Color change kinetics of lac dye as influenced by some food spoilage metabolites: validation for spoilage monitoring of strawberries

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Abstract

Purpose – The purpose of this paper was to study the color change kinetics of lac dye in response to aldehydes, carbon dioxide and other food spoilage metabolites for its potential application in intelligent food packaging.

Design/methodology/approach – UV–Vis spectroscopy was used to study the color change of dye solution. Ratio of absorbance of dye solution at 528 nm (peak of ionized form) to absorbance at 488 nm (peak of unionized form) was used to study the color change. Color change kinetics was studied in terms of change in absorbance ratio (A_{528}/A_{488}) with time using zero and first-order reaction kinetics. Lac dye-based indicator was prepared to validate the result of study for monitoring quality of strawberries.

Findings – Lac dye was orange-red in acidic medium and purple in alkaline medium. Color change of dye in response to benzaldehyde followed zero-order reaction kinetics, whereas for carbon dioxide first-order model was found best. No color change of dye solution was observed for alcohols, ketones and sulfur compounds. In the validation part, the color of the indicator label changed from purple to orange when the strawberries spoiled.

Originality/value – The study expands application area for lac dye as sensing reagent in intelligent food packaging for spoilage or ripeness detection of fruits and vegetables.

Keywords Natural dye, Lac dye, pH response, Intelligent packaging, Food spoilage metabolite

Paper type Research paper

1. Introduction

pH-sensitive dyes have attracted considerable attention in recent years because of their wide range of applications, including monitoring of chemical exposure in industries, assessment of food quality, sensitization of solar cell and damage and bacterial contamination (Rodríguez *et al.*, 2014; Taya *et al.*, 2016; Roy and Rhim, 2021). These pH-sensitive dyes respond to various analytes by changing color as a result of the reaction between the color component of the dye and the analyte (Miranda *et al.*, 2020). Intelligent packaging provides information to consumers about the status of food and its surrounding environment by detecting, sensing, tracing and recording the deterioration occurring inside a package (Müller and Schmid, 2019). pH-sensitive dyes are used as sensing reagent in intelligent food packaging.

During food spoilage, a large number of spoilage metabolites such as alcohols, organic acids, volatile nitrogen compounds, biogenic amines, gases and sulfur compounds are generated

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Pigment & Resin Technology © Emerald Publishing Limited [ISSN 0369-9420] [DOI 10.1108/PRT-12-2022-0151] (Lee *et al.*, 2019; Smolander, 2008). These metabolites could be used as indicators of food spoilage. When these spoilage metabolites react with the sensing reagent, they change the color of the dye.

Recently, various intelligent packaging indicators based on pH-sensitive dyes have been developed to detect spoilage metabolites using various synthetic dyes (bromothymol blue, methyl red, bromocresol green, bromocresol purple, bromophenol blue and chlorophenol red) and natural dyes of plant origin (anthocyanins) (Priyadarshi *et al.*, 2021). Natural dyes are safer for use in food applications because they are non-toxic (Zia *et al.*, 2021). However, natural dyes of plant origin have certain drawbacks, such as poor stability to light, enzymes, temperature, oxidants and metal ions, which limit their application as freshness indicators (Castañeda-Ovando *et al.*, 2009).

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Among the natural dyes, lac, a dye of insect origin, has great potential as an intelligent packaging indicator because of its stable physical and chemical properties, strong resistance to oxidation and pH sensitivity (Hong et al., 2011; Nacowong and Saikrasun, 2016). The dye is produced by the lac insect (Kerria lacca), which thrives on the sap of certain host trees. It is obtained during the washing of sticklac to obtain seedlac (a semirefined product of lac). It is sensitive to pH and possesses an orange-red color in acidic medium and reddish-violet in alkaline medium (Liu et al., 2019a, 2019b). Its principal color-imparting components are hydroxy-anthraquinone derivatives, designated as laccaic acids A, B, C, D and E (Sharma et al., 2020). Laccaic acid A (40.42%), E (20%) and B (17.66%) are major components of dye, whereas laccaic acid C (2.54%) and D (1.51%) are minor components (Hong et al., 2011). Lac dye is used as a natural food colorant (Divya et al., 2011), cosmetics colorant (Jimtaisong, 2020) and textile colorant (Wei et al., 2013).

