Effect of peppermint and citronella essential oils on properties of fish skin gelatin edible films

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Abstract. Fish skin gelatin films incorporated with peppermint and citronella essential oils at difference concentrations (10, 20 and 30% w/w) were prepared by solution casting. Addition of peppermint oil contributed to a significant decrease of tensile strength and Young’s modulus, while the percent elongation at break showed an obvious increase except at 30% w/w. On the other hand, addition of citronella oils promoted a great increase of tensile strength and young’s modulus, but an intense decrease of the percent elongation at break. At the predetermined content, the film incorporated with citronella oils outperformed the one with peppermint oils in term of water vapor transmission and solubility in water. Thermal properties of gelatin films with citronella oils exhibited an enhancement in heat stability, while the one with peppermint oils showed slight decrease in heat stability. The additions with both of essential oils exhibited excellent antibacterial properties against both Staphylococcus aureus and Escherichia coli.

1. Introduction

Gelatin is one kind of protein that is hydrolyzed from collagen. The gelatin is derived from bones or skin such as beef, chicken, pork, and fish. The edible gelatin films were prepared from bovine and porcine skin. They possess high strength, low deformation and high water vapor permeability compared with the one from fish skin as reported by Sobral [1].

Gelatin can be applied widely in many fields, such as food, pharmaceuticals, cosmetic manufacturing and packaging. Packaging made of gelatin can be edible. Typically most gelatins have hygroscopic properties and poor antibacterial properties [2]. Therefore our work is determined to improve gelatin by incorporation with essential oils for packaging application. This helps prolong the food freshness and reduce the perishable rate.

Aguirre A [3] studied the triticale protein films containing the oregano essential oil and found that the properties of the films were improved against the Gram positive bacteria Staphylococcus aureus and Gram negative bacteria (Escherichia coli and Pseudomonas aeruginosa). Gholamreza K [4] reported the Bovine gelatin films incorporated with Zataria multiflora essential oil having excellent antibacterial properties against both Gram-positive and Gram-negative bacteria. Mehraj A [5] reported the gelatin film from the skin of unicorn leatherjacket containing the bergamot and lemongrass oil at different concentrations, concluding that the addition of lemongrass oil in films contributed to lower

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water vapor permeability, and exhibited antibacterial properties against Gram-positive bacteria (S. aureus and L. monocytogenes) and Gram-negative bacteria (E. coli and S. typhimurium). Djamel D [6] studied the components of peppermint oil and its antibacterial properties against E. coli O157:H7 and S. aureus CECT 4459 in minced meat. They found that a significant drop in both bacterial growths in minced meat. Pires C. and Bárbara T [7, 8] showed the major components of citronella essential oil were Δ3-Carene (22.5%), β-Citronellal (11.9%) and β-Citronellol (9.00%). They reported that the citronella essential oil could be the substance against E. coli O157:H7 bacteria.

Even though a lot of work has been reported on film blended with various essential oils [3-8], no one has studied the effect of peppermint and citronella oil on the properties of the fish skin gelatin films. Therefore, this research is to investigate the incorporation of two types essential oils i.e., peppermint and citronella oils in fish skin gelatin film, at different amounts to improve the properties of water vapor resistance and antibacterial activity. In addition, the effect of essential oils on the mechanical, thermal properties and morphology of fish skin gelatin films was also studied.

2. Experimental

2.1. Materials
Fish skin gelatin powder (~ 250 blooms), glycerol was used as a plasticizer, tween 80 was used as a surfactant between gelatin and essential oils, citronella essential oil and peppermint essential oil were purchased from Chemipan Co., Ltd., Thailand.

2.2. Preparation of gelatin film solution casting
The gelatin powder was dissolved in distilled water to gain the protein concentration of 5% (w/v) and then heated at 60°C for 30 min under continuous stirring. After that, the glycerol was added at concentration 30% (w/w) of gelatin content. Then, each essential oil was mixed with tween 80 at 25% (w/w) of essential oil content. After that, the mixture was added into the gelatin solution at concentrations of 10%, 20%, and 30% (w/w) of gelatin content, respectively. The gelatin-essential oil solution was homogenized at 5,000 rpm for 3 minutes by using a homogenizer. For preparing the film, the gelatin-essential oil solution was cast onto a glass tray and conditioned at 40°C for 24 h to obtain gelatin-essential oil films.

