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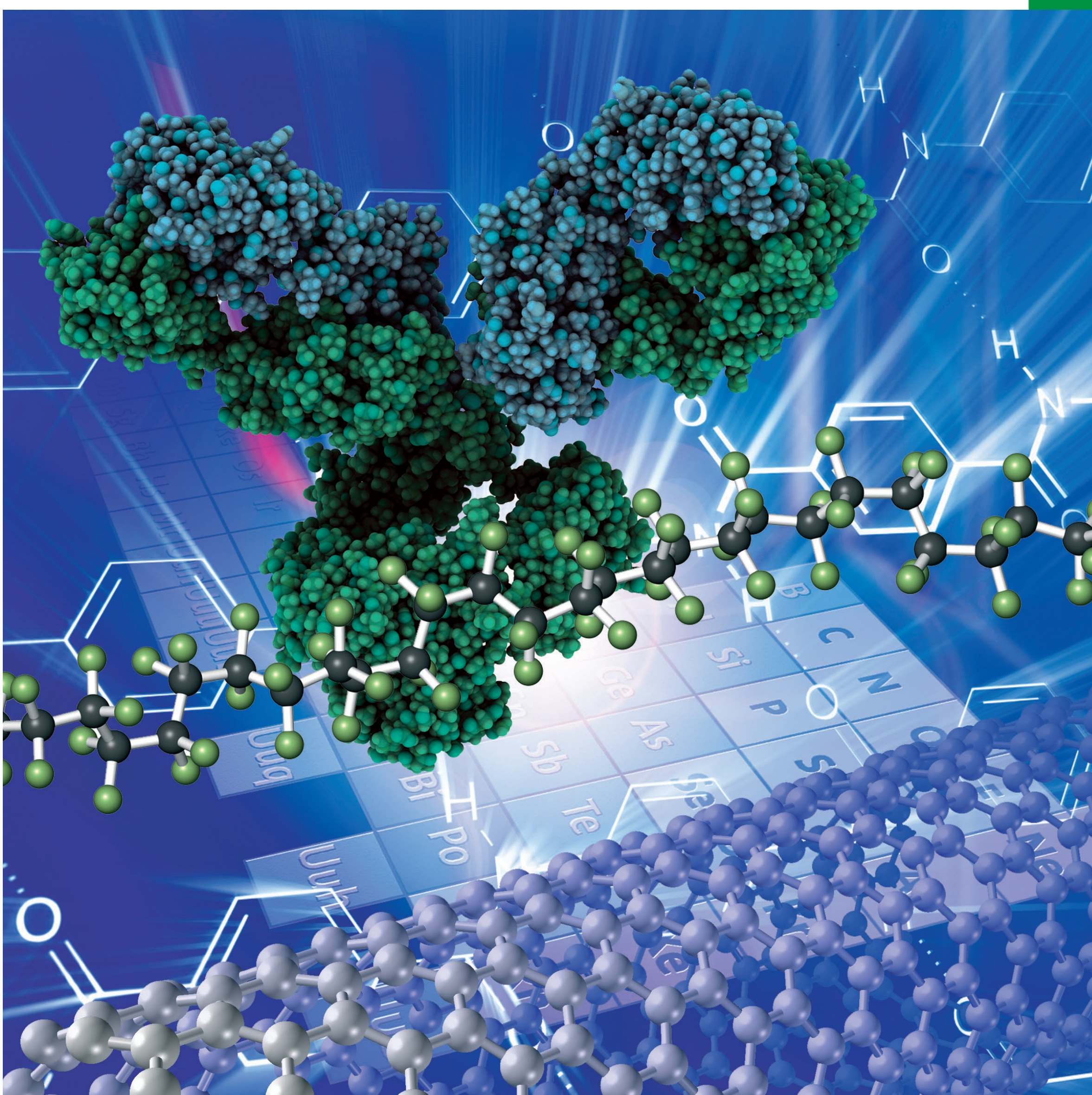

