République Algérienne Démocratique et Populaire
Université Abdelhamid Ibn Badis Mostaganem
Faculté Des Sciences de La Nature et de La Vie
Département de Biologie

Mémoire

Présenté pour l'obtention du diplôme de

MASTER EN SCIENCE BIOLOGIQUE

Spécialité : Pharmacotoxicologie

Par

AMURI SHERALY ZUBERI
&
MERABET NOUR ELYAKINE

Thème :

Développement d'un film alimentaire bioactif et intelligent à base d'amidon et de la curcumine

Soutenue publiquement le 04/07/2023 devant le jury composé de :

Président	Douichene Salima	MCA	Université de Mostaganem
Encadreur	Bakouri Hichem	MCB	Université de Mostaganem
Examinateur	Kribi Soraya	MCB	Université de Mostaganem
Co-encadreur	/	/	/

Année Universitaire : 2022/2023

DEMOCRATIC AND POPULAR REPUBLIC
OF ALGERIA
University of Abdelhamid Ibn Badis Mostaganem
Faculty of Natural science and life
Biology Department

Master Thesis

Presented to obtain the diplomas

MASTER IN BIOLOGIC SCIENCE

Specialty: Pharmacotoxicology

By

AMURI SHERALY ZUBERI
&
MERABET NOUR ELYAKINE

Theme:

Development of a Bioactive and Intelligent Food Film based on Starch and Curcumin

---

Publicly defended on 04th July 2023 in front of the jury:

President	Douichene Salima	MCA	Universiy of Mostaganem
Supervisor	Bakouri Hichem	MCB	Universiy of Mostaganem
Examinator	Kribi Soraya	MCB	Universiy of Mostaganem
Co-Supervisor	/	/	/

Academic Year 2022 - 2023

Acknowledgements

First and foremost, we are extremely grateful to our supervisor **Mr. Hichem BAKOURI** for his invaluable advice, continuous support, and patience during this research project. His immense knowledge and plentiful experience have encouraged us in all the time of our academic research and daily life.

We express our heartfelt gratitude to the board of examiners **Ms. Salima Douichene** the president of our jury and **Ms. Soraya Kribi** our examinator.

We are grateful to our parents for their understanding, encouragement, and support, which uplifted our spirits and enabled us to achieve our goals.

Lastly, we extend our regards and blessings to all those who supported us in any way during the completion of this endeavor.

Dedication

MERABET NOUR EL YAKINE

In loving memory of my dear aunt, Halima, whose beautiful soul we lost during the preparation of this work,
I would like to express my heartfelt gratitude to my beloved parents, who have always been my guiding lights and have made countless sacrifices for my sake,
To my cherished sisters and brother, your unwavering love and support have been invaluable throughout this journey.

AMURI SHERALY ZUBERI

I dedicate this work to my family and many friends. A special feeling of gratitude to my loving parents, my father Zuberi Amuri and my mother Hasina Ndunguru their words of encouragement and push for tenacity ring in my ears. My sister Maimuna, Zuhura and my brother-in-law John Mndala for their endless and tiedless support during my study.

Abstract

Active and intelligent films with antioxidant, antimicrobial and pH–responsive properties were developed by adding curcumin (Cur) and/or polyvinyl alcohol (PVA) into corn starch (CS) matrix. Functional properties of CS, CS/PVA, CS–Cur, and CS/PVA–Cur films were compared. Results showed the addition of Cur effectively changed the films colors when they are exposed to different pH mediums. By comparing different films, CS–Cur and CS/PVA–Cur films presented the stronger microbial growth inhibition as well as the potent free radical scavenging ability than CS and CS/PVA films. Notably, CS/PVA–Cur film packaging effectively demonstrated the monitoring of veal freshness and could be used as novel multifunctional packaging food industry.

Key words: Active packaging; Intelligent packaging; Starch; Polyvinyl alcohol; Curcumin

ملخص

تم تطوير أفلام نشطة وذكية ذات خصائص مضادة للأكسدة ومضادة للميكروبات ومتجاوبة مع قيمة
تمت .(CS) في مصفوفة نشا الذرة (PVA) و/أو كحول البولي فينيل (Cur) الحموضة بإضافة الكركمين
.CS/PVA–Cur ، وCS–Cur، CS/PVA، CS مقارنة التراكيب المجهرية والخصائص الوظيفية لأفلام
ينتج عنه تركيب مجهري CS في الفيلم القائم على PVA و Cur أظهرت النتائج أن الإضافة المتزامنة لـ
مختلفة. pH داخلي متماسك. وبالإضافة إلى ذلك، تغيرت الأفلام بشكل فعال عند تعرضها لمحاليل
قدرة أكبر على تثبيط نمو CS/PVA–Cur و CS–Cur بالمقارنة بين الأفلام المختلفة، أظهرت أفلام
يُذكر أن تغليف .CS/PVA و CS الميكروبات وقدرة قوية على التخلص من الجذور الحرة مقارنةً بأفلام
يمكنه مراقبة طازجية لحم العجل بشكل فعال ويمكن استخدامه كتغليف متعدد CS/PVA–Cur فيلم
.الوظائف جديد في صناعة الأغذية

الكلمات الرئيسية: التغليف النشط؛ التغليف الذكي؛ النشا؛ كحول البولي فينيل؛ الكركمين

Résumé

Des films actifs et intelligents dotés de propriétés antioxydantes, antimicrobiennes et réactives au pH ont été développés en ajoutant de la curcumine (Cur) et/ou de l'alcool polyvinylique (PVA) dans une matrice d'amidon de maïs (CS). Les microstructures et les propriétés fonctionnelles des films CS, CS/PVA, CS–Cur et CS/PVA–Cur ont été comparées. Les résultats ont montré que l'ajout simultané de Cur et de PVA dans le film à base de CS produisait une microstructure interne compacte. Par ailleurs, l'ajout de Cur modifiait efficacement les couleurs des films lorsqu'ils étaient exposés à différents milieux pH. En comparant les différents films, les films CS–Cur et CS/PVA–Cur présentaient une inhibition plus forte de la croissance microbienne ainsi qu'une capacité puissante à éliminer les radicaux libres par rapport aux films CS et CS/PVA. Notamment, l'emballage du film CS/PVA–Cur démontrait efficacement le suivi de la fraîcheur du veau et pourrait être utilisé comme nouvel emballage multifonctionnel dans l'industrie alimentaire.

Mots-clés : Emballage actif ; Emballage intelligent ; Amidon ; Alcool polyvinylique ; Curcumine

لا ترفض الافكار الجيده بحجة الانجاز
فهناك أفكار تزيد الإنتاجيه

Contents

List of Tables

List of Figures

List of Abbreviations

CS	Corn starch
Cur	Curcumin
PVA	Polyvinyl alcohol
PCL	polycaprolactone
PBSA	polybutyl succinic acid-butyl adipate
PBAT	polyadipate butylene terephthalate
SEM	Scanning electron microscope
DPPH	2,2-diphenyl-1-picrylhydrazyl
W/V	Weight/volume
V/V	Volume/volume
CFU	Colony Forming Unit

General Introduction

Synthetic plastics are extremely versatile and convenient materials that can be used to create a diversity of useful products, but their widespread use is causing increasing damage to the planet. One of the most effective strategies to reduce the problem of plastic pollution is to develop biodegradable materials that can replace it. At present, research on biodegradable materials mainly focuses on synthetic and natural polymers. Many synthetic polymers, including polylactic acid (PLA), polyvinyl alcohol (PVA), polycaprolactone (PCL), polybutyl succinic acid-butyl adipate (PBSA) and polyadipate butylene terephthalate (PBAT), are widely used biodegradable materials in daily life. In addition, a number of natural polymers are being used as raw materials for constructing biodegradable materials, such as proteins (whey protein, soy protein, silk fibroin), polysaccharides (chitosan, cellulose, starch), and lipids (beeswax, lauric acid). Among the natural biodegradable materials, starch-based biodegradable materials are widely studied and applied. This is because starch is naturally abundant, low cost, non-toxic, renewable, biocompatibility and can be used as a foodstuff packaging film. However, a number of technical challenges need to be overcome to increase the practical of starch-based films for manner applications.

Functional starch-based films have attracted extensive attention from researchers in recent years. Bioactive starch-based films with antibacterial, anti-oxidation, UV, oxygen, and water vapor barriers can reduce the reproduction of microorganisms and prolong the shelf life of packaged foods. Intelligent starch-based films with pH–, temperature–, magnetic field–, glucose–, and enzyme–responsive characteristics can be used to monitor the freshness of foods and control the delivery of functional ingredients and drugs. Therefore, the development of biodegradable bioactive and intelligent starch-based films is particularly important [1].

The addition of plant extracts, plant essential oils, and nanoparticles to starch films can improve their antibacterial, antioxidant, and barrier properties. The addition of anthocyanins to starch films can make the films pH–responsive, indicating the degree of spoilage in fish products and achieving the purpose of warning consumers. In the future, it is necessary to study other biologically active starch-based films, such as anti-fatigue, enzyme–responsive, and salt–responsive starch-based films.

Considering the pH sensitivity and antioxidant activity, as well as the antimicrobial activity of curcumin, novel bioactive and intelligent packaging films were prepared in the present work by adding turmeric extract (Cur) and/or polyvinyl alcohol (PVA) into corn starch (CS) matrix. We compared the structural and functional properties of CS, CS/PVA, CS–Cur, and CS/PVA–Cur films for the first time. Meanwhile, the developed film was evaluated to monitor veal freshness.

CHAPTER 01

LITERATURE REVIEW

1.1. Understanding Starch Structure

Starch constitutes a major energy supply for humans worldwide and is produced as a reserve carbohydrate in plants. The most important sources for humans are diverse cereals, rhizomes, roots and tubers. Storage starch is produced in amyloplasts as discrete granules with distinct morphology in different plants (Fig. 1.1), ranging from round, oval, ogival or elongated to flat, lenticular or polyhedral, and sizes from sub-microns to more than 100 µm in diameter [1]. Though the size and morphology of the granules is specific for each plant species, their internal structures have remarkably similar architecture.

