

Impact of Nano-Biomaterials and Essential Oils on Carboxymethyl Cellulose-Based Edible Films and Emulsions

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May 2025



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by

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fulfillment for the degree of*

Doctor of Philosophy

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Abstract

This research focuses on the formulation and application of sustainable edible coatings based on sodium carboxymethyl cellulose (CMC) and nano materials such as cellulose nanofibers (CNF), nanochitosan (N-Ch), and essential oils for enhancing the preservation of both fresh and dried food products. The first part of the study evaluated CMC/CNF-based coatings incorporated with varying concentrations of mandarin oil (MO) for their physicochemical properties and antifungal efficacy against *Botrytis cinerea*. Gas chromatography–mass spectrometry (GC-MS/MS) analysis identified β -pinene and limonene as major bioactive constituents of MO. The incorporation of MO significantly affected coating color, surface roughness, and antifungal performance, with 0.05% MO offering optimal reduction in strawberry weight loss and fungal infection. In a subsequent investigation, the combined use of mandarin peel extract (ME) and 1-methylcyclopropene (1-MCP) in CMC/CNF coatings further enhanced strawberry quality during 20-day storage at 5 °C by maintaining firmness, total soluble solids (TSS), citric acid, color, and total phenolic content. The CCM2-2 formulation yielded the most favorable metabolic and physical outcomes. Finally, the study extended to the preservation of dried silkworms (*Bombyx mori*) using coatings composed of CMC, CNF, N-Ch, and green pomelo peel oil (GPO). The CCG3 formulation, with a higher GPO concentration, improved coating elasticity, reduced thermal weight loss, and demonstrated the strongest antibacterial activity against *Staphylococcus aureus*, while enhancing porosity and metabolite retention in the dried product. Overall, this work highlights the potential of natural and nanostructured edible coatings to improve food quality, shelf life, and safety, while supporting circular economy principles through the valorization of agricultural by-products.

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Chapter 1: Introduction

1.1. Introduction of nanomaterials on edible films and coatings

In recent years, the growing concerns over environmental sustainability, food safety, and the reduction of synthetic packaging materials have fueled interest in biodegradable and edible alternatives for food preservation. Among these, edible coatings and films have gained significant attention due to their ability to act as protective barriers, improve shelf life, and serve as carriers for functional additives (Kowalczyk & Baraniak, 2014; Sharma et al., 2020). These coatings, often derived from natural biopolymers such as polysaccharides, proteins, and lipids, form thin layers on the surface of fruits, vegetables, and other perishable commodities, providing a partial barrier to moisture, gases, and microbial contaminants (Sogut, 2020). However, despite their benefits, conventional edible coatings often face limitations related to mechanical weakness, poor water vapor barrier properties, and limited antimicrobial or antioxidant activity. To overcome these challenges and enhance the functional performance of edible coatings, the incorporation of nanotechnology—particularly nanomaterials—has emerged as a transformative strategy (Muller et al., 2017).

Nanomaterials, defined by their nanoscale dimensions (typically less than 100 nanometers in at least one dimension), possess unique physicochemical properties that are not observed in their bulk counterparts. These properties include increased surface area, enhanced reactivity, and tunable optical, mechanical, and thermal behaviors (Chaudhary et al., 2021). When applied to edible coatings, nanomaterials can significantly improve structural integrity, barrier function, and the controlled release of active compounds such as essential oils, antioxidants, and antimicrobials (Hassan et al., 2018). Various types of nanomaterials have been explored in this context, including nanoclays, nanocellulose, nanoemulsions, and metal or metal oxide nanoparticles (e.g., silver, zinc oxide, titanium dioxide) (Shukla et al., 2021; Singh et al., 2021). Each of these nanostructures offers specific advantages, such as enhanced gas barrier properties, increased moisture resistance, or antimicrobial efficacy, making them valuable tools for food preservation.

The integration of nanomaterials into edible coatings also opens new avenues for the development of active and intelligent packaging systems. For instance, nanocarriers can be designed to respond to environmental stimuli, releasing bioactive agents in response to changes in temperature, pH, or microbial activity (Sirohi et al., 2020). Such functionalities contribute not only to extended shelf

life but also to improved food safety and quality assurance. Moreover, as nanomaterials can be derived from renewable resources or synthesized through green methods, their use aligns with the broader goals of environmental sustainability and circular economy practices in food packaging (Ravichandran, 2009).

Despite the potential benefits, the application of nanomaterials in edible coatings raises important considerations regarding safety, regulatory compliance, and consumer perception. Ensuring that these materials do not pose risks to human health or the environment is critical to their successful commercialization (Sekhon, 2010). Consequently, ongoing research is focused on evaluating the migration behavior, toxicity, and biodegradability of nanomaterials used in food systems.

This thesis explores the role of nanomaterials in enhancing the functional properties of edible coatings, with a particular focus on their integration with natural bioactive compounds such as essential oils. By examining the structural, physicochemical, and biological effects of incorporating nanomaterials into edible films, this work aims to contribute to the development of next-generation coating technologies that are safe, effective, and environmentally responsible.

1.1.1. Definition of nanomaterials

Nanomaterials are materials with at least one dimension in the nanometer range (1–100 nm). Due to their small size, they exhibit unique physical, chemical, and biological properties that differ significantly from their bulk counterparts. In the context of edible films and coatings, several types of nanomaterials have been explored:

- Nanoparticles (NPs): Metals (silver, zinc oxide), metal oxides (titanium dioxide), and biopolymer-based nanoparticles (chitosan, cellulose nanocrystals).
- Nanofibers: Electrospun fibers made from biopolymers that provide excellent barrier and mechanical properties.
- Nanocomposites: Matrices embedded with nanoscale fillers like clay nanoparticles, carbon nanotubes, or silica nanoparticles.
- Nano emulsions: Systems with nanosized droplets (e.g., essential oils in nano emulsion form) for improved antimicrobial and antioxidant activities.

The choice of nanomaterial depends on the intended functional enhancement, such as improved mechanical integrity, antimicrobial efficacy, or barrier performance.

1.1.2. Functional enhancements through nanomaterials

1.1.2.1. Mechanical and barrier properties

One of the primary motivations for incorporating nanomaterials into edible films is to improve their mechanical strength and barrier properties. Nanoparticles can act as reinforcing agents, creating a more cohesive and dense network within the polymer matrix. For instance, the inclusion of cellulose nanocrystals or montmorillonite clay has been shown to significantly enhance tensile strength, reduce water vapor permeability, and improve oxygen barrier properties. These enhancements are critical for prolonging the shelf life of perishable foods by limiting oxidative spoilage and moisture loss.

Moreover, the dispersion of nanomaterials at the nanoscale level leads to the creation of tortuous paths for gas and water molecule diffusion, effectively slowing down the transport process and improving the film's protective capabilities.

1.1.2.2. Antimicrobial activity

The integration of antimicrobial nanomaterials into edible coatings has gained particular attention in food safety applications. Metallic nanoparticles, such as silver nanoparticles (AgNPs) and zinc oxide nanoparticles (ZnO NPs), possess strong antimicrobial properties against a broad spectrum of pathogens, including bacteria, fungi, and viruses. When incorporated into edible films, these nanomaterials can inhibit microbial growth on the surface of food products, thereby reducing spoilage and foodborne illnesses.

In addition, natural nanoparticles, such as chitosan nanoparticles, offer antimicrobial activity while being biodegradable and biocompatible. Essential oil nano emulsions, such as those containing thyme or oregano oil, have also been employed to impart antimicrobial and antioxidant functionalities without introducing synthetic additives.

1.1.2.3. Controlled release of active compounds

Nanomaterials offer the possibility of designing edible films with controlled release mechanisms for active compounds. Through encapsulation at the nanoscale, bioactive agents such as

antimicrobials, antioxidants, and nutrients can be released gradually over time, maintaining their effectiveness throughout the food's storage period. This controlled release not only improves food preservation but also reduces the total amount of additives required, aligning with clean-label trends in the food industry.

For instance, nanoencapsulation of essential oils prevents their rapid evaporation and degradation, enabling prolonged antimicrobial activity on the food surface. Similarly, polymeric nanocarriers can modulate the release profile of encapsulated compounds in response to environmental stimuli like pH or temperature.

1.1.2.4. Safety and regulatory considerations

Despite the promising advantages of nanomaterials in edible films and coatings, their application raises important safety and regulatory concerns. The nanoscale size of these materials increases their reactivity and bioavailability, potentially leading to unforeseen toxicological effects when ingested.

Risk assessment studies have indicated that certain nanomaterials, such as silver nanoparticles, may accumulate in body tissues and cause cytotoxicity under specific conditions. Consequently, it is crucial to thoroughly evaluate the safety of each nanomaterial intended for use in edible applications. Parameters such as particle size, surface charge, dosage, and exposure route must be carefully considered.

Regulatory agencies, including the Food and Drug Administration (FDA) and the European Food Safety Authority (EFSA), are developing guidelines for the use of nanomaterials in food packaging and edible films. Approval processes typically require extensive data on material characterization, toxicology, migration behavior, and impact on human health.

1.1.2.5. Challenges and future perspectives

While the incorporation of nanomaterials into edible films holds significant promise, several challenges remain. Achieving uniform dispersion of nanoparticles within the polymer matrix is critical for maximizing functional benefits and minimizing agglomeration, which can compromise the film's performance. Moreover, scalability and cost-effectiveness are major considerations for commercial adoption.

Another important research direction involves the development of fully biodegradable and sustainable nanomaterials derived from renewable resources. For instance, cellulose nanocrystals, starch nanoparticles, and protein-based nanostructures offer an eco-friendly alternative to synthetic nanoparticles.

Additionally, "smart" edible coatings incorporating nanosensors or responsive materials could enable real-time monitoring of food freshness, further enhancing food safety and quality management.

Continued interdisciplinary research combining material science, food technology, toxicology, and regulatory science will be vital in unlocking the full potential of nanomaterials in edible film applications while ensuring consumer safety and environmental sustainability.

To summary, the integration of nanomaterials into edible films and coatings represents a transformative advance in food preservation technology. By enhancing mechanical strength, barrier properties, antimicrobial activity, and controlled release capabilities, nanomaterials offer solutions to many of the limitations faced by conventional edible films. However, careful consideration of safety, regulatory compliance, and environmental impact is essential to ensure the responsible development and application of these innovative materials. With ongoing research and technological advancements, nanomaterials are poised to play a key role in shaping the future of sustainable, safe, and functional food packaging systems.

1.2. Edible Coating and Edible Films

1.2.1. Definition of edible film and coating

Edible film and coating materials have been studied in recent years which are used to maintain the quality of fresh and processed food. Coating products have been used in 12th century by Chinese people who used wax to coat fruit and in 16th century England used to lard meat (Bourlieu *et al.*, 2008; KESTER and FENNEMA, 1989). Coatings are applied on the surface of products while films are prepared separately and applied latter. Edible coatings and films can be defined as a thin layer formed on the surface of the product and can be eaten as a part of product by consumers and provide a barrier to moisture, oxygen and/or solute movement in the food itself or between the food and its environment. The used of edible films are used in wide verity of products and its application is increasing overtime, now the total revenue of edible coating is exceeding \$100 million (Chemistry, 2000).

Edible films as defined earlier are thin sheets made of different materials, which are form separately. Edible films are not only characterized by their barrier properties (water vapor and oxygen permeability) but are also characterized by their structural properties such as; flexibility and stretch-ability for application on food and sensory characteristics; taste, smell, mouth feeling (Guilbert *et al.*, 1995; Bourlieu *et al.*, 2008). There are several kinds of components used to make films or are used to strengthen their structure and performance. These materials can be divided into 3 categories: proteins, polysaccharides and lipids and derivatives (Perez-Gago *et al.*, 2006; Bourlieu *et al.*, 2008); polymers which have the ability of making a continuous matrix. The most common materials are chitosan, starch, cellulose, sugars, pectin, vegetable gums, corn Zain, wheat gluten and derivatives, lipids, vegetable waxes, insect waxes etc. (Guilbert *et al.*, 1995). Generally its thickness is less than 0.3 mm (García *et al.*, 2009). Nowadays, scientists concentrate on chitosan and its deriviers combine with some additives to make edible coatings applying on agricultural products.

1.2.2. Materials

1.2.2.1. Carboxymethyl cellulose

Carboxymethyl cellulose (CMC), a water-soluble, anionic polysaccharide derived from the partial substitution of cellulose hydroxyl groups with carboxymethyl groups, has gained significant attention as a functional material in edible films and coatings. Due to its excellent film-forming ability, biodegradability, high viscosity at low concentrations, and recognized safety status (GRAS) by the United States Food and Drug Administration, CMC is widely applied in food preservation technologies (Dash et al., 2019). Its ability to form cohesive, flexible, and transparent films makes it a promising candidate for developing bio-based packaging alternatives aimed at improving food shelf life, quality, and safety.

The barrier properties of CMC-based films are critical for food preservation. Although CMC exhibits hydrophilic characteristics leading to moderate water vapor permeability, it provides substantial resistance to gas transmission, particularly oxygen and carbon dioxide (Azeredo, 2009). This selective gas barrier functionality is instrumental in slowing the metabolic processes of fruits and vegetables, delaying senescence, and reducing spoilage. Coatings based on CMC have been demonstrated to effectively reduce respiration rates and maintain postharvest quality parameters such as firmness, color, and nutritional content in a variety of produce, including strawberries (Ali et al., 2010), tomatoes (Rojas-Graü et al., 2009), and apples (Baldwin et al., 1999).

Beyond passive barriers, CMC serves as an effective carrier matrix for the incorporation of active compounds, facilitating the development of antimicrobial and antioxidant edible films. Incorporation of antimicrobial agents, such as nisin, chitosan, and essential oils (e.g., thyme, oregano, clove oils), into CMC matrices has shown synergistic effects in inhibiting bacterial and fungal growth on fresh and minimally processed foods (Ojagh et al., 2010). Similarly, embedding antioxidants like ascorbic acid, tocopherols, and polyphenolic extracts within CMC films mitigates oxidative deterioration, particularly lipid oxidation in fatty foods. Controlled release behavior of bioactive compounds from CMC matrices is governed by the polymer network structure, environmental factors (e.g., humidity, pH), and interaction dynamics between CMC and the incorporated molecules (Campos et al., 2011).

Nonetheless, the intrinsic hydrophilicity of CMC can limit its application in high-moisture environments. Strategies to enhance the functional performance of CMC films include blending with other biopolymers such as gelatin, starch, alginate, and chitosan. For instance, composite CMC-chitosan films demonstrate superior antimicrobial activity and reduced water vapor permeability compared to pure CMC films (Guerra et al., 2015). Plasticizers such as glycerol and sorbitol are typically added to improve film flexibility but must be carefully optimized to prevent excessive hydrophilicity.

Advances in nanotechnology have further expanded the application of CMC in food packaging. The incorporation of nanomaterials, including silver nanoparticles (AgNPs), zinc oxide nanoparticles (ZnO NPs), cellulose nanocrystals, and nanoemulsions of essential oils, into CMC matrices enhances mechanical strength, barrier properties, and antimicrobial functionality (Embuscado, 2011). For example, CMC films reinforced with cellulose nanocrystals exhibit higher tensile strength and lower oxygen permeability due to the creation of a more tortuous path for gas molecules. Meanwhile, AgNP-loaded CMC films have demonstrated significant antimicrobial efficacy against foodborne pathogens such as *Escherichia coli* and *Listeria monocytogenes* (Kanmani & Rhim, 2014).

Emerging trends in CMC-based edible films involve the development of active and intelligent packaging systems. Active films not only serve as barriers but also interact with the food or its environment to maintain or improve food quality. Intelligent films, incorporating natural pH-sensitive dyes like anthocyanins into the CMC matrix, have been explored for real-time monitoring of food freshness by indicating changes in pH associated with spoilage processes (Senturk Parreidt et al., 2018). These smart coatings hold promise for reducing food waste and enhancing consumer confidence.

In conclusion, Carboxymethyl cellulose presents a robust, versatile platform for edible films and coatings in food packaging. Its compatibility with a broad range of bioactive agents and nanomaterials, combined with its environmental friendliness and regulatory approval, positions it at the forefront of sustainable food packaging solutions. Future research should continue to address limitations related to moisture sensitivity and investigate the full potential of CMC in multi-functional, smart packaging systems capable of meeting the evolving demands of food safety, quality assurance, and environmental sustainability.

1.2.2.2. Plasticizers

Film properties and film structure were affected by film forming materials. Different kind of additive are used to modify the structure and functionality of the film; the most important additives are plasticizers, cross-linking agents, anti-oxidants, anti-microbials (Zhang et al., 2008).

Plasticsizers were used to make the film flexible. Plasticizers are any of a group of substances that are used to improve the viscosity, flexibility, softness, or other properties of a film (Janjarasskul & Krochta, 2016). Plasticizers are high boiling, non-separating organic solvent, which when added to another material, changes the physical and/or mechanical properties of that material. Plasticizers can be divided according to their function or their sources. Some of the functional categories are internal, external, primary, secondary, physical, chemical (HallStar, 2010). Plasticizers which are commonly used for polysaccharide-based films are polyols like; glycerol, sorbitol, xylitol (X), minnitol (M), eghylene glycol (EG) and lipids like; fatty acid, phospholipid etc (Janjarasskul & Krochta, 2016). Internal plasticizers chemically reacts with the polymer chain while external plasticizer interacts physically with polymer (HallStar, 2010).

Compatibility of the polymer-plasticizer is responsible for the homogeneity and stability of the solution during processing and cooling of the mixture respectively (Romero-Bastida *et al.*, 2005; Mali *et al.*, 2005). Therefore, plasticizer must be compatible with the polymer; Commonly glycerol and sorbitol are used in chitosan - based films as plasticizers. It should be mentioned that the amount of the plasticizers should be used in optimum concentration; this concentration is directly related to end use of the film/coating solution, if the concentration is not ideal it may have negative effect on the film barrier and other properties (López et al., 2008).

Plasticizers act as the barrier properties of the film in a significant level. Polymer without plasticizer are ridged and this raggedness is due to a coagulum of weak attachments along the chains which restricts the movement of adjacent polymers, when plasticizer interacts with these coagulum sites, resulting in a separation at the adjacent polymer chains, thus the intermolecular attraction between adjacent polymeric chains decreases, which improves film flexibility (Romero-Bastida ., 2005; HallStar, 2010).

Glycerol is a the most common polyol used as a plasticizer for natural polymers and for plant proteins, which include chitosan, starch, wheat gluten and soy protein. Glycerol is a nontoxic plasticizer which compatible with amylose and is most commonly used as a plasticizer for starch-

based film (Jansson and Thuvander, 2004; Mali *et al.*, 2005; Basiak *et al.*, 2018; Tian *et al.*, 2018). Glycerol is a hydrophilic component having a low molecular weight, which can interfere between the adjacent point of the polymer chain, thus reducing the intermolecular attraction and increasing the molecular mobility of polymer chains, producing a more flexible film (Farahnaky *et al.*, 2013; Chiumarelli and Hubinger, 2014; López *et al.*, 2008).

It has been reported that, if glycerol is used less than 30%, the produced films will be less flexible (Mohammadi Nafchi *et al.*, 2011), on the other hand if the concentration of glycerol exceeds 20g/L in a starch-based film, the film suspension will show stickiness which is due its migration to surface and making the films/coating hard to handle (López *et al.*, 2008) (García *et al.*, 2009) (García *et al.*, 1998).

1.2.2.3. Essential Oils

The demand for environmentally sustainable and health-conscious food packaging has catalyzed the development of bio-based edible films and coatings (EFCs). These materials not only act as barriers to moisture, gases, and microbial contamination but also extend the shelf life and quality of various perishable products. Among the natural additives incorporated into EFCs, essential oils (EOs) have garnered considerable attention due to their broad-spectrum antimicrobial, antioxidant, and antifungal properties. Derived from aromatic plants, EOs contain volatile bioactive compounds that can serve as natural preservatives and enhance the functional characteristics of edible packaging materials (Panahirad *et al.*, 2024; Moghimi *et al.*, 2024).

The integration of EOs into edible films offers a multifunctional approach to active packaging by combining the physical protection of films with the biological activity of natural extracts. However, despite their potential, the use of EOs in food packaging remains technically challenging due to their volatility, low water solubility, and strong organoleptic properties. In this chapter, the structural, functional, and application aspects of essential oils in edible films and coatings are examined, including recent technological advances and current research trends.

Essential oils are complex mixtures predominantly composed of terpenes (e.g., limonene, pinene), terpenoids (e.g., linalool, menthol), phenolic compounds (e.g., eugenol, thymol, carvacrol), aldehydes (e.g., cinnamaldehyde), and alcohols. These constituents are largely responsible for the biological activity of EOs. Phenolic compounds such as thymol and carvacrol exhibit membrane-

disruptive properties, leading to leakage of cellular contents and inhibition of microbial growth (Moghimi et al., 2024).

The antioxidant properties of EOs stem from their ability to neutralize free radicals, thereby reducing lipid oxidation and enzymatic browning in food systems. This activity makes EOs particularly effective in preserving the quality and sensory attributes of perishable commodities during storage. The specific chemical profile and potency of EOs vary depending on plant species, extraction methods, and environmental conditions.

1.2.2.3.1. Methods of Incorporation into Edible Films and Coatings

Several strategies have been developed to incorporate EOs into EFCs effectively while minimizing their negative sensory impact and maximizing their bioavailability. Direct incorporation into film-forming solutions is the simplest method, but it often leads to phase separation due to the hydrophobic nature of EOs.

To overcome this, nanoemulsion techniques have been extensively explored. Nanoemulsions are stable colloidal systems with droplet sizes typically below 200 nm, which enhance the dispersion and controlled release of EOs within the matrix. Soleimanian-Zad et al. (2024) reported that oregano EO nanoemulsions in chitosan films improved antibacterial activity and film uniformity. Alternatively, Pickering emulsions, stabilized by solid particles, and solid lipid nanoparticles have demonstrated high encapsulation efficiency and thermal stability (Akhtar et al., 2024).

Other encapsulation techniques include coacervation, spray drying, and the use of cyclodextrins or biopolymer microcapsules, which protect EOs from environmental degradation and provide sustained release properties.

1.2.2.3.2. Interaction with Film-Forming Materials

The interaction between essential oils and the film-forming matrix plays a critical role in determining the final properties of the film. Polysaccharides such as starch, pectin, and alginate, as well as proteins like gelatin and casein, are frequently used as base materials. The addition of EOs often modifies the mechanical properties, water vapor permeability (WVP), and optical characteristics of the films.

For example, incorporation of cinnamon EO in starch-based films reduced tensile strength but improved water resistance and antimicrobial properties (Asgher et al., 2024). Such trade-offs necessitate careful formulation optimization to balance mechanical performance and bioactivity.

1.2.2.3.3. Applications Across Food Categories

EO-enriched edible films and coatings have demonstrated efficacy in preserving a wide range of food products. In fresh produce, coatings containing thyme and clove EOs have significantly reduced microbial decay and retained firmness in strawberries and tomatoes (Panahirad et al., 2024). Similarly, films containing cinnamon or rosemary EOs have been successfully applied to apples, grapes, and citrus fruits, extending shelf life and preventing postharvest diseases.

In dairy applications, EO-based coatings have shown inhibitory effects against molds and spoilage bacteria on cheese surfaces. For instance, oregano EO in casein-based coatings helped maintain microbiological quality of hard cheese during cold storage (Moghimi et al., 2024). In meat and poultry, EO-infused films have effectively controlled lipid oxidation and pathogen growth. Chitosan films containing lemongrass and clove EOs inhibited *Listeria monocytogenes* and delayed discoloration in fresh chicken meat.

Bakery products have also benefited from EO applications. Bread wrapped in cinnamon EO-infused starch films showed delayed fungal growth without compromising sensory properties. Confectionery products like chocolates and candies have used edible coatings with peppermint or citrus EOs to add functional aroma and microbial protection.

1.2.2.3.4. Recent Advances

Recent innovations have focused on enhancing EO stability and functionality through advanced delivery systems. Smart packaging systems that respond to environmental triggers (e.g., temperature, pH) are being developed for controlled EO release. Active packaging combining EOs with other natural antimicrobials, such as bacteriocins and phenolic acids, has shown synergistic effects in reducing microbial loads.

Biopolymer blending is another area of progress, where combining different natural polymers enhances film flexibility, adhesion, and active agent compatibility. The integration of nanostructured carriers such as nanoclays, zeolites, or cyclodextrins also improves EO entrapment and release kinetics.

1.2.2.3.5. Challenges and Limitations

Despite their advantages, EO-based edible films face several challenges. One of the major concerns is the strong aroma and flavor imparted by EOs, which may not be acceptable in all food applications. Consumer acceptance is closely linked to formulation optimization to ensure that sensory attributes are not adversely affected.

Another limitation is the regulatory status of EOs, which varies by country. While many EOs are classified as GRAS (Generally Recognized As Safe) by the FDA, their permitted concentrations and food applications are strictly controlled. Furthermore, the natural variability in EO composition and the lack of standardized quality parameters complicate their industrial adoption.

Technologically, maintaining the stability of EOs during processing, storage, and application requires careful formulation. Environmental factors such as light, heat, and oxygen exposure can degrade EO constituents, reducing their efficacy.

1.2.2.3.6. Future Prospects

Looking forward, the development of smart, responsive edible films that release EOs based on environmental stimuli presents a promising direction. Multifunctional films that integrate EOs with biosensors or indicators could be used for real-time monitoring of food quality.

Consumer-centered research involving sensory testing and preference analysis will be essential for the commercial success of EO-infused EFCs. Moreover, sustainable sourcing and valorization of EO-rich agricultural by-products could reduce costs and environmental impacts.

Collaborative efforts between food scientists, material engineers, and regulatory agencies are needed to standardize EO film production and establish safety guidelines. Advances in nanotechnology, polymer chemistry, and food microbiology will continue to drive innovation in this field.

1.2.2.4. Lipids

On the other hand, chitosan films are hydrophilic; lipids are added to increase the hydrophobicity of the film which will result in decreasing their water affinity and will help in blocking microbial and physicochemical deterioration, the efficiency of lipid depends on its structure. This phenomenon has been demonstrated through evaluating water vapor and oxygen barrier of the film

(Debeaufort and Voilley, 2009). A wide range of these hydrophobic components have been used in film/coating solution, (Debeaufort & Voilley, 2009).

The functional property of lipid is mainly influenced by the structure, degree of saturation, chain length, physical state, shape and dimension of crystal, and distribution of lipids into the film (Cha & Chinnan, 2004). Lipids based on their function are classified into 2 groups polar and non-polar.

Non-polar lipids are completely insoluble in water and they do not spread on surface to form a monolayer, because they do not possess any polar set-up in their composition; like hydrocarbons, which would react with water, thus improving water vapor barrier of film more effectively compared to other kind of lipids. Waxes are considered as non-polar lipids and have many varieties among which carnauba, beeswax, paraffin, rice bran and candelilla have been used in combination with coating material (Cha & Chinnan, 2004).

Polar lipids are also non soluble in water but they will spread at the interface to form a stable monolayer. Triglycerides can be categorized in this class. The water affinity of this category depends on its structure; long-chain triglycerides are insoluble in water while short-chain triglycerides are partly soluble.

Monoglycerides are another type of lipid which can be classified as a polar or a non-polar lipid and this classification is based on their chain length. These compounds are used as emulsifier; for stabilizing emulsified film and increasing adhesion between two components with different hydrophobicity (Cha & Chinnan, 2004) (Lipid-based edible films).

Lipids interrupt the polymer matrix therefore it is important to use them in an appropriate ratio. Lipids are mainly used in coating compositions to improve barrier properties but, excess of lipid can have a undesirable effect on transparency and mechanical properties of the film (Jiménez et al., n.d.) (Fabra et al., 2009). The exceeded amount of lipid may migrate to the surface and this migration depends on the matrix microstructure. Therefore lipid-polymer ratio is also an important factor in formulating film/coating solution (Sapper & Chiralt, 2018). In starch based films lipid migration will not happen if an amount less than 5 g/L (sunflower oil) is used (Chemistry, 2000). At the same time (Lipid Based Edible Films) reported that in-cooperation of about 20% (w/w of polysaccharide) of edible oil to starch-based coating might enhance the shelf-life of high-oil foods.

1.2.2.5. Surfactants

Surfactants are solution compounds that have lower the surface tension (or interfacial tension) between two liquids, between a gas and a liquid, or between a liquid and a solid. Surfactants may act as detergents, wetting agents, emulsifiers, foaming agents, or dispersants. It sticks more to the container or surface than to itself (like benzene) and spreads itself as much as possible, which helps the surface to be coated to wet. It is important for the coating solution to cover all the surface, if it is not the case, the coating layer will not adhere on applied product, which would generate bubbles on the surface. These bubbles would cause opacity and ruin the strawberry of the product (Michale E. Kramer, 2009). Surfactants like Tween 20 or Tween 80 in cooperation with glycerol had showed a better effect of the physical properties of starch based edible films. (Rodríguez et al., 2006) Tween 20 also acts as an emulsifier which can stabilize an emulsion.

Research objective

1. To develop sustainable and environmentally friendly edible coatings using agricultural by-products (e.g., citrus and pomelo peels) and nanomaterials like cellulose nanofibers (CNF) and nano-chitosan (N-Ch), reducing reliance on synthetic materials.
2. To evaluate the antifungal and physical properties of edible coatings made from CMC/CNF and essential oils (e.g., mandarin or pomelo peel oil) for fruit preservation, especially for perishable fruits like strawberries.
3. To examine the effectiveness of incorporating 1-MCP into edible coatings in extending the shelf life and maintaining the freshness of non-climacteric fruits (e.g., strawberries), while reducing the limitations of current 1-MCP application methods.
4. To explore the use of edible coatings in the drying process of heat-sensitive samples like silkworm pupae, aiming to protect surface texture, nutritional compounds, and minimize quality deterioration.
5. To improve the mechanical, barrier, and antimicrobial properties of CMC-based films by incorporating nanomaterials and essential oils, enhancing their functionality in food packaging and preservation.
6. To assess the performance of edible coatings in real-world applications, including coating transferability, processing convenience, and effects under storage and heating conditions.

Chapter 2: Effect of edible coating incorporating cellulose nanofibers and self-produced Mandarin Oil based on sodium carboxymethyl cellulose on strawberries

2.1. Introduction

Fruit and vegetable residues contain high levels of nutrients and bioactive compounds, making them valuable resources for the food and pharmaceutical industries. Utilizing these residues not only helps reduce waste but also promotes sustainable development. Therefore, continuing researching and development efforts to explore sustainable applications of fruit and vegetable residues is crucial for promoting a circular economy (Jiang et al., 2021). Citrus trees are widely cultivated in many countries and serve as inexpensive sources of citrus fruits, which can be obtained in high yields (Anticona et al., 2021). The varieties of domestic mandarin produced in Japan primarily include Natsu-Mikan (*Citrus natsudaidai*) and Iyokan (*Citrus Iyo*). The Japanese tangerine/mandarin season typically spans from October to May. According to Foreign Agricultural Service (FAS) Tokyo, in the 2021–2022 period, mandarin production in Japan reached 924,000 tons, and approximately 90 % of tangerines are consumed fresh in the country (Valverde, 2021). Therefore, tangerine/mandarin peel holds great potential as a valuable by-product and a rich source of essential compounds for natural extraction. The application of biological compounds of citrus peels in food has been extensively studied because of their nutritional and health benefits (Yoo and Moon, 2016). Compounds such as flavonoids, carotenoids, and ascorbic acid present in mandarin oil (MO) show antioxidant, anti-inflammatory, anticancer, antibacterial, cardioprotective, neuroprotective, nephroprotective, and antiallergic activities (Anticona et al., 2021). In this study, the MO was extracted from mandarin peels obtained from Fukuoka, Japan. By taking advantage of locally available by-products, we simplified the preparation of edible coatings without relying on raw materials from other products.

Edible coatings have been a topic of research over the past few decades. Research on edible materials is for developing sustainable agriculture (He et al., 2019). While research on edible coatings has been widely published, it is essential to emphasize the need for ensuring the applicability and ease of transferability of the obtained results to actual production scenarios. There are still many limitations in the research and application of edible coatings. For example, use of chitosan-based edible coatings may require completely dissolution of chitosan, which can only be achieved under acidic conditions (Chen et al., 2018). This limitation has been acknowledged in

the actual production process because some agricultural products can react strongly under low-pH environmental conditions (Chen et al., 2018). Moreover, the time-consuming nature of adjusting the pH value poses significant challenges to practical applications of chitosan-based edible coatings (Su et al., 2007). Therefore, it is important to explore and develop alternative edible coatings that are both effective and easy to apply.

Sodium carboxymethyl cellulose (CMC) is a modified cellulose obtained via carboxymethylation (Lan et al., 2018). CMC is water-soluble, has good film-forming properties, and exhibits moderate air permeability (Nasibi et al., 2020). CMC has a stable internal network structure and can be used to improve the performance of composite membranes (Lan et al., 2018). Lan et al. (2018) also reported the positive effect of sodium alginate/chitosan/CMC on composite films, which demonstrate excellent mechanical properties and are used as the primary structure in membrane complexes. The combination of carboxymethylcellulose/cellulose nanofiber/potassium permanganate (CMC/CNF/KMnO₄) hydrogel films has been reported to extend the shelf life of bananas by using an active hydrogel; there KMnO₄ is a mineral which has strong oxidizing power. Applications of KMnO₄ include ethylene absorption, which can be used after harvest to increase the shelf life of fruits. According to the authors, hydrogels can extend the shelf life of bananas by simultaneously controlling ethylene and moisture in food packaging (Pirsa, 2021). Cellulose nanofibers (CNFs) were extracted from plants or bacterial cellulose via high-pressure homogenization. CNFs were used as reinforcement materials in the film matrix or as self-standing neat isotropic films (Miguel et al., 2021); CNF improved the droplet and fiber entanglements in the emulsification process, which led to a higher stabilization of fibers and droplets 3D network structure (Trinh et al., 2022). In this study, CNFs were utilized to stabilize the coating from CMC/MO emulsions, in addition to functioning as an oil/water (O/W) emulsion in these composites. Pirsa (2021) indicated that coating treatment of banana (climatic fruit) with the hydrogel films made from CMC/CNF/KMnO₄ absorbed the ethylene released from bananas, controlled the humidity inside the package, reduced ripening process, and improved shelf life and quality of banana fruit during storage. However, on strawberries (non-climatic fruit), there are no reports on the effect of base CMC/CNF and other combinations.

Therefore, in this study, the hydrogel-derived film from CMC and CNF combined with MO was made and applied on strawberry fruits. The main aim of this research was to determine the antifungal and physical properties of the new edible coating prepared by reusing natural by-

products, which is required in the field of preservation and packaging of agricultural products. The characteristics of the film were investigated and the quality preservation effect of the coating on strawberries was verified.

2.2. Materials and Methods

2.2.1. Chemical reagents

Sodium carboxymethylcellulose (CMC) with 6.5 % to 8.5 % Na, practical grade was obtained from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). CNF powder, produced from 2,2,6,6-tetramethylpiperidine-1-oxyl (TEMPO) catalytic oxidation-treated wood fiber, was obtained from Nippon Paper Industries Co., Ltd. (Tokyo, Japan). Oleic acid Wako first grade, polyoxyethylene 20 sorbitan monooleate molecular biology grade (Tween 80), glycerol guaranteed reagent grade, n-hexane Wako first grade, calcium chloride reagent grade, and sodium hypochlorite with practical grade were provided from FUJIFILM Wako Pure Chemical Co., Ltd. (Osaka, Japan). KOH/ MeOH 0.5 N, n-alkanes (C7-C30 saturated alkanes 1000 µg/mL), acenaphthene - d10 and EPA 524.2 solution were obtained from Sigma-Aldrich Japan G.K. (Tokyo, Japan). Potato dextrose agar (PDA) was obtained from Nissui Pharmaceutical Co., Ltd. (Tokyo, Japan). Distilled water was prepared using an Aquarius Advantech GS-200 automatic water distillation apparatus.

