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Development of Sustainable Paper Based Indicator Strip for Detection of Adulteration in Milk and Fish

Ajitha A[1a], Joshwin T[1b], Raveena S[1c], Vignesh V[1d], Antony Allwyn Sundarraj[2], Daniel Paul[3], Jayarathinam S[4] and U.S. Rammiya[5]

[1a ,1b,1c&1d]Bachelor of Food Technology and [2]Associate Professor, Department of Food Technology, JCT College of Engineering and Technology, Coimbatore, TamilNadu, India.

[3]Assistant Professor, Department of Food Technology, Nehru Institute of Technology, Coimbatore, Tamil Nadu, India.

[4]Assistant Professor, Department of Food Technology, Dhanalakshmi Srinivasan College of Engineering, Coimbatore, Tamil Nadu, India.

[5]Head of the Department, Department of Food Technology, Sree Sastha Institute of Engineering and Technology, Chennai, TamilNadu, India.

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KEYWORDS

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ABSTRACT:

The increasing incidence of chemical adulteration in highly perishable foods such as milk and fish demands rapid, accessible, and environmentally sustainable detection strategies. Conventional laboratory-based analytical techniques provide high accuracy but are often impractical for routine, on-site monitoring due to cost, complexity, and time constraints. This has encouraged the exploration of biodegradable and naturally derived sensing materials for food quality assessment. This review evaluates recent advancements in anthocyanin-based paper strip developed for the detection of common milk and fish adulterants. The literature indicates that anthocyanins, due to their pH-sensitive structural transformations, enable visible colorimetric responses when exposed to chemical contaminants. When immobilized onto cellulose substrates and biopolymer matrices such as chitosan, these natural pigments demonstrate improved functionality for low-cost, disposable sensing applications. Studies report promising sensitivity toward adulterants including urea, detergents, formaldehyde, and ammonia compounds. Despite their potential, limitations such as pigment instability under light, temperature, and prolonged storage conditions remain key challenges. Ongoing research focuses on enhancing stability, improving detection limits, and integrating these indicators into intelligent packaging and portable diagnostic platforms. Overall, anthocyanin-based paper strip represents a sustainable and innovative direction in food adulteration detection, offering a bridge between laboratory precision and field-level applicability, with strong potential for future commercialization and smart food monitoring systems.

Introduction:

Food adulteration, defined as the deliberate or accidental modification of food products resulting in reduced quality and safety, remains a pressing challenge in the global food industry. This problem increasingly affects highly consumed perishable items such as milk and fish, which are integral to human nutrition due to their high protein, fat, and micronutrient content. Milk is particularly susceptible to adulteration with substances such as urea, starch, detergents, formaldehyde, and melamine, often added to mimic higher protein content or improve physical properties. Such adulteration undermines consumer health and has been extensively reviewed as a significant global food safety concern requiring advanced analytical techniques [1]. Fish, another nutrient-rich food, is often treated with chemical preservatives like formaldehyde and ammonia to delay spoilage and maintain visual freshness, posing serious toxicological and health risks. The complexity of these adulteration practices highlights the limitations of conventional analytical methods, such as chromatography and spectroscopy, which although highly accurate require specialized equipment, trained personnel, and controlled laboratory conditions, making them impractical for rapid, infield screening. Studies have demonstrated that portable techniques, including multispectral sensing devices with machine learning–based analysis, show promise for real-time milk adulteration detection, emphasizing the need for rapid and accessible analytical tools [2]. Recent research trends demonstrate a shift toward biosensor and colorimetric based approaches that offer rapid, low-cost, and user-friendly alternatives. For instance, developments in optical and electrochemical biosensors have enabled sensitive detection of various milk adulterants including pH changes, urea, formaldehyde, and nitrates while addressing challenges such as matrix interference and portability [1]. However, many of these systems still rely on synthetic reagents or complex fabrication processes, raising concerns about environmental sustainability use.

Natural pigment–based sensors, particularly those utilizing anthocyanins extracted from plant sources, have emerged as an eco-friendly alternative. Anthocyanins display pH-dependent colour changes suitable for visual detection of spoilage and adulterants in food. Their incorporation into biodegradable matrices and paper substrates has been shown to enhance practical applicability and aligns with sustainable food safety technologies documented in recent anthocyanin sensor reviews [3]. This review aims to summarize recent developments in anthocyanin-based colorimetric strip for the detection of milk and fish adulteration, focusing on materials, mechanisms, challenges, and future directions toward sustainable food quality monitoring.

