

**DEVELOPMENT OF A REAL-TIME INTELLIGENT pH SENSITIVE COLOUR
CHANGE FRESHNESS INDICATOR USING ANTHOCYANIN-BIOPOLYMER FILM
FOR FOOD APPLICATIONS**

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KERALA AGRICULTURAL UNIVERSITY

Department of Processing and Food Engineering

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**KELAPPAJI COLLEGE OF AGRICULTURAL ENGINEERING AND FOOD
TECHNOLOGY**

TAVANUR-679573, MALAPPURAM

KERALA, INDIA

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PROJECT REPORT

Submitted in partial fulfilment of the requirement for the degree of

Bachelor of Technology

in

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Department of Processing and Food Engineering

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KERALA AGRICULTURAL UNIVERSITY

DEPARTMENT OF PROCESSING AND FOOD ENGINEERING

**KELAPPAJI COLLEGE OF AGRICULTURAL ENGINEERING AND FOOD
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DECLARATION

We hereby declare that this project report entitled "**DEVELOPMENT OF A REAL-TIME INTELLIGENT pH SENSITIVE COLOUR CHANGE FRESHNESS INDICATOR USING ANTHOCYANIN-BIOPOLYMER FILM FOR FOOD APPLICATIONS**" is a bonafide record of research work done by us during the course of research and that the report has not previously formed the basis for the award to me of any degree, diploma, associateship, fellowship or other similar title, of any other University or Society.

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CERTIFICATE

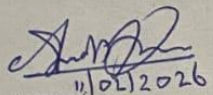
Certified that this project entitled "DEVELOPMENT OF REAL-TIME INTELLIGENT pH SENSITIVE COLOUR CHANGE BASED FRESHNESS INDICATORS USING ANTHOCYANIN-BIOPOLYMER FILM FOR FOOD APPLICATIONS" is a bonafide record of project work done by Rifa Thasneem (2022-06-016), Oxana Rison (2022-06-018), Yusra Jawad (2022-06-019), Ogha Lakshmi (2022-06-020) and Dhanish Rehman (2022-06-021) under my guidance and supervision and that it has not previously formed the basis for the award of any degree, fellowship or associateship to them.


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LIST OF SYMBOLS AND ABBREVIATIONS

°C	Degrees Celsius
mm	Millimeter
Å	Angstrom
kHz	Kilohertz
W	Watts
CMC	Carboxymethyl cellulose
PVA	Polyvinyl alcohol
CNC	Cellulose nanocrystals
NFC	Nanofibrilated cellulose
UAE	Ultrasound-assisted extraction
MAE	Microwave-assisted extraction
SFE	Supercritical fluid extraction
HPLC	High Performance Liquid Chromatography
SC-CO ₂	Supercritical carbon dioxide
XRD	X-ray diffraction
FTIR	Fourier Transform Infrared Spectroscopy
DSC	Differential Scanning Calorimetry
SEM	Scanning Electron Microscopy
UV-Vis	UV-Visible Spectroscopy
TPC	Total plate count
CFU	Colony forming units

TVB-N	Total Volatile Base Nitrogen
PCA	Plate count agar
NH ₃	Ammonia
H ₂ S	Hydrogen sulfide
SO ₂	Sulfur dioxide
TMA	Trimethylamine
NaOH	Sodium hydroxide
HCl	Hydrochloric acid
H ₂ SO ₄	Sulfuric acid
TCA	Trichloroacetic acid
ΔE*	Total color difference
T _g	Glass transition temperature
T _m	Melting temperature
T _c	Crystallization temperature
ΔH	Enthalpy change
RCE	Red cabbage extract
HBA	Hardy Kiwi Blossom Anthocyanin
ASKG	Artemisia sphaerocephala Krasch. gum
SPI	Soy protein isolate

SPVA	Starch/Polyvinyl alcohol
MOF	Metal-organic frameworks
Cu-MOF	Copper-based metal-organic frameworks
pH	Power of hydrogen
MW	Molecular weight
DF	Dilution factor
ϵ	Extinction coefficient
p	Pathlength
mg/L	Milligrams per liter
mg%	Milligrams percent
g/mol	Grams per mole
N/100	Normality 0.01
w/v	Weight/volume
w/w	Weight/weight
v/v	Volume/volume
GRAS	Generally Recognized as Safe
TAC	Total anthocyanin content
ASTM	American Society for Testing and Materials
$\lambda_{\max}$	Maximum absorption wavelength

2 θ	Two-theta angle (in XRD)
d-spacing	Distance between crystal planes
SEM	Scanning Electron Microscopy (also used for Solvent Extraction Method)
κ -carrageenan	Kappa-carrageenan

INTRODUCTION

CHAPTER I

INTRODUCTION

The packaging industry has undergone a significant transformation in recent years, focusing on the development of intelligent and sustainable packaging materials that not only protect and preserve food but also provide real-time information on product quality and safety (J. Zhang *et al.*, 2025). Conventional packaging works well for protection and containment, but it doesn't show if the product is fresh or spoiled (D. Liu *et al.*, 2022). This restriction has prompted research into intelligent packaging systems, especially those that use bio-based indicators and natural pigments to visibly convey changes in product quality (I. D. de L. Silva *et al.*, 2021). Among these approaches, anthocyanin-based colourimetric packaging systems have attracted particular attention because their pH-responsive pigments can provide a visible indication of food freshness while remaining environmentally friendly (Kumar *et al.*, 2026).

Intelligent packaging, which monitors food's conditions and provides the consumer with immediate information on its quality at the time of purchase, has arisen in response to the increased need for safe food (Yildirim *et al.*, 2018). Colorimetric indicator films have therefore emerged as an important component of intelligent food packaging systems (Silva *et al.*, 2021). These films change color in response to changes in the food, such as the release of volatile substances (J. Zhang *et al.*, 2025). Among these, pH-sensitive colourimetric films that respond to subtle changes in food surface chemistry have gained increasing attention in recent years (Kumar *et al.*, 2026). One of the most popular sources of dyes for making these indicator films is anthocyanins, which are naturally occurring pigments (Khoo *et al.*, 2017).

Red cabbage, blackberries, grapes, and purple sweet potatoes are just a few examples of the many fruits, vegetables, and flowers that contain anthocyanins, which are naturally occurring water-soluble pigments that belong to the flavonoid group (Khoo *et al.*, 2017). Since many food deterioration processes are accompanied by pH changes brought on by microbial activity or chemical interactions, their ability to change color under different pH settings allows them to serve as natural markers of food spoilage (Mustafa & Andreescu, 2020). Depending on the freshness of the food product, anthocyanins incorporated into packaging films can provide a visible colourimetric response, typically shifting from red to blue and eventually to green (C. Wang & Liu, 2024).

Anthocyanins have a number of benefits, including being biocompatible, typically nontoxic, and sourced from renewable resources, all of which are in line with sustainability objectives (Pereira *et al.*, 2021). They are perfect for signifying chemical changes linked to food decomposition since their color shifts span a wide range of pH values (usually red in highly acidic environments, pink to purple in neutral conditions, and blue to yellow in alkaline medium) (Kumar *et al.*, 2026).

The increasing concerns regarding synthetic dyes, plastic materials that do not degrade, and chemical indicators have underscored the necessity of investigating biodegradable, bio-based smart packaging options (Puebla-Duarte *et al.*, 2023). Indicators based on anthocyanins provide a safe and eco-friendly substitute, aligning with the global trend towards sustainable food packaging solutions that minimize waste and improve food safety tracking (Yildirim *et al.*, 2018). Researchers have explored different biopolymer matrices, including starch, chitosan, cellulose, and gelatin, to integrate anthocyanins, to achieve ideal mechanical properties, barrier performance, and color stability suitable for commercial usage (Remedio & Parada Quinayá, 2024).

Thus, research on anthocyanin-based intelligent packaging has increasingly focused on the development of colourimetric films, including the extraction and stabilization of anthocyanins, their incorporation into polymeric matrices, and their effectiveness in detecting spoilage in highly perishable foods such as dairy, fish, and meat (C. Wang & Liu, 2024).

Intelligent packaging based on anthocyanins offers a promising development in packaging systems because it can deliver a real-time, visual demonstration of food freshness and spoilage. Incorporating anthocyanins into polymeric films enables the development of colourimetric indicator films that combine freshness monitoring with the growing demand for sustainable and environmentally friendly packaging materials. Therefore, indicators based on anthocyanins could greatly enhance food quality monitoring, minimize food waste, and guarantee consumer safety. Accordingly, this work is to carry out to develop anthocyanin-based colourimetric films for intelligent food packaging, focusing on their sources, extraction, incorporation into biopolymer matrices, and applications in monitoring food freshness.

In this background the project entitled “Development of a Real-Time Intelligent pH Sensitive Colour Change Freshness Indicator Using Anthocyanin-Biopolymer Film for Food Applications” was undertaken at Kelappaji College of Agricultural Engineering and Food Technology (KCAEFT) Tavanur, Kerala, India with the following objectives:

- a) To develop a biodegradable smart packaging film incorporated with anthocyanin as natural colorimetric indicator.
- b) To evaluate the physical, mechanical, barrier and functional properties of the developed anthocyanin-incorporated biodegradable film.
- c) To develop and validate the anthocyanin-based pH sensitive biopolymer film as a freshness indicator for monitoring the freshness of food products.

REVIEW OF LITERATURE

CHAPTER II

REVIEW OF LITERATURE

This review synthesizes recent literature on anthocyanin-based biodegradable films, examining their development as pH-sensitive freshness indicators for food packaging. It encompasses sources and extraction methods of anthocyanins, biopolymer film matrices, evaluation of mechanical properties, and real-time food quality monitoring applications across perishable products.

2.1 ANTHOCYANINS: CHEMISTRY AND FUNCTIONAL PROPERTIES

Anthocyanins are water-soluble natural pigments whose colour is highly sensitive to environmental pH. These pigments may appear in different colours depending on the pH conditions of the medium from which they are extracted, typically ranging from red, orange and pink to blue. Their ability to undergo colour transitions across a broad pH range enables their use as natural indicators for detecting freshness changes in food systems (L. Zhao *et al.*, 2022).

Anthocyanins are naturally occurring organic compounds classified as flavonoids that are commonly found in fruits and vegetables. A growing body of research has demonstrated that anthocyanins exhibit a range of biological functions, including antioxidant, immunomodulatory, anti-tumor, hepatoprotective, anti-aging, and anti-inflammatory properties (Neves *et al.*, 2022). Anthocyanins are classified as water-soluble salts of flavyl compounds, and their structure is formed through the linkage of a sugar molecule with an anthocyanidin. They originate from anthocyanidins, which lack glycosylated groups. The role of anthocyanins in plants is to shield flowers and fruits from ultraviolet radiation and to inhibit the formation of harmful free radicals (Santamarina *et al.*, 2023).

Anthocyanins are polyphenolic O-glycosides that give plants, including a wide range of fruits and vegetables, their vivid red, purple, and blue hues. As a result, they have garnered significant attention from both industry and science as possible natural food coloring (Dangles, 2024).

2.1.1 STRUCTURE AND CHEMICAL CHARACTERISTICS

Structurally, anthocyanins are water-soluble salts of flavylium compounds formed through the linkage of sugar moieties to anthocyanidins. Anthocyanidins represent the aglycone form of these pigments and lack glycosylated groups. The fundamental structure of anthocyanins is based on the flavylium ion, which has a molecular weight of approximately 207.24 g/mol and the empirical formula $C_{15}H_{11}O^+$. The glycosylated derivatives of anthocyanidins are referred to as anthocyanins, and these conjugated structures are responsible for the characteristic red, blue and purple pigmentation observed in plants (Khoo *et al.*, 2017).

Anthocyanins are secondary (specialized) metabolites present in various plant species. Six major anthocyanidin types have been identified, namely delphinidin, cyanidin, petunidin, pelargonidin, peonidin, and malvidin. These compounds contribute to the red, purple, and blue pigmentation of flowers, fruits, and seeds, which plays an important role in attracting pollinators and seed-dispersing organisms. In addition, anthocyanins contribute to the red coloration observed in leaves during autumn and help protect plants from environmental stresses such as ultraviolet radiation, extreme temperatures, drought, and pathogen attack (Z. Lu *et al.*, 2024).

Anthocyanins are commonly classified according to their glycosylation pattern, which refers to the number and type of sugar molecules attached to the anthocyanidin structure. They may occur as monoglycosides (e.g., cyanidin-3-glucoside), diglycosides (e.g., delphinidin-3,5-diglucoside), or less commonly triglycosides, with sugars such as glucose, galactose, or arabinose contributing to structural diversity and stability. This glycosylation strongly influences pigment solubility, colour expression, and stability in plant tissues (Khoo *et al.*, 2017).

Acylation represents another important structural modification of anthocyanins. In this process, acyl groups—typically aromatic acids such as p-coumaric, caffeic, or ferulic acid, or aliphatic acids—are attached to the sugar moieties of anthocyanins. This modification stabilizes the pigment structure by enhancing intramolecular stacking, thereby protecting the molecule from degradation caused by pH fluctuations, light exposure, and temperature changes. Acylated anthocyanins generally exhibit improved colour stability, stronger antioxidant activity, and more intense pigmentation compared with non-acylated forms. Such stable pigments are commonly found in red cabbage, purple sweet potato, and black carrot, where their enhanced stability makes

them valuable for food colourants and smart packaging applications (Castañeda-Ovando *et al.*, 2009).

Anthocyanins can also be classified according to the number and arrangement of hydroxyl and methoxy groups present on the B-ring of the anthocyanidin structure. Higher hydroxylation levels, as observed in delphinidin, petunidin, and malvidin, generally enhance the blueness of the pigment but may reduce stability. In contrast, lower hydroxylation levels, such as in pelargonidin, produce more orange-red hues with greater stability. Methylation of hydroxyl groups, leading to compounds such as peonidin and malvidin, further improves pigment stability by reducing susceptibility to oxidation and enhancing colour intensity. These structural variations significantly influence the colour properties, antioxidant capacity, and stability of anthocyanins in food and biological systems (He & Giusti, 2010).

2.1.2 pH-DEPENDENT COLOR TRANSITIONS

Anthocyanins undergo structural transformations in response to changes in environmental pH, resulting in visible colour variations. Under highly acidic conditions ($\text{pH} < 2$), anthocyanins predominantly exist in the flavylium cation form, which produces a bright red colour. At moderately acidic to neutral pH values (approximately pH 3–6), the pigments may convert to carbinol pseudo-base (hemiketal) or chalcone structures, which appear colourless or pale yellow. Under alkaline conditions, anthocyanins transform into quinoidal base structures that typically exhibit blue or purple hues (S. Silva *et al.*, 2017).

Because many food spoilage processes involve pH changes resulting from microbial activity or chemical reactions, these colour transitions enable anthocyanins to function as natural freshness indicators in food systems.



Plate 2.1 pH-dependent colour transitions of anthocyanins

2.1.3 FACTORS AFFECTING ANTHOCYANINS

The stability of anthocyanins, in the context of a colourimetric packaging system, is strongly influenced by environmental factors. pH, temperature, light exposure and oxygen availability can affect the structural integrity and colour stability of these pigments.

Variations in these environmental parameters may lead to pigment degradation, colour fading, or structural changes that reduce the reliability of anthocyanin-based indicators. Consequently, controlling these factors is essential when incorporating anthocyanins into food packaging systems or indicator films designed for freshness monitoring. They are flavonoid pigments that dissolve in water, with their stability, color expression, and degradation being greatly affected by numerous intrinsic and extrinsic factors. Among these factors, pH is the most significant determinant, as it influences the structural form of anthocyanins and, in turn, their color; in highly acidic conditions, they appear as red flavylium cations, whereas at elevated pH levels they change into colourless or blue-purple forms. Temperature significantly influences degradation, as higher temperatures speed up hydrolysis and the breakdown into simpler substances, resulting in diminished color and antioxidant properties. Light, especially ultraviolet

radiation, causes photodegradation, leading to pigment loss, while oxygen facilitates oxidative processes that decrease stability and cause browning. Metal ions like iron, copper, and aluminium can stabilize or change anthocyanin color by forming complexes, but they can also promote degradation. The activity of enzymes, particularly polyphenol oxidase and peroxidase, results in the oxidation and degradation of anthocyanins within food systems. Furthermore, co-pigmentation with various phenolic compounds can improve color intensity and stability, while ascorbic acid typically speeds up degradation despite being an antioxidant. Sugars can enhance stability via molecular interactions, and factors such as time, temperature, and light and oxygen exposure considerably affect the total retention of anthocyanins. These elements together influence the effective functionality of anthocyanins in food uses, especially regarding their function as natural colorants and indicators of freshness. (Giusti & Wrolstad, 2003);(Castañeda-Ovando *et al.*, 2009);(Patras *et al.*, 2010)

2.2 NATURAL SOURCES OF ANTHOCYANIN

Anthocyanins are widely distributed natural pigments predominantly found in fruits, vegetables, cereals, legumes, and flowers, where they impart vibrant red, purple, and blue colors. These compounds belong to the flavonoid family and are synthesized via the phenylpropanoid pathway, making them abundant in many edible and non-edible plant species (Khoo *et al.*, 2017).

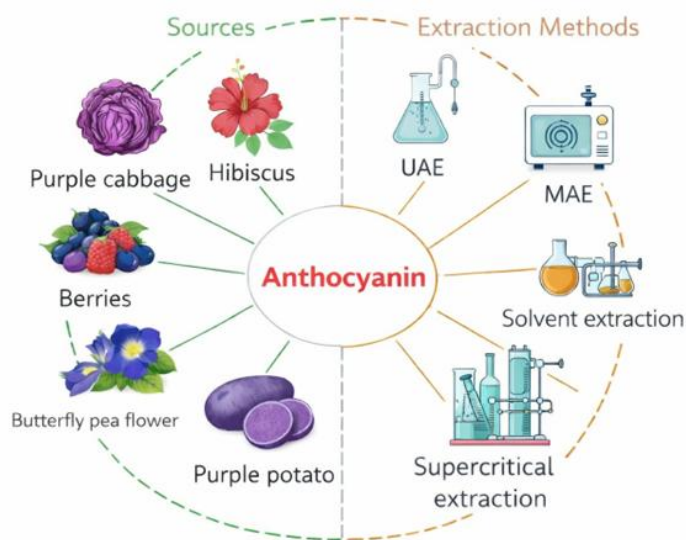


Plate 2.2. Anthocyanin sources and extraction methods

2.2.1 FRUITS

Fruits are the richest and most commonly utilized sources of anthocyanins. High concentrations occur in berries, such as blueberries, blackberries, strawberries, raspberries, elderberries, and cranberries (Tanaka & Ohmiya, 2008). Grapes—particularly red and black varieties—also contain significant anthocyanin levels, mainly in their skins (He & Giusti, 2010). Tropical fruits like pomegranates, cherries, plums, and blood oranges likewise serve as important sources. Because of their high anthocyanin content and ease of extraction, berry fruits remain the primary industrial source for natural pigment extraction (Giusti & Wrolstad, 2003).

2.2.2 VEGETABLES

Anthocyanins are detectable in a number of colored vegetables. Because of its stable and plentiful anthocyanin profile, red cabbage is one of the most researched sources and is frequently utilized in studies on intelligent pH-sensitive packaging (Castañeda-Ovando *et al.*, 2009). Purple sweet potatoes, eggplant peels, purple carrots, red onions, and beet varieties, though beetroot mostly includes betalains, are other noteworthy sources. Anthocyanins from purple sweet potato are considered particularly suitable for natural colourants and food-grade freshness indicators because of their relatively high stability (Truong *et al.*, 2007).

2.2.3 FLOWERS

Flowers with vibrant pigmentation, like Hibiscus sabdariffa and butterfly pea (*Clitoria ternatea*), are abundant in anthocyanins and have found extensive use in culinary applications, medicine, and smart packaging due to their antioxidant properties and pH-sensitive color characteristics (Juswardi *et al.*, 2023);(Hasanah *et al.*, 2023).

2.2.4 CEREALS AND GRAINS

Pigmented grains including black rice, purple corn, black barley, and black wheat also contain anthocyanins. Cyanidin-3-glucoside is a primary pigment found in purple maize, which is commonly employed in the creation of natural colorants (Petroni *et al.*, 2014). Anthocyanins derived from pigmented cereals are gaining increasing attention because of their relatively high yield and improved stability.

2.2.5 LEGUMES AND TUBERS

Anthocyanins are also found in several tubers and legumes. This includes potatoes with purple flesh, black beans, and red beans, all of which are high in derivatives of cyanidin and

delphinidin (Rein, 2005). Among these, purple-fleshed potatoes have attracted particular research interest due to their stability during processing and their potential as a pigment source.

Table 2.1. Overview of Anthocyanin Sources

Fruits/Vegetables /Legumes/Grains	Type	Predominant Anthocyanins (ACNs)	ACNs Content (mg/100 g)	Reference
Berries	Raspberries	Cyanidin-3-sophoroside	92–120	(Mullen <i>et al.</i> , 2002)
Stone fruits	Apricots (red varieties)	Cyanidin-3-glucoside	4.5–12.3	(Leccese <i>et al.</i> , 2007)
	Peaches (blood-flesh)	Cyanidin-3-rutinoside	18–42	(Gil <i>et al.</i> , 2002)
Tropical fruits	Açaí berries	Cyanidin-3-glucoside	320–450	(Schauss <i>et al.</i> , 2006)
Pome fruits	Pear (red-skinned)	Cyanidin-3-galactoside	6.8–15.4	(Fischer <i>et al.</i> , 2007)
Vegetables	Purple sweet potato	Peonidin-3-caffeoylsophoroside-5-glucoside	90–210	(Truong <i>et al.</i> , 2007)
	Purple potato	Malvidin-3-glucoside	35–98	(Lachman <i>et al.</i> , 2012)
	Red radish	Pelargonidin-3-sophoroside-5-glucoside	110–175	(Giusti <i>et al.</i> , 1998)
Cereals & grains	Purple wheat	Cyanidin-3-glucoside	25–70	(Abdel-Aal <i>et al.</i> , 2006)
	Red sorghum	Luteolinidin-5-glucoside	12–38	(Awika <i>et al.</i> , 2005)

Flowers	Butterfly pea (<i>Clitoria ternatea</i>)	Delphinidin-3,5- diglucoside	280–360	(Kazuma <i>et al.</i> , 2003)
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2.3 EXTRACTION OF ANTHOCYANIN

Extraction of anthocyanins from plant materials is commonly carried out using heat-assisted solvent-based techniques such as Soxhlet extraction, reflux extraction, acidified ethanol or methanol extraction, maceration, and conventional solvent extraction. These methods extract anthocyanins from plant matrices using polar organic solvents (which are frequently acidified) and high temperatures and times (Tan *et al.*, 2022).

