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Araştırma Makalesi / Research Article

Characterization of functional properties of edible methylcellulose films containing *Momordica charantia* L. ethanolic extract

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Abstract

Methylcellulose edible films were produced by using different concentrations (0.25, 0.50, 0.75, and 1% of *Momordica charantia* L. ethanolic extract (ME). Methylcellulose (MC) films were analyzed in terms of their physicochemical properties and antimicrobial (against *Bacillus cereus*, *Escherichia coli* ATCC 25922, *Staphylococcus aureus* 6538 IP, *Staphylococcus epidermis* ATCC 1228, *Vibrio parahemolyticus* ATCC 17802, *Yersinia pseudotuberculosis* ATCC 911, *Listeria monocytogenes*, *Enterococcus faecalis* ATCC 29212, *Salmonella* Typhimurium ATCC 14028, *Klebsiella pneumonia*, *Proteus vulgaris* ATCC 13315) and antioxidant activities. Fourier transform infrared (FTIR) spectroscopy analysis was used in determining the functional group interactions between the polymer and ME, whereas Thermal Gravimetric Analysis and Differential Scanning Calorimetry analysis were used in defining the physicochemical characterization. The highest antimicrobial effect against *Proteus vulgaris* (approximately 1.93 log CFU/mL) at the end of 24h was achieved with MC films containing 1% ME in comparison to the control film. While an inverse correlation was observed between increasing ME concentration and the tensile strength of the films, a significant decrease in water vapor permeability values, improvement in contact angle values and hydrophilic properties were determined. In light of all these results, the film samples of MCME demonstrated their suitability as viable candidates for biodegradable and edible food packaging applications.

Keywords: Bioactive edible film, Methylcellulose, Antioxidant activity, Antibacterial activity, Physicochemical properties

Momordica charantia L. etanol ekstraktı içeren yenilebilir metilselüloz filmlerin fonksiyonel özelliklerinin karakterizasyonu

Öz

Bu çalışmada, *Momordica charantia* L. etanol ekstraktının (ME) farklı konsantrasyonları (% 0.25, 0.50, 0.75 ve 1) kullanılarak metilselüloz yenilebilir filmler (MC) üretilmiştir. MC filmlerin fizikokimyasal özellikleri ile antimikrobiyal (*Bacillus cereus*, *Escherichia coli* ATCC 25922, *Staphylococcus aureus* 6538 IP, *Staphylococcus epidermis* ATCC 1228, *Vibrio parahemolyticus* ATCC 17802, *Yersinia pseudotuberculosis* ATCC 911, *Listeria monocytogenes*, *Enterococcus faecalis* ATCC 29212, *Salmonella Typhimurium* ATCC 14028, *Klebsiella pneumonia*, *Proteus vulgaris* ATCC 13315'e karşı) ve antioksidan aktiviteleri açısından analizleri yapılmıştır. Fourier dönüşümlü kızılötesi (FTIR) spektroskopisi analizi, polimer ve ME arasındaki fonksiyonel grup etkileşimlerini belirlemede kullanılırken, termal gravimetrik analiz (TGA) ve diferansiyel taramalı kalorimetri analizi ise fizikokimyasal karakterizasyonu tanımlamada kullanılmıştır. Kontrol filmlere kıyasla, 24 saat sonunda en yüksek antimikrobiyal etki *Proteus vulgaris*'e karşı (yaklaşık 1.93 log KOB/mL) %1 ME içeren MC filmlerinde elde edilmiştir. ME konsantrasyonunun artması ile film mukavemeti arasında ters bir ilişki gözlemlenirken, su buharı geçirgenlik değerlerinde önemli bir azalma, temas açısı değerlerinde iyileşme ve hidrofilik özelliklerde belirgin bir artış tespit edilmiştir. Tüm bu sonuçların ışığında, MCME film örnekleri biyolojik olarak parçalanabilir özellikte ve yenilebilir gıda ambalajlama uygulamaları için uygun bileşenler olarak nitelendirilmiştir.

Anahtar Kelimeler: Biyoaktif yenilebilir film, Metilselüloz, Antioksidan aktivite, Antibakteriyel aktivite, Fizikokimyasal özellikler

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1. Introduction

Besides the production, the protection of foods is also very important for meeting the needs of the growing world population. For this reason, edible films and coatings have been modified in order to prevent the quality losses and deterioration reactions in fresh, frozen, and processed foods, to prolong shelf life, and to protect the sensory properties (Krishna et al., 2012). Film technology such as edible coating is a promising alternative during processing and/or storage to improve food quality and preservation. In line with the wastes of the food industry, environmental problems, the increase in the cost of plastic disposal and consumer awareness, demand for edible films for natural, nutritious and healthy foods has begun to occur. They are biopolymers composed of protein, lipid or polysaccharide sources that are biodegradable and edible and can also act as carriers of active substances such as antimicrobials, flavorings, antioxidants and nutraceuticals (Umaraw and Verma, 2017). Films and coatings obtained with the same formulation act as a barrier depending on the specific requirements for food preservation. Coatings are applied to food products in liquid form, while films are prepared and applied as a solid layer (Cazón et al., 2017; Tavera-Quiroz et al., 2012; Ye et al., 2007). In many studies, the need to evaluate the thermal, mechanical (elasticity, tension), optical (gloss, opacity), morphological and wettability properties of edible films has been emphasized as they create a modified atmosphere that affects gas transfer and become a barrier to aromatics (Benbettaïeb et al., 2014; Yoo and Krochta, 2011). These properties depend on various parameters related to the type of additives added (antimicrobials, crosslinking agents, emulsifiers, plasticizers), preparation conditions (temperature, pH, component concentration, solvent), coating and film composition (Siracusa et al., 2018). Many types of edible films activated with cellulose, alginate, zein, pectin, soy protein, whey protein, and starch-based polymers have been examined to date. One of the most common renewable polymers in nature is cellulose. Cellulose, which is generally used as the raw material of biodegradable films, is known as a polymer with biocompatibility, non-toxicity, renewability, low cost, biodegradability and chemical stability (Wang et al., 2016). Besides being the most water-soluble cellulose derivative, methylcellulose is a more affordable and easily accessible polymer (Erdohan and Turhan, 2005). Moreover, methylcellulose is a good carrier for many active compounds (such as plant extracts and essential fatty acids) that have antimicrobial and antioxidant properties (Kalkan et al., 2020). Methylcellulose polymer was chosen as the film polymer used in our research due to its advantages.

In the previous studies, the natural active compounds such as malic acid, cinnamon essential oil, lemongrass oil, thymol, grape seed extract, and green tea extract were generally used in order to achieve bioactive properties in the film (Jin et al.,2009; Mastromatteo et al., 2009). *Momordica charantia* L. is a member of The Cucurbitaceae family. The most common name is the “bitter melon”,

which has been traditionally used as a medicinal food in many developing countries. As such, it is desirable to understand bitter melon's bioactive components depending on its medicinal properties and mechanism of action. *M. charantia L*. is a tropical plant, which is now cultivated throughout the world and is known to have therapeutic properties such as anti-diabetic, antioxidant, antiviral, and antineoplastic activities (Paul and Raychaudhuri, 2010). The objective of the present study is to determine the effect of *M. charantia L.* ethanolic extract on the physical and antioxidant capacity and antimicrobial activities of edible film samples. From this aspect, this is the first study to investigate the using possibility of *M. charantia L*. extracts as an additive in methylcellulose films for food packaging technology.

