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Extruded low density polyethylene-curcumin film: a hydrophobic ammonia sensor for intelligent food packaging

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Abstract

A hydrophobic film was developed using low density polyethylene (LDPE) and curcumin with the melting extrusion method. The hydrophobic nature of the LDPE endowed the film with good stability to pH buffer solutions, namely without obvious color change or curcumin leaching, when immersed in pH buffer solutions. The LDPE-curcumin composite film was sensitive to ammonia (NH_3), and the limit of detection (LOD) of LDPE-curcumin film to NH_3 at 90% RH was 0.18 μM. When the LDPE-curcumin film was used to monitor beef and silver carp spoilage at 4 °C, it showed light yellow-to-light brown color changes along with the storage time, resulting from the increasing TVB-N contents of the meat samples. As the developed LDPE-curcumin film was totally non-toxic and suitable for industrial production in a large scale, there will be a good potential for its application in intelligent food packaging.

Keywords

Hydrophobic film; low density polyethylene; curcumin; meat spoilage; intelligent packaging

1. Introduction

Intelligent food packaging has received great attention in modern food industry. It particularly aims to *in-situ* and real-time monitor the food quality in individual packages for food quality assessment and safety assurance. To date, various colorimetric sensors (Lin, et al., 2016; Long, et al., 2019; Zhai, et al., 2019) and indicators (Saliu & Della Pergola, 2018; Wang, Lu, & Gunasekaran, 2017; C. Zhang, et al., 2013), and wireless electronic sensors (Koskela, et al., 2015; Z. Ma, et al., 2018; Zhu, Desroches, Yoon, & Swager, 2017) have been developed for intelligent packaging, with advantages of good portability, low cost, and easy fabrication. Among them, colorimetric sensors have been widely studied, because their color changes can be directly assessed by naked eye or using digital imaging technology.

Meat spoilage during distribution can easily occur, due to enzymatic reaction and microbial contamination. Total volatile basic amines (TVB-N), such as trimethylamines, dimethylamines and ammonia, are widely regarded as a meat spoilage indicator. To achieve the real-time evaluation of meat spoilage, a number of studies have tried to use synthetic pH-sensitive dyes to develop the TVB-N sensors in intelligent packaging systems (Chun, Kim, & Shin, 2014; Domínguez-Aragón, Olmedo-Martínez, & Zaragoza-Contreras, 2018; Mo, et al., 2017; A. Pacquit, et al., 2006). Traditionally, considering of the potential toxicity of the synthetic dyes, pH-sensitive dyes are generally encapsulated into a polymer and then covered by a gas-permeable and ion-impermeable membrane (GPM) to prevent the leaching of synthetic dyes from the films to the humid food packaging environment (Kuswandi, et al., 2012; K. Lee, Baek, Kim, & Seo, 2019; Wells, Yusufu, & Mills, 2019). Of late, chemical covalent cross-linking (Jia, et al., 2019) and melting extrusion methods (Wells, et al., 2019) have also been proposed to embed the pH-sensitive dyes into solid films and simultaneously prevent the leaching of dyes, which provided new approaches to design colorimetric films for intelligent food packaging.

Although the synthetic pH-sensitive dyes showed good performance in developing meat quality indicators, it is still highly desirable to develop totally safe colorimetric films (S. J. Lee & Rahman, 2014). In recent years, natural edible pigments, such as anthocyanins (Dudnyk, Janeček, Vaucher-Joset, & Stellacci, 2018; Q. Ma & Wang, 2016; Zhai, et al., 2018; J. Zhang, et al., 2019), alizarin (Ezati, Tajik, & Moradi, 2019), betalains (Qin, Liu, Zhang, & Liu, 2020) and curcumin (Kuswandi, Jayus, Larasati, Abdullah, & Heng, 2011; Liu, et al., 2018; Q. Ma, Du, & Wang, 2017), have been used to develop pH-sensitive films to monitor meat quality. These pigments are generally embedded in hydrophilic polymer films, such as chitosan (X. Zhang, Lu, & Chen, 2014), gelatin (Chayavanich, Thiraphibundet, & Imyim, 2019), κ-carrageenan (Liu, et al., 2019), polyvinyl alcohol (Qin, et al., 2020) and so forth. However, the big concern is that the package with fresh meat has high relative humidity (RH), so that the hydrophilic polymer film could inevitably absorb water from the internal package environment, which induced the leaching of pigments from the film (Liu, et al., 2019; Liu, et al., 2018; Q. Ma, et al., 2017; Zhai, et al., 2020). Even though the leaching of natural pigments would not lead to food safety issues, it should have great effect on the indicating property of the film due to the unpredictable fluctuation of pigment content in the film caused by leaching (S. Huang, et al., 2019; Q. Ma, et al., 2017; Zhai, et al., 2017). Furthermore, the sensing ability of the hydrophilic films to volatile gases may be susceptible to the humidity of the package environment (Zhai, et al., 2020). Hence, there is an urgent need to reduce the leaching of the natural pigments and the effect of humidity on the sensing ability of the film.