Despite their popularity and ease of use, traditional solvent-based anthocyanin extraction techniques including maceration and heat-assisted extraction have a number of important drawbacks. Large amounts of organic solvents are frequently needed for these procedures, which raises questions concerning solvent toxicity, environmental effects, excessive solvent consumption, safety hazards related to solvent handling and disposal, and potential solvent residues in the finished extract (Tena & Asuero, 2022). Additionally, they often require lengthy extraction times and moderate-to-high temperatures, which not only lowers process efficiency but can also cause thermolabile anthocyanins to degrade thermally, changing color and decreasing bioactivity. Additionally, because anthocyanins are found inside plant cell vacuoles and the mass transfer barrier of cell walls restricts effective release into the solvent, traditional extraction frequently produces low yields. Poor selectivity is another significant drawback, where non-target substances like sugars and other phenolics are co-extracted, making downstream purification more difficult and degrading the quality of crude extracts. Lastly, compared to more sophisticated methods, the overall extraction efficiency is highly dependent on variables including solvent type, pH, particle size, and solid-to-liquid ratio, making repeatability unpredictable and optimization more difficult (Tena & Asuero, 2022).

2.3.1 SOLVENT EXTRACTION METHOD

Anthocyanin pigments are traditionally extracted from plant tissue (fruit skins, petals, berries, and vegetables) using a liquid solvent that dissolves and transports the colours into

solution. Because it is easy to use, scalable, and compatible with purification and downstream analysis (HPLC, spectrophotometry), it is widely employed (Cacace & Mazza, 2002).

Appropriate selection of extraction solvents is essential for efficient anthocyanin recovery. For the extraction of anthocyanins, methanol, ethanol, acidified water, or acidified ethanol are the most often utilized solvents (Muangrat *et al.*, 2018). According to a comprehensive analysis of earlier research, anthocyanins may be extracted by SEM with the following parameters: solid-to-liquid ratio of 1:15–1:30 g/mL, extraction temperature of 34.7–52.03 °C, and extraction period of 5 min–4.2 h (Antonio-Gomez *et al.*, 2021). Although SEM offers advantages such as simple equipment, straightforward operation, and ease of implementation, it also presents limitations including high solvent consumption, relatively low extraction efficiency, and long processing times.

2.3.2 SUPERCRITICAL FLUID EXTRACTION (SFE)

SFE is a type of liquid extraction in which a supercritical fluid - that is, a material above its critical point - replaces the typical liquid solvent phase. Due to its relatively low critical temperature and pressure, carbon dioxide is practically the only practical supercritical extraction solvent utilized among a large range of supercritical fluids (Sticher, 2008) (Sarker & Nahar, 2012) (Lutfun & Sarker, 2012) (Nahar & Sarker, 2006) (S. S. Handa *et al.*, 2008). Because anthocyanins are polar, high pressures and a high percentage of an organic co-solvent (methanol, ethanol, or water) are necessary for the extraction of anthocyanins utilizing supercritical CO₂ (SC-CO₂) methods (Ignat *et al.*, 2011).

A basic SFE system consists of an extraction vessel placed in an oven, a controller to maintain high pressure within the system, a trapping vessel, a mobile phase tank typically containing CO₂, a pump to pressurize the gas, and co-solvent vessels with an associated pump. Various meters, such as flow meters and dry/wet gas meters, may also be connected to the system.

SFE offers several advantages including high extraction efficiency, reduced solvent residues, environmental friendliness, and improved safety. Additionally, the SFE approach is particularly well-suited for the extraction of anthocyanins, which are heat-sensitive compounds, due to its low treatment temperature (Tian *et al.*, 2016). However, because of the expensive equipment costs and significant technological investment, this technology is still far from being

widely used in the food business. Furthermore, precise control of extraction parameters such as pressure and CO₂ flow rate can be technically challenging.

2.3.3 MICROWAVE ASSISTED EXTRACTION (MAE)

The frequency range of microwaves, which are non-ionizing electromagnetic radiation, is 300–300,000 MHz. To prevent interference with radio communications, 2450 MHz is typically used by both business and residential systems (S. Handa, 2008). They consist of two perpendicular oscillating fields, such as the magnetic and electric fields. The direct effects that microwaves have on polar materials form the basis of the microwave heating principle (Letellier & Budzinski, 1999). Ionic conduction and dipole rotation processes transform electromagnetic energy into thermal energy (Jain *et al.*, 2009). Because solvents with high dielectric constants (polar solvents) absorb more microwave radiation, the polarity of the extraction solvent plays a crucial role in improving extraction efficiency (Dai & Mumper, 2010);(Jain *et al.*, 2009);(Rawa-Adkonis *et al.*, 2003).

Microwave heating is instantaneous and takes place in the center of the sample because of the unique effects that microwaves have on matter (namely, ionic conductance and dipole rotation), which results in extremely quick extractions (Sticher, 2008). Unless the temperature inside the vessel increases excessively, microwave radiation does not degrade the extracted chemicals. Meanwhile, it has been discovered that microwaves have a particular impact on plant material (Sticher, 2008). They cause rapid heating and temperature increases by selectively interacting with the free water molecules in the solid matrix. This causes the plant tissue to rupture and release solutes into the solvent (L. Wang & Weller, 2006); (Mandal *et al.*, 2007).

2.3.4 ULTRASOUND ASSISTED EXTRACTION (UAE)

UAE uses ultrasonic frequencies between 20 kHz to 2000 kHz. The ultrasonic acoustic cavitation's mechanical action improves the permeability of cell walls and the surface contact between solvents and samples. When materials exposed to ultrasound undergo physical and chemical changes, the plant cell wall is disrupted, allowing chemicals to be released and improving the solvents' mass transfer into the plant cells (Dhanani *et al.*, 2017).

Compared with conventional solvent extraction methods, ultrasound treatment can enhance the extraction efficiency of natural anthocyanins. Therefore, the ultrasonic conditions (ultrasound power, extraction temperature, solid-to-liquid ratio, and extraction time) must be strictly controlled to give better play to the advantages of ultrasonic extraction (Tian *et al.*, 2016).

2.4 ANTHOCYANINS AS COLOURIMETRIC FRESHNESS INDICATORS

Anthocyanins have attracted considerable interest as natural colourimetric indicators because their molecular structure responds sensitively to changes in environmental pH. This property enables visible colour transitions that can be used to monitor chemical changes associated with food spoilage. In intelligent packaging systems, these pigments are incorporated into polymer matrices where they act as freshness indicators by responding to volatile compounds and pH changes produced during microbial deterioration.

Unlike conventional additives where anthocyanins are primarily used as colourants or nutraceutical compounds (Remedio & Parada Quinayá, 2024) recent research increasingly focuses on their role as visual indicators of food quality and safety. Their natural origin, strong colour response, and compatibility with biodegradable films make them particularly suitable for colourimetric freshness monitoring in intelligent food packaging systems.

2.4.1 INDICATOR MECHANISMS

The colourimetric response of anthocyanin indicators is primarily governed by structural transformations that occur when the surrounding pH changes during food spoilage. Microbial growth and biochemical reactions in perishable foods generate volatile compounds such as ammonia, amines, and other alkaline metabolites that alter the local pH environment.

These pH shifts cause anthocyanins to undergo reversible molecular transformations, resulting in visible colour transitions that can serve as a direct visual signal of freshness or spoilage. When incorporated into indicator films, these pigments therefore function as simple, instrument-free sensors capable of providing real-time information about food quality.

2.4.2 SYNTHETIC VS NATURAL INDICATORS

Numerous research studies have pointed out the limitations of synthetic chemical indicators in comparison to natural indicators derived from anthocyanins. Chemical indicators, such as phenolphthalein and methyl orange, are frequently harmful, not biodegradable, and can present environmental and health concerns due to their artificial nature and the dangers associated with their disposal. Additionally, these indicators tend to be more costly to manufacture and offer less sustainability since they depend on non-renewable petrochemical resources.

In contrast, anthocyanin-based indicators derived from fruits and vegetables are biodegradable, non-toxic, and environmentally friendly. They also exhibit a broader and more

distinct colour transition across different pH levels, which enhances their suitability for colourimetric freshness detection in food packaging systems (Tuslinah *et al.*, 2022).

2.4.3 ADVANTAGES OF ANTHOCYANIN INDICATORS

Anthocyanin indicators offer several advantages for intelligent food packaging applications. Their natural origin, strong pH sensitivity, and wide colour transition range allow clear visual monitoring of freshness without the need for analytical instruments. In addition, their compatibility with biodegradable polymers and renewable sources makes them attractive for sustainable packaging technologies aimed at improving food safety and reducing waste.

2.5 ANTHOCYANIN-BASED COLOURIMETRIC PACKAGING SYSTEMS

Smart packaging films incorporating pH-sensitive indicators have been extensively developed for rapid monitoring of food freshness. Anthocyanins exhibit colour-changing characteristics across a wide pH range, making them suitable natural sensors for evaluating food quality. Smart packaging is an emerging technology in the food sector that provides consumers with easily observable information regarding the quality and freshness of packaged food products. In cases of microbial contamination or chemical alterations in packaged foods, freshness can be evaluated through visible colour changes in the packaging indicator.

Recently, food packaging research has increasingly focused on environmentally friendly and biodegradable materials, with natural pigments such as anthocyanins gaining particular attention. Smart packaging films made from biodegradable polymers and incorporating anthocyanins are therefore advantageous for tracking food freshness. The typical biopolymers utilized in the development of such films include polysaccharides, proteins, and lipids (L. Zhao *et al.*, 2022).

Smart packaging technologies provide consumers with visible and comprehensive information about the freshness and quality of packaged foods. By monitoring chromatic changes, consumers can determine the freshness state of packaged food during microbial contamination or chemical deterioration (Pervaiz *et al.*, 2017). In addition, smart packaging systems may combine both active and intelligent functions to extend shelf life while simultaneously detecting spoilage in real time. Sustainable packaging systems composed of biopolymers and anthocyanin extracts

are therefore gaining increasing attention for monitoring microbial deterioration in food products (Neves *et al.*, 2022).

2.5.1 CONCEPT OF INTELLIGENT AND COLOURIMETRIC PACKAGING

A subclass of intelligent and active packaging known as pH-sensitive smart packaging uses colour changes in response to local pH variations to visually represent the chemical or microbiological state of food. These systems typically employ naturally occurring pH-responsive pigments—most notably anthocyanins—embedded in polymeric films or labels to detect volatile nitrogen compounds, microbial metabolites, or other spoilage indicators that alter the microenvironment during deterioration (L. Zhao *et al.*, 2022) (Prietto *et al.*, 2017).

The resulting colour transition provides consumers and supply-chain stakeholders with immediate and instrument-free information about food freshness, thereby improving food safety and reducing waste (Rawat *et al.*, 2023).

2.5.2 MECHANISMS OF FOOD SPOILAGE DETECTION

Food spoilage is generally associated with microbial metabolism that produces volatile alkaline compounds such as ammonia, amines, and total volatile basic nitrogen (TVB-N). These compounds accumulate during the deterioration of protein-rich foods including meat, fish, shellfish, fresh-cut fruits, and dairy products (Kaewprachu *et al.*, 2018) ;(J. Zhang *et al.*, 2021).

The release of these compounds increases the pH in the packaging headspace, which triggers structural transformations in anthocyanin molecules and results in visible colour changes in the indicator film. When evaluated for response time, sensitivity, colour range, mechanical performance, and migration properties, anthocyanin-based indicators have shown strong agreement with conventional freshness parameters such as microbial counts, pH measurements, and total volatile basic nitrogen.

2.5.3 FABRICATION OF ANTHOCYANIN INDICATOR FILMS

In practical applications, anthocyanin extracts are incorporated into polymer matrices through casting, extrusion, coating, or impregnation techniques to produce colourimetric indicator films or labels. These pigments are commonly embedded in biodegradable or synthetic matrices

such as carboxymethyl cellulose, starch, gelatin, chitosan, or polyvinyl alcohol (PVA) (Eghbaljoo *et al.*, 2025).

Anthocyanin-based indicator systems show strong potential for intelligent food packaging; however, challenges remain regarding pigment stability, leaching, polymer–matrix interactions, and the calibration of colour responses for quantitative freshness assessment. To address these limitations, recent studies have explored strategies such as co-pigmentation, acylation, encapsulation, layered protective coatings, and nanomaterial reinforcement to enhance pigment stability and sensor sensitivity (L. Wang *et al.*, 2023) ;(Benalaya *et al.*, 2024).

Similarly, anthocyanins can also be used in indicator films that respond to volatile compounds generated within the package headspace without direct contact with the food. During spoilage, microbial metabolism produces volatile metabolites that accumulate inside the package and induce colour changes in the indicator film. For protein-rich foods, the release of volatile basic nitrogen compounds increases the surrounding pH, triggering the colourimetric response of the anthocyanin indicator (Remedio & Parada Quinayá, 2024).

2.6 BIOPOLYMER MATRICES FOR ANTHOCYANIN INDICATOR FILMS

Biopolymer matrices play a crucial role in determining the mechanical strength, barrier properties, stability, and overall functionality of anthocyanin-based indicator films used in intelligent food packaging. Natural biopolymers are commonly selected as film-forming materials because they are biodegradable, non-toxic, capable of forming films, and compatible with bioactive compounds. Different biopolymer matrices provide distinct advantages in terms of encapsulation efficiency, pH responsiveness, mechanical behaviour, and interactions with anthocyanins, which ultimately influence the performance of colourimetric indicator films.

2.6.1 CELLULOSE-BASED FILMS

Long chains of glucose molecules make up cellulose, a complex carbohydrate and essential structural component of plant cell walls that gives plants strength and support. Since cellulose is the most common natural polymer, it is a great option for creating economically viable and environmentally friendly polymeric products that are often produced from synthetic polymers derived from petroleum. It is inexpensive, non-toxic, biodegradable, and the most abundant renewable substance in the biosphere. Because of its favourable physical and chemical

characteristics—including insolubility in water, semi-crystalline structure, high tensile strength, biodegradability, and chemical reactivity—cellulose has been widely explored for sustainable packaging applications.

Cellulose can also be converted into several derivative forms such as nanocellulose, fibres, films, and hydrogels, which further expand its applications across industries including packaging, bioplastics, biofuels, and biomedical materials (Marinho, 2025).

With a linear and high molecular weight structure, cellulose is the world's most abundant renewable resource. Cellulose exhibits a relatively ordered crystalline structure due to its linear arrangement, which results in films with considerable mechanical strength and stability. However, native cellulose has limited film-forming ability because of its high crystallinity and low solubility in most organic solvents. Therefore, cellulose derivatives such as cellulose acetate, methylcellulose, and hydroxypropyl methylcellulose are often used to produce films with improved film-forming ability, high modulus, and strong barrier properties suitable for freshness indicator packaging (Wagh *et al.*, 2023).

2.6.2 CHITOSAN-BASED FILMS

The second most prevalent natural polysaccharide, chitosan is an amino-polysaccharide derivative of chitin. Because of its distinct biological, chemical, and physical characteristics, chitosan has drawn a lot of attention and is useful for industrial, scientific, and medicinal applications. Only small amounts of chitosan are found, and it is usually produced by chemically or enzymatically deacetylating chitin. In the meantime, chitosan's morphology encourages its presence in a variety of forms, such as crystalline, semi-crystalline, and amorphous structures. Numerous bioactive characteristics, such as antihypertensive, anti-inflammatory, and antioxidant activities, are displayed by chitosan and its derivatives. Chitosan is frequently utilized in food preservation, cosmetics, and medicinal applications because of its safety profile (Sana *et al.*, 2025).

Chitosan, derived from deacetylated chitin, has an obvious inhibitory effect on bacteria and fungi. The positively charged $-NH_3^+$ group in chitosan molecules can adsorb negatively charged bacteria, disrupting the integrity of cell walls, increasing membrane permeability, and causing the leakage of cellular contents. Inside the cell, chitosan can adsorb and bind to proteins and nucleic acids, influencing the normal physiological functions of microorganisms and inhibiting their

growth and reproduction. Moreover, a large number of amino and hydroxyl groups exist on the molecular chain of chitosan and can selectively bind metal ions (such as Mg^{2+} and Ca^{2+}) on the outer membrane of bacteria, inhibiting the production of bacterial toxins. Furthermore, chitosan exhibits excellent film-forming properties and is transformed into highly transparent, non-toxic, and edible film. The mechanical properties, water resistance, and gas barrier of pure chitosan films are inadequate. The addition of anthocyanins to a chitosan film enhances its mechanical properties and air barrier performance. This improvement is primarily attributed to the interaction between the hydroxyl groups of anthocyanins and the hydroxyl and amino groups of chitosan. An active/intelligent film was developed by incorporating purple cabbage anthocyanins (PCAs) and purple sweet potato anthocyanins (PSAs) into chitosan/polyvinyl alcohol (CP), κ -carrageenan/polyvinyl alcohol (KP), and locust bean gum/polyvinyl alcohol (LP) matrices (L. Zhao *et al.*, 2022).

2.6.3 STARCH-BASED FILMS

Starch has emerged as one of the most promising materials for producing biodegradable polymer films due to its low cost and effective film-forming ability. The formulation of starch films strongly influences their barrier and mechanical properties.

Incorporating anthocyanin extracts can mitigate cracking and brittleness in starch films, improve flexibility and ductility, and enable the films to exhibit distinct colour changes in response to pH variations. Furthermore, starch can encapsulate anthocyanins through mechanisms such as electrostatic interactions, hydrogen bonding, and hydration, which may enhance pigment stability.

Additionally, the branched molecular structure of starch provides void spaces and adsorption sites that facilitate the encapsulation of anthocyanins. The release behaviour of anthocyanins from starch films can also be controlled by modifying the structure and size of starch molecules, which further influences the performance of indicator films (Schlindweinn *et al.*, 2022).

2.6.4 GELATIN-BASED FILMS

Gelatin is composed of repeating units of amino acids such as proline, hydroxyproline, and glycine. It is one of the most widely used hydrocolloids and can be derived from fish, poultry, and mammalian sources. In the food industry, gelatin is commonly used as a gelling, thickening, and emulsifying agent in various food products (Rather *et al.*, 2022).

Its molecular structure contains numerous functional groups capable of forming hydrogen bonds, enabling gelatin to produce stable colloidal systems in aqueous environments. This cross-linking capability allows the formation of flexible films with favourable barrier properties.

The formation of intermolecular hydrogen bonds between gelatin and anthocyanins can significantly improve the tensile strength, water vapour permeability, UV–visible barrier properties, and antioxidant activity of gelatin films. Incorporation of anthocyanins also enhances the pH-responsive colour change of gelatin films, enabling their application as intelligent indicators for monitoring food freshness (J. Wang *et al.*, 2022).

2.6.5 CMC-BASED FILMS

Sodium carboxymethyl cellulose (CMC) is frequently used as a matrix material for anthocyanin-based freshness indicators because it is biodegradable, food-grade, and capable of forming uniform and flexible films. Its hydroxyl and carboxymethyl functional groups contribute to good mechanical strength and enable uniform dispersion of hydrophilic anthocyanins within the film matrix.

Studies have shown that CMC–anthocyanin films respond effectively to food spoilage; for example, (Kaewprachu *et al.*, 2024) observed a distinct purple-to-green colour transition during shrimp degradation. However, the hydrophilic nature of CMC may increase moisture sensitivity and the risk of pigment migration (Remedio & Parada Quinayá, 2024).

To improve stability, researchers have incorporated additional polymers or encapsulation systems. For instance, (Ren *et al.*, 2025) demonstrated that κ -carrageenan/CMC films containing cabbage anthocyanins exhibited lower water-vapour transmission and improved colour stability, while (Jang & Koh, 2023) showed that maltodextrin–CMC microencapsulation significantly enhanced long-term pigment retention.

Overall, CMC provides a safe and effective matrix for colourimetric freshness sensing, although improving moisture resistance and pigment fixation remains essential for practical packaging applications (Li *et al.*, 2025) ; (L. Liu *et al.*, 2022).



Plate 2.3. Development of Anthocyanin based colorimetric pH sensitive intelligent packaging films

2.7 APPLICATIONS IN FOOD SYSTEMS

Anthocyanin-based indicator films have been widely explored for monitoring freshness in various food products, particularly highly perishable items where rapid spoilage detection is essential.

