EFFECT OF *PERILLA FRUTESCENS* VAR. *ACUTA* WATER EXTRACT ON THE SHELF LIFE OF COOKED BEEF PATTIES

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Abstract – This study investigated the effects of *Perilla frutescens* var. *acuta* water extract (WEP) on the shelf life of cooked beef patties. Three treatment groups were used: (1) beef patties without added antioxidant (control); (2) beef patties with 0.02% L-ascorbic acid (BAA); and (3) beef patties with 0.6% WEP (BWEP). The WEP contained phenolic compounds (80.65 mg gallic acid equivalents/g) and had half-maximal effective concentrations of 0.437 and 4.509 mg/mL for scavenging of DPPH and $ABTS^+$ radicals, respectively. Treatment with 0.6% WEP inhibited the growth of Escherichia coli O157:H7. When cooked beef patties were stored for 21 days at 4°C, the total number of aerobic bacteria in the BWEP group was lower than those in the control on all days except day 14 ($p < 0.05$). The thiobarbituric acid reactive substance values in the BWEP group were lower than those of controls on days 7, 14, and 21 ($p < 0.05$). We concluded that WEP can be used as a natural ingredient that improves the shelf life of meat products.

Key Words – *Perilla frutescens* var. *acuta*, beef patty, lipid oxidation, shelf life.

I. INTRODUCTION

The quality of processed meat product is deteriorated indubitably because they may be exposed to high temperatures and cross-contamination during production, which results in poor shelf life. A major contributor to the deterioration in the quality of processed meat products subjected to heat and storage is lipid oxidation. Lipid oxidation can be generally controlled with synthetic antioxidants which scavenge free radicals and chelating metal ions. However, the use of synthetic antioxidants in food is limited by safety concerns such as teratogenic and carcinogenic effects that have occurred in animal tests [1]. Therefore, alternative antioxidants in natural ingredients that can control lipid oxidation in processed meat products have been sought.

P. frutescens var. *acuta* is a naturalized edible plant and its extract contains various functional compounds with anti-inflammatory, antimicrobial, antioxidant activities [2, 3]. Therefore, this extract may control lipid oxidation and inhibit bacterial proliferation in processed meat products. However, few studies have been conducted to determine the effects of *P. frutescens var. acuta* extract on the quality of these products. Therefore, the objective of this study was to investigate the antioxidant and antibacterial activities of *P. frutescens var. acuta* extract and evaluate the effect of this extract on the shelf life of beef patties during refrigerated storage.

II. MATERIALS AND METHODS

Extraction of *Perilla frutescens var. acuta*

Dried mixture (50 g) of leaves and stems of *Perilla frutescens var. acuta* was added to 1.95 L of distilled water, then it was extracted for 24 h in a water bath at 70°C. Extract was centrifuged and the supernatant was filtered. The filtrate was lyophilized and stored until use in a -70°C deep freezer.

Antioxidative and antimicrobial potential of WEP

Total phenolic content of WEP was estimated by the Folin-Ciocalteu method [4]. The quantification of phenolics was based on the standard curve generated with the use of gallic acid, and expressed as gallic acid equivalent.

DPPH radical scavenging activity and ABTS+ reducing activity of WEP were determined as the method described by [5, 6], respectively. The percentage of DPPH radical scavenging was obtained from the following equation: Radical scavenging activity = [1 − (absorbance of sample / absorbance of control)] × 100. The percentage inhibition was calculated by the following

equation: ABTS+ reducing activity (%) = [(absorbance of control – absorbance of sample) / absorbance of control] × 100. The half maximal effective concentration (EC50) of WEP for DPPH radical scavenging and ABTS+ reducing activity was calculated by interpolation from the data and compared with that of L-ascorbic acid as a positive control.

