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ORIGINAL PAPER

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### DEVELOPMENT OF CHITOSAN EDIBLE FILM INCORPORATED WITH CHRYSANTHEMUM MORIFOLIUM ESSENTIAL OIL

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#### ABSTRACT

**Background.** Biodegradable food packaging has improved in quality with recent research incorporating natural extracts for functionality purposes. This research aims to develop chitosan film with *Chrysanthemum morifolium* essential oil to improve the shelf life of fresh raw chicken and beef.

**Materials and methods.** 1.5% (w/v) chitosan films with *Chrysanthemum morifolium* essential oil (0% to 6% (v/v)) were produced through homogenization, the casting of a film solution in a petri dish and convection drying. The edible film was evaluated in terms of its physical (color, thickness, water vapor permeability), mechanical (puncture strength, tensile strength, elongation at break) and chemical properties (antioxidant assay, Fourier Transform Infrared Spectroscopy (FTIR)).

**Results.** With an increasing concentration of *Chrysanthemum morifolium* in the chitosan film, the test values of physical properties such as tensile strength, puncture force, and elongation at break declined significantly. However, the thickness, water permeability, and color profile  $(L^*, a^*, b^*)$  values of the chitosan film increased. Similarly, the scavenging effect of antioxidant assay increased (from 4.97% to 18.63%) with a rise in *Chrysanthemum morifolium* concentration. 2%, 3%, and 4% of *Chrysanthemum morifolium* in the chitosan film showed a significant inhibition zone ranging from 2.67 mm to 3.82 mm against *Staphylococcus aureus*, a spoilage bacterium that is commonly found in chicken and beef products. The storage and pH tests showed that 4% of *Chrysanthemum morifolium* in the film maintained pH level (safe to consume), and the shelf life was extended from 3 days to 5 days of meat storage.

**Conclusion.** This study demonstrated that the incorporation of 4% (v/v) *Chrysanthemum morifolium* extract into 1.5% (w/v) chitosan film extends the storage duration of raw meat products noticeably by reducing *Staphylococcus aureus* activity. Therefore, it increases the quality of the edible film as an environmentally friendly food packaging material so that it can act as a substitute for the use of plastic bags. Future studies will be conducted on improving the tensile strength of the edible film to increase the feasibility of using it in the food industry. In addition, the microstructure and surface morphology of the edible film can be further determined.

Keywords: chitosan edible film, Chrysanthemum morifolium, Staphylococcus aureus

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#### INTRODUCTION

Current research has studied the innovation of edible film as a food packaging material to minimize plastic usage for ease of disposal management and environmental protection (Abdollahi et al., 2012; Galus and Kadzinska, 2015). Edible films are consumable, convenient, and act as barriers to decrease the permeability of water, gases, and flavor compounds to increase the shelf life of food products (Brunazzi et al., 2014; Salgado et al., 2015; Wang et al., 2018). Chitosan film is a non-toxic biopolymer that is commonly used in fruits, vegetables, and meat product packaging (Falguera et al., 2011; Suput et al., 2015). Chitosan film incorporated with essential oils such as oregano (Fournomiti et al., 2015), Asam keping (Zaman et al., 2018) and musk lime extract (Choong et al., 2019) prolonged the shelf life of food and improved antibacterial properties which prevent bacterial growth of organisms such as Escherichia coli, Listeria monocytogenes, Pseudomonas aeruginosa (Iwamoto et al., 2010), Salmonella enterica and Staphylococcus aureus (Mith et al., 2014), which are mainly found in meat and fish products (Vu et al., 2011). On the other hand, Chan et al. (2020) incorporated Melastoma malabatrichum extract with sodium alginate edible film to improve its functional properties.

