Comparative studies on properties and antioxidative activity of fish skin gelatin films incorporated with essential oils from various sources

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Abstract Properties of fish skin gelatin films incorporated with three essential oils from various sources (basil, plai and lemon) with a gelatin/essential oil ratio of 1:1 (w/w) were determined. Films containing different essential oils had lower tensile strength and elastic modulus, but higher elongation at break and thickness, compared with the control film (without essential oils) ($p < 0.05$). Lower water vapour permeability was observed when essential oils were incorporated, in comparison with the control, particularly when basil and lemon essential oils were used ($p < 0.05$). Films with essential oils had varying $L^*$, $a^*$, $b^*$ and $\Delta E^*$ values (total colour difference) and WI (whiteness). Amongst all films, that incorporated with plai essential oil showed the highest $b^*$- and $\Delta E^*$-values but lowest WI ($p < 0.05$). Lower light transmittance and higher transparency value, indicating more opaqueness, were observed when films were incorporated with essential oils ($p < 0.05$), especially for film added with lemon essential oil. Film containing basil essential oil showed the highest DPPH and ABTS radical scavenging activities ($p < 0.05$), whilst those incorporated with lemon essential oil had the highest chelating activity ($p < 0.05$). Thus, the incorporation of various essential oils from various sources determined properties and antioxidative activity of fish skin gelatin film differently.

Keywords Gelatin film · Essential oil · Basil · Plai · Lemon · Antioxidant activity

Abbreviations

| Acronym | Definition |
|---------|------------|
| ABTS    | 2,2-Azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) diammonium salt |
| DPPH    | 2,2-Diphenyl-1-picryl hydrazyl |
| EDTA    | Ethylenediaminetetraacetic acid |
| FFS     | Film forming solution |
| RH      | Relative humidity |
| TS      | Tensile strength |

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EM Elastic modulus
EAB Elongation at break
WVP Water vapour permeability
ASTM American Society for Testing and Materials
WI Whiteness
TE Trolox equivalent

Background

Potential processing and management are very important for fish industry, where great economic, nutritional and environmental values can be obtained by the better uses of byproducts. Fish processing generates solid wastes that can be as high as 50–80 % of the original raw material (Shahidi et al. 1994). About 30 % of the wastes consists of skin and bone with high collagen content (Wasswa et al. 2007). Gelatin can be obtained by partial hydrolysis or thermal denaturation of collagen in skin and bone. Moreover, gelatins have been widely used as film forming material, particularly for preparation of biodegradable or edible films (Gómez-Estaca et al. 2009). Edible films can be defined as thin continuous layer of biopolymer materials which can be applied as a coating on food, used as a wrap or made into pouch to protect foods against the external factors, e.g. water, oxygen, carbon dioxide and lipids (Krochta 1997). Although gelatins yielded transparent, colourless and highly extensible films, they have poor water resistance. This could be a drawback when they are applied to food products with high moisture content, because the films may swell, partially dissolve or disintegrate upon the contact with wet surface (Núñez-Flores et al. 2012). The use of essential oils from plants can be another approach to improve water vapour barrier property of gelatin films. Due to antioxidant and antimicrobial activities of essential oils (Burt 2004), they may make the film become active. As a consequence, smart films with varying properties can be produced, especially for shelf-life extension of foods. In our previous study, gelatin-based films incorporated with essential oils containing 30 % glycerol exhibited the higher antioxidative activity than those with 20 % glycerol, owing to their more loose structure of film matrix (Tongnuanchan et al. 2012). Moreover, essential oil as high as 100 % (w/w based on protein) could be incorporated into gelatin film and yielded the bilayer film with the lowest water vapour permeability with the highest antioxidative activity (Tongnuanchan et al. 2013b). Recently, Tongnuanchan et al. (2013a) reported that soy lecithin could be potentially used as a surfactant for gelatin film added with leaf essential oils, where homogeneous internal film network was formed.

Essential oils from different parts of plants or different plants showed varying antioxidative activity. Amongst essential oils from leaf, root and citrus peel, those from basil, plai and lemon showed the higher antioxidative activity, respectively (Tongnuanchan et al. 2012, 2013a, b). However, there is no information on the effect of essential oils from different parts of plants (leaf, root and citrus peel) on the properties of fish gelatin film. Thus, the objective of this study was to comparatively study the effects of three different essential oils (basil, plai and lemon) on the properties and antioxidative activity of film from fish skin gelatin.

