CURCUMIN-INCORPORATED COMPOSITE ACTIVE FILMS BASED ON CHITIN AND GLUCAN COMPLEXES EXTRACTED FROM *AGARICUS BISPORUS*

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ABSTRACT

CURCUMIN-INCORPORATED COMPOSITE ACTIVE FILMS BASED ON CHITIN AND GLUCAN COMPLEXES EXTRACTED FROM *AGARICUS BISPORUS*

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The primary objective of active packaging is to reduce food waste, energy consumption, and resource depletion by prolonging the shelf life of food products. The need for packaging materials derived from biodegradable, biocompatible, sustainable, and renewable resources is high due to their potential to help minimize harm to the environment and preserve limited natural resources. The objective of this study is to develop active packaging by the incorporation of curcumin into chitin, a biodegradable natural polymer. Chitin polymers were obtained from the cultivated mushroom, and curcumin was selected as the active ingredient based on its antimicrobial and antioxidant properties. Curcumin-loaded chitin glucan complex (CGC) was formed by filling β-glucan, which is covalently bound to chitin in the wall of *Agaricus bisporus*, with curcumin. Fabricated curcumin loaded CGC (CGC/Cu) films were characterized to evaluate their surface, optical, structural, barrier, mechanical, antioxidant, and antibacterial characteristics. The biodegradability of CGC/Cu films was assessed over a period of 14 days in a soil environment. The addition of curcumin had a notable impact on the surface morphology of the films, leading to enhanced light barrier characteristics, radical

scavenging activity, and total phenolic content. The CGC/Cu films with varying concentrations of curcumin exhibited antibacterial properties against *Escherichia coli*. However, no antibacterial activity was seen against *Staphylococcus aureus* in the films. Subsequently, the microbiological quality of the fresh skinless chicken breast sample was monitored over a refrigerated storage period of 10 days. Developed CGC/Cufilm resulted in a significant extension of the shelf-life of chicken samples, increasing it by a minimum of 40% when compared to the control sample wrapped in cling films.

Keywords: Biodegradable packaging, Antimicrobial activity, Active film, Curcumin, *Agaricus bisporus*

ÖZ

AGARICUS BISPORUS'TAN ELDE EDİLEN KİTİN ve GLUKAN KOMPLEKSLERİNE DAYALI CURCUMIN İÇEREN KOMPOZİT AKTİF FİLMLER

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Aktif paketlemenin birincil amacı, gıda ürünlerinin raf ömrünü uzatarak gıda israfını, enerji ve kaynak tüketimini azaltmaktır. Biyolojik olarak parçalanabilen, biyolojik olarak uyumlu, sürdürülebilir ve yenilenebilir kaynaklardan elde edilen ambalaj malzemelerine olan ihtiyaç, çevreye verilen zararı azaltmaya yardımcı olma ve sınırlı doğal kaynakları koruma potansiyelleri nedeniyle yüksektir. Bu çalışmanın amacı, biyolojik olarak parçalanabilen doğal bir polimer olan kitine kurkuminin dahil edilmesiyle aktif ambalaj geliştirmektir. Bu çalışmada, kültür mantarından kitin polimerleri elde edildi ve antimikrobiyal ve antioksidan özelliklerine göre aktif madde olarak kurkumin seçildi. *Agaricus bisporus*'un duvarında kitine kovalent olarak bağlı olan β-glukanın kurkumin ile doldurulmasıyla bir kurkumin-glukan kompleksi (CGC) oluşturuldu. Üretilen kurkumin yüklü CGC (CGC/Cu) filmleri, yüzey, optik, yapısal, bariyer, mekanik, antioksidan ve antibakteriyel özelliklerini değerlendirmek için karakterizasyona tabi tutuldu. CGC/Cu filmlerinin biyobozunurluğu, toprak ortamında 14 günlük bir süre boyunca değerlendirildi. Kurkumin ilavesinin, filmlerin yüzey morfolojisi üzerinde dikkate değer bir etkisi oldu ve bu da gelişmiş ışık bariyeri özelliklerine, antioksidan aktivitesine ve toplam fenolik içerikte artışa yol açtı. Farklı kurkumin konsantrasyonlarına sahip CGC/Cu

filmleri, *Escherichia coli*'ye karşı antibakteriyel özellikler sergiledi. Ancak filmlerde *Staphylococcus aureus*'a karşı herhangi bir antibakteriyel aktivite görülmemiştir. Daha sonra, taze derisiz tavuk göğsü örneğinin mikrobiyolojik özelliklerini değerlendirmek için bir analiz yapıldı, bu yüzden 10 gün boyunca buzdolabında saklanan tavuk örnekleri izlendi. Üretilen filmin kullanımı, streç film sarılı kontrol örneğine kıyasla piliç örneklerinin raf ömrünün önemli ölçüde uzamasına ve minimum %40 oranında artmasına neden oldu.

Anahtar Kelimeler: Biyobozunur ambalaj, Antimikrobiyal aktivite, Aktif film, Kurkumin, *Agaricus bisporus*

To my parents

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TABLE OF CONTENTS

ABSTRACT v

ÖZ vii

ACKNOWLEDGMENTS x

TABLE OF CONTENTS xii

LIST OF TABLES xv

LIST OF FIGURES xvi

CHAPTERS

1 INTRODUCTION 1

1.1 Sustainable and Eco-friendly Food Packaging Solutions 1

1.1.1 Biopolymers for Food Packaging Applications 2

1.1.2 Structure and Properties of Chitin 4

1.1.3 Cultivated Mushroom as Chitin Source 6

1.2 Active Packaging 9

1.2.1 Scavengers & Emitters 12

1.2.2 Antimicrobial Agents 14

1.2.3 Antioxidant Agents 16

1.3 Curcumin 17

1.3.1 Structure and Properties of Curcumin 17

1.3.2 Application of Curcumin as Food Packaging Material 19

1.4 Factors Affecting Properties of Active Film 21

1.4.1 Temperature 21

1.4.2 pH 23
1.5 Objectives of The Study 23
2 MATERIAL AND METHOD 25
2.1 Material 25
2.2 Methods 25
2.2.1 Preparation of Film 25
2.3 Characterization of Film 27
2.3.1 Light Barrier Properties 27
2.3.2 Total Phenolic Content (TPC) 27
2.3.3 DPPH Free Radical Scavenging Activity 28
2.3.4 Mechanical Properties 28
2.3.5 Water Solubility 29
2.3.6 Water Vapor Permeability (WVP) 30
2.3.7 Evaluation of Antimicrobial Activity 30
2.3.8 Microbiological Evaluation of Film on Fresh Chicken Breast 31
2.3.9 Biodegradation Test 32
2.3.10 Microstructural Analysis of Active Films 32
2.3.11 Analysis of Functional Groups in Active Films 32
2.3.12 Structural Analysis of Active Films 32
2.3.13 Porosity Determination 33
2.3.14 Thermal Analysis of Active Films 33
2.3.15 Statistical Analysis 33
3 RESULTS AND DISCUSSION 35
3.1 Microstructural Analysis of Active Films 35

3.2 Analysis of Functional Groups in Active Films 37
3.3 Structural Analysis of Active Films 39
3.4 Porosity of Active Films 41
3.5 Thermal Analysis of Active Films 42
3.6 Water solubility, Mechanical and Barrier Properties of Active Films 44
3.7 Light barrier properties of the active films 49
3.8 Phenolic compounds and Antioxidant Activity 50
3.9 Biodegradability of active films 52
3.10 Antibacterial activity evaluation 54
3.11 Application of films on fresh chicken meat 56
4 CONCLUSIONS AND RECOMMENDATIONS 59
REFERENCES 61
APPENDICES
A. Calibration Curves 89
B. Statistical Analysis 90

LIST OF TABLES

TABLES

Table 1-1 Active Packaging Systems and Food Applications (Miranda et al., 2016; Yildirim et al., 2018)..... 11
Table 3-1. Total porosity values of CGC/Cu films*..... 42
Table 3-2. Mechanical properties of CGC/Cu films*..... 47
Table 3-3 Water vapor permeability and thickness of CGC/Cu films*..... 48
Table 3-4 Water solubility of CGC/Cu films*..... 49
Table 3-5 Light Transmittance of CGC/Cu films*..... 50
Table 3-6 Total phenolic content and free radical scavenging activity of CGC/Cu films*..... 51
Table 3-7 The inhibition zone of CGC/Cu films against *E.coli* and *S.aureus**..... 55

LIST OF FIGURES

FIGURES

Figure 1.1.1 Chemical Structure of Curcumin 18
Figure 3.1. FESEM images of the surface morphologies for (a) CGC/Cu0, (b) CGC/Cu1, (c) CGC/Cu5, and (d) CGC/Cu10 films (scale bars: 30 µm) and images of cross-sectional morphologies for (e) CGC/Cu0, (f) CGC/Cu1, (g) CGC/Cu5, and (h) CGC/Cu10 films (scale bars: 100 µm). 37
Figure 3.2. FTIR spectra of curcumin, CGC/Cu0, CGC/Cu1, CGC/Cu5 and CGC/Cu10 films. 39
Figure 3.3. XRD patterns of curcumin, CGC/Cu0, CGC/Cu1, CGC/Cu5 and CGC/Cu10 films 41
Figure 3.4. (a) TGA thermograms (b) DTG analysis of curcumin, CGC/Cu0, CGC/Cu1, CGC/Cu5 and CGC/Cu10 films. 44
Figure 3.5 Biodegradability of the CGC/Cu films in the soil..... 53
Figure 3.6 Images of inhibition zones for (a) CGC/Cu0, (b) CGC/Cu1, (c) CGC/Cu5, (d) CGC/Cu10 films against *E. coli* and for (e) Ch, (f) CGC/Cu1, (g) CGC/Cu5, (h) CGC/Cu10 films against *S. aureus* 56
Figure 3.7 Changes in (a) TVC and (b) PBC of skinless chicken breast packed in cling film and CGC/Cu10 film during 10 days of storage at 4°C 58

CHAPTER 1

INTRODUCTION

1.1 Sustainable and Eco-friendly Food Packaging Solutions

Nowadays, a wide variety of materials are used for food packaging applications. Packaging is crucial to protect food from oxygen, light, and any damage during distribution and storage (Alves et al., 2022a; Becerril et al., 2020). Mainly, packaging materials are derived from non-renewable, petroleum-based sources like plastics (Hermann et al., 2011; Moshood et al., 2022). In the last six years, European plastic usage demand has increased day by day. This level of usage results in a high carbon footprint and blue water print, both of which have a significant environmental impact (Hermann et al., 2011). After a single use as food packaging, 40% of plastic Packaging ends up in landfills and accumulates in soils and the ocean (Groh et al., 2019). This marine and soil debris breaks down into micro and nano-sized particles, which might readily diffuse into living organisms, causing serious, long-term harm to people (Bhuyan, 2022). There has been a need to reduce the use of plastic, as consumer concerns for the environment have increased and the decomposition rate of petroleum-based materials in nature has exceeded for years. For these reasons, research studies have been started on the use of easily recyclable materials that cause lead to the usage of sustainable Packaging. According to The Australian Sustainable Packaging Alliance (SPA), four principles describe sustainable Packaging. First, Packaging needs to be functional and cost-effective for all consumers along the value chain. Efficiency, which refers to utilizing energy and material resources as effectively as possible, comes next. Moreover, they should be non-polluting and non-toxic and facilitate recovery, particularly through natural systems (Slusarczyk & Kot, 2018). Thus, they can act as manure and soil conditioner (Moshood et al., 2022; Rosentrater et al., 2018). This recovery is an important factor since, unlike recycling,

it does not need the usage of energy, which is expensive and sometimes polluting (Hurley et al., 2013). Even though all such attributes may be genuine and helpful, the single most crucial sustainability trait that Packaging must possess is missing from this list. Food packaging must accomplish the transporting and storing of foods without causing a sanitary risk to human health, as well as providing consumers with necessary information (Rosentrater et al., 2018). Recyclable materials such as paper, reusable materials such as glass, paperboard, and biodegradable materials such as biopolymers are used in sustainable Packaging (Koketso Ncube et al., 2020). According to the EU waste management hierarchy, the most desired solutions among these sustainable packaging alternatives are waste prevention, which involves using biodegradable and reusable materials (Kralj & Markič, 2008).

1.1.1 Biopolymers for Food Packaging Applications

Biopolymers are created by living organisms or obtained from biomass, and once used, they decompose in an acceptable amount of time without posing environmental pollution problems. Biopolymers refer to biodegradable polymers, and the capacity of a substance to decompose into natural elements such as carbon dioxide, water, and biomass because of microbial action is called biodegradability (Iversen et al., 2022). On the other hand, conventional polymers (polymers) are defined as compounds that are composed of monomers linked with each other by a chemical process (Namazi, 2017). Biopolymers have qualities and properties like conventional polymers and can be processed using a variety of techniques, such as solution casting, electrospinning, and thermal pressing (Koketso Ncube et al., 2020). Bio-based polymers are replacing conventional polymers in numerous applications but can also give new combinations of characteristics. For instance, a biopolymer can contribute to extending the shelf-life of food due to its antioxidative nature. Therefore, they have the potential to be very useful in food packaging applications. Furthermore, the wider use of biobased materials in Packaging brings in less plastic waste, lowers greenhouse gas emissions, and ensures the long-term utilization of

natural resources (Babaremu et al., 2023). These materials can be divided into 3 groups according to their production methods and sources. They can be chemically synthesized by using biobased components. Polylactic acids (PLA), which are biodegradable thermoplastic polyester, are an excellent example of this type of synthesis (Fabra et al., 2014). They are derived from a monomer lactic acid and are commonly used as an alternative to petroleum-based plastics (Aydogdu et al., 2019). Another production method of biopolymers is extraction from microorganisms. Polyhydroxyalkanoates (PHA), one of the most common examples of this method, are produced directly by fermentation of a carbon-based feedstock in a microorganism (Nduko & Taguchi, 2021). Both PLA and PHA are classified as bioplastics. The term bioplastic means plastics from bio-based sources or biodegradable plastics (Koketso Ncube et al., 2020). Bioplastics are a type of plastic that passes specific biodegradability standards. The length of biodegradability is determined by factors such as crystallinity, molecular weight, water absorption, stereoisomeric concentration, and environmental factors (Emadian et al., 2017). PLA, for example, has been shown to break down at a respectable pace in soil. Pure PLA degrades in the environment over a period of months to two years, whereas petroleum-based polymers take 500-1000 years (Chamas et al., 2020). PLA offers packaging uses for a broader range of items, such as films and food containers. PLA has a variety of desired qualities, like biocompatibility and desirable mechanical properties, and it can be shaped into a variety of forms (Nduko & Taguchi, 2021). A PLA bottle, for example, is equivalent to a PET bottle since its tensile strength and elastic modulus are similar (Koketso Ncube et al., 2020). As a third method, they can be directly extracted from biomass, such as polysaccharides (starch, cellulose, chitosan, pectin, and alginate) and proteins (caseinate, gluten, zein, soy protein, and whey protein) (Fabra et al., 2014). Polysaccharide-based packaging materials are the most prevalent because of their low cost, availability, and some functional or unique characteristics (G. Kaur et al., 2021). Depending on the type of polysaccharide, they show a good barrier to gas transference, good film-forming ability, and poor water solubility (Siracusa & Dalla, 2008). For instance, chitosan is a linear polysaccharide

consisting of (1,4)-linked 2-amino-deoxy-β-d-glucan and is widely used in sustainable film applications thanks to its non-toxic, antimicrobial, good film-forming, and mechanical properties. Chitosan is derived from chitin by the alkaline *N*-deacetylation of chitin (Elieh-Ali-Komi et al., 2016; Homez-Jara et al., 2018).

1.1.2 Structure and Properties of Chitin

Chitin (β-(1–4)-poly-N-acetyl-D-glucosamine) is the second most abundantly found bio-based and renewable polymer on the earth (Kaya et al., 2022). They have extremely good characteristics, including biodegradability, biocompatibility, and nontoxicity (Caz, 2017). Exoskeletons of crustaceans, cell walls of fungi, and yeast are prevalent sources of chitin (Kaya et al., 2022). The linear structure of chitin is composed of two hydroxyl groups with an acetamide group, and strong hydrogen bonding exists between these groups resulting in highly ordered crystalline chitin nanofibers (Kaya et al., 2022). These high-modulus and strength chitin nanofibers have been integrated into a protein matrix to generate biological nanocomposites with mechanical capabilities (Ofem et al., 2017; Rhim et al., 2013). Depending on the species and the orientation of the molecular chain, chitin nanofibers can differentiate into α, β, or γ-chitin (J. C. Silva & Borges, 2015). α-chitin is the most stable type of nanofiber and is mostly found in shells of crabs and shrimps, cuticles of insects, and cell walls of fungi. The maximum linkage among macromolecules via hydrogen bonds is observed due to the antiparallel arrangement of nanofibrils (Berezina, 2016; Chakravarty & Edwards, 2022). This antiparallel arrangement creates strong, steady fibrils with crystallinity higher than 80% (J. C. Silva & Borges, 2015). β-chitin is found commonly in squids and is in metastable form so it can be converted into α-chitin by chemical treatment. Its fibrils can achieve a crystallinity of 70% (J. C. Silva & Borges, 2015). A longer distance between molecules of β-chitin and parallel arrangement between nanofibrils causes weak intermolecular forces (Wu et al., 2019). This structure tends to make β-chitin more responsive and

vulnerable to solvent dissolution, as it allows easier access to water molecules. γ-chitin is known as a combination of both β-chitin and α-chitin in terms of molecular structure, which means it is made up of two parallel and one antiparallel chain, and its strength is like those of the β-type (Kumari & Kishor, 2020). Natural chitin nanofibrils have excellent mechanical characteristics like tensile strength (Wu et al., 2019). However, the physicochemical properties of chitin, which are crystallinity, molecular weight, moisture, protein, ash content, etc., vary depending on the chitin source's compositional differences and extraction techniques(Y. I. Cho et al., 1998; Pellis et al., 2022). Most chitin sources have 30 to 60% minerals, 0 to 14% lipids, 20 to 40% protein, 20 to 30% chitin, and in particular, about 90% contain calcium carbonate, protein, and chitin (S. Kaur & Dhillon, 2015). Proteins, minerals, and chitin are bonded; thus, a small quantity of protein and mineral is present in the polymer matrix. As a result, chitin isolation from the shell necessitates the removal of two major components, namely protein and minerals. Chitin can be extracted from its sources by chemical or biological methods. Chemical methods include acid and alkali treatments, which are needed to remove proteins and minerals by deproteinization and demineralization processes, respectively (Hassainia et al., 2018). Occasionally, an extra decolorization process is performed to remove pigment astaxanthin (Pellis et al., 2022). In the deproteinization process, alkali reagents like NaOH, Na_2CO_3, $NaHCO_3$, KOH, K_2CO_3, $Ca(OH)_2$, Na_2SO_3, $NaHSO_3$, $CaHSO_3$, and Na_3PO_4 are used (Gadgey & Dey, 2017). In the demineralization step, HCl, HNO_3, H_2SO_4, CH_3COOH, and HCOOH are commonly used (Percot et al., 2003). These reagents react with calcium carbonate and turn it into calcium chloride while emitting water and carbon dioxide. The application of chemicals with high temperatures and concentrations to get pure chitin from the exoskeletons of crustaceans produces harmful waste that damages the environment, raises the cost of chitin isolation, and leads to unwanted deacetylation and depolymerization (Chakravarty & Edwards, 2022; Ifuku et al., 2011; Kaya et al., 2022; Philibert et al., 2017). As a result, it is difficult to remove minerals and protein completely from chitin isolated from crustaceans without a harsh chemical and high-temperature treatment.

1.1.3 Cultivated Mushroom as Chitin Source

Global mushroom cultivation has reached an estimated value of over $75 billion by 2017, while half of the economic value of the global mushroom industry comes from edible mushrooms (Du et al., 2021). Because of their dietary and therapeutic benefits, interest in mushrooms has increased over the past years throughout the globe (Atila et al., 2017). The term "mushroom" is defined differently by individuals from various nations. For this reason, nobody can estimate the number of mushroom species in the world. A macrofungus with a recognizable fruiting body, known as a mushroom, generates spores that can be classified as epigeous or hypogeous, big enough to be seen with the unaided eye and picked by hand(S. Chang & Miles, 1992). According to this description, there are roughly 16,000 different species of mushrooms on earth, as defined by the International Code of Nomenclature (Hawksworth, 2012). The most prevalent varieties are *Agaricus bisporus*, *Lentinula eddoes*, *Pleurotus pulmonarius var. stechangii*, *Volvariela volvacea*, *Agaricus brasilienesis*, and *Ganoderma lucidum* (S. T. Chang & Wasser, 2017). Among these varieties, *Agaricus bisporus* commonly referred to as the button mushroom, the white mushroom, and the cultivated mushroom, has long been a favorite.

Polysaccharides constitute most of the fungal cell wall. Chitin is one of the skeletal fungal polysaccharides that give the cell wall its solidity and structure. In contrast to animal-derived chitin, fungal-sourced chitin includes significant amounts of glucan, protein, and other polysaccharides (W. M. F. B. W. Nawawi et al., 2020; Oliveira-Garcia & Deising, 2013). β-Glucan is a linear non-starch polysaccharide found in microbial and plant cell walls. It is composed of continuous β-(1 → 4)- D-glycoside bonds and non-continuous β-(1 → 3) bonds originating from D-glycoside linkages to glucose (J. Chang et al., 2019). Chitin-glucan complex in the wall structure is water-insoluble, hygroscopic, and has a high swelling capacity (Araújo et al., 2023). Chitin content in cell walls depends on the specie, crop, storage conditions, maturation of the mushroom, and part of the fruit body, and it can differ between 2% and 8.5% (to dry mass) for *Agaricus bisporus*. Three different *Pleurotus ostreatus*

species had chitin contents that ranged from 2.16% to 3.31% of dry mass (Vetter, 2007). Manzi et al. (2001) show that cooking *Agaricus bisporus* raised the percentage of chitin in both the deep-frozen and canned versions of the mushroom (control, 6.0%; cooked, 7.0%; deep-frozen, 3.4%; canned, 6.1%; cooked 7.4%). Moreover, studies demonstrate that the pileus (cap) of *A. bisporus* has a higher chitin content than the stipes (Vetter, 2007).

1.1.3.1 Extraction Methods of Chitin from Cultivated Mushroom

A. bisporus has great potential as a chitin source compared to crustacean shells since it is simple to cultivate and has a short growing cycle (Shwet et al., 2019). All these advantages piqued researchers' interest in extracting and characterizing chitin and chitosan from *A. bisporus*. Demineralization, deproteinization, and discoloration are the three major treatments typically used to extract chitin from crustacean shells (Poerio et al., 2020). Mushrooms are not calcified, so the demineralization process is not required. Deproteinization, which means the withdrawal of protein in the cell wall structure, is an essential step in the extraction of chitin (Goto & Teramoto, 2020; Younes & Rinaudo, 2015). Although deproteinization is less detrimental to chitin than demineralization, long-lasting treatments, high temperatures, and molar concentration of used chemicals can cause deacetylation (Percot et al., 2003). Decreases in hydrophobicity, film tensile strength, elasticity, solubility, cell adhesion, and an increase in the rate of biological degradation are all caused by higher deacetylation degrees (Y. W. Cho et al., 2000). A study used lyophilized mushrooms to extract chitin by treating 1 M NaOH at 80°C for 2 h (Hassainia et al., 2018). Milder extraction parameters were chosen because the porous fungal structure created by lipolysis facilitates sodium hydroxide access to the inner structures. In this way, partial deacetylation was minimized. The study resulted in 4 ± 1.2 % (stipes), 6.4 ± 1.4 % (pileus), and 5.9 ± 1.2% (gills) mass yield of chitin content. Ifuku et al. (2011) extracted the chitin from cultivated mushrooms by using 0.5 M NaOH for 1 day at 100°C and compared the N content in extracted chitin with that

in the commercial chitin. The results show that the N content of the *Agaricus bisporus* sample (6.19%) was lower than that of commercial chitin (6.89%) due to the covalently bounded chitin-glucan complex in the mushroom structure. After alkali application, chitin was treated with 2 M HCl to remove glucan, but no significant change in the amount of N was observed. The use of acid has been shown not to affect the extraction of pure chitin from mushrooms. Although there have been no studies on the biological production of chitin from mushroom varieties, it has been reported that the deproteinization step in the production of chitin from crustaceans is performed by the protease enzyme (Arbia et al., 2013). The use of enzymes rather than chemicals is less harmful to the environment and more promising in terms of preserving chitin's characteristic properties (Philibert et al., 2017). However, enzyme usage in extraction causes cost-related issues.

