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RESEARCH ARTICLE

Preparation of Red Algae Film Containing Grapefruit Seed Extract and Application for the Packaging of Cheese and Bacon

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Abstract Red algae (RA) film containing grapefruit seed extract (GSE) was used as a wrapping film for cheese and bacon. RA film containing 1% GSE was prepared to inhibit the growth of pathogenic bacteria such as *Escherichia coli* O157:H7 and *Listeria monocytogenes*. Wrapping of cheese and bacon with the film decreased the populations of *E. coli* O157:H7 and *L. monocytogenes*. After 15 days of storage, wrapping of cheese with the RA film reduced the populations of *E. coli* O157:H7 and *L. monocytogenes* by 1.21 and 0.85 log CFU/g, respectively, compared to control. Bacon wrapped with the RA film also decreased the populations of *E. coli* O157:H7 and *L. monocytogenes* by 0.45 and 0.76 log CFU/g, respectively. Wrapping of bacon with the RA film decreased peroxide and thiobarbituric acid values. These results suggest that RA film containing GSE is a useful wrapping material for extending the shelf lives of cheese and bacon.

Keywords: edible film, red algae, grapefruit seed extract, cheese, bacon

Introduction

Recently, studies of edible films have drawn considerable interest due to environmental concerns regarding plastic packaging materials (1). Edible films can extend the shelf life and improve the quality of food by providing moisture, gas, and lipid barriers for food and by inhibiting the growth of foodborne pathogens (2-4). Edible films are usually prepared using proteins, polysaccharides, and lipid residues and can serve as carriers of antioxidants and antimicrobials (5).

Red algae (RA), which contains large amounts of agar, is used as a food ingredient. The major component of RA is agarose, and it has been considered for use as food packaging due to its good film-forming properties (6). Previously, edible films consisting of extract from *Gelidium corneum* (GC), which is a type of red algae (6,7), were prepared and their mechanical properties were examined. However, the film manufactured from the GC extract had poor physical properties such as low tensile strength (8). Therefore, in the present investigation, to improve the mechanical properties of the films, the RA films using whole RA powder without extracting process of RA were prepared. This can make processing of film preparation simple as well as improving the mechanical properties of the films, compared to GC films.

Antimicrobial and antioxidant agents used in edible films include grapefruit seed extract (GSE), green tea extract, essential oils, tocopherol, organic acid, and chitosan (9,10). GSE is a natural product that contains tocopherol, citric acid, and ascorbic acid (11). GSE has antimicrobial and antioxidant activity and its film is used to preserve foods such as tomatoes, frankfurters, poultry products, and fish (12-14). The antioxidant activity of GSE is mainly due to flavonoids, and it can serve as a scavenger of free radicals, to chelate metals, and to reduce hydroperoxide formation (15).

The objectives of this study were to prepare a RA film containing GSE, and to apply the film in the wrapping of cheese and bacon as a typical processed food which are commonly consumed in the form of small quantity.

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Materials and Methods

Materials The red algae used in this study was harvested in Jeju Island, Republic of Korea. Sorbitol was purchased from Sigma-Aldrich (St. Louis, MO, USA). Grapefruit seed extract (GSE) was obtained from ABC Techno Inc. (Tokyo, Japan). Sliced cheese and bacon were purchased from a local market (Daejeon, Korea).

Preparation of the film-forming solution To prepare the red algae (RA) film forming solution, 2 g RA powder was dissolved in 100 mL distilled water and mixed with 1.5 g sorbitol, and various amounts (0.5, 0.7, 1.0, and 1.2%, w/w) of GSE were dispersed into the solution.

Film casting and drying The film-forming solutions were strained through cheesecloth and cast onto flat, Teflon-coated glass plates (24×30 cm). Uniform film thicknesses were produced by casting the same amount of film-forming solution onto each plate. Plates were dried at 25°C for 48 h, and the dried films were peeled intact from the casting surfaces. Specimens were cut for determination of water vapor permeability (2×2 cm) and tensile strength (TS, 2.54×10 cm).

Determination of film thickness The film specimens were conditioned in an environmental chamber at 25°C and at 50% relative humidity (RH) for 2 days. The thickness of each film was measured at 5 random positions using a micrometer (model No. 2046-08; Mitutoyo, Tokyo, Japan), and the mean value of the 5 measurements was determined.