The chrysanthemum flower is a plant which is commonly infused into beverages. According to the National Chrysanthemum Society in the USA, chrysanthemum essential oil is the aromatic liquid extracted from the Chrysanthemum morifolium plant, which is one of the 300 species in the Asteraceae (Compositae) group first cultivated in China as a flowering herb (Lin et al., 2019; Lograda et al., 2013). Chrysanthemum morifolium essential oil exhibits significant antigenotoxic, antibacterial, immunomodulatory humoral, and cellular activities (Wang et al., 2018). However, limited research has been conducted on the production of edible film using Chrysanthemum to wrap food products. A study conducted by Lin et al. (2019) stated that chitosan incorporated with Chrysanthemum has a significant inhibitory effect on Listeria monocytogenes. A few researchers have reported the effective antimicrobial activity of chrysanthemum flower against Escherichia coli, and Staphylococcus aureus (Cui et al., 2018; Matyjaszczyk and Smiechowska, 2019). Therefore, the main objective of this study is to test the chemical, mechanical and physical properties of chitosan edible film incorporated with *Chrysanthemum morifolium* and its antimicrobial properties when applied to raw meat products.

#### MATERIALS AND METHODS

### Preparation of *Chrysanthemum morifolium* chitosan film

Chitosan film was prepared by dissolving 1.5% (w/v) chitosan powder (low molecular weight (~3000 Da), VIS Food Tech, Malaysia) into a 1% (v/v) acetic acid aqueous solution with a magnetic stirrer for 24 hours at 45°C. The solution was filtered with Whatman no. 3 filter paper (Filters Fiorini, Sweden), followed by the addition of 0.75% (w/v) Glycerol (Chemolab, Malaysia) and 0.2% (v/v) Tween-80 (Chemolab, Malaysia) into filtered chitosan. Different concentrations of chrysanthemum essential oil (0%, 1%, 2%, 3%, 4%, 5%, and 6% (v/v)) (BF1, Kuala Lumpur) were added to chitosan filtrate by a homogenizer (HG-15A, Korea) at 9000 rpm for 4 minutes at room temperature. The mixture was poured into each petri dish (90×15 mm; Chemolab, Malaysia) and was placed in an oven (Memmert, USA) for 24 hours at 40°C. The dried edible films were peeled off with forceps and kept in a zip-lock plastic bag. The samples were kept in a desiccator until being removed further analysis.

## Physical properties of *Chrysanthemum morifolium* chitosan film

**Thickness.** The thickness of the rectangular shaped film was measured by a hand-held micrometer (JY, China) at five random locations at four side edges and center of the rectangle-shape film (Khaleque et al., 2016).

**Water vapor permeability.** In the cup container, 2 g of Silica gel (Chemolab, Malaysia) was covered by the film and kept for 24 hours at room temperature. The silica gel was used to produce 0% relative humidity conditions below the film. The transmission rate was obtained using a weight changes measurement according to the following equation (Bhopal et al., 2010; Li et al., 2013). Water vapor transmission rate:

$$W, \% = \frac{W_1 - W_2}{W_1} \times 100\%$$

where:

 $W_1$  – the initial weight of the film, g,

 $W_2$  – the weight of film after 24 h, g.

**Color.** The color test was carried out with a Hunter lab colorimeter (CFEZ0814, Hunterlab, Australia). The Hunter lab colorimeter was calibrated with a white and black calibration plate. An edible film with a diameter of 64 cm was placed on a Color Flex sample cup and rotated 90° after each reading. The total color difference ( $\Delta E$ ) of the film was determined based on the equation below:

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

The color of the edible film was expressed in  $L^*$  (brightness 100 or lightness 0),  $a^*$  (level of + red or – green) and  $b^*$  (level of + yellow or – blue)  $L^*$ ,  $a^*$  and  $b^*$  are the standard color parameter values ( $L^* = 93.82$ ,  $a^* = -1.2$ ,  $b^* = 1.61$ ) while L, a and b are the sample color parameter values (Saberi et al., 2016; Zaman et al., 2018).