The antimicrobial potential of WEP was measured on Escherichia coli O157:H7. E.O157:H7 was cultivated at 37°C for 18 h in a tryptic soy broth. 10 mL cultures of strain was transferred to tube and vortexed for 10 sec. The tube containing strain was centrifuged. The pellet was washed with sterile saline (0.85%), and suspended in saline to a final concentration of approximately 109 CFU/ml of the stock inoculum. The WEP solution with distilled water was added to tryptic soy broth and then inoculated with 1% culture suspension of E. O157:H7. The inoculated sample was incubated at 37°C for 48 hours, and smeared on tryptic soy agar. The plates were incubated at 37°C for 48 hours and the result was expressed as log of colony forming units (Log CFU)/g.

Preparation of beef patties

Meat batter for manufacture of beef patty was made by mixing ground bottom round of beef with ingredients (Table 1). The meat batter put in container and stored at 4°C for 24 h. After storage, 25 g of meat batter was modeled in a shape with diameter of 5.0 cm and thickness of 1 cm and the patties were cooked for 20 min at 180°C until they reached an internal temperature of 75°C. The patties were vacuum-packaged in a vacuum bags using a vacuum-packaging machine after cooling for 30min at room temperature and stored for 21 days at 4°C.

Microbiological analysis

Beef patties (10 g) were blended with sterile saline (90 mL) for 2 min by using a stomacher. A series of decimal dilutions was prepared using sterile saline. Each diluent (0.1 mL) was spread in triplicate on tryptic soy agar plates. The plates were incubated at 37°C for 48 h, and the microbial counts were expressed as Log CFU/g.

Lipid oxidation

The 2-thiobarbituric acid reactive substances (TBARS) of patties was measured with a method of

Table 1. Formulation of beef patties

	Treatment		
Ingredients	Control	l-ascorbic acid	WEP
Ground beef	75	75	75
Bread powder	5	5	5
Onion	5	5	5
Garlic	2	2	2
Egg-white powder	2	2	2
Salt	1	1	1
Water	10	10	10
Total	100	100	100
l-ascorbic acid		0.02	
WEP[1]			0.6

[1]Water extract of *Perilla frutescens* var. *acuta*

[7]. TBARS value was reported as mg malondialdehyde/kg meat.

Statistical analysis

All experiments in this study were performed five individual trials. The general linear model was performed using the raw data, and Tukey's multiple range test was used to compare significant differences between least square mean values ($p<0.05$). Least square mean values and standard error of the least square means (SEM) are reported. SAS software was used for all statistical analyses.

III. RESULTS AND DISCUSSION

Antioxidative and antimicrobial potential of WEP

The total phenolic content of WEP through five extractions was 80.65 ± 1.24 mg gallic acid equivalents/g (Table 2).

EC50 of WEP for scavenging DPPH and ABTS radicals were compared with those of L-ascorbic acid. Compared with those of WEP, the EC50 values of L-ascorbic acid for DPPH and ABTS radicals were approximately 12 times and 14 times higher, respectively. Therefore, we used 0.3% and 0.6% WEP as candidate for the BWEP group with the expectation of radical scavenging activity similar to that of L-ascorbic acid and two times higher than that of L-ascorbic acid.

The 0.3% and 0.6% WEP solution were used to measure the antimicrobial potential of WEP on E. coli O157:H7, a pathogenic microorganism. Significant difference was found only between the control and the 0.6% WEP treatment groups (data not shown). Given this result, we added 0.6% WEP to beef patties for our experiments.

Table 2. Total phenolic content (mg GAE[1]/g) and EC_{50}[2] value (mg/mL) for scavenging of DPPH and ABTS radical of water extract of *Perilla frutescens* var. *acuta* (WEP)

	Total phenolic content	EC_{50} value of scavenging	
		DPPH radical	ABTS radical
WEP	80.65 ± 1.24[3]	0.437[a]	4.509[a]
l-ascorbic acid	-	0.038[b]	0.326[b]
SEM[4]	-	4.1241	37.3739

[1]Gallic acid equivalent
[2]Half maximal effective concentration
[3]Standard deviation
[4]Standard errors of the least square means (n=10)
[a,b]Values with different letters within the same column differ significantly ($p < 0.05$).

