as diabetes and diverticulitis (Craig, Troup, Auerbach, & Frier, 1998; Topping & Clifton, 2001). Due to the health benefits of RS in the human diet, there has been a great interest in increasing its content in foods through various techniques.

Human α-amylase, as one of the key digestive enzymes in the digestive system, has been shown to function by breaking down starch into maltose and glucose (Sandip et al., 2008). Glucoamylase, also known as amyloglucosidase, is a biocatalyst capable of hydrolyzing α-1, 4 glycosidic linkages in raw or soluble starches and related oligosaccharides with the inversion of the anomeric configuration to produce β-glucose (Norouzian, Akbarzadeh, Scharer, & Moo, 2006). These two enzymes, which are the chief digestive enzymes, catalyze the hydrolysis of α-1, 6- and α-1, 4-glycosidic bonds in starch and other *gluco*-oligosaccharides, resulting in the formation of glucose (Shigechi et al., 2004). Great effort has been devoted to inhibit the activities of digestive enzymes in order to control the level of glucose. For instance, phenolic compounds can directly bind to digestive enzymes (amylases, sucrase, trypsin, and lipase), decreasing enzyme functionality (Le Bourvellec & Renard, 2005; Asquith & Butler, 1986; Vonk et al., 2000), and further slowing the rate of starch and protein digestion. Amylase inhibition by polyphenol binding could lead to lower blood glucose levels for diabetic patients. Interactions of polyphenols with enzymatic proteins subsequently change their molecular configuration, and this is known to reduce the catalytic activity of various enzymes (Bandyopadhyay, Ghosh, & Ghosh, 2012). In an in vitro study, He, Lv, and Yao (2007) discovered that tea polyphenols inhibit the activity of pepsin (31%) and other digestive enzymes like α-amylase (61%), trypsin (38%) and lipase (54%). This result suggests the possibility of antinutritional effects of tea polyphenols, in terms of reduction in activity of digestive enzymes. It was found that the dietary fiber materials as citrus pectin (Tsujita et al., 2003), pectin of high methylic esterification and guar gum (Isaksson, Lundquist, & Ihse, 1982) could contribute to inhibiting the activity of gastrointestinal tract enzymes. Espinal-Ruiz, Parada-Alfonso, Restrepo-Sánchez, and Narváez-Cuenca (2014) suggested that the inhibition of activity of enzymes by ingested pectic polysaccharides might play an important role to decrease the caloric intake.

Recent developments in bio-nanotechnology have had a large socioeconomic impact in bio-medical industrial sectors because the use of nanomaterials is constantly increasing in industrial activities such as biosensing, diagnostics, biomedicine, and therapeutics (Michalet et al., 2005; Zhang et al., 2008). The interaction of protein/enzyme molecules with nanomaterials is at the core of such applications. On interacting with nanoparticles, the enzyme molecules may alter their

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conformation, expose new epitopes on the protein surface, or even deviate from their normal function (Lynch, Dawson, & Linse, 2006). In a recent study, Ernest, Shiny, Mukherjee, and Chandrasekaran (2012) reported that silver nanoparticles showed an increased enzyme activity in the endohydrolysis of starch. Saha, Saikia, and Das (2015) investigated the in-depth interaction features of protein molecules with copper sulfide nanoparticles and they were able to extrapolate suitable parameters for developing functional bionanocomposites with the desired activity.

Cellulose nanocrystals (CNCs) is a material obtained from the acid hydrolysis (Incani, Danumah, & Boluk, 2013) or ultrasonic-assisted enzymatic hydrolysis (Cui, Zhang, Ge, Xiong, & Sun, 2016) of native cellulose. As a result of their high content of –OH groups, high aspect ratio and surface area, these nanocrystals could be used for polymer reinforcement, nanocomposite formulation and reinforcement, drug delivery and biomedical uses (de Lima et al., 2012; Male, Leung, Montes, Kamen, & Luong, 2012; Gaspar et al., 2014). To the best of our knowledge, however, there have been neither reports about the interaction of CNCs with α-amylase and glucoamylase nor the effect of CNCs on these enzyme activities. Therefore, in this work, we investigated this interaction using ultraviolet visible (UV–Vis), fluorescence, Fourier transform infrared (FTIR), and circular dichroism (CD) spectroscopic techniques. We also explored the effect of CNCs on the activities of α-amylase and glucoamylase. Further, we determined the impact of CNCs on starch digestibility, so as to ascertain whether our results could reasonably be extrapolated to CNCs during digestion.

