CARBOXYMETHYL CELLULOSE COATING INCORPORATED WITH LEMONGRASS ESSENTIAL OIL TO EXTEND SHELF LIFE OF TOMATO

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MASTER OF SCIENCE

IN

POSTHARVEST TECHNOLOGY AND INNOVATION

SCHOOL OF AGRO-INDUSTRY

MAE FAH LUANG UNIVERSITY

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ACKNOWLEDGEMENTS

First of all, I thank the Almighty Allah for given me stench, to peruse the program. I gratefully acknowledge International Cooperation Agency (TICA) for an opportunity in master's degree education, Mrs.Kaddy Bojang and my parents for given me courage to accepted this offer. I sincere gratitude to my humble supervisor Asst. Prof. Wirongrong Tongdeesoontorn Ph. D. for giving me excellent scientific guidance and kindly advices, motivations and support throughout my work. She encourages me to add my research presentation in the international conference.

I would express my gratitude to my co-supervisor Asst. Prof. Matchima Naradison Ph. D. for giving me precious advices and unconditional support to go on with my work. I am grateful to thank examining committees Assoc. Prof. Pornchai Rachtanapun, Ph. D. and Asst. Prof. Suthiwal Setha Ph. D.

I wish to use this opportunity to thank my humble friends Miss Wissuta Choeybundi and Mr.Kitaporn Ngiwngam for their timely responses anytime needed. I further extend my sincere gratitude to the scientist of Postharvest Technology and Packaging laboratory at Mae Fah Luang University for their assistances and advice in keeping my studies.

My special thanks go to S3 Technological instruments Centre which provided and supported me with materials equipment's, chemicals and microbial cultures. My special thanks go to Mae Fah Lang University for financial support. Thankful to all staff of international affairs for their guidance and support during my study period. I wish to thank my colleagues in School of Agro-industry, Mae Fah Luang university for their helps in my research.

Haddy Bah

Thesis Title Carboxymethyl Cellulose Coating Incorporated with Lemongrass Essential Oil to Extend Shelf Life of Tomato

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ABSTRACT

Tomato (*Solanum lycopersicon*) is the second most important crop nest to potato and a highly perishable climacteric fruits, with a relatively short postharvest life. The fruit decay by fungal diseases (*Aspergillus niger*) is one of common cause of tomato losses and its optimum storage cannot be prolonged. The aim of this study was to evaluate the edible carboxymethyl cellulose (CMC) coating accompanied with lemongrass essential oil (LGEO) against *A. niger* for prolonging shelf life of tomatoes during storage. Preliminary test was carried out with six different coating materials (LGEO, soy protein, guar gum, cassava starch and gelatin all at 3g except CMC which was 2g) were prepared and coated on tomatoes, stored at 13°C, 85% RH. Disease incidence and skin appearance were observed and finally CMC coated fruits showed low disease incidence and were able to maintained good appearance of skin during storage period. Consequently, CMC was used as an alternative to be continued in this experiment. Essential oils of (oregano, lemongrass essential oil and clove oil) were tested at different concentrations of 0.5%,1% and 2% on disk diffusion MIC and MFC methods. All these essential oils were able to inhibit *A. niger* at 1% and 2%

concentration. The sensory evaluation was also carried out by trained panelist on the smell of essential oils and appearance of coated fruits. Consequently, LGEO was used to be continue with this experiment with high evaluation scores for the appearance and low disease incidence on coated fruits for 30 days of storage at 13°C,85% RH. Finally, for the real experiment this work antimicrobial properties of LGEO were investigated by disk diffusion method, minimum inhibitory concentration (MIC) and minimum fungicidal concentration (MFC). The minimum inhibitory concentration (MIC) of LGEO against *A. niger* was 1% (w/v). The effect of CMC coating and CMC coating combined with LGEO on tomatoes qualities such as weight loss, total soluble solid (TSS), firmness, titratable acidity (TA), and disease incidence and severity were investigated. The results showed that application of CMC combined with 1% LGEO could reduce changes of weight loss, firmness, total soluble solid and disease incidence with good appearance for 40 days of storage at 13°C, 85 % RH. The CMC coating incorporated with 1% LGEO can extended the shelf life of tomato from 10 days to 30 days at 13°C, 85%RH. Therefore, CMC combined with LGEO can be used as the active coating for extending the shelf-life and delayed *A. niger* disease development in tomato fruits.

Keywords: Antifungal, Carboxymethyl Cellulose, Edible Coating, Lemongrass Essential Oil and Tomato

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ABBREVIATION AND SYMBOL

CMC	Carboxymethyl cellulose
DI	Disease incidence
DS	Disease severity
CRD	Completely randomize design
EOs	Essential oils
FAO	Food and Agriculture Organization
RH	Relative humidity
HPMC	Hydroxypropyl methyl cellulose
LGEO	Lemongrass essential oil
MIC	Minimum inhibitory concentration
MFC	Minimum fungicidal concentration
µL	Microliter
N	Newton
mL	Millimolar
TICA	Thailand International Cooperation Agency
NFCs	Natural flavoring complex
PPM	Parts per million
PDA	Potato dextrose agar
PDB	Potato dextrose broth
SPSS	Statistical package for social science
TSS	Total Soluble Solid
TA	Titratable Acidity
W/W	Weight by weight

CHAPTER 1

INTRODUCTION

1.1 Introduction to the Research Problems and Its Significance

Tomatoes (*Solanum lycopersicum* L) belongs to the family of Solanaceae one of the most popular vegetable crop worldwide. Tomatoes are highly perishable climacteric fruits, with a short post-harvest life (Gebeye, 2018). Tomato is one of the most versatile and widely-used food plants, being consumed both in natural (raw), and as a constituent of other products and dishes (Guchi, 2015). Nutritionally, tomato is rich in minerals, vitamins, and antioxidant compounds that support health benefits (Abushita, Hebshi, Daood & Biacs, 1997). Ripe tomatoes contain large amount of antioxidant lycopene which positive effects in preventing different types of cancer particularly prostate cancer (van Breemen & Pajkovic, 2008). Singh & Sharma (2018) stated that the post-harvest losses of tomatoes caused the main problems in low-income countries. These losses can either be cause by fungi decay by fungal disease the most common cause of tomato losses due to the physical characteristics, of the thin layer caused easily to attack by disease and pest.

Aspergillus niger is a highly pathogenic fungus of plants it has the tendency to enter the plant tissue through wounds caused by physical or natural openings, can result to fruit deterioration. Many authors reported about *A. niger* as a common infection in many perishable products during post-harvest handling (Aguilar, Palou & López-Malo, 2017). The quality maintenance of tomato is another challenge in postharvest handling. The loss of quality is quite common in developing countries. These losses occur due to inadequate post-harvest handling, poor transportation systems, fluctuating temperature, relative humidity (RH), storage gases, and postharvest diseases. Losses during postharvest handling are enormous and range from 25–50% in developing countries (Mahfoudhi, Chouaibi & Hamdi, 2014). The demand for highly nutritional and high-quality food is increasing due to rising health awareness.

Postharvest techniques have been developed such as edible coating, low temperature, control atmosphere etc., to reduced deterioration of tomato (Ajayi & Olasehinde, 2009). Edible coating is a common method for increasing shelf life of produce (Baldwin, Nisperos-Carriedo & Baker, 1995). Edible coatings generate a modified atmosphere within the fruit and delayed ripening processes (Konuk Takma & Korel, 2017). Edible coating is eco-friendly and non-toxic nature, and thus used as natural fungicide as an alternative in extending horticultural produce shelf life and controlling decay (Safari, Ding, Nakasha & Yusoff, 2020). There are four primary structural materials of edible coatings, i.e., polysaccharides, proteins, lipids, and composites coating (Nagata & Yamashita, 1992). Edible coatings are recommended of having active ingredients, such as antimicrobial agents, anti-browning agents, coloring, flavoring anent and even nutrients. Essential oils (EOs) from plant parts as natural antimicrobial agents has been increased in recent years. Gyawali, Hayek, and Ibrahim (2015) stated many essential oils (EOs) have been demonstrated to effects on controlling of microbes, fungi, and/or insecticidal activities Essential oil-edible coatings efficiently contribute to support the use in food by increasing their dispensability in areas where microorganisms grow. Essential oils have antimicrobial activity to reduce the impact on the quality of food during distribution (Donsì & Ferrari, 2016).

Carboxymethyl cellulose (CMC) is a derive from cellulose and considered as the most widely known as cellulose derivatives. CMC is water soluble polysaccharides that has a great importance in the industry and our everyday life (Biswal & Singh, 2004). The polysaccharide-based coating of CMC includes starch, alginates, carrageenan, and cellulose derivatives. Generally, CMC is characterized as odorless and tasteless, flexible, transparent, and nontoxic, therefore, it can be applied as coating material. Many types of research have applied CMC for prolonging shelf-life of fruits (Maftoonazad & Ramaswamy, 2005). CMC can be produced from agricultural waste such as durian rind (Rachtanapun, Luangkamin, Tanprasert & Suriyatem, 2012), sugar beet pulp cellulose for extended shelf life of peach and pear (Toğrul & Arslan, 2004) and papaya peel (Rachtanapun, Eitssayeam & Pengpat, 2010). The usage of chemical pesticides has a negative aspect such as high price, toxic to human, as well as pesticides that remain longest time in the environment (Paster & Bullerman, 1988).

General communities awaked of negative aspect involve in using hazard which has now increased the concerned in finding safer methods protectants as an alternative instead of using synthetic pesticides. The use of plant part is a common protectants pesticide with no or less compounds, less toxic and do not appear to cause problems in human and environment. Essential oil are extracts from different plant parts which are known to have different roles including conferring herbicides, pest and diseases resistance (Tzortzakis & Economakis, 2007).

Lemongrass (*Cympopogon citratus* L.) is a plant in the grass family that contains 1% to 2% essential oil on a dry basis with widely variation of the chemical composition depending on genetic diversity. Lemongrass essential oil (LGEO) are known for their bioactivities and uses in in pharmaceuticals, aromatherapy, food additives etc., (Carlson, Machado, Spricigo, Pereira & Bolzan, 2001). LGEO known to have high amount of citral (composed of neral and geranial isomers c. 69%), combination is used as a primary production of ionone, vitamin A and beta-carotene (Tzortzakis & Economakis, 2007). Many authors wrote about the inhibition of disease-causing microbe (even for human pathogenic fungi) by lemongrass oil (Appendinia & Hotchkiss, 2002). LGEO can be used to prevent the growth of microorganism such as molds, yeasts and bacteria both positive and negative bacteria gram (Mohd, Fomda, Ebenezer & Bhat, 2010). Limited research had been carried out to determine the effect of lemongrass essential oil combined into edible coatings on fruits and vegetables (Raybaudi & Martín, 2008).

The aim of this study was to test the effects of carboxymethyl cellulose CMC edible coating in combination with lemongrass essential oil on weight loss, firmness, total soluble solid content, titratable acidity, disease incidence and severity, and overall acceptance of fresh tomato fruit during storage.

1.2 Objectives of the Research

1.2.1 To evaluate the antifungal properties of lemongrass essential oil combined with carboxymethyl cellulose CMC coating solution.

1.2.2 To test the effectiveness of carboxymethyl cellulose (CMC) coating in cooperated with lemongrass essential oils on tomato during storage.

1.3 Scope of the Research

1.3.1 The accumulation of different essential oils was tested with different concentrations to determine the best essential oil which has high antifungal properties against *Aspergillus spp.* by disk diffusion method and minimum fungicidal concentration MFC determinations.

1.3.2 The optimal concentration of proper essential oil was mixed with carboxymethyl cellulose CMC coating solution and applied on tomato for retarding the qualities changes and decay during storage.

1.4 Research Outcome

1.4.1 The most effective coating treatment was CMC incorporated with lemongrass oil can be used as an alternative way for maintaining qualities and delaying the growth of *A. niger* on tomatoes during storage and distribution.

1.4.2 This innovation will be applicable on tomato producing areas.

1.4.3 Extend shelf-life of tomato by different coatings.

1.5 Research Outputs

The new antifungal fruit coating from CMC incorporated with lemongrass essential oil.

CHAPTER 2

LITERATURE REVIEW

2.1 Tomato

Tomatoes (*Solanum lycopersicum* L) belongs to the family of Solanaceae one of the most popular vegetable crop in the world. Food and Agriculture Organization (FAO) stated that nearly 162 million tons of tomatoes were produced worldwide, with China being the largest producer (more than 50 million tons during this period). Mexico produced 3,433,567 million tons with a yield of 355,254 kg/ha, placing it in the top ten leading producers of this fruit and as the top exporter (Ruiz-Martínez et al., 2020). Tomatoes are rich in nutrition which is minerals, vitamins and antioxidant compounds that promote health benefits (Guchi, 2015). Tomatoes are highly perishable climacteric fruit with short life due to physical/mechanical injury and microbiological decay (Gebeye, 2018).

2.1.1 Postharvest Losses of Tomatoes

Postharvest loss is a major problem of fruits and vegetables during the supply chain (Porat, Lichter, Terry, Roger & Buzby, 2018). These losses are encountered during: handling, storage, transportation, and processing resulting in a reduction in the quantity, quality, and market value of commodities (Emana et al., 2017). However, fruit decay by fungal diseases is the most common cause of tomato losses due to the physical characteristics of thin layer caused easily attack by pests and disease. After harvest, tomato fruit continuing metabolism process causes fruit losses during storage and transportation which may lead to economic losses.