2.7.1 FISH AND SEAFOOD

Fish and fisheries products are particularly perishable due to their high protein and lipid content, requiring sophisticated methods for quality assessment during processing, storage, and distribution. Traceability techniques to track freshness across the supply chain have been developed due to worries about seafood safety (Qin *et al.*, 2019). Because they can detect pH

changes brought on by the release of volatile basic compounds during fish spoiling, pH-sensitive films have emerged as viable intelligent packaging materials. This allows them to visually signal freshness by a characteristic color change (Lan *et al.*, 2021). Compared to chicken and red meat, fish rotting is mostly alkaline because the main gases emitted during this process are trimethylamine (TMA) and NH₃. Therefore, when fish deteriorate, anthocyanin-based intelligent packaging usually changes color from red to green or yellow. For instance, by adding *Phyllanthus reticulatus* anthocyanins to a chitosan/methylcellulose matrix, (Gasti *et al.*, 2021) created a pH-responsive intelligent film (CMPR). According to their research, the film accurately tracked fish freshness and extended the shelf life of seafood by turning pink in acidic settings and pale yellow in alkaline ones. In a similar vein, (Alizadeh-Sani *et al.*, 2021) demonstrated the viability of immobilizing *Myrica rubra* anthocyanins onto a chitin nanofiber/methylcellulose composite film by showing a color shift from red to light pink as the pH of fish samples increased. Despite the potential of anthocyanin-based intelligent packaging in fish preservation, various problems remain. First, anthocyanins are intrinsically unstable and prone to degradation under light, temperature, and pH variations, limiting their long-term usefulness in packaging applications. For instance, (Khezerlou *et al.*, 2023) created an anthocyanin-encapsulated freshness indicator for fish packaging, which successfully indicated real-time freshness variations but also emphasized the poor stability of anthocyanins, providing a key obstacle. Furthermore, anthocyanins have a narrow color response range, and often the color shift is too weak or imprecise to identify small spoilage changes. In order to solve this problem, (Wu *et al.*, 2022) created a red cabbage anthocyanin-polyvinyl alcohol composite film that may use color variations to track fish freshness.

However, further tuning is needed to boost stability and response accuracy, ensuring dependable food quality assessment across varied seafood products.

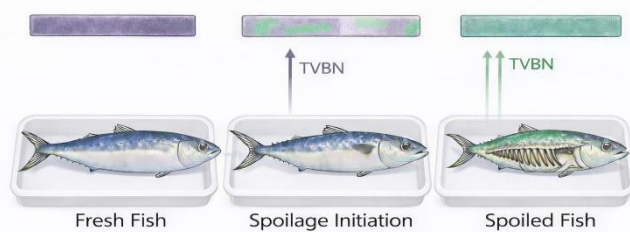


Plate 2.4. Colour change in the indicator film during spoilage of fish

2.7.2 SHELLFISH AND CRUSTACEANS

Shrimp and crabs are examples of crustacean seafood that must be immediately refrigerated or frozen after harvest due to their extreme perishability. Unlike other meats, their rotting involves not just protein degradation but also the breakdown of amino sugars in their exoskeletons, leading to a more complex composition of spoilage gases, including NH₃, H₂S and different amines. This intricacy needs more exact colorimetric responses in intelligent packaging for certain seafood goods. Developing novel packaging technologies that maintain flavor, increase shelf life, and check food quality has thus become a research emphasis. Acid-base indicators based on chemical or natural dyes have been frequently utilized for food quality assessment (Kuswandi *et al.*, 2011). Anthocyanins provide a natural substitute for synthetic dyes; their color changes are impacted by temperature, co pigmentation, pH, structure, and UV exposure, which makes them excellent for monitoring freshness in real time.

Anthocyanin-based intelligent packaging has shown considerable advantages in crustacean seafood applications, particularly in increasing shelf life and enabling real-time spoiling monitoring. The color of clever films usually changes from red to black or deep green because the spoiling of shrimp and crabs mostly releases sulfur-containing compounds. For example, (Tavassoli *et al.*, 2023) produced an indicator film employing pectin, polysaccharide nanofibers, and sumac anthocyanins, which efficiently monitored shrimp freshness through real-time color changes, giving a reliable approach for detecting seafood decomposition. According to recent research, anthocyanins can greatly improve the performance and responsiveness of packaging when combined with other materials, such as metal-organic frameworks (MOFs) and natural polysaccharides. For example, (Amin *et al.*, 2022) effectively combined red cabbage anthocyanins with a copper-based MOF (Cu-MOF) to create a pH-sensitive film that extended shelf life and delayed spoiling in shrimp packaging. Dual-layer colorimetric indicator films have also been created to improve freshness detecting accuracy. A sodium alginate–mulberry anthocyanin composite bilayer film was created using a casting/solvent evaporation technique by (Huang, Du, *et al.*, 2023). This film showed clear color variations during freshness monitoring of Chinese mitten crab, further indicating its potential for intelligent seafood packaging applications.

2.7.3 RED MEAT

Microbial growth, oxidative deterioration, and physicochemical changes are some of the major storage difficulties that affect the safety, quality, nutritional value, and sensory qualities of red meat and its products. Anthocyanins, owing to their high antioxidant and antibacterial characteristics, delay oxidation and limit microbiological growth, hence extending shelf life and enhancing food safety. Additionally, anthocyanins are frequently employed in intelligent food packaging to signal freshness due to their pH sensitivity. For instance, (Shi *et al.*, 2022) created a colorimetric intelligent film with anthocyanins that showed a clear color shift from purplish-red to deep blue as pork freshness decreased, indicating its potential for meat freshness monitoring. Similarly, (Jiao *et al.*, 2023) developed an antimicrobial absorbent pad with blueberry anthocyanins, which not only prolonged meat preservation but also visibly indicated decomposition.

Notably, myoglobin, which is abundant in red meat (such as beef and pork), oxidizes with time to become brownish metmyoglobin. Additionally, spoiling releases gases such as NH₃, H₂S and trimethylamine (TMA), all of which elicit anthocyanin-based color changes, enabling freshness identification. However, the color reaction time of intelligent packaging might be extended because lipid oxidation in red meat proceeds somewhat slowly. Studies have revealed that the antioxidant and antibacterial activity of packaging materials is favorably connected with anthocyanin content (Guo *et al.*, 2023). In order to check the freshness of frozen shrimp and pork, and (Huang, Xu, *et al.*, 2023) produced pH-responsive films with different concentrations (1%, 2%, and 3%, w/V) of Hardy Kiwi Blossom Anthocyanin (HBA) combined with guar gum/polyvinyl alcohol. According to the findings, films with 2% HBA performed the best in applications involving clever meat packaging. However, an excessive anthocyanin concentration does not necessarily promote pH responsiveness.

2.7.4 POULTRY PRODUCTS

With the increased emphasis on healthy eating, poultry meat has become a favorite choice due to its low fat, high protein, and rich supply of B vitamins and minerals. Compared to red meat, chicken includes a higher amount of unsaturated fatty acids, which are more prone to oxidation, resulting in rapid loss in flavor during storage (Mancinelli *et al.*, 2021). Poultry is also quite vulnerable to microbial contamination due to its high moisture content and moderate pH (usually 5.8–6.2). Therefore, stronger antioxidant and antibacterial techniques are needed to preserve red

meat. The use of anthocyanins in intelligent packaging has two advantages: they act as natural freshness indicators through pH-responsive color changes and as antioxidants and antimicrobials to increase shelf life.

The main gases emitted during the spoiling of poultry flesh are sulfur dioxide (SO₂), H₂S, and trace amounts of NH₃. Given the high amount of polyunsaturated fatty acids in poultry, lipid oxidation leads to the generation of aldehydes and ketones, resulting in more dramatic pH shifts. Consequently, the color shift in anthocyanin-based packaging for chicken deterioration is more sensitive and quicker than for red meat. For instance, (Ranjbar *et al.*, 2023) produced an edible coating employing sodium alginate, chitosan, and red beet anthocyanins for chicken fillets. The results revealed that this coating not only preserved freshness and suppressed microbial growth but also served as a spoiling indication.

Using anthocyanin-rich *Syzygium cumini* peel extract, (Talukder *et al.*, 2020) created a quality indicator that showed a direct association between color change and the quality of stored chicken patties, indicating its potential in intelligent food packaging. Research further suggests that anthocyanin-based intelligent packaging is not only effective in freshness monitoring but also environmentally friendly. For example, pH-responsive indicators made of microcrystalline cellulose, hydroxypropyl methylcellulose, and hibiscus anthocyanins demonstrated biodegradability and accurately reflected changes in food quality, demonstrating their potential in sustainable packaging applications (Boonsiriwit *et al.*, 2022); Additionally, the multifunctionality of intelligent packaging is improved by combining anthocyanins with other natural elements or composite materials like UiO-66 and UiO-66-br. For example, in a chicken breast freshness monitoring research, films comprising natural anthocyanins and UiO-66-br outperformed conventional films by delivering more exact colorimetric changes. This shows that anthocyanin-based intelligent packaging could grow beyond pH monitoring to broader applications in food freshness evaluation (D. Liu *et al.*, 2023).

2.7.5 MILK AND DAIRY

A perishable food with significant biological and nutritional value is milk. Lactic acid bacteria can produce lactic acids, which can cause acidification and degradation during the product's storage. Starch/PVA film with purple sweet potato anthocyanins was found by (B. Liu *et al.*, 2017) to be able to identify degradation in milk samples. The smart film changed from red hue for saved milk to purple color owing to pH fluctuations. In order to track the quality of milk

(Tirtashi *et al.*, 2019) assessed a starch-based film containing purple carrot anthocyanins. The film began as dark blue (fresh milk), changed to violet (medium fresh milk) after 24 hours, and then turned purple (deteriorated milk) after 48 hours, according to the authors' observations (Yong *et al.*, 2019) tested chitosan-based films containing anthocyanin-rich extracts from purple and black eggplant and exhibited a color shift when the film was immersed in spoilt milk during storage at 40° C for more than 10h. Therefore biopolymer films with anthocyanin can be used to monitor the deterioration of milk.

Table 2.2. Overview of Applications of Anthocyanin based Smart Packaging in Foods

Source of Anthocyanin	Film Forming Polymer	Extract/Polym er Ratio	Food Applicatio n	Colour Change	Characterization	Referen ce
Red cabbage	Gelatin / Pectin	2–5% w/w	Fresh meat (beef, fish, chicken) freshness indicator	Purple to yellow with increased pH / spoilage amines	FTIR, SEM; films functional as pH-indicator; antioxidant activity	(Cheng <i>et al.</i> , 2019)
	Gelatin / Pectin	1–3% (w/w)	Chicken & beef freshness monitoring	Purple to yellow as spoilage progresses	Mechanical, antioxidant activity, SEM	(Prietto <i>et al.</i> , 2017)
	Cellulose nanocrystals (CNC) + Nanofibrillated	30 wt% RCE (anthocyanin extract relative to CNC/NFC matrix)	Seafood freshness monitoring (e.g.,	Purple to green/yellow (with increasing pH/alkaline)	FTIR, mechanical (tensile, viscosity), optical & pH-responsiveness	(Shayan <i>et al.</i> , 2022)

	cellulose (NFC)		fish/shrimp packaging)			
Purple sweet potato	Gelatin	2% (w/w)	Fish freshness indicator	Red to blue to green (acidic to alkaline)	FTIR, mechanical properties, color response	(Zhai <i>et al.</i> , 2017)
Black rice	Chitosan	1–3% (w/w)	Smart food packaging indicator	Red to green with pH increase	FTIR, UV–Vis, mechanical & barrier properties	(Zeng <i>et al.</i> , 2023)
Grape pomace extract	Arrowroot starch		Smart/ Intelligent food packaging	Pink to greenish at higher pH		(Noguei ra <i>et al.</i> , 2024)
Butterfly pea flower	Polyvinyl alcohol (PVA)	1–5% (w/w)	Food spoilage indicator	Blue to green to yellow	Mechanical properties, color sensitivity	(Hidayat i <i>et al.</i> , 2021)
Food anthocyanin s (mixed food sources)	Starch / Polyvinyl alcohol (PVA)		Fresh shrimp deterioratio n indicator	Red to purple to greenish with increasing pH	FTIR, SEM, mechanical, UV- Vis pH response	(K. Zhang <i>et al.</i> , 2020)
Red barberry	Methylcellulos e (MC) + Chitin		Fresh fish spoilage indicator	Reddish to pink to yellow with increasing pH	Physico- mechanical, UV- Vis, barrier	(Sani <i>et al.</i> , 2021)

anthocyanins (RBAs)	nanofibers (CNFs)				properties; halochromic response	
Anthocyanins (plant extract)	Methylcellulose (MC) + Zinc alginate (ZA)	Anth3% (anthocyanin at 3% of total film matrix)	Freshness monitoring of pork & shrimp (smart packaging)	Reddish-pink to blue to blue-green as pH increases/ammonia exposure	Mechanical properties, thermal stability, UV-vis light transmission, colorimetric response to pH/ammonia gases	(C. Wang & Liu, 2024)
<i>Lycium ruthenicum</i> Murr. anthocyanins	Artemisia sphaerocephala Krasch. gum (ASKG) + Soybean protein isolate (SPI) + glycerol	Immersed in acidified ethanol containing anthocyanins at 1%, 2%, 3%, and 4% (g/mL) relative to film matrix	Fresh carp/meat freshness indicator	Red to lighter hues to yellowish-green/brown with spoilage & pH increase	FTIR, UV–Vis pH responses, SEM, mechanical & barrier	(Y. Zhao <i>et al.</i> , 2023)
Anthocyanins (from purple sweet potato) + Curcumin	Starch / Polyvinyl alcohol (SPVA) + Glycerol	Anthocyanin–curcumin blend (2:8, CR: ATH) incorporated into	Fish freshness monitoring (bighead carp)	Visible color change indicating fresh → medium → spoiled fish	FTIR, moisture content, solubility, WVP, thickness, ammonia response	(Chen <i>et al.</i> , 2020)

2.8 ADVANTAGES AND LIMITATIONS

2.8.1 ADVANTAGES

Because anthocyanin-based films are extremely sensitive to pH, they may serve as a real-time food freshness or deterioration indicator. These films help stop food deterioration from oxidation processes and food-borne microbes since they are safe, non-toxic, biodegradable, and typically have strong antioxidant and antibacterial qualities (L. Zhao *et al.*, 2022). Compared to many synthetic indicator dyes, anthocyanins are typically regarded as safe for food contact applications and have fewer toxicity issues because they are natural food colorants (found in berries, red cabbage, etc.) (Becerril *et al.*, 2021). The production of biodegradable/compostable indicator films that lessen plastic waste is made possible by the easy incorporation of anthocyanin extracts into biopolymers (starch, cellulose, pectin, and chitosan) (L. Zhao *et al.*, 2022).

When paired with active packaging techniques, anthocyanin-loaded films can offer antioxidant—and in some formulations, antimicrobial—activity that helps reduce oxidation and spoiling, potentially increasing shelf life (Zhu *et al.*, 2023). Anthocyanins are an economically appealing natural indicator because they may be derived from readily available and reasonably priced agricultural byproducts such as grape peel, berry wastes and red cabbage (Neves *et al.*, 2022).

2.8.2 LIMITATIONS

Food deterioration may result from the anthocyanin-based films' susceptibility to light, heat, water vapor, and oxygen penetration. Furthermore, a lot of composite films frequently have unsatisfactory mechanical characteristics. The primary drawback is that the films' capacity to track food freshness is lost due to anthocyanin degradation over time. Thus, further research should be done in the future to address these issues (L. Zhao *et al.*, 2022). The dependability and industrial significant limitation is their low heat and light durability, which causes anthocyanins to degrade and eventually fade color, decreasing their usefulness as visual indicators (M. Lu *et al.*, 2024).

Furthermore, anthocyanins experience irreversible color changes as a result of non-reversible structural alterations, which can limit their usefulness for repeated or long-term applications and undermine monitoring accuracy (Yong & Liu, 2020). Additionally, because of their hydrophilic nature, they are incompatible with many polymer matrices, which frequently results in uneven color distribution and increased brittleness in films or coatings (Sanches *et al.*, 2021).

Due to limited yields from plant sources, anthocyanin extraction is frequently coupled with high costs from a processing and economic standpoint, which limits large-scale and commercial application (Neves *et al.*, 2022). Additionally, anthocyanins are prone to auto-oxidation, which reduces the indicator system's effective lifespan and shortens its shelf life (Pereira Jr *et al.*, 2015). Food matrix interference, where changes in pH or ionic strength may result in inaccurate or misleading color responses, can also have an impact on the effectiveness of anthocyanin-based indicators (Neves *et al.*, 2022). Lastly, their usefulness across a variety of food products is limited by their relatively narrow pH sensitivity range, which makes them less appropriate for applications needing broader pH detection (L. Liu *et al.*, 2022).

2.9 CONCLUSIONS AND FUTURE DIRECTIONS

In response to growing demands for food safety, quality assurance, and waste reduction, smart packaging has become increasingly important in the food industry. Conventional packaging techniques mainly serve as passive barriers and do not provide information about the freshness or internal quality of food products. Intelligent packaging solutions capable of monitoring and communicating changes in packaged foods have therefore emerged as an effective alternative.

pH-responsive systems represent one of the most practical and consumer-friendly smart packaging strategies. These systems function by detecting chemical changes associated with food spoilage, such as variations in acidity or alkalinity resulting from microbial activity and metabolic processes. Because of concerns related to the sustainability, safety, and environmental impact of synthetic indicators, natural pigments are increasingly being explored for this purpose.

Many plant sources contain naturally occurring pigments known as anthocyanins that exhibit noticeable colour changes in response to pH variations. This distinctive colourimetric behaviour enables anthocyanins to function as visual indicators of food freshness in intelligent

packaging systems. In addition to their colourimetric properties, anthocyanins possess antioxidant and antimicrobial activities that may contribute to improved food quality and shelf-life extension. Their non-toxic and biodegradable nature further supports their suitability for food-contact applications.

The incorporation of anthocyanins into biodegradable polymer matrices has enabled the development of smart films with desirable functional characteristics. Biopolymers provide structural support, film-forming ability, and compatibility with hydrophilic pigments, allowing the production of flexible and responsive packaging materials. These films can display visible colour changes corresponding to variations in the freshness of perishable food products.

Anthocyanin-based smart packaging systems have demonstrated promising performance in monitoring the quality of a wide range of foods, particularly highly perishable products. The visual nature of these indicators enables consumers and supply-chain stakeholders to assess food freshness in real time without the need for specialized analytical equipment, thereby improving food safety and reducing unnecessary food waste.

By combining sustainability, safety, and functionality, anthocyanin-based smart packaging represents a promising advancement in intelligent food packaging technologies. The pH-responsive colour transitions of anthocyanins address a key limitation of traditional packaging systems by providing clear and easily interpretable freshness information. In addition, their natural origin and biodegradability align well with current environmental and regulatory demands for sustainable packaging materials.

However, several challenges still remain, including pigment instability, variability in film performance, and limited long-term colour stability under different environmental conditions. Addressing these limitations is essential to improve reliability and expand practical applications. Continued research focusing on material optimization, stabilization strategies, and improved film formulations will therefore be necessary.

Future research should focus on improving anthocyanin stability through encapsulation techniques, co-pigmentation strategies, and the incorporation of nanomaterials or protective polymer matrices. Further studies are also needed to develop multi-functional packaging systems

capable of simultaneously providing freshness indication, antimicrobial activity, and enhanced barrier properties. In addition, the development of standardized colour calibration methods and large-scale fabrication techniques will be essential for translating laboratory-scale indicator films into commercially viable packaging technologies.

Overall, anthocyanin-based smart packaging systems hold significant potential for improving food quality monitoring, enhancing consumer confidence, and supporting sustainable food supply chains. With continued advances in material science, extraction technologies, and intelligent packaging design, anthocyanin-based colourimetric indicators are expected to play an increasingly important role in next-generation food packaging systems.

MATERIALS AND METHODS

CHAPTER III

MATERIALS AND METHODS

The materials and experimental techniques used in the development and evaluation of anthocyanin-based smart packaging systems are described in this section.

3.1 SAMPLE COLLECTION

Purple cabbage (*Brassica oleracea* var. *capitata* f. *rubra*) was selected as natural source of anthocyanins for the study. Purple cabbage heads were procured from a local market and selected based on uniform purple coloration, firmness, and overall freshness. Purple cabbage samples were washed with distilled water, outer damaged leaves were removed, and the edible portions were chopped into small pieces. Purple cabbage pieces were then uniformly ground using a laboratory grinder to obtain a homogeneous sample, ensuring consistency in anthocyanin extraction. The ground samples were stored under refrigerated conditions at 8°C until further extraction and analysis.



Plate 3.1 Purple Cabbage

3.2 SOLVENT PREPARATION

The initial extraction conditions for anthocyanins from blueberry bagasse were established based on previously reported methods, using an aqueous solvent system containing 1% citric acid

(Lee and Wrolstad, 2004; Zardo, 2014).

Citric acid is used as a solvent for extraction because it provides an acidic environment (low pH), which stabilizes anthocyanins while also helping to break down plant cell walls for better release. It is favored for its ability to enhance extract stability, act as a safe/natural food-grade acidulant (GRAS), and its effectiveness, especially when combined with other methods or solvents like water/ethanol, yielding high-quality, stable colorants. The citric acid solvent was prepared by dissolving 1 g of citric acid in 100 mL of distilled water to obtain a 1% (w/v) aqueous solution.

3.3 EXTRACTION

Anthocyanin extraction from the uniformly ground purple cabbage samples was carried out using microwave-assisted extraction (MAE) and ultrasound-assisted extraction (UAE) techniques. Both methods were employed to evaluate the efficiency in recovering anthocyanins for smart packaging applications.

3.3.1 ULTRASOUND-ASSISTED EXTRACTION

Athena bath-type ultrasonicators has Stainless Steel (SS304/SS316) tanks, frequencies around 33-40 kHz, adjustable digital timers (up to 99 min), temperature control (often with heaters and optional chillers), and power varying by model (e.g., 120W to 650W+), with capacities from small lab sizes (2.5L) up to large industrial (100L+) units, using MOSFET-based generators for cavitation. The finely ground cabbage sample was combined with the extraction solvent, placed in a sealed sample container, and exposed to ultrasonic treatment to enhance mass transfer and promote efficient extraction of bioactive compounds.



Plate 3.2 Ultrasonicator

Table 3.1. Ultrasound – Assisted Extraction of Anthocyanin

Temperature	Solid-Solvent Ratio	Time	Ethanol	Reference
30°C	1:10	60min	60%	Wang <i>et al.</i> ,2016
35°C	1:30	30min	60%	Lopez <i>et al.</i> , 2018
45°C	1:20	40min	60%	Zou <i>et al.</i> ,2011

3.3.2 MICROWAVE ASSISTED EXTRACTION

Microwave-assisted extraction of purple cabbage was performed by dispersing the finely ground sample in the extraction solvent, transferring the mixture into a round-bottom flask, and exposing it to controlled microwave heating.



