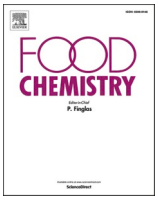
Food Chemistry 482 (2025) 144144

Contents lists available at ScienceDirect

Food Chemistry

journal homepage: www.elsevier.com/locate/foodchem



Biogenic amine-responsive ratiometric fluorescent microneedle sensor for real-time visualization of meat freshness

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ARTICLE INFO

Keywords:
Visualization
Ratiometric fluorescent sensor
Microneedle patch
Raw meat freshness
Biogenic amines

ABSTRACT

A ratiometric fluorescent hydrogel microneedle (MN) patch was developed for rapid raw meat freshness detection. The sensor integrated fluorescein isothiocyanate (FITC) as a biogenic amine (BAs) indicator and rhodamine B (RhB) as an internal reference within a silk fibroin methacryloyl (SilMA) hydrogel matrix. Leveraging FITC's specific response to BAs, the patch exhibited visible color changes proportional to histamine concentrations. Optimizing FITC/RhB concentrations enabled sensitive BAs detection in meat samples within 8 min. The FITC/RhB@SilMA MN patch demonstrated high accuracy and repeatability in freshness assessment for chicken breast, pork, and salmon stored at 0 °C and 4 °C. Freshness classification was established using G + R/(R + G + B) values: <0.60 (fresh), 0.60–0.65 (low quality), >0.65 (spoiled). These values showed strong correlation with the Biogenic Amine Index (BAI), confirming method reliability. This work presents a novel on-site strategy for real-time meat quality monitoring, offering practical applications in food safety control.

1. Introduction

Food safety is of vital importance to the food industry and human health, and has become a major concern in the worldwide (Li et al., 2024). Fish and meat are nutrient-rich foods and very common in people's daily diet. However, they are perishable and usually deteriorate rapidly in a few days (Kim et al., 2022). During fish and meat spoilage, biogenic amines (BAs) are producing by the degradation of amino acids, either through external microbial activity or endogenous tissue metabolism (Jia et al., 2019). The accumulation of BAs is linked to food spoilage and poisoning (Quan et al., 2021), making them important biomarkers for monitoring food freshness. Several methods are available for detecting BAs, such as chromatography (Munir & Badri, 2020; Onal et al., 2013), mass spectrometry (Wojnowski et al., 2019), spectroscopic techniques (Cheng & Sun, 2015; Moon et al., 2020) and electronic noses (Kim et al., 2022). Although these methods are effective, the requirements of specialized equipment and pretreatment steps, making them unsuitable for consumer use. Therefore, the development of food freshness sensors capable of on-site, in time detection of BAs with high sensitivity is crucial need.

Indicator labels have garnered widespread attention for their ability to monitor the freshness of fish/meat without sample pretreatment and in a non-destructive manner (Choi, Choi, Lee and Han, 2022; Guo et al., 2020). Indicator labels on packaging films effectively avoid dye migration and potential secondary contamination (Liu et al., 2020). However, they can only sense volatile substances at the top of the packaging and often provide delayed information. Direct-contact indicators can offer real-time and accurate information. Kim et al. immobilized bromocresol purple dye in highly hygroscopic materials such as polyvinyl alcohol (Kim et al., 2017). The indicator has pH-responsive activity and can monitor the freshness of chicken breast meat through direct surface contact. However, this type of direct-contact indicator label is at risk of dye leakage.

Visualized microneedle sensors have shown great potential for direct, real-time detection of biomarkers in situ. Microneedles containing arrays of miniaturized needles can extract biomarkers from sample tissues in a near nondestructive manner, using capillary force to drive in situ expansion within minutes (Yao et al., 2024). Owing to their

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https://doi.org/10.1016/j.foodchem.2025.144144
Received 20 November 2024; Received in revised form 7 March 2025; Accepted 29 March 2025
Available online 1 April 2025

portability, minimal sample pretreatment requirements, and fast response, microneedle sensors have been widely applied in the biomedical and healthcare fields (Wang et al., 2023). In recent years, their application has also extended into the realm of food safety. For example, Kim et al. reported a silk-based microneedle that can both sample fluids deep in food tissues and indicate the presence or absence of *E. coli* or food spoilage via colorimetric response (Kim et al., 2020). Two other colorimetric microneedle sensors were also built based on hydrogel and pH-responsive dyes for sensing meat/salmon spoilage (Wang et al., 2024; Wang, Chen, et al., 2023). Colorimetric sensors are generally considered less sensitive compared to electrochemical or fluorescence-based detection methods (Cheng et al., 2023; Gai et al., 2023). Development of portable devices using fluorescence signals as reporter have garnered increasing attention (Chang et al., 2016; Chang et al., 2018; Zhu et al., 2024).

An ideal portable optical sensor should be user-friendly, with a short analysis time, easy visualization, and high sensitivity (Kuswandi et al., 2011). Proportional fluorescence sensors consisting of two fluorescent agents, one used as an analysis indicator and the other as an internal reference, show great potential as optical sensors (Jia et al., 2019). By selecting different fluorescents, fluorescence color changes can be used for visual inspection in a reasonable manner. For instance, Jia et al. used cellulose-based proportional fluorescent materials to detect BAs using fluorescein isothiocyanate (FITC) as an indicator and protoporphyrin PpIX as an internal reference on cellulose acetate. The method allowed for rapid and sensitive color response to monitor the freshness of shrimp and crab visually (Jia et al., 2019). Similarly, Quan et al. designed a smart nanofiber proportional fluorescent sensor using FITC as a BA indicator for in-situ and visual detection of seafood freshness (Quan et al., 2021). Therefore, FITC exhibits excellent response to BAs as a biological response substance. However, ratiometric fluorescent microneedle sensors with FITC serving as an indicator for BA and detection of food freshness have not been reported.

Herein, FITC/RhB@SilMA MN patches were prepared by loading mixed fluorescent dyes into SilMA hydrogel substrates. The study first explored the specific recognition ability of FITC and Rhodamine B (RhB) fluorescent dyes for BAs and achieved sensitive detection of BAs levels in raw meat samples through the optimization of dye concentrations. The ratio fluorescence images were taken by the camera of the mobile phone and the RGB value was analyzed by the software of the mobile phone. The prepared FITC/RhB@SilMA MN patch showed good accuracy and repeatability by exhibiting visible yellow-yellow green-green color changes in freshness detection of chicken breast, pork, and salmon stored under different conditions. Moreover, the G + R/(R + G + B) values of the color pictures of the patch highly corresponded with the calculated results of the Biogenic Amine Index (BAI), confirming its effectiveness in assessing meat freshness. The innovation of this study lies in the combination of ratiometric fluorescent probe with MN patch to develop a new method for detecting the freshness of raw meat. Compared to traditional methods, this method is characterized by its simplicity, rapid detection, and high sensitivity, providing a new approach for on-site rapid detection of raw meat freshness.

