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A dual-mode sensor for colorimetric and fluorescent detection of nitrite in hams based on carbon dots-neutral red system

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ABSTRACT

Nitrite residue in hams was detected by a fluorescent and colorimetric sensor based on carbon dots (C-dots) and neutral red (NR). C-dots with green fluorescence was synthesized by a microwave-assisted method. This novel sensor was fabricated by C-dots as donors and NR as acceptors. The presence of nitrite led to decrease of absorbance and increase of fluorescence. Colorimetric and fluorescent methods for nitrite detection were developed with excellent correlation coefficients (R^2 = 0.995 and 0.991) and low limits of detection (196 nM and 0.518 nM). Moreover, nitrite residue in seven types of ham was detected by the colorimetric and fluorescent methods which were verified by a standard method. The results obtained by the proposed method were comparable and agree with that of the Griess-based method (relative errors < 5%). C-dots-NR system as a sensor has a potential application for nitrite detection in hams to monitor its quality and safety.

1. Introduction

Ham is a meat product highly consumed in the daily diet. Nitrite is a permitted additive in hams to inhibit the growth of spoilage bacteria, and it can improve recognizable colors and flavors of hams (Addiscott and Benjamin, 2004). High intake of nitrite has an adverse impact on human health for nitrite reacting with amines and amides can form a series of carcinogenic N-nitroso compounds (Honikel, 2008; James et al., 2009). In contrast, modest dietary intake of nitrite can mediate physiological effects including vasodilation, modulation of mitochondrial function, and protection from ischemia-reperfusion injury (Lundberg et al., 2011). Control of nitrite quantities is necessary to reduce the potential for N-nitroso compound formation and make nitrite have functions of mediating physiological effects. Therefore, accurate measurement of residual unreacted nitrite is required to guarantee quality and safety of hams.

Recently, various methods for nitrite detection have been developed including electrochemistry (Gayathri and Balasubramanian, 1999), chromatography (Li et al., 2003) and optical method (Gayathri and Balasubramanian, 1999; Li et al., 2003; Zhen et al., 2011). Chromatography and Griess-based method were standard methods for nitrite detection set by International Standardization Organization (ISO, 1975), Association of Official Analytical Chemists (AOAC, 1984), Chinese Ministry of Health (CHM, 2016) and the European Committee for Standardization (CEN, 1999). Chromatographic method is sensitive and reliable in the determination of nitrite. However, it is time-consuming with sophisticated operations and high-cost with expensive instrument. The Griess-based colorimetric method has a widespread application with advantages of simplicity and low cost. It is easily affected by colored pigments that may exist in hams due to relying on the color of azo-compounds. Moreover, it is not sensitive to trace-level nitrite. Development of a new method with high sensitivity, simplicity and low cost for nitrite detection is still a research focus.

Fluorescence-based methods have attracted increasing interest because of their high sensitivity, simplicity, and ease of operation. In addition, it possesses high selectivity without interference from color components. Carbon dots (C-dots) as inorganic fluorescent nanomaterials has gained considerable attention due to their excellent fluorescence properties, low toxicity, and low cost (Amjadi and Jalili, 2018; Wang and Hu, 2015; Yang et al., 2011). Fluorescence resonance energy transform (FRET) systems based on C-dots have been successfully developed for bioimaging (Chatzimarkou et al., 2018), biological sensing (Kudr et al., 2017), and chemical sensing (Wu et al., 2017). They have many advantages, such as fast response, low toxicity and high

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sensitivity (Liu et al., 2012). Therefore, FRET systems based on C-dots have potentials for nitrite detection.

A novel FRET system based on C-dots and NR was developed for nitrite detection in hams. C-dots is synthesized by a microwave-assisted method. C-dots-NR system displaying faint fluorescence is fabricated by C-dots as donors and NR as acceptors for the presence of NR can quench fluorescence emission. Colorimetric and fluorescent methods are developed for nitrite detection based on absorbance and fluorescence signals of C-dots-NR system. Nitrite in hams is detected by this colorimetric and fluorescent methods.

2. Materials and methods

2.1. Materials and instruments

All chemicals were of analytical grade. Polyethylene glycol 200 (PEG 200) was purchased from Sigma-Aldrich (St. Louis, MO, USA). The remaining reagents (sucrose, sodium nitrite ($NaNO_2$), sodium chloride, sodium bromide, and so on) were purchased from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). All aqueous solutions were prepared by using ultrapure water obtained from Milli-Q filter system (Millipore Co., USA) with a resistivity of 18.2 MΩ cm^{-1}. Seven types of ham at different levels were purchased from the local supermarket.

The size and morphology of C-dots were characterized by JEM-2100 transmission electron microscopy (TEM) (JEOL Ltd.) at a maximum accelerating voltage of 200 kV. A 5 μL of C-dots solution was drop-cast on carbon-coated copper grids and subsequently air-dried before TEM analysis. A portable apparatus composed of several commercially devices was used as ultraviolet-visible (UV-Vis) spectroscopy and fluorescence spectroscopy. It includes two light sources (Ocean optics, Halma Company), a USB 2000 spectrometer (Ocean optics, Halma Company), three fiber optics (Ocean optics, Halma Company), a 1-cm pathlength quartz cuvette (Ocean optics, Halma Company), and a tablet PC equipped with Spectrasuite software. Tungsten light or 365 nm laser light source is successively guided into a cuvette carriage through fiber optics for generation of absorbance or fluorescence signals. These signals are gathered by the micro spectrometer and transferred to the tablet PC for analysis (Fig. S1).

