International Food Research Journal 31(2): 443 - 453 (April 2024)
Journal homepage: *http://www.ifrj.upm.edu.my*

Combined effects of peanut oil and glycerol on whey protein-based edible film in extending fresh chicken meat shelf life

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Article history

Received:
13 February 2023
Received in revised form:
2 January 2024
Accepted:
22 January 2024

Keywords

edible film,
whey protein,
peanut oil,
chicken meat

Abstract

The present work aimed to develop a new antibacterial composite edible film of whey protein-based containing peanut oil (PO). Different concentrations of PO (0, 2, and 4% wt.), glycerol (Gly) (10, 15, and 20% wt.), and Tween 80 (2% wt.) were used as ingredients. The used of PO as antibacterial compound in the edible film was applied on fresh chicken meat during storage time at room temperature (25°C) and refrigerated temperature (-5°C) for 10 d. The characteristics of edible film, texture, and TPC on chicken meat were evaluated. Based on the results, the incorporation of Gly to the film increased the moisture content, while the incorporation of PO to the film decreased the moisture content, and slightly increased the thickness. The antibacterial effect of the composite film increased linearly with increasing PO concentration. The shelf life of chicken meat for film containing 4% wt. PO was the best during chilled temperature storage for 10 d.

DOI
https://doi.org/10.47836/ifrj.31.2.15

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Introduction

Chicken meat is an animal protein widely consumed by the population. Fresh chicken meat is highly susceptible to spoilage, and can be a perfect medium for microbial growth (Karimnezhad *et al*., 2019). Therefore, its preservation requires specialised packaging systems, during the distribution or transportation process till consumption, to maintain the quality of fresh chicken meat. Nowadays, meat companies are using synthetic packaging from non-degradable sources such as polyamide, polyethylene, polypropylene, and ethylene vinyl alcohol, which can be harmful to the environment (Siriwardana and Wijesekara, 2021).

Edible film is an alternative packaging that can be directly consumed with food products. Edible film can reduce non-organic waste which has been dominated by food packaging, besides the reduction of moisture loss from the packaged product (Bourtoom, 2008). The barrier requirements of edible films depend on the properties of the product and their applications (Mohamed *et al*., 2020). Films can be produced using natural biopolymers of polysaccharides, proteins, and lipids, either used alone or together, with the addition of plasticisers (Gennadios *et al*., 1993). The use of natural biopolymers as packaging materials can be enhanced with the incorporation of antimicrobial substances that help in the reduction of microbial loads (Soni *et al*., 2018).

Protein has the advantage of producing a film that is transparent, flexible, odourless, colourless, and has the property of retaining the aroma of the food product (Liaotrakoon and Raviyan, 2018; Mohamed *et al*., 2020). Various protein materials, including casein, collagen, corn zein, fish protein, ovalbumin, soy protein isolate, wheat gluten, and whey protein isolate have been verified (Ogur and Erkan, 2015). Whey protein is a pure protein source that contains protein concentrations of up to 90% or more. One of the potential sources of protein for food is nuts such

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as green beans, soybeans, and so on, which have been widely used as a source of protein. Protein isolates from whey protein containing α-lactalbumin and β-lactoglobulin are characterised by a transparent and flexible film (Liaotrakoon and Raviyan, 2018). The incorporation of peanut oil (PO) to edible films will provide excellent advantages in nutrition and shelf life. This is because PO contains up to 80% unsaturated fatty acids, especially oleic and linoleic acids (Dun *et al*., 2019). The development of edible films from whey protein combined with lemon and bergamot essential oils as carried out by Çakmak *et al*. (2020) produced films that had high antibacterial activity; they were able to inhibit the growth of *E. coli* and *S. aureus*. Emulsion-based films made using fatty acids can improve the water vapour barrier properties. Previous studies reported that oregano and thyme essential oil-coated edible film increased the shelf life of chicken meat patties (Soni *et al*., 2018). Soy protein film added with clove essential oil could effectively be resistant against *S. choleraesuis* and *S. sonnei* bacteria (Echeverría *et al*., 2016). The use of cinnamon essential oil in edible film solution showed significant increase in the elongation of the film, and extension of food shelf life (Zhou *et al*., 2021). Therefore, films formed from lipid-composite whey generally have a good moisture barrier because lipid compounds are hydrophobic. To increase the flexibility, mechanical, and barrier properties of edible films, plasticisers (*e.g*., glycerol (Gly), sorbitol, sucrose, mannitol, and polyethylene glycol) are mixed into film-forming solutions (Abdollahzadeh *et al*., 2021; Fahrullah *et al*., 2021).

The present work aimed to study the effect of the formulation of PO and Gly on the characteristics of the film, and to obtain the best combination of edible film as a packaging material for the shelf life of fresh chicken meat. In the present work, edible films were prepared by adding PO and Gly to the whey protein-based films.

