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ARTICLE

Preparation of perilla seed meal protein composite films containing various essential oils and their application in sausage packaging

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Abstract Perilla seed meal protein (PSMP) was extracted from perilla seed oil residue, and its composite films were developed using different amounts of red algae (RA) to prepare edible films. The PSMP composite film that contained 3 % RA had the highest tensile strength (11.52 MPa) and the lowest water vapor permeability (1.82×10^{-9} g m/m^2 s Pa). The incorporation of clove oil into the PSMP composite film had the highest antimicrobial activity against *Listeria monocytogenes*. Pork sausages packaged with the composite films containing clove oil showed antimicrobial and antioxidative activities during storage at 4 °C. The composite film containing 1.2 % clove oil reduced the microbial growth by 1.24 log CFU/g compared to the control, and thiobarbituric acid reactive substances and peroxide values were reduced by 40 and 37 %, respectively. These results suggest that the PSMP/RA composite film containing 1.2 % clove oil can be utilized in sausage packaging.

Keywords Composite film · Lipid oxidation · Perilla seed oil · Packaging

Introduction

Perilla (*Perilla frutescens*) seed has been commonly used as an edible oil source in several Asian countries. Perilla seed oil exhibits a number of biological activities such as antihypertensive, antioxidative, and immunostimulatory effects (Takenaka et al. 2010). Perilla seed consists of 51 % fat and 17 % protein, and after oil extraction, defatted perilla seed meal contains approximately 40 % protein (Longvah and Deosthale 1991). Despite being an abundant source of protein, defatted perilla seed meal is used as animal feed or fertilizer. Therefore, utilizing perilla seed meal as a value-added food ingredient like an edible film source could be worthwhile, although it may have undesirable smell due to its source when applied to food.

As the plastic packaging materials of foods are not environment friendly, protein-based edible films have been studied extensively (Lacroix and Vu 2014). However, protein films usually have poor mechanical properties due to their hydrophilic nature, and the composite films can be prepared (Giannelis 1996). It was suggested that *Gelidium corneum,* a red algae (RA), has excellent film-forming properties and can be used in food packaging applications (Ku et al. 2008; Hong et al. 2009). Furthermore, it has been reported that incorporation of RA into protein-based films resulted in better mechanical properties of the films (Shin et al. 2011).

Contamination of sausages with pathogenic bacteria such as *Listeria monocytogenes* during storage and marketing is a great concern in food industry (Degenhardt and Sant'Anna 2007; Daskalov et al. 2014). Thus, antimicrobial packaging can be an effective strategy for ensuring the microbial safety of the sausages. Considering that essential oils such as clove, rosemary, and lemongrass have exhibited antimicrobial properties, these substances could be added into the edible packaging materials as antimicrobial agents (Burt 2004; Holley and Patel 2005). Several studies have been conducted on incorporating essential oils into protein-based films without any safety issue, but no study has been undertaken regarding the application of essential oils in perilla seed meal protein film for sausage packaging.

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Therefore, this study was performed to investigate the mechanical properties of PSMP/RA composite films and to apply the PSMP composite films containing essential oils as an antimicrobial packaging of sausage.

Materials and methods

Materials

The perilla seed meals were obtained from a local company (On-Cheon Gireum, Korea). The essential oils (lemongrass, rosemary, and clove bud) were purchased from The Certification Academy for Holistic Aromatherapy (Korea). Pork sausage (pork, 70 %) and red algae were purchased from a local company (Korea).

Preparation of PSMP

Defatted perilla seed meal, which was obtained after oil extraction from perilla seed by the high pressure treatment under 60 MPa at 200 °C, was milled using a grinder (Osaka Chemical Co., Ltd., Japan). The PSMP was extracted from defatted perilla seed meal according to the method of Oita et al. (2008) with minor modifications. To the defatted perilla seed meal, phosphate buffer (20 mM, pH 7.5) containing 0.5 M NaCl was added (1:5, w:v) and blended for 2 h. The supernatants were obtained after centrifugation at 10,000×*g* for 1 h and dialyzed using a membrane (Spectrum Laboratories, USA). The dialyzed samples were then freeze dried.