Measurement of TS and elongation The TS and elongation at break (E) of the films were determined using an Instron Universal Testing Machine (model 4484; Instron Co., Canton, MA, USA) according to the ASTM standard method (16). The film specimens were conditioned in an environmental chamber at 25°C and 50% RH for 24 h. An initial grip distance of 5 cm and a cross-head speed of 50 cm/min were used. The TS was calculated by dividing the maximum load of a specimen by the initial cross-sectional area, and the elongation was expressed as a percentage of the change in the initial gauge length of a specimen at the point of sample failure. Five replicates of each film were tested.

Measurement of water vapor permeability (WVP) The WVP of the edible films were determined according to a modified method (7) at 25°C and 50% RH using a polymethylacrylate cup. The cup was filled to 1 cm with distilled water and was covered with a film specimen. The film specimens were conditioned in an environmental chamber at 25°C and 50% RH. The weight losses of the cups were measured over time. A linear regression analysis was performed to calculate the slope, and the WVP (ng·m/m^2·s·Pa) values were then calculated.

Culture preparation *Escherichia coli* O157:H7 (NCTC 12079) and *Listeria monocytogenes* (ATCC 19111) were cultured at 37°C for 24 h in 50-mL conical tubes containing 25 mL Luria-Bertani broth (BD, Detroit, MI, USA) or *Listeria* enrichment broth (Oxoid Ltd., Basingstoke, UK).

Diffusion test for antimicrobial activity *E. coli* O157:H7 (0.1 mL) was plated onto tryptic soy broth-bacto agar (BD), and *L. monocytogenes* (0.1 mL) was plated onto Oxford medium base agar (BD). Discs (10-mm in diameter) were cut from the films and placed onto the inoculated plates. After 3 h at 4°C to allow the GSE to diffuse, the *E. coli* O157:H7 plates were incubated at 37°C for 24 h, and the *L. monocytogenes* plates were incubated at 37°C for 48 h. Each microbial count was determined as the mean of 3 replicates, and the inhibition zone was measured in mm using a Digimatic caliper (model 500-181-20; Mitutoyo Co., Kawasaki, Japan).

Inoculation of pathogens on cheese and bacon *E. coli* O157:H7 and *L. monocytogenes* were incubated at 37°C in LB and *Listeria* enrichment broth, respectively, until they reached 10^6 CFU/mL. *E. coli* O157:H7 and *L. monocytogenes* were spread on the surfaces of cheese and bacon with a sterile glass rod and allowed to rest for 30 min. The initial inoculation levels of *E. coli* O157:H7 and *L. monocytogenes* in the cheese samples were 5.66 and 6.05 log CFU/g, and the bacon samples were 6.43 and 7.01 log CFU/g, respectively. Cheese and bacon were packed in direct contact with the RA film containing 1% GSE by wrapping. Samples packed in polyethylene terephthalate (PET) boxes were used as controls. All of the samples were stored at 4±1°C.

Microbiological analysis Samples (5 g) were removed using a sterile scalpel, homogenized for 3 min using a stomacher (MIX 2; AES Laboratoire, Combourg, France, 1,650 mL), filtered through sterile cheesecloth, and diluted 9 mL sample taken from the Stomacker bag with 0.1% peptone water for the measurement of microbial counts. Serial dilutions were performed on each selective agar plate. *E. coli* O157:H7 counts were determined by plating appropriately diluted samples onto MacConkey agar (BBL; BD), and *L. monocytogenes* were plated onto Oxford medium base agar (BD). For the determination of *E. coli* O157:H7 and *L. monocytogenes* populations, each plate was incubated at 37°C for 24 and 48 h, respectively. Each microbial count was the mean of 3 measurements, and microbial counts were expressed as log CFU/g.

Measurement of lipid oxidation The assessment of lipid oxidation was conducted using 2 different methods, the peroxide value (PV) and the thiobarbituric acid (TBA) assays. PV was determined according to method Cd 8-53 of AOCS (17). Bacon oil (2 g) was placed into a 250-mL Erlenmyer flask and 30 mL of a mixture of acetic acid/chloroform (3:2, v/v) was added. The solution was stirred to completely dissolve the lipids. A solution of potassium iodide was added and the flask was allowed to stand for 5 min in a dark room. Distilled water (30 mL) was added and titrated against sodium thiosulfate, with 1% starch as an indicator. The PV value was expressed as meq peroxide/kg sample. The TBA value was determined according to the method described by Vyncke (18). The sample (2 g) was mixed with 10 mL of 7.5% trichloroacetic acid and homogenized for 1 min. The suspension was filtered, and 5 mL of the filtrate was added to 5 mL of TBA reagent (0.02 M 2-thiobarbituric acid in distilled water). The mixture was immersed in a boiling water bath for 45 min, cooled with water, and the absorbance was measured at 539 nm. The TBA value was recorded as mg malonalehyde (MDA)/kg sample.