Plate 3.3 Microwave-assisted extraction

Table 3.2. Microwave-Assisted Extraction of Anthocyanin

Power	Solid Solvent Ratio	Time	Reference
200W	1:20	5min	Unzile Yigit <i>et al.</i> , (2021)
400W	1:28	8secs	Hong Kun Xue (2021)
700W	1:20	10min	Mora Pap <i>et al.</i> , (2012)

By making some alterations we kept ratio and extraction time constant as 1:10 and 10 minutes respectively. The extraction power was varied 20P, 40P and 60P. The suspension was subsequently filtered through a solvent resistance filter paper (Whatman No.1) to separate the solution.

3.4 pH RESPONSE of ANTHOCYANIN EXTRACT

The pH response of the extract was assessed by adding buffer solutions covering a pH range of 1–14. Buffer solutions of pH 4.5, 7.0, 9.2, and 11.0 were prepared using standard buffer tablets. The remaining buffer solutions were obtained by adjusting the pH of these buffers with HCl or NaOH as required, and confirming the final pH using a calibrated pH meter.

The pH responsiveness of the extract was evaluated by mixing 1 mL of the extracted sample with 1 mL of the corresponding buffer solution, followed by visual observation of the resulting color change.



Plate 3.4 pH responsiveness of the extract

3.5 TOTAL ANTHOCYANIN CONTENT



Plate 3.5 Spectrophotometer

The pH differential method was used to determine the total anthocyanin content (TAC). Two different buffer solutions were needed for the method: an acetate buffer (0.66 M) at pH 4.5 ± 0.05 and an aqueous buffer (0.025 M) at pH 1.0 ± 0.05 . Four pieces of each of the acetate and aqueous buffers were combined with one portion of the extract solution (Chua & Abd Wahab, 2023). A UV-vis spectrophotometer (Shmadzu UV-1800, Kyoto, Japan) was used to measure the mixture's absorbance at 520 and 700 nm.

Eq. (3.1) can be used to compute the difference in absorbances at various pH and wavelengths. Equation (3.2) can be used to calculate the concentration of monomeric anthocyanins.

$$A = (A_{520\text{nm}} - A_{700\text{nm}})_{\text{pH } 1.0} - (A_{520\text{nm}} - A_{700\text{nm}})_{\text{pH } 4.5} \quad \dots(3.1)$$

$$\text{TAC (mg/L)} = (A * \text{MW} * \text{DF} * 1000) / \epsilon * p \quad \dots(3.2)$$

where, A , difference in absorbance; MW (molecular weight), 449.2 g/mol for cyanidin-3-glucoside; DF, dilution factor; ϵ , extinction coefficient (26,900 L/mol/cm); p , pathlength (0.5 cm) (Chua L.S. *et.,al* (2024)).

3.6 POLYMER-BASED FILM FORMATION

In the polymer-based film formation, chitosan and carboxymethyl cellulose (CMC) were used as the film-forming matrices for incorporating the extracted anthocyanins. The prepared films were evaluated for their physical and mechanical properties.

A film-forming solution was prepared by blending carboxymethyl cellulose (CMC, 3.5 g) and gelatin (1.5 g) with 93–94 g of water. Glycerol (1 g) was added as a plasticizer. The mixture was subjected to continuous shaking and heated to 90 °C to ensure complete dissolution of the polymers. After heating, the solution was cooled to approximately 40 °C, followed by the incorporation of the anthocyanin extract. The resulting solution was then shaken to achieve uniform dispersion in a Rotary Stirrer, degassed to remove entrapped air bubble using an Ultrasonicator, cast onto a suitable surface which is a silicone mould, and dried to obtain the film in a Hot Air Oven at 40°C for 24-48hrs (Lupina *et al.*, 2022).

From this reference, the CMC was replaced with Chitosan as the wall material for the film formation and the properties were studied. The film-forming solution was prepared by dissolving chitosan (3.5 g) in 1.5%v/v of acetic acid aqueous solution, which served as the solvent for chitosan.

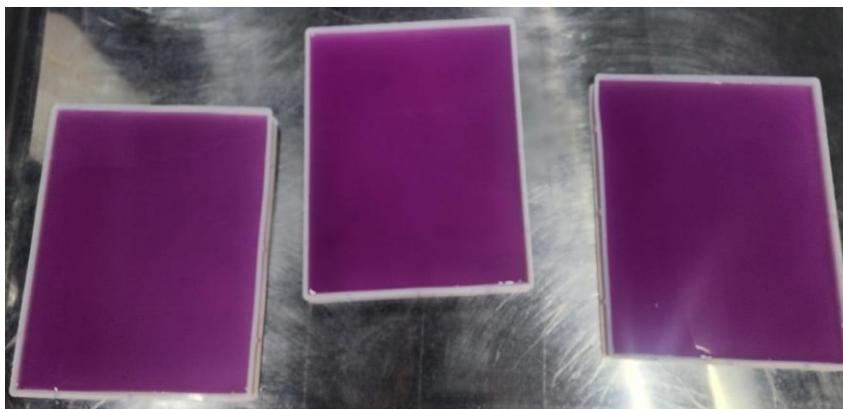


Plate 3.6 Film-forming solution

3.7 COLOUR

The colour of the Anthocyanin based film was evaluated to determine changes in visual appearance and to quantify colour variations, as colour is a key quality attribute influencing consumer acceptability. Colour measurements were carried out using a Lovibond handheld colorimeter (RM200, X-Rite, USA) which operates on the principle of reflectance colorimetry expressed in terms of CIE L*, a*, and b* colour values.



Plate 3.7 Lovibond colorimeter

In this colour space, L^* represents lightness (0 = black and 100 = white), a^* indicates the red (+) to green (–) component, and b^* indicates the yellow (+) to blue (–) component (Lee *et al.*, 2020).

For colour determination, one rectangular sheet of film was selected, cut into very small and uniform pieces, and filled into a clean cuvette up to the marked level to ensure complete coverage of the optical path and uniform light reflection. The cuvette was placed in the sample holding port of the colorimeter, and the colour reading was recorded. To minimize the effect of sample non-uniformity and ensure representative measurements, the position of the cuvette was adjusted and readings were taken at three different orientations, with the mean value considered as the final colour measurement. The cuvette was thoroughly washed with distilled water before and after each measurement to avoid cross-contamination and ensure reliable results.

The overall colour difference between samples was quantified using the total colour difference (ΔE^*) parameter, which is widely used to express perceptible colour changes in food products. The ΔE^* value was calculated using the following equation:

$$\Delta E^* = \sqrt{((\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2)} \quad \dots(3.3)$$

where L^* , a^* , b^* represent the colour values of the film sample, and L , a , b represents the corresponding colour values of the control sample. The ΔE^* value provides a single numerical representation of the magnitude of colour change, with higher values indicating greater deviation

from the control, and was used to evaluate the effectiveness of the treatments in preserving the colour stability of film (Lee *et al.*, 2020).

3.8 MECHANICAL PROPERTIES

The conditioned films were attached between two grips of a texture analyzer after being cut to precise proportions. The following formula was used to compute the mechanical property of films, such as tensile strength (TS), in accordance with the ASTM D882-02 standard (ASTM, 2002) (Nadi.M *et al.* (2023)).

Thickness is defined as the average distance between the two surfaces of the dried anthocyanin-based film and thickness was measured using a thickness gauge.

3.9 STORAGE STUDIES OF FRESH MACKEREL FISH AND PANEER USING ANTHOCYANIN-BASED SMART PACKAGING

The fresh mackerel fish and paneer was bought from a local market (Tavanur) and immediately transferred to the laboratory. The fish and paneer pieces were placed in sterile packaging boxes and each smart film ($2 \times 2 \text{ cm}^2$) was fixed on the internal space of its lids. These boxes were incubated at 4°C for 1 to 10 days, respectively. The photographic images of films' color changes were recorded by a digital camera. Moreover, the changes in pH, microbial loaded, and TVN-B of fish and paneer samples were determined during analysis.

3.9.1 Microbiological Analysis

For evaluation of the total plate count (TPC) of the samples, 10 g of fish and paneer was transferred to a sterile ringer solution (90 mL) and well homogenized. Then, serial dilutions were prepared and used for pour-plate culture on plate count agar (PCA). The number of colonies on the medium after 48 h of incubation at 37°C was reported as TPC.

$$\text{CFU/mL (or g)} = (\text{Number of Colonies} \times \text{Dilution Factor}) / \text{Volume Plated (mL or g)} \quad \dots (3.4)$$

3.9.2 Total Volatile Base Nitrogen (TVB-N)

It is a widely employed test for fish spoilage. In this method ammonia and other aliphatic amines are estimated.



Plate 3.8 Conway's Micro Diffusion Unit

TCA extract of the fish is prepared using 10 gm of finally grind sample and 10% 100 ml TCA solution. From this extract TVBN is liberated by treatment with alkali and the liberated TVBN is absorbed in standard acid in a Conway's Micro Diffusion Unit. From the amount of acid consumed, TVBN content is calculated.

Clean the Conway's unit and apply vacuum grease on the top of the Conway unit. Take 1 ml of N/100 H_2SO_4 in the inner chamber of the Conway's unit. Add 1 ml of TCA extract and 1 ml of saturated potassium carbonate in the outer chamber. Seal the unit with the glass lid. Gently rotate the unit to mix the solution in the chamber. Keep the unit undisturbed overnight. Remove the lid and add a drop of Tashiro's indicator to the inner chamber. Determine the amount of untreated acid in the inner chamber by titration with N/100 NaOH. A blank is run using 1 ml of TCA solution without using fish juice in the outer chamber.

Volume of N/100 NaOH used up by Blank

Volume of N/100 NaOH used up by Test

Amount of N/100 H_2SO_4 used by the base released from the fish juice

1 ml of N/100 $\text{H}_2\text{SO}_4 = 0.14 \text{ mg of N}_2$

Calculation

$$\frac{\text{Blank} - \text{Titre valued} \times 0.01 \times 14 \times \text{vol. made up} \times 100}{\text{Wt of sample} \times \text{vol. ppt.}} = \text{mg \%} \quad (3.5)$$

3.10 MATERIAL CHARACTERIZATION TECHNIQUES

Material characterization techniques were employed to investigate the structural, chemical, thermal, and morphological properties of the developed anthocyanin-based smart films. These analyses provide valuable information regarding the interaction between polymeric components and anthocyanin pigments, crystallinity, thermal stability, and surface morphology of the films. The characterization studies were carried out using X-ray Diffraction (XRD), Fourier Transform Infrared Spectroscopy (FTIR), Differential Scanning Calorimetry (DSC), and Scanning Electron Microscopy (SEM). The obtained results were used to correlate the physicochemical properties of the films with their performance as intelligent food packaging materials.

3.10.1 X-Ray Diffraction (XRD) Analysis

X-ray Diffraction (XRD) analysis was performed to determine the crystalline and amorphous nature of the developed film samples. The diffraction patterns were recorded using an X-ray diffractometer equipped with Cu-K α radiation ($\lambda = 1.5406 \text{ \AA}$). Film specimens were cut into appropriate dimensions and mounted on the sample holder. The samples were scanned over a 2θ range of 5° – 80° at a scanning rate of 2° min^{-1} under ambient conditions.

The diffraction peaks obtained were analyzed to evaluate the degree of crystallinity and structural modifications resulting from the incorporation of anthocyanin extract into the polymer matrix. The presence of sharp and intense peaks indicated crystalline regions, whereas broad peaks suggested an amorphous structure.

An increase in crystallinity indicates enhanced molecular ordering and stronger intermolecular interactions within the film matrix. A decrease in crystallinity suggests increased amorphous characteristics due to the incorporation of bioactive compounds or plasticizers.

3.10.2 Fourier Transform Infrared Spectroscopy (FTIR)

Fourier Transform Infrared Spectroscopy (FTIR) is a widely used analytical technique for identifying functional groups and investigating molecular interactions in polymeric materials. FTIR analysis helps in understanding the chemical structure of the developed films and confirms the incorporation of bioactive compounds through characteristic absorption bands.

The film samples were conditioned at room temperature prior to analysis. Small pieces of the film were placed directly on the sample holder of the FTIR spectrometer and scanned over the spectral range of 4000–400 cm^{-1} . The spectra obtained were recorded at appropriate resolution and number of scans. The characteristic absorption peaks corresponding to hydroxyl, carbonyl, amino, aromatic, and other functional groups were identified and compared among the different film formulations.

3.10.3 Differential Scanning Calorimetry (DSC) Analysis

Differential Scanning Calorimetry (DSC) was performed to evaluate the thermal behavior and stability of the developed smart films. Approximately 5–10 mg of film sample was accurately weighed and sealed in an aluminum pan. An empty aluminum pan was used as the reference. The samples were heated from 25°C to 300°C at a heating rate of 10°C min^{-1} under a nitrogen atmosphere. The heat flow associated with thermal transitions was continuously recorded as a function of temperature.

The thermograms obtained were analyzed to determine glass transition temperature (T_g), melting temperature (T_m), crystallization temperature (T_c), and enthalpy change (ΔH). Higher T_g values indicate restricted molecular mobility and stronger interactions within the film matrix. Increased melting temperature and enthalpy values suggest improved thermal stability and crystallinity. Changes in thermal transition temperatures after anthocyanin incorporation indicate interactions between the polymer matrix and bioactive compounds.

3.10.4 SCANNING ELECTRON MICROSCOPY (SEM)

Scanning Electron Microscopy (SEM) is a powerful analytical technique used to examine the surface morphology and microstructural characteristics of materials at high magnification.

SEM analysis provides detailed information regarding surface texture, homogeneity, porosity, cracks, phase separation, and distribution of additives within the film matrix.

Small sections of the developed film samples were cut into appropriate dimensions and mounted on aluminum stubs using double-sided conductive adhesive tape. The samples were then sputter-coated with a thin layer of gold to improve electrical conductivity and prevent charging during observation. The coated samples were examined using a Scanning Electron Microscope operated under suitable accelerating voltage and vacuum conditions. Micrographs were captured at different magnifications to evaluate the surface and cross-sectional morphology of the films.

The obtained SEM images were analyzed for the presence of pores, cracks, agglomerations, and uniformity of the film matrix. A smooth and homogeneous surface indicates good compatibility among film components, whereas the presence of cracks, voids, or particle aggregation suggests poor dispersion and weak intermolecular interactions. A compact and continuous film structure with minimal pores and cracks indicates improved mechanical strength and barrier properties. Uniform distribution of bioactive compounds within the polymer matrix confirms successful incorporation and compatibility of the additives. Increased surface roughness, cracks, or phase separation may indicate poor film integrity and reduced performance.

Changes in peak position, peak intensity, or the appearance and disappearance of absorption bands were evaluated to determine intermolecular interactions such as hydrogen bonding, electrostatic interactions, and compatibility between the film matrix and incorporated active compounds.

The presence of characteristic absorption bands confirms the functional groups present in the film matrix. Shifts in peak positions toward higher or lower wavenumbers indicate molecular interactions between film components. Increased broadening of the O–H stretching band generally suggests enhanced hydrogen bonding. The appearance of new peaks may indicate successful incorporation of active compounds, whereas disappearance of characteristic peaks may suggest chemical modifications or interactions occurring within the film matrix.

3.10.5 UV-VISIBLE SPECTROSCOPY

UV-visible spectroscopy is an analytical technique used to characterize the optical and structural properties of biopolymer films by measuring their light absorption across different wavelengths in the ultraviolet (UV) and visible regions of the electromagnetic spectrum (typically 200-800 nm).

When a biopolymer film is exposed to UV-visible light, the atoms and molecules within the film absorb photons at specific wavelengths corresponding to their electronic transitions. The spectrophotometer measures the transmittance and absorbance of light passing through the film, generating an absorption spectrum that reveals information about the film's composition, functional groups, and optical properties.

For protective packaging applications like anthocyanin-incorporated CMC films, UV-visible spectroscopy confirms the presence and functionality of active compounds, validates the color-changing indicator mechanism, and provides objective measurement of film degradation correlated with product spoilage, complementing visual observation with quantitative spectroscopic data.

RESULT AND DISCUSSION

CHAPTER IV

RESULT AND DISCUSSION

This chapter deals with the development, characterization and application of anthocyanin-based pH-sensitive colorimetric films as intelligent packaging materials. Further, the optimization of ultrasound-assisted extraction of anthocyanins, comparative evaluation of carboxymethyl cellulose (CMC) and chitosan as film-forming matrices, comprehensive characterization of the developed films using XRD, FTIR, DSC, SEM, UV-Visible spectroscopy etc and validation of the films for real-time freshness monitoring of perishable food products are also discussed in this chapter.

4.1 EXTRACTION

Purple cabbage (*Brassica oleracea* var. *capitata* f. *rubra*) was selected as natural source of anthocyanins for the study. Anthocyanin was extracted using Microwave assisted extraction and Ultrasound assisted extraction.

4.1.1 ULTRASOUND ASSISTED EXTRACTION(UAE)

Ultrasound-assisted extraction of bioactive compounds was studied under three different extraction conditions by varying temperature, solvent composition, extraction time, and ultrasonic parameters. In condition I extraction performed at 45 °C with a liquid-to-solid ratio of 20:1 for 40 minutes using 60% ethanol showed high extraction efficiency(Zou *et al.*,2011).Condition II involved higher temperatures 30 °C and longer extraction times 60 minutes using methanol–ethanol mixtures (60–70%)(Wang *et al.*,2016).In Condition III, extraction at 35 °C with optimized ultrasound power (243.7 W), 20 kHz frequency, and 59.9% ethanol for 60minutes (Lopez *et al.*, 2018).The conditions at 45 °C showed high extraction efficiency with a yield of 21.8mg/L anthocyanin extract.

4.1.2 MICROWAVE-ASSISTED EXTRACTION(MAE)

Microwave-assisted extraction (MAE) was investigated under varying conditions of temperature, solvent-to-solid ratio, microwave power, and extraction time to evaluate their effect on the recovery of bioactive compounds. The results demonstrated that MAE significantly enhanced extraction efficiency due to rapid volumetric heating and effective disruption of plant cellular structures.

Extraction conducted at moderate temperatures (45–50 °C) with solvent ratios ranging from 1:20 to 1:28 and ethanol concentrations of approximately 50–60% resulted in efficient recovery of bioactive compounds within short extraction times (5–20 minutes) (Unzile Yigit et.,al2021,Hong Kun Xue 2021,Mora Pap et.,al2012).Microwave-assisted extraction was optimized by keeping the solvent ratio (1:20) and extraction time (10 minutes) constant, while varying microwave power (20, 40, and 60). Extraction efficiency increased with power due to enhanced internal heating and improved cell wall disruption. The conditions at 20 power showed high extraction efficiency with a yield of 15.3mg/L anthocyanin extract.

The extraction yield demonstrated the superior effectiveness of ultrasound-assisted extraction, which produced the highest anthocyanin yield 21.8 mg/L under optimized conditions. This yield was significantly higher than that obtained through microwave-assisted extraction. The enhanced efficiency of ultrasound-assisted extraction is attributed to acoustic cavitation, which disrupts plant cell walls and improves solvent penetration, resulting in greater anthocyanin release. Overall, the results confirm ultrasound-assisted extraction as a mild, efficient, and effective method for anthocyanin extraction from red cabbage.

4.2 FILM FORMATION

A Chitosan and CMC–based film was prepared and initially kept in a hot air oven at 45 °C within 24 hours. After this period, the film was not completely dried. It was then kept for further drying, after which it dried properly and formed a thin, uniform film.

4.3 PH RESPONSE OF EXTRACT

The anthocyanins extracted from purple cabbage exhibited clear and characteristic pH-dependent color changes over a wide pH range from 1 to 14. In strongly acidic conditions (pH 1–2), the extract showed an intense red to deep pink color due to the predominance of the flavylium cation form of anthocyanins. As the pH increased to mildly acidic levels (pH 3–4), the color gradually changed to bright pink and magenta shades. Near neutral pH values (pH 5–7), the solution appeared pale pink to light purple, indicating structural transformation of the anthocyanin molecules. With the transition to alkaline conditions (pH 8–9), the color shifted from purple to bluish-green tones.

At moderately alkaline pH (pH 10–11), the extract exhibited a distinct green coloration. Under strongly alkaline conditions (pH 12–14), the color further changed to yellowish-green and yellow shades, suggesting significant molecular rearrangement and partial degradation of anthocyanin structures. These pronounced and reversible color transitions confirm the strong pH sensitivity of red cabbage anthocyanins, highlighting their suitability as natural pH indicators for incorporation into intelligent, pH-responsive film systems.