Methods

Chemicals

2,2-Azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) diammonium salt (ABTS), 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), 2,2-diphenyl-1-picrylhydrazyl (DPPH), glycerol, and soy lecithin were purchased from Sigma-Aldrich (St. Louis, MO, USA). Ethylenediaminetetraacetic acid (EDTA), 3-(2-pyridyl)-5,6-diphenyl-1,2,4-triazine-p,p'-disulfonic acid monosodium salt hydrate (ferrozine) and iron (II) chloride were obtained from Merck (Darmstadt, Germany). All chemicals were of analytical grade.
Fish gelatin and essential oils

Fish gelatin produced from tilapia skin (~ 240 bloom) was procured from LapiGelatineS.p.A (Empoli, Italy). Essential oils from basil (*Ocimum basilicum*), plai (*Zingiber montanum*) and lemon (*Citrus limonum*) were purchased from Botanicessence (Bangkok, Thailand).

Preparation of film from fish gelatin incorporated with different essential oils

To prepare film forming solution (FFS), gelatin powder was mixed with distilled water to obtain the protein concentration of 3.5 % (w/v). The mixture was heated at 70 °C for 30 min. Glycerol at 30 % (w/w) of protein content was used as a plasticiser. Prior to addition into solution, essential oils were mixed with soy lecithin at 25 % (w/w, based on essential oil) as surfactant. Thereafter, the prepared essential oils were added into the gelatin solution at gelatin/essential oil ratio of 1:1 (w/w). The obtained suspension was homogenised at 22,000 rpm for 3 min using a homogeniser (IKA Labortechnik homogeniser, Selangor, Malaysia). The dissolved air in the FFS was removed by a vacuum pump (Diaphragm vacuum pump, Wertheim, Germany) for 30 min at room temperature.

For film preparation, FFS (4 g) was cast onto a rimmed silicone resin plate (50 × 50 mm) and air-blown for 12 h at room temperature. The films were further dried at 25 °C and 50 ± 5 % RH for 24 h in an environmental chamber (WTB Binder, Tuttlingen, Germany). The resulting films were manually peeled off and subjected to analyses. Control films were prepared from FFS without essential oils and surfactants.

Determination of film properties

Prior to testing, films were conditioned for 48 h at 50 ± 5 % relative humidity (RH), at 25 ± 0.5 °C.

Film thickness

The thickness of film was measured using a micrometer (Mitutoyo, Model ID-C112PM, Serial No. 00320, Mitutoyo Corp., Kawasaki-shi, Japan). Five random locations around each film of ten film samples were used for average thickness determination.

Mechanical properties

Tensile strength (TS), elastic modulus (EM) and elongation at break (EAB) were determined as described by Iwata et al. (2000) with a slight modification using the Universal Testing Machine (Lloyd Instrument, Hampshire, UK) equipped with tensile load cell of 100 N. Ten samples (2 × 5 cm) with initial grip length of 3 cm were used for testing. Cross-head speed was set at 30 mm min^{-1}.

Water vapour permeability (WVP)

WVP was measured using a modified ASTM method (ASTM 1989) as described by Shiku et al. (2004). The film was sealed on an aluminum permeation cup containing dried silica gel (0 % RH) with silicone vacuum grease and a rubber gasket to hold the film in place. The cups were placed in a desiccator containing the distilled water at 30 °C. The cups were weighed at 1 h intervals over a 10-h period. WVP of the film was calculated as follows:

\[
WVP(\text{gm}^{-1}\text{sm}^{-1}\text{Pa}^{-1}) = \frac{wI^A^t^{-1}(P_2 - P_1)^{-1}}
\]

where \(w\) is the weight gain of the cup (g); \(I\) is the film thickness (m); \(A\) is the exposed area of film (m²); \(t\) is the time of gain (s); \(P_2 - P_1\) is the vapour pressure difference across the film (Pa).
Colour

Film samples were subjected to colour measurement using a CIE colourimeter (Hunter associates laboratory, Inc., VA, USA). \(D_{65}\) (day light) and a measure cell with opening of 30 mm was used. The colour of the films was expressed as \(L^*\)-value (lightness), \(a^*\)-value (redness/greenness), \(b^*\)-value (yellowness/blueness), total difference of colour (\(\Delta E^*\)) and whiteness (WI) were calculated as follows (Gennadios et al. 1996; Ghanbarzadeh et al. 2010):

\[
\Delta E^* = \sqrt{\left(\Delta L^*\right)^2 + \left(\Delta a^*\right)^2 + \left(\Delta b^*\right)^2}
\]

\[
WI = 100 - \sqrt{\left(100 - L^*\right)^2 + \left(a^*\right)^2 + \left(b^*\right)^2}
\]

where \(\Delta L^*, \Delta a^*\) and \(\Delta b^*\) are the differences between the colour parameter of the samples and the colour parameters of the white standard (\(L^* = 92.82, a^* = -1.21, b^* = 0.45\)).