Our previous study (Sakare *et al.*, 2022) investigated the color change kinetics of lac dye in response to pH, volatile nitrogen compounds, lactic acid and tyramine. In acidic pH, the dye solution showed a peak at 488 nm, whereas in alkaline pH, it was observed at 528 nm. The study suggested the potential application of lac dye in spoilage detection of seafood, meat, poultry and milk in intelligent food packaging systems. Present study is in continuation of our previous one (Sakare *et al.*, 2022).

In the present study, the color change of lac dye in response to other food spoilage metabolites such as aldehydes, carbon dioxide, alcohols, ketones and sulfur compounds was studied in detail to expand the application of lac dye as a sensing reagent in the intelligent packaging of other food products. In addition, the results of this study were validated for the quality monitoring of strawberries.

2. Materials and methods

2.1 Raw materials

Technical-grade lac dye was procured from Tajna Shellac Pvt. Ltd. Khunti, Ranchi, Jharkhand, India. The dye was analyzed for its dye content and adulteration of synthetic dye (if any) at the Quality Evaluation Laboratory, ICAR-National Institute of Secondary Agriculture (Formerly ICAR-Indian Institute of Natural Resins and Gums), Ranchi, Jharkhand, India. Dye content of the procured dye was 48%, and there was no evidence of adulteration. Chemicals used in the study were of analytical grade and purchased from HiMedia Laboratories Pvt. Ltd, Mumbai.

2.2 Color change of dye solution with pH

In total, 0.1% w/v dye solution was prepared by dissolving it into boiling distilled water. The pH of the dye solution was changed by adding 0.1 N NaOH. The color change of the dye solution was observed by capturing the images. About 20 mL of dye solution was poured onto a petri dish (8.5 cm diameter) and placed it on a white background. The photographs of the dye solution were captured in a wooden light box under uniform lighting using a smartphone camera (Galaxy M21, Samsung Electronics Co., Korea). The RGB color values were extracted from the image using Image J software and processed to CIELAB system. Color change of indicator was calculated using equation (1):

$$\Delta E = \sqrt{\left(L - L_0\right)^2 + \left(a - a_0\right)^2 + \left(b - b_0\right)^2}$$
(1)

Where L_0 , a_0 and b_0 are the initial color values of dye solution at pH 2.

L, a and b are the color values of dye solution at specific pH.

2.3 Color reversibility study of Lac dye

UV–Vis spectroscopy was used to study the color reversibility of dye solution. One mL of 0.1% dye solution was added to 20 mL distilled water. Initial absorbance and pH of the dye was recorded. In total, 5 mL of the diluted solution was taken and small amount (5 μ L) of 0.1 M NaOH was gradually added until the color change of dye solution was observed. Furthermore, in the same solution, 5–5 μ L of 0.1 M HCl was added gradually until the color of dye solution changed back to original color. Absorption spectrum of solution in the range of 400 nm– 700 nm after addition of NaOH or HCl was recorded with a UV–Vis spectrophotometer (UV Plus, Motras Scientific Instruments Pvt. Ltd., India).

2.4 Color change of lac dye solution in response to aldehyde

To study the color change of dye solution in response to aldehyde, benzaldehyde was taken as representative. Color change of dye solution was measured at three banzaldehyde concentrations (3, 4, 5 mol/L) and three dye concentrations (0.1%, 0.2% and 0.3% w/v). 100 mL benzaldehyde solution of desired molarity (3, 4, 5 mol/L) was prepared and poured in an Erlenmeyer flask. In another set of Erlenmeyer flasks, 0.1%, 0.2% and 0.3% w/v of dye solution (pH 9) were poured. Both set of flasks were connected through a glass connector to allow benzaldehyde to evaporate and react with dye solution. The reaction was carried out at $31 \pm 1^{\circ}$ C temperature and $80 \pm 2\%$ RH. Absorbance of dye solution was recorded at 488 nm and 528 nm at specific (1 h) time interval.