Materials and methods

Materials

Whey protein was purchased from Nutrifood Co., Ltd. (Jakarta, Indonesia), PO was purchased from Candle Lilla (Indonesia), Gly as a plasticising agent was purchased from Ecogreen Oleochemicals Co., Ltd. (Batam, Indonesia), and Tween 80 was purchased from KAO Indonesia Chemicals Co., Ltd. (Indonesia). All chemicals used in the present work were of analytical grade.

Methods

Preparation of edible films

Edible film solution was prepared by dissolving 15 g of whey protein in 100 mL of distilled water, and heating the mixture at 85°C for 10 min. The film-forming solution was formed by adding PO at different ratios (0, 2, and 4% (v/v) of whey protein), then 10, 15, and 20% (v/v) of Gly and 2% (v/v) of Tween 80 was added into the solution. The solution was stirred at 75°C (500 rpm, 20 min) to obtain a homogeneous solution using Ultra Turrax Homogenizer (IKA T10). After mixing, 25 mL of the solution was poured onto a 30 × 9 cm acrylic board, and then dried for 24 h. The composite film was peeled from the acrylic board, and stored in a desiccator at 25°C for 48 h before further characterisation.

Application of edible film on fresh chicken meat

Fresh chicken meat was obtained from a local slaughterhouse. Then, the skinless chicken breast meat were cut into 2 × 2 cm dimension. Each meat was wrapped with the edible film. All meat samples were stored in a refrigerator (-5°C) and room temperature (25°C) for 10 d. During storage, the quality of meat was observed and analysed for the changes of meat texture, colour, and bacterial load.

Total plate count

Two grams of chicken meat sample was diluted with 45 mL of 0.85% NaCl until 10^{-1}, 10^{-2}, 10^{-3}, 10^{-4}, 10^{-5}, and 10^{-6} g/mL of sample were obtained. One millilitre of diluted aliquots was inoculated on Petri dishes, and approximately 15 mL of plate count agar was added. After the agar was solidified, all Petri dishes were inverted and placed in an incubator at 30°C for 24 h.

Texture measurement

The hardness of chicken meat were measured using a GY-3 manual sclerometer with 11 mm diameter flat-head stainless steel cylindrical probe. Samples with 2 cm thick sections were evaluated by puncturing for three times, and the mean value was calculated.

Characterisation of edible films

Edible film colour analysis

The colour of the films was measured using a colorimeter FRU WR-10QC (Shenzhen Wave Optoelectronics Technology Co., Ltd, Shenzhen, China). Instrumental colour readings were L^* (lightness), a^* (red-green), and b^* (yellow-blue). The total colour difference was calculated using Eq. (1):

$$\Delta E = \sqrt{(L^* - L)^2 + (a^* - a)^2 + (b^* - b)^2} \quad \text{(Eq. 1)}$$

where, L^*, a^*, and b^* = standard values of white plate, and L, a, and b = values of samples measured.

Moisture content analysis

Measuring water content principle is by drying the material in an oven until achieving a constant weight (Apriliyani *et al*., 2020). Film samples were cut and placed into Petri dish, and their weights were recorded before (1 ± 0.0001 g) and after oven-drying at 105°C for 3 h. Then the sample was cooled in a desiccator, and moisture content was calculated using Eq. 2:

$$Moisture\ content = \frac{W_1 - W_2}{W_1 - W_0} \text{ x } 100\% \quad \text{(Eq. 2)}$$

where, W_0 = weight of the Petri dish (g); W_1 = weight of the Petri dish and film specimen before drying (g); and W_2 = weight of the Petri dish and film specimen after drying (g).

Tensile strength and percentage elongation analysis

Tensile strength (TS) and percentage elongation (%E) were measured using Brookfield CT 03 4500. TS was calculated by dividing the maximum load necessary to pull the specimen apart by the cross-sectional area of the specimen (Eq. 3). %E was calculated by dividing film elongation at the moment of rupture by initial gage length of specimens, and multiplying by 100% (Eq. 4) (Gennadios *et al*., 1993).

$$TS = \frac{F}{Ac} \quad \text{(Eq. 3)}$$

where, TS = tensile strength (Mpa); F = maximum load (N); and Ac = cross-sectional area of the sample (mm^2).

$$E = \frac{L}{L_0} \text{ x } 100\% \quad \text{(Eq. 4)}$$

where, E = elongation of edible film (%); L = length of the edible film at break (mm); L_0 = initial length (mm).

Microstructures

The surface and cross-sectional morphologies of the films were observed using JEOL JSM-6510 LA scanning electron microscope (Japan). The specimen was placed in a sample holder then sprayed to remove impurities, and coated with gold. The electron accelerating voltage was 15 kV, and each sample was observed at 250 and 3,000× magnifications (Suharyani *et al*., 2021).