2.1.2 Quality Changes of Fresh Tomato During Transportation

Fresh tomato fruit need manipulation at different stages from the orchard to the industry, before reaching the consumers (Al-Dairi, Pathare & Al-Yahyai, 2021). The composition and structural configuration related to damage and injuries worsen the physiological, chemical and microbial changes leading to quality losses. During transportation, tomatoes move through the supply chain are often subjected to rough handling and bruises hence loss of value (Idah, Ajisegiri & Yisa, 2007). However, Mashau, Moyane and Jideani (2012) stated that high temperature and low relative humidity conditions of tomato fruits lead to quality deterioration during transportation especially in area where there are no cooling facilities. Vursavuş and Ozguven (2004) stated that important factors that can cause the accelerated metabolic, enzymatic and poor temperature during transportation processes leading to a loss in the market value. Wounded tomatoes provide entry for spoilage and pathogenic microorganisms, this effect is not only limited to economic losses but also to health problems after consumption (Borah, Mathur, Srivastava & Agrawal, 2016). Mechanical damage of tomatoes during transportation is due to a variety of forces subjected to the fruits during freight which include: vibrational, abrasive, compression, and cutting forces. During transportation, vibrational forces from the vehicle due to sudden changes in the road profile causes the fruit to move randomly within the packaging units. These forces may reach thresholds that causes damage leading to quality loss (Al-Dairi et al., 2021). Vibration causes tomato fruit movement inside the package, rubbing against the surfaces of other fruit in the packaging units causing bruising which may leads to entry points for microbes. Cuts can occur when the fruit is pushed or rotated onto the sharp edges of the packaging units (Vursavuş & Ozguven, 2004). According to Vergano, Testin and Jr (2007) who stated that injuries can be externally visible and easily detectable (skin and flesh browning and off-flavors), or may not be visible externally. Therefore, both types of injuries result in quality reduction.

2.1.3 Microbial Spoilage

Microorganisms readily attack fresh produce and spread rapidly, owing to the lack of natural defense mechanisms in the tissues of fresh produce. Commercialization of tomato fruits is limited in developing countries caused by rotten (Singh & Sharma, 2018). Reduction in fruit quality occur because of diseases and possible microbial spoilage. Postharvest diseases of fruits and vegetables are caused by bacteria and fungi development between harvesting and consumption (Pinheiro, Gonçalves & Silva, 2013). Unripe tomatoes are more resistant to most decay pathogens than ripe fruit. Micro-organisms (P*enicillium* spp.), that normally are not considered decay pathogens of tomato may be attacked over-ripe fruit. Research has revealed that losses caused by microbial infections ranges between 50% and 90% in Africa (Bello, 2016). There are different types of fungi (*Alternaria solani, Alternaria alternata, Colletotrichum coccodes, Botrytis cinerea,* buckeye rot, *Phytophthora infestans* and *Rhizopus stolonifera*) that caused diseases in tomatoes. Bacterial decay, caused by *Pseudomonas syringae* and *Xanthomonas campestris,* may also have the tendency to developed in the fruit (Pinheiro et al., 2013). *Aspergillus niger* is a highly pathogenic fungi of plants since it is able to colonize through wounds or natural openings of the plant tissue. *A niger* has been reported as a major cause of infections in many perishable products, including tomatoes during storage and transportation (Ajayi & Olasehinde, 2009). As reported by (Jeddi et al., 2014) microbes might pose a serious health hazard to consumers as the occurrence of *Aspergillus* spp (which are carcinogenic) in water could be absorbed into the fruits through the osmotic potential. Proffering suitable solutions of controlling isolate and identify microbes associated with tomato fruits spoilage to safeguard human health.

2.2 Postharvest Treatments for Tomato

2.2.1 Temperature

Temperature control is the most common measures used to extend the shelf-life of fresh tomatoes during supply chains. Low-temperature storage is generally used since higher temperatures increase fruit respiration and shorten their shelf-life. The storage temperature of fresh tomato fruit depends on the maturity stage and cultivar. It has been generally known that temperatures lower than 13°C can induce chilling injury in tomato (Pinheiro et al., 2013). The kinetics of tomato quality reduction greatly depended on storage temperature and duration. High temperature, low relative humidity (RH), and extended storage conditions lead to the loss of valuable nutrients in tomatoes, especially vitamins (Sablani, Opara & Al-Balushi, 2006).

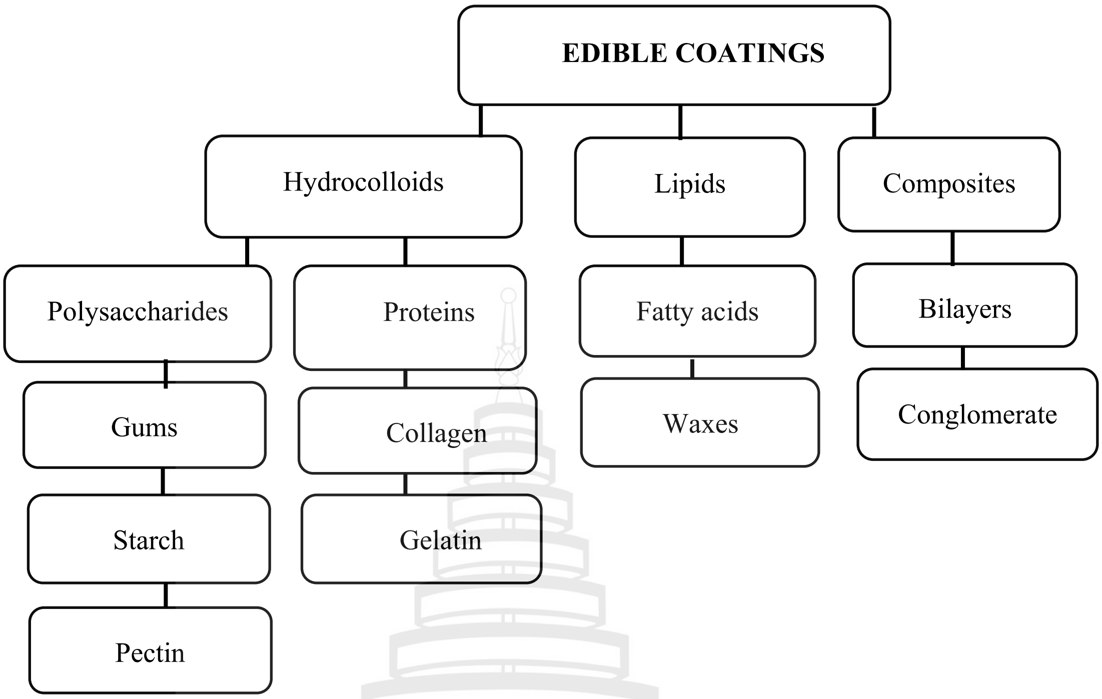
2.2.2 Edible Coating

Edible coatings are thin layers of edible material applied to the product surface. Edible coating can be used as a replacement for natural protective waxy coatings and to provide a barrier to moisture, oxygen, and solute movement for the food (Dhall, 2013). Edible coatings are applied directly to the food surface by dipping, spraying, or brushing to create a modified atmosphere. The material used for the preparation of edible films and coatings is Generally Recognized as Safe (GRAS). This approval was done by Elsabee & Abdou, (2013) and was conform to the regulations that apply to the food product concerned. An ideal coating is defined as one that can extend the storage life of fresh fruits and vegetables without causing anaerobiosis and reduces decay without affecting their quality. Previously, edible coatings have been used to reduce water loss, but recent developments of formulated edible coatings with a wider range of permeability characteristics have extended the potential for fresh produce application. Fruit-based coatings provide enhanced nutrition to products, which increases their market value. Edible and biodegradable coatings must meet some special functional requirements, such as moisture barrier, solute or gas barrier, water/ lipid solubility, color, appearance, mechanical characteristics, and should be nontoxicity, etc. The effect of coatings on fruits and vegetables depends greatly on temperature, alkalinity, thickness, type of coating, the variety and condition of fruit and vegetable (Dhall, 2013). According to Rojas-Graü, Soliva-Fortuny & Martin-Belloso, (2010) the main purpose of edible coating for fruits and vegetable is basically to increase the natural barrier.

Moreover, one of the most important things of this edible coating is the fact that they can be eaten together with fruits and vegetables. Edible coating provides an additional protection coating for fresh fruits and vegetables. Edible coating also provides the same effects as modified atmosphere storage in modifying internal gas composition. Recently, various edible coating was applied successfully for preserving fruits and vegetables such as orange, apples, grapefruits, cherries, cucumber, tomato capsicum, cherries and strawberry (Raghav, Agarwal & Saini, 2016). Coatings acts as semi-permeable barrier and extend shelf life by decreasing respiration rate and to reduce physiological disorders on products (Baldwin, Nisperos, Chen & Hagenmaier, 1996). Edible coating is consider as wrapping various fruits and vegetables to extend their shelf life and eaten together with foods, with or without removal. Edible coating is characterized mostly as odorless, colorless, tasteless and they should have good mechanical properties (Tesfay, Magwaza, Mditshwa & Mbili, 2017). Feature of edible coating is to increase shelf life of processed fruits and vegetables and protected it from post-harvest damages (Tharanathan, 2003). Edible coatings are used as a nutraceutical carrier of texture enhancer and antioxidants. Generally, Shiny appearance to fruits and vegetables are provided by edible films and the thickness is less than 0.3mm. (Montero-Calderón, Rojas-Graü & Martín-Belloso, 2008).

2.3 Classification of Edible Coatings

Edible coatings are having hydrophobic group, such as lipid-based and hydrocolloids group, such as polysaccharides-based, protein-based or combination of both groups to improve function of edible coating. Edible coatings are mostly applied as preservation of fruits and vegetables and for a good appearance. Edible coatings are not chemically synthesis and it is natural. Generally, edible coating is used for enhancing good appearance and preserving fruits and vegetables. Edible coatings are non-toxic in nature and its main advantages are cost effective. Edible coating generally made up of hydrocolloids (polysaccharides and protein-lipids), lipids and composite (Gupta & Prakash, 2019).



Source Raghav et al. (2016)

Figure 2.1 Types of Edible Film and Coating

2.3.1 Hydrocolloid

Hydrocolloids which are originated from animals, vegetables, microbial or synthetic, they are hydrophilic polymers. They have hydroxyl group and may be poly-electrolytes such as alginate, carrageenan, pectin, carboxymethyl cellulose, xanthan gum and gum arabic. Today, hydrocolloids are used in wide range as a coating forming solution or coat and control the color, texture, flavor and shelf life of fruits and vegetables. Generally, all hydrocolloids are partially or completely dissolving in water and principle use of this increase the viscosity of aqueous phase (continuous phase) i.e., gelling agent thickness (Raghav et al., 2016).

2.3.2 Polysaccharide-based Edible Coating

The most common polysaccharides used for edible coating of fruits and vegetables are chitosan, starch, alginate, cellulose, pullulan, carrageenan, gellan gum etc., (Park, 2003). Polysaccharides based edible coatings having poor moisture barrier properties, it is water soluble. Although this coating does not provide a good water vapor barrier, it can retard moisture or water loss from fruits and vegetable. However, polysaccharide coatings extend shelf-life of fruit and vegetable as coating can prevent dehydration, oxidation rancidity, and also surface browning to produce (Díaz-Galindo, Nesic, Bautista-Baños, Dublan García & Cabrera-Barjas, 2020).

2.3.3 Protein Based Edible Coating

Protein based edible coatings are derived from animals and plants. The plant-based protein edible coating material are milk protein casein, whey protein, zein from maize, gluten from wheat, soy protein and etc The animal protein includes egg albumen, collagen etc (Zhang, Liu & Rempel, 2018). Protein based edible coating has the excellent barrier properties for aroma, oil and oxygen but it is not effective barrier for moisture (Kouravand, Jooyandeh, Barzegar & Hojjati, 2020). The reason for its excellent barrier property for oxygen is its tightly packed hydrogen bonded structure (Raghav et al., 2016). Protein base edible coatings has good O_2 barrier property at low relative humidity. Protein based coating are not good barrier for water vapor due to its hydrophilic nature, but it consists good organoleptic and mechanical properties (Lacroix & Vu, 2014).

2.3.4 Lipid Based Edible Coating

The lipid based edible coatings have been used for many years for preservation of fruits and vegetables. They provide shiny and glossy appearance to food. Most common lipid-based coating materials are carnauba wax, bee wax, paraffin wax and mineral or vegetable oil. Lipids are having good water barrier capacity. Oil, fat and wax based coatings are not easily applied to surface of fruits and vegetables because of its greasiness and thickness and it gives rancid flavor (Morillon, Debeaufort, Blond, Capelle & Voilley, 2002).

2.3.5 Composites Based Edible Coating

Composites or multicomponent films and coatings contain combination of protein, polysaccharides and lipid-based material. This is used to enhance and improve mechanical strength, moisture and gas barrier properties of edible coatings and films (Pankaj, Wan & Keener, 2018). Furthermore, the coating with other ingredients has an effect on their barrier (such as water, oxygen and carbon dioxide) and also other mechanical properties (Mashau et al., 2012).

2.4 Application Method of Coating

2.4.1 Dipping

Dipping is the most common method of coating on the food product. It is the immersion of a food sample in the coating-forming dispersion. In the first step, the substrate is immersed in the coating emulsion/ solution at a constant speed, the dwelling ensures enough quantity of solution for wetting substrate and complete interaction between both substrate and coating matrix. The deposition process is used to develop thin layers of the precursor emulsion on the surface of food products. The excess surface liquid drains and removes by deposition (Costa, Conte & Del Nobile, 2014). During evaporation step, the solvent and excess liquid are evaporated from the surface of food products by using heating and drying procedure. The product will be dried either at room temperature or with the help of a dryer when the surplus coating is drained away (Andrade, Skurtys & Osorio, 2012). Thickness of liquid coated films has been shown to rely on the characteristics of a coating solution such as density, viscosity, surface tension as well as surface withdrawal rate (Cisneros-Zevallos & Krochta, 2003).