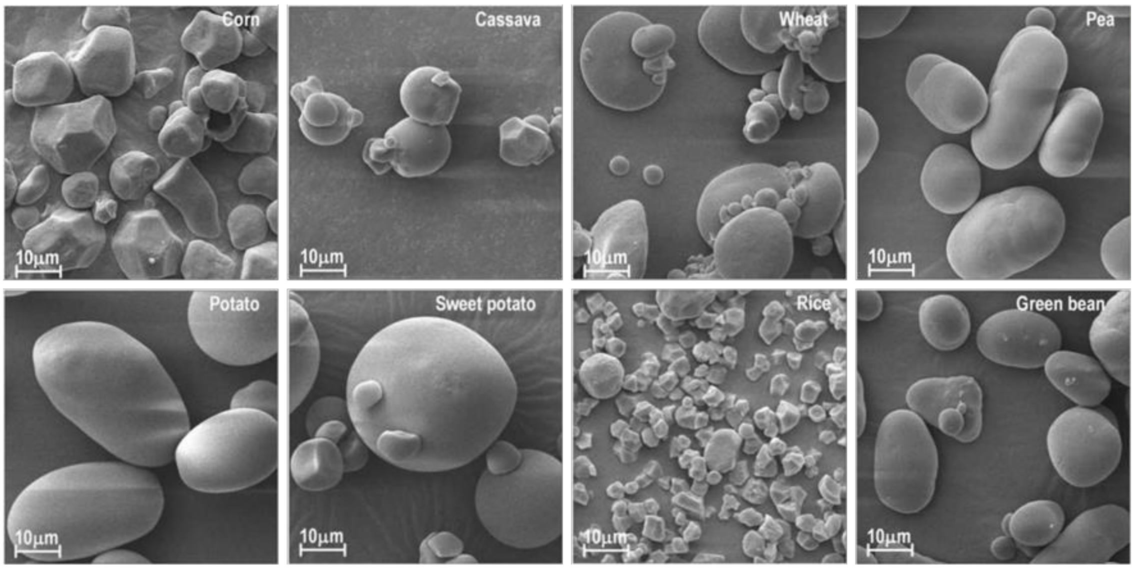


Figure 1. 1. SEM images of starch granules from different botanical sources [1].

Starch is composed of amylose (primarily linear molecule) and amylopectin (highly-branched molecule). The molecular structure of amylose is comparatively simple as it consists of glucose residues connected through α–(1,4)–linkages to long chains with a few α–(1,6)–branches (Fig. 1.2). Amylopectin, which is the major component, has the same basic structure, but it has considerably shorter chains and a lot of α–(1,6)–branches [2].

Starch granules are insoluble in cold water, but if they are heated above a certain temperature in excess of water, they would absorb water and expand, eventually leading to the disruption of the starch granules and the formation of a hydrophilic colloidal solution [3]. This process is known as the gelatinization of starch. After gelatinization, when a starch solution is cooled to a sufficiently low temperature, starch molecules reorganize, hydrogen bonds reform, and ordered structures are reestablished [4]. This process is known as retrogradation of starch.

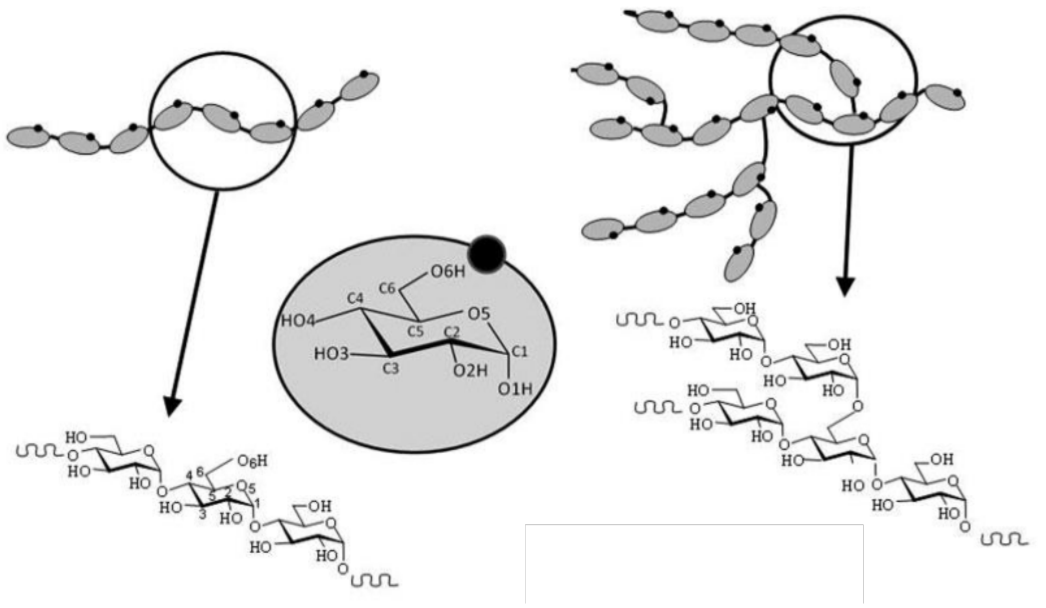


Figure 1. 2. Basic structural motifs of amylose and amylopectin.

1.2. Preparation of Starch–Based Films

The formation of starch films mainly depends on the retrogradation characteristics of starch. After starch granules are gelatinized, cast in a mold, and dried, the hydroxyl groups between the starches are combined through intermolecular hydrogen bonds to form a starch film. There are three main methods for preparing starch-based films: casting, extrusion/thermo-compression method, and blow molding method (Fig. 1.3).

1.2.1. Casting Method

The casting method is commonly used in laboratories due to limited throughput and laboratory space [5]. There are three steps in preparing starch-based film by casting. First, starch slurry is gelatinized while adding a plasticizer, and then starch paste is poured into a mold. The plasticizer is mainly glycerin to give the films flexibility. The last step is drying the paste to obtain a starch-based film. The film is balanced in a desiccator for several days.

Starch-based films are prepared by casting method with different sources of starch as film-forming materials. In the solution casting method, the concentration of starch is generally 5%–10%. Starch paste with the concentration lower than 5% is difficult to form film after drying. If the starch concentration is higher than 10%, the starch paste shows poor fluidity and is not conducive to pouring [6].

The advantage of using the casting method is that the film has high transparency and good flexibility. However, it is also characterized by high energy consumption and low production efficiency, which limits the application of starch-based films.

1.2.2. Extrusion/Thermo–Compression Method

Currently, extrusion/thermo-compression is the most commonly used method for producing plastic materials due to its fast temperature rise and simple operation. To prepare starch-based films by extrusion/ hot pressing, starch and plasticizer are mixed uniformly, extruded, and then thermoformed to form the films [7].

Extrusion can reach the required temperature in a short time, and the operation is simple. However, the operating conditions, such as temperature curve and screw speed, will seriously affect the performance of the material. Therefore, these conditions need to be optimized to make a good starch film. Moreover, this method requires that the polymers or added bioactive substance be able to withstand high temperatures.

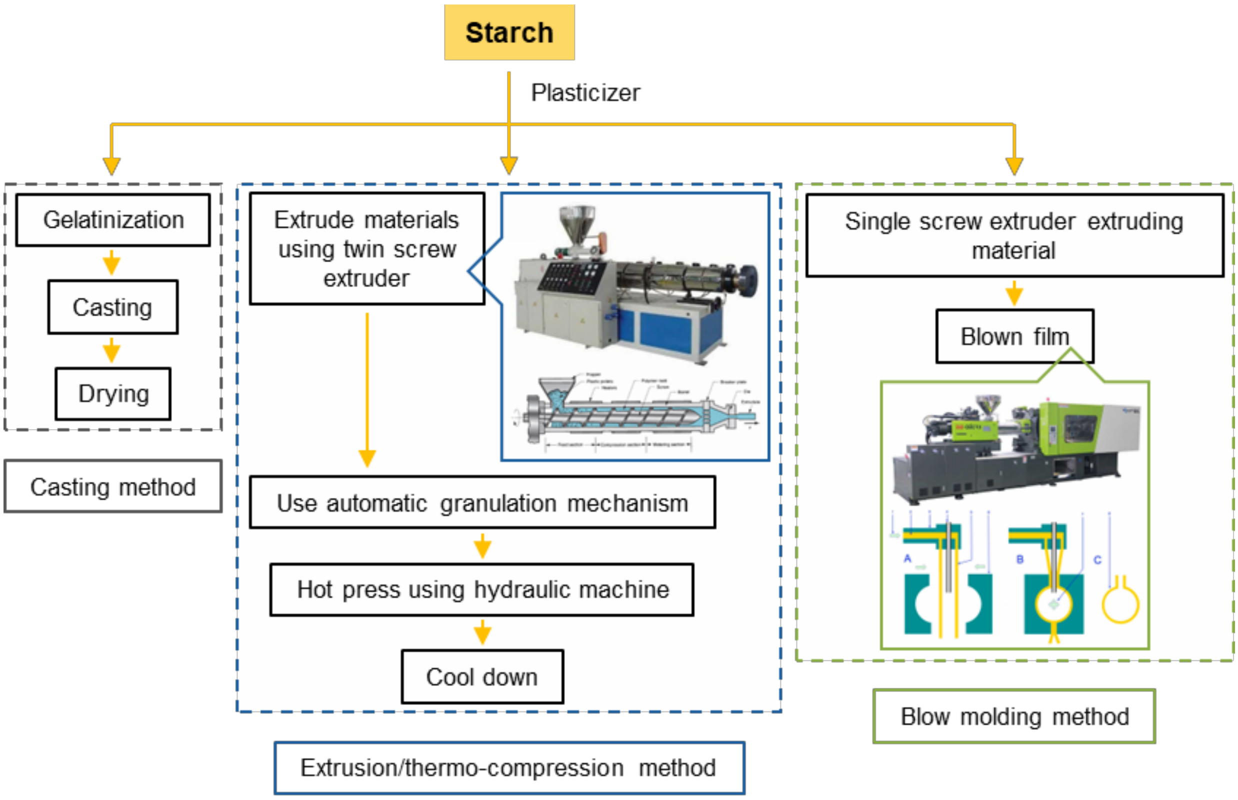


Figure 1. 3. Flow chart of three preparation methods of starch-based films.