2. Materials and Method

2.1. Preparation of *Momordica charantia L*. Extract

Momordica charantia L. was gathered from Osmaniye, Turkey, in September-October 2019. Ten grams of dried and powdered *Momordica charantia L*. (by using a drying oven at 40°C for 6 hours, fruit flesh only, without seeds) was extracted using 100 mL ethanolic (96 % w/w) by shaking for 24 h. The mixture was filtered using Whatman No. 1 filter paper and then the ethanolic in the extraction mixture was removed under vacuum using a rotary evaporator (Engin et al., 2018).

2.2. Elaboration of active MC films

Methylcellulose powder (MC; biological source: wood (pulp cellulose); powder; viscosity: 3,500-5,600 cP; Sigma Chemical, St. Louis, MO, USA) at the rate of 4% (w/v) was added to distilled water (100 mL) and dissolved using a magnetic stirrer. The mixture was heated to 90°C by continually blending (30 min). After that, glycerol (1.6 mL; Merck, Darmstadt, Germany) was added as a plasticizer and the solution was cooled to 50 ºC by stirring for 30 minutes. Finally, *Momordica charantia* extracts (ME) were put at the concentrations of 0%, 0.25%, 0.5%, 0.75 %, and 1%. To ensure a good homogenization in solution, the mixture was mixing up at 40°C for 1 h by using a hotplate magnetic stirrer (Model US152; Staffordshire, UK). Then, the final concentrations of all ingredients in the per 100 mL film-forming solution were 4 g MC, 1.6 mL glycerol, and different concentrations of ME (0.25; 0.50; 0.75, and 1 mL). All the film-forming solutions (12.5 mL) were casted into glass Petri dishes (r:90 mm) and dried at 37 ºC for 18 h. The resultant MC films were kept in a desiccator at ambient temperature and 50 ± 4% RH for 48 h for further analysis. Silica gels were

used as an RH control. Four groups of active MC films were formulated as a) without ME (MC-C); b) MCME -0.25; c) MCME -0.50; d) MCME -0.75; and e) MCME-1.00 (Kalkan et. al., 2020).

2.3. Antimicrobial activity of films

The antibacterial activity tests were used in investigating the foodborne spoilage and pathogenic bacteria (*Bacillus cereus*, *Escherichia coli* ATCC 25922, *Staphylococcus aureus* 6538 IP, *Staphylococcus epidermis* ATCC 1228, *Vibrio parahemolyticus* ATCC 17802, *Yersinia pseudotuberculosis* ATCC 911, *Listeria monocytogenes*, *Enterococcus faecalis* ATCC 29212, *Salmonella* Typhimurium ATCC 14028, *Klebsiella pneumonia*, *Proteus vulgaris* ATCC 13315), which were obtained from the American Type Culture Collection (ATCC) and Giresun University Food Engineering Department Culture Collection. For antimicrobial analysis, all film samples were cut into discs with a diameter of 1,6 cm using a sterile borer. To determine the antimicrobial effect in vitro of MCME films and control films were placed in the wells of cell-culture test plaques having flat bottoms. All pathogen cultures (1 mL) with diluted Mueller Hilton Agar (Merck, Germany) were placed into the wells at the concentration of 3 log CFU/mL. So as to make certain the bacterial growth in the medium, the cell-culture test plaques were covered and then they were kept at room temperature for 24 h. So as to make certain the bacterial growth in the medium, the cell-culture test plaques were covered and then they were kept at room temperature for 24 h. The first inoculum samples were taken on the 0th hour and the inoculum samples were taken at the 24th hour and spread out on Mueller Hinton Agar medium (Merck, Germany) and incubated for 24 h at 37 °C. After the incubation, the colonies were counted and denoted in log CFU/mL (Kalkan, 2018).

2.4. DPPH* antioxidant capacity of films

The free radical scavenging capacity of MC films was analyzed using the methodology designed by Kalkan et al. (2020). The MC films were dissolved in 10 mL of pure water to a final concentration of 50 µg/mL. Then, 3.9 mg of the DPPH radicals was dissolved in 100 mL ethanolic and 10^{-4} M DPPH solution was achieved. The solution was shaken at 250 rpm using a vortexer (Heidolph Reax top) for 10-15 s and then incubated at the ambient temperature for 1 h in the dark place. The absorbance against blank was measured at 517 nm. All analyses were run in triplicate.

DPPH antioxidant capacities of samples were measured as follows Equation (1) and expressed as AA (%):

$$DPPH\ scavenging\ activity\ (AA\%) = ((Ablank - Asample)\ /Ablank)\ x\ 100 \quad (1)$$

where, A_{blank} is the absorbance of the blank and A_{sample} is the absorbance of the sample.

2.5. Scanning Electron Microscopy (SEM)

Nova NanoSEM 200 electron microscope (FEI, Oregon) was used in determining the microstructures MC films and the surface micrographs were taken at 5 kV (Tavera-Quiroz et al., 2012).

2.6. Fourier transform infrared (FTIR) spectroscopy

FTIR spectrum analyses were made in order to determine the chemical structure of active films, as well as the possible interactions between MC-ME components. Dried film samples were analyzed using FT/IR-6600 spectrophotometer (Jasco Corporation, Tokyo, Japan) equipped with attenuated total reflection (ATR) unit and Spectra Manager software. The scanning procedure was performed at the resolution range of 4000 cm^{-1} and 4 cm^{-1}.

2.7. Thermal analysis

Thermal analysis (the glass transition temperature; Tg) was performed using a Hitachi DSC7020 dual furnace differential scanning calorimeter (DSC). Film samples weighing approx. 3 mg each were heated from - 90° to 200°C under nitrogen flow (20 mL min^{-1}).

2.8. Thermo-gravimetric analysis (TGA)

All the MC film samples were scanned using a thermo-gravimetric analyzer (LABSYS EVO STA; France) from 0 ° to 430 °C with the rate of 10 °C min^{-1}. For each analysis, 10 mg MC film samples were placed in the aluminum containers that were coated hermetically and heated under an argon atmosphere to avoid thermo-oxidative reactions.

2.9. Physical characteristics of MC films

The thickness of the films was measured by using a micrometer (Mitutoyo Corporation 293-821, Japan) with $1x10^{-3}$ micrometers precision. Measurements were performed at six different parts of each MC film. The average and standard deviation values were given by calculating the tensile and water vapor permeability (WVP) values of each film.