2. FOOD ADULTERATION IN MILK AND FISH

2.1Milk adulteration:

Milk is one of the most widely consumed food products globally, valued for its rich nutritional profile including proteins, fats, vitamins, and minerals. However, it is also one of the most frequently adulterated commodities, particularly in regions with limited regulatory oversight. Adulteration practices are often driven by economic incentives, where cheaper substances are intentionally added to increase volume or alter perceived quality (e.g., enhanced protein content) at the expense of safety and nutrition. Common adulterants include urea, starch, detergents, formalin (formaldehyde solution), hydrogen peroxide, and water, among others. These substances are added to mask dilution, alter density, improve appearance, or falsely elevate nutritional parameters such as non-fat solids. Recent field studies have documented widespread milk adulteration, with water dilution followed by addition of detergents and urea frequently observed in market samples, underscoring the prevalence of this issue in both rural and urban contexts [4]. The health risks associated with milk adulteration can be severe. For example, urea, when consumed in excess, places additional burden on renal function and can lead to kidney damage, urinary tract obstruction, and dehydration. The presence of detergents and hydrogen peroxide can irritate the gastrointestinal tract and disrupt normal digestion, while formalin, used improperly as a preservative, is a known toxicant with potential carcinogenic effects. Chronic exposure to formaldehyde has been linked to respiratory issues, immune dysfunction, and systemic toxicity [5].

Detecting adulterants in milk poses significant challenges for food safety authorities due to the complex composition of milk and the varying chemical properties of adulterants. Conventional laboratory methods such as chromatography, spectroscopy, and chemometric techniques though accurate are time-intensive, require costly instrumentation, and are unsuitable for rapid, point-of-care testing in field conditions. Spectroscopic methods combined with machine learning have recently shown high accuracy in detecting specific adulterants like formalin, but the complexity and cost of these methods limit their widespread application in routine monitoring [6].

2.2 Fish Adulteration:

Fish is a valuable source of high-quality protein, omega-3 fatty acids, and essential micronutrients. However, it is also susceptible to adulteration and spoilage due to its perishable nature. One of the most concerning practices involves the use of formaldehyde (formalin) and ammonia to preserve fish and delay spoilage. Formalin, a solution of formaldehyde in water, is often illicitly applied to fish to maintain a fresh appearance, texture, and odor during storage and transportation, especially in regions with limited cold chain infrastructure. Formaldehyde is classified as a Class I carcinogen and can cause irritation of the eyes, skin, and respiratory tract, and prolonged exposure has been associated with

increased cancer risk [7][8]. In addition to chemical adulteration, fish undergoes biochemical changes as it spoils. Spoilage indicators such as Total Volatile Basic Nitrogen (TVB-N) and changes in pH occur due to enzymatic degradation and microbial activity. Elevated TVB-N levels, resulting from the release of ammonia and other basic compounds, are widely used as quantitative measures of fish freshness and spoilage. High TVB-N values correlate with the breakdown of proteins and accumulation of off Odors, making them key parameters in fish quality assessment [8]. The public health impact of fish adulteration and spoilage is profound. Consumption of fish treated with formalin or contaminated with high levels of spoilage compounds can cause acute gastrointestinal disturbance, respiratory irritation, allergic reactions, and long-term health consequences due to carcinogenic exposure. Furthermore, reliance on adulterated fish in low-income communities exacerbates nutritional insecurity and elevates health risks among vulnerable populations [8].

3.CONVENTIONAL DETECTION METHODS

Conventional analytical methods remain essential for confirming food adulteration and ensuring food authenticity. These techniques broadly include chemical methods and instrumental methods.

3.1 Chemical methods:

Chemical methods for adulteration detection typically involve reagent-based reactions that produce observable changes (colour, precipitate, or turbidity) when specific adulterants are present [9]. For example,

i. Sugar test in milk using HCL and resorcinol.

ii. Pulverized soap using phenolphthalein as an indicator.

iii. Benzoic acid test by sulphuric acid and ferric chloride.

iv. Salicylic acid tested by H_2SO_4 and $FeCl_3$.

While chemical methods are straightforward and cost-effective, they are generally limited to qualitative or semi-quantitative results, may not detect low concentrations reliably, and often require multiple tests to identify different adulterant types. These limitations make chemical reagent tests suitable for preliminary screening but not definitive confirmation of adulteration [9].

3.2 Instrumental Methods:

Instrumental techniques provide high precision and specificity in detecting and quantifying food adulterants, especially when complex food matrices are involved.

1.**Fourier Transform Infrared Spectroscopy (FTIR):**

FTIR detects molecular vibrations of chemical bonds, enabling the identification of specific functional groups associated with adulterants. It is rapid, non-destructive, and requires minimal sample preparation. FTIR has been applied for identifying adulterants in milk and dairy products by comparing spectral fingerprints against authentic profiles. Such spectroscopic approaches, including near-infrared (NIR) and mid-infrared (MIR), offer fast analysis and can be coupled with chemometric tools for enhanced accuracy [6][10][42].

