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# Paper Substrate Optimization for Colorimetric Formaldehyde Detection Using Crude Betacyanin from Red Dragon Fruit Peel

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**ABSTRACT:** Red dragon fruit (*Hylocereus polyrhizus*) peel (RDFP) is an abundant agro-industrial by-product rich in water-soluble halochromic pigments (betacyanins and anthocyanins) applicable as natural colorimetric indicators for detecting illicit formaldehyde (formalin) in food. This study aimed to determine the optimal filter paper substrate for fabricating an RDFP pigment-based test strip and evaluate its sensitivity and applicability in food matrices. Five cellulose substrates—plain filter paper and ashless Whatman Grades 1, 40, 41, and 42—were immersed in crude acidified RDFP extract (1:2 w/v in 2% citric acid, macerated for 72 h) and evaluated for pigment absorption intensity using the digital cyan, magenta, yellow, and key/black (CMYK) magenta (M) parameter. Sensitivity was tested across standard formalin solutions (0%, 7%, 17%, 27%, and 37%), and validated against potassium permanganate ($KMnO_4$) in spiked sausages and commercial foods (tofu, wet noodles, meatballs, and salted fish). Quantitative data were analyzed via one-way ANOVA and Duncan's Multiple Range Test ($p < 0.05$). Whatman Grade 41 exhibited the highest magenta absorption intensity (55.0 ± 5.15) and concentration-dependent sensitivity ($p \leq 0.05$), reliably detecting formalin down to 17% in spiked sausages and identifying formalin contamination in commercial tofu and salted fish in full agreement with $KMnO_4$ testing. Whatman Grade 41 impregnated with crude RDFP extract provides a biodegradable, low-cost, and portable strip test for rapid food safety screening.

## 1. INTRODUCTION

Formaldehyde is a highly reactive low-molecular-weight aldehyde extensively utilized across industrial sectors as a chemical precursor for synthetic resins, a surface disinfectant, and a biological preservative (Crous and Haarhoff, 2011; Sun et al., 2024). In its aqueous solution form (37–40% w/v), commonly known as formalin, formaldehyde exhibits potent antimicrobial activity by cross-linking amino and sulfhydryl groups in microbial proteins, which has led to its illicit misuse as an adulterant to extend the shelf life of perishable foods. However, the addition of formalin to food products is strictly prohibited worldwide due to its severe acute and chronic toxicological hazards (Dembele et al., 2024; Sun et al., 2024). Human exposure to formaldehyde occurs via inhalation, dermal contact, and the ingestion of contaminated food items (Kotsiri et al., 2023; Sun et al., 2024). Chronic ingestion of formaldehyde-adulterated food induces gastrointestinal mucosal irritation, hepatotoxicity, neurotoxicity, and genotoxic DNA–protein cross-linking. Consequently, the International Agency for Research on Cancer (IARC) classifies formaldehyde as a Group 1 human carcinogen (Sun et al., 2024). These public health concerns underscore the urgent need for practical, sensitive, economical, and field-deployable analytical methods to strengthen routine food safety surveillance.

Conventional instrumental methods for formaldehyde determination, such as high-performance liquid chromatography (HPLC) and ultraviolet–visible (UV–Vis) spectrophotometry, offer high analytical accuracy, selectivity, and quantification capability (Dembele et al., 2024; Hasrianti et al., 2024). Nevertheless, their routine implementation is often constrained by the need for specialized laboratory instrumentation, multi-step sample preparation, hazardous chemical reagents, and trained analysts (Sayyad et al., 2024; Seebunrueng et al., 2024). These limitations pose a practical challenge for decentralized routine

screening, particularly in traditional markets and resource-limited environments. As an accessible alternative, colorimetric detection methods provide a straightforward approach by utilizing visible color transitions in response to the target analyte (Hama et al., 2024; Wardani et al., 2024). Colorimetric responses can be inspected directly by the naked eye or quantified using digital color-reading applications, thereby supporting rapid and objective preliminary screening systems (Conrado et al., 2025). In this context, natural pigment-based colorimetric sensors are particularly attractive because they integrate green analytical chemistry principles with the sustainable valorization of locally available biological resources (Carpena et al., 2023; Luo et al., 2023).

One promising natural pigment source for colorimetric sensor development is the peel of red dragon fruit (*Hylocereus polyrhizus*). Red dragon fruit peel (RDFP) is an abundant by-product of fruit processing that remains underutilized despite containing bioactive water-soluble pigments, notably nitrogenous betacyanins and phenolic anthocyanins (Nizori et al., 2020; Ash Shiddiqi and Karisma, 2021; Fitriyani et al., 2022). These pigments impart intense red-to-magenta coloration and exhibit halochromic optical properties that enable their use as indicators of chemical microenvironment changes (Basílio et al., 2021; Sousa et al., 2022). When formalin is introduced into the pigment extract or adsorbed onto an indicator strip, its intrinsic acidity—resulting from the oxidation of aqueous formaldehyde into formic acid—alters the local proton equilibrium and microenvironment around the immobilized chromophores, modifying their chemical state and visible absorption spectrum (Takemoto et al., 2002; Basílio et al., 2021; Liao, 2022). Furthermore, recovering natural pigments from RDFP adds value to agricultural waste while reducing organic disposal burdens. Utilizing crude acidified pigment extracts is also advantageous from a process efficiency standpoint because it avoids costly and solvent-intensive purification steps (Nizori et al., 2020; Sulistyorini et al., 2022). However, crude extracts contain a complex matrix of pigments, pectins, and organic acids that can influence color intensity, stability, and sensor reproducibility (Havlikova and Mikova, 1987; Nizori et al., 2020). Consequently, the compatibility between the crude extract and the solid carrier matrix must be carefully optimized.