The film samples were weighed (approximately 0.2 g; w1) to calculate the moisture content (mc) and then dried in an oven at 105 °C for 24 hours (w2). Moisture content was reported as the percentage of initial film weight lost during drying using Equation (2).

below:

$$mc\ (\%) = \left(\frac{w1-w2}{w1}\right) \times 100 \quad (2)$$

Where, w1 refers to the weight of the film sample before the drying (g) and w2 to the weight of the film sample after the drying (g). The edible film densities were defined using the specimen weight and volume. Film densities were reported using Equation (3) below:

$$\rho s = m / (A \times \gamma) \quad (3)$$

Where, ρs refers to density of film samples ($g.cm^{-3}$), m to dry mass weight (g), A to film surface area (cm^2), and γ to film thickness (cm). Thickness of film samples analyzed in the present study varied between 0.076 and 0.167 cm, whereas the radius was 4.5 cm.

The contact angle (hydrophobic or hydrophilic character of films containing ME extract) on the film surface in air was measured using a contact angle meter (Dataphysics OCA 15EC) after placing approx. 1 μL water was placed on the surface of film using a microsyringe. The contact angles were determined using the ImageJ free software. The process was triplicated for each film sample (Kalkan et al.,2020; Piñeros-Hernandez et al., 2017).

The swelling ratio of film samples was measured using the method introduced by Kalkan et al. (2020). Film samples (20 x 20 mm) were immersed in 30 mL distilled water for 2 min at room temperature. Measurements were made at the specified times until the weight gain of the Swollen films was reached, allowing them to dry slightly with filter paper for 1 minute to remove excess surface water before measuring. The ratio of the recovered water (g) to the total solids (g) in the film was expressed as the swelling ratio.

2.10. Water vapor permeability (WVP) of films

The WVP of the films was determined using the standard test method ASTM E96–80 (ASTM 1983) at 25±1°C. Special custom-manufactured Delrin test cups with 40 mm wall thickness were used in the analysis. The cup was filled with anhydrous calcium chloride (0% RH; Merck, Darmstadt, Germany) then a rubber joint was placed between the film and the cap to allow only water vapor to enter the Film. Each of the test cups was placed in a desiccator containing $MgNO_3$ (52 ± 2% RH; Merck, Darmstadt, Germany) for 12 h. The water vapor transmission rate (WVTR) of the films was measured using the graph of weight change over time. The weighing procedure was repeated subsequently 12 more times at 2 h intervals. WVP ($g\ Pa^{-1}\ s^{-1}\ m^{-1}$) was calculated using Equation (4):

$$WVP\ (g\ Pa^{-1}\ s^{-1}\ m^{-1}) = (W\ x\ X)\ (A\ x\ t\ x\ \Delta P) \quad (4)$$

where, W refers to the weight change of the cup (g), X to the film thickness (m), A to the area of exposed film (m^2), t to the time (s), and ΔP (Pa) to the partial vapor pressure difference of the atmosphere.

2.11. Mechanical properties of films

Elongation at break (E) and tensile strength (TS) textural properties of each film were evaluated using ASTM Method D638 with the aid of a texture analyzer (TA. XT Plus StableMicro Systems, Surrey, UK). Young's modulus (N/mm) of film sample was calculated by dividing the initial sample length by the film cross-section. Seven film specimens (80 x 25 mm) were prepared from each film sample. The average thickness of each film strips were used in estimating the cross-sectional area of sample. The film strips were conditioned in a desiccator containing magnesium nitrate (52 ± 2% RH) at 25 °C for 48 h. The crosshead speed of texture analyzer was set at 1.00 mm/s.

2.12. Color properties of films

The surface color of each edible films was measured using a Minolta portable chromameter (Minolta, Model CR-300 Osaka, Japan) which provided CIE L^*, a^* and b^* values. A chromameter explains color in three coordinates: L^* (openness), a^* (red-green) and b^* (yellow-blue). Total color differences (ΔE^*) of film samples were calculated using the following formula (Equation 5).

$$\Delta E^* = (\Delta L^2 + \Delta a^2 + \Delta b^2)^{0.5} \quad (5)$$

Where, $\Delta L = L^*_{std} - L^*_{sample}$, $\Delta a = a^*_{std} - a^*_{sample}$ and $\Delta b = b^*_{std} - b^*_{sample}$. The standard values of white plate were $L^* = 93.87$, $a^* = -0.55$, and $b^* = 5.13$ respectively.

Six measurements were taken on each film.

2.13. Statistical analysis

To determine the differences between the average values of film properties, the Duncan multiple Decembers test ($P<0.05$) and the analysis of variance (ANOVA) procedure, which has a random design, are SPSS (Version 19.0, SPSS Inc., Chicago, IL) was performed using the software.

3. Findings and Discussion

3.1. Antimicrobial activity results

Previous studies reported that ME extracts have antimicrobial activity against several pathogenic microorganisms (Engin et al., 2018). The antibacterial activity results of ME on MC films are presented in Table 1. MC-C films did not show any antimicrobial effects against the test microorganisms as expected. Pathogen microorganisms increased by MC-C films by approx. three logarithm units after 24 hours (Table 1). Despite the addition of ME to the MC films, no antimicrobial effects were detected against *B. cereus*, *S. epidermis*, and *K. pneumoniae*. The highest antimicrobial effect was achieved with MCME-1.00 films against *P. vulgaris* (approximately 1.93 log CFU/mL) compared to the control films at the end of 24 h. Moreover, MCME-1.00 films showed the lowest antimicrobial effect against *E. faecalis* (0.34 log CFU/mL). For pathogenic microorganisms detected during the inactivation, as the ME concentration did, inactivation raised considerably ($p \leq 0.05$) at all concentrations. These findings may be related to the increased ME solubility in the film matrix and a homogeny dispersion of the extract in the MC film. *Momordica charantia L.*, which has been used for many years due to its medicinal properties, is rich in secondary metabolites. In addition, its antimicrobial property has been determined in vitro (Braca et al., 2008). Reported by Leelaprakash et al. (2011), *Momordica charantia* extracts, which show a wide spectrum of antimicrobial activity, as well as various water, ethanolic and methanolic extracts of leaves, *Escherichia coli*, *Pseudomonas* spp. showed antibacterial activity against strains. Moreover, several studies have shown that extracts of *Momordica charantia L*. seed and seed oil obtained with various solvents have antimicrobial activity against *Escherichia coli*, *S. aureus*, *S. typhi* and *A. niger*. (Engin et al., 2018; Yaldız et al., 2015). Similarly, in the present study, ME preserves its antimicrobial property in films samples and MCME films samples have antimicrobial effect in variable rates depending on the microorganism types.

