PLANT SCIENCE TODAY
ISSN 2348-1900 (online)
Vol 11(sp4): 01–07
https://doi.org/10.14719/pst.5574

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RESEARCH ARTICLE

Application of santalin dye extracted from *Pterocarpus santalinus* (red sandalwood) as a natural colorimetric indicator for real-time monitoring of spoilage in ready-to-cook idly batter

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OPEN ACCESS

ARTICLE HISTORY

Received: 06 October 2024
Accepted: 15 October 2024
Available online
Version 1.0 : 16 December 2024

Check for updates

Additional information

Peer review: Publisher thanks Sectional Editor and the other anonymous reviewers for their contribution to the peer review of this work.

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Ranjita M, Balakrishnan M, Ramalakshmi A, Thirupathi V, Rammohan S, Rinshana P F. Application of santalin dye extracted from *Pterocarpus santalinus* (red sandalwood) as a natural colorimetric indicator for real-time monitoring of spoilage in ready-to-cook idly batter. Plant Science Today.2024;11(sp4):01-07. https://doi.org/10.14719/pst.5574

Abstract

The growing demand for natural and eco-friendly solutions in food quality monitoring has sparked significant interest in using natural dyes as sustainable alternatives to chemical dyes for detecting food freshness. In this study, a colorimetric indicator based on natural santalin dye was developed for real-time monitoring of spoilage in ready-to-cook (RTC) idly batter. Santalin, extracted from *Pterocarpus santalinus* (red sandalwood), is a stable red pigment renowned for its antioxidant, antimicrobial, and anti-inflammatory properties. The santalin dye was immobilised in a polyvinyl alcohol-methylcellulose matrix and applied to cellulose paper, creating a low-cost pH-sensitive colorimetric indicator label. The developed indicator label displayed a visible colour change from dark brown to lighter brown hues in response to increased carbon dioxide concentrations, corresponding with a decrease in pH from 5.3 to 4.0 and a rise in titratable acidity (TA) from 0.5% to 0.9 % during the spoilage of RTC idly batter. The colour changes were captured and converted into grayscale images for pixel intensity analysis using MATLAB. The results revealed a consistent decline in mean pixel intensity as fermentation and spoilage progressed, demonstrating the effectiveness of the santalin-based indicator. This novel, natural santalin dye-based indicator offers a promising alternative to synthetic indicators, addressing safety concerns such as chemical migration into food. It can thus serve as an "on-package sticker" for real-time monitoring of spoilage in RTC idly batter.

Keywords

santalin; red sandalwood; natural indicator; colorimetric label; RTC idly

Introduction

Natural dyes derived from plant sources are gaining increased attention for their use in intelligent food packaging, particularly as non-toxic and biodegradable colorimetric indicators for real-time monitoring of food spoilage. These indicators change colour in response to chemical reactions and microbial activity associated with food spoilage (1).

Chemical pH dyes such as methyl red, bromocresol purple, bromocresol green, and phenol red have been widely used as indicators in intelligent food packaging for numerous food products, including seafood, dairy, fruits, vegetables, meat, poultry, and kimchi (2–6). However, a significant issue with chemical colorimetric indicators is the potential leaching or migration of chemicals into food when the dye comes into direct contact with it. This concern can be mitigated by using natural dyes extracted from plants to develop safer, non-toxic colorimetric indicators.

Numerous studies have reported the development of natural colorimetric indicators as a reliable technology for monitoring food freshness. While various natural dyes have been investigated for their colorimetric properties, such as anthocyanin (7), curcumin (8), alizarin (9), betalain (10), shikonin (11), and phycocyanin (12), our comprehensive literature review reveals that santalin dye has not yet been studied as a colorimetric indicator.

Santalin is a red pigment extracted from the heartwood and bark of *Pterocarpus santalinus*, commonly known as red sandalwood. This plant is part of the Fabaceae family and is native to the southern regions of India, predominantly found in the tropical dry deciduous forests of Andhra Pradesh, Tamil Nadu, and parts of Karnataka. Santalin has high chromatic properties and contains bioactive compounds known for their stability, antioxidant, anti-inflammatory, antimicrobial, anti-cancer, and anti-diabetic properties (13).