2. Materials and methods

2.1. Materials

FITC, cadaverine (Cad), histamine (His), tyrosine (Tyro), tryptophan (Trp), threonine (Thr), serine (Ser), phenylalanine (Phe), methionine (Met) and isoleucine (Ile) were from Aladdin Biochemical Technology Co. (Shanghai, China). RhB was purchased from Beijing J&K Scientific Technology Co. (Beijing, China). Spermine (Spe), tryptamine (Try) and tyramine (Tyra) were purchased from Beijing Innochem Science & Technology Co. (Beijing, China). All reagents were of analytical grade and used without further purification. Chicken breast and pork samples were purchased from resources vanguard supermarket (Wuxi, China). Atlantic salmon (*Salmo salar*) samples were purchased from Shenzhen Feng Xian Food Co. (Shenzhen, China). Ultrapure water was purchased from Wahaha Group Co. (Hangzhou, China).

2.2. Response of RhB and FITC for biogenic amine

2.2.1. Excitation and emission spectra of RhB and FITC dyes

RhB (1.0 mg) and FITC (1.0 mg) were dissolved separately in dimethyl sulfoxide (1 mL) to obtain RhB and FITC stock solutions with 1 mg/mL. The solutions were then diluted with ultrapure water to 0.1 μg/mL, and the excitation and emission spectra of the mixed solution were measured using a fluorescence spectrophotometer (F-7000, Hitachi Limited, Japan) after shaking.

2.2.2. Fluorescent response of RhB and FITC dyes to biogenic amines and amino acids

RhB and FITC stock solutions (1 mg/mL) were diluted to 100 μg/mL using ultrapure water. Next, FITC or RhB solution (990 μL, 100 μg/mL) was added to each of the 13 centrifuge tubes. For BA fluorescence response, Spe, Cad, Try, Tyra, and His solution (10 μL, 0.1 mg/mL) were added to each of the five centrifuge tubes. For amino acid fluorescence response, Tyro, Trp, Thr, Ser, Phe, Met, and Ile solution (10 μL, 0.1 mg/mL) were added to each of the seven centrifuge tubes. Finally, ultrapure water (10 μL) was added to the last one centrifuge tube as a control. The centrifuge tubes were shaken to mix well and the emission spectra of the RhB and FITC solutions with different BAs / amino acids were sequentially measured using a fluorescence spectrophotometer (F-7000, Hitachi Limited, Japan).

2.3. Optimization of the mixed RhB/FITC concentrations

2.3.1. Visual fluorescence photos and intensity measurement of RhB and FITC dyes with histamine

Different volumes (0, 10, 30, 50, 70, 100 μL) of His (1 μg/mL) were added into six centrifuge tubes. RhB and FITC solutions (1 mg/mL) were diluted with ultrapure water to 500, 250, 100, 50, 25, 10, 1, and 0.5 μg/mL, respectively. Then, dilutions with different concentrations were added to the above centrifuge tubes to make the total volume of the solution to 1 mL. The solutions were then shaken for 10 min, irradiated with a 365 nm handheld UV lamp (ZF-5, JiaPeng, China), and photographed with an iPhone 12 Pro Max (Apple Computer Inc., America). The average fluorescence intensity of digital photographs was analyzed using Image J software.

2.3.2. Fluorescent response of the mixed RhB/FITC dyes to biogenic amines

The diluted solutions of RhB and FITC (100.0, 50.0 and 25.0 μg/mL) were respectively mixed with the same mass concentration in the volume ratios of 1:0, 5:1, 3:1, 2:1, 1:1, 1:2, 1:3, 1:5, and 0:1. Different volumes (0–100 μL) of His solution (1.0 mg/mL) were added to the mixed RhB/FITC dye solutions with different ratios (1,0, 5,1, 3,1, 2,1, 1:1, 1:2, 1:3, 1:5, and 0:1). The final concentration of histamine in the system was adjusted to 0, 10, 30, 50, 70, and 100 μg/mL. The solutions were then irradiated with a 365 nm handheld UV lamp (ZF-5, JiaPeng, China), and digital photographs of the solutions were captured. The RGB values of the digital photos were extracted using the mobile application "Color call", and then the G/R values were fitted to a nonlinear curve with the His concentration.

2.4. Preparation of FITC/RhB@SilMA MN patch

2.4.1. Fabrication of needle tips in MN patch

Preparation of needle tips in MN patch was according to the previous report. (Wang, Chen, et al., 2023).

2.4.2. Fabrication of backing layer in MN patch

To prepare the mixed fluorescent hydrogel solution, a mixed

fluorescent dye solution of RhB and FITC (500 μL, 200 μg/mL) with a ratio of 1:3 was added to SilMA hydrogel solution (420 μL, 14 wt%). Then, HRP (40 μL, 150 U/mL) and H_2O_2 (40 μL, 100 mM) were added sequentially and mixed thoroughly, resulting in the final solution.

The final fluorescent hydrogel solution (150 μL) was added dropwise onto the prepared tips in the mold, and then the mold was placed in a constant temperature drying oven at 37 °C for 36 h. After this procedure, the mixed fluorescent hydrogel solution (100 μL) was added dropwise again. The mold was placed in the drying oven again for hot blast drying at 37 °C for another 36 h. The FITC/RhB@SilMA MN patch was obtained by removing the mold and demolding.

2.4.3. Surface morphology

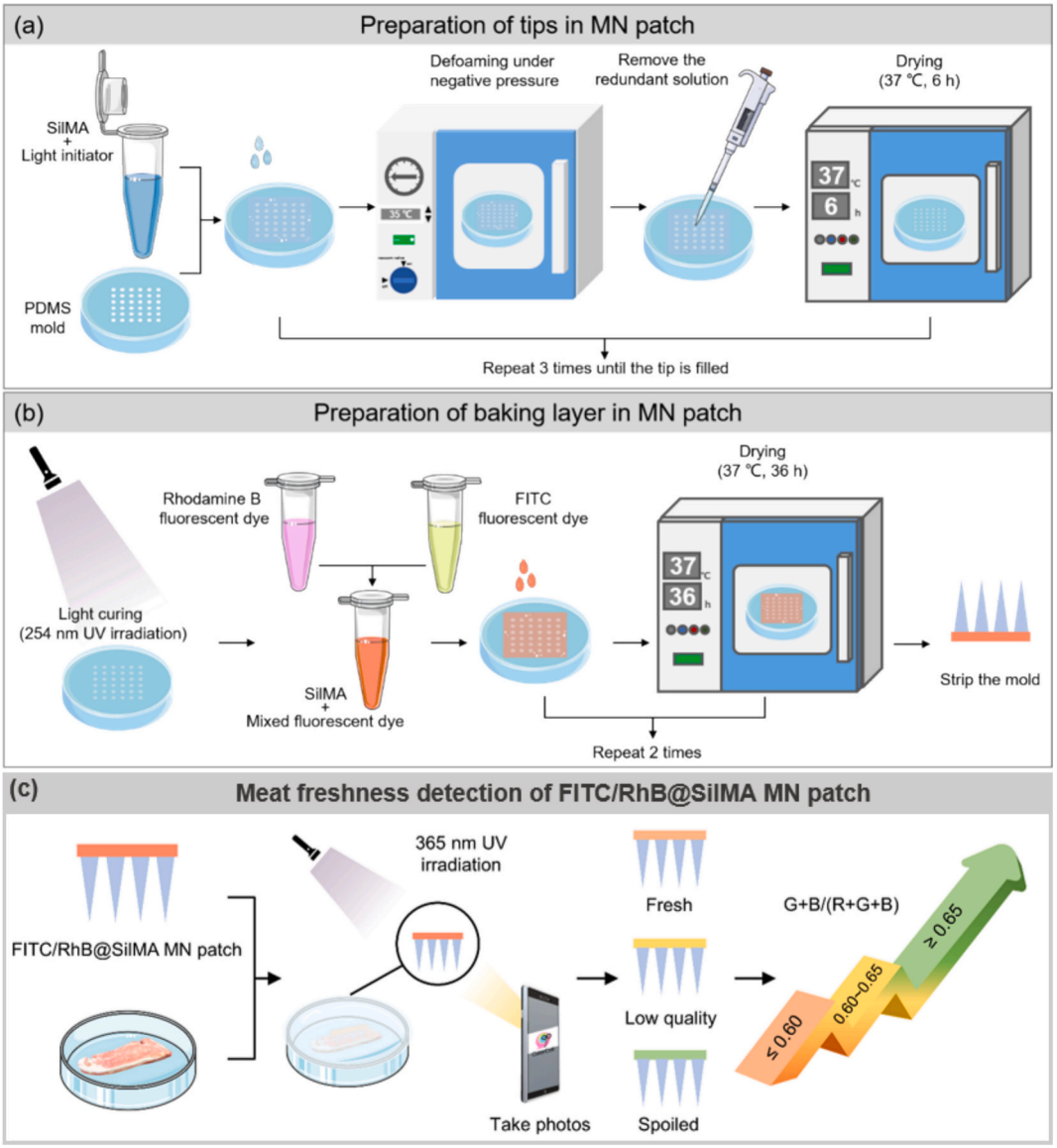
The morphology of the prepared MN patches was observed on a stereomicroscope (SGO-SZMN45TRB4, KWONG KUK, China).