Preparation of PSMP/RA composite film

To prepare the PSMP/RA composite films, PSMP and red algae (7:0, 6:1, 5:2, and 4:3, g/g) were dissolved in 100 mL of distilled water. Various plasticizers (glycerol, sorbitol, fructose, and sucrose) were used in the preliminary experiments. Subsequently, fructose (40 % of PSMP weight) was chosen and used as a plasticizer for the film-forming solution. To disperse the solution, it was stirred at 380 rpm for 1 h, followed by ultra-sonication for 8 min and homogenization at 14,000 rpm for 5 min. After homogenization, the solution was heated at 90 °C for 30 min. In addition, for the PSMP/RA composite films containing essential oils, various amounts (0.8, 1.0, and 1.2 g) of rosemary, lemongrass, and clove oils were added into the film-forming solution with 0.25 g Tween 20, respectively. The film-forming solutions were then filtered using cheese cloth, and 80 mL of PSMP/RA composite film-forming solution was cast onto glass plates and dried at 25 °C for 24 h. After drying, the films were peeled and partitioned for subsequent film measurements.

Measurement of color properties and transparency

The color of the film was determined using a colorimeter (Minolta, CR-400, Japan). Hunter L^*, a^*, and b^* were determined against a white standard plate ($L^* = 96.63$, $a^* = -0.14$, and $b^* = 2.09$). Each measurement was determined in triplicate. The total color difference (ΔE) was determined according to the method described by Srinivasa et al. (2002). The transparency of the film was determined by measuring the transmittance (%) at 660 nm using a spectrophotometer (UV-2450, Shimadzu Corporation, Japan).

Scanning electron microscopy (SEM)

The morphologies of the PSMP/RA composite films containing various amounts of RA were observed using a JSM-7000F field emission scanning electron microscope (JEOL, Japan). SEM images were obtained from the surface and cross section of PSMP composite films at a magnification of 300× and working distance of 25 mm. All specimens were coated with an osmium layer before SEM observation.

Measurement of mechanical properties

Tensile strength (TS) and elongation at break (E) of the films were measured using a tensiometer (M250-2.5 CT, The Testometric Company Ltd., UK) according to the ASTM method D638 M with a modification (Song et al. 2013). All SSMP films were incubated at a constant temperature of 25 °C and a relative humidity (RH) of 50 % for 48 h. The initial grip distance of 5 cm and the cross-head speed of 50 cm/min were used. Five replicates were tested for each film. The thickness of the film was determined at five random spots using a micrometer (Mitutoyo, Japan).

Measurement of water vapor permeability

The water vapor permeability (WVP) of the films was determined following the gravimetric method according to the method of Hong et al. (2009). All samples were carried out at least in triplicate. WVP was expressed as 10^{-9} g m/m^2s Pa.

Disk diffusion test for antimicrobial activity

Antimicrobial activity of the film was determined according to the method described by Song et al. (2013). *L. monocytogenes* (ATTC 19111) was cultured in *Listeria* enrichment broth (Oxoid Ltd., UK) at 37 °C for 24 h, and 0.1 mL of *L. monocytogenes* was plated onto Oxford medium base (Difco, USA). From the film containing essential oil, disks (diameter, 10 mm) were cut and placed onto the inoculated plates. The plates were then incubated at 37 °C for 48 h, and the inhibition zone was measured using a Digimatic caliper (Mitutoyo, Japan). Mean value of three replicates was determined.

Inoculation of *L. monocytogenes* on pork sausage and microbial count during storage

One milliliter of *L. monocytogenes* (10^6 CFU/mL) was evenly spread on the pork sausage surface. The inoculated sausage samples (10 g) were divided into the control without the PSMP composite film, the samples wrapped in direct contact with the PSMP composite film containing various essential oils, and the samples wrapped with the PSMP composite film without essential oils. The PSMP composite films containing each essential oil were used for wrapping the sausage samples, respectively. Each sample was then put in a sterile polyethylene bag (Lab Plas, Canada) and stored at 4 °C for 12 days. During storage of pork sausage, microbial count was measured every 3 days. Sausage samples (10 g) were taken after peeling off the films and homogenized for 3 min using a stomacher (AES Laboratoire, France), filtered, and diluted with peptone water for *L. monocytogenes* counts. The measurement of microbial count was performed in triplicate, and the counts were expressed as log CFU/g.