2.5 Color change of lac dye solution in response to carbon dioxide

To study the color change of the dye in response to CO_2 , gas was supplied from a CO_2 gas cylinder under a controlled flow rate. Gas flow rate was controlled using a flow-control valve, and the flow rate of the gas was measured using a flow meter. The color change of the dye solution was measured at two flow rates (0.2, 0.5 l/min) and three dye concentrations (0.5%, 1% and 1.5% w/v). Dye solution (100 mL, pH 9) of the desired concentration was prepared and poured into a 250 mL reaction flask. The reaction flask was closed from the top, and CO_2 gas was supplied at a set flow rate from the side arm of the reaction flask. The absorbance of the dye solution at 488 nm and 528 nm was recorded at 30 s time interval.

2.6 Color change of lac dye solution in response to alcohol, ketone and sulfur compounds

To study the color change of the dye solution in response to alcohol, ketone and sulfur compounds, ethanol, acetone and carbon disulfide were used as representatives, respectively. In Color change kinetics of lac dye

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addition to this color change in response to oxygen was also studied. A color change check was performed for all the abovementioned metabolites and gas at pH 3 and 9 of the dye solution (0.1% w/v). Color change in response to ethanol, acetone and carbon disulfide were determined by directly pouring representative metabolites into dye solution.

2.7 Kinetic modeling of color change

Kinetic modeling of color change was done to describe the reaction rate as a function of experimental variables. Several studies have reported the kinetics of color of food materials. The majority of these works report zero-order (rate of reaction is independent of the concentration of the material of materials) or first-order (rate depends on the reactant concentration raised to the first power) color change kinetics (Maskan, 2001). Therefore, in the present study, the change in absorbance ratio A_{528}/A_{488} of dye solution (which indicates the color change) with time was fitted to the zero-order model [equation (2)] and the first-order model [equation (3)]:

$$A_t = A_0 + kt \tag{2}$$

$$A_t = A_0 exp(kt) \tag{3}$$

Where t is the reaction time, k is the rate constant and A_t is the absorbance ratio at time t, and (Sakare *et al.*, 2022) A_0 represents the initial absorbance ratio.

2.8 Validation of dye for strawberry quality monitoring *2.8.1 Preparation of indicator*

Lac dye-based indicator as described in Sakare *et al.* (2022) was prepared by incorporating dye into agarose membrane and used for quality monitoring of strawberries.

2.8.2 Determination of quality parameters of strawberry

Fresh strawberries for the experiments were harvested from research farm of ICAR-Central Institute of Agricultural Engineering, Bhopal, MP, India. About 100 g of strawberry was placed in transparent PET punnet. The prepared indicator was stuck to the top cover of punnet. Samples were stored at $25 \pm 1^{\circ}$ C, and total soluble solids (TSS), firmness and headspace CO₂ concentration were determined at an interval of one day till five days of storage.

About 10 g of strawberry was homogenized in mortar and pestle and juice was extracted using a muslin cloth. TSS were measured from extracted juice using a digital refractometer (ATAGO INDIA Instruments Pvt. Ltd.) calibrated at 20°C to 0% with distilled water. Two readings each for 10 fruits were taken and averaged. The values are expressed as Brix at 20°C (Zhao, 2019).

The CO₂ concentration in the package headspace of the strawberry was measured using a headspace analyzer (Systech, GS3/P). A self-sealing septum was provided at the top surface of the package to facilitate the measurement of CO₂ concentration at regular time intervals (one day) using an injecting needle. The instrument was air calibrated before analyzing the package CO₂ concentration.

The firmness of the strawberry was measured using a Texture Analyzer (Stable Micro System Ltd., UK). Penetration was conducted using a cylindrical probe of 5 mm diameter at a speed of 1 mm/s during the pretest and puncture depth of 8 mm (Zhao, 2019). The maximum force was recorded as firmness. The penetration test was performed on six strawberry samples from each group on each storage day.

