People's Democratic Republic of Algeria
Ministry of Higher Education
and Scientific Research
University of Tissemsilt
Faculty of Science and Technology
Department of Natural and Life Sciences

Final dissertation for the award of the degree of academic Master in

Field**: Biology**

Specialty**: Applied biochemistry**

Presented by: **NOUBADJI Zahira**

BEDRANE Amel

Topic

Development and characterization of active/intelligent pectin edible films based on local natural extract

Defended on, June 18, 2023

Board of examiners:

Mr. BEGHALIA Mohamed	President	Prof	U-Tissemsilt
Mr. MOUSSAOUI Badreddine	Supervisor	M.C.A	U-Tissemsilt
Mr. HANAFI Tahar	Co-Supervisor	PhD Student	U-Blida
Mr. ZEMOUR Kamal	Co-Supervisor	M.C.B	U-Tissemsilt
Mrs. BENSAADI Nawal	Examiner	M.A.A	U-Tissemsilt
Mr. BENDAHMANE Mohamed	Examiner	M.C.A	U-Tissemsilt
Mr. MARAIF Amine	Examiner	Engineer	Boilait Company

Academic Year: 2022-2023

Acknowledgements

First of all, we would like to express our sincere gratitude to the Almighty, Allah, for providing us with health, courage, and the determination to complete our training and accomplish this humble work.

We would like to express our deepest gratitude and appreciation to our supervisor, Mr. MOUSSAOUI Badreddine, and Co-supervisors Mr.Hanafi T and Mr. Zemour K, for their guidance, support, and invaluable insights throughout the entire process of conducting this research. Their expertise, patience, and encouragement have been instrumental in shaping the direction and quality of this thesis.

Our sincere appreciation goes to Prof. Beghalia. M, for accepting graciously to chair the defense jury.

We would also like to express our utmost gratitude to Mrs. Bensaaid N, Mr. Bendahmane M and Mr. Marif A; for their invaluable participation as committee members and for honoring us by judging our work.

We would like to acknowledge Mr. LAFER Mohamed, for his expertise, knowledge, and technical guidance, which have been instrumental in the success of our experiments and the acquisition of accurate and reliable data.

Lastly, we extend our heartfelt thanks to our families, especially our parents, for their unwavering support throughout our studies.

Dedication

I dedicate this modest work:

***My mother**, who has worked for my success, through her love, her support, all the sacrifices made and her precious advice, for all her assistance and her presence in my life*

***My father** who surrounded me with love, affection and who does everything for my success, may God keep him*

*My brothers **Messaoud,Yacine, Mahieddine,Mustapha** who always been examples of perseverance, courage and generosity for me*

*My lovely sister **Zineb** I cannot find the right and sincere words to express my affection and my thoughts*

*My nephews **Salaheddine**, **Zakaria** and my niece **Mayar** who add joy and happiness to the family*

*My aunts especially **Ryma** and my cousin **Fatima** and brother's wife **Hamida**, source of hope and motivation*

*My dear friend **Amel** for her moral support, her patience and her understanding. and all her family*

To all those who are dear to me; All those who participated in the realization of this work

ZAHIRA

Dedication

I dedicate this humble work to:

***My mother**, whose unwavering dedication, love, and support have contributed immensely to my success. I am grateful for the sacrifices she has made and her invaluable advice that has shaped my life.*

***My father** can take pride in this achievement, as it is the culmination of years of selfless sacrifices, he made to help me progress in life. His love and unwavering support since my childhood have been instrumental in my journey.*

*My brother**, Mohammed El Amine**, has consistently been a source of inspiration and strength for me. His presence and unwavering determination have encouraged me to persevere.*

*My sisters, **Rachida** and **Wahiba**, I am immensely proud to have them as siblings. Their support and companionship have been invaluable to me.*

*My grandchildren, **Katre Ennada, Abd El Hak**, and **Khalil**, bring boundless joy to my life. They are a constant reminder of the beauty and blessings of family.*

*My cousins, **Amina** & **wadjila** and **Ikram,** have always been there for me. Their presence and support have made a significant impact on my journey.*

*My best friend, **Zahira**, and her wonderful family. Their friendship, love, and support have been a source of strength and happiness throughout my life.*

Lastly, to all those I hold dear and love, I extend my heartfelt gratitude. Your presence and support have played an integral role in shaping who I am today. Thank you all very much.

AMEL

Abstract

The invention of eco-friendly smart packaging represents currently an escape route to substitute the plastic that is relentlessly invading the globe without compassion. This work aims to design a smart packaging and labels that combine pectin as the base material, while incorporating anthocyanins sourced entirely from red cabbage plant. The films formed in conjunction with anthocyanins were thicker, denser and more moisturous. Yet, their features encompassed a lower wettability and transparency, all with inferior water solubility. The phyto-dye escaped with higher ease in low-fat food simulant, and the whole film degraded quite quickly in soil. The hued film shaped on patches was very effective as pH-change sensor by swinging mainly from purple to green color; a clue key for intensification of ammonia stemmed from stored chicken meat decay.

Keywords: pectin, anthocyanin, smart, packaging, pH-responsive.

Résumé

L'invention d'un emballage intelligent respectueux de l'environnement représente actuellement une voie de sortie pour remplacer le plastique qui envahit impitoyablement la planète sans compassion. Ce travail vise à concevoir des emballages et patchs intelligents en combinant la pectine comme matériau de base aux anthocyanes provenant entièrement du chou rouge. Les films formés en conjonction avec les anthocyanes étaient plus épais, plus denses et plus humides. Cependant, leurs caractéristiques comprenaient une mouillabilité et une transparence, ainsi qu'une hydro-solubilité amoindries. Le phyto-colorant s'échappait plus facilement dans un simulant d'aliments faibles en matières grasses, et l'ensemble du film se dégradait assez rapidement dans le sol. Le film teinté formé en patchs était très efficace en tant que capteur de changement de pH, passant principalement du violet au vert ; un indice clé pour l'intensification de l'ammoniac issue de la décomposition de la viande de poulet conservée.

Mots clés : pectine, anthocyane, intelligent, emballage, pH- sensible.

الملخص

تمثل التعبئة الذكية الصديقة للبيئة حاليًا ملجأ واعدا لاستبدال البلاستيك الذي يغزو العالم دون هوادة. يهدف هذا العمل إلى تصميم مواد تغليف وملصقات مستشعرة ذكية تستخدم البكتين كمادة أساسية، مع دمج الأنثوسيانين المستخرجة بالكامل من نبات الكرنب الأحمر. بينت النتائج ان الأفلام المحتوية على الأنثوسيانين أكثر سمكًا وكثافة ورطوبة، مع محدودية ذوبانها في الماء، تضمنها لشفافية اقل وقابلية منخفضة للتبلل. أكدت البيانات ايضا تسرب صبغة الأنثوسيانين بسهولة أكبر في المحلول المحاكي للأغذية قليلة الدهون، بالإضافة الى تحلل كامل وسريع لهذه الأفلام الملونة في التربة. في الاخير، تم الكشف على الفعالية الكبيرة لملصقات الأنثوسيانين كمستشعرات لتغير درجة الحموضة، بتغيرها اساسا من اللون الأرجواني إلى اللون الأخضر؛ نتيجة لوجود النشادر الناجم عن تلف لحم الدجاج المخزن.

الكلمات الرئيسية: بكتين، أنثوسيانين، ذكي، التغليف، حساس للحموضة.

List of abbreviations

%	Percentage
°	Degree
°C	Celsius degree
µl	Microliter
cm^2	Square centimeter
$CaCl_2$	Calcium chloride
cm	Centimeter
nm	Nanometer
g	Gram
h	Hour
HR	Relative humidity
M	Molar weight
µg	Microgram
m	Meter
mm^2	Square Millimeter
min	Minute
mL	Milliliter
mm	Millimeter
W/W	Weight/Weight
Pa	Pascale
pH	Hydrogen potential
S	Second
UV	Ultraviolet
WS	Water solubility
WVP	Water Vapor Permiability
SRGB	Sensitivity Red Green Bleu
RCA	Red Cabbage Anthocyanins
RCE	Red Cabbage Extract

List of tables

List of figures

Table of contents

Chapter II : Results and discussion

Introduction

Introduction

Changes in the safety and quality of food products can occur at various stages, including production, storage, distribution, shipment, and consumption. Food packaging plays a crucial role in maintaining the quality and safety of food by regulating the flow of oxygen and preventing the entry of harmful microorganisms. Additionally, it provides a protective barrier that supports the integrity of the food and extends its shelf life **(Abedi-Firoozjah, et *al*., 2022; Garavand et *al*., 2022; Garavand et *al*., 2017).**

While consumers typically rely on the shelf-life date printed on packaged foods to determine their freshness and quality, this approach may not be sufficient for assessing the freshness and quality of fresh fruits and vegetables. Moreover, the global concern over the environmental issues associated with petroleum-based plastic packaging, both in production and disposal, added to their failure to meet consumer demands for safe and high-quality food has led to an urge need for alternative solutions, and aroused the recent growing interest in using naturally biodegradable and edible materials such as proteins, polysaccharides and lipids for packaging purposes **(Abedi-Firoozjah, et *al*., 2022; Garavand et *al*., 2022; Khazaei et *al*., 2021).**

The development of modern bio-based packaging, for instance; intelligent packaging systems which consider the interplay between food products, packaging materials, internal and external environmental factors, and consumer needs, is now widely pursued worldwide in order to address the environmental and health concerns while improving packaging efficiency**.** These systems incorporate quality sensors, indicators, and traceability methods to provide consumers with valuable information regarding changes in food quality and safety throughout the entire food supply chain, and enable them consequently to make better decisions about the products they purchase (**Abedi-Firoozjah et *al*., 2022 ; Bahrami et *al*., 2022; Moghadam et *al*., 2021; Kuswandi et *al*., 2020 ; Rodriguez-Amaya et *al*., 2019).**

As such, smart colorimetric sensors and labels incorporating pH-sensitive natural colors are viewed as one of these promising and innovative alternatives due to their affordability, accessibility, abundance and reliable halochromic capacity**.** This last ability to change color in response to pH variations, representing the main trait of this type of smart films, serves as a visual indication of potential changes or spoilage in the food caused by chemical reactions or microbial growth, allowing, therefore, a timely quality assessment and ensuring consumer safety

(Abedi-Firoozjah, et *al*., 2022; Sani et *al*., 2021; Balbinot-Alfaro et *al*., 2019; Musso et *al*.,2016).