2.5. Detection of meat freshness with FITC/RhB@SilMA MN

Chicken breast, pork pieces and salmon samples of five grams each with uniform appearance were placed in sterile petri dishes (diameter 35 mm). Subsequently, the petri dishes were sealed with polyethylene film and refrigerated at 0 °C and 4 °C for 10 days. The samples were taken out for taking pictures every 24 h from day 0–10. The MN patch was inserted through the cling film to contact with the raw meat samples, and the color changes of the MN patch were recorded daily by taking pictures with a smartphone under the irradiation of a 365 nm handheld UV lamp.

To verify the response of the rapid test, the MN patch was inserted through the cling film and contacted with the raw meat samples after 10 days of storage. The MN patch was irradiated with a 365 nm handheld UV lamp and photographed with a phone every 2 min. The RGB values of the color images obtained from the above steps were analyzed using the phone application "Color call", and the G + B/(R + G + B) information was used to indicate the freshness of the raw meat.

BAs were determined by high-performance liquid chromatography (Waters e2695, Waters, USA) equipped with a UV–Vis detector and a Waters C18 column and according to the method of Yu et al. (Yu, Xia, Xu, & Jiang, 2016). The freshness of meat was evaluated according to the biogenic amine index (BAI = Put + Cad + Tyra + His) calculated from the sum of Put, Cad, Tyra and His, as proposed by Hernandez-Jover



Scheme 1. Schematic illustration for the design and preparation of FITC/RhB@SilMA MN patch (a, b) and the meat freshness detection using FITC/RhB@SilMA MN patch (c).

et al. (Hernandez-Jover et al., 1996), classifying products into three levels of freshness (acceptable meat, low quality meat and spoiled meat). When the BAI is ≤20 mg/kg, the meat is classified as acceptable consumption range. When the BAI is between 20 and 50 mg/kg, the meat is classified as low-quality meat. When the BAI value is ≥50 mg/kg, the meat is spoiled. The value is considered as 0 when none of the involved BAs is not detected (ND).

2.6. Data analysis

Each measurement was performed in triplicate with individually prepared samples, and the results were presented as mean standard deviation (SD). Curve fitting was carried out by existing mathematical models embedded in the Origin 2021 software (Origin Lab Co., Northampton, MA, USA). IBM SPSS Statistics 27 software was used for statistical analysis of the data, single factor ANOVA test was selected for analysis, and multiple comparisons were used to indicate significant differences between the lower-case marked data with different means of 95 % confidence intervals.

3. Results and discussion

3.1. Design, optimization, and preparation of FITC/RhB@SilMA MN

Scheme 1 a, 1b shows the schematic diagram of the preparation process for the FITC/RhB@SilMA MN patch using SilMA hydrogel materials with FITC and RhB fluorescent dyes. The tips and backing layer of microneedle patch were separately prepared using a PDMS mold. Fluorescent dyes were mixed with SilMA hydrogel to form the backing layer in microneedle patch. Scheme 1c elucidates the FITC/RhB@SilMA MN patch detecting the meat freshness with the aid of a handheld 365 UV lamp and a mobile phone. With the deterioration of meat quality and the increase of BA concentration, the color of the MN patch and the corresponding RGB value changed significantly.

3.1.1. Recognition and optimization of FITC and RhB

3.1.1.1. Specific recognition of biogenic amines by FITC and RhB. FITC and RhB are the two core elements in ratiometric fluorescent sensors. The excitation and emission wavelengths of FITC and RhB were investigated. As shown in Fig. S1, they had partially overlapping excitation bands and exhibited the same second largest excitation peaks at 350–360 nm. Thus, FITC and RhB could be simultaneously excited using a handheld 365 nm UV lamp. However, they showed different maximum fluorescence emission peaks (525 nm for FITC and 580 nm for RhB). Under excitation of 365 nm UV light, FITC emitted green fluorescence, while RhB emitted orange-red fluorescence and they could be visually differentiated.

Selectivity plays a crucial role in the rapid and accurate target identification for sensors (Quan et al., 2021). BAs are produced through microbial decarboxylation of proteins and amino acids, and all BAs derived from their corresponding amino acids (Kannan et al., 2020). To confirm the specific response of fluorescent dyes to the BAs, FITC and RhB were reacted with five BAs, respectively. Their corresponding amino acids were also reacted with FITC and RhB as the control experiment. Fig. S2 showed significant increases in fluorescence intensity when FITC was exposed to five BAs, indicating specific recognition of FITC towards BAs. However, there was no significant change in fluorescence intensity when FITC was exposed to seven amino acids, indicating its selectivity towards BAs. Fig. S3 shows that there was no significant change in fluorescence intensity of RhB when exposed to both BAs and amino acids. Therefore, FITC was chosen as the indicator for specific response to BAs while RhB was opted as the internal reference for ratiometric fluorescent sensor.

3.1.1.2. Optimization of RhB and FITC fluorescent dye concentrations. To optimize the color response performance, concentrations of the two fluorescent dyes were optimized. Firstly, different concentrations of RhB were reacted with different concentrations of histamine, and color changes were captured using a smartphone. The brightest orange-red fluorescence was observed at RhB concentrations of 100, 50, and 25 μg/mL after reacting with different concentrations of histamine (Fig. 1a). The average fluorescence intensity analysis was shown in Fig. 1b and the biggest intensity appeared in three groups with RhB of 100, 50, and 25 μg/mL concentrations. This result was consistent with the visual observation. Thus, RhB concentrations of 100, 50, and 25 μg/mL were selected for subsequent experiments.