In the development of paper-based colorimetric sensors, the choice of cellulose substrate is a critical factor governing analytical performance (Li and He, 2011; Zhang et al., 2017). Filter paper serves not only as a physical support for reagent immobilization but also regulates extract absorption, pigment distribution, drying uniformity, analyte–reagent mass transfer, and visual readability (Li and He, 2011; Sayyad et al., 2024). Differences in substrate physical properties—including thickness, pore size, capillary absorptivity, fiber composition, and surface topography—can produce significantly different color intensities even at identical pigment loadings (Li and He, 2011; Zhang et al., 2017). Previous studies have demonstrated that plant-derived pigments can be immobilized onto paper-based analytical devices to monitor chemical adulterants and food quality indicators (Shukla et al., 2016; Suryadnyani et al., 2021; Chen et al., 2024). For formaldehyde detection, substrate selection must ensure high pigment retention, distinct color contrast, and consistent responsiveness upon analyte contact without interference from ambient substrate variations (Fauziyah et al., 2024; Seebunrueng et al., 2024). Although the use of dragon fruit peel extracts for qualitative formalin testing has been reported in preliminary liquid and paper assays (Sulistyorini et al., 2022), a systematic evaluation comparing multiple filter paper grades immobilized with crude RDFP pigment extract for quantitative colorimetric formaldehyde sensing has not been thoroughly established. Therefore, this study aimed to optimize the filter paper substrate for colorimetric formaldehyde detection using crude betacyanin/anthocyanin pigment extract from red dragon fruit peel (*Hylocereus polyrhizus*), evaluate its analytical sensitivity across graded formalin concentrations, and validate its application for screening formalin in spiked and commercial food products.

## 2. MATERIALS AND METHODS

### 2.1 Preparation of Crude Pigment Extract

Fresh red dragon fruit (*Hylocereus polyrhizus*) peels displaying uniform magenta pigmentation were obtained from a commercial retailer in Semarang, Central Java, Indonesia. The peels were washed thoroughly with deionized water, diced into uniform fragments (5 × 5 mm), and homogenized using a commercial blender (HR2221/00; Philips, Amsterdam, Netherlands). The homogenized peel mass was weighed on an analytical balance (FSR-A220; Fujitsu, Tokyo, Japan) and extracted via solid–liquid maceration at a raw material-to-solvent ratio of 1:2 (w/v) using distilled water acidified with 2% (w/v) analytical-grade citric acid (Nizori et al., 2020). The extraction vessel was wrapped entirely in aluminum foil (Klinpak, Jakarta, Indonesia) to maintain dark conditions and incubated at ambient temperature (25 ± 2 °C) for 72 h. Following maceration, the resulting slurry was filtered through coarse cheesecloth to obtain a clear crude pigment filtrate exhibiting a deep reddish-purple to violet appearance

(**Figure 1**). The filtrate was stored in tightly sealed, light-impermeable plastic containers in a refrigerator at 4 °C (Polytron 211 S; PT Hartono Istana Teknologi, Kudus, Indonesia) prior to strip immobilization.



**Figure 1.** Visual appearance (deep reddish-purple to violet coloration) of the crude betacyanin/anthocyanin pigment extract obtained from red dragon fruit (*Hylocereus polyrhizus*) peel after 72 h dark maceration in acidified distilled water.

### 2.2 Fabrication of Test Strips

Test strip fabrication was conducted following the procedure described by Sahria et al. (2021) with minor modifications. Five distinct cellulose filter paper substrates were evaluated: qualitative plain filter paper (nominal pore size 11 µm) and ashless quantitative filter papers Whatman Grade 1 (11 µm), Whatman Grade 40 (8 µm), Whatman Grade 41 (20–25 µm), and Whatman Grade 42 (2.5 µm) (Cytiva Life Sciences, Marlborough, MA, USA). Paper sheets were cut into uniform rectangular strips (2.0 × 1.0 cm) and immersed completely in the crude RDFP pigment extract for 90 min at 28–35 °C. Following saturation, the strips were drained and air-dried in a dust-free enclosure under ambient dark conditions for 6 h.

### 2.3 Paper Color Absorption Intensity Testing

The pigment color intensity absorbed onto each filter paper grade was quantified digitally using the Color Picker from Image application based on the cyan, magenta, yellow, and key/black (CMYK) color space model. The magenta (M) channel value (%) was selected as the primary quantitative analytical parameter corresponding to the reddish-magenta chromophore of the RDFP extract. Each dried test strip was positioned on a flat, neutral surface under standardized diffuse illumination and photographed at a fixed working distance across five independent replicates.