2.**Gas Chromatography–Mass Spectrometry (GC–MS):**

GC–MS combines chromatographic separation with mass spectrometric identification, allowing detection and quantification of volatile and semi-volatile adulterants at trace levels. GC–MS is regarded as a “gold standard” for substance identification due to its high specificity and ability to characterize unknown compounds.

3. **High Performance Liquid Chromatography (HPLC):**

HPLC separates components in complex mixtures based on polarity and interaction with the stationary phase, making it suitable for non-volatile adulterants such as melamine or synthetic milk compounds. When coupled with UV-Vis or mass spectrometric detectors, HPLC can sensitively quantify adulterants even at low parts-per-million concentrations.

Other instrument-based methods, including nuclear magnetic resonance (NMR) spectroscopy and advanced imaging techniques (e.g., hyperspectral imaging), have also been explored for milk and food authentication, although these are still evolving for routine adulteration screening.

3.3 Limitations of conventional methods:

Although conventional laboratory techniques are often considered reliable and accurate, they have several notable limitations when applied to routine food adulteration detection:[10].

i. Cost and Infrastructure Requirements.

ii. Technical Expertise.

iii. Time-Consuming Procedures.

iv. Lack of Portability for Field Use.

v. Sensitivity vs Accessibility Trade-Off.

Conventional detection methods such as chemical tests, FTIR, GC–MS, and HPLC remain foundational tools for identifying and quantifying adulterants in milk and fish due to their precision and reliability. However, their high cost, operational complexity, and time requirements limit their suitability for rapid field-level screening. This underscores the need for alternative sensing systems, such as natural pigment–based paper indicators, that offer low-cost, rapid, and easily interpretable results without the need for sophisticated laboratory infrastructure.

4.NATURAL PIGMENTS IN FOOD SENSING:

Natural pigments derived from plants have gained increasing attention as eco-friendly and non-toxic colorimetric indicators in food quality monitoring. These pigments can respond to environmental changes such as pH variation, temperature, or volatile compounds produced during food spoilage, producing visible colour changes that indicate food freshness. Natural pigments are preferred over synthetic indicators due to their biodegradability, safety, and compatibility with food systems [12][13].

4.1 Classification of Natural Pigments:

Natural pigments used in food sensing can be broadly classified based on their chemical structure and colour forming mechanisms.

1. Anthocyanins:

Anthocyanins are water-soluble flavonoid pigments responsible for red, purple, and blue colours in many fruits and vegetables such as red cabbage, berries, grapes, and purple sweet potato. These pigments exhibit strong pH-dependent colour transitions, which makes them excellent natural pH indicators. At acidic pH (1–3) anthocyanins appear red, while at neutral pH (~7) they appear purple, and under alkaline conditions (pH 8–10) they turn blue or greenish due to structural transformations of the flavylium ion. Because of their visible colour transitions and sensitivity to chemical changes during food spoilage, anthocyanins are widely used in intelligent packaging and freshness indicators [14].

2. Betalains:

Betalains are nitrogen-containing water-soluble pigments found mainly in plants of the Caryophyllales family, including beetroot, amaranth, and dragon fruit.

Betalains are divided into two major groups:

- Betacyanin red to violet pigments
- Betaxanthins – yellow to orange pigments

These pigments can also respond to pH changes, allowing them to act as indicators for food freshness monitoring. However, betalains are less stable to heat and light compared with anthocyanins, which limits their application in some sensing systems [15].

3. Curcumin:

Curcumin is a natural polyphenolic pigment obtained from turmeric (Curcuma longa). It is widely used as a natural food colorant and possesses antioxidant and antimicrobial properties.

Curcumin also acts as a pH-responsive indicator. It appears bright yellow in acidic or neutral conditions (pH 3–7) and changes to orange-red or brown in alkaline conditions (pH > 8) due to deprotonation of the enolic hydroxyl group. Curcumin-based films have been used in smart packaging to detect volatile amines such as ammonia released during meat spoilage, which increase pH and trigger colour changes [16].

4. Chlorophyll:

Chlorophyll is the green pigment present in plants, responsible for photosynthesis. It consists mainly of two forms: chlorophyll a and chlorophyll b. Chlorophyll can undergo structural changes in response to pH, heat, and oxidation, forming derivatives such as pheophytin that cause colour changes from green to olive brown. While

chlorophyll can be used as a colour indicator, it is generally less sensitive to pH variations compared with anthocyanins, limiting its effectiveness as a sensing pigment in food freshness detection.

4.2 Why Anthocyanins Are Preferred:

Among all natural pigments, anthocyanins are the most widely used and studied indicators in food sensing systems.

1. Wide pH Sensitivity Range:

Anthocyanins exhibit a broad pH sensitivity range (approximately pH 1–10) with multiple visible colour transitions is shown in table 1. This wide range allows them to effectively detect changes in food environments caused by microbial growth or chemical spoilage [12][14].