1.1.3.2 Application of Chitin as Food Packaging Material

There is currently a lot of attention given to handling polymeric composite materials that contain small, rigid particles, commonly referred to as nanocomposites. This type of material has become increasingly popular due to significant advancements in its properties. To form a nanocomposite film, a suspension of polymers in a solvent is prepared and then dried. This process can be carried out using various methods, including solvent-casting, hot pressing, and sheet-making equipment (W. M. F. B. W. Nawawi et al., 2020). Casting is the most widely used of these methods. One of the most important reasons is that the films prepared by this method are stronger than those prepared by other methods since slow and controlled solvent removal may be utilized to form and protect a consistent percolation network (Gopalan Nair & Dufresne, 2003).

Although few studies have been conducted on chitin usage in nanocomposite films, chitin nanofibers provide good mechanical properties due to extensive fiber entanglement (Mushi, 2021). Furthermore, the matrix formed by the covalent bonding of fungi-derived chitin with glucan increases the strength and flexibility of

the chitin nanocomposite film (Fazli Wan Nawawi et al., 2019). Therefore, the use of chitin in nanocomposite film applications is promising. Nawawi et al. (2020) demonstrated that chitin nano papers derived from fungi-originated chitin have better tensile strength characteristics than crustacean counterparts. The existence of β-Glucan in the mushroom-chitin was specifically attributed to the increased strength. Besides, the ratio of glucan to chitin in the structure influences the surface and physicochemical properties of the formed film (Siroid et al., 2022). Furthermore, it has been proved that the yield of chitin-glucan complex obtained from mushrooms is higher than that of crustacean exoskeletons, which is associated with high tensile strength in mushroom-derived chitin films. Additionally, fungal-derived chitin shows lower wettability and hydrophilicity due to the lower polarity of the chitin-glucan complex, which contributes to the formation of nanocomposite film with good surface properties (W. M. F. W. Nawawi et al., 2020).

1.2 Active Packaging

In Europe, food waste amounts to approximately 89 million tons per year (Alves et al., 2022a). One potential solution to this problem is to extend the shelf life of foods with the goal of producing safer, and healthier food and thereby, reducing food waste. Nevertheless, food safety is a primary global concern for the food industry because of the rapid deterioration and degradation of highly perishable foods when they are not handled appropriately, packaged, and stored (Iversen et al., 2022; Mousavi Khaneghah et al., 2018). Food deterioration happens naturally due to its susceptibility to microbiological risks and biological reactions such as protein degradation and lipid oxidation, resulting in shorter shelf life (Gómez-Estaca et al., 2014). Therefore, innovative solutions are needed to fulfill the demands of industry and consumers and to contribute to sustainability.

Active packaging is a novel packaging method in which the package, the product, and the atmosphere interact to extend shelf life or improve safety or sensory attributes while preserving product quality (Chawla et al., 2021; Tripathi et al.,

2008). The primary goal of active packaging technologies is to change or modify the atmospheric conditions of packaged foods throughout their shelf life. On the other hand, adding active agents directly into the package may contribute to lowering the dosage of the agent required to achieve the targeted effect on the food. One of the reasons for this is that, even though active agents are added to the bulk of the food, deterioration occurs predominately on the surface first. As a result, the active substance in the package, which comes into direct contact with the food from the surface, will function more quickly and prevent surface deterioration (Yildirim et al., 2018). Moreover, adding active agents directly to food could diminish their activity due to food processing conditions (Chawla et al., 2021). Besides that, because these active agents are not added directly to the food, active packaging applications contribute to the clean label trend, which is gaining popularity today (Singh et al., 2021). Active Packaging is typically composed of active agents for food packaging and is classified based on their active functions, which include antimicrobial activity, antioxidant activity, carbon dioxide emission, oxygen extraction, etc. Table 1.1. shows some active packaging systems that are applied to different food categories. The active ingredients are discussed in detail below.

Table 1-1 Active Packaging Systems and Food Applications (Miranda et al., 2016; Yildirim et al., 2018)

Type of Active Packaging system	Use	Food Applications	References
Oxygen Scavengers	Controls the level of oxygen inside the packaging	Meat products Fried snacks Cheese	(Hurley et al., 2013) and (Tewari et al., 2002)
Carbon dioxide Emitters/ Releasers	Prevents the growth of microorganisms and slows down the rate of ripening	Fruits and vegetables Fresh Meat Coffee	(Nugraha et al., 2015) and (Cullen & Vaylen, 1994)
Ethylene Scavengers /Emitters	Controls the level of ethylene inside the Packaging	Fruits and Vegetables	(Murmu & Mishra, 2018)
Antioxidant Releasers	Protect the food from oxidation by improving oxidative stability	Fatty meat products Seeds and nuts Fried products Powdered food containing fat	(Guo et al., 2020) and (Marcos et al., 2014)
Moisture Absorbers	Reduce the rate of growth of microorganisms	Fish, poultry, meat Cereals Dried Foods	(Wang et al., 2017) and (D. Kim et al., 2017)
Antimicrobial Agent Releasers	Reduce the rate of growth of microorganisms	Fresh/cooked meat Dairy Products Ready-to-meal products Vegetables and fruits	(Khan et al., 2016) and (H. Kim et al., 2018)
Flavor/ Odor Absorbers	Eliminate unwanted gas molecules, metabolic byproducts, and respiration products.	Dairy Products Seafood Poultry Fried Snacks	(Brody et al., 2008) and (Abedi-Firoozjah et al., 2023)

1.2.1 Scavengers & Emitters

Scavengers remove unwanted substances from the packaging atmosphere to prolong shelf-life and protect product quality. There is no direct interaction/contact between scavengers and food. In contrast, conditions inside the Packaging are modified by absorbing oxygen, moisture, ethylene, or carbon dioxide (Mousavi Khaneghah et al., 2018).

One of the main scavenging systems is oxygen scavengers. They are mostly used in meat products (cooked or dried), bakery products, dried fish, and beverages (fruit juice, wine tomato-based products). The existence of oxygen inside the food packaging environment accelerates oxidation, which causes the deterioration of foods. It leads to the growth of aerobic microorganisms, the formation of undesirable odor and rancid taste, and a change in nutritional values (Roopa et al., 2022). Oxygen scavenging technology works in various ways, including chemical, biological, and enzymatic reactions. Each substance act as a different mechanism in scavenging. For instance, ascorbic acid is a reducing agent, and transition metals (e.g. cobalt, and palladium) catalyze oxidation reactions (Alves et al., 2022a). Usage of these two ingredients as a complex causes an extension of the shelf-life of bakery products. Also, this complex tends to show antimicrobial effects due to ascorbic acid. Iron, the most widely used active ingredient, is oxidized irreversibly to form stable ferric oxide trihydrate (Miltz & Perry, 2005). This oxidation reaction is triggered by moisture. Moreover, using iron nanoparticles instead of micro-sized particles increases the scavenger capacity of iron. Alpha-tocopherol nanoparticles, a natural antioxidant, and transition metal complex can also be given as another example (Byun et al., 2011). After transition metals initiate the formation of oxygen radicals, alpha-tocopherol absorbs these radicals to form stable radicals. Besides chemical reactions, biochemical mechanisms might work effectively in oxygen scavenging with the help of enzymes like laccase, glucose oxidase, and aerobic microorganism (Singh et al., 2021).

One another factor that causes microbial spoilage of food and a reduction in food quality and shelf life is excess moisture and in turn, high water activity. Moisture can be formed during storage inside the Packaging due to the chemical reactions occurring in the product, like respiration or water permeation from the atmosphere. Hence, moisture scavengers are commonly applied in foods. Frequently used moisture scavenger sachets are filled with absorbers, known as desiccants like sodium chloride, silica gels, zeolites, modified starch, and calcium oxide (Iversen et al., 2022; Yildirim et al., 2018). These sachets allow for the control of the moisture content level in Packaging, which inhibits mold growth but does not address the issue of water loss in the packaged product (Kõrge et al., 2020). Scavengers in sachet form are typically found in dried foods such as nuts, chips, instant coffee, and milk powder. Another scavenger type is the relative humidity controller. These are used in the form of pads and sheets under fresh products, like meat, poultry, and fish (Stroescu et al., 2019; Sung et al., 2013).

Ethylene is a colorless, volatile, and reactive hormone that stimulates the ripening of fruits and vegetables as it accelerates their respiration rates. A high-level ethylene concentration inside the package causes chlorophyll degradation, resulting in quality loss and reduced shelf-life (Ayhan, 2019). For this reason, it is necessary to remove ethylene from the packaging environment. Potassium permanganate embedded in silica is the most recognized, inexpensive, and widely used ethylene scavenger (Varghese et al., 2020). The silica helps to absorb ethylene, which is then oxidized by potassium permanganate to form ethylene glycol (Kirtiraj K. Gaikwad, 2017).

Emitters release active agents into the packaging environment to prevent destructive processes which cause spoilage, deterioration, loss of quality, and shelf-life reduction. They are aimed to preserve steady storability, and prolonged shelf-life, regulate carbon dioxide levels in the Packaging, inhibit the growth of microbial pathogens, and prevent rapid oxidation.

The presence of carbon dioxide plays a significant role in the growth of microorganisms in poultry and meat products. High carbon dioxide concentration

retards microbial growth and the respiration rate in fruits and vegetables. The antimicrobial effect of CO_2 depends on temperature, solubility, and partial pressure (Chaix et al., 2014). For instance, CO_2 is soluble in fatty medium or low temperatures (D. S. Lee, 2016). In addition, CO_2 emitters are a viable alternative to the modified atmosphere. Products are exposed to CO_2 over time, instead of just when they are packaged, thanks to the emitter. Emitters prevent bad odor and taste formation by ensuring excess CO_2.

1.2.2 Antimicrobial Agents

Microbial contamination is the most prevalent reason for food spoilage. Microbial contamination happens due to insufficient food processing, poor package barrier properties, or when food is exposed to the environment during storage and shipment (Mousavi Khaneghah et al., 2018). This contamination causes foodborne diseases and changes in sensorial characteristics like color and aroma. Thus, the current practice in food packaging applications is to develop more novel technologies to limit pathogenic microbial activities in products. Using antimicrobial agents in active packaging systems controls the growth of spoilage microorganisms, the resulting extension of shelf-life (Jung & Zhao, 2016). These agents can be listed as essential oils, enzymes and bacteriocins, organic acids and their derivatives, antimicrobial polymers, and metallic-based antimicrobial substances (Yildirim et al., 2018). These agents can be incorporated into the packaging system in different ways. These ways include incorporating a volatile component into the sachet, integrating the active agent directly into the film polymer structure, and chemical immobilization of antimicrobials in polymers (Alves et al., 2022b; Haktaniyan & Bradley, 2022; Mousavi Khaneghah et al., 2018).

Citric acid, sorbic acid, ascorbic acid, and benzoic acid are widely used organic acids in food packaging applications (Fite et al., 2004; Jung & Zhao, 2016; Yildirim et al., 2018). Variable concentrations of citric acid were combined with glucomannan and deacetylated chitin to form a nanocomposite film. It was observed that the

antimicrobial effect against *S. aureus* and *E. coli* enhanced as the citric acid concentration in the film increased (Jiang et al., 2020). The antimicrobial effect of an organic acid depends on the other polymers forming the film and the film fabrication method (Alves et al., 2022b). In another study, citric acid and fish gelatin interaction were investigated. Although the addition of citric acid showed an antimicrobial effect against *E. coli*, this effect was lower than the expected antimicrobial capacity of films. This unexpected result was explained by interactions between the formulation components' polar functional groups (Uranga et al., 2019). The inhibitory mechanism against microorganisms is primarily attributed to the penetration of protonated form of organic acid into the plasma membrane. The organic acid meets a higher pH in the cell and dissociates by releasing charged protons and anions that cannot cross the plasma membrane (Mousavi Khaneghah et al., 2018).

Essential oils are frequently used as organic antimicrobial agents in the food industry. Essential oils are volatile pure substances derived from aromatic plant parts such as seeds, flowers, fruit, roots, leaves, wood, fruits, as well as whole plants and are named according to the plant from where they're derived. These aromatic plant extracts comprise organic compounds like carbon, hydrogen, and oxygen. Because of the existence of various functional groups, they can be found in a wide range of forms, which include aldehydes, ketones, ethers, amines, and others. The existence and placement of functional groups on compounds can influence their activity against various pathogens (Varghese et al., 2020). These active ingredients bind to the cell's surface and penetrate the phospholipid bilayer of the cell membrane, causing the cell's structural integrity to deteriorate and necroptosis to occur. A study observed that the inclusion of black pepper oil damaged the *E. coli* cell membrane, causing cell death (J. Zhang et al., 2017). Due to their natural antimicrobial and antioxidant properties, essential oils are extensively employed in the food industry.

When essential oils are applied directly to the food material, they degrade rapidly because of the reactions between their unstable, volatile structure and environmental factors like light, oxygen, and heat. As a result, essential oils are used in active

Packaging in the form of films and coatings. Essential oils and their constituents have been incorporated into active films in various ways (Sharma et al., 2021). They have an antimicrobial effect on specific foodborne pathogenic bacterial species such as *Campylobacter jejuni, E. coli, Listeria monocytogenes, Salmonella enterica, and S. aureus.* Settani et al. (2012) reported that lemon essential oil could inhibit 42 strains of *L. monocytogenes* and *35 strains of S. aureus.*

1.2.3 Antioxidant Agents

After microbial growth, lipid oxidation is the primary factor in food spoilage. Foods with a high lipid content are particularly prone to degrading along. Food products that have had their lipids oxidized develop rancid-tasting flavors that make them unsafe to eat by humans (Gómez-Estaca et al., 2014). Various free radicals are produced during the early stages of lipid oxidation reactions. Antioxidative agents can prevent or delay oxidation by removing these radicals that cause lipid oxidation as soon as they occur (Lourenço et al., 2019). Natural antioxidants can improve the nutritional value and health benefits of foods and their storage stability by minimizing or preventing the formation of toxic compounds. By incorporating antioxidants into the packaging material, the food product is protected from oxidative damage, which can extend its shelf life. Antioxidants are generally recognized as safe, and their use in packaging materials is considered a natural way to preserve foods. Antioxidants could be synthetic or natural in origin. One of the benefits of using natural antioxidants in active packaging is that it can reduce the need for synthetic preservatives, such as BHA and BHT, which can have negative health effects (Yildirim et al., 2018). The main elements of naturally occurring plant-based antioxidants are tocopherols, carotenoids, flavonoids, lignans, and phenolic acids, which have powerful hydrogen-donating activity and, consequently, strong free radical scavenging activity (Tran., 2021; Xu et al., 2017). Besides from antioxidative effect, numerous of these natural antioxidants could slow down or stop the development of pathogenic bacteria like *Salmonella* spp. and *Escherichia coli*

(Cetin-Karaca & Newman, 2015). These antioxidants are often combined with other active packaging technologies, such as oxygen scavengers and moisture absorbers, to provide a synergistic effect that enhances the overall performance of the packaging. In a study, it was used a combination of chitosan nanoparticles and thyme essential oil , both of which have antioxidant and antibacterial properties. The results showed that the active packaging material significantly reduced lipid oxidation and the growth of bacteria in the meat burger samples compared to the control packaging. The active packaging was able to maintain the quality of the meat burger for a longer period and reduce the risk of microbial contamination (Ghaderi-Ghahfarokhi et al., 2016).

1.3 Curcumin

Curcumin is a polyphenolic substance naturally found in the turmeric plant's root (Curcuma longa), a member of the ginger family. It is the principal active ingredient responsible for turmeric's yellow color and has been used as an anti-inflammatory agent for centuries in conventional medicine (Kocaadam & Şanlier, 2017). Curcumin has been studied for its potential applications in the food industry, such as its use as a natural preservative and antimicrobial agent in food packaging materials and its health benefits.

1.3.1 Structure and Properties of Curcumin

Curcumin is a polyphenolic compound with a chemical formula of $C_{21}H_{20}O_6$ (Wahab et al., 2020). Curcumin's molecular structure is unique, with two aromatic rings and a ketone group. These rings have 2 main functional groups: methoxy groups ($-OCH_3$) and phenolic hydroxyl groups (-OH). Furthermore, these two aryl groups are linked by a seven-carbon chain in two enol and keto forms (S. Roy et al., 2022). Due to this structure, curcumin can display keto-enol tautomerism, which is influenced by the acidity of the solution. The dominant form of curcumin is keto in acidic and neutral

conditions, but in alkaline conditions, it is found in the enol form (Dutta et al., 2018). The chemical structure of curcumin is shown in Fig 1.1. Curcumin's antioxidant activity requires the presence of hydroxyl and methoxy groups (Galano et al., 2009; Rafiee et al., 2019). However, there is an ongoing debate about how these groups contribute to their antioxidant mechanisms. Some researchers believe that the phenolic part of curcumin donates electrons, allowing it to scavenge free radicals and inhibit lipid peroxidation (Rafiee et al., 2019; Venkatesan, 1998). Others, such as Jovanovic et al. (1999), propose that the central CH_2 group in the heptadienone link of curcumin keto form donates H-atoms as the primary antioxidant mechanism.

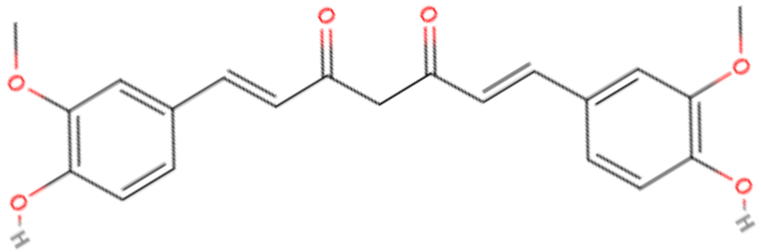


Figure 1.1.1 Chemical Structure of Curcumin

Curcumin has antimicrobial activity through a variety of mechanisms. It has the ability to disrupt the cell membrane of microorganisms, resulting in the leakage of cell contents and, eventually, cell death (Y. Zhang et al., 2018). Curcumin can also inhibit enzymes that are required for microorganism survival. It can interfere with protein synthesis by disrupting RNA (Munir et al., 2022). One such protein is Filamentous heat-sensitive protein Z (FtsZ), a prokaryotic version of the eukaryotic cytoskeletal protein tubulin that controls bacterial cell division. Curcumin restricts the assembly of FtsZ protofilaments and enhances the glycoside triphosphatase

(GTPase) activity of FtsZ, which can be lethal to bacteria as it impedes FtsZ assembly. By suppressing FtsZ assembly kinetics, curcumin can inhibit bacterial cell proliferation (D. Zheng et al., 2020). It can also interfere with DNA replication and transcription, resulting in decreased microbial growth. Studies have shown that curcumin can reduce bacterial gene expression, block the bacterial DNA damage response known as the SOS response, and interact with DNA molecules to accomplish bacteriostatic effects. Curcumin inhibited the reactivation of *Salmonella typhimurium* and *E. coli* phages by acting as an impactful SOS inhibitor (Rudrappa & Bais, 2008). Curcumin exhibits greater effectiveness in inhibiting the growth of Gram-positive bacteria compared to Gram-negative bacteria. The minimum concentration of curcumin needed to inhibit the growth of multidrug-resistant strains of *Staphylococcus aureus, S. haemolyticus, Escherichia coli,* and *Proteus mirabilis* has a value equal to or greater than 2000 µg/mL (S. Roy et al., 2022).

However, apart from these benefits, there are some limitations and drawbacks to consider. One of curcumin's main drawbacks is its low bioavailability (Jabczyk et al., 2021). This low bioavailability can be attributed to several factors. One is that curcumin is quickly metabolized by enzymes in the liver and intestinal wall, limiting its absorption. Another disadvantage is that curcumin is relatively insoluble in water, making it difficult for the body to absorb (Y. Chen et al., 2020). Several strategies have been developed to improve curcumin bioavailability. These include combining curcumin with other compounds that improve absorption or using formulations that improve solubility, such as liposomes or nanoparticles (X. Chen et al., 2015). Curcumin also has the disadvantage of being unstable and degrading quickly under certain conditions. It can degrade when exposed to light or heat, reducing its effectiveness (Rafiee et al., 2019; M. Zheng et al., 2015).

1.3.2 Application of Curcumin as Food Packaging Material

Recently, researchers have explored the possibility of utilizing curcumin as a food packaging material due to its unique antimicrobial and antioxidant properties.

Curcumin's ability to counteract various types of bacteria and fungi can be beneficial in inhibiting the growth of microorganisms in food products. Furthermore, its potent antioxidant properties help prevent lipid oxidation during food storage. In addition, curcumin has been demonstrated to possess favorable mechanical attributes, which are important characteristics of packaging materials, when it is embedded in a polymer matrix (S. Roy et al., 2022). Different techniques are commonly employed to produce curcumin-incorporated films, including solution casting, mold compression, and extrusion. Liu et al. (2016) developed chitosan blend films using solution casting. The molecular simulation results demonstrated that curcumin and chitosan exhibited superior compatibility due to hydrogen bonding. Compared to pure chitosan films, the thermal properties and X-ray diffraction patterns of the curcumin-incorporated films revealed a modification in structure and decreased crystallinity. The blend films had higher tensile strength and hydrophilicity. Curcumin inclusion in the films also resulted in encouraging antibacterial effects against *Staphylococcus aureus* and *Rhizoctonia solani.* In a study by Xie et al. (2021), the impact of curcumin on a composite film made of pectin and chitosan was examined. The addition of curcumin improved the barrier and mechanical properties of the film, while also demonstrating exceptional antioxidant activity in the resulting pectin/chitosan/curcumin film. The efficacy of this film was evaluated in pork packaging, and the findings revealed that the film exhibited antimicrobial properties against fresh pork and increased the shelf-life. Roy & Rhim (2020c) produced a gelatin/curcumin composite film by incorporating an emulsifier, sodium dodecyl sulfate. The outcome of the study revealed that curcumin was uniformly dispersed in the polymer matrix, resulting in a flexible film with a smooth surface. The mechanical and water vapor barrier characteristics of the film were greatly enhanced with the addition of curcumin, up to 1 wt%. Additionally, the gelatin/curcumin composite films displayed noteworthy antibacterial effects against foodborne pathogenic bacteria, specifically *E. coli* and *L. monocytogenes*, and strong antioxidant activity equivalent to ascorbic acid. In another report, Rachtanapun et al. (2021) investigated the influences of curcumin extract on chitosan film

characteristics. The findings indicate that incorporating curcumin into films made of chitosan raises light barrier properties. Analysis revealed interactions between the chitosan and the phenolic compounds of the extract, possibly having enhanced the mechanical properties and decreased the films' moisture content, water solubility, and water vapor barrier property. As the concentration of the curcumin extract increased, the films exhibited enhanced antioxidant activity.

1.4 Factors Affecting Properties of Active Film

A variety of factors can influence the properties of an active film. These factors can include the active ingredient's type and concentration, the matrix material used, the production process, and the environmental conditions to which the film is subjected. The active ingredient and its concentration can affect the film's efficacy, while the matrix material can affect the mechanical, barrier, and thermal properties (Tarique et al., 2021). The process parameters used during film production can also influence the structure and morphology of the film. These parameters include temperature and pH, which affect the stability and durability of the active film (Erna et al., 2022; X. Zhang et al., 2020).

1.4.1 Temperature

Solvent casting is a popular technique for creating active packaging materials (Siemann, 2005). The active ingredients, such as antimicrobial agents or antioxidants, are dissolved in a solvent before being cast onto a surface, which will form a film in the presence of a support polymer. Temperature influences the active ingredients' solubility, the solution's viscosity, the solvent evaporation rate of the active ingredients, the viscosity of the solution, and the rate of solvent evaporation during the solvent casting (M. Lee et al., 2009; Yang et al., 2010). Higher temperatures can make the active ingredients more soluble in the solvent. However,

if the temperature is too high, the solvent may evaporate too quickly, resulting in uneven distribution of the active ingredients.