## Mechanical properties of *Chrysanthemum morifolium* chitosan film

**Tensile strength and elongation at break.** The tensile strength was determined using a Dynamic Mechanical Thermal Analysis (DMTA) machine (TA Instruments, model RSA-3, USA). The film was cut into 20 mm  $\times$  70 mm strips and placed into the film-extension grips and clamped with an initial grip distance of 50 mm. The crosshead speed was adjusted to 20 mm/min. The tensile strength and elongation at break were determined based on the equations below (Dick et al., 2015; Saberi et al., 2016):

Tensile strength, MPa = 
$$\frac{\text{peak load, N}}{\text{cross-sectional area, mm}^2}$$
Elongation at break = 
$$\frac{\text{final length of film}}{\text{initial grip length, mm}} \times 100\%$$

**Puncture strength.** The puncture test was performed with a Texture Analyzer (TA.XT Plus, Stable Micro Systems, UK) with a 4 cm diameter disc-shaped piece of film that was fixed in an annular ring clamp (3 cm diameter). The film was given a vertical movement of 1.0 mm diameter with a spherical probe at a constant speed of 1 mm/s. The puncture strength (N) and deformation (mm) of the film were determined based on force-deformation curves to determine the mechanical resistibility of the films under sharp stress (Saberi et al., 2016).

### Chemical characteristics of *Chrysanthemum morifolium* chitosan film

Fourier transform infrared spectroscopy (FTIR). The absorbance spectra of the edible films were recorded and determined using Fourier Transform Infrared (FTIR) spectrometry (Thermo Fisher Scientific, Nicolet iS5FT-IR spectrometer, Waltham, MA, USA). Fourier transform infrared spectroscopy – FTIR was connected to the OMNIC Spectra Software in transmission mode. The films were placed in the sample holder and fixed by a tiny probe. The spectra of the films were obtained with a resolution of 4 cm<sup>-1</sup> as the average of 20 scans is within the range of 500 cm<sup>-1</sup> to 4000 cm<sup>-1</sup> against a background spectrum from an empty cell (Hromiš et al., 2015).

Antioxidant assay for *Chrysanthemum morifolium* chitosan film. DPPH (2,2-diphenyl-1-picrylhydrazyl) free radical scavenging assay (Sigma Aldrich, Germany) was conducted by mixing the film solution (25 mg film in 3 mL distilled water) with methanolic DPPH solution (4.3 mg DPPH in 3.3  $\mu$ l of methanol) and wrapped with aluminum foil (Ruiz-Navajas et al., 2013). The mixture was mixed using a vortex followed by incubation in the dark for 30 minutes, and the absorbance was determined using a UV-Vis Spectrophotometer at 517 nm with ethanol (Midlothian, UK) as a control. The scavenging effect was quantified through the equation:

$$\frac{\text{DPPH}}{\text{scavenging}} = \frac{\text{Abs of DPPH} - \text{Abs of sample}}{\text{Abs of DPPH}} \times 100\%$$

where:

Abs of DPPH – the absorbance before reaction,

Abs of the sample – the absorbance after the reaction has taken place for 30 minutes (Alam et al., 2013).

Antimicrobial properties of *Chrysanthemum morifolium* chitosan film. The disc diffusion method was applied to measure the antimicrobial properties of *Chrysanthemum morifolium* chitosan film (Zaman et al., 2018). Bacteria such as *Vibrio cholerae*, *Staphylococcus aureus*, *Escherichia coli*, and *Salmonella typhimurium* were prepared in Mueller Hinton Agar (MHA; Oxoid, UK) by the UCSI lab technician. Disc-shaped edible films of 6mm diameter were placed on MHA agar, and Penicillin and ethanol were used as positive and negative controls. The plates were incubated for 24 hours in an incubator (S1500, UK) at 37°C. The diameter of each inhibition zone to certain bacteria represents the antimicrobial activity of the film.

Storage test of meat products (chicken and beef) Preparation of meat products (chicken and beef).

The fresh meats (chicken and beef) were purchased from a local supermarket (Kepong, Kuala Lumpur) and were cleaned thoroughly. The meat samples were cut into 5 cm  $\times$  5 cm pieces with a knife and kept in a chiller at 4°C for the shelf-life test.