Analysis of moisture content The moisture contents of the samples was determined according to the method of AOAC (19). Samples were weighed and placed in an oven (C-DO; Chang Shin Scientific Co., Seoul, Korea) at 105±2°C for 24 h until a constant weight was reached. The moisture contents of the samples were then calculated using the sample weights before and after drying. The initial moisture contents of cheese and bacon were 54 and 73.67%, respectively.

Sensory evaluation During storage, samples were chosen randomly and analyzed for appearance, firmness, odor, and overall acceptability by 10 trained panelists (male: 4, female: 6, age: 23-28). Sensory qualities of the samples were evaluated subjectively on a 9-point hedonic scale. Sensory scores of 9-8 were very good, 7-6 good, 5-4 fair, 3-2 poor, and 1 very poor.

Statistical analysis Analyses of variance and Duncan's multiple range tests were performed using the SAS program (SAS Institute, Inc., Cary, NC, USA). Significant differences between treatments were determined at 95% confidence levels.

Results and Discussion

Physical and antimicrobial activities of RA films containing GSE The RA films containing various amounts of GSE were prepared to enhance the antimicrobial activity of the film. The thicknesses of the RA films having various amounts of GSE were similar, and TS of the RA films decreased as GSE was added (Table 1). The RA film containing 1% GSE had a TS of 7.2 MPa, while the film without GSE had a TS of 8.4 MPa. In contrast, Hong *et al.* (7) reported that TS in the GC-gelatin blend film was increased by the addition of GSE. This difference may be due to differences in the chemical nature of RA compared to GC-gelatin.

The E (elongation at break) values of the RA film increased by the addition of GSE (Table 1). The RA film containing 1.0% GSE had the highest E (47.9%), while the film without GSE had the lowest E (32.3%). These results are in sharp contrast with the results of Hong *et al.* (7), where they reported that the E value decreased with the addition of GSE in GC-gelatin blend film. This can be explained by the fact that there is a difference in the chemical nature between RA and GC-gelatin molecules. The WVP of the RA film containing GSE increased compared to the control film (0% GSE), and there were no significant ($p<0.05$) differences among the films containing GSE (Table 1).

The antimicrobial activity of the RA film containing GSE against *E. coli* O157:H7 and *L. monocytogenes* demonstrated increased inhibition zones of bacterial growth with increasing GSE concentration (Table 2). For the RA film containing 1.2% GSE, the inhibition zones against *E. coli* O157:H7 and *L. monocytogenes* were 16.4 and 32.5 mm, respectively. Heggers *et al*. (20) reported that GSE exhibited antimicrobial activity against Gram-positive and Gram-negative bacteria. Xu *et al*. (21) and Jang *et al*. (22) also reported the antimicrobial activity of GSE against *L. monocytogenes* and *E. coli*, and our results clearly

Table 1. Mechanical properties of the RA film containing various concentration of grapefruit seed extract (GSE)

GSE content (%)	Thickness (mm)	Tensile strength (MPa)	Elongation (%)	Water vapor permeability ($ng \cdot m/m^2 \cdot s \cdot Pa$)
0	0.024±0.00[a1)]	8.4±0.46[a]	32.3±1.24[b]	1.5±0.02[b]
0.5	0.024±0.00[a]	7.2±1.86[ab]	42.4±2.69[a]	1.8±0.05[a]
0.7	0.024±0.00[a]	7.2±0.47[ab]	42.4±2.09[a]	1.7±0.00[a]
1.0	0.025±0.01[a]	7.2±0.37[ab]	47.9±6.84[a]	1.8±0.04[a]
1.2	0.025±0.00[a]	7.0±0.28[b]	44.9±4.55[a]	1.8±0.06[a]

[1)]Mean values with different letters within a column are significantly different by Duncan's multiple range test at $p<0.05$.