### 2.4 Analytical Sensitivity Evaluation

Analytical sensitivity testing was performed on the optimal paper substrate selected from the absorption capacity assay by immersing the strips in graded standard formalin solutions, referring to the immersion protocol of Kotsiri et al. (2023) with modifications. Standard aqueous formalin solutions were prepared via serial dilution at concentrations of 37%, 27%, 17%, 7%, and 0% (deionized water control). Test strips were dipped into each standard formalin solution, and the resulting colorimetric transitions were photographed under standardized lighting. The intensity of the pink-to-magenta coloration developed on each strip was quantified by measuring the magenta (M) value in the CMYK color space using the Color Picker from Image application.

### 2.5 Validation in Food Matrices and Market Screening

Validation of the optimized RDFP test strips in food matrices was performed alongside a conventional potassium permanganate ($KMnO_4$) colorimetric reference test (Suryadnyani et al., 2021; Hasrianti et al., 2024). First, to establish the practical detection limit in a complex protein–lipid matrix, commercial sausage samples were deliberately soaked in formalin solutions at concentrations of 0%, 17%, 27%, and 37% for 8 h. Second, market screening was conducted on six commercial retail food products commonly susceptible to formalin adulteration—white tofu (Tofu 1 and Tofu 2), wet yellow noodles (Wet noodle 1 and Wet noodle 2), meatballs, and salted fish—collected from retail vegetable vendors in the Tembalang district, Semarang, Indonesia, via accidental sampling. For sample preparation, 5 g of each food sample was ground homogeneously using a

porcelain mortar and pestle, mixed with 10 mL of distilled water, and filtered to collect the aqueous sample filtrate in a glass test tube.

Formaldehyde screening was performed in triplicate by immersing the optimized RDFP test strip into the aqueous sample filtrate and comparing the result against a parallel aliquot reacted with 1 drop of 0.1 N $KMnO_4$ solution (Suryadnyani et al., 2021; Hasrianti et al., 2024). Food samples were classified as positive for formaldehyde when the RDFP test strip developed a distinct pink-to-magenta coloration and the $KMnO_4$ reference solution decolorized from purple to clear or pale brownish-yellow. Concordance between the RDFP strip response and the $KMnO_4$ reference assay was recorded qualitatively and documented photographically.

### 2.6 Statistical Analysis

Quantitative data obtained from the filter paper pigment absorption capacity assay and the formalin concentration sensitivity assay were expressed as mean ± standard deviation (SD) and analyzed statistically using one-way analysis of variance (ANOVA) in IBM SPSS Statistics for Windows (Version 26.0; IBM Corp., Armonk, NY, USA) at a 95% confidence level ($p < 0.05$). Whenever a significant main effect was detected ($p \leq 0.05$), post-hoc multiple comparisons among treatment means were conducted using Duncan's Multiple Range Test (DMRT). Data from the spiked sausage validation and commercial retail food screening assays were analyzed descriptively and qualitatively.

## 3. RESULTS AND DISCUSSION

### 3.1 Absorption Capacity of Filter Paper Substrates to Crude RDFP Pigment

One-way ANOVA revealed that the type of filter paper substrate significantly influenced the absorbed magenta color intensity ($p \leq 0.05$; **Table 1**). Quantitatively, Whatman Grade 41 (W41) achieved the highest magenta color intensity (55.0 ± 5.15), which was statistically comparable to qualitative plain filter paper (54.2 ± 3.56, $p > 0.05$) but significantly higher than Whatman Grade 1 (45.0 ± 6.89), Whatman Grade 40 (42.6 ± 4.16), and Whatman Grade 42 (40.8 ± 4.10) ($p < 0.05$). Visually, W41 produced a deep, intense magenta coloration and plain filter paper yielded a homogeneous pink surface, whereas the finer-pore quantitative grades (W1, W40, and W42) displayed noticeably paler pink hues (**Figure 2**). Because W41 provided both the highest quantitative pigment uptake and superior ashless matrix purity compared with ordinary qualitative paper, W41 was selected as the optimal substrate for subsequent analytical sensitivity and food validation experiments.

**Table 1.** Absorption capacity of evaluated filter paper substrates impregnated with crude pigment extract from red dragon fruit peel (RDFP).

| Filter paper substrate | Nominal pore size (µm) | Color intensity (M, %) | Visual color appearance |
|---|---|---|---|
| Plain filter paper | 11.0 | 54.2 ± 3.56$^{a}$ | Homogeneous pink |
| Whatman Grade 1 (W1) | 11.0 | 45.0 ± 6.89$^{b}$ | Light pink |
| Whatman Grade 40 (W40) | 8.0 | 42.6 ± 4.16$^{b}$ | Light pink |
| Whatman Grade 41 (W41) | 20.0–25.0 | 55.0 ± 5.15$^{a}$ | Dark pink / intense magenta |
| Whatman Grade 42 (W42) | 2.5 | 40.8 ± 4.10$^{b}$ | Pale pink |

Note: Data are presented as mean ± standard deviation ($n = 5$). Different superscript letters ($^{a,b}$) within the same column indicate statistically significant differences among filter paper types according to Duncan's Multiple Range Test ($p < 0.05$). RDFP: red dragon fruit peel; M: CMYK magenta channel intensity.