2.2.2. Methodology

2.2.2.1. Fruit preparation

The harvested strawberries (Amaou) with the size from 3.5 to 4.0 cm, the weight ranging from 20 to 23g, and mandarins (Miyagawa-wase) were transported directly from the Japan Agricultural Cooperative (JA) in Itoshima, Fukuoka, Japan, to the postharvest science laboratory on the day of the experiment. A total of 245 strawberries were graded and selected based on their freshness, color, uniformity, and size, any damaged or diseased strawberries were discarded. Of which 210 fruits were evaluated for quality and 35 fruits for antifungal evaluation. The selected strawberries were dipped in a 0.01 % sodium hypochlorite solution for approximately 2 min to remove dirt and microorganisms accumulated before and during harvesting and then air-dried at room temperature. The strawberries were divided as follows: in the control samples, the strawberries were dipped in distilled water for 1 min, and in the coating samples, the strawberries

were immersed in the coating solutions for 1 min. After dipping, the strawberries were allowed to air-dry at room temperature. The dried strawberries were placed in a polyethylene terephthalate (PET) pack (dimensions of 18 cm x 24 cm x 3 cm, six perforations, one padding layer, 15 fruits per pack) and stored at 5 °C and 85 ± 5 % relative humidity (RH) for 20 days in an incubator (PHCbi, MLR-352H, SciMed Pte LTD, Singapore). Strawberry samples were evaluated for quality at 1, 5, 10, 15 and 20 days with five replicates while antifungal experiments were evaluated at 2, 6, 10 and 15 with five replicates.

The mandarin fruits were washed, and the peels and pulp were separated. The mandarin peels were recovered and dried at 60 °C until the moisture did not change. Finally, the dried peels were ground into a powder using a grinder for oil extraction.

2.2.2.2. Experimental procedures

2.2.2.2.1. Oil extraction

The MO was extracted from the mandarin peels following the method described by Anticono et al. (2021), with modifications. A specific amount of mandarin peel powder was weighed and placed in a conical flask with a tight-fitting lid, and a specified quantity of n-hexane was added to the flask as a solvent. The n-hexane to mandarin powder ratio was 2:1. The flask was placed in a fixed position on a shaker (EYELA, MMS-3010, YamaP Co., Ltd, Japan) and was shaken at a speed of 230 rpm for 23 h. The mixture was allowed to settle for approximately 2 h, and the solution was filtered through Whatman filter papers number 41 Ashless with diameter 90 mm (Whatman International Ltd., UK). After filtration, the solution was separated to obtain pure oil using a rotary evaporator separation system (IKA Rotary Evaporator; IKA Japan K.K., Japan). This process was repeated twice because the authors found no further MO recovery after the third extraction. The extraction efficiency was calculated using the Eq. (1):

$$\text{Extraction efficiency (\%)} = \text{Oil yield (g)} \times 100 / \text{The amount of orange peel powder (g)} \quad (1)$$

2.2.2.2.2. Coating and film preparation

The base solution was prepared as follows: CMC (1.0 %, w/v) and CNF (0.5 %, w/v) powders were slowly added and stirred into distilled water. Glycerol (0.35 %, w/v) was added to the above solution with continuous stirring using a magnetic stirrer for 2 h. While the base solution was stirred, the oil solution was prepared by combining 0.05, 0.1, 0.2, 0.3, or 0.4 % (w/v) MO with

0.03 % (based on oil) Tween 80, 0.05 % oleic acid (based on oil) as an emulsifier, and distilled water. Subsequently, the MO solutions and the base solutions were combined and homogenized for 3 min at 20,000 rpm using a homogenizer (T-25 digital easy clean, IKA, Japan). The final solutions named CCM1 (CMC/CNF), CCM2 (CMC/CNF/MO 0.05 %), CCM3 (CMC/CNF/MO 0.1 %), CCM4 (CMC/CNF/MO 0.2 %), CCM5 (CMC/CNF/MO 0.3 %) and CCM6 (CMC/CNF/MO 0.4 %). The solutions were then de-bubbled in vacuum (Yamato ADP300, Japan) to ensure uniformity. For film casting, 15 mL of the solution was poured onto a square silicon plate and dried for 8 h at 40 °C in an incubator (EYELA WFO-520W, Tokyo Rikakikai Co., Ltd, Japan). The film was separated from the plate and kept at room temperature and 53 % RH in a desiccator containing calcium chloride as a moisture-retaining agent to achieve stable conditions for subsequent determinations.

2.2.2.3. Coating and film characteristics

2.2.2.3.1. pH, viscosities and emulsion property of the coatings

The pH of each coating was determined at room temperature using a compact pH meter (LAQUAtwin, pH-22, HORIBA Science, Japan) with five separate replicates.

A viscometer (Viscometer TV200, Toki Sangyo Co., Ltd, Japan) was used to measure the viscosity. 1.1 mL of the of the coating solution was poured into the screw tube. The screw tube containing the coating solution was used for measuring the pH, with three separate replicates.

The emulsion states of the coatings were assessed by observing the images captured at intervals of 10 days within 30 days of storage at room temperature.

2.2.2.3.2. Film characteristics

The film thickness (μm) was examined using a ratchet thimble digital micrometer (Series 102–707, Mitutoyo Corporation, Japan) to the nearest 0.001 mm, at five randomly selected points per film.

The film color was measured at different angles using a color reader (CR-20, Konica Minolta Inc., Japan), and each film contained five recorded values. The Commission Internationale de l'Eclairage (CIE) color scale includes the L^* , a^* , b^* and ΔE color systems (L^* values range from 0 to 100, indicating diffuse black reflective to white, though reflective white values can be higher; a^* values represent the color space between red and green; b^* values represent the color

space between yellow and blue). The value of ΔE (total color change) was calculated by Eq. (2) according to the method of Van et al. (2023):

$$\Delta E = \sqrt{(L_0 - L^*)^2 + (a_0 - a^*)^2 + (b_0 - b^*)^2}$$

(2) The values of L_0 , a_0 , and b_0 were for the control film (CCM1) while L^* , a^* , b^* were for the treatment films of triplicates.

Water vapor permeability of films

The water vapor permeability (WVP) of the films were evaluated using WVP cups (JIS Z-0208) with a permeable area of 28 cm². Briefly, 15 g of calcium chloride as moisture absorbents were placed in a cup then the cup was covered with a thin film. An incubator (ISUZU TPAV 120–20, Japan) was set and the cups were placed inside under conditions of 25 °C and 90 % RH. The cups were weighed every hour and changes in weight over time were recorded. The WVP was calculated according to Ep. (3):

$$WVP = (\Delta L \times TN) / (A \times \Delta T \times \Delta P) \quad (3)$$

where the unit of WVP is g mm /m² h kPa, ΔL is the weight increase of the cup (g), TN is the film thickness (mm), A is the exposed film area (m²), ΔT is the interval measurement time (h), and ΔP is the vapor pressure difference across the film (kPa). All films were measured in triplicate.

Scanning Electron Microscopy (SEM) and Atomic Force Microscopy (AFM)

The film surface and cross-section were examined using a scanning electron microscope (SEM, SU3500, Hitachi High-Technologies Corporation, Japan). A piece of the film was attached to conductive double-sided tape and coated with a thin layer of gold using a plasma-coating device osmium coater (HPC-1SW, Vacuum Device company, Japan). Pictures were taken and recorded under 15.0 kV voltage, 100x magnification, and 500 µm width for the surface, and under 15.0 kV, 4.00k magnification, and 10.0 µm width for the cross-section.

An atomic force microscope (AFM, Hitachi 5200S, Japan) operated by the Nano Navi application in tapping mode was used to determine the film morphology and roughness. The fixed samples were prepared by adding films (CCM1, CCM2, CCM3, CCM4, CCM5, and CCM6) to freshly cleaved mica. A type-SI-DF20 cantilever was used with a scanning frequency of approximately 0.84 Hz and a scanning area of 2 µm × 2 µm. Each film was measured 12

replications to determine the mean values of Ra (roughness average) and Rq (the root-mean square roughness).

2.2.2.4. Gas chromatography-mass spectrometry/mass spectrometry (GC-MS/MS) analysis

The primary chemical constituents of MO were identified using GC-MS/MS analysis. The GC (Nexis GC 2030, Shimadzu Corporation) was operated using a column (SH-PolarWax-MS, Shimadzu, Corp., USA) of 0.25 mm ID, 30 m length, and 0.25 μ m film thickness. The liquid injection system with a flow rate of 50 μ L/s was employed, and the injection port temperature was set at 250 °C. The column temperature was initially set at 50 °C for 5 min and then increased to 250 °C for 10 min, with a split ratio of 5.0 over 1 min. Helium (He) was used as the carrier gas with a linear velocity of 43.3 cm/s and a pressure of 83.5 kPa. The mass spectrometer was set to scan a range from 35 m/z to 400 m/z, with ion source temperature at 200 °C and the interface temperature at 250 °C. In the first step of the measurement, a smart aroma database set (Shimadzu, Corp., Japan) was used for the calibration of retention time using n-alkanes (10 μ g/mL) and for sensitive correction of mass-spectrometer, involving a combination of acenaphthene-d10 solution and EPA 524.2 solution in a 1:1 ratio. This set includes established calibration method files for n-alkanes and sensitive correction, along with a sample scanning method file which included 511 targeted compounds. After the calibration stages finished, the acquired result files were combined with the sample scanning method file from the smart aroma database to create a customized scanning method file for evaluating the samples. The subsequent procedure involved the performance of an acquisition and batch file for the measurement of the sample. Semi-quantitative concentrations of detected compounds were automatically calculated based on the sample scanning method and the dilution ratio used. The MO for analysis was prepared as previously described (Gago et al., 2022) with some modifications. Briefly, 0.5 mg of MO was diluted with 2 mL of n-hexane as normalization and 0.5 mL KOH/ MeOH 0.5 N as derivative of the fatty acids. Subsequently, 0.5 mL of the above solution was transferred to a 1.5 mL glass vial (GLCTV-901, Shimadzu, Corp., Japan) with a lid, and the vials were placed on the trays in sequence. The composition of the substances in the MO was automatically analyzed and calculated according to the Smart Aroma Database standard.

2.2.2.5. Antifungal activity

In vitro

The antifungal activities of the edible films were determined following a modified version of the method described by Sun et al. (2022). For the cultivation and collection of fresh and purebred mycelia, experiments were conducted in a sterilized incubator (BHC-T701II, A2 benchtop, AIRTECH, Japan); 15 mL of PDA as nutritional medium was poured into petri dishes and allowed to cool to solidify under UV light to prevent superinfection while cooling. Further, 5 mm of the mycelium of the *B. cinerea* strain (NBRC100717, NITE., Go., Japan) was transferred to the Petri dish containing the dried PDA. The dishes were covered with duct tape, and the mold was cultivated at 25 °C for 1 d in an incubator (EYELA LTE1010, Tokyo Rikakikai Co., Ltd, Japan). The one-day mycelial fungi were used to determine the fungal resistance of edible films.

A medium suitable for fungal testing was prepared by combining 15 ml of solid PDA with edible films. Five hundred microliters of edible coating or distilled water were poured onto the surface of the solid PDA placed in the Petri plate. The coating or distilled water on the surface of the PDA was dried overnight under UV light for 24 h. After the edible films or distilled water on the surface of the agar plate had completely dried, 5 mm of 1-day-old mycelium of the mold was transferred to the center of the surface of the plate with or without edible films, with five repetitions for each sample. The antifungal activity of the edible films was photographed, and the diameter of mycelia was measured using a digital caliper (Mitsutoyo Calipers, Japan). Data were recorded following the interval between the first and second days for a total of 2 days.

In vivo

The in vivo antifungal activity of the strawberries was also evaluated following the method of (Tran et al., 2021). The selected strawberries were dipped in a 0.01 % sodium hypochlorite solution for 1 min, then air-dried. Each fruit was wounded (5 mm depth x 2 mm width) using a sterilized pipette tip on the equatorial side and air-dried for 1 h. Approximately 6 µL of (5×10^6 spores/ µL) *B. cinerea* spore was inserted into the wound. After air-drying for 2 h, the wounded strawberries were immersed in the coating solution (treatments) or dipped in distilled water (control) for 1 min and subsequently dried. Perforated PET was used to pack both uncoated and coated fruits at 5 °C and 85 ± 5 % RH for 20 days. The rotten fruit diameter was measured in millimeters using a digital caliper (CD-15AXWW, Mitutoyo Digital ABS Calipers, Japan) on days 2, 6, 10, and 15, with five replicates per treatment.

2.2.2.6. Fruit quality changes

2.2.2.6.1. Weight Loss

Weight loss treatments were measured by weighing five fruits per treatment after the fruits were fully dried on days 1, 5, 10, 15, and 20 at 5 °C and 85 ± 5 % RH. Weight loss (%) was calculated by Eq. (4):

$$W_L = (W_0 - W_t) \times 100 / W_0$$

(4) where W_L is the weight loss (%), W_0 is the weight after drying on day 0 (g), and W_t is the weight at the interval time (g).

2.2.2.6.2. Color

The ΔE , L^* , a^* , and b^* color values were used to determine the color change by a colorimeter (CR-20, Konica Minolta Japan Co., Ltd.). The ΔE value was calculated following Eq. (2) with the values of L_0 , a_0 , and b_0 were for the initial day (0 day) while L^* , a^* , b^* were for each interval day, both sides of fruit and five fruits per treatment were recorded.

2.2.2.6.3. pH, citric acid, and TSS

A pocket Brix acidity meter was used to measure the total soluble solids (TSS) and citric acid (PAL-BX|ACID F5, Atago, Co., Ltd., Japan). A dilution of 1 g of fruit juice and 49 g of distilled water was used to examine the citric acid content. The pH was measured using 400 µL of strawberry fruit juice sample by a compact pH meter (LAQUAtwin-pH-22, HORIBA Scientific, Japan), $n = 3$ for each parameter.

2.2.2.6.4. Firmness

The firmness values were recorded using a creep meter (RE-3305, Yamaden Co., Ltd.). The strawberry was divided into two halves, and one half was placed on the holder of a creep meter with a 7 mm depth, a 10 mm/s speed, and a 6 mm probe.

2.2.2.6.5. Sensory analysis

The sensory testing phase involved a panel of ten participants, each of whom provided their consent to participate. We ensured a comprehensive understanding of the composition of the tested product by thoroughly explaining all contained substances. Before proceeding with their

comments and scoring, participants received brief training to establish a common evaluation framework.

To maintain impartial evaluations, samples were coded to prevent any biases. Participants then proceeded to rate the samples using a scale ranging from 1 to 5, where a score of 5 indicated poor quality and a score of 1 denoted excellent quality. The assessment encompassed several aspects, such as color, odor, texture, and taste. This evaluation was conducted at regular intervals spanning 1, 5, 10, 15, and 20 days.

2.2.2.7. Statistical data analysis

The R software (version 4.2.2) was used to analyze the results. The treatment effects were evaluated using analysis of variance (ANOVA). Tukey's test was used to determine significant differences ($P < 0.05$). The statistical analyses were performed by comparing the differences between samples at the same storage time or by comparing one sample at different storage times.

2.3. Results

2.3.1. Oil extraction

The extraction yield of MO was 1.76 %, which was higher than that obtained by the Nanfeng MO extraction method under optimum conditions (approximately 0.69 %) (Yi et al., 2018). Several factors affect the extraction yield of oil, including the size of the mandarin peel, solvent concentration, liquid-to-solid ratio, and soaking time. Yi et al. (2018) found that the oil yield was significantly affected by increasing or decreasing the amount of solvent and the soaking time. In contrast, the size of the mandarin peel and the liquid-to-solid ratio did not significantly affect the oil yield.

The results of the qualitative and quantitative analyses of the MO are presented (Fig. 2.1 and Table 2.1). Results were revealed with 30 MO identified compounds. The main components of MO include mainly pinene (alpha and beta) (total 423.878 ppm), limonene (285.121 ppm), p-cymene (133.152 ppm), methyl palmitate (182.233 ppm), linalool (57.417 ppm), and linoleic acid (21.599 ppm). The composition of MO in this study corresponded to that previously reported for other citrus species. Cheng et al. (2022) obtained similar results for pomelo oil. The authors reported that limonene is the main component of pomelo essential oil. Limonene was evaluated for its in vitro and in vivo antifungal activities, and the results demonstrated that limonene has the

potential to act as an antifungal agent. Moreover, d-limonene demonstrated the ability to inhibit fungal pathogens such as *Candida* (Muñoz et al., 2020; Yu et al., 2022), *Austropuccinia psidii* (Silva et al., 2020), *B. cinerea* (Umagiliyage et al., 2017), and *Penicillium digitatum* (du Plooy, Regnier, and Combrinck, 2009). Meanwhile, α - and β -pinene inhibited fungal pathogens such as *Rhizopus oryzae*, *Staphylococcus aureus*, and *Candida albicans* (Jaganathan and Supriyanto, 2012). In fact, MO might be caused by certain odors that affect the quality of strawberries, which can be assessed by comparing semi- quantitative concentrations obtained with standard thresholds of the main components. However, the results showed that the addition of this oil to the coating did not affect the sensory quality of the strawberries.

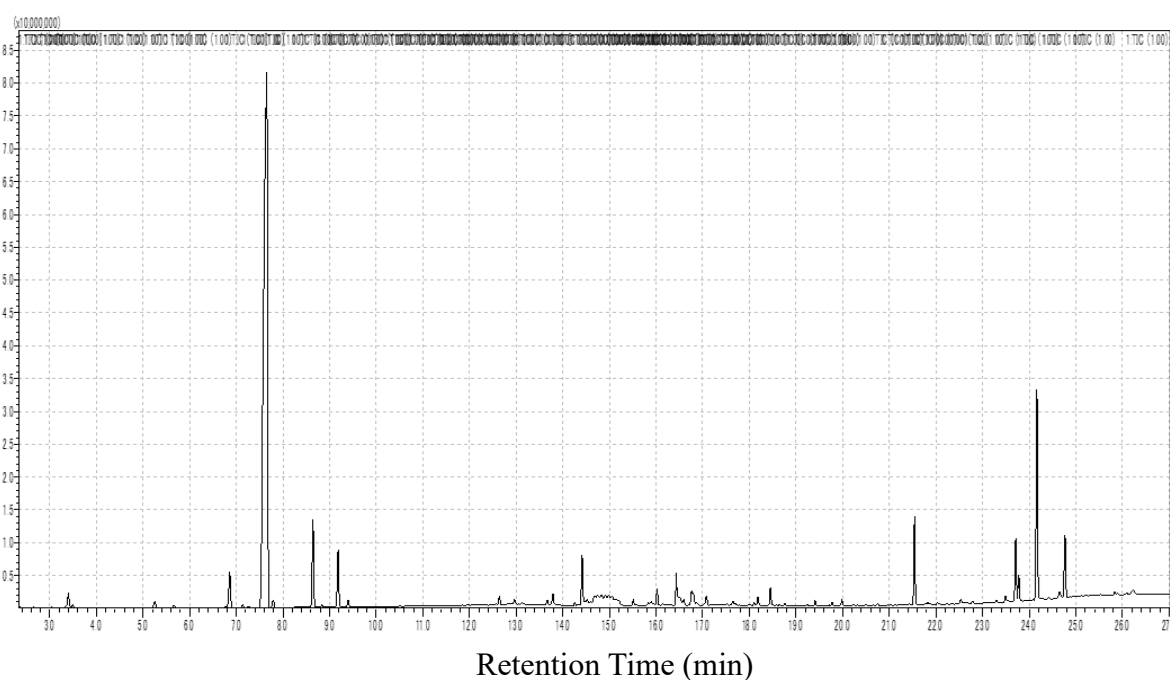


Fig. 2.1. Results of mandarin oil components on GCMS/MS

Table 2.1. Main components of mandarin oil

	Compound name	Semi-quantitative concentration (ppm)	Threshold (ppm)	Similarity (%)	Retention Index
1	α -Pinene	4.808	0.691 ^a	82	1016
2	β -Pinene	419.070	0.033 ^a	92	1170

3	Limonene	285.121	0.437 ^a	92	1207
4	Gamma-Terpinene	32.141	-	98	1249
5	β -Cymene	133.152	7.41E-05 ^b	99	1275
6	Octanal	1.776	4.17E-03 ^a	84	1301
7	2-Pentanone	1.161	-	78	1353
8	Nonanal	2.201	2.24E-03 ^a	86	1395
9	cis-Limonene oxide	2.319	-	94	1453
10	trans-Limonene oxide	3.190	-	92	1466
11	Linalool	57.417	0.054 ^a	98	1553
12	β -Caryophyllene	9.235	-	96	1606
13	α -Humulene	42.564	-	98	1682
14	Alloaromadendrene	2.882	-	87	1686
15	Naphthalene, 1,2,4a,5,6,8a-hexahydro-4,7-dimethyl-1-(1-methylethyl)-, (1.alpha.,4a.alpha.,8a.alpha.)	7.096	-	78	1735
16	Cyclohexane, 1-ethenyl-1-methyl-2-(1-methylethenyl)-4-(1-methylethylidene)	2.414	-	76	1745
17	Methyl laurate	16.385	-	86	1809
18	Geraniol	2.287	-	77	1855
19	3-Isopropoxy-1,1,1,7,7,7-hexamethyl-3,5,5-tris(trimethylsiloxy)tetrasiloxane	1.137	-	76	1824
20	Methyl myristate	13.561	-	97	2018

21	Perilla alcohol	1.304	-	78	2010
22	Tetradeca methyl hexasiloxane	1.431	-	75	2038
23	Hexasiloxane, tetradecamethyl-	1.416	-	75	2050
24	6-Octadecenoic acid, methyl ester	6.015	-	75	2147
25	Methyl palmitate	182.223	-	99	2226
26	7-Hydroxyfarnesen	3.716	-	85	2309
27	7,10-Octadecadienoic acid, methyl ester	2.974	-	83	2296
28	cis-10-Heptadecenoic acid, methyl ester	5.800	-	83	2356
29	Methyl stearate	15.311	-	91	2434
30	Linoleic acid, methyl ester	21.599	-	95	2509

^{a, b} the reported standardized odor threshold values in air following M. Devos et al. (1990), Schreiner et al. (2020).

2.3.2. Film characteristics

The physical properties of the CMC/CNF/MO-based edible films were observed based on their color and transparency, as shown in Fig. 2.2a. The CMC/CNF film was transparent and uniform. The transparency of the edible coating changed significantly in the presence of MO, and different concentrations of MO resulted in films with different dark-light colors.

The film thicknesses are listed (Table 2.2). The thickness of the CMC/CNF edible film was 0.033 ± 0.002 mm; the addition of MO significantly increased the thicknesses of the biopolymer films ($P < 0.05$). The higher the MO concentration, the higher the film thickness. However, when the MO concentration increased to 0.3 and 0.4 %, no significant increase was observed in the film thickness. The increase in the thickness of the films combined with the oil and solid phases may be correlated with higher levels of oil content in emulsion-based biopolymers (Fattahi et al., 2021).

WVP indicates the water vapor permeability across the biopolymer composition, as well as the moisture barrier properties of the film. In food packaging, moisture transmission between the

food and surrounding air is a critical factor to consider (Geng et al., 2023). Therefore, it is crucial to control the *WVP* of the biopolymers as much as possible. The measured *WVP* values are listed in Table 2.2. The *WVP* values increased significantly ($P < 0.05$) when the MO concentration increased; from 1.35 ± 0.08 g.mm/m². h. kPa in CCM1 to 1.65 ± 0.02 g.mm/m². h kPa in CCM6. Several studies have shown an inverse relationship between the water-blocking properties of emulsified biofilms and the droplet size of emulsions (Pérez-Gago et al., 2001). Furthermore, the *WVP* values depend on the diffusivity and solubility of water vapor in the biofilm matrix. Essential oils containing nonpolar components can decrease solubility by reducing the hydrophilic characteristics of the film composite; they can either reduce or maximize the diffusion coefficient depending on the type, droplet size, and absorption of EO in the film composition (Fattahi and Seyedain-Ardabili, 2021). Furthermore, EOs have a greasing effect, making the membrane matrix more accessible, thereby boosting the diffusion of water molecules (Rojas-Graü et al., 2007).

The visual differences in the colors of the edible coatings were further confirmed using L^* , a^* , b^* , and ΔE values (Supplement Table S2). The color parameters for different films changed significantly ($P < 0.05$). The L^* value ranged from 94.16 to 98.24, indicating a change in brightness; a^* ranged from -0.22 to 1.28, indicating an increase in the redness; b^* ranged from 4.80 to 45.16, indicating a strong increase in the yellow color. The value of ΔE , the total color differences, is based on the ΔL^* , Δa^* , and Δb^* color values. ΔE is measured on a scale from 0 to 100, with 0 indicating less color difference, and 100 indicating complete distortion. In this measurement, ΔE values ranged from 5.07 in CCM2 to 40.58 in CCM6 ($P < 0.05$). These results show that the MO strongly influences the color of the edible coatings.

SEM and AFM

The exterior and cross-section of the film obtained via a simple solution casting method are shown in Fig. 2.2b, 2.2c. The interactions between the film-forming compositions directly affect the properties of the film (Ghosh and Katiyar, 2022). The surface characteristics of the CMC/CNF-based films were formed from bio coatings that showed some deformities and heterogeneous textures owing to gelatinization (Fig. 2.2b). The MO-containing films (CCM2, CCM3, CCM4, CCM5, and CCM6) exhibited porous structures with several voids throughout their composition. Additionally, the higher concentration of MO into the film matrix, the greater heterogeneity the film is, accompanied by an increase in porosity. This integration led to notable

changes in droplet size and shape within the film (Fig. 2.2c). The availability of MO in the film matrix replaces strong interactions between polymer networks with weak interactions between the polymer matrix and EO, thus producing a film with less integrity, as shown in the images above. Azarifar et al. (2019) reported similar results for films produced from CMC and Ajowan essential oil; an increase in the concentration of Ajowan essential oil made the film heterogeneous and less cohesive.

The properties of the film layers were further clarified using AFM imaging (Figs. 2.2d (2D), and 2.2e (3D)). Comparable results for the original average mean square roughness (Rq) and roughness average (Ra) indices are also shown (Table 2.2). The base CCM1 film and CCM2 film showed uniform morphology (Ra: 3.11 ± 0.95 nm and Rq: 3.73 ± 0.722 nm; Ra: 1.35 ± 0.01 nm and Rq: 1.67 ± 0.05 nm, respectively) due to their the lowest roughness values ($P > 0.05$); similarly, the lowest roughness of the CMC films was reported by Fattahi et al. (2021). Additionally, the increase in MO resulted in a significant increase ($P < 0.05$) in film roughness, as illustrated by the higher Ra and Rq values, especially for the CCM5 and CCM6 films. Atarés et al. (2010) explained that the film distortions were due to the migration of oil droplets in the emulsion system, which led to surface roughness. The increase in roughness values indicates the influence of the oil concentration in the coating solution and the dispersion of the coating components during homogenization, casting, and drying. The increase in oil concentration with increased formation of intermolecular bonds was considered to be responsible for the increase in roughness values (Wigati et al., 2023). Furthermore, the images in Fig. 2d showed holes on the film surface, which represented the formation or aggregation of MO droplets. In addition, as the oil concentration increased from 0.05 % to 0.4 %, the aggregation of oil within the film structure became more pronounced. This could be attributed to the expansion of the oil droplet on the film surface after the drying process. This result is consistent with the obtained SEM cross-sectional results, and similar results have also been reported by Yan et al. (2023).

The results of AFM and SEM images showed the limitation of films when applying on fruits. The reason is the presence of oil in the matrix of the film, which creates micropores of different sizes on the fruit surface. The water vapor and gas permeability also depend on the surface structure of the film. These micropores affect fruit respiration, spoilage, and dehydration as well as tissue resistance, antioxidant enzyme activity, and fruit senescence (Perdones et al. 2012; Xing et al. 2016).

Table 2.2. Film characteristics

	CCM1	CCM2	CCM3	CCM4	CCM5	CCM6
Thickness (mm)	0.033 ± 0.002 ^d	0.034 ± 0.001 ^{cd}	0.036 ± 0.007 ^{bc}	0.039 ± 0.01 ^b	0.039 ± 0.009 ^a	0.041 ± 0.004 ^a
WVP (g.mm/m ² .h.kPa)	1.35 ± 0.08 ^d	1.36 ± 0.04 ^{cd}	1.51 ± 0.04 ^{bc}	1.50 ± 0.01 ^b	1.53 ± 0.02 ^a	1.65 ± 0.02 ^a
ΔE	-	5.07 ± 0.50 ^a	16.20 ± 2.38 ^{ab}	24.52 ± 4.56 ^b	38.73 ± 9.60 ^c	40.58 ± 8.72 ^c
L [*]	98.24 ± 0.16 ^a	97.74 ± 0.29 ^{ab}	96.88 ± 0.40 ^{ab}	96.08 ± 0.72 ^b	94.24 ± 1.43 ^c	94.16 ± 1.07 ^c
a [*]	0.20 ± 0.06 ^{abc}	-0.22 ± 0.1 ^c	-0.06 ± 0.08 ^{bc}	0.16 ± 0.24 ^{bc}	1.28 ± 0.97 ^{abc}	0.90 ± 0.67 ^{ab}
b [*]	4.80 ± 0.06 ^d	9.82 ± 0.48 ^{cd}	20.94 ± 2.36 ^{bc}	29.22 ± 4.52 ^b	43.3 ± 9.48 ^a	45.16 ± 8.64 ^a
Roughness (Ra)	3.11 ± 0.95 ^a	1.35 ± 0.01 ^a	5.60 ± 0.22 ^b	7.58 ± 0.74 ^c	8.09 ± 0.50 ^c	7.63 ± 0.58 ^c
Indices (nm) (Rq)	3.73 ± 0.722 ^{ab}	1.67 ± 0.05 ^a	7.33 ± 0.73 ^{bc}	7.33 ± 0.73 ^{bc}	6.54 ± 3.87 ^{abc}	9.37 ± 0.11 ^c

^{a,b,c,d} Means with different letters in the same row are statistically different by Tukey ($P < 0.05$)

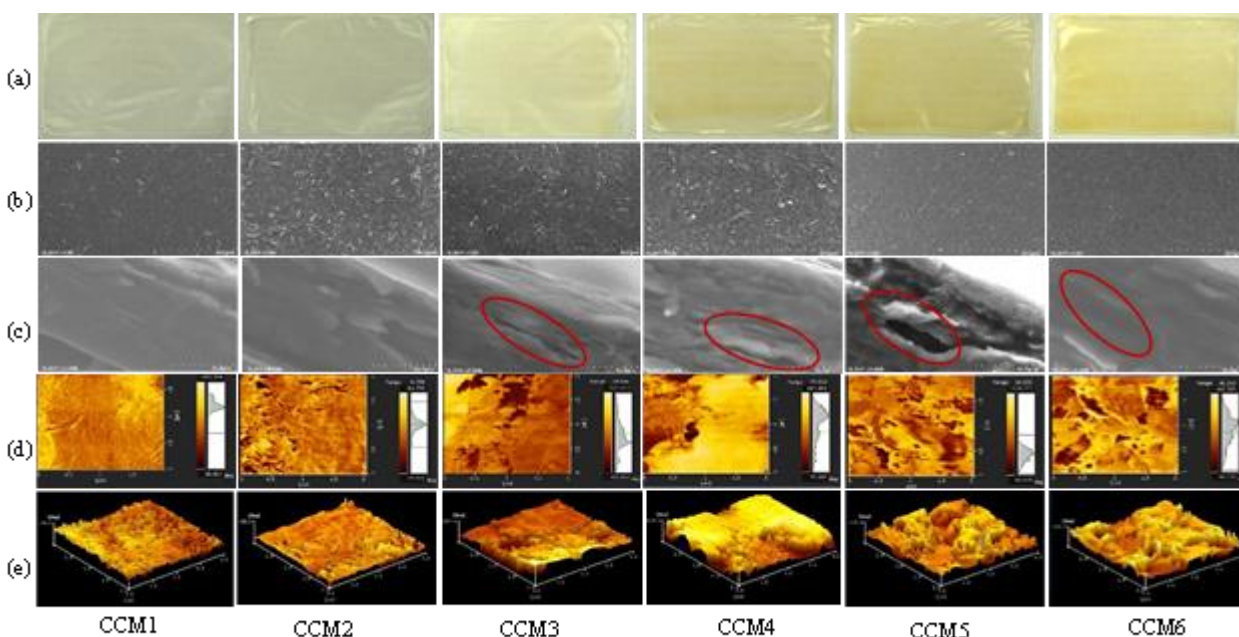


Fig. 2.2. Physical appearance (a); SEM images: surface (b) and cross – section (c); AFM images of surface roughness in 2D (d) and in 3D (e) of films; order is as follow CCM1, CCM2, CCM3, CCM4, CCM5 and CCM6; where CCM1 is CMC/CNF; CCM2, CCM3, CCM4, CCM5, CCM6

2.3.3. In vitro antifungal activities of the films

The antifungal effects of the edible films made from CMC/CNF/MO on days 1 and 2 of the culture are shown in Figs. 2.3a and 2.3b. After 1 day of culture, the MO-containing treatment group showed a significant inhibitory effect on *B. cinerea* compared to the control or CCM1 ($P < 0.05$). On day 2, the antifungal effects of the two groups were more pronounced than on day 1, with the highest inhibitory effect observed for CCM4, CCM5, and CCM6 ($P > 0.05$). The CCM2 and CCM3 films also showed good antifungal activity compared with the control and CCM1. Similar effects have been observed in films based on CMC and cinnamon essential oil (Fattahi et al., 2020). The mechanism of inhibition of fungal growth on the membranes is believed to be due to the presence of limonene compounds in MO. These findings are consistent with previously reported studies, such as the antimicrobial resistance of mandarin EO against *E. coli* ATCC 25922 (Raspo et al., 2020) and the resistance of mandarin EO against *Penicillium digitatum* and *P. italicum* (Jhalegar et al., 2015).

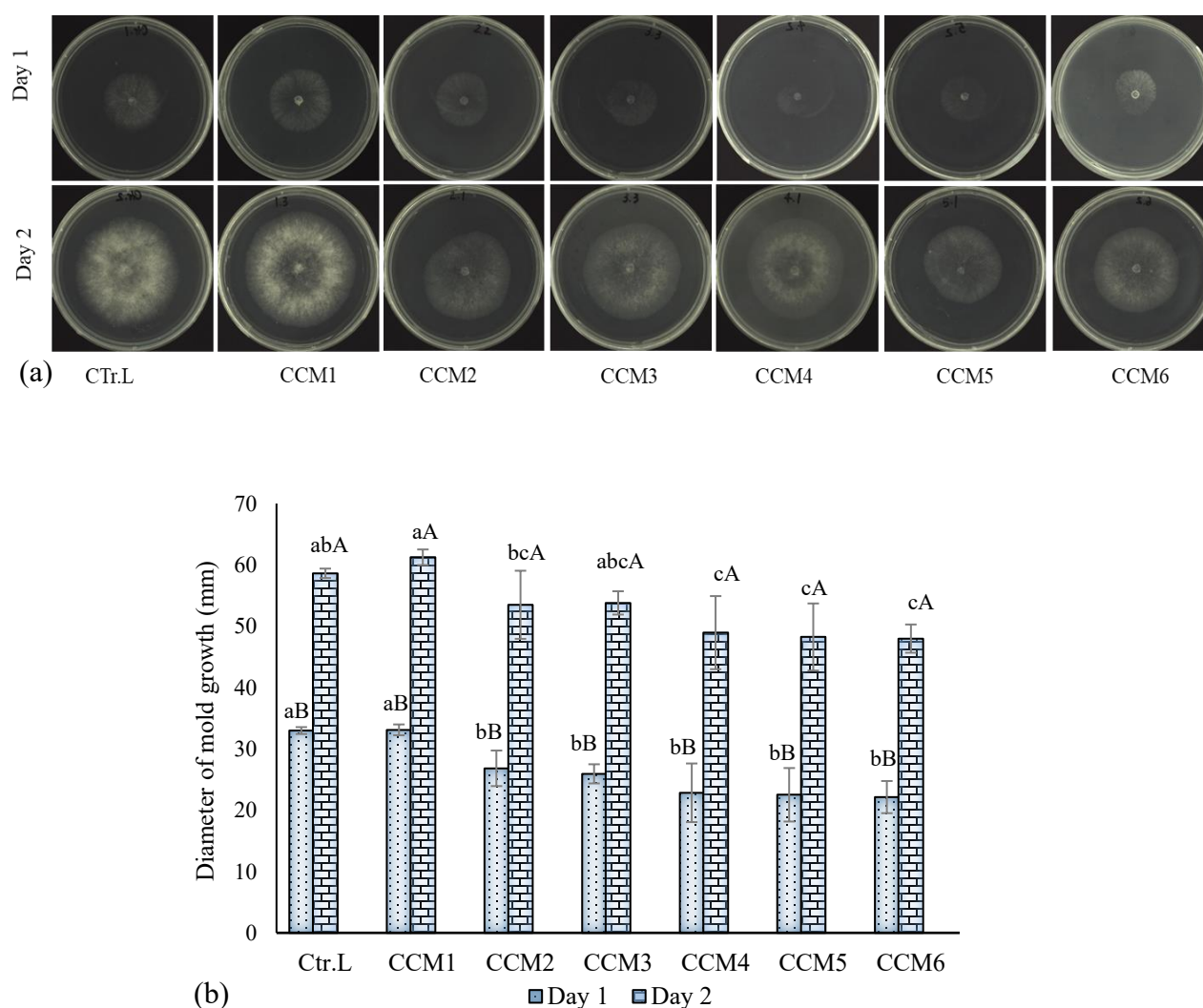


Fig. 2.3. Antifungal capacity of edible films set at 25 °C; (a) colony extension of mycelium radius, (b) statistical analysis of mycelial radius in day 1 and day 2; 5 replications.