Total aerobic bacteria

The number of total aerobic bacteria in the beef patties was monitored during refrigerated storage at 4°C for 21 days (Table 3). On day 0, the numbers of total aerobic bacteria in the control, BAA, and BWEP groups were 3.74, 3.33, and 2.85 Log CFU/g, respectively, with significant differences. Aerobic bacteria proliferated with the increase in storage days in all beef patties. The addition of WEP inhibited the proliferation of aerobic bacteria with significance compared with the control throughout the storage period ($p < 0.05$), with the exception of day 14. A previous study reported that the addition of plant extracts containing phenolic compounds retarded increases in aerobic bacteria in pork patties during storage [8]. The addition of WEP might improve the shelf life of beef patties. However, further studies are needed to elucidate the antimicrobial activity of WEP in beef patties given the inconsistent result on day 14.

Lipid oxidation

The TBARS values of beef patties are shown in Table 6. The initial TBARS values of the control, BAA, and BWEP groups were not significantly different. Compared to those of the control, the TBARS values of the BAA and BWEP groups were significantly lower on days 7, 14, and 21 ($p < 0.05$). The TBARS values of the BAA and BWEP groups did not changed with the increase in storage days, whereas that of the control increased after 7 days of storage. Previous studies have shown that the addition of plant extracts containing phenolic compounds inhibited lipid oxidation in beef patties during storage [9, 10]. However, no significant difference in TBARS value was found between the BAA and BWEP groups despite the expectation of lower lipid oxidation in the BWEP group was lower than did BAA because 0.6% WEP might have higher radical scavenging activity than that of 0.02% ascorbic acid based on these EC_{50} for DPPH and ABTS radical. Although reasons were unclear, the inconsistency result between radical scavenging activities of plant extracts and the inhibition effect of lipid oxidation in pork patty by adding plant extracts was found in the study from [8].

Table 3. Total aerobic bacterial number (Log CFU/g) of beef patty added with water extract of *Perilla frutescens* var. *acuta* (WEP) during storage for 21 days at 4°C

	Storage days				
Trt.	0	7	14	21	SEM[4]
Control	3.74[aY]	4.06[aY]	5.02[X]	4.89[aX]	0.171
BAA[1]	3.33[bY]	3.36[bY]	4.89[X]	5.31[aX]	0.125
BWEP[2]	2.85[cZ]	2.95[bZ]	4.93[X]	4.03[bY]	0.087
SEM[3]	0.067	0.140	0.074	0.201	

[1]BAA: beef patty added with 0.02% l-ascorbic acid
[2]BWEP: Beef patty added with 0.6% water extract of *Perilla frutescens* var. *acuta*
[3]Standard errors of the least square means (n=15), [4](n=20)
[a-c]Values with different letters within the same column differ significantly ($p < 0.05$).
[X-Z]Values with different letters within the same row differ significantly ($p < 0.05$).

Table 4. TBARS values of beef patty added with water extract of *Perilla frutescens* var. *acuta* (WEP) during storage for 21 days at 4°C

	Storage days				
Trt.	0	7	14	21	SEM[4]
Control	1.25[Y]	1.51[aX]	1.45[aX]	1.45[aX]	0.041
BAA[1]	1.16	1.29[b]	1.22[b]	1.18[b]	0.038

BWEP[2]	1.19	1.27[b]	1.23[b]	1.21[b]	0.025
SEM[3]	0.045	0.042	0.024	0.023	

[a,b]Values with different letters within the same column differ significantly ($p < 0.05$).
[X,Y]Values with different letters within the same row differ significantly ($p < 0.05$).

IV. CONCLUSION

The WEP produced in this study contained levels of phenolic compounds as high as 80.65 ± 1.24 mg gallic acid equivalents/g and showed antioxidative potential on DPPH and ABTS radicals and antimicrobial potential on E. coli O157:H7. The addition of WEP to beef patties inhibited lipid oxidation and the growth of aerobic bacteria in beef patties during storage for 21 days at 4°C. Therefore, we concluded that WEP can be used as a natural additive to improve the shelf life of meat products

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