Development of biodegradable hybrid polymer film for detection of formaldehyde in seafood products

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ABSTRACT

Despite the enormous accomplishments of current sensing methods, portable and sensitive sensing materials remains a challenging issue. Herein, a novel of a biodegradable hybrid polymer film was developed for quantitative analysis of formaldehyde seafood, including *Lutjanus erythropterus, Euthynnus affinis, Caranx indicus, and Penaeus monodon* at Sabah, Malaysia. In this research, starch and chitosan were introduced as the substrate to entrap Nash colorimetric reagents for the fabrication of biodegradable films for detection of formaldehyde. Under optimal conditions, excellent linearity (R^2 = 0.9918) of colorimetric response was obtained in formaldehyde concentration ranges of 100 to 0 ppm, with a limit of detection and quantification calculated to be 5 and 16.8 ppm, respectively. The developed film was successfully applied to the identification and quantification of formaldehyde in four different seafood samples with satisfactory recoveries, and RSD values obtained range between 98.80%–104.65% and 0.12%–1.21%, respectively. The present research demonstrated short response time (within 5 min) that provides reliable methods for application in biosensing, which exhibited the advantage of this well-performing platform for application in the food, environmental, and medical disciplines sensing.

1. Introduction

Formaldehyde is a colorless chemical compound that exists in gas form, while formalin is an aqueous solution contains about 30–40 w/% of formaldehyde solution added with methanol to prevent polymerization in storage condition [1,2]. Formaldehyde belongs to the aldehyde group, which has a pungent smell and commonly known as a toxic compound that has the possibility to affects human health. Specific properties of formaldehyde, such as preservation, disinfectant, and bleaching, have covered the way for the implementation of this organic compound in different sectors, including coating, cosmetics, food as well as adhesive. Furthermore, formaldehyde, a known carcinogen, has been found as an artificial preservative in various kinds of food, such as meat, vegetables, fruits, and seafood products [3,4]. Formaldehyde solution was purposely spraying or dip in fish is to prolong the shelf life, stiff and keeps them looking fresh and prevent spoilage.

However, formaldehyde able to stimulate dominant cells in the human body and may cause sleeplessness, nausea, neuralgia, and skin allergies [5]. Based on Food Safety and Quality Department 1985, Malaysian Food Regulations in 1985 and Regulations 148, 159 in 2006

recorded that small amount of formaldehyde is allowed in smoked meat and fish only during processing but not exceed 5 mg/kg [6]. Moreover, the European Food Safety Authority summarized the level formaldehyde in food, for example, meat and poultry is 5.7–20 mg/kg, fish is 6.4–293 mg/kg, milk and milk-based products is 0.01–0.80 mg/kg, sugar and sweeteners is 0.75 mg/kg, fruit and vegetables are 6–35 mg/kg, coffee is 3.4–16 mg/kg and alcohol beverages is 0.27–3.0 mg/kg [4].

In recent years, various approaches have been established for monitoring the level of formaldehyde, including spectrophotometry, electrochemistry, and chromatography, which are accurate and consistent, but required a complicated procedure, high-cost, and time-consuming. Hence, it is urgently needed to discover a new finding to quantify the level of formaldehyde concentration in food products [7,8]. A colorimetric sensor is one of the most popular techniques, such as portable, visually identify, fast detection, and easy to handle [9]. Generally, the colorimetric sensor is applied in combination with UV-spectrophotometer techniques for the quantitative analysis of target analyte. Nash [10] developed colorimetric techniques by following the Hantzsch reaction principle that involves formaldehyde as the target

analyte with a reagent containing acetylacetone, acetic acid as well as ammonium acetate, resulting in the production of diacetyldihydrolutidine (DDL) which is a yellow derivative of formaldehyde that comes with a high extinction coefficient.