Light transmittance and transparency value

The light transmittance of films was measured at the ultraviolet and visible ranges (200–800 nm) using a UV–Vis spectrophotometer (UV-1800, Shimadzu, Kyoto, Japan) as described by Shiku et al. (2004). The transparency value of film was calculated using the following equation (Han and Floros 1997):

\[
\text{Transparency value} = -\log T_{600}/x
\]

where \(T_{600}\) is the fractional transmittance at 600 nm and \(x\) is the film thickness (mm). The greater transparency value represents the lower transparency of film.

Antioxidative activity

Films were solidified using liquid nitrogen in a mortar and ground with a pestle. Ground film (0.25 g) was mixed with 5 ml of methanol and stirred vigorously for 3 h. The mixture was centrifuged at 2,700 \(\times\) g for 10 min using a centrifuge (Beckman Coulter, Avanti J-E Centrifuge, Beckman Coulter, Inc., Palo Alto, CA, USA). The supernatant obtained was determined for antioxidative activities.

DPPH radical scavenging activity

DPPH radical scavenging activity was determined as described by Binsan et al. (2008) with a slight modification. Sample (1.5 ml) was added with 1.5 ml of 0.15 mM 2,2-diphenyl-1-picryl hydrazyl (DPPH) in 95 % ethanol. The mixture was mixed vigorously using a mixer (Vertex-Genie 2, Model G-560E, Scientific Industries, inc., Bohemia, New York, USA) and allowed to stand at room temperature in dark for 30 min. The absorbance of the resulting solution was measured at 517 nm using a spectrophotometer. Sample blank was prepared in the same manner except that 95 % methanol was used instead of DPPH solution. A standard curve was prepared using Trolox in the range of 10–60 \(\mu\)M. The activity was calculated after the sample blank subtraction and expressed as \(\mu\)mol Trolox equivalents (TE)/g dried film.

ABTS radical scavenging activity

ABTS radical scavenging activity was assayed as per the method of Arnao et al. (2001) with a slight modification. The stock solutions included 7.4 mM ABTS solution and 2.6 mM potassium persulphate solution. The working solution was prepared by mixing the two stock solutions in equal quantities. The mixed solutions were allowed to react for 12 h at room temperature in dark. The solution was then diluted by mixing 1 ml of ABTS solution with 50 ml of methanol in order to obtain an absorbance of 1.1 \(\pm\) 0.02 units at 734 nm.
using a spectrophotometer. ABTS solution was prepared freshly prior to assay. Sample (150 μl) was mixed with 2,850 μl of ABTS solution and the mixture was left at room temperature for 2 h in dark. The absorbance was then measured at 734 nm using a spectrophotometer. Sample blank was prepared in the same manner except that methanol was used instead of ABTS solution. A standard curve of Trolox ranging from 50 to 600 μM was prepared. The activity was calculated after sample blank subtraction and was expressed as μmol Trolox equivalents (TE)/g dried film.

Ferrous ion chelating activity

Ferrous ion chelating activity was measured by the method of Thiansilakul et al. (2007). Diluted sample (4.7 ml) was mixed with 0.1 ml of 2 mM FeCl₂ and 0.2 ml of 5 mM ferrozine. The reaction mixture was allowed to stand for 20 min at room temperature. The absorbance was then read at 562 nm. EDTA with the concentration range of 0–50 μM was used as the standard. Ferrous ion chelating activity was expressed as μmol EDTA equivalents (EE)/g dried film.

Statistical analysis

All experiments were run in triplicate with different three lots of films. Data were subjected to analysis of variance (ANOVA) and mean comparisons were carried out by Duncan’s multiple range test. Analysis was performed using the SPSS package (SPSS for windows, SPSS Inc., Chicago, IL, USA).