To meet this requirement, in this study, we tried to develop a hydrophobic colorimetric film by incorporating natural pigment curcumin in LDPE using the melting extrusion method. On one hand, curcumin is a low molecular-weight phenolic compound obtainable from the rhizomes of turmeric (*Curcuma longa Linn.*), and has been widely used as a food coloring agent owing to its intense yellow color (Xiang, Sun-Waterhouse, Cui, Wang, & Dong, 2018). On the other hand, LDPE is a nontoxic polymer and LDPE films have been widely used in food packaging due to its good mechanical and water barrier properties. A recent study has shown the feasibility of developing LDPE-curcumin films by using the melting extrusion method (Zia, Paul, Heredia-Guerrero, Athanassiou, & Fragouli, 2019). However, to our best knowledge, the indicating property of the LDPE-curcumin film, as a colorimetric gas sensor, for intelligent food packaging has not been reported yet. The experimental results showed that the LDPE-curcumin film could prevent the leaching of curcumin in buffer solutions. Meanwhile, the film was sensitive to NH_3 and could exhibit visible color changes when used to monitor meat spoilage. As the LDPE-curcumin film is nontoxic, cost effective and fabricated by using the melting extrusion method (Fig. S1), a classical polymer technological process for large scale industrial production, it presented a promising method to develop natural pigment based colorimetric films for intelligent packaging.

2. Materials and methods

2.1. Materials and reagents

Fresh beef and live silver carp were bought from a local market (Zhenjiang, China). LDPE powder was purchased from Shanghai Jiexun Plasticizing Co., Ltd. (Shanghai, China). Curcumin, magnesium chloride, magnesium nitrate, sodium chloride, potassium sulphate, citric acid, sodium citrate, hydrochloric acid, sodium hydroxide, methyl red, methylene blue, and boric acid were purchased from Sinopharm Chemical Reagent Co., Ltd (Shanghai, China). Gaseous NH_3 was purchased from Thorpe Chemical Co., Ltd (Zhenjiang, China).

2.2. Preparation of the LDPE-curcumin film

The preparation of LDPE-curcumin film was according to a previous literature with slight modification (Zia, et al., 2019). First, 1 g of curcumin powder and 100 g of LDPE powder were mixed by mechanical stirring. The curcumin-covered LDPE powder was then pelletised using a twinscrew extruder (Baopin International Precision Instruments Co., Ltd.) with screws of 20 mm diameter, and length to diameter ratio of 40. The operating temperatures were set at 90, 110, 120, 130 and 130 °C for the feeding, heating zones 1-3 and pelletising die, respectively. The extruder screw speed was 80 rpm throughout the extrusion process. The obtained LDPE-curcumin pellets were then extruded into a thin, clear yellow-colored plastic film with operating temperatures of 130 °C, using a single-screw extruder (Baopin International Precision Instruments Co., Ltd.) with a screw of 20 mm diameter and length to diameter ratio of 28. The thickness of the obtained film was ~ 50 μm. The control LDPE film without curcumin was also prepared following the same procedures.

2.3. Response to pH buffer solutions

The pH buffer solutions in the range of 2-12 were prepared by using 0.2 M disodium hydrogen phosphate, 0.2 M citric acid and 0.2 M sodium hydroxide solutions with different proportions. Curcumin powder was added to the prepared pH buffer solutions with the addition of 20% ethanol as curcumin has an extremely low solubility. Then, the UV-Vis spectra of curcumin solutions were recorded. The release of curcumin from the film to buffer solutions was investigated by immersing 4 g of LDPE-curcumin film in 100 mL of the buffer solution at 25 °C. The film was taken out from the buffer solution after every 24 h, and the UV-Vis spectra of the solutions were recorded.

2.4. Response to NH_3 under different RH

First, the LDPE-curcumin film (30 × 10 mm) was adhered on the inner surface of the cuvette. The cuvettes were put in desiccators with silica gels, NaBr saturated solution and Na_2HPO_4 saturated solution for 24 h at 25 °C. The actual RH in the desiccators were determined to be 6% (silica gels), 55% (NaBr saturated solution), and 95% (Na_2HPO_4 saturated solution), by using a commercial hygrometer (AR837, SMART SENSOR). The cuvettes were sealed with rubber caps before they were taken out from the desiccators. A certain amount of NH_3 was injected into the cuvettes by using needle syringes. Here, the concentration of NH_3 was determined by trapping NH_3 with 10 mL 2% aqueous solution of boric acid and 3 droplets of mixed indicator produced from dissolution of 0.2 g of methyl red and 0.1 g of methylene blue to 100 mL of ethanol. After that, the boric acid solution was titrated with a 0.01 M hydrochloric acid solution, and NH_3 was quantified by the hydrochloric acid used.

The reversibility of the film when reacting with NH_3 was also investigated (Khattab, Dacrory, Abou-Yousef, & Kamel, 2019). The film was first reacted with 80 μM NH_3 under 90% RH for 30 min, and then was put in air for 1 h at 25 °C. The UV-vis spectra of the film before reacting with NH_3, after reacting with NH_3, and after recovering in air were detected. This process was repeated for 5 times.

2.5. Stability test of the film at different temperatures

The film (30 × 10 mm) was adhered on the inner surface of the cuvette. The cuvette was placed in a constant temperature and humidity incubator with a fluorescent lamp. The absorbance at 420 nm of the LDPE-curcumin film was recorded by using the UV-Vis spectrometer.