2. Materials and methods

2.1. Materials

The commercial wheat cellulose (95%) that is derived from wheat straw was provided by Shanghai NuoShen Food Trading Co., Ltd. (China). Normal maize starch (with an amylose content of approximately 31.0%), pea starch (with an amylose content of approximately 40.0%), potato starch (with an amylose content of approximately 26.3%), and soluble starch (from potato) were obtained from the Zhucheng Xingmao Corn Development Co., Ltd. (Shandong, China). Porcine pancreatic α-amylase (from porcine pancreas, ~30 U/mg) and glucoamylase (≥300 U/mL), supplied by Sigma-Aldrich (Shanghai) Trading Co., Ltd., were diluted with distilled water into pancreatic α-amylase solution (290 U/mL) and amyloglucosidase solution (500 U/mL). All other chemicals used in the present study were of analytical grade.

2.2. Preparation of cellulose nanocrystals

The CNCs were prepared using the method described by Silvério, Neto, Dantas, and Pasquini (2013), with some modifications. Briefly, the cellulose was milled with a blender and passed through a 35-mesh screen before being used for the extraction of nanofibers through acid hydrolysis. For each gram of cellulose, 30 mL of H_2SO_4 64% (w/w) was used. Following this, the hydrolysis was performed for 90 min at 45 °C while it was constantly and vigorously stirred. Immediately following the hydrolysis, the suspension was diluted 10-fold with cold water to stop the hydrolysis reaction, and then centrifuged twice for 15 min at 7000 rpm to remove the excess acid. The precipitate was washed several times with deionized water until neutrality was reached. The resulting suspension was subsequently treated for 5 min at 20,000 rpm using a disperser type UltraTurrax, and sonicated for 5 min before being freeze-dried to obtain the CNC samples.

2.3. Transmission electron microscopy (TEM)

Transmission electron micrographs of the CNCs samples were taken with a Hitachi 7700 (Tokyo, Japan) transmission electron microscope with an acceleration voltage of 80 kV. A drop of diluted CNC aqueous suspension was deposited on a Cu microgrid (200 mesh) and allowed to dry. The grid was negatively stained with a 3% (w/w) solution of uranyl acetate and dried at room temperature.

2.4. X-ray diffraction

The X-ray diffraction of samples was determined using an X-ray diffractometer (AXS D8 ADVANCE, Bruker, Karlsruhe, Germany) with Cu Kα radiation (λ = 0.1542) at 44 kV and 26 mA. The samples were scanned at 5°/min with a 2θ angle range from 5° to 40°. The crystallinity index value was computed according to Segal, Creely, Martin, and Conrad (1959) to quantify the crystallinity of the samples. The crystallinity index is defined by:

$$\mathrm{CIr}(\%) = \frac{(I_{002} - I_{am})}{I_{002}} \times 100 \quad (1)$$

where I_{002} is the peak intensity corresponding to crystalline, and I_{am} is the peak intensity of the amorphous fraction.

2.5. Interaction of enzyme with CNCs

The CNCs was dispersed in 50 mL deionized water by ultrasonic mixing. Then, CNC suspensions at various concentrations (0, 0.04, 0.08, 0.12, and 0.16%) were incubated with α-amylase or glucoamylase at a constant 0.1% (w/v) concentration at room temperature for 20 min on a rotary shaker (300 rpm). After the period of interaction, characterization studies were performed with the help of UV–vis, fluorescence, FTIR, and CD spectroscopy.

2.6. UV–vis spectroscopy

Following the interaction between CNCs and α-amylase or glucoamylase, about 3 mL of each sample was used to record UV–vis spectra using a UV–vis spectrophotometer (Shimadzu-2600, Kyoto, Japan) from 240 to 350 nm. The final spectra were baseline-corrected by subtracting the corresponding CNCs spectra obtained under the same condition.

2.7. Fluorescence spectra analysis

After interacting with CNCs, α-amylase or glucoamylase fluorescence was measured using a F2500 Spectrofluorimeter (Hitachi, Tokyo, Japan). The samples were subjected to analysis at the excitation wavelength of 285 nm and at emission spectra in the range of 300–460 nm. A quartz cuvette (4 cm × 1 cm × 1 cm) with a 1 cm path length was used for measurements.

2.8. Fourier transform infrared

The FTIR spectra of α-amylase, glucoamylase, and the mixture of the enzymes and CNCs were recorded on an FTIR spectrophotometer (NEXUS-870; ThermoNicolet Corporation, Madison, WI, USA) within the wavenumber range of 4000–600 cm^{-1}. The background obtained from a KBr scan was automatically subtracted from the sample spectra. A total of 32 scans were obtained and the resolution was 4 cm^{-1}.

2.9. Circular dichroism spectroscopy

The CD spectroscopy was performed to analyze the changes that occurred in the secondary structure of α-amylase and glucoamylase. An aliquot of α-amylase or glucoamylase was incubated for 20 min with various concentrations of CNCs (0, 0.04, 0.08, 0.12, and 0.16%). Then, CD spectra were analyzed using a JASCOJ-715 CD spectroscopy (Welltech Enterprises, Maryland, United States). The secondary structure contents of the samples were estimated using Dichroweb: the online Circular Dichroism Website http://dichroweb.cryst.bbk.ac.uk