Starch components are well dissolved, more comfortable to handle, and environmentally friendly. Different types of natural polymers, including starch, cellulose, collagen, chitosan, gelatin, and others, either from their mixtures or from isolated amylose and amylopectin components, are often used to produce biodegradable films. Hence, films are obtained from materials with thermoplastic characteristics in which they can be melted or rubbery through thermal processing at a lower temperature than the breakdown temperature [11,12]. Previously, Wongniramaikul [13] have optimized a variety of starch, including tapioca and corn-starch, rice as well as glutinous flour. Based on the findings, they found that tapioca starch showed a transparent homogeneous film with stable in room temperature during casting compared to the corn, rice, and glutinous flour, which forming brown film.

Chitosan is a helical polysaccharide discovered in the crustacean exoskeleton, such as shrimp, crab, lobster, and prawns. Chitosan has a wide variety of distinctive applications, including biodegradable film formation and water purification. Also, chitosan is well known as the second most plentiful natural biopolymer following cellulose and a biomaterial generated mainly from chitin's alkaline deacetylation (40–50% NaOH). A significant amount of crustacean is thrown away through the seafood market and company due to rise up of population growth and seafood consumption of humankind [14].

Hence, in this present study, environmentally friendly colorimetric films were fabricated using natural biodegradable polymers as the substrate for the detection of formaldehyde in the different matrix of seafood samples. Biodegradable films fabricated from starch and chitosan were used to entrap the Nash colorimetric reagent. Digital image analysis was then carried out to facilitate on-site detection instead of the conventional spectrophotometric method. Recently, digital image measurement gains overwhelming attention owing to the simplicity and rapidity [15]. The analysis was carried out according to the basic red/green/blue (RGB) color models of the image of the colorimetric outcome. In this research, the on-site quantification of formaldehyde is cost-effective and convenient as only an ordinary smartphone was required. Finally, high selectivity and sensitivity, less energy consumption, cost-effective, portable and straightforward detection method of formaldehyde in real samples has been proposed by using the developed biodegradable film that has a unique sensing mechanism.

2. Materials and methods

2.1. Materials

Tapioca starch was bought from the local supermarket at Kota Kinabalu, Sabah, Malaysia. Formaldehyde, acetylacetone, ammonium acetate, acetic acid, and chitosan were obtained from Merck (Germany) and Sigma-Aldrich (USA). Standard formaldehyde solutions were freshly prepared each day through serial dilution of the stock solution to appropriate concentrations. Nash reagent was prepared by mixing 0.75 mL of acetylacetone, 1 g of ammonium acetate, and 0.1 mL of acetic acid. Milli-Q® ultrapure water with a resistivity of 18.2 MΩ cm was used to prepare all the working solutions. All the chemicals were of analytical grade and used without further purifying process.

2.2. Fabrication of biodegradable film

The biodegradable film was fabricated, as illustrated by Wongniramaikul [13], with some adjustments. 0.4 g of tapioca starch and 0.1 g of chitosan was heated in 10 mL of ultrapure water and continuously stirred until forming a clear, viscous solution. The mixture was then left to cool down. The hybrid polymer mixed with Nash reagent with ratio 2:1 to obtain the optimal sensor system. The mixture

was stirred to form a homogeneous solution. About 100 μL of the solution was transferred into the cap of 1.5 mL centrifuge tube and heated at 120 °C for 30 min.

2.3. Structural properties

2.3.1. Moisture content analysis (MC)

The films were freshly prepared and dry in the oven for 24 h at 105 °C. The films were weighed before and after to obtain the wet weight (W_w) and dry weight (W_d), respectively. The MC of the different biodegradable films was determined by using the procedure as explained by AOAC [16] and the following equation:

$$MC\,(\%) = \frac{W_w - W_d}{W_d} \times 100$$

2.3.2. Water solubility analysis (WS)

The biodegradable films were prepared and dried for 24 h at 105 °C for the water solubility analysis. The weight of the films was recorded as the initial (W_i). Ultrapure water was added into the centrifuge tube and kept at 25 °C for 24 h. The film was filtered to separate the insoluble portions before drying at 105 °C overnight. The weight of the insolubilized dry matter (W_f) was obtained by reweighing the oven-dried samples. The solubility was expressed as a percentage of solubilized material, and determined based on the formula:

$$WS\,(\%) = \frac{W_i - W_f}{W_i} \times 100$$

2.3.3. Water absorption (WA)

Water absorption of the biodegradable film prepared was determined by using the procedure explained by Ludueña [17] A total of five replicates were prepared and dried overnight at 105 °C. The initial dry weight was recorded as $W_{t=0}$. The dried films were placed into containers with a relative humidity of approximately 57%. Controlled temperature and pressure were fixed at 25 °C and 1 atm, respectively, throughout this analysis. The final weight of films after 24 h in a stationary state was measured and recorded as $W_{t=24}$.

$$WA\,(\%) = \frac{W_{t=24h} - W_{t=0}}{W_{t=0}} \times 100$$

2.3.4. Swelling analysis (SW)

The swelling property of the developed films was obtained based on the approach represented by Liu [18]. The prepared films were cut to get a circle shape with 5 mm diameter ($Ø_i$). The film discs were immersed in tubes containing 20 mL of ultrapure water after the drying process. The sealed tubes were put in a controlled temperature (25 °C) for 24 h. The changes in diameter ($Ø_f$) of the discs were recorded.

$$SW\,(\%) = (Ø_f - Ø_i) \times Ø_i^{-1} \times 100$$

2.4. Mechanical analysis

2.4.1. Film thickness

The thickness of the developed biodegradable films was obtained by using a hand-held micrometer (Wilkens-Anderson Co.) with a precision of 0.001 mm. The analysis was made at least five random spots on each film.

2.4.2. Density

Density was obtained by using each film discs (12 mm diameter – Ø) as specified by Herniou [19]. The film was dried for 24 h at 105 °C, after the initial weight (W_i), thickness (e) and area (A) were measured. The final weight (W_f) was obtained after the drying process completed for one day. Density was calculated using the following equation:

$$\rho = \frac{W_i - W_f}{\left(\frac{\varnothing}{2}\right)^2 \times \pi \times e}$$

2.4.3. Biodegradability

The biodegradability of the film proposed was determined by using the protocol as described by Gutiérrez [20]. Concisely, the black soil was crushed and put into the container up to a height of approximately 5 cm. The films were cut with diameters of 5 mm and buried approximately 10 mm depth into the container containing black soil. The containers were weighed and labeled as initial weight (m_i). The containers were unearthed and weighed every day to obtain the final weight (m_f) of films for one week.

Biodegradability $(\%) = ((W_i - W_f)/W_i) \times 100$

2.5. Optical analysis

Analysis of the changes in color intensity was done by an RGB imaging system by using an iPhone 10 camera equipped with color scanning application. The results have been validated by the standard colorimetric method by colorimeter (Kinoca Minolta Inc., Tokyo, Japan). The hue angle ($h°$) of the color changes was calculated and recorded in degree, as shown in the following equation [21]:

$h° = 60° (2 + (B-R)/(MAX-MIN))$.Where;B: Blue, R: Red, MAX: Maximum number of R/G/B, MIN: Minimum number of R/G/B.

2.6. Real samples analysis

Local seafood such as L. *erythropterus, E. affinis, C. indicus,* and *P. monodon* was collected at the wet market, Tuaran, Sabah, Malaysia. All the samples were extracted by following Sani [22] with slight modification. A total of 30 g from each sample were cut into small pieces and then homogenized into 60 mL of trichloroacetic acid by using a blender for 30 min. This method was used to prepare the fish extract sample. Whatman filter paper No. 1 was used to filter the resulting liquid to remove the digested tissues. The seafood samples were spiked with different concentrations of formaldehyde for recovery study analysis. The concentration of formaldehyde was calculated using the linear regression equations. The rate of recoveries (%) was calculated according to the equation below;

Rate of recoveries $(\%) = (\text{Found}/\text{Expected}) \times 100$

2.7. Statistical analysis

SPSS (Version 25, Chicago, USA) and ANOVA software were used to interpreted and analyze the data for all tests. A posthoc analysis was conducted by using a multiple-range Tukey's test to determine the differences detect significant differences ($p \leq .05$) in measured properties of biodegradable films. All the analyses were carried out using five replicates, and the data obtained was recorded in mean ± standard deviation.