**Application of** *Chrysanthemum morifolium* chitosan film to meat products (chicken and beef). The meat products wrapped with chitosan-*Chrysanthemum morifolium* film and unwrapped meat (control) were placed into a polyethylene plastic bag and stored in a chiller at 4°C for a five-day of storage test.

**pH values of meat products (chicken and beef).** pH tests were conducted on the meat stock solution (meat homogenized with 100 mL of peptone water) with a calibrated pH meter (3505 pH meter, Singapore) (Sujiwo et al., 2018).

**Microbial count of** *Staphylococcus aureus.* Total Plate Count (TPC) of *Staphylococcus aureus* was conducted using the spread plate method with Columbia blood sheep agar (Connolly et al., 2017; Remya et al., 2016). The meat stock solution was serially diluted from  $10^{-1}$  to  $10^{-6}$  in a test tube, and each dilution was inoculated onto an agar plate. The plates were incubated

at 37°C, and TPC (log CFU/mL) were conducted during each day of storage (day 0 to day 5).

#### Statistical analysis of data

The analyses were carried out in triplicate (n = 3), and the data were analyzed using IBM SPSS version 20. The results were expressed in mean values ±standard deviation by using the One Way ANOVA Tukey test. The significance level was set at 95% interval  $(p \le 0.05)$ .

#### **RESULTS AND DISCUSSION**

Based on the preliminary optimization result, the chitosan films with higher *Chrysanthemum morifolium* essential oil extract (>5% (v/v)) were viscous and oily. As a result, the films were hard to shape and stuck to the disc puncture with brownish-gold clusters of oil seen throughout the surface of the film. Meanwhile, previous studies showed that 1.5% (w/v) chitosan produced the optimal physical and mechanical properties for an edible film (Choong et al., 2019; Zaman et al., 2018). Therefore, 1.5% (w/v) chitosan filtrate with 0% to 4% (v/v) of *Chrysanthemum morifolium* essential oil was used in the storage test to determine the effects of the film on the microbiological properties of chicken and beef (Fig. 1).

#### Physical properties of films

Film thickness and water vapor permeability (WVP) test. According to Table 1, the thickness of the films increased from 0.05 mm to 0.15 mm with an increasing concentration of *Chrysanthemum morifolium* essential oil. The results show a significant difference  $(p \le 0.05)$  in terms of film thickness from 0% to 4% (v/v) of the *Chrysanthemum morifolium* film. Similar results were found by Khaleque et al. (2016) and Ahmed et al. (2016), who found that film thickness increased with the addition of different concentrations of clove oil and cinnamon oil, respectively. Zaman et al. (2018) reported that essential oil filled a chitosan matrix caused the value of thickness to increase.

Based on Table 1, the incorporation of essential oil (0% to 4% (v/v)) showed an increasing trend of the WVP ranging from 18.99% to 38.83%. With an increase of 2% to 4% (v/v) of essential oil, the value of WVP significantly increased from 26.76% to 38.83%

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**Fig. 1.** Appearance of chitosan film with: A - 5% (v/v) of *Chrysanthemum morifulium* CH film, B - 4% (v/v) *Chrysanthemum morifolium* CH film; CH – chitosan

 $(p \le 0.05)$  indicating a decrease in moisture resistance of the films (Choong et al., 2019; Li et al., 2013). This can be explained by the reduction of the association between chitosan molecules, resulting in the loss of the film's spatial structure when the concentration of herbal extracts increased (Saberi et al., 2016). **Color test.** Based on Table 2, the  $\Delta E$  values ranged from 5.61 to 62.69, and the lightness ( $L^*$  value) of the films reduced from -5.25 to -16.17 when the

Table 2. Color test of chitosan i	incorporated v	with different
concentrations of Chrysanthemun	m morifolium	essential oil

