A visual indicator based on curcumin with high stability for monitoring the freshness of freshwater shrimp, *Macrobrachium Rosenbergii*

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Abstract:

An ink-free printing method of multifunction pH indicator was provided based on agar and polyvinyl alcohol (AP) incorporated with curcumin for monitoring of shrimp freshness. The physical properties results showed that the addition of curcumin increased elongation at break of hydrogel films from 47.02% to 68.69% but reduced their water content. The curcumin film showed a greater colorimetric stability at 4 ˚C which *ΔE* value was 3.93. The hydrogel films exhibited obvious color changes to ammonia while the electrochemical writing pattern was well-stable acts as a reference. Meanwhile, the intelligent indicator presented a highly color change from yellow to red with the increasing of shrimp storage time. Last but not the least, the printed information not only provides the basic information of packaging (production date, shelf-life, etc.) but also has the reference function for an indicator.

Keywords: curcumin; hydrogel film; shrimp; electrochemical writing; color stability; non-destructive method

1. Introduction

Macrobrachium rosenbergii is one of the most economically important freshwater shrimps with fast growth and high fecundity in agricultural development on a global scale (Li et al., 2019). However, *Macrobrachium rosenbergii* is susceptible to contamination by bacteria and mildew triggering rapid death and severe economic losses (Soares et al., 2013). Therefore, it is indispensable to monitor its freshness for better health protection of the consumers. Generally, the decomposition of protein in *Macrobrachium rosenbergii* produces various volatile nitrogenous compounds such as ammonia, dimethylamine, and trimethylamine (Zhang et al., 2019). Then the total volatile basic nitrogen (TVB-N) level is increasing and bringing alteration to the pH of packaging environment. Recently, the visual pH indicators for food freshness attract widespread attention. Novel pH indicators have been developed for 'on-package' which can track food quality in real time by naked eyes without any sample destruction (Choi et al., 2017; Huang et al., 2019). Likewise, the color of curcumin shows visual change from yellow to orange-red with the pH increasing (Liu et al., 2018). Curcumin, which exhibits antiviral, antimicrobial, antioxidant, and anticancer properties, is a natural dye comprising of a dike tone compound extracted from the Curcuma rhizomes (Pereira and Andrade, 2017). Nowadays, several works have reported the use of curcumin as a pH indicator to indicate food spoilage (Luo et al., 2012; Musso et al., 2016; Ma et al., 2017a). For instance, an indicator film was developed based on κ-carrageenan and curcumin to monitor the freshness of pork and shrimp samples (Liu et al., 2018).

Previously, in order to immobilize natural pH dyes, several film-forming materials such as polyvinyl alcohol, chitosan and agar have been prepared (Mannozzi et al., 2018; Ebrahimi Tirtashi et al., 2019). Agar is extracted from Gelidiaceae and Gracilariaceae families of seaweeds and has well-defined phase barriers in the gel-forming process. Polyvinyl alcohol (PVA) is a synthetic vinyl polymer with a C-C chain backbone and polyhydroxyl. The hydroxyl groups in PVA may cause high solubility and weak water resistance. Thus, it is imperative to incorporate PVA with another natural polymer that could enhance its physical properties (Lyons et al., 2009).

The traditional inks used for printing are usually toxic which limits their suitability for food products. Therefore, a new method about ink-free printing on hydrogel films fetches the attention of food industries. Previous work reported that printing on chitosan and agarose film has provided valuable information about fish freshness with electrochemistry analysis (Wu et al., 2018). Besides, Zhai and co-workers have developed an edible film combined with electrochemical writing for milk and carp freshness (Zhai et al., 2018). However, as the mentioned indicators above, they showed lower stability owing to the degradation of the anthocyanins which directly related to the coloration of the indicators (Kara and Erçelebi, 2013). Therefore, in our study, the curcumin was used as the pH dye, and agar was bound with PVA as the film-forming materials to prepare the hydrogel film indicator. And we aimed to provide a multifunction curcumin indicator. The ink-free printed information not only provides the basic information on packaging (production date, shelf-life, etc.) but also has the reference function. This indicator was similar as a ratio indicator, the curcumin film was used as a sensor for meat freshness and the imprinted character was used as a reference. Interestingly, the imprinted was written on the hydrogel film based on electrochemical analyzer and a robotic arm was provided for an assistant device of an automatic ink-free printing method.

2. Material and Methods

2.1 Materials

Shrimp (*Macrobrachium Rosenbergii*) was purchased from the local supermarket in Zhenjiang city of Jiangsu province, China. Polyvinyl alcohol (molecular weight about 77000) and agar were acquired from Shanghai Natural Wild-insect Kingdom Co., Ltd. Glycerin (EG, 99.9%) and Ethyl alcohol (≥ 99.5%) were obtained from Sinopharm Chemical Reagent Co., Ltd (Shanghai, China). Curcumin powders were purchased from Macklin Biochemical Co., Ltd (Shanghai, China). Disodium citrate was purchased from Jiangsu Thorpe Group Co. Ltd (Jiangsu, China). Buffers with the pH range of 3.0-11.0 were acquired with citric acid/disodium hydrogen phosphate. Glass molds were acquired from Sigma Chemical Co. Ltd (St. Louis, MO, USA).