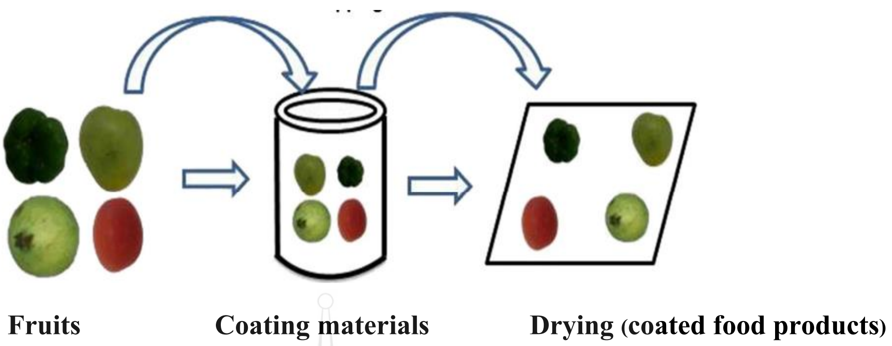
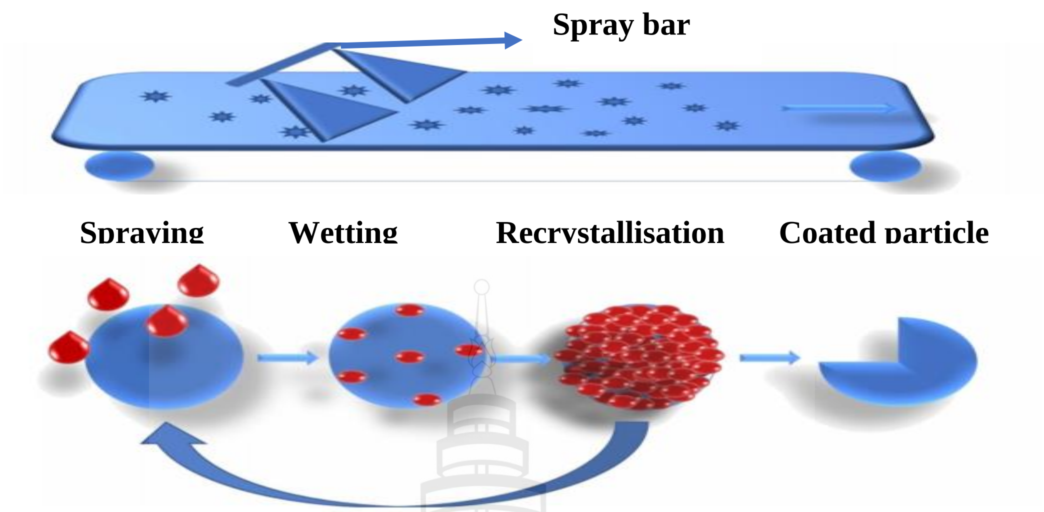


Figure 2.2 Dipping Method for Coating

2.4.2 Spraying

This method is used to apply thin layers of coating material to dry particles of exceptionally low density and/ or small size (Solís-Morales, Sáenz-Hernández & Ortega-Rivas, 2009). The coating solution and suspension are sprayed onto the fluidized powder surface via a number of nozzles to form a shell-like structure in a fluidized coating process. Fluidized-bed process method is classified into three categories: top spray, bottom spray, and rotary fluidized bed. In the food industry, however, the traditional top spraying method is more commonly used (Jacquot & Pernetti 2004). The powder agglomeration of the fluidized bed coating technique promotes the dispersion and solubility of the coating material. At the same time, the liquid binder is used for the enhancing stability of the material; the process is done under heat treatment before sprinkled on foods. This process results in the adhesion, aggregation, and drying of the particles. The commonly used nozzles in the fluidized-bed coating are pneumatic or binary: the fluid is delivered at low pressure and the air is sheared into the nozzles (Aydin, Kahve & Ardic, 2017).

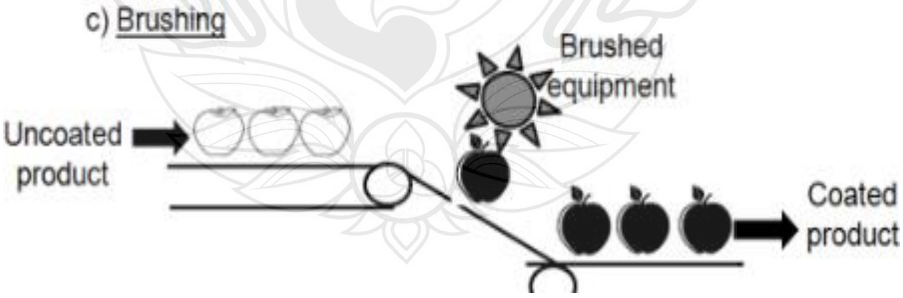


Source Suhag, Kumar, Petkoska and Upadhyay (2020)

Figure 2.3 Spraying Application Method of Edible Coating

2.4.3 Brushing

The brushing method is the application of film or coating to fruits and vegetable which is better than wrapping and dipping methods in terms of reducing the moisture loss (Dhanapal et al., 2012).



Source García, Burgos, Jimenez and Garrigós (2015)

Figure 2.4 Brushing Method for Coating

2.5 Carboxymethyl Cellulose (CMC)

The most consider and widely cellulose derivatives used is carboxymethyl cellulose (CMC) derive from cellulose. It has a great importance in the industry and our everyday life and it is water soluble polysaccharides (Biswal & Singh, 2004). CMC is a polysaccharide-based coating which includes starch, alginates, carrageenan, and cellulose derivatives. CMC is generally characterized as odorless and tasteless, flexible, transparent, and nontoxic. CMC coatings is able to preserved flavor, improve textural quality, reduce oxygen uptake with increasing carbon dioxide level of coated fruits (Perdones, Escriche, Chiralt & Vargas, 2016). Moreover, CMC is environmentally friendly and has no harmful effects on human health (Corbo et al., 2015).

Table 2.1 Carboxymethyl Cellulose (CMC) Coating on Fresh Produce

CMC coating	**Fresh produce**	**Results**	**References**
Carboxymethyl cellulose (CMC)	Mandarin	The application of CMC on mandarin was able to suppress the increased, TSS, ripening index substantially higher TA, and ascorbic acid on coated fruits mean it can increase storage life alongside with qualities.	(Ali et al., 2021)
Carboxymethyl cellulose (CMC) with corn starch	Cucumber	CMC in combination with corn starch on cucumber fruits increase qualities (weight loss, firmness, TSS, ascorbic acid and less microbial counts and finally increase shelf life.	(Adetunji, Arowora, Fawole, Adetunji & Ar, 2013)
CMC with moringa leaves and seed extracts	Avocado	Treated fruit had a significantly lower mass loss, ethylene and respiration rate compared to the untreated control.	(Tesfay et al., 2017)

Table 2.1 (continued)

CMC coating	Fresh produce	Results	References
CMC and Chitosan	Citrus fruit	The effect of CMC and alginate in combination with brewer yeast was coated on grapefruits. The result showed that the increase in weight loss, decreased TSS of was lower on coated grape of was lower on coated grape and maintain of antioxidant properties compare to control.	(Arnon, Zaitsev, Porat & Poverenov, 2014)
CMC and γ-irradiation	Peach	Carboxymethyl cellulose (CMC) disease incidence for the storage irradiation prevents.	(Hussain, Suradkar, Wani & Dar, 2016)
CMC and Alginate + yeast	Grape	The effect of CMC and alginate in combination with brewer yeast was coated on grapefruits. The result showed that the increased in weight loss, decreased TSS of was lower on coated grape and maintain of antioxidant properties compare to control.	(Fan et al., 2009; Yinzhe & Shaoying, 2013)
CMC+ Marin yeast	Jujubes	The result showed that CMC incorporated with Marin yeast had no significant effect on the qualities' such as color, TSS and TA but can control postharvest decay on jujube fruits during the storage period.	(Wang et al., 2011)

Essential Oil and Application

From ancient times, herbs and spices have been part of the human diet to enhance the flavor, color and aroma of foods. These spices, together with their essential oils EOs were also a major form of therapy due to their antimicrobial and antiseptic properties (Moore-Neibel, Gerber, Patel, Friedman & Ravishankar, 2012). Essential oils have different biological activities and therapeutic effects which can be applied in different industries and treat several disorders in human, animals, plants and foods. Essential oils EOs are characterized as oily, hydrophobic, aroma and volatile liquids that can be extracted from plants. EOs maybe obtain from specific groups within particular parts of the plant, such as stem, leaves, the foliage, back, wood, fruit, seeds and rhizomes (Mehdizadeh & Moghaddam, 2018). Essential oils EOs are usually obtained by steam distillation. EOs are complex mixtures of components, including terpene derivatives, with well-known aromatic properties. and contain a range of oxygenated and non-oxygenated terpene hydrocarbons. Various essential oils, extracted from cinnamon, clove, eucalyptus, lemon, lemongrass, lime, mint, orange, alma rosa, rosemary, basil and wintergreen have been traditionally used by people for various purposes in different parts of the world. Essential oils enter the body in different ways such as inhalation, absorption via skin and ingestion, and exert their properties in the body. Different essential oils from plants indicate several useful therapeutic characteristics, such as analgesic (peppermint, lemongrass, clove, rosemary); antibiotic (tea tree, lavender), antifungal (tea tree, lemongrass); anti-inflammatory (yarrow, German chamomile, lavender, clove) antiseptic (tea tree, lavender), antiviral (Melissa, tea tree, lavender, lemon) (Mehdizadeh & Moghaddam, 2018). In general, to achieve a significant antimicrobial and/or antioxidant activity, a relatively high concentration of EOs is required. Thus, EO from oregano, thyme, garlic, lemongrass, bay leaf, rosemary and clove, can be used alone or in combination with other preservatives, to improve the shelf life of food products. Although the use of plant essential oils has been primarily studied in the medical fields. The natural properties of essential oils and their effectiveness in new applications have been explored. EOs developed newly, especially in food, cosmetic, and public health fields in particular essentials oils have been studied for their good use as possible remedies for the treatment of many infectious disease (Shaaban & El-Ghorab, 2012). Essential oils from different herbs and spices have been newly accepted as food flavorings and preservatives agents because of their inherent aromatic and antimicrobial constituents. EOs have great potential to natural safety that can be added in almost all foods, due to their high efficiency at very low concentrations (Roller & Seedhar, 2002).

Food safety is usually ensured by the addition of antimicrobials that prevent, or considerably retard, microbial organisms promoting spoilage. A variety of different synthetic fungicides and chemicals, such as benzimidazoles, aromatic hydrocarbons, and sterol biosynthesis inhibitors, with various degrees of persistence for many years. These are employed as antifungal agents to inhibit the growth of plant pathogenic fungi. In many areas around the world, the extensive use of these chemicals has led to the development of resistance. To overcome this problem, higher concentrations of these chemicals were used, but the widespread use of pesticides has significant drawbacks including cost, handling hazards, pesticide residues, and threats to human health and the environment. Moreover, synthetic pesticides can also cause environmental pollution, because of their slow biodegradation in the environment, and harm human health (Isman, 2006). The development of new safe and biodegradable alternatives as natural fungicides has increased. Therefore, there has been a growing interest in finding safer alternative materials to replace synthetic chemical products, of natural and plant secondary metabolites. These alternatives can be plant-based essential oils extracts for pest and disease control in agriculture, which may be less damaging for pest and disease control. Monoterpene hydrocarbons and their oxygenated derivatives are the major components of plant's essential oils. These components inhibit potential antimicrobial activities and strongly inhibit microbial pathogens. Reports were made on some monoterpenes possess antifungal potentials against plant pathogenic fungi and many fungicidal (Nakatsu, Lupo, Chinn, & Kang, 2000), bactericidal properties (Lo Cantore, Shanmugaiah & Iacobellis, 2009). The natural pesticidal properties of some monoterpenes make them a potential alternative pest control agent. Also, it act as a good leading compound for the development of safe, effective, and fully biodegradable pesticides .

The increasing incidence of food-borne diseases as a result of contamination of foods with undesirable microorganisms during food processing, storage and distribution poses a serious public health problem (Oussalah, Caillet, Saucier & Lacroix, 2007). Microorganisms grow on food products, producing toxins that are hazardous to human health, some are capable of causing food spoilage and food-borne diseases in processed foods. Yeast and molds are capable of producing toxins, such as aflatoxin and citrinin, generate off-flavors, and cause discoloration and proteolysis.

Aflatoxins are produced by certain strains of *Aspergillus flavus* and are the most dangerous mycotoxins, thus the need to control fungal growth and mycotoxin formation. Preventing fungal decay in food products after harvest is increasingly challenging and novel preservation approaches that comply with food standards need to be developed.

The use of synthetic food preservatives to curb the deleterious effects of microorganisms is beset by problems of microbial resistance and food safety concerns, creating renewed interest in the preservation of food by natural preservatives. Preliminary experiments have shown that the EOs of various plants possess antifungal and antimicrobial activities with little or no risk of microbial resistance as they contain different antimicrobial substances having different modes of action (Paranagama, Abeysekera, Abeywickrama & Nugaliyadde, 2003). The antifungal and antibacterial activities of EO of lemongrass against different microbial species have been documented. In a report by Silveira et al. (2012) lemongrass essential oil was found to be most active compared with other EOs against 12 bacterial strains: *Staphylococcus aureus, Lactobacillus plantarum, Listeria monocytogenes, Enterococcus faecalis*, *Bacillus cereus*, *Bacillus subtilis*, *Escherichia coli*, *Salmonella typhimurium*, *Proteus vulgaris*, *Enterobacter aerogenes*, *Pseudomonas aeruginosa*, and *Yersinia enterocolitica*. Their results were consistent with reports by Mohd et al., (2010) who reported that lemongrass essential oil was effective against both Gram-positive and Gram-negative bacteria. These results demonstrate that the application of lemongrass EO in food products may provide better food safety and longer shelf life. The use of lemongrass essential oil (LGEO) were reported to have a significant reduction in the colony development of key postharvest pathogens such as *Colletotrichum coccodes, Botrytis cinerea*, *Cladosporium herbarum*, *Rhizopus stolonifer* and *Aspergillus niger* (Tzortzakis & Economakis, 2007).