Measurement of lipid oxidation during storage

Peroxide value (PV) and thiobarbituric acid (TBA) assay were determined according to the method of Song et al. (2012). Each sample (2 g) after peeling off the films was taken during storage and used for the measurement. The PV value and TBA value were expressed as meq O_2 and mg malonaldehyde (MDA) per kg sample, respectively.

Statistical analysis

To analyze the experimental data, Duncan's multiple range tests were performed using the SAS program version 8.1 (SAS Institute, Inc., USA), and significant differences between treatments at a 95 % confidence level were determined. All results are expressed as the mean ± standard deviation.

Results and discussion

Mechanical properties of the PSMP/RA composite films

The TS of the PSMP/RA composite film increased with the increasing RA content (Table 1). The PSMP (7 g) film without RA had the lowest TS (1.79 MPa), while the PSMP/RA composite film containing 3 g RA had the highest TS (11.52 MPa). It has been reported that the TS of the sunflower seed meal protein composite film increased with the addition of RA (Song et al. 2013). The reason for this increment by the addition of RA was mainly due to the strong favorable interactions between PSMP and RA molecules. In addition, the incorporation of RA decreased the WVP values. In particular, the WVP of PSMP without RA was 2.07×10^{-9} g m/m^{2}s Pa, whereas the WVP of PSMP composite film containing 3 g RA was 1.82×10^{-9} g m/m^{2}s Pa (Table 1). Letendre et al. (2002) also reported that the WVP was decreased with the addition of agar for the milk protein films. The E values of the PSMP/RA composite films also showed a decreasing trend with the addition of RA. The PSMP composite film containing 3 g RA had the lowest E (15.18 %). These results were well corresponding with the study of Song et al. (2013), where the *E* values of sunflower seed meal protein films were decreased with the addition of RA. Therefore, it is suggested that the increase of the TS and decreases of the WVP and *E* with RA concentration should be mainly due to the internal structure changes with RA, which was well presented in the film microstructure using SEM (Fig. 1).

Table 1 Physical properties of PSMP/RA composite films

PSMP (g)	Red algae (g)	Tensile strength (MPa)	Elongation (%)	WVP ($\times 10^{-9}$ g m/m^{2}s Pa)
7	0	$1.79 \pm 0.36^{a,1}$	56.84 ± 1.26^{a}	2.07 ± 0.07^{a}
6	1	5.30 ± 0.51^{b}	29.31 ± 3.01^{b}	1.83 ± 0.02^{b}
5	2	9.60 ± 0.48^{c}	15.99 ± 1.17^{c}	1.83 ± 0.01^{b}
4	3	11.52 ± 0.75^{d}	15.18 ± 1.73^{c}	1.82 ± 0.02^{b}

[1] Values in a column followed by different superscript letters are significantly different ($p < 0.05$)

Optical properties

The incorporation of RA had a significant effect on the optical properties of the PSMP film (Table 2). The addition of RA into the PSMP composite film increased the lightness (L^*) and ΔE value, while it decreased the redness (a^*) and the yellowness (b^*). The increase of the L^* value of sunflower seed meal protein by the addition of RA was also reported by a previous study (Song et al. 2013). The reason for the change of the color properties of the films with the addition of RA could be due to the miscibility between the PSMP and RA molecules, resulting in increase of lightness. Similar to this report, Tian et al. (2011) also reported that soy protein isolates and agar molecules were miscible because of the presence of free hydroxyl functional groups in both molecules.