2.2 Preparation of hydrogel films

The hydrogel films were prepared following a series of processes. Firstly, 100 mL of distilled water containing 1.6 g agar and 0.4 g PVA (AP) was heated and stirred with a magnetic stirrer (F-101S, YUHUA, China) at 100 ˚C for 60 minutes (Lyons et al., 2009). Afterward, the curcumin with different concentrations (0-3% of the AP, w/w) was dissolved in a series of ethanol solutions (5 mL, ethanol/water = 4:1, v:v). Then, the mixture was added to the AP solution at 60 ˚C expressed as CAP1, CAP2, and CAP3, respectively. The mixed solution was stirred until it was utterly homogenized. Each hydrogel film was prepared by casting 10 g of mixing solution into a smooth glass mold (90×90 mm) at room temperature for 10 minutes. The prepared hydrogel films were stored at 4 ˚C for further analysis.

2.3 UV-Vis spectroscopy measurement

The UV-Vis spectra of curcumin in the range of pH 3.0-11.0 was measured using an Agilent CARY 100 UV-Vis spectrophotometer (Varian Corporation, USA). The absorbance of solutions was measured in the range of 400-800 nm and the solution without curcumin was served as blank.

2.4 Electrochemical writing on hydrogel films.

The cathode made of a platinum wire counter electrode (diameter 0.5 mm) contacted onto the surface of the hydrogels and the anode (platinum sheet) was placed underside of the hydrogel. The constant applied voltage was set at -10V, then OH^- was produced at the cathode. With the increasing of the local pH, the color of the imprinted characters changed from yellow to red at the cathode. As shown in Fig. 1, the moving cathode on the hydrogel was controlled by a robotic arm (Yue Jiang Technology Co., Ltd., Shenzhen, China). The required characters or motifs were printed by electrochemical writing and controlled by an in-built computer program attached to the system. In this study, the characters "JU" (Jiangsu University) were printed on hydrogel films and the size of the letter was controlled to 25.66×17.00 mm. Finally, the hydrogel film was dried with excess water being evaporated in an oven at 50 ˚C for 6 h.

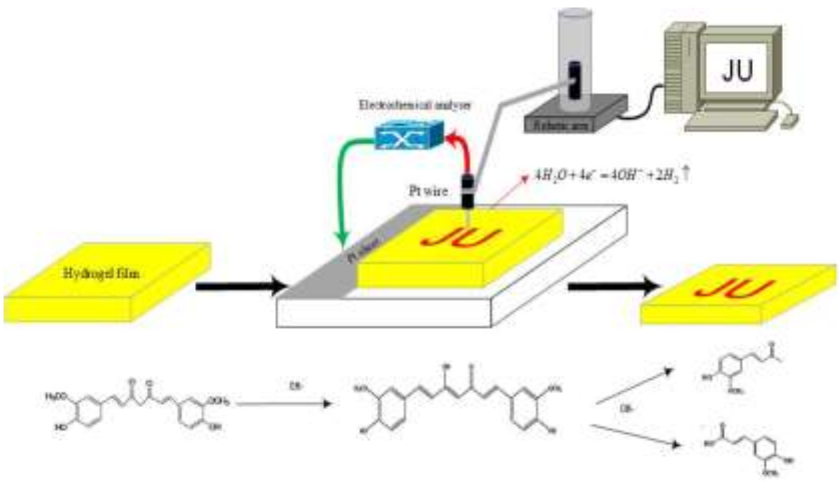

Fig. 1. Schematic diagram of electrochemical printing

2.5 Characteristics of the hydrogel films

2.5.1 Morphology and structure of hydrogel films

Fourier transform infrared (FT-IR) spectra of the hydrogel films with different curcumin concentrations were carried out on a Nicolet 50 infrared spectrometer (Boston, USA). The spectra were acquired at a resolution 4 cm^{-1} in the range of 4000 and 650 cm^{-1} with the ATR mode and three scans. Then, the cross-section of hydrogel films was examined by a scanning electron microscopy (SEM, JSM-7800F, Japan electronics, Japan). Prior to analysis, the samples were dried, and then attached onto the slide of aluminum stubs. All of the samples were coated with a thin layer of gold under vacuum at an accelerating voltage of 15 kV.

2.5.2 The physical characteristics of hydrogel films.

The thickness of the hydrogel films was recorded by a digital micrometer (Sanfeng Group Co., Taiwan, China). The tensile strength (TS) and elongation at break (EB) were measured using a universal texturometer (Model 4500, Instron Corporation, Canton, MA, USA) according to the method of ASTM D882-00. Prior to analysis analysis, each film was cut into 60 mm length and 20 mm width. The crosshead speed was set to 0.06 mm/s and the initial grip separation was set to 40 mm with 150 kg of a load cell.

The water content (WC) of hydrogel films was measured by moisture drying method at 105 ˚C according to the following equation:

$$WC\ (\%) = 100 \times (M_i - M_f) / M_i \quad (1)$$

Where M_i was the initial weight of hydrogel (g) and M_f was the final weight of hydrogel at 105 ˚C (g).

2.5.3 The stability of curcumin film and the electro-writing with letter "JU"

The hydrogel films were stored in an incubator at 4 and 25 ˚C with 75% relative humidity (RH). The color of films was determined by using a Scanjet G4050 optical scanner (HP, China) for two weeks. The stability of the curcumin film and the imprinted characters "JU" were both defined as the color change based on the following equations: Lab model is a kind of color pattern published by the International Commission on illumination (CIE) in 1976. Lab mode is also composed of three channels. The value of L* is the lightness, a* is red to green, and b* is yellow to blue.

$$\Delta E = \sqrt{(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2} \quad (2)$$

Where $\Delta L^*=L^*- L_0^*$; $\Delta a^*=a^*- a_0^*$; $\Delta b^*=b^*- b_0^*$; L_0^*, a_0^* and b_0^* were the initial color values of the films, L^*, a^* and b^* were the color after storage.