Some active agents used in active packaging may lose effectiveness as temperatures rise. Degradation is one of the most significant effects of temperature on active agents. Curcumin is susceptible to degradation at high temperatures, especially when exposed to light, oxygen, or moisture (B. Zheng & McClements, 2020). Exposure to high temperatures can cause the active packaging to lose its antioxidant and antimicrobial activity. Generally, curcumin has been reported to degrade at temperatures above 200°C, with some studies indicating a range of 220-280°C (Esatbeyoglu et al., 2015). Curcumin stability in an LDPE film at different temperatures (4°C, 25°C, and 37°C) over time was examined. Curcumin concentration gradually decreased at all temperatures, with a faster degradation rate at higher temperatures (Zhai et al., 2020). Temperature can also influence the release of curcumin from active packaging. Higher temperatures can cause curcumin to be released from the packaging material more quickly and extensively. In a study, it was investigated of antioxidant activity and release kinetics of curcumin from tara gum/ polyvinyl alcohol active film at different temperatures (25°C, 35°C, and 40°C), and it was revealed that higher temperatures accelerated curcumin release (Ma et al., 2017).

Lower temperatures, on the other hand, can reduce the solubility of the active ingredients. This results in the formation of clumps or aggregates of the active ingredients. Lower temperatures also can slow the solvent evaporation rate, resulting in a more even distribution of the active ingredients on the film (F. A. G. S. Silva et al., 2020). As a result, the optimal temperature to produce an active package is determined by the specific active ingredients and solvent used, as well as the desired final product properties.

1.4.2 pH

The pH of a film-forming solution could influence the mechanical strength, water solubility, and permeability of the film (Jancikova et al., 2020). The pH of the film-forming solution could also affect its charge, which influences the interactions between carbohydrate molecules and other solution components. The research examined how different solution pH affects the properties of pullulan-chitosan films. Chitosan molecules were found to have an elongated conformation at pH 4.0, which promoted stronger intermolecular interactions with pullulan than their more tightly coiled structure. As a result, the film had higher tensile strength and barrier properties, as the film-forming solution became viscous (Li et al., 2015). Furthermore, the pH of a chitin-based film-forming solution could have a considerable impact on the properties of the films. Chitin becomes more soluble at lower pH, which leads to greater crosslinks and polymer degradation. However, the chitin molecules deacetylate at higher pH levels, converting into chitosan, which has different properties than chitin. As a result, the ideal pH for chitin-based film-forming solutions is determined by the desired properties of the resulting films. Chitin starts to deprotonate at pH values higher than 6, which leads to increased solubility and swelling. Since the increased solubility enables chitin's dissolution in solvents, pH range of 6-7 is suitable for producing chitin-based films with good mechanical and barrier properties (J. C. Roy et al., 2017).

1.5 Objectives of The Study

Millions of packaged foods are discarded every year, and this number continues to rise. This food waste has detrimental effects on natural resources and causes numerous ethical and economic issues. In addition, waste from conventional fossil-based and non-biodegradable packaging materials contributes to global environmental degradation. These issues can be resolved by extending the storage life of products and utilizing environmentally friendly packaging materials. CGCs

extracted from cultivated mushrooms are abundant, renewable, and biodegradable. Use of such biomaterials is in line with the objective of reducing environmental pollution and promoting sustainable packaging practices. Chitin has good filming forming ability and barrier properties, including resistance to water vapor, oxygen, and ultraviolet radiation. When incorporated into packaging films, chitin complexed with glucan can help preserve the quality and freshness of packaged food by minimizing the penetration of moisture and oxygen, thereby extending shelf life. It is simple to combine CGCs with other bioactive substances, such as curcumin, to produce composite films with enhanced functionalities. Curcumin is derived from turmeric. It is considered a safe and natural substance, making it suitable for food packaging. Using curcumin in packaging corresponds with the rising demand for eco-friendly and natural ingredients across multiple industries. It has antimicrobial and antioxidant properties. By integrating curcumin into packaging materials, it is possible to reduce the risk of foodborne illnesses and inhibit the growth of microorganisms. As a hydrophobic material, curcumin-containing active films are commonly used to prevent microbial growth in meat and poultry products. In addition, as a potent antioxidant, it helps reduce lipid oxidation in foods.

In the literature, numerous studies use curcumin as an active ingredient to prevent microbial spoilage and prolong shelf-life. However, there are limited studies on the extraction of CGCs from cultivated mushroom *(Agaricus Bisporus*) and its use as a film-forming material. In addition, the application of biodegradable nanocomposite films containing curcumin to food samples is limited. Therefore, the objective of this study is to fabricate curcumin incorporated CGCs nanocomposite active films and to evaluate physical, morphological, microbial and antioxidant properties of films, and monitor its effect on the shelf-life of fresh chicken samples.

CHAPTER 2

MATERIAL AND METHOD

2.1 Material

White button mushrooms, *Agaricus Bisporus*, were purchased from a local market (Ankara, Turkey). Curcumin (1,7-Bis [4-hydroxy-3- methoxyphenyl]-1,6-heptadiene-3,5, dione), gallic acid, plate count agar, and DPPH (2,2-diphenyl-1-picrylhydrazyl) were obtained from Sigma-Aldrich Chemical Co. (St. Louis, MO, USA). Sodium hydroxide, ethanol, sodium carbonate anhydrous, hydrochloric acid fuming (37 %,) and glycerol (99 %) were purchased from ISOLAB (Wertheim, Germany). Folin-Ciocalteu reagent and nutrient broth were provided by Merck (Darmstadt, Germany). Baird Parker agar and chromogenic E. coli coliform agar were obtained from DiaTek (Diagnostic Products and Technical Consultancy, Turkey).

2.2 Methods

2.2.1 Preparation of Film

2.2.1.1 Chitin Extraction from Cultivated Mushroom

Extraction was performed using the hot alkaline treatment method reported by Fazli Wan Nawawi et al. with slight modifications (Fazli Wan Nawawi et al., 2019). 150 g of frozen mushrooms were thawed and washed with distilled water to remove any coarse impurities. The mushrooms were blended with a blender (Arçelik K 1252 HBS Cheffo 400 W, Istanbul, Turkey) for 5 min to increase the surface area. Distilled water was added to the blended mushroom until the total volume was 600 mL. The

solution was stirred by a magnetic stirrer (MaxTir 500, Daihan Scientific, Seoul, Korea) at 500 rpm and 85 ◦C for 30 min to remove water-soluble substances. The suspension was centrifuged (Nuve-Bench Top Centrifuge, NF 1200R, Turkey) at 8000 rpm for 20 min. Afterward, the obtained pellet was soaked and stirred in an alkaline solution (450 mL of 1 M NaOH) at 500 rpm and 65 ◦C for 3 h to deproteinize and remove alkaline-soluble substances. The solution was then centrifuged at 8000 rpm for 10 min, and the precipitate was rinsed with distilled water several times. Later, the precipitate was suspended in 400 mL of distilled water, and the pH of the solution was adjusted to 7.00 ± 0.02 by using 2 M HCl. The solution was centrifuged at 8000 rpm for 10 min, and then the supernatant was discarded. Next, the pellet was freeze-dried (Christ Alpha 2–4 LD Plus, Martin Christ, Germany) and stored at 4 ◦C before use.

2.2.1.2 Preparation of Chitin Glucan Complex (CGC) Nanocomposite Film with Curcumin

Active CGC nanocomposite films were prepared by using the solvent casting method. Firstly, the neutral pellet was resuspended in DI water (50 % w/v) using a high-speed homogenizer (IKA T18 Digital Ultra Turrax, Staufen, Germany) at 22,000 rpm for 1 min. This suspension was then mixed with different concentrations of curcumin (0, 0.2, 1, and 2 wt% on a pellet basis) and glycerol (25 % wt on pellets) dissolved in ethanol. The solution without curcumin addition was used to fabricate the control films. The film-forming solutions were prepared using a high-speed homogenizer (IKA T18 Digital Ultra Turrax, Staufen, Germany) at 22,000 rpm for 5 min to get a homogenous solution. Later, solutions were placed into an ultrasonic bath (ISOLAB; Laborgerate GmbH, Wertheim, Germany) at 37 kHz for 20 min to remove the air bubbles in the solutions. 35 mL of the final film-forming solutions were poured into Teflon plates with a diameter of 14 cm and allowed to dry at 40 ◦C in a drying oven (Binder ED 56, Tuttlingen, Germany) for 36 h. The dried films were peeled off from the plates and kept in a ventilated climate chamber (TK120, Nuve,

Ankara, Turkey) at 25 ◦C with 50 % relative humidity (RH) for 48 h. The prepared films containing 0, 0.2, 1, and 2 wt% of the dried pellet were designated as CGC/Cu0, CGC/Cu1, CGC/Cu5, and CGC/Cu10 films, respectively.

2.3 Characterization of Film

2.3.1 Light Barrier Properties

Light barrier properties of films were investigated by using a UV-Vis spectrophotometer (Optizen UV/Vis spectrophotometer, Mecasys Co. Ltd., Seoul, South Korea). The films of 4 cm×1 cm size were placed in quartz cuvettes. The absorbance at 600 nm was recorded, and the light transmittance value was calculated. The measurements were replicated three times.

2.3.2 Total Phenolic Content (TPC)

Total phenolic content (TPC) assay was performed following the procedure reported by Yildiz, Bayram, et al. (2021) with slight modifications**.** The film of 0.5 g was cut into smaller pieces and mixed with a methanol solution (10% v/v). The mixture was homogenized with a high-speed homogenizer (IKA T18 Digital Ultra Turrax, Staufen, Germany) at 22,000 rpm for 10 min. Afterward, it was centrifuged (Nuve-Bench Top Centrifuge, NF 1200R, Turkey) at 8000 rpm for 20 min. 0.5 mL was taken from the collected supernatant to mix with 2.5 mL 0.2 N Folin-Ciocalteu reagent. Later, the mixture was kept in the dark for 5 min. Then, 2 mL sodium carbonate solution (7.5% w/v) was added and allowed to stand for 2 h in the dark at room temperature. After storage, the absorbance values were measured at 760 nm by UV-vis spectrophotometer (Optizen UV/Vis spectrophotometer, Mecasys Co. Ltd., Seoul, South Korea). A gallic acid calibration curve was prepared in methanol solution (10% v/v) and the total phenolic content of the films was reported as mg

gallic acid equivalent (GAE). A standard curve was generated with the following equation:

$$TPC\ \left(\frac{mg\ GAE}{g\ film}\right) = \frac{C \times V \times D}{W} \qquad 1$$

where C was the concentration obtained from the calibration curve (mg/L), V was the volume of film solution (L), D was the dilution rate, and W was the weight of film (g). The calibration curve is given in Appendix (Figure A.1).

2.3.3 DPPH Free Radical Scavenging Activity

The antioxidant activity of the films was determined according to the method described by Yildiz, Sumnu, et al. (2021) with some modifications. 0.5 g of the film was dissolved in methanol solution (90% v/v) and then, centrifuged (Nuve-Bench Top Centrifuge, NF 1200R, Turkey) at 8000 rpm for 20 min. 0.2 mL of the diluted film extract was mixed with 0.1 mmol 2,2-diphenyl-1-picrylhydrazyl (DPPH) solution. The mixture was kept in the dark for 30 min at room temperature and the absorbance value was measured at 517 nm by using a UV-Vis spectrophotometer (Optizen UV/Vis spectrophotometer, Mecasys Co. Ltd., Seoul, South Korea). The measurements were replicated three times. DPPH radical scavenging activity was evaluated by using the following equation:

$$DPPH\ Radical\ Scavenging\ Activity\ (\%) = \frac{Abs_{DPPH} - Abs_s}{Abs_{DPPH}} \times 100 \qquad 2$$

where Abs_{DPPH} was the absorbance of the DPPH solution and Abs_s was the absorbance of the film samples

2.3.4 Mechanical Properties

Tensile strength (TS) and elongation at break values (EB) of films were measured using a Zwick/Roell testing machine (Zwick/Roell Z250 UTM, Germany) according

to the ASTM D 882-09 standard method. The dumbbell-shaped films (4 cm×0.4 cm) were prepared with a manual cutting press (ZCP 020, Zwick GmbH&Co., Ulm, Germany) and before testing the samples were conditioned at 25 ℃ with 50% RH for 48 h. 20 mm grip separation and 0.3 mm/s crosshead speed was set to perform the test. The measurements were replicated three times.

TS and EB values were calculated by the following equations:

$$TS(MPa) = \frac{F_{max}}{A} \qquad EB(\%) = \frac{\Delta L}{L_0} \times 100 \tag{3}$$

where F_{max} was peak load (N), A was the cross-sectional area of the film (mm^2), ΔL was the change in length at the breaking point (mm), L_0 was the initial length of the film (mm)

2.3.5 Water Solubility

The solubility of the films was measured according to the method of (Gontard et al., 1994). The 2 cm diameter films were cut, and their weight was measured. Then the films were kept in a drying oven (BLULAB BKHS KP 550, Turkey) at 105℃ for 24 h to reach constant weight. The dried films were weighed to calculate the initial moisture content of films by the following equation:

$$MC = \frac{W_i - W_f}{W_i} \tag{4}$$

where W_i was the initial weight of films, W_f was the weight of films after drying at 105 ℃, MC was the initial moisture content of films.

Meanwhile, films were cut into square pieces of 2 cm × 2 cm and weighed to determine water solubility. The films were soaked in 50 mL of distilled water and stirred by a magnetic stirrer at 120 rpm for 24 h. After filtration, the filtrates were dried at 105 ℃ for 24 h. The final weight of dried films was recorded. The experiment was performed triplicate. Water solubility was calculated by the following equation:

$$WS(\%) = \frac{W_i \times (1-MC) - W_f}{W_i \times (1-MC)} \times 100 \quad 5$$

where W_i was the initial weight of 2 cm × 2 cm films, and W_f was the weight of 2 cm × 2 cm films after drying at 105 ℃.

2.3.6 Water Vapor Permeability (WVP)

WVP values of the films were measured by using the standard method E96-95 ASTM reported by McHugh et al. (1993). Cylindrical permeability cups with a 40 mm internal diameter were filled with 35 mL of distilled water to reach 100% RH inside the test cups. The thickness of the films was measured from five different points with a digital micrometer (LYK 5202, Loyka, Ankara, Turkey). The films were sandwiched between the cups and the lid rings of the cups. The initial weight of the cups was recorded. The cups were placed into a silica-gel-filled desiccator with 1% RH. The weight of the samples, temperature, and RH in the desiccator was recorded by using a temperature logger (LOG200, Dostmann electronic GmbH, Wertheim-Reicholzheim, Germany) for 10 hours at 1-hour intervals. The WVP was calculated as g/m.s.Pa (McHugh et al., 1993).

The WVP was calculated by the following equation:

$$WVP\ (g/Pa \times s \times m) = \frac{S * x}{A \times P \times (R_i - R_d)} \quad 6$$

where S was slope (change in film weight over time), x was film thickness (m), A was area (m^2), P *was* saturated water vapor pressure (Pa), R_i was initial relative humidity of test cups, and R_d was relative humidity in the desiccator.

2.3.7 Evaluation of Antimicrobial Activity

The antimicrobial activity of films was evaluated by following the disk diffusion assay against *Escherichia coli (E. coli)* and *Staphylococcus aureus (S. aureus).* The film discs of 2 cm diameter were prepared, and then the surface of the films was

sterilized by UV light exposure at 254 nm for 5 min. *S. aureus* and *E. coli* were cultured overnight in the nutrient broth (Merck, Darmstadt, Germany) at 37ºC. They were diluted to 0.5 McFarland standard (equivalent to 3×10^8 CFU/mL), which is in a range of 0.1 and 0.2 absorbance values at 625 nm (Akman et al., 2019; Sohbatzadeh et al., 2010). 0.1 mL of inoculums was plated on the selective agars, and the film samples were placed on inoculated agars. Selective chromogenic E. coli coliform agar for *E. coli* and Baird Parker agar for *S. aureus* were used. They were incubated at 37°C for 24 h. The antimicrobial activity of the films was determined by measuring the size of the inhibition zones and reported in mm.

2.3.8 Microbiological Evaluation of Film on Fresh Chicken Breast

The effect of CGC/Cu10 film on the shelf-life extension of the chicken breast sample was determined by using the method reported by Konuk Takma & Korel (2019) with some modifications. 10 grams of fresh chicken samples were prepared in aseptic conditions and wrapped with the active film sample of CGC/Cu10. Control chicken samples were covered with the cling film to prevent direct exposure of the sample to air. The actively packed chicken samples and cling film-packed control chicken samples were kept in a refrigerator at 4°C for 10 days. Every day both chicken samples of actively packed and cling film packed were homogenized in 90 mL distilled water using a stomacher (WES-400, WiseMix®; Daihan, Wonju, Korea) for 2 min. A series of decimal dilutions were performed from 10^{-1} to 10^{-6} with nutrient broth. Plate count agar (Sigma-Aldrich Chemical Co., St. Louis, MO, USA) was used with the pour plate method.1 mL of each dilution was poured plated on Plate Count Agar, and the inoculated plates were incubated at 30 °C for 48 h for total bacteria count and at 4 °C for 7 days for psychotropic bacteria aerobically. The results were expressed as log colony forming unit per gram sample (log CFU/g).

2.3.9 Biodegradation Test

The biodegradability assessment of each film in soil was conducted by following the procedure described by da Silva Filipini (2020) with slight modifications. The soil was poured into plastic containers with an 11 cm internal diameter and 10 cm height. The film samples were cut into square pieces of 2 cm×2 cm and placed into a support material to locate them easily during the measurements. Then, they were buried in the soil until fully covered. The containers were stored at room temperature ($23^{o}C$) and hydrated with 20 mL of water every other day. The results were obtained by taking weekly images of the films for 2 weeks.

2.3.10 Microstructural Analysis of Active Films

The surface morphology of films was evaluated by using Scanning Electron Microscopy (SEM) (Quanta 400 F, USA). Film samples were coated with a thin layer of gold-palladium, then cross-section and surface images were collected. To monitor the surface chemical bond interaction and changes, an analysis of films was carried out by the attenuated total reflection.

2.3.11 Analysis of Functional Groups in Active Films

Fourier transform infrared spectroscopy (ATR-FTIR) (IRSpirit FTIR Spectrometer, Shimadzu, Japan). The results were recorded from 4000 to 600 cm^{-1} at a resolution of 16 cm^{-1} with 32 scans.

2.3.12 Structural Analysis of Active Films

The crystalline structure of curcumin and films was determined using an X-ray diffractometer (XRD) (Rigaku Ultima IV X-ray diffractometer, Japan). Film samples were prepared by cutting into 1 cm×1 cm square shapes. It was operated at room

temperature, from 5^0 to 40^0 (2θ) with 0.02/30 s speed. The crystalline index (CrI; %) was estimated using Eq. (6) according to the literature (Hassainia et al., 2018).

$$CrI\ (\%) = \frac{I_{110} - I_{am}}{I_{110}} \times 100 \qquad 7$$

where I_{110} is the maximum peak intensity at 2θ ≅ 20◦, which represents the diffraction of the (110) lattice, and I_{am} is the minimum intensity at 2θ.

≅ 16◦, which corresponds to the amorphous region.

2.3.13 Porosity Determination

Porosity tests were accomplished by using a mercury porosimeter (Quantachrome Corporation, Poremaster 60, Austria). The surface tension of mercury and the contact angle was set as 0.48 N/m and 140°, respectively.

2.3.14 Thermal Analysis of Active Films

Thermal behaviors of films were analyzed by a Thermogravimetric Analyzer (TGA) (SDT 650, TA Instruments, New Castle, USA) at a range from 40 ℃ to 600 ℃ with a 10℃/min heating rate under an inert atmosphere (flow rate 20 mL/min).

2.3.15 Statistical Analysis

All experiments were conducted in triplicate. Collected data were analyzed using the statistical analysis software (MINITAB, version 20, State College, PA, USA) with ANOVA. If a significant difference was determined, Tukey's Multiple Comparison tests were used. Statistical significance was taken for the differences where the p-value was less than 5% ($p<0.05$).

CHAPTER 3

RESULTS AND DISCUSSION

3.1 Microstructural Analysis of Active Films

FESEM analysis was performed to evaluate the surface, and cross-sectional morphologies of CGC nanocomposite films, and the images were presented in Fig. 3.1. In the CGC/Cu0 control films, a more agglomerated surface structure was observed, which led to the formation of a denser structure (Fig. 3.1a). When curcumin was added to the CGC nanocomposite film, the surface of the film changed dramatically. The agglomerates on the film surface steadily diminished as the curcumin content increased, resulting in a smoother surface. The agglomerates on the surface entirely disappeared at the CGC/Cu10 film with the highest curcumin concentration, and a fully smooth surface formed (Fig. 3.1d). In a previous study by Hassainia et al. (2018), SEM images of chitin derived from *Agaricus bisporus* gills revealed a similar structure with a macroporous morphology, accompanied by the presence of granules at the micro-scale. The porous microstructure might give additional pathways for gas or liquid material diffusion, which can assist in minimizing the overall rate of migration, as well as more surface area or volume for these materials to interact with the film. In other words, this structure might be expected to provide the film with good barrier properties and encapsulation capacity.

The cross-sectional images of the films are shown in Fig. 3.1e, 3.1f, 3.1g, and 3.1h. The presence of whitish globular structures was detected in CGC/Cu0, CGC/Cu1, and CGC/Cu5 films. These globular structures can be linked with the β-glucan, which is found by covalently bonded to chitin in the wall of the *Agaricus bisporus*. Since harsh chemicals are not used to remove glucan during chitin extraction from white button mushrooms, it can be expected to observe glucan in the film structure. In the literature, SEM analysis of white button mushrooms treated with alkali

revealed a network shape comparable to that of the CGCs (H. Kim et al., 2021). However, in these images, chitin nanofibers were not observed. The reason for this can be explained as the use of whole mushrooms containing significant amounts of glucan in the production of the films (Fazli Wan Nawawi et al., 2019). The addition of curcumin to the films changed the size of these glucan particles significantly. When the curcumin concentrations increased in the nanocomposite films, CGC started to become smaller, and finally, it completely disappeared (Fig. 3.1h.) The phenomenon described above may be attributed to the progressive substitution of indigenous inter- and intramolecular hydrogen bonding contacts inside curcumin-loaded glucan complexes (CGCs) with intermolecular interactions, notably hydrophobic and hydrogen bonding, between curcumin and glucan following encapsulation of curcumin within the glucan matrix. (H. Kim et al., 2021; Le et al., 2016). Curcumin has the potential to occupy glucan pores and form a complex with glucan molecules. An increase in curcumin concentration resulted in the generation of a glucan-curcumin complex with enhanced glucan-curcumin interaction while concurrently leading to a reduction in the porosity of the glucan structure. Previous studies have shown the efficacy of using glucan particles as a carrier for curcumin. (Plavcová et al., 2019).