2.3.4. Strawberry application

2.3.4.1. Weight loss

The rate of weight loss of strawberries, increased significantly after storage for 5, 10, 15, and 20 days ($P < 0.05$), as shown in Fig. 2.4. The results show a significant difference ($P < 0.05$) among the three groups of fruits treated with different coating formulations. The three groups were CCM2, CCM6; CCM3, CCM5; and control, CCM1, CCM4. The CCM2 and CCM6 group exhibited the lowest weight loss rate, with a 20th-day weight loss rate of 5.08 % and 5.81 %, respectively ($P > 0.05$). The CCM3 and CCM5 indicated moderate weight loss rate ($P > 0.05$). The group with the lowest efficiency was the control (7.66 %), CCM1 (8.10 %), and CCM4 (8.69 %).

%) ($P > 0.05$). Natural water loss or a decrease in the weight of agricultural products depends on transpiration, respiration, or loss of polysaccharide components (Haghighi et al., 2019). Edible coating combined with oils has the advantage of increasing resistance to moisture loss due to increased hydrophobicity content (Tran et al., 2021). However, in this study, we found that increasing oil concentration did not significantly affect the weight loss of strawberries, especially at high oil concentrations (CCM3, CCM4, and CCM5). One of the reasons for this might be the low coverage ability of the film on the strawberry surface. The cause of reasons might be the heterogeneous structure of the films, which resulted in significant differences in roughness values. In this research, the weight loss of CCM6 was also higher than that of CCM2 but lower than that of the others. This might be explained by the ratio of hydrophobic and hydrophilic substances in the coating composition affecting its covering ability. Dong and Wang (2017) reported the effect of carboxymethyl cellulose combined with garlic essential oil on the weight loss of strawberries (*Fragaria ananassa*). The authors confirmed that when the oil concentration exceeded a certain level, the weight loss of strawberries increased. The reason might be that adding a higher concentration of essential oil could weaken the intermolecular interactions, causing the structure to lose integrity and leading to increased water vapor transmission. On the other hand, it might be that the main components of the oil were terpene compounds instead of lipids (Rojas-Graü et al., 2007a).

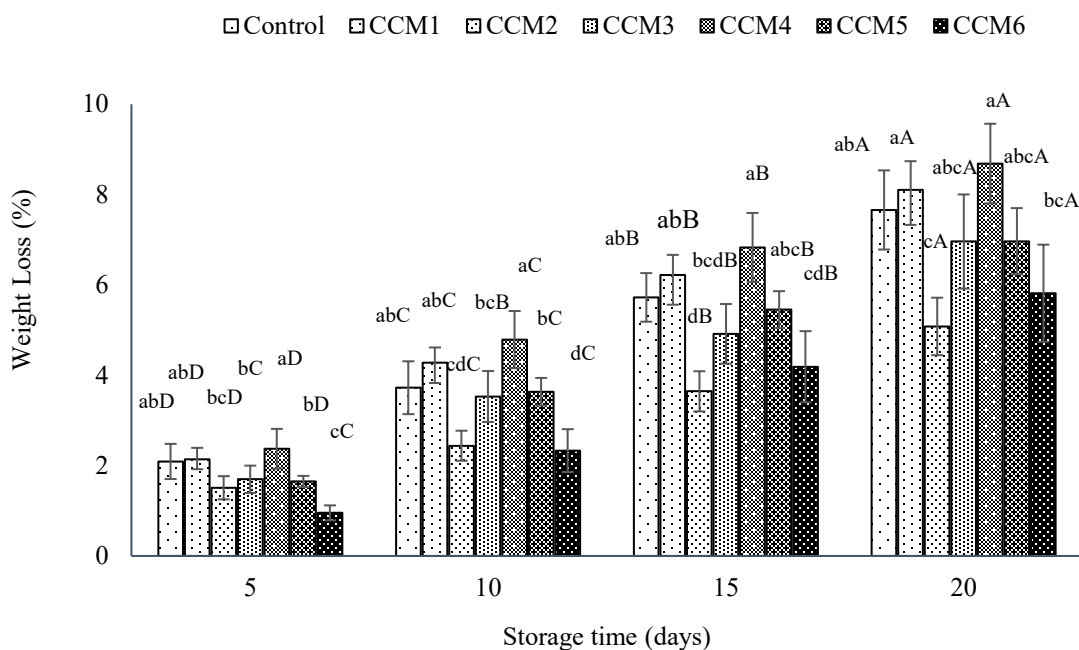


Fig. 2.4. Weight loss of uncoated (control) and coated strawberry named CCM1, CCM2, CCM3, CCM4, CCM5, and CCM6 storage at 5 °C, 85 ± 5 % RH (n = 5). On the column, significant distinction in storage time and treatments were shown by the capital letters a

2.3.4.2. Strawberry color

Results showed the changes in the color of the strawberry samples with storage time (Table 2.3). For all samples, no significant differences were observed in the values of ΔE , L^* , a^* , and b^* , except for the control sample. After 20 days of storage, the color of the control sample showed a significant change ($P < 0.05$), while most of the samples showed no significant color change ($P > 0.05$). This indicates that the presence of MO in the coatings slowed the color change of strawberries during storage. The red color indicates the typical color of strawberries because of the anthocyanin content in the ripe fruit. Changes in pigment color depend on storage conditions such as temperature and respiration intensity (Dhital et al., 2018). After 20 days of storage, strawberries with MO-additive coating showed a higher L^* values than the uncoated strawberries, the color of the coated fruits was lighter than that of the uncoated fruit. Meanwhile, ΔE values for all strawberries showed no significant differences during storage period. Overall, the color of strawberries at the end of storage was light red for coated fruits and dark red for uncoated fruits. This indicated the effectiveness of MO in reducing anthocyanin accumulation and delaying the fruit aging process, which is consistent with similar results reported by Shehata et al. (2020).

Table 2.3. Color changes of undipped (control) and dipped strawberry by CCM1, CCM2, CCM3, CCM4, CCM5, and CCM6 storage at 5 °C, 85 ± 5 % RH.

Parameters	Storage days	Treatments						
		Control	CCM1	CCM2	CCM3	CCM4	CCM5	CCM6
L*	0	86.36±6.88 ^a A	84.59±5.74 ^a A	88.18±7.42 aA	82.72±6.17 aA	83.82±6.59 aA	84.03±6.74 aA	82.23±6.48 aA
	5	85.91±3.86 aA	84.29±5.43 aA	80.42±4.96 aA	84.13±7.98 aA	82.57±6.18 aA	86.23±6.73 aA	87.96±7.13 aA
	10	86.66±4.36 aAB	84.29±4.75 aA	83.03±4.31 aA	85.16±4.53 aA	84.54±4.79 aA	82.67±6.51 aA	83.92±6.50 aA
	15	81.39±4.90 aAB	82.41±3.57 aA	82.23±4.86 aA	81.93±6.96 aA	82.35±5.51 aA	79.68±7.42 aA	83.35±7.94 aA
	20	78.14±3.69 aB	82.07±7.87 aA	82.8±7.23 ^{aA}	78.46±6.08 aA	80.46±6.28 aA	81.44±7.19 aA	81.93±7.34 aA
a*	0	65.99±3.60 aB	67.68±4.07 aB	68.98±3.83 aBC	66.20±4.09 aAB	67.09±3.49 aAB	65.57±4.51 aB	64.68±3.71 aB
	5	77.73±3.78 aA	76.35±5.29 aA	75.70±4.90 aA	73.08±6.36 aA	74.15±4.76 aA	75.22±4.67 aA	76.33±5.66 aA
	10	65.08±6.19 aB	68.46±5.45 aB	68.32±5.45 aBC	63.95±7.80 aB	66.18±6.18 aB	66.98±7.77 aAB	68.96±6.20 aAB
	15	61.01±8.11 aB	61.31±6.61 aB	64.11±3.48 aC	59.71±7.19 aB	64.20±6.87 aB	60.95±6.95 aB	66.13±8.15 aB
	20	65.75±8.08 aB	68.59±7.17 aAB	72.42±6.43 aAB	63.94±7.44 aB	65.81±6.52 aB	66.70±7.74 aAB	69.22±8.82 aAB
b*	0	51.81±9.51 aAB	48.07±7.63 aAB	52.36±8.36 aA	47.76±7.14 aAB	48.52±7.37 aA	49.46±9.07 aAB	45.89±7.27 aA
	5	55.31±5.77 aA	53.03±8.40 aA	50.84±7.79 aAB	54.33±10.7 0 ^{aA}	50.20±8.32 aA	55.12±10.2 2 ^{aA}	55.59±11.6 8 ^{aA}
	10	42.23±6.19 aC	44.72±6.13 aAB	44.21±6.31 aAB	41.74±9.53 aB	44.02±8.13 aA	46.20±11.4 5 ^{aAB}	45.34±9.46 aA

	15	38.67±7.22 aC	38.33±6.09 aB	40.86±5.55 aB	39.11±9.64 aB	42.64±8.68 aA	40.00±8.74 aB	44.20±10.9 1 aA
	20	43.29±6.06 aBC	45.84±10.2 6 aAB	48.39±9.26 aAB	41.29±9.24 aB	42.73±7.20 aA	45.40±9.90 aAB	44.85±9.75 aA
ΔE	0	-	-	-	-	-	-	-
	5	19.05±5.31 aA	14.64±8.17 aA	15.36±5.77 aA	17.39±12.7 2 aA	17.63±9.08 aA	16.52±9.74 ^a A	21.66±13.3 0 aA
	10	13.09±5.41 aA	14.58±5.72 aA	17.43±7.05 aA	13.94±6.55 aA	12.92±5.11 aA	17.38±8.82 aA	17.79±7.82 aA
	15	18.26±8.60 aA	18.14±10.2 9 aA	18.88±10.2 1 aA	19.95±9.88 aA	13.17±10.9 1 aA	21.06±9.41 aA	19.66±10.7 8 aA
	20	18.30±10.5 2 aA	18.91±9.12 aA	20.31±7.36 ^a A	24.11±7.36 aA	18.44±9.23 aA	20.05±6.32 aA	17.16±9.23 aA

Mean values were letters whereas the uppercases showed a significant difference in storage time and the lowercases indicated a significant difference in treatment according to Tukey ($P < 0.05$)

2.3.4.3. Physicochemical characteristics

The changes in pH, TSS, citric acid, and firmness are shown (Table 2.4). The pH values of all strawberry samples decreased from the first day to the last day of storage. After 20 days of storage, the pH value of the control was the lowest (3.25), whereas the highest values were observed in the CCM1 and CCM6 groups (both are 3.55) ($P < 0.05$). The TSS value of the samples peaked on day 5 and then gradually decreased with storage time, except for CCM1 and CCM4.

Citric acid is the main acid present in strawberries (Kilic et al., 2021), and during storage, the citric acid content slightly increased on days 5 and 10, followed by a decrease on the last day of storage. The highest amounts of citric acid were observed on day 5 in CCM1 and CCM6 (contained 2.10 % and 2.06 % citric acid, respectively); day 10 in control (2.04 %), CCM2 (2.04 %), CCM4 (2.07 %), and CCM5 (2.09 %). After 20 days of storage, the citric acid content decreased compared with the first day in all samples. The citric acid content of strawberry showed an increasing and decreasing trend in relation to the initial red to full red and then the dark red stages (Yang et al., 2023). The decrease in citric acid has several causes. The most important causes is the use of citric acid as a respiratory substrate in the tricarboxylic acid (TCA) cycle (Yao et al.,

2018); or the degradation of citric acid might have resulted in the synthesis of other amino acids such as iso citric acid (Gupta et al., 2012).

The firmness value of a fruit is indicative of its freshness after storage (Totad et al., 2019). Therefore, firmness is an essential parameter for assessing fruit quality. The firmness of the control sample reached a maximum on day 5 of storage, while the firmness values of CCM1, CCM5, and CCM6 peaked on day 10 of storage, and those of CCM2, CCM3, and CCM4 on the 15th day. After reaching a maximum, the firmness value of all strawberry samples decreased gradually; however, on the last day of storage, the value was higher than that for the first day of storage. These results demonstrated that CMC/CNF/MO reduced the degradation of fruit quality. Similarly, Ban et al. (2020) reported that CMC/chitosan/zingiber essential oils maintained the quality of jujube fruits.

Table 2.4. pH, TSS, citric acid and firmness changes of undipped (control) and dipped strawberry by CCM1, CCM2, CCM3, CCM4, CCM5, and CCM6 storage at 5 °C, 85 ± 5 % RH.

Parameters	Storage days	Treatments						
		Control	CCM1	CCM2	CCM3	CCM4	CCM5	CCM6
pH	1	3.70±0.03 ^{aA}	3.66±0.05 ^b B	3.76±0.07 ^{aA}	3.68±0.03 ^{aB}	3.83±0.11 ^{aA}	3.79±0.08 ^{aB}	3.81±0.01 ^{aB}
	5	3.76±0.03 ^{bA}	3.86±0.10 ^a bA	3.90±0.10 ^{ab} A	3.89±0.09 ^{abA}	3.97±0.04 ^{abA}	3.99±0.02 ^{aA}	4.00±0.02 ^{aA}
	10	3.48±0.02 ^{bB}	3.55±0.03 ^a B	3.55±0.01 ^{aB}	3.60±0.04 ^{aBC}	3.57±0.06 ^{aB}	3.54±0.03 ^{aC}	3.54±0.03 ^{aC}
	15	3.44±0.01 ^{abcB}	3.49±0.01 ^a B	3.38±0.02 ^{cB}	3.47±0.02 ^{aC}	3.39±0.03 ^{bcB}	3.51±0.02 ^{aC}	3.46±0.02 ^{abD}
	20	3.25±0.03 ^{bc}	3.55±0.02 ^a B	3.51±0.01 ^{aB}	3.51±0.01 ^{aC}	3.49±0.01 ^{aB}	3.52±0.01 ^{aC}	3.55±0.03 ^{aC}
TSS	1	10.00±0.16 ^{ab} AB	9.80±0.16 ^b A	10.10±0.14 ^a bBC	10.26±0.13 ^{abA} B	10.05±0.05 ^{abAB}	10.13±0.12 ^{abB}	10.56±0.34 ^{aAB}
	5	10.50±0.16 ^{abA}	10.53±0.09 ^{abA}	10.77±0.09 ^{aA}	10.73±0.26 ^{aA}	10.24±0.14 ^{bAB}	10.60±0.08 ^{abA}	10.70±0.00 ^{aA}

Citric acid (%)	10	10.23 ± 0.24 bAB	9.94 ± 0.05 bB	10.26 ± 0.05 abB	10.07±0.05 ^{bB}	10.22±0.10 ^{bAB}	10.20±0.08 ^{bB}	10.67±0.13 ^{aA}
	15	9.47±0.05 ^{cC}	10.03±0.05 abB	9.93 ± 0.05 bcd	10.17±0.09 ^{aB}	10.03±0.05 ^{abB}	9.53±0.05 ^{cC}	9.63±0.09 ^{cC}
	20	9.93 ± 0.09 bcBC	10.07±0.05 abB	9.77±0.13 ^{cD}	9.90±0.08 ^{bcB}	10.30±0.08 ^{aA}	9.67±0.05 ^{cC}	10.10±0.08 ^{abBC}
	1	1.97±0.00 ^{bcB}	1.74 ± 0.02 ^c D	1.95 ± 0.03 ^{bc} B	2.09±0.01 ^{abA}	2.13±0.02 ^{bcA}	2.09±0.01 ^{abA}	2.27±0.04 ^{aA}
	5	2.03±0.01 ^{bcA}	2.10 ± 0.01 ^c A	1.93 ± 0.01 ^{bc} BC	2.05±0.02 ^{abAB}	1.68±0.01 ^{bD}	1.82±0.01 ^{abC}	2.06±0.01 ^{aB}
	10	2.04±0.01 ^{bA}	1.97 ± 0.00 ^c B	2.04±0.01 ^{bA}	2.04±0.01 ^{bBC}	2.07±0.01 ^{aB}	2.09±0.01 ^{aA}	1.98±0.01 ^{cC}
	15	1.97±0.01 ^{dB}	1.86 ± 0.01 ^f C	1.91 ± 0.00 ^{eB} C	2.01±0.02 ^{cC}	2.00±0.01 ^{cC}	2.09±0.01 ^{aA}	2.05±0.00 ^{bB}
	20	1.93±0.01 ^{bc}	1.70 ± 0.01 ^c E	1.89±0.01 ^{cC}	1.68±0.01 ^{eD}	2.05±0.01 ^{aB}	1.90±0.00 ^{cB}	1.82±0.01 ^{dD}
	1	0.142±0.023 ^a bB	0.185±0.04 1 ^{aA}	0.170 ± 0.00 6 ^{abB}	0.172 ± 0.016 ^{ab} B	0.203±0.035 ^{abA}	0.213 ± 0.055 ^{ab} A	0.215±0.045 ^{bAB}
	5	0.253±0.074 ^a A	0.213±0.02 0 ^{aA}	0.209 ± 0.02 7 ^{aAB}	0.230±0.036 ^{aA}	0.196±0.017 ^{aA}	0.193±0.011 ^{aA}	0.205±0.016 ^{aAB}
Firmness (N)	10	0.209±0.018 ^a AB	0.223±0.01 9 ^{aA}	0.202 ± 0.03 5 ^{aAB}	0.224±0.012 ^{aA}	0.202±0.021 ^{aA}	0.208±0.024 ^{aA}	0.257±0.037 ^{aA}
	15	0.183±0.045 ^b AB	0.195±0.01 7 ^{abA}	0.216 ± 0.00 7 ^{abA}	0.237±0.018 ^{aA}	0.210±0.015 ^{abA}	0.193 ± 0.013 ^{ab} A	0.212±0.008 ^{abAB}
	20	0.174±0.009 ^a AB	0.197±0.03 0 ^{aA}	0.197 ± 0.01 2 ^{aAB}	0.211±0.016 ^{aA} B	0.196±0.014 ^{aA}	0.192±0.024 ^{aA}	0.185±0.022 ^{aB}

Mean values were letters whereas the uppercases showed a significant difference in storage time and the lowercases indicated a significant difference in treatment according to Tukey ($P < 0.05$)

2.3.4.4. Strawberry sensorial analysis

The sensory analysis of strawberries after 20 days of storage is shown (Fig. 2.5). The results showed that the edible coatings containing CMC/CNF/MO were more effective in preserving sensory quality than the control, particularly in terms of texture and taste. Moreover, after 20 days of storage, CCM6 showed better overall acceptability than the control and CCM1 ($P < 0.05$, Tukey test). However, evaluation by R- Friedman for overall acceptance at 20 days did not show any difference among treatment groups (the obtained P -value was 0.08). Similar effects have been reported by Ban et al. (2020), who documented the effects of CMC/chitosan and ginger essential oil on the sensory quality of jujube fruits.

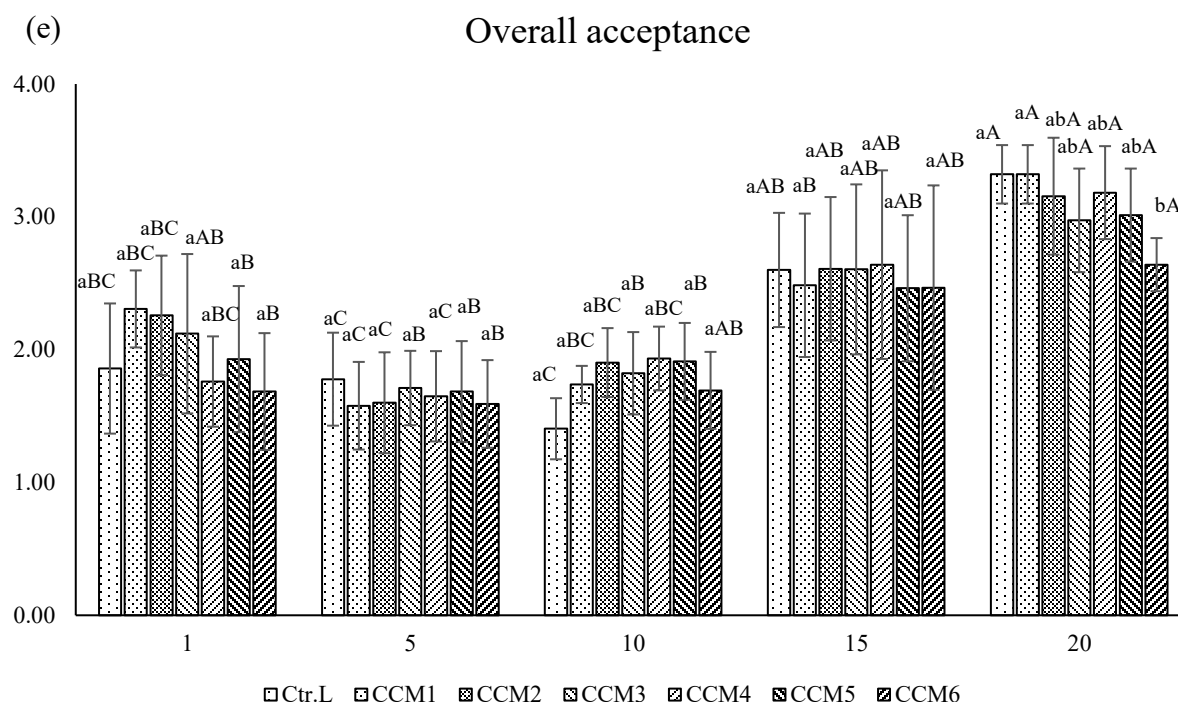
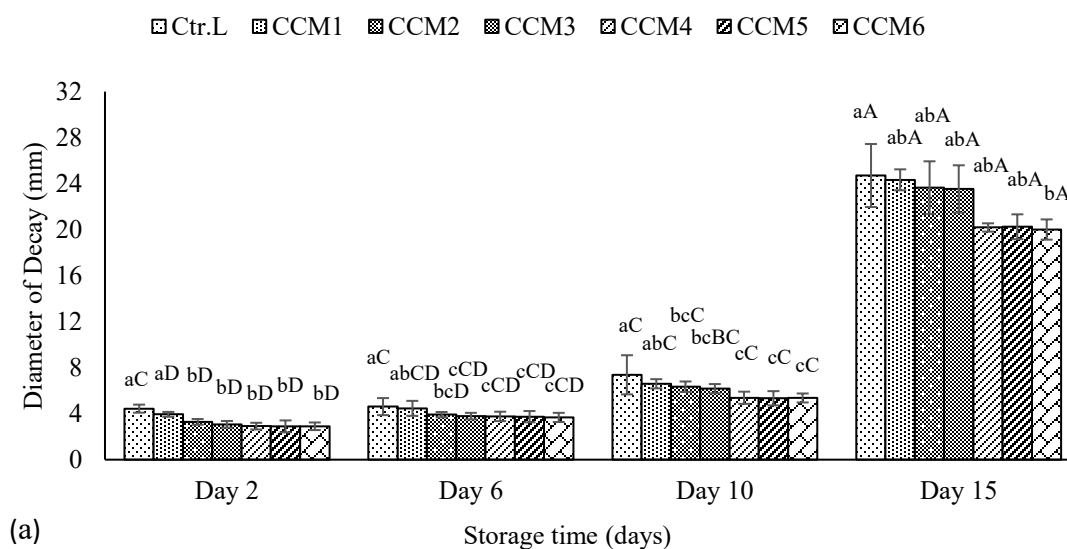


Fig.2.5. Sensory evaluation of strawberry during storage: (a) color; (b) odor, (c) texture; (d) taste and (e) overall acceptance; samples named control, CCM1, CCM2, CCM3, CCM4, CCM5 and CCM6.

2.3.4.5. In vivo antifungal activity

The results of fungal spoilage of strawberries are shown in Fig. 2.6, where the damaged zones were measured based on the perimeter of the fungal damaged zone on the fruit exterior. There was a significant difference in spoilage diameter between the coating group without MO (control) and the coating group with MO. Considering the percentage of fungal rot on the last day

of the samples as 100 %, on the 6th day of monitoring, CCM2 (16.55 %) and CCM3 (16.11 %) exhibited the lowest rot rates, while the control (18.67 %) exhibited the highest rot rate ($P < 0.05$). Similar results were obtained on storage days 10 and 15, with the highest fungal decay rates observed in the control and CCM1 group. No significant difference was observed in the rate of fungal decay when the MO concentration was increased from 0.05 to 0.4 % in the coating formulations. These results illustrate the significant effect of MO in inhibiting the spoilage caused by *B. cinerea*. The antifungal effect of MO has been demonstrated using the active ingredient limonene (Fattahi et al., 2020). In addition, pinene also shows activity against many fungi (Feng et al., 2020; Shi et al., 2019). Currently, several theories exist to explain the mechanism by which fungal growth inhibition leads to decay. It has been suggested that terpene components break the lipid synthesis of cell wall bacteria, leading to the breakdown of cell membranes, degeneration of cellular proteins, and eventually cell lysis and cell death (Fisher and Phillips, 2008). The antibacterial activities of EOs were originally believed to result from components such as citral, limonene, perillaldehyde, geraniol, linalool, α -terpineol, and carvacrol, and their effects against *E. coli*, *S. enterica* serovar *Typhimurium*, *L. monocytogenes*, and *Vibrio vulnificus* (Kim et al., 1995). A plausible explanation for this is that terpene components interact with the proteins and phospholipids of fungal membranes, leading to the inhibition of the cellular respiratory chain, disruption of oxidative phosphorylation, breakdown of nucleic acid synthesis, and loss of metabolites (Angane et al., 2022).



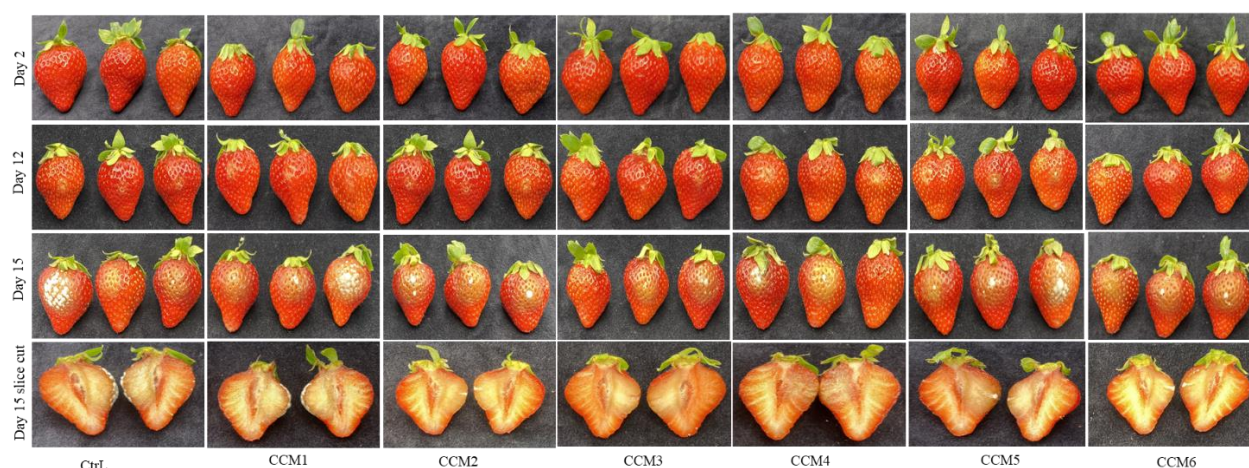


Fig. 2.6. The diameter decay of uncoated and coated strawberry caused by *B. cinerea*, (a) static analysis, (b) visible images of strawberry at 5 °C, 85 ± 5 % RH.

2.4. Conclusion

This paper reports the characteristics of CMC/CNF/MO films and the effects of edible coatings on maintaining the quality of strawberries. The GC-MS/MS analysis results showed that the main components of MO included monoterpenes, such as β -pinene and limonene. Incorporation of MO into the CMC/CNF film resulted in significant changes in color and average roughness of films. These properties had significant effects on its application to strawberry where the rate of fruit weight loss was significantly reduced except for CCM4, and the color of the fruit remained stable. Addition of MO to the CMC/CNF matrix enhanced the antifungal activity, inhibited fungal growth and protecting the fruit from fungal infection compared to the control. However, the addition of MO at high concentrations did not yield a more meaningful effect on strawberry quality compared to the lower MO concentration, despite its effectiveness in antifungal activity. Overall, this study showed that MO addition at a concentration of 0.05% gave the best effect in controlling weight loss and effectiveness in antifungal against *B. cinerea*. Extensive research on other subjects is recommended to confirm the effectiveness of the coating in the future.

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Chapter 3: Effect of coatings incorporating cellulose nanofibers and 1-methylcyclopropane or mandarin peel extract on the freshness and metabolic profiles of cold stored strawberry

3.1. Introduction

Freshness is an important indicator of the quality of harvested agricultural products. Freshness is evaluated using indicators such as changes in fruit colour, weight loss, total soluble solids (TSS), pH, and texture (Arce et al., 2012; Suriati and Suardani, 2021). Strawberries (*Fragaria × ananassa*) are rich in minerals and vitamins and their fresh taste makes them popular among consumers. The colour of fresh strawberries is the most important factor in selection decisions; in fact, taste is less important than colour in the USA (C. He et al., 2021). Strawberry qualities include colour, size, firmness, flavour, and shelf life (Mendoza et al., 2019), but consumers are most concerned about external colours (red) and mild flavours (Aoki and Akai, 2023). Strawberries are sensitive fruits with a short shelf life. They are easily damaged after harvesting because of their soft texture and thin peel, leading to negative effects such as economic losses and food waste. Thus, specific technologies or treatments are required to avoid spoilage, maintain quality, and prolong shelf life.

One strategy is to coat the surface of strawberries with consumer-safe materials, such as polysaccharides, proteins, lipids, and waxes. The choice of a material can be based on its function, benefits generated, and availability (Wigati et al., 2023b). In a previous study, the effects of edible coatings made from sodium carboxymethyl cellulose/cellulose nanofibers/mandarin peel extract (CMC/CNF/ME) on strawberries were analysed for their antifungal activities and quality changes, revealing significant effects on both antifungal properties and weight loss parameters (Van, et al., 2023). In this study, coatings were applied to form a thin film on the surface of fruits to create a barrier to prevent ethylene exchange and alter respiration and ripening times, leading to changes in the shelf life of the fruits. 1-methylcyclopropane (1-MCP) was added to the coatings to develop and enhance the freshness and metabolism of strawberries. CMC water-soluble can form films that are moderately air-permeable (Nasibi et al., 2020). A critical factor in improving the mechanical and barrier properties of coatings is the incorporation of nanoparticles such as cellulose derivatives into the coating formulations (Azeredo, 2009). Coatings are stabilised by CNF extracted from plants or bacteria using high-pressure homogenisation (Azeredo, 2009). The composition of ME was evaluated and prepared using a gas chromatograph-triple-quadrupole

tandem mass spectrometer system (GC-MS/MS). Analysed results revealed that the main components of ME were β -pinene, limonene, and methyl palmitate. These compounds have demonstrated antifungal and antibacterial properties in fruits. Detailed qualitative and quantitative results of the ME are described in our previous report (Van et al., 2023).

Assessing strawberry freshness after storage is important because it indicates acceptable fruit quality. Although strawberries were previously considered to be less sensitive to ethylene, some strawberries have recently become ethylene-sensitive (Merchante et al., 2013; Sun et al., 2013). Coatings with 1-MCP could have positive effects because 1-MCP interacts with internal ethylene by binding to ethylene receptors and blocking their receptors, thereby preventing the absorption of external ethylene (Reis et al., 2020). Therefore, it could help extend the shelf life and protect fruits against physical damage. 1-MCP can inhibit ethylene signaling in climacteric fruits, which prolongs the storage time of fruits (Steelheart et al., 2019), beside, 1-MCP also effectively delays quality decay in non-climacteric fruits such as strawberries (Reis et al., 2020). 1-MCP is non-toxic, effective, easy to use, and significantly affects the preservation of respiratory fruits, such as apples and kiwis (H. Huang et al., 2019; Vidyapeeth et al., 2014; Kumar et al., 2023). Coatings have been combined using various methods, owing to concerns regarding the rapid release of 1-MCP, which could reduce its effectiveness. Coating with a carnauba wax base, followed by 1-MCP fumigation, is an effective method for preserving Fuji apples. This strategy significantly reduces the amount of weight loss and putrefaction, limits the malondialdehyde content, retains ascorbic acid, and significantly increases TSS (Li, 2020). Coating with a film containing 1% chitosan (*w/V*) then exposed with 1 μ L/L 1-MCP for 24 h, improved the quality of preserved passion fruit by reducing the weight loss rate, the shrinkage index, inhibiting peroxidase activity (POD) and reducing that of ascorbate peroxidase (APX) (You et al., 2022). Formulations, including simple 1-MCP immersion, can prolong the shelf life of fruits and maintain the same quality as the solubility, fumigation, or exposure methods (Agehara et al., 2018; Manganaris et al., 2008; Shadowing and Organisationnelle, 2013). Immersing mulberries in low concentrations of 1-MCP maintained their sensory quality (Oz and Ulukanli, 2014). Although 1-MCP becomes gaseous when dissolved in water, some of it should remain in water because it has a solubility of 137 mg/L at 20 °C (FAO, 2019). Poly-1-*p*-menthene serves in the immersion method as a non-ionic wetting agent in the immersion method to adhere 1-MCP to the fruits. Most single-preservation methods are implemented step-by-step and take a long time because of concerns

regarding the effectiveness of 1-MCP treatment. To the best of our knowledge, no coating solutions have been dissolved in 1-MCP. Further research is required to clarify the effects of incorporating 1-MCP into coating solutions to slow its release from the emulsion solution before application to fruits, thereby prolonging their shelf life. 1-MCP residues may require further investigation because of rising health concerns among consumers, and the safety of 1-MCP for human health has been confirmed (Huang et al., 2019; Vidyapeeth et al., 2014; Kumar et al., 2023).

In this study, coating solutions containing dissolved 1-MCP were concurrently applied as a novel method to extend the shelf life of strawberries, reducing processing time while maintaining quality when both treatments were administered. This study aimed to determine the effects of dissolving CMC/CNF-based coatings in 1-MCP/ME on strawberry freshness and metabolic profiles during cold storage. Environmentally friendly films designed to meet the requirements for agricultural product preservation and enhance the efficiency of the cooling system chain, particularly in packaging, were prepared. Most of the materials used in this study were generated from waste and byproducts of agricultural processing. This helps reduce product costs and protect the environment.