Table 2. Antimicrobial activity of the grapefruit seed extract-red algae (GSE-RA) film against the pathogenic bacteria

GSE (g)	Inhibition zone (mm)	
	E. coli O157:H7	*L. monocytogenes*
0.5	13.0±0.49$^{c1)}$	28.0±0.41^{c}
0.7	13.6±1.04^{b}	27.9±1.53^{c}
1.0	15.8±0.97^{a}	29.8±1.50^{b}
1.2	16.4±2.06^{a}	32.5±0.77^{a}

[1)]Mean values with different letters within a column are significantly different by Duncan's multiple range test at $p<0.05$.

indicate that GSE had higher antimicrobial activity against Gram-positive than Gram-negative bacteria.

Based on the mechanical properties such as TS, E, and WVP of RA film containing GSE, the optimal GSE concentration for use in RA film wrapping for cheese and bacon was determined to be 1.0%, since it had the best TS, E, and WVP.

Microbiological analysis of cheese and bacon packed with RA film The populations of inoculated *E. coli* O157:H7 and *L. monocytogenes* in cheese and bacon wrapped with RA film containing 1.0% GSE were determined during storage at 4ºC (Table 3, 4). The initial populations of *E. coli* O157:H7 and *L. monocytogenes* inoculated on cheese were 5.7 and 6.1 log CFU/g, respectively, while the initial populations of *E. coli* O157:H7 and *L. monocytogenes* inoculated on bacon were 6.4 and 7.0 log CFU/g, respectively. The populations of *E. coli* O157:H7 and *L. monocytogenes* on the cheese and bacon decreased for the first 3 days after inoculation and then increased during 15 days of storage. Similarly, Corrals *et al*. (23) reported that the population of *B. thermosphacta* in pork loins packed with GSE enriched film decreased for the first 4 days of storage and then increased. The reduction of the bacterial populations might be occurred due to the high phenolic compound content of GSE. The phenolic compounds act on the surfaces of food product and inhibit bacterial growth by inhibiting the function of bacterial cell membranes (24). After 15 days of storage, the population of *E. coli* O157:H7 on the control cheese was 6.0 log CFU/g, while the population of the bacteria on the cheese wrapped with the film was 4.7 log CFU/g. For bacon, the population of *E. coli* O157:H7 on the control was 7.0 log CFU/g, whereas the population of the bacteria on the bacon wrapped with the film was 6.6 log CFU/g. Hong *et al*. (7) reported that the population of *E. coli* O157:H7 on pork loins packed with the GC-gelatin blend film containing 1% GSE decreased during storage at 4ºC, compared to the control.

In addition, after 15 days of storage, the population of *L. monocytogenes* on the control cheese was 6.7 log CFU/g, while cheese wrapped with the film had 5.9 log CFU/g. In the case of bacon, the population of *L. monocytogenes* on the control was 8.4 log CFU/g, whereas the samples wrapped with the film had 7.6 log CFU/g. According to Sivarooban *et al*. (25), GSE effectively reduced the population of *L. monocytogenes* on turkey frankfurters compared to the control. The results in the present investigation are similar to previous results for strawberries packed with rapeseed protein-gelatin film containing GSE, for which a reduction in the population of *L. monocytogenes* was observed during storage (22). Therefore, these results suggest that processed foods wrapped with RA film containing GSE may reduce microbial populations during

Table 3. Change in the populations of *E. coli* O157:H7 and *L. monocytogenes* in cheese during storage at 4°C (log CFU/g)

Microorganism	Type of packaging	Storage time (day)					
		0	3	6	9	12	15
E. coli O157:H7	Control	5.7±0.05$^{Ab1)}$	5.1±0.07^{Ac}	5.5±0.19^{Ab}	5.6±0.24^{Ab}	5.7±0.20^{Aab}	6.0±0.02^{Aa}
	GSE-RA film	5.7±0.05^{Aa}	3.9±0.17^{Be}	4.3±0.13^{Bd}	4.5±0.01^{Bc}	4.5±0.02^{Bc}	4.7±0.05^{Bb}
L. monocytogenes	Control	6.1±0.07^{Ac}	5.8±0.03^{Ad}	6.0±0.18^{Ac}	6.3±0.11^{Ab}	6.4±0.02^{Ab}	6.7±0.02^{Aa}
	GSE-RA film	6.1±0.07^{Aa}	5.1±0.17^{Ad}	5.3±0.17^{Bcd}	5.5±0.11^{Bbc}	5.7±0.13^{Bb}	5.9±0.05^{Ba}

[1)]Mean values with different letters within a column (A-B) and row (a-d) are significantly different by Duncan's multiple range test at $p<0.05$.