The dye is extracted from the heartwood and bark using various extraction techniques such as microwave-assisted, ultraviolet, ultrasound, and gamma radiation. It primarily consists of two pigments: Santalin A [6- (3.4-dihydroxy benzyl) - 2.1 O-dihydroxy-5- (4-hydroxy-2-metho -xyphenyl) -1,3-dimethoxybenzo[a]xanthen-9-one] and Santalin B [corresponding 6- (4-hydroxy-3-methoxybenzyl) derivative] (14). These anthraquinones impart a deep red colour to the dye, which is known for its stability and resistance to fading, making it a popular choice for colouring food and pharmaceuticals. Commercially, santalin dye is widely used in the textile, pharmaceutical, cosmetic, and food industries as food colorant (15). However, its potential as a colorimetric indicator in intelligent food packaging has not been explored.

Santalin-based indicators are non-toxic and biodegradable, offering a safer alternative to synthetic dyes, which may pose health risks due to chemical migration into food. Additionally, these natural indicators demonstrate comparable sensitivity and specificity in detecting spoilage while being environmentally friendly, addressing consumer demand for safer food packaging solutions.

This study aims to explore the use of santalin as a natural colorimetric indicator to monitor food freshness or spoilage. A novel colorimetric indicator label was developed by blending dye with polyvinyl alcohol and methylcellulose, using cellulose paper as the base material, and applying the solvent coating method. The developed indicator label was tested for its ability to monitor spoilage in ready-to-cook idly batter.

Materials and Methods

Materials

Red sandalwood (RSW) bark samples were collected from the Forest College and Research Institute, Tamil Nadu Agricultural University, Mettupalayam, India (Fig. 1). Rice and black gram were bought from a local market in Chennai, Tamil Nadu, India. The chemicals used in the study, including sodium hydroxide, hydrochloric acid, polyvinyl alcohol, methylcellulose, polyethylene glycol 400 and the pH indicator dye bromocresol green, were sourced from Thermo Fisher Scientific, Chennai, India. The 12µPET/60µPE (PET outer, PE inner) and PLA film were supplied by ITC Packaging and Printing Division, Chennai, Tamil Nadu, India.

Fig 1. Bark of *Pterocarpus santalinus* (red sandalwood).

Preparation of plant material

The red sandalwood bark was collected, cleaned, and shade-dried to remove moisture. The dried material was then powdered using an electrical mortar, and the resulting powder was sieved to remove coarse particles. The fine powder was collected and stored in an airtight container until further use.

Aqueous extraction of santalin dye

The aqueous extraction of RSW bark was conducted using a magnetic stirrer at 85 rpm. For the extraction, 10 g of the airdried bark powder was mixed with 100 mL of distilled water. The mixture was stirred at 70°C for 6 h, as aqueous extraction preserves bioactive compounds, minimizes chemical degradation, and ensures controlled temperature extraction (70°C). After extraction, the solution was filtered through muslin cloth. The resulting extract was stored at 5°C in airtight containers to prevent oxidation and enhance stability (Fig. 2) (16).

Fig 2. Santalin dye extract solution stored at 5°C.

Preparation of indicator label

The santalin colorimetric indicator was developed using a modified solvent coating method (17). One millilitre of santalin extract solution was mixed with 30 mL of 10% polyvinyl alcohol and stirred on a magnetic stirrer at 40°C for 30 min to ensure homogenous mixing of the dye. The desired coating solution was prepared by combining 20 mL of the indicator solution with 3% methylcellulose (as a binder) and 1% polyethylene glycol 400 (as a plasticizer). The pH of the mixture was adjusted to 7 ± 0.5 using NaOH solution and homogenised at 700 ± 10 rpm. PEG and methylcellulose served as binding agents, enabling controlled dye release in response to pH changes, which enhanced the indicator's sensitivity and longevity.