The concentrations of FITC fluorescent dye were similarly optimized. Conspicuous green fluorescence was observed at 100, 50, and 25 μg/mL of FITC after reacting with 0–100 μg/mL of histamine (Fig. 1c). The corresponding average fluorescence intensities were relatively prominent in Fig. 1d, and the concentrations of 100, 50, and 25 μg/mL were selected for subsequent experiments.

In order to get a wide color range, the RhB and FITC exhibiting two different fluorescent colors with concentrations of 100, 50, and 25 μg/mL were mixed in different ratios, ranging from 1:0 to 0:1. These mixed solutions were then reacted with various concentrations of His (0–100 μg/mL) to obtain solutions with different colors. The results showed that the solutions mixed with 1:2, 1:3, 1:5 ratios had wider range of color development compared with other ratios after reacting with different concentrations of His (Fig. 2a, c, e). Among those, the solution with dye concentrations of 100 μg/mL mixed with ratio 1:3 was the most excellent, showing color change from orange-red to yellow and then to green with His concentration increasing. The non-linear curve fitting for the G/R values obtaining from fluorescent pictures were also presented in Fig. 2b, d, f. The 1:3 ratio curve showed that R^2 was 0.997 with the greatest across span. This result also supported the choice of 1:3 mixture with 100 μg/mL RhB and FITC solutions for subsequent experiments.

3.1.2. Preparation and characterization of FITC/RhB@SilMA MN

The preparation of FITC/RhB@SilMA MN patch was in two parts according to the previous literature with some modification (Wang, Chen, et al., 2023). The as-prepared MN patch was composed of an 8 × 8 array of needles and appeared orange-red color in natural light (Fig. 3a). The needle tips were conical and regularly arranged (Fig. 34c). Under 365 nm UV light irradiation, the MN patch appeared light yellow color (Fig. 3b).

To test the penetration capability of microneedles in FITC/RhB@SilMA MN patch under different temperature conditions, MN patches were inserted into chicken breast tissues at 0, 4, and 25 °C (Fig. S4). The results demonstrated that under varying test temperatures, the microneedle patch could be successfully inserted and achieved optimal conformability with chicken breast tissue. In addition, when the microneedle patch was inserted into chicken tissue at 4 °C for ten consecutive days (Fig. S5), apart from minor bending of the needle tips due to water absorption-induced softening, the overall structural integrity remained intact.

To evaluate potential leakage of fluorescent dyes loaded in FITC/RhB@SilMA MN patches, the patches were inserted into meat-simulating gelatin hydrogel for ten consecutive days (Fig. S6). Imaging under 365 nm UV illumination revealed no detectable changes in fluorescence intensity compared to control hydrogel without patch insertion. These results showed that the embedded fluorescent dyes maintain stable retention without leakage over 10 days.

3.2. Application of FITC/RhB@SilMA MN patch for raw meat freshness detection

3.2.1. Color development effect of FITC/RhB@SilMA MN patch

Firstly, the color response of the FITC/RhB@SilMA MN patch with time-varying for rapid detection of raw meat freshness was verified.

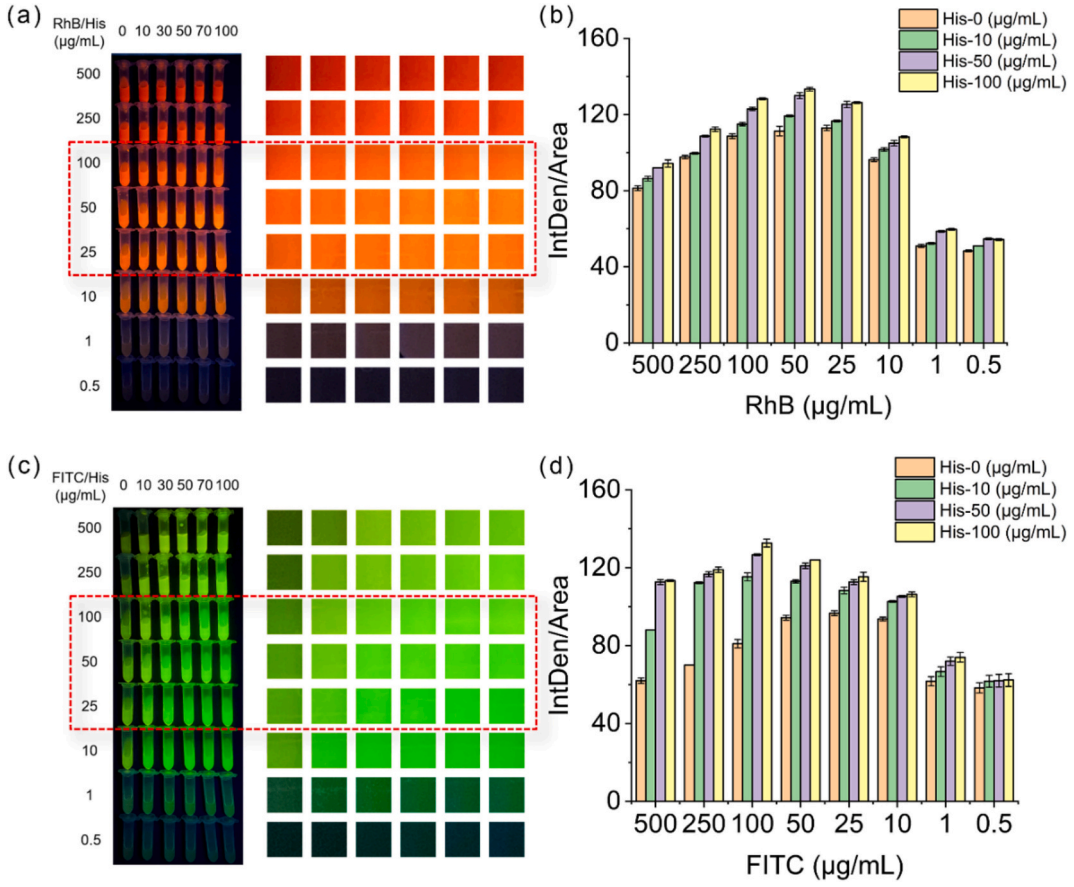


Fig. 1. Color pictures of RhB (a) and FITC (c) reacting with different concentrations of histamine under 365 nm UV excitation. (b, d) Mean fluorescence intensities calculated from (a) and (c).

Chicken breast (Fig. 4a), pork (Fig. 4b) and salmon (Fig. 4c) samples stored at 0 °C and 4 °C after 10 days were inserted with the patch, and the color changes were recorded every 2 min. The RGB signal (G + B/(R + G + B)) of the color images in those samples all significantly increased in the first 6 min and stabilized after 8–20 min. The color of the MN patch was stable after 8 min, indicating complete extraction of liquids from the sample. The result demonstrated the MN patch could achieve rapid detection of sample freshness/spoilage for raw meats in a few minutes.