Table 4. Change in the populations of *E. coli* O157:H7 and *L. monocytogenes* in bacon during storage at 4°C (log CFU/g)

Microorganism	Type of packaging	Storage time (day)					
		0	3	6	9	12	15
E. coli O157:H7	Control	6.4±0.15$^{Acd1)}$	6.2±0.10^{Ae}	6.3±0.03^{Ade}	6.6±0.24^{Abc}	6.7±0.03^{Ab}	7.0±0.04^{Aa}
	GSE-RA film	6.4±0.15^{Ab}	5.8±0.11^{Bd}	6.0±0.02^{Bd}	5.2±0.11^{Bc}	6.4±0.07^{Bb}	6.6±0.05^{Aa}
L. monocytogenes	Control	7.0±0.06^{Ad}	7.0±0.01^{Ad}	7.7±0.01^{Ac}	7.9±0.07^{Ab}	8.0±0.04^{Ab}	8.4±0.07^{Aa}
	GSE-RA film	7.0±0.06^{Ad}	6.8±0.14^{Ae}	7.0±0.01^{Bd}	7.2±0.02^{Bc}	7.5±0.03^{Bb}	7.6±0.05^{Ba}

[1)]Mean values with different letters within a column (A-B) and row (a-e) are significantly different by Duncan's multiple range test at $p<0.05$.

Table 5. Change in peroxide value (PV) of bacon during storage at 4°C (meq peroxide/kg sample)

Type of packaging	Storage time (day)					
	0	3	6	9	12	15
Control	0.31±0.01$^{Af1)}$	0.82±0.01^{Abe}	1.02±0.02^{Ad}	1.93±0.01^{Ac}	2.88±0.02^{Ab}	3.76±0.02^{Aa}
GSE-RA film	0.31±0.01^{Af}	0.71±0.03^{Be}	0.87±0.02^{Bd}	1.31±0.03^{Bc}	1.96±0.03^{Bb}	2.43±0.03^{Ba}

[1)]Mean values with different letters within a column (A-B) and row (a-f) are significantly different by Duncan's multiple range test at $p<0.05$.

Table 6. Change in thiobarbituric acid (TBA) value of bacon during storage at 4°C (mg MDA/kg sample)

Type of packaging	Storage time (day)					
	0	3	6	9	12	15
Control	0.62±0.00$^{Af1)}$	1.01±0.01^{Ae}	1.56±0.01^{Ad}	1.89±0.01^{Ac}	2.01±0.01^{Ab}	2.21±0.02^{Aa}
GSE-RA film	0.62±0.00^{Af}	0.92±0.01^{Be}	1.18±0.01^{Bd}	1.35±0.01^{Bc}	1.53±0.03^{Bb}	1.74±0.02^{Ba}

[1)]Mean values with different letters within a column (A-B) and row (a-f) are significantly different by Duncan's multiple range test at $p<0.05$.

Table 7. Change in moisture contents of cheese and bacon during storage at 4°C (%)

Sample	Type of packaging	Storage time (day)					
		0	3	6	9	12	15
Cheese	Control	54.00±0.00$^{Aa1)}$	52.67±0.58^{Ab}	50.33±0.58^{Ac}	47.67±0.57^{Ad}	45.67±0.50^{Ae}	43.00±0.00^{Bf}
	GSE-RA film	54.00±0.00^{Aa}	53.33±0.58^{Aa}	51.00±0.00^{Ab}	48.67±0.58^{Ac}	46.00±0.00^{Ad}	44.33±0.57^{Ae}
Bacon	Control	73.67±1.15^{Aa}	70.67±1.15^{Ab}	68.33±0.58^{Ac}	66.67 ±1.53^{Acd}	65.00±1.00^{Bde}	64.33±1.15^{Ae}
	GSE-RA film	73.67±1.15^{Aa}	72.33±0.58^{Aa}	70.67±1.53^{Ab}	68.67±0.58^{Ac}	67.33±0.58^{Ac}	65.50±0.50^{Ad}

[1)]Mean values with different letters within a column (A-B) and row (a-e) are significantly different by Duncan's multiple range test at $p<0.05$.

storage. However, it should be noted that the degree of microbial reduction is not great than expected, most probably due to the limited antimicrobial activity of the film.