4.4 THICKNESS GAUGE READING

Table 4.1. Thickness Gauge Reading

CMC (mm)	CHITOSAN (mm)
0.196	0.443
0.407	0.194
0.405	0.413
0.516	0.110
0.273	0.135
0.279	0.303
0.508	0.329
0.409	0.153
0.341	0.144
0.252	0.276

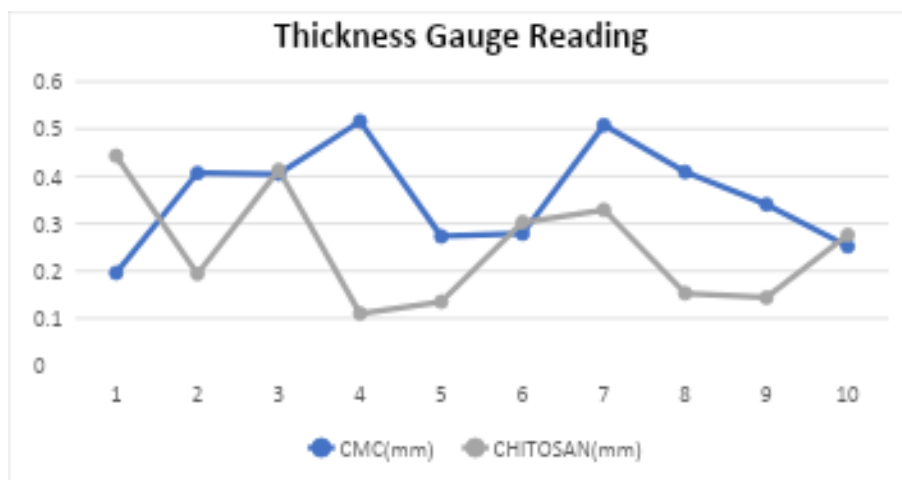


Fig 4.1. Thickness Gauge Measurement of Biopolymer Film

The average thickness of the developed pH-sensitive indicator films showed noticeable variation between the two polymer matrices. The CMC-based film exhibited a higher average thickness of 0.36 mm, whereas the chitosan-based film showed a comparatively lower average thickness of 0.25 mm. Film thickness is a crucial parameter for indicator applications, as it influences pigment retention, color intensity, visibility of pH-induced color changes, and ease of handling. Since the films were designed to function only as visual indicators and not as complete packaging materials, adequate thickness is essential to ensure clear and stable color responses.

Thicker indicator films enhance the readability of anthocyanin color changes and reduce pigment leaching during use. Based on these considerations, CMC was selected as the indicator matrix due to its higher average thickness, which supported better anthocyanin incorporation and resulted in more distinct and stable pH-responsive color changes. Therefore, CMC-based films were found to be more suitable for use as anthocyanin-based pH-sensitive smart packaging indicators.

4.5 TENSILE STRENGTH TEST

The tensile properties of chitosan and CMC-based films were evaluated to understand their suitability as pH-sensitive indicator films. The chitosan film showed an average tensile force of 14.33 N with a relatively low average elongation of 6.33 mm, indicating a stiffer and less flexible structure. In contrast, the freshly dried CMC film (CMC film I) exhibited a lower average force of

12.0 N but a very high average elongation of 33 mm, reflecting excellent flexibility. High elongation is a desirable property for indicator films, as it improves handling and reduces the risk of cracking during application.

Table 4.2 Tensile Strength

Chitosan film		CMC film I		CMC film II	
Force (N)	Elongation (mm)	Force (N)	Elongation (mm)	Force (N)	Elongation (mm)
6.5	4	4.5	36	23	13
14.5	7	13	31	34	10
22	8	18.5	32	26	12

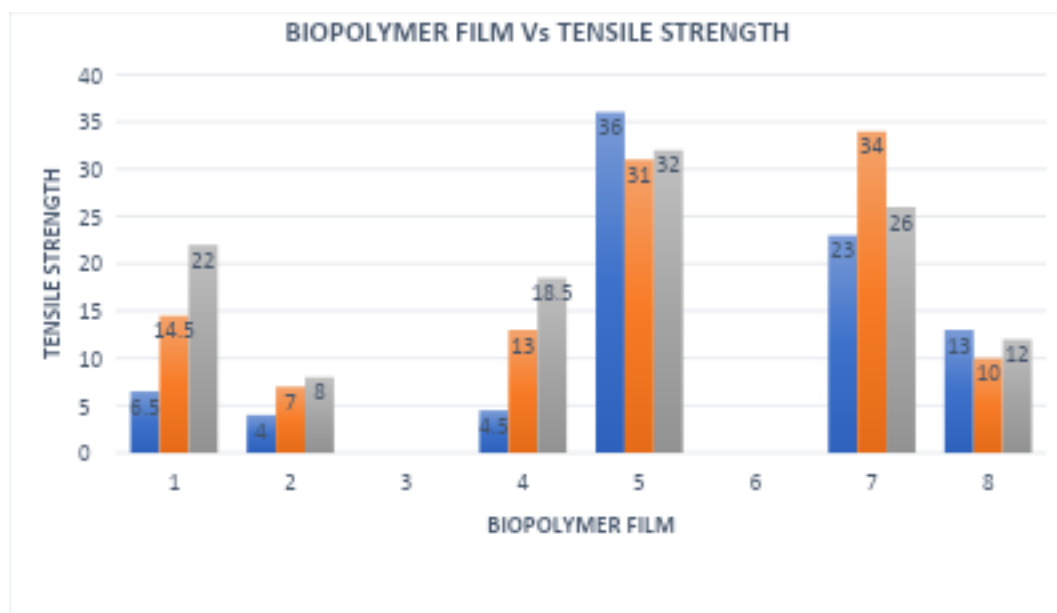


Fig 4.2. Tensile Strength of Anthocyanin-Biopolymer Films

After keeping the CMC film over time (CMC film II), the average force increased significantly to 28.5 N, while the average elongation decreased to 11.5 mm. This change suggests that ageing or moisture loss over time led to increased rigidity and reduced flexibility of the CMC film. Despite this reduction, CMC films still showed higher elongation than chitosan films.

Therefore, CMC was selected as the base material, primarily due to its superior elongation and flexibility, making it more suitable for use as an anthocyanin-based pH-sensitive indicator rather than as a complete packaging material.

4.6 pH CHANGE IN FILM

The pH sensitivity of the prepared films was evaluated by adding solutions of different pH directly onto the dried films and observing the resulting colour changes. Both CMC and chitosan films exhibited visible colour variations in response to changes in pH, confirming their suitability as pH-sensitive indicator films.

The CMC-based film showed distinct, sharp, and easily recognizable colour changes across the tested pH range. Under acidic conditions, the film exhibited reddish to pink hues, while gradual changes to purple, greenish, and yellowish tones were observed as the pH shifted towards neutral and alkaline conditions. The colour changes were uniform and visually prominent, allowing clear differentiation between different pH levels.

In contrast, the chitosan-based film displayed comparatively dull and less intense colour transitions, especially under neutral and alkaline conditions. Although pH responsiveness was evident, the colour changes were less uniform and harder to distinguish when compared with the CMC film. This reduced visual clarity limits its effectiveness as a practical pH indicator.

Based on the comparative analysis, the CMC film demonstrated superior pH sensitivity and clearer visual colour response than the chitosan film. Therefore, CMC was finally selected as the suitable film matrix for the development of anthocyanin-based pH-sensitive indicator films.

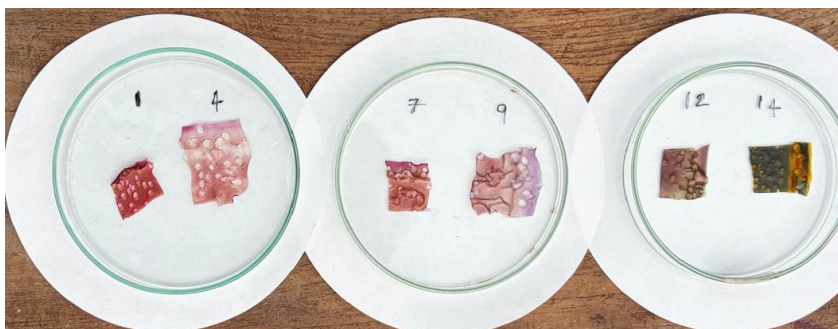


Plate 4.1 pH response of anthocyanin incorporated film



Plate 4.2 Anthocyanin incorporated CMC-based Film

4.7 COLOUR PARAMETERS OF FILM

The colour parameters of the CMC–cabbage and chitosan–cabbage films revealed noticeable differences, highlighting the influence of the polymer matrix on visual appearance. The CMC–cabbage fresh film showed a higher lightness value ($L^* = 34.83$) than the chitosan–cabbage film ($L^* = 26.26$), indicating that the CMC film was brighter and less dark. This higher lightness is generally desirable for packaging and indicator films, as it improves visual clarity and consumer acceptability, suggesting that the CMC matrix provides a more uniform and visually appealing background for cabbage pigments.

Although the chitosan–cabbage film exhibited higher redness ($a^* = 19.13$) and colour saturation ($c^* = 21.33$), the CMC–cabbage film displayed a more balanced combination of redness ($a^* = 11.23$) and yellowness ($b^* = 12.93$), resulting in a pleasant and stable colour tone. The higher hue angle of the CMC–cabbage film ($h^* = 35.01^\circ$) compared to the chitosan–cabbage film (24.33°) indicates a shift toward a yellowish-red region, which is often considered more visually acceptable and less intense than deep red tones.

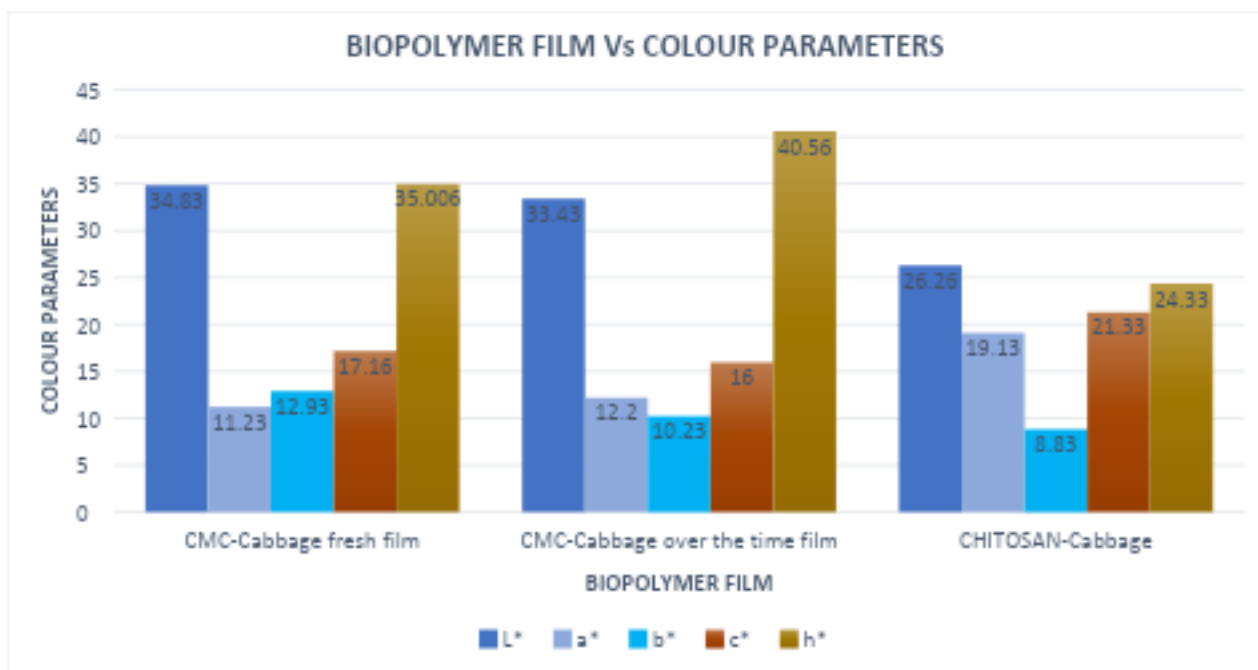


Fig 4.3. Colour Parameters of Anthocyanin-Biopolymer Films

Table 4.3. Colour Parameters CMC – Cabbage film(fresh)

Parameters	Value
L*	34.83
a*	11.23
b*	12.93
C*	17.16
h*	35.006

Table 4.4. Colour Parameters CMC – Cabbage film(aged)

Parameters	Value
L*	33.43
a*	12.2
b*	10.23
C*	16
h*	40.56

Table 4.5. Colour Parameters CHITOSAN – Cabbage film

Parameters	Value
L*	26.26
a*	19.13
b*	8.83
C*	21.33
h*	24.33

Overall, the CMC–cabbage film can be considered superior when compared to the chitosan–cabbage film in terms of colour quality, as it exhibited higher lightness, moderate chromatic values, and a more uniform hue. These characteristics suggest that CMC is a better film-forming material for incorporating cabbage pigments, providing improved visual appearance and suitability for applications where clarity and aesthetic quality are important.

4.8 STORAGE STUDIES OF FRESH MACKEREL FISH AND PANEER USING ANTHOCYANIN-BASED SMART PACKAGING FILMS

4.8.1 MICROBIOLOGICAL ANALYSIS

The storage study of CMC film used for fish and paneer was evaluated by monitoring microbial quality through total plate count (TPC) analysis. Microbial enumeration was carried out using the serial dilution technique followed by the pour plate method, which is widely accepted for determining total viable microbial load. This approach enabled effective assessment of microbial growth on fish and paneer packaged samples during the storage period and provided a clear indication of the protective efficiency of the CMC film.

The effect of anthocyanin incorporated CMC film as a real-time freshness indicator during storage is presented in Table 4.6. The observations were recorded at 2-day intervals to monitor the progressive colour changes of the indicator film and to correlate these changes with the microbiological quality of the packaged food products.

The anthocyanin-incorporated CMC film exhibited a distinct and progressive colour transition throughout the storage period as a result of biochemical changes associated with microbial spoilage. Initially, the indicator film exhibited a purple colour. As storage progressed, the film gradually changed to dark purple, indicating the onset of product deterioration. With further storage, the indicator underwent a final colour transition to dark green, signifying advanced spoilage of the packaged food product. This colour transformation can be attributed to the interaction of anthocyanin pigments with volatile basic compounds and other metabolites produced during microbial growth, which alter the chemical environment surrounding the packaged food.

Both fish and paneer samples packaged with the anthocyanin-incorporated CMC film exhibited a similar pattern of colour change during storage. The visual changes observed in the indicator film corresponded closely with the increase in microbial population determined through microbiological analysis. The microbiological results confirmed that the progressive colour transition accurately reflected the deterioration of the food products.

By the seventh day of storage, when the indicator film had completely changed from purple to dark green, the total microbial counts had reached the maximum permissible limits specified by the Food Safety and Standards Authority of India (FSSAI), namely 1×10^7 CFU g⁻¹ for fish and 3.5×10^5 CFU g⁻¹ for paneer. These results confirmed that the food products had reached the spoilage stage and were no longer suitable for consumption. The close agreement between the visual colour change of the indicator film and the microbiological analysis demonstrates the reliability of the anthocyanin-based indicator for monitoring product freshness.

Overall, the anthocyanin-incorporated CMC film functioned effectively as an intelligent real-time freshness indicator by providing a simple visual assessment of food quality without the need for laboratory analysis. The sequential colour transition from purple to dark purple and finally to dark green enabled continuous monitoring of product freshness throughout the storage period and provided a clear visual warning when the products exceeded the microbiological safety limits. These findings demonstrate the potential of anthocyanin-incorporated CMC film as an economical, reliable and environmentally friendly smart packaging material for monitoring the freshness and safety of highly perishable food products.

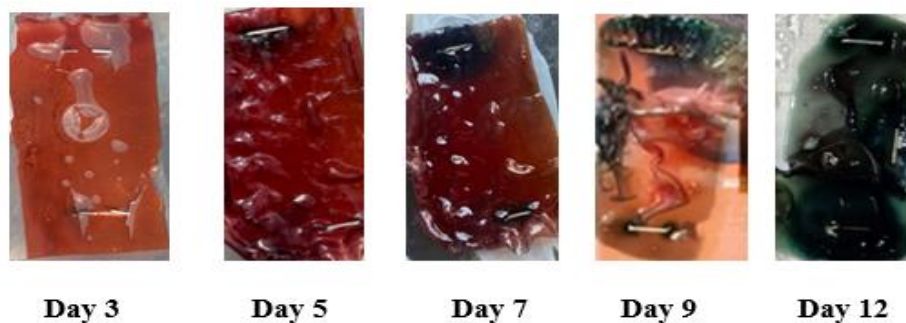


Plate 4.3 Colour change response of Anthocyanin incorporated CMC film during storage study of Fish

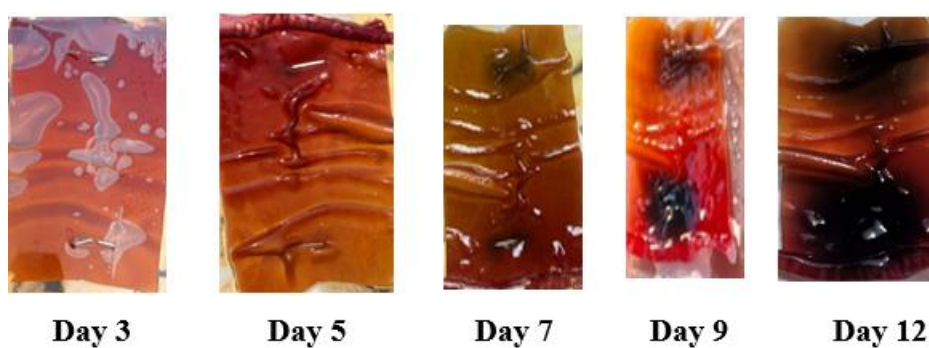


Plate 4.4 Colour change response of Anthocyanin incorporated CMC film during storage study of Paneer

Table 4.6 Colour change response of Anthocyanin incorporated CMC film

Day	Fish	Paneer
3	Purple	Purple
5	Dark Purple	Red -Orange
7	Dark Red	Pale Brown
9	Light brown to Green	Rusty Orange
12	Dark Green	Green

Table 4.7. Microbiological Analysis of Fish

Dilution	Day 3 (CFU/g)	Day 5 (CFU/g)	Day 7 (CFU/g)	Day 9 (CFU/g)	Day 12 (CFU/g)
10 ⁻⁴	6.5×10 ³	9.2 × 10 ⁴	7.6 × 10 ⁶	6.5×10 ⁷	9.2 × 10 ⁸
10 ⁻⁵	3.2×10 ²	4.6 × 10 ³	3.8 × 10 ⁵	3.2×10 ⁶	4.6 × 10 ⁷
10 ⁻⁶	1.4×10 ¹	1.8 × 10 ²	1.4 × 10 ⁴	1.4×10 ⁵	1.8 × 10 ⁶

Table 4.8. Microbiological Analysis of Paneer

Dilution	Day 3 (CFU/g)	Day 5 (CFU/g)	Day 7 (CFU/g)	Day 9 (CFU/g)	Day 12 (CFU/g)
10 ⁻⁴	4.8 × 10 ⁴	5.4 × 10 ⁴	6.8 × 10 ⁵	8.5×10 ⁵	8.9 × 10 ⁶
10 ⁻⁵	2.8 × 10 ³	4.2 × 10 ³	3.4 × 10 ⁴	4.2×10 ⁴	4.2 × 10 ⁵
10 ⁻⁶	1.2 × 10 ²	2.0 × 10 ²	1.3 × 10 ³	2.0×10 ³	2.0 × 10 ⁴

4.8.2 TOTAL VOLATILE BASE NITROGEN (TVB-N)

The TVB-N test is used to evaluate the freshness and spoilage level of protein-rich foods, especially fish and seafood. It measures volatile nitrogenous compounds such as ammonia, trimethylamine, and dimethylamine formed due to microbial and enzymatic degradation. An increase in TVB-N value indicates progressive spoilage and loss of freshness. TVB-N is commonly expressed as mg nitrogen per 100 g of sample. Lower TVB-N values during storage indicate better preservation and delayed spoilage. The test is widely used to assess the effectiveness of packaging materials in extending the shelf life of perishable foods.

Table 4.9. Total Volatile Base Nitrogen (TVB-N)

Sl. No.	Sample code	Weight of sample (g) (m)	Normality of HCl used	Vol of 0.01 N HCl for blank(ml) (v₀)	Titre value (v₁)	TVB-N = ((v₁-v₀)*0.01*14*1000*100)) (50*100) (mg%)
1.	Day 1	10	0.01	1	0.96	5.60

2.	Day 3	10	0.01	1	0.95	7.00
3.	Day 5	10	0.01	1	0.93	9.80
4.	Day 7	10	0.01	1	0.87	18.20
5.	Day 9	10	0.01	1	0.74	36.40
6.	Day 12	10	0.01	1	0.67	46.20

4.9 MATERIAL CHARACTERIZATION TECHNIQUES

4.9.1 UV-Visible Spectroscopy

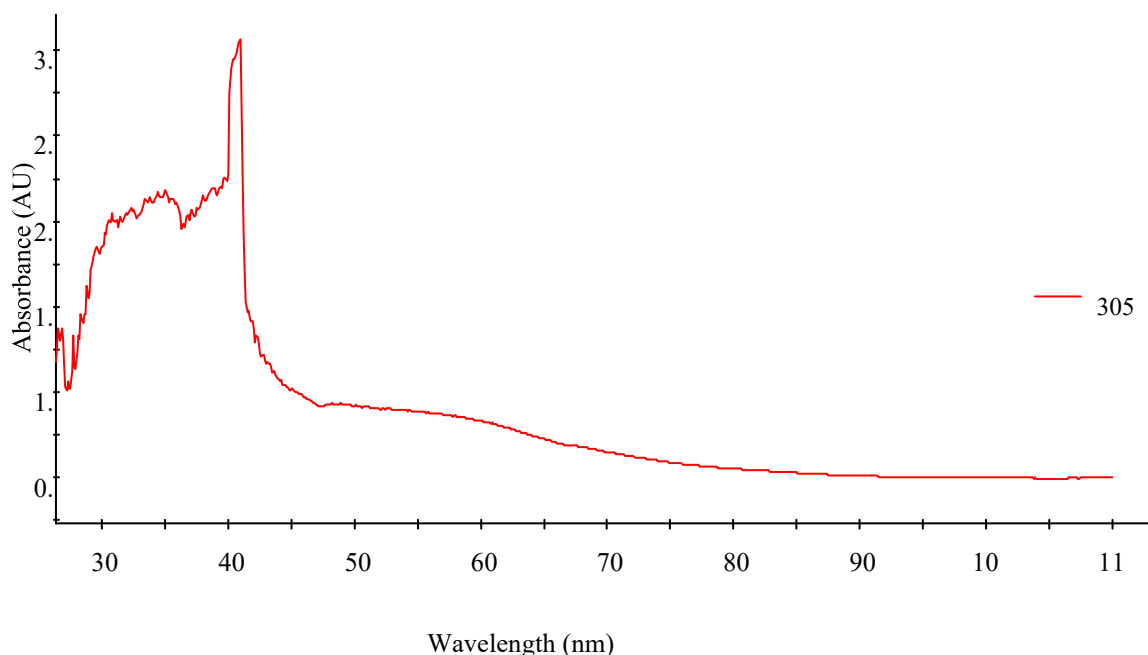


Fig.4.4 UV-Visible Absorption Spectrum (Wavelength vs. Absorbance)

The UV–Visible absorbance spectrum of the anthocyanin-incorporated CMC film showed strong absorption in the UV region (280–400 nm), attributed to the phenolic compounds present in anthocyanins, indicating excellent UV-blocking properties. A characteristic absorption peak was observed around 420–430 nm, confirming the successful incorporation of anthocyanins into the CMC matrix. The shift from the typical anthocyanin absorption wavelength may be due to interactions between anthocyanins and the polymer matrix. Beyond 450 nm, absorbance gradually decreased, indicating greater transparency in the visible and near-infrared regions. These results

suggest that the developed film is suitable for intelligent food packaging by providing UV protection while maintaining desirable optical properties.