Results and discussion

Thickness, mechanical and physical properties

Thickness

Thickness of fish skin gelatin films incorporated with different essential oils (basil, plai and lemon) and control film (without essential oils) is shown in Table 1. The thickness of all films containing essential oils was higher than that of the control film (p < 0.05). Essential oil droplets might insert and localise themselves in the film network. As a result, the interaction between gelatin chains could be impeded. The loss of compact network and the decrease in ordered alignment of gelatin chains might bring about the protruded structure as indicated by the increased thickness. Moreover, it was noted that films prepared using different types of essential oils had varying thickness (p < 0.05). Amongst all essential oils used, lemon essential oil yielded the film with the highest thickness (p < 0.05), followed by those added with basil and plai essential oils, respectively. This might be governed by the differences in surface tension or size of oil droplets. Additionally, the essential oils might have different compositions, which could interact with gelatin chain in the film matrix differently. As a result, the alignment of gelatin molecules in the film matrix might be different, leading to the differences in film thickness.

| Essential oils | TS (MPa) | EAB (%) | EM (MPa) | WVP (×10⁻¹¹ g⁻¹ s⁻¹ Pa⁻¹) | Thickness (mm) |
|---------------|---------|---------|----------|-----------------|---------------|
| Control       | 33.13 ± 2.13<sup>A</sup> | 51.10 ± 6.66<sup>a</sup> | 910.03 ± 76.09<sup>a</sup> | 1.84 ± 0.06<sup>a</sup> | 0.055 ± 0.002<sup>d</sup> |
| Basil         | 23.34 ± 0.64<sup>b</sup> | 127.53 ± 9.16<sup>b</sup> | 367.10 ± 23.63<sup>b</sup> | 0.71 ± 0.01<sup>d</sup> | 0.115 ± 0.008<sup>b</sup> |
| Plai          | 19.61 ± 2.16<sup>c</sup> | 140.80 ± 5.31<sup>a</sup> | 294.10 ± 32.34<sup>c</sup> | 1.01 ± 0.01<sup>b</sup> | 0.106 ± 0.007<sup>c</sup> |
| Lemon         | 15.27 ± 0.75<sup>d</sup> | 113.78 ± 3.43<sup>c</sup> | 277.51 ± 34.63<sup>c</sup> | 0.75 ± 0.04<sup>c</sup> | 0.125 ± 0.004<sup>c</sup> |

Control film was prepared from FFS without addition of essential oils
Different lowercase superscripts in the same column indicate significant differences (p < 0.05)
<sup>A</sup> Mean ± SD (n = 3)
**Mechanical properties**

Mechanical properties expressed as tensile strength (TS), elastic modulus (EM) and elongation at break (EAB) of gelatin films incorporated with different essential oils are shown in Table 1. Fish skin gelatin films with and without essential oils were flexible and visually homogeneous. Oil exudation was not found on the surface of films containing essential oils in spite of high amount of oil added. Addition of all types of essential oils led to the change in mechanical properties of films. Films with essential oils incorporated had lower TS and EM, but higher EAB, as compared with the control film ($p < 0.05$). TS of films were decreased by 29.5, 40.8 and 53.9 % when basil, plai and lemon essential oils were incorporated, respectively, compared with that of control film. EM of films also decreased by 59.7, 67.7 and 69.5 %, when basil, plai and lemon essential oils were added, respectively. Amongst all essential oils incorporated, basil essential oil yielded the film with the highest TS and EM ($p < 0.05$), followed by those containing plai and lemon essential oils, respectively. However, EAB of films incorporated with basil, plai and lemon essential oils were increased by 149.6, 175.5 and 122.6 %, respectively. The highest EAB was obtained in film added with plai essential oil, followed by basil and lemon essential oils ($p < 0.05$). It was found that the incorporation of essential oil affected TS, EM and EAB of resulting films and the value varied with types of essential oils incorporated.

Several parameters such as the characteristics of the lipid and their capability to interaction with protein molecules in film network affected properties of the resulting emulsion films (Gontard et al. 1994). In general, the incorporation of lipids decreased TS and puncture strength of protein-based film from gelatin (Limpisophon et al. 2010), whey protein (Soazo et al. 2013) and soy protein isolate (Guerrero et al. 2011). This result was in agreement with Zinoviadou et al. (2009) who reported that the incorporation of oregano essential oil (0.5–1.5 % w/w in FFS) decreased EM and TS with the concomitant increase in EAB of whey protein isolate films. The changes were in a dose-dependent manner. Tongnuanchan et al. (2013b) also reported the decrease in tensile resistance and the increase in stretch-ability of gelatin-based film when root essential oils were added. Atarés et al. (2010b) studied the mechanical properties of soy protein isolate incorporated with cinnamon and ginger essential oils at different concentrations (protein-to-oil mass ratios of 1:0.025, 1:0.050, 1:0.075 and 1:0.100). A slight decrease in EM was observed as the oil content increased. Normally, simple protein-based films are stronger and higher extensibility than protein/lipid emulsion films (Chen 1995). Interference of protein–protein interaction by the replacement of lipids occurred in film network. According to Yang and Paulson (2000), the interactions between non-polar molecules and polar polymers molecules are much lower than those between polar polymer molecules. Essential oil contains high amount of non-polar molecules or hydrophobic compounds, especially monoterpene hydrocarbon, which could reduce the compactness of film network as evidenced by the decreased TS and EM. With high proportions of essential oil used, they showed high ability to reduce the rigidity of film or acted as plasticiser. Increasing plasticising agent content yields a decrease in the resistance to breakage with the increased deformability. However, the incorporation of cinnamon essential oil resulted in an increase in TS of soy protein isolate film (Atarés et al. 2010b). Different types of essential oils plausibly affected the rearrangement of protein molecule in film matrix differently as evidenced by varying TS, EM and EAB. Essential oils contain aldehyde, ketone or phenolic compounds as major constituents (Bakkali et al. 2008). Those compounds were reported to interact with protein and enhance the mechanical properties of film (Hernández-Muñoz et al. 2004; Hoque et al. 2011). Essential oils containing various compounds might interact with protein, thereby affecting characteristic of film network. In the present study, film containing lemon essential oil was more stretchable, compared with those containing basil and plai essential oils ($p < 0.05$). Lipid addition generally could not enhance the cohesiveness and uniformity of film network formation (Péroyal et al. 2002). Therefore, essential oils from different sources affected the mechanical property of film differently.