The homogenised solution was then degassed for 10 min, and the prepared coating solution was applied twice onto pure cellulose paper using a coating rod. Cellulose paper was selected as the substrate due to its cost-effectiveness and wide availability. Its porous structure allows for effective dye absorption and retention, facilitating clear colour changes. The coated paper was dried in a hot air oven at 40°C for 4–5 h. The prepared indicator label was stored in a dark place until use.

The indicator label was cut into 10 × 10 mm squares and affixed to a semi-transparent polylactic acid layer (as a background) using transparent cellophane tape. These labels were then placed on top of the packaging to monitor the spoilage of idly batter stored at 30°C.

Preparation and storage of idly batter

Rice and black gram were soaked for 5–6 h, and the soaked mixture was ground in a 3:1 ratio with water added at 1.5 times the initial weight. Additionally, 2% salt (w/w) was incorporated during grinding to prepare the ready-to-cook (RTC) idly batter. The freshly prepared batter, weighing 500 ± 5 g, was packaged in polyethylene terephthalate/ polyethylene (PET/PE) film (250 mm × 150 mm) with a total packaging volume of 1300 mL. The film used had a water vapor transmission rate (WVTR) of 10 $g/m^2/day$.

The freshly prepared batter samples were then subjected to analysis, with fermentation occurring at a controlled temperature of 30°C. Each package was stored vertically to prevent the batter from contacting the inner walls, maintaining an initial headspace volume of 850 mL. The natural colorimetric indicator was placed in the headspace of each package. Fermentation was monitored at regular intervals, with spoilage analysis conducted every 4 h for samples undergoing fermentation at 30°C. The temperature was selected to replicate practical conditions that consumers might encounter when storing these products at home or in retail settings.

UV-Vis spectra measurement of santalin extract

The colour characterisation of the santalin extract (SE) solution was conducted using a UV-Visible spectrophotometer (UV-1800, SHIMADZU, Japan). The absorption spectra of SE solutions with pH values ranging from 4.0–11.0 were recorded in the 200–500 nm range (Fig. 3). 1 mL of SE was dissolved in 30 mL of distilled water, and the pH was adjusted to 4–11 using 0.1 N HCl and NaOH solutions. Distilled water was used as the blank, and the SE solution across the pH range of 4 to 11 was scanned using the UV-Vis. The pH of the SE solutions was measured using a digital pH meter (Mettle Toledo, India) (16).

Headspace gas composition of RTC idly batter

The headspace gas composition within the packaged ready -to-cook (RTC) batter was analysed using a gas analyser (CheckMate II, PBI Dansensor, Denmark). Before taking measurements, the instrument was calibrated with a standard atmosphere. For each sample, the analyser's probe was inserted into the package's headspace to quantify the gaseous components, which were expressed as percentages by volume (4).

pH and titratable acidity RTC idly batter

The fermentation process of the ready-to-cook (RTC) idly batter was monitored at 4 h intervals until spoilage was observed, with pH and titratable acidity (TA) as the primary parameters. A 10 g sample of the RTC idly batter was homogenised with 100 mL of distilled water and subjected to centrifugation at 5000 rpm for 20 min. The resulting supernatant was then used for subsequent analysis. The pH of the supernatant was measured using a calibrated pH meter (Mettle Toledo, India), which was standardized before each use with deionized water and pH 4, 7, and 9 buffer solutions. To determine titratable acidity, aliquots of the supernatant were titrated against 0.1 N sodium hydroxide solution, using phenolphthalein as the endpoint indicator. The total acidity of the batter was then determined using Equation 1 (18).