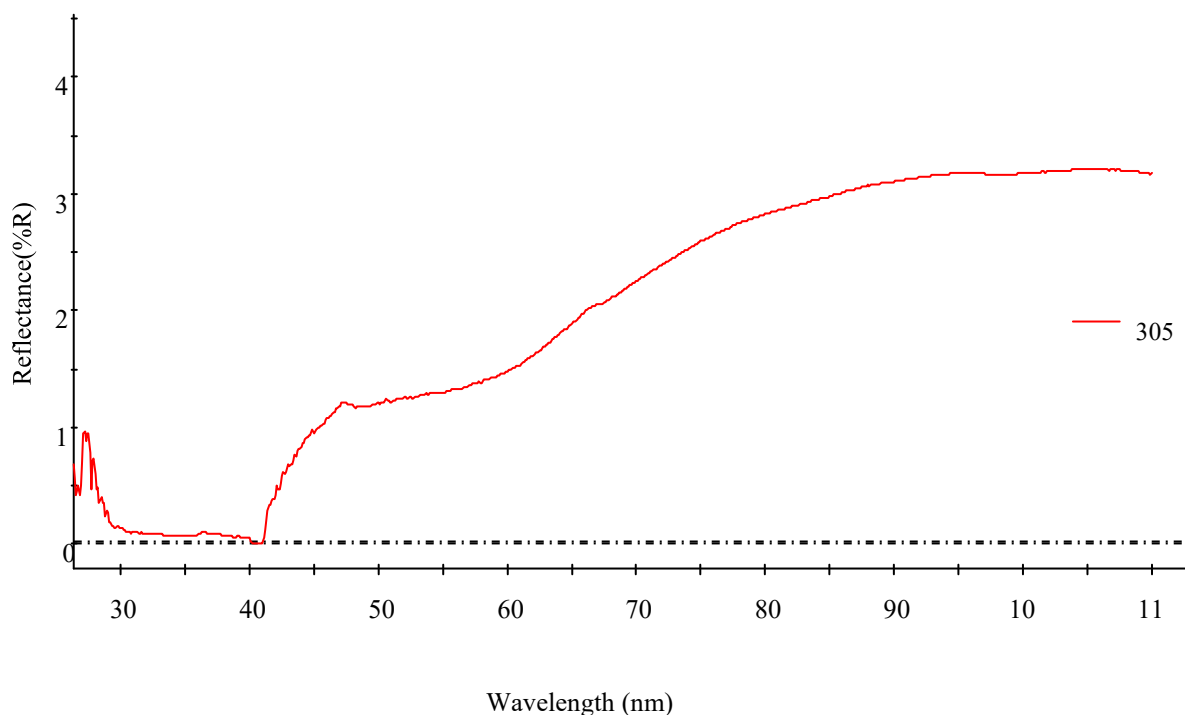


Fig 4.5 UV-Visible Reflectance Spectrum (Wavelength vs. Reflectance)

The reflectance spectrum of the anthocyanin-incorporated CMC film showed very low reflectance in the 300–450 nm region, indicating strong absorption of UV light and confirming its effective UV-shielding ability. Reflectance gradually increased beyond 450 nm and reached approximately 35–37% in the 700–1100 nm region, indicating reduced light absorption and greater reflection at longer wavelengths. These findings suggest that the film effectively protects packaged foods from UV-induced photo-oxidation while maintaining suitable optical properties for intelligent food packaging applications.

The UV–Visible analysis confirmed the successful incorporation of anthocyanins into the CMC film, as evidenced by strong UV absorption and low reflectance in the ultraviolet region. The film exhibited excellent UV-blocking properties while maintaining transparency at higher wavelengths, making it a promising material for intelligent food packaging with potential for pH-based freshness indication.

4.9.2 X-RAY DIFFRACTION (XRD) ANALYSIS

The X-ray diffraction (XRD) analysis was carried out to evaluate the crystalline and amorphous nature of the developed anthocyanin-based biopolymer film.

4.9.2.1 XRD 1

The XRD pattern of the sample showed characteristic diffraction peaks at 9.680° , 19.086° , 21.969° , 28.409° , 30.657° , 31.679° , and 44.598° , corresponding to d-spacing values ranging from 9.12960 Å to 2.03010 Å.

These peaks indicate the semi-crystalline structure of the developed film. The presence of both sharp and broad peaks confirms that the film contains both crystalline and amorphous regions.

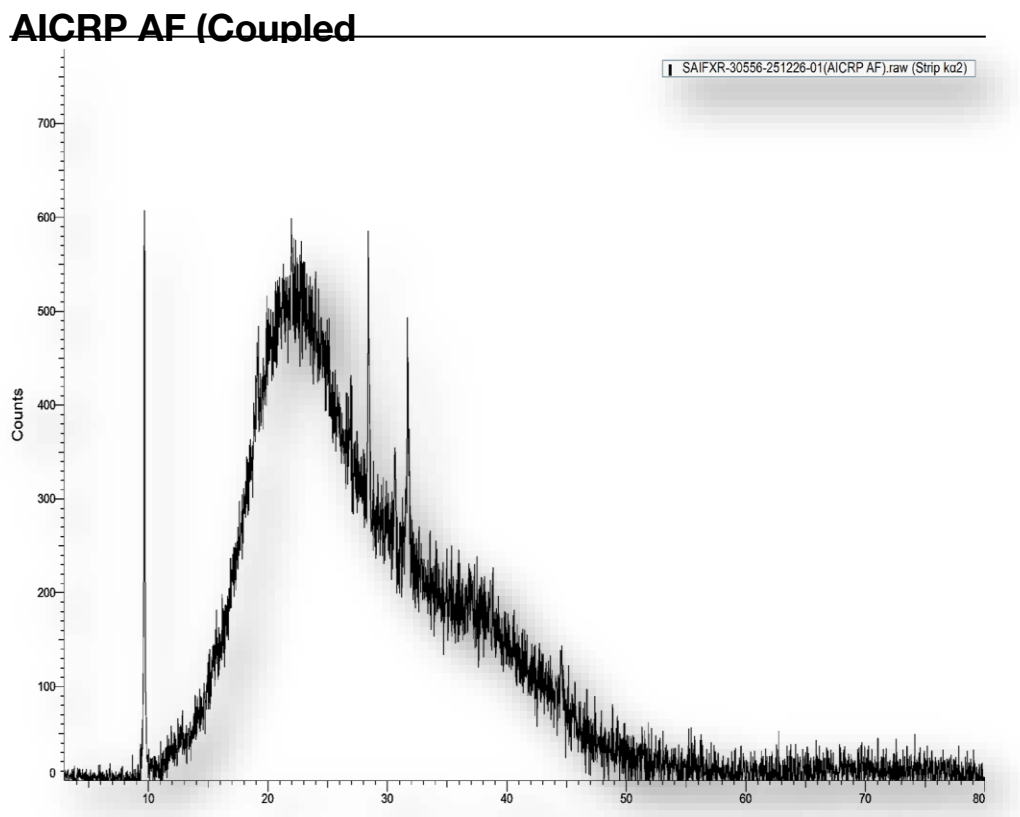


Fig 4.6. X – Ray Diffraction (XRD) Pattern of Control Biopolymer Film

The moderate intensity peaks suggest that incorporation of anthocyanin into the biopolymer matrix resulted in partial disruption of the ordered crystalline structure, leading to improved flexibility and film-forming ability.

This semi-crystalline nature is advantageous for intelligent packaging applications, as it provides a balance between mechanical strength and flexibility.

4.9.2.2 XRD 2 (DETAILED PEAK ANALYSIS)

Further analysis of the diffraction pattern confirms that the film exhibits characteristic peaks associated with polymer–anthocyanin interactions.

The peaks observed at lower angles indicate larger d-spacing values, suggesting less compact molecular arrangement, while peaks at higher angles correspond to tighter molecular packing. The reduction in crystallinity compared to pure polymer films indicates successful incorporation of anthocyanins into the matrix, which disrupts hydrogen bonding and intermolecular interactions. This structural modification enhances the responsiveness of the film to environmental changes such as pH variations, making it suitable for smart packaging applications.

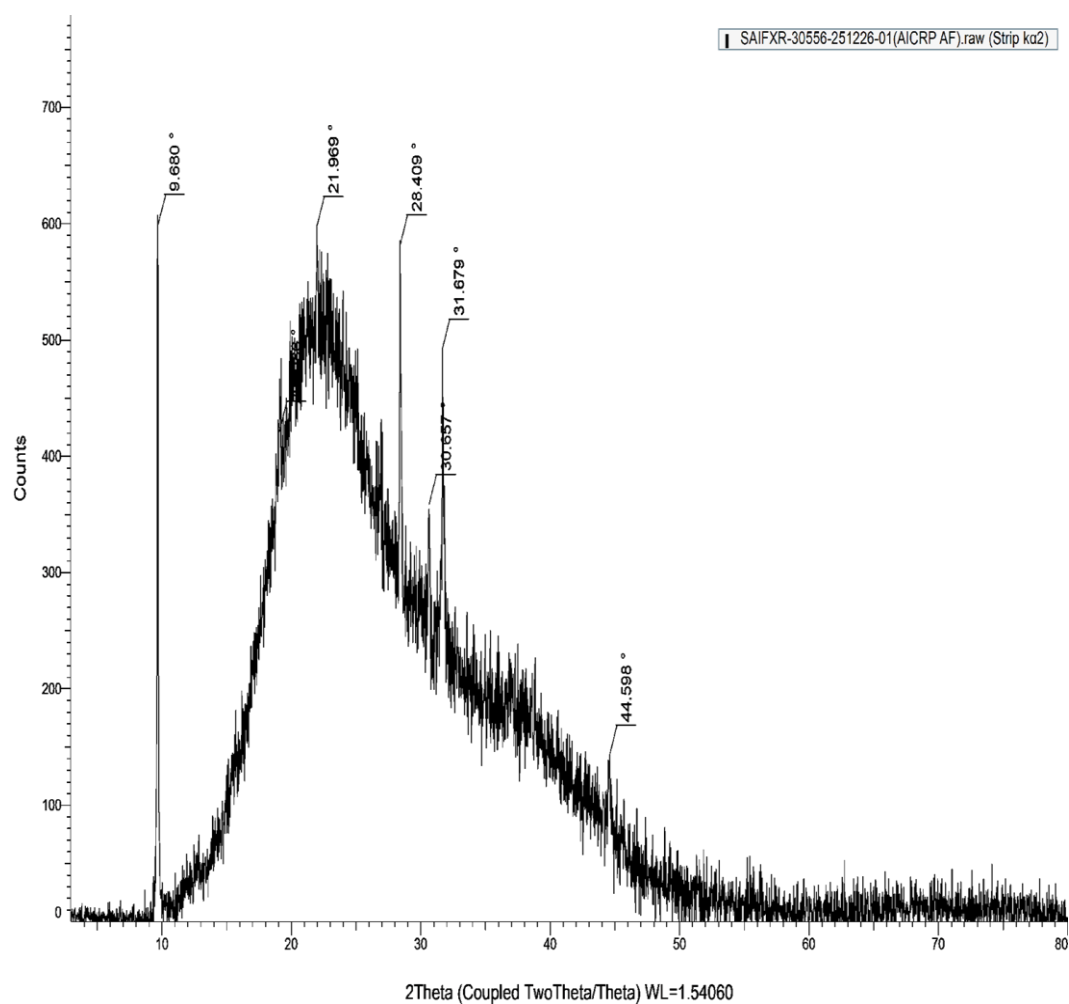


Fig 4.7 X – Ray Diffraction (XRD) Pattern of Anthocyanin Loaded Biopolymer Film

Angle	d Value
9.680 °	9.12960 Å
19.086 °	4.64619 Å
21.969 °	4.04262 Å
28.409 °	3.13912 Å
30.657 °	2.91390 Å
31.679 °	2.82217 Å
44.598 °	2.03010 Å

Table 4.10. Peak List of XRD

4.9.3 FOURIER TRANSFORM INFRARED (FTIR) SPECTRUM ANALYSIS

FTIR analysis was performed to identify functional groups and confirm the interaction between anthocyanin and the biopolymer matrix.

The FTIR spectrum showed characteristic absorption bands corresponding to hydroxyl (–OH), carbonyl (C=O), and aromatic groups present in anthocyanins. The broad peak observed around the hydroxyl region indicates strong hydrogen bonding interactions between anthocyanin molecules and the polymer matrix. Shifts in peak positions compared to the pure polymer confirm successful incorporation of anthocyanins into the film. These interactions contribute to improved film stability, enhanced pH sensitivity, and better colorimetric response.

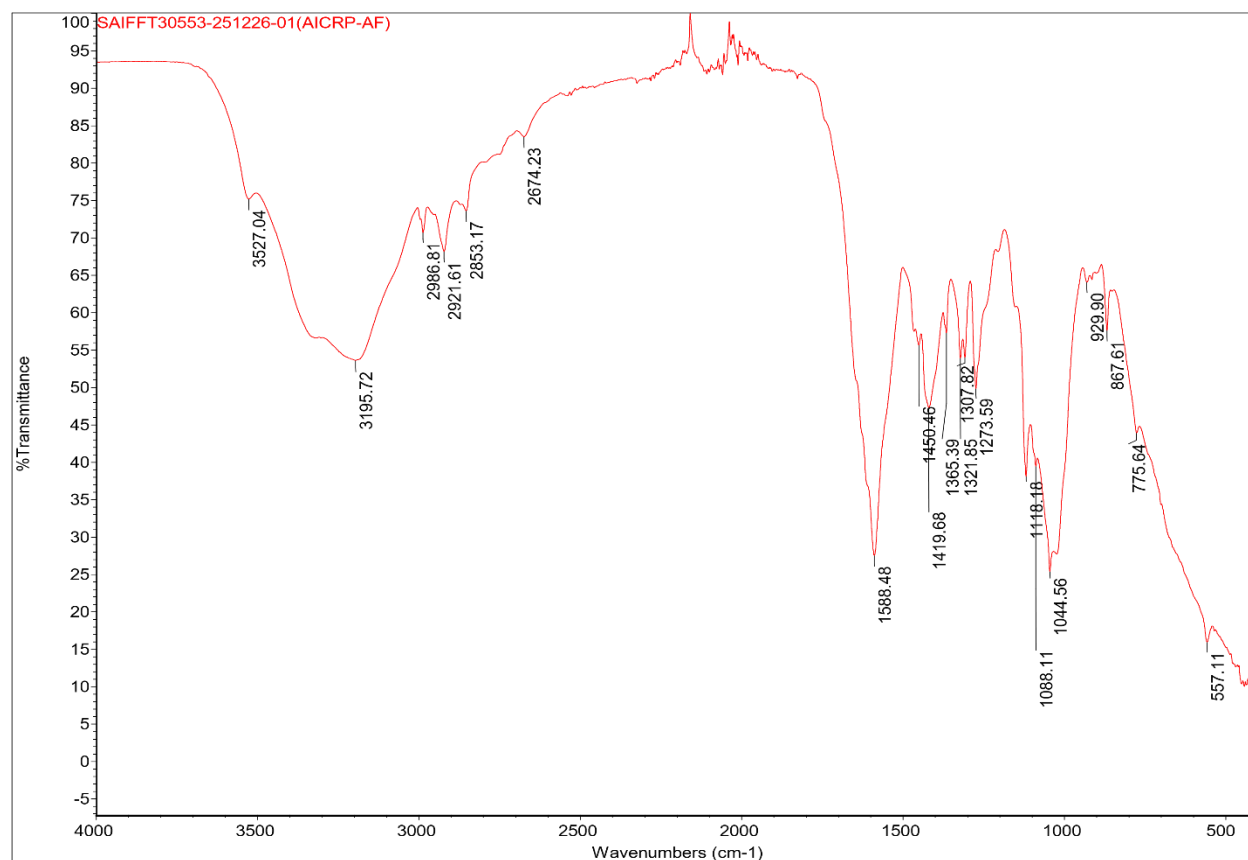
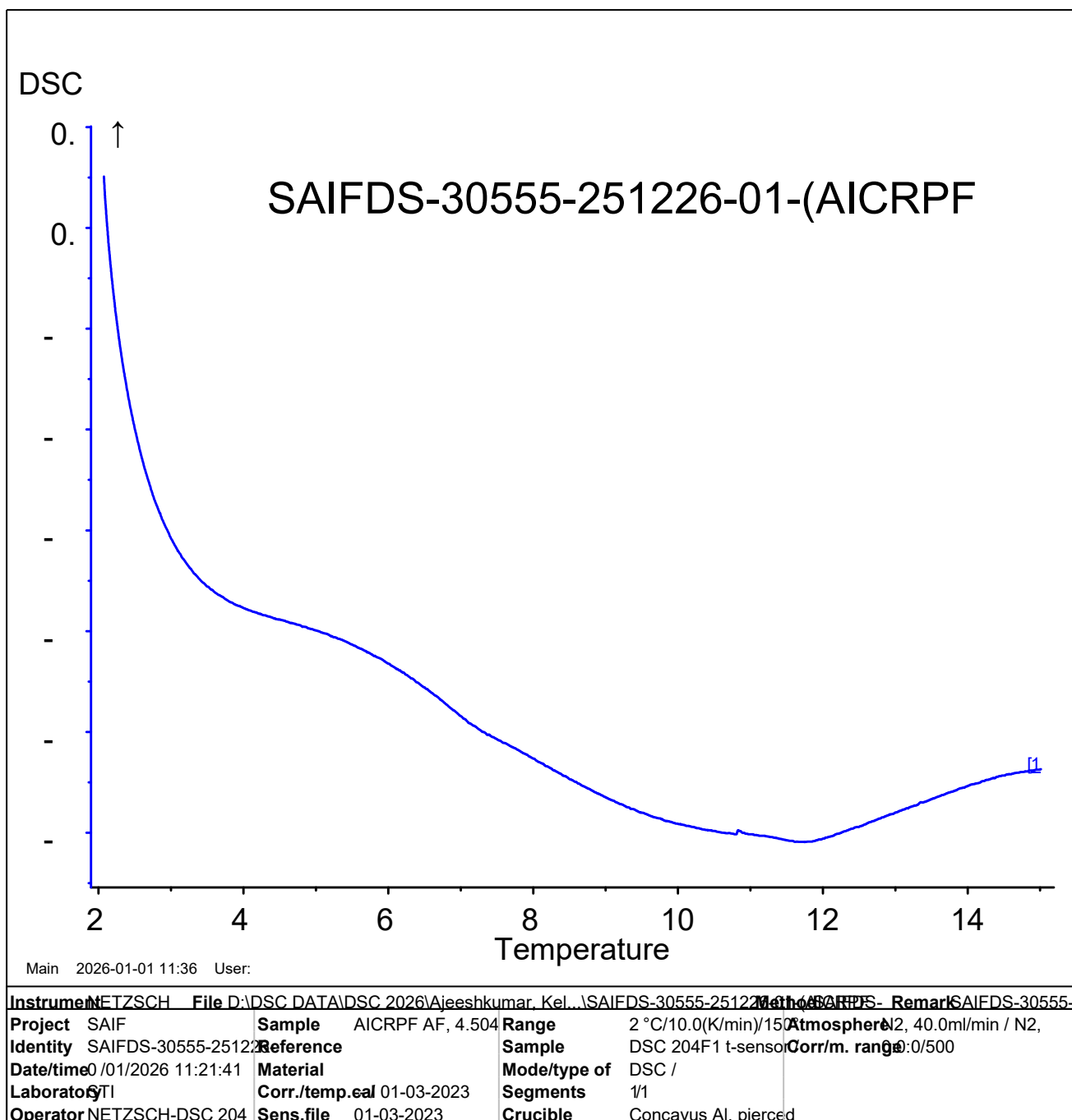


Fig 4.8 Fourier Transform Infrared (FTIR) Spectrum of Anthocyanin-Biopolymer

4.9.4 DIFFERENTIAL SCANNING CALORIMETRY (DSC) ANALYSIS

DSC was conducted to evaluate the thermal behavior and stability of the developed film. The DSC thermogram showed endothermic and exothermic transitions corresponding to moisture evaporation and polymer degradation.

The presence of a broad endothermic peak indicates the loss of bound water and possible structural rearrangement within the film. The thermal stability of the film was slightly reduced after anthocyanin incorporation, which is expected due to the natural and heat-sensitive nature of anthocyanins. However, the film maintained sufficient thermal stability for food packaging applications under normal storage conditions.



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**Fig 4.9 Differential Scanning Calorimetry (DSC) Thermogram of Anthocyanin–
Biopolymer Film**

4.9.5 SCANNING ELECTRON MICROSCOPY (SEM) ANALYSIS

SEM analysis was performed to study the surface morphology of the developed film.