**Water vapour permeability**

WVP of films incorporated with three essential oils is presented in Table 1. Films containing all essential oils had lower WVP than the control film, irrespective of the types of essential oil added ($p < 0.05$). WVP of films containing basil, plai and lemon essential oils were decreased by 61.4, 45.1 and 59.2 %, respectively, compared with that of control film. These results suggested that the increasing amount of hydrophobic substance such as essential oils more likely increased the hydrophobicity of film. As a result, the adsorption and
permeation of water vapour through the films containing essential oils were lowered. The rate of adsorptivity as well as diffusivity of water vapour in the films depends on the hydrophilicity/hydrophobicity ratio of the film components. It has been known that protein-based films had relatively poor water vapour barrier properties because of their hydrophilicity of polar amino acids in protein molecules (Krochta 2002). Monoterpenes (C_10) and sesquiterpenes (C_15) are highly hydrophobic substances found in essential oils, in which the content varied with types of essential oils (Turina et al. 2006). Amongst all essential oils incorporated, basil essential oil had the highest efficiency in lowering WVP of film (p < 0.05), followed by lemon and plai essential oils, respectively. The difference in WVP amongst films might be due to the difference in hydrophobic substances in essential oils. Thus, WVP of films was varied with types of essential oils used. Hydrophobic materials such as essential oils has been incorporated to improve water vapour barrier property of protein-based films, e.g. film from hake protein by thyme essential oil (Pires et al. 2011), film from soy protein isolate by cinnamon and ginger essential oils (Atarés et al. 2010b). Tongnuanchan et al. (2012) reported that WVP of fish skin gelatin film decreased markedly from 3.11 to 1.88, 1.89 and 2.45 × 10^{-11} \text{g} \text{s}^{-1} \text{Pa}^{-1} (p < 0.05), when films were incorporated with ginger, turmeric and plai, respectively, at a level of 100 %, in which WVP of film was reduced by 39.5, 39.2 and 21.2 %, respectively. No difference in WVP was found amongst films from sodium casinate added with cinnamon and ginger essential oils at the concentration of 2.5–7.5 % (w/w) (Atarés et al. 2010a). Moreover, dispersion of essential oil in hydrophillic based film could limit the diffusion of water vapour through the film via increasing tortuosity factor for water transfer by the discontinuities in film matrix. Thus, incorporation of essential oil could markedly enhance water vapour barrier property of gelatin film, in which the capability was dependent on types of essential oils used.

**Colour**

Table 2 presents the colour (\(L^*\), \(a^*\), \(b^*\), \(\Delta E^*\)- and WI-values) of gelatin films incorporated with three types of essential oils. Films containing all essential oils showed lower \(L^*\)-value (lightness) and WI (whiteness) with the coincidental increases in \(b^*\)- and \(\Delta E^*\)-values (p < 0.05). Film added with basil essential oil had the highest WI, compared with those incorporated with other essential oils (p < 0.05). Amongst all film samples, that incorporated with plai essential oil had the highest of \(b^*\)- and \(\Delta E^*\)-values with the lowest \(L^*\)-value and WI (p < 0.05). Higher \(\Delta E^*\)-value was in agreement with higher \(b^*\)-value. This might be associated with the yellowish colour of plai essential oil. Plai essential oil had the highest yellowness as evidenced by the highest \(b^*\)-value, compared with other essential oils (data not shown). The difference of colouring pigments/compounds and their contents in essential oils might determine the different colour of resulting films. Therefore, incorporation of essential oils from different plants and parts had direct impact on the colour of resulting film. This was in agreement with Salarbashi et al. (2014) who reported that films prepared from soluble soybean polysaccharide and incorporated with both *Zataria multiflora* Boiss and *Mentha pulegium* essential oils had lower \(L^*\)-value and WI but higher \(b^*\)- and \(\Delta E^*\)-values, especially with increasing oil concentration. Peng and Li (2014) also reported that the incorporation of lemon, thyme, cinnamon and their mixtures in chitosan-based film could increase \(b^*\)- and \(\Delta E^*\)-values, whilst \(L^*\)-value decreased.