Table 1. Antimicrobial activity of MC films with ME against test microorganisms

Microorganisms	Exposure time (h)	Antimicrobial effect (log CFU/mL)*				
		MC-C	MCME-0.25	MCME-0.50	MCME-0.75	MCME-1.00
Bacillus cereus	0	3.09±0.22^{a}	3.09±0.22^{a}	3.09±0.22^{a}	3.09±0.22^{a}	3.09±0.22^{a}
	24	5.98±0.13^{a}	6.10±0.13^{a}	5.90±0.12^{a}	5.97±0.24^{a}	6.04±0.07^{a}
Escherichia coli	0	3.17±0.11^{a}	3.17±0.11^{a}	3.17±0.11^{a}	3.17±0.11^{a}	3.17±0.11^{a}
	24	6.06±0.09^{a}	5.67±0.38^{ab}	5.55±0.36^{ab}	5.48±0.42 ^{ab}	5.19±0.06^{b}
Staphylococcus aureus	0	3.14±0.16^{a}	3.14±0.16^{a}	3.14±0.16^{a}	3.14±0.16^{a}	3.14±0.16^{a}
	24	6.34±0.25^{a}	5.36±0.23^{b}	5.17±0.23^{b}	5.02±0.08^{b}	4.60±0.07^{c}
Staphylococcus epidermis	0	3.15±0.13^{a}	3.15±0.13^{a}	3.15±0.13^{a}	3.15±0.13^{a}	3.15±0.13^{a}
	24	6.16±0.05^{a}	6.13±0.08^{a}	6.07±0.13^{a}	6.14±0.09^{a}	6.13±015^{a}
Vibrio parahemolyticus	0	3.21±0.04^{a}	3.21±0.04^{a}	3.21±0.04^{a}	3.21±0.04^{a}	3.21±0.04^{a}
	24	6.08±0.10^{a}	5.29±0.31^{b}	5.04±0.08^{bc}	4.93±0.07^{c}	4.47±0.19^{d}
Yersinia pseudotuberculosis	0	3.09±0.15^{a}	3.09±0.15^{a}	3.09±0.15^{a}	3.09±0.15^{a}	3.09±0.15^{a}
	24	6.07±0.11^{a}	5.90±0.13^{ab}	5.64±0.28^{abc}	5.49±0.42^{bc}	5.25±0.07^{c}
Listeria monocytogenes	0	3.21±0.18^{a}	3.21±0.18^{a}	3.21±0.18^{a}	3.21±0.18^{a}	3.21±0.18^{a}
	24	6.34±0.25^{a}	5.36±0.23^{b}	5.17±0.20^{b}	5.02±0.08^{b}	4.60±0.07^{c}
Enterococcus faecalis	0	3.03±0.07^{a}	3.03±0.07^{a}	3.03±0.07^{a}	3.03±0.07^{a}	3.03±0.07^{a}
	24	5.76±0.21^{a}	5.64±0.10^{ab}	5.63±0.10^{ab}	5.48±0.05^{ab}	5.42±019^{b}
Salmonella Typhimurium	0	3.11±0.09^{a}	3.11±0.09^{a}	3.11±0.09^{a}	3.11±0.09^{a}	3.11±0.09^{a}
	24	5.69±0.11^{a}	5.57±0.15^{ab}	5.50±0.20^{ab}	5.43±0.18^{ab}	5.27±0.13^{b}
Klebsiella pneumoniae	0	3.32±0.11^{a}	3.32±0.11^{a}	3.32±0.11^{a}	3.32±0.11^{a}	3.32±0.11^{a}
	24	5.56±0.41^{a}	5.52±0.43^{a}	5.62±0.54^{a}	5.53±0.38^{a}	5.58±0.27^{a}
Proteus vulgaris	0	3.08±0.11^{a}	3.08±0.11^{a}	3.08±0.11^{a}	3.08±0.11^{a}	3.08±0.11^{a}
	24	6.31±0.23^{a}	5.42±0.10^{b}	5.14±0.29^{bc}	4.94±0.14^{c}	4.37±0.25^{d}

*Mean ± standard deviation (n=3) with different superscript letters for microorganisms are significantly different at p≤0.05; MC-C: Control methylcellulose films; MCME-0.25: Methylcellulose films containing 0.25% (w/w) ME; MCME-0.50: Methylcellulose films containing 0.50 % (w/w) ME; MCME-0.75: Methylcellulose films containing 0.75% (w/w) ME; MCME-1.00: Methylcellulose films containing 1.00 % (w/w) ME

3.2. DPPH radical scavenging ability results

The radical scavenging capacity of MC films was evaluated using the DPPH assay (Figure 1). As expected, MC-C film samples did not show any noticeable antioxidant activity. The DPPH antioxidant capacity increased considerably (p≤0.05) from 4.6 % for the MCME-0.25 film to 30.4 % for the MCME-1.00 film. The antioxidant and antibacterial properties of MC films added with ME extracts could be related to the bioactive compounds such as phenolics acids and flavonoids obtained from the *Momordica charantia* L. (Svobodova et al., 2017). In fact, the antioxidant mechanisms of natural antioxidants can be attributed to their ability to chelate with metals, to scavenge superoxide, hydrogen peroxide, and free radicals, and to their hydrogen donor capacity. When the antioxidants are added into the MC films, interactions with the MC can also affect their antioxidant activity. There are no studies in the literature carried out on the strong antioxidant activities related with ME content in film. However, there are many studies in the literature on the total phenolic and flavonoid content of ME, which brings high antioxidant properties (Tan et al., 2014).