3. Results and discussion

3.1 Color change of dye solution with pH

Optical image of aqueous dye solutions with varying pH values (2-10) is shown in Figure 1. The lac dye solution was orangered in acidic medium and turned purple in alkaline medium. Figure 2 shows the color difference (ΔE) of the dye solution with pH based on the L^* , a^* and b^* coordinates. It can be observed that the color difference of the dye solution increased with increase in pH. Change in the color of the indicator is attributed to deprotonation of the phenolic and carboxylic acid groups of dye molecules at high pH values, which results in different electron configurations and, consequently, different colors (Van der Schueren and De Clerck, 2012; Chairat *et al.*, 2004).

Figure 2 Color difference of dye solution with pH









Source: Authors' own work

3.2 Reversibility study of dye solution

The color reversibility of dye solution was analyzed to observe the reversible color change capacity of the coloring compounds of dye. Figure 3 shows the absorption spectra of dye solution after addition of NaOH solution. It can be observed that the absorption band of dye solution, which was at 488 nm shifted to 528 nm after addition of NaOH solution. Shift in the absorption band was observed immediately after addition of small amount (5 μ L) of NaOH solution. This indicates that a small quantity of OH⁻ could break the equilibrium of chemical structure of dye solution. Absorption band at 528 nm increased in intensity as the amount (5–15 μ L) of NaOH in solution increased. Further addition of NaOH in dye solution does not result in increase in the absorption intensity. This may be attributed to deprotonation the dye molecules. Figure 4 shows

Figure 3 UV-Vis absorption spectra of dye solution after addition of different amount of NaOH



Source: Authors' own work

Dye solution (20 µl NaOH added) 0.8 5 ul 10 µl 15 µl 0.7 20 µl 25 µl 0.6 30 µl Absorbance 0.5 0.4 0.3 0.2 0.1 0.0 400 450 500 550 600 650 700 Wavelength (nm)

Figure 4 UV–Vis absorption spectra of dye solution after addition of different amount of HCl

Figure 5 Change in absorbance ratio (A_{528}/A_{488}) of dye in response to benzaldehyde



Source: Authors' own work

the absorption spectra of dye solution after addition of HCl solution. It can be observed that absorption band around 528 nm decreased in intensity and shifted to 488 nm when the pH was decreased by adding HCl solution. Absorption band slightly decreased in intensity as the amount of HCl increased gradually form 5 to $20 \,\mu$ L. Further addition of HCl in dye solution led to hypsochromic shift of absorption band. These changes were due to the deprotonation and protonation of the main coloring compounds upon addition of NaOH and HCl, respectively (Chairat *et al.*, 2004; Etxabide *et al.*, 2021). It was also observed that the dye solution loses its ability to reverse color in extreme alkaline solution. This might be due to degradation of coloring compound of dye in extreme alkaline condition.

3.3 Color change of lac dye in response to aldehydes

Figure 5 shows the change in absorbance ratio A_{528}/A_{488} with time at different concentrations of benzaldehyde and lac dye. The color of the dye solution changed from purple to orangered when exposed to benzaldehyde vapor. Figure 5(a) shows that as the molarity of benzaldehyde solution increased, the time required to change the color of dye solution (0.1%)decreased. Similar trends [Figure 5(b) and (c)] were observed for dye solutions of higher concentrations (0.2% and 0.3%). This may be due to the fact that at constant temperature when the molarity of solution increases vapor pressure also increases (Raoult's law). The vapor pressure determines the number of banzaldehyde molecules that are able to react with the dye (Kim et al., 2018). Similarly, as the dye concentration increased, time required to change the color of dye also solution increased. Absorbance ratio decreased as the benzaldehyde vapor reacted with dye solution. This may be due to reduction in pH of dye solution on reaction with benzaldehyde leading to protonation of dye molecules. Benzaldehyde is an α -hydrogen aldehyde. It shows the color change of dye solution by Cannizzaro reaction (Morrison and Boyd, 1992). The color change reaction can be described by equation (4):

$$R - CHO + OH^{-} \leftrightarrow R - COOH + R - OH + R - COO^{-}$$
(4)

Rate of change in absorbance ratio of dye solution were obtained from the zero and first-order kinetic model fitting. Results of the model fitting are shown in Table 1. Zero order model was found better ($R^2 = 0.93-0.99$) to describe the change in absorbance ratio with time at different benzaldehyde and dye concentration. Reaction rate constant (k) was found to increase with increase in concentration of ammonia and decreased with increase in dye concentration.