**Table 2** Colour and transparency value of films from fish skin gelatin containing basil, plai and lemon essential oils

| Essential oils | Colour | Transparency value |
|---------------|--------|--------------------|
|               | \(L^*\) | \(a^*\) | \(b^*\) | \(\Delta E^*\) | WI | Transparency value |
| Control       | 90.76 ± 0.06\(^a\) | −1.36 ± 0.02\(^a\) | 0.93 ± 0.04\(^d\) | 2.13 ± 0.05\(^d\) | 90.61 ± 0.06\(^a\) | 0.86 ± 0.06\(^d\) |
| Basil         | 89.38 ± 0.09\(^b\) | −2.81 ± 0.02\(^b\) | 11.28 ± 0.06\(^c\) | 11.46 ± 0.08\(^c\) | 84.26 ± 0.10\(^b\) | 1.09 ± 0.04\(^c\) |
| Plai          | 88.42 ± 0.17\(^c\) | −3.52 ± 0.02\(^d\) | 20.25 ± 0.13\(^a\) | 20.39 ± 0.10\(^a\) | 76.41 ± 0.08\(^d\) | 2.32 ± 0.06\(^a\) |
| Lemon         | 89.34 ± 0.07\(^a\) | −2.84 ± 0.03\(^b\) | 13.34 ± 0.08\(^b\) | 13.15 ± 0.07\(^b\) | 82.69 ± 0.03\(^c\) | 4.49 ± 0.10\(^b\) |

Control film was prepared from FFS without addition of essential oils

Different lowercase letters in the same column indicate significant differences (p < 0.05)

\(^a\) Mean ± SD (n = 3)
Transmission of UV and visible light in the wavelength range of 200–800 nm of gelatin films incorporated with different essential oils is shown in Fig. 1. The transmission of UV light was not observed at 200 nm for films incorporated with and without essential oils. However, films containing essential oils had much lower light transmittance at wavelength lower than 280 nm, as compared to control film, regardless of types of essential oils added. It was suggested that the incorporation of essential oils into gelatin film could enhance the barrier property against UV light. Therefore, gelatin films effectively protected the transmission of UV light, regardless of essential oils incorporated. Generally, protein films exhibited the excellent UV barrier properties due to their high amount of aromatic amino acids that absorb UV light (Hamaguchi et al. 2007). Jongjareonrak et al. (2006) also reported higher UV light barrier capacity of gelatin film from cuttlefish and brownstripe red snapper and bigeye snapper, respectively. Light transmission of visible range (350–800 nm) of film without essential oil incorporated (control) ranged from 42.84 to 89.12 %, whereas lower values were observed for film incorporated with essential oils (6.13–80.38 %), regardless of essential oil types. This result suggested that the light transmission of films was considerably decreased by the incorporation of essential oils. Essential oil droplets localised in film matrix possibly inhibited the light transmission for both UV and visible ranges of resulting films. Amongst all types of essential oils used, lemon essential oil yielded the film with the lowest light transmittance in visible range, indicating the highest opacity. Essential oils in films might cause light scattering to different degrees. Moreover, light transmission of film was most likely governed by the arrangement or alignment of polymer in film network (Limpan et al. 2010).