To this end, natural colorants, primarily those derived from plants, have been suggested to supersede safely to detrimental synthetic dyes. In this context, hydro-soluble naturel flavonoid pigments, named Anthocyanins, found in many fruits, vegetables, and flowers and known for light reflectance in the red-blue range of the visible spectrum, have been extensively employed as valuable visual indicators and sensors in smart packaging **(Abedi-Firoozjah, et *al*., 2022; Garavand et *al*., 2021 ; Sani et *al*., 2021 ; Realini & marcos., 2014).** On the top of that, they exhibit strong antimicrobial and antioxidant properties, making them ideal candidates for extending the shelf life of food products, and justifying their multitasking inclusion to ensure both quality preservation and safety enhancement **(Chen et *al*., 2020; Garavand., 2016).**

As mentioned above, the integration of Anthocyanins derived from plant sources represents currently the modern tendency for the purpose of designing and creating active and/or intelligent packaging. Likewise, red cabbage known scientifically as (*Brassica oleracea* L.), is an abundant natural source of anthocyanins, beside its packed richness in micronutrients and phytochemicals with nutritional and human health benefits: oligosaccharides, minerals, vitamins, flavonols, and glucosinolates **(Drozdowska et *al*., 2020).**

Besides, in order to meet the environmental and customer's requirements, the conception of harmless reusable or compostable packaging endowed with nutritional/curative aspects, leads to recommend various polysaccharides including "chitosan, starch, cellulose, pectin, and natural gums" and proteins such as "zein, soy protein isolate, and gelatin" to be used usually as the primary or combined matrix materials for anthocyanin-rich packaging films. Fundamentally, the pectin is one of the favorite natural substances recommended by the US Environmental Protection Agency (EPA) to made biopackaging. It is a complex carbohydrate found in the cell walls of plants, particularly in fruits such as apples and citrus fruits, and may act as a gelling agent, thickener, and stabilizer in various food and pharmaceutical products. (**Abedi-Firoozjah et *al*., 2022; Falguera et *al*., 2011)**.

The aim of this study is to develop smart and biodegradable pectic films, incorporating red cabbage anthocyanins as pH-change responsive indicator. Afterward, the formulated films undergo a set of characterization tests, followed thereafter, by assaying their practicability in form of patches (stickers) applied on conserved food.

Experimental part

Chapter I:

Materials and methods

I.1.Objectives

The main objectives of this work are to:

- Elaborate, at low-cost, a smart pH-responsive film from abundantly naturel sources.
- Assess the physico-chemical, mechanical and optical features of the film, and highlight its environmental biodegradation
- Evaluate the effectiveness of the developed film as a sensor patch on conserved chicken.

I.2. Plant material

I.2.1. Red cabbage

The red cabbage used in this study was purchased between January and February 2023 from the supermarket "Familly Shop" in the Wilaya of Tissemsilt. Then, the raw material was immediately kept in the refrigerator until its use. The pectin powder was obtained from a local confectionery store.

I.3. Laboratory equipment

Table 1 illustrates the chemicals, glassware and apparatus used in this work:

Table 1. Materials and reagents used

Devices	Chemicals	Glass
- Water bath	- Pectin	- Magnetic bars
- Analytical balance	- Distilled water	- Beakers
- Mincer	- Ethanol C_2H_6O	- Watch glass
- Comparator	- Glycerol	- Filter paper
- Desiccator	- Citric acid $C_6H_8O_7$	- Strainer
- Ventilated oven (Memmert)	- Calcium chloride $CaCl_2$	- Molds
- Mixer	- Sodium acetate $C_2H_3NaO_2$	- Funnels
- pH meter (HANNA)	- Acetic acid CH_3COOH	- Test tubes
- Heating plate	- Ammonia NH_3	- Volumetric flask
- Vacuum pump (KNF)	- Sodium hydroxide NaOH	- Yellow tips
- Refrigerator	- Hydrogen chloride HCl	- Spatula
- Rotavapor (Bushi R_200)	- Di-Sodium hydrogen phosphate Na_2HPO_4	- Flasks
- Spectrophotometer UV/VIS (JENWAY 7305)	- Potassium chloride KCl	
- Adjustable micropipette		

I.4. Experimental protocol

The following diagram schematizes the overall methodology adopted in this study, where each analysis is repeated 3 times:

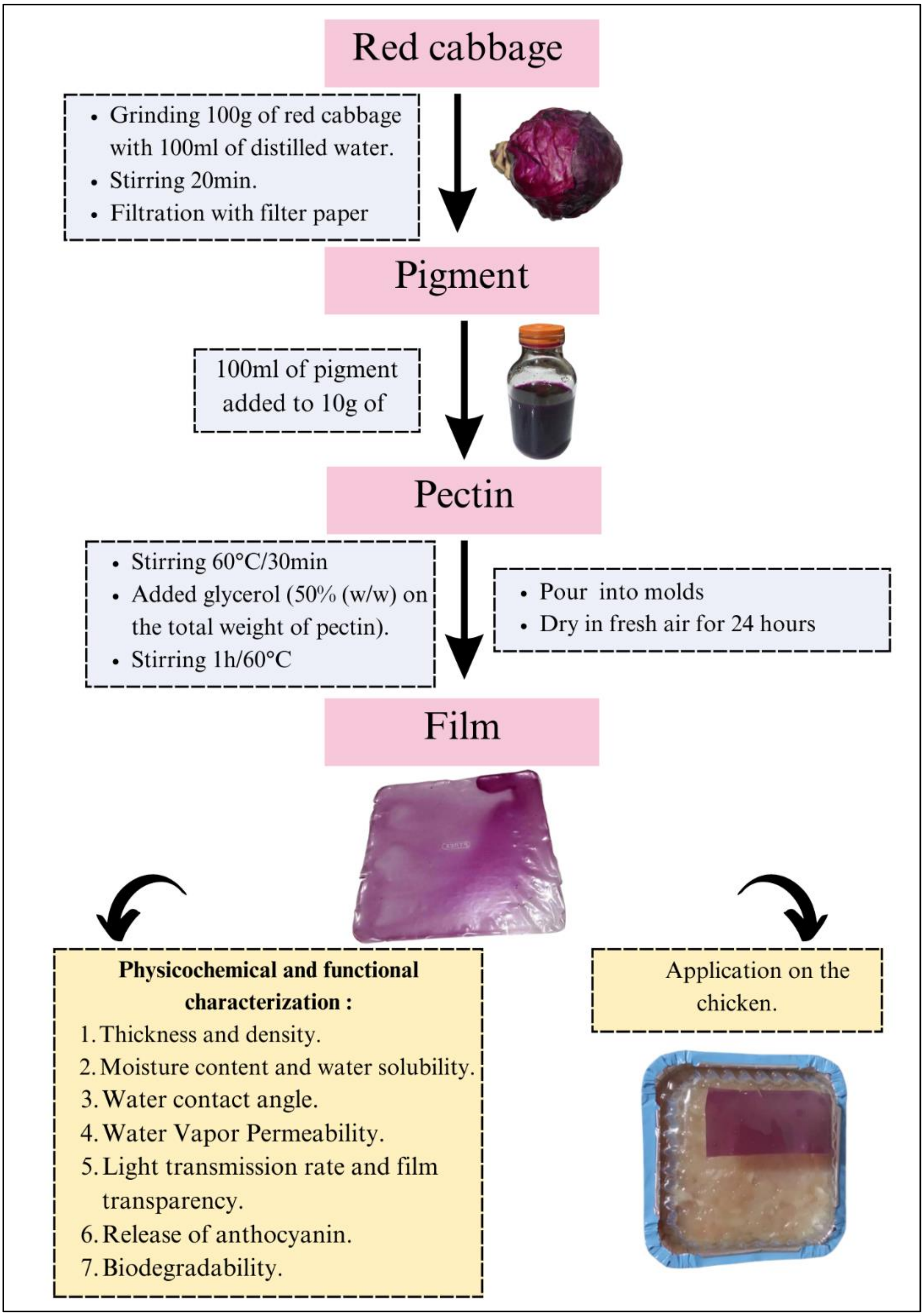


Figure 1. Diagram of the experimental protocol **(Original).**

I.5. Red cabbage anthocyanins

I.5.1. Extraction

According to the modified method of **Chandrasekhar et *al*. (2012),** 100 g of red cabbage was chopped and added by 100 ml of distilled water. The resultant mixture was agitated for 30 minutes and then strained through using a strainer to get rid of any big particles. The mixture is then shaken once again for an additional 15 minutes.

I.5.2. Determination of total anthocyanin content (TAC)

The pH differential method as outlined by **Halász and Csóka, (2018)** with minor adjustments was used to determine the Total Anthocyanin Content (TAC) of the obtained extract. Briefly, 0.2 mL of the red cabbage extract was added to 10 mL of potassium chloride buffer (pH = 1.0) or 10 mL of sodium acetate buffer (pH = 4.5). The solutions were then incubated in the dark for 30 minutes before measuring the absorbance at 510 and 700 nm using a UV-Visible spectrophotometer. The TAC was calculated using the equations thereafter **(Moghadam et *al*., 2021)**:

$$A = [(A_{510} - A_{700})_{\text{pH } 1.0} - (A_{510} - A_{700})_{\text{pH } 4.5}]$$

$$TAC\ (\text{mg/l}) = \frac{A \times MW \times DF \times 1000}{\varepsilon \times L}$$

Where: *A* is the absorbance, *MW* is the molecular weight of cyanidin-3-glucoside (449.2 g/mol), *DF* is the dilution factor, ε is the molar absorptivity (26,900), and *L* is the cell path length (1.0 cm) .