2.6. Application of the film in monitoring meat spoilage

Live silver carp was pretreated by removing its innards, head, tail and feathers. Then, it was cleaned by water and diced. A quantity of 500 g of diced fresh beef and silver carp were put into a polyethylene terephthalate (PET) box with a buttoned lid. The dimensions of the box is 165 × 115 × 43 mm (L × W × H). The LDPE-curcumin film was cut to square (20 × 20 mm) and was put in the headspace of the box. The experimental setup was according a previous studies (Chun, et al., 2014; Alexis Pacquit, et al., 2007). As the LDPE-curcumin film is transparent, which is not good for *in-situ* color measurement, it was put on a white cellulose acetate filter paper (25 × 25 mm) that served as a background. Then, the filter paper was tightly adhered onto the internal surface of the PET lid by using adhesive tape (see Fig. S4). The above processes were conducted in a clean bench. Finally, the PET boxes were placed in an incubator at 4 °C in a refrigerator. The color parameters of the films were detected by using a portable colorimeter (CM-2300d, Konica Minolta, Inc., Japan). The chromatic parameters of ΔE and ΔC* were calculated according to following equations:

$$\Delta E = \sqrt{(L_t^* - L_0^*)^2 + (a_t^* - a_0^*)^2 + (b_t^* - b_0^*)^2} \quad (1)$$

$$\Delta C^* = \sqrt{(a_t^* - a_0^*)^2 + (b_t^* - b_0^*)^2} \quad (2)$$

where L_t*, a_t* and b_t* were the color parameters of the LDPE-curcumin film after a certain storage time, and L_0*, a_0* and b_0* were the initial color parameters of the LDPE-curcumin film.

The total volatile basic nitrogen (TVB-N) content of meat samples was measured according to a previous literature (Cai, Chen, Wan, & Zhao, 2011), following the

Chinese Standard (GB 5009. 228-2016). Briefly, 20 g of meat sample was put in a beaker with 100 mL of distilled water, and then crushed by using a tissue homogenizer. The homogenate was filtered with filter papers to obtain the filtrate. Then, 10 mL of filtrate was transferred to a Kjeldahl distillation unit. The volatile biogenic amines were distilled, and the distillate was collected with 10 mL of boric acid solution (20 g/L) containing 5 droplets of mixed indicator that was made by dissolving 0.2 g of methyl red and 0.1 g methylene blue in 300 mL ethanol. Finally, the distillate was titrated with 0.01 M HCl solution. The TVB-N content was calculated according to the amount of HCl used during titration, and expressed as mg/100 g.

The total viable counts (TVC) of meat samples were measured using the Plate Count Agar (Mehdizadeh, Tajik, Langroodi, Molaei, & Mahmoudian, 2020). Meat samples (25 g) were added to 225 mL of phosphate buffer solution (PBS), and then homogenized by using a homogenizer (IKA, Germany). The homogeneous solution was then serially diluted at a ratio of ten (V/V) by using the PBS. 1 mL of the diluted solution was spread on the surface of the plate count agar medium in Petri dishes, which were further incubated at 35 °C for 2 days. The bacterial counts were expressed as colony-forming units (CFU) per gram of meat sample and then transformed to base 10 logarithm values, namely $\log_{10}$(CFU/g).

2.7. Statistical analysis

All the experiments were conducted in triplicate and the data was expressed as means ± standard deviation.

3. Result and discussion

3.1. Stability of curcumin during extrusion process

In this work, curcumin was embedded into LDPE through extruding method, during which LDPE powder was heated (≤ 130 °C) to molten state and to mix with curcumin. Therefore, the stability of curcumin under heating should be investigated. The curcumin powder was first heated in oven in air, and then exposed to NH_3. As shown in Fig. 1, the curcumin powder did not show obvious color changes after 1 h heating at 130 °C, but exhibited yellow-to-brown color changes when subsequently reacted with NH_3.

This result indicated that the curcumin powder maintained its sensitivity to NH_3 after being heated under 130 °C for 1 h in air. However, it presented yellow-to-dark brown color change when heated at 180 °C for 1 h, while did not show obvious color changes exposing to NH_3. This can be due to that the curcumin powder was intensely oxidized and decomposed to ferulic acid and 4-vinyl guaiacol under 180 °C (Esatbeyoglu, Ulbrich, Rehberg, Rohn, & Rimbach, 2015), and therefore lost the reactivity with NH_3. Hence, the melting temperature (≤ 130 °C) during the extruding process would not significantly induce the oxidative decomposition of curcumin. In addition, the pelletizing process was conducted in vacuum condition, which can prevent curcumin from being oxidized. As shown in Fig. 1, the LDPE-curcumin film presented a light yellow color, and turned to light brown in response to NH_3, indicating its sensing ability to NH_3. Furthermore, the incorporation of curcumin had no significant effect on the microstructure of LDPE film. As shown in Fig. S2, the LDPE-curcumin film had a dense cross section similar to that of the LDPE film. No big particle agglomerates, air gaps, cracks, or detachment zones were evident, which was in line with a previous study (Zia, et al., 2019). These results indicated that curcumin, as a natural pigment, can be embedded into LDPE through extruding technology, and the fabricated LDPE-curcumin film was sensitive to NH_3.

3.2. Response of curcumin and LDPE-curcumin film to pH buffer solutions

As mentioned, the package with fresh meat generally has high RH, and the volatile water vapor would be adsorbed on the surface of the film fixed on the headspace of the packages. As a result, some non-neutral gases, such as volatile amines (primary and secondary amines), carbon dioxide and hydrogen sulfide, generated from meats would partially dissolve and then hydrolyze or ionize in the adsorbent water before they directly diffused into the films. This would therefore make the film exposed to a non-neutral aqueous microenvironment. Therefore, the stability of the LDPE-curcumin film in pH buffer solutions which simulated the non-neutral aqueous microenvironment was investigated.