3. Results and discussion

3.1. Morphological characterization of biodegradable film

The morphology of the prepared biodegradable film was characterized by a scanning electron microscope (SEM), as shown in Fig. 1. The chitosan was well-dispersed and showed high porosity (Fig. 1a) in the presence of starch (Fig. 1b) and Nash reagent (Fig. 1c). The biodegradable film clearly showed homogeneously arranged in the presence of Nash reagent. Likewise, current research conducted by Ali [23] has published that starch films show reasonably a smooth surface. Fig. 1d showed the SEM photograph of biodegradable starch film's surface without incorporated with Nash reagent. Without the addition of Nash reagent, the starch film has a colorless surface. In contrast, the yellow marks or pores of Nash reagent can be identified within the film's matrix after incorporated with Nash reagent. It can be seen that the film with Nash reagent in Fig. 1e appeared as yellowish color and fibrous. The biodegradable colorimetric film used for detection of formaldehyde in this experiment was prepared by a thin natural starch polymer film with entrapment of the colorimetric reagent within its matrix. Nash reagent was used as a colorimetric reagent and combined with the starch–chitosan mixture before casting because it can selectively respond with formaldehyde compounds. The incorporated Nash reagent on starch film, which is yellowish in color pores randomly distributed over the surface of the thin starch film. The starch film was observed in a porous structure with many nanopores entrapped within starch matrix. The irregular shape pores were distributed randomly over the film.

3.2. Mechanism reaction

The biodegradable colorimetric film sensing of formaldehyde detection well explained by Hantzsch reaction in samples by spectrophotometry and fluorometry method. Hantzsch reaction required the cyclization of amine, aldehyde, and B-diketone and produced a dihydropyridine derivative product in yellowish color [24]. In this experiment, the formaldehyde reacts with the acetylacetone in the presence of ammonia, forming a yellowish product of 3,5-diacetyl-2,6-dihydrolutidine (DDL) which allow colorimetry detection of formaldehyde present in samples shown in Fig. 2. The acetylacetone act as B-diketone which reacts faster with formaldehyde and produces a yellow color.

3.3. Optical analysis

The reaction between formaldehyde and Nash reagent within the biodegradable film resulting in color changes from colorless to yellow solution. In order to obtain the RGB values, the photographs of color changes were captured by using a smartphone (iPhone 10) equipped with color scanning application [13] and validated by the standard colorimetric method by colorimeter under same condition. According to Maxwell's color theory, the RGB model is an additive color spaced based on three primary colors, which are red, green, and blue [21]. Theoretically, the hue angle values for the developed biodegradable film will be increased as the formaldehyde concentration increased due to the reaction between formaldehyde and acetylacetone, which was selected as the β-diketone and resulting in a yellow product. The intensity of the yellow color obtained is directly proportional to the formaldehyde concentration that will absorb more light. As shown in Table 1, the hue angle for each concentration analyzed lies in the yellow region, which is in the range of 53° to 67° as illustrated in the CIE-LAB hue sequence and hue angle orientation [25]. However, the hue angle of film containing Nash reagent is significantly higher than the film control. Nash reagent as an indicator used to quantify the absorbance of formaldehyde [3]. Light-sensitivity of Nash reagent nature will give the respective absorbance when it reacted with different concentrations of formaldehyde measured at 415 nm by using UV–Vis spectrophotometer.