3.2. Materials and Methods

3.2.1. Chemicals

CMC, CNF, oleic acid (first grade), Tween 80 (molecular biology grade), glycerol (guaranteed reagent grade), calcium chloride (grade of reagent), ribitol (special grade), pyridine (reagent grade), methanol (reagent grade), chloroform (reagent grade) and sodium hypochlorite (practical grade) were purchased from Fujifilm Wako Pure Chemical Industries Ltd. (Tokyo, Japan). 1-MCP (SmartFresh™, AgroFresh Solutions Inc., Philadelphia, USA) was used for post harvest treatment. ME was obtained from mandarin peels at the laboratory of post harvest science, Kyushu University, Fukuoka city, Japan. ME was extracted using the solvent method (Van, et al., 2023). Previously analysed results indicated that the main components of ME include β -pinene and limonene. Furthermore, an edible coating complex of ME on CMC/CNF enhanced the antifungal activity, inhibited fungal growth, and protected the fruit from fungal infection compared with the control (Van et al., 2023). Methoxyamine-HCl, *N*-methyl-*N*-trimethylsilyltrifluoroacetamide (MSTFA), and qualitative retention time index (*n*-alkanes, 100-200 μ g/mL in hexane) were obtained from GL Sciences, Co., (Japan) and Restek Corp., (USA),

respectively. Millipore water was purified using Autopure WT101 UV (Yamato Corp., Japan). Distilled water was collected using an Aquarius Advantech GS-200 automatic water distillation apparatus (Toyo Seisakusho Kaisha, Ltd., Japan).

3.2.2. Fruit preparation

Amaou strawberries (Japan Agricultural Cooperative (JA), Itoshima, Japan) were harvested, and 225 fruits were selected based on freshness, colour, approximately 3.5 cm in diameter, and 20 g in weight, and delivered to the laboratory on the day of the experiment. The strawberries were decontaminated by immersion in 0.01% sodium hypochlorite for 2 min, then drained at room temperature until completely dried. The fruits were then separated into five groups of 45 fruits each. The control group was immersed in distilled water for 1 min, and four groups were immersed in the coating solutions for 1 min. Then all samples were completely dried at room temperature and placed in *polyethylene terephthalate* (PET) boxes with 18 cm x 24 cm x 3 cm sizes, 15 strawberries per one, and then stored at 5 °C, 85% relative humidity (RH) for 20 days in a climate test chamber (MLR-352H, SCIMED PTE., Ltd., Singapore). The interval times for fruit evaluation were 1, 10, 15, and 20 d.

3.2.3. Experimental procedures

3.2.3.1 Coating and film preparation

CMC (1.0% w/V) and CNF (0.5% w/V) were dissolved by slowly pouring the powder into distilled water, adding 0.35% (w/V) glycerol, and agitating the mixture with a magnetic stirrer for 2 h. A solution of ME and/or 1-MCP was prepared at concentrations of 0.05% and/or 0.1% (w/V), then 0.03% (ME-based) Tween 80 and 0.05% oleic acid (ME-based) as an emulsifier with distilled water. The stock substrate was homogenised with or without ME or 1-MCP for 3 min at 20,000 rpm using an IKA T-25 easy clean ULTRA-TURRAX homogeniser (IKA-Werke, Staufen, Germany, Japan). The final coatings were as follows: control (distilled water); CCM1-1 (CMC and CNF); CCM1-2 (CMC, CNF, and 1-MCP 0.1%); CCM2-1 (CMC, CNF, and ME 0.05%); and CCM2-2 (CMC, CNF, ME 0.05%, and 1-MCP 0.1%). The coatings were defoamed in an ADP300 incubator (Yamato Scientific Co. Ltd., Tokyo, Japan) to ensure homogeneity. Casting films were done by pouring 25 mL of each coating onto a square silicon sheet then drying them for 8 h at 40 °C in an EYELA WFO-520W incubator (Tokyo Rikakikai Co., Ltd., Tokyo, Japan). The film was

then separated from the sheets and stored at room temperature at 53% RH in a desiccator containing a moisture-retaining agent, calcium chloride, before the following properties were assessed:

3.2.3.2. Film characteristics

The thickness of films (μm) was evaluated by a digital micrometer of ratchet thimble (Series 102–707, Mitutoyo Corporation, Japan), five replicates per film.

A CR-20 colour reader (Konica Minolta Inc., Japan) was used to determine the colour of the films with five replicates per film. The Commission Internationale de l'Eclairage (CIE) colour scale includes the L^* , a^* , b^* and ΔE colour systems (L^* indicated black reflective to white, though reflective white values can be higher; a^* values represent the red to green; b^* values represent yellow to blue colour. The value of ΔE (total colour change) was calculated by Eq. (1), according to the method of (Van et al., 2023):

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

(1)

The values of L_0 , a_0 , and b_0 were white standard plates, with $L_0 = 98.90$, $a_0 = 0.00$, and $b_0 = 3.90$, respectively.

3.2.3.3. Atomic Force Microscopy (AFM)

Film morphology and roughness of the films were measured using a 5200S AFM (Hitachi Solutions Ltd., Tokyo, Japan) in the tapping mode controlled by a Nano Navi Application (Hitachi Solutions Ltd.). Film samples CCM1-1, CCM1-2, CCM2-1 and CCM2-2, were prepared by adding a film of appropriate diameter to freshly cleaved mica and inserting it into a sample holder. A type-SI-DF20 cantilever had a scanning frequency of ~ 0.84 Hz, and scan area $2 \times 2 \mu\text{m}^2$. The average roughness values, including the root mean square deviation (Rq) and arithmetic mean deviation (Ra) from the mean, were determined from two-dimensional (2D) images and calculated from 10 different areas using Eq. (2) and (3), as follows:

$$Rq = \sqrt{\frac{1}{n} \sum_{j=1}^n z_j^2}$$

(2)

$$Ra = \frac{1}{n} \sum_{j=1}^n |Z_j| \quad (3)$$

where Z_j is the height deviation of j and n is the total number of data points.

3.2.3.4. Fourier transform infrared (FTIR)

Functional group characteristics of the coatings were assessed using an FT/IR-620 spectrometer (JASCO, Tokyo, Japan) at a range of 500–4000 cm^{-1} with average of 32 scans by the attenuated total reflectance method. Initially, the system was calibrated with air before performing the measurements.

3.2.4. Determination of fruit quality

3.2.4.1. Weight loss

The measurement of weight loss for each treatment involved coated and uncoated strawberries and five fruits from each group after 0, 10, 15, and 20 d of storage. The weight loss ratio (%) was calculated using Eqs. (4):

$$W_L = (W_0 - W_1) \times 100 / W_0 \quad (4)$$

where W_L is weight loss (%), W_0 is weight after drying on day 0 (g), and W_1 is weight at each time interval (g).

3.2.4.2. Color

Changes in pericarp colour were evaluated in two fixed positions on each of five fruits per treatment using the L^* , a^* , b^* , and ΔE colour system and a CR-20, colorimeter (Konica Minolta Japan Co., Ltd., Tokyo, Japan) in the CIELAB colour mode. The difference colour of ΔE value was calculated following Eq. (1), and L_0 , a_0 , and b_0 are the colours of the strawberries on the first day.

3.2.4.3. Citric acid, TSS, and pH,

Citric acid and TSS levels were measured using a pocket Brix acidity meter (PAL-BX ACID F5; Atago Co. Ltd., Tokyo, Japan) (Tran et al., 2021). The TSS was measured three times at each storage interval. Citric acid levels were determined by dissolving strawberry fruit juice in 1

g of fruit juice and 49 g of distilled water, and measuring the resulting mixture using a digital balance (Atago, Co., Ltd.) with three replicates.

To measure the pH value, strawberry fruit juice was analysed using a compact pH meter LAQUAtwin-pH-22 (Horiba Ltd., Japan), three replications, 400 μ L solution for each.

3.2.4.4. Firmness

Firmness is defined as the maximum force that penetrates the fruit peel. Firmness values were recorded using an RE-3305 creep meter (Yamaden Co. Ltd., Kyoto, Japan). Each strawberry sample was sliced in half, and one half was positioned in the sample holder of the creep meter. The sliced shoulder zone was then penetrated to a depth of 7 mm at a speed of 10 mm/s using a 6-mm probe.

3.2.4.5. Sensory analysis

Sensory testing was performed as described by Wang et al. (2020). Ten semi-educated participants agreed to participate in sensory testing. Ethical permission was not required to conduct a human sensory study in the institution and/or country. These explanations ensured a comprehensive understanding of the composition of strawberries and the coatings for all the substances contained. Before proceeding with their comments and scoring, brief training was provided to the participants to establish a common framework evaluation.

Samples were washed with tap water, dried, and encoded to prevent bias. Participants evaluated the samples using a scale ranging from 1 to 5, where a score of 5 indicated inferior quality, and a score of 1 denoted excellent quality. The assessment involved several aspects including colour, odour, texture, and overall acceptance.

3.2.4.6. Total phenol content and DPPH assays

The total phenol content (TPC) of the coatings was determined by the Folin-Ciocalteu method. Coating solutions (450 μ L each) (CCM1-1, CCM1-2, CCM2-1, and CCM2-2) were diluted with 7,200 μ L of distilled water, then 450 μ L of Folin-Ciocalteu reagent (0.25 M) was added, followed by 900 μ L of Na₂CO₃ (1 M). The mixture was homogenized for 30-40 seconds using a Digital Vortex-Genie 2 Mixer (Scientific Industries, Bohemia, NY, USA) and incubated for 2 h in the dark at room temperature. The absorbance was measured at 760 nm using a Model V-730 UV/VIV spectrophotometre (Jasco Co., Tokyo, Japan). The data shown in milligrams of

gallic acid per gram of coating solution are the averages of three replicates from three separate measurements of the initial sample.

Antioxidant activity was evaluated using DPPH, as described (Zhang et al., 2021), with some modifications: a fresh stock solution of DPPH (2 mM) was prepared by dissolving 7.8 mg in 99.5% ethanol (100 mL). A control DPPH solution (A control) was prepared by mixing the DPPH stock solution with 99.5% ethanol at a ratio of 1:4 (*V/V*). Thereafter, extracts from the samples (0.6 mL) were mixed with 2.4 mL of 0.1 M Tris-HCl buffer (pH 7.5), followed by 3 mL DPPH solution (A samples). The mixture was then incubated in the dark at room temperature for 30 min and the absorbance was measured at 517 nm. A blank control comprised 99.5% ethanol (1.2 mL) and 0.8 mL of 0.1 M Tris-HCl buffer (pH 7.5). The absorption values were recorded, and the DPPH radical scavenging activity was calculated.

3.2.5. Metabolic profile of strawberry

The metabolic constituents of strawberry were identified on GC-MS/MS system (Shimadzu, Kyoto, Japan), GC (Nexis GC 2030, Shimadzu Corporation) was operated using a column (DB-5, Shimadzu, Corp., USA) with an inner diameter of 0.25 mm, length of 30 m, and film thickness of 1.0 μm . A liquid injection system operating at a flow rate of 50 $\mu\text{L/s}$ was used, with the injection port temperature maintained at 250 $^{\circ}\text{C}$. The column temperature was set at 100 $^{\circ}\text{C}$ for 4 min and 320 $^{\circ}\text{C}$ for 11 min in splitless mode. The carrier gas was helium, with a linear velocity of 39.0 cm/s and a pressure of 83.7 kPa. The mass spectrometer ranged from 45 *m/z* (mass-to-charge ratio) to 600 *m/z*, and ion source temperature was 200 $^{\circ}\text{C}$, while the interface temperature was 280 $^{\circ}\text{C}$. The injection port temperature was set to 280 $^{\circ}\text{C}$.

In the initial step of the measurement, a smart metabolism database file (Shimadzu Corp., Japan) was utilised to calibrate the retention time with *n*-alkanes at a concentration of 100 $\mu\text{g/mL}$. Once the calibration stages were completed, the acquired result file was merged with the sample scanning method file from the smart metabolism database to generate a customised scanning method file for sample evaluation. This file contains the 467 targeted compounds. The next steps involved creating an acquisition and batch file to measure the samples, including blank and strawberry sample measurements. Blank vials were first analysed to ensure that there was no contamination; then, sample measurements were continued with three replications per sample. The relative percentages of the tentatively detected compounds were calculated using the height ratio

of ribitol as an internal standard to the detected components. Freeze-dried strawberry samples for analysis were prepared by freeze-drying on the first day and at the end of storage (20 days). As for sample scanning preparation, briefly, 5 mg of freeze-dried strawberries were diluted with 10 μ L of ribitol at a concentration of 1 mg/mL, then 1 mL of Millipore water, and a mixture of methanol and chloroform at ratio of 1:2.5:1 (*V/V/V*), was added to the above samples. The samples were grinded in a grinder (Micro smash MS- 100 R, Tomy Seiko, Japan) at 4600 rpm/ 5 min, then centrifuged at 15000 rpm/ 3 min/ 4 °C (Kubota 5922, Yama P Co. Ltd, Japan). In the next step, 900 μ L sample and 400 μ L Millipore water were added, stirred, centrifuged. Then, 200 μ L of the solution was taken out, heated, and stirred to separate methanol in sample in a speed vacuum concentrator (SPD1030A-115, Thermo Fisher Scientific, United State). For derivatisation, 100 μ L of methoxyamine - HCl and pyridine at 20 mg/mL concentration were added to the above dried samples. After that, the sample was then heated continuously and shaken to dissolve at 30 °C / 1200 rpm/ 90 min. Next, 50 μ L of MSTFA solution was added and continued to be shaken at 37 °C / 1200 rpm/ 30 min. The solution was then centrifuged at 20 °C / 15000 rpm/ 3 min. Finally, 100 μ L of the solution was introduced into the vial insert (Shimadzu, Corp., Japan) with a lid, and the vials were placed on the trays in sequence. The profiles of the substances in the strawberry samples were automatically analysed and calculated using the Smart Metabolism Database. The samples were analysed in triplicate.

3.2.6. Statistical analysis

The results were analysed using R software (version 4.2.2), and the metabolic data were analysed using Metaboanalyst 5.0. Treatment effects were assessed using analysis of variance (ANOVA), and Tukey's test was used to identify significant differences ($P < 0.05$). Statistical analyses involved comparing the differences between samples at the same storage time or evaluating a single sample across different storage times.

3.3. Results and Discussion

3.3.1. Film characteristics

3.3.1.1. Film appearance

Table 3.1 and Fig. 3.1 shows the properties of the films. The films containing ME were distinctly yellow compared to the transparent films containing CMC/CNF. Film thickness plays

an important role in the quality of agricultural products (Pajak et al., 2019). Table 3.1 shows the proposed thickness indices. The addition of 1-MCP significantly increased the film thickness compared to CCM1-1 and CCM1-2 ($P < 0.05$); however, the CCM2-1 and CCM2-2 groups did not significantly differ. The effect of 1-MCP on the film thickness has not yet been reported. Nonetheless, the combination of 1-MCP with a component in the coating may have widened the structure of the film, thereby thickening it. Indeed, 1-MCP has been confirmed to significantly increase the cell wall thickness of cherry tomatoes (Taye et al., 2019).

Table 3.1 presents our evaluation of the film colour. The colours of the films significantly differed between the coatings without ME (CCM1-1, CCM1-2) and those with ME (CCM2-1, CCM2-2) ($P < 0.05$). In addition, the ΔE , a^* , and b^* values significantly changed between the CCM2-1 and CCM2-2 groups ($P < 0.05$), whereas these values did not change on CCM1-1 and CCM1-2 ($P > 0.05$). These findings indicated that both 1-MCP and ME strongly influenced the colour of the coating.

Table 3.1. Film characteristics

		CCM1-1	CCM1-2	CCM2-1	CCM2-2
Thickness (mm)		0.043 ± 0.002^b	0.053 ± 0.002^a	0.045 ± 0.004^{ab}	0.050 ± 0.002^{ab}
ΔE		11.481 ± 0.630^c	1.505 ± 0.300^c	30.883 ± 3.620^b	41.417 ± 2.090^a
L^*		993.500 ± 0.780^a	93.567 ± 0.210^a	89.967 ± 0.370^b	88.500 ± 0.290^b
a^*		0.033 ± 0.050^c	0.067 ± 0.090^c	2.067 ± 0.420^b	4.033 ± 0.370^a
b^*		5.133 ± 0.190^c	5.300 ± 0.220^c	34.500 ± 3.580^b	44.800 ± 2.050^a
Roughness	Ra	2.86 ± 0.29^b	2.73 ± 0.42^b	7.05 ± 0.75^a	6.69 ± 0.22^a
	Rq	3.58 ± 0.39^b	3.58 ± 0.39^b	8.90 ± 0.75^a	8.08 ± 0.57^a

^{a,b,c} Means with different letters in the same row are statistically different by Tukey test ($P < 0.05$).

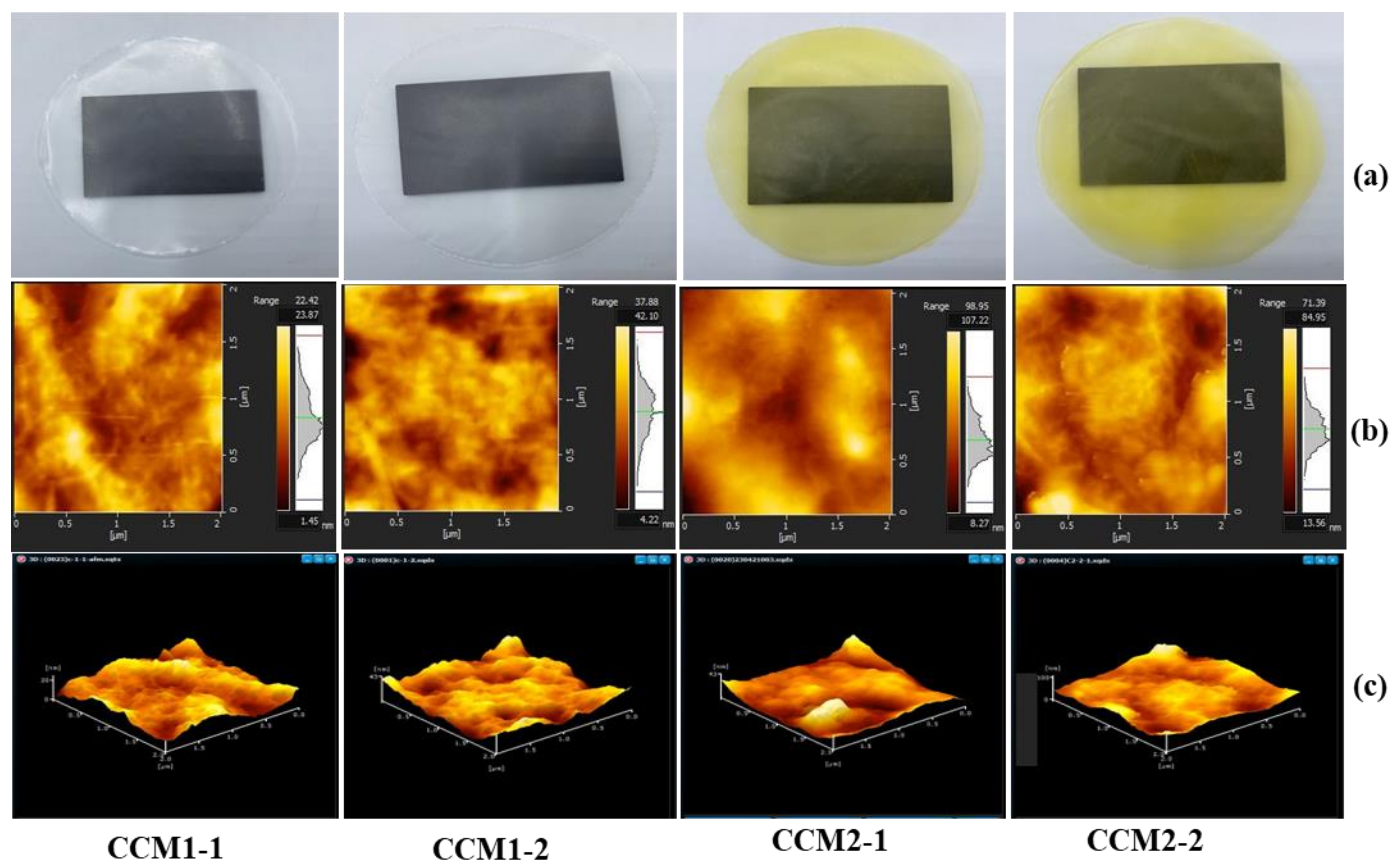


Fig. 3.1. The physical appearance (a) and structure properties of films through AFM images where (b) indicates 2D pictures and (c) indicates 3D pictures of CCM1-1, CCM1-2, CCM2-1, CCM2-2.

3.3.1.2. AFM

The physical and structural features of the film layers were analysed by AFM (Fig. 3.1). The differences in the roughness index (R_a) and original mean square roughness (R_q) were investigated (Table 3.1). Atomic force microscopy (AFM) images showed that the films had a heterogeneous surface structure. The R_a and R_q values differed significantly between the CCM2-1 and CCM2-2 groups (containing ME) and the CCM1-1 and CCM1-2 groups (ME-free coatings) ($P < 0.05$). However, the surface properties of CCM1-1, CCM1-2, CCM2-1, and CCM2-2 did not differ significantly. Thus, the main cause of the significantly increased R_a and R_q values was ME owing to the formation of droplets in the film structure (Shektaei et al., 2023). However, small internal holes on the surfaces of the CCM1-1 and CCM1-2 structures were evident in the AFM images. The accumulation of CNF at high concentrations can lead to the formation of aggregates or holes on the surface and film structure (Bagheri et al., 2019; Sartori et al., 2018). The increased

film roughness could also be caused by macro- and nano-emulsions of oil in the film-forming materials (Fattahi et al., 2020).

3.3.1.3. FTIR

Fig. 3.2 shows the FTIR spectra of the interactions between the functional and intermolecular groups in film constituents CCM1-1, CCM1-2, CCM2-1, and CCM2-2. The results revealed that the hydroxyl group O-H, C=C elongated at 3313.1, and 3293.8 cm^{-1} and C-O at 1018 cm^{-1} are the dominant linkage bands, representing the characteristics of CMC. Moreover, the C-H₂, C-C-H, C=C, C-H₂ bonds extend at bands 2915.8, 2848.3, 1589.1, and 1317.1 cm^{-1} , respectively, indicating of the ME component. The spectra of CCM1-2 and CCM2-2 revealed a more significant absorption peak near 3313.1 cm^{-1} , suggesting that 1-MCP, which causes binding strain oscillations, resulted in more extensive associations than usual (Xie et al., 2020). In contrast, the CCM2-1 group exhibited stronger flexural transmission than the CCM1-1, CCM1-2, and CCM2-2 groups, with peaks at 2915.8 and 2848.3 cm^{-1} . This may be related to the degree of flexural transmission, the influence of C=C on the EO surface, or the crosslinking reaction between CMC and CNF. The CCM2-1 group had an additional peak at 1259.3 cm^{-1} indicating an OH bond in the cellulose structure. The spectra of the membrane materials prepared with CMC and ginger essential oil were similar (Ban et al., 2020), indicating that most of the configuration spectra were comparable. The authors suggested that the structural variation in the microcapsules of the membrane could be due to variations in the composition of the substances in the layer of the material constituting the membrane. Changes in cellulose composition indicate that 1-MCP directly modifies cellulose content, leading to increased or decreased cellulose content at peaks I1740/I1508/I1379 (Xie et al., 2020).

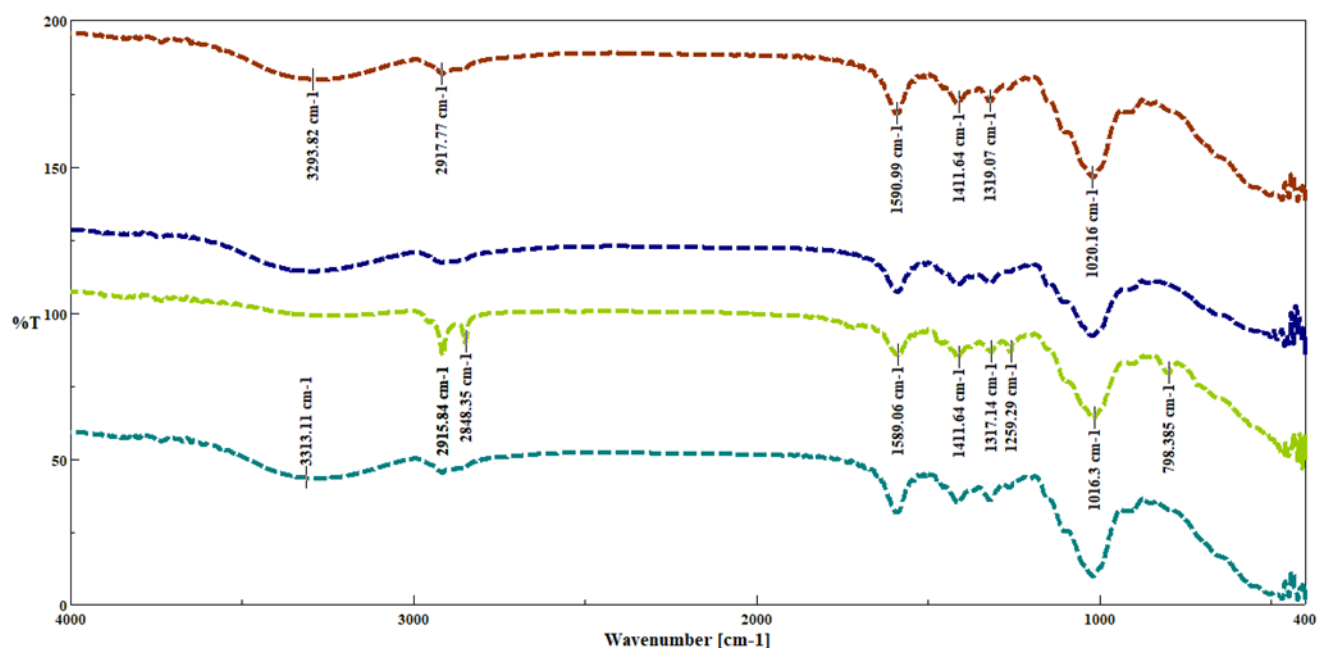


Fig. 3.2. The physical and structure properties of films through FTIR analysis of CCM1-1, CCM1-2, CCM2-1, CCM2-2.

3.3.2. Effects on strawberries

3.3.2.1. Weight loss and firmness

Strawberry weight loss

Weight loss is an important parameter used to evaluate the quality of agricultural products after storage. The rate of weight loss reflects changes in fruit quality over time. Fig. 3.3 shows the effects of the 1-MCP and ME films on the mass change. Weight loss differed significantly between the groups containing 1-MCP and ME. Specifically, the CCM1-2 and CCM2-2 groups lost the least amount of weight (5.08% and 5.63%, respectively), which was lower than that of the control (7.66%) and CCM1-1 (8.10%) after 20 d ($P < 0.05$). Groups containing ME and/or 1-MCP did not differ on the final day of storage. These differences might be due to the powerful impact of 1-MCP and ME on metabolism and respiration and their ability to maintain the structure, leading to less weight loss in CCM1-2, CCM2-1, and CCM2-2. Others have found that 1-MCP maintains hardness and reduces respiration and decay incidence, which leads to less weight loss compared to untreated fruits (Win et al., 2021; Xu et al., 2019). In addition, ME acts as a hydrophobic barrier to the stomata of fruits, preventing moisture loss from the fruit surface (Oliveira Filho et al., 2022a).

Strawberry firmness

External and internal ethylene concentrations and treatment time affect fruit firmness (Sisler and Wood, 1988; Xu et al., 2019). Fig. 3 shows that the firmness of strawberries did not significantly differ between storage days 1, 10, and 15. However, on day 20, the firmness of strawberries coated with a solution containing 1-MCP was 1.3-fold higher than that of the control sample and 1.1-fold higher than that of the single-coated samples ($P < 0.05$). The addition of ME to the coating did not improve the firmness of strawberries compared to that of strawberries coated with 1-MCP. The firmness of kiwifruit improved 7.5-fold on day 20 when treated with 1-MCP and lysozyme (Xu et al., 2019). Maintaining a combination of 1-MCP in the coatings may improve the firmness of the fruit surface (Xu et al., 2019). Furthermore, 1-MCP inhibits ethylene activity, thus delaying kiwifruit softening (Zhang et al., 2014).

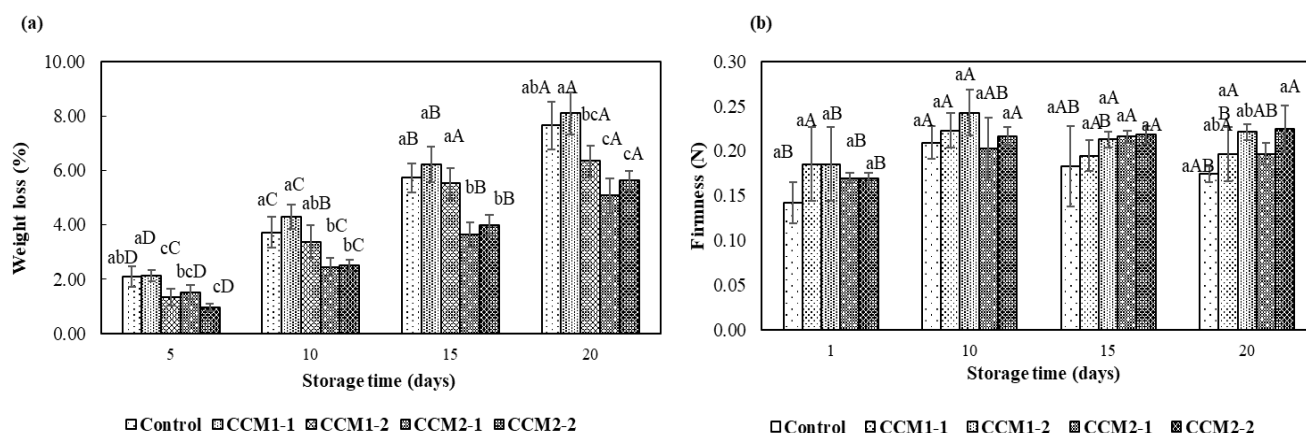


Fig. 3.3. Effect of coatings on weight loss (a) and firmness (b) of strawberry during storage at 5 °C, 85 % RH, 20 days.

3.3.3.2. Strawberry color

The colour of strawberries is closely associated with their ripeness, and changes are associated with anthocyanin content during ripening, when redness starts to spread from the crown of the fruit to the surface until it is completely red and fully ripe (Cao et al., 2015; Hong and Eum, 2020). Table 3.2 shows the colour changes of the strawberries. The colours of the coated and uncoated strawberries did not differ significantly during storage. This might be because the strawberries were mature and the colour was stable during storage.

Table 3.2. Effect of coatings on color changes of strawberry during storage at 5 °C, 85 % RH, 20 days.

Parameters	Storage days	Treatments				
		Control	CCM1-1	CCM1-2	CCM2-1	CCM2-2
<i>L*</i>	0	86.36±6.88 ^{aA}	84.59±5.74 ^{aA}	82.22±4.00 ^{aA}	88.18±7.42 ^{aA}	83.07±5.97 ^{aA}
	10	83.92±4.36 ^{aAB}	86.66±4.75 ^{aA}	83.86±6.06 ^{aA}	84.29±4.53 ^{aA}	84.57±6.79 ^{aAB}
	15	81.39±4.90 ^{aAB}	82.41±3.57 ^{aA}	81.23±3.73 ^{aA}	82.23±6.96 ^{aA}	79.96±4.49 ^{aB}
	20	78.14±3.69 ^{aB}	82.07±7.87 ^{aA}	80.85±6.32 ^{aA}	82.80±7.23 ^{aAB}	82.15±5.63 ^{aAB}
<i>a*</i>	0	65.99±3.60 ^{aA}	67.68±4.07 ^{aA}	65.99±3.99 ^{aA}	68.98± 3.83 ^{aAB}	66.25± 4.09 ^{aA}
	10	65.08±6.19 ^{aA}	68.46±5.45 ^{aA}	66.45±4.91 ^{aA}	68.32±5.45 ^{aAB}	67.41±7.48 ^{aA}
	15	61.01±8.11 ^{aA}	61.31±6.61 ^{aA}	61.56±6.12 ^{aA}	64.11±3.48 ^{aB}	61.92±6.26 ^{aA}
	20	65.75±8.08 ^{aA}	68.59±7.17 ^{aA}	66.52±7.87 ^{aA}	72.42±6.43 ^{aA}	69.52±8.03 ^{aA}
<i>b*</i>	0	51.81±9.51 ^{aA}	48.07±7.63 ^{aA}	48.02± 7.88 ^{aA}	52.36± 8.36 ^{aA}	47.73±7.13 ^{aA}
	10	42.23±6.19 ^{aB}	44.72±6.13 ^{aAB}	42.93±7.61 ^{aA}	44.21±96.31 ^{aAB}	44.12± 9.36 ^{aAB}
	15	38.67±7.22 ^{aB}	38.33±6.09 ^{aB}	39.51±6.73 ^{aA}	40.86±5.55 ^{aB}	38.83±7.48 ^{aB}
	20	43.29±6.06 ^{aAB}	45.84±10.26 ^{aAB}	42.74±9.40 ^{aA}	48.39±9.26 ^{aAB}	44.39±7.85 ^{aAB}
<i>ΔE</i>	0	-	-	-	-	-
	10	11.98±3.91 ^{aB}	13.62± 4.08 ^{aB}	15.10± 3.11 ^{aB}	16.66±5.82 ^{aB}	11.67±3.81 ^{aB}
	15	18.26±2.43 ^{aB}	15.41±7.08 ^{aB}	17.81±5.58 ^{aB}	18.33±5.80 ^{aB}	14.21±5.85 ^{aB}
	20	8.17±1.95 ^{aA}	9.93± 4.17 ^{aA}	10.08±2.52 ^{aA}	12.65±2.78 ^{aA}	7.49±1.82 ^{aA}

Small and capital letters indicated the significant in treatment and storage time by Tukey test ($P < 0.05$).

3.3.2.3. Physicochemical (pH, TSS, Citric acid) characteristics

Table 3.3 shows changes in pH, TSS, and citric acid content of strawberries. The pH of the coated and uncoated strawberries decreased during storage, but was significantly lower in the control after 20 d of cold storage ($P < 0.05$). The pH was the highest in the CCM1-1 and CCM2-1 groups (3.55 and 3.51, respectively; $P > 0.05$). The decreased pH of strawberries is primarily caused by respiration or the activity of fungi that cause rotting fruit (Oliveira Gre et al., 2022b).

The TSS content of strawberries varied with storage duration. The control, CCM2-1, and CCM2-2 had the highest TSS content on day ten of storage (10.40, 10.27, and 10.37, respectively), whereas those of CCM1-1 and CCM1-2 were maximal on the last day of storage (10.07) and on day 15 (10.13), respectively. After reaching a peak, the TSS content decreased and was mostly below that on the first day of storage, except for CCM1-1 and CCM1-2. The comparison of the

TSS values between samples with (CCM1-2, CCM2-2) and without (CCM1-1 and CCM2-1) the 1-MCP combination was found that the TSS was generally better in the group with, than without 1-MCP, except on storage day 20. This result showed that 1-MCP in the coating significantly helped maintain the TSS content in strawberries. Combining 1-MCP with several coatings retards the physicochemical composition, such as weight loss, TSS, and acidity, compared with either 1-MCP or coating alone (Yang et al., 2020; W. Zhang et al., 2022).

The change in citric acid composition in fruits is associated to the respiration process owing to the use of citric acid as a substrate in the tricarboxylic acid (TCA) cycle, or it might be related to the synthesis of other amino acids, leading to changes in the amount of citric acid in the fruit (Yao et al., 2018). In this experiment, the citric acid levels increased in all samples after 10 days. However, at a storage time of 15 d, the citric acid content in all samples decreased compared to that 10th day, except for the CCM1-2 and CCM2-2 samples, which still increased. After 20 d of storage, most samples showed a decrease compared to the first day, except for CCM1-2. This might indicate that the presence of 1-MCP slowed down citric acid metabolism in the CCM1-2 and CCM2-2 samples compared to the rest of the samples. This result is similar to the 1-MCP treatment study on strawberries that showed the highest concentration of citric acid (Taş et al., 2021).

Table 3.3. Effect of coatings on pH, TSS, citric acid changes of strawberry during storage at 5 °C, 85 % RH, 20 days.