Firstly, the colors and UV-Vis spectra of curcumin solutions were investigated, as shown in Fig. 2. Curcumin solution showed nearly the same yellow color at pH 2-8, while turned to brown at pH 9-10 and then reddish brown at pH 11-12 (Fig. 2A), which was line with a previous study (Pourreza & Golmohammadi, 2015). Correspondingly, the UV-Vis spectra of curcumin solution showed a maximum absorption peak at nearly 420 nm at pH 2-8, and the absorbance values slightly decreased with the increase of pH (Fig. 2B). When pH increased from 9 to 12, the maximum absorption peak gradually shifted to 474 nm, and simultaneously the absorbance values rose. The color and spectral changes of curcumin solutions with the increase of pH was due to the deprotonation of curcumin molecular, as described in previous studies (Kotha & Luthria, 2019; Liu, et al., 2018; Priyadarsini, 2014).

However, the LDPE-curcumin film did not show color changes even being immersed in pH buffer solutions for 6 h (Fig. 2C). This insensitivity of LDPE-curcumin film to pH buffer solutions was further confirmed by the UV-Vis spectra. As shown in Fig. 2D, the LDPE-curcumin films showed nearly the same spectra at pH 2-12, and the range value of the maximum absorbance at 420 nm (A_{420}) was lower than 0.01 (Fig. 2D inset). The L^*, a^* and b^* values of the film were shown in Table S2, indicating no significant difference between the colors of the films. These results could be due to that curcumin was embedded in LDPE molecular to reduce the contact between curcumin and water molecular. Similar phenomenon was also found in an extruded LDPE-bromophenol blue film (Wells, et al., 2019). Apart from this, the presence of curcumin could also reduce the water permeability of LDPE film (Zia, et al., 2019), which could also reduce the contact between curcumin and water.

To further investigate the stability of the LDPE-curcumin film in pH buffer solutions, the LDPE-curcumin film was immersed into buffer solutions for 5 d, and the UV-Vis spectra of the lixiviums were determined every day. Fig. 2E shows the photo of lixiviums of the LDPE-curcumin films at the 5th d. The lixiviums were nearly colorless and transparent at pH 2-10, but showed weak yellow at pH 11-12. The corresponding A_{420} values of the lixiviums were shown in Fig. 2F. All the lixiviums showed low A_{420} values that were below 0.03 with weak fluctuations within 5 d, indicating the low curcumin contents in the lixiviums. The slightly higher A_{420} values at pH 11 and 12 might be due to the better solubility of curcumin at basic condition (Priyadarsini, 2014). In addition, the color of the LDPE-curcumin film remained yellow at the 5th d, even at pH 11 and 12 (Fig. 2F inset). These results indicated that the leaching of curcumin from the LDPE-curcumin to the pH solution was extremely low.

3.3. Response of LDPE-curcumin film to NH_3 under different relative humidity

The response of LDPE-curcumin film to NH_3 under different relative humidity (RH) was shown in Fig. 3A. The films turned from light yellow to brown after exposed to NH_3 at 6%, 55% and 95% RH (Fig. 3A inset). The corresponding UV-Vis spectra showed that the maximum absorption peak shifted from ~ 420 nm to ~ 460 nm. It can be also seen that a relative lower RH is good for the reaction between LDPE-curcumin film and NH_3, as LDPE-curcumin showed a deeper brown color and higher absorbance value at ~ 460 nm after exposed to NH_3 under 6% RH than 55% RH, and followed by 95% RH.

As mentioned above, the LDPE-curcumin film was insensitive to pH buffer solutions, but sensitive to NH_3. These results indicated that the reaction between curcumin and NH_3 is a solid-gas interaction. Although this does not exclude the possibility of there being some water present, the water content is likely to be very small (Mills, Wild, & Chang, 1995). The proposed reaction mechanism was shown in Fig. 3B. The diketo group of curcumin could exhibit keto-enol tautomerism, and the enal form contains three labile protons (Priyadarsini, 2014). When NH_3 diffused into the LDPE-curcumin film (Karim, Hijaz, Kastner, & Smith, 2011; Wells, et al., 2019), it could make the deprotonation of these three active groups of curcumin, and thus inducing the color and the spectral changes. This reaction could be described as Equations 3 and 4 (Mills, et al., 1995). Hence, the reason that a lower RH contributed to the reaction between LDPE-curcumin film and NH_3 may be that more NH_3 molecular had dissolved into water under a higher RH to form $NH_3 \cdot H_2O$, and then was ionized into NH_4^+ and OH^-, while the OH^- in water drop could not easily diffuse into the film and make the color changes of curcumin.

$$NH_3(g) \underset{}{\overset{K_H}{\rightleftarrows}} NH_3(dis) \quad (3)$$

$$HR + NH_3(dis) \underset{}{\overset{K_1}{\rightleftarrows}} NH_4^+R^- \quad (4)$$

where NH_3 (g) is the gaseous ammonia, K_H is Henry's constant for gaseous ammonia in the plastic film. NH_3 (dis) is the gaseous ammonia dissolved in the plastic film. HR is the protonated form of curcumin. K_1 is an ion-pair formation constant (which depends strongly upon the dissociation equilibrium constant, K_a, of curcumin) and $NH_4^+R^-$ is the ion-pair formed between the dissolved ammonia and HR.