3.4. Structural properties

The structural properties of the biodegradable film were summarized, as shown in Table 2. Moisture content for the developed biodegradable film containing Nash reagent higher compared to control with the value of 0.246 and 0.097, respectively. Based on the analysis, film with colorimetric reagent contain a high amount of hydroxyl groups, which results in a more exceptional ability to take up water molecules from the environment, which further increases the moisture content. Chitosan showed a positive impact on the film-forming solutions, which
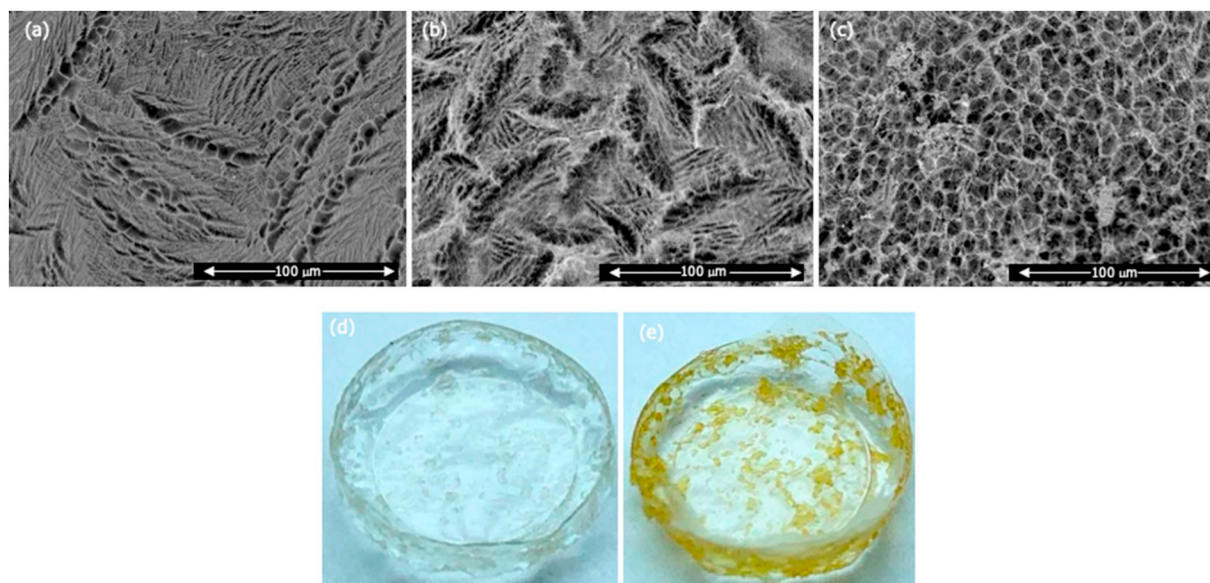
Fig. 1. SEM of biodegradable film (a) chitosan (420×), (b) chitosan/starch (500×), and (c) starch/chitosan/Nash (500×). Naked eye observation (d) without Nash reagents and (e) contain Nash reagents.

increases intermolecular forces and resulting in a higher viscosity starch solution. The structural gaps existing in the polysaccharide chain were filled up by the addition of Nash reagent as it entrapped within the film matrix, which produces a denser chitosan film. The starch/chitosan/Nash was showed statistically significant ($p > .05$), which can be seen that present of Nash reagent increased permeability because of the structural modification of the polymer. The channels for water transport across the film choked as the interstitial space in the chitosan matrix were reduced [26].

Water absorption symbolizes moisture transport through the biodegradable colorimetric film. The WA of starch/chitosan film showed higher compared to starch/chitosan/Nash with a value of 0.058% and 0.025%, respectively. A thick film due to the addition of Nash reagent into film containing starch/chitosan has successfully inhibited the water molecules from moving in and out of the films, which reducing the WA values [27]. Besides, the films also have compact structures when the Nash reagent was incorporated [28]. Moreover, the swelling effect in the control chitosan film was significant than starch/chitosan/Nash film, which is 164.00 and 144.00, respectively. The swelling ability reduced as a higher crosslinking density and network rigidity within the film [29]. The polymer is hydrophilic, and it will swell faster when in contact with water. Hence, a less dense structure is obtained as

Table 1
Hue angle (°) based on L*(lightness), a* (redness), b* (yellowness) and R* (red) G* (green) B* (blue) color system.