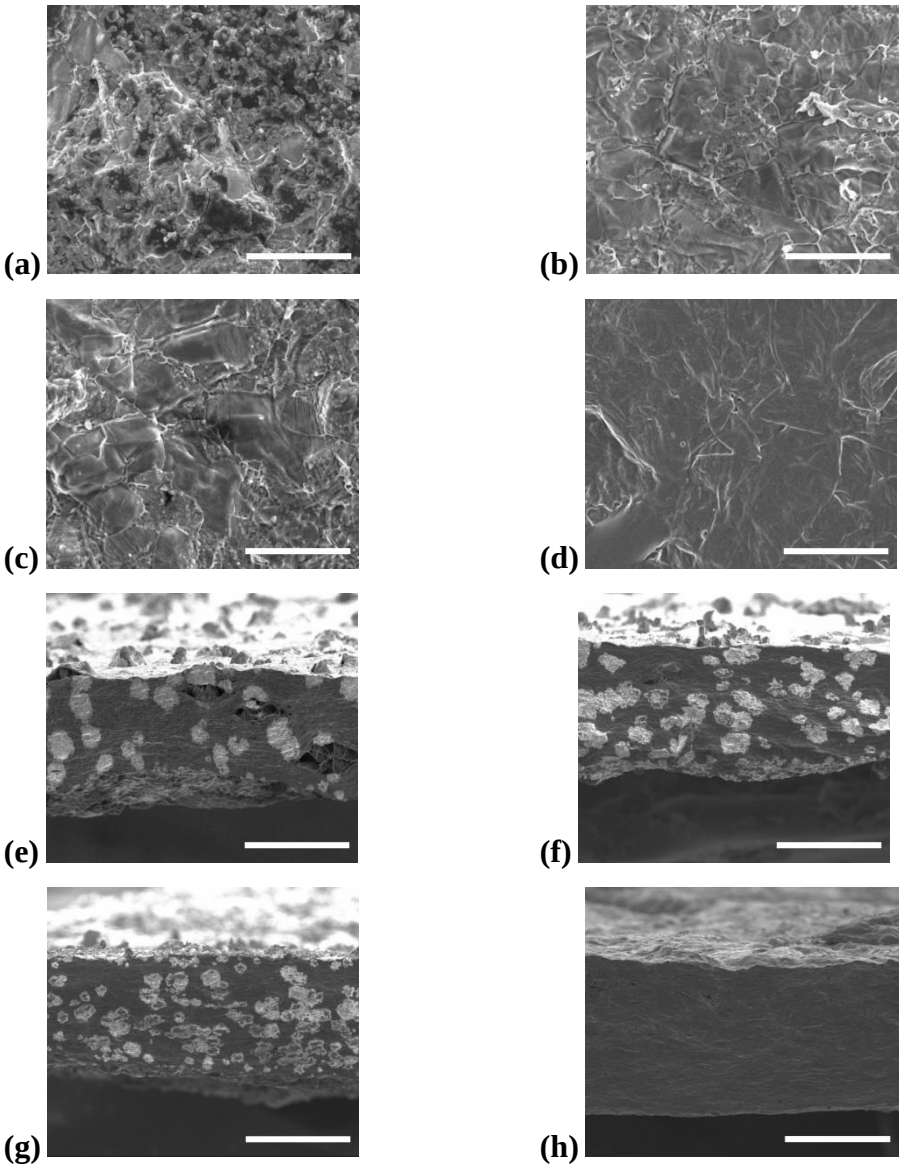


Figure 3.1. FESEM images of the surface morphologies for (a) CGC/Cu0, (b) CGC/Cu1, (c) CGC/Cu5, and (d) CGC/Cu10 films (scale bars: 30 μm) and images of cross-sectional morphologies for (e) CGC/Cu0, (f) CGC/Cu1, (g) CGC/Cu5, and (h) CGC/Cu10 films (scale bars: 100 μm).

3.2 Analysis of Functional Groups in Active Films

FTIR analysis is conducted to analyze the chemical composition of the films since it identifies the existence of numerous functional groups in the film structure. The FTIR spectra of CGC nanocomposite films and curcumin-incorporated CGC

nanocomposite films are illustrated in Fig 3.2. All film samples had a broader peak compared to commercial chitin between 3150 and 3450 cm^{-1} associated with O–H stretching (Hassainia et al., 2018). This broad peak can be explained by the existence of glucan in the chitin structure, as glucan forms additional hydroxyl groups (Fazli Wan Nawawi et al., 2019; Nawawi et al., 2020). Moreover, the presence of an amorphous structure in the films might result in the appearance of broad O–H stretching peaks since amorphous substances often have hygroscopic properties. (Mikhailov et al., 2009).

Hygroscopicity refers to a material's capacity to absorb moisture from its surroundings. Similarly, it was observed that chitin nanofibers derived from *Agaricus bisporus* displayed broader O–H stretching peaks compared to commercially available chitin (Ifuku et al., 2011). On the other hand, this broad O–H stretching peak prevented the identification of the chitin's characteristic N–H stretching peak at 3270 cm^{-1}. Furthermore, the spectral analysis revealed different peaks in all films. The peak between 1620 and 1670 cm^{-1} was identified as amide I, specifically associated with C—O stretching vibrations. The peaks observed at 1550 cm^{-1} and 1000 cm^{-1} were attributed to amide II, which corresponds to N–H bending vibrations and C–O stretching, respectively. FTIR spectrum of commercial curcumin powder was also presented in Fig 3.2. The peak at 3509 cm^{-1} displays the existence of phenolic hydroxyl groups in the curcumin molecule. At 1626 cm^{-1}, the peak corresponds to aromatic C-C stretching vibrations, defining the connectivity of carbon atoms in the aromatic ring structure. The peak at 1602 cm^{-1} and 1506 cm^{-1} indicates the stretching vibrations of the benzene ring and the presence of C-O and C-C stretching vibrations, respectively. Moreover, the peaks observed at 1427 cm^{-1}, 1271 cm^{-1}, and 1151 cm^{-1} correspond to olefinic C-H bending vibrations, indicating the presence of hydrogen-carbon bonds in the olefinic region of curcumin, aromatic C-O stretching vibrations, indicating the involvement of oxygen atoms within the aromatic ring, and C-O-C stretching, respectively (X. Chen et al., 2015; Tiwari et al., 2017). Incorporating curcumin into the CGC nanocomposite films resulted in insignificant changes in the locations and intensities of the observed peaks. This

finding is consistent with the study conducted by Rotrekl et al. (2020), which found that encapsulating curcumin with glucan particles did not lead to significant changes in the FTIR spectrum compared to pure glucan particles. As a result, it can be concluded that adding curcumin did not form new functional groups on the film surface. Instead, it can be suggested that curcumin promotes physical interactions rather than chemical interactions inside the nanocomposite film matrix.

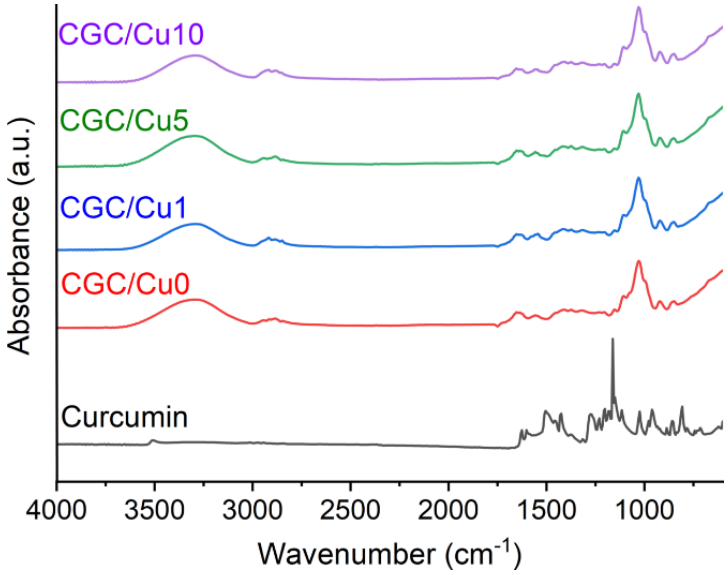


Figure 3.2. FTIR spectra of curcumin, CGC/Cu0, CGC/Cu1, CGC/Cu5 and CGC/Cu10 films.

3.3 Structural Analysis of Active Films

XRD has the potential to offer helpful information on the crystallinity as well as the molecular structure of the active components within the film. XRD can detect the presence of crystalline regions in the structure of a film by quantifying the diffraction pattern of X-rays passing through it. The arrangement and order of the crystalline elements within the film can be determined by the intensity and location of the diffraction peaks (Bajpai et al., 2015). Figure 3.3. displays the XRD patterns of curcumin and CGC nanocomposite films with varying concentrations of curcumin. The XRD analysis of the curcumin sample showed specific crystalline peaks at 8.04°, 9.01°, 12.34°, 14.67°, 17.44°, 23.62°, 24.76°, 25.80°, and 29.24°. The

presence of distinct peaks in the curcumin sample indicates that it was in the crystalline form. Notwithstanding the low resolution, the CGC/Cu0, CGC/Cu1, and CGC/Cu5 films exhibited a noticeable peak at approximately 20°, a characteristic chitin peak (Kumari & Kishor, 2020). Broad structures observed in the XRD patterns of the CGC/Cu0, CGC/Cu1, and CGC/Cu5 films were likely attributable to the existence of amorphous chitin, which is in line with the XRD patterns reported in CGC that originate from mushrooms and yeast (Farinha et al., 2015; Hong & Ying, 2019; H. Kim et al., 2021). However, these films did not exhibit any distinctive crystalline peaks of curcumin. No appearance of a curcumin peak might be due to the encapsulation of curcumin within amorphous glucan structures (Nieto-Suaza et al., 2019). Particularly, the absence of a distinctive peak for crystalline β-glucan at $2\theta = 6°$ endorses the idea that β-glucan in the CGC films is found in an amorphous state (Hassainia et al., 2018). Notably, the CGC/Cu10 film exhibited two additional peaks at 9.01° and 14.07°, showing the presence of crystallinity. At 9.02° and 14.66°, these peaks corresponded to curcumin's characteristic crystalline diffraction. These peaks indicate that the glucans in the film structure could not completely encapsulate excess curcumin. CGC/Cu0, CGC/Cu1, CGC/Cu5, and CGC/Cu10 films had respective crystallinity indices (CrI) of 64.5%, 55.7%, 56.8%, and 54.5%. Although the CrI of the CGC/Cu0 control films approached the published value for chitin derived from *A. bisporus* stipe (63.2%), it was observed that the CrI values of all film samples were significantly lower compared to chitin sourced from shrimp (85%) (Hassainia et al., 2018; H. Kim et al., 2021). The lower CrI values observed are due to amorphous β-glucan in the CGC, resulting in a lower degree of crystallinity in chitin compared to that seen in crustaceans. The incorporation of curcumin into the film matrix led to a further reduction in film crystallinity, leading to a drop in the density of polymer chains. This may be due to the formation of new intermolecular interactions between curcumin and biopolymer, which impede the crystallization process of the complex system, thus leading to a decrease in its crystallinity. (Jiang et al., 2020). Furthermore, curcumin can disrupt the crystalline structure of the film matrix, which will result in a lower crystallinity index.

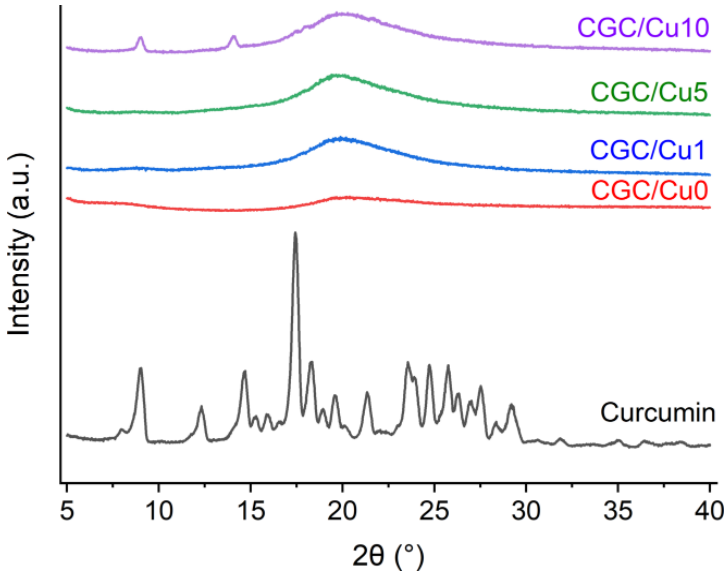


Figure 3.3. XRD patterns of curcumin, CGC/Cu0, CGC/Cu1, CGC/Cu5 and CGC/Cu10 films

3.4 Porosity of Active Films

The porosity values of each film are shown in Table 3.1. it was observed that the inclusion of curcumin into the CGC nanocomposite film caused a significant decrease in porosity, from 17.05% to 0.95% *($p < 0.05$).* The observed decrease in porosity as curcumin concentration increases may be attributed to the encapsulating of curcumin by β-glucan. (Plavcová et al., 2019). As an additional component, curcumin can serve as a filler within the film matrix. It fills the voids between the chitin and glucan complexes, leading to a denser structure with less porosity (Konwar et al., 2015). Furthermore, curcumin can interact with the chitin and glucan complexes, resulting in increased intermolecular adhesion and film cohesion. This improved interaction may reduce the number of voids and result in a reduction in porosity. These results are consistent with the film microstructures observed in the SEM analysis, as shown in Figure 3.1. The SEM images most likely revealed a more compact and dense structure in the curcumin-incorporated films.

Table 3-1. Total porosity values of CGC/Cu films*

Film Samples	Apparent Porosity (%)
CGC/Cu0	17.05±0.028[a]
CGC/Cu1	2.15 ±0.043[b]
CGC/Cu5	1.71 ±0.007[c]
CGC/Cu10	0.95 ±0.014[d]

* The values are presented as mean ± standard deviation. Different letters in the same column indicate significant differences ($p < 0.05$)

3.5 Thermal Analysis of Active Films

TGA is widely used to investigate a material's thermal stability and decomposition behavior. It measures the variations in a sample's weight when it is heated. As shown in Figure 3.4a, the temperature range of 140°C to 220°C detected in the TGA results indicates the first stage of degradation, which can be associated with the thermal decomposition of glycerol. Typical glycerol decomposition reactions include dehydration, fragmentation, and the development of volatile products (Smith et al., 2022). Due to the emission of volatile decomposition products related to glycerol, the TGA curve shows a weight loss in this temperature range (Dou et al., 2009). The second degradation phase occurred between 250°C and 370°C. This degradation step might be explained by the polymer degradation of chitin-based films (H. Kim et al., 2021). This degradation temperature range could differ depending on factors such as the integrity of chitin and the presence of additional elements in the film. However, the observed temperature range in this study is consistent with the reported thermal degradation behavior of chitin-based materials. The decomposition temperatures of chitin and β-glucan derived from fungi were determined to be 309°C and 275°C, respectively. In addition, it was reported that chitin extracted from *A. Bisporus* showed a weight loss between 200°C and 400°C, which is related to the chain degradation of chitin (Hassainia et al., 2018). This range corresponds to the second degradation stage of chitin-based films in this study. In

contrast to CGC films, curcumin did not exhibit moisture loss up to 200°C since it is prevented by the hydrophobic nature of curcumin (Marcolino et al., 2011). This suggests that curcumin is not susceptible to significant water evaporation or degradation at lower temperatures, thereby emphasizing its stability under these conditions. The weight loss of curcumin began at 260°C and accelerated at 400°C. The DTG curves shown in Figure 3.4b presented more details regarding the maximal weight loss rates of the various films. In this study, the addition of curcumin to CGC films caused the decomposition peaks to move to lower temperatures compared to CGC/Cu0 control films. The presence of curcumin may influence the thermal degradation behavior of the films since it has the potential to modify the decomposition kinetics and rate of weight loss. Fernandez-Marin et al. (2022) investigated the properties of smart films comprised of chitosan/chitin nanocrystals with curcuma oil and anthocyanins, and they reported similar findings. The results of these investigations show that the incorporation of curcumin or bioactive substances can influence the thermal behavior and weight loss rates of composite films, probably because of interactions between film components or matrix modifications.

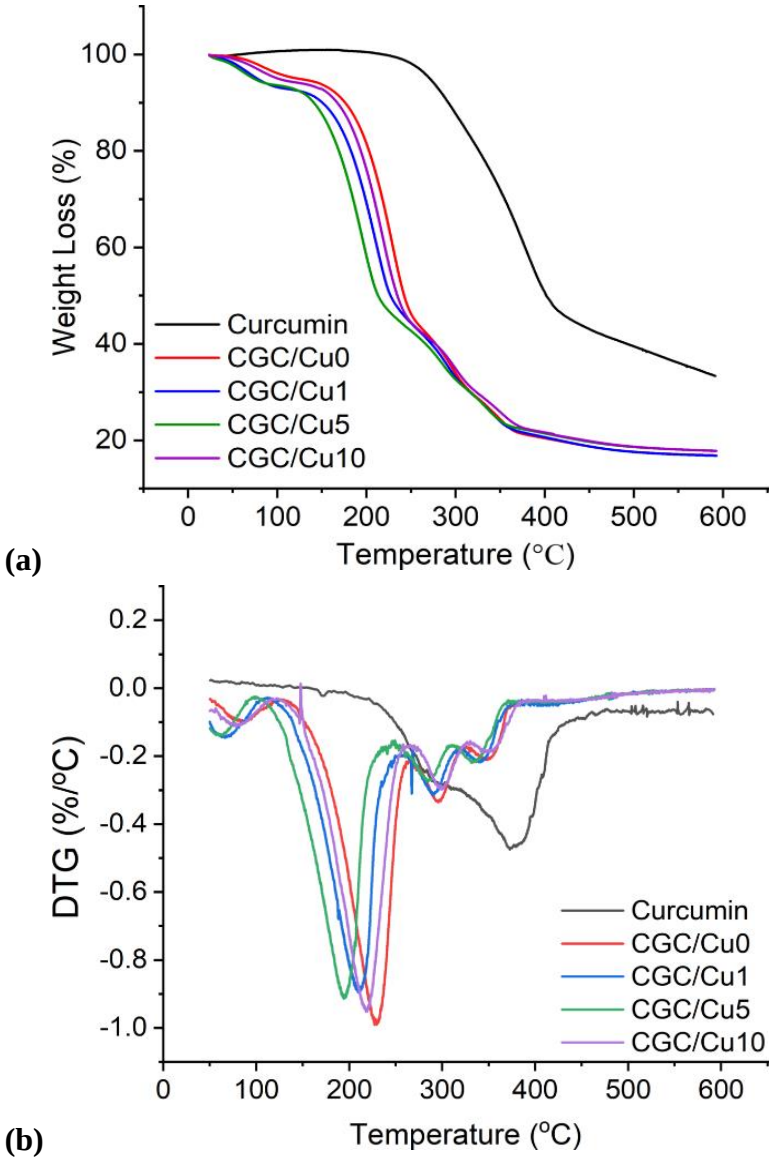


Figure 3.4. (a) TGA thermograms (b) DTG analysis of curcumin, CGC/Cu0, CGC/Cu1, CGC/Cu5 and CGC/Cu10 films.

3.6 Water solubility, Mechanical and Barrier Properties of Active Films

Tensile strength and elongation at break are critical parameters for the effective functioning of biodegradable films. Tensile strength is the maximum force a film can withstand before breaking or fracturing. This characteristic is critical for ensuring the film will not break apart under stress. Elongation at break, on the other hand,

refers to a film's ability to stretch before breaking. This property is essential in applications where the film must conform to irregular shapes or absorb shocks without rupturing.

Table 3.2 presents the results of the TS and EB tests conducted on CGC films incorporated with curcumin. The CGC/Cu0 film showed a TS value of 2.17±0.01% MPa and an EB value of 42.56±0.43%. The results revealed that the TS of the CGC film obtained from mushrooms was lower than that of films made from other natural sources such as crabs, shrimp, and lobsters. Earlier studies in the literature have reported that films obtained from shrimp and lobsters showed TS values within a range of 110 and 150 MPa, whereas surface-deacetylated chitin films derived from crabs showed TS values of 157 MPa (Duan et al., 2013; Wu et al., 2019). Our findings demonstrate the dependence of chitin sources on the strength of CGC films. The presence of residual substances after chitin extraction could cause this strong dependency. Proteins and minerals, for example, may have a significant impact on the mechanical properties of the chitin-based film. In crustaceans, chitin is found to bounded to protein, and after extraction, residual protein is very low (Jones et al., 2020). However, in fungal sources, chitin is covalently linked to glucan, and after the extraction process, it can contain a high amount of glucan (Nawawi et al., 2020). Furthermore, the degree of chitin deacetylation (DA), which refers to the extent to which acetyl groups are removed from chitin molecules, is an important factor that influences the strength of chitin-based films. Chitin with a higher degree of deacetylation forms more stable and stronger films than chitin with a lower degree of deacetylation. Poerio et al. (2020) reported that chitin from mushroom sources had the lowest DA value among different sources. Despite these outcomes, research on the characteristics of chitin from various sources is still being conducted (Azuma et al., 2014). The EB value of the CGC/Cu0 film was 42.56±0.43%. In a previous study, Koc et al. (2020) reported that the chitosan/fungal extract films had a TS value of 2.1 MPa and an EB value of 35%, comparable to the current study's findings.

Curcumin incorporation resulted in an increase in TS and a decrease in EB values at low concentrations, even though these changes were not significant. However, the

film with the highest amount of curcumin showed a significant difference in both TS and EB values ($p < 0.05$) compared to the CGG/Cu1 and CGC/Cu5 films. CGC/Cu10 film had a TS value of 1.25±0.01 MPa and an EB value of 50.72±3.05%. Curcumin may interfere with the chain association of chitin nanofibrils, resulting in a decrease in the density of intramolecular interactions. This interference can reduce the contact between chitin nanofibrils, resulting in decreased fracture resistance and a loss in tensile strength. The presence of high curcumin concentrations can be linked to these results. Moreover, incorporating hydrophobic compounds such as curcumin into the chitin nanofibrils might cause structural discontinuities, leading to decreased chain mobility. This can also contribute to the creation of weak areas in the film structure, which might result in the failure of the material under strain (Souza et al., 2015). Those outcomes were corroborated by the XRD pattern (Figure 3.3.), and cross-sectional SEM images of the nanocomposite films (Figure 3.1e, 3.1f, 3.1g, 3.1h). The analysis of CGC/Cu1 and CGC/Cu5 films revealed that the curcumin molecules were completely encapsulated in the glucan particles, so no significant changes were observed in these films' TS and EB values. However, non-encapsulated curcumin molecules were detected in the CGC/Cu10 film. The excess curcumin molecules accumulated and precipitated outside of the glucan particles, which may cause structural discontinuities in the structure of the film. It's likely that the discontinuous polymer network resulted in an increase in the EB value of the CGC/Cu10 films with the highest curcumin concentration. The increase in film flexibility might be attributed to an increase in the mobility of polymer chains in the presence of these structural interruptions (R. Zhang et al., 2019). As observed in the CGC/Cu10 films in this study, it has been observed in the literature that the addition of hydrophobic substances such as curcumin to the active polysaccharide films reduces the TS values of the films. According to Souza et al. (2015), adding hydrophobic quercetin to chitosan films significantly reduced their TS. Likewise, Rubilar et al. (2013) showed that the TS values of chitosan films containing the hydrophobic agent carvacrol decreased when the concentration of carvacrol increased. The presence of hydrophobic materials disrupts the films' cohesive structure, leading to a decrease in

TS. The reason for this disruption is the incompatibility of the hydrophobic agents with the polysaccharide molecules, which are responsible for the strength and structure of the film. The existence of these agents reduces intermolecular interactions while increasing free volume inside the film matrix. As a result, the films become less cohesive and more prone to breaking under stress by reducing their tensile strength.

Table 3-2. Mechanical properties of CGC/Cu films*

Film Samples	**TS (MPa)**	**EB (%)**
CGC/Cu0	2.17±0.01[a]	42.56±0.43[b]
CGC/Cu1	2.32±0.04[a]	37.46±0.03[b]
CGC/Cu5	2.29±0.06[a]	36.92±1.78[b]
CGC/Cu10	1.25±0.05[b]	50.72±3.05[a]

* The values are presented as mean ± standard deviation. Different letters in the same column indicate significant differences ($p < 0.05$)

Water vapor permeability is an indicator rate of a material's ability to allow water vapor to diffuse. It is a critical parameter because it influences the shelf life and quality of the product (Meritaine da Rocha et al., 2018). WVP results are illustrated in Table 3.3. The WVP of the CGC/Cu0 film was measured to be 7.38±0.58x10^{-10} g Pa^{-1} s^{-1} m^{-1}. With the incorporation of curcumin into the films, a reduction was observed in the WVP. The WVP values for the CGC/Cu1 and CGC/Cu5 films were calculated as 6.49±0.58x10^{-10} g Pa^{-1} s^{-1} m^{-1} and 5.84±0.51x10^{-10} g Pa^{-1} s^{-1} m^{-1}, respectively. Nevertheless, the WVP of CGC/Cu10 film increased to 7.31±0.67x10^{-10} g Pa^{-1} s^{-1} m^{-1}. Although WVP measurements showed a similar trend to the WS results, no statistically significant difference was observed after the addition of curcumin to CGC nanocomposite films. This finding is in line with the literature, where the incorporation of curcumin into the biomolecule-based film did not affect the WVP of the film (Roy & Rhim, 2020c). However, the decreasing behavior in

WVP due to curcumin addition on CGC nanocomposite film can be explained by the fact that curcumin creates a more cohesive and less porous structure in films which does not allow for the diffusion of water and curcumin's hydrophobic nature, which prevents moisture uptake. Furthermore, an increase in the CGC/Cu10 can be linked with the presence of excess curcumin, resulting in a discontinuous structure.