The effect of humidity on the hydrophobicity LDPE-curcumin film was quite different from the hydrophilic films incorporated with curcumin that were reported in recent studies (Liu, et al., 2018; Q. Ma, et al., 2017), where it was proposed that NH_3 firstly combined with H_2O in the hydrophilic films or the environment surrounding the films, and then the ionized OH^- induced the color change of curcumin. As a result, the higher RH was beneficial to the generation of more OH^-, accelerating the color change of the hydrophilic films (Q. Ma, et al., 2017). However, the reaction mechanism between NH_3 and curcumin in the presence or absence of water may need to be further investigated.

3.4. Sensitivity of the film to NH_3

The sensitivity of the film to NH_3 was determined at 90% RH, because the packages containing fresh meats (with water coefficients ~ 0.99) generally have high RH. Fig. 4A shows the UV-Vis spectra of the film in response to increasing contents of NH_3. The maximum absorption peak gradually shifted from ~ 420 nm to ~ 474 nm, which was consistent with the changes of UV-Vis spectra of curcumin solutions with increasing pH (see section 3.2). The maximum absorbance value at 420 nm showed a continuous decrease while the absorbance value at 540 nm increased, with the increase of NH_3 concentration. Generally, visible color absorbs light of wavelengths corresponding to its complementary color (Choi, Lee, Lacroix, & Han, 2017). Yellow color absorbs light of wavelength corresponding to its complementary color, blue (~ 400-480 nm), and red color absorbs light of the wavelength corresponding to its complementary color, green

(~ 495-570 nm). Hence, the absorbance ratio at 540 nm versus 420 nm (A_{540}/A_{420}), wavelength of each complementary color, could indicate the increase of red color intensity compared to yellow color intensity, with a deeper red color when A_{540}/A_{420} value was high. As shown in Fig. 4B, the A_{540}/A_{420} value increased with the NH_3 concentration, indicating that the color of the film gradually presented a yellow-to-red transition. In addition, the calibration curves showed that the A_{540}/A_{420} value had good linear relationship with the NH_3 concentration in the range of 0-80 μM and 100-240 μM, with R^2 of 0.9906 and 0.9897, respectively. Accordingly, the limit of detection (LOD) of the LDPE-curcumin film to NH_3 was determined to be 0.18 μM, by using Equation 5 (Courbat, Briand, Damon-Lacoste, Wöllenstein, & de Rooij, 2009):

$$LOD = \frac{3K}{N} \tag{5}$$

where K is the standard deviation of blank measurements and N is slope of the calibration curve (0.9906).

The LOD of LDPE-curcumin film was comparable to that of some other colorimetric sensors (see Table S2). It is generally expected that the sensing film could have high sensitivity to NH_3. There are several key factors of the pigments (or dyes) based colorimetric film that determine the sensitivity: (1) The acid dissociation constant (pK_a) value of a pigment. Generally, a lower pKa of the pigment is good for its color change (Mills, et al., 1995). (2) The content of pigments. A relatively lower content of pigment in the film was conducive to the sensitivity (Zhai, et al., 2017). (3) The microstructure of the film. For example, porous structure generally could improve the sensitivity because it could provide much more contact areas between the pigments and target gases (Luo & Lim, 2020). However, it should be noted that when more attention have been paid to improving the sensitivity of the films, their stabilities (e.g. under oxygen and high RH environment during storage and usage) ought to be considered at the same time.

The response of the film to NH_3 was reversible, namely the film could recover its color after it was separated from NH_3. As shown in Fig. S3, the initial A_{540}/A_{420} value of the LDPE-curcumin film was 0.4232, while it increased to 0.7302 after reacting with NH_3 for the first time. Then, the A_{540}/A_{420} value decreased to 0.4203 when the film was separated from NH_3 and put in air for 1 h. This meant that the film could almost recover to its original color. This reaction cycle was repeated for 5 times. Finally, when the film was reacted with NH_3 and then recovered in air, the A_{540}/A_{420} values were 0.7172 and 0.4343, respectively, which were close to A_{540}/A_{420} values of the first reaction cycle. Hence, the response of the film to NH_3 was reversible.

3.5. Stability of the film during storage

A good stability for a colorimetric film during storage is important. The A_{420} values of the LDPE-curcumin film stored under visible light along with time were shown in Fig. 5. The A_{420} value of the film showed gradual decrease with storage at 4, 25 and 37 °C. This instability of the film could be due to the photodecomposition by loss of two hydrogen atoms from the curcumin molecule, according to a previous study (Tønnesen, Karlsen, & van Henegouwen, 1986). It was obvious that a lower storage temperature was conducive to the stability of the film. The A_{420} values decreased by 4.2%, 7.3% and 15.3% after respectively stored at 4, 25 and 37 °C for 16 d. Hence, the LDPE-curcumin film was better to be stored or used at a low temperature to reduce its decomposition.