Concentration (ppm)	L*a*b* (°)	R*G*B* (°)	Color
	Starch/Chit/ Nash/FA	Starch/Chit/Nash/ FA	
0	69.18	60.00	Philippine gray
5	71.20	60.50	Citron
10	74.49	60.89	Old gold
20	73.26	61.57	Citron
30	74.61	62.14	Wattle
40	73.45	62.33	Goldenrod
50	73.80	62.48	Goldenrod
60	76.42	63.00	Citrus
70	75.51	63.50	Celery
80	76.52	64.03	Golden brown
90	72.70	64.48	Earls green
100	76.42	65.14	Celery

both ends of the molecule chain in the film are relatively more mobile since the absorbed water molecules modified the matrix of the film [30]. The capacity of swelling also reflects the water absorption
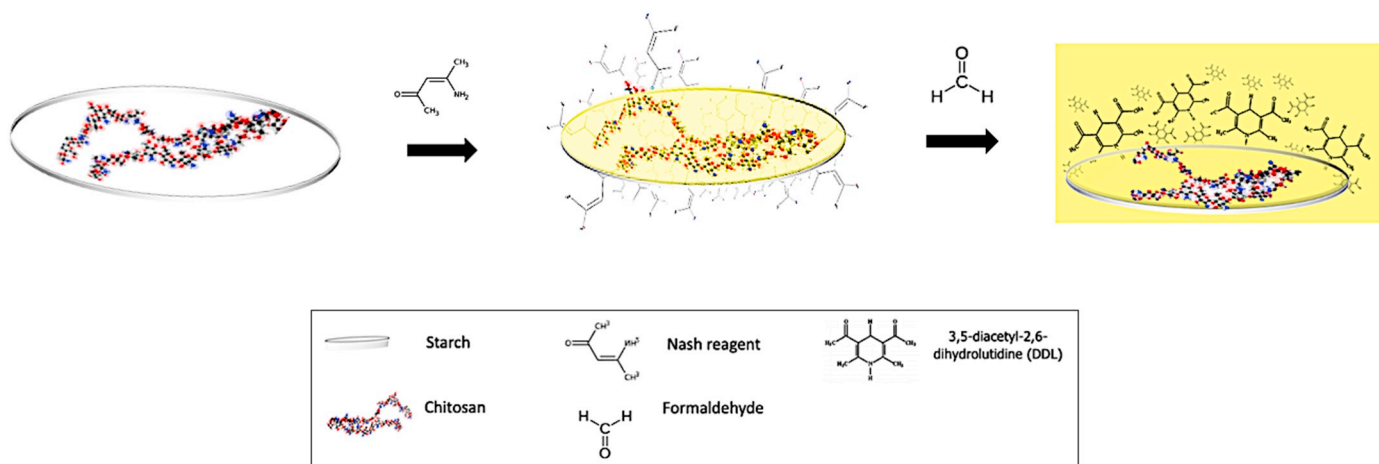

Fig. 2. (a) Schematic representation of the biodegradable film and (b) color changing in different concentrations of formaldehyde.

Table 2
Characterization of different parameters of the biodegradable film.

Parameters	Starch/Chitosan	Starch/Chitosan/Nash
Moisture content (%)	0.097 ± 0.036	0.246 ± 0.063
Water solubility (%)	0.160 ± 0.042	0.255 ± 0.022
Water absorption (%)	0.058 ± 0.043	0.025 ± 0.0005
Swelling (%)	164.0 ± 40.99	144.0 ± 35.78
Thickness (mm)	0.054 ± 0.016	0.064 ± 0.014
Density (g/cm^3)	0.787 ± 0.389	3.915 ± 3.624
Biodegradability (%)	0.156 ± 0.019	0.180 ± 0.037

capacity of the film prepared.