1.2.3. Blow Molding Method

The blow molding method requires the use of a twin-screw extruder to pelletize the starch and additives and then a single-screw blown-film extruder to prepare a starch-based film.

The extrusion blow molding process is mainly used to produce food plastic packaging materials. In recent years, the use of the extrusion blow molding to prepare starch-based films has received widespread attention. To obtain starch-based films, plasticizers and thermoplastic biopolymers must be added. Garcia et al. [8] used sericin as a compatibilizer for starch and poly (butylene–adipate–co–terephthalate) to prepare films. First, the components are mixed uniformly, and pellets are obtained through a laboratory twin-screw extruder, and then the pellets are passed through a mono-screw extruder to obtain blown films.

Starch-based films prepared by the extrusion blow molding method have good mechanical strength and show less breakage, but the transparency of the film is low, which limits its application as a transparent food packaging material.

1.3. Functions of Starch–Based Films

1.3.1. Antibacterial Activity

There are many natural active substances in nature that have antibacterial effects, and they are widely used as antibacterial agents. Research has shown that starch films themselves exhibit no antibacterial activity [9]. However, starch films prepared by adding antibacterial substances to a starch matrix can be used as an active antibacterial packaging material, which can effectively inhibit the proliferation of bacteria, prolong the shelf life of food, and reduce the occurrence of bacterial diseases. The following sections describe the bacteriostatic properties of starch-based films. Table 1.1 summarizes the inhibition zones of antibacterial starch-based films on different bacteria.

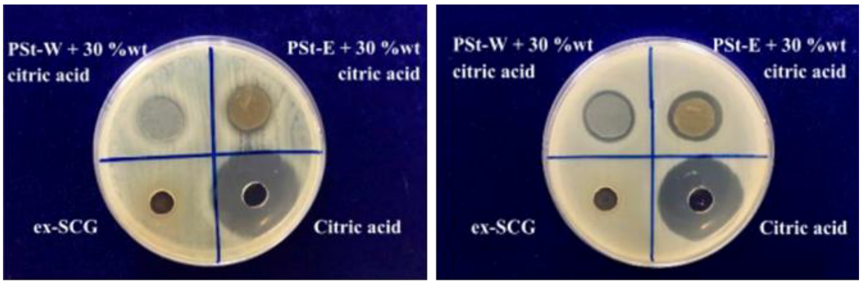
Table 1. 1. Diameters of inhibition zone of different starch-based films.

Types of starch	Antibacterial substance	The diameter of inhibitory zone (mm)			Ref
		S. aureus	*E. coli*	*L. monocytogenes*	
Starch/gelatin	Tea tree oil	27.8 ± 0.29	18.4 ± 0.20	ND	[10]
Cassava starch	Cinnamon oil	10.75 ± 0.50	15.25 ± 1.85	ND	[11]
Foxtail starch	Clove leaf oil	ND	ND	14.79 ± 0.10	[12]
Potato starch	Coconut oil	25.17 ± 0.017	24.11 ± 0.016	25.20 ± 0.016	[13]
Rice starch	Propolis extract	8.2 ± 1.2	ND	ND	[14]
Potato starch	Nano–SiO_2	6.2 ± 0.07	8.0 ± 0.08	ND	[15]

ND: No data were presented in the manuscript.

There are natural antibacterial substances in plant extracts, such as polyphenols, flavonoids, tannins, and alkaloids. In general, these substances have antibacterial effects and can damage cell membranes, interfere with active transport, and inhibit enzyme activity.

Moreover, adding the by-products produced in the food production process to the starch-based film can also have antibacterial effects. PVA/starch film with the addition of waste coffee grounds and 30% citric acid has the largest inhibition zone against *E. coli* and *Staphylococcus aureus* (Fig. 1.4) [16]. The use of by-products from food processing as antibacterial substances is a trend in the preparation of active starch-based films. The addition of by-products could provide a low-cost way for antibacterial film preparation, while they are also environment-friendly due to the recycle of industry waste.



Escherichia coli *Staphylococcus aureus*

Figure 1. 4. Antibacterial activity of film samples [16].

Many studies have added essential oils to starch-based films to improve their antibacterial and antioxidant capabilities. Plant essential oils, as natural active antibacterial substances, are of great significance when used in active food packaging materials. In general, among essential oils, phenolic substances are the main antibacterial substances, including carvacrol and thymol.

Additionally, because nano–silica (nano–SiO_2), nano titanium dioxide, and nano–clay have excellent properties, such as an extremely porous structure and high surface activity, they are added to starch-based films as bacteriostatic agents [15, 17]. In general, nano–SiO_2 is chemically inert and biocompatible, which can prevent bacteria from proliferating. Furthermore, nano-SiO_2 has an inhibitory effect on bacteria because it can be adsorbed on the cell walls of bacteria, further destroying the structure of the cytomembrane and organelles and achieving bacteriostatic effects [18].

1.3.2. Antioxidant Activity

Antioxidant activity is also an important function of starch-based films as food packaging materials. Adding antioxidant ingredients to starch-based films gives the starch-based film anti-oxidation properties to extend the shelf life of packaged foods. Table 1.2 summarizes the free radical removal capabilities of several starch-based films. The following describes the antioxidant effect of starch-based films.

Essential oils are known to have antioxidant effects and have been widely used in starch-based films to enhance their antioxidant properties. Antioxidant active packaging appears to be a promising application of essential oils as natural antioxidants. That’s because synthetic antioxidants are potentially harmful to human health. Essential oil molecules can react with colored free radicals ($DPPH^{\bullet}$) to counteract the oxidative stress caused by free radicals. In starch-based film packaging materials, essential oil vapor can diffuse in the internal atmosphere, directly contact with food, and produce antioxidant protection [19].

Table 1. 2. Antioxidant properties of starch-based films containing different antioxidants.

Types of starch	Antioxidant ingredients	Antioxidant capacity	Ref
Potato starch	Tea tree oil	The total antioxidant capacity is 985.7 ± 84.3 nmol/cm^2.	[10]
Potato starch	Lavender oil	The total antioxidant activity is improved by 88.97%, as compared with the control film.	[20]
Foxtail starch	Clove leaf oil	The DPPH free radical scavenging activities are 40.81%.	[12]
Apple starch	Ellagic acid	The oxidation resistance increased from 200 to 2200 μmol TEAC/g dry film.	[21]
Rice starch	Curcumin	The DPPH antioxidant activity is 85.60%.	[22]
Hydroxypropyl starch	Tea polyphenols	Gallic acid equivalent is increased from 0 to 15 mg/g film.	[9]
Adzuki bean starch	Cocoa bean extract	The DPPH radical scavenging activities reach 94.9%.	[23]

Due to the health benefits of polyphenols, including antioxidants, anti-inflammatory, and anti-atherosclerotic effects, there is growing interest in adding them to starch-based films. In addition to the widely used essential oils and polyphenol antioxidants, carotenoids and ascorbic acid can also be used as antioxidants in starch-based films.

1.4.Intelligent Response Starch–Based Films

1.4.1. pH–Responsive Starch–Based Films

In recent years, intelligent packaging materials have received increasing attention. Intelligent pH–responsive starch films as packaging materials can monitor the status of perishable foods and remind consumers to pay attention to pathogen contamination. In intelligent food packaging materials, visual sensors are mainly pH indicators. As the pH of foods change, intelligent pH–responsive starch films show different colors to indicate the deterioration of the foods. Currently, the research on pH–sensitive starch-based intelligent packaging films is still in the early stages, mainly using the pH–sensitive properties of anthocyanins in natural fillers or adding acid–base indicators to starch-based films to achieve pH response. In this context, natural anthocyanins are safe, non-toxic, water-soluble pigments that are sensitive to changes in pH. In the process of fruit production, a substantial amount of waste residues rich in anthocyanins are often produced, such as grape waste residue in wine production and blueberry waste residue in blueberry juice production. These waste residues can be reprocessed and utilized. For example, anthocyanins extracted from waste residues can be used as pH indicators. Table 1.3 summarizes the color changes and applications of pH–responsive starch-based films at different pH values.

Table 1. 3. Color changes and applications of different types of pH–responsive starch-based films.

Types of starch/films	Additives	Color changes	Application	Ref
Guinea pueraria lobata starch	Grape waste powder	At pH = 1, pink hue; At pH = 7, blue.	ND	[24]
Potato starch	Purple sweet potato anthocyanin	Red turns green.	Monitored meat spoilage	[25]
Starch/polyvinyl alcohol	Red dragon fruit peel extract	Pink turns yellow.	Monitored the shrimp deterioration process	[26]
rice starch-based	Blueberry agro-industrial waste	Red at acidic pH and blue at alkaline pH.	Specific sensors and/or indicators	[27]
Sodium carboxymethyl starch	Mulberry anthocyanin extract	Red becomes dark blue.	Indicated the freshness of the fish	[28]
Potato starch	Butterfly pea flower	Light pink to green.	Monitored the freshness of shrimp and seafood samples	[29]

Choi et al. [25] used anthocyanin extract from purple sweet potato and agar/potato starch to prepare an intelligent pH indicator film to monitor meat spoilage (Fig. 1.6). As the pH of pork samples changes, the pH indicator film changes from red to green. Moreover, Liu et al. [30] prepared an intelligent starch/PVA film containing anthocyanins and limonene (Fig. 1.7).