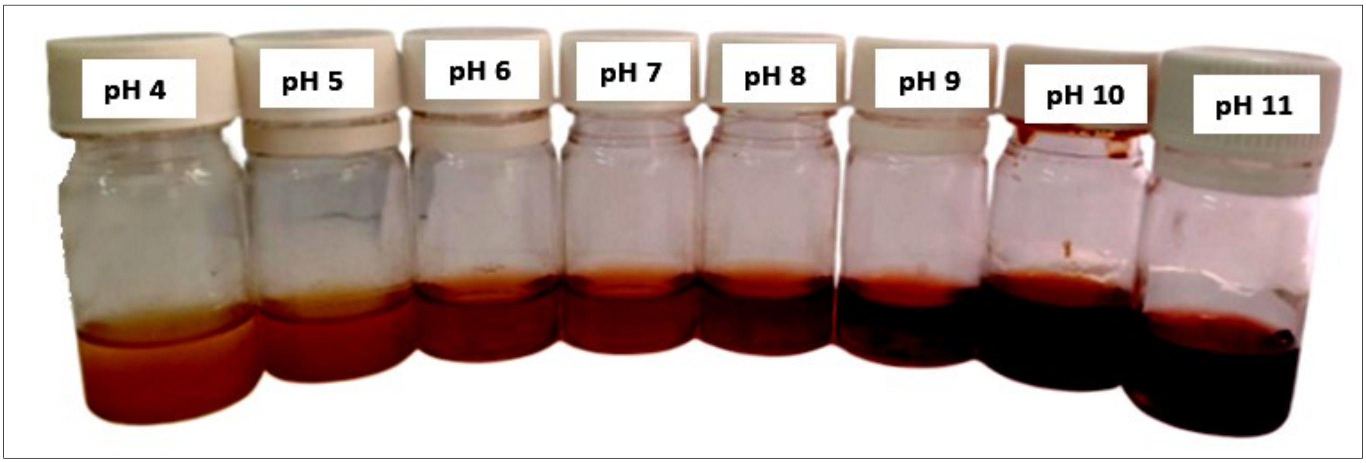

Fig 3. Santalin extract solutions with varying pH values of 4.0 – 11.0.

Titratable acidity (%) =

$$\frac{\text{Equivalent weight of acid x N of NaOH x Titre value}}{\text{Weight of the sample x 1000}} \times 100$$

.........(Eqn. 1)

Scanning electron microscopy analysis of label

The surface morphology of the pure cellulose paper and the colorimetric indicator label coated with santalin dye was analysed using a scanning electron microscope (SEM; Quanta 250 FEI, Czech Republic) at an accelerating voltage of 10 kV and a magnification of 1000X. Before the analysis, the samples were coated with gold (20 nm) using a sputter coater (19) .

Colour change of the indicator label for monitoring spoilage of RTC idly batter

The prepared indicator label was attached to the headspace of the RTC idly batter package. The colour change of the developed indicator label was monitored throughout the fermentation period until spoilage occurred. Images of the colorimetric indicator labels were captured in natural lighting using a Galaxy A34 smartphone every 4 h until spoilage was observed. Multiple replicate samples were analysed at each time point. The captured RGB images of the labels were converted to grayscale using MATLAB (version R2024a, MathWorks, USA). The mean pixel intensity was calculated using Equation 5 (20).

$$\Delta R = |R_0 - R_1| \text{.........(Eqn. 2)}$$

$$\Delta G = |G_0 - G_1|| \text{.........(Eqn. 3)}$$

$$\Delta B = |B_0 - B_1|| \text{.........(Eqn. 4)}$$

$$S = \frac{\Delta R + \Delta G + \Delta B}{R_0 + G_0 + B_0} \text{ X } 100\%$$

......(Eqn. 5)

Where, the initial RGB colour components of the sample are denoted as R_0, G_0, and B_0, while R_1, G_1, and B_1 represents the colour changes in the indicator label in response to fluctuations in headspace CO_2 concentration and pH levels within the stored RTC idly batter.

Statistical analysis

The experiments were statistically analysed using one-way analysis of variance (ANOVA) in Microsoft Excel 2016 (Windows 10.0) and SPSS 25.0 (SPSS Inc, Il, USA). The experimental data were conducted in triplicate (n=3). The mean and standard deviation ($p<0.05$) were calculated using Duncan's multiple-range test (SPSS 25.0). The quality of fit was assessed using the coefficient of determination R^2. Image analysis of the colorimetric indicator label was performed with MATLAB R2024a (MathWorks, USA).

