The Combination Effect of Rosemary Extract, Organic Acid Salts and Modified Atmosphere Packaging on the Storage Quality of Ready-to-Eat Hamburger Steak During Refrigerated Storage

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Abstract- **This research was conducted to evaluate the combination effect of MAP and the addition of both rosemary extract and organic acids to the quality of Ready-to-Eat Hamburger steak during refrigerated storage. Two groups of Hamburger steak were prepared those are control and treatment groups. The Hamburger steaks of treatment group were added with rosemary extract (500 ppm), sodium acetate (3000 ppm) and calcium lactate (1500 ppm). Two MAP were used in this study, those are 0%O_2:30%CO_2:70%N_2 (30% CO_2-MAP) and 0%O_2:0%CO_2:100%N_2 (100% N_2-MAP). The Hamburger steaks were stored at 5 ^oC for 14 d. The pH value of treated group was lower ($P<0.05$) compared to control group. The addition of rosemary and organic acid salts reduced ($P<0.05$) the aerobic and anaerobic bacterial counts both in 30% CO_2-MAP and 100% N_2-MAP. The 30% CO_2-MAP also showed more detaining ability to microbial growth compared to 100% N_2-MAP, more over 30% CO_2-MAP in combination with additives resulted lowest bacterial counts during storage. The treated Hamburger steaks were darker (lower CIE L*), lower in redness (CIE a*) and higher in yellowness (CIE b*), and in contrast control Hamburger steak packed with 30% CO_2-MAP maintained highest redness during storage. The 30% CO_2-MAP decreased ($P<0.05$) lipid oxidation from 8 to 14 d of storage regardless the addition of additives. The use of 30% CO_2-MAP in combination with rosemary and organic acids can maintain and prolonged the self-life of Ready-to-Eat Hamburger steak during refrigerated storage.**

Keywords-**Ready-to-Eat Hamburger Steak, MAP, Antioxidants**

I. INTRODUCTION

Hamburger is a molded mixture of ground lean and fatty beef, prepared with or without added salt and seasonings [1]. As the changing of life style (convenient foods), ready-to-eat (RTE) products have become popular meat products. It is necessary to find the suitable packaging methods to maintain the shelf-life and to prevent the quality deterioration of RTE Hamburger steak.

Modified atmosphere packaging (MAP) has been used by meat industry to extend the shelf-life as well as to keep the quality of meat products. CO_2 is utilized in MAP gas composition because of its antibacterial effects. [2] suggested that 20-60% of in MAP is required to detain the growth of bacteria.

Organic acid salts used in meat as an antioxidant, includes calcium lactate and sodium acetate. Calcium lactate is generally recognized as the safe food ingredient, and is commonly used in meat industry, as an antibacterial agent [3]. Sodium acetate combined with potassium lactate showed a synergistic effect on improving color stability in pork [4]. Rosemary has been used by the researcher as well as the meat industry due to its antioxidant activity in meat products. The antioxidant activity of rosemary attributed to their carnosic acid, carnosol and rosmarinic acid component [5]. Previous studies reported the antioxidant ability of rosemary in pork patties [6], and cooked pork patties [7].

This study was conducted to evaluate the combination effect of MAP and the addition of both rosemary extract and organic acids to the quality of Ready-to-Eat Hamburger steak during refrigerated storage.

II. MATERIALS AND METHODS

A. Sample preparation

The Hamburger steak was prepared by mixing 34.49% of beef, 37.53% of pork foreleg, 6.49% of beef fat, and other ingredients. The ingredients were mixed well

using a mixer for 10 min. A hundred grams of mix materials were molded in the size of 10 cm dia. and 1 cm thickness using a hand molder. For treatment, Hamburger steak was added with 500 ppm of rosemary extract, 3000 ppm of sodium acetate and 1500 ppm of calcium lactate. The Hamburger steak were heated at 115 C for 7 min on a room heater (FM 2011 E3, Forno Misto, Italy) followed by cooling in chilled room at 7 C for 10 min. The Hamburger steaks were packed with two different gas composition, those are 0% O_2: 30% CO_2: 70% N_2 (30%CO_2-MAP), and 0% O_2: 0% CO_2: 100% N_2 (100% N_2-MAP).

