

Effect of Ozone and Potassium Lactate on Color Stability and Survival of *Listeria Monocytogenes* on Fresh Pork

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Abstract—This research evaluated the effect of ozone gas and potassium lactate on color stability and survival of *Listeria monocytogenes*. Samples were treated with three levels of potassium lactate (KL) inoculated with *L. monocytogenes* and then packed in airtight polyethylene bags with and without ozone. Ozone gas was introduced into the plastic bags at four levels of injection and then the samples were chilled. Color and microbial loads were determined every five days. Ozone and KL treatment had no effect on L-value but increase a-value, *L. monocytogenes* were sensitive to high concentrations of KL and ozone. Ozone and potassium lactate were shown to inhibit *L. monocytogenes* on fresh pork and improved color stabilization.

Keywords—fresh pork; ozone; potassium lactate; *Listeria monocytogenes*

I. INTRODUCTION

Pork is a source of quality protein for humans. Two important factors affecting consumer perceptions and purchasing are freshness and color. Still there are often reports of pork contaminated with pathogen microorganisms such as *Salmonella sp.*, *Listeria monocytogenes* and *Escherichia coli* [4, 12, 19]. *L. monocytogenes* is potential threat to the meat processing industry and the consumers [10, 17]. In an attempt to eliminate the pathogens from carcasses, various applications like the application of potassium lactates are widely used to extend shelf life and increase the safety of meat and poultry products [1, 9, 11]. The United States Department of Agriculture (USDA) approved the use of potassium salts (up to 4.8% w/w) in meat products as an antimicrobial ingredient to control pathogens [16]. Ozone is considered an antimicrobial substance due to its potential oxidizing capacity and it was granted the Generally Recognized As Safe (GRAS) status [18]. Ozone use has many advantages in the food industry [21]. The aim of this research was to study the effect of ozone gas and potassium lactate on color stability and survival of *L. monocytogenes* on fresh pork.

II. MATERIALS AND METHODS

A. Materials

Raw pork samples were purchased from a local meat shop in Maha sarakham, Thailand. Ozone gas was generated

by a laboratory corona discharge ozone generator (HGOZ-1000, Enaly M&E, China) with a working voltage of 220 volt, 50 Hz using oxygen gas as a substrate. *Listeria monocytogenes* were obtained from a stock culture of the Foodborne Pathogen Research Laboratory (FBRL), Maha sarakham University, Thailand.

B. Bacterial strain

L. monocytogenes was activated in tryptic soy broth (Criterion, Santa Maria, USA) containing 0.6% yeast extract and grown in tryptic soy agar (Criterion, Santa Maria, USA) at 30°C for 24h. [13]. Cell suspension was prepared by adding 0.1% sterile peptone water onto culture plates. This suspension was further diluted with 0.1% sterile peptone water to obtain a cell density of 9 log CFU/ml.

C. Inoculation and treatment

Sample of fresh pork were cut into pieces approximately (8×5×0.6) cm in size, then (0, 2 and 4%) potassium lactate (KL), (Fluka, Netherlands) inoculated with 0.5 ml *S. typhimurium* suspension was applied to each side. The samples were then packed in a customized airtight polyethylene plastic bags sizing 6×8 Inc with a thickness of 87.5 µm. Ozone gas was introduced into the plastic bags at a rate of 0, 200, 500 and 1,000 mg/h. All samples were stored at 8°C on 15 days. Color analysis (Hunter Lab) and survival of *L. monocytogenes* was determined every 5 days.

D. Instrument color and survival study

On days 0, 5, 10 and 15, CIE L, a, and b value measurements of the samples were made with a chroma meter Hunter Lab (CR-300, Dp-301 Data processor, Konica Minolta, Canada). Survival of *L. monocytogenes* was determined applied by [3].

E. Statistics

A two way analysis of variance (ANOVA) and Sheffe's multiple range tests were performed to test the statistical differences in the color value and numerical value of *L. monocytogenes* between treatments using SPSS software (version 12). The significance level was p<0.01.

III. RESULTS AND DISCUSSION

A. Instrument color

Lightness (L-value) for all treatment were not different ($p>0.01$) on days of storage similar [15]. The a-value was not different ($p>0.01$) on day 0 but increased had significantly ($p<0.01$) on day 5 in samples used 200mg/h ozone with 4%KL and samples used 1,000 mg/h without KL had the same resulted on days 10-15. Redness (a-value) results on samples that used ozone may have been due to oxygen from ozone degradation stimulated oxymyoglobin [8] and similar

reported by [15]. The effects of 4% lactate with 200mg/h ozone on pork-exhibited redness. Observation indicated that lactate improved surface redness of samples and these observations are in agreement with [6, 7, 14], who reported that the color stabilizing activity of lactate is modulated through a lactate-induced increase in lactate dehydrogenase activity and metmyoglobin reducing activity. Yellowness (b-value) were not significantly ($p>0.01$) in all treatments on each day of storage but showed an increase on day 5 ($p<0.01$) followed by decrease on day10 and 15, similar reported by [15].