For some food products (such as fruits), it is an indicator of ripeness (Kim *et al.*, 2018). Sensitivity of lac dye to benzaldehyde suggests that lac dye-based indicators can be used for ripeness monitoring of such foods in intelligent packaging system.

3.4 Color change of lac dye solution in response to carbon dioxide

Figure 6 shows the change in absorbance ratio A_{528}/A_{488} with time at different flow rates of CO₂. The color of the dye solution changed from purple to orange-red upon reaction with CO₂. Figure 6(a)–(c) shows that, as the flow rate of CO₂ increased, the time required to change the color of dye solution (0.5%) decreased. Similar trends were observed for dye solutions of higher concentrations (1% and 1.5%). Absorbance ratio decreased as the CO₂ reacted with dye solution. This may be attributed to reduction in pH of dye solution is due to formation of carbonic acid. The color change reaction can be described by equations (5) and (6):

$$\mathrm{CO}_2 + \mathrm{H}_2\mathrm{O} \leftrightarrow \mathrm{H}^+ + \mathrm{HCO}_3^-$$
 (5)

$$[\mathrm{H^{+} In^{-}}] + [\mathrm{H^{+} + HCO_{3}^{-}}] \leftrightarrow \mathrm{HIn} + \mathrm{H_{2}CO_{3}} \qquad (6)$$

Rate of change in absorbance ratio were obtained from the zero and first-order kinetic model fitting. The results of model fitting are listed in Table 2. The first-order model was found to be better ($R^2 = 0.86-0.97$) for describing the change in absorbance ratio with time at different dye concentrations and CO₂ flow rates. The reaction rate constant (k) increased with increase in flow rate of CO₂ and decreased with increasing dye concentration.

Carbon dioxide (CO_2) is generally known to be produced as a result of respiration of fruits and vegetables and during microbial growth (Chen *et al.*, 2018; Nopwinyuwong *et al.*, 2010). Sensitivity of lac dye to CO_2 suggests that lac dye-based indicators can be prepared for quality monitoring of such food in intelligent packaging system.

 Table 1
 Parameters of kinetic model for color change of dye in response to benzaldehyde

Dye concentration (%)	Benzaldehyde concentration (mol/L)	Zero-order reaction kinetics				First-order reaction kinetics			
		k	A ₀	R ²	RMSE	k	A ₀	R ²	RMSE
0.1	3	-0.02409	1.19	0.9946	0.0064	-0.02266	1.194	0.9924	0.0076
	4	-0.02806	1.185	0.9885	0.0089	-0.0261	1.188	0.986	0.0097
	5	-0.03892	1.168	0.974	0.0143	-0.03697	1.171	0.9693	0.0156
0.2	3	-0.01715	1.282	0.9315	0.0240	-0.01471	1.284	0.9155	0.0267
	4	-0.02356	1.275	0.9588	0.0199	-0.0205	1.278	0.9452	0.0229
	5	-0.03242	1.243	0.9874	0.0118	-0.02965	1.249	0.984	0.0133
0.3	3	-0.01615	1.289	0.9644	0.0179	-0.01393	1.293	0.9512	0.0211
	4	-0.02032	1.275	0.9654	0.0189	-0.01787	1.279	0.9527	0.0222
	5	-0.02444	1.239	0.991	0.0097	-0.02259	1.246	0.9843	0.0128
Source: Authors' own work	< C								





Source: Authors' own work

3.5 Color change of lac dye in response to alcohols, ketones and sulfur compounds

A color change check was performed for alcohols, ketones, sulfur compounds and oxygen in unionized and ionized form of dye solution. No color change in response to the abovementioned metabolites was observed. Alcohols are amphoteric, implying that they can act as both acids and bases. However, alcohols are weaker acids than water. This suggests that the dye solution is a better proton donor (i.e. a stronger acid) than the alcohol. Therefore, the color of the dye solution did not change at a basic pH. Alcohols can also act as Brønsted bases. This is due to the presence of unshared electron pairs on oxygen, which make them proton acceptors. However, alcohols are weak bases that do not react with weak acids. Therefore, it did not change the color of the dye solution at acidic pH. A similar concept of Brønsted acids and bases is applicable for ketones and sulfur compounds. Similar results of no color change in response to alcohol and ketone were observed by (Kim et al., 2018) for methyl red, bromocresol purple, bromocresol green and methyl orange dyes.