Concentration %	$L^*$	<i>a</i> *	<i>b</i> *	$\Delta E$
0	31.14 ±0.26°	$-0.80 \pm 0.06^{a}$	$\begin{array}{c} 0.92 \\ \pm 0.38^{a} \end{array}$	$\begin{array}{c} 0.00 \\ \pm 0.26^{d} \end{array}$
1	$-5.25 \pm 0.61^{b}$	0.16 ±0.02 <sup>b</sup>	1.98 ±0.27 <sup>b</sup>	5.61 ±0.51ª
2	$-14.41 \pm 0.17^{a}$	$0.54 \pm 0.05^{b}$	3.46 ±0.31°	$14.83 \pm 0.18^{\rm b}$
3	$-15.27 \pm 0.78^{a}$	1.10 ±0.07°	$\begin{array}{c} 8.50 \\ \pm 0.31^{d} \end{array}$	17.51 ±0.31°
4	$^{-16.17}_{\pm 0.45^{a}}$	1.52 ±0.07°	10.97 ±0.62°	19.60 ±0.40°

**Table 1.** Physical and mechanical properties of chitosan ed-ible film with different concentrations of *Chrysanthemum morifolium* essential oil

Water vapor permeability

%

 $18.99 \pm 0.68^{\mathrm{a}}$ 

 $19.23 \pm 1.93^{a}$ 

Thickness

mm

 $0.05 \pm 0.01^{\rm a}$ 

 $0.07 \pm 0.01^{b}$ 

Results are expressed as means ±standard deviation, and col-			
4	0.15 ±0.01°	$38.83 \pm 1.06^{\rm d}$	
3	$0.12 \pm 0.01^{\rm d}$	$31.88\pm\!0.62^\circ$	
2	$0.10\pm 0.01^{\circ}$	$26.76 \pm 1.41^{b}$	

umns with different superscripts (a, b, c, d, e) differ significantly ( $P \le 0.05$ ) from each other.

Concentration

%

0

1

concentration of the essential oil increased from 1% to 4%, which is similar to reports by Zaman et al. (2018) and Ahmed et al. (2016). This might be due to the color nature of *Chrysanthemum morifolium*, which reduced the transparency and induced yellowness. This can be observed through a noticeable increment in the  $b^*$  parameter, which indicates a high-intensity yellow hue. In addition, the addition of the yellow color of *Chrysanthemum morifolium* essential oil in a concentration-dependent manner resulted in low transparency and high opacity of the film. Li et al. (2013) also reported that the transparency of the films was reduced as the absorbance of the film with different concentrations of essential oils increased.

## Mechanical properties of *Chrysanthemum morifolium* chitosan film

Based on Table 3, the values of elongation at break and puncture strength are inversely proportional to *Chrysanthemum morifolium* concentration. The 0% *Chrysanthemum morifolium* film showed the highest value of tensile strength (15.477 MPa), elongation at break (17.877%) and puncture force values (24.240 N) by a significant margin ( $p \le 0.05$ ). The addition of essential oil resulted in lower intermolecular interactions, which caused the film to be brittle and fragile (Saberi et al., 2016). On the other hand, the 4% *Chrysanthemum morifolium* film showed the lowest value of tensile strength (5.120 MPa), elongation at break

**Table 3.** Tensile strength, elongation at break and puncture force of chitosan film with different concentrations of *Chrysanthemum morifolium* 

Concen- tration %	Tensile strength MPa	Elongation at break %	Puncture force N
0	$15.477 \pm 0.130^{\circ}$	17.877 ±0.201°	$24.240 \pm \! 0.532^{\rm d}$
1	$8.813 \ {\pm} 0.055^{\text{b}}$	16.180 ±0.225°	$17.347 \pm 0.484^{\circ}$
2	$7.777 \ \pm 0.042^{\rm b}$	$14.073 \pm \! 1.634^{\rm b}$	$8.067 \pm \! 0.794^{\rm b}$
3	$5.870\pm\!\!0.207^{\rm a}$	$10.723 \pm 0.168^{b}$	$6.800 \pm 0.815^{a}$
4	$5.120\pm\!\!0.020^a$	$7.770 \pm 0.178^{\rm a}$	$6.777 \pm 0.206^{a}$

Results are expressed as means  $\pm$ standard deviation and columns with different superscripts (a, b, c, d) differ significantly ( $P \le 0.05$ ) from each other.