I.5.3. Response to pH changes

In order to evaluate the capacity of anthocyanins extract to function as calorimetric indicator, (use volume en µL) were poured into 5 ml of buffer solutions spanning from pH 1 to pH 12. Photos of the color changes were taken after a 10-second exposure to the buffer solutions **(Rawdkuen et *al*., 2020)**.

The pH array of (2.0-8.0) was obtained by phosphate-citrate buffer (0.50 M citric acid/ 0.5 M di-sodium phosphate. The pH =1.0 was attained through adjustment of the previous buffer by HCl (0.1M). In the other hand, NaOH solution (0.1M) was added to the phosphate-citrate buffer solution to reach the alkaline pH range (pH=9.0-12.0).

I.6. Smart films

I.6.1. Preparation

The filmogenic solution was prepared by a gradual solubilization of 10 g of pectin powder in 100 ml of anthocyanins extract, with a permanent stirring at 60°C in a water bath until a complete dissolution. Once dissolved, the agitation was maintained at the same conditions for one hour. After what, glycerol was added as plasticizer up to 50% (w/w) based on the total weight of pectin. A final homogenization was carried out at 60°C/30 min **(Ngo et *al*., 2020; Shahrampour et *al*., 2020)**.

The final mixture was poured into molds (0.16 ml/cm^2) and left to dryness in ambient air for 24 hours. The recovered films were stabilized in desiccator containing silica gel for 6 hours then stored at hermitic boxes until their use **(Ngo et *al*., 2020; Shahrampour et *al*., 2020)**.

The films without anthocyanins extract, which was supplanted by distilled water, followed the same synthesis process and lead to the formation of the control film (uncolored film).

I.6.2. Characterization

I.6.2.1. Thickness and density

The film's thickness was measured at ten different points using a comparator for each film according to **Gheribi et *al*. (2018)**.

To determine the films density, the technique of **Ramos et *al*. (2012)** was employed. A 2cm^2 portion of the film (s), with a known thickness (e) determined from earlier measurements, was weighed (m), and the density (d) was calculated as described **(Li et *al*., 2020):**

$$d = \frac{m}{s.e}$$

I.6.2.2. Moisture content and water solubility

The determination of the relative humidity (RH) of films was performed following the assay of **Jouki et *al*., (2013)**. To do so, 2cm^2 samples were weighed initially (mi), then dried in an oven at 90°C for 24 hours before being weighed again (mf). The moisture content was then calculated using the equation hereafter **(Gheribi et *al*., 2018) :**

$$HR = \frac{mi - mf}{mi} \times 100$$

To test the water solubility (w_s) of the films, pre-weighed oven-dried pieces of 2 cm^2 (m_i), were immersed in 50ml of distilled water at 25°C for 30 minutes. The undissolved fragments were then oven-dried (90°C/24h) and reweighed (m_f) after cooling to room temperature **(Jouki et *al*., 2013).** The water solubility of the films was then calculated using the formula developed by **Gheribi et *al*. (2018):**

$$ws = \frac{mi - mf}{mi} \times 100$$

I.6.2.3. Water contact angle

The hydrophobicity of the film's surface was evaluated by measuring the contact angle with water (°). This involved using Image J software **(Joonas, 2021 64-bit Java 8)** to determine the angle between the baseline of a 4µL water droplet deposited on the film surface and the tangent line at the point of contact **(Gheribi et *al*., 2018).**

I.6.2.4. Water Vapor Permeability (WVP)

An acrylic cup containing $CaCl_2$ was sealed with films. The assembly was weighed and placed in a chamber with 500 ml of distilled water at 20°C to maintain the relative humidity at around 99% during the experiment. The weight gain was monitored every hour over a period of 6 hours to calculate the water vapor transmission rate (WVTR) of the films**.** The water vapor permeability WVP was calculated according to the following equation **(Gonzalez et *al*., 2019; Zhang et *al*., 2016):**

$$WVP = \frac{WVTR}{A}\frac{e}{\Delta Pv}$$

Where:

WVTR: the water vapour transmission rate ($g.s^{-1}$) is the slope of the linear regression weight gain = f (time), calculated by plotting the final weight minus the initial weight of the sample (W_f - W_0) against time (t);

e: the average film thickness (m);

A: the transfer area (m^2);

ΔPυ: the water vapor pressure difference between the $CaCl_2$ atmosphere and the chamber atmosphere (2337 Pa).

I.6.2.5. Light transmission rate and film transparency

The UV-visible spectrophotometer was used to measure the absorbance and transmittance of (0.5cm x 4.0cm) film strips placed in a quartz cell at 600 nm **(Zhang et *al*., 2016)**. The transparency of the films was calculated using the ratio described by **Han et *al*. (1997):**

$$\boldsymbol{Transparency = \frac{A600}{S} = \frac{-\log T600}{S}}$$

Where:

A600 and T600: the absorbance and transmittance at 600 nm, respectively;

S: the thickness of the film**.**

I.6.2.6. Release of anthocyanins in food simulants

Film samples (2 cm × 2 cm) were immersed in 25 mL of food simulants (water, 10%, 50%, and 95% ethanol solutions) and agitated gently. 2mL of sample was obtained predetermined time intervals (30, 60, 90, 120, 180, 240 min) and the absorbance was measured using a UV-vis spectrophotometer at 520 nm. The rate of anthocyanins release was estimated through the recorded absorbance (Optical density OD) **(Alizadeh-Sani et *al*., 2021).**

I.6.2.7. Biodegradability

In order to investigate how easily films break down in soil, experiments were conducted using a procedure on a small scale **(Sanhawong et *al*., 2017)**. Two film samples, each measuring 3cm × 3cm, were placed in a plastic cup filled with horticultural soil, about 10 cm below the surface. The samples were first dried at 105°C for 24 hours and weighed to establish their initial weight (M_0). The weight of the film was then measured every three days (M_1). To calculate how much weight the sample had lost, the following equation was used:

$$\boldsymbol{Weight\ loss}\ (\%) = \boldsymbol{\frac{M0 - M1}{M0} \times 100}$$

I.6.3. Film application

I.6.3.1. Ammonia sensitivity test

To assess the sensitivity of the film to ammonia, a 2x2 cm sample was suspended in a beaker at a distance of 1 cm from an ammonia solution (8 mM, 80 mL) for 30 minutes at 25°C. The color change of sample was recorded at 5-minute intervals throughout the 30-minute period **(Alizadeh-Sani et *al*., 2021).**

Using the Pixie program for Windows, the R (red), G (green), and B (blue) values of the film were determined, and the film's responsiveness to volatile ammonia vapor was computed utilizing the subsequent formula **(Alizadeh-Sani et *al*., 2021):**

$$\boldsymbol{SRGB}(\%) = \frac{(\boldsymbol{Ri}-\boldsymbol{Rf})+(\boldsymbol{Gi}-\boldsymbol{Gf})+(\boldsymbol{Bi}-\boldsymbol{Bf})}{\boldsymbol{Ri}+\boldsymbol{Gi}+\boldsymbol{Bi}} \times \mathbf{100}$$

Where*:*

SRGB*:* the Sensitivity Red Green Bleu*;* ***Ri, Gi, Bi*** and ***Rf, Gf, Bf*** were the initial and final values of the red, green, and blue values of the film, respectively**.**

I.6.3.2. Smart patch efficiency test

Rectangular film samples with dimensions of (1cm×3 cm) were pasted at the inner side of packaging containers lids containing 50 g of ground chicken. The containers were divided into two groups to be stored for 72 hours either at room temperature (25°C) or in a refrigerator (4°C). The observable change in films color was registered by taking photos at 6 hour intervals **(Alizadeh-Sani et *al*., 2021).**

Chapter II:

Results and discussion

II.1. Red cabbage anthocyanins

II.1.1. Total anthocyanin content (TAC) of red cabbage extract

Measuring the total anthocyanin content provides valuable information about the quality, nutritional value, and potential health benefits of a particular sample. Red cabbage is a widely available and abundant source of anthocyanins **(Chen et al., 2021)**.

The total amount of extracted anthocyanin in red cabbage reached a value of 13.19 ±0.89 mg/L. Our findings indicated a lower quantity compared to that reported by **Hematian et al., (2023)** for red cabbage of Iran, which was 35.66+3.14 mg /L.

This difference may be correlated to the cabbage species, growing climate, and cultivation region. Different red cabbage species exhibit varying levels of anthocyanins, with a natural superiority of some of them. The growing climate plays a crucial role, since a cooler temperatures and sufficient sunlight promote higher anthocyanin content, while excessive heat or inadequate light can reduce their production **(Mineykina et *al*., 2021).**

The cultivation region, including soil composition and environmental conditions, influences anthocyanin levels also. Favorable soil conditions and nutrient availability, aside with certain geographic locations or microclimates can provide optimal conditions for higher anthocyanin levels. Therefore, managing these factors is essential for producing red cabbage with desirable anthocyanin content **(Mineykina et *al*., 2021).**

Moreover, it was confirmed that anthocyanins are very affected by the extraction factors and might demonstrate a high sensitivity towards the pH, the light, and the temperature. Due to that reactivity, they had a tendency to degrade or undergo reactions with other components present in the film matrix, leading to the formation of colorless or brown-colored compounds **(Chaiyasut et al., 2016).**

II.1.2. Halochromic (pH-Sensitive) properties of Red Cabbage Anthocyanins

In plants, anthocyanins are natural phyto-colorants responsible for the red, purple, and blue colors **(Abedi-Firoozjah et *al*., 2022)**. The color alteration of buffer solutions containing the red cabbage anthocyanin rich extract was depicted in Figure 2.