Statistical analysis

Mean and standard deviation (SD) of the data were calculated using MSExcel. One-way ANOVA was applied to identify any significant differences of the various properties of the film formulations (PO and Gly concentrations) on the moisture content, thickness, colour parameters, tensile strength, elongation, microstructure, TPC, and texture measurement. Duncan's tests were performed as *post hoc* analysis. Statistical significance level was set at $p < 0.05$. Statistical tests were conducted using the IBM SPSS for Windows, version 26.0 (IBM Corp., Armonk, NY, USA).

Results and discussion

Effect of PO and Gly incorporation on physical properties of edible film

Table 1 shows the physical properties of the whey protein films containing PO and Gly. Moisture content (MC) of the whey protein film increased linearly with the incorporation of Gly (10, 15, and 20% (w/w)). As a hydrophobic plasticiser, Gly has water-holding properties which can hold the hydrophilic molecule, and retain the moisture. Liaotrakoon and Raviyan (2018) also suggested that higher Gly contents increased MC in edible films. Furthermore, high concentration of Gly promotes retention of water molecules in the matrix, and supports hydrogen bonding between Gly and water. Abdolshahi *et al*. (2022) reported similar results in an edible film from *Lallemantia peltata* (L.) seed gum. The mechanical properties of the films improved as the Gly content increased. However, the MC of whey protein film gradually decreased when PO was

Table 1. Moisture content (MC) and thickness of whey protein-based edible films incorporated with peanut oil (PO) and glycerol (Gly) at different concentrations.

Film sample	MC (%)	Thickness (mm)
10% Gly	0.18 ± 0.03^{abc}	0.22 ± 0.01^{ab}
15% Gly	0.19 ± 0.04^{abc}	0.21 ± 0.02^{ab}
20% Gly	0.24 ± 0.03^{a}	0.20 ± 0.01^{b}
2% PO-10% Gly	0.12 ± 0.06^{cd}	0.25 ± 0.07^{ab}
2% PO-15% Gly	0.17 ± 0.02^{bcd}	0.21 ± 0.01^{ab}
2% PO-20% Gly	0.20 ± 0.05^{ab}	0.19 ± 0.06^{b}
4% PO-10% Gly	0.11 ± 0.05^{d}	0.29 ± 0.01^{a}
4% PO-15% Gly	0.14 ± 0.02^{bcd}	0.22 ± 0.03^{ab}
4% PO-20% Gly	0.19 ± 0.03^{abc}	0.23 ± 0.04^{ab}

Values are mean ± standard deviation of triplicates (n = 3). Means followed by different lowercase superscripts in the same column significantly different between the film samples ($p < 0.05$).

incorporated into the film. The increase in PO concentration (2 and 4% (w/w)) affected the amount of water in the film. Moreover, the incorporation of PO also affected the hydrogen bonds in the matrix which became less hydrophobic, and subsequently reduced water solubility.

The thickness of the whey protein films ranged from 0.21 ± 0.01 to 0.29 ± 0.01 mm. These results did not show any significant change ($p > 0.05$) in thickness of film properties, although the thickness increased with higher concentrations of PO (2 and 4% (w/w)). The thickness of 10% (w/w) Gly film without PO was 0.22 ± 0.01 mm. The incorporation of PO into the film with 2 and 4% (w/w) slightly increased the thickness of films from 0.25 ± 0.07 to 0.29 ± 0.01 mm. Kadzińska *et al*. (2020) and Sani *et al*. (2021) emphasised that the incorporation of vegetable oils altered the thickness and microstructure of the films. Moreover, the thickness of film is also affected by the size and distribution of essential oils in the matrix, which resulted in increased free volume of the film. Tügen *et al*. (2020) demonstrated the effect of essential oil incorporation on the thickness of edible film. They noted that high concentration of lemon essential oil increased the thickness of gelatine/chitosan edible film. Li *et al*. (2021) also found an increasing trend in the thickness of the chitosan and fish skin gelatine-based films with the increasing of orange peel essential oils. Han *et al*. (2018) reported similar results in the sodium alginate/carboxymethyl cellulose films incorporated with cinnamon essential oil. In contrast, the interaction between Gly and PO in the whey protein film resulted in insignificant change of thickness values of film. These results contradicted the study of Rocha *et al*. (2013) and Nordin *et al*. (2020), where the incorporation of Gly increased the thickness properties of films.