Table 1: Typical colour transitions

pH Range	colour
1-3	Red
4-6	Pink/Purple
7	Violet
8-10	Blue/Green
>10	Yellow

These clear colour shifts enable easy visual detection without analytical instruments.

2. Strong Colour Visibility:

Anthocyanins produce bright and distinct colours across the visible spectrum, making them ideal for colorimetric sensing applications. Their visible transitions allow consumers to easily interpret food freshness indicators in intelligent packaging [13].

3. Simple Extraction from Natural Sources:

Anthocyanins can be easily extracted using simple solvent extraction methods from many plant materials such as:

1. Red cabbage
2. Blueberries
3. Purple sweet potato
4. Butterfly pea flower

Because these sources are widely available and inexpensive, anthocyanins are suitable for large-scale applications. Compare with Anthocyanins from other natural sources, red cabbage anthocyanins are of great interest in food packaging because, they represent an acceptable colour spectrum over a board range of pH values [43]. Brassica oleracea var capitata rubra is noteworthy due to its physiological functions and applications [44]. The colours of anthocyanins extracted from red cabbage vary from red at low pH to blue and green at high ph. Thus, this broad colour change makes it attractive for application as natural PH indicators [45].

4. Additional Functional Properties:

Besides colour indication, anthocyanins also possess antioxidant and antimicrobial properties, which can help improve food stability when incorporated into packaging materials [13].

5. Pigments:

Pigments were compared with other anthocyanins, provide wider pH responsiveness, stronger colour changes, and better suitability for smart food sensing systems, making them the most preferred natural indicator is shown in table 2.

Table 2: Comparison of various pigments colour range and stability

Pigment	pH sensitivity	Colour range	Stability
Anthocyanins	Very high	Red-purple-blue-green	Moderate
Betacaine	Moderate	Yellow-red-violet	Lower stability
Curcumin	Moderate	Yellow - orange/red	Good
Chlorophyll	Low	Green - brown	Sensitive to heat

5. Chemistry and Mechanism of Anthocyanins:

Anthocyanins are water-soluble flavonoid pigments responsible for the red, purple, and blue colours found in many fruits, vegetables, and flowers. Chemically, anthocyanins consist of an anthocyanidin aglycone linked to one or more sugar moieties, which improves their solubility and stability in aqueous systems. The fundamental chromophore responsible for the colour of anthocyanins is the flavylium cation structure, which undergoes several reversible transformations depending on environmental conditions such as pH, temperature, light exposure, and oxygen. These structural transformations produce different molecular species that result in distinct colour changes. Because of these chemical properties, anthocyanins are widely used as natural colorants and pH-sensitive indicators in food systems and intelligent packaging [17],[18].

5.1 Structure and Forms of Anthocyanins:

Anthocyanins exist in multiple structural forms in aqueous solutions, which are interconverted through reversible chemical reactions is shown in fig.1 and fig.2. The most important structural forms include:

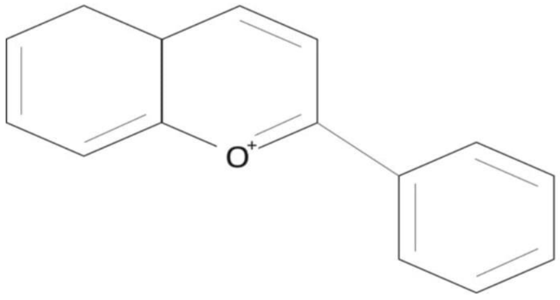

Figure 1: Structure of anthocyanins

1. Flavylium Cation:

The flavylium cation is the dominant structure under strongly acidic conditions (pH < 3). In this form, the anthocyanin molecule carries a positive charge on the oxygen atom of the central ring, producing a bright red colour. This form is considered the most stable coloured species in acidic environments [18].

2. Quinonoid Base:

When the pH increases slightly (around pH 4–6), the flavylium cation undergoes deprotonation, forming the quinonoidal base. This structure produces purple or blue coloration and contributes to the colour of many flowers and fruits [19].

3. Carbinol Pseudo base (Hemiketal):

At intermediate pH values, the flavylium ion can react with water through hydration, forming a carbinol pseudo base (hemiketal). This structure is colourless, which causes fading of anthocyanin pigments in neutral environments [20].

4. Chalcone Form:

Further structural rearrangement of the carbinol pseudo base leads to the formation of chalcone, which appears pale yellow or colourless is shown in table 3. Chalcone formation occurs through ring opening of the anthocyanin molecule [21].