B. The pH measurement

Briefly, 10 g of sample was added with 100 mL distilled water and then homogenized at 10,000 rpm for 60 sec using a homogenizer (PH91, SMT Co. Ltd., Japan), and the pH was measured using a pH meter (SevenEasy pH, Mettler-Toledo GmbH, Switzerland).

C. Thiobarbituric acid reactive substance (TBARS) value analysis

The TBARS value was measured according to [8]. Briefly, 0.5 g sample was mixed with 3 drops of antioxidant solution, 3 mL of TBA solution, and 17 mL of 25% Trichloroacetic acid. The mixture was heated at 100^{o}C for 30 min, and centrifuged at 3,500 rpm for 30 min. An absorbance of supernatant was measured at 532 nm using a spectrophotometer (UV-mini-1240, Shimadzu, Japan). The results were calculated as mg malonaldehyde (MA) per kg sample.

D. Instrumental color measurement

CIE Lightness (L*), redness (a*) and yellowness (b*) was measured using a color difference meter (CR-400, Konica Minolta Sensing Inc., Japan) and an illuminant C. The color instrument was calibrated using white plate (Illuminant C: Y=93.6, x=0.3134, and y=0.3194).

E. Microbial analysis

The plate count agars (Difco, USA) were used for aerobic and anaerobic bacterial counts. Agar plates were prepared according to the manufacturer's instruction. Samples were incubated for 48 h at 35^{o}C. Microbial populations were counted in 30-300 colonies and expressed as Log CFU/ gram of sample.

F. Statistical analysis

All data were analyzed using SPSS 14.0 for Windows Evaluation Version (2005).

III. RESULTS AND DISCUSSION

A. The pH value

The pH value (Table 1) of treated Hamburger steak were lower ($P<0.05$) compared to control Hamburger steak regardless the MAP gas composition from 0 to 4 d of storage, and it might be related with the addition of organic acid salts, and the pH lowering ability of it only can be found at the early storage time. For instance, [9] reported the decreased pH level in pork by increasing the concentration of calcium lactate. In addition [10] noted a lower pH value in cooked beef added with rosemary extract packed with 30% CO_2-MAP.

B. TBARS value

The addition of rosemary extract and organic acid salts lowered the lipid oxidation only on 2 d of storage ($P<0.05$) (Table 2). The 30% CO_2-MAP detained the lipid oxidation both in control and treatments group. In control Hamburger steak, 30% CO_2-MAP lowered the TBARS value on 8 to 12 d of storage, and 8 to 10 d in treated Hamburger steak. The effects of packaging methods (MAP) was more superior compared to rosemary and organic acids salts addition in detain the lipid oxidation. The lower TBARS value in 30% CO_2-MAP may be attributed to the lower bacterial counts in 30% CO_2-MAP. [11] mentioned some factors affecting the lipid oxidation are the presence of pro- and antioxidant, and the presence of enzymes (microbiology).

C. Instrumental Color

In the control Hamburger steak, 100% N_2-MAP tended to be a lighter than 30% CO_2-MAP (Table 4). In general, addition of rosemary extract and organic acid salts resulted a lower CIE L* value compared to control groups. Similar results were reported by [12] who noted the darkening effect of lactates and rosemary on beef *longissimus.*

Table 1. The combination effect of rosemary extract, organic acid salts and modified atmosphere packaging on pH value of ready-to-eat Hamburger steak