2.6 The sensitivity of hydrogel films to ammonia.

The response of the hydrogel films to ammonia was determined according to the method described by Kuswandia (Kuswandia et al., 2012) with slight modification. The hydrogel films were cut into squares then sealed on the conical flask (500 mL) by a rubber band containing ammonia for 24 minutes at room temperature. The concentration of the ammonia was 100 mmol/L. An image was captured by the camera which fixed on the top of the light box (50×50×50 cm) every 2 minutes (Fig. S1). On both sides of the light box, there are two fluorescent lights with a fixed position, incident angle and intensity kept (details in the Supplementary material). Then the R, G, B of the picture of films were obtained by using the MATLAB (Version 2013, Math Works, USA). The response sensitivity (*S*) of the hydrogel films was calculated according to the following equations (Huang et al., 2015; Zhai et al., 2018):

$$\Delta R = |R_a - R_b|$$

$$\Delta G = |G_a - G_b|$$

$$\Delta B = |B_a - B_b| \qquad (3)$$

$$S = \frac{\Delta R + \Delta G + \Delta B}{R_b + G_b + B_b} \times 100\%$$

where R_a, G_a, B_a were the initial values of the red, green and blue, R_b, G_b, B_b were the color of detection under ammonia

2.7 The application test of hydrogel films for shrimp

2.7.1 Gas chromatography-mass spectrometer (GC-MS) analysis

Before the GC-MS analysis, the shrimp samples were homogenized into mince and then pretreated by Solid-phase micro-extraction (SPME). The samples of 6 g minced shrimp were put into the extraction bottle (15 mL). A screw cup and silicone septum were on the extraction bottle to make it airtight (ANPEL Laboratory Technologies Inc., Shanghai, China). The samples were equilibrated for 10 minutes at 60 °C. Then the volatiles were extracted for 20 minutes at 60 °C onto the SPME fiber assembly (50/30 μm DVB/CAR/PDMS, 1 cm, gray; ANPEL Laboratory Technologies (Shanghai) Inc.).

These volatile nitrogenous compounds were detected using the Trace Ultra ITQ1100 GC–MS system (Thermo Scientific, Waltham, MA). After extraction, the fiber desorbed the splitless into the GC injector at 250 ˚C for 5 minutes. The separation was carried out by using a column Agilent DB-WAX (60 m length×0.25 mm I.D.×0.25 um film thicknesses; Agilent Technologies, Folsom, CA, USA) with a gas Helium flow rate of 1 mL/min. The initial temperature program was set at 40 ˚C with 4 minutes and then warmed to 100 ˚C with 10 ˚C/min, finally followed by a ramp of 6 ˚C/min to 220 ˚C and then held for 3 minutes. The MS spectrometer was set at -70 eV electron energy with ion source setting at 230 ˚C by the Agilent 5973 MSD. The temperature for quadrupole was set at 200 ˚C. The identification of the volatile nitrogenous compounds was compared to the mass spectra with National Institute of Standards and Technology (NIST) library (Zhai et al., 2019).

2.7.2 The shrimp spoilage trial

The hydrogel film indicator was used to evaluate the freshness of the shrimp. Firstly, 50 g of shrimp samples were placed into a crisper with a CAP2 film indicator fixing on it. The size of each hydrogel film has a diameter of 40 mm. The crisper box was placed at 4 ˚C and 75% RH. The film was captured every 12 hours for three days with a CR-400 portable Chromameter (KONICA MINOLTA, Japan). The TVB-N of shrimp was measured according to the method described by the semi-micro *Kjeldahl* method (Zhang et al., 2019).

2.8 Statistical analysis

All the analyses were performed in triplicate independent experiments and reported as average ± std. Mean differences on a completely randomized design were performed with the analysis of variance (ANOVA) procedure in Statistic Package for Social Science (SPSS) software (Version 21, SPSS Inc) followed by Duncan's multiple range test for mean comparison. The significance was defined to be at a P value of less than 5%.

3. Results and discussion

3.1 UV-vis spectra and color of curcumin

The color of curcumin and UV-vis spectra are shown in Fig. 2. As can be seen, the Fig. 2A demonstrates a noticeable change of curcumin color from light yellow to orange-red as the pH values increased from 3.0 to 11.0. Curcumin was light yellow when the pH was less than 5.0, yellow-orange at pH 6.0-7.0, and reddish-orange at pH 10.0-11.0. The changes assigned to the phenolic compounds and unsaturated bonds structure of curcumin (Rupesh Kumar et al., 2011). Thus, Fig. 2B shows that the absorption peak of curcumin was at about 448 nm (pH<5). With increasing pH, the maximum absorption peak shifts to 462 nm and another new absorption peak produced at 388 nm in the range of pH 6.0-9.0. Similar pH-sensitive color changes of curcumin were observed by Liu et al., 2018. Under acidic conditions, the variation of curcumin peak location depended on its chemical structure. It has two distinct structures, one exists in an enol form the other presence of the diketone (Fig. 2C). As alkalinity further increasing, the intramolecular charge transferred from the phenyl ring towards the carbonyl and changed the moiety diketone structure of curcumin (Zsila et al., 2003). The electronegativity was enhanced to produce a dark effect, causing the changes in maximum absorption wavelength and intensity (Hazzah et al., 2015). Moreover, a significant change in the spectra was observed in the range of 10.0-11.0, showing two new absorption peaks at 336 nm and 464 nm. These new peaks were probably caused by the degradation of curcumin to ferulic acid and its methane forms (Fig. 2D).