Natural flavor complexes (NFCs) such as lemongrass oil exert their effects by binding at extremely low levels of exposure to the gustatory or olfactory systems receptor proteins to produce an identifiable sensation. However, at higher levels these molecules and their metabolites may become toxic; hence, NFCs are evaluated for safety based on the chemical composition and variability of their composition in commercial products (Hallagan & Hall, 2009).

According to the 22nd publication of Federal Emergency Management Agency expert panel, lemongrass oil is GRAS affirmed. This is because very low amounts of the oil are used as flavoring, hence the toxic congeneric components of the oil consumed are usually below threshold (1.80 mg/person/day). The higher concentrations of these congeneric components also effectively detoxified to yield metabolites that are eliminated from the body (Hallagan & Hall, 2009). Considering the various reports on the antimicrobial, antifungal, and organoleptic conserving activities of the LGEO against many spoilage microorganisms, it can be concluded that the oil possesses features that make it a good candidate for the bio-preservation and storage of food.

2.6 Lemongrass Essential Oil

Lemongrass essential oil (LGEO) is still as one of the most important EOs produced in the world, even though it has lost its commercial importance due to the production of synthetic citral. The EO of lemongrass is generally considered as safe and contains aromatic and flavoring components. Also, its antimicrobial properties have been widely investigated (Mishra & Dubey, 1994).

When added to food stuffs, the oil helps retain organoleptic properties and retard microbial activity by food spoilage microorganisms responsible for reducing food duality, spoilage and economic loss These properties suggest the potential use of lemongrass oil as a food preserving agent in an industry that has become complex due to the increasing number of food products requiring longer shelf life and the negative consumer perceptions of artificial preservatives. The main active ingredients of lemongrass EO 65-85% i.e., citral, neral, and geraniol have been shown to inactivate foodborne pathogens (Moore-Neibel et al., 2012).

Figure 2.5 Commercial Lemongrass Essential Oil

Table 2.2 Chemical Composition of Lemongrass Essential Oil (LGEO)

Chemical name	Composition
Linalool	1.34%
Geraniol	5.00%
Citronellol, nerol	2.20%
Cineole, citronellal	0.37%
linalyl acetate, geranyl acetate	1.95%
α-pinene	0.24%
Limonene	2.42%
Caryophyllene, β- pinene, β- thujene, myrcene	0.46%
β- ocimene	0.06%
Terpinolene	0.05%
Methyl heptanone	1.50%
α-terpineol	0.24%

Source Joy, Skaria, Mathew, Mathew and Joseph (2006)

Edible Coatings Combined with Essential Oils (EOs)

Edible coating made of polysaccharides/lipids bilayer is suitable to increase fresh and fresh-cut fruit storage life and to carry many functional ingredients such as antimicrobial and antioxidant agent Antunes, Gago, Cavaco & Miguel, (2012). The main objective of incorporating essential oils and their constituents into edible coatings is to use their antioxidant and antimicrobial capacity to improve edible coatings in horticultural commodities by increasing their shelf life.

One of the first works by (Pranoto, Rakshit & Salokhe, 2005) concerning the utilization of essential oils into edible coatings was explored. The authors reported that the antibacterial activity of garlic oil added to alginate-based film against *Staphylococcus aureus* and *B. cereus*, using agar diffusion method. Garlic oil was also assayed by incorporation in whey protein isolate films, demonstrated to be much less active than oregano essential oil against *Escherichia coli.*

The fight against molds and total flora was also surveyed by using essential oils of diverse aromatic plants as well as pure components incorporated in functionalized chitosan-based edible coating on strawberries. They found out that formulations based on modified chitosan containing limonene and tween 80 performed better than other formulations in strawberries (Vu, Hollingsworth, Leroux, Salmieri & Lacroix, 2011). Other diseases caused by a fungus (*Colletotrichum* spp.) include anthracnose which is responsible for considerable losses on tropical fruits. *In vivo* studies revealed that gum arabic combined with cinnamon oil was the best for controlling decay due to the infection by *C. musae* and *C. gloeosporioides* in artificially inoculated bananas and papayas, respectively. As seen in the table below different essential oil were coated on different fruits and vegetables.

Table 2.3 Lemongrass Essential Oils (LGEO) in Edible Coating on Fresh Produce

Lemongrass Essential Oil	**Fresh produce**	**Results**	**References**
LGEO & flaxseed Gum	pomegranate	Reduced weight loss, decayed ripening and disease incidence total soluble solid, pH and titratable acidity significantly varied over storage period.	(Yousuf & Srivastava 2017)
cassava starch	papaya MJ9	Inhibit the microbial and reduce mold & yeast during storage.	(Praseptiangga, Utami, Khasana & Kawiji, 2017)
LGEO + chitosan	mango, papaya and guava	The combination inhibits mycelial growth of *Colletotrichum species* (*C. asiarum*, *C. Siamese*, *Combination C. fructicola*, *C. tropicale* and *C.karstii*)	(Oliveiraa,Vieira, Câmara & Souzaa, 2018)
LGEO +cinnamon essential oil	papaya & banana	Coated fruit had lower weight and firmness loss, as well as lower reduction in titratable acidity and soluble sugar content during storage. Cinnamon and LGEO coated fruits had higher score for overall acceptability.	(Maqbool et al., 2011)
LGEO, chitosan + thyme oil	strawberry	Reduced weight loss and delays the change in anthocyanin content and microbial growth. Coating maintained firmness, TA, ascorbic acid and overall acceptability of strawberry fruits but no change on TS.	(Am et al., 2017)

CHAPTER 3

METHODOLOGY

3.1 Materials

3.1.1 Raw Materials

Fresh mature reddish-green tomatoes (*Lycopersicun esculentum*) (Figure 3.1) were collected from Lan Muang market with uniform size, color, maturity without any signs of mechanical damage or fungal decay. The surfaced of fruit were disinfected with 0.02% for 3 min and gently rinsed with distilled water, then air-dried for 40 min (Jiang & Li, 2001).

Figure 3.1 Maturity Stages of Tomato

3.1.2 Chemicals

All chemicals used in this study are shown in table 3.1.

Table 3.1 Chemicals Used in this Experiment

Chemical Agent	Sources
Carboxymethyl cellulose (CMC)	Nof corporation (Japan)
Lemongrass essential oil	Lapis Tropical Spa Production (China)
Tween 80	MERCK India
Ethanol	Merck KGA New Zealand
Potato dextrose agar (PDA)	M&P Impax India
Potato dextrose broth (PDB)	M&P Impax India
Glycerol	Thai Glycerin Co., Ltd. (Thailand)

3.2 Methods

3.2.1 Screening of Edible Coating Type

Preliminary research was conducted to investigate the potential biopolymer coating material from different protein and polysaccharides such as carboxymethyl cellulose (CMC), chitosan, guar gum, gelatin, cassava starch and soy protein. Firstly, CMC 2g in powder form was weighed and dissolved in 1L distilled water on a hot plate at 70 ℃ with continuous stirring using overhead stirrer (IKA-Labor Technik, Germany) at 2,000 rpm for 45 min followed by the addition 1% glycerol (w/w) as plasticizer. Stirring was continued for another 10 min at 70 ℃ and allowed to cool down at room temperature prior to addition of LGEO, (1% v/v-based CMC solution) and 1% (v/v) tween 80 to avoid oxidation with continuing stirring for 30 minutes at room temperature (Dashipour et al., 2015).

Guar gum coating solution was prepared by weighing 3g in 1L distilled water. Mixing was done with magnetic stirrer continuously for 30 min at 30 ℃ . After stirring, the samples were stored for 4 h to obtain homogenous state of gaur gum solution (Anjum, Zuber, Zia, Anjum & Aftab, 2021).Gelatin powder (3%) was weighed and dissolved in distilled water on a hot plate at 45 °C for 10 min followed by addition of 1% (w/w) glycerol and volume was adjusted to 1L. Powdered soy protein isolate (3%) was dissolved in 1L distilled water and solutions were prepared by dispersing the power in distilled water at room temperature for 1 h, followed by heating the dispersion on a hot plate at 85 °C for 30 min. Glycerol at 1% level was added as plasticizer. The mixtures were stirred for 1 h, at room temperature to make up 1L final volume and employed for coating. (Alves, Gonçalves & Rocha, 2017).

Furthermore, cassava starch powder 3% was dispersed in 1L distilled water placed on a hot plate at 70 ◦C with constant stirring using overhead stirrer and added with 1% glycerol followed by continuous stirring for 15min with 1500 rpm to make volume of 1L (Oriani, Molina, Chiumarelli, Pastore & Hubinger, 2014). Chitosan 3% was dispersed in distilled water and heated at 40 ◦C for 2h and added with 2g of lactic acid to make final volume of 1L (Jiang & Li, 2001).

3.2.2 Screening of Type and Concentration of Essential Oils for Tomato Coating

Three kinds of essential oils (lemongrass, oregano, and clove essential oil) at 0.5%, 1% and 2% were used for *in vitro* antifungal property study against *A. niger* by disk diffusion method and also coated on tomatoes for consumer acceptance test. The sensory evaluation of three essential oil were evaluated by 10 trained panelist and hedonic scoring test was performed to evaluate samples. The results of score range test was converted into 0-1 point represented extremely dislike, 2 was fair, 3-4 is good, 5 was excellent. The essential oil with the highest antifungal property and consumer acceptance were chosen for the next experiment.

3.2.3 Screening for Minimal Inhibitory Concentration of Essential Oil against *A. Niger*

3.2.3.1 Disk diffusion

The disk diffusion assay was determined using the method of Bauer, Kirby, Sherris and Turck (1966). Materials utilized in this experiment were all sterilized before use, *A. niger* were bought from Thailand Bioresource Research Center (TBRC). This fungus was sub-cultured on sterilize a sterilize potato dextrose agar plates for 5 days before used. Different concentration of essential oils at 0.5%, 1% and 2% were prepared. One hundred µL of each leveled of prepared EOs were mixed with sterilized potato dextrose agar (PDA) and allowed the combination to solidify on petri dishes which took 30 min in a laminar flow cabinet.

The spores of the fungal from *A. niger* were suspended in sterile distilled water. Following serial dilution and next to spore counted with the use of hemocytometer. The spore suspension was serially diluted to attain 10^6spores/ml of *A. niger* and 25mL of diluted suspension was mixed with 150 ml of potato dextrose agar (PDA). PDA inoculated *A. niger* was poured into the sterile petri dish. Furthermore, sterilized filter paper disk (0.6 cm diameter) were loaded with different concentration of 0.5, 1, and 2% oregano, clove and LGEO at (50 µL). These disks were placed on a petri dish and incubated at 25 ^{o}C for 48 h which the clear zone indicted the inhibitory area.

3.2.3.2 Minimal Inhibitory Concentration (MIC) and Minimal Fungicidal Concentration (MFC)

MIC and MFC assay were investigated using the method of Tayel, Moussa, Salem, Mazrou & El-Tras (2016) with some modifications. Ten µL of EOs were mixed with 100 µL potatoes dextrose broth (PDB) and 10 µL of fungi were prepared and loaded into sterile 96-well microplate and incubation at 25^{o}C for 48 h. Absorbance of turbidity induced by fungal growth was measured using microplate reader to know MIC values of different levels of EOs.

MFC was determined by using the same concentration of EOs tested for MIC The selected concentrations from MIC with the lowest concentration were sub-cultured on a PDA plate, and stored at 25 °C for 48 h to know the MFC values of different levels of tested EOs. The level of EOs showing lowest concentration against the growth of fungi was recorded and was used in this experiment as (Tayel et al., 2016).

3.2.4 Coating Solution Preparation

Coating solutions were prepared as described by Dashipour et al. (2015) with some modifications. CMC solutions were prepared by dissolving 2% of CMC in 1L of distilled water on hot plate using overhead stirrer (IKA-Labor Technik, Germany) at 2,000 rpm for 45 min followed by addition of 1% glycerol (w/w) as plasticizer. Stirring was continued for another 10 min at 70 ℃ and allowed to cool down at room temperature prior to addition of LGEO, 1% (v/v-based CMC solution) and 1% (v/v) tween 80 to avoid oxidation with continuing stirring for 30 min at room temperature. As seen in Table 3.2 entails compositions of each treatment.

Table 3.2 Compositions of Edible Coatings

Treatments	LGEO % (v/v)	CMC % (w/w)	Tween 80 %(v/v)	Glycerol % (w/w solid)
Control (non-coating)	-	-	-	-
2% CMC	-	2	1	1
1% LGEO	1	-	1	1
2% CMC + 1%%LGEO	1	2	1	1

3.2.5 Coating Application

Tomatoes were divided into four batches. Each batch consisted of four replications and each replication contained four fruit. Untreated batch was used control, while treated batches were coated with 2% CMC, 1% LGEO, and 2% CMC+ 1% LGEO, respectively. Prior to coating, tomatoes were soaked 3 min in 0.02% sodium hypochlorite solution as disinfectant and dried at room temperature for

1 h. CMC along with LGEO coating with applied by dipping method. Tomatoes were dipped in coating solutions for 1 min, drained, and dried at room temperature for 2 h. Tomatoes were then placed in pet clamshell boxes and stored at 13 ℃ and 85% RH.

For *in vivo* antifungal assay, tomatoes were punctured with an inoculated needle containing 10 µL drop of fungal spore suspension of *A. niger* (10^6 spores/mL). Finally, the fruits were placed in pet clamshell boxes and stored in the refrigerator at 13℃ and 85% RH for 30 days. Quality changes such as weight loss color, firmness, total acidity (TA), total soluble solid (TSS), disease incidence, disease severity and sensory evaluation were determined with an interval of 5 days up to 45 days of postharvest storage.