TABLE I. L-VALUE OF FRESH PORK AT 8°C 15 DAYS

Treatment		Days of storage			
		0 days	5 days	10 days	15 days
Ozone (mg/h)	KL (%)	L- value			
Control (0)	(0)	42.18±3.06 ^{nsA}	46.05±3.23 ^{nsA}	41.73±0.79 ^{nsA}	29.99±1.54 ^{nsB}
0	2	41.98±0.79 ^A	45.37±1.77 ^A	41.80±2.26 ^A	42.40±3.10 ^A
0	4	41.47±0.43 ^A	45.84±2.39 ^A	42.48±1.11 ^A	40.76±2.29 ^A
200	0	41.21±1.29 ^A	44.28±0.57 ^A	40.00±1.61 ^A	42.02±3.28 ^A
200	2	41.13±1.64 ^A	46.70±1.16 ^A	40.60±1.79 ^A	37.59±5.24 ^A
200	4	41.94±1.75 ^A	41.15±1.64 ^A	37.66±4.02 ^A	38.24±2.12 ^A
500	0	41.00±3.85 ^A	45.97±0.27 ^A	41.45±1.64 ^A	38.87±3.12 ^A
500	2	43.11±1.26 ^A	47.62±2.33 ^A	41.81±2.38 ^A	39.99±1.87 ^A
500	4	44.27±1.00 ^A	42.90±2.29 ^A	36.13±3.80 ^A	36.49±3.11 ^A
1,000	0	41.12±1.88 ^A	44.27±2.36 ^A	37.42±4.27 ^A	36.85±3.79 ^A
1,000	2	44.04±1.68 ^{AB}	45.97±0.15 ^A	37.31±2.91 ^B	37.99±1.54 ^B
1,000	4	40.86±0.97 ^{AB}	44.92±0.65 ^A	39.77±1.17 ^B	41.64±1.55 ^{AB}

^{ns} Mean the same column are not significant different at ($P > 0.01$).

^{AB} Means within different letters in a rows present significant different at ($P < 0.01$)

TABLE II. A-VALUE OF FRESH PORK AT 8°C 15 DAYS

Treatment		Days of storage			
		0 days	5 days	10 days	15 days
Ozone (mg/h)	KL (%)	a- value			
Control (0)	(0)	12.29±2.15 ^{nsA}	11.04±1.66 ^{dA}	12.26±1.22 ^{bA}	12.23±1.48 ^{nsA}
0	2	13.26±1.17 ^A	14.18±0.43 ^{cdA}	13.90±1.11 ^{abA}	12.86±0.99 ^A
0	4	13.32±1.73 ^A	13.63±2.61 ^{cdA}	13.93±3.47 ^{abA}	13.58±1.19 ^A
200	0	13.22±0.57 ^A	15.53±0.72 ^{bcdA}	14.60±1.26 ^{abA}	13.87±1.74 ^A
200	2	13.15±0.64 ^A	16.14±1.28 ^{bcdA}	15.37±1.72 ^{abA}	14.28±1.46 ^A
200	4	12.99±1.41 ^A	21.92±1.52 ^{abB}	19.33±0.99 ^{abB}	17.61±0.26 ^B
500	0	13.85±0.25 ^A	15.90±1.08 ^{bcdA}	14.53±1.26 ^{abA}	14.56±0.95 ^A
500	2	12.63±0.71 ^A	14.42±0.40 ^{cdA}	15.12±1.47 ^{abA}	14.23±0.89 ^A
500	4	11.87±2.45 ^A	18.12±0.57 ^{abcB}	17.74±0.88 ^{abB}	15.59±1.37 ^{AB}
1,000	0	14.28±0.30 ^A	20.21±0.70 ^{abB}	20.38±1.32 ^{abB}	16.81±1.42 ^{AB}

1,000	2	12.91±1.52 ^A	18.22±0.29 ^{abcA}	14.51±1.01 ^{abA}	14.70±3.21 ^A
1,000	4	12.72±0.83 ^A	15.69±0.83 ^{bcdA}	15.24±0.86 ^{abA}	13.44±0.68 ^A

^{a, b, c...d} Values with different letters in a column represent significant difference (P < 0.01).

^{AB} Means within different letters in a rows present significant different at (P < 0.01), ns Mean the same column are not significant different (p>0.01).