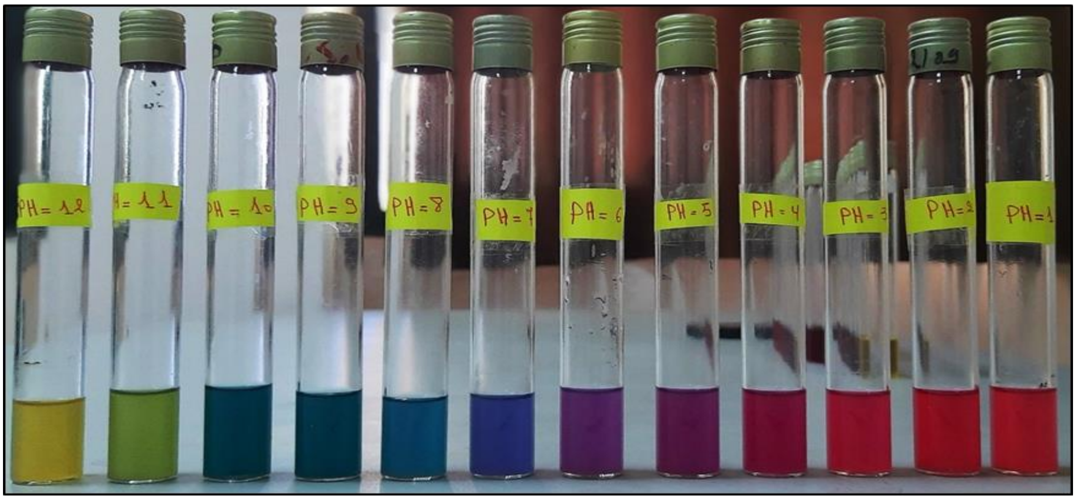


Figure 2. Color change of anthocyanin extract at a pH range (1-12) **(Original).**

An advantage of utilizing anthocyanin as a pH indicator is its ability to demonstrate a conspicuous change in color in response to various pH values. Therefore, utilizing this pH-responsive propertie could be beneficial in creating intelligent packaging systems (**Abedi-Firoozjah et *al*., 2022)**. Identically to the results of **Chen et al. (2021),** the red cabbage anthocyanins pigment in this study displayed a red color at pH 1 and 2, pink at pH 3, violet at pH 4-6, blue at pH 7 and 8, green at pH 9-11, and yellow at pH 12, which make it highly suitable for use in films as indicator of changes in pH levels.

According to the interpretation of **Abedi-Firoozjah et *al*., (2022)**, anthocyanins are a type of phenolic compound that can dissolve in water, and have the ability to produce a broad spectrum of hues (such as blue, purple, orange, and red) depending on the pH level of the solution they are in. This means that anthocyanins come in different colors and forms, which can be used to monitor the quality of food products and keep track of their shelf-life. The reversible color characteristics of solutions containing anthocyanins are linked to the origin, composition, and structure of these compounds. Thus, there are four distinct chemical forms of anthocyanins, each with varying colors that are observable at different pH levels of the solution **(figure 2)**.

The color of the anthocyanins extract changes gradually from red to green/yellow as the pH increases from acidic to alkaline conditions. The properties causing this variation are named hyperchromism and bathochromism. At pH values below 2, the extract appears mostly red due to the flavylium cation. As the pH increases slightly to between 2 and 4, the color changes to a purple/blue quinoidal base. In the slightly acidic to near-neutral range, the extract becomes colorless carbinol pseudo-base. However, at higher pH values above 7, the extract becomes less stable and generates a green-yellow color due to chalcone formation (**Abedi-Firoozjah et *al*.,2022)**.

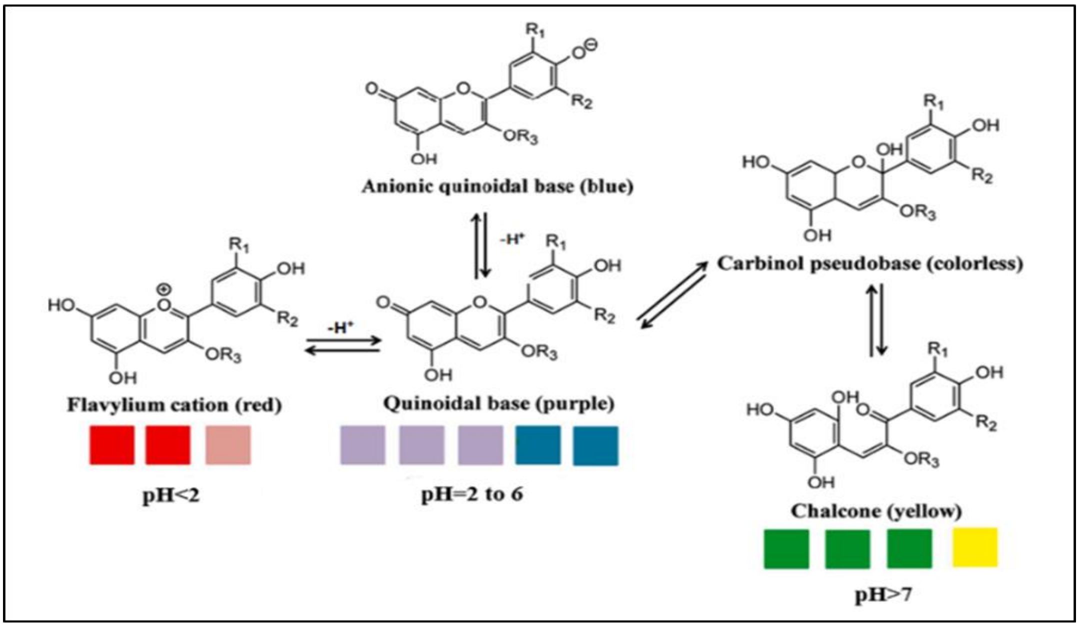


Figure 3. Colors change of red cabbage anthocyanin-rich extract at different pH values **(Abedi-Firoozjah et *al*.,2022).**

II.2. Smart films

II.2.1. Physico-chemical properties

Table 2 illustrates the results recorded for the characterization of physicochemical properties of colored and uncolored intelligent film based on pectin and red cabbage:

Table 2. Physico-chemical properties of smart films.

Properties	Smart films	
	Colored	**Control**
Thickness (mm)	0.158±0.006	0.100±0.008
Density (g/ml)	0.210±0.008	0.155±0.003
Moisture (%)	31.74±0.11	22.58±0.023
Solubility (%)	53.48±0.05	99.99±0.005
Contact angle (°)	62.29±0.06	74.76±0.132
Water vapor permeability (WVP) ($g.m^{-1}. s^{-1}. Pa^{-1}$)	$2.39\times10^{-2}\pm2.08\times10^{-4}$	$1.82\times10^{-2}\pm1.76\times10^{-4}$
Transparency (%)	1.686±0.046	1.908±0.028

II.2.2. Thickness and density

The thickness of packaging films plays a significant role in determining the mechanical strength, light transmission, and gas barrier abilities of the produced composites. This characteristic is greatly affected by the composition, dispersibility, and flow properties of the film **(Abedi-Firoozjah et *al*., 2022)**.

The colored films were thicker than their homologous uncolored (0.158±0.006mm against 0.100±0.008mm), which reflected the effect of the presence of anthocyanins extract on the thickness increase of films. According to **Abedi-Firoozjah et *al*. (2022)**, incorporating excessive quantities of anthocyanins into the film can potentially compromise the structural integrity of the matrix and impact the thickness of the film**.** The studied films were thicker than those prepared by **Halis et Hassouni, (2022)** with (0.031±0.0012 to 0.053±0.001mm), but thinner than films of pectin/chitosan and curcumin extract of 0.426±0.084 mm prepared by **Xie et *al*. (2021)**.

Regarding the film density, **Li et *al*. (2020)** claimed its direct impact on tensile strength, elongation and water vapor permeability (WVP)**.** The colored films in this work had a higher density compared to the uncolored films, with respective measurements of 0.210±0.008 g/ml and 0.155±0.003 g/ml. The density of the anthocyanin-based films was similar to the density of films made with pear peel mucilage and potato Husk Starch with glycerin reported in the study of **Ayquipa-Cuellar et *al.* (2021).** These results suggested an influence of the formulation composition and thickness on density **(Ayquipa-Cuellar et *al*., 2021)**

Figure 4. Film thickness measurement by the comparator **(Original).**

II.2.3. Moisture content of the film

Moisture absorption is a crucial consideration in the development of pH-sensitive smart packaging systems seeing its obvious action on the efficiency of color response within the film. This is particularly important for hydrophilic composite films that are sensitive to water, as changes in moisture content and water activity can significantly impact their structure and functionality **(Abedi-Firoozjah et *al*., 2022).** Conversely, the elevated levels of water vapor permeability can be attributed to the increased water content present in the films, as stated by **Chiou et *al*. (2009).**

Based on the findings in the table 3, the moisture content of the colored film was equal to 31.74±0.11%, compared to lesser rate for uncolored film exhibiting only 22.58±0.023%. These ascertainments aligned closely within the results obtained by **Che Hamzah et *al*. (2022),** in which, the moisture content of their films made with starch and red cabbage attained 23.95% to 40.38%.