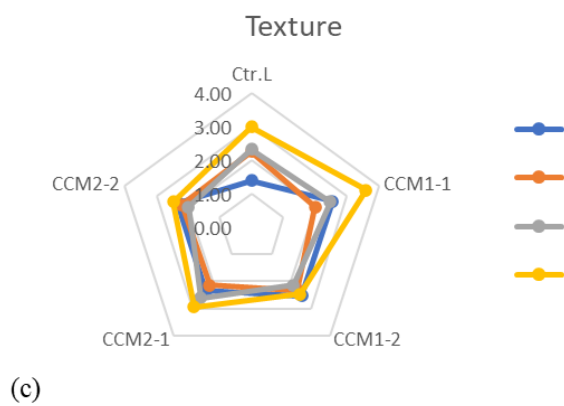
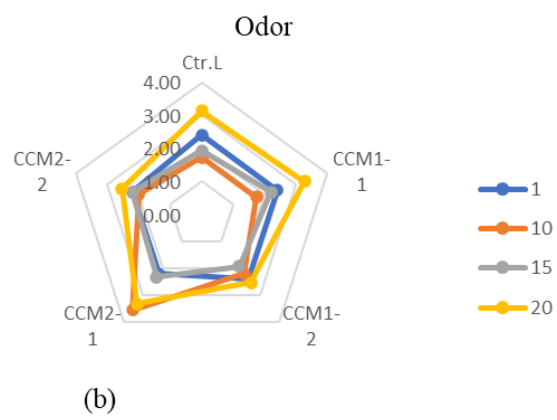
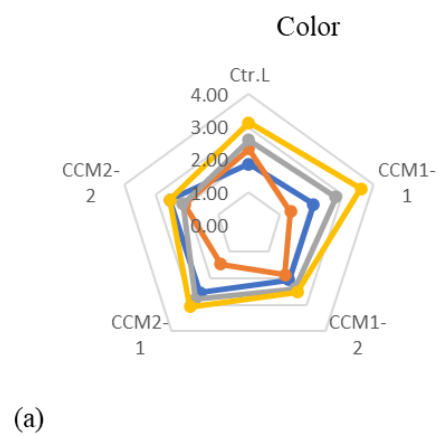
Parameters	Storage days	Treatments				
		Control	CCM1-1	CCM1-2	CCM2-1	CCM2-2
pH	1	3.70±0.03 ^{aA}	3.66±0.05 ^{aA}	3.64±0.05 ^{aA}	3.76±0.07 ^{aA}	3.78±0.07 ^{aA}
	10	3.48±0.02 ^{bB}	3.55±0.03 ^{abB}	3.60±0.02 ^{aAB}	3.55±0.01 ^{abB}	3.53±0.03 ^{abB}
	15	3.44±0.01 ^{abB}	3.49±0.01 ^{abB}	3.44±0.02 ^{bC}	3.38±0.02 ^{cC}	3.44±0.02 ^{abB}
	20	3.25±0.03 ^{bC}	3.55±0.02 ^{aB}	3.46±0.07 ^{aBC}	3.51±0.01 ^{aB}	3.49±0.04 ^{aB}
TSS	1	9.93±0.09 ^{aB}	9.83±0.12 ^{aA}	9.77±0.17 ^{aB}	10.10±0.14 ^{aAB}	10.13±0.12 ^{aB}
	10	10.40±0.08 ^{aA}	9.90±0.08 ^{bA}	9.93±0.05 ^{bAB}	10.27±0.05 ^{aA}	10.37±0.05 ^{aA}
	15	9.47±0.05 ^{cC}	10.03±0.05 ^{abA}	10.13±0.05 ^{aA}	9.93±0.05 ^{bBC}	10.10±0.00 ^{aB}
	20	9.93±0.09 ^{abB}	10.07±0.05 ^{aA}	9.87±0.09 ^{abAB}	9.77±0.12 ^{bC}	9.77±0.05 ^{bC}
Citric acid	1	1.97±0.00 ^{aB}	1.74±0.02 ^{bC}	1.75±0.01 ^{bC}	1.96±0.02 ^{aB}	1.95±0.02 ^{aB}

10	2.04±0.01 ^{bA}	1.97±0.00 ^{cA}	1.97±0.00 ^{cA}	2.04±0.01 ^{bA}	2.17±0.00 ^{aA}
15	1.97±0.00 ^{bB}	1.86±0.00 ^{dB}	1.98±0.01 ^{bA}	1.91±0.00 ^{cC}	2.19±0.00 ^{aA}
20	1.93±0.01 ^{abC}	1.70±0.00 ^{dC}	1.92±0.00 ^{bB}	1.89±0.00 ^{cC}	1.94±0.01 ^{aB}

Small and capital letters indicated the significant in treatment and storage time by Tukey test ($P < 0.05$).

3.3.2.4. Sensorial evaluation

Regarding the effect of 1-MCP on human health, there are no reports on the consumption of 1-MCP in humans. However, it has been confirmed the consumption of 1-MCP in mammals does not have a classification of acute toxicity through oral, dermal, or inhalation routes, and it does not cause skin or eye irritation or skin sensitisation, even at a maximum dosage mass content of up to 5% in weight (Arena et al., 2018). The evaluation of sensorial parameters is presented in Figs. 3.4 (a, b, c, d). The extrinsic variables analysed included colour, odour, texture, and overall acceptance. Accordingly, the results did not differ significantly among the coated strawberries on days 1, 10, and 15 ($P > 0.05$) for all the parameters. However, strawberries coated with 1-MCP differed significantly from the other groups on storage day 20 ($P < 0.05$) in terms of overall acceptance. The evaluation criteria showed that the sensory scores for CCM 1-2 and CCM 2-2 were less than 2.7 points. Compared to a failure score of 5 points, 2.7 points was considered an acceptable threshold after 20 days of storage. Similar differences were observed in the sweetness and overall preference of pears treated with 1-MCP and lemongrass essential oil nanocoatings (Gago et al., 2022).



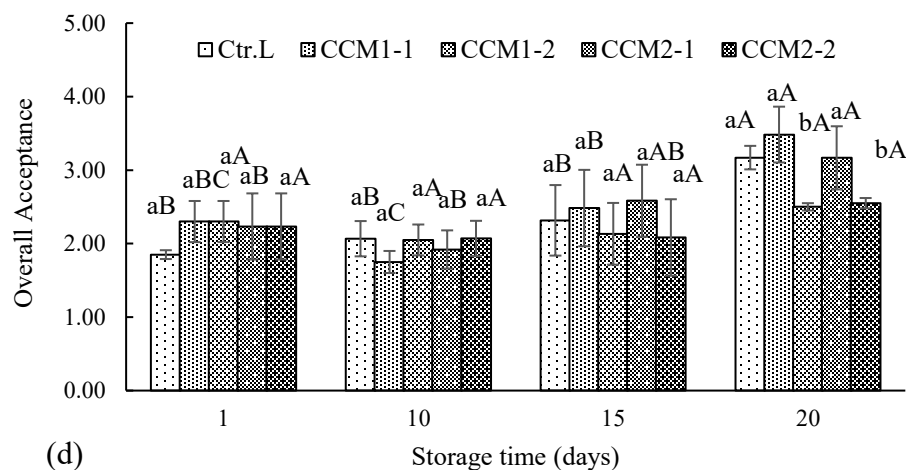


Fig.3.4. Strawberry sensory analysis stored at 5 °C, 85 % RH, 20 days. Vertical bars represented the standard deviation of the mean value from replicates. Inside (a) represented color change, (b) represented odor change, (c) represented texture change, (d) represented overall acceptance changes.

3.3.2.5. Total phenol content and DPPH assay

Phenolic compounds are important secondary metabolites that improve the antioxidant properties of fruits by removing reactive oxygen. These are essential for maintaining fruit quality characteristics such as firmness, colour, and flavour (Y. Yu et al., 2021). Fig. 3.5a shows that the total phenol content initially increased during strawberry storage and then decreased, except for CCM1-2, which might be related to the degree of strawberry maturity. Strawberries were harvested after ripening, when phenolic substances accumulated over the short term. The antioxidant capacity of strawberries gradually weakens during prolonged storage, resulting in oxidation and gradual decomposition of phenolic substances (Y. Yu et al., 2021). By storage day 20, the TPC was higher for CCM1-2 and CCM2-1 than for the other samples ($P < 0.05$), which was 1.3-fold higher than that of the control. Other studies have shown that 1-MCP promotes antioxidant capacity and slows total phenol depletion in dragon fruits (Xu et al., 2019).

The antioxidant properties of fruits are closely associated with their effectiveness as oxygen free radical scavengers. Therefore, an effective post harvest strategy to promote the accumulation of antioxidant components in fruits is of practical significance for improving their antioxidant capacity (Y. Yu et al., 2021). Fig. 3.5b shows that the ability to remove DPPH from strawberries at low temperatures rapidly decreased on day 10 of storage and then gradually increased again, except for CCM2-2. Our results agree with the increased capacity of tomatoes

coated with 1-MCP to remove DPPH (Wang et al., 2010), which contributes to the maintenance of nutritional value and delayed fruit ripening. Furthermore, chitosan results in higher total phenol content and DPPH removal capacity in Jonagold apples, thus increasing antioxidant activity and maintaining fruit quality (Y. Ma et al., 2019).

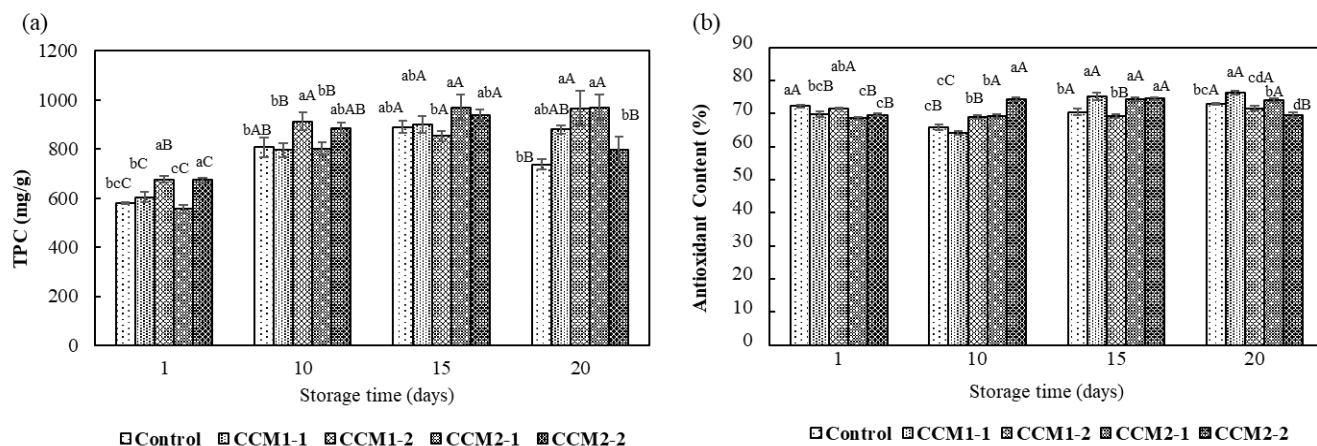


Fig. 3.5. Total phenol content and antioxidant content of strawberry stored at 5 °C, 85 % RH, 20 days. Inside (a) indicated TPC changes and (b) indicated Antioxidant content changes.

3.3.4. Metabolic analysis of uncoated and coated strawberry

In this study, the primary metabolites of uncoated and coated strawberries were specified based on the component profiles from the Shimadzu Metabolomic Smart database, resulting in a total of 102 components (Table 3.4) that were tentatively identified, including organic acids, fatty acids, esters, sugars, sugar alcohols, nitrogenous compounds, sugar acids, and other major metabolites. When strawberries were coated with different coatings, the relative percentage of each component varied. The sugar and sugar alcohol groups comprised 44 components, represented by monosaccharides as the dominant metabolite type. Among these components, mannose-meto-5TMS, psicose-meto-5TMS, tagatose-meto-5TMS, galactose-meto-5TMS, sucrose-8TMS, and glucose-meto-5TMS had the highest relative percentages in all strawberry samples. Remarkably, CCM2-2 exhibited a significantly elevated relative percentage of sucrose-8TMS, lactose-meto-8TMS, fructose 6-phosphate-meto-6TMS, mannose 6-phosphate-meto-6TMS, and maltose-meto-8TMS compared with the other samples ($P < 0.05$). Mannose-meto-5TMS, lactose-meto-8TMS, and sucrose-8TMS were commonly employed in the food industry, and being a rare sugar used as a low-calorie sweeteners (Kwiecień et al., 2008; S. Huang et al., 2022; Baky et al., 2022). There were no differences between the first- and final-day samples for sugar components; however, the

relative percentages of the samples fluctuated. The high sugar content of fruits and vegetables reflects their nutritional value and contributes to their pleasant taste (Baky et al., 2022). The sugar alcohol xylitol-5TMS was detected in all samples. Xylitol-5TMS is a low-calorie alternative sweetener which has been used to promote dental health, exert prebiotic effects, and reduce calorie intake (Grembecka, 2015). Additionally, 27 organic acids, including oxalic acid-2TMS, ascorbic acid-4TMS, tartaric acid-4TMS, and malic acid-3TMS, were detected in all five samples at relatively high concentrations. Overall, the relative concentration of organic acids in the first-day strawberries was lower than that in the final storage days, except for malic acid-3 TMS and ribonic acid-5TMS ($P < 0.05$). Fatty acids/esters, including myristic acid-TMSs, stearic acid-TMSs, and palmitic acid-TMSs, were identified as important primary metabolites. These acids are considered saturated fatty acids that have negative effects on health (Mensink, 2005). Analysis of the composition results on the first day showed that citric acid-4TMS, ureidosuccinic acid-3TMS, 3-Aminoglutaric acid-2TMS, malic acid-TMS, 2-Aminopimelic acid-3TMS, tartaric acid-4TMS, citramalic acid-3TMS, and ribonic acid-5TMS accounted for higher relative percentages than the other acids. The results also revealed the presence of four types of sugar acids, among which CCM2-2 exhibited a significantly higher relative percentage than the other samples in terms of gluconic acid-6TMS and glucaric acid-6TMS ($P < 0.05$); except for glucuronic acid-meto-5TMS and galacturonic acid-meto-5TMS ($P > 0.05$). The analytical results showed that the quantitative and relative percentages of substances and qualitative compounds on the first day of storage were lower than those on the 20th day. Based on these analyses, the use of coatings and/or 1-MCP/ME significantly affected strawberry metabolism.

The heatmap of the metabolic profiles (Fig. 3.6a) shows that the strawberry samples were divided into six groups: A (initial day), B (CCM1-2), C (CCM-2-1), D (CCM-1-1), E (CCM-2-2), and F (control). The colours of the heatmap reflect the normalised peak intensities of the spectrum, with the dark red colour representing high intensity and the dark blue colour indicating low intensity. The heatmap revealed that the peak intensities of most metabolites in CCM2-2 samples were higher than those in the other samples.

To identify and comprehensively evaluate distinct metabolites, Principal Component Analysis (PCA) with an unsupervised statistical technique was used for dimensionality reduction, and PC (Principal Component) were used to elucidate the overarching trends in metabolite changes in uncoated and coated strawberries. The PCA score plot (Fig. 3.6b) shows the influence of various

treatments on the metabolic components of strawberries. Notably, the CCM2-2 samples exhibited a more pronounced effect than the other samples. The CCM2-2 aligned predominantly along PC2 (15.9%), whereas the 0-day sample aligned predominantly along PC1 (29.7%), and demonstrating marked separation from the remaining groups. The PCA biplot (Fig. 3.6c) provides insights into the metabolic profiles of the samples. Specifically, the metabolic signatures of the CCM2-2, CCM1-2, and CCM2-1 groups were characterised by the presence of alose-meto-5TMS, sucrose-8TMS, sorbose-meto-5TMS, and ribose-meto-4TMS, whereas the control group was associated with organic acids. The CCM1-1 group, on the other hand, the 0-day samples showed distinct metabolites, such as ascorbic acid-4TMS and sucrose-8TMS. The components involved in primary metabolism mainly consist of compounds such as esters, acids, and sugars. Most compounds undergo changes over time due to storage conditions, such as temperature, humidity, or treatment methods. In this experiment, most substances increased over the storage period, and there were differences in the relative percentages between samples at the same storage time. This indicated the influence of the coating formulations on the strawberry samples. One of the main reasons for this could be that fresh strawberries were treated with different coating formulations and stored over time, and the biological processes inside the fruits continue to occur during these processes. Processes such as respiration, enzyme activity, ripening, and senescence convert organic acids into sugars or sugars into organic acids to provide energy and regulate physiological processes within cells (Osorio et al., 2020). These metabolic processes change the composition of substances, such as sugars, acids, and aromatic compounds. As the samples were treated using different coating methods, the basic metabolism of each substance varied. The flavour, structure, and composition of the samples were altered after storage.

Partial least squares-discriminant analysis (PLS-DA) was used to assess metabolic differences between coated and uncoated strawberries. The PLS-DA model showed clear data segregation and a robust predictive capability. Fig. 3.7a shows a 2D score plot revealing the distinct separation of samples based on different treatments at 20 days of storage and on the initial day. The corresponding information, shown in Fig. 3.7b, highlights the 15 primary metabolite components that contributed to this separation. These components included glucuronic acid-5TMS, ribonic acid, glucaric acid, inositol-6TMS, ribulose-meto, aspartic acid, malic acid-3TMS, uridine-3TMS, glyceric acid, fructose 6-phosphate-meto-6TMS, rhamnose-meto, mannose 6-phosphate-meto-6TMS, putrescine-4TMS, galacturonic acid, fructose-1phosphate-meto-6TMS which

aligned with the observations made in the earlier PCA results. Additionally, in the CCM2-2 group, inositol-6TMS, fructose 1-phosphate-meto-6TMS, fructose 6-phosphate-meto-6TMS, and mannose 6-phosphate-meto-6TMS were the main sugars with higher metabolomic concentrations, whereas on the initial day, ribonic acid-5TMS and malic acid-3TMS exhibited elevated concentrations.

Table 3.4. Metabolomic profile on GC-MS/MS of coated and uncoated strawberry fruits

	0 day	Control	CCM1-1	CCM1-2	CCM2-1	CCM2-2
ORGANIC ACIDS						
Oxalic acid-2TMS	0.029 ^b ±0.002	0.032 ^b ±0.004	0.038 ^a ±0.004	0.030 ^b ±0.004	0.040 ^a ±0.004	0.039 ^a ±0.001
Oxalic acid-13C2-2TMS	0.003±0.002	0.005±0.001	0.006±0.001	0.005±0.001	0.006±0.001	0.006±0.000
Glyceric acid-3TMS	0.048 ^c ±0.001	0.095 ^a ±0.004	0.081 ^b ±0.003	0.083 ^b ±0.003	0.077 ^b ±0.002	0.085 ^b ±0.001
5-Aminovaleric acid-2TMS	0.009±0.000	0.021±0.012	0.026±0.002	0.024±0.000	0.018±0.008	0.028±0.000
Citramalic acid-3TMS	0.047±0.002	0.044±0.001	0.043±0.001	0.041±0.000	0.043±0.000	0.044±0.001
Malic acid-3TMS	0.418 ^a ±0.014	0.369 ^b ±0.007	0.364 ^b ±0.009	0.358 ^b ±0.006	0.376 ^b ±0.003	0.382 ^b ±0.003
3-Aminoglutaric acid-2TMS	1.050 ^b ±0.041	1.478 ^a ±0.057	1.523 ^a ±0.068	1.521 ^a ±0.028	1.497 ^a ±0.034	1.566 ^a ±0.037
Aspartic acid-d3-3TMS	0.005 ^b ±0.000	0.008 ^a ±0.000	0.008 ^a ±0.000	0.008 ^a ±0.000	0.008 ^a ±0.000	0.009 ^a ±0.000
Aspartic acid-3TMS	NI	0.023±0.001	0.025±0.001	0.026±0.000	0.024±0.000	0.025±0.000
Threonic acid-4TMS	0.153±0.001	0.179±0.008	0.173±0.007	0.178±0.007	0.173±0.008	0.185±0.003
Tartaric acid-4TMS	0.009±0.000	0.003±0.000	0.003±0.000	0.003±0.000	0.003±0.000	0.003±0.000
Lauric acid-d3-TMS	0.003±0.000	0.003±0.000	0.003±0.000	0.003±0.000	0.003±0.000	0.003±0.000
Ureidopropionic acid-3TMS	0.023 ^b ±0.000	0.030 ^a ±0.001	0.026 ^a ±0.001	0.024 ^b ±0.000	0.023 ^b ±0.000	0.022 ^c ±0.000
2-Ketoglutaric acid-oxime-3TMS	0.005 ^b ±0.000	0.007 ^a ±0.000	0.007 ^a ±0.000	0.006 ^a ±0.000	0.007 ^a ±0.000	0.006 ^a ±0.000
2-Ketoadipic acid-meto-2TMS	0.052 ^c ±0.001	0.055 ^b ±0.004	0.056 ^b ±0.004	0.060 ^a ±0.003	0.061 ^a ±0.002	0.061 ^a ±0.003
Threo-b-hydroxyaspartic acid-4TMS	0.002±0.001	0.002±0.002	0.003±0.000	0.003±0.000	0.003±0.000	0.003±0.000
Ureidopropionic acid-2TMS	0.003±0.000	0.003±0.000	0.003±0.000	0.003±0.000	0.003±0.000	0.003±0.000
Ureidosuccinic acid-3TMS	1.634±0.075	1.689±0.023	1.669±0.061	1.629±0.059	1.666±0.011	1.630±0.012
Aconitic acid-3TMS	0.023±0.000	0.025±0.001	0.023±0.002	0.024±0.001	0.024±0.000	0.024±0.002

Ribonic acid-5TMS	0.055 ^a ±0.003	NI	0.053 ^a ±0.003	0.050 ^a ±0.001	0.049 ^a ±0.000	0.026 ^b ±0.019
Dihydroorotic acid-3TMS	0.156±0.106	0.234±0.006	0.152±0.103	0.005±0.000	0.006±0.001	0.082±0.109
Shikimic acid-4TMS	0.004±0.000	0.002±0.002	0.003±0.000	0.001±0.001	0.004±0.000	0.004±0.000
2-Aminopimelic acid-3TMS	0.747±0.031	0.779±0.018	0.786±0.034	0.783±0.026	0.816±0.015	0.826±0.012
Isocitric acid-4TMS	0.009±0.000	0.009±0.000	0.009±0.001	0.008±0.001	0.009±0.000	0.009±0.000
Citric acid-d4-4TMS	0.020±0.001	0.021±0.000	0.022±0.001	0.020±0.002	0.018±0.002	0.022±0.000
Citric acid-4TMS	1.138±0.075	1.137±0.019	1.128±0.049	1.133±0.000	1.140±0.018	NI
Ascorbic acid-4TMS	0.010±0.011	0.016±0.010	0.193±0.119	0.141±0.130	0.029±0.001	0.033±0.011
FATTY ACIDS AND ESTERS						
Myristic acid-TMS	0.021±0.002	0.022±0.004	0.021±0.001	0.018±0.001	0.024±0.005	0.028±0.000
Stearic acid-TMS	0.601±0.013	0.705±0.114	0.652±0.118	0.590±0.030	0.733±0.133	0.861±0.134
Palmitic acid-TMS	1.396±0.046	1.591±0.209	1.466±0.284	1.304±0.043	1.599±0.299	1.793±0.285
NITROGENOUS						
4-Hydroxyphenylpyruvic acid-meto-2TMS	0.173 ^b ±0.008	0.178 ^b ±0.005	0.182 ^a ±0.005	0.183 ^a ±0.006	0.194 ^a ±0.004	0.195 ^a ±0.002
2-Hydroxybutyric acid-2TMS	0.022±0.020	0.025±0.004	0.030±0.004	0.024±0.003	0.031±0.003	0.031±0.001
SUGAR ACID						
Glucuronic acid-meto-5TMS	0.054 ^b ±0.017	0.056 ^b ±0.022	0.049 ^c ±0.016	0.048 ^c ±0.024	0.072 ^a ±0.001	0.057 ^b ±0.022
Galacturonic acid-meto-5TMS	0.041 ^b ±0.015	0.025 ^b ±0.003	0.033 ^b ±0.005	0.042 ^b ±0.012	0.049 ^a ±0.002	0.037 ^b ±0.020
Gluconic acid-6TMS	0.058 ^a ±0.002	0.066 ^a ±0.002	0.064 ^a ±0.003	0.067 ^b ±0.001	0.072 ^b ±0.001	0.075 ^b ±0.004
Glucaric acid-6TMS	0.037 ^d ±0.002	0.058 ^a ±0.002	0.051 ^b ±0.002	0.051 ^b ±0.001	0.045 ^c ±0.001	0.057 ^a ±0.001
SUGARS AND SUGAR ALCOHOLS						
Erythrulose-meto-3TMS	0.036±0.001	0.035±0.001	0.034±0.001	0.033±0.001	0.034±0.000	0.034±0.000
Glycerol-3TMS	0.053±0.000	0.049±0.004	0.052±0.010	0.056±0.013	0.049±0.006	0.054±0.007
Threitol-4TMS	NI	0.002±0.002	0.002±0.000	0.002±0.001	0.003±0.001	0.003±0.001
Xylose-meto-4TMS	0.091±0.002	0.124±0.006	0.082±0.055	0.080±0.053	0.082±0.055	0.043±0.054
Lyxose-meto-4TMS	0.244 ^c ±0.003	0.288 ^a ±0.018	0.286 ^b ±0.014	0.296 ^a ±0.005	0.302 ^a ±0.006	0.285 ^b ±0.010

Arabinose-meto-4TMS	0.110 ^c ±0.002	0.146 ^a ±0.007	0.136 ^b ±0.008	0.136 ^a ±0.001	0.141 ^a ±0.003	0.132 ^b ±0.004
Ribulose-meto-4TMS	0.007 ^b ±0.000	0.011 ^a ±0.000	0.010 ^a ±0.001	0.010 ^a ±0.000	0.011 ^a ±0.000	0.011 ^a ±0.000
Ribose-meto-4TMS	0.110±0.002	0.103±0.067	0.136±0.008	0.136±0.001	0.141±0.003	0.132±0.004
Xylulose-meto-4TMS	0.007±0.000	0.008±0.001	0.006±0.000	0.013±0.011	0.006±0.000	0.006±0.000
Xylitol-5TMS	0.008±0.000	0.012±0.002	0.009±0.001	0.010±0.000	0.011±0.001	0.011±0.000
1,6-Anhydroglucose-3TMS	0.608±0.002	0.595±0.003	0.589±0.005	0.578±0.003	0.568±0.003	0.563±0.002
Arabitol-5TMS	0.600±0.001	0.600±0.005	0.615±0.005	0.626±0.002	0.632±0.003	0.641±0.004
Rhamnose-meto-4TMS	0.004 ^b ±0.000	0.006 ^a ±0.000	0.005 ^a ±0.000	0.005 ^a ±0.000	0.005 ^a ±0.000	0.006 ^a ±0.000
Dihydroxyacetone phosphate-meto-3TMS	0.006±0.004	0.007±0.002	0.006±0.004	0.004±0.000	0.002±0.000	0.006±0.004
Glycerol 3-phosphate-4TMS	0.012±0.001	0.011±0.000	0.011±0.000	0.011±0.000	0.011±0.000	0.012±0.001
2-Deoxy-glucose-meto-4TMS	0.022±0.016	0.032±0.001	0.032±0.001	0.021±0.015	0.023±0.016	0.032±0.000
2-Deoxy-glucose-4TMS	0.034±0.019	0.057±0.009	0.028±0.016	0.017±0.001	0.017±0.001	0.031±0.019
Psicose-meto-5TMS	8.933 ^a ±0.227	9.177 ^a ±1.100	8.293 ^b ±0.905	7.405 ^c ±0.155	8.764 ^b ±0.898	8.447 ^b ±0.754
Tagatose-meto-5TMS	8.333 ^a ±0.158	9.167 ^a ±0.158	8.341 ^a ±0.871	6.774 ^b ±0.165	6.492 ^b ±0.662	6.753 ^b ±0.080
Glucono-1,5-lactone-4TMS	7.325±0.355	7.388±0.174	7.231±0.177	7.058±0.243	7.437±0.131	7.580±0.063
Sorbose-meto-5TMS	4.252 ^c ±0.152	4.681 ^a ±0.153	4.539 ^a ±0.102	4.392 ^b ±0.087	4.498 ^b ±0.102	4.222 ^c ±0.048
Allantoin-4TMS	0.020 ^c ±0.000	0.021 ^b ±0.000	0.021 ^b ±0.001	0.022 ^b ±0.001	0.023 ^a ±0.000	0.023 ^a ±0.000
Fructose-meto-5TMS	4.304 ^c ±0.135	4.750 ^b ±0.093	4.745 ^b ±0.146	4.746 ^b ±0.075	5.049 ^a ±0.083	4.973 ^b ±0.052
Allose-meto-5TMS	6.618±1.928	4.244±0.134	4.139±0.124	5.482±0.989	4.270±0.086	6.335±1.787
Mannose-meto-5TMS	9.222±1.596	8.696±1.219	8.665±1.327	10.265±0.372	10.800±0.158	10.686±0.084
Glucono-1,4-lactone-4TMS	0.010±0.001	0.010±0.002	0.010±0.000	0.011±0.002	0.011±0.000	0.009±0.001
Galactose-meto-5TMS	7.705 ^c ±2.071	9.500 ^a ±2.119	9.476 ^a ±2.249	7.940 ^c ±2.137	8.402 ^b ±2.077	6.940 ^d ±0.050
Glucose-meto-5TMS	7.188 ^b ±0.247	7.739 ^a ±0.094	7.834 ^a ±0.225	7.747 ^a ±0.279	8.095 ^a ±0.094	7.883 ^a ±0.053
Tyrosine-3TMS	0.214±0.009	0.201±0.034	0.202±0.003	0.213±0.011	0.208±0.008	0.184±0.016
Mannitol-6TMS	0.028±0.001	0.028±0.005	0.027±0.001	0.029±0.002	0.028±0.001	0.025±0.001
Sorbitol-6TMS	0.008±0.001	0.008±0.003	0.009±0.001	0.007±0.003	0.020±0.009	0.017±0.009
Galactitol-6TMS	0.007±0.000	0.009±0.003	0.008±0.001	0.007±0.002	0.019±0.009	0.015±0.009

Inositol-6TMS	1.399 ^a ±0.019	1.165 ^c ±0.038	1.091 ^c ±0.068	1.049 ^c ±0.007	1.036 ^c ±0.025	1.373 ^b ±0.026
Fructose 1-phosphate-meto-6TMS	0.053 ^c ±0.002	0.151 ^a ±0.012	0.170 ^a ±0.011	0.151 ^a ±0.005	0.166 ^a ±0.004	0.138 ^b ±0.045
Fructose 6-phosphate-meto-6TMS	0.003 ^c ±0.000	0.008 ^b ±0.000	0.009 ^b ±0.001	0.008 ^b ±0.000	0.009 ^b ±0.000	0.010 ^a ±0.000
Mannose 6-phosphate-meto-6TMS	0.031 ^c ±0.001	0.075 ^b ±0.005	0.092 ^b ±0.011	0.085 ^b ±0.002	0.080 ^b ±0.091	0.102 ^a ±0.011
Glucose 6-phosphate-meto-6TMS	0.009±0.000	0.024±0.008	0.031±0.002	0.027±0.000	0.046±0.037	0.034±0.002
Sucrose-8TMS	4.560 ^a ±0.142	0.921 ^b ±0.021	0.783 ^b ±0.055	0.711 ^b ±0.018	0.790 ^b ±0.043	5.249 ^a ±0.039
Lactose-meto-8TMS	0.024 ^c ±0.002	0.026 ^b ±0.001	0.025 ^b ±0.002	0.024 ^c ±0.002	0.025 ^b ±0.001	0.029 ^a ±0.001
Trehalose-8TMS	0.217 ^b ±0.002	0.208 ^b ±0.008	0.214 ^b ±0.010	0.198 ^b ±0.002	0.207 ^b ±0.002	0.254 ^a ±0.006
Lactitol-9TMS	0.032±0.001	0.030±0.001	0.030±0.002	0.027±0.002	0.030±0.001	0.037±0.002
Maltose-meto-8TMS	0.248 ^b ±0.002	0.236 ^c ±0.009	0.244 ^b ±0.013	0.228 ^c ±0.003	0.238 ^c ±0.004	0.290 ^a ±0.095
Maltitol-9TMS	0.022 ^d ±0.000	0.271 ^b ±0.014	0.265 ^b ±0.016	0.309 ^a ±0.005	0.286 ^b ±0.003	0.142 ^c ±0.003
Isomaltose-meto-8TMS	0.004±0.000	0.022±0.015	0.028±0.027	0.038±0.029	0.022±0.013	0.017±0.001
OTHERS						
Hydroxylamine-3TMS	0.163 ^b ±0.011	0.183 ^b ±0.025	0.218 ^a ±0.028	0.174 ^b ±0.022	0.235 ^a ±0.024	0.238 ^a ±0.008
Sarcosine-d3-2TMS	0.007 ^b ±0.000	0.007 ^b ±0.001	0.009 ^b ±0.001	0.007 ^b ±0.001	0.010 ^b ±0.001	0.029 ^a ±0.002
Leucine-2TMS	0.053±0.001	0.068±0.010	0.055±0.003	0.060±0.002	0.054±0.001	0.055±0.002
Serine-3TMS	0.962 ^c ±0.029	1.180 ^a ±0.030	1.058 ^b ±0.070	1.109 ^b ±0.009	0.943 ^c ±0.012	1.020 ^c ±0.017
Acetylglycine-2TMS	0.006 ^a ±0.000	0.006 ^a ±0.000	0.005 ^b ±0.000	0.006 ^a ±0.000	0.005 ^b ±0.000	0.006 ^a ±0.000
4-Hydroxyproline-3TMS	0.103 ^b ±0.002	0.120 ^b ±0.007	0.126 ^b ±0.011	0.122 ^b ±0.006	0.131 ^b ±0.023	0.155 ^a ±0.016
Asparagine-4TMS	0.015±0.000	0.021±0.000	0.019±0.001	0.018±0.000	0.019±0.000	0.017±0.001
Phenylalanine-2TMS	0.367 ^c ±0.004	0.481 ^a ±0.011	0.446 ^a ±0.015	0.431 ^a ±0.012	0.444 ^a ±0.011	0.430 ^b ±0.012
Homocysteine-3TMS	0.004±0.002	0.005±0.000	0.004±0.000	0.004±0.000	0.004±0.000	0.004±0.000
Asparagine-3TMS	0.634 ^c ±0.016	0.652 ^b ±0.058	0.685 ^b ±0.018	0.713 ^a ±0.015	0.740 ^a ±0.006	0.697 ^b ±0.031
Putrescine-4TMS	0.049 ^c ±0.002	0.096 ^a ±0.013	0.076 ^b ±0.006	0.067 ^b ±0.004	0.071 ^b ±0.001	0.062 ^b ±0.005
Glutamine-3TMS	1.336 ^b ±0.036	1.217 ^b ±0.111	1.409 ^a ±0.055	1.542 ^a ±0.082	1.337 ^b ±0.081	1.235 ^b ±0.116

Ornithine-d7-4TMS	0.013±0.005	0.014±0.003	0.011±0.000	0.017±0.003	0.014±0.004	0.017±0.004
Arginine-3TMS	0.013±0.001	0.015±0.001	0.014±0.001	0.014±0.000	0.013±0.001	0.013±0.001
N-Acetylglutamine-3TMS	0.031±0.001	0.032±0.001	0.032±0.001	0.032±0.001	0.034±0.001	0.033±0.001
Glucosamine-5TMS	0.039 ^c ±0.003	0.043 ^b ±0.007	0.040 ^b ±0.010	0.041 ^b ±0.008	0.051 ^a ±0.027	0.030 ^c ±0.005
N-Acetylmannosamine-meto-4TMS	0.082 ^a ±0.001	0.077 ^b ±0.002	0.072 ^b ±0.003	0.069 ^b ±0.001	0.065 ^c ±0.002	0.079 ^a ±0.002
Tryptophan-3TMS	0.149 ^d ±0.017	0.191 ^c ±0.030	0.213 ^c ±0.010	0.308 ^a ±0.017	0.230 ^b ±0.010	0.240 ^b ±0.023
Oleamide-TMS	0.008±0.005	0.012±0.006	0.011±0.003	0.008±0.004	0.011±0.006	0.007±0.003
Uridine-4TMS	0.003±0.001	0.003±0.000	0.002±0.000	0.003±0.000	0.002±0.000	NI
Uridine-3TMS	0.019 ^b ±0.002	0.033 ^a ±0.004	0.029 ^a ±0.001	0.027 ^a ±0.001	0.026 ^a ±0.002	0.029 ^a ±0.002
Monostearin-2TMS	0.575±0.012	0.621±0.049	0.635±0.103	0.573±0.030	0.686±0.121	0.802±0.133

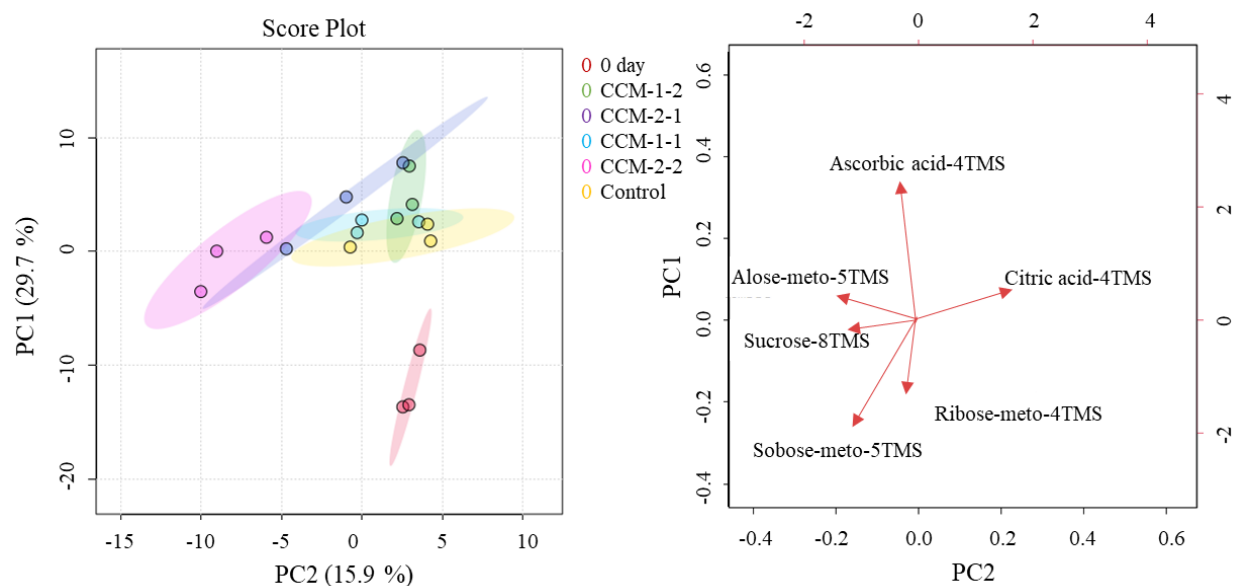


Fig. 3.6. Heatmap (a), PCA score plot (b), biplot (c) of uncoated and coated strawberry.

