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Sodium hydroxide-mediated hydrogel of citrus pectin for preparation of fluorescent carbon dots for bioimaging

Xi Juan Zhao[a,b], Wen Lin Zhang[a], Zhi Qin Zhou[a,b,*]

[a] College of Horticulture and Landscape Architecture, Southwest University, Chongqing 400716, China
[b] Key Laboratory of Horticulture Science for Southern Mountainous Regions, Ministry of Education, Chongqing 400715, China

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ABSTRACT

The citrus process industry produces annually a huge amount of pomace, which is a rich source of citrus pectin. Here, we report the hydrogel of citrus pectin mediated by sodium hydroxide can be used to prepare fluorescent carbon dots (CDs). The introduction of hydrogel can not only make the temperature of the hydrothermal reaction down to 100 °C, but also avoid visually carbonized precipitates in the synthesis process even up to 180 °C. The as-synthesized CDs are well dispersed in water with an average size of 2.7 nm and show cyan fluorescence with high photostability, good biocompatibility. Furthermore, the CDs can act as a potential fluorescent probe for cell imaging. Citrus pectin as a non-toxic carbonaceous precursor for preparation of fluorescent CDs provides a new approach for the efficient utilization of citrus germplasm in future.

1. Introduction

Citrus is one of the most commonly consumed fruits in the world with an annual production of more than 100 million tons [1]. Citrus fruits can be consumed freshly or processed into dessert, juice and jam, and are good resources of vitamin C, folic acid, flavonoids, dietary fiber and many other health-promotion substances [2]. The citrus process industry produces annually a huge amount of pomace (usually 50% of the raw fruits). Besides the use as animal feed, the pomace can provide a rich source of pectin for food and other industries. Pectin is a naturally water-soluble polysaccharide with low production cost. Its backbone comprises linear chains of 1,4-linked α-D-galacturonic acid residues with some rhamnogalacturonic acid residue and α-L-rhamnopyranose by α-1-2 linkage, and contains many carboxyl groups and some methyl esters [3]. Owing to its biocompatibility, biodegradability and non-toxicity, pectin has been widely used in the pharmaceutical and food industries, e.g., acting as a carrier and coating material [4–6], as well as a gelling and thickening agent [7]. Nevertheless, the efficient use of citrus pomace has still been a problem unresolved. In this work, pectin is reported to be a non-toxic carbonaceous precursor for preparation of fluorescent carbon dots (CDs). The efficient use of citrus pectin will promote future germplasm utilization study of the genus *Citrus* L.

CDs have attracting considerable attention since the discovery of fluorescent carbon nanoparticles in 2004 [8]. Different from semiconductor quantum dots with the known toxicity and potential ecosystem hazard [9,10], CDs have their fascinating properties such as low cytotoxicity and good biocompatibility for fluorescent labeling [11]. To date, various routes for preparing CDs have been reported [12–14], e.g., laser ablation of graphite [15], electrochemical oxidation of multiwalled carbon nanotubes [16], hydrothermal reaction of some carbonaceous precursors such as C_{60} [17], *Bombyx mori* silk [18] and soy milk [19]. Among them, the hydrothermal route is popular owing to the simple reaction process and renewable carbonaceous precursors. And bio-precursors are particularly attractive considering the eco-friendly effects. Zhou et al. have synthesized fluorescent CDs from the hydrothermal carbonization of peach gum polysaccharide under the temperature of 180 °C for 12 h [20]. Such a high temperature can facilitate the carbonization of the precursors [21], but simultaneously lead to a lot of visually black particles in the products. While Sahu et al. reported that orange juice by the hydrothermal treatment of 120 °C can produce highly luminescent CDs [22]. However, orange juice is a very nutritious drink, which is not suitable for mass production of CDs compared with the pectin that can be extracted from plenty of the residual pomace in the citrus processing. Here, we report a new kind of fluorescent CDs synthesized from the sodium hydroxide-mediated

* Corresponding author at: Key Laboratory of Horticulture Science for Southern Mountainous Regions, Ministry of Education, College of Horticulture and Landscape Architecture, Horticulture Post-Doctoral Research Station, Southwest University, Chongqing, 400716, China. Tel.: +86 23 68250229; fax: +86 23 68251274.
E-mail addresses: xijuanzh@swu.edu.cn (X.J. Zhao), zhangwenlin88519@126.com (W.L. Zhang), zqzhou2013@swu.edu.cn (Z.Q. Zhou).

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hydrogel of citrus pectin at relatively low temperatures down to 100 °C. The involvement of hydrogel plays an important role in lowering the reaction temperature and can avoid visually carbonized precipitates even up to 180 °C.

2. Materials and methods

2.1. Reagents and chemicals

All reagents were obtained from commercial sources and used as received if no additional statement. Pectin from citrus peel (galacturonic acid ≥74.0%, dried basis) was purchased from Sigma–Aldrich Co. LLC. (USA). Ultrapure water (18.2 MΩ) prepared with a Milli-Q system (Millipore, Bedford, MA, USA) was used throughout the experiments.

2.2. Apparatus and characterization

Transmission electron microscopy (TEM) and high-resolution transmission electron microscopy (HRTEM) were recorded with the Tecnai G2 F20 S-TWIN microscopy (FEI, USA). Elemental and functional groups analysis were made on an ESCALAB 250 X-ray photoelectron spectrometer and a FTIR-8400S Fourier transform infrared spectrometer (Shimadzu, Japan), respectively. Raman spectrum was taken on the Ag substrate using a LabRAM HR 800 Raman spectrometer (Horiba Jobin Yvon Inc., France). The zeta potential was recorded with Nano-ZS spectrometer (Malvern, UK) using the CDs after dialysis and in such case, the pH value of the CDs solution was determined to be about 7.0 with the pH test paper in order to make sure that residual NaOH and other small molecules have been detached. Fluorescence imaging was carried out with a DSU live-cell confocal microscope (Olympus, Japan) system. Absorption and fluorescence spectra were measured at room temperature with a U-3010 spectrophotometer (Hitachi, Japan) and an F-2500 fluorescence spectrophotometer (Hitachi, Japan), respectively.