The moisture content of films increased with an increase in film thickness, which could be allotted to the availability of hydroxyl groups resulting from the interaction between ingredients. However, the positive action of red cabbage anthocyanin (RCA) on water content observed in this study, is contradictory to other studies suggesting no significant effect of the anthocyanins addition on water content of films **(Piñeros-Hernandez et *al*., 2017)**

It is worth noting that the incorporation of RCA can enhance the hydrophilic characteristics of biopolymer films. Nevertheless, due to limited evidence, determining the precise impact of anthocyanins on moisture absorbency is challenging **(Abedi-Firoozjah et *al*., 2022).**

II.2.4. Water solubility

The water solubility of films indicates their susceptibility to water. Packaging films that exhibit excellent water resistance are preferred for preserving food items with moderate to high moisture content. In contrast, intelligent films with low water resistance can dissolve rapidly leading to significant colorimetric agent loss and release **(Abedi-Firoozjah et *al*., 2022).**

It is evident that the uncolored film showed a higher hydrosolubility as it completely dissolved (99.99±0.005%), whereas the colored film had a lower water dissolution rate going down approximately to the half (53.448%±0.05%). These results are far from the findings of **Xie**

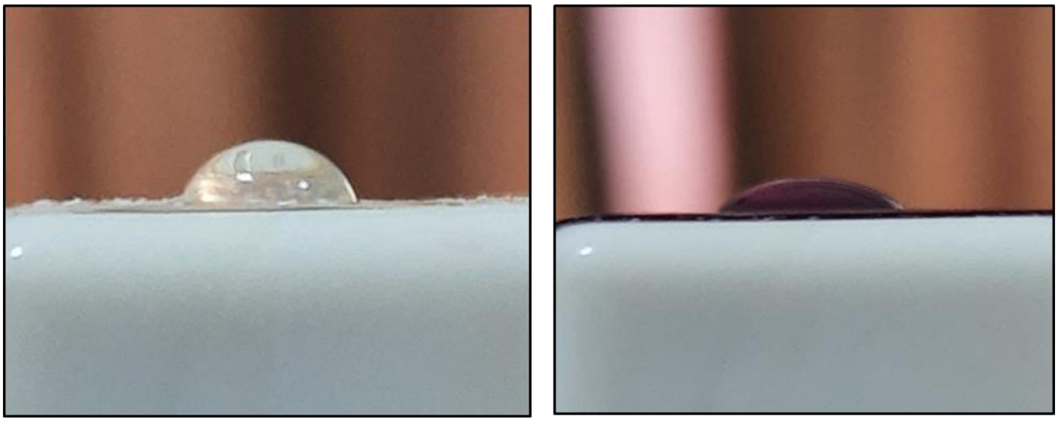
et *al*. (2021), where the difference between the two types was very slight with an overtaking for control uncolored films (51.9% vs 53.65%).

The water solubility of the films recognized a significant decrease upon the addition of anthocyanins, as a likely response to their H-bounds forming property. Conversely, **Prietto et *al*. (2017)** and **Kuswandi et *al*. (2020)** noticed an enhancement of films hydrosolubility after anthocyanins adding.

II.2.5. Water contact angle

The contact angle provides an assessment of the interaction between a solvent and the material's surface. Consequently, higher contact angles observed between water droplets and the film surface indicate hydrophobicity resulting from the film's composition. In opposition, lower contact angles indicate a hydrophilic film's composition **(Manrich et *al*., 2017).**

Here, the pectin uncolored films exhibited a larger contact angle compared to the anthocyanic films, measuring successively 74.76±0.132° and 62.29°±0.06°. These outcomes contradict the results of **Ngo et *al*. (2020),** as they reported a higher contact angle for the active film (84.8°) compared to the control film (62.1°). Therefore, the studied films are more hydrophilic than those tested by **Ngo et *al*. (2020)**.



A **B**

Figure 5. Shape of the water droplet on the A) control film, B) colored film **(Original).**

Anthocyanins are generally hydrophilic compounds. They have an affinity for water and are soluble in aqueous solutions. Accordingly, the hydrophilicity of anthocyanins is attributed to their molecular structure containing polar groups such as hydroxyl (-OH) and sugar moieties. These polar groups interact with water molecules through hydrogen bonding, facilitating their dissolution in water and enhancing their solubility **(Abedi-Firoozjah et *al*., 2022).**

II.2.6. Water Vapor Permeability (WVP)

Young et *al*. (2019) emphasized the importance of water vapor permeability (WVP) as a significant parameter that gauges a film's effectiveness in blocking the passage of water vapor. WVP measures the amount of water that permeates a given area over time ($g.m^{-1}.s^{-1.}Pa^{-1}$). Typically, the primary aim is to develop films that minimize WVP values to prevent moisture transfer between food and the surrounding environment, as highlighted by **Riaz et *al*. (2018).**

The control of WVP is crucial for prolonging the shelf life of packaged food by maintaining the desired moisture balance and preventing physical or chemical deterioration. **(Abedi-Firoozjah et *al*., 2022).**

The results indicated an inferior WVP of uncolored films in comparison with colored films ($1.82\times10^{-2}\pm1.72\times10^{-4}$ against $2.39\times10^{-2}\pm2.08\times10^{-4}$ $g.m^{-1}.\ s^{-1}.\ Pa^{-1}$). **Yong et al. (2019)** discovered slight increases in WVP values when anthocyanins from purple and black eggplant extracts were incorporated.

These films are more permeable than the films of **Bared et Belhaouas, (2021)** (from $0.53x10^{-6}$ to $0.1x10^{-5}$ $g.m^{-1}.s^{-1}.Pa^{-1}$). In contrast, **Chen et *al*. (2021)** demonstrated that incorporating anthocyanins into composite films resulted in a significant reduction in water vapor permeability (WVP). They attributed these changes to the formation of hydrogen bonds between the composite film and the pigment.

II.2.7. Light transmission rate and film transparency

The transparency of a film plays a crucial role in determining its barrier properties against UV-visible light, which can be harmful to food during storage. Film transparency is an important factor in assessing the ability of a packaging material to block light and preserve the visual appearance of the packaged product **(Zam et *al*., 2022).**

The transparent films exhibited higher clarity compared to the colored smart films, with transparency levels of 1.908±0.028% and 1.686±0.046% respectively. Our films demonstrate lower transparency compared to the films prepared by **Zam et *al*. (2022),** which exhibited a range of transparency levels going from 1.32% to 7.91%.

Consistent with the research conducted by **Gutierrez and Alvarez (2018)** and **Yun et *al*. (2019),** they observed that the UV-Vis light barrier effectiveness of anthocyanin-rich films increased as the anthocyanin content was raised. This enhancement can be attributed to the ability of the aromatic rings present in anthocyanins to absorb UV-Vis light.

II.2.8. Release of anthocyanins

The purpose of esteeming the anthocyanin release was primordially to investigate the interactions between the film matrix and the pigment **(Alizadeh-Sani et *al*., 2021)**. The figure 6 displays the release of anthocyanins from smart films when immersed in various food simulants (10%, 50% and 95% ethanol solutions, distilled water) for duration of 240 minutes. The release of anthocyanins was fastest in distilled water, followed subsequently by 10% then 50% alcohol solutions. The 95% alcohol solution implicated the slowest speed of liberation.

The release of active compounds is affected by factors such as the water solubility of the polymer, the type of food simulant used, and the diffusion of active compounds from the film into the simulant, as indicated by **(Lee et *al*., 2018)**. In the case of red cabbage anthocyanin extract (RCAE), its high solubility in water promotes fast release when in contact with distilled water. Conversely, the low solubility of RCAE in alcohol solutions and the insolubility of the films in ethanol solutions contribute to a slower release of RCAE from the films when exposed to other simulants **(Cheng et *al*., 2022).**

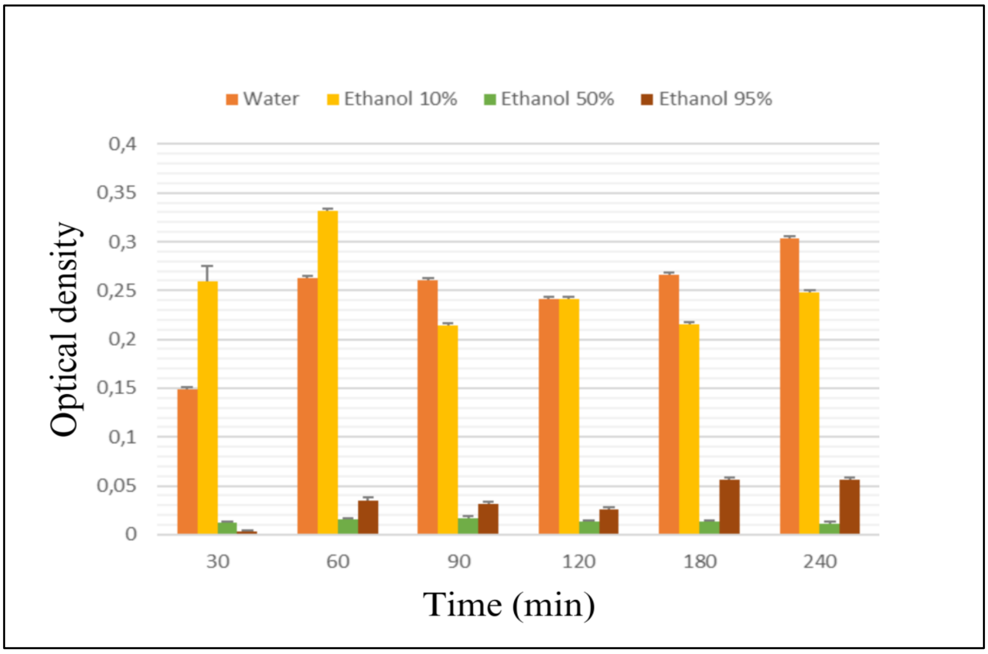


Figure 6. The release of anthocyanins in different food stimulants **(Original).**

Anthocyanins act as a defense mechanism against environmental stressors such as intense sunlight or low temperatures. Also, they may attract pollinators or serve as a warning signal to herbivores, deterring them from consuming the plant **(Kossyvaki et *al*., 2022)**. Additionally, these pigments protect the plant by absorbing harmful UV radiation and acting as antioxidants. Anthocyanins, have shown similarly significant antimicrobial properties when incorporated into active packaging materials. This can be attributed to their ability to penetrate the cell membranes of microorganisms, inhibit extracellular enzymes, and disrupt the cytoplasmic membranes of these microbes **(Ozdemir et Floros, 2004)**.