Results and Discussion

UV-Vis colour characterisation of santalin dye extract

The spectrophotometric analysis of the santalin dye extracted from red sandalwood at varying pH levels revealed significant shifts in the absorption maxima and colour transitions, which can be attributed to changes in the molecular structure under acidic and basic conditions. The UV -visible absorption spectra (Fig. 4) demonstrated distinct absorbance peaks at different pH values, reflecting the dye's sensitivity to pH variations. At pH 4, the dye exhibited high absorbance at around 350 nm, corresponding to a yellowish hue, which can be attributed to the protonation of santalin molecules. As the pH increased to 5 and 6, the absorbance peaks shifted slightly to longer wavelengths, and the colour transitioned from yellow to a more orange tint, indicating gradual deprotonation and structural rearrangement of the dye molecules, consistent with the behaviour of other natural pigments, such as anthocyanins (20).

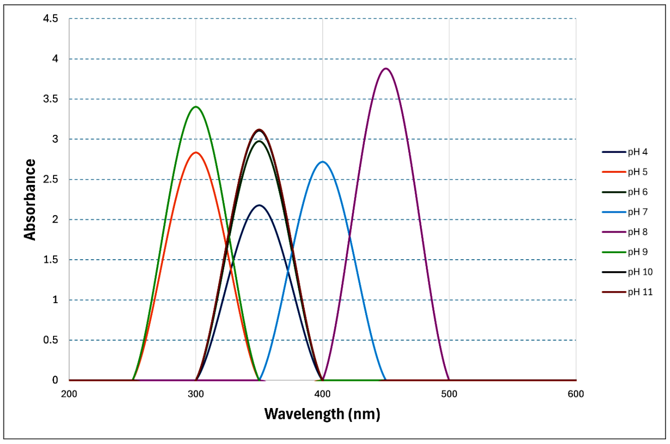

Fig. 4. Absorbance peaks of santalin extract solutions across different pH values of 4.0–11.0.

At neutral pH 7, the dye displayed a further shift towards a brown tint in the absorption spectrum, peaking at 400 nm with a significant color change to red, marking a notable transition. This suggests the formation of a stable quinoidal base, typical of santalin and other natural anthraquinonoid dyes in near-neutral conditions. The red hue is characteristic of the quinoidal form of the pigment, which is also observed in other pH-sensitive natural dyes, such as carminic acid and anthocyanins (21).

At pH 9–11, the absorption peak shifts to the visible region around 300–350 nm, where the dye solution appears dark brown to nearly red. This shift is likely due to further deprotonation and the formation of quinoidal anhydride structures, which are less stable at higher pH levels. The absorbance decreases beyond pH 10, indicating the onset of santalin degradation under strongly alkaline conditions, a phenomenon also observed in the degradation pathways of anthocyanins and other polyphenolic compounds in alkaline media (22). These shifts result from changes in electron delocalisation within the santalin molecule as protons are added or removed from key functional groups. The hydroxyl (OH) group on the aromatic ring in the santalin structure loses their hydrogen in basic conditions, resulting in a negative charge on the oxygen ($-O^-$). In acidic conditions, these groups remain protonated. The carbonyl groups (C=O) become protonated at the oxygen in highly acidic conditions, forming an $-OH^+$group. Consequently, the santalin dye exhibits a visible colour change in both alkaline and acidic conditions, making it suitable for use as a pH-sensitive colorimetric indicator.

Change in headspace CO2 concentration of RTC idly batter

The headspace concentrations of oxygen (O_2) and carbon dioxide (CO_2) (%, v/v) in ready-to-cook (RTC) idly batter stored at 30°C were monitored over time until spoilage and correlated with the colour change in the santalin dye-based indicator (Fig. 5). Initial, the oxygen (O_2) concentration in the headspace was approximately 19.8%, but it steadily declined to 0% by the 30th h, showing a strong linear relationship with time, as indicated by a high correlation coefficient (R^2 = 0.965). In contrast, the carbon dioxide (CO_2) concentration increased progressively from 0% to 50.65% over the same period, with a similarly strong correlation to time (R^2 = 0.9356). Oxygen was fully depleted and CO_2 levels surpassed 50% at around 28 h of fermentation. This complete oxygen depletion indicates the onset of anaerobic conditions, which promote rapid microbial growth and eventual spoilage. The significant rise in CO_2 concentration marks the end of the optimal fermentation phase and the beginning of spoilage. As the CO_2 concentration increases, it reacts with the developed indicator to form carbonic acid, which alters the pH inside the RTC batter package and influences the colour change of the indicator label.