Table 3: Summary of Structural Forms

Structural form	pH range	Colour
Flavylium cation	<3	Red
Quinonoidal base	4-6	Purple / blue
Carbinol pseudo base	5-7	Colourless
Chalcone	>7	Yellowish

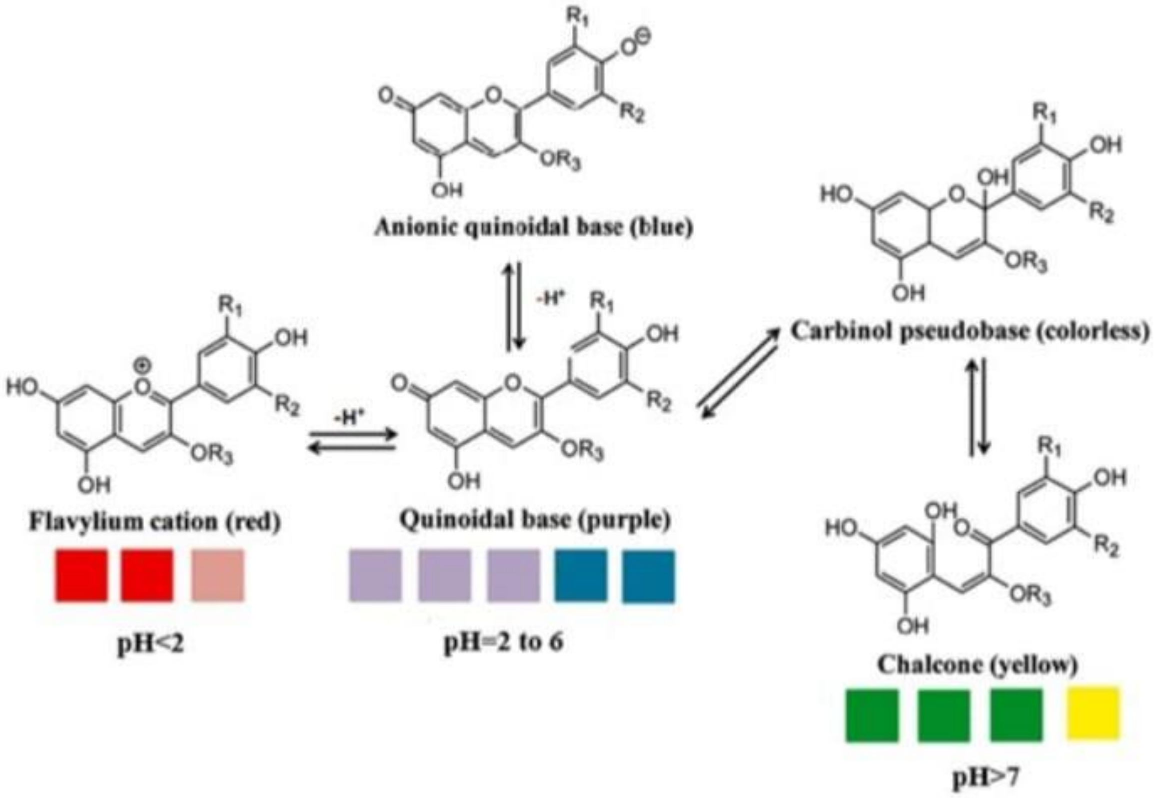

Figure 2: Anthocyanin colour mechanism

These structural transformations create the dynamic colour changes observed in anthocyanin-based indicators.

5.2 pH-Dependent Colour Transition Mechanism:

The colour changes of anthocyanins are mainly caused by protonation–deprotonation and hydration reactions of the flavylium ion.

***Acidic Conditions**

Under acidic conditions, the molecule remains in the flavylium cation state, which absorbs light in the visible spectrum around 520 nm, producing a red colour.

***Neutral Conditions**

As the pH increases, deprotonation occurs, converting the flavylium ion into the quinonoidal base. This transformation shifts the electronic structure of the molecule and changes its light absorption characteristics, resulting in purple or blue coloration [22].

***Slightly Alkaline Conditions**

At moderate pH levels, water molecules attack the flavylium ring, producing the carbinol pseudo base, which is colourless due to the disruption of the conjugated chromophore system.

***Alkaline Conditions**

Under alkaline conditions, the carbinol pseudo base undergoes ring opening to form chalcone, which appears yellowish and represents a degraded pigment form. Thus, the equilibrium among these molecular species determines the colour of anthocyanins at different pH values [18].

5.3 Stability Factors of Anthocyanins:

The stability of anthocyanins is strongly influenced by environmental conditions such as light, temperature, oxygen, and PH.

1. Effect of Light:

Exposure to light can cause photodegradation of anthocyanins, leading to fading of colour. Light energy promotes oxidation reactions that break down the anthocyanin structure, reducing pigment stability.

2. Effect of Temperature:

High temperatures accelerate hydrolysis and degradation reactions, converting anthocyanins into phenolic acids and other colourless compounds. Thermal degradation reduces both pigment intensity and stability.

3. Effect of Oxygen:

Oxygen can react with anthocyanins through oxidative reactions, especially in the presence of enzymes such as polyphenol oxidase. These reactions lead to pigment breakdown and colour loss.