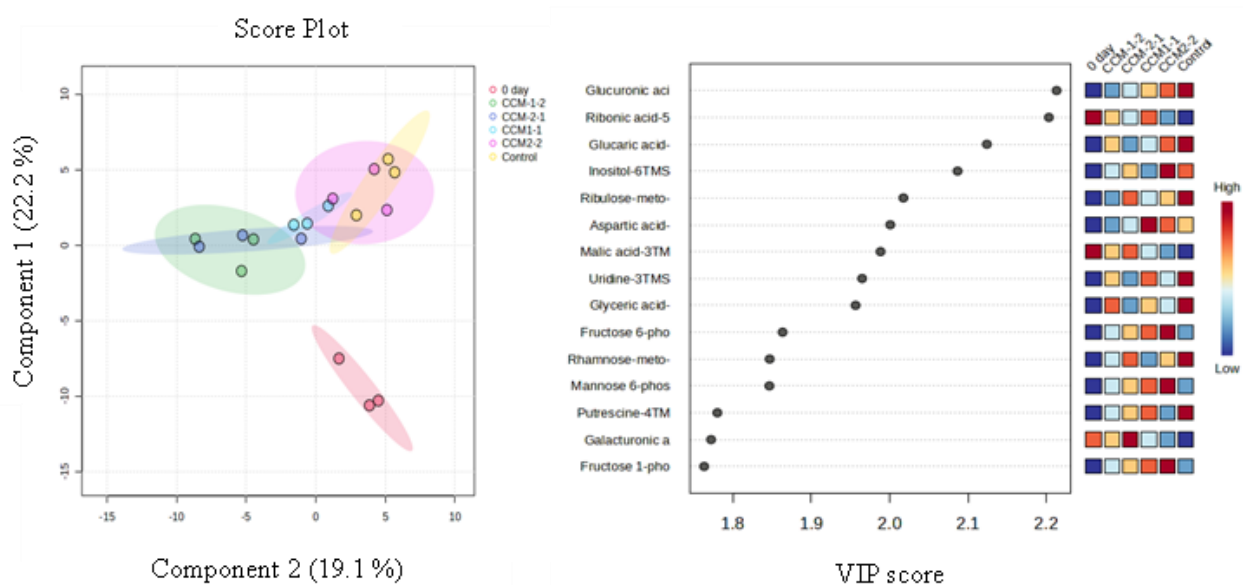


Fig. 3.7. PLS-DA score plot (a) and important features (b) of uncoated and coated strawberry.

3.4. Conclusions

The inclusion of 1-MCP/ME in the coating solutions had notable effects on both coatings and strawberries. The application of 1-MCP contributed to the preservation of the coating colour for up to 50 d. The addition of ME increased the Ra and Rq values, indicating that the films had a significantly rougher surface. Coatings enhanced with ME and/or 1-MCP maintained the post harvest freshness of strawberries by reducing weight loss and maintaining firmness, TSS, citric

acid content, colour, and TPC. Moreover, the developed coatings influenced the profiles and relative percentages of the metabolic components. Strawberries coated with CCM2-2 exhibited superior quality and metabolic outcomes. This study substantiated the efficacy of 1-MCP using a dilution method. Consequently, incorporating 1-MCP into coating solutions enhances its effects, streamlines the process, and improves the future preservation of agricultural products. However, the dilution of 1-MCP powder in coatings raises concerns about potential 1-MCP residues affecting human health. Therefore, further research is necessary to confirm this effect.

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Chapter 4: Application of green pomelo peel essential oil-based carboxymethylcellulose coatings reinforced with nano chitosan and nano cellulose fibers during the drying process on dried silkworms

4.1. Introduction

Edible insects have been studied for their potential as a food source providing essential nutrients for humans (Ardoin, 2021). Silkworm pupae are the main byproduct after the process of spinning silk threads on cocoons. Silkworm provides a high protein source (48.31%–67.39%) including all essential amino acids (0.17% – 3.92%) (Hirunyophat & Chalermchaiwat, 2022). Drying silkworm helps prolong the shelf life of preservation which is an intermediate step for other uses. However, the drying condition might alter compounds, or change the sensory properties of the dried product (Dandadzi et al., 2023). For example, drying methods differentially altered the main volatile components of edible grasshoppers and silkworms, including the change in appearance, flavor, texture. The change rate varies depending on each drying method (Dandadzi et al., 2023). To prevent the nutritional value of dried silkworm, a convenient and suitable drying method is a requirement for scientific research nowadays. Besides, a novelty method which could assist silkworm drying process is also needed. One of recommendations is that the use of edible coating to cover the surface of silkworms and protect them from the heat of the drying process. The mechanism of the covering is creating a thin film on the sample surface to protect samples from unshielded contact with heat or airflow within the drying system. In this study, the purpose of the coating is to cover the surface of silkworm before drying process so it can prevent samples from heat and airflow during drying, thereby minimizing damage to surface texture, appearance, retaining the most nutritional compounds of dried products.

The environmental and human health problems associated with petroleum-based synthetic plastic materials used in the food sector were attracting the attention of scientists and people around the world. Choosing coatings from natural and edible materials is a priority in the current time. Nanomaterials show better improved mechanical properties such as gas barrier properties, improved dimensional stability, and better thermal expansion (Zafar et al., 2016). Nano-chitosan (N-Ch) is a particle with a small molecular size of a few hundred nm. The roles of N-Ch were to provide excellent properties that improve the functional characteristics, and strengthen the chitosan film, it also have a positive effect on the antibacterial activity of the membrane (Sheerzad et al.,

2024). To perform its role as filler in the membrane complex, N-Ch must not be dissolved in the system, and the protonation of weakly basic amine groups requires the pH of the coating solution to be maintained at a higher level than its pKa ($pK_a \approx 6.3-6.5$) (Aditya et al., 2023). Through carboxymethylation, sodium carboxymethyl cellulose was produced⁷. This modified cellulose can be soluble in water, offers advantageous properties in film-forming, and provides moderate air penetrability (Nasibi et al., 2020). Due to its robust internal network structure, this material is considered an active hydrogel, contributing to enhanced composite membrane performance⁷. Combination of natural oils or nanomaterials in CMC based could alter the water-resistant characteristics of CMC films and improve their mechanical and antibacterial properties (Al-Tayyar, Youssef, & Al-hindi, 2020)-(Al-Tayyar, Youssef, & Al-Hindi, 2020).

Essential oil in general plays an important role in both antibacterial, antioxidant and film-forming dispersions, especially in essential oil concentration (Sánchez-González et al., 2011) (Sánchez-González et al., 2010). These authors also mentioned that the nature or higher concentration of essential oils such as tea tree, bergamot, and lemon essential oils into chitosan or hydroxypropyl methylcellulose could lead to higher antibacterial effect and film physico-chemical characteristic changes such as the lower sorption of moisture or the water vapor permeability. However, these are some authors who were concerned about the negative effects of the essential oils on food properties such as sensory properties and consumer acceptance (Yousuf et al., 2021). Therefore, the selection of essential oil combining with edible coatings also plays a crucial role in the application. In this study, green pomelo peel essential oil (GPO) was selected because of its regional plant area specificity, which was extracted from green pomelo peel in Ben Tre province, Vietnam. The main components of GPO were confirmed containing limonene, α -pinene, β -myrcene and sabinene (Ngan et al., 2022), those components play an important role in antimicrobial contamination and maintaining the quality of agricultural products (Van, Thi, et al., 2023).

The use of deep learning techniques, such as instance segmentation algorithms, is increasingly prevalent in agriculture. These algorithms, like YOLO, identify and classify objects in images by assigning class labels, unique instance IDs, and pixel-wise masks, providing detailed object information (Lin et al., 2014). YOLOv8, an improvement over YOLOv5, advances in structural design, loss computation, data augmentation, training strategies, and inference processes, making it highly versatile across visual domains (Terven et al., 2023). For instance, combining

YOLOv8 with an enhanced DeepLabv3+ achieves 90.8% mean intersection over union in plant leaf segmentation (K. Yang et al., 2023), while its application with CSP and C2f modules reaches 99.5% accuracy in classifying fruit ripeness (Xiao et al., 2024). Previous studies revealed that YOLOv8 was applied to detect multi-stage apple fruit in farm (B. Ma et al., 2024) or fruit freshness (Wei et al., 2023) with high accuracy. Besides, X-ray CT is a useful non-destructive technique which was applied to detect the change of internal structure of persimmon and strawberry²² (Thi Hang Phuong et al., 2024). This study used X-ray CT imaging and deep learning to analyze porosity differences in the inner structure of silkworms before and after drying, comparing coated and uncoated samples to find out the effect of treatments.

In previous studies, we determined the effect of CMC/NCF combined with mandarin peel extraction and/or 1-MCP. The results revealed that the addition of mandarin peel extraction or 1-MCP plays a vital role to control strawberry weight loss, reduce the fungal growth, and significant changes in color and average roughness values of the film. However, there were still some disadvantages such as the increase of the peel extraction concentration did not show the higher effect on prolonging the shelf life of strawberries or the dilution method of 1-MCP might increase the concern about human health (Van, Wardana, et al., 2023) (Van et al., 2024). From our perspectives, there were no reports which determined the effect of edible coatings on drying process, especially on dried silkworms which must suffer a heat treatment until now. Therefore, this study aimed to combine GPO based on CMC and N-Ch/NCF without any emulsifiers into the edible coating to clarify the effect of this oil on bacterial resistance, film forming, and drying process, to check whether it has any effect to prevent the quality change on silkworm under heated condition.

4.2. Material and methodology

4.2.1. Chemicals

CMC and NCF were industrial standard and sourced from Wako Pure Chemical Industries and Nippon Paper Industry (Japan). N-Ch with purity 99.9 %, average particle size was 80 - 100 nm, molecular weight at 161 (g/mol) was obtained from Nano Research Element (Haryana, Indian). Glycerol was guaranteed standard, ribitol was special standard, while pyridine, methanol, and chloroform were reagent standard. N-hexane was Wako first grade, and calcium chloride was reagent grade. These chemicals were provided from FUJIFILM Wako Pure Chemical Co., Ltd.

(Japan). N-methyl-N-trimethylsilyltrifluoroacetamide (MSTFA), methoxyamine-HCl, and n-alkanes (the qualitative retention time index, 100-200 $\mu\text{g/mL}$ in hexane) were procured through GL Sciences, Co., (Japan), and Restek Corp., (USA), respectively. PDA (potato dextrose agar) and PDB (potato dextrose broth) were obtained through Nissui Pharmaceutical Co., Ltd. (Japan). To ensure high purity for experimental procedures, Millipore water was processed through an Autopure WT101 UV system provided by Yamato Corp., Japan. Additionally, distilled water used in the study was obtained utilizing the automatic water distillation apparatus of Aquarius Advantech GS-200 manufactured by Toyo Seisakusho Kaisha, Ltd., (Japan). These materials and apparatuses were carefully selected to maintain the integrity and reliability of the experimental outcomes.

4.2.2. Preparation of coatings and films

The stock mixture was developed as follows: 1.0% (w/v) of CMC, 0.5% (w/v) of NCF and 0.1%, (w/v) of N-Ch powders. These materials were slowly poured into stirred distilled water by water bath (EWS-100RD, AS ONE, Japan) at 850 rpm, 85 °C in 1 h. Then, 0.35% (v/v) of glycerol was added to the above solution during the stirring step. After the stirring step finished, the coating solution was kept in the room temperature for cooling down, then, the GPO was added in ratio 0.01; 0.1; 1.0% (v/v). Subsequently, the homogeny step by T-25, IKA, (Japan) was used to get final solutions in 10 min and 20,000 rpm. The targeted solutions named CCG0 (CMC, N-Ch/ NCF), CCG1 (CMC, N-Ch/ NCF, GPO 0.01%), CCG2 (CMC, N-Ch/ NCF, GPO 0.1%), and CCG3 (CMC, N-Ch/ NCF, GPO 1.0%). A vacuum Yamato ADP300 (Japan) was used to de-bubbled and ensure uniformity of solutions. To make films, the solution was poured onto a square silicon plate with 20 mL of volume, dried for 8 h at 40 °C in an oven EYELA WFO-520W (Japan). After drying process, the dried film was removed from the plate and kept in the plastic desiccator at room temperature and 53% RH with calcium chloride to reach constant state for subsequent determinations.

4.2.3. Silkworm preparation

A total of 125 silkworms and the 3.5 kg wet powder of mulberry were obtained from Ehine Sansyu Co. Ltd., (Japan). The wet powder scroll of mulberry was used to feed the silkworms in 15 days. After the silkworms transfer to the cocoon, the silkworm pupa was collected, then they were divided into five groups as follows: control, CCG0, CCG1, CCG2, CCG3. In this step, these

silkworm pupas were treated with distilled water and four kinds of coatings, respectively. After that, they were dried in an incubator (Isuzu Seisakusho Co., Ltd, Japan) at 70 °C, 20% RH, and flow rate at 2.7 m/s in 5.5 h. For metabolism analysis, the dried silkworm pupa was grilled by a grinder (Philips HL-3258, Japan) in 1 min, and then the silkworm powder was kept in a freezer (NF-300 HC, Nihon Freezer, Japan) at -60 °C to prevent of degradation of quality until further experiments.

4.2.4. Analytical methods

4.2.4.1. Characteristics of the coatings and films

pH, viscosity of coatings

A LAQUA twin pH meter (Horiba, Japan) and a viscometer (Viscometer TV200, Toki Sangyo Co., Ltd, Japan) were used to measure the pH and viscosity of the emulsions. Basically, 1 mL for pH and 1.1 mL for viscosity of coatings was used to measure the value at room temperature, three replications.

Color, thickness of films

The color of the film was characterized at various angles by a CR-20 color reader (Konica Minolta Inc., Japan), with five values recorded for each film sample. The measurements were based on the Commission Internationale de l'Eclairage (CIE) color scale, where the L^* , a^* , b^* , and ΔE color systems included. Specifically, L^* values are from 0 to 100, representing the spectrum from diffuse from black to white, with white values potentially exceeding 100. The a^* values describe the color between red and green, while the b^* values indicate the color between yellow and blue. The total color change (ΔE) was determined using Equation (1), described by Van, et al. (Van, Thi, et al., 2023). This comprehensive approach allows for precise quantification of color changes in the film, facilitating a better understanding of its optical properties.

$$\Delta E = \sqrt{(L_o - L^*)^2 + (a_o - a^*)^2 + (b_o - b^*)^2} \quad (1)$$

The values of L_o , a_o , and b_o were for the white plate, meanwhile L^* , a^* , and b^* were for the membranes, five replications.

As for thickness, a digital micrometer (Series 102–707, Mitutoyo Corporation, Japan) was eliminated to record the film thickness (μm), using to the accuracy at 0.001 mm and five points randomly per film.

Scanning Electron Microscopy (SEM)

The cross-section and surface of films were clarified by SEM system (SU3500, Hitachi High-Tech, Corp., Japan). Basically, a small portion of the film was put into nitrogen liquid for freezing, then, the films were broken out to several pieces. Next steps, the broken films were marked to record the broken points. For imaging, the fractured films were mounted on conductive double-sided tape and coated with a fine gold layer using a plasma coater (HPC-1SW, Vacuum Device Co., Japan). Images were captured at 15.0 kV voltage, with a magnification of 300x and a width of 100 μm for the surface view, and at 15.0 kV, 1.00 k magnification, and 50.0 μm width for the cross-sectional view.

Atomic force microscopy (AFM)

Film roughness was measured by AFM system (Hitachi 5200S, Japan) conducted in tapping mode of Nano Navi Application. Coatings were similarly formulated by adding them (CCG0, CCG1, CCG2, and CCG3) to the fresh carbon tape before imaging. The cantilever of SI-DF3P2 was chosen with a scanning frequency around 1 Hz, and an area scanning of 5 $\mu\text{m} \times 5 \mu\text{m}$. There were two types of measurements including roughness surface and force mapping. Before the measurement, the performance of Q-curve in DFM mode was evaluated and performed the approach to get the standard curve. After that, AFM and the force mapping mode were set up to measure the force curve and elastic modulus of film samples. The time for analyzing one sample was 5 h, which depended on the scan area of sample setting. The force curve values indicated the indentation force acting on the sample at a given penetration depth which can represent the stiffness and adhesion properties of the sample, characterized by F values (nN). The modulus of elasticity (E_s , MPa) was calculated by converting the measured force curve into a load-indentation curve and fitting a predetermined DMT contact model to the load indentation curve. Measurements were taken from 10 distinct areas, and the values were calculated using Eq. (2) to (3) as detailed below.

$$F = \alpha \cdot \delta^{\frac{3}{2}} + F_{ad} \quad (2)$$

$$E_s = \frac{1 - V_s^2}{\frac{4\sqrt{R}}{3\alpha} - \frac{1 - V_t^2}{E_t}} \quad (3)$$

F : Force load

δ : Penetration depth

R : Probe curvature radius

α : Coefficient

F_{ad} : Adhesion force

E_t : Probe elastic modulus

V_s : Sample poisson's ratio

E_s : Sample elastic modulus

V_t : Probe poisson's ratio

Thermal analysis

The thermogravimetry (TG) and differential thermogravimetric (DTG) curve were measured with a thermogravimetric analyzer (TGAQ500, TA Analyzer, USA) under a nitrogen (N₂) atmosphere. The sample mass was about 5 mg, and the test temperature range was from 50 °C to 450 °C.

4.2.4.2. Determination of antimicrobial activity

The bacterium *Staphylococcus aureus* was selected for testing. *Staphylococcus aureus* was shown to alive (but may not grow) at low-moisture foods and environments for extended periods of time (Beuchat, L.; Komitopoulou, E.; Betts, R.; Beckers, H.; Bourdichon, F.; Joosten & S.; ter Kuile, 2011). Besides, *Staphylococcus aureus* is the most well studied in silkworm models (Hamamoto, 2024). Firstly, the bacteria were cultured in a sterile environment using tryptic soy broth (TSB) at the recommended concentration of manufacturer for 24 h at 37°C. Subsequently, 10 µL of the bacterial solution at a concentration of 5 x 10⁵ (spores/µL) was used to test the antibacterial resistance of the coating solutions. To prepare for the experiment, 10 mL of sterilized tryptic soy agar (TSA) solution combined with 5 mL of sterilized distilled water (control) or 5 mL of the coating solution, and then 10 µL of the bacterial solution, were placed in the same test tube. The solution in the test tube was stirred at 1200 rpm for 10 s for homogenization. Then, the solution in the test tube was transferred onto plastic petri dishes, incubated at 37°C, and the count of colony-forming units was examined after 24 h. The colony count was automatically determined using colony counter software (Shashin Kagaku PSF-1000, Japan).

4.2.4.3. Silkworm pupae application

4.2.4.3.1. Moisture content

The moisture content of samples was determined by drying samples at 105°C in 24 h. The weight in initial and final day was recorded, then moisture content was calculated by Eq. (4):

$$M_C = (M_0 - M_1) \times 100 / M_0 \quad (4)$$

where M_C is the moisture content (%), M_0 is the weight before drying (g), and M_1 is the weight of final drying time (g).

4.2.4.3.2. Color change

The color changes of silkworm were measured using CR-20 (Konica Minolta Japan Co., Ltd.) by evaluating ΔE , L^* , a^* , and b^* values. The ΔE value was determined using Eq. (1), where L_0 , a_0 , and b_0 represent the values before coating while L^* , a^* , and b^* correspond to the values after coating and drying, five replications.

4.2.4.3.3. Metabolism profile of dried silkworm

The metabolism process of silkworms was reflexed using a GC-MS/MS system (Shimadzu, Kyoto, Japan). The gas chromatography (Nexis GC 2030, Shimadzu Corporation) was outfitted with a DB-5 column (Shimadzu Corp., USA), with specifications of a 0.25 mm inner diameter, a 30 m length, and a film thickness of 1.0 μm . A liquid injection system delivering 50 $\mu\text{L/s}$ was employed, with the injection port temperature set at 250 °C. The column temperature program began with a 4 min hold at 100 °C, then rose to 320 °C over 11 min in spitless mode. Helium served as the carrier gas, moving at a linear velocity of 39.0 cm/s and a pressure of 83.7 kPa. Mass spectrometry was performed across a range of 45 to 600 m/z, with the ion source and interface temperatures at 200 °C and 280 °C, respectively. The temperature of injection port was maintained at 280 °C. All the setting was followed by Van et al. (Van et al., 2024).

In the initial analysis phase of analysis, a smart metabolite database file from Shimadzu Corporation (Tokyo, Japan) was employed to calculate retention times using 100 $\mu\text{g/mL}$ of n-alkanes. After calibration, the generated data file was integrated with the sample scanning method file from the smart metabolite database to create a customized scanning protocol for sample analysis. The original method targeted 467 specific compounds. Subsequent steps involved

generating acquisition and batch files to measure the samples, which included both blank and silkworm samples. Blank vials were analyzed first to verify no contamination, followed by sample measurements, with each sample tested in triplicate. Relative proportions of the tentatively identified compounds were determined by the height ratio of ribitol, (the internal standard) to the detected components. Silkworm powders were prepared by grinding samples with a grinder for further analysis.

For sample preparation, fresh silkworms and dried silkworms were grinded by hand grinder prior to scanning. Then, 10 mg of grinded silkworms were initially diluted with 10 μ L of ribitol solution (1 mg/mL). Then, 1 mL of Millipore water and a methanol-chloroform mixture (1:2.5:1 *V/V/V*) were added. Samples were ground using Micro Smash MS-100 R grinder (Tomy Seiko, Japan) at 4600 rpm for 5 min, then centrifugation by Kubota 5922 (Yama P Co. Ltd, Japan) at 15000 rpm for 3 min at 4 °C. Subsequently, 900 μ L of the sample and 400 μ L of Millipore water were added, stirred, and centrifuged again. Next, the supernatant (200 μ L) was extracted, heated, and stirred to evaporate methanol using SPD1030A-115 equipment (Thermo Fisher Scientific, USA), and a speed vacuum concentrator. In derivatization step, methoxyamine-HCl (100 μ L) in pyridine (20 mg/mL) was incorporated into the vacuumed samples. The mixture was heated, and shaken at 30 °C and 1200 rpm for 90 min. Afterward, MSTFA solution (50 μ L) was introduced, and the mixture was further shaken at 37 °C and 1200 rpm for 30 min. After a final centrifugation at 20 °C and 15000 rpm for 3 min, 100 μ L of the solution was transferred to a glass vial with a lid (Shimadzu Corp., Japan), and the vials were arranged in sequence on the tray holder. The profiles of the compounds present in the silkworms were instantly analyzed using the smart metabolite database. Each sample was processed in triplicate.

4.2.4.3.4. Analyzing X-ray CT images by using instance segmentation and image processing

X-ray CT imaging plays a crucial role in non-destructive, non-invasive 3D imaging which allows researchers to visualize and quantify internal modifications in samples, such as changes in tissue density, cell wall integrity, and porosity, without damaging the samples. With this purpose, X-ray CT was invaluable function for studying the microstructural changes of biomaterials over time ⁵⁴, (Schoeman et al., 2016). In this study, the drying process had directly affected internal structure of silkworm which was caused by the water loss and microstructure change such as porosity rate. Specifically, the porosity indicates the void space or the pore space volume as a

fraction of the total sample volume (Mendoza et al., 2007). Moreover, the change of internal structure was represented by ratio of porosity inner samples before and after being dried. After taking the X-ray CT images, images were analyzed by using AI and image processing which was confirmed by high accuracy and faster than manual. The workflow was shown on Fig. S2.

Image collection

The purpose of this method is to determine the differences between the white and black pixel ratio in the silkworm samples, which represented the solid and empty parts in cell tissue structure after the drying process. The process of X-ray CT imaging of fresh and dried silkworms was performed using a CosmoScan FX scanner (Rigaku Corporation, Tokyo, Japan). The operational parameters were configured with a voltage of 90 kV, a current of 88 μ A, a 0.06-mm Cu filter, a 0.5-mm Al filter, and a field of view (FOV) of 72 mm for standard CT scanning. The samples were positioned in holding tubes, and the scanning duration was set at 2 min with a pixel size of 144 μ m. A total of 2500 images for all samples with resolutions of 256 x 256 were collected and analyzed. There were five samples including control, CCG0, CCG1, CCG2 and CCG3. These images were taken automatically using X-ray CT system.

Image annotation

In this study, there were two objectives which were determined including the holder and the sample. After annotation (a total of 557 images), the images were augmented and splitting in the dataset. To facilitate model training and validation, images were divided randomly into training and validation with 486 images for training and 71 images for validation, respectively.

Model training

In this study, the YOLOv8s model was selected for training AI model. The model was trained via transfer learning (Zhuang et al., 2021) by fine-tuning pre-trained weights learned on the COCO dataset (Lin et al., 2014). Firstly, the AI model was trained using the PyTorch 2.1.0 deep learning framework on an Ubuntu 20.04 operating system. The training process involved utilizing CUDA version 11.8 with the Intel(R) Core(TM) i7-11800H, 2.30GHz serving as the central processing unit (CPU), and the NVIDIA 3070 was handling the graphics processing unit (GPU) tasks for image training. In addition to the training process, the YOLOv8s model, with 11.2 million parameters, was configured with an initial learning rate of 0.01, whereas the momentum

and weight decay used were 0.937 and 0.0005 respectively. The training batch size was set to 8 images, and the total number of iterations was fixed at 150. These parameter settings were chosen to optimize the speed of the training process while minimizing the chances of overfitting the model to the training dataset.

Model evaluation: the calculation was performed by Eq. (5) (6) (7) (8) as following Sapkota et al. (Sapkota et al., 2024)

$$\text{Precision} = \frac{TP}{TP+FP} \quad (5)$$

$$\text{Recall} = \frac{TP}{TP+FN} \quad (6)$$

$$\text{IoU} = \frac{\text{Area Overlap}}{\text{Area Union}} = \frac{TP}{FP+TP+FN} \quad (7)$$

$$\text{mAP} = \left(\frac{1}{K}\right) \sum_{i=0}^k (AP)n \quad (8)$$

In the calculation, TP , FP , and FN deputize true positive, false positive, and false negative object instances respectively. In addition, k deputizes the total number of objects while $(AP)n$ represents the average precision calculation for n objects with AP refers to the area of the precision-recall curve for a given object.

Deployment/ testing

After the training model, the resulting model was deployed on the remaining images. The use of image processing, then, was applied to calculate the porosity and solid area on images after segmented images. The porosity area on silkworm samples were calculated following Eq. (9):

$$Po = \frac{\text{The porosity area inside segmented samples}}{\text{Total area segmented samples}} \times 100 \quad (9)$$

Where Po is ratio of the porosity area of segmented images.

4.2.5. Statistical analysis

R software (version 4.2.2) was used to analyze the results. Treatment effects were assessed through analysis of variance (ANOVA), and significant differences were determined using Tukey's test ($P < 0.05$). Metabolic profile was analyzed using Metaboanalyst software 5.0.

4.4. Results

4.4.1. Coating and film characteristics

4.4.1.1. Physical characteristics

pH parameters (Table 4.1) range from 9.02 to 9.30, the solutions have similar pH, in which CCG1 solution has the highest pH of 9.30. The pH value of the solution depends on the components present such as CMC, CNF, N-CH and GPO, where the pH value of GPO is 3.43. The pH value was confirmed that it affects to mechanical characteristics, color, antibacterial, and antioxidant of edible films (Mir et al., 2023).

One of the important parameters that affects the film thickness forms on a food product, is the viscosity of coating solution. In this study, the coating solution viscosity ranges from 141.77 to 167.90 mPas (Table 4.1). The lowest viscosity value was 141.77 observed for coating solutions including CMC/CNF/N-CH, while viscosity increased for coating solutions with GPO added. Our pervious researches also represented the same the results (Van, Thi, et al., 2023) (Wardak et al., 2024). Previous studies explained that the increase in viscosity upon the addition of essential oils to edible coating solution might be due to molecular interactions between essential oils and polymer matrix. Because essential oils in general or GPO are complex mixtures of hydrophobic compounds such as terpenes, phenols, and alcohols that can interact with the polymer matrix of the edible coating such as polysaccharides, proteins, or lipids. These interactions can lead to the formation of hydrogen bonds, or hydrophobic interactions, which increase the overall resistance to flow, thereby increasing viscosity (Sánchez-González et al., 2009). Other reasons might be that essential oils have hydrophobic nature, and their incorporation into aqueous-based edible coatings can cause phase separation or aggregation. The hydrophobic interactions between oil molecules and the polymer chains can create a more rigid network, further increasing viscosity (Atarés & Chiralt, 2016). In addition, the addition of essential oils can restrict the movement of polymer chains in the coating solution, leading to increased viscosity (Perdones et al., 2012).

The average film thickness (Table 4.1) was 0.050–0.064 mm in this study. The incorporation of different materials which added to films might affect to the thickness of the films (Van, Wardana, et al., 2023). The current results showed that the highest thickness value was found in films CCG3 which was made of CMC/CNF/N-CH and the highest concentrations of GPO, while the lowest value was found in the film with the lowest concentration of GPO ($P > 0.05$).

The nature of GPO is colorless, and it has a light smell of pomelo peel. After being analyzed, the results revealed that there were no significant differences among the color of films (Table 4.1). This might be the results of the film-based materials and GPO. Some studies demonstrated that edible coatings changed the color of the stored objects such as mandarin extract coatings affected the color of stored strawberry (Van, Thi, et al., 2023), pectin-based coatings affected tomato color during low temperature storage (Pholsin et al., 2024).

Table 4.1. Coating and film characteristics

	CCG0	CCG1	CCG2	CCG3
pH	9.02 ^b ±0.04	9.30 ^a ±0.02	9.26 ^a ±0.01	9.24 ^a ±0.02
Viscosity	141.77 ^b ±5.76	160.77 ^a ±2.00	161.53 ^a ±0.24	167.90 ^a ±2.63
TSS	1.67 ^a ±0.05	1.60 ^b ±0.00	1.60 ^b ±0.00	1.60 ^b ±0.00
Film thickness (mm)	0.086 ^a ±0.008	0.084 ^a ±0.003	0.088 ^a ±0.006	0.090 ^a ±0.004
Ra	8.501 ^a ±0.772	5.859 ^b ±0.707	3.725 ^{bc} ±0.996	3.212 ^c ±0.253
<i>L</i> *	98.12 ^a ±0.10	98.28 ^a ±0.10	98.14 ^a ±0.17	98.00 ^a ±0.40
<i>a</i> *	0.32 ^a ±0.10	0.34 ^a ±0.05	0.40 ^a ±0.06	0.40 ^a ±0.17
<i>b</i> *	6.96 ^a ±0.15	7.18 ^a ±0.12	7.20 ^a ±0.13	7.38 ^a ±0.41
ΔE	3.02 ^a ±0.16	3.20 ^a ±0.10	3.26 ^a ±0.17	3.49 ^a ±0.46

4.4.1.2. SEM

The cross-section and surface structure of the films were illustrated in Fig.4.1. According to Ghosh and Katiyar. (Ghosh & Katiyar, 2022), the film's properties are directly influenced by the interactions among the components involved in film formation. The surface features of films based on CMC/CNF exhibited irregularities and varied textures due to gelatinization. Films containing GPO (CCG1, CCG2, and CCG3) displayed porous structures with numerous voids distributed throughout. Furthermore, an increase in GPO concentration resulted in greater heterogeneity and increased porosity in the film, leading to noticeable alterations in shape and droplet size within the film. Incorporations of GPO into the film composite replaced robust interactions among polymer networks with lesser interactions between the polymer composite and GPO. This substitution

ultimately led to a film with reduced integrity, as evidenced by the presented images. This situation might due to the distribution between hydrophilic and hydrophobic layer in the film matrix (W. J. Shi et al., 2016). Similar outcomes in films produced were observed from CMC and Ajowan essential oil, noting that an elevated concentration of Ajowan essential oil resulted in a heterogeneous and less cohesive film (Azarifar et al., 2019). Furthermore, Zhang et al. (Y. Zhang et al., 2024) confirmed the effect of essential oils on polysaccharide base film of sodium alginate matrix with holes were detected on surface and ellipse microspores on cross-section of SEM. The author argued that lipid aggregation might lead to this phenomenon.