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An Intelligent Label for Freshness of Fish Based on a Porous Anodic Aluminum Membrane and Bromocresol Green

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A sensitive and intuitive freshness intelligent label was fabricated for indicating the freshness of fish based on porous anodic aluminum (PAA) membrane and bromocresol green. This label is fabricated by the bromocresol green (BCG) immobilized on PAA membrane, which responds through visible color change to the total volatile basic nitrogen (TVBN) released during fish spoilage period. The result showed that the fish is inedible when it was stored at 25 °C for 6 h (TVBN > 20 mg/100 g). Under this condition, the color of BCG/PAA label changed from yellow to green obviously. In addition, BCG/PAA membrane label and the electrospinning nanofiber label were used to evaluate the fish freshness. As a result, the color of BCG/PAA membrane label is more obvious and sensitive. Thus, BCG/PAA membrane label can be considered as a new promising method for the assessment of fish freshness.

Introduction

Freshness is one of the main attributes used to determine the food quality. As the improvement of living level and the enhancement of health consciousness of people, food quality control has become more and more important. The freshness of fishery products is commonly evaluated based on sensory aspects, such as the investigation of the scale condition, pupil color, flesh elasticity, and skin odor. Odor is usually considered to be an important parameter for determination of fish freshness and can be easily determined by consumers.[1] However, we need a simpler and more accurate method to access the shelf life of fish products.

Traditional analytical methods[2–4] for detecting the freshness of aquatic products, such as gas chromatography and liquid chromatography, have been widely used. These methods are high sensitivity and low detection limit. Nevertheless, they are costly and sophisticated. Moreover, they are not applicable for real-time determination. One simple method[5–7] is using color changes to identify the freshness of fish. The total volatile basic nitrogen (TVBN) compounds contents are recognized as an index of seafood spoilage, and volatile amines are directly correlated to the sensorial quality of fish. The color of freshness indicator will be changed as a result of increasing TVBN contents released during the fish storage. At present, there are many publications which have built indicators or sensors for assessing the freshness of food. For example, Hu et al.[8] developed a nanotube-based fluorescent sensor, which is highly sensitive, portable and expedient. Kuswandi et al.[9] developed a chemical sensor for monitoring fish freshness based on polyaniline film, which is simple, low-cost and rapid. Moreover, Pacquit et al.[10] and Chun et al.[1] prepared a freshness indicator for monitoring fish freshness based on cellulose acetate membrane, which is fast, sensitive and non-invasive. Zhang et al.[11] constructed a time-temperature indicator for corruptible products by dynamically programmable Ag overgrowth on Au nanorods, which is sensitive, low cost and convenient. However, this indicator fails to reflect the food quality truly, because the color change depends on the time and temperature at which the product is stored, rather than the composition of the product.

Though the methods above are cheap, the indicators are disordered which may cause the nonuniform color change. However, the porous anodic aluminum (PAA) membrane is characterized by large surface area, high regularity and porosity,[12–13] facilitating the larger adsorption capacity and the more uniform distribution of volatile gases and pH indicator compared with the former carriers. Furthermore, the PAA membrane prepared in phosphoric acid was white, so that the color change was better to be observed when the membrane was immersed in pH indicator solution.

In this study, a simple and intuitive freshness intelligent indicator was fabricated by using PAA membrane containing pH-sensitive dye bromocresol green (BCG) to indicate the content of TVBN. This label showed a definite color change