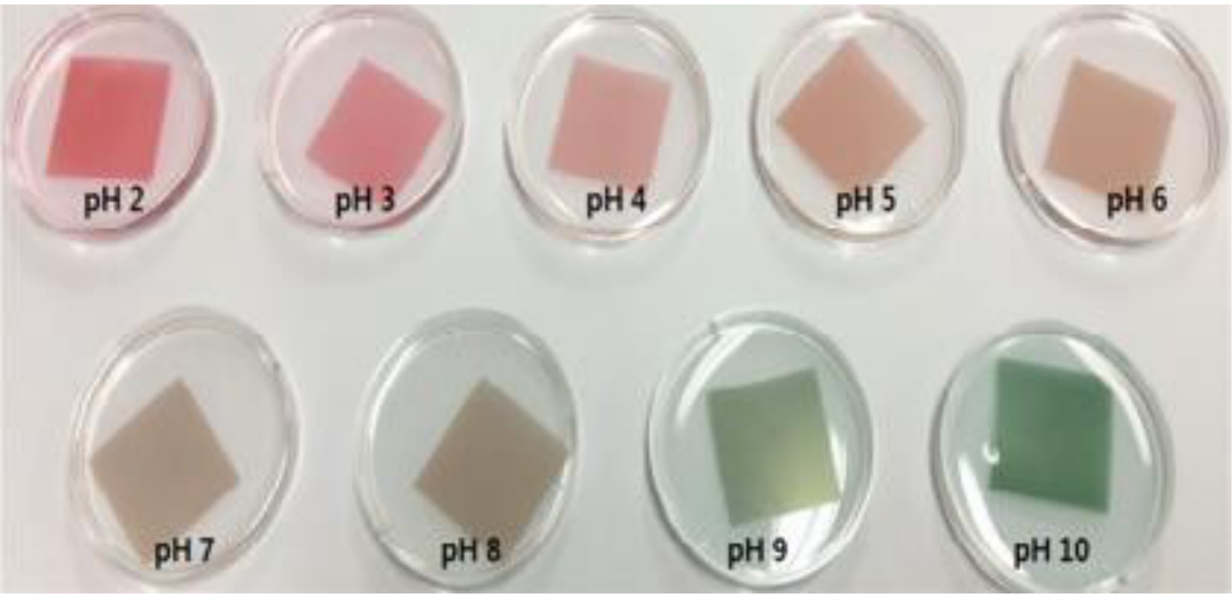


Figure 1. 5. pH indicator of agar/potato starch film with purple sweet potato extract [25].

As expected, the film showed good color indication and antibacterial activity for pasteurized milk, indicating that the film has a good ability to signal food deterioration. In general, fish and shrimp are extremely perishable. The enzymatic hydrolysis of trimethylamine oxide by putrefaction organisms increases volatile nitrogen compounds in fish. These compounds are alkaline gases that increase the pH of fish samples. Therefore, consumers can monitor the freshness of fish by the color change on the packaging [31].

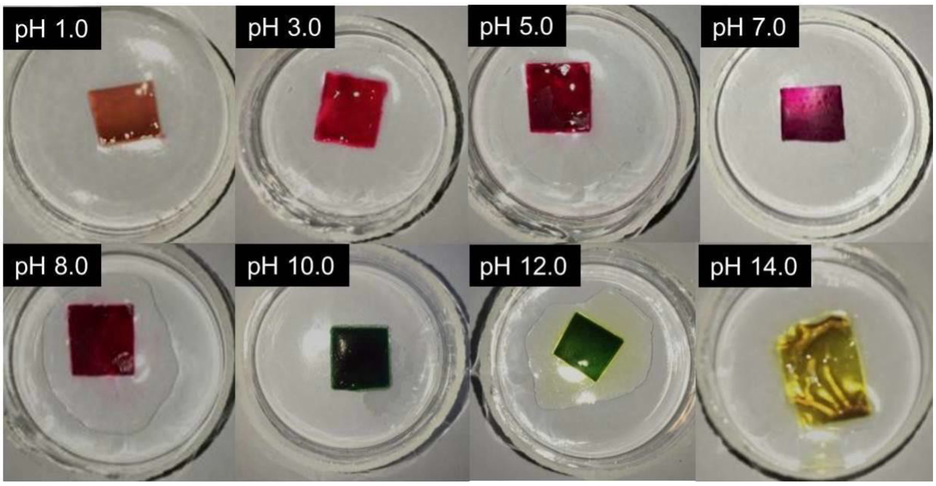


Figure 1. 6. Colorimetric response of the STA–PVA–ANT–LIM film at different pH conditions [30].

1.4.2. Temperature–Responsive Films

Poly(N–isopropylacrylamide) (PNIPAAm) is one of the most popular heat–sensitive polymers. It has a lower critical solution temperature when it is close to human body temperature, so it is suitable for use in temperature–responsive starch hydrogels for drug delivery. Poorgholy et al. [32] used itaconic anhydride modified starch, iron oxide nanoparticles (Fe_3O_4 NPs), and N–isopropylacrylamide to prepare temperature–responsive magnetic nanohydrogels. When the temperature increased from 25 °C to 40 °C, the diameter of the magnetic nanohydrogels decreased from 79 nm to 67 nm, which was mainly due to the change in the hydrophilic–hydrophobic properties of the thermos–responsive polymer.

1.6. Application of Starch–Based Films

1.6.1. Application in Food Packaging

Bioactive starch-based films have broad application prospects as food packaging materials. Starch-based films are accessible, affordable, biodegradable, and environment-friendly material for food packaging. Through adding other compounds such as polyphenols, essential oils, and nanoparticles, the films can also show desirable antibacterial, antioxidation, ultraviolet barrier, water vapor barrier, and oxygen barrier properties. These characteristics are extremely beneficial to food preservation, especially perishable foods, such as fruits and vegetables, aquatic meat, and other foods. As shown in Table 4, the application of starch-based films in food preservation is summarized.

Table 1. 4. Application of starch-based films in food preservation.

Types of starch	Additives	Foods	Consequents	Ref
Corn starch	Corncob cellulose and cassia seed oil	Green grapes	Composite film decay index (11%) was significantly lower than that of the blank group (32%).	[33]
Pinhao starch	Feijoa peel flour, citric acid pectin	Apples	Maintained a constant weight after five days of storage.	[34]
Tapioca starch	Chitosan nanoparticles	Cherry tomatoes	Inhibited the growth of microorganisms compare to neat starch film.	[35]
Potato starch	Chitosan	Apples	Maintained the quality of apples for four weeks.	[36]
Cassava starch	Pumpkin extract residue and OEO	Ground beef	Prevented lipid oxidation when tested in meat until the third day of the test.	[37]
Rice starch	Glycerin and OEO	Fish fillets	Packaged fish fillets show less microbiological growth in 6 days of storage.	[36]
Corn starch	Lecithin and oleic acid	Sunflower oil	Prevented sunflower oil oxidation even after 53 days of storage at 30 °C.	[38]

1.6.2. Fruit Preservation

Fruits are highly perishable foods, and prolonging their shelf life is a significant challenge. Bioactive starch-based films with antibacterial and antioxidant properties are widely used in fruit preservation. In general, bananas are highly prone to blackening and spoilage. A starch- based film with lactic acid bacteria, sodium carboxymethyl cellulose, and glycerin demonstrated a good preservation effect on bananas, preventing them from browning and turning black [39]. Moreover, Francisco et al. [40] prepared acetylated tapioca starch and hydroxyethyl cellulose films as guava coatings, prolonging the shelf life of guava up to 13 days (fig 1.9).

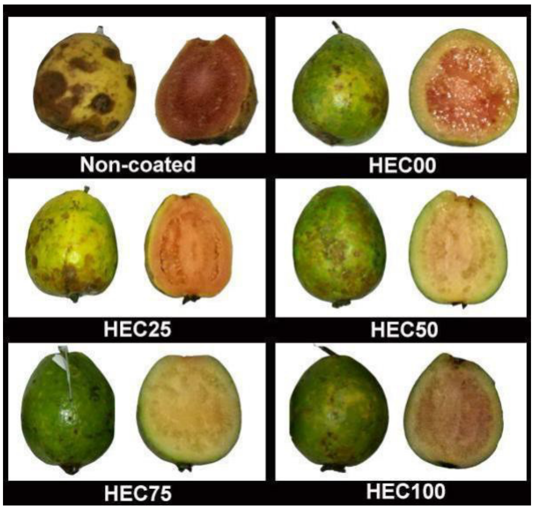


Figure 1. 7. Guava non-coated or coated with films of acetylated cassava starch (ACS) and hydroxyethyl cellulose (HEC) mixtures within 13 days [40].

1.6.3. Meat and Aquatic Products Preservation

As a packaging material, antimicrobial active starch-based film not only keeps food fresh but also monitors spoilage microorganisms. For instance, Anthocyanin pigment extracted from sweet potato and red cabbage was incorporated into corn starch/PVA blend to prepare an intelligent pH–sensitive starch-based film, which could be used as an intelligent pH indicator to monitor the freshness of shrimp and seafood samples [41]. During the storage of the shrimp food samples for 24 h, the color of the film gradually changed from pink to dark blue, clearly indicating the deterioration of the food material (Fig. 1.10).