2.3. Preparation of CDs

The CDs were synthesized using pectin by a hydrothermal method. In a typical synthesis, 20 mL Teflon-lined stainless steel autoclave was cleaned in a bath of fresh aqua regia and rinsed thoroughly in H_2O before using. Then, 2 mL of 10 g/L pectin and 2 mL of 1 mol/L NaOH were added to the autoclave. In such case, the hydrogels of pectin formed. And the autoclave was maintained at 100 °C, 120 °C, 150 °C or 180 °C for 2 h. After the autoclave cooled down naturally, a homogeneously light brown solution was obtained, indicating the formation of CDs. Next, the light brown solution was centrifuged at 15,000 rpm for 10 min to get the upper solution. Through a dialysis membrane (1000 MWCO), residual NaOH and other small molecules will be detached. The obtained CDs solution was then freeze-dried under vacuum in order to get solid powder for further use.

As for the contrast experiments, there is no NaOH-mediated hydrogel in the synthesis procedure. The details are shown as below. 2 mL of 10 g/L pectin and 2 mL of H_2O were added to the 20 mL autoclave. After mixing thoroughly, the autoclave was maintained at 100 °C, 120 °C, 150 °C or 180 °C for 2 h. When the autoclave cooled down naturally, we can obtain the corresponding products with different states.

2.4. Biocompatibility testing

The HEp-2 cells (2×10^5 cells/mL) in RPMI 1640 culture medium supplemented with 10% fetal bovine serum (FBS) were added to each well of a 96-well plate (100 μL per well). The cells were first cultured for 24 h in an incubator (37 °C, 5% CO_2), and for another 24 h, the culture medium was replaced with 100 μL of RPMI 1640 (2% FBS) containing 10 μL of the CDs prepared at 180 °C with different concentrations (0, 0.01, 0.05, 0.1, 0.2, 0.5, 1 mg/mL). Cells cultured in the medium without adding CDs were taken as control. After that, the cells were washed with PBS buffer solution twice, and then, 90 μL PBS buffer and 10 μL of CCK-8 solution were added to each well and incubated for another 30 min. The optical density (OD) of the mixture was measured at 450 nm with a Microplate Reader Model. The cell viability was estimated by the ratio of absorbance of the cells incubated with CDs to that of the cells incubated with the culture medium only.

2.5. Intracellular uptake and fluorescent imaging

The HEp-2 cells in RPMI 1640 culture medium containing 10% fetal bovine serum were added to each well of a 24-well plate (300 μL per well). The cells were first cultured for 24 h in an incubator (37 °C, 5% CO_2), and for another 24 h after the culture medium was replaced with 270 μL of RPMI 1640 culture medium containing 30 μL CDs obtained at 180 °C (1 mg/mL). After that, followed removing the culture medium, each well was washed with PBS buffer for three times. Then the cells were fixed with 4% paraformaldehyde for 30 min, and mounted with glycerol on microscope slides for imaging.

3. Results and discussion

3.1. Synthesis of the fluorescent CDs

The aqueous solution of pectin shows a distinct absorption band centered at 286 nm with a shoulder peak at around 350 nm (Fig. 1A). Here, when an appropriate amount of the aqueous solution of NaOH is added to the pectin solution, hydrogel of pectin forms immediately accompanied with the color change to light-green. However, the absorption spectrum of pectin has no significant changes as shown in Fig. 1A. After the hydrogel was transferred to Teflon-lined stainless steel autoclave maintaining at 100 °C, 120 °C, 150 °C or 180 °C for 2 h, respectively, the hydrogel will be destroyed and the color of the product turns yellow or light brown to some extent (Fig. 1C), indicating the formation of CDs. Also, the absorption spectra have been changed. The featured absorption peak at 286 nm disappeared and a new one centered at 260 nm appeared gradually with increasing the temperature (Fig. 1B). Under 365 nm UV lamp light, the CDs can exhibit varying degrees of cyan fluorescence emission. And the higher the temperature, the stronger the fluorescence intensity will be (Fig. 1D). Since either pectin or the hydrogel has no fluorescence emission, the emission comes from the new generated CDs. Furthermore, the fluorescence spectra of the corresponding CDs are dependent of the excitation wavelength (Fig. 2 and Supporting Information, Figs. S1–S3). This excitation-dependent emission property is in accordance with that of CDs in previous reports. As shown in Fig. 2, the CDs obtained at 180 °C as an example have a broad UV absorption band with a featured peak at 260 nm and exhibit strong emission centered at around 460 nm when excited at 360 nm. The emission peaks can shift from 460 nm to 554 nm with the increase of the excitation wavelength. And the quantum yield is calculated to be approximately 1.1% with quinine sulfate as the reference, which is comparable to that of the CDs obtained from natural gas soot (0.43%) as reported [13].

However, if no hydrogel is mediated in the synthesis procedure, no fluorescent CDs can be formed from pectin under the temperature of 100 °C and 120 °C for 2 h (Supporting Information, Fig. S4). The color of the pectin solution has no changes, and under 365 nm UV lamp light, the aqueous solution shows very weak fluorescence