2.3. Film characterization

2.3.1. Mechanical properties. The mechanical properties of the film samples were measured by using a Universal Testing Machine. Films were cut into strips with a dimension of 150×25.4 mm, then placed at 25°C for 48 h and 50 ± 5% relative humidity (RH) before testing. The test was carried out by the ASTM D 882 standard method, with the initial grip separation of 100 mm, a crosshead speed of 10 mm/min, and load cell of 5 kN. The final value represented the average of six samples.

2.3.2. Film thickness. Film thickness was measured using a micrometer (SM-112, Teclock Corporation, Japan). The final value represented the average of five random measurements taken at different locations of the film.

2.3.3. Water vapor transmission rates. Water vapor transmission rate (WVTR) was measured using a modified ASTM E96 method. The glass bottles containing dried silica gel (0% RH) were closed at the mouth of the bottle by the film samples, placed at 25°C and 75% RH in desiccator. The weight of the glass bottles was measured at 1 h intervals during 24 h. The result demonstrated the relationship between the weight change (g) and time (h). WVTR of the film was calculated by equation (1):

\[ \text{WVTR (g/m}^2\text{.h)} = \frac{G}{tA} = \frac{(G/t)}{A} \]  

(1)
Where $G$ is weight change (from the straight line), $g$; $t$ is time, $h$; $G/t$ is calculated from slope of the straight line, $g/h$; and $A$ is test area (bottle mouth area), $m^2$.

2.3.4. **Solubility in water.** Film solubility in water was measured according to the method proposed by Gontard and Wang et al. [9, 10], and the samples were cut into small pieces and the films were dried in oven at 100°C for 24 h to a constant weight. Each dried film sample was immersed in 50 ml of distilled water for 24 h. After 24 h, all of the samples were removed from the solutions and the films were dried at 100°C for 24 h. The final weight was recorded and the solubility was calculated by equation (2):

$$\text{Solubility} = \left( \frac{\text{Initial Weight} - \text{Final Weight}}{\text{Initial Weight}} \right) \times 100\% \tag{2}$$

2.3.5. **Thermo gravimetric analysis (TGA).** Samples of approximately 5 mg were scanned using a thermo-gravimetric analyzer (TGA: TGA/DSC 1, Mettler Toledo, Switzerland) from 50 to 600°C at a rate of 10°C/min under a nitrogen flow of 30 mL/min. Weight losses of samples were measured as a function of temperature.

2.3.6. **Antibacterial activity test using colony counting.** Bacteria reduction method was used to investigate antibacterial efficacy of films. All of the film samples were cut into circular shape (with a diameter of 1.2 cm). In the first step, the plastic film was placed on the bottom of each bottle. Each prepared sample was placed on the plastic film. The active bacterial solution, McFarland Standard No. as 0.5, was dropped on the sample and covered them by plastic film again. After 24 h in an incubator, phosphate buffered saline (PBS) was added to each bottle. And 0.1 ml of each solution was sampled and diluted by PBS for 10 times. In each dilution, the solution was dropped into the prepared agar plate and kept in an incubator at 37°C for 18 h. Finally, the colony of bacteria in each plate was calculated.

2.3.7. **Statistical analysis.** Analysis of variance (ANOVA) was used to compare the mean differences of the samples and the differences between the means were determined by the least significant difference test at $p < 0.05$. The statistical software package (SPSS ver. 17.0, SPSS Inc. Chicago, IL, USA) was used to analyze the experimental data.

3. **Results and discussion**

3.1. **Mechanical properties and thickness**

Table 1 demonstrated the properties of tensile strength (TS), percent elongation at break (%EB), Young’s modulus (YM) and the thickness of fish skin gelatin films with peppermint and citronella oils at various contents (10, 20 and 30% w/w). From the measurements, the thickness of gelatin films was found to have no differences when either peppermint or citronella essential oils were presented at the contents of 10% to 30% w/w compared with the pure gelatin film. However, the fish skin gelatin incorporated with 50% w/w root essential oils indicated the increase in film thickness because the interaction of essential oils between peptide chains could reduce the ordered alignment of gelatin chains and the compact network could not be developed [11].

The addition of peppermint oil into the gelatin films at the contents of 10% to 30% w/w demonstrated a significant decrease ($p < 0.05$) in TS compared with the pure gelatin film due to the effect of plasticized behavior of peppermint oil. This could reduce or even hinder the interaction between gelatin chains, thus diminishing the TS of the gelatin films [12].