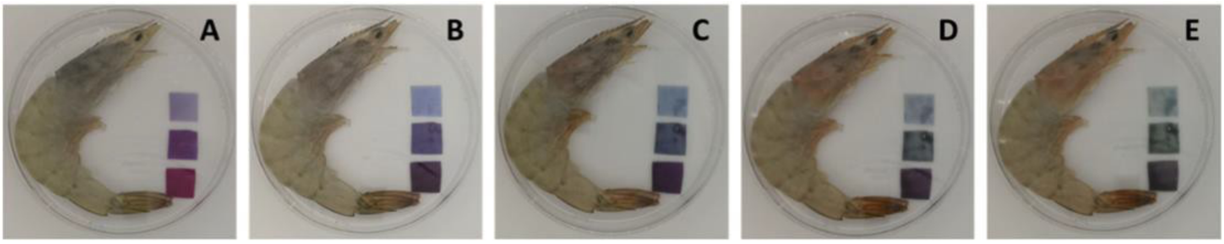


Figure 1. 8. Color changes of pH–sensitive films during shrimp spoilage at 25 °C for (A) 0 h, (B) 6 h, (C) 12 h, (D) 18 h, and (E) 24 h [41].

1.7. Curcumin as an Additive

Curcumin, a yellow polyphenolic pigment from the Curcuma longa L. (turmeric) rhizome, has been used for centuries for culinary and food coloring purposes, and as an ingredient for various medicinal preparations, widely used in Ayurveda and Chinese medicine. In recent decades, their biological activities have been extensively studied. Thus, this review aims to offer an in-depth discussion of curcumin applications for food and biotechnological industries, and on health promotion and disease prevention, with particular emphasis on its antioxidant, anti-inflammatory, neuroprotective, anticancer, hepatoprotective, and cardioprotective effects. Bioavailability, bioefficacy and safety features, side effects, and quality parameters of curcumin are also addressed. Finally, curcumin's multidimensional applications, food attractiveness optimization, agro-industrial procedures to offset its instability and low bioavailability, health concerns, and upcoming strategies for clinical application are also covered [45].

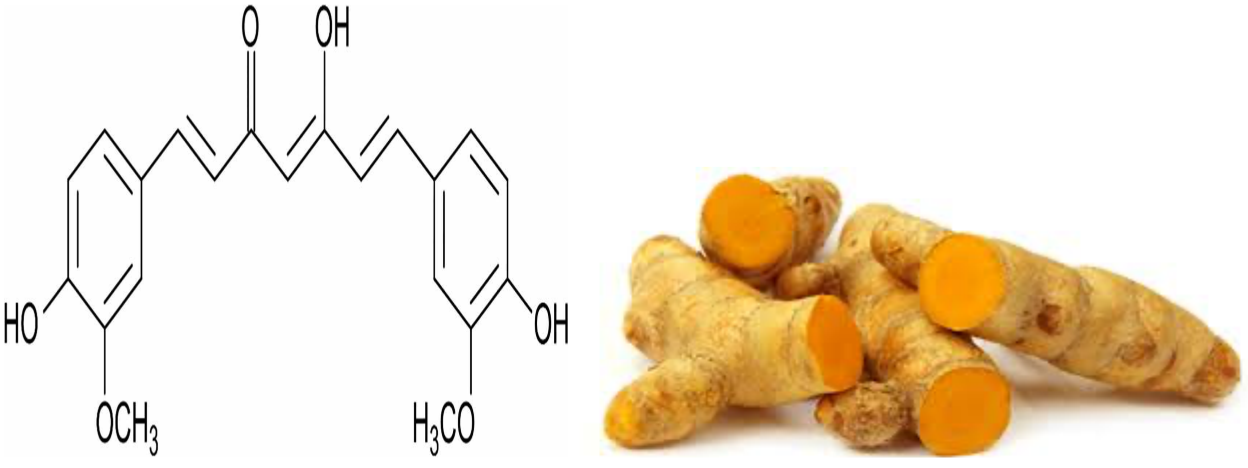

(a) (b)

Figure 1.9 Curcumin (a) Chemical structure of curcumin; (b) Turmeric rizhome.

CHAPTER 02

MATERIALS AND METHODS

2.1. Materials

Dried rhizomes of turmeric (*Curcuma longa* L.) and Fresh fish (*Sparus aurata*) were purchased from the local supermarket (Mostaganem, Algeria).

Two foodborne pathogenic species strains, bacteria (*Staphylococcus aureus*) and fungus (*Rhizoctonia solani*) for estimation of the antimicrobial activity of the films were kindly supplied by the Microbial Laboratory of Djillali Liabes University (Sidi Bel Abbes, Algeria).

2.2.Solvents and Reagents

All the materials including, solvents, chemicals, and reagents were purchased from Sigma–Aldrich (St. Louis, MO, USA), Merck (Darmstadt, Germany) Chemicals Company. All other chemical reagents were of analytical grade.

2.3.Extraction Procedures

The rhizomes of turmeric were dried in oven at 105 °C for 3 h. Dried rhizomes were triturated using mortar and screened through a sieve with mesh 80 to obtain uniform powder with particle size of 0.18 mm. The turmeric powder was stored in refrigerator to prevent moisture uptake. The Soxhlet extraction, as the reference method, was performed as follows: 15 g ground turmeric powder was weighed and embedded in a thimble and put in the Soxhlet apparatus which was gradually filled with acetone as the extraction solvent. The extraction experiment was carried out at 60 °C within 8 h. Upon completion of the extraction, the acetone was separated from the extract using rotary evaporator under vacuum at 35 °C and the residue (curcumin) was dried.

2.4.Development of CS, CS/PVA, CS–Cur, and CS/PVA–Cur Films

To prepare active packaging films, CS, PVA and Cur solutions were first separately prepared. Aqueous CS (2%, w/v) solution was prepared by dissolving 1.0 g of CS in 50 mL of distilled water 75 °C for 30 min until completely gelatinized. Meanwhile, PVA (3%, w/v) aqueous solution was prepared by dissolving 1.5 g PVA in 50 mL of distilled water at 90 °C for 2h until a clear solution was obtained, while Cur (0.25%, w/v) solution was prepared by dissolving 0.05 g of Cur in 20 mL of 80% ethanol aqueous solution (v/v) at room temperature. Subsequently, PVA and/or Cur solutions were added into CS solution, which was followed by addition of 30% (v/v) of glycerol. After being degassed, approximately 20 g of composite film forming solutions were cast onto a horizontal glass plate and dried in a thermostatic drier chamber at 30 °C and 50 % relative humidity (RH) for 2 days. Then, the films were peeled intact from the glass plates and conditioned in a tight container prior to further testing. CS film with PVA, Cur and both of them were termed as CS/PVA film, CS–Cur film and CS/PVA–Cur film, respectively. The resulting films are shown in Fig. 2.1.

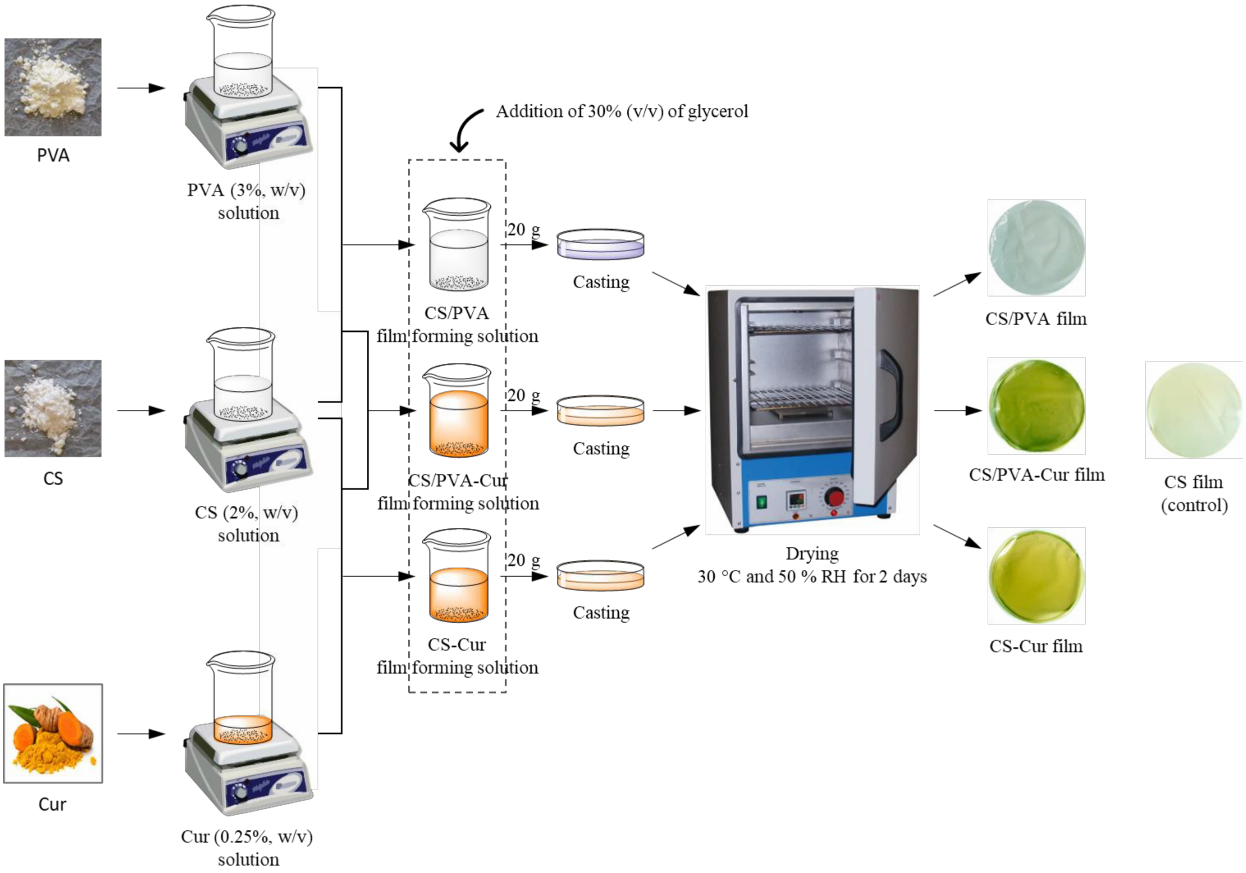


Figure 2. 1. Preparation routes of different films.