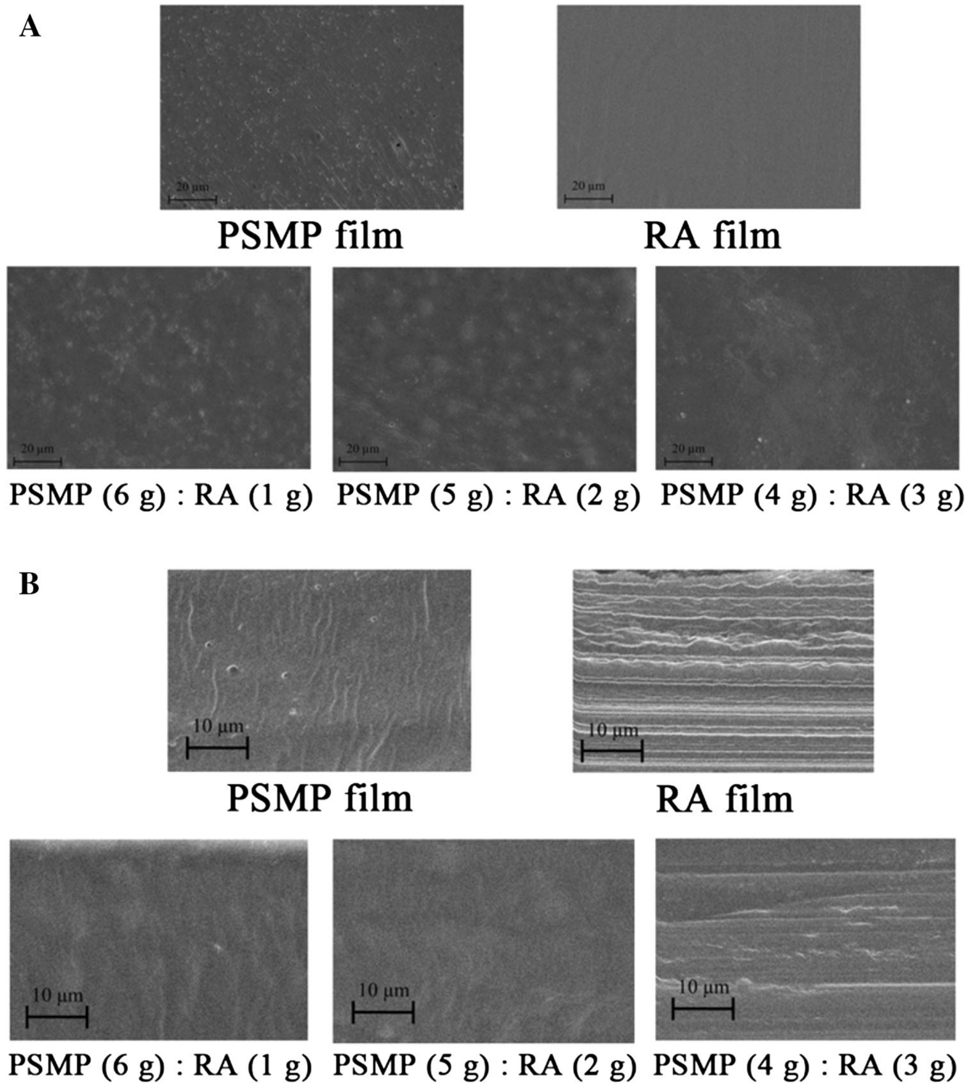

Fig. 1 Surface and cross-sectional SEM images of PSMP/RA composite films. **a** Surface image, **b** Cross-sectional image

Table 2 Optical properties of the PSMP/RA composite films

PSMP (*g*)	Red algae (*g*)	*L**	*a**	*b**	Δ*E*	Transparency (%)
7	0	$62.27 \pm 0.55^{a,1}$	8.30 ± 0.14^{a}	32.28 ± 0.18^{a}	–	39.58 ± 1.13^{a}
6	1	68.35 ± 0.26^{b}	4.63 ± 0.12^{b}	32.16 ± 0.12^{a}	7.10 ± 0.28^{a}	48.59 ± 1.03^{b}
5	2	69.29 ± 0.15^{c}	4.05 ± 0.05^{c}	31.45 ± 0.13^{b}	8.25 ± 0.15^{b}	56.80 ± 0.66^{c}
4	3	73.63 ± 0.13^{d}	2.02 ± 0.05^{d}	30.37 ± 0.05^{c}	13.12 ± 0.12^{c}	63.23 ± 2.48^{d}

[1] Values in a column followed by different superscript letters are significantly different ($p < 0.05$)

The transparency of PSMP/RA composite films is shown in Table 2. The transparency of the films was increased by the incorporation of RA. With the addition of 3 g RA, the transmittance of PSMP/RA composite film increased from 39 to 63 %. Rattaya et al. (2009) also found that the transmittance of fish gelatin film increased after the addition of seaweed extract, similar to RA in this study. The increase in the transparency in the PSMP/RA composite films mainly comes from the miscibility between PSMP and RA molecules, which form a uniform network to facilitate the light passage through the film.