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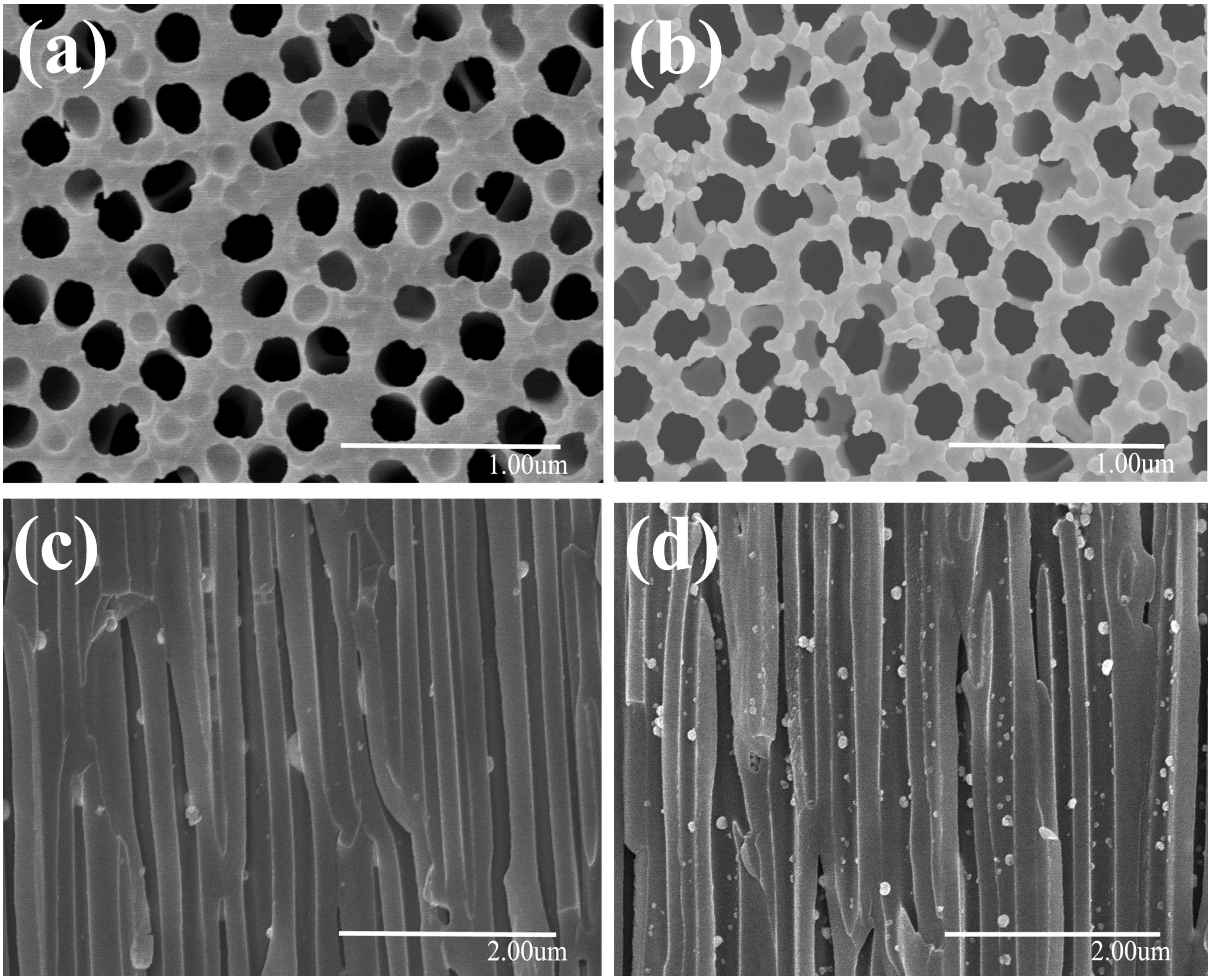

Figure 1. Scanning electron microscopy (SEM) of PAA membrane and BCG/PAA membrane. (a)The surface of PAA membrane; (b) The surface of BCG/PAA membrane; (c) The cross-section of PAA membrane; (d) The cross-section of BCG/PAA membrane.

from yellow to blue that easily reflected fish spoilage. In addition, TVBN contents were also assessed for correlation with bacterial growth.