TABLE III. B- VALUE OF FRESH PORK AT 8°C 15 DAYS

Treatment		Days of storage			
		0 days	5 days	10 days	15 days
Ozone (mg/h)	KL (%)	b- value			
Control(0)	(0)	3.09±0.55 ^{nsA}	10.11±0.20 ^{nsC}	4.79±0.39 ^{nsB}	3.10±0.12 ^{nsA}
0	2	2.68±0.30 ^A	9.84±0.74 ^B	3.84±0.65 ^A	3.33±0.84 ^A
0	4	2.13±0.31 ^A	9.28±0.23 ^B	3.46±0.49 ^A	2.82±0.68 ^A
200	0	3.75±0.82 ^A	10.96±0.71 ^B	4.07±0.93 ^A	3.43±0.45 ^A
200	2	2.40±0.58 ^A	10.67±1.10 ^B	4.14±0.50 ^A	3.87±0.80 ^A
200	4	2.87±0.36 ^A	13.10±0.68 ^C	6.52±0.49 ^B	4.85±0.45 ^{AB}
500	0	3.47±1.13 ^A	11.59±0.50 ^B	5.12±0.19 ^A	3.53±1.04 ^A
500	2	3.52±0.25 ^A	11.39±0.89 ^B	5.43±0.44 ^A	3.86±0.29 ^A
500	4	3.05±0.97 ^A	11.76±1.59 ^B	5.42±0.61 ^A	3.68±1.13 ^A
1,000	0	3.73±0.82 ^A	13.08±1.64 ^B	6.57±1.47 ^A	4.43±0.52 ^A
1,000	2	3.44±0.65 ^A	10.88±1.63 ^B	4.38±0.40 ^A	3.03±0.22 ^A
1,000	4	3.02±0.35 ^A	10.90±1.17 ^B	4.64±1.15 ^A	3.04±0.44 ^A

^{AB} Means within different letters in a rows present significant different at (P < 0.01).

^{ns} Mean the same column are not significant different (p>0.01)

B. Survival study

L. monocytogenes had 8.6 log CFU/g on day 0 and were not significantly (p>0.01) in all treatments. The *L. monocytogenes* level did however decrease significantly (p<0.01) on day 5 in samples used 4% KL with and without ozone and 1,000 mg/h ozone without KL. On days 10 and 15

the results were similar to those on day 5. The 4% KL inhibited *L. monocytogenes* due to the content of lactate ions described by [20]. However, results indicated a small decrease in overall survival similar reported by [2]. This may have been because the performance of ozone can decrease when used in foods that contain fat and protein [5].

TABLE IV. SURVIVAL OF *L. MONOCYTOGENES* ON FRESH PORK AT 8°C 15 DAYS

Treatment		Days of storage			
		0 days	5 days	10 days	15 days
Ozone(mg/h)	KL (%)	log CFU/g			
Control (0)	(0)	8.6±0.03 ^{nsA}	8.8±0.04 ^{bb}	9.0±0.06 ^{bc}	9.1±0.02 ^{bcC}
0	2	8.6±0.07 ^A	8.7±0.08 ^{ba}	9.0±0.02 ^{bb}	9.0±0.03 ^{bcB}
0	4	8.5±0.04 ^C	8.0±0.05 ^{aa}	8.2±0.03 ^{aaB}	8.2±0.02 ^{ab}
200	0	8.6±0.01 ^A	8.7±0.07 ^{bb}	9.0±0.02 ^{bc}	9.1±0.01 ^{cC}
200	2	8.6±0.05 ^A	8.7±0.02 ^{bb}	8.9±0.04 ^{bc}	9.1±0.02 ^{bcD}
200	4	8.5±0.07 ^C	8.1±0.01 ^{aa}	8.2±0.03 ^{aaB}	8.3±0.01 ^{ab}
500	0	8.5±0.07 ^A	8.7±0.02 ^{bb}	8.9±0.05 ^{bbc}	9.0±0.04 ^{bcC}
500	2	8.5±0.07 ^A	8.6±0.08 ^{ba}	8.9±0.04 ^{bb}	9.0±0.02 ^{bb}
500	4	8.6±0.01 ^C	8.1±0.02 ^{aa}	8.2±0.03 ^{aa}	8.3±0.02 ^{ab}

1,000	0	8.5±0.08 ^B	8.1±0.04 ^{aA}	8.1±0.01 ^{aA}	8.3±0.01 ^{aA}
1,000	2	8.5±0.07 ^C	8.1±0.01 ^{aA}	8.1±0.01 ^{aAB}	8.3±0.03 ^{aB}
1,000	4	8.6±0.04 ^C	8.0±0.07 ^{aA}	8.1±0.01 ^{aB}	8.2±0.03 ^{aB}

^{a, b, c, d} Values with different letters in a column represent significant difference ($P < 0.01$).

^{ABC} Means within different letters in a rows present significant different at ($P < 0.01$), ns Mean the same column are not significant different ($p > 0.01$)

IV. CONCLUSION

This research study found that ozone and potassium lactate could act to inhibit *L. monocytogenes* in fresh pork and improve color stabilization and may provide a viable alternative to contemporary food preservation technologies and improve the overall quality and safety of current meat preservation practices.

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