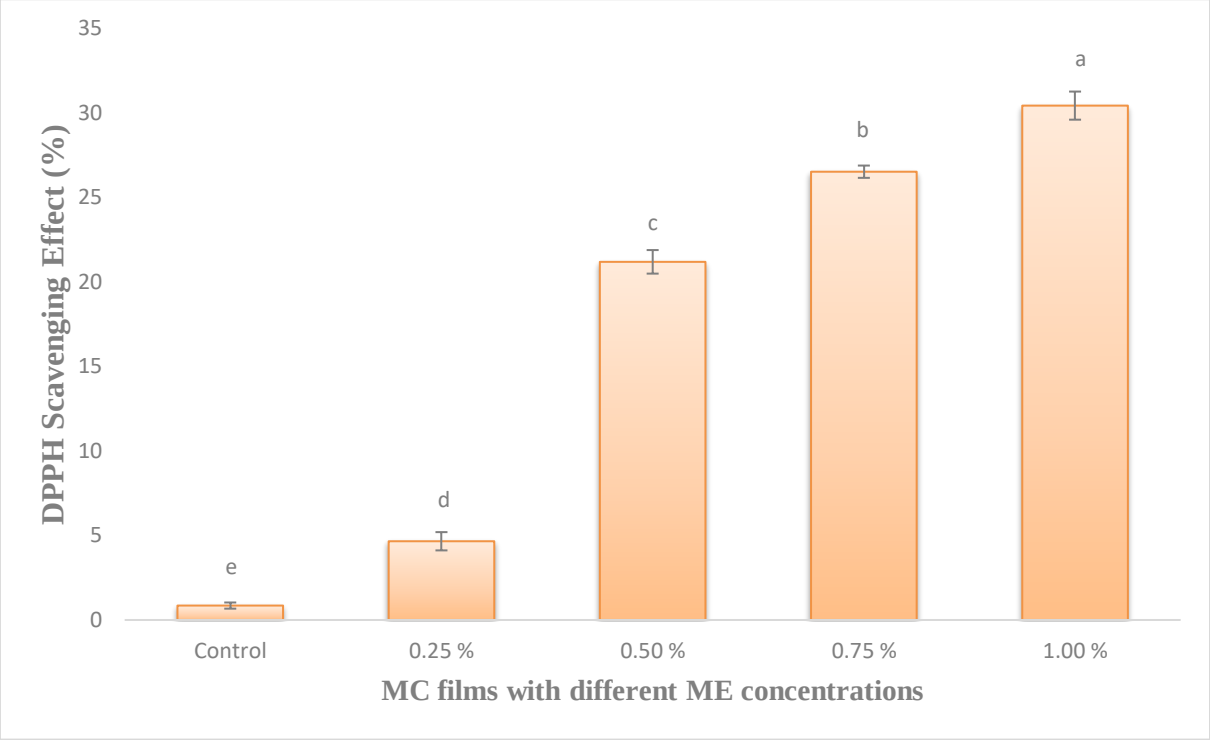

Figure 1. DPPH radical scavenging activity of MC films with different ME concentrations

3.3. SEM analysis

The morphological analysis of MC films was performed by scanning electron microscopy. SEM analyses of the surface films with and without ME were presented in Figure 2. The surface of the control MC film was homogeneous and smooth and had small irregularities when compared to other films, as shown in Figure 2. Nevertheless, ME-added film samples had a heterogeneous surface and porous structure. Although the ME addition rates were very low, the increase of ME was enough to make this impact more considerable. The surface of the film incorporating ME at the rates of 0.50, 0.75, and 1% had numerous apparent pores. These pores were responsible for the higher WVP values when compared to the control film that had a smooth surface. Moreover, these results might be related to the microphase separation of methylcellulose and ME. Furthermore, the surface roughness of film samples was related to the transparency values. As similarly, findings regarding SEM analysis were also reported in some researches (Kalkan et al., 2020; Mohsenabadi et al., 2018).

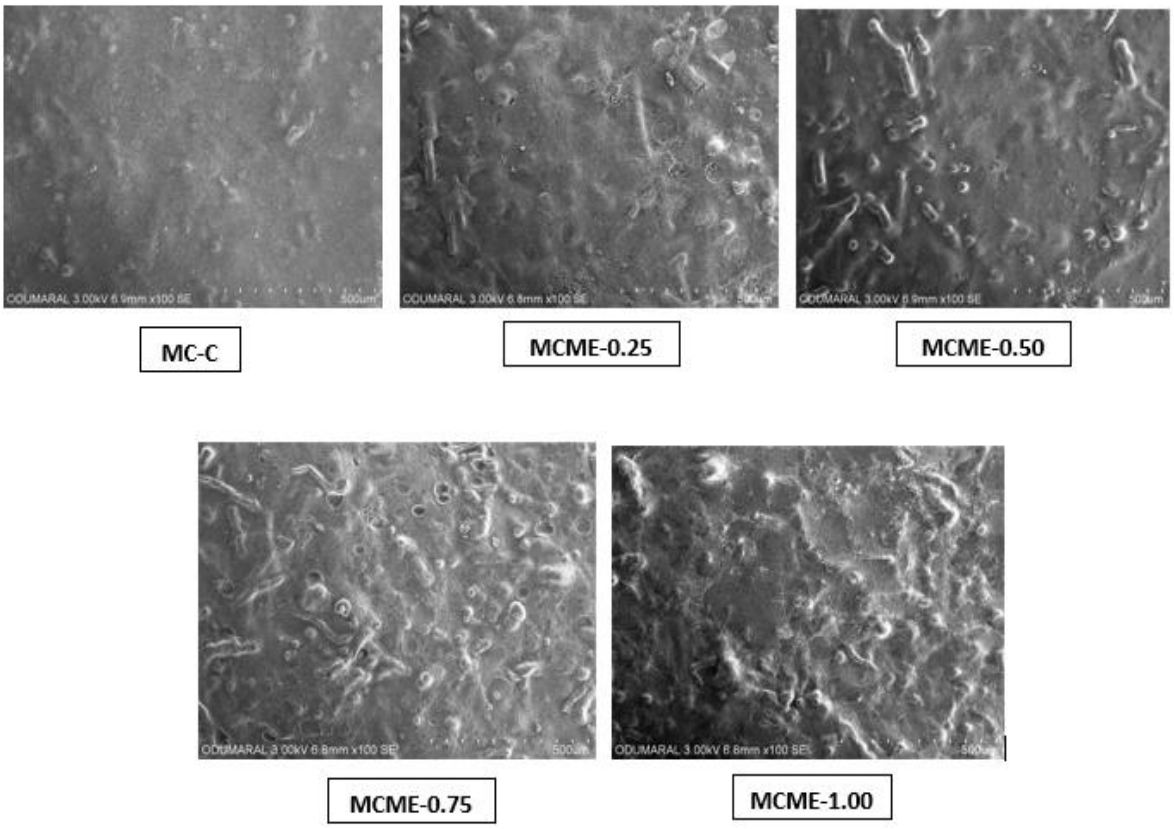

Figure 2. Scanning electron microscope (SEM) images of the surface of MC film with different ME concentrations

3.4. Physical features of edible films

Hydration characteristics, especially the water solubility, are one of the major features of edible films prepared using biodegradable polymers. Film integrity and water resistance should be improved for the potential application of MC films as food packaging (Bertuzzi et al., 2007). All the physical (color properties) and hydration properties (density, swelling behavior, humidity, and contact angle) of film samples are presented in Table 2. Significant changes were observed in the swelling index in terms of the ME concentration of film samples ($p \leq 0.05$) (Table 2). MC films containing 1% ME showed higher water absorbency properties with 39.32±2.46. It is desired to absorb extra water from the outer surface for the high-moisture foods. Therefore, a higher swelling index may be a desired feature. According to the results of Moradi et al. (2012) and Mayachiew and Devahastin (2010), the swelling rate of the film samples depends on the nature of the intermolecular chain interactions and the drying temperature. Researchers have revealed that molecular properties of phenolic compounds significantly affect the strength of film matrix.