3.6. Application of films in monitoring meat spoilage

The LDPE-curcumin film was used to monitor silver carp and beef spoilage in a PET box, as shown in Fig. 6A and 6B, respectively. The color of the LDPE-curcumin film was shown in Table S3. The initial $L^*$of the film was 86.89, which decreased to 68.96 after 1 day storage of silver carp, while a^* and b^* did not significantly change. Similar phenomenon was also observed from the film used for beef. This was because the filter paper and the LDPE-curcumin film were wetted by water vapor, leading to a decrease of light of the film. For both the silver carp and beef packaging, the corresponding a^* value of the LDPE-curcumin film increased, while the b^* value decreased, indicating a gradually stronger red color and a weaker yellow color. Hence, the chromatic parameter ΔC^*, which depends on the changes of a^* and b^*, was used to describe the color changes of the film. It can be seen that the Δ*C** values of the film used for silver carp gradually increased from 0 to 8.25 after storage for 7 days. Meanwhile, the TVB-N value of silver carp increased from 5.95 to 29.31 mg/100 g. Similarly, the Δ*C** values of the film used for beef gradually increased from 0 to 8.25 after storage for 7 days as well, and the TVB-N value of beef increased from 6.34 to 26.21 mg/100 g. The increase of TVB-N with storage time for silver carp and beef was in line with previous studies (Ezati, et al., 2019; Kachele, Zhang, Gao, & Adhikari, 2017). These results indicated that the LDPE-curcumin film could present a yellow-to-brown color change resulting from reacting with the volatile amines of the meats.

According to Chinese Standard GB 2707-2016, the rejection limits of TVB-N are 20 and 15 mg/100 g for silver carp and beef, respectively. In this study, the TVB-N value of silver carp increased to 20 mg/100 g at nearly 5.3 d, when the Δ*C** value of the film was nearly 3.5 (Fig. 6C). This indicated that if the Δ*C** value of the film was higher than 3.5, then the silver carp sample should not be consumed. Similarly, the TVB-N value of beef sample rose to 15 mg/100 g at nearly 4.2 d, when the Δ*C** of the film was nearly 2.2 (Fig. 6D), indicating that the beef sample should be discarded at this point.

TVC is generally used to evaluate the meat spoilage as well (L. Huang, Zhao, Chen, & Zhang, 2013). The TVC of silver carp and beef during storage at 4 °C was shown in Table S3. The TVC of silver carp and beef increased respectively from 3.31 to 9.65 $\log_{10}$(CFU/g), and from 3.55 to 9.49 $\log_{10}$(CFU/g). Similar outcomes have been found in previous studies (Mehdizadeh, et al., 2020; Zhai, et al., 2019). According to International Commission on Microbiological Specifications for Food (ICMSF) (Gould, 1990), the maximum acceptable limit for fresh fish is 10^7 CFU/g. In this study, the TVC of silver carp increased to 7 $\log_{10}$(CFU/g) at nearly 5 d. This indicated that silver carp could not be consumed after 5-days storage, which was similar to the result of the TVBN analysis. In addition, according to the European legislation (European Commission, 2007), the maximum acceptable limit for raw meats is 5×10^6 CFU/g. In this study, the TVC of beef increased to $\log_{10}(5\times10^6$ CFU/g), namely 6.7 $\log_{10}$(CFU/g), just after 4 d. This indicated that the beef sample could not be consumed after 4 d, which was also similar to the result of the TVBN analysis.

It is generally expected that the film could indicate the meat spoilage as early as possible, and the color changes are highly visible for naked eye for practical application. In this study, the color differences of the film could be clearly seen after 5 days. Hence, improving the gas sensitivity of the film to make the film a better indicating property would be the focus of our further study.

4. Conclusions

LDPE-curcumin film was successfully developed through the melting extrusion method. The LDPE-curcumin film, as a hydrophobic film, could prevent the leaching of curcumin under high RH environment. The LDPE-curcumin film was sensitive to NH_3, and a lower RH was conducive to the sensitivity. The LOD of LDPE-curcumin film to NH_3 was determined to be 0.18 μM at 90% RH. The LDPE-curcumin film showed yellow-to-brown color changes with the storage of silver carp and beef at 4 °C. As the film is safe, low cost and suitable for industrial production, it would have a good potential for application in intelligent food packaging.

Declaration of competing interest

None

Acknowledgments

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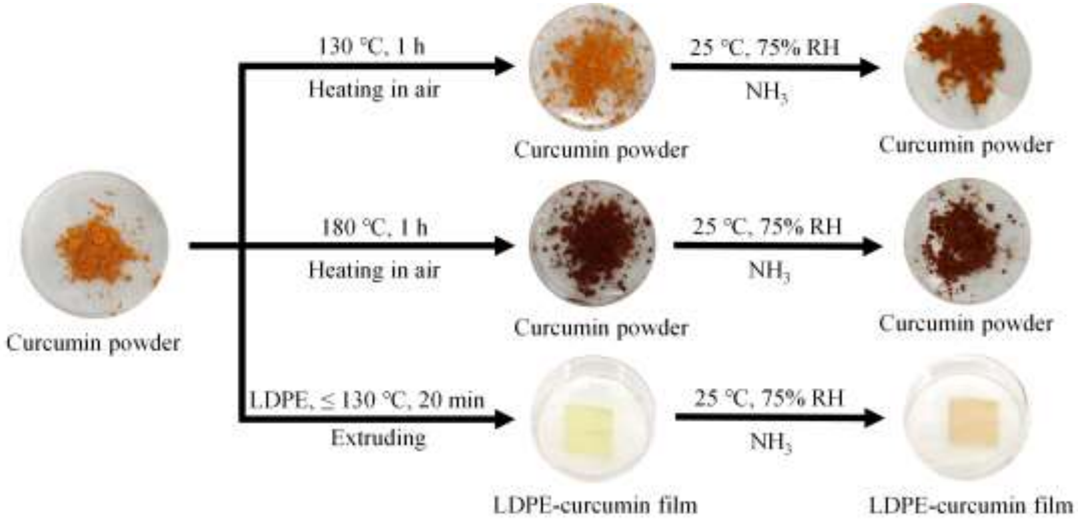

Fig. 1. The photos of curcumin powder and LDPE-curcumin film.