On the other hand, the EB of gelatin films demonstrated an intense increase ($p < 0.05$) from 10% to 20% w/w of peppermint oils due to plasticizing effect. This led to the chains slippage during film stretching. However, the addition of peppermint oils at 30% w/w caused the EB reduction to 8.26 % due to the fact that an excessive amount of peppermint oils led to more interaction reduction between gelatin chains and even easy rupture [5]. Moreover, the presence of excess essential oils in gelatin
matrix could interfere in the interactions between polymer chains and reduce the flexibility of the gelatin matrix [13].

In terms of Young’s modulus, YM, the addition of peppermint oils in gelatin films with concentrations of 10% to 20% w/w contributed to a significant decrease ($p < 0.05$) in YM from 1777 to 1621 MPa respectively due to plasticizing effect. Polymer chains could be able to move, making the film flexible according to Zinoviadou et al. [14]. Their work was conducted on the addition of oregano oil and found that the YM of whey protein isolate films was decreased.

The addition of the citronella oils into the gelatin films at 10% promoted a reduction in TS and YM but an increase in EB. However, TS of fish gelatin film showed a significant increase from 27.78 MPa at 10% w/w concentration to 31.15 MPa at 30% w/w, while a significant decrease in EB from 15.33% at 10% w/w to 4.68% at 30%w/w citronella oils was observed. These could be explained by the presence of β-citronellal in citronella oils. Such component could interact with proteins which promoted their cross-linking in gelatin film, resulting in the rigidity behavior in gelatin film [15]. This led to an increase in both TS and YM of fish skin gelatin films.

### Table 1. Mechanical properties and thickness of fish skin gelatin films containing peppermint and citronella essential oils at 10, 20 and 30% w/w.

| Essential oils | Content (%) | TS (MPa) | EB (%) | YM (MPa) | Thickness (mm) |
|---------------|-------------|----------|--------|----------|---------------|
| Pure          | -           | 33.46 ± 0.40$^a$ | 3.44 ± 0.40$^a$ | 1896.78 ± 30.14$^a$ | 0.1228 ± 0.0025$^a$ |
| Peppermint    | 10          | 30.38 ± 0.72$^b$ | 7.38 ± 0.97$^b$ | 1777.58 ± 44.21$^b$ | 0.1233 ± 0.0021$^a$ |
|               | 20          | 28.38 ± 0.69$^c$ | 12.61 ± 0.92$^c$ | 1621.05 ± 34.16$^c$ | 0.1241 ± 0.0025$^a$ |
|               | 30          | 25.59 ± 0.74$^d$ | 8.26 ± 0.59$^d$ | 1696.76 ± 42.53$^d$ | 0.1249 ± 0.0017$^a$ |
| Citronella    | 10          | 27.78 ± 0.84$^e$ | 15.33 ± 0.54$^e$ | 1522.41 ± 33.87$^e$ | 0.1235 ± 0.0018$^a$ |
|               | 20          | 29.60 ± 0.78$^f$ | 13.10 ± 0.66$^f$ | 1647.33 ± 25.36$^f$ | 0.1245 ± 0.0027$^a$ |
|               | 30          | 31.15 ± 0.46$^g$ | 4.68 ± 0.77$^g$ | 1791.56 ± 37.82$^g$ | 0.1254 ± 0.0021$^a$ |

*aThe values are presented as means ± standard deviation.

*bDifferent letters in the same column indicate significant differences ($p < 0.05$), and the same letters indicate no significant differences.

### Table 2. Water vapor transmission rates (WVTR) and solubility in water of fish skin gelatin films incorporated with peppermint and citronella essential oils at 10, 20 and 30% w/w.

| Essential oils | Content (%) | WVTR (g/m².day) | Solubility in water (%) |
|---------------|-------------|-----------------|-------------------------|
| Pure          | -           | 65.13 ± 1.22$^a$ | 61.72 ± 1.12$^a$       |
| Peppermint    | 10          | 59.82 ± 1.17$^b$ | 66.39 ± 0.88$^b$       |
|               | 20          | 56.51 ± 1.75$^c$ | 70.13 ± 1.01$^c$       |
|               | 30          | 53.86 ± 0.57$^d$ | 78.55 ± 1.28$^d$       |
| Citronella    | 10          | 56.68 ± 0.99$^e$ | 67.78 ± 0.92$^e$       |
|               | 20          | 53.03 ± 1.25$^f$ | 64.49 ± 0.69$^f$       |
|               | 30          | 43.92 ± 1.30$^g$ | 62.28 ± 0.77$^g$       |

*aThe values are presented as means ± standard deviation.

*bDifferent letters in the same column indicate significant differences ($p < 0.05$), and the same letters indicate no significant differences.