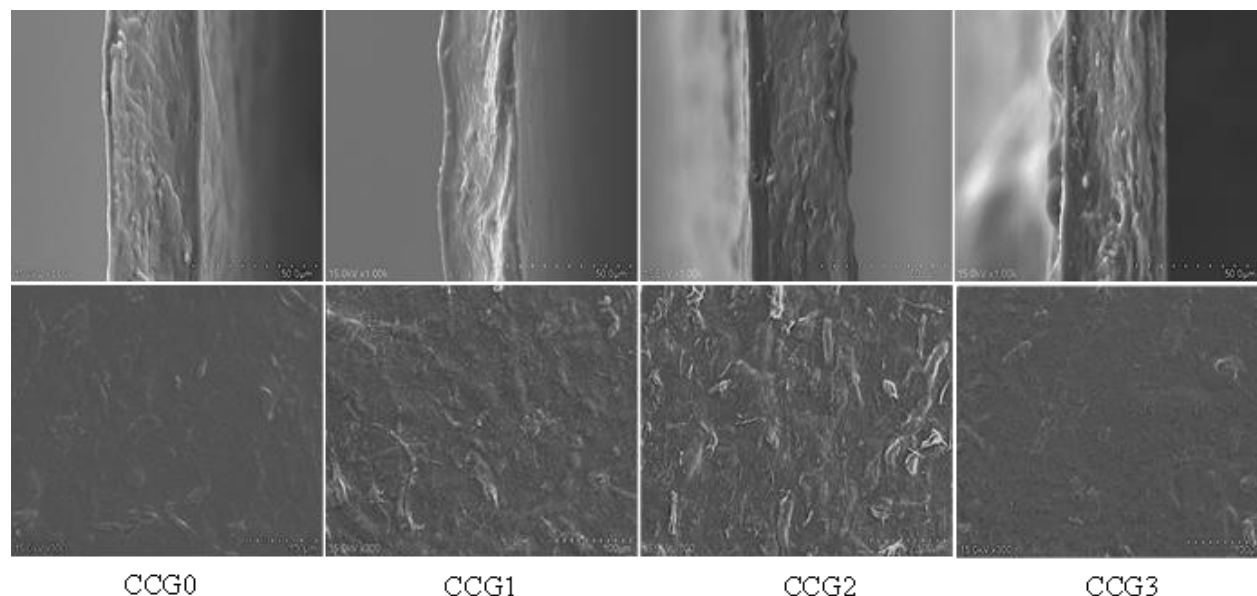


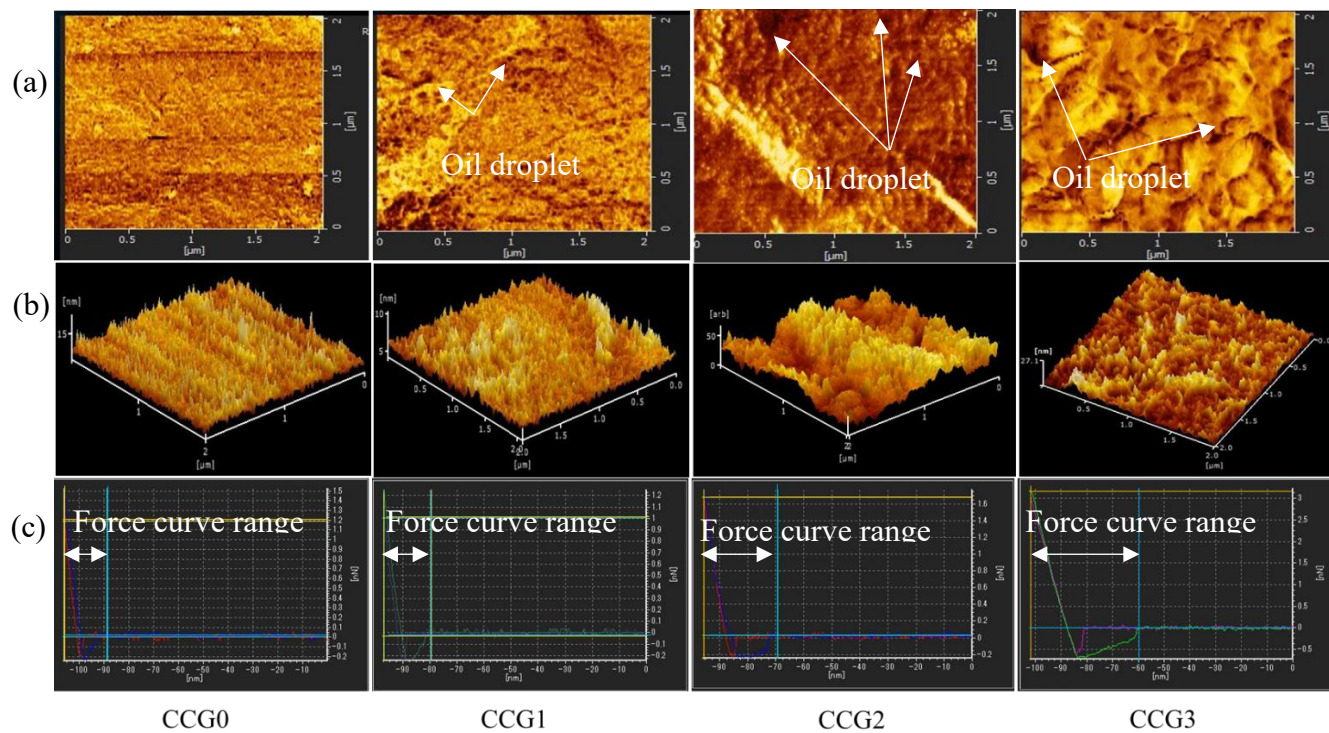
Fig. 4.1. Cross-section (a) and surface (b) SEM images of the films with and without GPO

4.4.1.3. Atomic force microscopy

The characteristics of the film layers were elucidated through AFM imaging, depicted in Figs. 4. 2a, 2b, with corresponding results of force curve and elastic modulus indices presented in Figs. 4. 2c, 2d. From the images, the CCG0 films showed uniform morphology. Conversely, an increase in GPO concentration, particularly in the CCG2 and CCG3 films, led to a substantial increase in inhomogeneous film surface. The film structure was affected by the adding of the oils in the matrix which was confirmed by a lots of studies (Van, Thi, et al., 2023) (Van et al., 2024) (Y. Zhang et al., 2024) (Yin et al., 2021) which explained that the ratio of saturated fatty acid and unsaturated fatty acid in edible oil also affected to the film surface characteristics. Wongphan et al. (Wongphan et al., 2023) explained that the size and numbers of the particles reduced when adding

essential oil in the film matrix, leading to the change in film surface properties. The elevated roughness values might indicate the impact of oil concentrations in the coating film and the dispersion of coating components during homogenization, casting, and drying processes.

The AFM system captures surface images and evaluates nanoscale material properties, assessing film adhesion through force curves. Adhesion force is measured during probe retraction by the maximum attractive force separating the probe from the film surface. The difference among force curves depend on the material properties and film surface characteristics, which represent by the distance between the surface of samples and cantilever probe, it also reflects how harness the film was (E. Sun et al., 2024). Additionally, the elastic modulus reflects a material's resistance to elastic deformation, adhesion signifies the pull-off force necessary for the cantilever to overcome adhesive interactions between the tip and the sample, dissipation measures the energy dissipated during tip-surface interaction, and deformation encompasses both elastic and plastic deformation (K. Wang et al., 2021). In this study, results indicated that the force curve and elastic modulus increase significantly ($P < 0.05$) with films contain GPO such as CCG1, CCG2 and CCG3 compared to CCG0 (without GPO). The force curve and elastic modulus values of CCG3 were highest compared to other films ($P < 0.05$). A study on the role of sunflower oil in hydroxypropyl methylcellulose based oleogels in chocolate indicated that the harder texture need the greater force to penetrate on it (Espert et al., 2021). The increase in force curve values indicated the changes in the molecular structure among film samples (E. Sun et al., 2024), where the concentration of lighter molecules such as aroma or oil components increased. In addition, Yin et al. (Yin et al., 2021) explained that the oil with high saturated fatty acid content led to reduce the cutting force. In this study, the force curve of films increases along with the rise of oil concentration, this might suggest that GPO contains more higher unsaturated fatty acids than saturated fatty acids. The increasing of elastic modulus values might indicate that the films without GPO were stiffer than the films contained GPO. These results were in accordance with the appearances of 2D and 3D AFM images which represented the effects of GPO on surface and cross-section of the films.



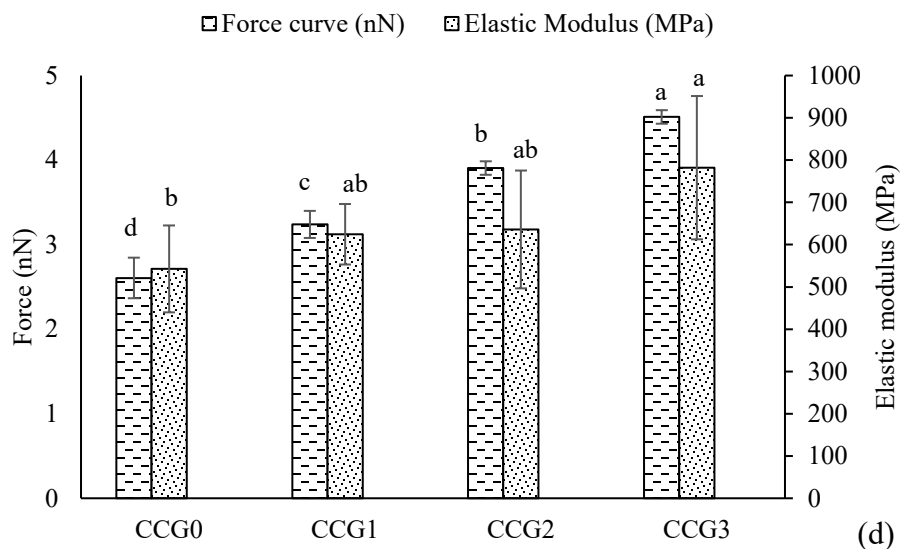


Fig. 4.2. AFM visual images (a) – (2D), (b) – (3D), (c) – force curve visual, and (d) - force curve analysis

4.4.1.4. Thermal analysis

The thermal properties of films were shown in Fig. 4.3 and Table 4.2 which indicated the weight loss of all films dramatically increases following by the temperature rise. The results indicated that the weight loss process was divided into three steps following by a light difference in thermal temperature of each film. Firstly, the temperature rose from 45 °C to 246 °C, which was dominantly due to the loss of free and bound water as well as the loss of their molecular weights through evaporation, or volatile components in materials (Y. Zhang et al., 2024). Secondly, the range varied at 246 °C - 328 °C. In this step, the weight loss significantly increased, this might cause from the thermal activity associated with natural CMC/CNF/N-CH base, which were main content of the film matrix. At this point, the hydrogen bond chain is broken with the evaporation of glycerol and polyoxyethylene (20) sorbitan, and the sugar in the polysaccharide is broken down. Finally, the temperature was observed from 328 °C to 486 °C, which related to the monosaccharides such as glucose, mannose with decomposition of the carbon residue produced in the second step. After finishing the heating process, the weight loss values of CCG0, CCG1, CCG2, and CCG3 were 74.6%, 70.88%, 70.35%, and 61.93%, respectively, which represented that the value of CCG0 was highest while CCG3 was lowest. The causes might be the cross-linking reaction among materials in the film matrix (Wigati et al., 2023b). This result suggested that the CCG3 film might have the highest stability against the heating process.

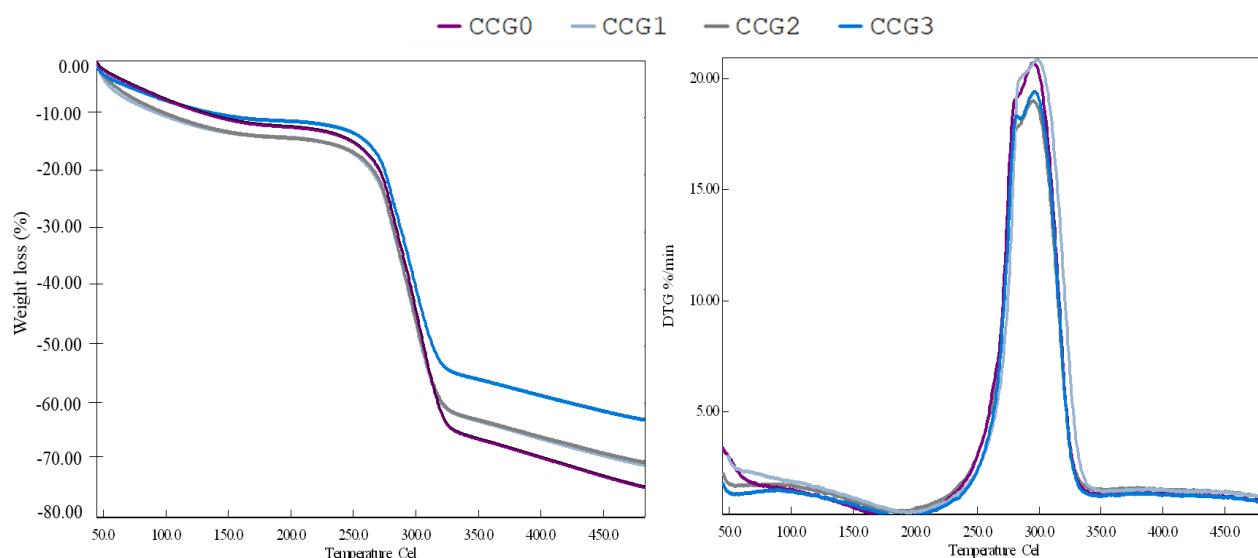


Fig. 4.3. Thermal analysis TG and DTG curves of cellulose edible films

Table 4.2. Temperature and weight loss related to stages of TG curve on cellulose edible films.

Films	First stage		Second stage		End	
	Temperature (°C)	Weight loss (%)	Temperature (°C)	Weight loss (%)	Temperature (°C)	Weight loss (%)
CCG0	45–246.4	13.65	246.4 - 327.5	64.01	327.5 - 486.0	74.6
CCG1	45–245.3	15.68	245.3 - 323.0	60.67	323.0 - 484.3	70.88
CCG2	45–242.3	15.26	242.3-328.3	61.34	328.3-484.4	70.35
CCG3	45–249.0	11.58	249.0-325.6	53.05	325.6-484.0	61.93

4.4.2. Antimicrobial activity

The antibacterial efficacy of edible coatings derived from CMC/CNF/GPO after 24 h of cultivation is illustrated in Figs. 4. 4a and 4b. After one day of cultivation at 37 °C, the inhibition effect of Control and CCG2 samples was higher than the CCG0 and CCG1 ($P < 0.05$) while CCG3 exhibited the highest inhibitory effect on *L. aureus* compared to other groups ($P < 0.05$). The reason why the inhibition effect on *L. aureus* of CCG0 and CCG1 was lower than the other group

might be from the concentration of GPO in CCG0 and CCG1, which was negative or not enough to inhibit the growth of bacteria in their optimism environment. Similar observations have been reported from incorporating cinnamon essential oil and CMC (Fattahi et al., 2020). These findings align with previous studies, mandarin EO against *E. coli* ATCC 25922 (Raspo et al., 2020) and *P. digitatum* and *P. italicum* (Jhalegar et al., 2015).

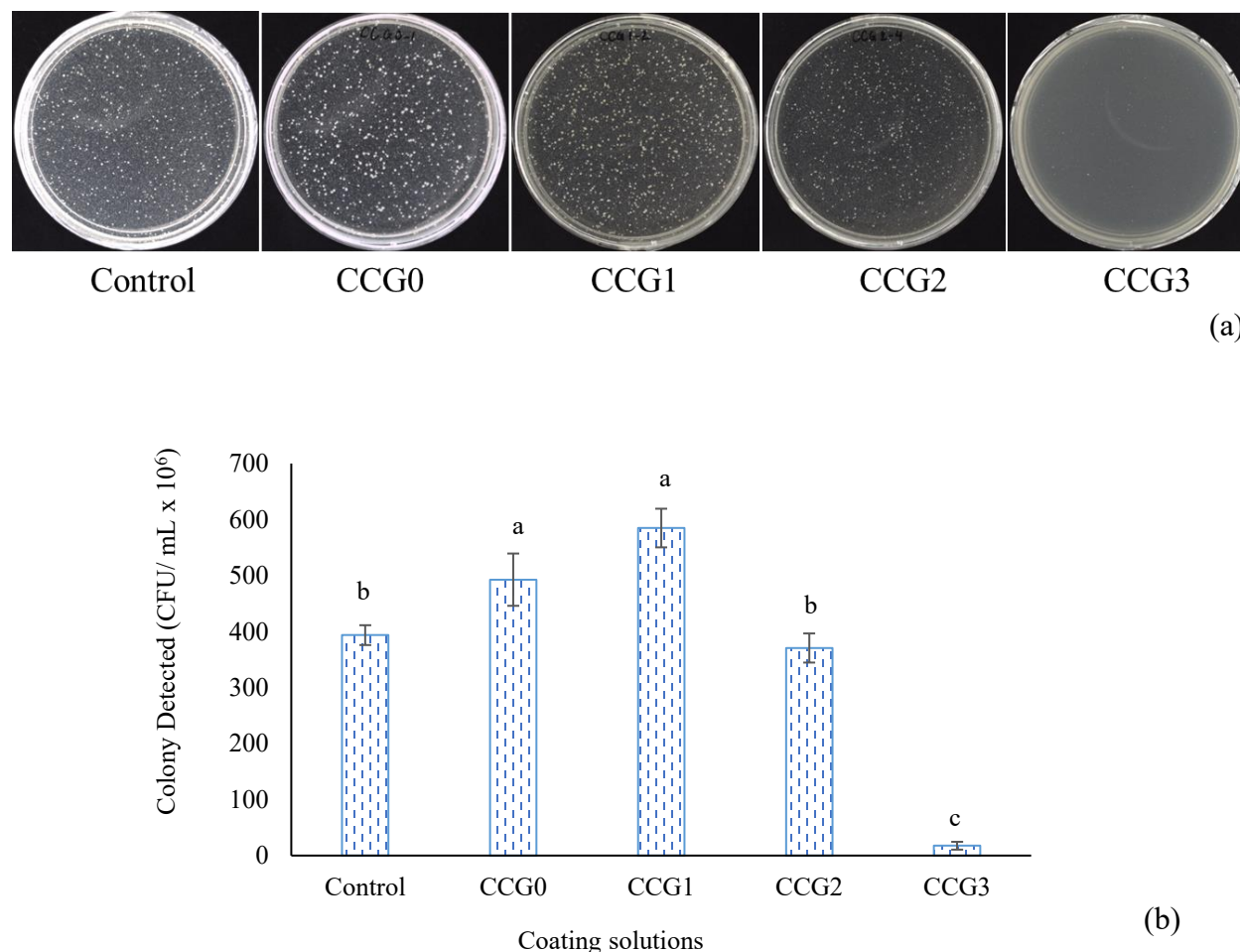


Fig. 4.4. Antibacterial of edible coatings (a) – visual recognize, (b) – colony detected.

4.4.3. Silkworm pupae application

4.4.3.1. Moisture content of silkworms

The result of moisture content of silkworm was shown on Fig. 4. 5. As a result of the mass loss percentage increased throughout the drying process, the moisture value of the fresh sample was highest (71.5%) compared to dried samples. The moisture of dried samples gradually decreased from the control to CCG3. Specifically, the moisture of dried silkworms fluctuated from

19.28% in CCG1 (the highest moisture content of dried-samples) and 15.58% in CCG3 (the lowest moisture content among dried-samples) ($P < 0.05$). This result indicated the effect of coatings on silkworm during drying process, specifically, all samples were treated with the same drying condition such as temperature, time, and airflow forced. CCG3 indicated the lowest moisture content compared to the other coatings. This result might reveal that the coating formular showed good characteristics to prevent shrinkage and retain the original cell structure during moisture loss in drying process. This result also suggests that the coating could save more energy and time to reach optimized moisture in the drying process. Ando et al. (Y. Ando, T. Watanabe, T. Orikasa, 2024) compared two different drying methods of freeze-drying and hot air-drying on Silkworm Larvae (*Bombyx mori*). The author reported that freeze-drying can keep the original silkworm appearance while significant shrinkage structure occurred in hot air-drying. Therefore, the application of coating dried products opens a new direction for agriculture sustainability.

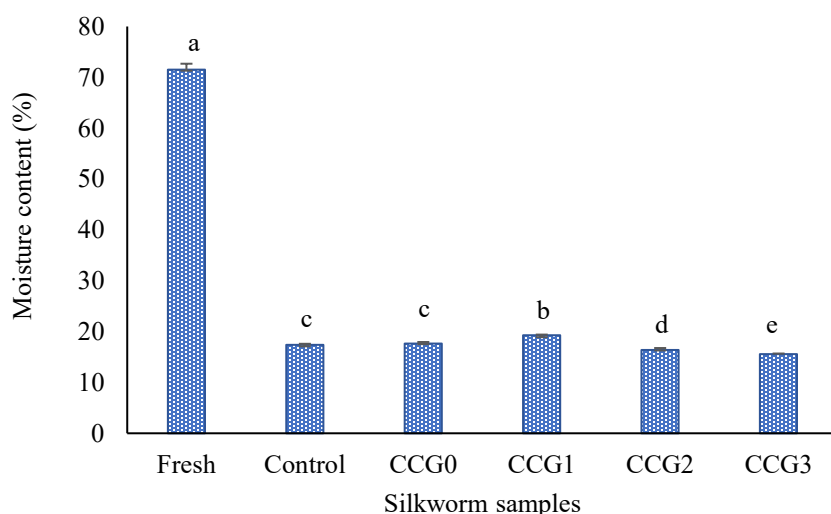


Fig. 4. 5. Moisture content of silkworm samples

4.4.3.2. Metabolism profile of dried silkworm

In this study, the primary metabolic process of silkworms was identified according to the metabolomic database profiles. 109 ingredients were detected (Table 4.3), mainly organic acids, fatty acids, esters, sugars, nitrogenous compounds, and other important metabolites. Silkworms were coated with different solutions, resulting in differing relative percentages of each component. The sugar and sugar alcohol group included 34 components, with monosaccharides being the most prevalent type of main metabolite. Among these components, trehalose-8TMS, maltose-meto-

8TMS, arabitol-5TMS, xylitol-5TMS, glucose-meto-5TMS, and sorbitol-6TMS occupied higher relative percentages in all silkworm samples. Besides, a total of 18 organic acids, including phosphoric acid-3TMS, fumaric acid-2TMS, malic acid-3TMS, 2-aminopimelic acid-3TMS, oxalic acid-2TMS, and citric acid-4TMS, were detected in all samples at high relative concentrations. In general, fresh silkworms had a lower relative concentration of organic acids compared to dried silkworms ($P < 0.05$). Key primary metabolites identified included fatty acids/esters like stearic acid-TMS, kynurenic acid-TMS, and palmitic acid-TMS. Silkworms were characterized by high sources of protein, fat, minerals and vitamins with 21.5 % of protein sources, 13.0 % of fat, and 6.70 % of carbohydrate per 100 % edible part (Wu et al., 2021). The released results revealed that there were significant differences between two groups of CCG2, CCG3 and the others. Specifically, CCG2 and CCG3 represented higher relative concentrations ($P < 0.05$) during metabolite process in comparison to other groups with components such as Phosphoric acid-3TMS, Malic acid-3TMS, 2-Hydroxyglutaric acid-3TMS, Glucuronic acid-meto-5TMS, Xylitol-5TMS, Allose-meto-5TMS, Galactose-meto-5TMS, Glycerol-3TMS, Glutamine-3TMS, Lysine-4TMS, Uracil-2TMS, Serine-3TMS, Leucine-2TMS, and Tryptophan-3TMS. In detail, CCG2 was more dominant in amino groups (Leucine-2TMS, Tryptophan-3TMS) while CCG3 was more dominant in carbohydrates and nitrogenous (Malic acid-3TMS, Galactose-meto-5TMS, 2-Hydroxyglutaric acid-3TMS). Additionally, the analysis indicated that both the quantity and relative percentage of substances, as well as the qualitative, were lower in fresh samples compared to dried samples ($P < 0.05$). Some components, such as succinic acid-2TMS and glutamine-3TMS, showed higher concentrations in coated silkworms than in uncoated ones ($P < 0.05$). These differences showed the influences of thin film layer on the surface of silkworm during drying process, which were shown by increasing the relative concentration of main components. The drying process was confirmed by the major change in sensory properties (Bonazzi & Dumoulin, 2011) or texture, volatiles, and appearance of dried products (Nöfer et al., 2018). The most significant factor leads to these changes was from Maillard response. This reaction also resulted in metabolism changes which was confirmed by the relative percentage alternatives of groups such as fresh and dried silkworms (CCG0, CCG1, CCG2, CCG3) in this study. The result suggests the application of coating for drying process had a significant effect to prevent the nutrient degradation of dried products.

The metabolic profile heatmap (Fig. 4. 6a) shows that the silkworm samples were divided into six groups: group A (fresh), group B (CCG3), group C (CCG2), group D (Control), group E (CCG0), and group F (CCG1). The heatmap colors represent the normalized peak intensities of the spectrum, where dark red signifies high intensity, and dark blue represents low intensity. It showed that the peak intensities for most metabolites were greater in the CCG2 and CCG3 samples compared to the other samples.

PCA, an unsupervised statistical method for dimensionality reduction, was performed to identify and thoroughly assess the distinct metabolites, highlighting the overall trends in metabolite changes between uncoated and coated dried silkworms. The PCA score plot (Fig. 4. 6b) illustrated how different treatments affected the metabolic components of silkworms. Notably, the CCG1, CCG2, and CCG3 samples showed a more significant impact compared to the other treatments. The CCG1 sample was mainly aligned with PC2 (8.3%), while the fresh sample predominantly aligned with PC1 (63.1%), clearly distinguishing silkworms before and after being dried. The PCA biplot (Fig. 4. 6c) offered further insight into the metabolic profiles of silkworms. Specifically, the metabolic profile of the CCG2 group was characterized by the presence of cysteine-3TMS, while the control group was linked to galactiol-4TMS. In contrast, the CCG1 group featured unique metabolites, including putrescine-4TMS and glycerol-3TMS. These results indicated the significant effect of coatings on the drying process due to their ability to cover the surface of silkworms, which was confirmed to be influenced by direct exposure with heat or airflow in the dryer (Y. Ando, T. Watanabe, T. Orikasa, 2024). The reasons for the significant distinguishing might be from the Maillard reaction leading to the chemical component changes such as oxidation, degradation, or water loss (Mishyna et al., 2020). This metabolism modified the ratio composition on dried products, that were affected by different coatings. These results were confirmed by the change in the moisture content, porosity ratio in this study.

Partial least squares-discriminant analysis (PLS-DA) was employed to assess the metabolic distinctions among silkworms which were shown in Fig. 6d and e. The PLS-DA model showed clear data segregation and robust predictive capability of fresh and dried samples. Fig. 4. 6d shows a 2D score plot, revealing distinct data separation and strong predictive performance between fresh and dried samples. Specifically, fresh silkworm was detected along component 1 at 62.5% while dried samples of control and CCG3 were illustrated along component 2 at 7%. The data presented in Fig. 4. 6e identified fifteen key metabolite components that played a significant role in this

separation such as glutamine-3TMS, glutamine-3TMS, aspartic acid, glyceraldehyde acid, guanine-3TMS, ornithine-3TMS, glycine-3TMS, tryptophan-3TMS, aconitic acid, and succinic acid. Among samples, CCG2 showed the highest intensity while the fresh show the lowest metabolic profile compared to others. These results are consistent with PCA conclusion.

Table 4.3. Metabolomic profile on GC-MS/MS of coated and uncoated silkworm

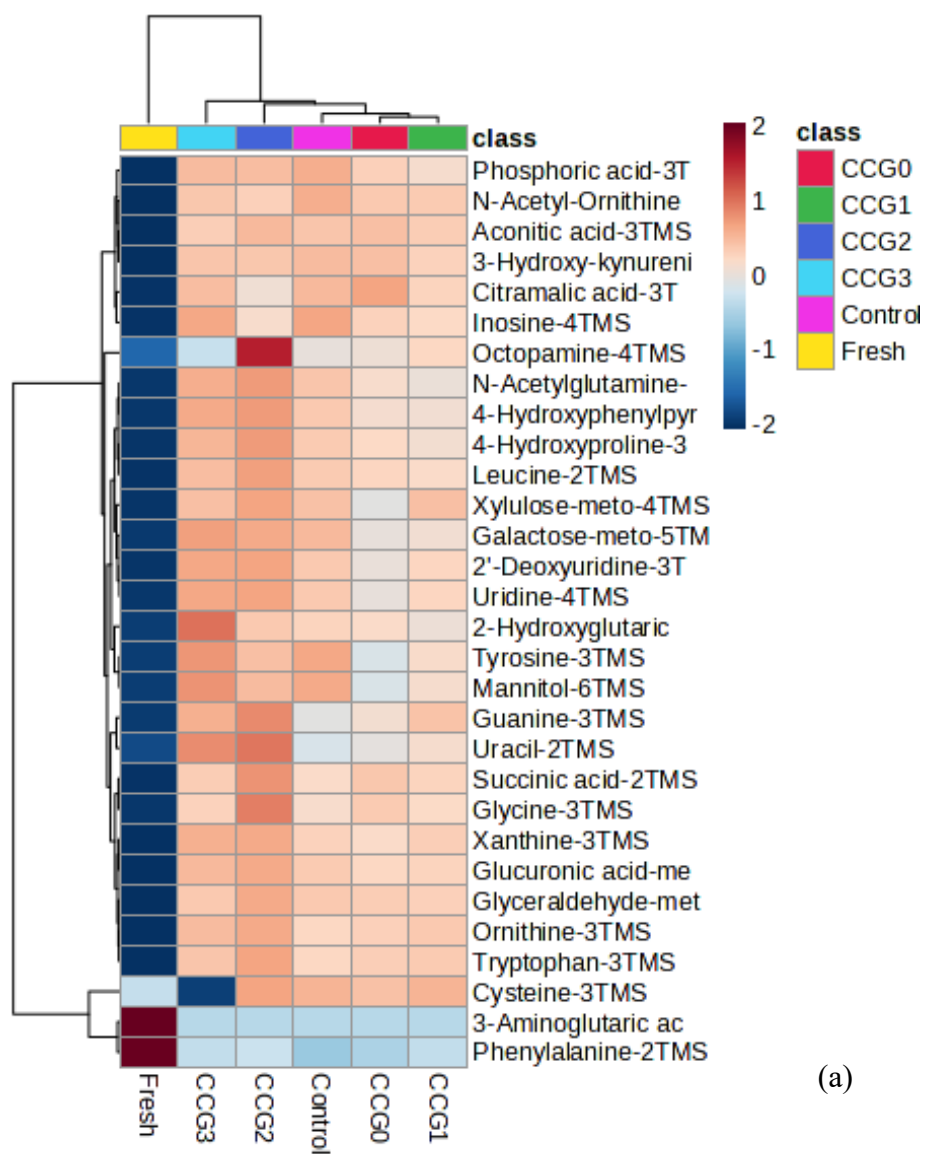
	Fresh	Control	CCG0	CCG1	CCG2	CCG3
ORGANIC ACIDS						
Oxalic acid-2TMS	0.047±0.006	0.051±0.010	0.047±0.007	0.045±0.006	0.048±0.009	0.053±0.009
Phosphoric acid-3TMS	3.515 ^c ±0.364	8.838 ^a ±0.565	7.950 ^a ±0.138	7.540 ^b ±0.585	8.425 ^a ±0.210	8.466 ^a ±0.489
Succinic acid-2TMS		0.025 ^c ±0.001	0.029 ^b ±0.001	0.026 ^b ±0.001	0.038 ^a ±0.001	0.028 ^b ±0.002
Glyceric acid-3TMS	0.012±0.000	0.043±0.002	0.040±0.000	0.039±0.002	0.054±0.002	0.045±0.004
Fumaric acid-2TMS	0.027 ^c ±0.004	0.188 ^a ±0.032	0.195 ^a ±0.019	0.128 ^b ±0.038	0.137 ^b ±0.025	0.185 ^a ±0.023
2-Aminopimelic acid-3TMS	0.564±0.254	1.429±0.089	1.361±0.071	1.246±0.028	1.460±0.005	1.311±0.042
Citramalic acid-3TMS		0.010 ^b ±0.001	0.012 ^a ±0.001	0.009 ^c ±0.001	0.007 ^c ±0.000	0.010 ^b ±0.000
Malic acid-3TMS	0.027 ^c ±0.006	0.078 ^a ±0.005	0.091 ^a ±0.006	0.067 ^b ±0.003	0.059 ^b ±0.002	0.077 ^a ±0.003
Aspartic acid-3TMS	0.007±0.000	0.017±0.001	0.020±0.001	0.019±0.001	0.023±0.001	0.019±0.001
Threonic acid-4TMS	0.015±0.004	0.041±0.002	0.034±0.001	0.034±0.003	0.036±0.001	0.033±0.002
Ureidopropionic acid-3TMS	0.011 ^b ±0.000	0.030 ^a ±0.002	0.031 ^a ±0.002	0.021 ^a ±0.000	0.016 ^b ±0.000	
Aconitic acid-3TMS		0.012 ^b ±0.001	0.012 ^b ±0.000	0.012 ^b ±0.000	0.013 ^a ±0.001	0.011 ^b ±0.001
Isocitric acid-4TMS	0.013 ^b ±0.000	0.020 ^a ±0.001	0.018 ^a ±0.001	0.016 ^a ±0.001	0.018 ^a ±0.001	0.017 ^a ±0.001
Citric acid-4TMS	0.773 ^b ±0.345	1.923 ^a ±0.119	1.825 ^a ±0.103	1.660 ^a ±0.041	1.926 ^a ±0.008	1.717 ^a ±0.051
Citric acid-d4-4TMS	0.013 ^b ±0.006	0.032 ^a ±0.002	0.030 ^a ±0.002	0.028 ^a ±0.000	0.033 ^a ±0.000	0.028 ^a ±0.001
Myristic acid-TMS	0.019±0.001	0.024±0.004	0.016±0.001	0.015±0.004	0.016±0.002	0.021±0.004
Pantothenic acid-3TMS	0.034±0.008	0.085±0.005	0.075±0.003	0.075±0.004	0.078±0.003	0.074±0.006
FATTY ACIDS AND ESTERS						

Stearic acid-TMS	0.644±0.099	0.699±0.129	0.554±0.008	0.550±0.096	0.559±0.029	0.664±0.119
Kynurenic acid-2TMS	0.066±0.012	0.116±0.010	0.100±0.001	0.090±0.010	0.100±0.002	0.102±0.008
Palmitic acid-TMS	1.322±0.199	1.476±0.296	1.131±0.022	1.141±0.193	1.232±0.077	1.447±0.292
NITROGENOUS						
2-Hydroxyglutaric acid-3TMS	0.010 ^e ±0.000	0.384 ^c ±0.014	0.338 ^d ±0.011	0.266 ^d ±0.029	0.444 ^b ±0.011	1.297 ^a ±0.053
2-Hydroxybutyric acid-2TMS	0.037±0.005	0.041±0.008	0.037±0.005	0.036±0.005	0.037±0.007	0.041±0.007
Dihydroorotic acid-3TMS	0.187 ^b ±0.075	0.460 ^a ±0.024	0.424 ^a ±0.022	0.371 ^a ±0.010	0.431 ^a ±0.001	0.391 ^a ±0.015
4-Hydroxyphenylpyruvic acid-meto-2TMS		0.022 ^a ±0.001	0.017 ^b ±0.001	0.018 ^b ±0.005	0.031 ^a ±0.004	0.028 ^a ±0.003
SUGAR ACID						
Gluconic acid-6TMS	0.009±0.000	0.010±0.001	0.008±0.001	0.008±0.001	0.014±0.001	0.011±0.003
Glucuronic acid-meto-5TMS		0.032 ^b ±0.003	0.029 ^c ±0.003	0.031 ^b ±0.005	0.040 ^a ±0.006	0.036 ^a ±0.004
SUGARS AND SUGAR ALCOHOLS						
Erythrulose-meto-3TMS	0.051±0.009	0.034±0.019	0.007±0.001	0.041±0.005	0.043±0.000	0.026±0.020
Lyxose-meto-4TMS	0.007 ^b ±0.006	0.034 ^a ±0.002	0.035 ^a ±0.002	0.038 ^a ±0.004	0.044 ^a ±0.003	0.033 ^a ±0.003
Xylose-meto-4TMS	0.011 ^b ±0.003	0.022 ^a ±0.001	0.023 ^a ±0.002	0.025 ^a ±0.003	0.028 ^a ±0.002	0.021 ^a ±0.002
Ribulose-meto-4TMS	0 ^b	0.007 ^a ±0.001	0.007 ^b ±0.000	0.008 ^a ±0.001	0.010 ^a ±0.001	0.009 ^a ±0.000
Ribose-meto-4TMS	0.011 ^b ±0.005	0.022 ^a ±0.002	0.023 ^a ±0.001	0.025 ^a ±0.002	0.029 ^a ±0.002	0.023 ^a ±0.002
Xylulose-meto-4TMS	0 ^d	0.013 ^b ±0.001	0.009 ^c ±0.002	0.014 ^b ±0.002	0.017 ^a ±0.002	0.014 ^b ±0.002
Xylitol-5TMS	0.713 ^b ±0.006	0.718 ^b ±0.019	0.698 ^b ±0.008	0.711 ^a ±0.012	0.737 ^a ±0.010	0.742 ^a ±0.007
1,6-Anhydroglucose-3TMS	0.595 ^a ±0.003	0.584 ^a ±0.009	0.563 ^b ±0.005	0.571 ^b ±0.006	0.587 ^a ±0.004	0.589 ^a ±0.003
Glycerol 2-phosphate-4TMS		0.006 ^c ±0.000	0.008 ^b ±0.000	0.007 ^c ±0.000	0.009 ^b ±0.000	0.012 ^a ±0.001