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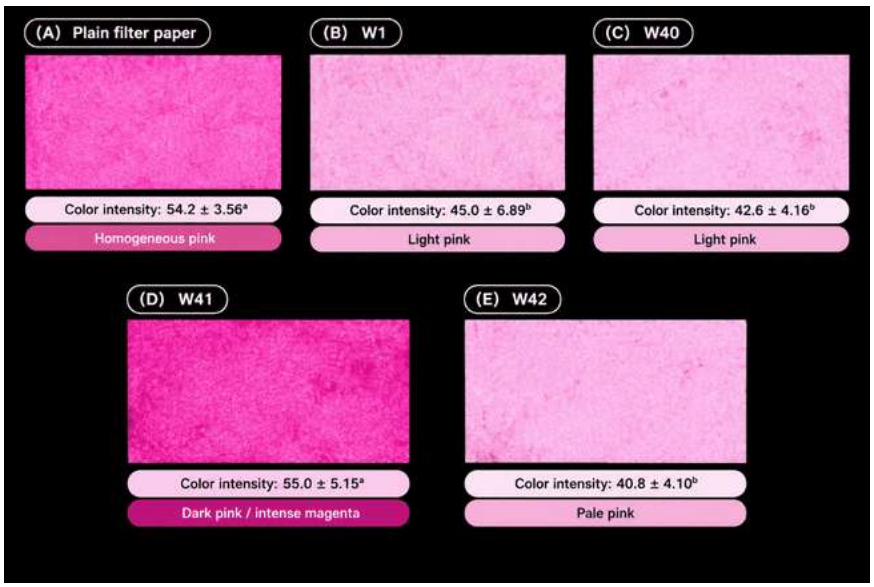


**Figure 2.** Visual comparison of crude pigment absorption from red dragon fruit peel across five filter paper substrates: (A) plain filter paper; (B) Whatman Grade 1 (W1); (C) Whatman Grade 40 (W40); (D) Whatman Grade 41 (W41); and (E) Whatman Grade 42 (W42).

### 3.2 Analytical Sensitivity of Whatman Grade 41 Test Strips to Formalin Solution

Based on its superior pigment absorption capacity, Whatman Grade 41 (W41) was evaluated for its colorimetric sensitivity across standard aqueous formalin concentrations of 0%, 7%, 17%, 27%, and 37% (**Table 2**). Visually, the magenta-pink color of the W41 test strip intensified progressively with increasing formalin concentration and faded markedly as formalin concentration decreased toward 0% (**Figure 3**). Quantitative digital color analysis confirmed that formalin concentration exerted a highly significant effect on the magenta color intensity of the W41 test strip ($p \leq 0.05$), with DMRT post-hoc analysis distinguishing all five concentration levels into statistically distinct groups ($^{a–e}$). Specifically, color intensity rose from 21.4 ± 1.67 at 0% formalin (pale faded pink) and 37.2 ± 2.86 at 7% formalin (faint pink) to a sharp, clearly discernible pink at 17% (62.8 ± 2.68), moderate pink at 27% (68.8 ± 2.86), and concentrated magenta at 37% (73.4 ± 5.13). Because 17% formalin marked the threshold at which an unmistakable visual pink transition (>60% magenta intensity) occurred relative to the faded control, 17% was identified as the reliable visual detection threshold for the unmodified crude strip.

**Table 2.** Quantitative colorimetric sensitivity of Whatman Grade 41 test strips across graded concentrations of standard formalin solution.

| Formalin concentration (%) | Color intensity (M, %) | Visual color appearance |
|---|---|---|
| 0 | 21.4 ± 1.67$^{e}$ | Pale pink / faded |
| 7 | 37.2 ± 2.86$^{d}$ | Faint pink |
| 17 | 62.8 ± 2.68$^{c}$ | Distinct pink |
| 27 | 68.8 ± 2.86$^{b}$ | Moderate pink |
| 37 | 73.4 ± 5.13$^{a}$ | Concentrated magenta |

Note: Data are presented as mean ± standard deviation ($n = 5$). Different superscript letters ($^{a–e}$) within the same column indicate statistically significant differences among formalin concentrations according to Duncan's Multiple Range Test ($p < 0.05$). M: CMYK magenta channel intensity.