Fig. 4d shows the color changes of FITC/RhB@SilMA MN patches after insertion of raw meat samples stored at 0 °C and 4 °C from day 0 to day 10. As storage time increase, the color of the FITC/RhB@SilMA MN patch on chicken breast stored at 0 °C changed from khaki to darkkhaki during the first 6 days and turned mediumaquamarine on day 7, and remained this green until the end. The MN patch color on chicken breast stored at 4 °C was khaki on day 0 and yellowgreen on day 1, turning darkolivegreen on days 2–4 and lightgreen on days 5–6, then appeared mediumaquamarine on days 7–10. The color changes of the MN patches inserted on the pork and salmon samples were also recorded. For pork stored at 0 °C, the MN patch color was darkkhaki on days 0–2, tan on days 3–6, and mediumaquamarine on days 7–10. Meanwhile, for pork stored at 4 °C, the MN patch color was darkkhaki on days 0–1, darkolivegreen on day 2, and turning mediumaquamarine on days 3–10. For salmon stored at 0 °C, the MN patch color was darkkhaki on days 0–1, darkolivegreen on days 2–7, and mediumaquamarine on days 8–10. While for salmon stored at 4 °C, the MN patch color was darkkhaki on day 0, tan on days 1–3, and mediumaquamarine on days 4–10. The distinct color changes with storage conditions indicated the potential of the FITC/RhB@SilMA MN patch for freshness detection of raw meats.

3.2.2. Biogenic amine index analysis of FITC/RhB@SilMA MN patch in freshness testing of raw meats

To assess the quality of meats, the BAs levels (Balamatsia, Paleologos, Kontominas, & Savvaidis, 2006) were tested. BAs can be used individually or in combination to evaluate meat and meat product quality and rancidity (Ruiz-Capillas & Jimenez-Colmenero, 2004). Therefore, Hernandez-Jover et al. proposed the BAI, calculated as the sum of Put, Cad, Tyr, and His, to evaluate the freshness of meat including chicken, pork, and fish (Chmiel et al., 2022; Huang et al., 2014; Ozogul & Ozogul, 2006; Ruiz-Capillas & Jimenez-Colmenero, 2004).

The BA content changes of chicken breast, pork and salmon samples storing at 0 °C and 4 °C for 10 days were listed in the Table S1-S6. As shown in Table S1 and S2, only Put was detected in chicken breast meat on day 0 both during storage at 0 °C and 4 °C. Cad was detected on day 4 at 0 °C and on day 1 at 4 °C, then His and Tyra were subsequently detected. With storage time extension, the contents of these BAs increased in different degrees, and this result is consistent with the report by Chmiel et al. (Chmiel et al., 2022). On day 4 at 0 °C, the BAI of chicken breast was 14.77 mg/kg (Table S1), within the range of fresh and edible meat (BAI $\leq$ 20 mg/kg). However, on day 5 and day 6, the BAI increased to 40.70 mg/kg and 42.08 mg/kg, respectively, indicating low-quality meat (BAI: 20–50 mg/kg). On day 7, the BAI of chicken breasts was 62.24 mg/kg, indicating spoiled meat (BAI $\geq$ 50 mg/kg). Meanwhile, when stored at 4 °C, the BAI of chicken breast was $\leq$20 mg/kg for 0–1 day, indicating fresh and edible meat. While on 2–4 days, the chicken breast was of low-quality meat (BAI: 20–50 mg/kg). However, the BAI increased to 59.52 mg/kg on the 5th day, indicating spoiled meat (BAI $\geq$ 50 mg/kg) (Table S2).

The freshness of pork and salmon samples was also evaluated based on the BAI results. For pork stored at 0 °C, it was fresh and edible meat

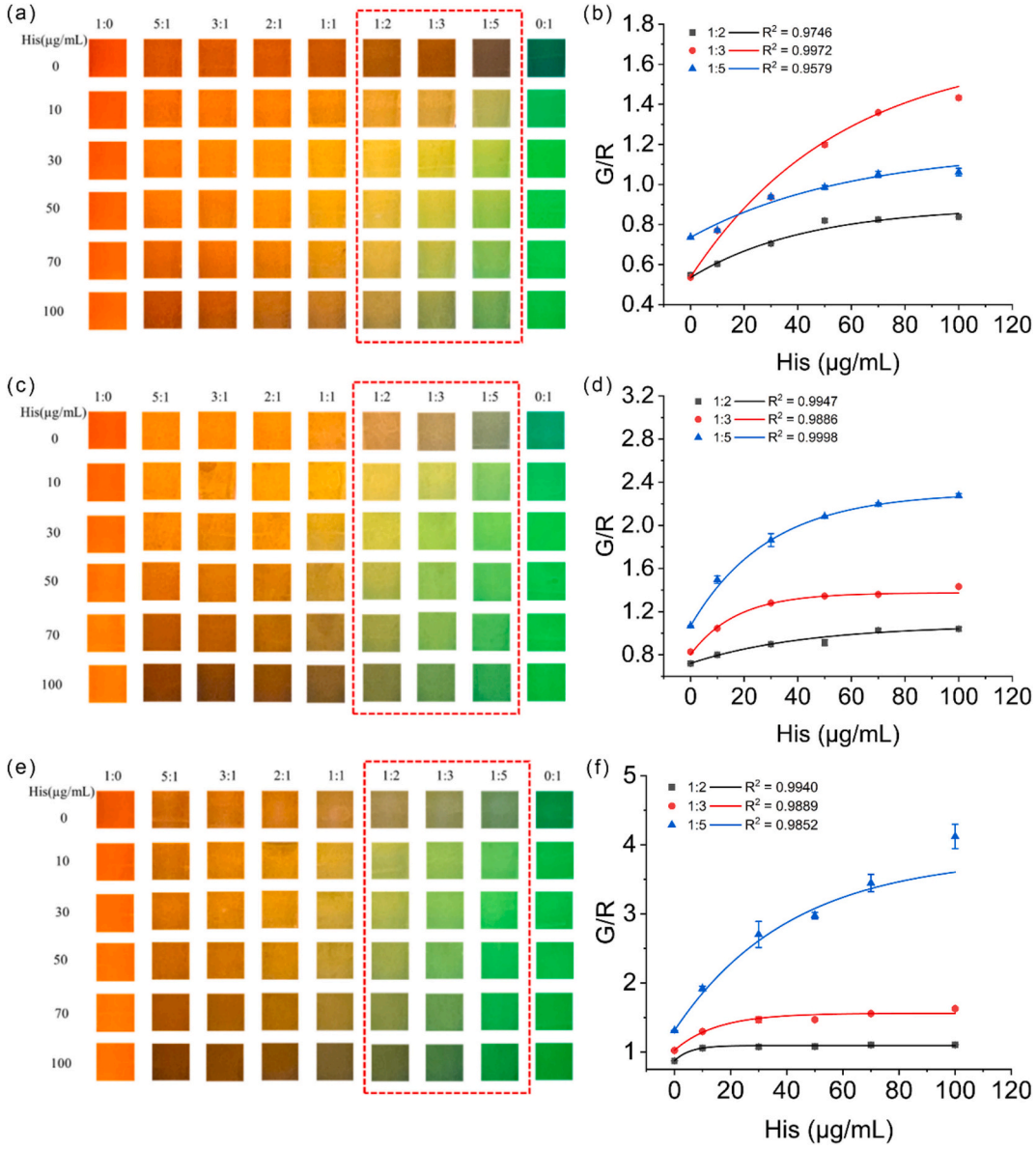


Fig. 2. Photographs of RhB, FITC solutions with mass concentrations of (a) 100 μg/mL, (c) 50 μg/mL, (e) 25 μg/mL mixing in different proportions and reacting with different concentrations of histamine; The nonlinear curve fitting relationships between G/R value and histamine with (b) 100 μg/mL, (d) 50 μg/mL, (f) 25 μg/mL RhB and FITC dyes mixing at ratios of 1:2, 1:3, 1:5.