### 3.2. Water vapor transmission rates (WVTR)

WVTR of fish skin gelatin films containing peppermint and citronella essential oils at various contents is shown in Table 2. Incorporations of peppermint oils and citronella oils at 10% ~ 30% w/w in gelatin films caused a significant decrease ($p < 0.05$) of WVTR from 59.82 to 53.86 g/m²h and from 56.68 to 43.92 g/m²h, respectively. These could be due to the different hydrophobicity of its components in peppermint [7] and citronella [8] essential oils, for instance, monoterpenes, a highly hydrophobic component [16]. Different amounts of monoterpenes could influence the hydrophobicity. In other words, monoterpenes is the governing factor in determining the hydrophobicity of gelatin film. As a result, the water vapor transmission through the gelatin films would be different between the films.
with peppermint and citronella oils. With the same concentration of essential oils, the fish skin gelatin film incorporated with citronella oils possessed lower WVTR than its counterpart with peppermint oils due to hydrophobicity of its component.

3.3. Solubility in water

The water solubility of fish skin gelatin films incorporated with peppermint and citronella oils at 10, 20 and 30% w/w is shown in table 2. The increase of peppermint oil from 10% to 30% w/w caused a significant increase \( (p < 0.05) \) of films solubility in water. The presence of peppermint oil in gelatin matrix may reduce the interaction between gelatin chains, which led to the weakening of the interactions between gelatin films and allowed to an increase of its leaching out [15].

The addition of citronella oil into gelatin films at concentration of 10% led to an increase of water solubility of films in comparison with pure gelatin film. Water solubility of fish skin gelatin films was decreased with the increasing amount of citronella oils from 10% to 30% w/w. These results were supported by the fact that the cross-linking between protein chains by β-citronellal component caused a significant decrease of solubility in water of films.

3.4. Thermo gravimetric analysis (TGA)

The results of degradation temperatures \( (T_d) \), weight loss \( (\Delta w) \) and residue of fish skin gelatin films incorporated with peppermint and citronella oils at different contents (10, 20 and 30% w/w) are shown in table 3. All film samples showed three stages of degradation temperatures \( (T_d) \) and weight loss \( (\Delta w) \). The first stage at the range of up to 100 °C, \( T_{d1} \) and the \( \Delta w_1 \) of all films may be due to the loss of free water or other volatile compounds from the gelatin film. Gelatin films containing peppermint and citronella oils at any content (10, 20 and 30%) outperformed pure gelatin film on the weight loss due to the low ability in water absorption by the hydrophobic components in both peppermint and citronella essential oils [17].

Table 3. Thermal degradation temperatures \( (T_d, °C) \) and weight loss \( (\Delta w, \%) \) of fish skin gelatin films incorporated with peppermint and citronella essential oils at 10, 20 and 30% w/w.

| Essential oils types | Content (%) | \( \Delta_1 \) | \( T_{d1,onset} \) | \( \Delta w_1 \) | \( T_{d2,onset} \) | \( \Delta w_2 \) | \( T_{d3,onset} \) | \( \Delta w_3 \) | Residue (%) |
|----------------------|------------|----------------|----------------|----------------|----------------|----------------|----------------|----------------|-------------|
| Pure                 | -          | 77.12          | 8.05           | 220.08         | 25.24          | 295.42         | 48.28          | 20.73          |
| Peppermint           | 10         | 80.43          | 7.98           | 222.89         | 25.90          | 294.80         | 48.51          | 17.23          |
|                      | 20         | 79.32          | 6.47           | 228.18         | 25.71          | 292.52         | 51.29          | 15.97          |
|                      | 30         | 74.25          | 2.72           | 226.12         | 25.44          | 291.85         | 56.83          | 14.07          |
| Citronella           | 10         | 69.27          | 6.43           | 227.75         | 25.66          | 294.63         | 46.19          | 21.54          |
|                      | 20         | 74.35          | 6.36           | 228.90         | 24.13          | 302.12         | 44.79          | 22.92          |
|                      | 30         | 71.20          | 2.53           | 233.59         | 24.01          | 302.82         | 42.35          | 24.45          |

The second stage, \( T_{d2} \) and the \( \Delta w_2 \), of all films indicated the loss of glycerol component and small protein molecule in gelatin films. The work was reported by Hoque et al. [18] that the degradation temperature in the range of 196.30–216.71°C appeared in cuttlefish skin gelatin film.