Table 3-3 Water vapor permeability and thickness of CGC/Cu films*

Film Samples	**Thickness (mm)**	**WVP×10^{-10} (g Pa^{-1} s^{-1} m^{-1})**
CGC/Cu0	0.21±0.01[a]	7.38±0.58[a]
CGC/Cu1	0.22±0.01[a]	6.49±0.58[a]
CGC/Cu5	0.20±0.01[a]	5.84±0.51[a]
CGC/Cu10	0.22±0.01[a]	7.31±0.67[a]

* The values are presented as mean ± standard deviation. Different letters in the same column indicate significant differences ($p < 0.05$)

The water solubility of films is important since it indicates their water resistance and hydrophilicity (Pereda et al., 2011). In some instances, high water solubility is advantageous, particularly when the film is designed to be edible. However, low water solubility is essential for the packaging of foods with high moisture. As shown in Table 3.4, the WS of CGC nanocomposite films first decreased significantly when curcumin concentration increased (p <0.05). CGC/Cu1 and CGC/Cu5 films showed a considerable decrease compared to control CGC/Cu0 films. This behavior can be attributed to the curcumin's natural hydrophobic structure (Fernández-Marín et al., 2022). Rubilar et al. (2013) reported similar results for the WS values of chitosan films, including the hydrophobic agent carvacrol. However, no significant change was observed between CGC/Cu1 and CGG/Cu5 films, as WS depends on the curcumin concentration. On the other hand, CGC/Cu10 film with the 2 wt% curcumin showed a different trend, and its solubility increased. The WS value of CGC/Cu10 was determined to be 34.87±1.72%, close to the WS value of the control

CGC/Cu0 films measured to be 35.07±2.99%. This result might be explained by the fact that the structural integrity of the films was weakened by the precipitation of curcumin outside the glucan particles, as previously discussed. Due to this structural deformation, the permeability of water vapor through the film matrix has increased, leading to increased WS.

Table 3-4 Water solubility of CGC/Cu films*

Film Samples	**WS (%)**
CGC/Cu0	35.07±2.99[a]
CGC/Cu1	28.09±1.89[b]
CGC/Cu5	28.07±2.32[b]
CGC/Cu10	34.87±1.72[a]

* The values are presented as mean ± standard deviation. Different letters in the same column indicate significant differences ($p < 0.05$)

3.7 Light barrier properties of the active films

The light barrier function of active films is critical since light can cause photochemical reactions and accelerate the oxidation of oils in foods, resulting in the development of off-flavors and loss of nutrients. Active films with good light barrier properties can reduce the transmission of light. Table 3.5 shows the light transmittance of curcumin-loaded CGC nanocomposite films. It was observed that the incorporation of curcumin into the CGC nanocomposite films led to a significant reduction in light transmittance at 600 nm ($p < 0.05$). CGC/Cu10 film had the lowest transmittance to the light at 9.73%. These results indicate that curcumin exhibits effective light barrier properties. The reduction in light transmittance could be attributed to the presence of phenolic groups in curcumin. Curcumin is a polyphenolic compound with several aromatic rings connected by conjugated double bonds (Esatbeyoglu et al., 2012). These conjugated double bonds form a network of delocalized electrons that can absorb light in the visible spectrum (Stanić, 2017). When light strikes the phenolic groups of curcumin, it excites these electrons,

causing them to jump to higher energy levels. This causes higher light absorption and lower light transmittance (Bitencourt et al., 2014). Similar findings have been reported in the literature, where the addition of curcumin to chitosan and carrageenan films resulted in a significant reduction in light transmittance. The observed decrease in both UV and visible light transmittance was mainly ascribed to the light absorption property of curcumin (S. Roy & Rhim, 2020a)**.**

Table 3-5 Light Transmittance of CGC/Cu films*

Film Samples	**T_{600} (%)**
CGC/Cu0	13.95±0.25[a]
CGC/Cu1	11.82±0.06[b]
CGC/Cu5	11.09±0.07[b]
CGC/Cu10	9.73±0.38[c]

* The values are presented as mean ± standard deviation. Different letters in the same column indicate significant differences ($p < 0.05$)

3.8 Phenolic compounds and Antioxidant Activity

The Folin-Ciocalteu assay was used to determine total phenolic content (TPC). The reaction of phenolic compounds with the Folin-Ciocalteu reagent results in the formation of a blue-colored complex that can be measured spectrophotometrically. DPPH radical scavenging activity methods were used to assess the antioxidant activity of the nanocomposite films. The determination of TPC and radical scavenging activity in active films is critical as it provides information about the film's potential antioxidant and antimicrobial properties. Table 3.6 denotes the phenolic content and antioxidant activity of curcumin-loaded CGC nanocomposite films. The results showed that when curcumin concentrations were increased, a significant increase in TPC and radical scavenging activity of the films was observed ($p < 0.05$). This suggests that curcumin has a beneficial effect on both the TPC and antioxidant activity of the CGC nanocomposite films. Curcumin is a naturally occurring polyphenol with a conjugated structure composed of phenolic hydroxy and

β-diketone groups in an enol form (Yildiz, Sumnu, et al., 2021). Therefore, it exhibits radical scavenging as well as chain-breaking antioxidant activity. Apart from the β-diketone group, reactions between curcumin and DPPH via the sequential proton loss electron transfer are responsible for the antioxidant activity of curcumin (Galano et al., 2009). A study investigating carboxymethyl cellulose films containing curcumin showed a similar trend between the curcumin concentration and the films' antioxidant activity. Increasing concentrations of curcumin resulted in a corresponding increase in the antioxidant activity of the films (S. Roy & Rhim, 2020b). Nevertheless, the findings of this study indicated that the curcumin-free CGC/Cu0 films still exhibited a considerable level of TPC and scavenging activity with 0.87±0.081 mg GAE/g film and 2.62±0.28%. This indicates the presence of phenolic compounds in *A.bisporus* extract. In previous studies, it was reported that *A. bisporus* has phenolic contents and radical scavenging activity. The research has identified gallic, caffeic, and ferulic acids as the main phenolic compounds found in A. *bisporus* extract, while oxalic, succinic, and citric acids have been identified as the primary organic acids with antioxidant activity (Gąsecka et al., 2018). In addition, a study by Ashraf Khan et al. (2017) reported that β-glucan extracted from *Agaricus bisporus* exhibited DPPH radical scavenging activity. These findings are consistent with the observed TPC and scavenging activity in the CGC/Cu0 films of the current study.

Table 3-6 Total phenolic content and free radical scavenging activity of CGC/Cu films*

Film Samples	**TPC (mg GAE/g film)**	**DPPH (%)**
CGC/Cu0	0.87 ± 0.081^{d}	2.62 ± 0.28^{d}
CGC/Cu1	2.60 ± 0.09^{c}	9.66 ± 0.75^{c}
CGC/Cu5	4.70 ± 0.19^{b}	50.55 ± 1.96^{b}
CGC/Cu10	14.25 ± 0.21^{a}	70.96 ± 1.54^{a}

* The values are presented as mean ± standard deviation. Different letters in the same column indicate significant differences ($p < 0.05$)

3.9 Biodegradability of active films

Active films composed of biodegradable materials contribute to decreasing waste and positively impact the environment. They can naturally degrade over time because of the activity of organisms such as bacteria, fungi, and other living things. The biodegradability of a film is affected by its thickness, composition, and environmental conditions. The biodegradability evaluation of curcumin-loaded CGC nanocomposite films was conducted using soil as a testing environment for 14 days, and the results are illustrated in Figure 3.5. The degradation of all types of films containing varying amounts of curcumin was negligible until day 7, after which the degradation of films was accelerated. All films exhibited a significant level of decomposition in the soil, whereas the CGC/Cu10 film lost its initial shape on day 7. By the end of the 14th day, the observation was made that all films experienced a total loss of integrity. This biodegradation behavior of CGC nanocomposite films observed in the soil environment might be attributed to the existence of chitin-degrading microorganisms in the soil, which produce the enzyme chitinase (Pereira et al., 2021).

Chitinase breaks down the glycosidic linkage in N-acetyl-D-glucosamine units of chitin. Thus, it contributes to the acceleration of film degradation (Saima et al., 2013). Actinobacteria are Gram-positive bacteria recognized by their branching filamentous structure. They are typically found in soil and significantly affect organic matter breakdown, and they synthesize chitinolytic enzymes to a great extent (Veliz et al., 2017). Streptomyces species among Actinobacteria in the soil is crucial in soil ecology, as it can hydrolyze a wide variety of polysaccharides like chitin by producing chitinase (Barka et al., 2016). Furthermore, the existence of other chitinase-producing genera found in soil environments, such as *Pseudomonas, Bacillus, Vibrio,* and *Serratia,* also expedites the process of chitin breakdown (Saima et al., 2013). In addition to the chitinase enzyme in the soil, curcumin incorporated film structure also contributed to the degradation. It was observed that as the concentration of curcumin in the film increased, the degradation of the film integrity

also accelerated. The film with the highest curcumin concentration, CGC/Cu10, showed the most degradation. The reason for this might be explained by the fact that curcumin addition to the structure causes the weakening of the intermolecular forces that form the film matrix. In addition, studies have demonstrated that the presence of curcumin in the medium promotes the growth of Actinobacteria (Jabczyk et al., 2021). Therefore, as the curcumin concentration in the films increased, the Actinobacteria in the soil and therefore the chitinase enzyme increased, and degradation accelerated.

Studies have shown that enzymes that contribute to the hydrolysis of chitosan are less common in the soil environment than chitinase enzymes (Makarios-Laham & Lee, 1995). For this reason, preferring chitin instead of chitosan, which is frequently used in eco-friendly, biodegradable active packaging studies, could be a superior option in terms of environmental sustainability. In the literature, the degradation rate of both chitin and chitosan-incorporated polyethylene films were compared, and it was reported that the incorporation of chitin into polyethylene films accelerated the degradation of films in the soil (Makarios-Laham & Lee, 1995)

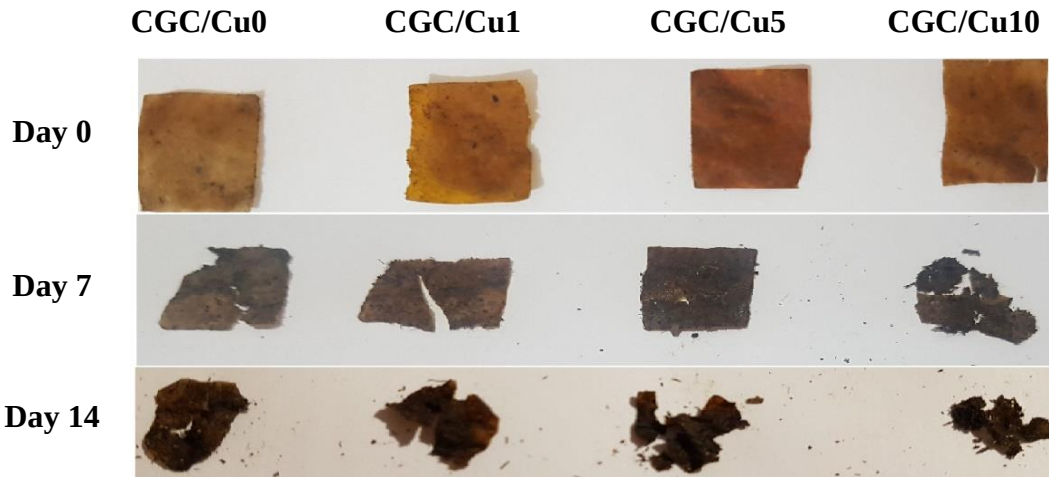


Figure 3.5 Biodegradability of the CGC/Cu films in the soil

3.10 Antibacterial activity evaluation

Antibacterial activities of CGC nanocomposite films against *Escherichia coli* (Gram-negative) and *Staphylococcus aureus* (Gram-positive) were determined by the disc diffusion method. Clear zones around the films formed because of the diffusion of curcumin into the agar were measured, and results are listed in Table 3.7, and corresponding images are presented in Figure 3.6. *E. coli* and *S. aureus* were selected to test the antimicrobial activity of films because they are the most prevalent pathogens. Curcumin has a strong and wide range inhibitory effect on both gram-positive and gram-negative bacteria. Its inhibitory effect might be linked to its ability to damage the membrane's phospholipid bilayer and DNA structure of target microorganisms and promote oxidative stress. However, this effect against pathogens depends on the bacterial strains (Adamczak et al., 2020). The research shows that some bacterial strains are more susceptible to curcumin since each strain could have variations in its cell wall structure and composition (Cetin-Karaca & Newman, 2015). As shown in Figures 3.6a, 3.6b, 3.6c, and 3.6d, films displayed good antimicrobial activity against *E. coli* depending on curcumin concentration in nanocomposite films. CGC/Cu10 film with 2% wt curcumin on a dry pellet basis was the only film that showed a significant difference in terms of inhibition zones ($p < 0.05$). The reason is that curcumin affects the growth and division of cells by interacting with the FtsZ protein which plays a critical role in bacterial cell division. It also interferes with the polymerization reaction of the FtsZ protein which results in the inhibition of bacterial growth (Adamczak et al., 2020; Rai et al., 2008). Furthermore, an inhibition zone was observed in CGC/Cu0 film. The formation of an inhibition zone around CGC/Cu0 films indicates the presence of antibacterial agents in *A. bisporus*. A study conducted by Gasecka et al. (2016) showed that *A. bisporus* has an antimicrobial effect against *E. coli* due to the presence of organic acids in its structure. In addition to organic acids, other bioactive compounds like phenolic compounds found in *A. bisporus* play a role in its antimicrobial properties.

Table 3-7 The inhibition zone of CGC/Cu films against *E.coli* and *S.aureus**

Film Samples	**Inhibition Zone (mm) for *E. coli***	**Inhibition Zone (mm) for *S. aureus***
CGC/Cu0	7.92±0.14[b]	n.e.**
CGC/Cu1	8.44±0.21[b]	n.e.
CGC/Cu5	8.73±0.06[b]	n.e.
CGC/Cu10	10.13±0.5[a]	n.e.

* The values are presented as mean ± standard deviation. Different letters in the same column indicate significant differences ($p < 0.05$)

**n.e: no effect

Although curcumin exhibits an inhibitory effect against gram-positive and gram-negative bacteria, none of the CGC/Cu films showed any inhibitory effect against *S. aureus* in this study (Figure 3.6e, 3.6f, 3.6g, 3.6h). The strain-specific antimicrobial activity of curcumin can support these results. Further, curcumin has been reported to have inhibitory activity against some strains of *S. aureus* with a minimum inhibitory concentration of 6 g/L (Niamsa & Sittiwet, 2009). Nevertheless, in this study, the film with the highest curcumin concentration had a curcumin content of 1.5 g/L in a film-forming solution, which is lower than the required concentration to show inhibitory effects against *S. aureus*. When the result was evaluated for the CGC/Cu0 films, extraction methodology should be considered. The methodology might affect the antibacterial effect of *A. bisporus*. In the literature, *A.bisporus* were subjected to extraction, which uses methanol as solvent, and the extract demonstrated antibacterial activity against *S. aureus* (M. Alves et al., 2012). However, a hot alkaline extraction method was performed in this study, which does not include methanol usage. This deviation in the methodology might be one of the reasons why CGC films did not show any antibacterial activity against S. *aureus*. Moreover, the bacterial growth medium may affect the diffusion of curcumin from the film structure. In this study, different and species-specific selective agars for *E. coli* and *S. aureus* were used. The difference in these agars may have affected the

diffusion of curcumin and caused no zones to be observed in *S.aureus* (Yildiz et al., 2022).

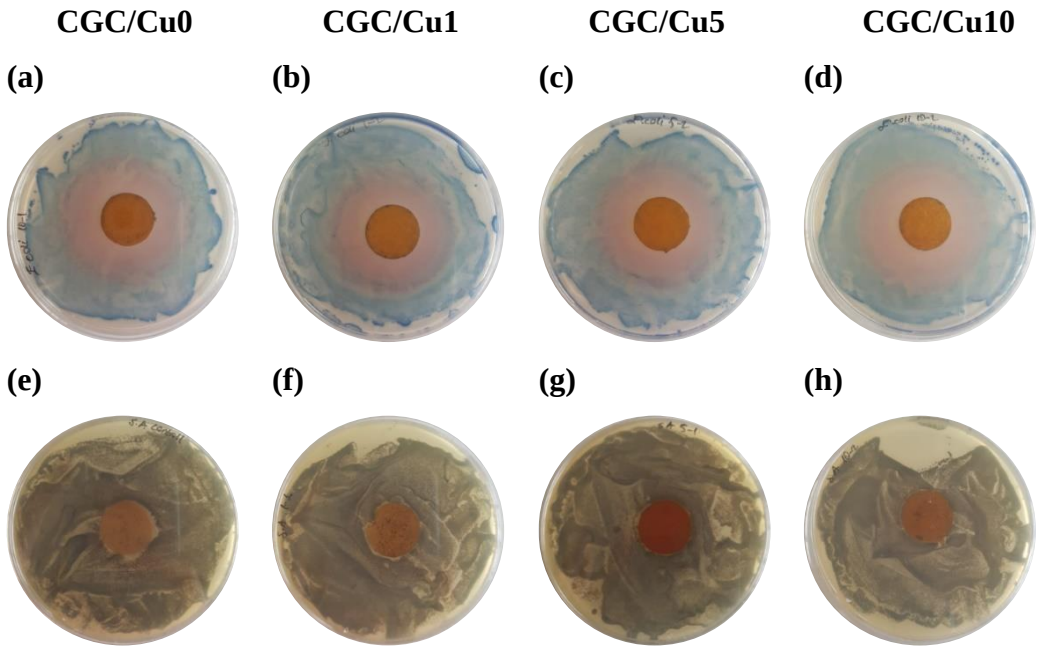


Figure 3.6 Images of inhibition zones for (a) CGC/Cu0, (b) CGC/Cu1, (c) CGC/Cu5, (d) CGC/Cu10 films against *E. coli* and for (e) Ch, (f) CGC/Cu1, (g) CGC/Cu5, (h) CGC/Cu10 films against *S. aureus*

3.11 Application of films on fresh chicken meat

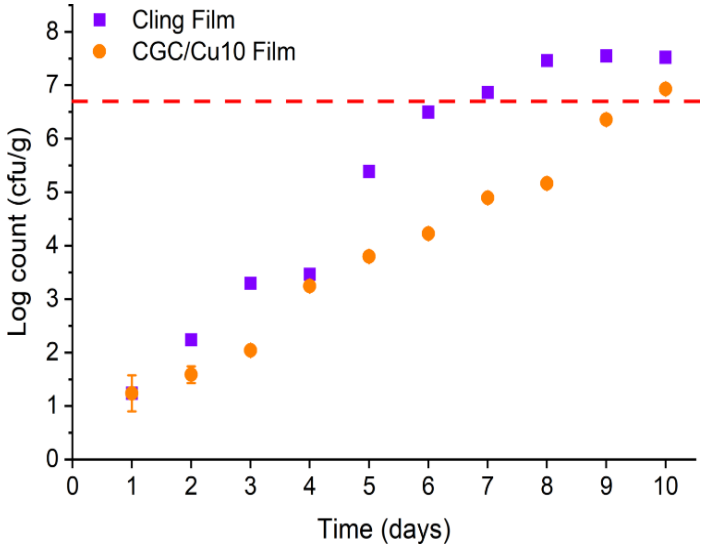
Total viable count (TVC) refers to the total number of living microorganisms found in products that can grow and reproduce under specified conditions. It is one of the critical parameters evaluating microbial safety and quality of the foods. In this study, CGC/Cu10 film was chosen to investigate its suitability for food packaging applications since it demonstrated maximum growth inhibition in the antimicrobial activity experiments. To assess the effect of CGC/Cu10 film on TVC, skinless chicken breast samples were packed with CGC/Cu10 film and cling film, which was served as a control. The results are illustrated in Figure 3.7. On day 1, the TVC value of the chicken breast sample was 2.30 log CFU.g^{-1}, which proved that the sample was of good microbial quality at the beginning of the experiment. TVC values for both CGC/Cu10 and cling-film packed samples were monitored for 10 days at 4°C.

Although TVC increased for both samples for 10 days, the TVC level of the cling film packed sample increased rapidly compared to CGC/Cu10 packed samples.

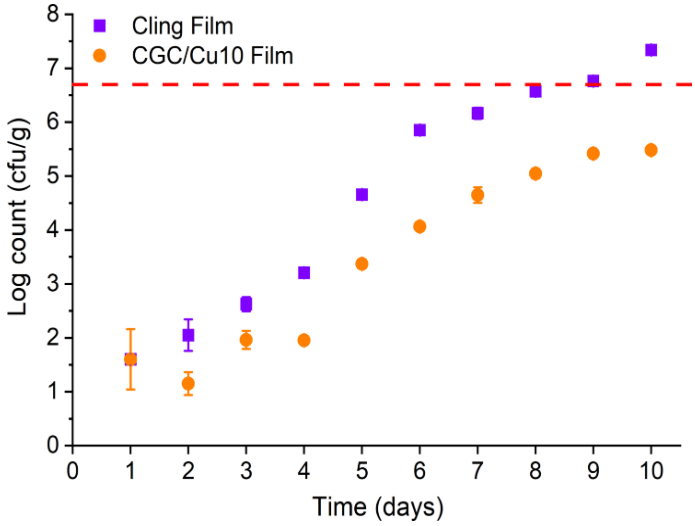
According to European Union and Turkish legislations, the acceptable microbiological upper limit for raw chicken breast is 6.7 log CFU.g^{-1} (Kilic et al., 2022). The control chicken breast sample packed with cling film reached and exceeded the critical microbiological threshold on day 7 of storage. This finding is consistent with research by Amjadi et al. (2019), which evaluated the freshness of chicken fillets. In contrast, the samples packed by CGC/Cu10 film reached the maximum TVC limit with the value of 6.82 log CFU.g^{-1} on day 10, which was 3 days later than control samples, which indicates that the usage of CGC/Cu10 as food packaging material provided approximately 40% extension in chicken breast's shelf-life. Similar results have been reported by Bojorges et al. (2020). The TVC values of chicken breast stored in refrigerated conditions at 4°C and wrapped with curcumin-loaded films were monitored, and it was observed that the shelf life of the chicken sample was increased to 12 days thanks to the use of curcumin-incorporated films.

In addition to TVC, in the literature, it has been suggested that the temperature of the poultry carcass differs between mesophilic to psychotropic throughout the slaughtering process, which causes to the formation of ideal conditions for the growth of psychrotrophic microorganisms (Hematizad et al., 2021). Psychrotrophic microorganisms in poultry cause a serious food safety threat, as they are able to generate heat-stable toxins that are not destroyed by standard cooking temperatures (Ercolini et al., 2009). Because of these reasons, in this study, the antibacterial activity of CGC/Cu10 films was tested against psychrotrophic bacteria, and the result of psychrotrophic bacteria count (PBC) is presented in Figure 3.7b. When the CGC/Cu10 films were compared with the cling film samples for each day, it was observed that the psychrotrophic microbial growth of CGC/Cu10 films was considerably lower than control films. The PBC value was recorded at 1.6 log CFU.g-1 on day 1 for the control samples. It progressively increased over time, reaching 7.3 log CFU.g^{-1} on day 10. Nevertheless, it was recorded that the PBC values of chicken samples packed with CGC/Cu10 films reached the 5.5 log

CFU.g^{-1} after 10 days of storage, as seen in Figure 3.7b. The outcomes of this study suggest that the use of CGC/Cu10 has a promising ability to suppress microbial growth on skinless fresh chicken samples and extend the shelf-life of the products by at least 40% under refrigerated conditions.