on days 0–2 (BAI: 7.34–16.60 mg/kg), low-quality meat on days 3–6 (BAI: 23.28–48.53 mg/kg), and spoiled meat on days 7–10 (BAI: 51.01–102.14 mg/kg) (Table S3). Meanwhile, for pork stored at 4 °C, the first day (BAI: 15.26 mg/kg) was acceptable fresh edible meat, the 2nd day (BAI: 43.85 mg/kg) was low-quality meat, and the 3rd day (BAI: 69.73 mg/kg) was spoiled and deteriorated meat (Table S4). For salmon stored at 0 °C, it was fresh and edible meat on days 0–1 (BAI: 6.96–14.85 mg/kg), low-quality meat on days 2–7 (BAI: 23.99–46.85 mg/kg), and spoiled meat on days 8–10 (BAI: 54.19–76.00 mg/kg) (Table S5). While for salmon stored at 4 °C, the 1st-3rd day (BAI: 26.43–42.33 mg/kg) was low-quality meat, and the 4th day (BAI: 52.55 mg/kg) started to rot and was spoiled meat (Table S6).

The G + R/(R + G + B) values were calculated from the color pictures of the FITC/RhB@SilMA MN patch. Different colors were labeled "fresh – low quality – spoiled" differently, according to the BAI and G + R/(R + G + B) data (Table 1, Table S7-S11). On the whole, meat could be defined as fresh when the G + R/(R + G + B) value of the patch color was less than 0.60. When the G + R/(R + G + B) value was in the range of 0.60–0.65, meat could be defined as low quality. When the G + R/(R + G + B) value was greater than 0.65, the meat was considered to be spoiled. As a result, the FITC/RhB@SilMA MN patch can make good use of color display for detecting the freshness of raw meats.

The values are expressed as mean ± standard deviation (n = 3). Different small letters (a-e) represent significant differences within the same column ($P < 0.05$). BAI, biogenic amine index.

4. Conclusion

In summary, we have successfully developed a novel FITC/RhB@SilMA microneedle patch for the rapid detection of the raw meat freshness. By selecting and optimizing the concentrations of FITC and RhB fluorescent dyes, we achieved high selectivity and sensitivity in the response of biogenic amines (BAs), which is crucial for assessing the meat freshness. The results indicated that the FITC/RhB@SilMA microneedle patch could intuitively indicate the freshness of meat through color changes in 8 min, and the G + R/(R + G + B) values of

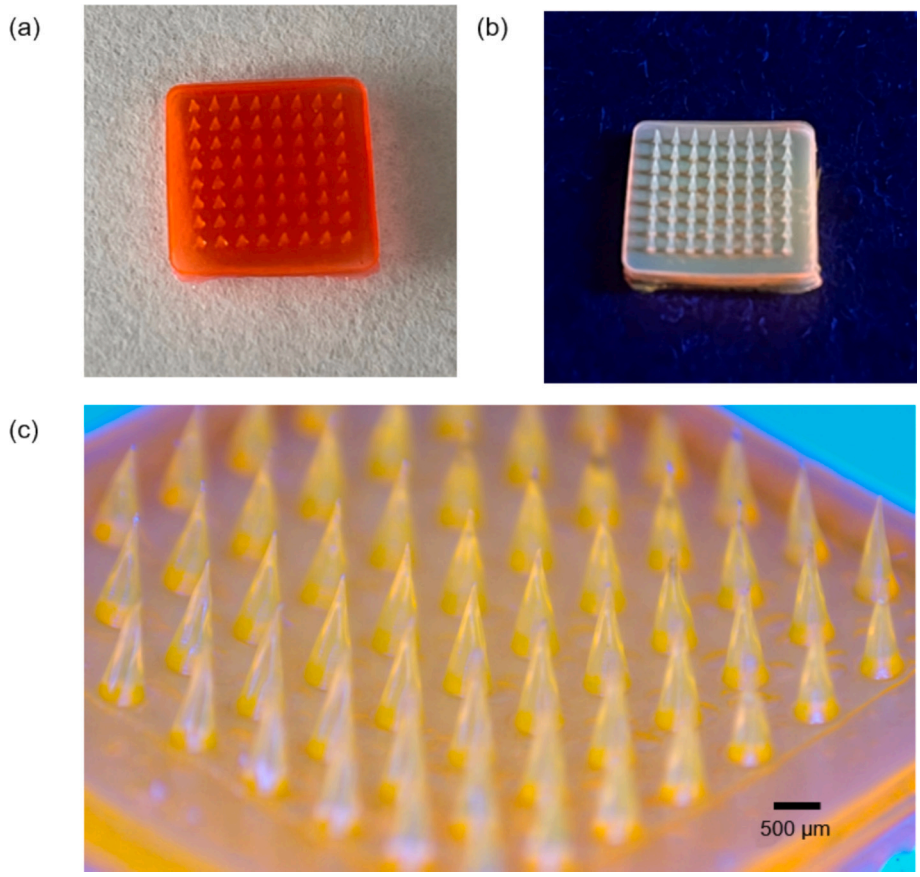


Fig. 3. Photograph of FITC/RhB@SilMA MN patch (a) under natural light and (b) under 365 nm UV light. (c) Microscopy image of FITC/RhB@SilMA MN.