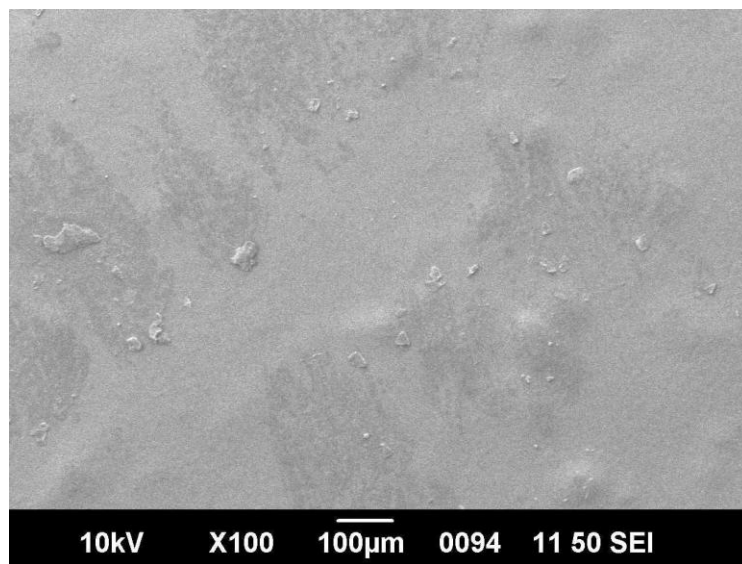


Plate 4.5 SEM 1

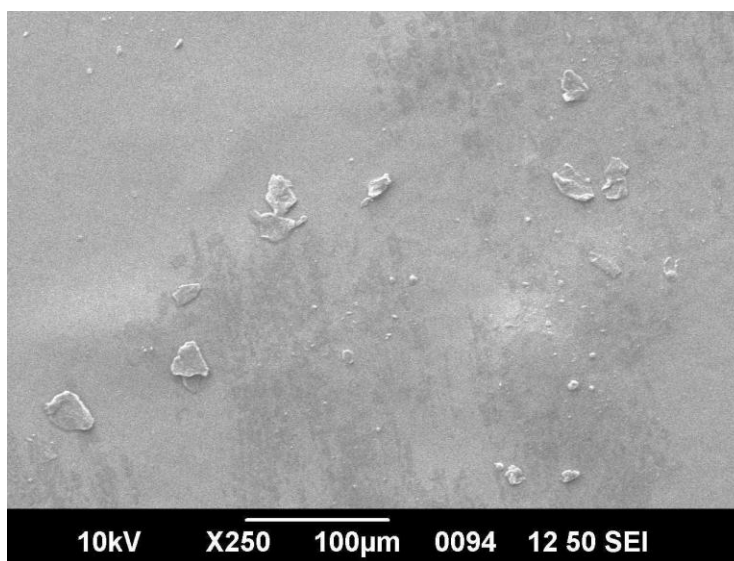


Plate 4.6 SEM 2

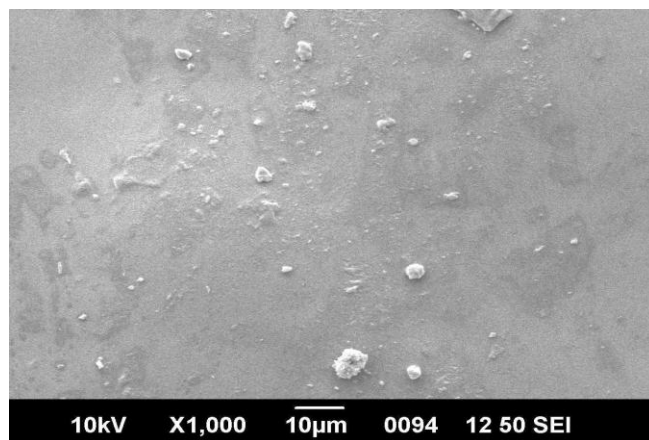


Plate 4.7 SEM 3

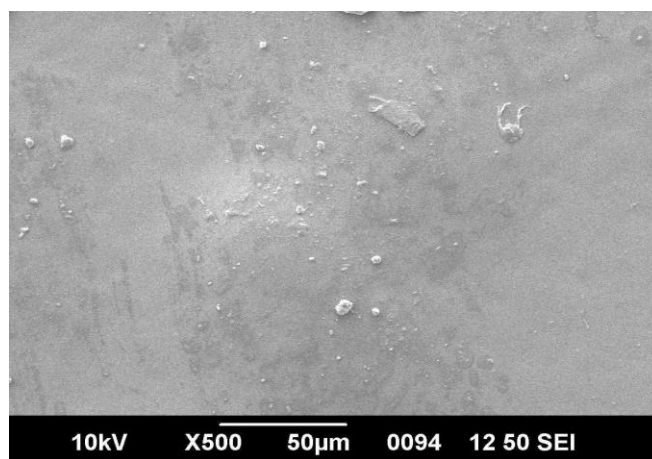


Plate 4.8 SEM 4

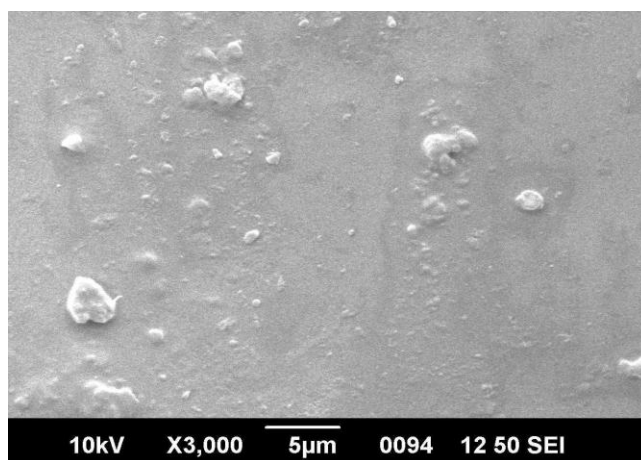


Plate 4.9 SEM 5

The SEM micrographs revealed that the film possessed a relatively smooth, continuous, and homogeneous surface structure, indicating uniform film formation. The anthocyanin pigment

appeared to be well distributed throughout the polymer matrix without any significant phase separation, suggesting good compatibility between the components.

No major cracks, pores, or structural discontinuities were observed, which confirms the integrity of the film. However, minor surface irregularities were noticed in certain regions, which may be attributed to drying effects during film formation or localized aggregation of anthocyanin molecules. Despite these minor variations, the overall morphology remained consistent and stable. The observed uniformity in surface structure plays a crucial role in ensuring consistent color response and reliable pH sensitivity of the film. Additionally, the absence of structural defects contributes to improved mechanical strength and durability. Therefore, the SEM analysis confirms that the developed film exhibits a stable, well-integrated structure suitable for intelligent packaging applications.

SUMMARY AND CONCLUSION

CHAPTER V

SUMMARY AND CONCLUSION

Food spoilage is a major cause of food loss worldwide, leading to significant economic losses and potential health risks. Conventional food packaging primarily acts as a protective barrier but does not provide information on the freshness or quality of packaged food. Growing consumer demand for safe, high-quality and sustainable food products has driven the development of intelligent packaging technologies. Among these, pH-sensitive colourimetric indicators have gained considerable attention because they provide real-time visual information on food freshness through observable colour changes. Anthocyanins are particularly suitable for such applications owing to their excellent pH sensitivity, non-toxic nature, biodegradability, antioxidant properties and compatibility with biodegradable polymers. Consequently, anthocyanin-based intelligent packaging offers a sustainable alternative to conventional synthetic packaging materials.

Based on this concept, the study entitled "Development of a Real-Time Intelligent pH Sensitive Colour Change Freshness Indicator Using Anthocyanin-Biopolymer Film for Food Applications" was undertaken to develop a biodegradable intelligent packaging film capable of providing real-time freshness information through visible colour changes. The study also evaluated the physicochemical, mechanical, structural, thermal and functional properties of the developed films and validated their application for monitoring the freshness of highly perishable food products.

Anthocyanins were extracted from purple cabbage using ultrasound-assisted extraction (UAE) and microwave-assisted extraction (MAE). UAE yielded 21.8 mg/L anthocyanins compared to 15.3 mg/L by MAE using 1% citric acid, demonstrating superior extraction efficiency. The extracted anthocyanins exhibited excellent pH responsiveness across pH 1–14, changing from intense red/deep pink in acidic conditions to purple near neutral pH and yellowish-green under alkaline conditions, confirming their suitability as natural colourimetric indicators.

Biodegradable films were prepared using carboxymethyl cellulose (CMC) and chitosan with glycerol as plasticizer and anthocyanin extract as the active indicator. The CMC film exhibited a greater thickness (0.36 mm) than the chitosan film (0.25 mm), providing better pigment

retention and clearer colour visibility. Mechanical analysis showed that chitosan films had a tensile force of 14.33 N and elongation of 6.33 mm, whereas freshly prepared CMC films exhibited 12.0 N tensile force with a significantly higher elongation of 33 mm, indicating superior flexibility. After conditioning, the tensile force of CMC increased to 28.5 N while elongation decreased to 11.5 mm because of moisture loss, although flexibility remained higher than that of chitosan. CMC films also showed better lightness, colour uniformity and pH-responsive colour transitions, making CMC the preferred polymer matrix for intelligent packaging. The developed films were comprehensively characterized using UV–Visible spectroscopy, FTIR, XRD, DSC and SEM. UV–Visible analysis showed strong UV absorption between 280–400 nm with a maximum absorbance at 420–430 nm, indicating excellent UV-blocking ability while maintaining adequate transparency in the visible region. FTIR confirmed successful anthocyanin incorporation through hydrogen-bond interactions with the polymer matrix. XRD analysis revealed reduced crystallinity, demonstrating successful pigment integration into the CMC network. DSC showed acceptable thermal stability despite the slight heat sensitivity of anthocyanins, while SEM revealed a smooth, homogeneous and crack-free surface with uniform anthocyanin dispersion, confirming good structural integrity and compatibility.

The practical applicability of the developed anthocyanin-incorporated CMC film as an intelligent freshness indicator was evaluated using fish and paneer during refrigerated storage. The film exhibited distinct and progressive colour transitions from purple (fresh) to dark red (intermediate stage) and finally to green (spoiled condition) in response to pH changes associated with food deterioration, thereby providing a simple visual indication of freshness. To validate the reliability of these visual colour changes, microbial quality was assessed by Total Plate Count (TPC), while fish spoilage was further evaluated using Total Volatile Base Nitrogen (TVB-N) analysis. The observed colour transitions closely corresponded with increasing microbial counts and TVB-N values, thereby confirming its effectiveness as an intelligent freshness indicator.

Overall, the study demonstrated that anthocyanin-incorporated biodegradable CMC films successfully integrated real-time freshness indication, biodegradability, UV protection, favourable mechanical properties, structural stability, antimicrobial activity and pH-responsive colour change within a single intelligent packaging system. The superior performance of CMC over chitosan was confirmed by its higher flexibility (33 mm elongation), optimum thickness (0.36 mm), improved

optical properties and effective extension of fish shelf life to the 7th day. The findings provide strong scientific evidence for the development of sustainable intelligent food packaging systems and establish a foundation for future work aimed at improving anthocyanin stability, moisture resistance and commercial scalability.

REFERENCES

CHAPTER VI

REFERENCES

1. Abdel-Aal, E.-S. M., Young, J. C., & Rabalski, I. (2006). Anthocyanin composition in black, blue, pink, purple, and red cereal grains. *Journal of Agricultural and Food Chemistry*, 54(13), 4696–4704.
2. Alizadeh-Sani, M., Tavassoli, M., McClements, D. J., & Hamishehkar, H. (2021). Multifunctional halochromic packaging materials: Saffron petal anthocyanin loaded-chitosan nanofiber/methyl cellulose matrices. *Food Hydrocolloids*, 111, 106237.
3. Amin, U., Khan, M. K. I., Maan, A. A., Nazir, A., Riaz, S., Khan, M. U., Sultan, M., Munekata, P. E., & Lorenzo, J. M. (2022). Biodegradable active, intelligent, and smart packaging materials for food applications. *Food Packaging and Shelf Life*, 33, 100903.
4. Antonio-Gomez, M. V., Salinas-Moreno, Y., Hernández-Rosas, F., Martínez-Bustos, F., Andrade-González, I., & Herrera-Corredor, J. A. (2021). Optimized extraction, microencapsulation, and stability of anthocyanins from *Ardisia compressa* K. fruit. *Polish Journal of Food and Nutrition Sciences*, 71(3), 299–310.
5. Awika, J. M., Rooney, L. W., & Waniska, R. D. (2005). Anthocyanins from black sorghum and their antioxidant properties. *Food Chemistry*, 90(1–2), 293–301.
6. Becerril, R., Nerín, C., & Silva, F. (2021). Bring some colour to your package: Freshness indicators based on anthocyanin extracts. *Trends in Food Science & Technology*, 111, 495–505.
7. Benalaya, I., Alves, G., Lopes, J., & Silva, L. R. (2024). A review of natural polysaccharides: Sources, characteristics, properties, food, and pharmaceutical applications. *International Journal of Molecular Sciences*, 25(2), 1322.
8. Boonsiriwit, A., Itkor, P., Sirieawphikul, C., & Lee, Y. S. (2022). Characterization of natural anthocyanin indicator based on cellulose bio-composite film for monitoring the freshness of chicken tenderloin. *Molecules*, 27(9), 2752.
9. Cacace, J., & Mazza, G. (2002). Extraction of anthocyanins and other phenolics from black currants with sulfured water. *Journal of Agricultural and Food Chemistry*, 50(21), 5939–5946.
10. Castañeda-Ovando, A., de Lourdes Pacheco-Hernández, M., Páez-Hernández, M. E., Rodríguez, J. A., & Galán-Vidal, C. A. (2009). Chemical studies of anthocyanins: A review. *Food Chemistry*, 113(4), 859–871.
11. Chen, H., Zhang, M., Bhandari, B., & Yang, C. (2020). Novel pH-sensitive films containing curcumin and anthocyanins to monitor fish freshness. *Food Hydrocolloids*, 100, 105438.
12. Cheng, J., Wang, H., Kang, S., Xia, L., Jiang, S., Chen, M., & Jiang, S. (2019). An active packaging film based on yam starch with eugenol and its application for pork preservation. *Food Hydrocolloids*, 96, 546–554.
13. Dai, J., & Mumper, R. J. (2010). Plant phenolics: Extraction, analysis and their antioxidant and anticancer properties. *Molecules*, 15(10), 7313–7352.

14. Dangles, O. (2024). Anthocyanins as natural food colorings: The chemistry behind and challenges still ahead. *Journal of Agricultural and Food Chemistry*, 72(22), 12356–12372.
15. Dhanani, T., Shah, S., Gajbhiye, N., & Kumar, S. (2017). Effect of extraction methods on yield, phytochemical constituents and antioxidant activity of *Withania somnifera*. *Arabian Journal of Chemistry*, 10, S1193–S1199.
16. Eghbaljoo, H., Alizadeh Sani, M., Sani, I. K., Maragheh, S. M., Sain, D. K., Jawhar, Z. H., Pirs, S., Kadi, A., Dadkhodayi, R., & Zhang, F. (2025). Development of smart packaging halochromic films embedded with anthocyanin pigments; recent advances. *Critical Reviews in Food Science and Nutrition*, 65(4), 770–786.
17. Fischer, T. C., Gosch, C., Pfeiffer, J., Halbwirth, H., Halle, C., Stich, K., & Forkmann, G. (2007). Flavonoid genes of pear (*Pyrus communis*). *Trees*, 21(5), 521–529.
18. Gasti, T., Dixit, S., D'souza, O. J., Hiremani, V. D., Vootla, S. K., Masti, S. P., Chougale, R. B., & Malabadi, R. B. (2021). Smart biodegradable films based on chitosan/methylcellulose containing *Phyllanthus reticulatus* anthocyanin for monitoring the freshness of fish fillet. *International Journal of Biological Macromolecules*, 187, 451–461.
19. Gil, M. I., Tomás-Barberán, F. A., Hess-Pierce, B., & Kader, A. A. (2002). Antioxidant capacities, phenolic compounds, carotenoids, and vitamin C contents of nectarine, peach, and plum cultivars from California. *Journal of Agricultural and Food Chemistry*, 50(17), 4976–4982.
20. Giusti, M. M., Ghanadan, H., & Wrolstad, R. E. (1998). Elucidation of the structure and conformation of red radish (*Raphanus sativus*) anthocyanins using one- and two-dimensional nuclear magnetic resonance techniques. *Journal of Agricultural and Food Chemistry*, 46(12), 4858–4863.
21. Giusti, M. M., & Wrolstad, R. E. (2003). Acylated anthocyanins from edible sources and their applications in food systems. *Biochemical Engineering Journal*, 14(3), 217–225.
22. Guo, N., Song, M., Liu, W., Zhang, F., & Zhu, G. (2023). Preparation of an elderberry anthocyanin film and fresh-keeping effect of its application on fresh shrimps. *Plos One*, 18(11), e0290650.
23. Handa, S. (2008). An overview of extraction techniques for medicinal and aromatic plants. *Extraction Technologies for Medicinal and Aromatic Plants*, 1(1), 21–40.
24. Handa, S. S., Khanuja, S. P. S., Longo, G., & Rakesh, D. D. (2008). Extraction technologies for medicinal and aromatic plants (United Nations Industrial Development Organisation and the International Centre for Science and High Technology). *International Centre for Science and High Technology-United Nations Industrial Development Organization, Area Science Park Padriciano*, 99, 34012.
25. Hasanah, N. N., Mohamad Azman, E., Rozzamri, A., Zainal Abedin, N. H., & Ismail-Fitry, M. R. (2023). A systematic review of butterfly pea flower (*Clitoria ternatea* L.): Extraction and application as a food freshness pH-Indicator for polymer-based intelligent packaging. *Polymers*, 15(11), 2541.
26. He, J., & Giusti, M. M. (2010). Anthocyanins: Natural colorants with health-promoting properties. *Annual Review of Food Science and Technology*, 1(1), 163–187.

27. Hidayati, N., Wijaya, M., Bintoro, V., Mulyani, S., & Pratama, Y. (2021). Development of biodegradable smart packaging from chitosan, polyvinyl alcohol (PVA) and butterfly pea flower's (*Clitoria ternatea* L.) anthocyanin extract. *Food Research*, 5(3), 307–314.
28. Huang, X., Du, L., Li, Z., Xue, J., Shi, J., Tahir, H. E., Zhai, X., Zhang, J., Zhang, N., & Sun, W. (2023). A visual bi-layer indicator based on mulberry anthocyanins with high stability for monitoring Chinese mitten crab freshness. *Food Chemistry*, 411, 135497.
29. Huang, X., Xu, F., Yun, D., Li, C., Kan, J., & Liu, J. (2023). Development and application of intelligent packaging films based on guar gum, polyvinyl alcohol and hyacinth bean (*Lablab purpureus* (L.) sweet) anthocyanins. *International Journal of Biological Macromolecules*, 251, 126369.
30. Ignat, I., Volf, I., & Popa, V. I. (2011). A critical review of methods for characterisation of polyphenolic compounds in fruits and vegetables. *Food Chemistry*, 126(4), 1821–1835.
31. Jain, T., Jain, V., Pandey, R., Vyas, A., & Shukla, S. (2009). Microwave assisted extraction for phytoconstituents—an overview. *Asian Journal of Research in Chemistry*, 2(1), 19–25.
32. Jang, Y., & Koh, E. (2023). Characterisation and storage stability of aronia anthocyanins encapsulated with combinations of maltodextrin with carboxymethyl cellulose, gum Arabic, and xanthan gum. *Food Chemistry*, 405, 135002.
33. Jiao, X., Xie, J., Du, H., Bian, X., Wang, C., Zhou, L., & Wen, Y. (2023). Antibacterial smart absorbent pad with Janus structure for meat preservation. *Food Packaging and Shelf Life*, 37, 101066.
34. Juswardi, J., Yuliana, R., Tanzerina, N., Harmida, H., & Aminasih, N. (2023). Anthocyanin, antioxidant and metabolite content of butterfly pea flower (*Clitoria ternatea* L.) based on flowering phase. *Jurnal Pembelajaran Dan Biologi Nukleus*, 9(2), 349–360.
35. Kaewprachu, P., Amara, C. B., Oulahal, N., Gharsallaoui, A., Joly, C., Tongdeesoontorn, W., Rawdkuen, S., & Degraeve, P. (2018). Gelatin films with nisin and catechin for minced pork preservation. *Food Packaging and Shelf Life*, 18, 173–183.
36. Kaewprachu, P., Jaisan, C., Rawdkuen, S., & Osako, K. (2024). Colorimetric indicator films based on carboxymethyl cellulose and anthocyanins as a visual indicator for shrimp freshness tracking. *Heliyon*, 10(11).
37. Kazuma, K., Noda, N., & Suzuki, M. (2003). Malonylated flavonol glycosides from the petals of *Clitoria ternatea*. *Phytochemistry*, 62(2), 229–237.
38. Khezerlou, A., Alizadeh Sani, M., Tavassoli, M., Abedi-Firoozjah, R., Ehsani, A., & McClements, D. J. (2023). Halochromic (pH-Responsive) indicators based on natural anthocyanins for monitoring fish freshness/spoilage. *Journal of Composites Science*, 7(4), 143.
39. Khoo, H. E., Azlan, A., Tang, S. T., & Lim, S. M. (2017). Anthocyanidins and anthocyanins: Colored pigments as food, pharmaceutical ingredients, and the potential health benefits. *Food & Nutrition Research*, 61(1), 1361779.
40. Kumar, P., Tripathi, S., Deshmukh, R. K., Meshram, B. P., & Gaikwad, K. K. (2026). Emerging smart packaging materials and technologies for ethylene detection and freshness monitoring in fresh produce. *Sustainable Food Technology*.