Film microstructure

SEM analysis was carried out to have a better insight on the homogeneity and morphology of the PSMP films. The

Table 3 Physical properties of PSMP/RA composite films containing various concentrations of essential oils

Essential oil	Tensile strength (MPa)	Elongation (%)	WVP ($\times 10^{-9}$ g m/m^{2}s Pa)
None[1]	11.52 ± 0.75[a,2]	15.18 ± 1.73[a]	1.82 ± 0.02[a]
Rosemary			
0.8 %	10.43 ± 0.24[b]	20.45 ± 2.82[b]	1.94 ± 0.01[ab]
1.0 %	9.83 ± 0.59[b]	27.16 ± 1.52[c]	2.00 ± 0.18[ab]
1.2 %	9.60 ± 0.33[b]	32.74 ± 2.43[d]	2.09 ± 0.06[b]
Lemongrass			
0.8 %	10.79 ± 0.84[ab]	18.87 ± 1.89[b]	2.04 ± 0.02[b]
1.0 %	9.74 ± 0.61[b]	21.69 ± 2.75[bc]	2.05 ± 0.01[b]
1.2 %	9.18 ± 0.32[c]	26.98 ± 3.89[c]	2.11 ± 0.03[c]
Clove			
0.8 %	11.31 ± 0.43[a]	20.93 ± 1.68[b]	1.99 ± 0.18[ab]
1.0 %	10.80 ± 0.17[ab]	24.72 ± 0.70[c]	1.99 ± 0.17[ab]
1.2 %	9.80 ± 0.80[b]	29.70 ± 2.76[d]	2.08 ± 0.06[b]

[1] PSMP/RA composite film without essential oil

[2] Values in a column followed by different superscript letters are significantly different ($p < 0.05$)

Table 4 Antimicrobial activities of the PSMP/RA composite films containing essential oils against *L. monocytogenes*

Essential oil (%)	Inhibition zone (mm)
Rosemary	
0.8	11.70 ± 0.19[a1]
1.0	12.06 ± 0.48[a]
1.2	14.47 ± 0.29[b]
Clove	
0.8	16.36 ± 0.36[c]
1.0	21.67 ± 0.53[d]
1.2	25.63 ± 0.60[e]
Lemongrass	
0.8	12.27 ± 0.17[a]
1.0	17.63 ± 0.32[c]
1.2	19.13 ± 0.73[d]

[1] Values in a column followed by different superscript letters are significantly different ($p < 0.05$)

SEM images of the PSMP/RA composite films are shown in Fig. 1. The surface SEM images clearly indicate that the PSMP film without RA had a heterogeneous structure having pores and cracks. However, as the addition of RA increased, pores and cracks disappeared and the films became more homogeneous and smoother, indicating that the addition of RA into the PSMP film increased the transparency of the films. The increase of transparency with the addition of RA was also supported by the transparency data of the PSMP/RA composite films (Table 2). In addition, the cross-sectional SEM micrograph of the RA film showed a layered structure with roughness, whereas the PSMP film had a non-layered structure with relatively smoother texture than RA film. Therefore, the PSMP/RA composite film became more layered and showed increased roughness, as the amount of RA increased.

Mechanical properties of PSMP/RA composite film containing essential oils

The addition of essential oils to the PSMP/RA composite films affected the mechanical properties (Table 3). The PSMP/RA composite film without essential oils had a TS of 11.52 MPa, whereas the TS of PSMP/RA composite film decreased with the incorporation of essential oils (rosemary, lemongrass, and clove). The decrease of TS is mainly due to the breakdown of molecular interactions in the films caused by the addition of essential oils. Similarly, Pranoto et al. (2005) also reported the decrease in TS of chitosan film with the addition of garlic oil. In contrast, the E of PSMP/RA composite films containing essential oils increased, compared with the control. The PSMP/RA composite film without essential oils had the lowest E (15.18 %), whereas the composite film containing 1.2 % rosemary oil had the highest E (32.74 %). The reason for the increase in E of the films with the increase of essential oil content was mainly due to the increase of water molecules caused by the breakdown of molecular interactions. Incorporation of essential oils also increased the WVP values of the films. The PSMP/RA composite film without essential oils had a WVP value of 1.82×10^{-9} g m/m^{2}s Pa, whereas all PSMP/RA composite films containing essential oils showed higher WVP values. Hosseini et al. (2009) also reported the increase of WVP of chitosan film with the addition of essential oils, which was caused by the change in the hydrophilicity of the film matrix.