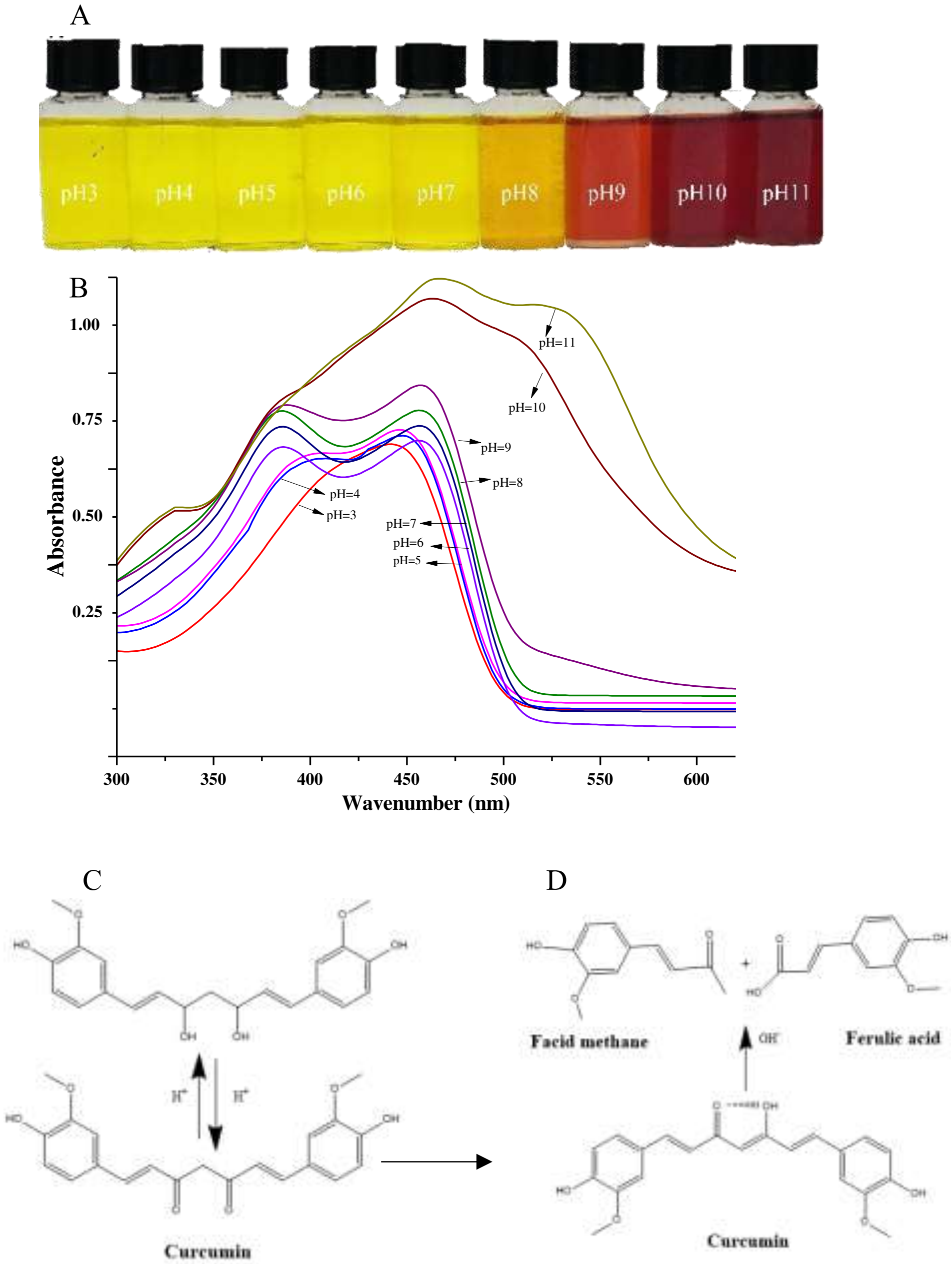

Fig. 2. (A) Colors, and (B) UV–vis spectra of curcumin solutions at pH 3–11; (C) structure of curcumin in acidic conditions (D) degrades of curcumin in alkaline conditions