3.2.6 Qualities Determination of Tomatoes

3.2.6.1 Weight loss of tomatoes

Tomatoes without and with coating treatments were weighed using digital balance (Presica 400c, Zurich, Switzerland) with an interval of 5 days from day 0 to 45 days of storage. The results were calculated as percentage of total weight loss between initial and final weight with the following equation (1):

$$\text{Weight loss} = \frac{\text{initial weight} - \text{final weight}}{\text{initial weight}} \times 100\% \qquad (1)$$

3.2.6.2 Color Measurement

The color of tomato was measured by colorimeter, Ectrophotometer CM-600d (Konica minolta, inc. Japa)). The color values were expressed as L* (lightness), a*(redness (+)/ greenness (-), and b* (yellowness (+) (Batu, 2004).

3.2.6.3 Firmness

Fruit firmness was evaluated by measuring newton force (N) punctured onto sample surface using TA-XT plus texture analyzer equipment (Stable Micro System, Texture Technologies, England). The measurement was done by using 6 mm cylinder probe with 2 kg load cell for 70 mm depth and maximum puncture speed at 15 mm/min (García, Ventosa, Diaz, Silvia & Casariego, 2014).

3.2.6.4 Total Soluble Solids (TSS) and Total Acidity (TA)

Tomatoes were cut and blended to extract juice from each treatment and analyzed for TSS measurement by using an automatic refractometer (Atago., Ltd., Japan). The equiptment was standardized using distilled water before each measurement and the results were expressed as ˚Brix (Al-Dairi et al., 2021). The total acidity was analyzed using 1 mL of the juice diluted in 49 mL of distilled water. Then the acid percentage of diluted solution was accessed using pocket acidity meter (PAL-Easy Acid F5).

3.2.6.5 Disease Incidence for Un-Inoculated Fruit

Disease incidence, was measured by counting the infected tomatoes and divided by the total number of tomato fruit (Mathews, Basha & Reddy, 2011). The percentage of disease incidence was calculated by using formula (2):

$$\%\ \text{disease incidence} = \frac{\text{Infected fruit}}{\text{Total fruit}} \text{x} 100 \quad (2)$$

3.2.6.6 Disease severity

Tomato samples treated with different coating materials were evaluated for disease severity (DS) with slightly modifications (Nešić et al., 2019). Scores between 0 to 4 were given by 10 trained panelists, such as, 0 = no visible symptoms of disease on the fruits; 1=1-25% of area covered by slight necrotic and fungi mycelia; 2 = 26-50% of area covered by slight necrotic and fungi mycelia; 3 = 51-75% of the fruits infected; 4 = 76-100% of fruits with fungal mass and the appearance of fruit softness and decay. Percentage of disease severity was calculated with the following equation (3):

$$\%\ \text{disease severity} = \frac{\text{sum of all disease scores}}{\text{infected fuits } \times \text{ maximum scores}} \times 100$$

3.2.6.7 Sensory evaluation

Sensory evaluation was conducted for acceptability of fruit appearance (color, skin appearance, and glossiness) was accessed by 10 trained panelists as per the method of (Ali, Ong & Forney, 2014) with some modifications. Hedonic scale of 0-9 points was employed to evaluate samples in which 0-2 point represented extremely dislike, 3-5 is fair, 6-8 is good, 9 is excellent (Abera, 2013).

3.2.7 Statistical Analysis

All the experiment were carried out in a completely randomize design (CRD) with three coating treatments and 16 replications for each treatment. The analysis of variance was performed using statistical software SPSS (version 16, SPSS Inc., Chicago, IL), Duncan’s multiple range test was used at the level of significance t $p \leq 0.05$ for comparing differences among the mean value

CHAPTER 4

RESULTS AND DISCUSSIONS

4.1 Evaluation of Essential Oils

From the preliminary study, out of six edible coatings tested cassava starch, gelatin, guar gum, soy protein isolate, chitosan, gelatin and CMC for the determination of the best adhesiveness with good appearance of coating on fruit surface. Hence, CMC was selected as the coating material for tomatoes in this research. For adding antimicrobial property to the coating, antimicrobial activity against *Aspergillus niger* of three essential oils lemongrass, thyme, clove oil were determined by disk diffusion method and then checked the odor acceptability were evaluated by 10 people when applied on tomato. Consequently, lemongrass essential oil LGEO was selected for this study Figure 4.1

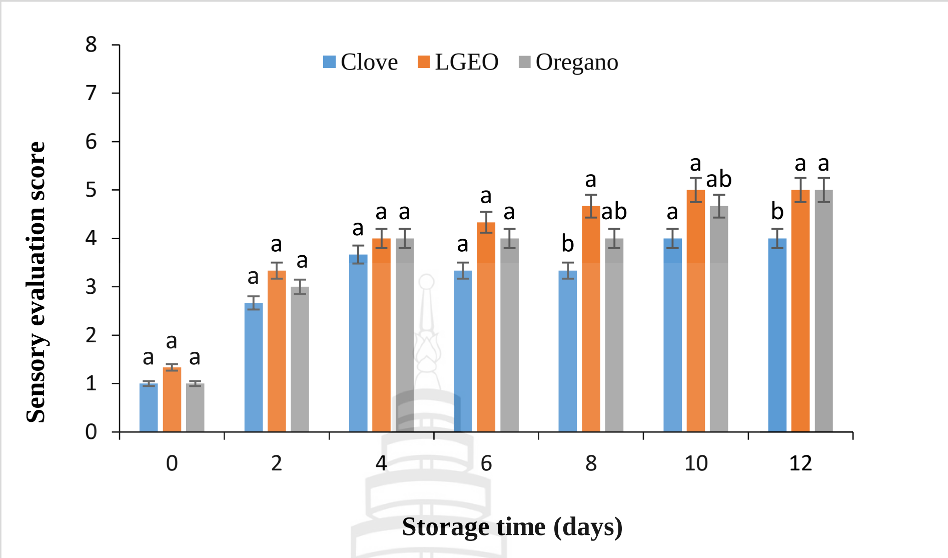


Figure 4.1 Screening of Different EOs for CMC Coating in Tomatoes

4.2 Antifungal Activity of Lemongrass Essential Oil

The antifungal activity of lemongrass essential oil (LGEO) were investigated using disk diffusion test by Bauer et al., (1966) at various concentrations of LGEO (0.5%, 1% and 2%) against *A. niger* (Table 4.1). The disk diffusion result represented that 1% and 2% (v/v) LGEO could inhibit the growth of *A. niger*. Both of MIC and MFC of LGEO against *A. niger* was 0.5 % (v/v). Therefore, 1% LGEO was used with CMC coating application in tomatoes.

Fungal mycelial growth was rapid for the control as seen on the PDA plate without LGEO. LGEO at 1% and 2% level treated plates showed the lower fungal growth than the plates with 0.5% LGEO and control without any treatment. The combination of 2% CMC and 1% LGEO was more effects against fungal growth as compared to 2% CMC coated fruits and 1% LGEO, respectively. EOs combined with polysaccharides have been documented to suppress the fungal due the strong aroma of volatile bioactive compounds on fruit surface (Ali, Noh & Mustafa, 2015).

Table 4.1 Antifungal Activity of Different LGEO Concentrations Against *A. Niger* Determined by Disk Diffusion Method

LGEO (%)	Disk diffusion diameter of clear zone (mm)
Control	N.D.
0.5% LGEO	5.6
1% LGEO	14.0
2% LGEO	23.1
0.1% Carbendazim	27.4

Therefore, the results of disk diffusion confirmed that 1% and 2% LGEO (v/v) could inhibit the growth of *A. niger* (Table 4.1). The MIC and MFC results also showed that 1% LGEO was the minimal concentration that could inhibit the growth of *A. niger*. Thus, 1% LGEO was supplemented in CMC coating of tomatoes.

4.3 Effects of CMC Coating Incorporated with LGEO on the Qualities of Tomato during Storage

Incorporation of essential oils into polysaccharides based edible coatings has gained interest in the agricultural science owing to the antibacterial and fungicidal properties associated with these volatile compounds (Mehdi, Asgar & Alderson, 2010). The antimicrobial activity of LGEO has been demonstrated against a range of microbes, and incorporated it in edible coatings applied on banana and papaya fruits (Maqbool et al., 2011). Moreover, LGEO has been successfully used in various concentration for the control of anthracnose on avocado (Mpho, Sivakumar, Sellamuthu & Bautista-Baños, 2013).

In this experiment, the effect of CMC coating incorporated with LGEO was employed to assess the qualities of tomatoes. The sample was divided into 4 treatments: control or uncoated, 2% CMC coated, 1% LGEO coated, and 2% CMC +

1% LGEO coated fruits. The qualities of tomato fruit such as weight loss, fruits firmness, TSS, TA, color change, disease incidence and severity were determined during storage at 13℃ and 85% RH for 30 days. The antifungal effect of coating was also investigated in the inoculated tomatoes, stored at 13℃, 85% RH for 45 days.

4.3.1 Weight Loss

Weight loss is one of the main problem that could affect the quality of fresh products during postharvest storage (Arreola, Raposo, Morais & Morais, 2009). The results attained weight loss in without and with coatings of tomatoes at 13ºC and 85% RH are presented in Fig 4.2. The weight loss of the control fruits (7.86%) increased significantly ($p<0.05$) and was lower in 2% CMC coated fruits (6.53%). Similarly, 1% LGEO had less weight loss than control sample. However, percent weight loss was observed to increased gradually in all treatments during the storage period of 30 days at 13 ºC and 85% RH. Fruits coated with 2% CMC showed lower weight loss compared to 1% LGEO coated fruits (7.06%). The percent weight loss obtained in 2% CMC+1% LGEO sample (6.26%) was significantly reduced due to potential barrier properties and efficacy of LGEO against fungal decay during storage. These results were in line with the reduces weight loss of fruits coated with chitosan and cinnamon oil (Xing, Xu, Che, Li & Li, 2011). The combination of essential oils and polysaccharides could delay the ripening and reduce the weight loss of fruits (Ghaouth, Arul, Grenier & Asselin, 1992). Similar results had been reported about the decreases in moisture loss of coated fruits with chitosan and LGEO because of the higher water vapor barrier properties (Am et al., 2017). The mechanism for these beneficial effects is based on the hygroscopic properties that could act as a barrier to diffusion of moisture between the fruit and the environment (Morillon et al., 2002). The reduction in weight loss might be also referred to the semi-permeable barrier properties of polysaccharide coatings with essential oils against O_2, CO_2, solute movement and moisture migration that could delay respiration rates, water loss and redox reactions during storage (Al Juhaimi, Ghafoor & Babiker, 2012). In general, the increase in weight loss of fresh fruits during storage may be due to loss in moisture content of the fruit and through respiration process (Amal, El-Mogy, Aboul Anean &

Alsanius, 2010). Therefore, these results can be concluded that CMC coatings combine with 1% LGEO has prevented percent weight loss during 30 days of storage at 13 ºC and 85%RH.

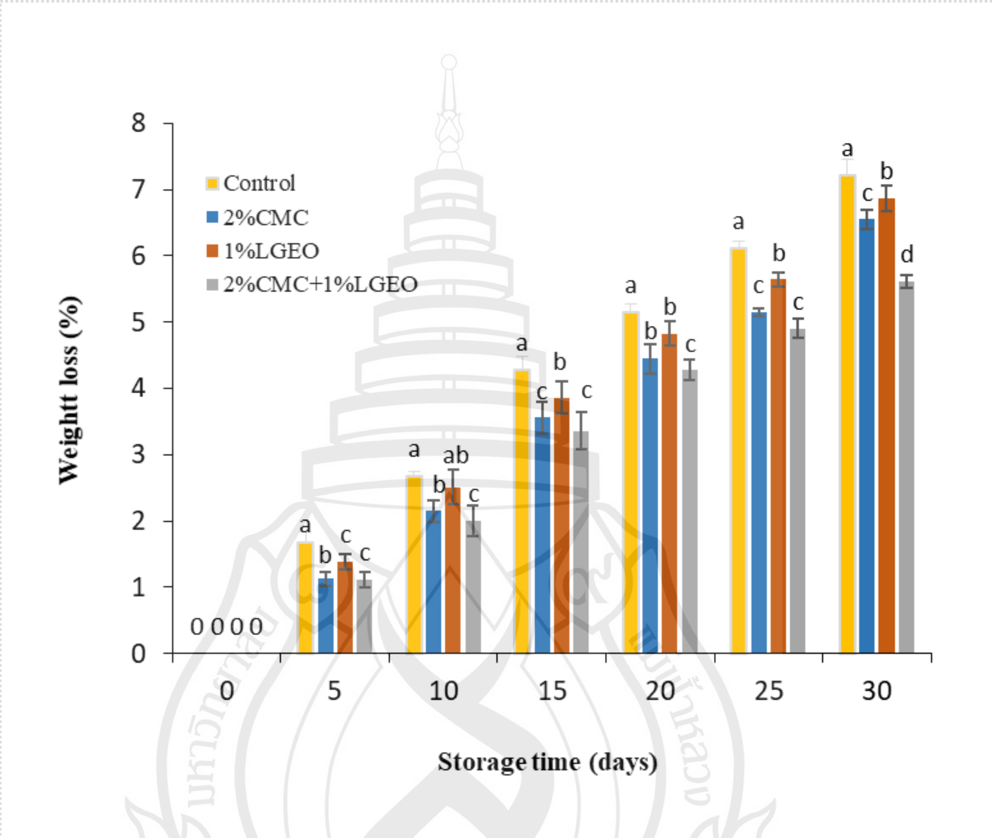


Figure 4.2 Effect of Edible Coating on Weight loss of Tomatoes during 30 Days of Storage at 13 °C and 85% RH

4.3.2 Color Values

Color values were measured to determine the color charges during storage (Figure 4.3). Color changes during ripening of tomatoes undergoes chlorophyll degradation and lycopene development during postharvest storage. Color changes were observed for control and treated samples throughout storage and lower color changes were observed in the combined treatment of LGEO+CMC coated fruits.