3.5. Mechanical properties

The mechanical properties of the biodegradable film were summarized, as shown in Table 2. The biodegradable films were prepared homogeneous, flexible, and has smooth surfaces to evaluate the thickness of the films. The thickness values of the films varied from 0.054 to 0.064 mm (Table 2). The thickness of chitosan films was increasing in the presence of Nash reagent because of the higher content of dry solids. According to references, the addition of chitosan leads to more hydrophilic groups susceptible to interacting with water, resulting in more high thickness. The density of chitosan film incorporated with Nash reagent is higher compared to the control chitosan film, which is 3.915 and 0.787, respectively. However, the density of film added with colorimetric reagent was not significantly higher ($p < .05$) than control.

The biodegradability properties of the films were studied buried in soil for one week. The film content colorimetric reagent showed fast degradation because of low water activity in films at a particular time compared to the control film shown in Table 2. The main reason is because of the small interaction within the higher content of hydroxyl groups, resulting in an in compact structure, which was more natural to decompose and utilized by the microorganisms. Based on the results, the weight loss of the control and developed colorimetric films were all much higher, representing excellent biodegradability properties of the developed hybrid colorimetric films. Instability of intermolecular interaction help to increase the water absorption of polymer materials,

Table 3
Recovery of spiked of different real samples using biodegradable film.

Sample	Added (ppm)	Found (ppm)	Expected (ppm)	Recovery rate (%)	RSD (%)
Lutjanus erythropterus	0	45.32	–	–	–
	5	50.78	50.32	100.92	1.06
	50	99.74	95.32	104.64	1.21
	100	147.59	145.32	101.57	0.78
Euthynnus affinis	0	45.21	–	–	–
	5	52.21	50.21	103.99	0.80
	50	99.64	95.21	104.65	0.90
	100	150.91	145.21	103.93	0.46
Caranx indicus	0	55.54	–	–	–
	5	59.97	60.54	99.07	0.35
	50	104.27	105.54	98.80	0.53
	100	157.39	155.54	101.19	0.65
Penaeus monodon	0	89.73	–	–	–
	5	94.62	94.73	99.88	0.25
	50	142.37	139.73	101.89	0.12
	100	190.03	189.73	100.16	0.54

which promotes disintegration by reducing particle sizes. Eventually, degrade entirely and does not cause any contamination or pollution to the environment [20]. Overall, all the materials studied had completely degraded after four days, which indicated that the film was easily biodegraded in soil and showed no significant differences in each other.