The last attributes matter the most when the anthocyanins release in foods is evoked, since they are responsible for the important part of biological activity of the active packaging.

The highest release rate of anthocyanins was remarked in the ethanol solution 10% (OD=0.33) followed by distilled water (OD=0.30%), indicating their high solubility in aqueous solutions. However, the colored films freed their anthocyanins more hardly for ethanol solutions 50% (OD=0.018) and 95% (OD=0.058).

The results were very similar to those found by **Cheng et *al*. (2022)** the percentages of the release of the active compounds in distilled water, 10%, 50%, and 95% ethanol solutions were found to be 32.08%, 25.79%, 12.48%, and 0.23% respectively.

It can be said that the anthocyanins of red cabbage are easily released from pectin films in low-fat and water-rich foods. Nevertheless, this phenomenon occurs in an inversely proportional manner along with increase of fat portion in the food.

II.2.9. Biodegradability

The importance of film biodegradability lies in its ability to rapidly decompose when discarded in the environment, typically through natural chemical or biochemical processes **(Sani et *al*., 2021).** The obtained results of weight loss of colored/uncolored films after 15 days in soil are summarized in table 3:

Table 3. Weight loss percentage of colored and uncolored films.

Days	Weight loss	
	colored film	Uncolored film
1	28.83% ±0.026	48.78% ±0.027
3	70.97% ±0.027	56.09% ±0.030
9	99.99% ±0.005	68.78% ±0.026
12	99.99% ±0.008	74.63% ±0.014
15	99.99% ±0.006	89.26% ±0.020

Table 3 shows an almost linear loss of initial weight for the both films. The uncolored film lost nearly their half weight (48.78%) only after 24 hours, but it underwent a progressive degradation until day 15 reaching a threshold of 89.26%. Similarly but more intensively, the colored film lost 28.83% after the first 24 hours to disappear completely (total degradation) by the 9th day.

The results reported by **Zam et *al*. (2022)**, supported the previous findings where both films biodegradation occurred positively. However, an interesting distinction is the pace of films degradation in this work was faster and initiated from the 3rd day, whereas their films began to degrade from the 5th day.

Pectin is widely acknowledged for its biodegradability and eco-friendly characteristics making it a popular choice as a component in biodegradable films. Its affordability, abundance and biodegradable nature have led to its utilization in film construction**.** When combined with anthocyanins, pectin-based edible films demonstrate a notable attribute of high-water vapor permeability. As a result, these films hold promise in contributing to the biodegradability of packaging materials **(Sani et *al*., 2021).**

II.3. Film application

II.3.1. Ammonia sensitivity test

Meat consists of water, protein, fat, and carbohydrates, which can be decomposed by enzymes and bacteria, leading to the production of volatile gases such as ammonia, hydrogen sulfide, and ethanethiol. During the spoilage process, the concentrations of these gases increase exponentially. By utilizing ammonia gas sensors, we can detect these gases and assess the freshness of the meat. The freshness of meat can be determined by considering factors such as the growth time of microorganisms and the food poisoning index **(Eom et *al*., 2014).**

The purpose of testing the sensitivity to ammonia vapor is to appreciate the detection/indication ability of smart film food packaging in presence of ammonia gas, which allows an early warning in case of any potential spoilage or contamination of the packaged food. The smart film can change color or display a visible indicator in the presence of ammonia vapor, alerting consumers or handlers to the compromised state of the packaged food **(Chia et *al*., 2022) .**

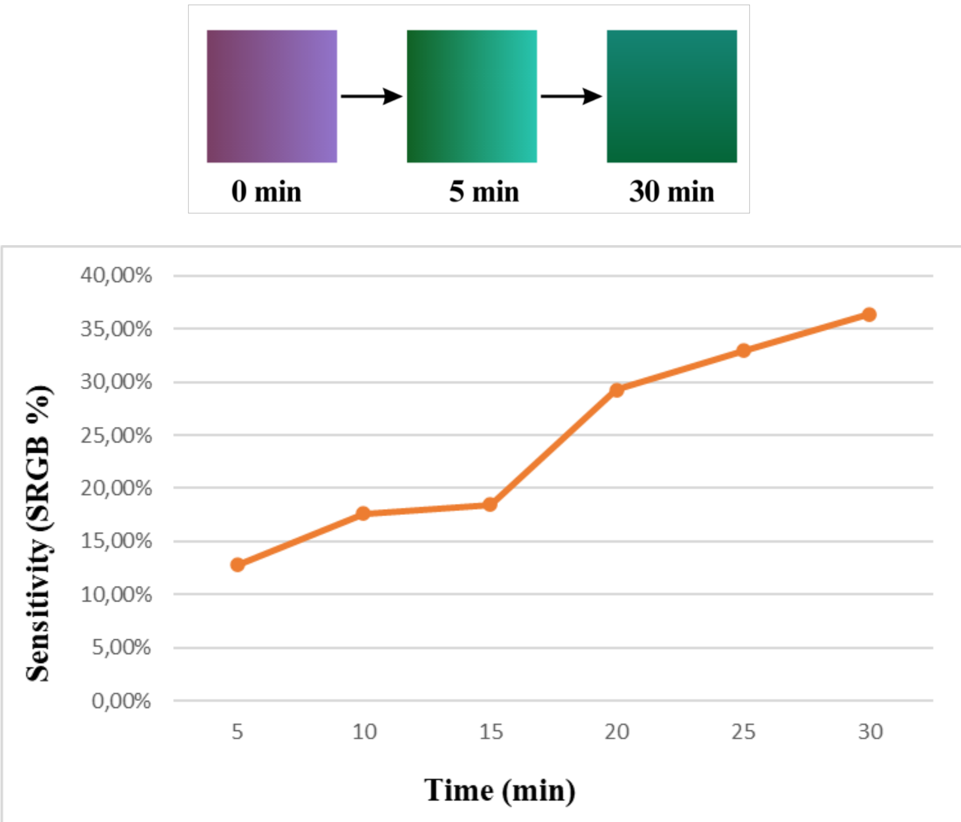


Figure 7. The sensitivity of the pectin/anthocyanins film to ammonia vapor.

The figure 7 describes the color transition of pectin-based films integrated with RCA and exposed to NH_3 for 30 minutes at 25°C. In the initial 5-minute period, these films changed rapidly from purple to green, indicating a swift and noticeable color transition in agreement with results of **Freitas et *al*. (2020)**. However, our film exhibits a faster color change within 5 minutes, whereas their film takes 15 minutes for the same change in color.

A substantial increase in the sensitivity of the pH-sensible film (SRGB) to ammonia vapor was observed over time. The SRGB coefficient rose from 12.78% at 5 minutes to 18.46% at 15 minutes, then escalated further to 36.36% at the 30-minute mark. Parallelly, **Alizadeh-Sani et *al*. (2021)** found an SRGB coefficient increase from 11.05% to 37.09% before touching 70.61% at 5, 15 and 30 min.

The color transition of the films is attributed to the interaction between NH_3 molecules and water molecules that are both present on the film's surface and within the polymer chains. This interaction leads to the formation of NH_4 and OH^- species. The presence of OH^- triggers modifications in the structures of anthocyanins, ultimately resulting in the color transition of the films **(Zhai et *al*., 2017).**

II.3.2. Smart patch efficiency test

During storage, the degradation of products can lead to the production of basic volatile nitrogen in the form of ammonia gas **(Wang et *al*.,2021; Lougovois., 2005)**. The transition of food pH during this degradation process serves as an indicator of the product's quality **(Sani et *al*., 2021).** Table 4 presents the film's color variations as the chicken's pH changes over storage time. Thoroughly, the film color transited from purple to green on the edges of the patch after 30 hours at 25°C, whereas the same color change occurred lately (after 60 hours) for the refrigerator container.

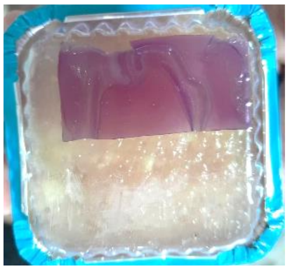
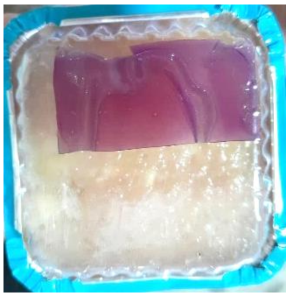
For the container stored at 25°C, a noticeable color transformation occurs in the film since the first hours, turning it from light purple into a dark purple shade, which signified a shift in acidity towards a mild acidic pH of 6. After 36 hours, another color change takes place, shifting from dark purple to dark green, expressing a shift from an acidic environment (pH 6) to a basic environment (pH 10). By the end of the 72 h, yellow hues appeared on the edges of the patch as a result of an extremely alkaline pH of 12 corresponding to a visible worms development in the ground chicken.

Regarding the container stored at 4°C, a slower rate of change was noticed compared to the package stored at 25°C. The color shift to dark green was observed after 60 h, but across the entire surface. This indicated a change in the pH from 6 to 10, accompanied by a clear unpleasant odor, without any visible worms growth. The dark green color state persisted until the 72h.

The results of pectin-based films and red cabbage extract as a pH-responsive patch applied on stored ground chicken similar to the results of **Vo et *al*., (2019).** The researchers obtained a change of film colors from pink (acidic pH) to yellow-pale green (basic pH) after 24h of contact with pig meat in the open air. Also, **Zam et *al*., (2022)** conducted an analogous experiment using fish stored in refrigerator. They unveiled color change from pink to yellow, indicating a shift from acidic to basic pH, concomitantly to bacterial contamination. This color change persisted for up to six days.