Results and discussion

Characterization of PAA, BCG/PAA membrane

PAA and BCG/PAA membranes were characterized by scanning electron microscopy (SEM), with particular attention to the morphology of their top and cross-sectional surfaces. Figure 1 (a) and (c) show the SEM images of the surface morphology and cross-sectional views of PAA membrane after two-step anodizing and pores widening treatment. The surface image [Figure 1 (a)] shows pores are fairly uniform in shape and diameter, with a diameter of about 200 nm. The cross section image [Figure 1 (c)] clearly shows the parallel alignment of the pore. Figure 1 (b) and (d) show the SEM images of the surface morphology and cross-sectional views of BCG/PAA membrane, respectively. And BCG is uniformly distributed on the PAA membrane.

Behavior of the intelligent label

The color of the label will change due to the increase in the amount of TVBN which released during fish spoilage. The color of the dye molecules BCG on the intelligent label will change from yellow to blue, indicating the freshness of the fish. The change of the freshness intelligent label with different pH (2.50, 2.65 and 2.80) and BCG concentrations (0.02%, 0.04%, 0.06%, 0.08% and 0.1%) in response to TVBN levels in fish spoilage at 25 °C for 24 h. The color changes from 0–12 h are shown in Figure 2, and the rest of changes (14-24 h) are depicted in Supporting Information (Figure S1). Five fish samples and fifteen intelligent labels were used for this experiment. 0.02% BCG-0.1% BCG represents the BCG concentration, which was used to prepare the intelligent label. Before using BCG, the PAA membrane was immersed in a phosphoric acid-ethanol solution