2.5.Functional Properties of the Films

2.6.1. Antioxidant Activity

The 2,2–diphenyl–1–picrylhydrazyl (DPPH) radical scavenging rate was used to evaluate the antioxidant activity of the developed CS, CS/PVA, CS–Cur, and CS/PVA–Cur films. A method reported by Kaya et al. [42] with some modifications was used for the study of DPPH scavenging activity. Briefly, 0.1 mmol/L DPPH solution was prepared by dissolving 4 mg of DPPH with 100 mL of methanol. Next, 20 mg of each film specimen was taken and dissolved in 4 mL of freshly prepared DPPH solution. The mixture was then incubated in the dark under ambient conditions for 24 h, and the absorbance of all the extracted solutions (reaction products) was recorded at the wavelength of 517 nm using UV–Vis spectrophotometer (Shimadzu UV–1800, Tokyo, Japan). The DPPH radical scavenging rate of both CS, CS/PVA, CS–Cur, and CS/PVA–Cur films was calculated using the formula:

$$\boldsymbol{DPPH\ radical\ scavenging\ rate}\ (\%) = \frac{\boldsymbol{A_{DPPH} - A_{EXTRACT}}}{\boldsymbol{A_{DPPH}}} \times \mathbf{100}$$

where A_{DPPH} and $A_{Extract}$ is the absorbance value of the methanolic solution of DPPH and sample extracts at 517 nm, respectively. Pure methanolic solution without DPPH was used as a blank. Each sample was assayed in triplicates.

2.6.2. Antimicrobial Activity

The study of antimicrobial activity of the films was performed by measuring the diameter of the inhibition zone, expressed in millimeters. The zone of inhibition was determined in triplicates using the diffusion technique, with values representing the average zone of inhibition. The nutrient broth medium (50 mL) was prepared and sterilized in autoclave at 121 °C for 15 min. Bacteria (*Staphylococcus aureus*) were inoculated in tubes of nutrient broth, whereas the fungal cultures (*Rhizoctonia solani*) were inoculated in tubes of potato dextrose agar, and incubated at 37 °C for 24 h; then the suspension was centrifuged at 8000× g for 5 min, the pellet was suspended in double–distilled water, and the cell density was standardized spectrophotometrically ($A_{610\ nm}$). All of the microbial cultures were adjusted to 1.5 McFarland standards, which is visually comparable to a microbial suspension of approximately 1.5×10^8 CFU/mL.

The bacterial specie, chosen as representative of spoilage and pathogenic specie commonly found contaminating surfaces and workplaces, was used to evaluate the antibacterial activities of the prepared films. The bacteria were maintained on Muller Hilton broth media at 37 °C. Then, Muller Hilton agar plates were uniformly seeded with 100 μL inocula of the test microorganism and kept for 15 min for absorption. Afterward, sterilized film samples (12 mm in diameter) were placed on the agar plate's surface. Then the plate was incubated at 37 °C for 24 h.

2.6.3. pH–Sensitivity

pH–sensitivity of CS/PVA–Cur film was monitored by immersing the film sample in different buffer solutions (pH 3.0 to 10.0) at room temperature for 5 min. The color changes of the film were recorded using a digital camera.

2.6.Potential Application

CS/PVA–Cur film was selected to monitor veal freshness. Fresh meat samples with a weight of 50 g were placed in a sterilized Petri dishes (15 cm in diameter) and covered with a piece of CS/PVA–Cur film (11 cm in diameter, CS film as control). Subsequently, the dishes were sealed with polyethylene film and stored at 25 °C for 4 days. The color changes of the films were performed daily until the end of storage.

CHAPTER 03
RESULTS AND DISCUSSION

3.1. Analysis of Curcumin

- The obtained product is having yellow color.
- It is insoluble with water.
- 2 ml of curcumin solution turns red when 2 ml of sulfuric acid is added and stirred.
- A piece of filter paper is wetted with curcumin solution and dried. A few drops (2 to 3 drops) of hydrochloric acid, followed by a few drops (2 to 3 drops) of boric acid solution are dropped onto the piece of filter paper. Upon drying by heating, it turns cherry red. When a few drops (2 to 3 drops) of ammonia solution is added, it turns blue.

The above Results show that the product obtained is curcumin.

3.2. Antioxidant Activity

Antioxidant packaging films has received special attention since they can reduce lipid oxidation and enhance the shelf life of food by releasing the antioxidant agent into the food package [43]. Hence, it was essential to evaluate the antioxidant activity of developed films by DPPH free radical scavenging assay. The hydrogen donating potential of the antioxidant decides the degree of the color change of DPPH radical into a yellow–colored DPPH compound. As shown in Fig. 3.1, an intense color change can be seen from purple to yellow on incorporating the Cur. H–atoms donation from the phenolic groups in curcumin have been considered strong antioxidants due to their capacity to capture and react with free radicals to yield highly stable macromolecular radicals. This was evident qualitatively that developed films possessed a strong antioxidant activity. DPPH radical scavenging rate of the different films is illustrated in Fig. 3.1. Our results confirmed that CS/PVA–Cur offers the highest antioxidant property with 81.51% DPPH scavenging activity. The scavenging activity of CS–Cur was found to be 81.45% which does not change significantly compared to CS/PVA–Cur. CS film exhibited no scavenging activity as there was no antioxidant agent present in the film. Moreover, no significant change was observed in the DPPH scavenging activity of CS film when PVA was added, further confirming PVA did not possess antioxidant activity. Thus, the Cur used in the current study for the development of CS and CS/PVA films proved to be an effective antioxidant agent.

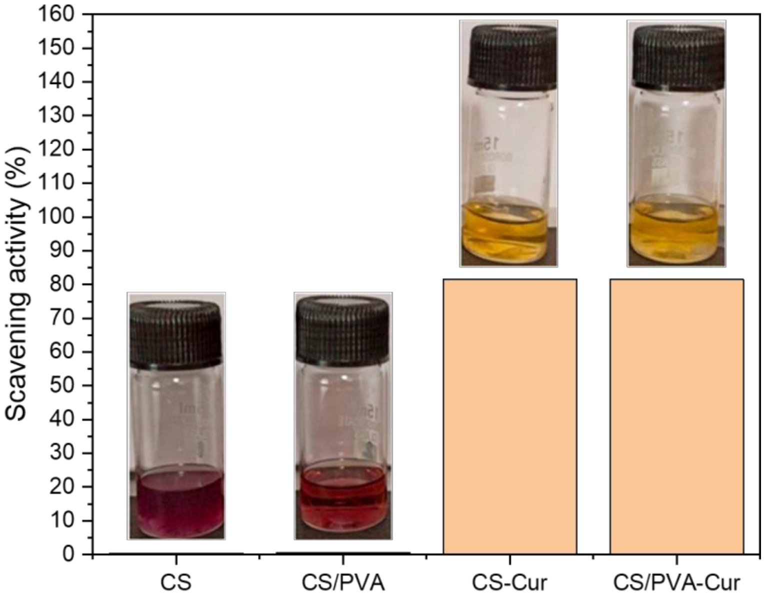


Figure 3. 1. Color change of DPPH radical scavenging activity of different films.

3.3.Antimicrobial Activity

Microbial pathogens are responsible for a large portion of food spoilage and food–related diseases. Hence, active antimicrobial packaging has become one of the most studied food packaging science topics [44]. *S. aureus*, a classic food poisoning microorganism, was selected as a model bacteria. The antimicrobial activities of different films are shown in Fig. 3.2 and Table 3.1. As shown in Fig. 3.2, CS and CS/PVA films showed no inhibition zone among the tested films. Meanwhile, *S.aureus* grew slightly on the surface of the films, demonstrating that CS and CS/PVA films have no antimicrobial activity. By contrast, the addition of Cur could remarkably enhance the antimicrobial activity of the films. Several antimicrobial mechanisms of Cur film have been proposed: (1) the hydrophobic substance of curcumin can directly act on the microbial cell membranes, leading to leakage of proteins and other intracellular components; (2) Cur can inhibit microbial growth by generated polyphenols that interact with the essential cell division–initiating protein FtsZ. However, it is required that the exact antimicrobial mechanism of Cur needs to be further investigated. In summary, these results proved that CS and CS/PVA films supplemented with Cur had the potential to inhibit target microorganisms.

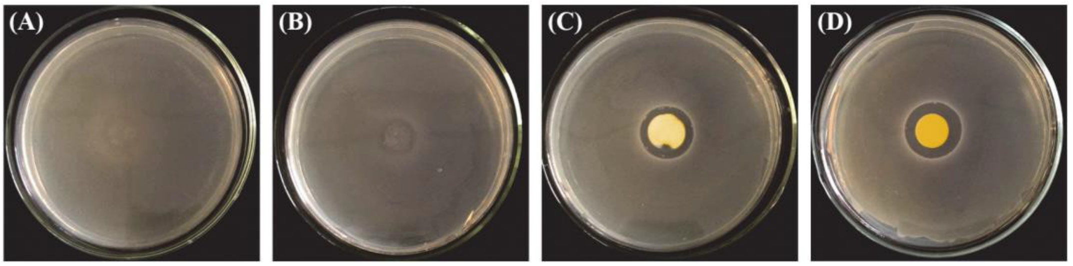


Figure 3. 2. Antibacterial activity of CS (A), CS/PVA (B), CS–Cur (C), and CS/PVA–Cur (D) films.

Table 3. 1. Inhibition zone values for different films.

Film	Inhibition zone (mm)
CS	Not detected
CS/PVA	Not detected
CS-Cur	14.79 ± 0.35
CS/PVA–Cur	15.11 ± 0.04

3.4.Intelligent pH Responsivity

For the evaluation of the pH sensing function, the typical CS/PVA–Cur film was immersed in buffer solutions with different pH values (pH at 3.0–10.0) and the corresponding visual images were presented in Fig. 3.3. The color of CS/PVA–Cur film varied from bright yellow to orange and orange–red. Significant decrease was observed in lightness upon increase in the solution pH, suggesting the color darkness with increase of alkalinity.