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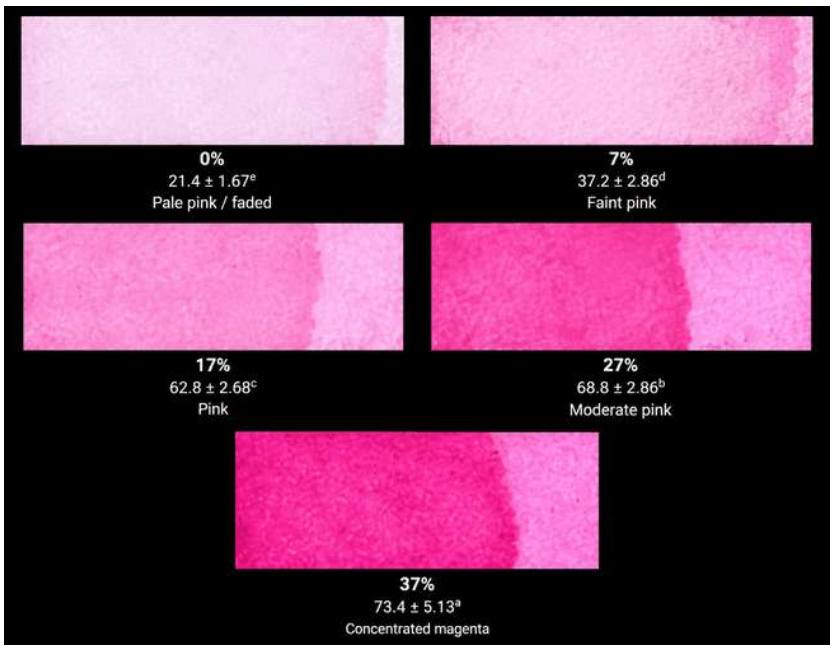


**Figure 3.** Visual color transitions and quantitative CMYK magenta intensity values of Whatman Grade 41 test strips following immersion in standard formalin solutions at concentrations of 0%, 7%, 17%, 27%, and 37%.

### 3.3 Application of Test Strips for Detecting Formaldehyde in Spiked and Commercial Foods

To verify analytical performance in a complex food matrix, the optimized W41 RDFP test strip was evaluated on sausage samples deliberately soaked in 0%, 17%, 27%, and 37% formalin solutions alongside the standard $KMnO_4$ redox indicator (**Table 3**; **Figure 4**). In unspiked control sausages (0% formalin), the RDFP test strip exhibited no pink chromophoric development, while the $KMnO_4$ solution retained a purplish-red hue, confirming the absence of formaldehyde. Conversely, in sausages spiked with 17%, 27%, and 37% formalin, the RDFP test strip consistently developed a positive pink-to-intense magenta response that intensified proportionally with formalin concentration. Parallel reactions with $KMnO_4$ corroborated these findings, showing progressive oxidative reduction of permanganate from light brown (17%) and pale faded brown (27%) to a completely decolorized clear solution (37%) (**Figure 4**).

**Table 3.** Comparative detection of formaldehyde in formalin-spiked sausage samples using Whatman Grade 41 RDFP test strips and standard $KMnO_4$ reagent.

| Formalin concentration (%) | RDFP strip visual response | $KMnO_4$ solution reaction | Test interpretation |
|---|---|---|---|
| 0 | No pink coloration | Purplish red | Negative |
| 17 | Distinct pink | Light brown | Positive |
| 27 | Moderate pink | Pale brownish / faded | Positive |
| 37 | Intense magenta | Clear / decolorized | Positive |

Note: RDFP: red dragon fruit peel; $KMnO_4$: potassium permanganate.

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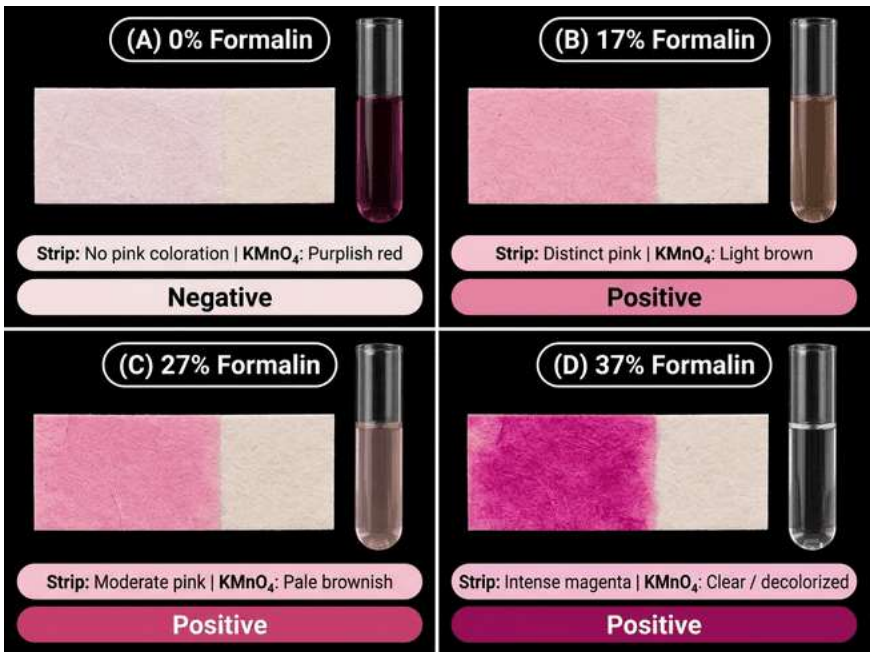


**Figure 4.** Comparative detection of formalin in spiked sausage extracts using Whatman Grade 41 RDFP test strips (left panels) and standard $KMnO_4$ colorimetric solution (right panels) across formalin concentrations: (A) 0%; (B) 17%; (C) 27%; and (D) 37%.