3.6. Sensing application

3.6.1. Analytical performance of the biodegradable film

A gradual color change from colorless to yellow color was observed in Fig. 3 in the presence of a different concentration of formaldehyde. The images of sample solutions were captured according to an increase in concentrations order from 0 to 100 ppm. A minimal discrepancy was noted between batches, with a near-identical absorption being observed. The calibration curve relationship between absorbance and concentration shows a highly linear response with $R^2 = 0.9918$. The LOD and LOQ values were calculated to be 5 ppm and 16.8 ppm, respectively. The LOD and LOQ were measured by estimated using formulas ($3 \times$ standard deviation/slope) and ($10 \times$ standard deviation/slope) from the slope of the plotted calibration curve. The World Health
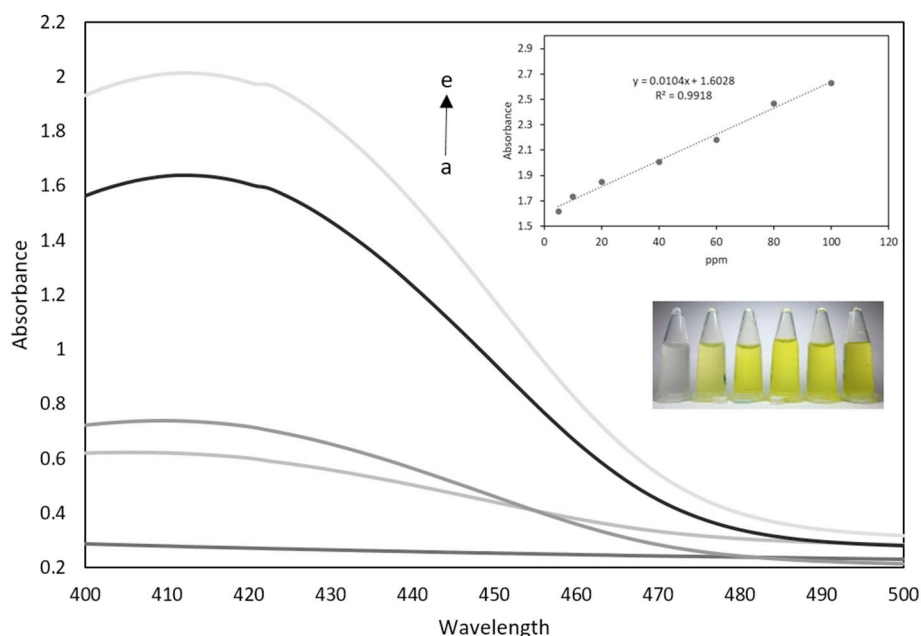

Fig. 3. A biodegradable colorimetric film on the lid of a centrifuge tube with colorimetric reaction products from different concentrations of formaldehyde (a–e: 0, 5, 10, 30, 40 ppm).

Organization (WHO) has stated the original contents of formaldehyde found in fish were ranged from 1 to 98 mg/kg, and with these performance characteristics of the sensor, it was adequate to the identification of the formaldehyde contaminants in foods [31,32].

3.6.2. Real samples analysis

The performance of the film was further analysis in real samples that usually adulterated by formaldehyde. A total of four samples, including L. *erythropterus, E. affinis, C. indicus,* and *P. monodon,* were purchased from the local wet market at Tuaran, Sabah, Malaysia. All the samples were prepared and spiked at different concentration 5 ppm, 50 ppm, and 100 ppm to investigate the extraction recoveries. The sensor was directly applied in several food samples for the determination of the presence of formaldehyde in samples to validate the potential application of the developed film. Table 3 shows typical response profiles obtained from the blank and spiking samples. In order to assess the accuracy and precision of the proposed approach, the recoveries analysis was done by spiking standard formaldehyde of 5, 50, and 100 ppm into each of real samples. The recovery ranged values was found from 98.79% to 104.65%, with RSD values between 0.12% and 1.21% ($n = 5$). The results indicated that the detection of formaldehyde in complex food samples with negligible interferences done accurately and precisely by the developed colorimetric sensor film.

4. Conclusions

In the present study, the developed biodegradable films were successfully entrapped Nash reagent for the colorimetric quantification of formaldehyde residues in seafood samples. The biodegradable film was in-situ designed onto the lids of small centrifuge tubes, and this promotes easy portability and in-tube detection by directly added the sample solutions. The biodegradable film was dissolved and directly showed yellowish when the presence of formaldehyde. In combination with RGB, the fast detection of formaldehyde with cost-effective portable kit was developed for on-site application in the food safety field. A low detection limit achieved as a wide linear range with excellent linearity in the calibration graph constructed. The developed biodegradable film was successfully applied in real samples to detect the presence of formaldehyde with satisfactory recoveries values. The results obtained did not significantly differ with an impractical for the portable field used standard laboratory method. The findings concluded that the successful quantification of formaldehyde done by an environmentally friendly colorimetric sensor developed.

Declaration of Competing Interest

We declare that there are no conflicts of interest.

Acknowledgments

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