4. Effect of pH:

Anthocyanins are most stable in acidic environments, where the flavylium cation predominates. As the pH increases toward neutral or alkaline conditions, anthocyanins become unstable and transform into less coloured or colourless forms [18].

6. Paper-Based Sensor Technology

Paper-based sensor technology has emerged as an innovative and low-cost platform for rapid chemical and biological detection. These sensors utilize porous paper substrates as microfluidic platforms where liquids can flow through the fibres via capillary action without external pumps. Due to their advantages such as low cost, portability, biodegradability, and ease of fabrication, paper-based sensors are widely applied in environmental monitoring, food safety analysis, and medical diagnostics. In food analysis, paper-based sensors are particularly useful because they allow visual colorimetric detection, enabling rapid identification of contaminants or adulterants without the need for sophisticated laboratory equipment [23].

6.1 Concept of Paper-Based Analytical Devices (PADs):

Paper-based analytical devices (PADs), also known as microfluidic paper-based analytical devices (μPADs), are miniaturized analytical platforms fabricated on paper substrates. These devices operate based on capillary action, which allows fluids to flow through the cellulose fibres of the paper without the need for external pumps or power sources. The concept of PADs was popularized in 2007 when researchers introduced patterned paper as a platform for low-cost bioassays. Since then, PADs have been widely used for applications such as chemical sensing, medical diagnostics, and food safety testing. PADs typically consist of hydrophilic channels and hydrophobic barriers that guide the flow of liquid samples to specific detection zones. When the analyte interacts with reagents embedded in the paper, a measurable signal such as colour change, fluorescence, or electrochemical response is produced.

The main advantages of PADs include:

i. Low manufacturing cost.
ii. Portable and disposable design.
iii. Minimal sample and reagent consumption.
iv. Rapid detection without complex instruments.

Because of these advantages, PADs are increasingly used for point-of-care testing and on-site environmental or food analysis [24].

6.2 Types of Paper Substrates:

The performance of paper-based sensors strongly depends on the type of paper substrate used. Paper substrates differ in their porosity, thickness, capillary flow rate, and chemical compatibility, which influence the sensitivity and accuracy of the sensor.

1. Whatman No.1 Filter Paper

One of the most commonly used substrates in paper-based sensors is Whatman No.1 filter paper. This cellulose-based filter paper has a uniform Fiber structure, moderate flow rate, and excellent absorption capacity, which makes it suitable for microfluidic applications [25]

Whatman No.1 paper typically has:

- Thickness: approximately 180 μm.
- Pore size: about 11 μm.

- Moderate liquid retention and capillary flow properties.

These characteristics allow the paper to efficiently transport liquid samples through the device while maintaining good reagent retention. Because of these properties, Whatman No.1 filter paper is widely used in paper-based biosensors, chromatography, and colorimetric analytical devices [26]. Several studies have demonstrated the use of Whatman No.1 paper as a substrate for microfluidic analytical devices capable of detecting microorganisms and chemical compounds through visible colour changes [28].

2. Bamboo Paper

Bamboo paper is another emerging paper substrate used in sustainable sensor fabrication. Bamboo fibres provide renewable, biodegradable, and environmentally friendly cellulose materials that can serve as an alternative to conventional filter paper.

Bamboo-derived paper possesses several advantageous properties:

i. High cellulose content.
ii. Good mechanical strength.
iii. Adequate porosity for capillary flow.
iv. Sustainable and biodegradable nature.

These characteristics make bamboo paper suitable for developing eco-friendly paper-based analytical devices. Due to its natural Fiber structure, bamboo paper can effectively absorb liquid samples and facilitate reagent interaction, enabling colorimetric detection in sensing applications.

6.3 Role of Biopolymers (Chitosan):

Biopolymers are frequently incorporated into paper-based sensors to improve stability, sensitivity, and functional performance. Among these biopolymers, chitosan is widely used because of its biocompatibility, film-forming ability, and antimicrobial properties. Chitosan is a natural polysaccharide obtained through the deacetylation of chitin, which is commonly found in crustacean shells such as shrimp and crab [29].

1. Film Formation

One of the key properties of chitosan is its excellent film-forming ability. When dissolved in acidic solutions, chitosan can form thin films or coatings on paper substrates. These films create a uniform matrix that immobilizes sensing reagents and improves the stability of the sensor. In paper-based analytical devices, chitosan coatings can enhance the mechanical stability of the paper and provide functional groups for chemical interactions [27].

2. Binding and Stabilization

Chitosan contains free amino groups ($–NH_2$) that can bind with various biomolecules such as enzymes, antibodies, and dyes through electrostatic interactions or covalent bonding. This property makes chitosan an excellent material for immobilizing sensing elements on paper substrates. By immobilizing the sensing molecules on the paper surface, chitosan improves:

- Reagent retention.
- Sensor sensitivity.
- Stability of the sensing system

Therefore, chitosan plays a crucial role in enhancing the functionality of paper-based sensors.