3.2 Characterization of the hydrogel films

3.2.1 Morphology and structure of films

Fig. 3 illustrates the FT-IR spectra of curcumin powders, AP, CAP1, CAP2, and CAP3 films. The peaks of the curcumin spectrum at 3505 cm^{-1}, 1620 cm^{-1} and 1601 cm^{-1} were ascribed to the free vibration of -OH phenolic stretching, C=O stretching and C–H bending bound to methyl groups, respectively (Mohan et al., 2012; Mangolim et al., 2014). The bands located at 1501 cm^{-1} and 1280 cm^{-1} corresponding to stretching vibrations of C=C and C=O of aromatic rings (Silva-Pereira et al., 2015). The bands presented at 1207 cm^{-1}, 1426 cm^{-1} and 1028 cm^{-1} were assigned to stretching vibrations of C-C, C-O and C-O-C bending (Zhou and Tang, 2016). The spectra of hydrogel films were characterized in the dehydrated state of the hydrogel film. A shift in the spectral region with an increased intensity of 1043 cm^{-1} was described to a C-C stretching mode of PVA crystallinity. The intense peaks at 1647 cm^{-1} and 1417 cm^{-1} were associated with C=C stretching of the phenyl ring. Furthermore, the peaks around the -OH stretching vibration at 3365 cm^{-1} moved to 3358 cm^{-1}, 3347 cm^{-1} and 3342 cm^{-1} with the addition of curcumin from 0, 1, 2, and 3% of the AP, respectively. Also, the intensity decreased with the curcumin solution increasing. These results were probably assigned to the hydrogen bonding interaction between the agar/PVA matrix and curcumin. Curcumin is a hydrophobic active component and the addition of curcumin weakened the hydrogen bonding interaction (Wang et al., 2016).

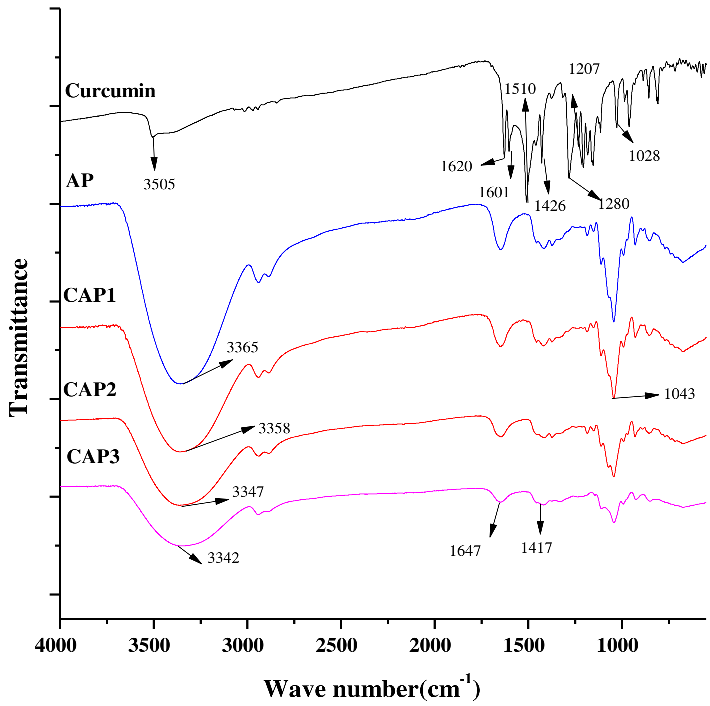

Fig. 3. FTIR of curcumin and hydrogel films with Curcumin contents at: 0%, 1%, 2%, 3%.

The cross-section of AP, CAP1, CAP2 and CAP3 films by SEM are shown in Fig. 4. The AP film exhibited excellent compatibility which has a smooth and flat surface. The results indicated that film-forming materials have no apparent phase separation as shown in Fig. 4A. After the addition of curcumin, the micro-phase of CAP1 film was well distributed. However, the curcumin crystal distribution in the surface of CAP2 film deteriorated and the structure was not as smooth as AP and CAP1. The structure showed typical irregularities associated with the hemimorphic crystals form (Cano et al., 2015). The curcumin crystals became more poorly distributed and precipitated with the increasing concentration of curcumin. The amorphous phases of CAP3 film showed the least favorable structure with evident agglomeration as illustrated in Fig. 4D. Meanwhile, the whole cross-section presented irregularities in a crystalline structure which was caused by decreasing solubility promoting crystal formation. As a result, low curcumin content may be well-distributed in the film-forming materials, but higher curcumin content is related to a high degree of crystallinity. The phenomenon was consistent with the observation made by Liu et al。

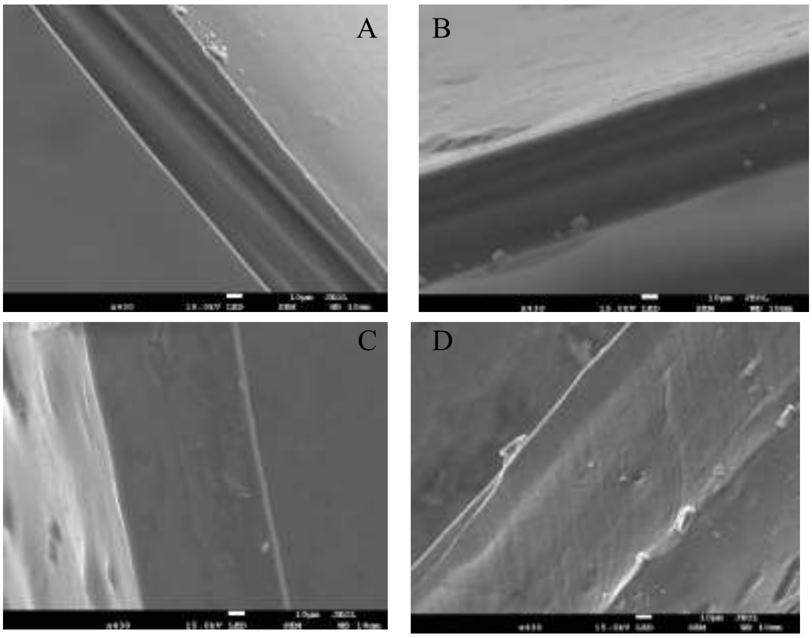

Fig. 4. SEM images of hydrogel films: AP (A), CAP1 (B), CAP2 (C), and CAP3 (D)