Changes in color indicated the progression of fruit ripening related to both chlorophyll degradation and lycopene development (Saad, Ibrahim & El-Bialee, 2016). The combination of 2% CMC + 1% LGEO played a significant role in the retardation of color changes. Changes in color for the 1% LGEO treatment were slightly different from the control.

At 30 days of storage, L* of all fruits decreased and a* value gradually inclined as seen in Figure 4.3A and 4.3B. Control group show the highest a* increase compared to the other treated samples coated with CMC, 1% LGEO, and their combination. Additionally, 2% CMC+ 1% LGEO treatment effectively delayed color changes evidenced by the lower a* and higher in L* values compared to the control and other treatments. Thus, the incorporation of CMC along with 1% LGEO coating potentially delayed the color charges and ripening process of tomatoes fruits during 30 days of storage at 13 °C and 85%RH.

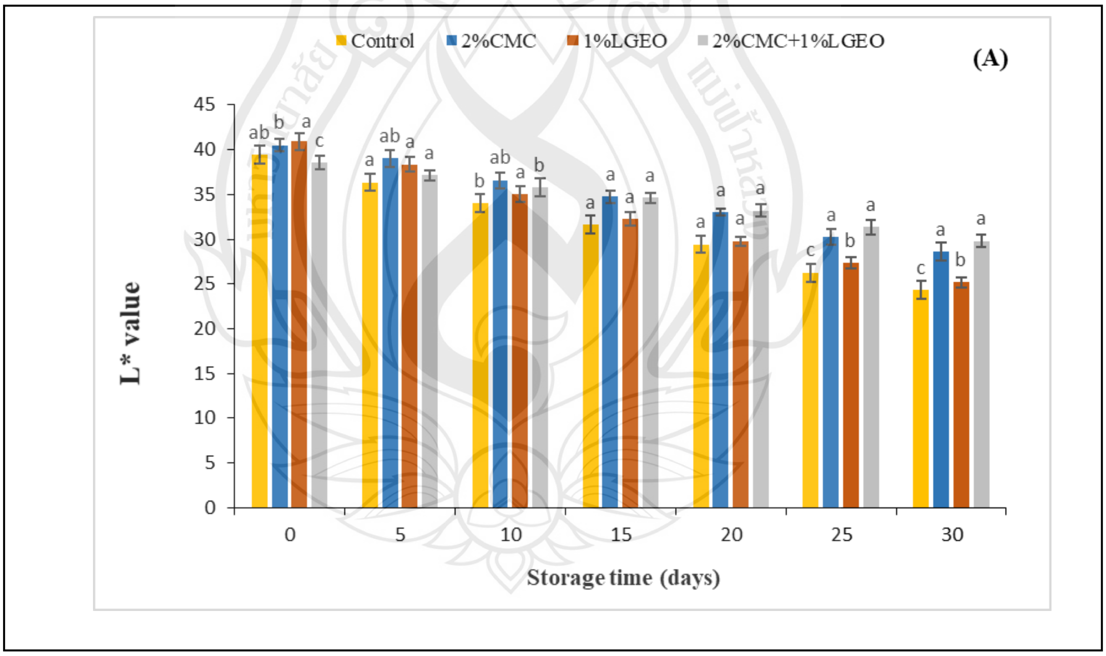


Figure 4.3 Effect of Edible Coating on (A) L* and (B) a* of Tomatoes during 30 Days of Storage at 13 °C, 85% RH

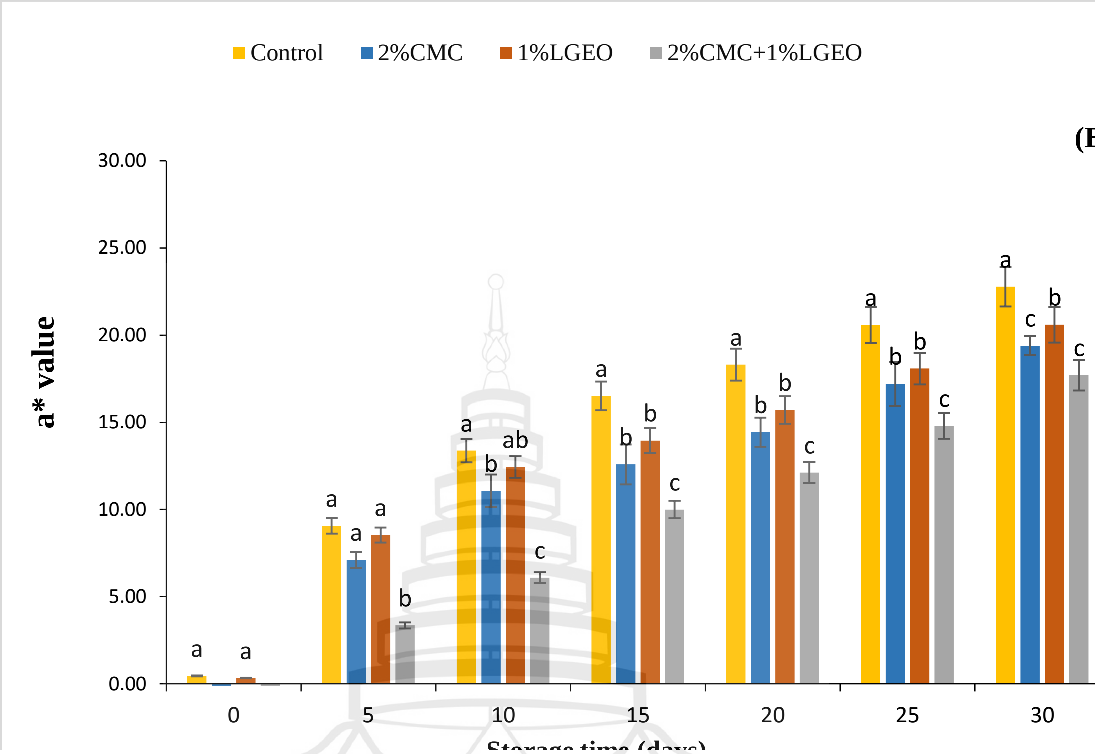


Figure 4.3 (continued)

4.3.3 Total Soluble Solids (TSS) and Total Acidity (TA) Content

The values of TSS content treated samples showed a significant difference compared to control (Figure 4.4 A). The increment in TSS was reported due to ripening process during storage (Tasdelen & Bayindirli, 1998). Initially, TSS content in the control sample was lower (2.42%) compared to the end of the storage (4.47%) ($p<0.05$). From day 0 to day 30 of storage, TSS of the fruits treated with 2% CMC ranged between 2.42% to 3.95%, respectively. LGEO treated fruits indicated initial TSS of 2.45% and raised to 4.15% during storage. Fruits treated with CMC+ 1% LGEO exhibited the lowest TSS from day 0 (2.42%) to day 30 (3.7%) of storage (Figure 4.4 B).

The TA content in the control samples was significantly higher at day (05.7%) and was lower at day 30 (2.12%) of storage. Similarly, TA of fruit treated with 2% CMC ranged between 5.8% to 2.70% during the 30 days of storage. LGEO treated fruit indicated initial TA of 5.72% final TA values of 2.45% at day 30 of storage. The results showed that TA content was higher in the coating with CMC + 1% LGEO due to delayed ripening process that gave the lowest TSS values compared to the control and other treatments during 30 days of storage.

The lower TSS and higher TA contents obtained in the combined coating treatment could be correlated with the least rate of respiration and metabolic process. Moreover, the interactions between the essential oil and cell membrane of fruit could affect the fruit metabolic processes (Perdones et al., 2016). Similar results TA content were reported in tomatoes in which TA and TSS was minimum in the tested fruits (Raffo, Leonardi, Fogliano, Ambrosino & Quaglia, 2002). It has been also reported that decreases in acidity and increases TSS of tomato fruits as a result of conversion of organic acids into sugars during respiratory metabolism as evidenced by the degradation of carbohydrates, pectin, partial hydrolysis of protein, and decomposition of glycosides into sub-units during respiration (Anthon, Strange & Barrett, 2011; Hernández Suárez, Rodríguez Rodríguez & Díaz Romero, 2008) documented that the physio-chemical like TSS and TA contents may be also influenced by several other factors such as cultivar, regions and season of production. In this experiment the acid content of the whole tomato fruit decreased at the end of the storage period. Edible coating could delay the changes of acidity, TSS, slowed lower gas transfer with increased CO_2 atmosphere might retard ethylene production and delayed fruit ripening during storage. Therefore, tomato fruit coated with 2% CMC + 1% LGEO had greater acid content than the control and other treatment.

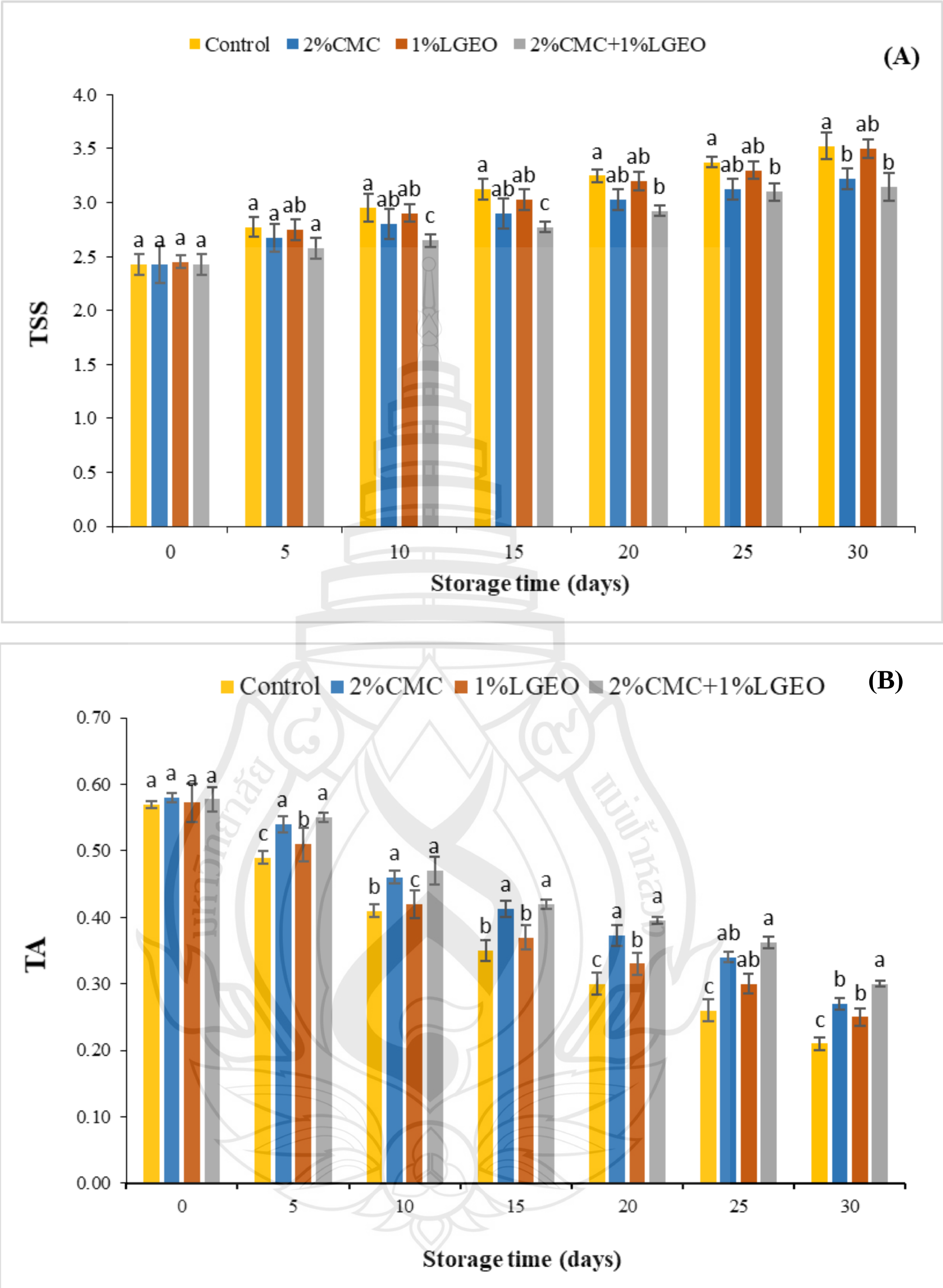


Figure 4.4 Effect of Edible Coating on (A) Total Soluble Solid (TSS) and (B) Total Acidity (TA) of Tomatoes during 30 days of Storage at 13 °C, 85% RH

4.3.4 Firmness

The firmness of control fruits significantly ($p \leq 0.05$) decreased during storage time. Fruits coated with 2% CMC+ 1% LGEO had significantly ($p \leq 0.05$) higher firmness values during storage than the control and fruit firmness decreased gradually during the storage period. The results obtained indicated that CMC+ 1% LGEO significantly ($p \leq 0.05$) retained the firmness of fruits (Figure 4.5). At the end of the storage control clearly showed the lowest firmness. Softening of fruit usually occurred due to deterioration a factor that affect firmness of fruits due to the cell structure, cell wall composition and intracellular materials. The biochemical process involving the hydrolysis of pectin and starch by enzymes are wall hydrolases (Ali, Muhammad, Sijam & Siddiqui, 2011). As the process of fruit ripening progresses, shortening of chain length of pectin substances occurs with an increase in pectin esterase and polygalacturonase activities (Maftoonazad & Ramaswamy, 2005) . The coating of fruits can be expected to modify the internal gas composition of fruits especially reducing the oxygen concentrations and elevating carbon dioxide concentration which might explain the lower textural changes in the coated fruits (Maftoonazad, Ramaswamy & Marcotte, 2008) in this experiment, polysaccharides coatings were able to retained firmness compared to control. Similarly, (Am et al., 2017) reported that refrigerated strawberries coated with wheat gluten-base film retained their firmness better than control fruit. The results of this experiment can be concluded that CMC incorporated with LGEO coatings-maintained firmness of tomato fruits for 30 days of storage at 13 ºC ,85% RH.