2.2. Detection of nitrite residue in hams using Griess-based colorimetric method

A Griess-based colorimetric method was used to detect nitrite residue (NO_2^-) in seven types of ham according to GB 5009.33-2016 in China (CHM, 2016) and ISO 2918-1975 (ISO, 1975). The preprocessing procedures for extraction and isolation of nitrite in hams included: (1) 5 g of homogenized ham was macerated with 14 mL borax solution and 100 mL of distilled water (70 °C); (2) the mixture was heated in boiling water for 15 min; (3) 5 mL ferrous potassium cyanide and 5 mL zinc acetate solution were subsequently added; (4) the solution was made up to a final volume of 200 mL with water followed by filtration through 0.45 μm micro-filter. The filtrates were stored at 4 °C for analysis.

Sodium nitrite standard solution or ham extraction was added into the Griess reagent containing sulfanilic acid solution (0.4 mL, 2 g/L) and N-(1-naphthyl) ethylenediamine dihydrochloride solution (0.2 mL, 1 g/L) incubating for 15 min. The concentration of $NaNO_2$ was 0, 0.02, 0.04, 0.06, 0.08, 0.10, 0.15, 0.20, 0.25 μg/mL, respectively. Then absorbance spectra of those mixtures were obtained. A linear calibration curve was established by plotting absorbance values at 538 nm to the corresponding $NaNO_2$ concentrations (0–0.25 μg/mL). The absorbance values of ham extraction at 538 nm were recorded and the nitrite concentration in ham was detected according to Eq. (1). All measurements were performed in triplicate on different days.

$$Q = \frac{(A_{538} - 0.0098) \times 5 \times 200}{6.076 \times 1.371 \times 5} (\text{mg/kg}) \quad (1)$$

Where Q is the nitrite residue in hams, A_{538} represents the absorbance value at 538 nm.

2.3. Development of colorimetric and fluorescent methods for nitrite detection

2.3.1. Synthesis of C-dots

Synthesis of C-dots was according to a microwave-assisted method with minor modifications (Gong et al., 2014). Sucrose solution (1 mL, 30% (*w/v*)) and concentrated H_2SO_4 (200 μL) were sequentially added to 6 mL of PEG in a 10 mL glass tube. The mixture was heated in a 900 W domestic microwave oven (Midea, China) for 15 s. It turned golden yellow indicating the formation of C-dots. The C-dots solution was centrifuged to remove the insoluble substance, and small molecules were removed using a dialysis membrane (molecular weight cut-off = 1000) for 24 h. The resultant C-dots was diluted into 100 mL and stored at 4 °C for further characterization and utilization.

2.3.2. Optimization of experimental conditions

The concentration of C-dots, concentration of NR, pH and reaction time were optimized to achieve the maximum sensitivity for nitrite detection. Different volumes of C-dots (1, 2, 3 and 4 mL) were analyzed to select the optimal volume of C-dots. C-dots (1 mL) mixing with different amounts of NR (0, 40, 80, 120, 160, 200 and 300 μL) was used for selecting the optimal amount of NR. Different pH values (0.9, 1.0, 1.2, 1.7, 2.0, 3.0, 4.0, 5.0, and 6.0) were investigated in presence of 0.10 μg/mL nitrite, 1 mL C-dots and 160 μL NR. The mixture (1 mL C-dots, 160 μL NR, 100 μL HCl and 0.10 μg/mL nitrite) was incubated for 5, 10, 15 and 20 min to select the optimal reaction time. All solutions were diluted into 5 mL with water before spectroscopy measurements.

2.3.3. Colorimetric and fluorescent methods for nitrite detection

Colorimetric and fluorescent methods were developed for nitrite detection. NR and of C-dots were mixed to form C-dots-NR system. Then $NaNO_2$ solution and HCl were added into the C-dots-NR system followed by diluted to 5.0 mL. The final concentration of $NaNO_2$ were 0, 0.005, 0.01, 0.015, 0.02, 0.03, 0.04, 0.05, 0.06, 0.07, 0.08, 0.1, 0.12, 0.015, 0.2, 0.25, and 0.3 μg/mL, respectively. Absorbance and fluorescence spectra were acquired as an average of three independent measurements. Absorbance values and fluorescence intensities were recorded to establish linear calibration curves. The response of C-dots-NR system to other ions was also studied to validate the specificity for NO_2^- detection. All experiments were performed in triplicate on different days.

2.4. Colorimetric and fluorescent detection of nitrite residue in ham

Nitrite residue in seven types of ham was detected by the colorimetric and fluorescent methods based on C-dots-NR system according to the Section 2.3.3. The unknown concentration was calculated by putting fluorescent intensity or absorbance value into the corresponding calibration curve. All experiments were performed in triplicate on different days (sample size is 12 for each ham).

3. Results and discussion

3.1. Nitrite in hams detected by the Griess-based method

Nitrite residue (NO_2^-) levels in hams detected by Griess-based colorimetric method were between 0.102 and 22.276 mg/kg (Fig. 1). Absorbance values enhanced with increasing concentration of NO_2^- (Fig. 1a). Excellent linearity between nitrite concentrations (0–0.25 μg/L) and absorbance values at 538 nm was observed with correlation