Table 2. Hydration, mechanical, water vapor barrier properties and color parameters and of MC films with different ME concentrations

	Film samples*				
Parameters	**MC-C**	**MCME-0.25**	**MCME-0.50**	**MCME-0.75**	**MCME-1.00**
Thickness (µm)	76.0±0.0^{a}	123.5±0.0^{b}	137.8±0.0^{b}	138.6±0.0^{b}	167.1±0.0^{c}
Swelling index (%)	25.1±1.5^{a}	28.2±9.6^{ab}	29.1±4.1^{ab}	30.6±0.2^{ab}	39.2±2.4^{b}
Moisture content (%)	10.8±5.1^{a}	10.5±1.8^{a}	9.2±5.6^{b}	8.5±0.8^{b}	6.3±3.0^{c}
Density (g.cm^{-3})	1.9±0.3^{a}	2.0±0.3^{a}	2.0±0.1^{a}	2.0±0.2^{a}	2.1±0.1^{a}
Contact angle (θ°)	81.2±3.7^{c}	72.4±14.4^{bc}	65.4±9.6^{bc}	58.1±19.4^{b}	16.1±0.4^{a}
Tensile strength (MPa)	26.9±15.0^{a}	21.0±3.6^{ab}	17.5±7.0^{ab}	17.7±4.6^{ab}	15.3±2.2^{b}
Young's Modulus (MPa)	97.3±0.2^{bc}	103.2±2.6^{c}	83.0±12.4^{b}	45.8±5.6^{a}	59.5±14.4^{a}
Elongation (%)	43.4±19.3^{ab}	41.8±6.1^{a}	47.5±17.9^{ab}	55.8±2.6^{a}	68.8±3.7^{b}
Water vapor permeability (10^{-6} g.m^{-1}.s^{-1}.Pa^{-1})	3.2±0.0^{c}	2.6±0.0^{bc}	2.7±0.0^{bc}	2.4±0.0^{b}	1.4±0.0^{a}
***L**	93.3±0.1^{a}	89.6±1.9^{b}	88.9±0.1^{b}	87.0±2.2^{bc}	86.4±1.1^{c}
***a**	2.2±0.0^{a}	2.0±0.0^{a}	2.2±0.0^{a}	1.4±0.4^{a}	1.4±0.1^{a}
***b**	-12.3±0.5^{a}	-2.8±0.2^{b}	0.6±0.8^{b}	9.5±6.3^{c}	16.0±6.0^{c}
***ΔE**	18.5±0.4^{ab}	13.6±0.6^{a}	13.0±0.2^{a}	15.5±4.7^{ab}	19.5±4.5^{b}

* Mean ± standard deviation (n=3); a–c : Values with the same superscript letters in the same rows are not significantly different ($p < 0.05$). MC-C: Control methylcellulose films; MCME-0.25: Methylcellulose films containing 0.25% (w/w) ME; MCME-0.50: Methylcellulose films containing 0.50 % (w/w) ME; MCME-0.75: Methylcellulose films containing 0.75% (w/w) ME; MCME-1.00: Methylcellulose films containing 1.00 % (w/w) ME

As represented in Table 2, the moisture content of edible films ranged between 10.84 and 6.32%. Similar to the results reported by Kalkan et al. (2020), the moisture content of the films decreased significantly due to the rate of ME added to the films ($p \leq 0.05$). Pastor et al. (2013) stated that the moisture content of MC films enriched with trans-resveratrol was 5.9-7.0%. In a different study, it was found that the moisture content of chitosan edible films varied between 8.87 to 14.87 (Rubilar et al., 2013). The etheric groups in the methylcellulose polymer are believed to cause high humidity in film samples (Kalkan et al., 2020). In this research, the density values of samples ranged from 1.97 and 2.19. Similarly, Wang et al. (2013) stated that the density of chitosan edible films were slightly decreased from 1.23 to 1.10 ($p \leq 0.05$) by raising the proportion of tea phenolics. Kalkan et al. (2020) reported that the density of methylcellulose film samples containing *Rheum ribes L*. extract between 1.48 and 1.22. The higher density values found in the present study are thought to result from the use of different plant extracts in the film formulation.

Contact angle (CA) measurements of MC film enriched with ME were performed using a static sessile drop method. The results are shown in Table 2. As can be seen, CA values significantly decreased with increasing the rate of ME ($p \leq 0.05$). MC-C film had a water contact angle of 81.23°. However, it remarkably decreased from 81.23° in MC-C to 16.16° in MCME-1.00 composite film due to the addition of RE and this result suggests an increase in the hydrophilicity of composite surface (Liu et al., 2017). As known, the quantitative definition of relative terms "hydrophobic" and "hydrophilic" surfaces has been made according to their water contact angle of $\theta > 65^{\circ}$ and $\theta < 65^{\circ}$,

respectively (Karbowiak et al., 2006). MC has a hydrophilic character as a polysaccharide. This feature causes the packaging materials produced using MC polymer to have low water vapor and gas barrier characteristics. In the present study, CA values revealed similar results with the previous studies (Liu et al., 2017).

3.5. Water vapor permeability of films (WVP)

In general, polysaccharide-based films such as methylcellulose polymer have higher WVP values than commercial synthetic materials. WVP values of the MC edible and biodegradable films with separate concentrations of ME were examined and all results are shown in Table 2. The WVP of films decreased from 3.2 to 1.4 (10^{-6} $g.m^{-1}.s^{-1}.Pa^{-1}$) with an increasing concentration of ME from 0% to 1%. Lower WVP values in comparison with control film were achieved by adding ME into MC film. It might be because the polyphenolic compounds of extract comply with the MC matrix and establish cross-links through hydrogen bond or hydrophobic interaction with reactive groups of MC. Moreover, the high moisture content of film samples may indicate the same effect on the WVP values (Wu et al., 2013). Similarly, Rattaya et al. (2009) reported that WVP of the films decreased with increasing concentrations of seaweed extract. Teixeira et al. (2014) stated that adding clove essential oil remarkably reduced the water vapor permeability of films, whereas no statistical differences were determined between films containing garlic or origanum essential oils.

3.6. Mechanical properties of films

The percentage of elongation (%E) and tensile strength (TS) values, which determine the strength and flexibility of biodegradable films, are specified as the mechanical properties of the films, and these values are generally desired to be high. The mechanical properties of MC films incorporated ME at different concentrations and control films were shown in Table 2. When the ME was added into the film, the TS values decreased with ME concentrations increasing from 26.98 to 15.38 MPa ($p \leq 0.05$). Polymer-polyphenol interactions decreased the mechanical properties of MC films and the addition of extract as an active component increased film fragility (Kalkan et al., 2020; Çağrı-Mehmetoğlu, 2010). Gutierrez-Pacheco et al. (2016) reported that the factor that caused a significant decrease in TS and YM was the addition of essential oils such as clove, cinnamon and allspice bud to the film formulation. As seen in Table 2, YM values decreased from 97.30 to 59.57. Similarly, the mechanical properties of hydroxypropyl methylcellulose-based edible films were determined to be 19.3–36.2 MPa (TS) 62.5–71.4 MPa (YM) (Moghimi et al., 2017). In our study, E (%) values increased from 43.4 to 68.8. As similar our study, Kavoosi et al. (2014) reported that the addition of

cinnamon and zataria multifloral essential fatty acids in the films resulted in a significant reduction in TS and an increase in E (%). Esmaeilli and Ebrahimzadeh Fazel (2016) reported that TS values were between 21.3 and 50.2, whereas E (%) values were between 10.2 and 28.0 of MC film samples. Ayana and Turhan (2009) determined that TS and E (%) values were found to be 17.3-24.0 and 27.0-46.5, respectively.