3.2.2 The physical characteristics of hydrogel films

The thickness, TS, EB and WC of hydrogel films are shown in Table S1. The results obviously indicate that the thickness of the hydrogel films slightly increases with the addition of curcumin content. The increasing content of curcumin changed the interior structure of the hydrogel-forming matrix and the increasing in the spatial distance between curcumin and the polymer film-forming material (Liu et al., 2016). WC was investigated to evaluate the water resistance of the packaging films. The WC of CAP film decreased significantly compared to the control film ($p< 0.05$), of which values decreased from 42.99% to 40.73%, 27,25% and 12.20% with the concentration of curcumin from 0% to 3% of AP, respectively. The altered WC of CAP film may have been caused by the hydrophobicity of curcumin. In addition, the molecular interaction between the base materials and the curcumin of hydroxyl groups was a possible barrier to moisture diffusion through the CAP films (Musso et al., 2016). Generally, the addition of active compounds can decrease the TS of films because of the weakened interaction between film-forming materials and curcumin (small molecule compound) (Noronha et al., 2014). However, the TS of CPA3 film was higher than the CAP1 film because of film discontinuities, but it was still lower than the AP (control film). It can also be seen that the EB of hydrogel films increases from 47.02% to 62.91%, 66.52% and 68.69% with the concentration of curcumin from 0% to 3% of AP, respectively. Therefore, these behaviors of films were due to the following two reasons: the curcumin into the agar/PVA matrix increased the polymer mobility and hydrogen bond interaction (Luo et al., 2012).

Table S1 Summary of the mechanical properties of CAP films.

Samples	Film thickness (mm)	TS (MPa)	EB (%)	WC (%)
AP	0.087 ± 0.007[b]	7.54 ± 0.54[a]	47.02 ± 0.83[c]	42.99 ± 2.61[a]
CAP1	0.093 ± 0.002[b]	4.38 ± 0.08[b]	62.91 ± 1.42[b]	40.73 ± 1.28[a]
CAP2	0.104 ± 0.001[a]	5.22 ± 0.49[b]	66.52 ± 1.19[a]	27.25 ± 2.84[b]
CAP3	0.106 ± 0.001[a]	6.94 ± 0.14[a]	68.69 ± 0.50[a]	12.20 ± 1.28[c]

Notes: data with the same superscript letter in the same column indicate that statistically different ($p < 0.05$). The data (mean ± standard) result from three replicates. Where: TS: tensile strength; EB: elongation at break; WC: water content

3.2.3 The stability of hydrogel films

The color stability of curcumin films and the imprinted characters "JU" was a crucial role of the color performance in relation to freshness detection. The color variations of curcumin films and the characters "JU" are shown in Fig. 5. Compared to the color of imprinted characters, the curcumin films showed a higher stability. Generally, the imprint character region was produced due to the OH^- at the cathode (Electrochemical Writing) and then the phenol hydroxyl groups easily converted to a phenolic oxygen anion of curcumin. Besides that, the localized curcumin was more easily oxidized and reduced the stability of the imprint characters (Zhai et al., 2018). Fig. 5A shows that the curcumin films stored at 4 ˚C had lower ΔE values which was 3.93 at the 14th day, indicating that they had more excellent color stability. By contrast, the color of the curcumin films changed for two weeks at a higher temperature (25 ˚C). The more inferior stability of curcumin was due to oxidization reaction at a higher temperature (Ma et al., 2017b). The difference was also recorded by the color stability between the different content of curcumin in the hydrogel films. It can be seen that the color stabilities of the curcumin films and the imprint character “JU” both improved with the increase of curcumin content. The results indicated that the hydrogel films with more curcumin content possessing higher color stabilities at 4 ˚C. The hydrophobic of the curcumin modified the retention of active ingredients in hydrogel films. Hence, the phenolic hydroxyl group of hydrogel films with high curcumin concentration could not be readily formed into phenoxide anion (Musso et al., 2017). However, there was no similar pattern found at 25 ˚C. The irregular change of Δ*E* values was related to the accelerated polymer mobility of curcumin at a higher temperature (Kuorwel et al., 2013). The instability of indicators at high temperature has been shown as the previous studies (Zhang et al., 2019;Huang et al., 2019; Mohammadalinejhad et al., 2020).

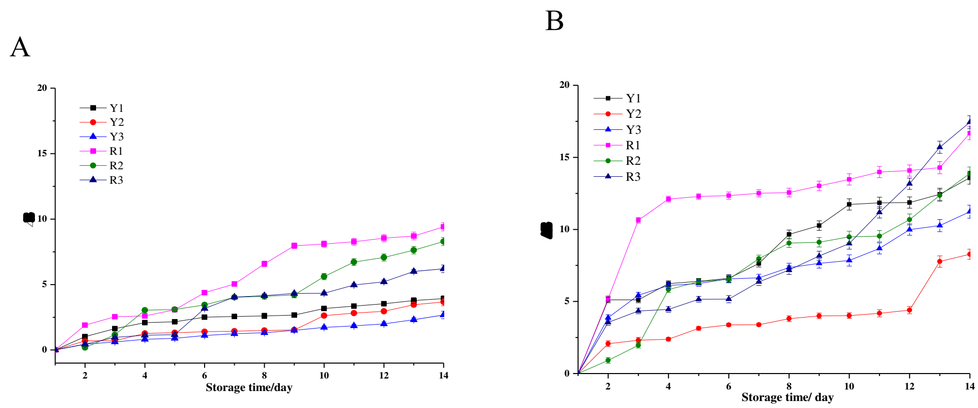

Fig. 5. The relative color change (ΔE values) of the hydrogels stored at (A) 4˚C, (B) 25 ˚C for 14 days;