(7.770%), and puncture force (6.777 N), as shown in Table 3. This might be due to the existence of an incompatible substance in the essential oil caused by the discontinuous structure and irregular matrix of chitosan (Saberi et al., 2016).

# Chemical properties of *Chrysanthemum morifolium* chitosan film

Fourier transform infrared spectroscopy (FTIR). The FTIR test was carried out to study the interaction between chitosan and Chrvsanthemum morifolium essential oil. Figure 2 shows a similar pattern of FTIR spectra among all the chitosan films incorporated with various levels of Chrysanthemum morifolium essential oil. The 0% to 4% Chrysanthemum morifolium in the chitosan film showed 4 similar peaks which were around 3281 cm<sup>-1</sup>, 2880 cm<sup>-1</sup>, 1551 cm<sup>-1</sup>, and 1150 cm<sup>-1</sup>. The absorption peak of around 3281 cm<sup>-1</sup> was caused by the stretching of O-H and N-H bond bonds (Qin et al., 2015). Meanwhile, an absorption area of 2880 cm<sup>-1</sup> was due to -CH<sub>2</sub> stretching, followed by 1551 cm<sup>-1,</sup> and 1150 cm<sup>-1</sup> which were caused by C-N and -NH stretching (amide II) and C-O (carbonyl group), respectively (Breda et al., 2017; Choong et al., 2019; Liu et al., 2008). N-H and C-HO stretching was representative of the chemical structure of chitosan specifically (Breda et al., 2017; Qin et al., 2015), and other peak patterns were representative of the chemical characteristics of Chrysanthemum morifolium and chitosan edible film. Jiang et al. (2013) reported that Chrvsanthemum morifolium extract showed similar peaks of 3384 cm<sup>-1</sup>, which is representative of the O-H stretching mode, and 2921 cm<sup>-1</sup>, which is representative of the aldehydic C-H stretching mode. This is similar to studies conducted by Liu et al. (2008) on different types of Chrysanthemum essential oil, which specified that the contents of CH<sub>2</sub> in different Chrysanthemum samples are very different.

Antioxidant and antimicrobial properties of *Chrysanthemum morifolium* chitosan film. According to Table 4, the readings of the scavenging effect increased from 4.97% to 18.63% as the concentration of essential oil increased from 1% to 4%. This trend is similar to results found by Hromiš et al. (2015) that the antioxidant activity of chitosan film increased from 28% to 90% with the incorporation of 0% to 3% of





**Fig. 2.** The FTIR spectra for chitosan film with different concentrations of *Chrysanthemum morifolium*: CH – chitosan, EO – essential oil

Table 4. Scavenging effect and microbial inhibition of chitosan-Chrysanthemum morifolium

Concentration, %	Scavenging effect, % —	Inhibition zone, mm	
		Staphylococcus aureus	Escherichia coli
0	$4.971 \pm 0.685^{a}$	$0.00 \pm 0.00^{a}$	$0.00\pm 0.00^{\mathrm{a}}$
1	$5.412 \pm 0.69^{\rm a}$	$0.00 \pm 0.00^{a}$	$0.00\pm 0.00^{\mathrm{a}}$
2	$9.421 \pm 1.201^{b}$	$2.667 \pm 0.58^{\text{b}}$	$0.00\pm 0.00^{\mathrm{a}}$
3	$11.626 \pm 1.09^{b}$	$3.167 \pm 0.29^{b}$	$1.033 \pm 0.06^{\rm b}$
4	18.632 ±2.471°	$3.822\pm0.29^{b}$	$1.100 \pm 0.17^{\rm b}$

Results are expressed as means ±standard deviation and columns with different superscripts (a, b, c) differ significantly ( $P \le 0.05$ ) from each other.

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caraway essential oil, respectively. The 4% *Chrysanthemum morifolium* film showed a significant difference ( $p \le 0.05$ ) compared to the other concentrations of essential oil.