3.Antimicrobial Property

Another important property of chitosan is its natural antimicrobial activity. Chitosan can inhibit the growth of bacteria and fungi due to its positively charged amino groups, which interact with negatively charged microbial cell membranes. This antimicrobial activity helps to:

- Prevent microbial contamination of the sensor.
- Extend the shelf life of the device.
- Improve the reliability of the sensing system.

7.Recent Research Trends

In the past decade, research on natural pigment-based sensors—especially those using anthocyanins has expanded significantly due to the increasing demand for rapid, low-cost, and environmentally friendly food quality monitoring systems. Modern developments focus on improving sensor performance through nanotechnology, digital detection systems, and integration into intelligent food packaging. These advancements aim to enable real-time monitoring of food

freshness and enhance food safety during storage and distribution.

Recent studies highlight four major research directions: nanocomposite anthocyanin sensors, smartphone-based detection systems, intelligent packaging technologies, and commercialization potential [12][31].

7.1 Nanocomposite Anthocyanin Sensors:

Nanotechnology has become an important approach for enhancing the performance of anthocyanin-based sensors. Researchers have incorporated nanomaterials such as nanocellulose, silica nanoparticles, and cellulose nanocrystals into polymer matrices to improve sensor sensitivity, stability, and mechanical strength. For example, a recent study developed an anthocyanin-based intelligent packaging film reinforced with cellulose nanocrystals and chitosan for monitoring shrimp freshness. The nanocomposite film demonstrated improved tensile strength and reduced oxygen and water-vapor permeability while maintaining strong pH-responsive colour changes during food spoilage [32].

- Improved mechanical and barrier properties of sensor films.
- Increased colour stability and sensitivity.
- Enhanced interaction between pigments and polymer matrices.

These improvements significantly increase the practical applicability of anthocyanin-based sensors for food quality monitoring [33].

7.2 Smartphone-Based Detection:

Another emerging trend is the use of smartphone-assisted colorimetric detection systems. Instead of relying solely on visual observation, smartphone cameras and mobile applications can analyse colour changes quantitatively using RGB (Red-Green-Blue) image analysis [46].

In smartphone-based sensing, the colour change produced by a natural pigment sensor is captured using the phone camera. Image processing software then converts the colour values into numerical data, enabling more accurate detection of food spoilage [47].

Studies have shown that smartphone-assisted systems can:

- Improve measurement accuracy and sensitivity.
- Provide quantitative analysis of colour changes.
- Allow real-time monitoring using portable devices.

For instance, a plant-based freshness sensor developed for milk spoilage detection demonstrated that colour changes of natural pigments could be analysed using smartphone cameras to evaluate milk quality [48]. Additionally, smartphone-compatible colorimetric sensors have been proposed for monitoring pH changes using simple digital imaging and RGB analysis without sophisticated laboratory instruments [49].

These developments enable low-cost and user-friendly food monitoring systems accessible to consumers and food industries.

7.3 Intelligent Packaging Integration:

Intelligent packaging represents one of the most promising applications of natural pigment sensors. Unlike traditional packaging, intelligent packaging systems provide real-time information about food quality and freshness. Anthocyanins are widely used in intelligent packaging because they exhibit distinct colour transitions in response to pH changes caused by microbial spoilage. When incorporated into biodegradable polymers such as chitosan, starch, gelatine, or cellulose, these pigments form films or indicators that visually signal food deterioration. Recent research has demonstrated the integration of anthocyanin-based sensors into packaging materials for various foods such as:

i. Fish and seafood
ii. Meat products
iii. Milk and dairy products
iv. Fruits and vegetables

These packaging systems respond to spoilage compounds such as volatile amines and organic acids, producing clear colour changes that indicate the freshness status of the packaged food [37]. Such technologies allow continuous monitoring of food quality throughout the supply chain, improving food safety and reducing food waste.

7.4 Commercial Potential:

The commercial potential of natural pigment-based sensors is gaining attention due to their environmental

sustainability and consumer safety. Unlike synthetic dyes, natural pigments such as anthocyanins are biodegradable, non-toxic, and derived from renewable plant sources [14].

Commercial adoption is driven by several advantages:

i. Low-cost production using plant extracts
ii. Easy integration into biodegradable packaging materials
iii. Visible colour changes that consumers can easily interpret
iv. Compatibility with digital monitoring systems such as smartphones

In addition, intelligent packaging technologies based on natural pigments have the potential to reduce food spoilage, enhance food safety, and improve supply-chain monitoring. However, several challenges remain before large-scale commercialization, including improving pigment stability, extending shelf life of sensors, and developing standardized calibration methods [36].