These color changes observed at different storage intervals (violet to green, and eventually to yellow) indicated the degradation of the samples due to external factors like microbial spoilage and enzyme-assisted reactions. Furthermore, those processes contributed to the autolysis of the chicken meat **(Wang et *al*.,2021; Lougovois., 2005).**

Table 4. Evolution of color change of packaging smart films.

Time (h)	Smart film color change	
	Stored in refrigerator (4°C)	Stored at room temperature (25°C)
6h		
12h		
18h		
24h		
30h		

36h		
42h		
48h		
54h		
60h		
66h		
72h		

In response to customer demand for fresher and longer-lasting food items, the food industry has invested in smart packaging placed on store shelves. These innovative packages incorporate pH indicators, aiming to create a deliberate connection between food and packaging in order to improve product quality. By observing the color of the packaging, consumers can easily detect pH changes in the food, which are closely linked to its deterioration **(Sani et *al*., 2021).** This application offers a successful and practical approach to continuously assess the freshness of food products throughout their shelf life (**Abedi-Firoozjah et *al*.,2022)**.

Conclusion

Conclusion

Smart and active film packaging materials derived from natural sources have significant potential in food industry, enhancing food quality, safety, and shelf-life while minimizing waste. Natural pigments can be utilized as indicators of freshness, quality, or safety, undergoing color changes in response to alterations in pH, gas levels, or temperature. The primary advantage of these materials is their ability to continuously monitor product freshness and safety without opening the package, providing information on past exposure to light, oxygen, pH, or temperature fluctuations **(Sani et *al*., 2021).**

Thus, smart systems and sensors offer numerous advantages, including traceability through meta-information and serialized packaging, real-time data for improved quality and safety management, reduced analysis costs, and enhanced connections between food processors, the market and consumers **(Abedi-Firoozjah et *al*., 2022).**

Incorporating red cabbage anthocyanins (RCA) characterized by halochromic properties into pectin-based films has augmented their thickness, density, moisture content and amplified their water vapor transmission. Though, this pigment conducted to a lesser hydrosolubility, an enhanced hydrophobic surface status with a better UV barrier impact.

Furthermore, the liberation of anthocyanins will probably be facilitated by low-fat and water-rich foods according to their release in food mimetic models. Their presence ameliorated the biodegradability of the films through shortening of required time to get a complete vanishing in soil. The application of pectin/anthocyanins as pH-responsive patch on chicken meat container, revealed a color shift from purple to green and even to yellow, according to the concentration of ammonia vapor produced gradually during the deterioration phases. This reaction was in concordance with the test of exposition to vapor of ammonia solution.

The formulated colored films produced with safe inexpensive and available materials, highlighted the potent use of similar active/smart food packaging labels in monitoring the freshness and the quality of food products and likely consumer safety. Equipped with adaptable reactivity towards food, pharmaceutical products, and numerous other types of goods, these trending packaging systems ensure continuous monitoring and offer new business opportunities based on digitization, aligning themselves with the broader field of industry. However, the commercialization of smart food packaging systems faces challenges related to regulations, cost-effectiveness, sustainability, security, data privacy, and acceptance by the supply chain and end-users **(Abedi-Firoozjah et *al*., 2022).**

Reference list

Reference list

1. Abedi-Firoozjah, R., Yousefi, S., Heydari, M., Seyedfatehi, F., Jafarzadeh, S., Mohammadi, R., Rouhi, M., & Garavand, F. (2022). Application of Red Cabbage Anthocyanins as pH-Sensitive Pigments in Smart Food Packaging and Sensors. Polymers, 14, 1629.
2. Alizadeh-Sani, M., Mohammadian, E., Rhim, J.-W., & Jafari, S.M. (2020). pH-sensitive (halochromic) smart packaging films based on natural food colorants for the monitoring of food quality. Trends in Food Science & Technology, 105, 93-144.
3. Alizadeh-Sani, M., Tavassoli, M., Mohammadian, E., Ehsani, A., Khaniki, G. J., Priyadarshi, R., & Rhim, J. W. (2021). pH-responsive color indicator films based on methylcellulose/chitosan nanofiber and barberry anthocyanins for real-time monitoring of meat freshness. International Journal of Biological Macromolecules, 166, 741-750.
4. Ayquipa-Cuellar, E., Salcedo-Sucasaca, L., & Azamar-Barrios, J. A. (2021). Assessment of Prickly Pear Peel Mucilage and Potato Husk Starch for Edible Films Production for Food Packaging Industries. Waste and Biomass Valorization, 12, 321-331.
5. Bahrami, R., Zibaei, R., Hashami, Z., Hasanvand, S., Garavand, F., Rouhi, M., Jafari, S.M., Mohammadi, R. (2022). Modification and improvement of biodegradable packaging films by cold plasma; a critical review. Critical Reviews in Food Science and Nutrition, 62, 1936-1950.
6. Bared, S. Belhaouas, A. (2021). Élaboration et caractérisation d'un biofilm comestible à base de mucilage des cladodes d'opuntia ficus indica de la région de Tissemsilt. Université de Tissemsilt, Tissemsilt.
7. Balbinot-Alfaro, E., Craveiro, D.V., Lima, K.O., Costa, H.L.G., Lopes, D.R., Prentice, C. (2019). Intelligent packaging with pH indicator potential. Food Engineering Reviews, 11, 235–244.
8. Chaiyasut, C., Sivamaruthi, B. S., Pengkumsri, N., Sirilun, S., Peerajan, S., Chaiyasut, K., & Kesika, P. (2016). Anthocyanin profile and its antioxidant activity of widely used fruits, vegetables, and flowers in Thailand. Asian Journal of Pharmaceutical and Clinical Research, 9(6), 218-224.
9. Chandrasekhar, J., Madhusudhan, M.C., Raghavarao, K.S.M.S. (2012). Extraction of anthocyanins from red cabbage and purification using adsorption. Food and Bioproducts Processing, 90(4), 615-623.
10. Che Hamzah, N. H., Khairuddin, N., Muhamad, I. I., Hassan, M. A., Ngaini, Z., & Sarbini, S. R. (2022). Characterisation and Colour Response of Smart Sago Starch-Based

Packaging Films Incorporated with Brassica oleracea Anthocyanin Membranes. Membranes, 12, 913.

11. Chen, M., Yan, T., Huang, J., Zhou, Y., & Hu, Y. (2021). Fabrication of halochromic smart films by immobilizing red cabbage anthocyanins into chitosan/oxidized-chitin nanocrystals composites for real-time hairtail and shrimp freshness monitoring. International Journal of Biological Macromolecules, 179, 90-100.
12. Chen, S., Wu, M., Lu, P., Gao, L., Yan, S., Wang, S. (2020). Development of pH indicator and antimicrobial cellulose nanofibre packaging film based on purple sweet potato anthocyanin and oregano essential oil. International Journal of Biological Macromolecules, 149, 271–280.
13. Cheng, M., Yan, X., Cui, Y., Han, M., Wang, Y., Wang, J., Zhang, R., & Wang, X. (2022). Characterization and Release Kinetics Study of Active Packaging Films Based on Modified Starch and Red Cabbage Anthocyanin Extract. Polymers, 14, 1214.
14. Chia, M.-R., Ahmad, I., Phang, S.-W. (2022). Starch/Polyaniline Biopolymer Film as Potential Intelligent Food Packaging with Colourimetric Ammonia Sensor. Polymers, 14, 1122.
15. Drozdowska, M., Leszczyńska, T., Koronowicz, A., Piasna-Słupecka, E., Domagała, D., & Kusznierewicz, B. (2020). Young shoots of red cabbage are a better source of selected nutrients and glucosinolates in comparison to the vegetable at full maturity. European Food Research and Technology, 246, 2505-2515.
16. Eom, K.-H., Hyun, K.-H., Lin, S., & Kim, J.-W. (2014). The Meat Freshness Monitoring System Using the Smart RFID Tag. International Journal of Distributed Sensor Networks, 10(7).
17. Freitas, P. A. V., Silva, R. R. A., Oliveira, T. V. de, Soares, R. R. A., Junior, N. S., Moraes, A. R. F., Pires, A. C. dos S., & Soares, N. F. F. (2020). Development and characterization of intelligent cellulose acetate-based films using red cabbage extract for visual detection of volatile bases. LWT, Volume 132, 109780.
18. Garavand, F. (2016). Changes in bioactive compounds, quality attributes and rheological behavior of black grape juice caused by microwave and conventional heating. Nutrafoods, 15, 285–292.
19. Garavand, F., Cacciotti, I., Vahedikia, N., Rehman, A., Tarhan, Ö., Akbari-Alavijeh, S., Shaddel, R., Rashidinejad, A., Nejatian, M., Jafarzadeh, S. (2022). A comprehensive review on the nanocomposites loaded with chitosan nanoparticles for food packaging. Critical Reviews in Food Science and Nutrition, 62, 1383-1416.