(a)



(b)

Figure 3.7 Changes in (a) TVC and (b) PBC of skinless chicken breast packed in cling film and CGC/Cu10 film during 10 days of storage at 4ºC

CHAPTER 4

CONCLUSIONS AND RECOMMENDATIONS

This study aims to produce active and sustainable food packaging using curcumin and CGC extracted from *Agaricus Bisporus* by solution casting method. Since CGC can be found abundantly in nature, it was used in this study as the support polymeric matrix. Curcumin is a natural antioxidant recognized for its ability to scavenge free radicals and prevent oxidative stress. SEM analysis revealed that curcumin was encapsulated by β-glucan in the CGC film matrix and formed a glucan-curcumin complex. Porosity and agglomerations in the film structure decreased by increasing curcumin concentration. The incorporation of curcumin into CGC films significantly enhanced the antioxidant activity and total phenolic content of the films. Furthermore, it provided an opaque layer for foods to be protected from light. Besides their functional properties, the mechanical strength of the CGC/Cu films was also evaluated. It was revealed that films have promising properties for food packaging applications.

Moreover, the biodegradability of films was tested in the soil environment. They exhibited good biodegradation properties, highlighting their eco-friendly characteristics and potential to minimize pollution. Even though the CGC/Cu films with all concentrations of curcumin demonstrated antibacterial activity against *Escherichia coli*, antibacterial activity was not observed against *Staphylococcus aureus*. Additionally, fresh skinless chicken breast meat wrapped with CGC/Cu10 film was tested for both total viable and psychrotrophic microorganisms, and it was observed that the shelf-life of chicken breast samples extended by approximately 40 %. As a result, the developed films can be used as active packaging material to increase the shelf-life of food that are susceptible to microbial spoilage.

The release behavior of curcumin from the film matrix might be examined in future studies. A different source may be used to extract chitin to eliminate the presence of glucan, which can improve the release of curcumin from the matrix. Sensory tests might also be performed to determine whether the curcumin that passes from the film to the food changes the food's taste, smell, and appearance. Furthermore, a hydrophilic active agent can be preferred to show antimicrobial activity for a broad range of microorganisms.

REFERENCES

Abedi-Firoozjah, R., Salim, S. A., Hasanvand, S., Assadpour, E., Azizi-Lalabadi, M., Prieto, M. A., & Jafari, S. M. (2023). Application of smart packaging for seafood: A comprehensive review. *Comprehensive Reviews in Food Science and Food Safety*, *22*(2), 1438–1461. https://doi.org/10.1111/1541-4337.13117

Adamczak, A., Ożarowski, M., & Karpiński, T. M. (2020). Curcumin, a natural antimicrobial agent with strain-specific activity. *Pharmaceuticals*, *13*(7), 1–12. https://doi.org/10.3390/ph13070153

Akman, P. K., Bozkurt, F., Balubaid, M., & Yilmaz, M. T. (2019). Fabrication of Curcumin-loaded Gliadin Electrospun Nanofibrous Structures and Bioactive Properties. *Fibers and Polymers*, *20*(6), 1187–1199. https://doi.org/10.1007/s12221-019-8950-8

Alves, J., Gaspar, P. D., Lima, T. M., & Silva, P. D. (2022a). What is the role of active packaging in the future of food sustainability? A systematic review. In *Journal of the Science of Food and Agriculture*. https://doi.org/10.1002/jsfa.11880

Alves, J., Gaspar, P. D., Lima, T. M., & Silva, P. D. (2022b). What is the role of active packaging in the future of food sustainability? A systematic review. *Journal of the Science of Food and Agriculture*. https://doi.org/10.1002/jsfa.11880

Amjadi, S., Emaminia, S., Nazari, M., Davudian, S. H., Roufegarinejad, L., & Hamishehkar, H. (2019). Application of Reinforced ZnO Nanoparticle-Incorporated Gelatin Bionanocomposite Film with Chitosan Nanofiber for Packaging of Chicken Fillet and Cheese as Food Models. *Food and Bioprocess Technology*, *12*(7), 1205–1219. https://doi.org/10.1007/s11947-019-02286-y

Araújo, D., Rodrigues, T., Roma-Rodrigues, C., Alves, V. D., Fernandes, A. R., & Freitas, F. (2023). Chitin-Glucan Complex Hydrogels: Physical-Chemical Characterization, Stability, In Vitro Drug Permeation, and Biological Assessment in Primary Cells. *Polymers*, *15*(4). https://doi.org/10.3390/polym15040791

Arbia, W., Arbia, L., Adour, L., & Amrane, A. (2013). Chitin Extraction from Crustacean Shells Using Biological Methods – A Review. *Chitin Recovery Using Biological Methods, Food Technol. Biotechnol*, *51*(1), 12–25.

Ashraf Khan, A., Gani, A., Masoodi, F. A., Mushtaq, U., & Silotry Naik, A. (2017). Structural, rheological, antioxidant, and functional properties of β–glucan extracted from edible mushrooms Agaricus bisporus, Pleurotus ostreatus and Coprinus attrimentarius. *Bioactive Carbohydrates and Dietary Fibre*, *11*, 67–74. https://doi.org/10.1016/J.BCDF.2017.07.006

Atila, F., Owaid, M. N., & Shariati, M. A. (2017). *THE NUTRITIONAL AND MEDICAL BENEFITS OF AGARICUS BISPORUS : A REVIEW*. *2008*, 281–286. https://doi.org/10.15414/jmbfs.2017/18.7.3.281-286

Aydogdu, A., Yildiz, E., Ayhan, Z., Aydogdu, Y., Sumnu, G., & Sahin, S. (2019). Nanostructured poly(lactic acid)/soy protein/HPMC films by electrospinning for potential applications in food industry. *European Polymer Journal*, *112*, 477–486. https://doi.org/10.1016/J.EURPOLYMJ.2019.01.006

Ayhan, Z. (2019). Packaging and the Shelf Life of Fruits and Vegetables. In *Reference Module in Food Science*. Elsevier. https://doi.org/10.1016/b978-0-08-100596-5.22649-8

Azuma, K., Ifuku, S., Osaki, T., Okamoto, Y., & Minami, S. (2014). Preparation and biomedical applications of chitin and chitosan nanofibers. *Journal of Biomedical Nanotechnology*, *10*(10), 2891–2920. https://doi.org/10.1166/jbn.2014.1882

Babaremu, K., Oladijo, O. P., & Akinlabi, E. (2023). Biopolymers: A suitable

REPLACEMENT for plastics in product packaging. *Advanced Industrial and Engineering Polymer Research*, *xxxx*. https://doi.org/10.1016/j.aiepr.2023.01.001

Bajpai, S. K., Chand, N., & Ahuja, S. (2015). Investigation of curcumin release from chitosan/cellulose micro crystals (CMC) antimicrobial films. *International Journal of Biological Macromolecules*, *79*, 440–448. https://doi.org/10.1016/J.IJBIOMAC.2015.05.012

Barka, E. A., Vatsa, P., Sanchez, L., Gaveau-Vaillant, N., Jacquard, C., Meier-Kolthoff, J. P., Klenk, H.-P., Clément, C., Ouhdouch, Y., & van Wezel, G. P. (2016). Correction for Barka et al., Taxonomy, Physiology, and Natural Products of Actinobacteria. *Microbiology and Molecular Biology Reviews*, *80*(4). https://doi.org/10.1128/mmbr.00044-16

Becerril, R., Nerín, C., & Silva, F. (2020). Encapsulation systems for antimicrobial food packaging components: An update. *Molecules*, *25*(5). https://doi.org/10.3390/molecules25051134

Berezina, N. (2016). Production and application of chitin. *Physical Sciences Reviews*, *1*(9), 1–8. https://doi.org/10.1515/psr-2016-0048

Bhuyan, M. S. (2022). Effects of Microplastics on Fish and in Human Health. *Frontiers in Environmental Science*, *10*(March), 1–17. https://doi.org/10.3389/fenvs.2022.827289

Bitencourt, C. M., Fávaro-Trindade, C. S., Sobral, P. J. A., & Carvalho, R. A. (2014). Gelatin-based films additivated with curcuma ethanol extract: Antioxidant activity and physical properties of films. *Food Hydrocolloids*, *40*, 145–152. https://doi.org/10.1016/j.foodhyd.2014.02.014

Bojorges, H., Ríos-Corripio, M. A., Hernández-Cázares, A. S., Hidalgo-Contreras, J. V., & Contreras-Oliva, A. (2020). Effect of the application of an edible film with turmeric (Curcuma longa L.) on the oxidative stability of meat. *Food Science and Nutrition*, *8*(8), 4308–4319. https://doi.org/10.1002/fsn3.1728

Brody, A. L., Bugusu, B., Han, J. H., Sand, C. K., & McHugh, T. H. (2008). Innovative food packaging solutions. *Journal of Food Science*, *73*(8). https://doi.org/10.1111/j.1750-3841.2008.00933.x

Byun, Y., Darby, D., Cooksey, K., Dawson, P., & Whiteside, S. (2011). Development of oxygen scavenging system containing a natural free radical scavenger and a transition metal. *Food Chemistry*, *124*(2), 615–619. https://doi.org/10.1016/j.foodchem.2010.06.084

Caz, P. (2017). *Food Hydrocolloids Polysaccharide-based fi lms and coatings for food packaging : A review*. *68*, 136–148. https://doi.org/10.1016/j.foodhyd.2016.09.009

Cetin-Karaca, H., & Newman, M. C. (2015). Antimicrobial efficacy of plant phenolic compounds against Salmonella and Escherichia Coli. *Food Bioscience*, *11*, 8–16. https://doi.org/10.1016/j.fbio.2015.03.002

Chaix, E., Guillaume, C., & Guillard, V. (2014). Oxygen and Carbon Dioxide Solubility and Diffusivity in Solid Food Matrices: A Review of Past and Current Knowledge. *Comprehensive Reviews in Food Science and Food Safety*, *13*(3), 261–286. https://doi.org/10.1111/1541-4337.12058

Chakravarty, J., & Edwards, T. A. (2022). *Innovation from waste with biomass-derived chitin and chitosan as green and sustainable polymer : A review*. *8*(September). https://doi.org/10.1016/j.nexus.2022.100149

Chamas, A., Moon, H., Zheng, J., Qiu, Y., Tabassum, T., Jang, J. H., Abu-Omar, M., Scott, S. L., & Suh, S. (2020). Degradation Rates of Plastics in the Environment. *ACS Sustainable Chemistry and Engineering*, *8*(9), 3494–3511. https://doi.org/10.1021/acssuschemeng.9b06635

Chang, J., Li, W., Liu, Q., Zhou, Y., Chen, X., Lyu, Q., & Liu, G. (2019). Preparation, properties, and structural characterization of β-glucan/pullulan blend films. *International Journal of Biological Macromolecules*, *140*, 1269–1276. https://doi.org/10.1016/J.IJBIOMAC.2019.08.208

Chang, S., & Miles, P. (1992). Mushroom biology — A new discipline. *Topics in Catalysis*, *6*(2), 64–65. https://doi.org/10.1016/S0269-915X(09)80449-7

Chang, S. T., & Wasser, S. P. (2017). *The Cultivation and Environmental Impact of Mushrooms* (Issue March).

Chawla, R., Sivakumar, S., & Kaur, H. (2021). Antimicrobial edible films in food packaging: Current scenario and recent nanotechnological advancements- a review. *Carbohydrate Polymer Technologies and Applications*, *2*(August 2020), 100024. https://doi.org/10.1016/j.carpta.2020.100024

Chen, X., Zou, L. Q., Niu, J., Liu, W., Peng, S. F., & Liu, C. M. (2015). The stability, sustained release and cellular antioxidant activity of curcumin nanoliposomes. *Molecules*, *20*(8), 14293–14311. https://doi.org/10.3390/MOLECULES200814293

Chen, Y., Lu, Y., Lee, R. J., & Xiang, G. (2020). Nano encapsulated curcumin: And its potential for biomedical applications. *International Journal of Nanomedicine*, *15*, 3099–3120. https://doi.org/10.2147/IJN.S210320

Cho, Y. I., No, H. K., & Meyers, S. P. (1998). *Physicochemical Characteristics and Functional Properties of Various Commercial Chitin and Chitosan Products*. *8561*(97).

Cho, Y. W., Jang, J., Park, C. R., & Ko, S. W. (2000). Preparation and solubility in acid and water of partially deacetylated chitins. *Biomacromolecules*, *1*(4), 609–614. https://doi.org/10.1021/bm000036j

Cullen, John S. Vaylen, N. E. (1994). *United States Patent (19). 19*.

da Silva Filipini, G., Romani, V. P., & Guimarães Martins, V. (2020). Biodegradable and active-intelligent films based on methylcellulose and jambolão (Syzygium cumini) skins extract for food packaging. *Food Hydrocolloids*, *109*, 106139. https://doi.org/10.1016/J.FOODHYD.2020.106139

Dou, B., Dupont, V., Williams, P. T., Chen, H., & Ding, Y. (2009). Thermogravimetric kinetics of crude glycerol. *Bioresource Technology*, *100*(9), 2613–2620. https://doi.org/10.1016/J.BIORTECH.2008.11.037

Du, X., Sissons, J., Shanks, M., & Plotto, A. (2021). Aroma and flavor profile of raw and roasted Agaricus bisporus mushrooms using a panel trained with aroma chemicals. *LWT*, *138*, 110596. https://doi.org/10.1016/J.LWT.2020.110596

Duan, B., Chang, C., Ding, B., Cai, J., Xu, M., Feng, S., Ren, J., Shi, X., Du, Y., & Zhang, L. (2013). High strength films with gas-barrier fabricated from chitin solution dissolved at low temperature. *Journal of Materials Chemistry A*, *1*(5), 1867–1874. https://doi.org/10.1039/c2ta00068g

Dutta, T., Bagchi, D., & Pal, S. K. (2018). Bimetallic zeolitic imidazolate framework as an active excipient of curcumin under physiological condition. *Biomedical Physics and Engineering Express*, *4*(5). https://doi.org/10.1088/2057-1976/aad29e

Elieh-Ali-Komi, D., Hamblin, M. R., & Daniel, E.-A.-K. (2016). Chitin and Chitosan: Production and Application of Versatile Biomedical Nanomaterials HHS Public Access. In *Int J Adv Res (Indore)* (Vol. 4, Issue 3).

Emadian, S. M., Onay, T. T., & Demirel, B. (2017). Biodegradation of bioplastics in natural environments. *Waste Management*, *59*, 526–536. https://doi.org/10.1016/j.wasman.2016.10.006

Ercolini, D., Russo, F., Nasi, A., Ferranti, P., & Villani, F. (2009). Mesophilic and psychrotrophic bacteria from meat and their spoilage potential in vitro and in beef. *Applied and Environmental Microbiology*, *75*(7), 1990–2001. https://doi.org/10.1128/AEM.02762-08

Erna, K. H., Felicia, W. X. L., Vonnie, J. M., Rovina, K., Yin, K. W., & Nur'aqilah, M. N. (2022). Synthesis and Physicochemical Characterization of Polymer Film-Based Anthocyanin and Starch. *Biosensors*, *12*(4).

https://doi.org/10.3390/bios12040211

Esatbeyoglu, T., Huebbe, P., Ernst, I. M. A., Chin, D., Wagner, A. E., & Rimbach, G. (2012). Curcumin-from molecule to biological function. *Angewandte Chemie - International Edition*, *51*(22), 5308–5332. https://doi.org/10.1002/anie.201107724

Esatbeyoglu, T., Ulbrich, K., Rehberg, C., Rohn, S., & Rimbach, G. (2015). Thermal stability, antioxidant, and anti-inflammatory activity of curcumin and its degradation product 4-vinyl guaiacol. *Food and Function*, *6*(3), 887–893. https://doi.org/10.1039/c4fo00790e

Fabra, M. J., López-Rubio, A., & Lagaron, J. M. (2014). Biopolymers for food packaging applications. In *Smart Polymers and their Applications* (pp. 476–509). Woodhead Publishing. https://doi.org/10.1533/9780857097026.2.476

Farinha, I., Duarte, P., Pimentel, A., Plotnikova, E., Chagas, B., Mafra, L., Grandfils, C., Freitas, F., Fortunato, E., & Reis, M. A. M. (2015). Chitin-glucan complex production by Komagataella pastoris: Downstream optimization and product characterization. *Carbohydrate Polymers*, *130*. https://doi.org/10.1016/j.carbpol.2015.05.034

Fazli Wan Nawawi, W. M., Lee, K. Y., Kontturi, E., Murphy, R. J., & Bismarck, A. (2019). Chitin Nanopaper from Mushroom Extract: Natural Composite of Nanofibers and Glucan from a Single Biobased Source. *ACS Sustainable Chemistry and Engineering*, *7*(7), 6492–6496. https://doi.org/10.1021/acssuschemeng.9b00721

Fernández-Marín, R., Fernandes, S. C. M., Sánchez, M. Á. A., & Labidi, J. (2022). Halochromic and antioxidant capacity of smart films of chitosan/chitin nanocrystals with curcuma oil and anthocyanins. *Food Hydrocolloids*, *123*. https://doi.org/10.1016/j.foodhyd.2021.107119

Fite, A., Dykhuizen, R., Litterick, A., Golden, M., & Leifert, C. (2004). Effects of Ascorbic Acid, Glutathione, Thiocyanate, and Iodide on Antimicrobial

Activity of Acidified Nitrite. *Antimicrobial Agents and Chemotherapy*, *48*(2), 655–658. https://doi.org/10.1128/AAC.48.2.655-658.2004

Gadgey, K. K., & Dey, S. (2017). Development of chitin and chitosan from narmada riverside crab shells. *International Journal of Mechanical Engineering and Technology*, *8*(7), 298–307.

Galano, A., Álvarez-Diduk, R., Ramírez-Silva, M. T., Alarcón-Ángeles, G., & Rojas-Hernández, A. (2009). Role of the reacting free radicals on the antioxidant mechanism of curcumin. *Chemical Physics*, *363*(1–3), 13–23. https://doi.org/10.1016/J.CHEMPHYS.2009.07.003

Gąsecka, M., Magdziak, Z., Siwulski, M., & Mleczek, M. (2018). Profile of phenolic and organic acids, antioxidant properties and ergosterol content in cultivated and wild growing species of Agaricus. *European Food Research and Technology*, *244*(2), 259–268. https://doi.org/10.1007/s00217-017-2952-9

Ghaderi-Ghahfarokhi, M., Barzegar, M., Sahari, M. A., & Azizi, M. H. (2016). Nanoencapsulation Approach to Improve Antimicrobial and Antioxidant Activity of Thyme Essential Oil in Beef Burgers During Refrigerated Storage. *Food and Bioprocess Technology*, *9*(7), 1187–1201. https://doi.org/10.1007/s11947-016-1708-z

Gómez-Estaca, J., López-de-Dicastillo, C., Hernández-Muñoz, P., Catalá, R., & Gavara, R. (2014). Advances in antioxidant active food packaging. *Trends in Food Science and Technology*, *35*(1), 42–51. https://doi.org/10.1016/j.tifs.2013.10.008

Gontard, N., Duchez, C., Cuq, J. - L, & Guilbert, S. (1994). Edible composite films of wheat gluten and lipids: water vapour permeability and other physical properties. *International Journal of Food Science & Technology*, *29*(1), 39–50. https://doi.org/10.1111/j.1365-2621.1994.tb02045.x

Gopalan Nair, K., & Dufresne, A. (2003). Crab shell chitin whisker reinforced natural rubber nanocomposites. 1. Processing and swelling behavior.

Biomacromolecules, *4*(3), 657–665. https://doi.org/10.1021/bm020127b

Goto, K., & Teramoto, Y. (2020). Distribution of the Degree of Deacetylation of Surface-Deacetylated Chitin Nanofibers: Effects on Crystalline Structure and Cell Adhesion and Proliferation. *ACS Applied Bio Materials*, *3*(12), 8650–8657. https://doi.org/10.1021/acsabm.0c01040

Groh, K. J., Backhaus, T., Carney-Almroth, B., Geueke, B., Inostroza, P. A., Lennquist, A., Leslie, H. A., Maffini, M., Slunge, D., Trasande, L., Warhurst, A. M., & Muncke, J. (2019). Overview of known plastic packaging-associated chemicals and their hazards. *Science of the Total Environment*, *651*, 3253–3268. https://doi.org/10.1016/j.scitotenv.2018.10.015

Guo, Z., Wu, X., Zhao, X., Fan, J., Lu, X., & Wang, L. (2020). An edible antioxidant film of Artemisia sphaerocephala Krasch. gum with sophora japonica extract for oil packaging. *Food Packaging and Shelf Life*, *24*(December 2019), 100460. https://doi.org/10.1016/j.fpsl.2019.100460

Haktaniyan, M., & Bradley, M. (2022). Polymers showing intrinsic antimicrobial activity. *Chemical Society Reviews*, *51*(20), 8584–8611. https://doi.org/10.1039/d2cs00558a

Hassainia, A., Satha, H., & Boufi, S. (2018). Chitin from Agaricus bisporus: Extraction and characterization. *International Journal of Biological Macromolecules*, *117*, 1334–1342. https://doi.org/10.1016/J.IJBIOMAC.2017.11.172

Hawksworth, D. L. (2012). Global species numbers of fungi: Are tropical studies and molecular approaches contributing to a more robust estimate? *Biodiversity and Conservation*, *21*(9), 2425–2433. https://doi.org/10.1007/s10531-012-0335-x

Hematizad, I., Khanjari, A., Basti, A. A., Karabagias, I. K., Noori, N., Ghadami, F., Gholami, F., & Teimourifard, R. (2021). In vitro antibacterial activity of gelatin-nanochitosan films incorporated with Zataria multiflora Boiss essential

oil and its influence on microbial, chemical, and sensorial properties of chicken breast meat during refrigerated storage. *Food Packaging and Shelf Life*, *30*. https://doi.org/10.1016/j.fpsl.2021.100751

Hermann, B. G., Debeer, L., De Wilde, B., Blok, K., & Patel, M. K. (2011). To compost or not to compost: Carbon and energy footprints of biodegradable materials' waste treatment. *Polymer Degradation and Stability*, *96*(6), 1159–1171. https://doi.org/10.1016/j.polymdegradstab.2010.12.026

Homez-Jara, A., Daza, L. D., Aguirre, D. M., Muñoz, J. A., Solanilla, J. F., & Váquiro, H. A. (2018). Characterization of chitosan edible films obtained with various polymer concentrations and drying temperatures. *International Journal of Biological Macromolecules*, *113*, 1233–1240. https://doi.org/10.1016/j.ijbiomac.2018.03.057

Hong, Y., & Ying, T. (2019). Characterization of a chitin-glucan complex from the fruiting body of Termitomyces albuminosus (Berk.)Heim. *International Journal of Biological Macromolecules*, *134*. https://doi.org/10.1016/j.ijbiomac.2019.04.198

Hurley, B. R. A., Ouzts, A., Fischer, J., & Gomes, T. (2013). PAPER PRESENTED AT IAPRI WORLD CONFERENCE 2012 Effects of Private and Public Label Packaging on Consumer Purchase Patterns. *Packaging and Technology and Science*, *29*(January), 399–412. https://doi.org/10.1002/pts

Ifuku, S., Nomura, R., Morimoto, M., & Saimoto, H. (2011). Preparation of chitin nanofibers from mushrooms. *Materials*, *4*(8), 1417–1425. https://doi.org/10.3390/ma4081417

Iversen, L. J. L., Rovina, K., Vonnie, J. M., Matanjun, P., Erna, K. H., 'Aqilah, N. M. N., Felicia, W. X. L., & Funk, A. A. (2022). The Emergence of Edible and Food-Application Coatings for Food Packaging: A Review. *Molecules*, *27*(17). https://doi.org/10.3390/molecules27175604

Jabczyk, M., Nowak, J., Hudzik, B., & Zubelewicz-Szkodzí Nska, B. (2021).