Effect of PO and Gly incorporation on colour change of edible film

Colour is an important parameter in food packaging that will maintain the quality and affect the appearance of the packaged product. The colour values (L^*, a^*, b^*, and ΔE) of all films are presented in Table 1. The increase in PO concentration resulted in a decrease in lightness (L^*) value of the films. The incorporation of PO introduced phenolic compounds into the film formulation thus, increasing its darkness. Pranoto *et al*. (2005) pointed out that L^* values decreased by incorporating garlic oil into the film composition. In another report, increasing essential oil concentration resulted in decreased lightness, and change the films from transparent to pale yellow (Zhou *et al*., 2021). Similarly, in the present work, the incorporation of Gly at various concentrations into film solution decreased the lightness value. Opposite results were observed when Gly and PO were incorporated; total chromatic difference (ΔE) values increased as the amount of Gly and PO incorporated increased. Edible film with 4% (w/w) PO and 20% (w/w) Gly showed the highest ΔE values (41.43 ± 0.22) compared with others. These results might have been caused by the oil compound which formed an emulsion when incorporated to the film solution. This result agreed with other researchers who found the

increase in *ΔE* value of the films containing essential oils (Rocha *et al*., 2013; Désiré *et al*., 2018; Farajpour *et al*., 2020).

Tensile strength, elongation, and morphology of edible film

Figure 1 shows the tensile strength and elongation of edible film at different concentrations of PO and Gly. The tested films were rectangular strips, 30 mm in length and 5 mm in width. Films containing 2 and 4% (w/w) PO had decreased film tensile strength from 0.15 to 0.13 MPa at 15% of Gly (Figure 1a). However, the opposite result was that the elongation of film increased from 37.33 to 45.50 MPa for the film containing PO with 2 and 4% (w/w) (Figure 1b). Tensile strength could significantly be decreased with the incorporation of essential oil. Zhou *et al*. (2021) pointed out that essential oil could easily penetrate biopolymer network which reduced the intra- and inter-molecular interactions. Therefore, the tensile strength of film decreased with the incorporation of PO. The decrease in tensile strength as a result of increasing essential oil concentration has also been reported in composite edible films using clove oil (Fahrullah *et al*., 2021) and cinnamon-perilla essential oil (Zhao *et al*., 2022). Incorporating higher oil concentrations imparts better elasticity to the film. Results indicated that the elongation values of film increased with increasing PO content. The observed elongation values showed that a more stretchable matrix was formed when the hydrophobic portion increased in the emulsion-based films. A previous study also reported that increasing palm oil concentration (6, 7, and 8%) decreased tensile strength and increased elongation of WPI-palm oil composite films (Liaotrakoon and Raviyan, 2018). Similarly, Galus and Kadzińska (2016) reported an increase in tensile strength at lower almond and walnut oils. We also found these similar results in our study. Moreover, further increase in plasticiser decreased the film tensile strength ($p < 0.05$). Increasing Gly concentration in composite film significantly increased the mobility of polymer network chains by decreasing the interaction between the molecules of the basic ingredients the edible film (Rusli *et al*., 2017; Malik *et al*., 2022). The large number of hydroxyl groups, small molecule size, and hydrophilic nature of Gly make the molecules penetrate between the polymer chains, and weaken the intermolecular forces. This would alter and result in greater cross-linking and barrier ability than other plasticisers (Gennadios *et al*., 1993; Ebrahimi *et al*., 2016; Zhang *et al*., 2016).

In contrast, with increasing plasticiser concentrations, elongation of the films also increased. Results showed that the presence of Gly in whey protein based films clearly resulted in a decrease of tensile strength around 29%, and an increase in elongation at break around 20% (Figure 1b). Zhang *et al*. (2016) reported that increasing Gly and sorbitol concentrations as plasticisers in a concentration of 15 - 45% (w/w) of gum ghatti films increased film tensile strength but decreased its elongation. An increase in elongation at break and decrease in tensile strength as a result of Gly presence in films from cassava starches varieties has been reported previously (Désiré *et al*., 2018).

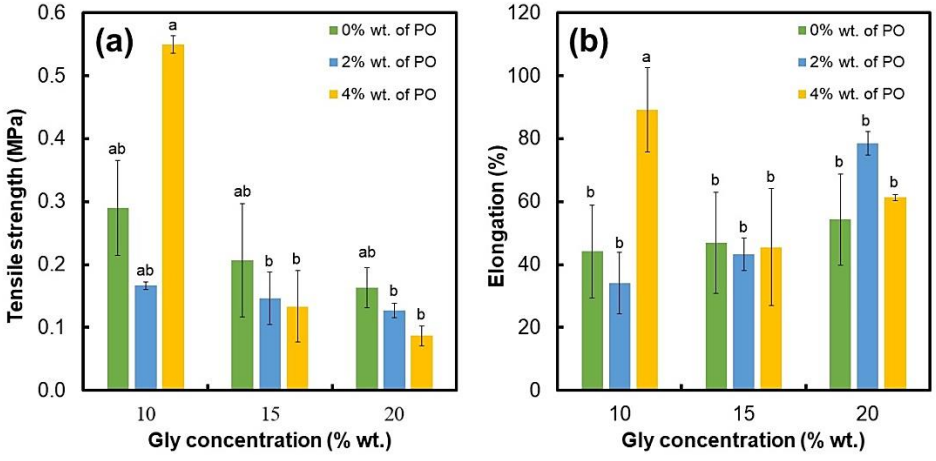

Figure 1. Physical properties of whey protein-based edible films incorporated with peanut oil (PO) and glycerol (Gly) at different concentrations: **(a)** tensile strength, and **(b)** elongation.