8. STRIP BASED INDICATORS:

Recent advances in paper-based analytical devices (PADs) and intelligent packaging have further expanded the potential of natural pigment sensors. Integration with biopolymers such as chitosan, nanocomposite materials, has improved the sensitivity, stability, and applicability of these sensors for real-time food monitoring. Among the different sensing formats, strip-based indicators offer several advantages, which justify their selection for practical applications. Strip indicators are simple, portable, low-cost, and easy to fabricate, making them suitable for rapid on-site testing without the need for complex instrumentation. The porous structure of paper allows efficient absorption of liquid samples and rapid interaction with the sensing dye, resulting in quick and visible colour responses. In addition, strip-based indicators require only small sample volumes, making them highly convenient for routine food quality assessment. Another important advantage is their compatibility with natural pigments and biopolymers, which can be easily immobilized on paper substrates to form stable sensing matrices. These strips can also be incorporated into intelligent food packaging systems, enabling real-time monitoring of food freshness during storage and transportation. Therefore, strip-based anthocyanin indicators represent a practical, environmentally friendly, and consumer-friendly approach for rapid detection of milk spoilage and fish freshness. Their simplicity, sensitivity, and cost-effectiveness make them promising candidates for future applications in food safety monitoring, smart packaging, and point-of-care food quality testing.

9.Future Scope

Natural pigment-based sensors, particularly those utilizing anthocyanins, have shown significant potential for rapid and eco-friendly food quality monitoring. However, further research is required to improve their performance and enable large-scale commercial applications. One important area for future research is enhancing pigment stability. Anthocyanins are sensitive to environmental factors such as light, oxygen, and temperature, which can lead to degradation and reduced sensor accuracy. Researchers are exploring strategies such as microencapsulation, incorporation into nanocomposite materials, and immobilization within biopolymer matrices (e.g., chitosan, starch, and cellulose) to improve the stability and durability of anthocyanin-based sensors [38]. Another promising direction is the development of nanocomposite and hybrid sensing systems. The incorporation of nanomaterials such as cellulose nanocrystals, graphene oxide, and metal nanoparticles can enhance mechanical strength, sensitivity, and colour stability of intelligent packaging films [39]. These materials may significantly improve the reliability of natural pigment sensors for real-time monitoring of food freshness.

Future research is also focusing on digital detection technologies, particularly smartphone-based monitoring systems. Smartphone cameras combined with image-analysis applications can quantify colour changes using RGB analysis, allowing more precise evaluation of food spoilage compared to visual observation alone [40]. In addition, the integration of natural pigment sensors into intelligent food packaging systems is expected to play a major role in the future. Such packaging can continuously monitor food quality during storage and transportation, providing consumers with real-time information about product freshness and helping reduce food waste [41]. Overall, advancements in nanotechnology, biopolymer engineering, and smart packaging technologies are expected to improve the

performance and commercial viability of anthocyanin-based sensing systems for food safety monitoring.

Conclusion:

In conclusion, the reviewed literature clearly demonstrates that anthocyanin-based paper strip indicators represent a promising, sustainable, and user-friendly approach for the rapid detection of adulteration in highly perishable foods such as milk and fish. The inherent pH-responsive colour-changing properties of anthocyanins, combined with their natural origin, biodegradability, and safety, make them highly suitable for developing eco-friendly sensing platforms. When immobilized onto paper substrates and enhanced with biopolymers such as chitosan, these indicators offer significant advantages including low cost, portability, ease of use, and rapid visual detection without the need for sophisticated instrumentation. Compared to conventional analytical techniques, which are accurate but limited by cost, complexity, and lack of field applicability, anthocyanin-based strip sensors effectively bridge the gap between laboratory precision and real-time, on-site monitoring. Their demonstrated sensitivity toward common adulterants such as urea, detergents, formaldehyde, and ammonia highlights their practical relevance in food safety assurance. However, challenges related to pigment stability under environmental conditions such as light, temperature, and oxygen exposure remain a critical limitation for long-term application and commercialization. Addressing these issues through advanced material engineering approaches, including nanocomposites, encapsulation, and improved biopolymer integration, is essential for enhancing sensor durability and reliability.

Furthermore, the integration of these natural indicators into intelligent packaging systems and smartphone-based detection platforms offers exciting opportunities for real-time, quantitative, and consumer-accessible food quality monitoring. Such innovations not only improve food safety and supply chain transparency but also contribute to reducing food waste and promoting sustainable practices. Overall, anthocyanin-based paper strip indicators hold strong potential as next-generation tools in food adulteration detection, supporting the transition toward smart, sustainable, and accessible food quality monitoring systems. Continued interdisciplinary research and technological advancements will be key to overcoming current limitations and enabling their widespread adoption in both industrial and consumer applications.

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Conflicts of Interest:

The authors declare that there are no conflicts of interest associated with this publication. All opinions, analyses, and conclusions presented in this review are solely those of the authors and do not necessarily reflect the views of the funding agencies or affiliated institutions.

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