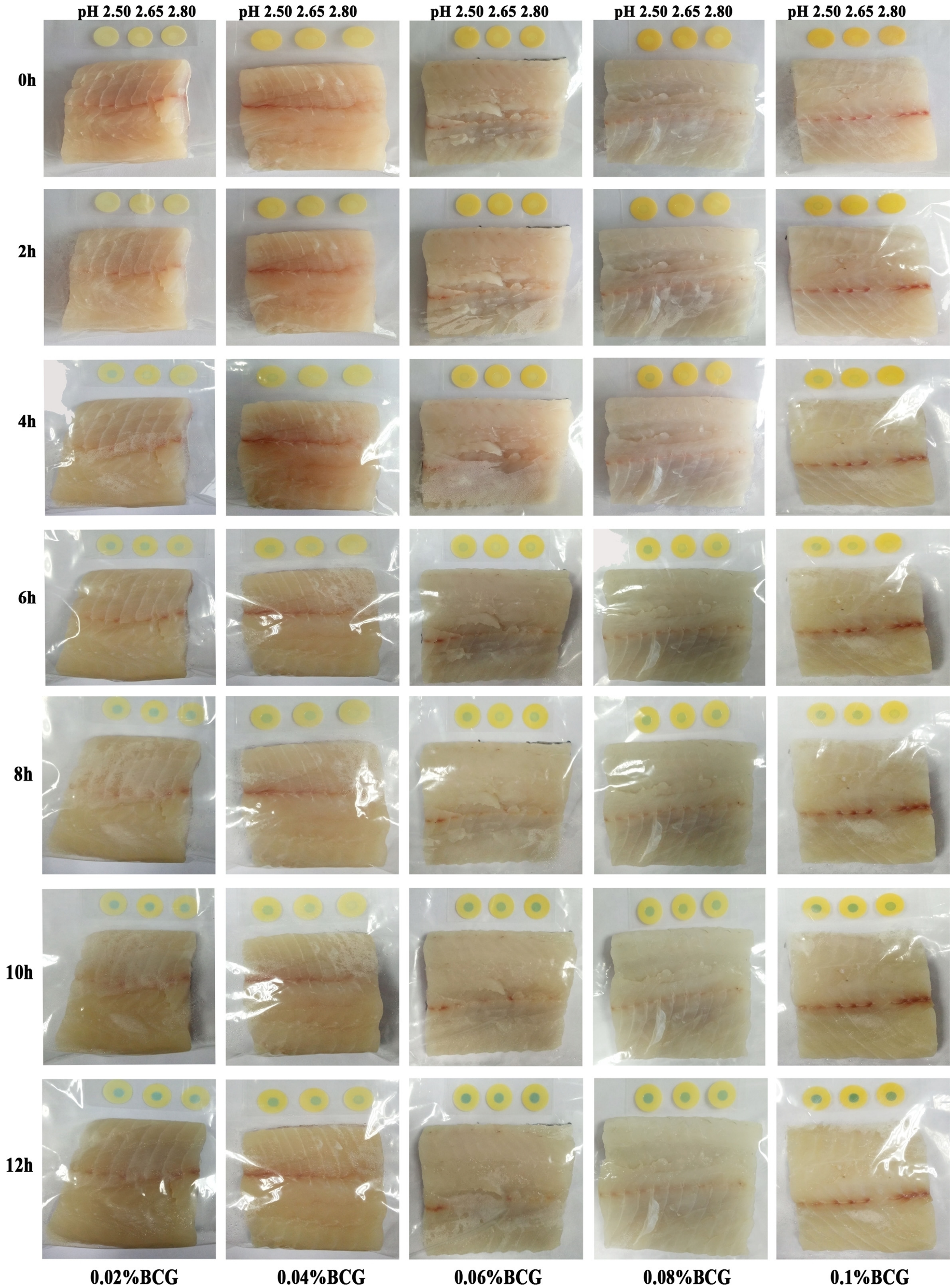

Figure 2. The intelligent label with different pH (2.50, 2.65 and 2.80) and BCG concentrations (0.02%, 0.04%, 0.06%, 0.08% and 0.1%) in response to TVBN levels in fish spoilage at 25 °C. The range of time is 0–12 h. The experiment was repeated five times.

at a pH of 2.50, 2.65 and 2.80. Finally, each of the BCG concentrations corresponds to the three labels were used to monitor each fish sample. The color of the label (0.02% and 0.04% BCG) changes from yellow to green gradually, which is not obvious. The color of the label (0.06%, 0.08% and 0.1% BCG) changes from yellow to green and finally into blue with time, which is obvious. But the label (0.06% and 0.08% BCG) changes without regularity. In addition, the discoloration time of the smart labels prepared under different pH conditions is different, among which the discoloration time of pH 2.50, pH 2.65 and pH 2.80 are corresponded to the storage time of 4 h, 6 h and 6 h, respectively. Compared with the TVBN value by measured in our experiment, the color of the label (pH 2.65) shows the most obvious sequential color change to the naked eye. The color of the label (0.1% BCG) changes more obviously, and is related to TVBN contents. Therefore, pH 2.65 and 0.1% BCG were selected in this experiment. In order to show its applicability in different temperature condition, the freshness of fish in refrigerated and or iced situation were also monitored by the labels. The results show that our labels can also be used in chiller or iced fish condition (Supporting information, Figure S2 and Figure S3).

Color changes of the intelligent label during fish spoilage trial

The color of the intelligent label was detected at 25 °C using a colorimeter. The result is shown in Figure 3. The value of a represents the sample color change from red (a value is positive) to green (a value is negative). The value of b represents the color change from yellow (b value is positive) to blue (b value is negative). Figure 3 (a) shows a value is remained negative and the trend is decreased with time. This indicates the label turns to green with time and is easier to be observed by the naked eye. Figure 3 (b) shows b value changes from positive to negative and the trend is decreased while the label color getting blue. The label is yellow at initially 4 h, then turn to green (6 h), ultimately is blue (14 h).