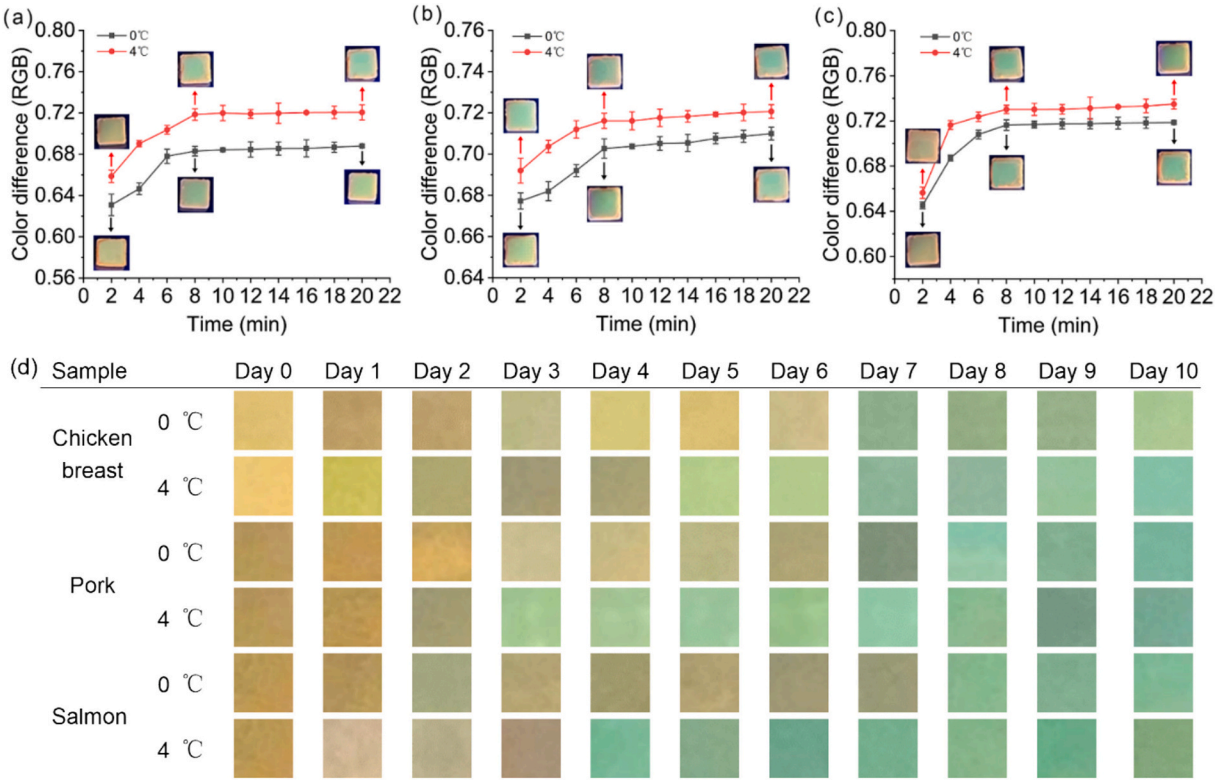


Fig. 4. Color responses of FITC/RhB@SilMA MN patch for (a) chicken breast, (b) pork, and (c) salmon stored at 0 °C and 4 °C on day 10. (d) Color changes of MN patch inserted on three raw meats stored at 0 °C and 4 °C for 10 days. The values are expressed as mean ± standard deviation ($n = 3$).

Table 1
The correlation of RGB values of color pictures from MN patches and the calculated BAI of chicken breast during storage at 0 °C for 10 days.

Chicken breast 0 °C	BAI (mg/kg)	Freshness	Color of MN patch	
			Picture	G + B/(R + G + B)
Day 0	4.79	Fresh		0.586 ± 0.001[a]
Day 1	5.07	Fresh		0.588 ± 0.001[a]
Day 2	5.45	Fresh		0.589 ± 0.002[ab]
Day 3	6.09	Fresh		0.592 ± 0.004[ab]
Day 4	14.77	Fresh		0.595 ± 0.003[b]
Day 5	40.70	Low-quality		0.635 ± 0.001[c]
Day 6	42.08	Low-quality		0.647 ± 0.010[d]
Day 7	62.24	Spoiled		0.673 ± 0.002[d]
Day 8	65.96	Spoiled		0.673 ± 0.001[d]
Day 9	86.78	Spoiled		0.675 ± 0.001[d]
Day 10	100.83	Spoiled		0.687 ± 0.002[e]

these color pictures corresponded well with the calculated results of BAI. The freshness of meat samples can be categorized as fresh when the G + R/(R + G + B) value is less than 0.60, low quality when it is between 0.60 and 0.65, and spoiled when it exceeds 0.65. The microneedle patch showed good accuracy and repeatability using ratio fluorescence images in freshness detection of chicken breast, pork, and salmon when stored at 0 and 4 °C. Moreover, the method is simple to operate and does not require complex equipment, only needs a handheld 365 nm UV lamp and a mobile phone, making it suitable for on-site rapid testing. The FITC/RhB@SilMA microneedle patch provides an innovative solution for the rapid detection of raw meat freshness, mainly animal and poultry meat with fur removed and fish with scales removed. Compared to traditional BA detection methods, this visualized microneedle sensor, with its portability, minimal sample pretreatment requirements, and fast response, has potential for commercial applications and may significantly impact food safety and quality control.

CRediT authorship contribution statement

Li-Jian Chen: Writing – review & editing, Supervision, Project administration, Conceptualization. **Jiang-Yue Wang:** Writing – original draft, Methodology, Formal analysis. **Xu Zhao:** Writing – review & editing, Methodology, Funding acquisition. **Xiu-Ping Yan:** Resources, Methodology, Funding acquisition.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

The authors appreciate the financial support from the National Natural Science Foundation of China (No. 21934002), the Natural Science Foundation of Jiangsu Province, China (No. BK20231491) and Collaborative Innovation Center of Food Safety and Quality Control in Jiangsu Province.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.foodchem.2025.144144.

Data availability

Data will be made available on request.

References

Chang, J., Li, H., Hou, T., Duan, W., & Li, F. (2018). Paper-based fluorescent sensor via aggregation induced emission fluorogen for facile and sensitive visual detection of hydrogen peroxide and glucose. *Biosensors and Bioelectronics, 104*, 152–157. https://doi.org/10.1016/j.bios.2018.01.007

Chang, J., Li, H., Hou, T., & Li, F. (2016). Paper-based fluorescent sensor for rapid naked-eye detection of acetylcholinesterase activity and organophosphorus pesticides with high sensitivity and selectivity. *Biosensors and Bioelectronics, 86*, 971–977. https://doi.org/10.1016/j.bios.2016.07.022

Cheng, J.-H., & Sun, D.-W. (2015). Rapid and non-invasive detection of fish microbial spoilage by visible and near infrared hyperspectral imaging and multivariate analysis. *LWT - Food Science and Technology, 62*(2), 1060–1068. https://doi.org/10.1016/j.lwt.2015.01.021

Cheng, W., Yao, Y., Li, D., Duan, C., Wang, Z., & Xiang, Y. (2023). Asymmetrically split DNAzyme-based colorimetric and electrochemical dual-modal biosensor for detection of breast cancer exosomal surface proteins. *Biosensors and Bioelectronics, 238*, Article 115552. https://doi.org/10.1016/j.bios.2023.115552

Chmiel, M., Roszko, M., Hac-Szymanczuk, E., Cegielka, A., Adamczak, L., Florowski, T., Pietrzak, D., Bryla, M., & Swider, O. (2022). Changes in the microbiological quality and content of biogenic amines in chicken fillets packed using various techniques and stored under different conditions. *Food Microbiology, 102*, Article 103920. https://doi.org/10.1016/j.fm.2021.103920

Choi, I., Choi, H., Lee, J. S., & Han, J. (2022). Novel color stability and colorimetry-enhanced intelligent CO_2 indicators by metal complexation of anthocyanins for monitoring chicken freshness. *Food Chemistry, 404*(Part A), Article 134534. https://doi.org/10.1016/j.foodchem.2022.134534

Gai, P., Pu, L., Wang, C., Zhu, D., & Li, F. (2023). CeO_2@NC nanozyme with robust dephosphorylation ability of phosphotriester: A simple colorimetric assay for rapid and selective detection of paraoxon. *Biosensors and Bioelectronics, 220*, Article 114841. https://doi.org/10.1016/j.bios.2022.114841