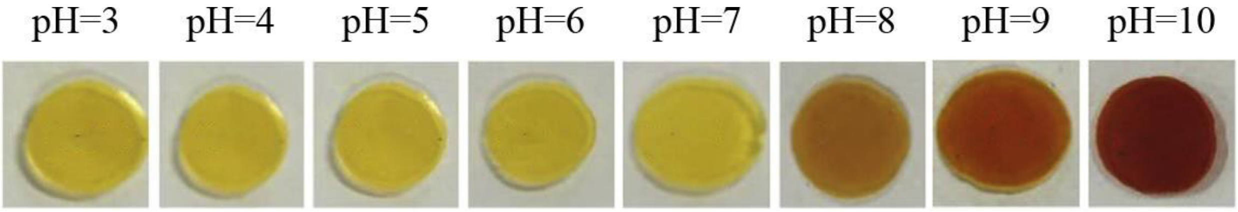


Figure 3. 3. Color changes of CS/PVA–Cur film at different pH conditions.

As shown in Fig. 3.3, a similar shade of redness was observed in the range of pH 3.0–7.0. However, an immediate change was observed as the pH increased from 7.0 to 10.0, indicating a strong color–shift under alkaline condition. Such a phenomena can be related to pH–induced change in the chemical structure of Cur (Fig. 3.4). The visible color shift of Cur was caused by the gradual conversion of keto form (yellow) to enol form (orange–red) under alkaline conditions. The above results suggested that the developed CS/PVA–Cur packaging film might serve to monitor seafood freshness.

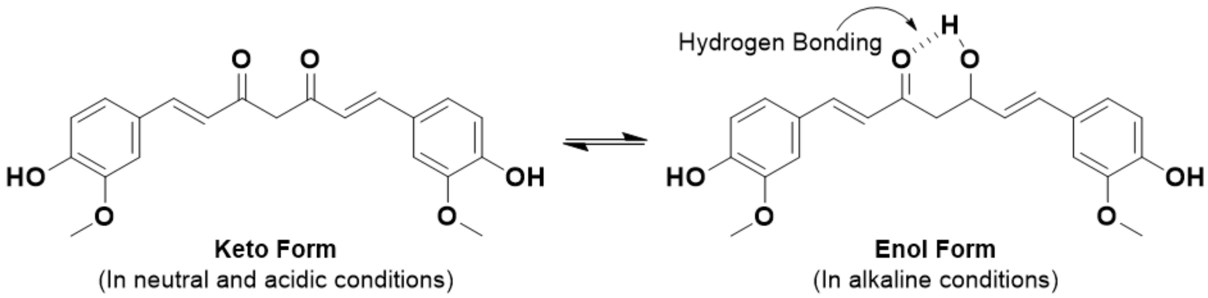


Figure 3. 4. Changes in curcumin structure due to pH changes, adapted from.

3.5.Potential Application

Generally, the spoilage of most animal-based foods is related to enzymes' action and protein decomposition that occur during storage. Various volatile nitrogen compounds including ammonia, dimethylammonium and trimethylamine are formed, which increases the pH environment inside the sealed package. Based on this phenomenon, CS/PVA–Cur film was employed as a pH sensing indicator to monitor veal freshness, thus demonstrating the potential for food application. The typical results were shown in Fig. 3.5. As expected, the control film showed no obvious change in color during the storage. In a remarkable contrast, the color of CS/PVA–Cur film clearly turned from yellow (0th day) to orange (3rd day), and the redness shades of CS/PVA–Cur film significantly increased after 3 days, suggesting the complete meat spoilage point. The color variations can be explained by the increase in volatile nitrogen compounds content of meat samples during storage. Therefore, the CS/PVA–Cur film can be used as a promising intelligent packaging to monitor meat freshness via direct naked-eye inspection.

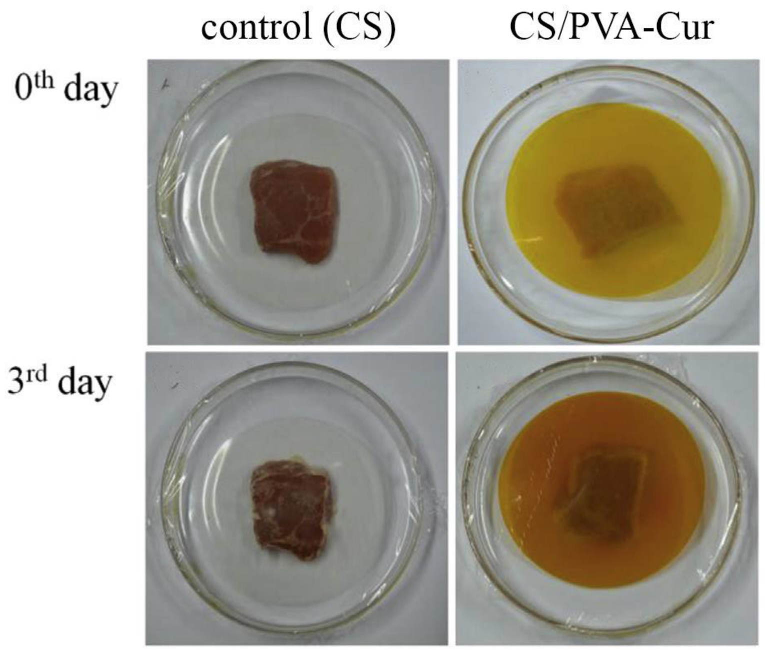


Figure 3. 5. Applications of sensing film for monitoring veal freshness.

General Conclusion

In conclusion, due to the abundant, non-toxic, degradable, and biocompatible characteristics of starch, the development of starch-based films has attracted great researcher interest. Bioactive and intelligent starch-based films can be prepared through simple laboratory methods, such as the “casting” and “extrusion/hot pressing” methods, and with the industrial “blow molding” method. Films with antibacterial, antioxidation, and pH responsiveness could be used as packaging materials for food preservation. Moreover, starch-based films can also be used as carriers for the controlled release of biomedicines. Additionally, the addition of anthocyanins could endow starch-based films with pH–responsive properties, but anthocyanins in nature are unstable and easily degraded. Thus, the application of anthocyanins in smart food packaging is limited due to their instability.

In this study, Active and intelligent packaging films were successfully developed. The addition of Cur into CS or CS/PVA blend films could interact with the matrix achieving a higher compact microstructure. The films containing Cur showed strong antioxidant activity, better antimicrobial activity and intelligent pH responsivity. Notably, CS/PVA–Cur had superior structural and functional properties, which can be used as a novel packaging material to extend the shelf life of animal-based protein foods and monitor their freshness.

Currently, the functions of bioactive starch-based films are relatively limited, especially responsive films. In future, additional studies are needed to develop multifunctional and multi-reactive starch-based films with, for example, anti-fatigue properties, enzyme responsiveness, and salt responsiveness. Multi-responsive starch-based materials that respond to pH, temperature, and amylase are mainly used to release drugs. Functional food ingredients loaded into multi-responsive starch films also require further investigation.

References

[1] S. Khalid, L. Yu, L. Meng, H. Liu, A. Ali, L. Chen, Poly (lactic acid)/starch composites: Effect of microstructure and morphology of starch granules on performance, J. Appl. Polym. Sci. 134 (2017) 45504.

[2] E. Bertoft, Understanding starch structure: Recent progress, Agronomy. 7 (2017) 56.

[3] Y. Liu, C. Chao, J. Yu, S. Wang, S. Wang, L. Copeland, New insights into starch gelatinization by high pressure: Comparison with heat-gelatinization, Food Chem. 318 (2020) 126493.

[4] C. Li, Y. Hu, E. Li, Effects of amylose and amylopectin chain-length distribution on the kinetics of long-term rice starch retrogradation, Food Hydrocoll. 111 (2021) 106239.

[5] O. Ochoa-Yepes, L. Di Giogio, S. Goyanes, A. Mauri, L. Famá, Influence of process (extrusion/thermo-compression, casting) and lentil protein content on physicochemical properties of starch films, Carbohydr. Polym. 208 (2019) 221–231.

[6] Z. Żołek-Tryznowska, A. Kałuża, The influence of starch origin on the properties of starch films: Packaging performance, Materials (Basel). 14 (2021) 1146.

[7] C. Herniou, J.R. Mendieta, T.J. Gutiérrez, Characterization of biodegradable/non-compostable films made from cellulose acetate/corn starch blends processed under reactive extrusion conditions, Food Hydrocoll. 89 (2019) 67–79.

[8] P.S. Garcia, F.R.B. Turbiani, A.M. Baron, G.L. Brizola, M.A. Tavares, F. Yamashita, D. Eiras, M.V.E. Grossmann, Sericin as compatibilizer in starch/polyester blown films, Polímeros. 28 (2018) 389–394.

[9] M. Feng, L. Yu, P. Zhu, X. Zhou, H. Liu, Y. Yang, J. Zhou, C. Gao, X. Bao, P. Chen, Development and preparation of active starch films carrying tea polyphenol, Carbohydr. Polym. 196 (2018) 162–167.

[10] E. Jamróz, L. Juszczak, M. Kucharek, Development of starch-furcellaran-gelatin films containing tea tree essential oil, J. Appl. Polym. Sci. 135 (2018) 46754.

[11] B. Iamareerat, M. Singh, M.B. Sadiq, A.K. Anal, Reinforced cassava starch based edible film incorporated with essential oil and sodium bentonite nanoclay as food packaging material, J. Food Sci. Technol. 55 (2018) 1953–1959.

