APPLICATION OF SINGLE-ATOM NANOZYMES IN THE DETECTION AND MONITORING OF FRESHNESS IN FRESH MEAT

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I. INTRODUCTION

Freshness is a critical indicator for assessing the quality of fresh meat. However, achieving precise, real-time, and portable freshness detection and monitoring remains a significant challenge and a pressing demand in meat science research [1-3]. Traditional detection methods, such as microbiological testing (total bacterial count) and physicochemical testing (volatile basic nitrogen), are limited by long processing times, complex procedures, and the lack of capability for online detection and monitoring [4,5]. To address these limitations, nanomaterials with catalytic properties have been extensively explored for accurate, real-time, and portable sensing and identification of fresh meat freshness [6]. The objective of this research is to develop an online, real-time, and rapid evaluation method for meat freshness using single-atom nanozymes as catalytic sensing materials.

II. MATERIALS AND METHODS

This study is divided into four main sections. Firstly, a single-atom iron nanozyme (SAFe-N-C nanozyme) with high peroxidase-like activity is synthesized through a host-guest strategy. Subsequently, a colorimetric-fluorescent dual-mode sensor is developed by integrating the nanozyme with fluorescent carbon quantum dots. The sensor is designed to accurately detect volatile biogenic amines, which serve as key markers for assessing meat freshness. Secondly, the detection performance of the SAFe-N-C nanozyme and fluorescent carbon quantum dots is evaluated. Simulated systems with an ammonia concentration of 1 mM are used, followed by validation with real meat samples, including pork, beef, lamb, and chicken, each weighing 80 g. Thirdly, a paper-based sensor (2×2 cm) is developed by immobilizing the SAFe-N-C nanozyme and fluorescent carbon quantum dots on a Whatman No.1 paper substrate. Finally, approximately 20 g samples of pork, beef, lamb, and chicken meat were weighed and evenly divided into four portions (triplicates per group). Each portion was transferred to a 90 mm diameter petri dish. A colorimetric-fluorescence dual-signal biosensor was attached to the lid of each petri dish, and the assembly was sealed in a sterile plastic bag. All samples were stored at 25°C. The gray values and fluorescence intensity of the biosensor, along with the total volatile basic nitrogen (TVB-N) values of the meat samples, were recorded at storage intervals of 0, 6, 12, 24, 36, and 42 hours. By establishing a predictive model that correlates the gray value/fluorescence intensity ratio with the actual TVB-N values, a portable and quantitative method for rapid freshness detection and real-time monitoring was developed.

III. RESULTS AND DISCUSSION

A colorimetric-fluorescent dual-signal biosensor was developed for food freshness detection by integrating SAFe-N-C nanozyme with carbon quantum dots. The dual-signal biosensor exhibited response times of 6 min and 15 min in colorimetric and fluorescent modes, respectively, upon exposure to ammonia (Figure 1). The detection limits for the two modes were 0.98 ppm and 0.08 ppm, respectively (Figure 2). The colorimetric-fluorescent dual-signal biosensor was used to monitor freshness changes of pork, beef, lamb, and chicken at 25℃ without damaging the sample. As shown in Figure 3, visible and fluorescent colour photographs of the colorimetric-fluorescent dual-signal biosensor during the storage period of 42 hours were recorded. The grey value and fluorescence intensity values of the colorimetric-fluorescent dual-signal sensor were quantified and displayed in Figure 4a, b. The total volatile basic nitrogen (TVB-N) value was measured to monitor freshness changes in meat with a limit of ≤ 15 mg/100 g for raw meat according to national standard methods (GB 5009.228-2016, GB 2707-2016). It was observed that the values of TVB-N in pork, beef, lamb, and chicken increased from 0.92, 2.23, 0.031, and 4.37 mg/100 g to 52.8, 65.9, 48.7, and 72.6 mg/100 g, respectively, with storage from 0 h to 42 h. The spoilage times of pork beef, lamb, and chicken were 30 h, 24 h, 36 h, and 12 h, respectively. The visible and fluorescent colours of the control group did not change until the end of storage. Furthermore, quantitative models between

the grey value, fluorescence intensity of colorimetric-fluorescent the dual-signal sensor and TVB-N value of the meat samples were built as shown in Figure 4c, d. The correlation coefficients were 0.9196 and 0.9360, respectively. In addition, the colorimetric-fluorescent dual-signal biosensor had good stability when stored at 25°C (Figure 5a) and was valid for three years (Figure 5b). It can be reused four times or more, and its performance was much better than that of natural horseradish peroxidase (one more) (Figure 5c). All the above results proved that the colorimetric-fluorescent dual-signal biosensor has good stability, reusability, and higher economic benefits.

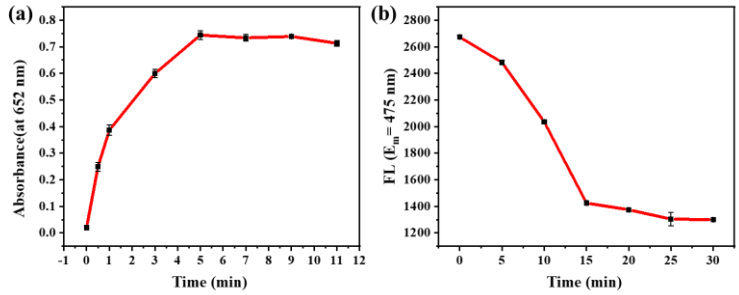

Figure 1. Optimization of the optimal response time of the colorimetric-fluorescent dual-signal biosensor to ammonia. (a) Colorimetric system. (b) Fluorescent system.

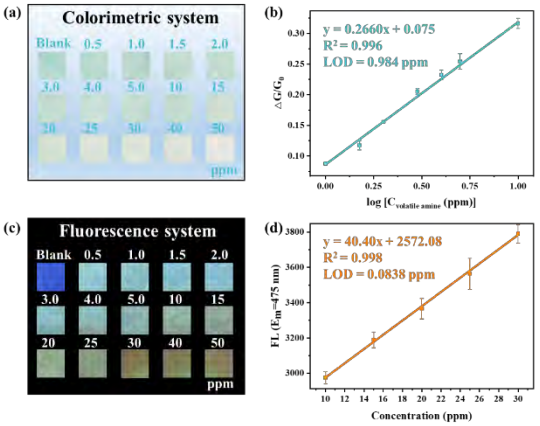

Figure 2. Sensitivity of the colorimetric-fluorescent dual-signal biosensor. (a) Visible response of the dual-signal biosensor to different concentrations of ammonia. (b) Linear correlation between the grey values and the concentration of ammonia. (c) Fluorescence response of the dual-signal biosensor to different concentrations of ammonia. (d) Linear correlation between the fluorescence intensity values and the concentration of ammonia.

IV. CONCLUSION

In summary, a colorimetric-fluorescent dual-signal biosensor was successfully constructed for food freshness detection. The response times are 6 mins and 15 mins, respectively, when exposed to volatile alkaline gases such as ammonia for colorimetric mode and fluorescent mode, respectively. The LOD values for these two modes were 0.9840 ppm and 0.0838 ppm, respectively. The dual-signal sensor enables rapid optical signal acquisition through a portable phone-based program, making it highly suitable for monitoring volatile alkaline gases in meat. This study proposes a design strategy for developing a portable, accurate, and rapid multi-signal detection method to assess food freshness.

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