Antimicrobial properties of PSMP/RA composite films

The effects of essential oils on the antimicrobial property of PSMP/RA composite film are shown in Table 4. The presence of essential oils in the films gave rise to varying level of antimicrobial activities. The antimicrobial property of the

Table 5 Change in the populations of *L. monocytogenes* inoculated in sausage during storage at 4 °C

(log CFU/g)

Packaging film	Storage time (day)				
	0	3	6	9	12
Control	5.16 ± 0.09[A1a2]	6.07 ± 0.04[Ba]	6.49 ± 0.17[Ca]	7.83 ± 0.09 [Da]	7.85 ± 0.11[Da]
PSMP composite	5.16 ± 0.09[Aa]	6.04 ± 0.13[Ba]	6.56 ± 0.12[Ca]	7.78 ± 0.12 [Da]	7.81 ± 0.15[Da]
PSMP/clove oil	5.16 ± 0.09[Aa]	5.31 ± 0.15[Ac]	5.36 ± 0.17[Ac]	6.37 ± 0.25[Bc]	6.61 ± 0.23[Bc]
PSMP/rosemary oil	5.16 ± 0.09[Aa]	5.83 ± 0.07[Bb]	6.06 ± 0.24[Bb]	7.20 ± 0.27[Cb]	7.25 ± 0.36[Cb]
PSMP/lemongrass oil	5.16 ± 0.09[Aa]	5.65 ± 0.07[Bb]	5.86 ± 0.09[Cb]	7.00 ± 0.07[Db]	7.02 ± 0.08[Db]

[1] Values in a row followed by different superscript letters are significantly different ($p < 0.05$)

[2] Values in a column followed by different superscript letters are significantly different ($p < 0.05$)

PSMP/RA composite film largely depended on the type and the amount of essential oil. The antimicrobial activities of clove, lemongrass, and rosemary oil were attributed to the their functional components: clove (eugenol and eugenyl acetate), lemongrass (geranial, neral, and limonene), and rosemary (α-pinene, bornly acetate, camphor, and 1,8-cineole) (Daferera et al. 2000). The hydrophobic compounds of essential oils could disturb the membrane structure and cause leakage of cell contents (Burt 2004). The PSMP/RA composite films containing clove oil had a larger inhibition zone than those of the PSMP/RA composite films containing other essential oils. In addition, the inhibition zone against *L. monocytogenes* increased with the increasing concentration of essential oils. In particular, the PSMP composite film containing 1.2 % clove oil had the biggest inhibition zone of 25.63 mm. Similarly, other studies regarding the different antimicrobial activities of essential oils were reported (Oussalah et al. 2007; Viuda-Martos et al. 2010).

Quality change in pork sausage packed with PSMP composite film during storage

The populations of *L. monocytogenes* inoculated on pork sausage wrapped with the PSMP composite film containing various essential oils were determined during storage (Table 5). The population of *L. monocytogenes* inoculated on pork sausage was initially 5.16 log CFU/g. The sausages wrapped with the PSMP composite film had an increase in the populations of *L. monocytogenes* until 9 days of storage, while the population of *L. monocytogenes* in the sausages wrapped with the PSMP composite film containing clove oil did not increase until 6 days, but started to increase after 9 days of storage. The microbial growth pattern depends on the growth temperature, relative humidity, available nutrients, etc. After 12 days of storage, the populations of *L. monocytogenes* in the control and the sausage wrapped with the PSMP composite film were 7.85 and 7.81 log CFU/g, respectively, whereas the population in the sausage wrapped