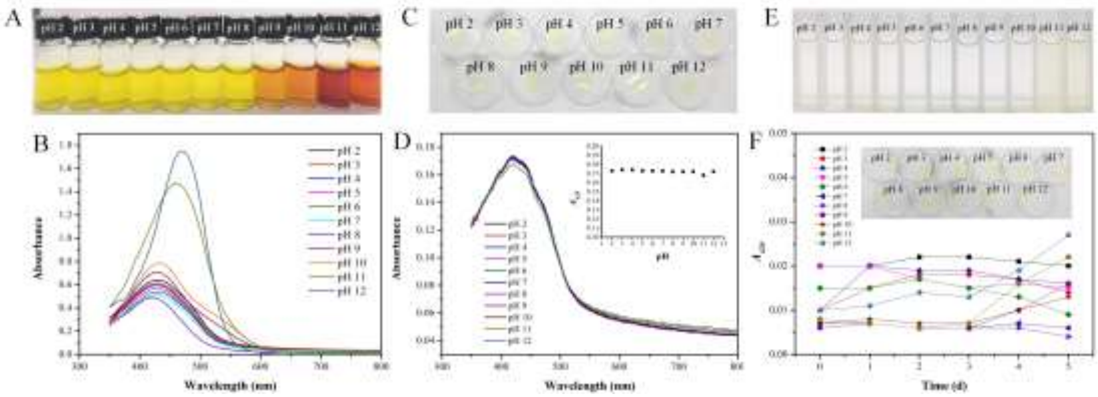

Fig. 2. (A) The photos and (B) UV-Vis spectra of curcumin solution at pH 2-12. (C) The photos and (D) UV-Vis spectra of LDPE-curcumin film immersed in buffer solutions, and inset of (D) is the A_{420} of the film immersed in buffer solution. (E) The photos and (F) A_{420} of lixiviums of LDPE-curcumin film immersed in buffer solution, and inset of (F) is the photo of LDPE-curcumin film after immersed in buffer solution for 5 d.

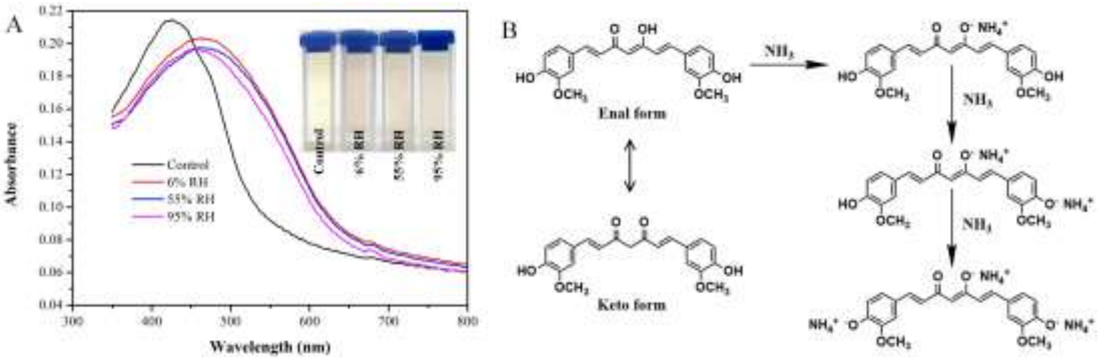

Fig. 3. (A) UV-Vis spectra and photos (inset) of LDPE-curcumin film after exposed to 200 μM of NH_3, and (B) the proposed reaction mechanism between curcumin and NH_3

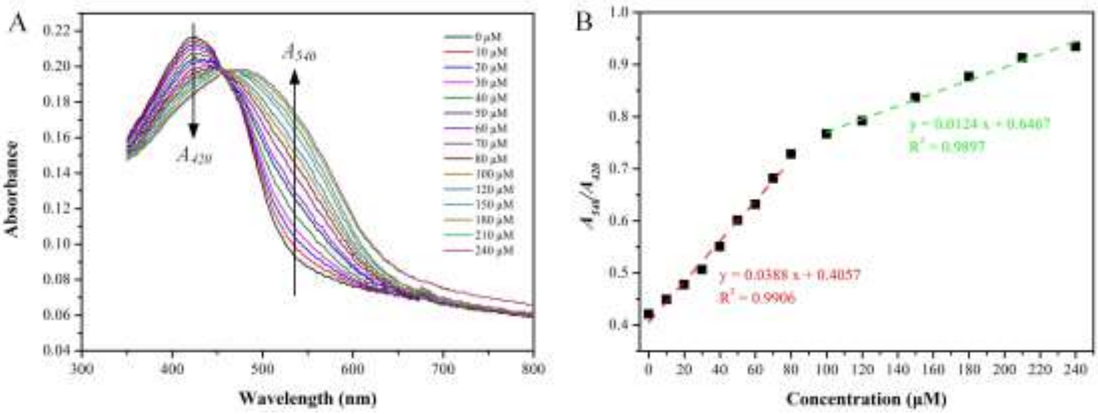

Fig. 4. (A) The UV-Vis spectra and (B) A_{540}/A_{420} of LDPE-curcumin film in response to NH_3 with concentrations of 0-240 μM.