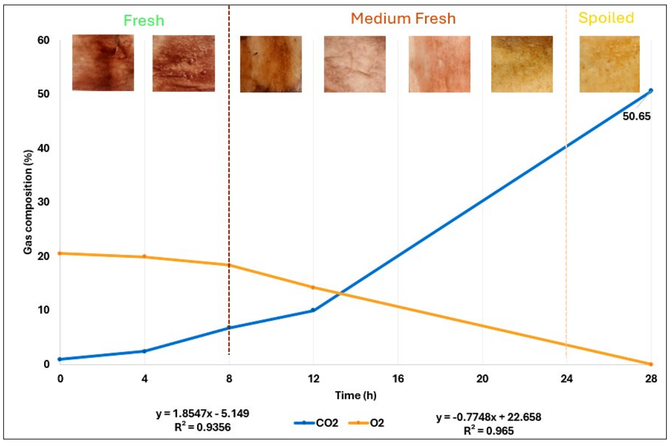

Fig. 5. Gas concentration (%, v/v) of oxygen (O_2) and carbon dioxide (CO_2) in ready-to-cook (RTC) idly batter stored at 30°C.

Initially, CO_2 levels rise slowly during the first 4–6 h of fermentation. Afterward, there is a steady increase in CO_2 concentration until spoilage. During fermentation, the lactic acid bacteria in the batter metabolize carbohydrates, producing lactic acid and CO_2 as byproducts. This process leavens the batter and increases the acidity, which is essential for the development of flavor and texture in idly. However, excessive CO_2 levels results in undesirable flavor and texture, making the idly sour and unpalatable (23).

Effect of pH and titratable acidity of RTC idly batter on colour change of developed indicator label

The changes in pH and TA of the idly batter during storage were correlated with the colour change in the developed santalin dye-based indicator label, as shown in Fig. 6. The spoilage point of the RTC idly batter was determined through sensory evaluation based on appearance, smell, touch and taste. The pH of the RTC idly batter decreases from 5.3 ± 0.04 to 4.0 ± 0.02 at a storage temperature of 30° over 28 h, transitioning from fresh to spoiled batter. Correspondingly, the TA increased from 0.55% to 0.9%. This inverse relationship between pH and TA is strongly supported by high correlation coefficients (R^2 = 0.93 and R^2 = 0.96, respectively), indicating the typical lactic acid fermentation process occurring within the batter.

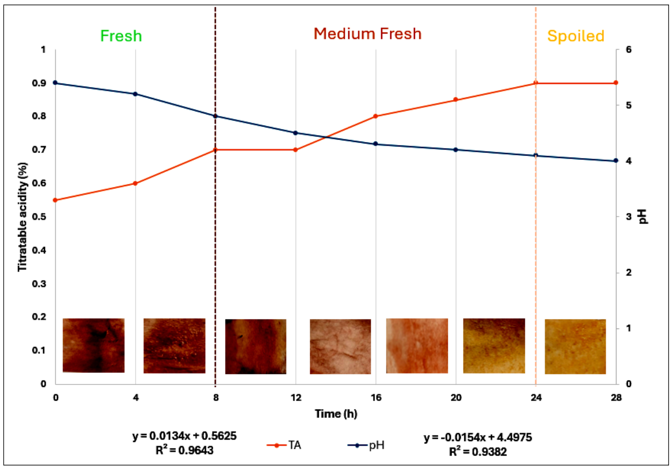

Fig. 6. Correlation of colour changes of the indicator label with pH and titratable acidity of RTC idly batter at 30°C.

As fermentation progresses, lactic acid bacteria (LAB) such as *Leuconostoc mesenteroids* and *Streptococcus thermophilus* proliferate and convert carbohydrates into lactic acid. This breakdown occurs through the LAB's glycolysis metabolic pathway, where glucose is converted into pyruvate, generating a small amount of ATP. Pyruvate is further converted into lactic acid and CO_2 through lactic acid fermentation (24). The accumulation of lactic acid in the batter causes the pH of the batter to decrease, contributing to the souring and spoilage of the batter.