3.6 Validation of dye for strawberry quality monitoring

Different quality attributes (headspace CO₂ concentration, TSS and firmness) were analyzed for spoilage detection of strawberry. Figure 7 shows that headspace CO_2 concentration increased, whereas TSS and firmness decreased during storage. The strawberries spoiled after five days at $25 \pm 1^{\circ}$ C where the headspace CO2 concentration, TSS and firmness were $7.13 \pm 0.56\%$, 3.93 ± 0.12 °Brix and 3.65 ± 0.75 N, respectively. The increase in the headspace CO₂ concentration during storage was attributed to high respiration rate of strawberry. High respiration rate had also increased the water transpiration, which resulted in reduction in firmness. Reduction in the TSS can be explained by hydrolysis of sugars to keep normal respiration in the senescence process of strawberries (Zhao, 2019). The color of indicator label was purple at Day 0 and turned orange at Day 5 when strawberry gets spoiled (Figure 8). The color change of the indicator was easily distinguishable by naked eye.

4. Conclusion

In the present study, the color change kinetics of lac dve in response to food spoilage metabolites was investigated in detail to explore potential application of dye in intelligent food packaging systems. Color change kinetics of the dye solution in response to benzaldehyde and carbon dioxide was studied in terms of change in absorbance ratio (A_{528}/A_{488}) with time using zero order and first-order reaction kinetics. Color change of lac dye solution in response to alcohols, ketones and sulfur compounds was also observed by taking representative of each group. Color change of dye solution in response to benzaldehyde followed zero-order kinetics, whereas first-order model was found appropriate for carbon dioxide. No color change of the dye solution was observed for alcohols, ketones and sulfur compounds. Reaction rate constant was found to be largely dependent on the concentration of spoilage metabolite and dye. The results of this study were validated to monitor the quality of packaged strawberries during storage. Changes in the color of indicator labels were correlated with the quality attributes (headspace CO₂ concentration, TSS Color change kinetics of lac dye

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Dye concentration (%)	Carbon dioxide flow rate (l/min)	Zero-order reaction kinetics				First-order reaction kinetics			
		k	A ₀	R ²	RMSE	k	A ₀	R ²	RMSE
0.5	0.2	-0.001835	1.256	0.973	0.0192	-0.001645	1.26	0.9753	0.0184
	0.5	-0.005515	1.251	0.9441	0.0569	-0.005194	1.258	0.9628	0.0464
1	0.2	-0.001644	1.258	0.9702	0.0249	-0.001529	1.267	0.9751	0.0228
	0.5	-0.002994	1.218	0.837	0.0627	-0.002852	1.226	0.8631	0.0574
1.5	0.2	-0.001586	1.228	0.902	0.0415	-0.001525	1.239	0.9208	0.0373
	0.5	-0.002662	1.211	0.8381	0.0641	-0.002618	1.222	0.8654	0.0584
Source: Authors' own work									

 Table 2
 Parameters of kinetic model for color change of dye in response to carbon dioxide

Figure 7 Changes in quality parameters and headspace CO₂ concentration of strawberry during storage



Source: Authors' own work

Figure 8 Color change of the indicator during strawberry spoilage



Source: Authors' own work

and firmness) of strawberries. During storage, the headspace CO_2 concentration increased from 3.23% to 7.13% as the strawberries spoiled. Similarly, TSS and firmness decreased from 6.6 °Brix to 3.9 °Brix and 7.38 N to 3.65 N, respectively. The color of the indicator changed from purple to orange when the strawberry spoiled.

The sensitivity of lac dye to benzaldehyde and carbon dioxide suggests that the dye can be used as a sensing reagent in spoilage or ripeness detection of fruits and vegetables in intelligent food packaging systems.

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