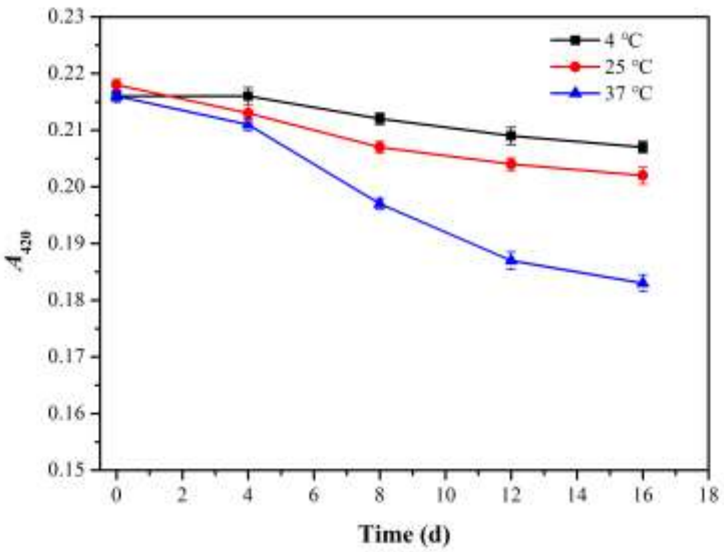

Fig. 5. The changes of A_{420} of the LDPE-curcumin film stored at 4, 25 and 37 °C.

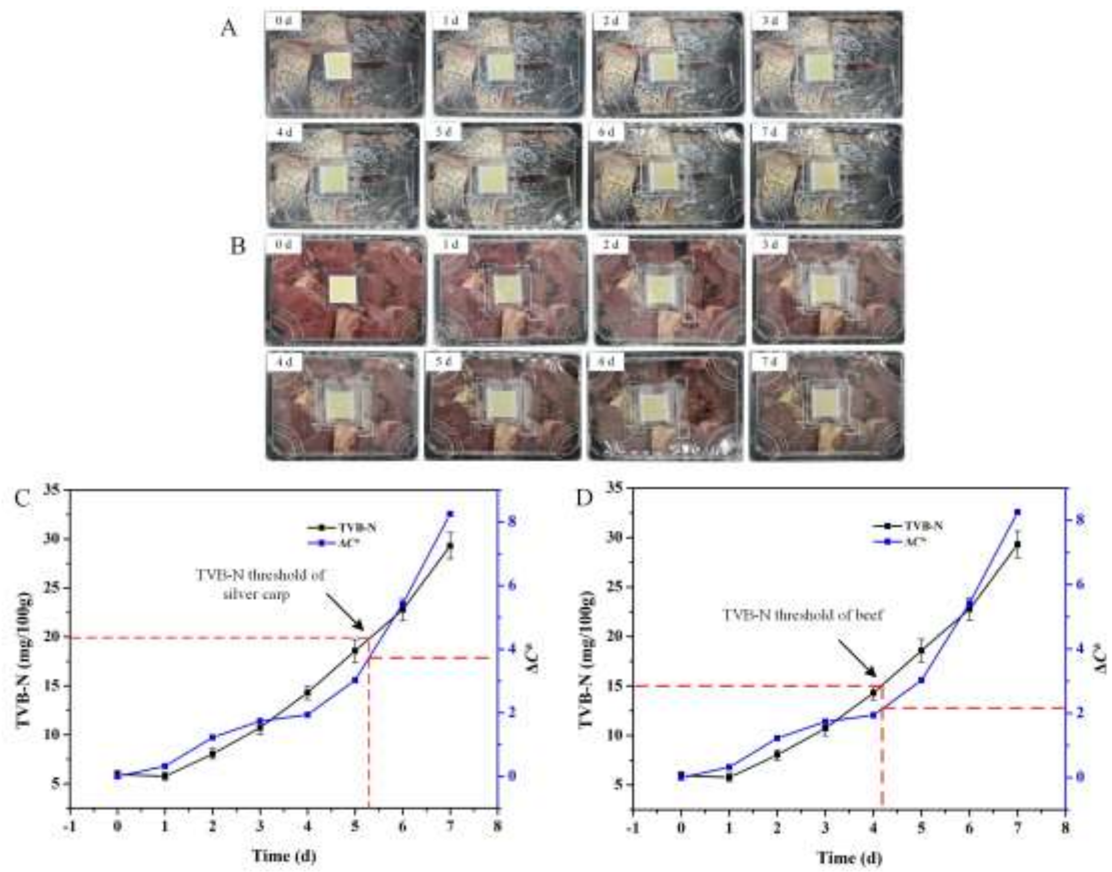

Fig. 6. The photos of the packages with (A) silver carp and (B) beef, and the relation between TVB-N and ΔC^* of LDPE-curcumin film for (C) silver carp and (D) beef.