3.7. Color results

In Table 2, *L**, *a**, *b** values and total difference in film color are shown. All color parameters (*L**, *a** and *b**) were influenced by the addition of ME. Actually, the film color shifts towards red and blue with positive and smaller a* and b* values, respectively, and the color lightness decreases as a result of increasing the extract concentration. These color changes may be elucidated by the increase in the smoothness of the surface of the film by factors that cause surface irregularities such as the addition of plant extract in the films, the migration of droplets or the coagulation of the extract during the drying of the films. This result possibly indicates that the elements causing surface roughness can cause light hitting the surface to reflect from different angles, causing it to appear more opaque and hazy (Kalkan et al., 2020). The difference of total color (*ΔE*) is similar for samples MCME-0.25 and MCME-0.50 (non-statistically different; $p \leq 0.05$). Moreover, MCME-1.00 films have the highest *ΔE* values with 19.59±4.56. As stated in previous studies, the addition of herbal extract also makes a difference to film color parameters depending on the amount and type of herbal extract used (Atarés et al., 2010). The addition of herbal extract shifted the indigenous color of edible film samples and the color change of film samples addition ME in comparison to MC-C films is evident in our present study.

3.8. Fourier transform infrared (FTIR) spectroscopy analysis

The FT-IR spectrums of the films are shown in Figure 3. The peak at 3420 cm^{-1} corresponds to the OH longitudinal vibrational mode of the methylcellulose alcohol group and the R-OH groups of glycerol. Moreover, it represents the OH bonds of alcohols at the peaks between 3220 and 3540 cm^{-1}. Phenols and carboxylic acid groups are present in ME. While these characterize the methylcellulose and glycerol structures at the peaks, they are associated with the peak aliphatic -CH groups around 2850 cm^{-1}. While the bending stress of the water molecule can be observed with the battery around 1650 cm^{-1}, the vibrations of the MC groups can correspond to the peaks at 1270 cm^{-1} and 1500 cm^{-1}. The region from 1720 to 1820 cm^{-1} originates from carbonyl groups such as ketones, aldehydes,

esters, and carboxylic acids. Absorption refers to nitro groups from 1550 to 1640 and 1350 cm^{-1} (Pavia et al., 2013). Each of the spectra was found to be consistent with Khatib et al. (2012).

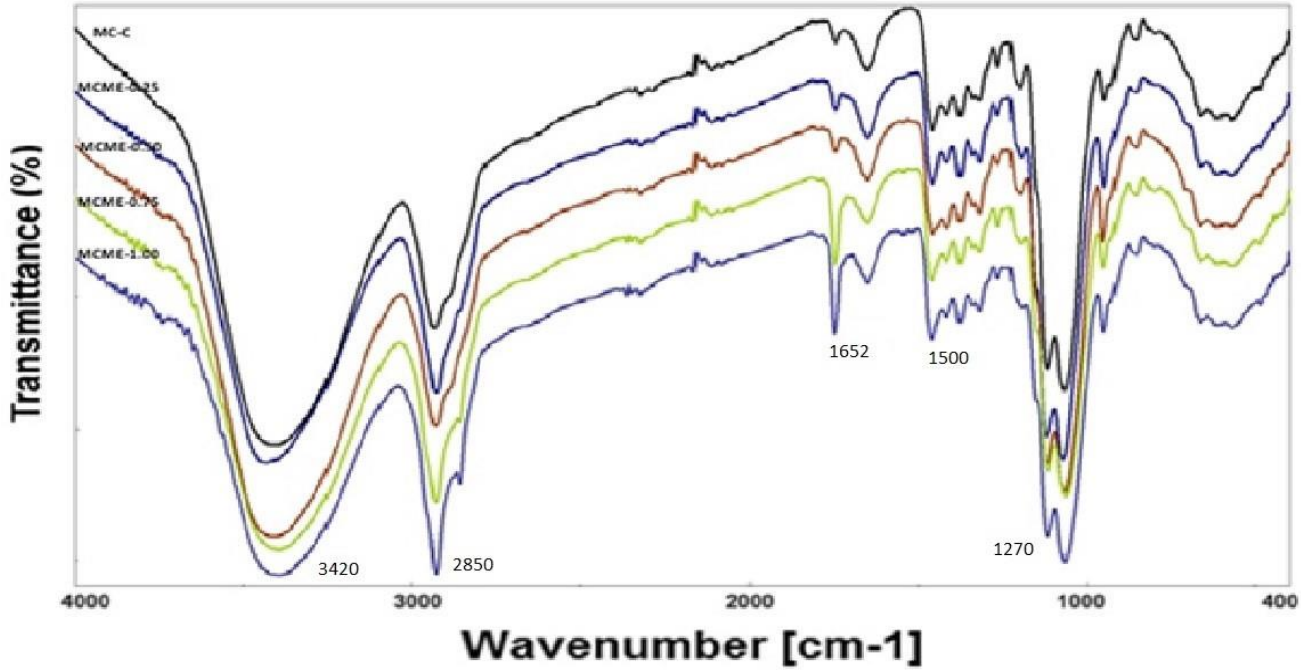

Figure 3. FT-IR spectra of methylcellulose, Glycerol, and *Momordica charantia L.* extract

3.9. Thermogravimetric analysis (TGA)

The first derivative function was performed on the TGA curves to find the thermal decomposition temperatures of all different rates of edible films (Figure 4a). Thermal decomposition of the developed biofilms took place in three main stages. While this initial phase bulk reduction can be expressed by evaporation of water from composition, mass loss starting below 100 °C is due to water adsorbed on edible film Roy and Rhim, 2020). The second phase was monitored at about 210 °C and might be related to the disruption of the structure of glycerol (Beghetto et al., 2020; Huntrakul and Harnkarnsujarit, 2020). The original bulk reduction was determined at approx. 325 °C and was caused by the decomposition of MC structure and plant extracts (Rodrigues Filho et al., 2007).

3.10. Differential scanning calorimetry (DSC) analysis

Plasticizers are used in to reduce the glass transition temperature of polymer films and increase their flexibility (Mali et al., 2005). In the present study, glass transition temperatures of methylcellulose / glycerol film samples were -76.9 ± 1.4 °C for MC-C, -78.9 ± 1.3 °C for MCME-0.25, -76.5 ± 2.5 °C for MCME-0.50, -81.8 ± 3.2 °C for MCME-1.00, and -80.1 ± 2.8 °C for MCME-0.75. Results close to the findings obtained in this study have been found in previous studies. Averous

et al. (2000) and Buera et al. (1999) evaluated the glass transition temperature of glycerol using the DSC technique and results were reported to be −78 °C and −77 °C, respectively. In the present study, use of herbal extracts was shown to decrease Tg values and raise the flexibility of films. Consequently, the decrease in Tg with decreasing proportion of MC is consistent with the decreasing extent of crosslinking (Su et al., 2010). The differential scanning calorimetry (DSC) analysis results obtained in the present study are shown in Figure 4b.