Subsequently, the RDFP test strips and $KMnO_4$ reference reagent were applied to screen six commercial retail food products collected from local markets (**Table 4**; **Figure 5**). Three of the six retail samples—Tofu 1, Tofu 2, and Salted fish—tested positive for formaldehyde adulteration, as evidenced by a distinct pink coloration on the RDFP test strip accompanied by complete decolorization (clear solution) in the $KMnO_4$ assay. In contrast, Wet noodle 1, Wet noodle 2, and Meatball samples tested negative: the RDFP strips did not transition to bright pink but instead retained a faded violet-purple appearance, while the $KMnO_4$ indicator shifted only to reddish-brown or dark brown due to mild interaction with endogenous food matrices rather than rapid aldehyde reduction. The 100% qualitative agreement between the W41 RDFP strip test and the $KMnO_4$ reference assay across all six commercial samples demonstrates the reliability of the natural test strip for field screening of high-risk proteinaceous foods (Hama et al., 2024).

**Table 4.** Field screening of commercial retail food products using Whatman Grade 41 RDFP test strips and $KMnO_4$ reference reagent.

| Commercial food sample | RDFP strip visual response | $KMnO_4$ solution reaction | Qualitative result |
|---|---|---|---|
| Tofu 1 | Pink coloration | Clear (decolorized) | Positive |
| Tofu 2 | Pink coloration | Clear (decolorized) | Positive |
| Salted fish | Pink coloration | Clear (decolorized) | Positive |
| Wet noodle 1 | Faded violet / purple | Dark brown | Negative |
| Wet noodle 2 | Faded violet / purple | Dark brown | Negative |
| Meatball | Faded violet / purple | Reddish brown | Negative |

Note: RDFP: red dragon fruit peel; $KMnO_4$: potassium permanganate.

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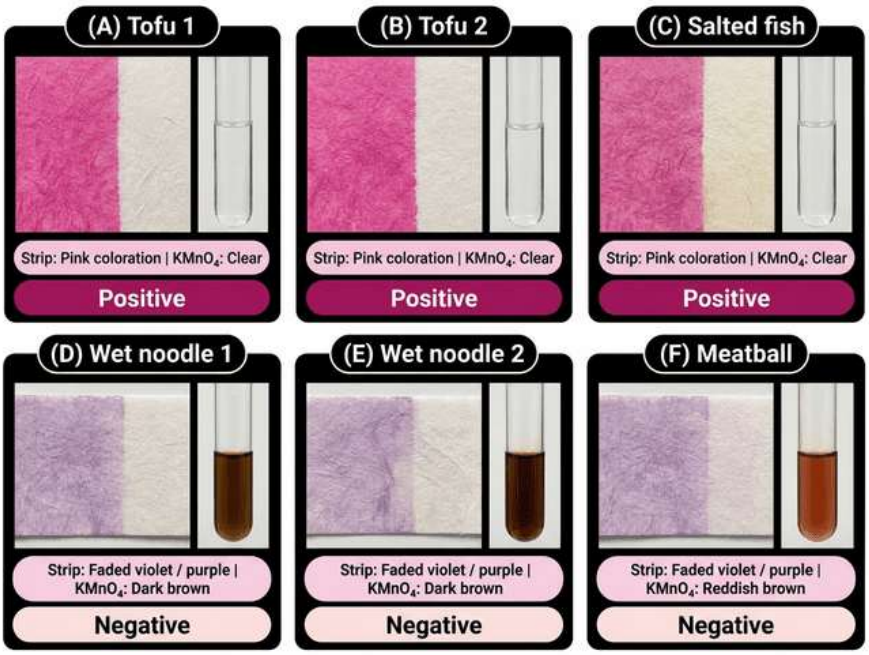


**Figure 5.** Field application of Whatman Grade 41 RDFP test strips (left panels) and $KMnO_4$ reference solution (right panels) for screening formaldehyde contamination in commercial retail food products: (A) Tofu 1; (B) Tofu 2; (C) Salted fish; (D) Wet noodle 1; (E) Wet noodle 2; and (F) Meatball.

### 3.4 Mechanism of Colorimetric Response and Substrate Performance

This study successfully extracted crude water-soluble pigments from RDFP characterized by a vivid reddish-purple coloration (**Figure 1**), which were immobilized onto cellulose substrates to assemble a user-friendly prototype formalin test kit displaying a clear magenta indicator response (**Figure 6**). The utilization of natural plant pigments—including anthocyanins and betalains/betacyanins—as solid-state colorimetric reagents has garnered substantial interest due to their biocompatibility, non-toxicity, and pronounced halochromic responsiveness (Carpena et al., 2023; Luo et al., 2023; Wardani et al., 2024). Natural pigment indicators provide a sustainable green analytical alternative to hazardous synthetic chromophores (such as pararosaniline in Schiff's reagent or acetylacetone in Nash's reagent), enabling portable, instrument-free screening of food adulterants and spoilage markers (Shukla et al., 2016; Wardani et al., 2024).