Figure 2 shows the surface microstructure and cross-section of whey protein-based films. The microstructure of edible film was tested using a scanning electron microscope (SEM) at a magnification of 500× to evaluate the homogeneity of surface structure. The surface of film depends on the interaction between the components contained in the film-forming solution. The surface and cross-sectional structure of the film changed with the increase in PO concentration.

Table 2 shows the changes in the colour parameters of the whey protein-based edible films. The incorporation of PO (2 and 4% wt.) increased roughness, wrinkles, and cracks on the surface, with some holes appearing (Figures 2d and 2g) compared to the PO-free surface (Figure 2a). Zhou *et al*. (2021)

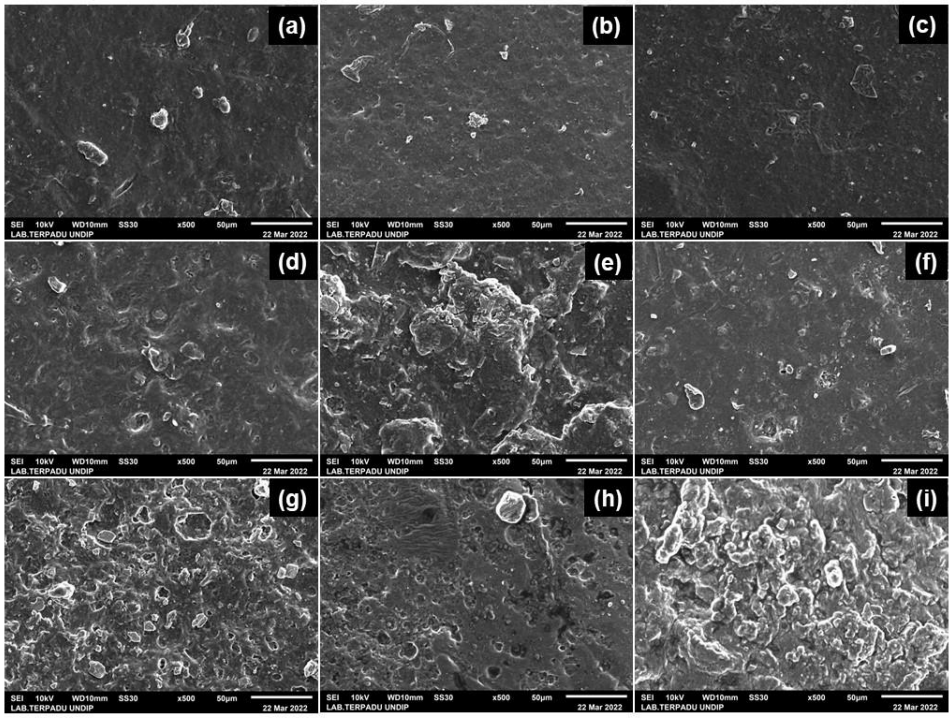

Figure 2. Morphology of whey protein-based edible films incorporated with peanut oil (PO) and glycerol (Gly) at different concentrations under SEM: **(a)** 10% wt. Gly; **(b)** 15% wt. Gly; **(c)** 20% wt. Gly; **(d)** 2% wt. PO-10% wt. Gly; **(e)** 2% wt. PO-5% wt. Gly; **(f)** 2% wt. PO-20% wt. Gly; **(g)** 4% wt. PO-10% wt. Gly; **(h)** 4% wt. PO-5% wt. Gly; and **(i)** 4% wt. PO-20% wt. Gly.

Table 2. Changes in colour parameters of whey protein-based edible films incorporated with peanut oil (PO) and glycerol (Gly) at different concentrations.