[12] S. Yang, L. Cao, H. Kim, S. Beak, K. Bin Song, Utilization of foxtail millet starch film incorporated with clove leaf oil for the packaging of queso blanco cheese as a model food, Starch-Stärke. 70 (2018) 1700171.

[13] Z. Fangfang, B. Xinpeng, G. Wei, G. Wang, Z. Shi, C. Jun, Effects of virgin coconut oil on the physicochemical, morphological and antibacterial properties of potato starch-based biodegradable films, Int. J. Food Sci. Technol. 55 (2020) 192–200.

[14] R. Suriyatem, R.A. Auras, C. Rachtanapun, P. Rachtanapun, Biodegradable rice starch/carboxymethyl chitosan films with added propolis extract for potential use as active food packaging, Polymers (Basel). 10 (2018) 954.

[15] R. Zhang, X. Wang, M. Cheng, Preparation and characterization of potato starch film with various size of nano-SiO2, Polymers (Basel). 10 (2018) 1172.

[16] A. Ounkaew, P. Kasemsiri, K. Kamwilaisak, K. Saengprachatanarug, W. Mongkolthanaruk, M. Souvanh, U. Pongsa, P. Chindaprasirt, Polyvinyl alcohol (PVA)/starch bioactive packaging film enriched with antioxidants from spent coffee ground and citric acid, J. Polym. Environ. 26 (2018) 3762–3772.

[17] E. Arezoo, E. Mohammadreza, M. Maryam, M.N. Abdorreza, The synergistic effects of cinnamon essential oil and nano TiO2 on antimicrobial and functional properties of sago starch films, Int. J. Biol. Macromol. 157 (2020) 743–751.

[18] F. Bi, X. Zhang, J. Liu, H. Yong, L. Gao, J. Liu, Development of antioxidant and antimicrobial packaging films based on chitosan, D-α-tocopheryl polyethylene glycol 1000 succinate and silicon dioxide nanoparticles, Food Packag. Shelf Life. 24 (2020) 100503.

[19] R. Amorati, M.C. Foti, L. Valgimigli, Antioxidant activity of essential oils, J. Agric. Food Chem. 61 (2013) 10835–10847.

[20] E. Jamróz, L. Juszczak, M. Kucharek, Investigation of the physical properties, antioxidant and antimicrobial activity of ternary potato starch-furcellaran-gelatin films incorporated with lavender essential oil, Int. J. Biol. Macromol. 114 (2018) 1094–1101.

[21] J.M. Tirado-Gallegos, P.B. Zamudio-Flores, J. de J. Ornelas-Paz, C. Rios-Velasco, G.I. Olivas Orozco, M. Espino-Díaz, R. Baeza-Jiménez, J.J. Buenrostro-Figueroa, M.A. Aguilar-González, D. Lardizábal-Gutiérrez, Elaboration and characterization of active apple starch films incorporated with ellagic acid, Coatings. 8 (2018) 384.

[22] N.S. Said, N.M. Sarbon, Response surface methodology (RSM) of chicken skin gelatin based composite films with rice starch and curcumin incorporation, Polym. Test. 81 (2020) 106161.

[23] S. Kim, S.-K. Baek, E. Go, K. Bin Song, Application of adzuki bean starch in antioxidant films containing cocoa nibs extract, Polymers (Basel). 10 (2018) 1210.

[24] T.J. Gutiérrez, C. Herniou-Julien, K. Álvarez, V.A. Alvarez, Structural properties and in vitro digestibility of edible and pH-sensitive films made from guinea arrowroot starch and wastes from wine manufacture, Carbohydr. Polym. 184 (2018) 135–143.

[25] I. Choi, J.Y. Lee, M. Lacroix, J. Han, Intelligent pH indicator film composed of agar/potato starch and anthocyanin extracts from purple sweet potato, Food Chem. 218 (2017) 122–128.

[26] Y. Qin, Y. Liu, X. Zhang, J. Liu, Development of active and intelligent packaging by incorporating betalains from red pitaya (Hylocereus polyrhizus) peel into starch/polyvinyl alcohol films, Food Hydrocoll. 100 (2020) 105410.

[27] C.L. Luchese, N. Sperotto, J.C. Spada, I.C. Tessaro, Effect of blueberry agro-industrial waste addition to corn starch-based films for the production of a pH-indicator film, Int. J. Biol. Macromol. 104 (2017) 11–18.

[28] C. Zhang, G. Sun, L. Cao, L. Wang, Accurately intelligent film made from sodium carboxymethyl starch/κ-carrageenan reinforced by mulberry anthocyanins as an indicator, Food Hydrocoll. 108 (2020) 106012.

[29] S.K. Mary, R.R. Koshy, J. Daniel, J.T. Koshy, L.A. Pothen, S. Thomas, Development of starch based intelligent films by incorporating anthocyanins of butterfly pea flower and TiO 2 and their applicability as freshness sensors for prawns during storage, RSC Adv. 10 (2020) 39822–39830.

[30] B. Liu, H. Xu, H. Zhao, W. Liu, L. Zhao, Y. Li, Preparation and characterization of intelligent starch/PVA films for simultaneous colorimetric indication and antimicrobial activity for food packaging applications, Carbohydr. Polym. 157 (2017) 842–849.

[31] G. Jiang, X. Hou, X. Zeng, C. Zhang, H. Wu, G. Shen, S. Li, Q. Luo, M. Li, X. Liu, Preparation and characterization of indicator films from carboxymethyl-cellulose/starch and purple sweet potato (Ipomoea batatas (L.) lam) anthocyanins for monitoring fish freshness, Int. J. Biol. Macromol. 143 (2020) 359–372.

[32] N. Poorgholy, B. Massoumi, M. Jaymand, A novel starch-based stimuli-responsive nanosystem for theranostic applications, Int. J. Biol. Macromol. 97 (2017) 654–661.

[33] H. Chen, Y. Yuan, Q. Li, Preparation and characterization of corn starch-based composite films containing corncob cellulose and cassia oil, Starch-Stärke. 72 (2020) 1900209.

[34] W.G. Sganzerla, G.B. Rosa, A.L.A. Ferreira, C.G. da Rosa, P.C. Beling, L.O. Xavier, C.M. Hansen, J.P. Ferrareze, M.R. Nunes, P.L.M. Barreto, Bioactive food packaging based on starch, citric pectin and functionalized with Acca sellowiana waste by-product: Characterization and application in the postharvest conservation of apple, Int. J. Biol. Macromol. 147 (2020) 295–303.

[35] R.A. Shapi'i, S.H. Othman, N. Nordin, R.K. Basha, M.N. Naim, Antimicrobial properties of starch films incorporated with chitosan nanoparticles: In vitro and in vivo evaluation, Carbohydr. Polym. 230 (2020) 115602.

[36] J.C.M. da Costa, K.S.L. Miki, A. da Silva Ramos, B.E. Teixeira-Costa, Development of biodegradable films based on purple yam starch/chitosan for food application, Heliyon. 6 (2020) e03718.

[37] K. dos Santos Caetano, N.A. Lopes, T.M.H. Costa, A. Brandelli, E. Rodrigues, S.H. Flôres, F. Cladera-Olivera, Characterization of active biodegradable films based on cassava starch and natural compounds, Food Packag. Shelf Life. 16 (2018) 138–147.

[38] E. Talón, M. Vargas, A. Chiralt, C. González-Martínez, Antioxidant starch-based films with encapsulated eugenol. Application to sunflower oil preservation., Lwt. 113 (2019) 108290.

[39] S. Li, Y. Ma, T. Ji, D.E. Sameen, S. Ahmed, W. Qin, J. Dai, S. Li, Y. Liu, Cassava starch/carboxymethylcellulose edible films embedded with lactic acid bacteria to extend the shelf life of banana, Carbohydr. Polym. 248 (2020) 116805.

[40] C.B. Francisco, M.G. Pellá, O.A. Silva, K.F. Raimundo, J. Caetano, G.A. Linde, N.B. Colauto, D.C. Dragunski, Shelf-life of guavas coated with biodegradable starch and cellulose-based films, Int. J. Biol. Macromol. 152 (2020) 272–279.

[41] K. Zhang, T.-S. Huang, H. Yan, X. Hu, T. Ren, Novel pH-sensitive films based on starch/polyvinyl alcohol and food anthocyanins as a visual indicator of shrimp deterioration, Int. J. Biol. Macromol. 145 (2020) 768–776.

[42] M. Kaya, S. Khadem, Y.S. Cakmak, M. Mujtaba, S. Ilk, L. Akyuz, A.M. Salaberria, J. Labidi, A.H. Abdulqadir, E. Deligöz, Antioxidative and antimicrobial edible chitosan films blended with stem, leaf and seed extracts of Pistacia terebinthus for active food packaging, RSC Adv. 8 (2018) 3941–3950.

[43] M. Ramos, A. Beltrán, M. Peltzer, A.J.M. Valente, M. del Carmen Garrigós, Release and antioxidant activity of carvacrol and thymol from polypropylene active packaging films, LWT-Food Sci. Technol. 58 (2014) 470–477.

[44] C. Vilela, R.J.B. Pinto, J. Coelho, M.R.M. Domingues, S. Daina, P. Sadocco, S.A.O. Santos, C.S.R. Freire, Bioactive chitosan/ellagic acid films with UV-light protection for active food packaging, Food Hydrocoll. 73 (2017) 120–128.

[45] J.Sharifi-Rad, Y. El Rayess , A.A Rizk , C.Sadaka , R. Zgheib, W. Zam, S.Sestito, S. Rapposelli, K.Neffe-Skocinska , D. Zielinska, B. Salehi, W.N. Setzer, N.S. Dosoky, Y.Taheri , M.El Beyrouthy , M.Martorell, Turmeric and Its Major Compound Curcumin on Health: Bioactive Effects and Safety Profiles for Food,Pharmaceutical,Biotechnoogical and Medicinal Applications. Frontiers in Pharmacology. 11 (2020) 01021.