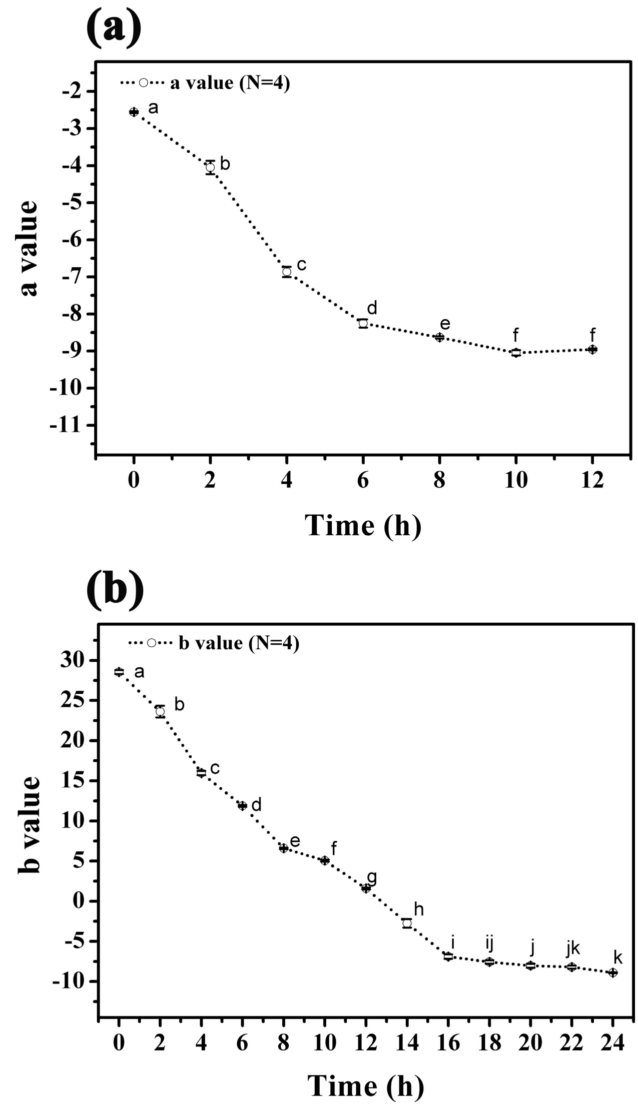

Figure 3. The chromophotometer detected the color change of the intelligent label. (a) The chromophotometer measured the intelligent label to obtain a value; (b) Chromophotometer measured the intelligent label to obtain b value. The temperature of the experiment was 25 °C. The error bars are standard error of the mean. The experiment was repeated four times ($N = 4$, $P < 0.05$).

TVBN analysis

The total volatile base nitrogen (TVBN) was composed of trimethylamine (TMA), dimethylamine (DMA) and ammonia (NH_3) and other volatile amine compounds which were generated by microbial degradation. Hence, we can utilize the TVBN value because it can indirectly reflect the deterioration of the fish.[14–16] Figure 4 shows the TVBN content of fish. The TVBN content was gradually increased and exceeded 20 mg/100 g at 6 h. And the color of the intelligent label was observed from yellow to green during the same period, finally to blue as shown in Figure 2. Furthermore, the color of the label was observed from yellow to blue when the TVBN values are increasing. The TVBN content with 20 mg/100 g is defined as the baseline of fish freshness, the fish sample with TVBN content exceed 20 mg/100 g was defined as "stale" and otherwise the "fresh" one according to Chinese Standard GB 2733–2015. Therefore, TVBN contents exceeded the recommended limit at 6 h after the fish died in our experimental condition.

Microbial analysis of fish samples

There are many elements affect the bacterial counts, such as capture season, capture ripeness, geographic position and fish varieties. Soit is difficult to use reported bacterial counts to define exact spoilage thresholds. The levels of iced fish specific spoilage bacteria reached $10^8 \sim 10^9$ cfu/g indicating the fish have been corrupted which was advised by Gram and Huss et al..[17] The TVC and *Pseudomonas* values of fresh fish samples up to 10^7cfu/g indicating that the samples reached the end of