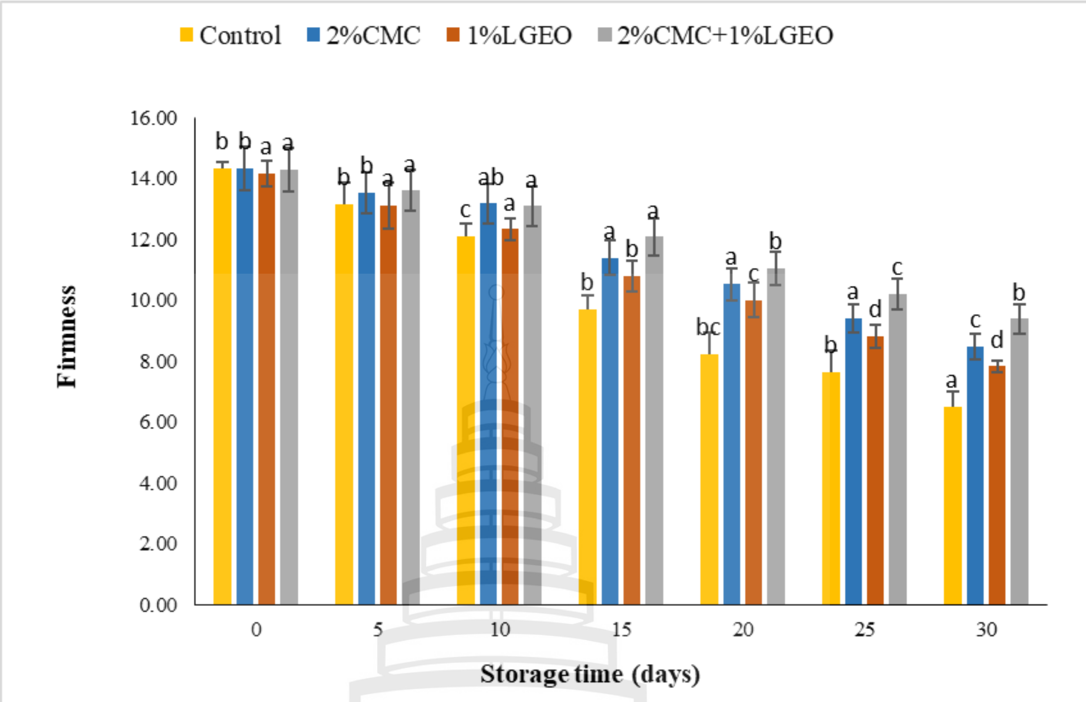


Figure 4.5 Effect of Edible Coating on Firmness of Tomatoes during 30 Days of Storage with 13 °C and 85% RH

4.3.5 Diseases Incidence and Severity of Coated Fruits without *A. Niger* Inoculation

Disease incidence and severity of non-inoculated tomatoes without and with coating treatments are presented in Figure 4.6. It was noted that disease incidence increased with the advancement of storage period in all the treatments. However, the disease incidence was higher in control fruits without any treatment. At first, diseased sports appeared on the on the day 15 of storage on the control fruit was 12.25 %, while at day 45 of storage 100 % of the fruit surface was infected ($p<0.05$) compared to the CMC, 1% LGEO and CMC + 1% LGEO samples. Fruits coated with CMC showed 12.5% of disease incidence at day 20 and 68.25 % at day 45 of storage, respectively. The 1% LGEO coated fruit had a disease incidence of 6.25 % at 25 days and 37.5% at 45 days of storage. Moreover, CMC + 1% LGEO coated fruits showed lowest disease incidence (6.25%) at day 30, while as it reached to a limit of 31.25%

up to 45 days of storage (Figure 4.6 A). The film contained 1% LGEO had the lowest disease incidence because of the antimicrobial properties of the oil against pathogens. Furthermore, it was evidenced that 1% LGEO and CMC coated fruits had very strong antimicrobial efficacy to prevent disease incidence of tomatoes during extended storage time.

Disease severity was found to be highly significant between all the treatments during the entire storage. The lowest severity was seen in coated fruits compare to the control ($p<0.05$) during storage. Among all treated fruits, higher disease incidence was observed in CMC treatment with LGEO and the lowest was observed in CMC + 1% LGEO coated fruits (Figure 4.6 B). This indicate that CMC did not have antimicrobial activity and was not effective to prevent the diseased spots on fruit body during extended storage time. Therefore, tomatoes treated with 2% CMC with 1% LGEO can inhibit microbial growth up to 30 days of storage and also maintain the disease-free quality of tomato at 13 °C and 85% RH.

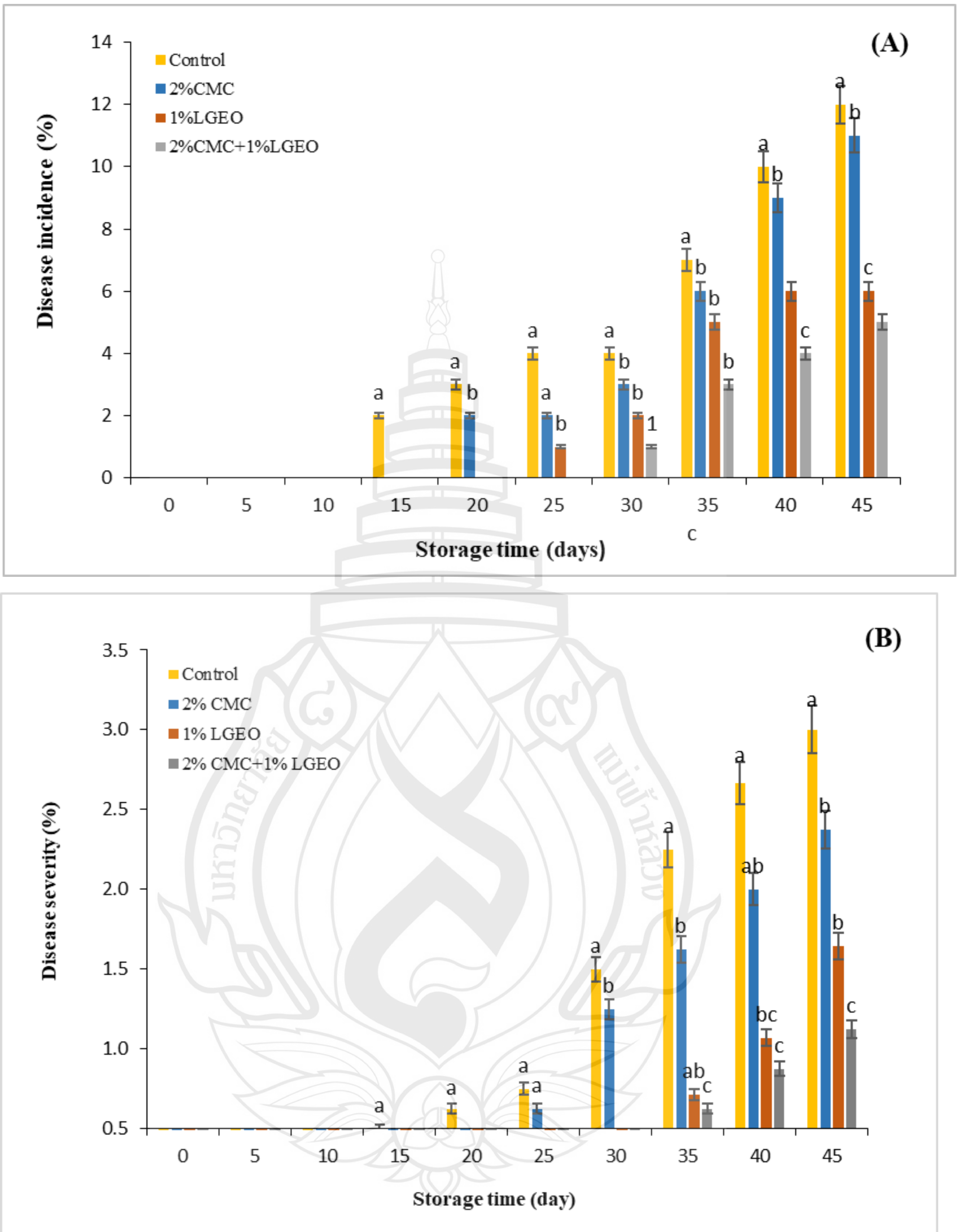


Figure 4.6 Effect of CMC Coating Combined with (LGEO) on (A) Disease Incidence of Non-Inoculated Tomatoes and (B) Disease Severity of Non-Inoculated Tomatoes during 45 Days of Storage at 13 °C, 85% RH

4.3.6 Appearance of *A. Niger* Inoculated and Non-Inoculated Tomatoes During Storage

The photographs displaying the external appearances of inoculated and non-inoculated tomato fruits such as control, CMC, LGEO and CMC + LGEO coating treatments have been shown in Figure 4.7 and 4.8, respectively. Tomatoes coated with CMC, 1% LGEO and showed lower disease incidence than control. These results reconfirmed that CMC+ 1% LGEO coating could delay the color changes and incidence of disease in tomatoes at 13ºC and 85%, RH.

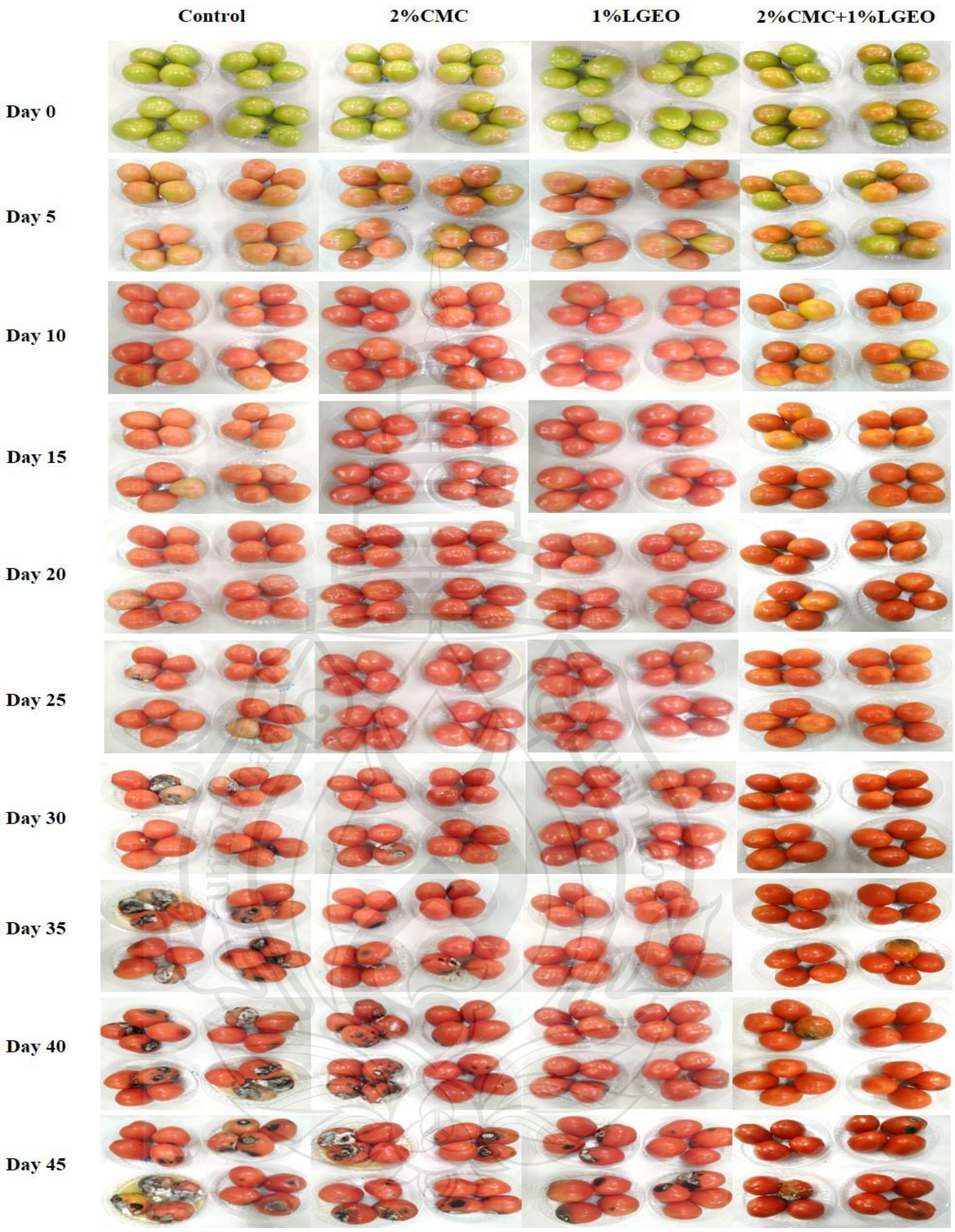


Figure 4.7 Effect of CMC Coating Combined with LGEO on the Appearance of Tomatoes during 45 Days of Storage at 13°C and 85% RH

4.3.7 Disease Severity of Inoculated Tomatoes

Disease severity values of tomatoes with prior inoculation of *A. niger* are shown in Figure 4.9 C. Fungal growth in tomatoes inoculated with A. *niger* began in control sample at day 15 of storage. The growth of A. *niger* on CMC inoculated treatment started at day 20 after which showed the highest severity among the coated treatment. The severity of inoculated sample treated with 1% LGEO began at 25 days of storage followed by inoculated fruits coated with CMC + 1% LGEO began at day 30 of storage. Therefore, use of essential oils (EOs) in coatings could delay spoilage and extend the shelf-life of fresh tomatoes due to active release of antifungal compounds supplemented in polysaccharide-based coatings (Sivakumar & Bautista-Baños, 2014). Moreover, inoculated control sample shower higher fungal growth compared to the inoculated tomatoes with CMC during storage. The storage study of disease incidence tomatoes of was continued up to 45 days in which, inoculated fruits such as control and CMC treated samples were totally destroyed at day 45 of storage (Figure 4.9 A and B). CMC can only act as a semi-permeable membrane film preventing ripening and senescence but lacks antimicrobial activities (Kumar, Baswal, Ramezanian Gill & Mirza, 2021). In this experiment A. *niger* showed rapid growth on of the inoculated tomatoes without any treatment in comparison to the delayed growth in 1% LGEO coated tomatoes ($p<0.05$). Thus, CMC with 1% LGEO coating could inhibit microbial growth on the pre-inoculated tomato sample with *A. niger* for a period of 30 days of storage.