Curcumin and Its Potential Impact on Microbiota. https://doi.org/10.3390/nu13062004

Jancikova, S., Dordevic, D., Jamroz, E., Behalova, H., & Tremlova, B. (2020). Chemical and physical characteristics of edible films, based on κ- And ι-carrageenans with the addition of lapacho tea extract. *Foods*, *9*(3). https://doi.org/10.3390/foods9030357

Jiang, H., Sun, J., Li, Y., Ma, J., Lu, Y., Pang, J., & Wu, C. (2020). Preparation and characterization of citric acid crosslinked konjac glucomannan/surface deacetylated chitin nanofibers bionanocomposite film. *International Journal of Biological Macromolecules*, *164*. https://doi.org/10.1016/j.ijbiomac.2020.08.138

Jones, M., Kujundzic, M., John, S., & Bismarck, A. (2020). Crab vs. Mushroom: A review of crustacean and fungal chitin in wound treatment. In *Marine Drugs* (Vol. 18, Issue 1). https://doi.org/10.3390/md18010064

Jovanovic, S. V., Steenken, S., Boone, C. W., & Simic, M. G. (1999). H-atom transfer is a preferred antioxidant mechanism of curcumin. *Journal of the American Chemical Society*, *121*(41), 9677–9681. https://doi.org/10.1021/ja991446m

Jung, J., & Zhao, Y. (2016). Antimicrobial Packaging for Fresh and Minimally Processed Fruits and Vegetables. In *Antimicrobial Food Packaging*. Elsevier Inc. https://doi.org/10.1016/B978-0-12-800723-5.00018-8

Kaur, G., Sharma, S., Mir, S. A., & Dar, & B. N. (2021). Nanobiocomposite Films: a “Greener Alternate” for Food Packaging. *Food and Bioprocess Technology*, *14*, 1013–1027. https://doi.org/10.1007/s11947-021-02634-x/Published

Kaur, S., & Dhillon, G. S. (2015). Recent trends in biological extraction of chitin from marine shell wastes: A review. *Critical Reviews in Biotechnology*, *35*(1), 44–61. https://doi.org/10.3109/07388551.2013.798256

Kaya, E., Kahyaoglu, L. N., & Sumnu, G. (2022). Development of curcumin incorporated composite films based on chitin and glucan complexes extracted from Agaricus bisporus for active packaging of chicken breast meat. *International Journal of Biological Macromolecules*, *221*(July), 536–546. https://doi.org/10.1016/j.ijbiomac.2022.09.025

Khan, A., Gallah, H., Riedl, B., Bouchard, J., Safrany, A., & Lacroix, M. (2016). Genipin cross-linked antimicrobial nanocomposite films and gamma irradiation to prevent the surface growth of bacteria in fresh meats. *Innovative Food Science & Emerging Technologies*, *35*, 96–102. https://doi.org/10.1016/J.IFSET.2016.03.011

Kilic, B., Dogan, V., Kilic, V., & Kahyaoglu, L. N. (2022). Colorimetric food spoilage monitoring with carbon dot and UV light reinforced fish gelatin films using a smartphone application. *International Journal of Biological Macromolecules*, *209*(PA), 1562–1572. https://doi.org/10.1016/j.ijbiomac.2022.04.119

Kim, D., Lee, S., Lee, K., Baek, S., & Seo, J. (2017). Development of a pH indicator composed of high moisture-absorbing materials for real-time monitoring of chicken breast freshness. *Food Science and Biotechnology*, *26*(1), 37–42. https://doi.org/10.1007/s10068-017-0005-6

Kim, H., Beak, S. E., Yang, S. Y., & Song, K. Bin. (2018). Application of an antimicrobial packaging material from chicken bone gelatine and cinnamon bark oil to mozzarella cheese. *International Journal of Food Science and Technology*, *53*(3), 619–625. https://doi.org/10.1111/ijfs.13636

Kim, H., Kang, S., Li, K., Jung, D., Park, K., & Lee, J. (2021). Preparation and characterization of various chitin-glucan complexes derived from white button mushroom using a deep eutectic solvent-based ecofriendly method. *International Journal of Biological Macromolecules*, *169*, 122–129. https://doi.org/10.1016/J.IJBIOMAC.2020.12.081

Kirtiraj K. Gaikwad, Y. S. L. (2017). Current Scenario of Gas Scavenging Systems Used in Active Packaging - A Review. *Korean Journal of Packaging Science and Technology*, *23*(2), 109–117. https://doi.org/10.20909/kopast.2017.23.2.109

Koc, B., Akyuz, L., Cakmak, Y. S., Sargin, I., Salaberria, A. M., Labidi, J., Ilk, S., Cekic, F. O., Akata, I., & Kaya, M. (2020). Production and characterization of chitosan-fungal extract films. *Food Bioscience*, *35*, 100545. https://doi.org/10.1016/J.FBIO.2020.100545

Kocaadam, B., & Şanlier, N. (2017). Curcumin, an active component of turmeric (Curcuma longa), and its effects on health. *Critical Reviews in Food Science and Nutrition*, *57*(13), 2889–2895. https://doi.org/10.1080/10408398.2015.1077195

Koketso Ncube, L., Ude, A. U., Nifise Ogunmuyiwa, E., Zulkifli, R., & Beas, I. N. (2020). materials Environmental Impact of Food Packaging Materials: A Review of Contemporary Development from Conventional Plastics to Polylactic Acid Based Materials. *Materials*, *13*. https://doi.org/10.3390/ma13214994

Konuk Takma, D., & Korel, F. (2019). Active packaging films as a carrier of black cumin essential oil: Development and effect on quality and shelf-life of chicken breast meat. *Food Packaging and Shelf Life*, *19*, 210–217. https://doi.org/10.1016/J.FPSL.2018.11.002

Konwar, A., Gogoi, N., Majumdar, G., & Chowdhury, D. (2015). Green chitosan-carbon dots nanocomposite hydrogel film with superior properties. *Carbohydrate Polymers*, *115*, 238–245. https://doi.org/10.1016/j.carbpol.2014.08.021

Kõrge, K., Bajić, M., Likozar, B., & Novak, U. (2020). Active chitosan–chestnut extract films used for packaging and storage of fresh pasta. *International Journal of Food Science and Technology*, *55*(8), 3043–3052.

https://doi.org/10.1111/ijfs.14569

Kralj, D., & Markič, M. (2008). Building materials reuse and recycle. *WSEAS Transactions on Environment and Development*, *4*(5), 409–418.

Kumari, S., & Kishor, R. (2020). Chapter 1. Chitin and chitosan: origin, properties, and applications. In *Handbook of Chitin and Chitosan*. INC. https://doi.org/10.1016/B978-0-12-817970-3.00001-8

Le, M. H., Do, H. D., Thi, H. H. T., Dung, L. V., Nguyen, H. N., Thi, H. N. T., Nguyen, L. D., Hoang, C. K., Le, H. C., Thi, T. H. Le, Trinh, H. T., & Ha, P. T. (2016). The dual effect of curcumin nanoparticles encapsulated by 1-3/1-6 β-glucan from medicinal mushrooms Hericium erinaceus and Ganoderma lucidum. *Advances in Natural Sciences: Nanoscience and Nanotechnology*, *7*(4). https://doi.org/10.1088/2043-6262/7/4/045019

Lee, D. S. (2016). Carbon dioxide absorbers for food packaging applications. *Trends in Food Science and Technology*, *57*, 146–155. https://doi.org/10.1016/j.tifs.2016.09.014

Lee, M., Park, J. K., Lee, H. S., Lane, O., Moore, R. B., McGrath, J. E., & Baird, D. G. (2009). Effects of block length and solution-casting conditions on the final morphology and properties of disulfonated poly(arylene ether sulfone) multiblock copolymer films for proton exchange membranes. *Polymer*, *50*(25), 6129–6138. https://doi.org/10.1016/j.polymer.2009.10.023

Li, Y., Yokoyama, W., Wu, J., Ma, J., & Zhong, F. (2015). Properties of edible films based on pullulan-chitosan blended film-forming solutions at different pH. *RSC Advances*, *5*(128), 105844–105850. https://doi.org/10.1039/c5ra21876d

Liu, Y., Cai, Y., Jiang, X., Wu, J., & Le, X. (2016). Molecular interactions, characterization and antimicrobial activity of curcumin–chitosan blend films. *Food Hydrocolloids*, *52*, 564–572. https://doi.org/10.1016/J.FOODHYD.2015.08.005

Lourenço, S. C., Moldão-Martins, M., & Alves, V. D. (2019). Antioxidants of natural plant origins: From sources to food industry applications. In *Molecules* (Vol. 24, Issue 22). https://doi.org/10.3390/molecules24224132

Ma, Q., Ren, Y., & Wang, L. (2017). Investigation of antioxidant activity and release kinetics of curcumin from tara gum/ polyvinyl alcohol active film. *Food Hydrocolloids*, *70*, 286–292. https://doi.org/10.1016/j.foodhyd.2017.04.018

Makarios-Laham, I., & Lee, T. C. (1995). Biodegradability of chitin- and chitosan-containing films in soil environment. *Journal of Environmental Polymer Degradation*, *3*(1), 31–36. https://doi.org/10.1007/BF02067791

Manzi, P., Aguzzi, A., & Pizzoferrato, L. (2001). *Nutritional value of mushrooms widely consumed in Italy*. *73*.

Marcolino, V. A., Zanin, G. M., Durrant, L. R., De Toledo Benassi, M., & Matioli, G. (2011). Interaction of Curcumin and Bixin with β-Cyclodextrin: Complexation Methods, Stability, and Applications in Food. *J. Agric. Food Chem*, *59*, 3348–3357. https://doi.org/10.1021/jf104223k

Marcos, B., Sárraga, C., Castellari, M., Kappen, F., Schennink, G., & Arnau, J. (2014). Development of biodegradable films with antioxidant properties based on polyesters containing α-tocopherol and olive leaf extract for food packaging applications. *Food Packaging and Shelf Life*, *1*(2), 140–150. https://doi.org/10.1016/j.fpsl.2014.04.002

McHUGH, T. H., AVENA- BUSTILLOS, R., & KROCHTA, J. M. (1993). Hydrophilic Edible Films: Modified Procedure for Water Vapor Permeability and Explanation of Thickness Effects. *Journal of Food Science*, *58*(4), 899–903. https://doi.org/10.1111/j.1365-2621.1993.tb09387.x

Meritaine da Rocha, de Souza, M. M., & Prentice, C. (2018). Biodegradable Films: An Alternative Food Packaging. In *Food Packaging and Preservation* (pp. 307–342). Academic Press. https://doi.org/10.1016/b978-0-12-811516-

9.00009-9

Mikhailov, E., Vlasenko, S., Martin, S. T., Koop, T., & Pöschl, U. (2009). Amorphous and crystalline aerosol particles interacting with water vapor: Conceptual framework and experimental evidence for restructuring, phase transitions and kinetic limitations. *Atmospheric Chemistry and Physics*, *9*(24), 9491–9522. https://doi.org/10.5194/acp-9-9491-2009

Miltz, J., & Perry, M. (2005). Evaluation of the performance of iron-based oxygen scavengers, with comments on their optimal applications. *Packaging Technology and Science*, *18*(1), 21–27. https://doi.org/10.1002/pts.671

Miranda, J. M., Mondragón, A. C., Lamas, A., Roca-Saavedra, P., Ibarra, I. S., Rodriguez, J. A., Cepeda, A., & Franco, C. M. (2016). Effect of Packaging Systems on the Inactivation of Microbiological Agents. *Antimicrobial Food Packaging*, *December*, 107–116. https://doi.org/10.1016/B978-0-12-800723-5.00008-5

Moshood, T. D., Nawanir, G., Mahmud, F., Mohamad, F., Ahmad, M. H., & AbdulGhani, A. (2022). Sustainability of biodegradable plastics: New problem or solution to solve the global plastic pollution? *Current Research in Green and Sustainable Chemistry*, *5*, 100273. https://doi.org/10.1016/J.CRGSC.2022.100273

Mousavi Khaneghah, A., Hashemi, S. M. B., & Limbo, S. (2018). Antimicrobial agents and packaging systems in antimicrobial active food packaging: An overview of approaches and interactions. *Food and Bioproducts Processing*, *111*, 1–19. https://doi.org/10.1016/j.fbp.2018.05.001

Munir, Z., Banche, G., Cavallo, L., Mandras, N., Roana, J., Pertusio, R., Ficiarà, E., Cavalli, R., & Guiot, C. (2022). Exploitation of the Antibacterial Properties of Photoactivated Curcumin as ‘Green’ Tool for Food Preservation. *International Journal of Molecular Sciences*, *23*(5). https://doi.org/10.3390/ijms23052600

Murmu, S. B., & Mishra, H. N. (2018). Selection of the best active modified atmosphere packaging with ethylene and moisture scavengers to maintain quality of guava during low-temperature storage. *Food Chemistry*, *253*(May 2017), 55–62. https://doi.org/10.1016/j.foodchem.2018.01.134

Mushi, N. E. (2021). A review on native well-preserved chitin nanofibrils for materials of high mechanical performance. *International Journal of Biological Macromolecules*, *178*, 591–606. https://doi.org/10.1016/j.ijbiomac.2021.02.149

Namazi, H. (2017). Polymers in our daily life. *BioImpacts*, *7*(2), 73–74. https://doi.org/10.15171/bi.2017.09

Nawawi, W. M. F. B. W., Jones, M., Murphy, R. J., Lee, K. Y., Kontturi, E., & Bismarck, A. (2020). Nanomaterials Derived from Fungal Sources-Is It the New Hype? *Biomacromolecules*, *21*(1), 30–55. https://doi.org/10.1021/acs.biomac.9b01141

Nawawi, W. M. F. W., Lee, K. Y., Kontturi, E., Bismarck, A., & Mautner, A. (2020). Surface properties of chitin-glucan nanopapers from Agaricus bisporus. *International Journal of Biological Macromolecules*, *148*, 677–687. https://doi.org/10.1016/j.ijbiomac.2020.01.141

Nduko, J. M., & Taguchi, S. (2021). Microbial Production of Biodegradable Lactate-Based Polymers and Oligomeric Building Blocks From Renewable and Waste Resources. *Frontiers in Bioengineering and Biotechnology*, *8*(February), 1–18. https://doi.org/10.3389/fbioe.2020.618077

Niamsa, N., & Sittiwet, C. (2009). Antimicrobial activity of Curcuma longa aqueous extract. *Journal of Pharmacology and Toxicology*, *4*(4), 173–177. https://doi.org/10.3923/jpt.2009.173.177

Nieto-Suaza, L., Acevedo-Guevara, L., Sánchez, L. T., Pinzón, M. I., & Villa, C. C. (2019). Characterization of Aloe vera-banana starch composite films reinforced with curcumin-loaded starch nanoparticles. *Food Structure*,

22(September 2018), 100131. https://doi.org/10.1016/j.foostr.2019.100131

Nugraha, B., Bintoro, N., & Murayama, H. (2015). Influence of CO2 and C2H4 Adsorbents to the Symptoms of Internal Browning on the Packaged 'Silver Bell' Pear (Pyrus communis L.). *Agriculture and Agricultural Science Procedia*, *3*, 127–131. https://doi.org/10.1016/j.aaspro.2015.01.025

Ofem, M. I., Anyandi, A. J., Ene, E. B., Iver, C. R. R., & Of, U. N. I. V. (2017). *PROPERTIES OF CHITIN REINFORCES COMPOSITES : A REVIEW*. *36*(1), 57–71.

Oliveira-Garcia, E., & Deising, H. B. (2013). Infection structure-specific expression of β-1,3-glucan synthase is essential for pathogenicity of Colletotrichum graminicola and evasion of β-Glucan-triggered immunity in maize. *Plant Cell*, *25*(6), 2356–2378. https://doi.org/10.1105/tpc.112.103499

Pellis, A., Guebitz, G. M., & Nyanhongo, G. S. (2022). *Chitosan : Sources , Processing and Modification Techniques*. 5–25.

Percot, A., Viton, C., & Domard, A. (2003). Optimization of Chitin Extraction from Shrimp Shells. *Biomacromolecules*, *4*(6), 12–18.

Pereda, M., Ponce, A. G., Marcovich, N. E., Ruseckaite, R. A., & Martucci, J. F. (2011). Chitosan-gelatin composites and bi-layer films with potential antimicrobial activity. *Food Hydrocolloids*, *25*(5), 1372–1381. https://doi.org/10.1016/j.foodhyd.2011.01.001

Pereira, D. G. M., Vieira, J. M., Vicente, A. A., & Cruz, R. M. S. (2021). Development and characterization of pectin films with salicornia ramosissima: Biodegradation in soil and seawater. *Polymers*, *13*(16). https://doi.org/10.3390/polym13162632

Philibert, T., Lee, B. H., & Fabien, N. (2017). Current Status and New Perspectives on Chitin and Chitosan as Functional Biopolymers. *Applied Biochemistry and Biotechnology*, *181*(4), 1314–1337. https://doi.org/10.1007/s12010-016-2286-

2

Plavcová, Z., Šalamúnová, P., Saloň, I., Štěpánek, F., Hanuš, J., & Hošek, J. (2019). Curcumin encapsulation in yeast glucan particles promotes its anti-inflammatory potential in vitro. *International Journal of Pharmaceutics*, *568*, 118532. https://doi.org/10.1016/J.IJPHARM.2019.118532

Poerio, A., Petit, C., Jehl, J. P., Arab-Tehrany, E., Mano, J. F., & Cleymand, F. (2020). Extraction and physicochemical characterization of chitin from cicada orni sloughs of the south-eastern French mediterranean basin. *Molecules*, *25*(11). https://doi.org/10.3390/molecules25112543

Rachtanapun, P., Klunklin, W., Jantrawut, P., Jantanasakulwong, K., Phimolsiripol, Y., Seesuriyachan, P., Leksawasdi, N., Chaiyaso, T., Ruksiriwanich, W., Phongthai, S., Sommano, S. R., Punyodom, W., Reungsang, A., & Ngo, T. M. P. (2021). Characterization of chitosan film incorporated with curcumin extract. *Polymers*, *13*(6), 1–15. https://doi.org/10.3390/polym13060963

Rafiee, Z., Nejatian, M., Daeihamed, M., & Jafari, S. M. (2019). Application of different nanocarriers for encapsulation of curcumin. *Critical Reviews in Food Science and Nutrition*, *59*(21), 3468–3497. https://doi.org/10.1080/10408398.2018.1495174

Rai, D., Singh, J. K., Roy, N., & Panda, D. (2008). Curcumin inhibits FtsZ assembly: An attractive mechanism for its antibacterial activity. *Biochemical Journal*, *410*(1), 147–155. https://doi.org/10.1042/BJ20070891

Rhim, J. W., Park, H. M., & Ha, C. S. (2013). Bio-nanocomposites for food packaging applications. *Progress in Polymer Science*, *38*(10–11), 1629–1652. https://doi.org/10.1016/J.PROGPOLYMSCI.2013.05.008

Roopa, H., Panghal, A., Kumari, A., Chhikara, N., Sehgal, E., & Rawat, K. (2022). Active Packaging in Food Industry. *Novel Technologies in Food Science*, *8*(5), 375–404. https://doi.org/10.1002/9781119776376.ch10

Rosentrater, K. A., Jiménez, A., Guillard, V., Gaucel, S., Fornaciari, C., Angellier-Coussy, H., Buche, P., & Gontard, N. (2018). *The Next Generation of Sustainable Food Packaging to Preserve Our Environment in a Circular Economy Context*. *5*, 121. https://doi.org/10.3389/fnut.2018.00121

Rotrekl, D., Devriendt, B., Cox, E., Kavanová, L., Faldyna, M., Šalamúnová, P., Baďo, Z., Prokopec, V., Štěpánek, F., Hanuš, J., & Hošek, J. (2020). Glucan particles as suitable carriers for the natural anti-inflammatory compounds curcumin and diplacone – Evaluation in an ex vivo model. *International Journal of Pharmaceutics*, *582*, 119318. https://doi.org/10.1016/J.IJPHARM.2020.119318

Roy, J. C., Salaün, F., Giraud, S., Ferri, A., Chen, G., & Guan, J. (2017). Solubility of Chitin: Solvents, Solution Behaviors and Their Related Mechanisms. *Solubility of Polysaccharides*, *November*. https://doi.org/10.5772/intechopen.71385

Roy, S., Priyadarshi, R., Ezati, P., & Rhim, J. W. (2022). Curcumin and its uses in active and smart food packaging applications - a comprehensive review. *Food Chemistry*, *375*(December 2021), 131885. https://doi.org/10.1016/j.foodchem.2021.131885

Roy, S., & Rhim, J. W. (2020a). Preparation of carbohydrate-based functional composite films incorporated with curcumin. *Food Hydrocolloids*, *98*(August 2019), 105302. https://doi.org/10.1016/j.foodhyd.2019.105302

Roy, S., & Rhim, J. W. (2020b). Carboxymethyl cellulose-based antioxidant and antimicrobial active packaging film incorporated with curcumin and zinc oxide. *International Journal of Biological Macromolecules*, *148*, 666–676. https://doi.org/10.1016/j.ijbiomac.2020.01.204

Roy, S., & Rhim, J. W. (2020c). Preparation of antimicrobial and antioxidant gelatin/curcumin composite films for active food packaging application. *Colloids and Surfaces B: Biointerfaces*, *188*.

https://doi.org/10.1016/j.colsurfb.2019.110761

Rubilar, J. F., Cruz, R. M. S., Silva, H. D., Vicente, A. A., Khmelinskii, I., & Vieira, M. C. (2013). Physico-mechanical properties of chitosan films with carvacrol and grape seed extract. *Journal of Food Engineering*, *115*(4), 466–474. https://doi.org/10.1016/J.JFOODENG.2012.07.009

Rudrappa, T., & Bais, H. P. (2008). Curcumin, a known phenolic from Curcuma longa, attenuates the virulence of Pseudomonas aeruginosa PAO1 in whole plant and animal pathogenicity models. *Journal of Agricultural and Food Chemistry*, *56*(6), 1955–1962. https://doi.org/10.1021/jf072591j

Saima, Kuddus, M., Roohi, & Ahmad, I. Z. (2013). Isolation of novel chitinolytic bacteria and production optimization of extracellular chitinase. *Journal of Genetic Engineering and Biotechnology*, *11*(1), 39–46. https://doi.org/10.1016/j.jgeb.2013.03.001

Settanni, L., Palazzolo, E., Guarrasi, V., Aleo, A., Mammina, C., Moschetti, G., & Germanà, M. A. (2012). Inhibition of foodborne pathogen bacteria by essential oils extracted from citrus fruits cultivated in Sicily. *Food Control*, *26*(2), 326–330. https://doi.org/10.1016/j.foodcont.2012.01.050

Sharma, S., Barkauskaite, S., Jaiswal, A. K., & Jaiswal, S. (2021). Essential oils as additives in active food packaging. *Food Chemistry*, *343*(August 2020), 128403. https://doi.org/10.1016/j.foodchem.2020.128403

Shwet, K., VP, S., Mamta, G., Anupam, B., & Manjit, S. (2019). Genetics and breeding of white button mushroom, Agaricus bisporus (Lange.) Imbach. - A comprehensive review. *Mushroom Research*, *28*(1). https://doi.org/10.36036/mr.28.1.2019.91938

Siemann, U. (2005). Solvent cast technology - A versatile tool for thin film production. *Progress in Colloid and Polymer Science*, *130*(June), 1–14. https://doi.org/10.1007/b107336

Silva, F. A. G. S., Dourado, F., Gama, M., & Poças, F. (2020). Nanocellulose bio-based composites for food packaging. *Nanomaterials*, *10*(10), 1–29. https://doi.org/10.3390/nano10102041

Silva, J. C., & Borges, J. P. (2015). *Chitin-Based Nanocomposites : Biomedical Applications*. *July*. https://doi.org/10.1007/978-81-322-2473-0

Singh, A. K., Ramakanth, D., Kumar, A., Lee, Y. S., & Gaikwad, K. K. (2021). Active packaging technologies for clean label food products: a review. *Journal of Food Measurement and Characterization*, *15*(5), 4314–4324. https://doi.org/10.1007/s11694-021-01024-3

Siracusa, V., & Dalla, M. (2008). *Biodegradable polymers for food packaging : a review*. *19*, 634–643. https://doi.org/10.1016/j.tifs.2008.07.003

Siroid, O., Klechak, I., & Duhan, O. (2022). Prospects of Industrial Production of Chitin-Glucan Complexes From Fungal Cultures. *Food Science and Technology*, *15*(4), 69–76. https://doi.org/10.15673/fst.v15i4.2259

Slusarczyk, B., & Kot, S. (2018). Solution for sustainable development: Provisions limiting the consumption of disposable plastic carrier bags in Poland. *Journal of Security and Sustainability Issues*, *7*(3), 449–458. https://doi.org/10.9770/jssi.2018.7.3(7)

Smith, L. R., Douthwaite, M., Mugford, K., Dummer, N. F., Willock, D. J., Hutchings, G. J., & Taylor, S. H. (2022). Recent Advances on the Valorization of Glycerol into Alcohols. *Energies*, *15*(17). https://doi.org/10.3390/en15176250