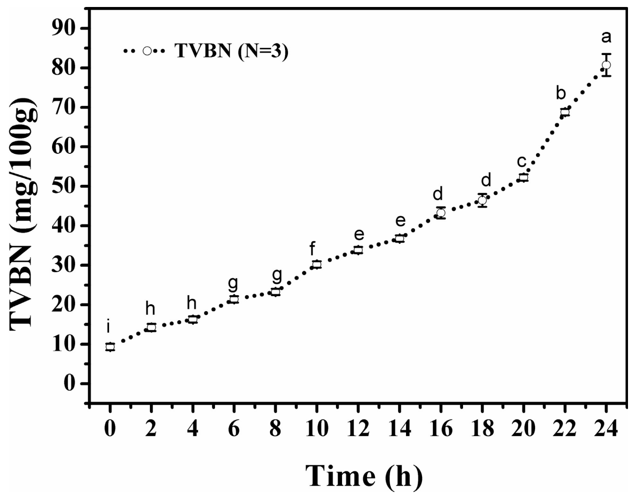

Figure 4. TVBN values of fish with time. The temperature of the experiment was 25 °C. The error bars are standard error of the mean. The experiment was repeated three times (N = 3, P < 0.05).

shelf life, which were reported by Koutsoumanis[18] and Olafsdottir et al..[19]

In this experiment, three replicate experiments were conducted to determine the number of microorganisms in tilapia at 25 °C for 24 h. The results are shown in Figure 5. The

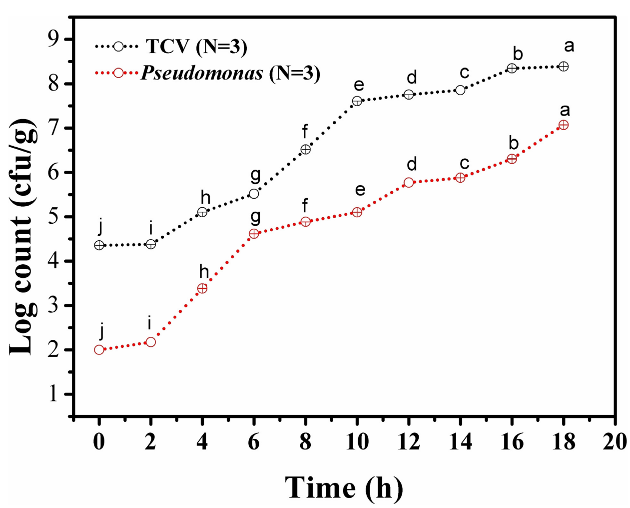

Figure 5. Microbial populations in the fish (TVC and *Pseudomonas* SPP.) change over time. The experiment temperature was 25 °C. The error bars are standard error of the mean. The experiment was repeated three times (N = 3, P < 0.05).

number of TVC (blank line) is about 2.3×10^4 cfu/g at 2 h, then gradually increases, and reaches the counts about 4.1×10^7 cfu/g at 10 h. In addition, the number of *Pseudomonas* (red line) was about 1.5×10^2 cfu/g, increasing to about 1.2×10^7 cfu/g at 18 h. The fish samples deteriorated about 10 h in our experiment. And the color of the intelligent label was observed from yellow to green during the same period, finally to blue as shown in Figure 2. Furthermore, the color of the label was observed from yellow to blue when microbial populations are increasing.

Different labels based on different carriers to monitor the freshness of fish

SEM images of electrospinning nanofiber membranes (containing 0.1% BCG) at a magnification of 10,000 times and 30000 times are shown in Figure 6 (a) and (b), respectively. The fiber