Surface morphology of colorimetric indicator

The surface morphology of pure cellulose paper before and after coating with the santalin dye indicator solution is depicted in Fig. 7. The microstructure of the paper plays a crucial role in its interaction with the indicator solution. Before coating, the cellulose paper displays a fibrous structure with numerous voids (Fig. 7A). However, after coated with the dye, the surface becomes smoother with fewer voids due to the deposition of dyes (Fig. 7B). SEM images confirm that the porous structure of the cellulose paper facilitates effective dye absorption, resulting in smoother surfaces post-coating. This enhances dye retention and improves the indicator's sensitivity to pH changes.

Image processing of colour change of indicator labels

The RGB values of the developed colorimetric indicator labels were recorded at regular intervals during the fermentation process until the spoilage of the ready-to-cook (RTC) idly batter (Fig. 8). The images were converted from RGB to grayscale using MATLAB, and the mean pixel intensity was calculated to track colour changes in the indicator, which ranged from 0 to 255 (darkest to lightest). Initially, the mean pixel intensity of the indicator was 97.30 ± 0.5 (dark brown) during the early stages of batter freshness

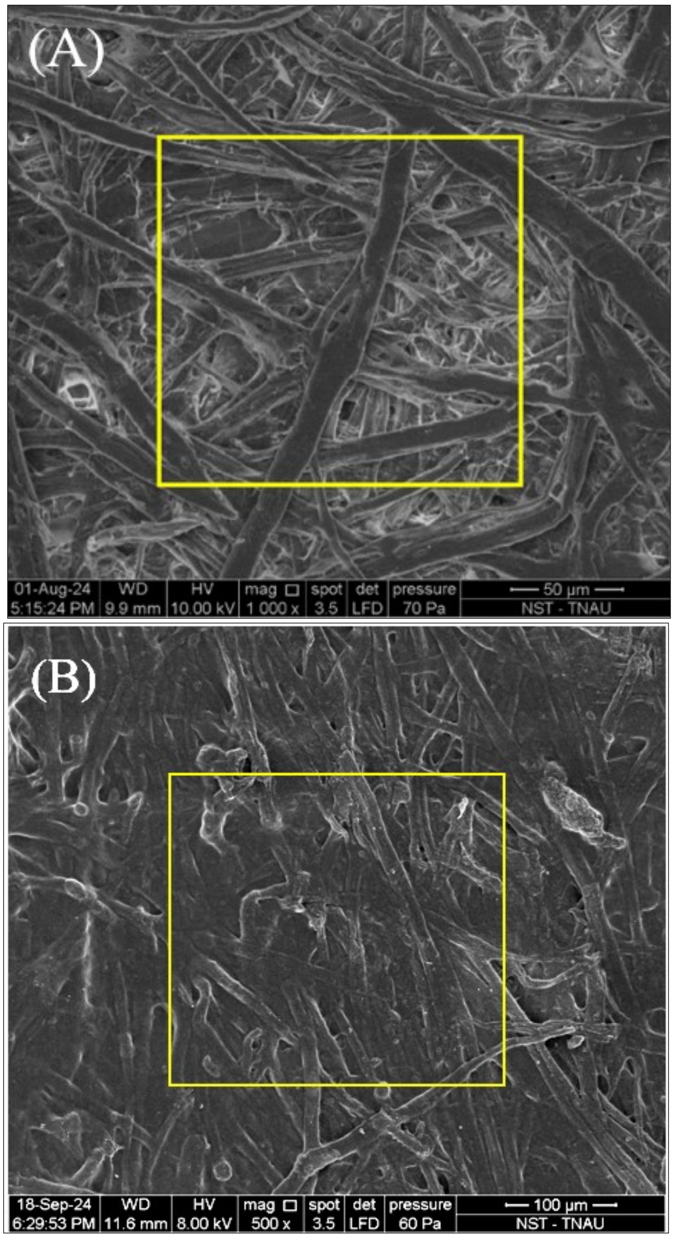

Fig. 7. SEM surface morphology showing (**A**) Pure cellulose paper with more void space (**B**) Indicator coated on pure cellulose paper with less void space and smoother surface.