Arabitol-5TMS	0.692±0.003	0.690±0.001	0.686±0.003	0.689±0.001	0.693±0.003	0.687±0.003
2-Deoxy-glucose-meto-4TMS	0.027 ^b ±0.01	0.061 ^a ±0.003	0.055 ^a ±0.003	0.048 ^a ±0.000	0.054 ^a ±0.001	0.048 ^a ±0.001
2-Deoxy-glucose-4TMS	0.018 ^b ±0.006	0.039 ^a ±0.002	0.036 ^a ±0.002	0.032 ^a ±0.000	0.039 ^a ±0.000	0.033 ^a ±0.001
Tagatose-meto-5TMS	0.031 ^b ±0.002	0.093 ^a ±0.005	0.080 ^a ±0.002	0.076 ^a ±0.021	0.082 ^a ±0.004	0.074 ^a ±0.002
Psicose-meto-5TMS	0.032 ^c ±0.001	0.094 ^a ±0.008	0.082 ^a ±0.002	0.076 ^b ±0.020	0.083 ^a ±0.003	0.075 ^b ±0.003
Fructose-meto-5TMS	0.015 ^d ±0.001	0.061 ^a ±0.003	0.052 ^b ±0.001	0.049 ^c ±0.012	0.061 ^a ±0.004	0.056 ^b ±0.003
Sorbose-meto-5TMS	0.015 ^b ±0.001	0.061 ^a ±0.002	0.053 ^a ±0.002	0.051 ^a ±0.013	0.063 ^a ±0.005	0.057 ^a ±0.004
Allose-meto-5TMS	0.291 ^c ±0.054	1.169 ^a ±0.452	0.797 ^b ±0.237	0.862 ^b ±0.162	1.498 ^a ±0.073	1.038 ^a ±0.354
Glucono-1,5-lactone-4TMS	0.153 ^d ±0.032	0.667 ^c ±0.027	0.530 ^c ±0.022	0.546 ^c ±0.147	0.973 ^a ±0.120	0.859 ^b ±0.100
Mannose-meto-5TMS	0.242 ^d ±0.083	1.208 ^b ±0.379	0.859 ^c ±0.198	0.908 ^c ±0.093	1.596 ^a ±0.104	1.157 ^b ±0.279
Galactose-meto-5TMS	0.273 ^e ±0.081	1.566 ^c ±0.158	1.089 ^d ±0.013	1.185 ^d ±0.171	1.725 ^b ±0.098	1.821 ^a ±0.083
Glucose-meto-5TMS	0.343 ^d ±0.066	1.484 ^b ±0.365	1.007 ^c ±0.211	1.052 ^c ±0.126	1.822 ^a ±0.107	1.432 ^b ±0.275
Mannitol-6TMS	0.182 ^d ±0.028	0.909 ^a ±0.137	0.552 ^c ±0.013	0.666 ^c ±0.039	0.814 ^b ±0.018	0.989 ^a ±0.041
Sorbitol-6TMS	0.145±0.099	0.008±0.000	0.205±0.279	0.006±0.000	0.007±0.001	0.357±0.493
Glycerol 3-phosphate-4TMS	0.027 ^b ±0.000	0.114 ^a ±0.006	0.138 ^a ±0.007	0.127 ^a ±0.011	0.143 ^a ±0.003	0.204 ^a ±0.017
Galactitol-6TMS	0.188±0.030	0.815±0.000	0.555±0.017	0.716±0.000	0.799±0.000	0.948±0.000
Glucose 6-phosphate-meto-6TMS	0.090 ^b ±0.065	0.382 ^a ±0.014	0.278 ^a ±0.032	0.359 ^a ±0.026	0.318 ^a ±0.006	0.392 ^a ±0.021
Sucrose-8TMS	0.035±0.012	0.085±0.057	0.072±0.045	0.046±0.021	0.023±0.012	0.017±0.001
Trehalose-8TMS	5.611 ^b ±2.259	10.881 ^a ±0.572	9.052 ^a ±0.606	7.965 ^a ±0.201	9.123 ^a ±0.262	9.740 ^a ±0.409
Maltose-meto-8TMS	1.567 ^b ±0.602	2.869 ^a ±0.150	2.405 ^a ±0.139	2.103 ^a ±0.069	2.401 ^a ±0.060	2.553 ^a ±0.096
Glycerol-3TMS	0.051 ^c ±0.011	0.085 ^b ±0.001	0.089 ^b ±0.009	0.097 ^b ±0.005	0.212 ^a ±0.004	0.209 ^a ±0.009
2-Aminoethanol-3TMS	0.019 ^d ±0.001	0.169 ^a ±0.011	0.136 ^c ±0.002	0.150 ^b ±0.000	0	0

Inositol-6TMS	0.196 ^d ±0.041	0.383 ^a ±0.036	0.325 ^b ±0.006	0.298 ^c ±0.033	0.333 ^b ±0.008	0.343 ^b ±0.025
AMINE GROUPS						
Hydroxylamine-3TMS	0.283±0.035	0.320±0.067	0.290±0.043	0.282±0.034	0.297±0.060	0.332±0.060
N-Acetylglutamine-3TMS	0	0.012 ^b ±0.001	0.010 ^b ±0.001	0.009 ^b ±0.002	0.016 ^a ±0.002	0.014 ^a ±0.001
Tryptamine-2TMS	0	0.008±0.001	0.007±0.000	0.007±0.000	0.007±0.000	0.007±0.001
Glutamine-3TMS	0.331 ^c ±0.140	0.700 ^b ±0.109	0.848 ^a ±0.061	0.875 ^a ±0.152	1.162 ^a ±0.176	0.952 ^a ±0.136
Glucosamine-5TMS	0.010±0.001	0.008±0.001	0.007±0.000	0.007±0.000		
Octopamine-4TMS		0.018 ^b ±0.001	0.020 ^b ±0.000	0.026 ^b ±0.005	0.189 ^a ±0.015	0.011 ^c ±0.003
Dopamine-4TMS	0.013 ^c ±0.000	0.130 ^a ±0.017	0.100 ^a ±0.005	0.052 ^b ±0.009	0.050 ^b ±0.002	0.068 ^b ±0.008
N-Acetylmannosamine-meto-4TMS	0.017±0.007	0.037±0.006	0.037±0.011	0.028±0.001	0.034±0.014	0.027±0.016
Cystamine-nTMS	0.018 ^b ±0.000	0.023 ^a ±0.002	0.021 ^a ±0.005	0.031 ^a ±0.007	0.019 ^a ±0.002	0.024 ^a ±0.004
O-Phosphoethanolamine-4TMS	0.033 ^b ±0.019	0.151 ^a ±0.013	0.138 ^a ±0.014	0.147 ^a ±0.003	0.151 ^a ±0.006	0.146 ^a ±0.008
Putrescine-4TMS	4.094±2.141	8.156±0.308	7.715±0.823	8.462±0.501	8.567±0.154	8.958±0.445
AMINO GROUPS AND OTHERS						
Lysine-4TMS	0.559 ^c ±0.099	1.282 ^a ±0.067	1.050 ^a ±0.036	1.013 ^b ±0.042	1.133 ^a ±0.074	1.110 ^a ±0.086
Histidine-3TMS	2.611 ^b ±1.149	9.379 ^a ±0.247	9.294 ^a ±0.649	9.361 ^a ±0.530	9.768 ^a ±0.607	9.995 ^a ±0.847
Tyrosine-3TMS	0.027 ^c ±0.003	0.130 ^a ±0.019	0.080 ^b ±0.002	0.097 ^b ±0.005	0.114 ^a ±0.002	0.140 ^a ±0.006
Proline-2TMS	1.996 ^b ±0.550	3.660 ^a ±0.130	3.466 ^a ±0.130	3.184 ^a ±0.134	3.357 ^a ±0.181	3.274 ^a ±0.225
Glycine-3TMS	1.868 ^c ±0.404	5.412 ^b ±0.223	5.970 ^b ±0.086	5.536 ^b ±0.221	7.962 ^a ±0.245	5.796 ^b ±0.392
Uracil-2TMS	0.014 ^c ±0.001	0.026 ^b ±0.003	0.027 ^b ±0.001	0.028 ^b ±0.001	0.038 ^a ±0.001	0.036 ^a ±0.001
Serine-3TMS	3.076 ^c ±0.764	3.630 ^b ±0.167	3.644 ^b ±0.076	3.351 ^b ±0.110	5.293 ^a ±0.082	4.181 ^a ±0.260
Acetylglycine-2TMS	0.017 ^b ±0.004	0.049 ^a ±0.003	0.044 ^a ±0.001	0.040 ^a ±0.003	0.050 ^a ±0.002	0.046 ^a ±0.004

Threonine-3TMS	0.347 ^b ±0.076	0.931 ^a ±0.057	0.834 ^a ±0.012	0.774 ^a ±0.047	0.956 ^a ±0.042	0.857 ^a ±0.064
Glyceraldehyde-meto-2TMS		0.018 ^b ±0.001	0.018 ^b ±0.001	0.017 ^b ±0.000	0.022 ^a ±0.001	0.018 ^b ±0.002
Leucine-2TMS	0.903 ^c ±0.114	2.655 ^b ±0.142	2.528 ^b ±0.099	2.465 ^b ±0.221	3.110 ^a ±0.133	2.788 ^b ±0.181
Methionine-2TMS	0.030 ^b ±0.003	0.068 ^a ±0.003	0.079 ^a ±0.003	0.075 ^a ±0.001	0	0
4-Hydroxyproline-3TMS	0.139 ^a ±0.016	0.385 ^b ±0.020	0.364 ^b ±0.012	0.348 ^b ±0.013	0.456 ^a ±0.010	0.417 ^a ±0.027
5-Oxoproline-2TMS	0.012±0.004	0.030±0.014	0.039±0.001	0.034±0.002	0.042±0.002	0.042±0.000
Cysteine-3TMS	0.052 ^c ±0.009c	0.120 ^a ±0.008	0.107 ^b ±0.003	0.121 ^a ±0.007	0.137 ^a ±0.008	0.010 ^b ±0.001
Ornithine-3TMS	0.039 ^c ±0.003	0.216 ^b ±0.019	0.225 ^b ±0.015	0.236 ^b ±0.018	0.287 ^a ±0.034	0.257 ^b ±0.022
Phenylalanine-2TMS	0.492 ^a ±0.054	0.043 ^c ±0.009	0.047 ^c ±0.004	0.055 ^b ±0.004	0.058 ^b ±0.006	0.055 ^b ±0.004
Xanthine-3TMS		0.023 ^b ±0.002	0.021 ^b ±0.000	0.023 ^b ±0.000	0.029 ^a ±0.001	0.028 ^a ±0.000
Guanine-3TMS		0.012 ^c ±0.001	0.015 ^c ±0.002	0.019 ^c ±0.002	0.029 ^a ±0.001	0.022 ^b ±0.001
Kynurenine-3TMS	0.030 ^d ±0.011	0.102 ^a ±0.007	0.065 ^c ±0.006	0.090 ^b ±0.008	0.063 ^c ±0.004	0.054±0.003
Tryptophan-3TMS	0.398 ^c ±0.096	2.055 ^b ±0.116	2.193 ^b ±0.092b	2.247 ^b ±0.191	2.848 ^a ±0.186	2.313 ^b ±0.136
Spermidine-5TMS	0.018 ^b ±0.000	0.023 ^a ±0.002	0.022 ^a ±0.006a	0.032 ^a ±0.008	0.019 ^a ±0.001	0.024 ^a ±0.004
2'-Deoxyuridine-3TMS		0.017 ^b ±0.002	0.013 ^c ±0.001	0.016 ^b ±0.001	0.022 ^a ±0.001	0.022 ^a ±0.002
Uridine-4TMS		0.029 ^b ±0.003	0.021 ^b ±0.002	0.026 ^b ±0.002	0.038 ^a ±0.003	0.037 ^a ±0.003
Uridine-3TMS	0.009 ^d ±0.001	0.012 ^c ±0.001	0.012 ^c ±0.001	0.039 ^a ±0.024	0.024 ^b ±0.015	0.013 ^c ±0.001
Inosine-4TMS		0.036 ^a ±0.002	0.027 ^b ±0.004	0.026 ^b ±0.006	0.024 ^b ±0.001	0.036 ^a ±0.003
Adenosine-4TMS	0.012 ^b ±0.000	0.046 ^a ±0.004	0.030 ^a ±0.004	0.046 ^a ±0.001	0.034 ^a ±0.006	0.038 ^a ±0.003
Cytidine-4TMS	0.022 ^b ±0.001	0.138 ^a ±0.004	0.107 ^a ±0.006	0.128 ^a ±0.004	0.094 ^a ±0.010	0.110 ^a ±0.005
Monostearin-2TMS	0.510±0.093	0.487±0.015	0.418±0.021	0.433±0.056	0.490±0.032	0.538±0.076
Ornithine-d7-4TMS	0.134±0.089	0.021±0.002	0.022±0.002	0.021±0.001	0.018±0.001	0.019±0.001
Arginine-3TMS	0.102±0.002	0.015±0.001	0.015±0.000	0.016±0.001	0.015±0.000	0.013±0.001



(a)

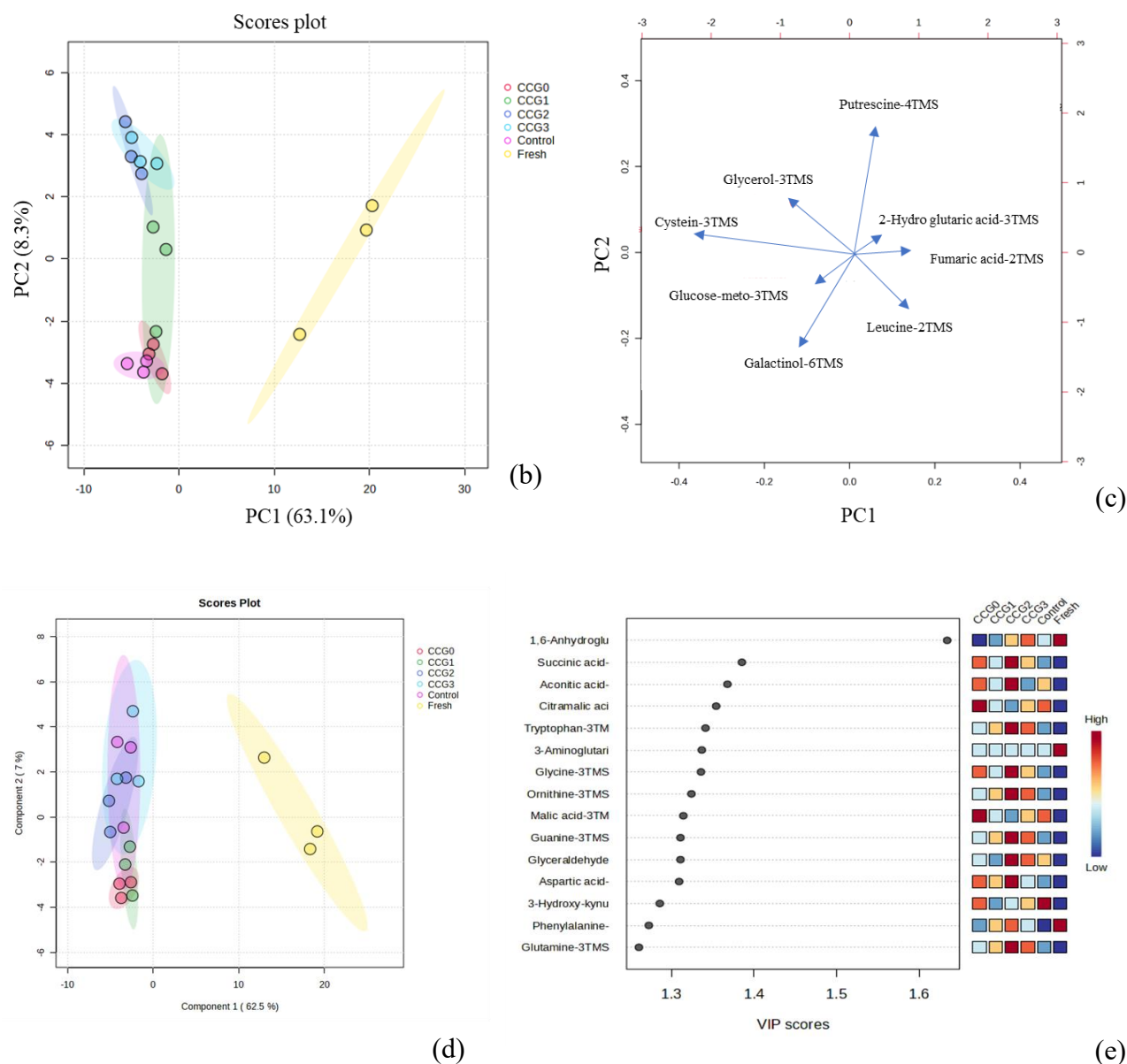


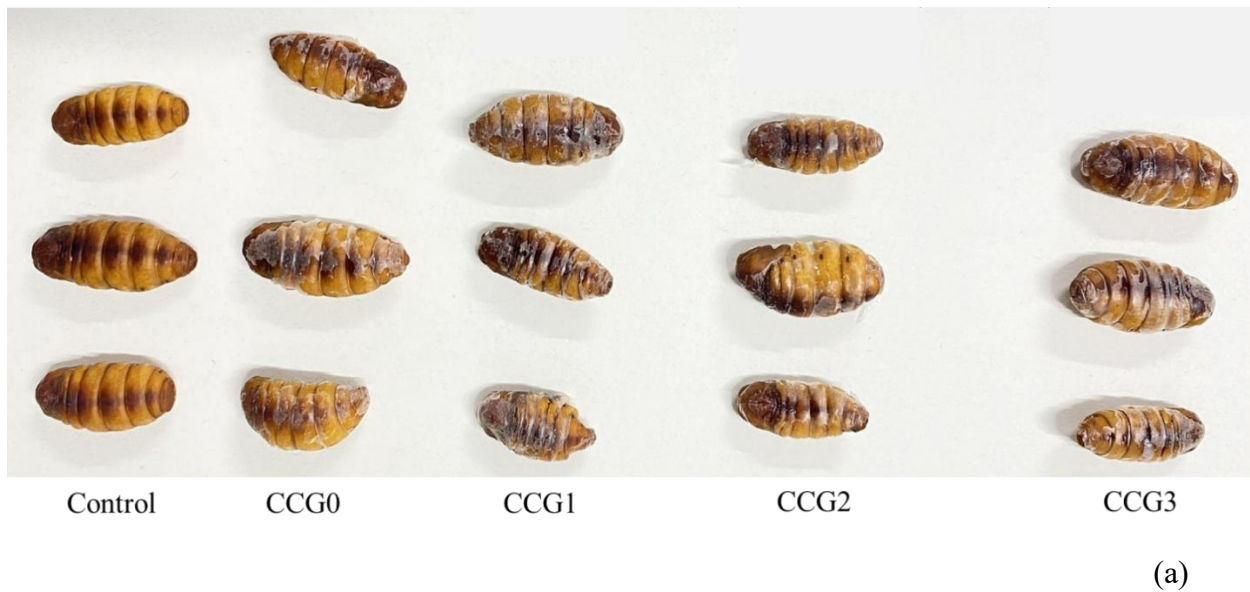
Fig. 4. 6. Heatmap (a), PCA score plot (b), biplot (c), PLS-DA score plot (d) and important features (e) of uncoated and coated silkworms

4.4.3.3. Color change

The visual appearance of silkworm after coating was shown on Fig. 4. 7. According to, silkworms were covered by thin film which was clearly seen by eye. The color change results of silkworms (Fig. S1b) showed significant differences in the silkworm samples before and after drying compared to the original sample. Overall, all values of L^* , a^* and b^* decrease after drying process. Specifically, the a^* and b^* values of coated silkworms decreased significantly in comparison with these values of the control sample, as shown by the significant differences among

the results obtained, especially CCG2 and CCG3 ($P < 0.05$), meanwhile, there was no difference in L^* values.

The ΔE values revealed a significant difference between before and after drying process ($P < 0.05$) as it was calculated from L^* , a^* and b^* values. In particular, CCG1, CCG2, and CCG3, the a^* and b^* values were significantly lower than those of Control and CCG0, meanwhile ΔE values were significantly increase. These results suggested that coating components played an important role in the color change of silkworm before and after drying. Further, this result also demonstrated the effect of coating on drying process. The reason for this change might from the Maillard reactions, leading to the formation of melanoid, brown color nitrogenous polymers, and co-polymers, or pigment degradation, which are responsible for the changing of the redness and brightness of dried silkworm (Piazza et al., 2023).



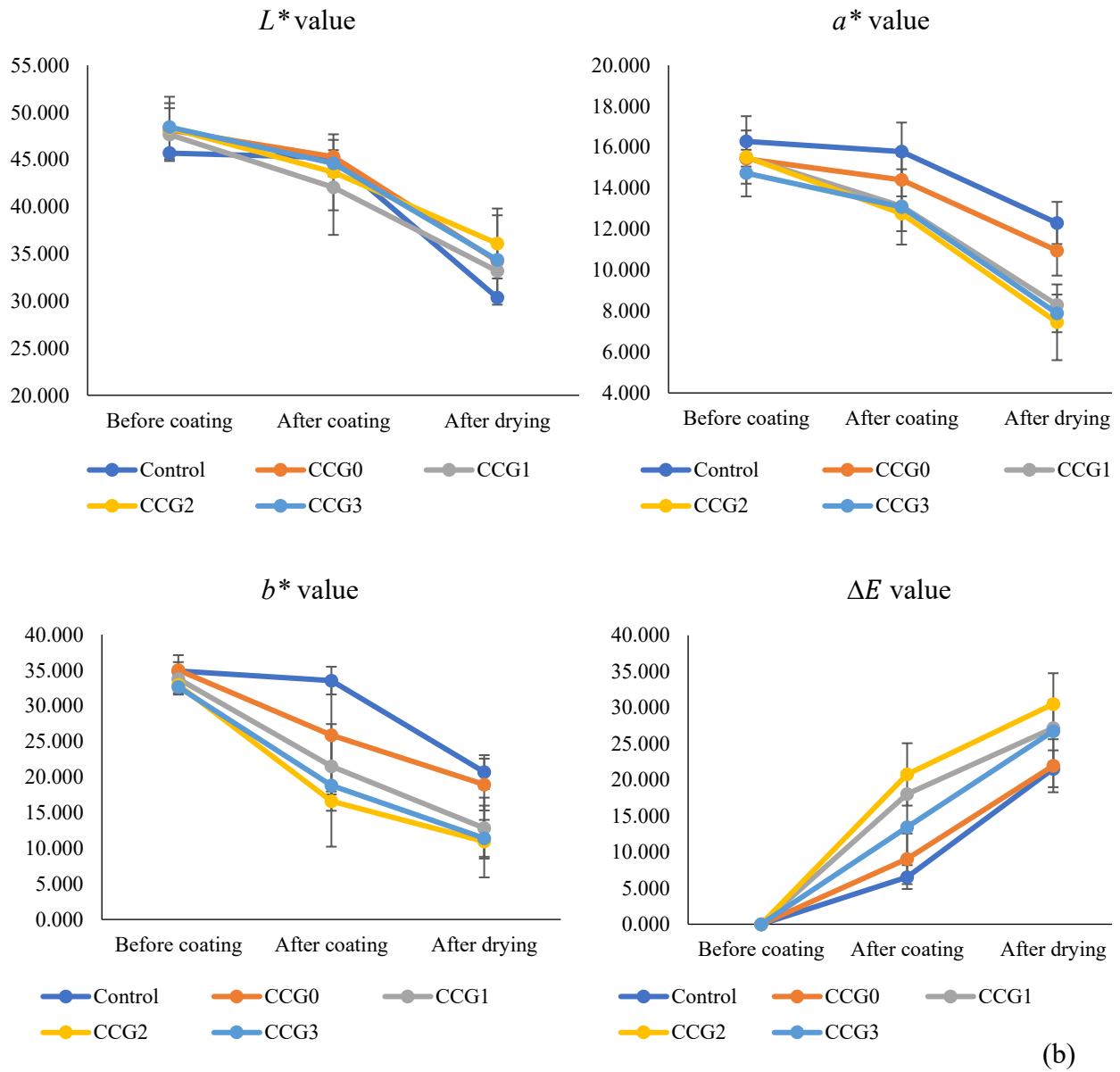


Fig. 4.7. Visual (a) and color change (b) of silkworm samples before and after drying process

4.4.3.4. Instance segmentation application on X-ray CT image analysis

The accuracy of image segmentation process

From previous studies, YOLOv8 represented advantage results in both single and multiple objective classification (Sapkota et al., 2024). In this study, two objectives include the holder and sample which were segmented. As a result, it was shown in Figs. 4. 8, all classes reached a precision of 1.00 at confidence threshold of 0.829 (Fig. 4. 8a) while the recall achieved 1.00 at the lowest confidence threshold (Fig. 4. 8b). In addition, the result about precision-recall curve (Fig.

4. 8c) revealed that mAP values (a mean average precision) reached 0.995 over all classed objects at IoU value (an intersection over union) of 0.5, which were detailed for sample holder at 0.995 and sample 0.994. These results demonstrated a high accuracy in image segmentation of YOLOv8. This finding was similar to the result of Sapkota et al. (Sapkota et al., 2024), which was confirmed by several segmentation methods in both single and multiple segmentation. This YOLOv8 also demonstrated a higher accuracy performance in the condition of low light and time sensitive tasks, which plays an important role in agricultural product quality by machine learning.

The instance segmentation analysis of silkworm samples

The structural changes of the dried product depend on the product itself, the drying method, and the drying conditions such as temperature, humidity, airflow speed, and drying time. The application of coating film on silkworm surface was first reported in this study. The evaluation of the microstructural changes in fresh and dried silkworms demonstrated the influence of the coating film on the silkworm quality and the efficiency of the drying process. The microstructure of the cell changes based on the temperature difference between the drying airflow and the surface or core temperature of the cell tissue, as a result, the porosity of the cells also varies (Prawiranto et al., 2019). In this study, all silkworm samples were dried under the same conditions. After that, X-ray images were evaluated using image processing. The results of X-ray CT in this study indicated the different sizes of the red color which represented for the porosity ratio in images (Fig. 4. 9a) and average porosity ratios (Fig. 4. 9b) among silkworm samples. As can be seen from the Figs., the porosity ratio of the fresh sample was very low and almost unobservable (1.80%). In contrast, the porosity area of dried samples was 10 times higher, with the CCG3 and CCG0 samples being the largest at 23.06% and 22.90%, respectively, while the control and CCG1 samples were smallest at 21.40% and 20.20%. These results suggest that using edible coatings for fresh samples might reduce the time of drying process and save energy. Ando et al. explained the difference in temperature of the drying airflow and the surface of tissue leads to altering the cell shrinkage during drying process, and stiffness in the cell tissue quickly (Y. Ando, T. Watanabe, T. Orikasa, 2024). This limits the water evaporation of internal moisture and hinders the dehumidification of the sample during drying process through the cell (Y. Ando, T. Watanabe, T. Orikasa, 2024). Based on this hypothesis, the mechanism of edible coating might be that the covering of a thin film on the sample surface, leading to reduce the temperature difference between the hot airflow stream and the cell surface, preventing the stiffness of the cell, and promoting dehumidification adequately.

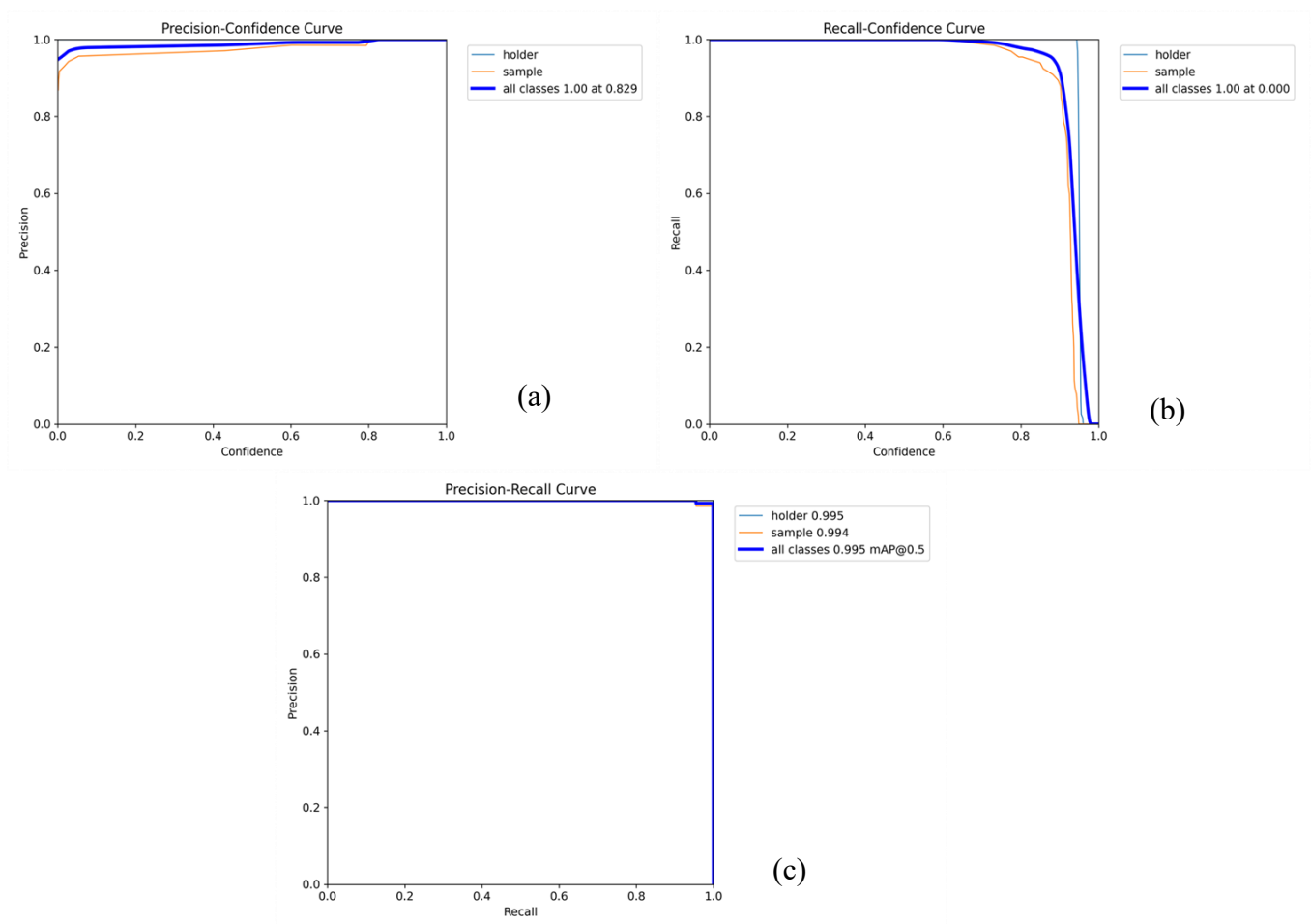


Fig. 4.8. Precision confidence (a), recall confidence (b), and precision-recall curves (c) of silkworm images

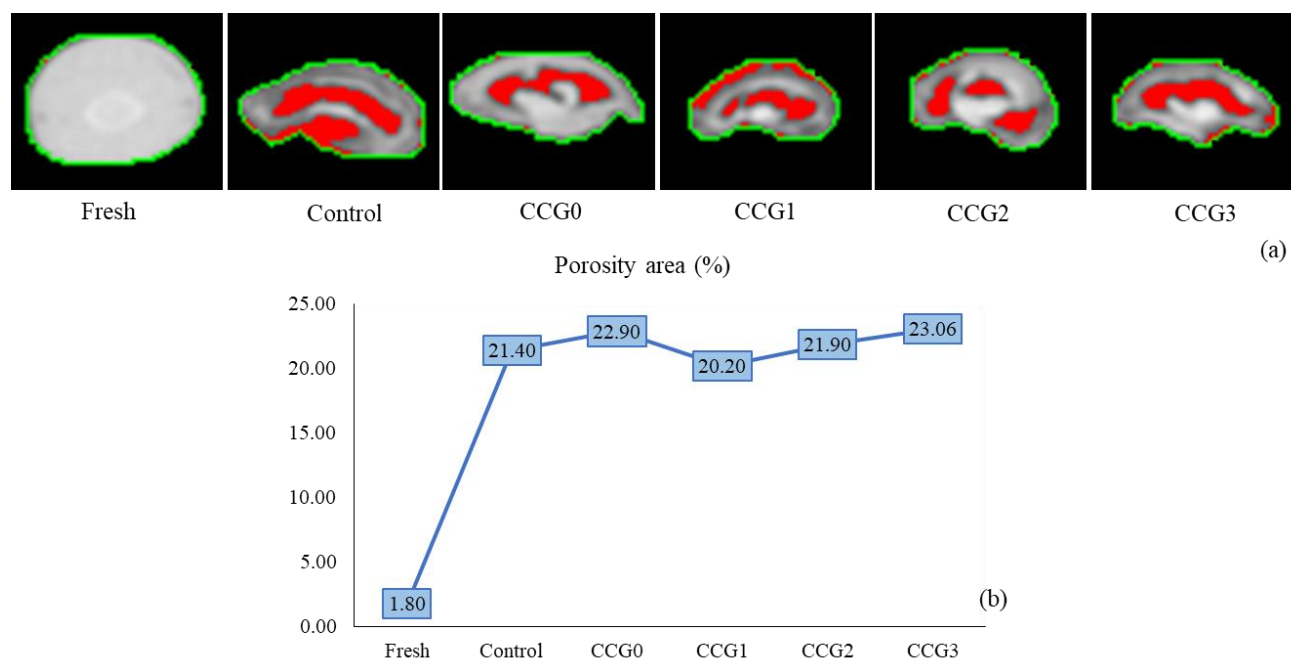


Fig. 4. 9. The porosity area of silkworms (as red color), boundary of segmented sample (as green color), images after segmentation and image processing (a), and data analysis of porosity area (b)

4.5. Conclusion

In this study, the effect of the combination of CMC/NCF/N-Ch and GPO on silkworm drying process was determined. The antibacterial activities of these edible coating were also clarified. The results showed that the various concentrations of GPO affected the physicochemical characteristics of the coating such as pH, TSS, and viscosity values, especially force curve and elastic modulus values which represent the elastic on the surface of the film. In addition, the result of thermal analysis reveals reduced weight loss with higher GPO concentrations, particularly in the CCG3 film. The CCG3 film exhibited the strongest antibacterial effects against *Staphylococcus aureus* while effectively maintaining lower moisture content than others. Additionally, CCG3 displayed more extensive metabolic conversion of compounds and showed greater porosity values in dried silkworm. These findings suggest that edible films on dried products offer significant potential, advancing product quality, energy efficiency, and sustainable practices. However, besides positives, the study remained some drawbacks on the film characteristic such as inhomogeneous surface structure, or low antibacterial effects which require high concentration of GPO. Therefore, studies on other essential oils combined with these bases should be deployed in the future.

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Chapter 5: Summary and Conclusion

This study highlights the promising potential of carboxymethyl cellulose (CMC)-based edible coatings reinforced with nanomaterials and essential oils in enhancing the preservation and quality of agricultural products. The incorporation of mandarin peel essential oil (MO) into CMC/cellulose nanofiber (CNF) matrices significantly improved antifungal activity and helped reduce weight loss in strawberries, with the 0.05% MO concentration proving most effective. The integration of 1-methylcyclopropene (1-MCP) and ME into coatings not only extended color stability and improved surface morphology but also preserved key quality parameters such as firmness, total soluble solids, titratable acidity, and antioxidant capacity in strawberries. Furthermore, CMC/CNF/nanochitosan (N-Ch) coatings enriched with green pomelo oil (GPO) demonstrated strong antibacterial effects and improved drying performance in silkworm applications, particularly in the CCG3 formulation. Despite these advances, limitations such as the need for high essential oil concentrations, inhomogeneous film surfaces, and concerns about potential 1-MCP residues indicate that further optimization and safety evaluations are necessary. Overall, the findings support the development of multifunctional, biodegradable edible coatings using agro-waste-derived components and nanotechnology for improved postharvest management and sustainable food systems.