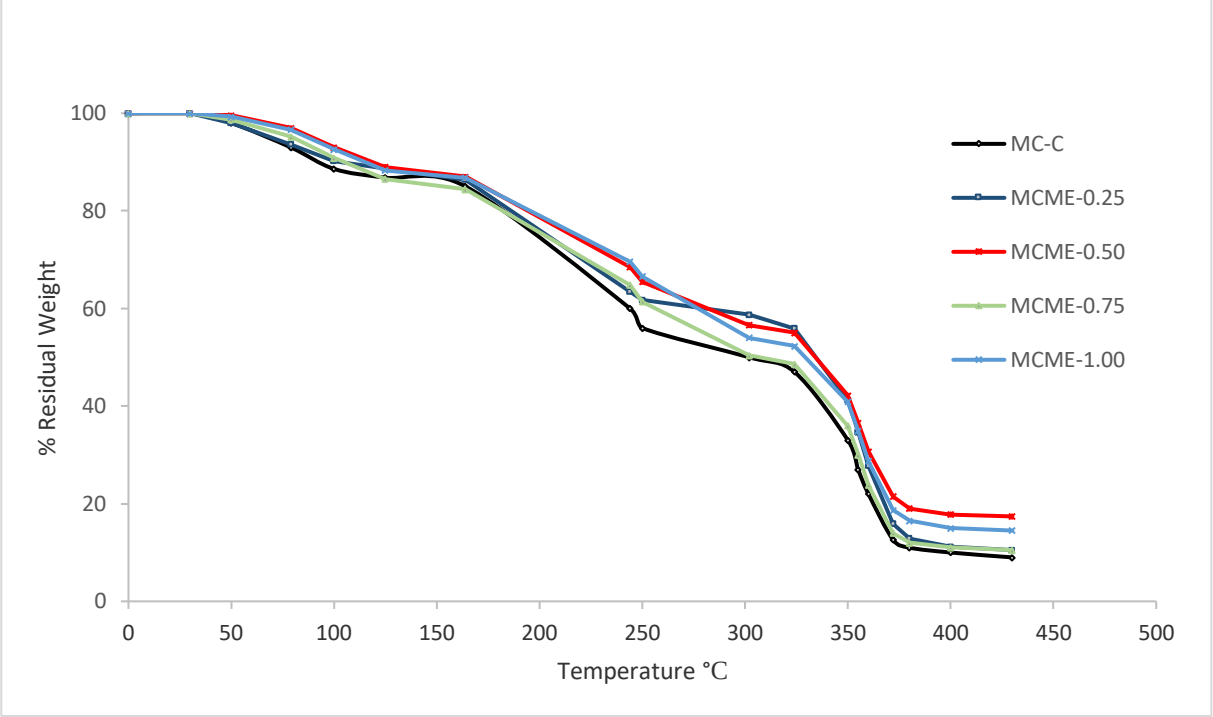

Figure 4a. TGA of MCME polymer blends

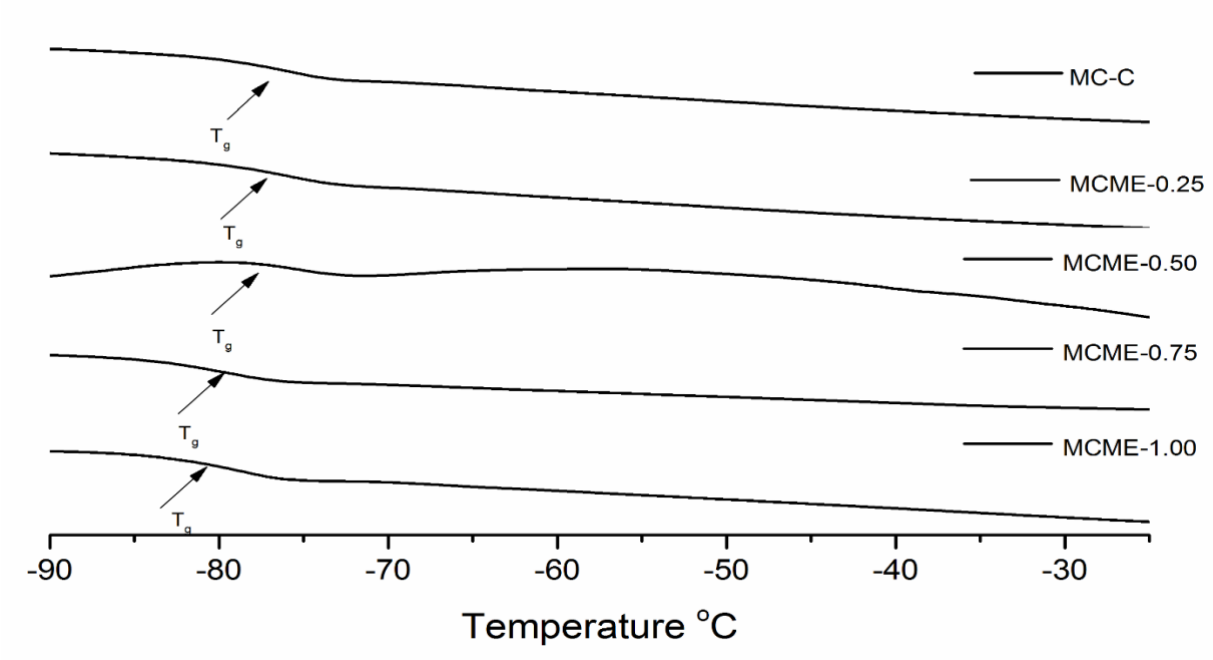

Figure 4b. Differential scanning calorimetry (DSC) analysis result of MC film samples

4. Conclusions and Recommendations

In conclusion, the active edible films containing methylcellulose, glycerol, and *Momordica charantia L*. ethanolic extract were successfully achieved. MCME films showed antimicrobial effects against pathogenic bacteria. It was determined that the highest antimicrobial effect was achieved against *P. vulgaris*, *S.aureus*, and *L. monocytogenes*. However, MCME films had no antimicrobial effect against *B. cereus*, *S. epidermis*, and *K. pneumoniae*. It was noted that the free-radical scavenging activities increased due to the amount of ME concentration in films and MCME-1.00 film samples showed the highest DPPH radical scavenging activity with 33.4%. Since the water vapor permeability and tensile strength of the MC film samples were reduced significantly after the addition of ME. While the MC-C films have a non-porous surface structure in accordance with the texture of film surface surveying, the smoothness was impaired due to adding ME to the film polymer. Further, ME adding was altered the natural color of MC films. The consequences of this study represent that methylcellulose films containing *Momordica charantia L.* ethanolic extract showed impressive characteristics (water vapor permeability, hydration properties, antioxidant capacity and antibacterial activities) as appropriate candidates for use in biodegradable and edible food packaging applications.

Authors' Contributions

All authors contributed equally to the study.

Statement of Conflicts of Interest

There is no conflict of interest between the authors.

Statement of Research and Publication Ethics

The author declares that this study complies with Research and Publication Ethics.

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