Film sample	*L**	*a**	*b**	ΔE
10% Gly	43.37 ± 0.67^{a}	2.00 ± 0.04^{c}	3.57 ± 0.33^{c}	22.02 ± 0.65^{h}
15% Gly	41.25 ± 0.33^{b}	1.81 ± 0.08^{d}	3.71 ± 0.10^{bc}	24.14 ± 0.32^{g}
20% Gly	39.34 ± 0.37^{c}	1.26 ± 0.07^{e}	0.55 ± 0.16^{f}	26.03 ± 0.37^{f}
2% PO-10% Gly	42.49 ± 0.76^{ab}	1.78 ± 0.15^{d}	5.00 ± 0.06^{a}	23.03 ± 0.75^{gh}
2% PO-15% Gly	32.90 ± 0.50^{e}	2.32 ± 0.08^{b}	4.08 ± 0.38^{b}	32.50 ± 0.52^{d}
2% PO-20% Gly	26.01 ± 0.56^{g}	2.43 ± 0.03^{b}	1.78 ± 0.11^{e}	39.33 ± 0.56^{b}
4% PO-10% Gly	37.48 ± 0.51^{d}	1.98 ± 0.06^{c}	5.25 ± 0.20^{a}	28.04 ± 0.49^{e}
4% PO-15% Gly	30.47 ± 1.94^{f}	2.42 ± 0.08^{b}	4.13 ± 0.44^{b}	34.94 ± 1.92^{c}
4% PO-20% Gly	23.92 ± 0.22^{h}	2.61 ± 0.11^{a}	3.00 ± 0.04^{d}	41.43 ± 0.22^{a}

Values are mean ± standard deviation of triplicates (n = 3). Means followed by different lowercase superscripts in the same column significantly different between the film samples ($p < 0.05$).

demonstrated that the incorporation of essential oil caused the changes in film microstructure, decreased the tensile strength, and led to higher water vapour permeability. Similar observations were previously reported by Tügen *et al*. (2020) for the films based on gelatine/chitosan incorporated with lemon essential oil, and was confirmed by Liaotrakoon and Raviyan (2018) for the films based on whey protein isolate with palm oil; the samples incorporated with higher oil content had holes, porosity, and rough surface. On the other hand, the whey protein isolate edible films incorporated with Gly did not result in significant differences in the microstructure (Figures 2a, 2b, and 2c).

Effect of edible film on chicken meat texture

Table 3 shows the texture parameters of the chicken meat during the storage at -5°C. The texture of fresh meat is often used by consumers as an indicator to identify the quality of meat. The incorporation of PO to film-forming significantly decreased the hardness of the meat. Zhao *et al*. (2022) also found the incorporation of essential oil slowly decreased the hardness of chicken meat. In the texture test of each sample over the 10 d, there was an increasing texture value of the chicken meats. For chicken meat wrapped in composite film with PO at refrigerated temperature, the hardness of the sample stored for 10 d showed little increment (0.06%) compared with the film without PO (0.12%) ($p < 0.05$). Figure 3 shows the effect of whey protein-based edible films containing 15% wt. of Gly with different concentrations of PO (0 - 4% wt.) on bacterial growth of chicken meat after 10 d storage at different temperatures. The hardness was not tested beyond 10 d due to spoilage. Edible films with PO were always superior as food product packaging since PO significantly maintained the hardness of chicken meat during storage.

Effect of edible film on chicken meat bacterial load

Bacterial analysis of chicken meat packaged with edible films during 10 d storage at room temperature (25°C) and refrigerated temperature (-5°C) are shown in Figure 4. The bacterial counts in all chicken meats packed under the treatment of edible film and control increased during storage. After 5 d storage under two conditions, TPC of untreated sample increased exponentially to 9.4 log CFU/g. Meanwhile at day 10, the bacterial populations were too numerous to count (TNTC) plus growth of maggots in chicken meat. Results showed that PO incorporation affected the antibacterial properties of the edible films. Bacterial loads in chicken meats after 10 d storage at 0 and 2% wt. PO incorporation were 7.23 and 7.15 log CFU/g, respectively. Meanwhile, at higher PO concentration, 6.76 log CFU/g was recorded. After 10 d storage at refrigerated temperature (Figure 4b), the inhibition effect of whey protein-based edible film on bacterial growth was high compared to 25°C storage temperature. Zio *et al*. (2021) concluded that the incorporation of essential oil with high phenolic compounds and antioxidant substances on the edible film caused antimicrobial, lipid-lowering, anti-carcinogenic, and anti-inflammatory effects of edible film. Therefore, higher concentration of PO was proven to exert significant effect in extending chicken meat shelf life.

Conclusion

Results revealed that increasing the incorporation of PO and Gly concentrations affected the mechanical properties of the composite film. The higher the concentration of PO and Gly in solution, the lower L^* and thickness, and the higher a^*, ΔE, and %E. SEM showed that the incorporation of PO led to rough, cracked, and porous surface of the films. The incorporation of PO to the film also inhibited bacterial growth, and reduced the hardness of sample compared with the control and without PO. These results indicated that these films acted as a natural protective film in extending the shelf life of product. However, further investigation of the effectiveness of composite film on the preservation of product, especially against contamination and other environmental factors is warranted.