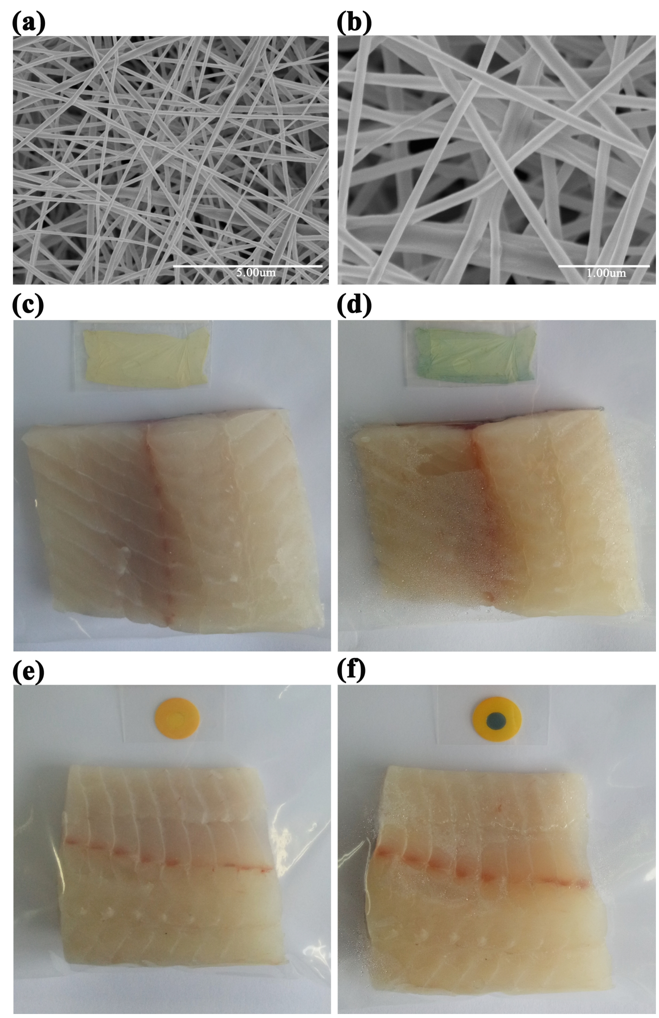

Figure 6. Comparison of PAA membrane label and electrospinning fiber membrane label. SEM images of the electrospinning nanofiber membrane at a magnification of 10,000 times (a) and 30000 times (b); The monitoring the fish freshness by the electrospinning nanofiber membrane label: (c) The time was 0 h; (d) The time was 14 h; The monitoring the fish freshness by PAA membrane label: (c) The time was 0 h; (d) The time was 14 h. The experiment temperature was 25 °C. The experiment was repeated three times.

of membrane possesses excellent continuity and uniform distribution and with an average diameter of 27 nm.

The BCG/PAA membrane label and the electrospinning fiber membrane label were placed with fish in the sealed bag to monitor the freshness of fish under the same conditions. The results are shown in Figure 6. Figure 6 (c) and (e) show the initial state of the electrospinning nanofiber membrane label and the BCG/PAA membrane label when placed in a sealed bag, respectively. As can be seen from the figure, the color of the label is yellow. Figure 6 (d) and (f) show that the electrospinning nanofiber membrane label and the BCG/PAA membrane label was placed in a sealed bag to monitor the fish freshness for 14 hours, respectively. And the colors of the label have changed, but the color of the BCG/PAA membrane label is more obvious, uniform and easier to observe than the electrospinning nanofiber membrane label. The reason is that the PAA membranes are porous and ordered nanomaterials, while the electrospinning nanofiber membranes are disordered.

Conclusions

We have developed a novel intelligent label based on PAA membrane as a cheap and visual indicator to assess the fish freshness. The label is sensitive to volatile amines. When the fish was stored at 25 °C for 6 h, the amount of TVBN exceeded 20 mg/100 g, resulting in the color of the label changed from yellow to green. This color change indicates that the fish cannot be edible. Under this condition, the color of BCG/PAA membrane label changed from yellow to blue obviously, which was more visual, sensitive and simpler than the electrospinning nanofiber membrane label. Accordingly, the BCG/PAA membrane label can be considered as a novel promising method to evaluate the freshness of fish.

Acknowledgments

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Conflict of Interest

The authors declare no conflict of interest.

Keywords: bromocresol green · fish · freshness intelligent label · porous anodic aluminum membrane

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