The transparency value of all films is shown in Table 2. Films incorporated with essential oils had higher transparency values than the control film, regardless of essential oil types \( (p < 0.05) \). The lower transparency value indicated that the film was more transparent. Thus, films containing essential oils were more opaque and less transparent, compared with the control film. For the films prepared with various essential oils, those incorporated with lemon essential oil showed the highest transparency value \( (p < 0.05) \), followed by those added with plai and basil essential oils, respectively. It was noted that film containing lemon essential oil had the highest opaqueness, compared with other essential oils used. Peng and Li (2014) also reported that lemon essential oil was the most efficient in lowering the opacity of chitosan film. Transparency value of films incorporated with essential oils varied, depending on types of essential oils. This might be governed by the differences in size or the distribution of essential oil droplets localised in the film network. This result was in agreement with Shojaee-Aliabadi et al. (2013) who reported that emulsified films based on carrageenan and essential oil became more opaqueness, probably associated with the higher light scattering of lipid droplets. Therefore, the incorporation of essential oils had pronounced impacts on light transmittance and transparency of films.
Antioxidative activities expressed as DPPH radical scavenging activity, ABTS radical scavenging activity and chelating activity of basil, plai and lemon essential oils are presented in Table 3. Basil essential oil had the highest DPPH and ABTS radical scavenging activities \( (p < 0.05) \), followed by plai and lemon essential oils, respectively. Nevertheless, chelating activity of lemon essential oil was highest \( (p < 0.05) \), followed by plai and basil essential oils, respectively. In general, plant essential oils have been known as antioxidants (Wu et al. 1982). Several compounds in essential oils have the structure mimicking the well-known plant phenols with antioxidant activity. Phenolics are organic compounds consisting of hydroxyl group \((-\text{OH})\) attached directly to a carbon atom that is a part of aromatic ring. The hydrogen atom of hydroxyl group can be donated to free radicals, thereby preventing other compounds to be oxidised (Nguyen et al. 2003). Lemon essential oil was mainly composed of monoterpenic hydrocarbons (\(\alpha\)-pinene, \(\alpha\)-fenchene, limonene and camphene) and oxygenated monoterpenes (citronellal, \(cis\)-carveol, \(\alpha\)-citral, carvacol, terpinol, thymol, carvacrol and citral) (Mohamed et al. 2010). Methylchavicol, 3-methoxycinnamaldehyde, methyleugenol, \(\gamma\)-cadinene and \(\gamma\)-muurolene were the dominant compounds identified in basil essential oil (Teixeira et al. 2013). Thymol and carvacrol were reported to possess the highest antioxidant activity (Dapkevicius et al. 1998). Methylchavicol exhibited high antioxidant activity (Teixeira et al. 2013). The antioxidant activity is generally related to the major active compounds in essential oils. However, the other compounds in essential oils also have antioxidant activity, but their amounts are probably too low to exhibit antioxidant activity (Ruberto and Baratta 2000).

The control film showed radical scavenging activities and chelating activity to some extent (Table 4). Gelatin exhibited antioxidant activities, in which peptide fraction containing particular amino acids such as glycine and proline had high activity (Kim et al. 2001). Gómez-Estaca et al. (2009) also reported that edible film from tuna-skin and bovine-hide gelatin exhibited antioxidant activities. Films incorporated with essential oils had the marked increase in antioxidant activities and chelating activity \( (p < 0.05) \). DPPH and ABTS radical scavenging activities of film incorporated with basil essential oil were highest \( (p < 0.05) \), followed by those added with plai and lemon essential oils, respectively. For chelating activity, film containing lemon

| Essential oils | Antioxidant activity (\(\mu\text{mol Trolox equivalents mL}^{-1}\)) | Chelating activity (\(\mu\text{mol EDTA equivalents mL}^{-1}\)) |
|---------------|-------------------------------------------------|-------------------------------------------------|
|               | DPPH | ABTS                        |                                   |
| Basil         | 34.23 ± 0.05<sup>Aa</sup> | 1,921.27 ± 14.40<sup>a</sup> | 1.53 ± 0.18<sup>a</sup> |
| Plai          | 3.03 ± 0.02<sup>b</sup>  | 60.12 ± 3.18<sup>b</sup>   | 3.91 ± 0.47<sup>b</sup>  |
| Lemon         | 0.49 ± 0.09<sup>c</sup>  | 3.96 ± 0.85<sup>c</sup>    | 30.51 ± 0.54<sup>c</sup> |

Different lowercase letters in the same column indicate significant differences \( (p < 0.05) \)

<sup>A</sup> Mean ± SD \((n = 3)\)

| Essential oils | Antioxidant activity (\(\mu\text{mol Trolox equivalents/g dried film}\)) | Chelating activity (\(\mu\text{mol EDTA equivalents/g dried film}\)) |
|---------------|-------------------------------------------------|-------------------------------------------------|
|               | DPPH | ABTS                        |                                   |
| Control       | 0.09 ± 0.02<sup>Ad</sup> | 0.84 ± 0.16<sup>d</sup>   | 0.25 ± 0.01<sup>d</sup>  |
| Basil         | 10.38 ± 0.06<sup>a</sup>  | 129.65 ± 1.69<sup>a</sup> | 21.72 ± 0.51<sup>a</sup> |
| Plai          | 0.64 ± 0.01<sup>b</sup>  | 10.93 ± 0.08<sup>b</sup>   | 55.02 ± 0.03<sup>b</sup>  |
| Lemon         | 0.53 ± 0.01<sup>c</sup>  | 5.05 ± 0.53<sup>c</sup>    | 58.12 ± 0.13<sup>c</sup> |