Acknowledgement

The authors are grateful to the Chemical Engineering Department, Diponegoro University for the edible film solution preparation using IKA T10 Ultra Turrax Homogenizer. The authors also thank the Institute for Research and Community Service of Diponegoro University for providing the English editing service on the manuscript.

Table 3. Effect of different concentrations of peanut oil (PO) and glycerol (Gly) on the texture of chicken meat during storage (mean ± SD).

Treatment	Storage temperature at (-5°C)						Storage temperature at (25°C)					
	Day 0	Day 1	Day 3	Day 5	Day 7	Day 10	Day 0	Day 1	Day 3	Day 5	Day 7	Day 10
Control	4.50 ± 0.46^{a}	4.80 ± 0.60^{a}	4.13 ± 0.21^{cd}	4.00 ± 0.20^{bcd}	5.23 ± 0.45^{a}	5.33 ± 0.31^{abc}	3.93 ± 0.64^{ab}	4.73 ± 0.15^{a}	4.00 ± 0.20^{c}	3.70 ± 0.30^{c}	3.27 ± 0.31^{d}	3.23 ± 0.25^{e}
10% Gly	3.23 ± 0.32^{b}	3.48 ± 0.19^{cd}	5.40 ± 0.20^{a}	4.42 ± 0.28^{abc}	5.35 ± 0.31^{a}	5.13 ± 0.25^{bcd}	4.33 ± 0.51^{ab}	4.62 ± 0.13^{ab}	5.42 ± 0.18^{a}	5.20 ± 0.36^{a}	5.50 ± 0.10^{b}	5.80 ± 0.26^{a}
15% Gly	3.57 ± 0.21^{b}	3.80 ± 0.20^{bc}	5.83 ± 0.60^{a}	5.00 ± 0.36^{a}	5.73 ± 0.42^{a}	5.07 ± 0.26^{cd}	3.68 ± 0.03^{bc}	3.7 ± 0.05^{c}	4.97 ± 0.15^{b}	5.32 ± 0.13^{a}	6.47 ± 0.32^{a}	4.88 ± 0.08^{b}
20% Gly	3.25 ± 0.35^{b}	4.33 ± 0.25^{ab}	4.48 ± 0.18^{ef}	4.67 ± 0.35^{a}	4.23 ± 0.35^{b}	5.63 ± 0.21^{ab}	4.50 ± 0.17^{a}	4.80 ± 0.30^{a}	4.60 ± 0.30^{b}	5.20 ± 0.36^{a}	5.55 ± 0.18^{b}	5.87 ± 0.31^{a}
2% PO-10% Gly	3.40 ± 0.35^{b}	3 ± 0.20^{ef}	3.42 ± 0.26^{b}	4.03 ± 0.15^{bcd}	4.15 ± 0.35^{b}	4.45 ± 0.23^{e}	2.83 ± 0.15^{d}	2.67 ± 0.45^{d}	3.80 ± 0.46^{c}	3.15 ± 0.13^{d}	3.66 ± 0.25^{d}	4.33 ± 0.15^{c}
2% PO-15% Gly	3.52 ± 0.33^{b}	4.62 ± 0.19^{a}	4.77 ± 0.06^{a}	4.53 ± 0.15^{ab}	5.35 ± 0.31^{a}	4.80 ± 0.26^{cde}	2.80 ± 0.26^{d}	3.63 ± 0.25^{c}	4.67 ± 0.21^{b}	4.63 ± 0.21^{b}	4.55 ± 0.31^{c}	5.63 ± 0.25^{a}
2% PO-20% Gly	3.12 ± 0.19^{b}	3.25 ± 0.15^{cde}	3.75 ± 0.13^{de}	4.70 ± 0.46^{a}	5.28 ± 0.30^{a}	5.78 ± 0.16^{a}	3.17 ± 0.35^{cd}	4.57 ± 0.74^{ab}	3.98 ± 0.15^{c}	4.50 ± 0.30^{b}	4.87 ± 0.21^{c}	4.73 ± 0.32^{bc}
4% PO-10% Gly	2.10 ± 0.70^{c}	2.8 ± 0.36^{efg}	3.87 ± 0.25^{de}	3.63 ± 0.45^{de}	3.67 ± 0.15^{c}	5.03 ± 0.26^{cd}	2.97 ± 0.15^{cd}	3.98 ± 0.68^{bc}	3.83 ± 0.21^{c}	4.33 ± 0.29^{b}	4.60 ± 0.10^{c}	5.48 ± 0.23^{a}
4% PO-15% Gly	1.83 ± 0.15^{c}	2.43 ± 0.21^{fg}	2.90 ± 0.20^{f}	3.13 ± 0.35^{e}	3.40 ± 0.36^{c}	4.67 ± 0.15^{de}	2.43 ± 0.75^{de}	2.20 ± 0.10^{d}	3.03 ± 0.32^{d}	3.60 ± 0.20^{c}	3.05 ± 0.13^{e}	3.85 ± 0.13^{d}
4% PO-20% Gly	2.17 ± 0.21^{c}	2.33 ± 0.55^{g}	3.60 ± 0.61^{de}	3.83 ± 0.25^{cd}	2.57 ± 0.31^{d}	3.77 ± 0.55^{f}	1.83 ± 0.38^{e}	2.33 ± 0.45^{d}	2.97 ± 0.29^{d}	3.75 ± 0.15^{c}	2.97 ± 0.35^{e}	3.73 ± 0.32^{d}