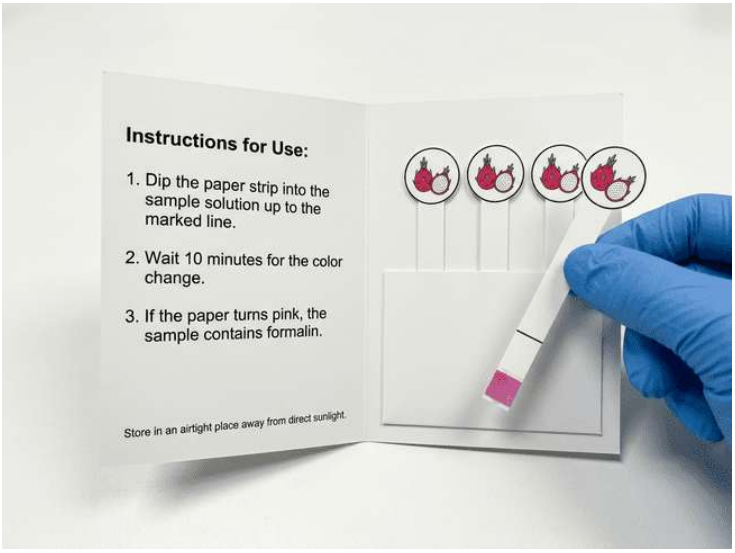


**Figure 6.** Prototype packaging design, fabricated Whatman Grade 41 test strips, and user instruction card of the red dragon fruit peel pigment-based test kit for rapid on-site formaldehyde screening.

A central finding of this investigation is that the physical microstructure of the cellulose filter paper substrate dictates pigment immobilization efficiency and subsequent colorimetric readability (**Table 1**; **Figure 2**). Although all five evaluated filter papers exhibited pink-to-magenta hues following 90 min impregnation, digital CMYK analysis revealed significantly higher pigment uptake on Whatman Grade 41 (nominal pore size 20–25 μm) and plain filter paper (11 μm) than on the tighter-pore quantitative grades W1 (11 μm), W40 (8 μm), and W42 (2.5 μm). Crude aqueous extracts of *Hylocereus polyrhizus* peel contain not only water-soluble betacyanin and anthocyanin chromophores but also high-molecular-weight pectic polysaccharides and mucilage co-extracted under acidic maceration (Nizori et al., 2020; Ash Shiddiqi and Karisma, 2021). In ultra-fine, dense cellulose matrices such as W42 (2.5 μm) and W40 (8 μm), high capillary flow resistance and steric hindrance from viscous pectic macromolecules restrict deep, uniform penetration of the chromophores into the fiber network, resulting in pale surface coloration (40.8 ± 4.10 and 42.6 ± 4.16, respectively). Conversely, the macroporous architecture and rapid wicking rate of W41 (20–25 μm) facilitate unhindered capillary transport and high volumetric loading of the viscous crude extract throughout the cellulose fibers, yielding an intense, uniform magenta surface (55.0 ± 5.15) (Li and He, 2011; Zhang et al., 2017). Furthermore, coupling substrate optimization with digital CMYK magenta channel quantification mitigates subjective visual bias and aligns with modern smartphone-assisted colorimetric sensing workflows (Conrado et al., 2025).

The colorimetric transition of the RDFP test strip upon formalin exposure is governed by the proton-dependent structural equilibrium of the immobilized natural pigments (Basílio et al., 2021; Sousa et al., 2022). In aqueous formalin solutions, dissolved formaldehyde readily undergoes autoxidation and Cannizzaro-type disproportionation to form formic acid, imparting a distinctly acidic pH (typically pH 2.8–4.0 depending on formaldehyde concentration) (Takemoto et al., 2002; Liao, 2022). Under neutral or unbuffered aqueous conditions (0% formalin control), partial hydration and deprotonation of the immobilized pigment chromophores—combined with pigment leaching into the neutral aqueous phase—cause the strip color to fade to a pale pinkish-violet (M = 21.4 ± 1.67). Conversely, when immersed in increasingly concentrated formalin solutions (7% to 37%), higher proton activity at the paper–solution interface drives the halochromic equilibrium toward the thermodynamically favored cationic flavylium state of anthocyanins (pH < 3) and stabilizes the protonated betanidin chromophore of betacyanins, resulting in progressive magenta-red intensification (M = 37.2 ± 2.86 to 73.4 ± 5.13) (Havlikova and Mikova, 1987; Basílio et al., 2021; Sousa et al., 2022). In addition, electrophysiological and nucleophilic interactions between formaldehyde carbonyl groups and electron-rich hydroxyl or amino moieties on the pigment ring system may further stabilize the reddish-magenta chromophore complex on the cellulose matrix (Hama et al., 2024). Because natural halochromic pigments exhibit broad pH transition windows, colorimetric sensitivity diminishes at low formalin concentrations (7%) as interfacial acidity approaches neutrality (Suthar and Saran, 2020; Fauziyah et al., 2024).