The third stage, \( T_{d3} \) and the \( \Delta w_3 \), of all film samples referred to the degradation of the larger molecules of protein in gelatin films [17]. The addition of peppermint oil at 10% to 30% w/w into gelatin films demonstrated caused a slight decrease of \( T_{d3} \) from 294.80 to 291.85°C and an increase of \( \Delta w_3 \) from 48.51 to 56.83%, respectively. The addition of peppermint oil at all contents led to the results of \( T_{d3} \) to have the lower temperature compared with pure gelatin film. In contrast, \( \Delta w_3 \) of fish skin gelatin film at all contents of peppermint oil was higher than that of the pure gelatin film. These could be explained by the plasticizing effect of peppermint oil. These may interfere in the interaction between protein chains in the gelatin films. On the other hands, the addition of citronella oil from 10% to 30% w/w into gelatin films contributed to an increase of \( T_{d3} \) from 294.63 to 302.82 °C but a decrease of \( \Delta w_3 \) from 46.19 to 42.35%, respectively. This could be explained by the presence of β-citronellal, a component in citronella oil, which might cause the cross-linking between protein chains in gelatin.
films. Therefore, the gelatin film incorporated with citronella oil exhibited an improvement in the thermal stability.

3.5. Antibacterial activity test using colony counting

Antibacterial activities of fish skin gelatin films incorporated with peppermint and citronella oils at 10, 20 and 30% w/w are shown in table 4. As per our studies, pure gelatin film or the fish skin gelatin film without essential oil showed no activity against both E. coli and S. aureus. For both essential oils, the gelatin films incorporated with either peppermint or citronella oils at 30% showed a significant increase of antibacterial activity against both E. coli and S. aureus compared with its counterpart incorporated with either essential oil at 10%.

According to the report of Zivanovic et al. [19], the phospholipids of cellular membrane were attacked by the phenolic components of essential oils. Consequently, an increase in permeability and loss of cytoplasm took place. Antibacterial activity occurred when the phenolic components found in the essential oil interacted with such enzymes localized in the cell wall, which caused catastrophic incidents to the membranes. Most of the essential oils comprised phenolic components. Therefore, the gelatin films incorporated with peppermint and citronella essential oils exhibited activities against both E. coli and S. aureus. However, gelatin films incorporated with peppermint essential oils or citronella essential oils are more effective to S. aureus (Gram-positive) than to E. coli (Gram-negative) due to the presence of lipopolysaccharides, since the outer cell wall of Gram-negative bacteria was composed of lipopolysaccharides and could act as the protective membranes against the phenolic components from essential oils [20].

As for the films with the same amount of essential oil, the gelatin film with peppermint oil exhibited better antibacterial activity than its counterpart with citronella oil for both types of microorganisms since a higher amount of phenolic components existed in the peppermint oil [7] than in the citronella oil [8].

**Table 4. Antibacterial reduction of fish skin gelatin films incorporated with peppermint and citronella essential oils at different concentrations.**

| Essential oils types | Content (%) | % Antibacterial Reduction |   |   |
|----------------------|-------------|---------------------------|---|---|
|                      |             | E. coli                  | S. aureus |   |
| Pure                 | -           | 0.00                     | 0.00       |
| Peppermint           | 10          | 81.17                    | 84.13       |
|                      | 20          | 82.63                    | 85.07       |
|                      | 30          | 84.02                    | 85.52       |
| Citronella           | 10          | 79.97                    | 83.92       |
|                      | 20          | 81.91                    | 84.47       |
|                      | 30          | 83.59                    | 85.27       |

4. Conclusion

The peppermint essential oil can act as plasticizer in gelatin films. When increasing the amount of peppermint oil, the mechanical and thermal properties of fish gelatin films are likely to decrease but solubility in water is increased. β-Citronellal component in citronella essential oils can act as the cross-linker between gelatin chains and will increase the mechanical and thermal properties of fish gelatin films, but the solubility in water will decrease when the amount of citronella oils increases. The outcome of antibacterial properties and water vapor permeability of gelatin films depends on the amount of phenolic and hydrophobic component in each essential oil. The fish skin gelatin film with citronella oil shows to be more effective in term of WVP than the film with peppermint oil because of the crosslinking of gelatin chains by β-Citronellal component in citronella oil. Incorporations of either peppermint or citronella oil into the fish skin gelatin films could improve the antibacterial activity
against both *E. coli* (Gram-negative) and *S. aureus* (Gram-positive). But, the gelatin film with peppermint oil outperforms its counterpart with citronella oil for antibacterial activity due to its phenolic component.

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