Control film was prepared from FFS without addition of essential oils

Different lowercase letters in the same column indicate significant differences \( (p < 0.05) \)

<sup>A</sup> Mean ± SD \((n = 3)\)

**Antioxidative activities**

Antioxidative activities expressed as DPPH radical scavenging activity, ABTS radical scavenging activity and chelating activity of basil, plai and lemon essential oils are presented in Table 3. Basil essential oil had the highest DPPH and ABTS radical scavenging activities \( (p < 0.05) \), followed by plai and lemon essential oils, respectively. Nevertheless, chelating activity of lemon essential oil was highest \( (p < 0.05) \), followed by plai and basil essential oils, respectively. In general, plant essential oils have been known as antioxidants (Wu et al. 1982). Several compounds in essential oils have the structure mimicking the well-known plant phenols with antioxidant activity. Phenolics are organic compounds consisting of hydroxyl group \((-\text{OH})\) attached directly to a carbon atom that is a part of aromatic ring. The hydrogen atom of hydroxyl group can be donated to free radicals, thereby preventing other compounds to be oxidised (Nguyen et al. 2003). Lemon essential oil was mainly composed of monoterpenic hydrocarbons (\(\alpha\)-pinene, \(\alpha\)-fenchene, limonene and camphene) and oxygenated monoterpenes (citronellal, \(cis\)-carveol, \(\alpha\)-citral, carvacol, terpinol, thymol, carvacrol and citral) (Mohamed et al. 2010). Methylchavicol, 3-methoxycinnamaldehyde, methyleugenol, \(\gamma\)-cadinene and \(\gamma\)-muurolene were the dominant compounds identified in basil essential oil (Teixeira et al. 2013). Thymol and carvacrol were reported to possess the highest antioxidant activity (Dapkevicius et al. 1998). Methylchavicol exhibited high antioxidant activity (Teixeira et al. 2013). The antioxidant activity is generally related to the major active compounds in essential oils. However, the other compounds in essential oils also have antioxidant activity, but their amounts are probably too low to exhibit antioxidant activity (Ruberto and Baratta 2000).

The control film showed radical scavenging activities and chelating activity to some extent (Table 4). Gelatin exhibited antioxidant activities, in which peptide fraction containing particular amino acids such as glycine and proline had high activity (Kim et al. 2001). Gómez-Estaca et al. (2009) also reported that edible film from tuna-skin and bovine-hide gelatin exhibited antioxidant activities. Films incorporated with essential oils had the marked increase in antioxidant activities and chelating activity \( (p < 0.05) \). DPPH and ABTS radical scavenging activities of film incorporated with basil essential oil were highest \( (p < 0.05) \), followed by those added with plai and lemon essential oils, respectively. For chelating activity, film containing lemon
essential oil exhibited the highest activity ($p < 0.05$), followed by those added with plai and basil essential oils. Different active compounds in various essential oils affected antioxidative activities and chelating activity of resulting films. Furthermore, some active compounds in different essential oils could interact with gelatin film matrix differently. This led to the varying release of antioxidant compounds from films. The availability of free active compounds in those films matrix could be varied. Thus, the incorporation of selected essential oils from various sources such as leaf, root and peel into gelatin-based film could enhance antioxidative activity.

**Conclusion**

Incorporation of different essential oils from various sources including basil, plai and lemon into fish gelatin film decreased TS and EM with coincidentally increased EAB via plasticising effect. Essential oils effectively improved the water vapour barrier property of film, especially that containing basil and lemon essential oils. However, those essential oils could affect the colour and light transmittance of resulting films. Amongst essential oils added, basil essential oil could be appropriately used to enhance water vapour barrier property and yielded film with antioxidative activity. Therefore, plant essential oils could be used as natural additives, which could improve physical and functional properties of gelatin film. For the commercial production of film based on fish gelatin incorporated with essential oils, thermal processing such as film extrusion, film blowing, etc., can be implemented. Nevertheless, characteristics and properties of films may be varied. Thus, further study is still required.

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**Conflict of interest**  The authors declare that they have no competing interests.

**Author’s contributions**  SB and TP formulated the hypothesis and designed the study. PT conducted the experiments and analysis of data. PT wrote the manuscript with assistance from SB and TP. All authors read and approved the final manuscript.

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