Lipid oxidation in bacon during storage PV and TBARS value represent the degree of lipid oxidation in foods. The PV and TBA values of bacon samples increased during storage (Table 5, 6). After 15 days of storage, the PV values of bacon wrapped with the control treatment and the GSE-RA film were 3.76 and 2.43 meq peroxide/kg, respectively. Bacon wrapped with the RA film containing GSE had lower PV and TBA values during storage than the control. After 15 days of storage, the TBA values of bacon wrapped with RA film decreased by 0.47 mg MDA/kg sample compared to the control. The results in the present investigation are similar with those of Gómez-Estaca *et al*. (26), who found that the PV and TBA values of salmon samples packed with gelatin film containing oregano and rosemary extract were lower than those of the control during storage. Hong *et al*. (7) reported that the TBA value of pork loin samples packed with the *Gelidium corneum*-gelatin blend film containing GSE was lower than that of the control during storage. Ha *et al*. (27) also reported that the TBA value of ground beef packed with LDPE containing 0.5 and 1.0% GSE increased more slowly during storage than those packed according to the control treatment. In the present investigation, the results clearly indicate that lipid oxidation in bacon and cheese during storage is delayed by wrapping them with RA film containing GSE.

Analysis of moisture content and sensory evaluation The moisture content of cheese and bacon wrapped with RA film containing GSE during storage is shown in Table 7. The moisture content of the cheese and bacon samples wrapped with the film decreased less than those of the control during storage at 95% confidence level. Similarly, according to of Fajardo *et al*. (28), the moisture content of the cheese coated with chitosan based edible film decreased less during storage compared to the control sample.

Sensory evaluations such as color, odor, firmness, and overall acceptability of cheese were examined on a 9-point hedonic scale. The sensory scores of cheese decreased with increasing storage time for all treatments (Table 8). However, cheese samples wrapped with the RA film containing GSE had higher scores than the control, suggesting that the film may improve the quality of cheese products during storage. Similarly, Lim *et al*. (30) and Jang *et al*. (22) reported that strawberries wrapped with edible film containing GSE had higher scores than those of controls.

In summary, RA films containing 1% GSE may be applied to the wrapping of small quantity of cheese and

Table 8. Sensory evaluation of cheese during storage at 4°C

Organoleptic parameter	Treatment	Storage time (day)					
		0	3	6	9	12	15
Color	Control	9.00±0.00$^{Aa1)}$	8.40±0.55^{Aa}	7.40±0.55^{Ab}	6.81±0.45^{Ab}	5.40±0.55^{Ac}	5.60±0.54^{Ad}
	GSE-RA film	9.00±0.00^{Aa}	8.70±0.45^{Aa}	7.60±0.54^{Ab}	7.20±0.45^{Ab}	6.00±0.71^{Ac}	5.20±0.45^{Ad}
Odor	Control	9.00±0.00^{Aa}	8.60±0.55^{Aab}	8.20±0.45^{Ab}	6.42±0.54^{Bc}	5.63±0.55^{Bd}	4.60±0.54^{Be}
	GSE-RA film	9.00±0.00^{Aa}	8.81±0.45^{Aab}	8.40±0.55^{Ab}	7.00±0.00^{Ac}	6.60±0.54^{Ac}	5.60±0.55^{Ad}
Firmness	Control	9.00±0.00^{Aa}	9.00±0.00^{Aa}	8.21±0.44^{Ab}	6.80±0.45^{Ac}	6.00±0.71^{Bd}	5.19±0.84^{Be}
	GSE-RA film	9.00±0.00^{Aa}	9.00±0.00^{Aa}	8.39±0.55^{Ab}	7.40±0.55^{Ac}	7.20±0.44^{Ac}	6.20±0.44^{Ad}
Overall	Control	9.00±0.00^{Aa}	8.20±0.44^{Ab}	7.40±0.534^{Ac}	6.40±0.55^{Bd}	5.40±0.55^{Bd}	4.00±0.00^{Bd}
	GSE-RA film	9.00±0.00^{Aa}	8.81±0.45^{Aab}	7.80±0.44^{Ab}	7.00±0.00^{Ac}	6.41±0.54^{Ad}	5.38±0.54^{Ae}

[1)]Mean values with different letters within a column (A-B) and row (a-e) are significantly different by Duncan's multiple range test at $p<0.05$.

bacon product to prevent lipid oxidation and moisture loss.

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