20. Garavand, F., Eghbal, N., Nooshkam, M., Miraballes, I., Jafari, S.M. (2021). Salt, spices, and seasonings formulated with nano/microencapsulated ingredients. In Application of Nano/Microencapsulated Ingredients in Food Products. Amsterdam, The Netherlands: Elsevier, pp. 435–467.
21. Garavand, F., Rouhi, M., Razavi, S.H., Cacciotti, I., Mohammadi, R. (2017). Improving the integrity of natural biopolymer films used in food packaging by crosslinking approach: A review. International Journal of Biological Macromolecules, 104, 687-707.
22. Gheribi, R., & Khwaldia, K. (2019). Mucilage de cactus pour des applications d'emballage alimentaire. Revêtements, 9(10), 655.
23. Gheribi, R., Puchot, L., Verge, P., Jaoued-Grayaa, N., Mezni, M., Habibi, Y., & Khwaldia, K. (2018). Development of plasticized edible films from Opuntia ficus-indica mucilage: A comparative study of various polyol plasticizers. Carbohydrate Polymers, 190, 204-211.
24. Gutiérrez, T. J., & Alvarez, V. A. (2018). Bionanocomposite films developed from corn starch and natural and modified nano-clays with or without added blueberry extract. Food Hydrocolloids, 77, 407–420.
25. Halis, S. Hassouni, I. (2022). Élaboration et caractérisation d'un emballage comestible actif à base de mucilage d'Opuntia ficus indica et de la gamme de caroube. Université de Tissemsilt, Tissemsilt.
26. Halász, K., Csóka, L. (2018). Black chokeberry (Aronia melanocarpa) pomace extract immobilized in chitosan for colorimetric pH indicator film application. Food Packaging and Shelf Life, 16, 185-193.
27. Hematian, F., Baghaei, H., Mohammadi Nafchi, A., & Bolandi, M. (2023). Preparation and characterization of an intelligent film based on fish gelatin and Coleus scutellarioides anthocyanin to monitor the freshness of rainbow trout fish fillet. Food Science & Nutrition, 11, 379-389.
28. Joonas, K., Janne, L., & Markku, J. (2021). Un modèle de canal en visibilité directe pour la bande de fréquences 100–450 gigahertz. Journal EURASIP sur les communications et les réseaux sans fil, 2021(1).
29. Jouki, M., Yazdi, F. T., Mortazavi, S. A., & Koocheki, A. (2013). Physical, barrier and antioxidant properties of a novel plasticized edible film from quince seed mucilage. International Journal of Biological Macromolecules, 62, 500-507.

30. Jouki, M., Yazdi, F. T., Mortazavi, S. A., & Koocheki, A. (2013). Propriétés physiques, barrières et antioxydantes d'un nouveau film alimentaire plastifié à partir de mucilage de graines de coing. Journal international des macromolécules biologiques, 62, 500-507.
31. Khazaei, A., Nateghi, L., Zand, N., Oromiehie, A., Garavand, F. (2021). Evaluation of Physical, Mechanical and Antibacterial Properties of Pinto Bean Starch-Polyvinyl Alcohol Biodegradable Films Reinforced with Cinnamon Essential Oil. Polymers, 13, 2778.
32. Kossyvaki, D., Contardi, M., Athanassiou, A., Fragouli, D. (2022). Colorimetric Indicators Based on Anthocyanin Polymer Composites: A Review. Polymers, 14, 4129.
33. Kuswandi, B., Asih, N. P., Pratoko, D. K., Kristiningrum, N., & Moradi, M. (2020). Edible pH sensor based on immobilized red cabbage anthocyanins into bacterial cellulose membrane for intelligent food packaging. Packaging Technology and Science, 33, 321-332.
34. Kuswandi, B., Asih, N.P., Pratoko, D.K., Kristiningrum, N., Moradi, M. (2020). Edible pH sensor based on immobilized red cabbage anthocyanins into bacterial cellulose membrane for intelligent food packaging. Packaging Technology and Science, 33, 321–332.
35. Lee, M.H., Kim, S.Y., Park, H.J. (2018). Effect of halloysite nanoclay on the physical, mechanical, and antioxidant properties of chitosan films incorporated with clove essential oil. Food Hydrocolloids, 84, 58-67.
36. Li, S., Ma, Y., Ji, T., Sameen, D. E., Ahmed, S., Qin, W., ... & Liu, Y. (2020). Edible films of cassava starch/carboxymethylcellulose embedded with lactic acid bacteria for extending the shelf life of banana. Carbohydrate Polymers, 248, 116805.
37. Lougovois, V. (2005). Freshness Quality and Spoilage of Chilled-Stored Fish. Hauppauge, NY, USA: Nova Science Publishers, Inc.
38. Manrich, A., Moreira, F. K. V., Otoni, C. G., Lorevice, M. V., Martins, M. A., & Mattoso, L. H. C. (2017). Hydrophobic edible films made from tomato cutin and pectin. Carbohydrate Polymers, 164, 83-91.
39. Mineykina, A., Bondareva, L., Soldatenko, A., Domblides, E. (2021). Androgenesis of Red Cabbage in Isolated Microspore Culture In Vitro. Plants, 10, 1950.
40. Moghadam, M., Salami, M., Mohammadian, M., & Emam-Djomeh, Z. (2021). Development and characterization of pH-sensitive and antioxidant edible films based on mung bean protein enriched with Echium amoenum anthocyanins. Journal of Food Measurement and Characterization, 15, 2984-2994.

41. Moghadam, M.A., Mohammadi, R., Sadeghi, E., Mohammadifar, M.A., Nejatian, M., Fallah, M., Rouhi, M. (2021). Preparation and characterization of poly (vinyl alcohol)/gum tragacanth/cellulose nanocomposite film. Journal of Applied Polymer Science, 138, 50672.
42. Musso, Y.S., Salgado, P.R., Mauri, A.N. (2016). Gelatin based films capable of modifying its color against environmental pH changes. Food Hydrocolloids, 61, 523–530.
43. Ngo, T. M. P., Nguyen, T. H., Dang, T. M. Q., Tran, T. X., & Rachtanapun, P. (2020). Characteristics and antimicrobial properties of active edible films based on pectin and nanochitosan. International Journal of Molecular Sciences, 21(6), 2224.
44. Ozdemir, M., Floros, J.D. (2004). Active Food Packaging Technologies. Critical Reviews in Food Science and Nutrition, 44, 185-193.
45. Piñeros-Hernandez, D., Medina-Jaramillo, C., López-Córdoba, A., Goyanes, S. (2017). Edible cassava starch films carrying rosemary antioxidant extracts for potential use as active food packaging. Food Hydrocolloids, 63, 488–495.
46. 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.
47. Prietto, L., Pinto, V. Z., El Halal, S. L. M., de Morais, M. G., Costa, J. A. V., Lim, L. T., Dias, A. R. G., & Zavareze, E. D. R. (2018). Ultrafine fibers of zein and anthocyanins as natural pH indicator. Journal of the Science of Food and Agriculture, 98, 2735-2741.
48. Ramos, M., Beltran, A., Fortunati, E., Peltzer, M. A., Cristofaro, F., Visai, L., Valente, A. J. M., Jiménez, A., Kenny, J. M., & Garrigós, M. C. (2020). Controlled release of thymol from poly(lactic acid)-based silver nanocomposite films with antibacterial and antioxidant activity. Antioxidants, 9(5), 395.
49. Realini, C.E., Marcos, B. (2014). Active and intelligent packaging systems for a modern society. Meat Science, 98, 404–419.
50. Riaz, A., Lei, S., Akhtar, H. M. S., Wan, P., Chen, D., Jabbar, S., Abid, M., Hashim, M. M., & Zeng, X. (2018). Preparation and characterization of chitosan-based antimicrobial active food packaging film incorporated with apple peel polyphenols. International Journal of Biological Macromolecules, 114, 547-555.
51. Rodriguez-Amaya, D.B., Mérillon, J., Ramawat, K. (2019). Natural food pigments and colorants. In Bioactive Molecules in Food, Reference Series in Phytochemistry; Springer: Cham, Switzerland.

52. 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.
53. Sani, M.A.; Azizi-Lalabadi, M.; Tavassoli, M.; Mohammadi, K.; McClements, D.J.(2021). Recent Advances in the Development of Smart and Active Biodegradable Packaging Materials. Nanomaterials, 11, 1331
54. Sanhawong, W., Banhalee, P., Boonsang, S., Kaewpirom, S. (2017). Effect of concentrated natural rubber latex on the properties and degradation behavior of cotton-fiber-reinforced cassava starch biofoam. Industrial Crops and Products, 108, 756-766.
55. Shahrampour, D., Khomeiri, M., Razavi, S. M. A., & Kashiri, M. (2020). Development and characterization of alginate/pectin edible films containing Lactobacillus plantarum KMC 45. LWT, 118, 108758.
56. Vo, T. V., Dang, T. H., & Chen, B. H. (2019). Synthesis of Intelligent pH Indicative Films from Chitosan/Poly (vinyl alcohol)/Anthocyanin Extracted from Red Cabbage. Polymers, 11, 1088.
57. Wang, Z.-C., Yan, Y., Nisar, T., Sun, L., Su, P., Chen, D.-W., & Guo, Y. (2018). Influence of postmortem treatment with nitric oxide on the muscle color and color stability of tilapia (Oreochromis niloticus) fillets. Nitric Oxide, 76, 122-128.
58. Xie, Q., Zheng, X., Li, L., Ma, L., Zhao, Q., Chang, S., & You, L. (2021). Effect of curcumin addition on the properties of biodegradable pectin/chitosan films. Molecules, 26, 2152.
59. 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.
60. 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.
61. Yun, D., Cai, H., Liu, Y., Xiao, L., Song, J., & Liu, J. (2019). Development of active and intelligent films based on cassava starch and Chinese bayberry (Myrica rubra Sieb. et Zucc.) anthocyanins. RSC Advances, 9(53), 30905–30916.
62. Zam, M., Niyumsut, I., Osako, K., & Rawdkuen, S. (2022). Fabrication and characterization of intelligent multi-layered biopolymer film incorporated with pH-sensitive red cabbage extract to indicate fish freshness. Polymers, 14, 4914.

63. 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, Volume 69, 308-317.
64. Zhang, S. Q., Bi, H. M., & Liu, C. J. (2016). Extraction of bioactive components from Rhodiola sachalinensis under ultrahigh hydrostatic pressure. Separation and Purification Technology, 57(2), 277-282.