Guo, L., Wang, T., Wu, Z., Wang, J., Wang, M., Cui, Z., Ji, S., Cai, J., Xu, C., & Chen, X. (2020). Portable food-freshness prediction platform based on colorimetric barcode combinatorics and deep convolutional neural networks. *Advanced Materials, 32*(45), 2004805. https://doi.org/10.1002/adma.202004805

Hernandez-Jover, T., Izquierdo-Pulido, M., Veciana-Nogues, M. T., & Vidal-Carou, M. C. (1996). Biogenic amine sources in cooked cured shoulder pork. *Journal of Agricultural and Food Chemistry, 44*(10), 3097–3101. https://doi.org/10.1021/jf960250s

Huang, X. W., Zou, X. B., Shi, J. Y., Guo, Y., Zhao, J. W., Zhang, J., & Hao, L. (2014). Determination of pork spoilage by colorimetric gas sensor array based on natural pigments. *Food Chemistry, 145*, 549–554. https://doi.org/10.1016/j.foodchem.2013.08.101

Jia, R., Tian, W., Bai, H., Zhang, J., Wang, S., & Zhang, J. (2019). Amine-responsive cellulose-based ratiometric fluorescent materials for real-time and visual detection of shrimp and crab freshness. *Nature Communications, 10*(1), 795. https://doi.org/10.1038/s41467-019-08675-3

Kannan, S. K., Ambrose, B., Sudalaimani, S., Pandiaraj, M., Giribabu, K., & Kathiresan, M. (2020). A review on chemical and electrochemical methodologies for the sensing of biogenic amines. *Analytical Methods, 12*(27), 3438–3453. https://doi.org/10.1039/d0ay00358a

Kim, D., Cao, Y., Mariappan, D., Bono, M. S., Hart, A. J., & Marelli, B. (2020). A microneedle technology for sampling and sensing bacteria in the food supply chain. *Advanced Functional Materials, 31*(1), 2005370. https://doi.org/10.1002/adfm.202005370

Kim, D., Lee, S., Lee, K., Baek, S., & Seo, J. (2017). Development of a pH indicator composed of high moisture-absorbing materials for real-time monitoring of chicken breast freshness. *Food Science and Biotechnology, 26*(1), 37–42. https://doi.org/10.1007/s10068-017-0005-6

Kim, K. H., Moon, D., An, J. E., Park, S. J., Seo, S. E., Ha, S., … Song, H. S. (2022). Wireless portable bioelectronic nose device for multiplex monitoring toward food freshness/spoilage. *Biosensors and Bioelectronics, 215*, Article 114551. https://doi.org/10.1016/j.bios.2022.114551

Kuswandi, B., Wicaksono, Y., Jayus Abdullah, A., Heng, L. Y., & Ahmad, M. (2011). Smart packaging: Sensors for monitoring of food quality and safety. *Sensing and Instrumentation for Food Quality and Safety, 5*(3–4), 137–146. https://doi.org/10.1007/s11694-011-9120-x

Li, Y., Jiang, X., Li, Y., Yan, X., Tang, L., Sun, X., Zhong, K., Li, X., & Li, J. (2024). A smartphone-adaptable fluorescent probe for visual monitoring of fish freshness and its application in fluorescent dyes. *Food Chemistry, 458*, Article 140239. https://doi.org/10.1016/j.foodchem.2024.140239

Liu, X., Chen, K., Wang, J., Wang, Y., Tang, Y., Gao, X., & Li, J. (2020). An on-package colorimetric sensing label based on a sol-gel matrix for fish freshness monitoring. *Food Chemistry, 307*, Article 125580. https://doi.org/10.1016/j.foodchem.2019.125580

Moon, E. J., Kim, Y., Xu, Y., Na, Y., Giaccia, A. J., & Lee, J. H. (2020). Evaluation of salmon, tuna, and beef freshness using a portable spectrometer. *Sensors, 20*(15), 4299. https://doi.org/10.3390/s20154299

Munir, M. A., & Badri, K. H. (2020). The importance of derivatizing reagent in chromatography applications for biogenic amine detection in food and beverages. *Journal of Analytical Methods in Chemistry, 14*, 5814389. https://doi.org/10.1155/2020/5814389

Onal, A., Tekkeli, S. E., & Onal, C. (2013). A review of the liquid chromatographic methods for the determination of biogenic amines in foods. *Food Chemistry, 138*(1), 509–515. https://doi.org/10.1016/j.foodchem.2012.10.056

Ozogul, F., & Ozogul, Y. (2006). Biogenic amine content and biogenic amine quality indices of sardines (sardina pilchardus) stored in modified atmosphere packaging and vacuum packaging. *Food Chemistry, 99*(3), 574–578. https://doi.org/10.1016/j.foodchem.2005.08.029

Quan, Z., He, H., Zhou, H., Liang, Y., Wang, L., Tian, S., Zhu, H., & Wang, S. (2021). Designing an intelligent nanofiber ratiometric fluorescent sensor sensitive to biogenic amines for detecting the freshness of shrimp and pork. *Sensors and Actuators B: Chemical, 333*, Article 129535. https://doi.org/10.1016/j.snb.2021.129535

Ruiz-Capillas, C., & Jimenez-Colmenero, F. (2004). Biogenic amines in meat and meat products. *Critical Reviews in Food Science and Nutrition, 44*(7–8), 489–499. https://doi.org/10.1080/10408690490489341

Wang, J., Lu, Z., Cai, R., Zheng, H., Yu, J., Zhang, Y., & Gu, Z. (2023). Microneedle-based transdermal detection and sensing devices. *Lab on a Chip, 23*, 869. https://doi.org/10.1039/D2LC00790H

Wang, J., Xia, L., Liu, H., Zhao, C., Ming, S., & Wu, J. (2024). Colorimetric microneedle sensor using deep learning algorithm for meat freshness monitoring. *Chemical Engineering Journal, 481*, Article 148474. https://doi.org/10.1016/j.cej.2023.148474

Wang, J. Y., Chen, L. J., Zhao, X., & Yan, X. P. (2023). Silk fibroin-based colorimetric microneedle patch for rapid detection of spoilage in packaged salmon samples. *Food Chemistry, 406*, Article 135039. https://doi.org/10.1016/j.foodchem.2022.135039

Wojnowski, W., Namiesnik, J., & Płotka-Wasylka, J. (2019). Dispersive liquid-liquid microextraction combined with gas chromatography–mass spectrometry for in situ determination of biogenic amines in meat: Estimation of meat's freshness. *Microchemical Journal, 145*, 130–138. https://doi.org/10.1016/j.microc.2018.10.034

Yao, S., Zhang, C., Ping, J., & Ying, Y. (2024). Recent advances in hydrogel microneedle-based biofluid extraction and detection in food and agriculture. *Biosensors and Bioelectronics, 250*, Article 116066. https://doi.org/10.1016/j.bios.2024.116066

Zhu, L., Zhang, J., Zhang, H., Zhang, M., & Wang, J. (2024). Paper-based chip integrated with vitrified silk fibroin microneedle array for colorimetric detection of histamine in food. *Sensors and Actuators B: Chemical, 412*, Article 135819. https://doi.org/10.1016/j.snb.2024.135819