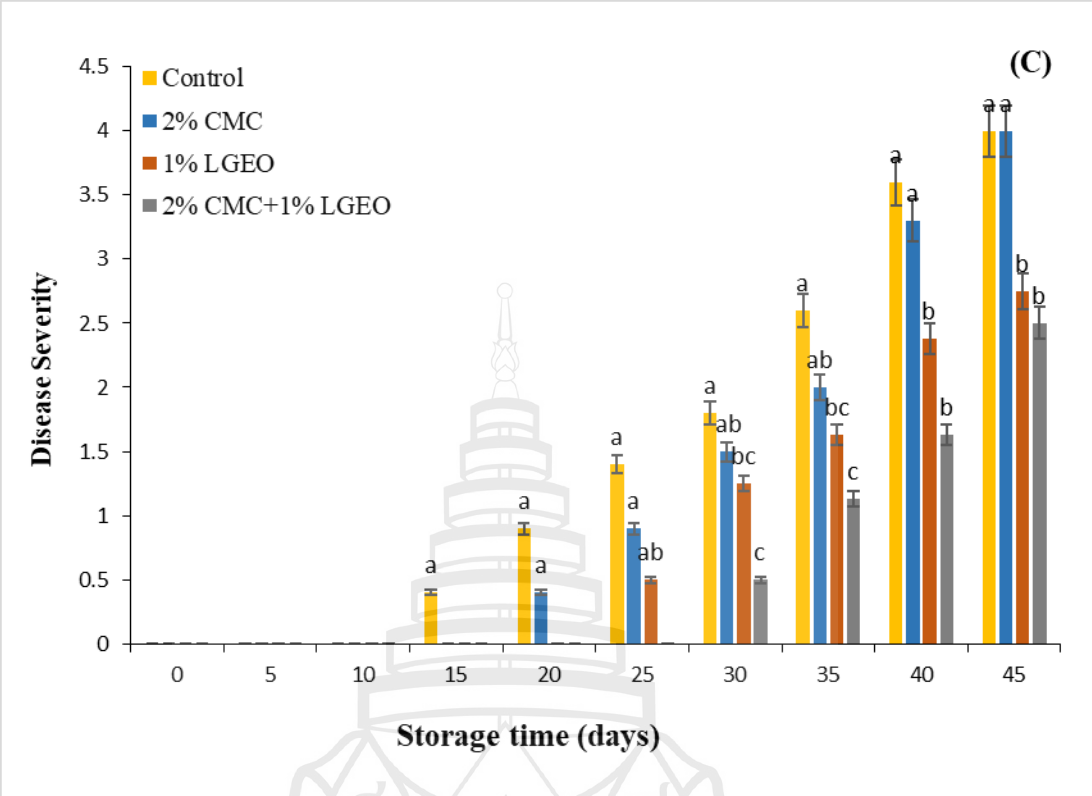


Figure 4.8 Effect of CMC Coating Combined with LGEO on (C) Disease Severity of Inoculated Tomatoes during 45 days of Storage at 13 °C, 85% RH

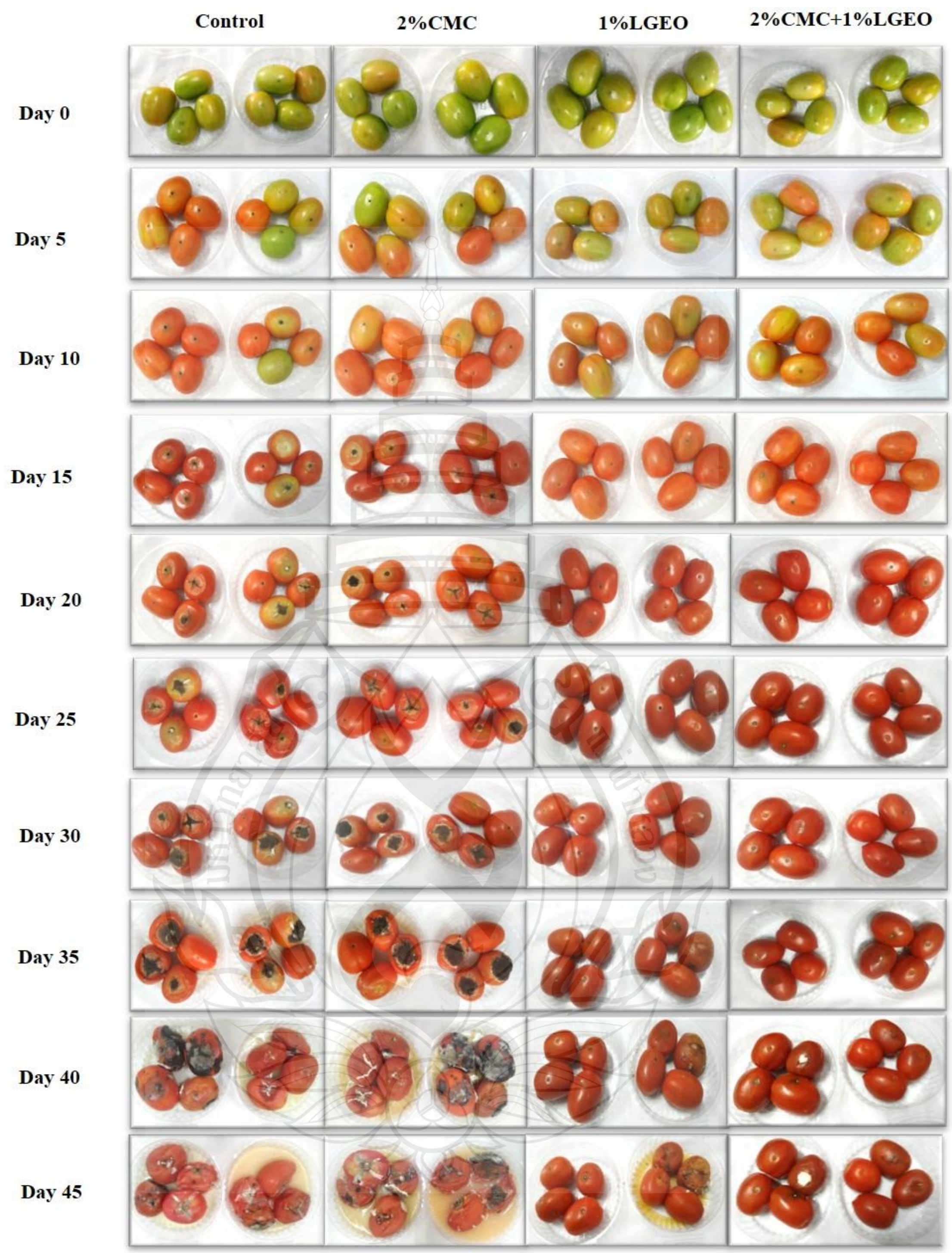


Figure 4.9 Effect of Edible Coating on Disease Severity of Inoculated Tomatoes during 45 Days of Storage at 13℃, 85% RH

4.3.8 Sensory Evaluation

Coatings supplemented with LGEO improved the sensory quality and extended the shelf life of tomatoes when compared to control. Control was acceptable up to 10 days while fruit samples coated with 2% CMC and 1% LGEO and their combination 2% CMC with 1% LGEO were had the satisfactorily color, skin appearance and overall quality within 30 days of postharvest storage. The control sample spoiled and was completely unacceptable after 10 days, whereas 2% CMC coated fruits retained their quality up to 20 days of storage. Additionally, 1% LGEO coated fruits quality retained quality until 25 days and the combination of CMC with LGEO samples had the good quality attributes up to 30 day of storage. In general, all the treated samples showed no differences between sensory quality up to 10 days with that of the control fruits. The control sample started deterioration from day 10 of storage. There were significantly differences ($p<0.05$) between fruits treated with 2% CMC, 1% LGEO and CMA+LGEO, respectively (Figure 4.10). Among the treated fruits, CMC alone showed less protection, thereby resulted in shorter storage life compared to LGEO coated fruits with antimicrobial properties. LGEO has been reported to coagulate the cytoplasm of spoilage and pathogenic microbes due to the presence of monoterpenes in lemongrass oil (Edirisinghe, Ali, Maqbool & Alderson, 2014). CMC possesses film-forming properties which allowed it to act as a barrier on tomato surface (Nešić et al., 2019). Thus, the combined effect of CMC+LGEO coating preserved the sensorial quality of tomatoes up to 30 days of postharvest storage.

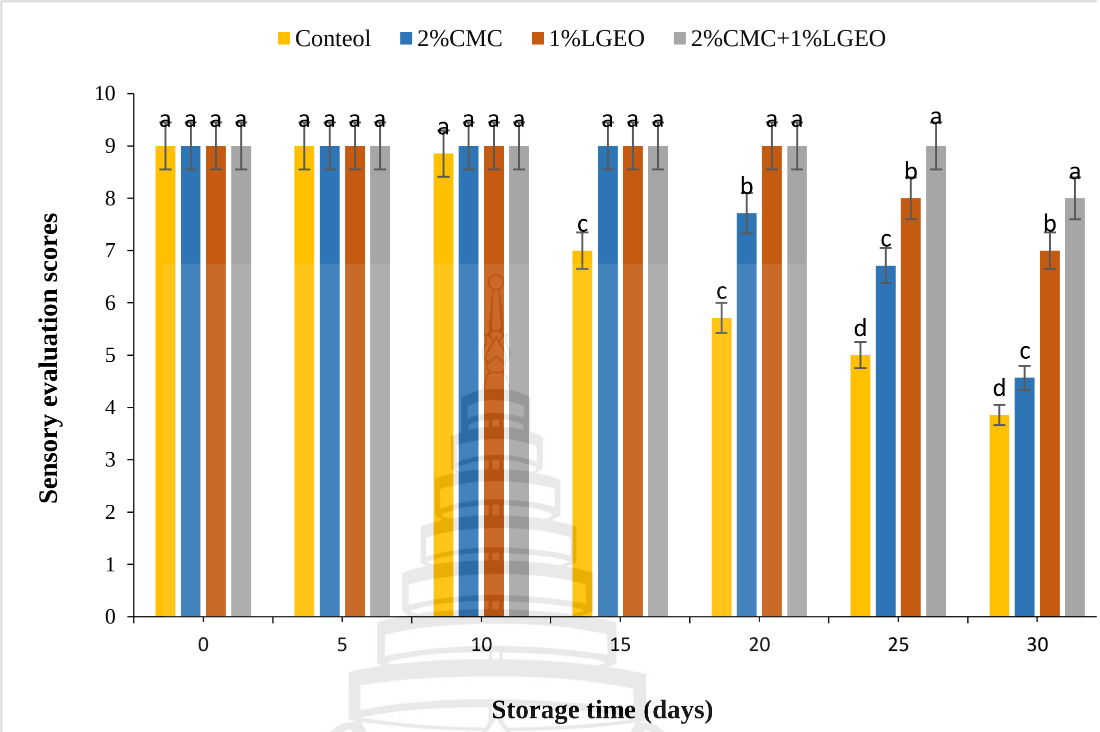


Figure 4.10 Sensory Evaluation of Tomatoes with and Without Edible Coating during 45 Days of Storage at 13 °C and 85% RH

CHAPTER 5

CONCLUSIONS AND SUGGESTIONS

5.1 Conclusions

CMC coating fortified with 1% LGEO can be an effective method to improve the quality and extending the shelf-life of tomatoes. CMC and LGEO proved to be a potential moisture barrier, thereby preventing the weight loss and delaying fungal growth on the coated fruits. Edible coating maintained the firmness, color, TSS and TA of all the coated. CMC and 1% LGEO coated samples exhibited higher sensorial scores. The combination of CMC coating with LGEO can extended the shelf life of tomato from 10 days to 30 days at 13°C and 85% RH.

5.2 Suggestion

The coating combines with CMC+ LGEO was successful to reduce fungal growth in disease development in tomatoes, Therefore, it should be applied to other fresh fruits and vegetables.

The other tropical fruits were susceptibility to pathogens; thus, antimicrobial activities should be determined against various types of microorganisms in further studies.

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Bah, H., Naradisorn, M., Choeybundit, W. & Tondeesoontorn, W. (2021). Carboxymethyl cellulose coating with lemongrass essential oil (*Cymbopogon citratus*) to reduce postharvest losses of tomato. *Proceeding of the International Conference on Food innovation and sustainability through Bio-Circular -Green economy*. 17-18 June 2021 (Online Bitec, Bangkok, Thailand).