41. Kuswandi, B., Wicaksono, Y., Jayus, Abdullah, A., Heng, L. Y., & Ahmad, M. (2011). Smart packaging: Sensors for monitoring of food quality and safety. *Sensing and Instrumentation for Food Quality and Safety*, 5(3), 137–146.
42. Lachman, J., Hamouz, K., Orsák, M., Pivec, V., Hejtmánková, K., Pazderů, K., Dvořák, P., & Čepl, J. (2012). Impact of selected factors—Cultivar, storage, cooking and baking on the content of anthocyanins in coloured-flesh potatoes. *Food Chemistry*, 133(4), 1107–1116.
43. Lan, W., Wang, S., Zhang, Z., Liang, X., Liu, X., & Zhang, J. (2021). Development of red apple pomace extract/chitosan-based films reinforced by TiO₂ nanoparticles as a multifunctional packaging material. *International Journal of Biological Macromolecules*, 168, 105–115.
44. Leccese, A., Bartolini, S., & Viti, R. (2007). Total antioxidant capacity and phenolics content in apricot fruits. *International Journal of Fruit Science*, 7(2), 3–16.
45. Letellier, M., & Budzinski, H. (1999). Microwave assisted extraction of organic compounds. *Analysis*, 127(3), 259–270.
46. Li, C., Tao, Y., Lu, J., Hu, J., Du, J., & Wang, H. (2025). Light stable carboxymethyl cellulose-based smart packaging film for real-time visual detecting the freshness of food. *Journal of Future Foods*.
47. Liu, B., Xu, H., Zhao, H., Liu, W., Zhao, L., & Li, Y. (2017). Preparation and characterization of intelligent starch/PVA films for simultaneous colorimetric indication and antimicrobial activity for food packaging applications. *Carbohydrate Polymers*, 157, 842–849.
48. Liu, D., Zhang, C., Pu, Y., Chen, S., Liu, L., Cui, Z., & Zhong, Y. (2022). Recent advances in pH-responsive freshness indicators using natural food colorants to monitor food freshness. *Foods*, 11(13), 1884.
49. Liu, D., Zhong, Y., Li, X., Pu, Y., Chen, S., & Zhang, C. (2023). Indicator films based on anthocyanins loaded on metal-organic framework carriers and BP neural network for monitoring meat freshness. *Food Hydrocolloids*, 145, 109106.
50. Liu, L., Wu, W., Zheng, L., Yu, J., Sun, P., & Shao, P. (2022). Intelligent packaging films incorporated with anthocyanins-loaded ovalbumin-carboxymethyl cellulose nanocomplexes for food freshness monitoring. *Food Chemistry*, 387, 132908.
51. Lu, M., Cai, Y., Chen, X., Wang, Y., & Yuan, G. (2024). A novel anthocyanin indicator film with rosmarinic acid copigmentation having enhanced stability and pH indicator ability for monitoring pork freshness. *Journal of the Science of Food and Agriculture*, 104(5), 2641–2650.
52. Lu, Z., Wang, X., Lin, X., Mostafa, S., Zou, H., Wang, L., & Jin, B. (2024). Plant anthocyanins: Classification, biosynthesis, regulation, bioactivity, and health benefits. *Plant Physiology and Biochemistry*, 217, 109268.
53. Lutfun, N., & Sarker, S. (2012). *Supercritical fluid extraction in natural products analyses*.
54. Mancinelli, A. C., Silletti, E., Mattioli, S., Dal Bosco, A., Sebastiani, B., Menchetti, L., Koot, A., Van Ruth, S., & Castellini, C. (2021). Fatty acid profile, oxidative status, and content of volatile organic compounds in raw and cooked meat of different chicken strains. *Poultry Science*, 100(2), 1273–1282.

55. Mandal, V., Mohan, Y., & Hemalatha, S. (2007). Microwave assisted extraction—An innovative and promising extraction tool for medicinal plant research. *Pharmacognosy Reviews*, 1(1), 7–18.
56. Marinho, E. (2025). Cellulose: A comprehensive review of its properties and applications. *Sustainable Chemistry for the Environment*, 100283.
57. Muangrat, R., Pongsirikul, I., & Blanco, P. H. (2018). Ultrasound assisted extraction of anthocyanins and total phenolic compounds from dried cob of purple waxy corn using response surface methodology. *Journal of Food Processing and Preservation*, 42(2), e13447.
58. Mullen, W., Stewart, A. J., Lean, M. E., Gardner, P., Duthie, G. G., & Crozier, A. (2002). Effect of freezing and storage on the phenolics, ellagitannins, flavonoids, and antioxidant capacity of red raspberries. *Journal of Agricultural and Food Chemistry*, 50(18), 5197–5201.
59. Mustafa, F., & Andreescu, S. (2020). *Nanotechnology-based approaches for food sensing and packaging applications*. *RSC Adv* 10: 19309–19336.
60. Nahar, L., & Sarker, S. D. (2006). Supercritical fluid extraction. In *Natural products isolation* (pp. 47–76). Springer.
61. Neves, D., Andrade, P. B., Videira, R. A., de Freitas, V., & Cruz, L. (2022). Berry anthocyanin-based films in smart food packaging: A mini-review. *Food Hydrocolloids*, 133, 107885.
62. Nogueira, G. F., Meneghetti, B. B., Soares, I. H. B. T., Soares, C. T., Bevilacqua, G., Fakhouri, F. M., & de Oliveira, R. A. (2024). Multipurpose arrowroot starch films with anthocyanin-rich grape pomace extract: Color migration for food simulants and monitoring the freshness of fish meat. *International Journal of Biological Macromolecules*, 265, 130934.
63. Patras, A., Brunton, N. P., O'Donnell, C., & Tiwari, B. K. (2010). Effect of thermal processing on anthocyanin stability in foods; mechanisms and kinetics of degradation. *Trends in Food Science & Technology*, 21(1), 3–11.
64. Pereira Jr, V. A., de Arruda, I. N. Q., & Stefani, R. (2015). Active chitosan/PVA films with anthocyanins from Brassica oleraceae (Red Cabbage) as Time–Temperature Indicators for application in intelligent food packaging. *Food Hydrocolloids*, 43, 180–188.
65. Pereira, P. F., de Sousa Picciani, P. H., Calado, V., & Tonon, R. V. (2021). Electrical gas sensors for meat freshness assessment and quality monitoring: A review. *Trends in Food Science & Technology*, 118, 36–44.
66. Pervaiz, T., Songtao, J., Faghihi, F., Haider, M. S., & Fang, J. (2017). Naturally occurring anthocyanin, structure, functions and biosynthetic pathway in fruit plants. *J. Plant Biochem. Physiol*, 5(2), 1–9.
67. Petroni, K., Pilu, R., & Tonelli, C. (2014). Anthocyanins in corn: A wealth of genes for human health. *Planta*, 240(5), 901–911.
68. Prietto, L., Mirapalhete, T. C., Pinto, V. Z., Hoffmann, J. F., Vanier, N. L., Lim, L.-T., Dias, A. R. G., & da Rosa Zavareze, E. (2017). pH-sensitive films containing anthocyanins extracted from black bean seed coat and red cabbage. *Lwt*, 80, 492–500.
69. Puebla-Duarte, A. L., Santos-Sauceda, I., Rodríguez-Félix, F., Iturralde-García, R. D., Fernández-Quiroz, D., Pérez-Cabral, I. D., & Del-Toro-Sánchez, C. L. (2023). Active and intelligent

packaging: A review of the possible application of cyclodextrins in food storage and safety indicators. *Polymers*, 15(21), 4317.

70. Qin, Y., Liu, Y., Yong, H., Liu, J., Zhang, X., & Liu, J. (2019). Preparation and characterization of active and intelligent packaging films based on cassava starch and anthocyanins from *Lycium ruthenicum* Murr. *International Journal of Biological Macromolecules*, 134, 80–90.
71. Ranjbar, M., Tabrizad, M. H. A., Asadi, G., & Ahari, H. (2023). Investigating the microbial properties of sodium alginate/chitosan edible film containing red beetroot anthocyanin extract for smart packaging in chicken fillet as a pH indicator. *Heliyon*, 9(8).
72. Rather, J. A., Akhter, N., Ashraf, Q. S., Mir, S. A., Makroo, H. A., Majid, D., Barba, F. J., Khaneghah, A. M., & Dar, B. (2022). A comprehensive review on gelatin: Understanding impact of the sources, extraction methods, and modifications on potential packaging applications. *Food Packaging and Shelf Life*, 34, 100945.
73. Rawa-Adkonis, M., Wolska, L., & Namieśnik, J. (2003). Modern techniques of extraction of organic analytes from environmental matrices. *Critical Reviews in Analytical Chemistry*, 33(3), 199–248.
74. Rawat, S., Acharya, P., Bhutia, P. O., Pandey, A., Kumar, D., Joshi, R., & Bhatt, I. D. (2023). Changes in nutritional, physicochemical, phytochemical composition and antioxidant potential of *Mahonia nepalensis* fruits during ripening. *International Journal of Food Properties*, 26(1), 1062–1078.
75. Rein, M. (2005). *Copigmentation reactions and color stability of berry anthocyanins*.
76. Remedio, L. N., & Parada Quinayá, C. (2024). Intelligent packaging systems with anthocyanin: Influence of different polymers and storage conditions. *Polymers*, 16(20), 2886.
77. Ren, M., Wang, N., Lu, Y., & Wang, C. (2025). Preparation and characterization of Antioxidative and pH-sensitive films based on κ -carrageenan/Carboxymethyl cellulose blended with purple cabbage anthocyanin for monitoring Hairtail freshness. *Foods*, 14(4), 694.
78. Sana, S. S., Gangadhar, L., Sofi, M. A., Sudarshan, K., & Oh, T. H. (2025). Chitosan as a wall biomaterial for the encapsulation of essential oils: A comprehensive review of pharmaceutical breakthroughs and future directions. *International Journal of Biological Macromolecules*, 148551.
79. Sanches, M. A. R., Camelo-Silva, C., da Silva Carvalho, C., de Mello, J. R., Barroso, N. G., da Silva Barros, E. L., Silva, P. P., & Pertuzatti, P. B. (2021). Active packaging with starch, red cabbage extract and sweet whey: Characterization and application in meat. *Lwt*, 135, 110275.
80. Sani, M. A., Tavassoli, M., Hamishehkar, H., & McClements, D. J. (2021). Carbohydrate-based films containing pH-sensitive red barberry anthocyanins: Application as biodegradable smart food packaging materials. *Carbohydrate Polymers*, 255, 117488.
81. Santamarina, A. B., Calder, P. C., Estadella, D., & Pisani, L. P. (2023). Anthocyanins ameliorate obesity-associated metainflammation: Preclinical and clinical evidence. *Nutrition Research*, 114, 50–70.
82. Sarker, S. D., & Nahar, L. (2012). An introduction to natural products isolation. *Methods in Molecular Biology*, 864, 1–25.

83. Schauss, A. G., Wu, X., Prior, R. L., Ou, B., Patel, D., Huang, D., & Kababick, J. P. (2006). Phytochemical and nutrient composition of the freeze-dried Amazonian palm berry, *Euterpe oleraceae* Mart.(Acai). *Journal of Agricultural and Food Chemistry*, 54(22), 8598–8603.
84. Schlindweinn, E. B., Chacon, W. D. C., Koop, B. L., de Matos Fonseca, J., Monteiro, A. R., & Valencia, G. A. (2022). Starch-based materials encapsulating anthocyanins: A review. *Journal of Polymers and the Environment*, 30(9), 3547–3565.
85. Shayan, M., Gwon, J., Koo, M. S., Lee, D., Adhikari, A., & Wu, Q. (2022). pH-responsive cellulose nanomaterial films containing anthocyanins for intelligent and active food packaging. *Cellulose*, 29(18), 9731–9751.
86. Shi, C., Ji, Z., Zhang, J., Jia, Z., & Yang, X. (2022). Preparation and characterization of intelligent packaging film for visual inspection of tilapia fillets freshness using cyanidin and bacterial cellulose. *International Journal of Biological Macromolecules*, 205, 357–365.
87. Silva, I. D. de L., Oliveira, F. S. M. de, Andrade, M. F. de, Brito, A. M. S. S., Hallwass, F., & Vinhas, G. M. (2021). Evaluation of the potentials of jurema preta (*Mimosa tenuiflora*) and cajueiro (*Anacardium occidentale* L.) extracts for use in antimicrobials and antioxidants active packaging. *Matéria (Rio de Janeiro)*, 26, e12924.
88. Silva, S., Costa, E., Calhau, C., Morais, R., & Pintado, M. (2017). Anthocyanin extraction from plant tissues: A review. *Critical Reviews in Food Science and Nutrition*, 57(14), 3072–3083.
89. Sticher, O. (2008). Natural product isolation. *Natural Product Reports*, 25(3), 517–554.
90. Talukder, S., Mendiratta, S., Kumar, R., Agrawal, R., Soni, A., Luke, A., & Chand, S. (2020). Jamun fruit (*Syzgium cumini*) skin extract based indicator for monitoring chicken patties quality during storage. *Journal of Food Science and Technology*, 57(2), 537–548.
91. Tan, J., Han, Y., Han, B., Qi, X., Cai, X., Ge, S., & Xue, H. (2022). Extraction and purification of anthocyanins: A review. *Journal of Agriculture and Food Research*, 8, 100306.
92. Tanaka, Y., & Ohmiya, A. (2008). Seeing is believing: Engineering anthocyanin and carotenoid biosynthetic pathways. *Current Opinion in Biotechnology*, 19(2), 190–197.
93. Tavassoli, M., Khezerlou, A., Moghaddam, T. N., Firoozy, S., Bakhshizadeh, M., Sani, M. A., Hashemi, M., Ehsani, A., & Lorenzo, J. M. (2023). Sumac (*Rhus coriaria* L.) anthocyanin loaded-pectin and chitosan nanofiber matrices for real-time monitoring of shrimp freshness. *International Journal of Biological Macromolecules*, 242, 125044.
94. Tena, N., & Asuero, A. G. (2022). Antioxidant capacity of anthocyanins and other vegetal pigments: Modern assisted extraction methods and analysis. *Antioxidants*, 11(7), 1256.
95. Tian, X., Dong, Y., & Zhao, D. (2016). Optimization of extraction process for anthocyanins from black rice and research on its stability. *China Brewing*, 35(6), 161–164.
96. Tirtashi, F. E., Moradi, M., Tajik, H., Forough, M., Ezati, P., & Kuswandi, B. (2019). Cellulose/chitosan pH-responsive indicator incorporated with carrot anthocyanins for intelligent food packaging. *International Journal of Biological Macromolecules*, 136, 920–926.

97. Truong, V., McFeeters, R., Thompson, R., Dean, L., & Shofran, B. (2007). Phenolic acid content and composition in leaves and roots of common commercial sweetpotato (*Ipomea batatas* L.) cultivars in the United States. *Journal of Food Science*, 72(6), C343–C349.
98. Tuslinah, L., Yuliana, A., Arisnawati, D., & Rizkuloh, L. R. (2022). Comparison of Color Change to pH Range and Acid-Base Titration Indicator Precision Test of Multiple Ethanol Extracts. *Trends in Sciences*, 19(1), 1446–1446.
99. Wagh, R. V., Khan, A., Priyadarshi, R., Ezati, P., & Rhim, J.-W. (2023). Cellulose nanofiber-based multifunctional films integrated with carbon dots and anthocyanins from *Brassica oleracea* for active and intelligent food packaging applications. *International Journal of Biological Macromolecules*, 233, 123567.
100. Wang, C., & Liu, C. (2024). A pH-sensitive intelligent packaging film harnessing anthocyanin for food freshness monitoring. *Food and Bioprocess Technology*, 17(12), 5312–5323.
101. Wang, J., Sun, X., Zhang, H., Dong, M., Li, L., Zhangsun, H., & Wang, L. (2022). Dual-functional intelligent gelatin based packaging film for maintaining and monitoring the shrimp freshness. *Food Hydrocolloids*, 124, 107258.
102. Wang, L., & Weller, C. L. (2006). Recent advances in extraction of nutraceuticals from plants. *Trends in Food Science & Technology*, 17(6), 300–312.
103. Wang, L., Yang, C., Deng, X., Peng, J., Zhou, J., Xia, G., Zhou, C., Shen, Y., & Yang, H. (2023). A pH-sensitive intelligent packaging film harnessing *Dioscorea zingiberensis* starch and anthocyanin for meat freshness monitoring. *International Journal of Biological Macromolecules*, 245, 125485.
104. Wu, H., Jiao, C., Li, S., Li, Q., Zhang, Z., Zhou, M., & Yuan, X. (2022). A facile strategy for development of pH-sensing indicator films based on red cabbage puree and polyvinyl alcohol for monitoring fish freshness. *Foods*, 11(21), 3371.
105. Yildirim, S., Röcker, B., Pettersen, M. K., Nilsen-Nygaard, J., Ayhan, Z., Rutkaite, R., Radusin, T., Suminska, P., Marcos, B., & Coma, V. (2018). Active packaging applications for food. *Comprehensive Reviews in Food Science and Food Safety*, 17(1), 165–199.
106. Yong, H., & Liu, J. (2020). Recent advances in the preparation, physical and functional properties, and applications of anthocyanins-based active and intelligent packaging films. *Food Packaging and Shelf Life*, 26, 100550.
107. Yong, H., Wang, X., Zhang, X., Liu, Y., Qin, Y., & Liu, J. (2019). Effects of anthocyanin-rich purple and black eggplant extracts on the physical, antioxidant and pH-sensitive properties of chitosan film. *Food Hydrocolloids*, 94, 93–104.
108. Zeng, F., Ye, Y., Liu, J., & Fei, P. (2023). Intelligent pH indicator composite film based on pectin/chitosan incorporated with black rice anthocyanins for meat freshness monitoring. *Food Chemistry: X*, 17, 100531.
109. Zhai, X., Shi, J., Zou, X., Wang, S., Jiang, C., Zhang, J., Huang, X., Zhang, W., & Holmes, M. (2017). Novel colorimetric films based on starch/polyvinyl alcohol incorporated with roselle anthocyanins for fish freshness monitoring. *Food Hydrocolloids*, 69, 308–317.

110. Zhang, J., Huang, X., Shi, J., Liu, L., Zhang, X., Zou, X., Xiao, J., Zhai, X., Zhang, D., & Li, Y. (2021). A visual bi-layer indicator based on roselle anthocyanins with high hydrophobic property for monitoring griskin freshness. *Food Chemistry*, 355, 129573.
111. Zhang, J., Zhang, J., Zhang, L., Qin, Z., & Wang, T. (2025). Review of Recent Advances in Intelligent and Antibacterial Packaging for Meat Quality and Safety. *Foods*, 14(7), 1157.
112. Zhang, K., Huang, T.-S., Yan, H., Hu, X., & Ren, T. (2020). Novel pH-sensitive films based on starch/polyvinyl alcohol and food anthocyanins as a visual indicator of shrimp deterioration. *International Journal of Biological Macromolecules*, 145, 768–776.
113. Zhao, L., Liu, Y., Zhao, L., & Wang, Y. (2022). Anthocyanin-based pH-sensitive smart packaging films for monitoring food freshness. *Journal of Agriculture and Food Research*, 9, 100340.
114. Zhao, Y., Gao, L., Wang, J., Xue, Z., Zhang, M., Ma, X., Wang, G., & Lv, S. (2023). Preparation and Application of pH-Sensitive Film Containing Anthocyanins Extracted from *Lycium ruthenicum* Murr. *Materials*, 16(10), 3828.
115. Zhu, B., Zhong, Y., Wang, D., & Deng, Y. (2023). Active and intelligent biodegradable packaging based on anthocyanins for preserving and monitoring protein-rich foods. *Foods*, 12(24), 4491.

**DEVELOPMENT OF A REAL-TIME INTELLIGENT pH SENSITIVE COLOUR
CHANGE FRESHNESS INDICATOR USING ANTHOCYANIN-BIOPOLYMER FILM
FOR FOOD APPLICATIONS**

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ABSTRACT OF THESIS

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ABSTRACT

Food spoilage represents a critical challenge in the global food supply chain, necessitating innovative solutions for real-time freshness monitoring. This study successfully developed a biodegradable intelligent packaging film that provides visual indication of food freshness through pH-dependent colour transitions, addressing the limitations of conventional passive packaging systems. Anthocyanins extracted from purple cabbage were selected as the natural colourimetric indicator due to their pH sensitivity, non-toxicity, biodegradability, and sustainability advantages over synthetic indicators. Anthocyanin extraction was optimized using ultrasound-assisted extraction (UAE) and microwave-assisted extraction (MAE) techniques, with UAE yielding significantly higher anthocyanin content (21.8 mg/L) compared to MAE (15.3 mg/L). The extracted anthocyanins exhibited excellent pH responsiveness across pH 1–14, displaying distinct colour transitions from red in acidic conditions to purple at neutral pH and yellow-green under alkaline conditions, confirming their suitability as natural colourimetric sensors. Biodegradable films were fabricated using carboxymethyl cellulose (CMC) and chitosan as polymer matrices through solvent casting methodology. Comprehensive characterization revealed that CMC-based films demonstrated superior properties: higher thickness (0.36 mm), enhanced flexibility (33 mm elongation), better colour uniformity, and superior pH-responsive behaviour compared to chitosan films. Advanced analytical techniques including UV-Visible spectroscopy, FTIR, XRD, DSC, and SEM confirmed successful anthocyanin incorporation with excellent structural integrity and thermal stability. Storage studies on fresh mackerel fish and paneer over 12 days in refrigerated condition demonstrated that the anthocyanin-incorporated CMC film exhibited progressive colour changes from purple (fresh) to dark red (intermediate) to green (spoiled), accurately correlating with microbiological analysis and Total Volatile Base Nitrogen (TVB-N) values, thereby confirming its effectiveness as an intelligent freshness indicator. This study established that anthocyanin-biopolymer films represent a promising sustainable alternative to synthetic packaging, effectively combining environmental responsibility with functional freshness monitoring capability for highly perishable foods.