Sohbatzadeh, F., Hosseinzadeh Colagar, A., Mirzanejhad, S., & Mahmodi, S. (2010). E. coli, P. aeruginosa, and B. cereus Bacteria Sterilization Using Afterglow of Non-Thermal Plasma at Atmospheric Pressure. *Applied Biochemistry and Biotechnology*, *160*(7), 1978–1984. https://doi.org/10.1007/s12010-009-8817-3

Souza, M. P., Vaz, A. F. M., Silva, H. D., Cerqueira, M. A., Vicente, A. A., & Carneiro-da-Cunha, M. G. (2015). Development and Characterization of an Active Chitosan-Based Film Containing Quercetin. *Food and Bioprocess Technology*, *8*(11), 2183–2191. https://doi.org/10.1007/s11947-015-1580-2

Stanić, Z. (2017). Curcumin, a Compound from Natural Sources, a True Scientific Challenge – A Review. *Plant Foods for Human Nutrition*, *72*(1), 1–12. https://doi.org/10.1007/s11130-016-0590-1

Stroescu, M., Isopencu, G., Busuioc, C., & Stoica-Guzun, A. (2019). *Antimicrobial Food Pads Containing Bacterial Cellulose and Polysaccharides*. https://doi.org/10.1007/978-3-319-77830-3_3

Sung, S. Y., Sin, L. T., Tee, T. T., Bee, S. T., Rahmat, A. R., Rahman, W. A. W. A., Tan, A. C., & Vikhraman, M. (2013). Antimicrobial agents for food packaging applications. *Trends in Food Science and Technology*, *33*(2), 110–123. https://doi.org/10.1016/j.tifs.2013.08.001

Tarique, J., Sapuan, S. M., & Khalina, A. (2021). Effect of glycerol plasticizer loading on the physical, mechanical, thermal, and barrier properties of arrowroot (Maranta arundinacea) starch biopolymers. *Scientific Reports*, *11*(1), 1–17. https://doi.org/10.1038/s41598-021-93094-y

Tewari, G., Jeremiah, L. E., Jayas, D. S., & Holley, R. A. (2002). Improved use of oxygen scavengers to stabilize the colour of retail-ready meat cuts stored in modified atmospheres. *International Journal of Food Science and Technology*, *37*(2), 199–207. https://doi.org/10.1046/j.1365-2621.2002.00557.x

Tiwari, A., Singh, A., Garg, N., & Randhawa, J. K. (2017). Curcumin encapsulated zeolitic imidazolate frameworks as stimuli responsive drug delivery system and their interaction with biomimetic environment. *Scientific Reports*, *7*(1), 1–12. https://doi.org/10.1038/s41598-017-12786-6

Tran, & N, T.-N. (2021). Active Antioxidant Additives in Sustainable Food

Packaging. In *Sustainable Food Packaging Technology* (pp. 349–367). https://doi.org/10.1002/9783527820078.ch13

Tripathi, S., Mehrotra, G. K., & Dutta, P. K. (2008). Chitosan based antimicrobial films for food packaging applications. *E-Polymers*, *093*, 1–7. https://doi.org/10.1515/epoly.2008.8.1.1082

Uranga, J., Puertas, A. I., Etxabide, A., Dueñas, M. T., Guerrero, P., & de la Caba, K. (2019). Citric acid-incorporated fish gelatin/chitosan composite films. *Food Hydrocolloids*, *86*, 95–103. https://doi.org/10.1016/j.foodhyd.2018.02.018

Varghese, S. A., Siengchin, S., & Parameswaranpillai, J. (2020). Essential oils as antimicrobial agents in biopolymer-based food packaging - A comprehensive review. *Food Bioscience*, *38*(October 2019), 100785. https://doi.org/10.1016/j.fbio.2020.100785

Veliz, E. A., Martí Nez-Hidalgo, P., & Hirsch, A. M. (2017). Chitinase-producing bacteria and their role in biocontrol. *AIMS Microbiology*, *3*(3), 689–705. https://doi.org/10.3934/microbiol.2017.3.689

Venkatesan, N. (1998). Curcumin attenuation of acute adriamycin myocardial toxicity in rats. *British Journal of Pharmacology*, *124*(3), 425–427. https://doi.org/10.1038/sj.bjp.0701877

Vetter, J. (2007). Food Chemistry Chitin content of cultivated mushrooms Agaricus bisporus , Pleurotus ostreatus and Lentinula edodes. *Food Chemistry*, *102*, 6–9. https://doi.org/10.1016/j.foodchem.2006.01.037

Wahab, N. A. A., Lajis, N. H., Abas, F., Othman, I., & Naidu, R. (2020). Mechanism of anti- cancer activity of curcumin on androgen- dependent and androgen- independent prostate cancer. *Nutrients*, *12*(3), 1–34. https://doi.org/10.3390/nu12030679

Wang, H. J., An, D. S., Rhim, J. W., & Lee, D. S. (2017). Shiitake mushroom

packages tuned in active CO2 and moisture absorption requirements. *Food Packaging and Shelf Life*, *11*, 10–15. https://doi.org/10.1016/j.fpsl.2016.11.002

Wu, Q., Jungstedt, E., Šoltésová, M., Mushi, N. E., & Berglund, L. A. (2019). High strength nanostructured films based on well-preserved β-chitin nanofibrils †. *Nanoscale*, *11*. https://doi.org/10.1039/c9nr02870f

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*(8). https://doi.org/10.3390/molecules26082152

Xu, D. P., Li, Y., Meng, X., Zhou, T., Zhou, Y., Zheng, J., Zhang, J. J., & Li, H. Bin. (2017). Natural antioxidants in foods and medicinal plants: Extraction, assessment and resources. *International Journal of Molecular Sciences*, *18*(1), 20–31. https://doi.org/10.3390/ijms18010096

Yang, Z., Peng, H., Wang, W., & Liu, T. (2010). Crystallization behavior of poly(ε-caprolactone)/layered double hydroxide nanocomposites. *Journal of Applied Polymer Science*, *116*(5), 2658–2667. https://doi.org/10.1002/app

Yildirim, S., Röcker, B., Pettersen, M. K., Nilsen-Nygaard, J., Ayhan, Z., Rutkaite, R., Radusin, T., Suminska, P., Marcos, B., & Coma, V. (2018). Active Packaging Applications for Food. In *Comprehensive Reviews in Food Science and Food Safety* (Vol. 17, Issue 1, pp. 165–199). https://doi.org/10.1111/1541-4337.12322

Yildiz, E., Bayram, I., Sumnu, G., Sahin, S., & Ibis, O. I. (2021). Development of pea flour based active films produced through different homogenization methods and their effects on lipid oxidation. *Food Hydrocolloids*, *111*, 106238. https://doi.org/10.1016/J.FOODHYD.2020.106238

Yildiz, E., Sumnu, G., & Kahyaoglu, L. N. (2021). Monitoring freshness of chicken breast by using natural halochromic curcumin loaded chitosan/PEO nanofibers as an intelligent package. *International Journal of Biological*

Macromolecules, *170*, 437–446. https://doi.org/10.1016/j.ijbiomac.2020.12.160

Yildiz, E., Sumnu, G., & Kahyaoglu, L. N. (2022). Assessment of curcumin incorporated chickpea flour/PEO (polyethylene oxide) based electrospun nanofiber as an antioxidant and antimicrobial food package. *Food and Bioproducts Processing*, *135*, 205–216. https://doi.org/10.1016/j.fbp.2022.08.002

Younes, I., & Rinaudo, M. (2015). Chitin and chitosan preparation from marine sources. Structure, properties and applications. *Marine Drugs*, *13*(3), 1133–1174. https://doi.org/10.3390/md13031133

Zhai, X., Wang, X., Zhang, J., Yang, Z., Sun, Y., Li, Z., Huang, X., Holmes, M., Gong, Y., Povey, M., Shi, J., & Zou, X. (2020). Extruded low density polyethylene-curcumin film: A hydrophobic ammonia sensor for intelligent food packaging. *Food Packaging and Shelf Life*, *26*(March), 100595. https://doi.org/10.1016/j.fpsl.2020.100595

Zhang, J., Ye, K. P., Zhang, X., Pan, D. D., Sun, Y. Y., & Cao, J. X. (2017). Antibacterial activity and mechanism of action of black pepper essential oil on meat-borne escherichia coli. *Frontiers in Microbiology*, *7*(JAN), 1–10. https://doi.org/10.3389/fmicb.2016.02094

Zhang, R., Wang, W., Zhang, H., Dai, Y., Dong, H., & Hou, H. (2019). Effects of hydrophobic agents on the physicochemical properties of edible agar/maltodextrin films. *Food Hydrocolloids*, *88*, 283–290. https://doi.org/10.1016/J.FOODHYD.2018.10.008

Zhang, X., Xu, S., Shen, L., & Li, G. (2020). Factors affecting thermal stability of collagen from the aspects of extraction, processing and modification. *Journal of Leather Science and Engineering*, *2*(1). https://doi.org/10.1186/s42825-020-00033-0

Zhang, Y., Chen, X., Gueydan, C., & Han, J. (2018). Plasma membrane changes

during programmed cell deaths. *Cell Research*, *28*(1), 9–21. https://doi.org/10.1038/cr.2017.133

Zheng, B., & McClements, D. J. (2020). Formulation of more efficacious curcumin delivery systems using colloid science: Enhanced solubility, stability, and bioavailability. *Molecules*, *25*(12), 1–25. https://doi.org/10.3390/molecules25122791

Zheng, D., Huang, C., Huang, H., Zhao, Y., Khan, M. R. U., Zhao, H., & Huang, L. (2020). Antibacterial Mechanism of Curcumin: A Review. *Chemistry and Biodiversity*, *17*(8). https://doi.org/10.1002/cbdv.202000171

Zheng, M., Liu, S., Guan, X., & Xie, Z. (2015). One-Step Synthesis of Nanoscale Zeolitic Imidazolate Frameworks with High Curcumin Loading for Treatment of Cervical Cancer. *ACS Applied Materials and Interfaces*, *7*(40), 22181–22187. https://doi.org/10.1021/acsami.5b04315

APPENDICES

A. Calibration Curves

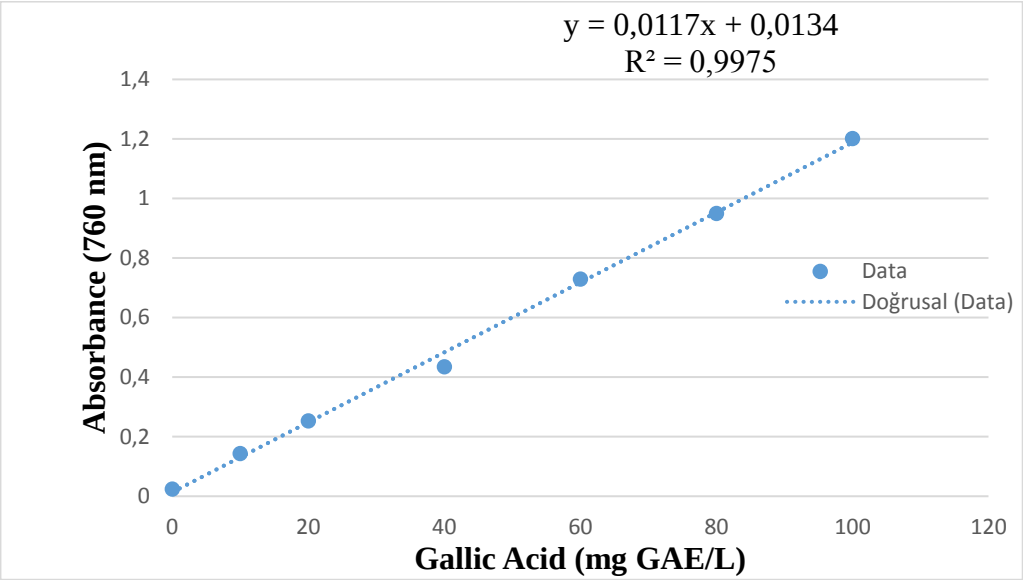


Figure A.1 Calibration curve prepared by gallic acid in methanol:water mixture (10:90 v/v) for determination of total phenolic contents

B. Statistical Analysis

Table 1: Water Solubility of CGC/Cu films

Method

Null hypothesis	All means are equal
Alternative hypothesis	Not all means are equal
Significance level	$\alpha = 0.05$

Equal variances were assumed for the analysis.

Factor Information

Factor	Levels	Values
Type of Film	4	CGC/Cu; CGC/Cu1; CGG/Cu10; CGC/Cu5

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Type of Film	3	0.014252	0.004751	9.11	0.006
Error	8	0.004172	0.000522		
Total	11	0.018424			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.0228374	77.35%	68.86%	49.05%

Means

Type of Film	N	Mean	StDev	95% CI
CGC/Cu0	3	0.3507	0.0299	(0.3203; 0.3811)
CGC/Cu1	3	0.2809	0.0189	(0.2505; 0.3113)
CGC/Cu10	3	0.34871	0.01724	(0.31830; 0.37911)
CGC/Cu5	3	0.2807	0.0232	(0.2503; 0.3111)

Pooled StDev = 0.0228374

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

Type of Film	N	Mean	Grouping	
CGC/Cu0	3	0.3507	A	
CGC/Cu10	3	0.34871	A	
CGC/Cu1	3	0.2809		B
CGC/Cu5	3	0.2807		B

Means that do not share a letter are significantly different.

Table 2: Thickness of CGC/Cu films

Method

Null hypothesis	All means are equal
Alternative hypothesis	Not all means are equal
Significance level	$\alpha = 0.05$

Equal variances were assumed for the analysis.

Factor Information

Factor	Levels	Values
Type of Film	4	CGC/Cu; CGC/Cu1; CGG/Cu10; CGC/Cu5

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Type of Film	3	0.000864	0.000288	3.88	0.055
Error	8	0.000593	0.000074		
Total	11	0.001457			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.0109095	59.28%	44.01%	8.38%

Means

Type of Film	N	Mean	StDev	95% CI
CGC/Cu0	3	0.21187	0.00502	(0.20040, 0.22333)
CGC/Cu1	3	0.22413	0.00831	(0.21267, 0.23560)
CGC/Cu10	3	0.22073	0.00991	(0.20927, 0.23220)
CGC/Cu5	3	0.20227	0.01021	(0.19080, 0.21373)

Pooled StDev = 0.00861297

Grouping Information Using the Tukey Method and 95% Confidence

Type of Film	N	Mean	Grouping
CGC/Cu1	3	0.22413	A
CGC/Cu10	3	0.22073	A
CGC/Cu0	3	0.21187	A
CGC/Cu5	3	0.20227	A

Means that do not share a letter are significantly different.

Table 3: Water Vapor Permeability of CGC/Cu films

Method

Null hypothesis	All means are equal
Alternative hypothesis	Not all means are equal
Significance level	$\alpha = 0.05$

Equal variances were assumed for the analysis.

Factor Information

Factor	Levels	Values
Type of Film	4	CGC/Cu; CGC/Cu1; CGG/Cu10; CGC/Cu5

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Type of Film	3	4.778	1.5928	4.62	0.037
Error	8	2.759	0.3449		
Total	11	7.538			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.587298	63.39%	49.66%	17.63%

Means

Type of Film	N	Mean	StDev	95% CI
CGC/Cu0	3	7.377	0.581	(6.595; 8.159)
CGC/Cu1	3	6.493	0.576	(5.711; 7.274)
CGC/Cu10	3	7.311	0.671	(6.529; 8.093)
CGC/Cu5	3	5.845	0.51	(5.063; 6.626)

Pooled StDev = 0.587207

Grouping Information Using the Tukey Method and 95% Confidence

Type of Film	N	Mean	Grouping
CGC/Cu0	3	7.377	A
CGC/Cu1	3	6.493	A
CGC/Cu10	3	7.311	A
CGC/Cu5	3	5.845	A

Means that do not share a letter are significantly different.

Table 4: Tensile Strength of CGC/Cu films

Method

Null hypothesis	All means are equal
Alternative hypothesis	Not all means are equal
Significance level	$\alpha = 0.05$

Equal variances were assumed for the analysis.

Factor Information

Factor	Levels	Values
Type of Film	4	CGC/Cu0; CGC/Cu1; CGC/Cu10; CGC/Cu5

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Type of Film	3	1.57054	0.523513	265.06	0.000
Error	4	0.00790	0.001975		
Total	7	1.57844			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.0444418	99.50%	99.12%	98.00%

Means

Type of Film	N	Mean	StDev	95% CI
CGC/Cu0	2	2.16946	0.01166	(2.08221; 2.25671)
CGC/Cu1	2	2.3243	0.0405	(2.2370; 2.4115)
CGC/Cu10	2	1.2478	0.0462	(1.1605; 1.3350)
CGC/Cu5	2	2.2930	0.0631	(2.2058; 2.3803)

Pooled StDev = 0.0444418

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

Type of Film	N	Mean	Grouping	
CGC/Cu1	2	2.3243	A	
CGC/Cu5	2	2.2930	A	
CGC/Cu0	2	2.16946	A	
CGC/Cu10	2	1.2478		B

Means that do not share a letter are significantly different.

Table 5: Elongation at break of CGC/Cu films

Method

Null hypothesis	All means are equal
Alternative hypothesis	Not all means are equal
Significance level	$\alpha = 0.05$

Equal variances were assumed for the analysis.

Factor Information

Factor	Levels	Values
Type of Film	4	CGC/Cu0; CGC/Cu1; CGC/Cu10; CGC/Cu5

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Type of Film	3	245.48	81.828	25.66	0.004
Error	4	12.75	3.189		
Total	7	258.24			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
1.78569	95.06%	91.36%	80.24%

Means

Type of Film	N	Mean	StDev	95% CI
CGC/Cu0	2	42.560	0.427	(39.054; 46.066)
CGC/Cu1	2	37.458	0.254	(33.953; 40.964)
CGC/Cu10	2	50.72	3.06	(47.21; 54.22)
CGC/Cu5	2	36.92	1.78	(33.41; 40.42)

Pooled StDev = 1.78569

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

Type of Film	N	Mean	Grouping	
CGC/Cu10	2	50.72	A	
CGC/Cu0	2	42.560		B
CGC/Cu1	2	37.458		B
CGC/Cu5	2	36.92		B

Means that do not share a letter are significantly different.

Table 6: Light Transmittance of CGC/Cu films

Method

Null hypothesis	All means are equal
Alternative hypothesis	Not all means are equal
Significance level	$\alpha = 0.05$

Equal variances were assumed for the analysis.

Factor Information

Factor	Levels	Values
Type of Film	4	CGC/Cu0; CGC/Cu1; CGC/Cu10; CGC/Cu5

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Type of Film	3	18.6107	6.20357	115.16	0.000
Error	4	0.2155	0.05387		
Total	7	18.8262			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.232097	98.86%	98.00%	95.42%

Means

Type of Film	N	Mean	StDev	95% CI
CGC/Cu0	2	13.949	0.250	(13.493; 14.404)
CGC/Cu1	2	11.8169	0.0577	(11.3612; 12.2725)
CGC/Cu10	2	9.731	0.380	(9.276; 10.187)
CGC/Cu5	2	11.0919	0.0722	(10.6362; 11.5475)

Pooled StDev = 0.232097

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

Type of Film	N	Mean	Grouping		
CGC/Cu0	2	13.949	A		
CGC/Cu1	2	11.8169		B	
CGC/Cu5	2	11.0919		B	
CGC/Cu10	2	9.731			C

Means that do not share a letter are significantly different.

Table 7: Total phenolic content of CGC/Cu films

Method

Null hypothesis	All means are equal
Alternative hypothesis	Not all means are equal
Significance level	α = 0.05

Equal variances were assumed for the analysis.

Factor Information

Factor	Levels	Values
Type of Film	4	CGC/Cu0; CGC/Cu1; CGC/Cu10; CGC/Cu5

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Type of Film	3	320.980	106.993	4296.62	0.000
Error	8	0.199	0.025		
Total	11	321.180			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.157803	99.94%	99.91%	99.86%

Means

Type of Film	N	Mean	StDev	95% CI
CGC/Cu0	3	0.8751	0.0815	(0.6650; 1.0852)
CGC/Cu1	3	2.6002	0.0979	(2.3901; 2.8103)
CGC/Cu10	3	14.252	0.212	(14.042; 14.462)
CGC/Cu5	3	4.702	0.196	(4.492; 4.912)

Pooled StDev = 0.157803

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

Type of Film	N	Mean	Grouping			
CGC/Cu10	3	14.252	A			
CGC/Cu5	3	4.702		B		
CGC/Cu1	3	2.6002			C	
CGC/Cu0	3	0.8751				D

Means that do not share a letter are significantly different.

Table 8: Antioxidant activity of CGC/Cu films

Method

Null hypothesis	All means are equal
Alternative hypothesis	Not all means are equal
Significance level	$\alpha = 0.05$

Equal variances were assumed for the analysis.

Factor Information

Factor	Levels	Values
Type of Film	4	CGC/Cu0; CGC/Cu1; CGC/Cu10; CGC/Cu5

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Type of Film	3	9646.92	3215.64	1869.54	0.000
Error	8	13.76	1.72		
Total	11	9660.68			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
1.31150	99.86%	99.80%	99.68%

Means

Type of Film	N	Mean	StDev	95% CI
CGC/Cu0	3	2.620	0.282	(0.874; 4.366)
CGC/Cu1	3	9.662	0.752	(7.916; 11.408)
CGC/Cu10	3	70.960	1.541	(69.214; 72.706)
CGC/Cu5	3	50.55	1.96	(48.80; 52.29)

Pooled StDev = 1.31150

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

Type of Film	N	Mean	Grouping			
CGC/Cu10	3	70.960	A			
CGC/Cu5	3	50.55		B		
CGC/Cu1	3	9.662			C	
CGC/Cu0	3	2.620				D

Means that do not share a letter are significantly different.

Table 9: Antimicrobial zone of CGC/Cu films against *E. coli*

Method

Null hypothesis	All means are equal
Alternative hypothesis	Not all means are equal
Significance level	α = 0.05

Equal variances were assumed for the analysis.

Factor Information

Factor	Levels	Values
Type of Film	4	CGC/Cu0; CGC/Cu1; CGC/Cu10; CGC/Cu5

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Type of Film	3	5.3873	1.79578	26.04	0.004
Error	4	0.2759	0.06897		
Total	7	5.6632			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.262615	95.13%	91.48%	80.52%

Means

Type of Film	N	Mean	StDev	95% CI
CGC/Cu0	2	7.918	0.145	(7.403; 8.434)
CGC/Cu1	2	8.4408	0.0209	(7.9252; 8.9563)
CGC/Cu10	2	10.135	0.500	(9.620; 10.651)
CGC/Cu5	2	8.7305	0.0643	(8.2149; 9.2461)

Pooled StDev = 0.262615

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

Type of Film	N	Mean	Grouping	
CGC/Cu10	2	10.135	A	
CGC/Cu5	2	8.7305		B
CGC/Cu1	2	8.4408		B
CGC/Cu0	2	7.918		B

Means that do not share a letter are significantly different.

Table 10: Porosity of CGC/Cu films

Method

Null hypothesis	All means are equal
Alternative hypothesis	Not all means are equal
Significance level	$\alpha = 0.05$

Equal variances were assumed for the analysis.

Factor Information

Factor	Levels	Values
Type of Film	4	CGC/Cu0; CGC/Cu1; CGC/Cu10; CGC/Cu5

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Type of Film	3	359.2983	119.7661	168092.79	0.000
Error	4	0.0028	0.0007		
Total	7	359.3012			

Model Summary

S	R-sq	R-sq(adj)
0.02669	100.00%	100.00%

Means

Type of Film	N	Mean	StDev	95% CI
CGC/Cu0	2	17.0500	0.0283	(16,9966; 17.1034)
CGC/Cu1	2	2.1500	0.0424	(2.0966; 2.2034)
CGC/Cu5	2	1.7150	0.0071	(1.6616; 1.7684)
CGC/Cu10	2	0.9500	0.0141	(0.8966; 1.0034)

Pooled StDev = 0.0267

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

Type of Film	N	Mean	Grouping			
CGC/Cu0	2	17.0500	A			
CGC/Cu1	2	2.1500		B		
CGC/Cu5	2	1.7150			C	
CGC/Cu10	2	0.9500				D

Means that do not share a letter are significantly different.