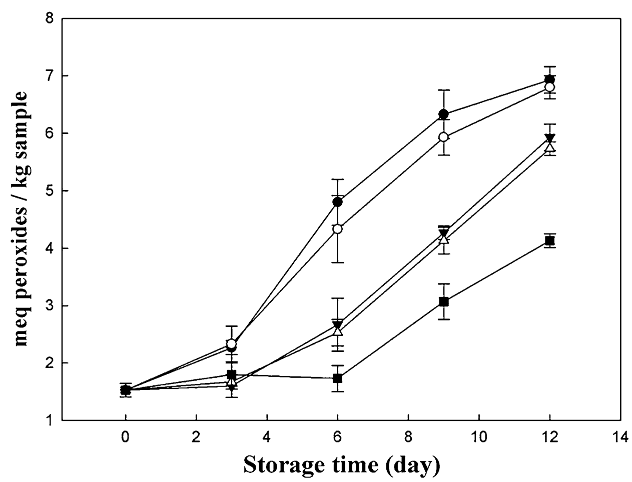

Fig. 2 Change in PV values of pork sausage during storage at 4 °C. *Filled circle* control; *open circle* PSMP composite film; *filled inverted triangle* PSMP composite film containing lemongrass oil; *open triangle* PSMP composite film containing rosemary oil; *filled square* PSMP composite film containing clove oil

with the PSMP composite film containing 1.2 % clove oil was 6.61 log CFU/g, indicating a 1.2 log CFU/g reduction in the microbial content. Miladi et al. (2010) also reported that the population of *L. monocytogenes* in fresh-cut salmon containing 2 % clove oil was reduced by 2.35 log CFU/g after 15 days of storage, compared to the salmon without clove oil. These antibacterial effects of the films containing essential oils are mainly due to the release of antimicrobial components from the incorporated essential oils toward packaged foods during storage, which may be probably by diffusion mechanism.

The PV and TBA values of pork sausage wrapped with the PSMP composite film containing various essential oils were determined during storage (Figs. 2, 3). The PV and TBA values of the pork sausage increased during storage. However, the pork sausage wrapped with the PSMP composite film containing essential oils had lower PV and TBA

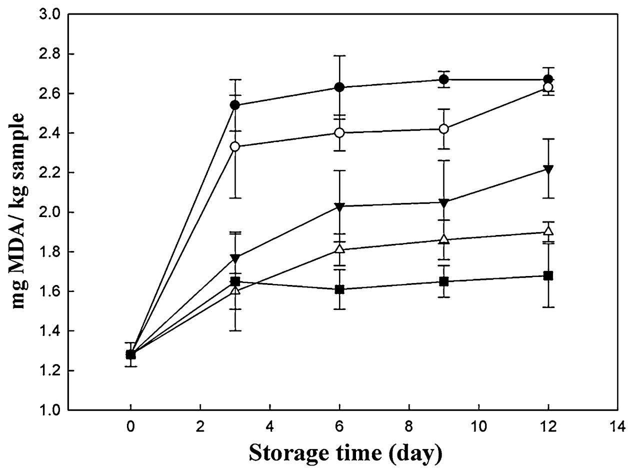

Fig. 3 Change in TBA values of pork sausage during storage at 4 °C. *Filled circle* control; *open circle* PSMP composite film; *filled inverted triangle* PSMP composite film containing lemongrass oil; *open triangle* PSMP composite film containing rosemary oil; *filled square* PSMP composite film containing clove oil

than the pork sausage wrapped with the film without essential oils. In particular, the PSMP composite film containing clove oil showed the best antioxidative property. After 12 days of storage, the PV of pork sausage wrapped with the PSMP composite film containing clove oil decreased by 40 %, compared to the control (Fig. 2). And the TBA value also decreased by 37 % (Fig. 3). The type and the amount of the incorporated essential oils were the two main factors affecting the antioxidative properties of the composite films. It appears that the presence of phenolic compounds contributed to the antioxidant capacities of the essential oils (Shan et al. 2005).

In summary, as an edible packaging material, PSMP in combination with 3 % RA has shown the most suitable mechanical properties in terms of TS, WVP, E, and transparency. Among the essential oils incorporated into the composite film, clove oil exhibited the highest level of antimicrobial property. A reduction of microbial growth along with very high level of antioxidative activities was found in the pork sausage packaged with the composite film containing clove oil. Therefore, this study has meaningful results in terms of utilization of perilla seed meal as an edible film source and suggests that PSMP/RA composite film incorporated with clove oil could be a potential candidate in sausage packaging.

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