Values are mean ± standard deviation of triplicates (n = 3). Means followed by different lowercase superscripts in the same column significantly different between the film samples ($p < 0.05$).

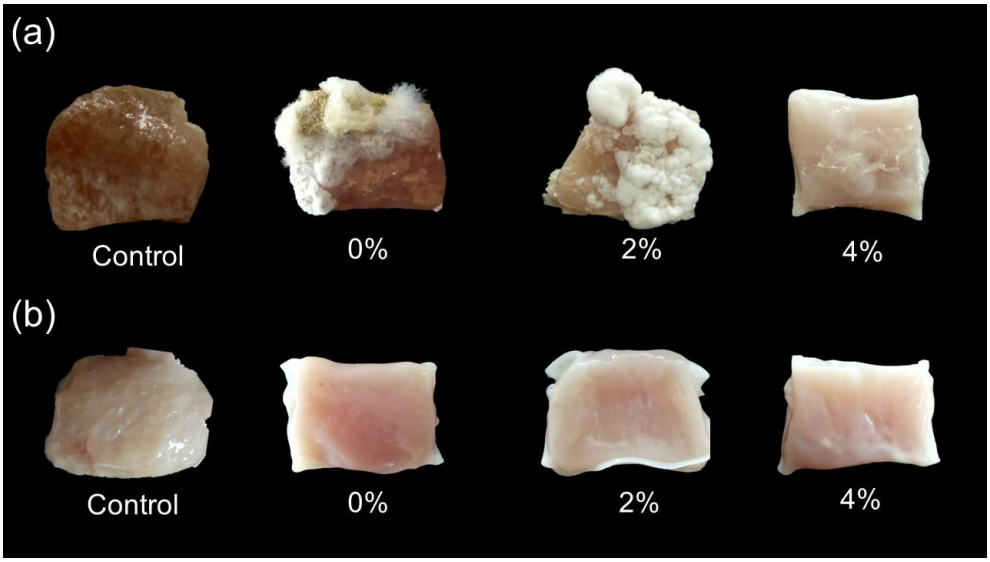

Figure 3. Effect of whey protein-based edible films containing 15% wt. of glycerol and peanut oil at different concentrations (0 - 4% wt.) on microbial growth of chicken meat after 10 days of storage at different temperatures: **(a)** at 25°C; and **(b)** -5°C.

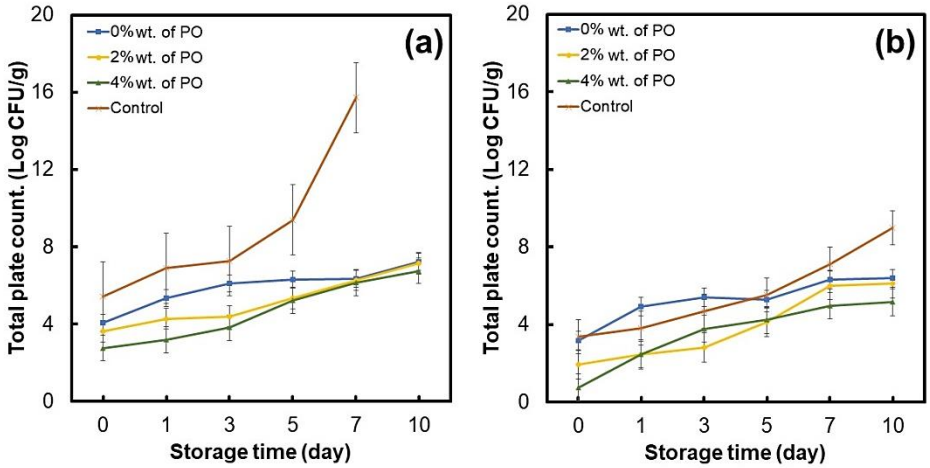

Figure 4. Microbial growth profile of chicken meat treated with whey protein-based edible films containing 15% wt. of glycerol and peanut oil (PO) at different concentrations (0 - 4% wt.): **(a)** at 25°C; and **(b)** at -5°C.

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