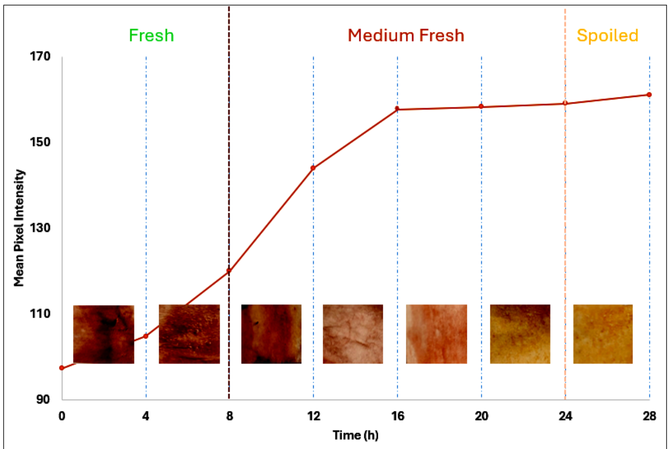

Fig. 8. Mean pixel intensity of the developed natural colorimetric indicator label at different fermentation time.

and gradually increased to 161.05 ± 0.43 (light brown) as the batter reached spoilage. This reduction in intensity corresponds with similar studies where indicators effectively monitored the spoilage process through visible colour shifts (25). The decline in pixel intensity over time signifies a transition from darker to lighter shades, correlating with the batter's decreasing pH and increasing acidity.

The colour intensity change of the developed indicator demonstrated that santalin displayed comparable pH sensitivity to other natural dyes, such as anthocyanins, but with improved stability in neutral conditions. Unlike anthocyanins, which degrade more rapidly when exposed to light and high temperatures, santalin exhibited greater stability range over a broad range of conditions with reduced degradation under moderate conditions.

However, the developed santalin indicator has some limitations. Its specificity to fermented RTC idly batter may restrict its use with foods that spoil through different mechanisms. Additionally, the study's duration was relatively short, and the long-term stability of the indicator was not fully explored. Higher temperatures may accelerate the indicator's colour change, and high humidity could affect the integrity of the cellulose paper substrate. Further research with a variety of food products and storage conditions is required to extend the indicator's potential applications.

Conclusion

A natural santalin dye-based colorimetric indicator was developed to monitor the real-time freshness and spoilage of RTC idly batter. The indicator reacts to decreasing pH and increasing acidity, displaying a colour change from dark (indicating fresh batter) to light (signaling spoiled idly batter). This cost-effective, eco-friendly label can be applied as an on-package sticker for fermented food products, providing a simple and reliable tool for both consumers and the food industry. Its potential can be further explored for other fermented foods, dairy products, or meat packaging, and for large-scale commercial applications. Future research could focus on combining the santalin indicator with other natural dyes to create a more versatile colour-changing system applicable to a broader range of food products.

Acknowledgements

The authors thank the Department of Food Process Engineering, Tamil Nadu Agricultural University, Coimbatore, for the support in carrying out the research work. The authors also acknowledge ITC Limited Packaging and Printing Division, Chennai, Tamil Nadu, for their guidance and support in extending the facility to carry out the research work. The authors also acknowledge the Reliance Foundation for Postgraduate Scholarship.

Authors' contributions

MR carried out the experiment, took observations, analysed and interpreted the data and drafted the manuscript. MB guided the research by formulating the research concept, project administration, and editing the final manuscript. AR contributed by guiding the formal analysis, data interpretation and reviewing the manuscript. VT helped in revising the manuscript. SR helped formulate the conception and methodology. PFR helped in data analysis and editing of the manuscript. All authors read and approved the final manuscript.

Compliance with ethical standards

Conflict of interest: Authors do not have any conflict of interests to declare.

Ethical issues: None

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