In practical food testing, the W41 RDFP test strip demonstrated complete qualitative concordance with the standard $KMnO_4$ oxidation assay across both spiked sausages (**Table 3**) and commercial retail products (**Table 4**). In the $KMnO_4$ reference test, formaldehyde acts as a reducing agent that reduces purple permanganate ions ($MnO_4^-$, $Mn^{+7}$) into colorless $Mn^{2+}$ or brown manganese dioxide ($MnO_2$, $Mn^{+4}$) while oxidizing the aldehyde into formic acid (Narayanan Kutty and Vasudeva Murthy, 1970; Takemoto et al., 2002). However, liquid $KMnO_4$ reagents suffer from intrinsic instability in aqueous solution, corrosiveness, potential false positives from other reducing food components, and chemical waste disposal requirements (Narayanan Kutty and Vasudeva Murthy, 1970; Suryadnyani et al., 2021). While previous natural indicator studies have focused primarily on purified anthocyanins from purple sweet potato (*Ipomoea batatas*), *Coleus* leaves (*Solenostemon scutellarioides*), butterfly pea flower (*Clitoria ternatea*), or *Ocimum sanctum* (Padri and Umponstira, 2017; Li et al., 2019; Suthar and Saran, 2020; Suryadnyani et al., 2021), the present work demonstrates that unpurified crude RDFP extract immobilized on Whatman Grade 41 filter paper provides an effective, zero-waste valorization route for dragon fruit processing by-products. Nevertheless, several limitations warrant consideration: (i) the visual detection threshold of the unmodified crude strip remains relatively high (17% formalin), (ii) strongly acidic non-formalin food additives could induce cross-reactive halochromic shifts (Fauziyah et al., 2024), and (iii) long-term storage stability of crude natural pigments against photothermal degradation requires further enhancement. Future investigations should explore co-pigmentation, buffered polymer co-immobilization, or vapor-phase headspace configurations (Seebunrueng et al., 2024) to lower the limit of detection to trace regulatory levels.

## 4. CONCLUSION

This study demonstrated that crude water-soluble pigment (betacyanin/anthocyanin) extracted from red dragon fruit (*Hylocereus polyrhizus*) peel (RDFP) can be effectively immobilized onto cellulose filter paper to fabricate a natural colorimetric test strip for

formaldehyde screening. Substrate optimization across five filter paper grades revealed that filter paper microstructure significantly governs pigment uptake ($p \leq 0.05$), with macroporous ashless Whatman Grade 41 yielding the highest magenta color intensity (55.0 ± 5.15). Quantitative sensitivity testing confirmed a statistically significant, concentration-dependent magenta intensification across graded formalin solutions (0–37%, $p \leq 0.05$), with a clear visual detection threshold down to 17% formalin. When validated in formalin-spiked sausages and commercial retail foods, the Whatman Grade 41 RDFP strip accurately detected formaldehyde adulteration in commercial white tofu and salted fish with 100% qualitative concordance against the standard $KMnO_4$ reference test. Compared with liquid $KMnO_4$ reagents, the developed RDFP test strip offers distinct advantages in user safety, biodegradability, portability, and ease of on-site operation. Further research is recommended to enhance trace-level sensitivity below 7%, evaluate selectivity against acidic food additives, and improve long-term pigment storage stability through co-pigmentation or polymer matrix encapsulation.

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## AUTHOR CONTRIBUTIONS

**Siti Susanti:** Conceptualization, Methodology, Funding Acquisition, Investigation, Writing—Original Draft.

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## CONFLICT OF INTEREST

The authors declare that there is no conflict of interest regarding the publication of this manuscript.

## ETHICS STATEMENT

Ethical approval was not required for this study as it did not involve human participants, clinical trials, or animal subjects.

## DATA AVAILABILITY STATEMENT

All data generated or analyzed during this study are included in this published article. Raw datasets supporting the findings are available from the corresponding author upon reasonable request.

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## FIGURE LEGENDS

**Figure 1.** Visual appearance (deep reddish-purple to violet coloration) of the crude betacyanin/anthocyanin pigment extract obtained from red dragon fruit (*Hylocereus polyrhizus*) peel after 72 h dark maceration in acidified distilled water.

**Figure 2.** Visual comparison of crude pigment absorption from red dragon fruit peel across five filter paper substrates: (A) plain filter paper; (B) Whatman Grade 1 (W1); (C) Whatman Grade 40 (W40); (D) Whatman Grade 41 (W41); and (E) Whatman Grade 42 (W42).

**Figure 3.** Visual color transitions and quantitative CMYK magenta intensity values of Whatman Grade 41 test strips following immersion in standard formalin solutions at concentrations of 0%, 7%, 17%, 27%, and 37%.

**Figure 4.** Comparative detection of formalin in spiked sausage extracts using Whatman Grade 41 RDFP test strips (left panels) and standard $KMnO_4$ colorimetric solution (right panels) across formalin concentrations: (A) 0%; (B) 17%; (C) 27%; and (D) 37%.

**Figure 5.** Field application of Whatman Grade 41 RDFP test strips (left panels) and $KMnO_4$ reference solution (right panels) for screening formaldehyde contamination in commercial retail food products: (A) Tofu 1; (B) Tofu 2; (C) Salted fish; (D) Wet noodle 1; (E) Wet noodle 2; and (F) Meatball.

**Figure 6.** Prototype packaging design, fabricated Whatman Grade 41 test strips, and user instruction card of the red dragon fruit peel pigment-based test kit for rapid on-site formaldehyde screening.