Meanwhile, inhibitory zone tests were conducted for four bacteria: Vibrio cholera, Staphylococcus aureus, Escherichia coli, and Salmonella typhimurium. However, 0% to 4% Chrysanthemum morifolium chitosan film showed no significant inhibitory effects on Vibrio cholera and Salmonella typhimurium. Therefore, Table 4 showed the microbial inhibition of *Chrysanthemum morifolium* towards *Staphylococcus aureus* and *Escherichia* coli. The control film (0% *Chrysanthemum morifolium* chitosan film) and 1% *Chrysanthemum morifolium* chitosan film did not have inhibitory effects on the two bacteria. However, the inhibition zone of *Staphylococcus aureus* increased from 2.667 mm to 3.822 mm as the *Chrysanthemum morifolium* concentration increased from 2% to 4% (Fig. 4). However, there was no significant difference



**Fig. 3.** pH changes from the first day to the fifth day of storage for chicken (A) and beef (B) samples: control – chitosan film without *Chrysanthemum morifolium* essential oil, CH – chitosan

(p > 0.05) in the effects of different concentrations of essential oil on *Staphylococcus aureus*. 2%, 3% and 4% of *Chrysanthemum morifolium* in *the* chitosan film showed the highest inhibition zone results and were applied to the food products to proceed with the storage test.

#### Storage of chicken and beef

**pH.** According to Figure 3, the control sample showed deterioration in terms of quality, with a rise in pH value from day 0 to 5 of storage time in chicken (5.86 to 6.77) and beef (5.53 to 7.06) samples, accordingly. Poultry meat is considered to be of good quality



**Fig. 4.** TPC of *Staphylococcus aureus* found at: A – chicken, B – beef at the control and *Chrysanthemum morifolium* edible film treatment; CH – chitosan, TPC – total plate count

at a pH of 6.2 and is considered to be inedible when the pH is higher than 6.2 (Sujiwo et al., 2018). Meanwhile, beef meat with a pH of 5.6 to 6.0 is safe for consumption, and the darkening of beef meat occurs when the pH is higher than 6.0 (Kuswandi and Nurfawaidi, 2017). The control sample was unsafe for consumption as a pH value above 6.7 on the fourth and fifth days of storage. However, 2%, 3%, and 4% *Chrysanthemum morifolium* chitosan films were able to retain a pH below 6.7 from the first day (5.59) to the fifth day (6.25) of storage.

#### Total plate count (TPC) of Staphylococcus aureus.

Based on Figure 4, the growth rate of Staphylococcus aureus in chicken and beef gradually increased with continuing days of storage. Throughout the five days of the storage test, 4% Chrysanthemum morifolium in chitosan film was the most effective in controlling the growth of Staphylococcus aureus in both chicken and beef compared to the control group, 2% and 3% of essential oil. A similar study by Salgado et al. (2015) on edible film with clove essential oil showed a reduction in the total plate count. The Food Safety Authority of Ireland stated that the threshold level of Staphylococcus aureus is 10<sup>5</sup> log CFU/mL as the toxin is produced at that detectable level, and it is unsafe for consumption. With 4% Chrysanthemum morifolium in the film, the total plate count of Staphylococcus aureus in beef and chicken from the first day to the fifth day of storage was 3.6 to 4.7 log CFU/ml, which is safe for consumption. Therefore, 4% Chrysanthemum morifolium chitosan film improved the shelf life of meat products up to the fifth day of storage compared to the control group (without film) that reached the limit point and only kept until the third day of storage.

#### CONCLUSION

The addition of *Chrysanthemum morifolium* essential oil increased the values of thickness, water vapor permeability, scavenging effect, and total color difference of chitosan films. On the other hand, transparency, mechanical properties, tensile strength, elongation at break, and puncture force values decreased as the concentration of the essential oil increased. Chitosan film with different concentrations of *Chrysanthemum morifolium* revealed inhibitory activity towards *Staphylococcus aureus*, followed by *Escherichia coli*, which is commonly found in meat products. Based on the storage test and pH values, chitosan with 4% *Chrysanthemum morifolium* essential oil extended the storage life of chicken and beef up to 5 days in the chiller.

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