Where: Y1: CAP1; Y2: CAP2; Y3: CAP3; R1: Red letter of CAP1; R2: Red letter of CAP2; R3: Red letter of CAP3

3.2.4 Sensitivity of hydrogel films to ammonia

In order to evaluate the sensor function of the hydrogel films, the curcumin films were exposed in an ammonia environment. As shown in Fig. 6, the curcumin films presented visible response sensitivity to ammonia at different response times. However, the character imprint “JU” had small response sensitivity within 24 minutes. Curcumin showed a strong redness-shift under the alkaline condition, the redness color was enhanced with the increase of reaction time. The decomposition of curcumin was accelerated in the alkaline concentration. Meanwhile, the phenoxide anion was easily formed and produced the color changes (Ma et al., 2017a). It can be seen that the *S* values of films improved with the increase of curcumin content. According to the sensitivity test to ammonia, the curcumin film was used as a sensor for shrimp freshness and the imprint character was used as a reference.

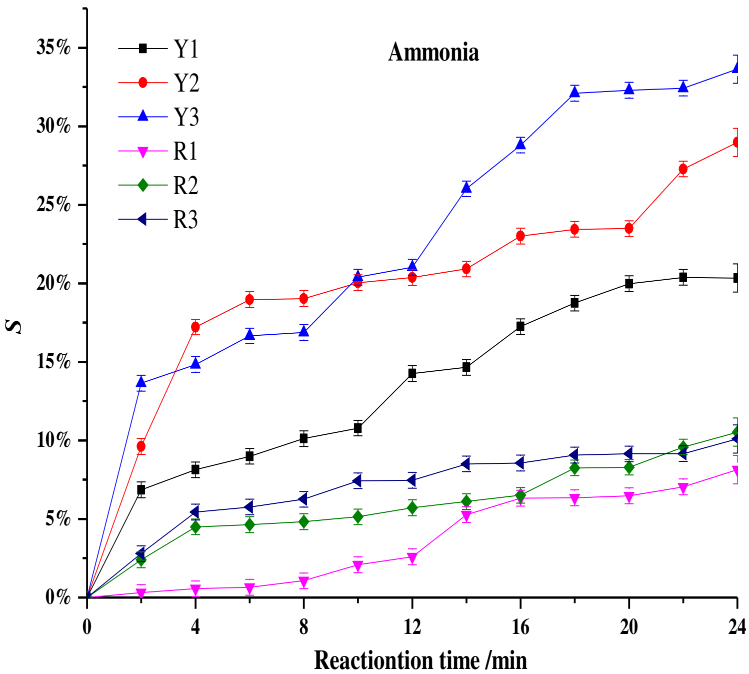

Fig. 6. The color response of the curcumin film and the letter “JU” toward ammonia gas.

3.3 Application of hydrogel films to shrimp

The CAP2 hydrogel film was employed to monitor the shrimp freshness due to its excellent mechanical properties and higher color variation rate. The curcumin film presented visible *S* values while the imprint character did not show significantly change in the ammonia environment. Therefore, the curcumin film was used as a sensor for shrimp freshness and the imprinted character was used as a reference. As shown in Fig. 7A, the curcumin film gradually changed from yellow to orange-red with the decline of shrimp freshness. By SPME/GC-MS analysis, the Table S2 shows that volatile compounds contents of the freshness sample have a little amount. Only six compounds were produced in the first day of the *Macrobrachium Rosenbergii* and there were no decomposition odors in the freshness sample. While as shown in Table S3, abundant compounds approximately eighteen were identified in the spoiled sample after the storage time of shrimp. And the results indicated that the most obvious compounds were trimethylamine, 2-Octanol and phenol in a spoiled sample. The results were corresponding to the detections of the TVB-N levels (Zhang et al., 2019). The TVB-N values of shrimp increased from 8.7 to 18.6 mg/100 g at 24 h. According to the Chinese standard GB2733-2015, the limit of TVB-N level in shrimp is 20 mg/100 g. The results indicated that the shrimp could be consumed on the first day. Meanwhile, the ΔE value was 13.6. The TVB-N level was 31.6 mg/100 g and the ΔE value of the curcumin film increased to 19.8, suggesting that the shrimp was not fresh at 36 h. However, at this point, the sensory evaluation of shrimp was still acceptable to the consumers. And ΔE values was greater than 12, imply the color belongs to different space. Thus, the color of pH indicator were more sensitive to sensory evaluation of the shrimp. Then the shrimp sample had a deeper putrefaction with the increasing storage time. The curcumin film showed an obvious color changes which the ΔE values were increased to 36.5 at 60 h. Correspondingly, TVB-N levels of shrimp were 56.8 mg/100 g. The color of the film induced by the increasing TVB-N levels and the correlation were also established between the TVB-N level and the ΔE value:

$$y = -0.0066x^2 + 1.1107x - 6.4409 \qquad R^2 = 0.9731 \qquad (4)$$

The positive correlation was observed between the changed color and the TVB-N level with a coefficient of 0.97. Maybe this low robustness of data could be improved in future studies by using some more data analysis. The correlation was responding to the Δ*E* value and TVB-N level of packaging and then providing information of shrimp real-time status for consumers.

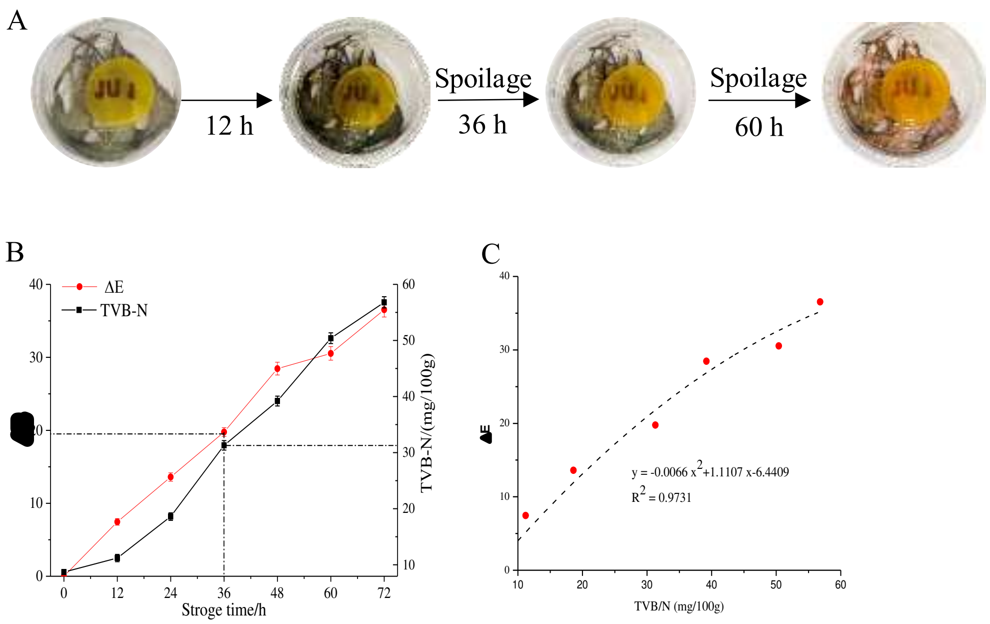

Fig. 7. (A) Images of CAP2 film with red character "JU" of the shrimp from fresh to spoilage; (B) Images of CAP2 film with red characters imprint of the shrimp from fresh to spoilage; (C) The correlation between TVB-N of the shrimp and ΔE values of the curcumin film

Table S2 The GC-MS analysis results of freshness shrimp volatile compound

Number	Retention time	Compounds	Area%
1	6.97	Hexane	1.97%
2	9.86	Cyclotrisiloxane	8.23%
3	13.38	Cyclotetrasiloxane	4.11%
4	15.54	Toluene	3.43%
5	18.07	Cyclopentasiloxane	4.54%
6	27.63	Trimethylsilyl	1.02%
7	30.02	Cyanocyclohexene	2.26%
8	31.83	Indole	31.87%
9	31.87	Indole	27.92%
10	32.55	Oxime-, methoxy-phenyl	14.65%

Table S3 The GC-MS analysis results of spoilage volatile compound

Number	Retention time	Compounds	Area%
1	6.77	Trimethylamine	6.34%
2	7.4	Methanethiol	1.67%
3	8.21	Dimethyl sulfide	0.39%
4	9.26	Acetone	1.86%
5	11.76	Cyclopentasiloxane	0.40%
6	11.96	Methylene Chloride	0.20%
7	12.79	Heptane	0.24%
8	15.51	Toluene	0.75%
9	15.76	Ethanethioic acid,	1.97%
10	16.63	sulfuretted hydrogen	3.46%
11	18.05	Cyclopentasiloxane	2.10%
12	20.52	1-Butanol, 3-methyl	2.13%
13	21.69	Cyclohexasiloxane	2.46%
14	25.05	2-Octanol	44.40%
15	25.57	2-Nonanone	0.29%
16	25.81	Dimethyl trisulfide	0.89%
17	26.81	Acetic acid	1.73%
18	34.21	Cyclohexasiloxane	1.97%
19	37.82	Phenol	26.74%

4. Conclusion

In this study, the visual indicator was successfully developed and then provided an effective non-destructive means for shrimp freshness. The characters "JU" were successfully printed on a hydrogel film by using an electrochemical analyzer. The SEM images revealed that at lower content, curcumin was well-distributed in the film-forming materials. The color stability results indicated that the curcumin films possessed higher color stability within 14 days which ΔE values was 3.93% at 4 °C. And the response sensitivity of curcumin film showed visible color changes in the presence of ammonia. Finally, the changed color from yellow to red/orange of curcumin film was easily recognizable by the naked eyes with the deterioration process of the shrimp. Also a positive correlation was established between the TVB-N of shrimp and the ΔE value with a coefficient of 0.97. Future studies should focus on the imprint characters of the hydrogel film that can be used as a sensor under an acidic environment (i.e. red back to yellow) and curcumin as a reference to explore a new multifunctional intelligent packaging in food packaging systems. These intelligent systems will help improve food safety and shelf life for consumers and producers.

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Declarations of interest

All authors declare that they have no conflicts of interest.

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