Storage time (d)	Treatment			
	30% CO_2	100% N_2	30% CO_2+RO	100% N_2+RO
0	6.65 ± 0.01^{aBCD}	6.65 ± 0.01^{aB}	6.52 ± 0.02^{bE}	6.52 ± 0.02^{bE}
2	6.79 ± 0.05^{aA}	6.81 ± 0.03^{aA}	6.65 ± 0.02^{bC}	6.64 ± 0.04^{bC}
4	6.82 ± 0.01^{aA}	6.78 ± 0.01^{bA}	6.66 ± 0.01^{dB}	6.73 ± 0.01^{cB}
6	6.80 ± 0.11^{aA}	6.79 ± 0.06^{aA}	6.72 ± 0.05^{aA}	6.77 ± 0.02^{aA}
8	6.61 ± 0.05^{aCD}	6.41 ± 0.07^{bD}	6.47 ± 0.01^{bF}	6.46 ± 0.01^{bF}
10	6.76 ± 0.12^{aAB}	6.65 ± 0.04^{bB}	6.61 ± 0.03^{bC}	6.63 ± 0.01^{bC}
12	6.55 ± 0.10^{aD}	6.57 ± 0.06^{aC}	6.59 ± 0.02^{aD}	6.59 ± 0.01^{aD}
14	6.67 ± 0.10^{aBC}	6.52 ± 0.07^{bC}	6.57 ± 0.01^{bD}	6.56 ± 0.01^{bD}

[a-b] Values within each row with different superscripts are significantly different (p<0.05). [A-F] Values within each column with different superscripts are significantly different (p<0.05)

Table 2. The combination effect of rosemary extract, organic acid salts and modified atmosphere packaging on the TBARS value (mg MA/kg sample) of ready-to-eat Hamburger steak

Storage time (d)	Treatments			
	30% CO_2	100% N_2	30% CO_2+RO	100% N_2+RO
0	0.58 ± 0.07^{aB}	0.58 ± 0.07^{aC}	0.59 ± 0.04^{aB}	0.59 ± 0.04^{aD}
2	0.69 ± 0.07^{aA}	0.70 ± 0.09^{aAB}	0.62 ± 0.04^{bB}	0.61 ± 0.07^{bD}
4	0.66 ± 0.08^{aA}	0.65 ± 0.7^{aBC}	0.63 ± 0.05^{aB}	0.64 ± 0.08^{aC}
6	0.68 ± 0.09^{aA}	0.63 ± 0.11^{aBC}	0.62 ± 0.07^{aB}	0.64 ± 0.08^{aC}
8	0.65 ± 0.07^{bA}	0.70 ± 0.08^{aAB}	0.63 ± 0.08^{bB}	0.70 ± 0.06^{aB}
10	0.64 ± 0.07^{bA}	0.72 ± 0.11^{aAB}	0.65 ± 0.07^{bB}	0.71 ± 0.09^{aB}
12	0.64 ± 0.09^{bA}	0.76 ± 0.15^{aA}	0.64 ± 0.10^{bB}	0.69 ± 0.16^{abB}
14	0.72 ± 0.08^{aA}	0.73 ± 0.13^{aAB}	0.78 ± 0.07^{aA}	0.79 ± 0.10^{aA}

[a-b] Values within each row with different superscripts are significantly different (p<0.05). [A-D] Values within each column with different superscripts are significantly different (p<0.05)

The addition of rosemary extract and organic acid salts lowered (P<0.05) the redness (CIE a*) value both in 30% CO_2-MAP and 100% N_2-MAP in whole day of storage. On the control group, CIE a* value of Hamburger steak packed with 30% CO_2-MAP was higher (P<0.05) compared to Hamburger steak packed with 100% N_2-MAP during storage. The control Hamburger steak were lower (P<0.05) in CIE b* value (yellowness) compared to treatment group. In the control groups the 30% CO_2-MAP tended to have a higher CIE b* value in early storage time and at the end of storage were lower compared to 100% N_2-MAP.

D. Microbiology

The aerobic bacterial counts were increased as the increased of storage time in all the treatments of Hamburger steak. The 30% CO_2-MAP detained the growth of aerobic and anaerobic bacteria (P<0.05) both in control group and treatment group during the storage. The aerobic bacterial growth of control Hamburger steak packed with 100% N_2-MAP was faster than other treatments, in which the aerobic bacterial counts reached 6.21 Log CFU/units on 4 d of storage. Furthermore, the antibacterial effects of CO_2 are well documented by [13,14]. The addition of rosemary extract and organic acid salts effectively detained (P<0.05) the growth of aerobic bacteria of ready-to-eat Hamburger steak during storage both in 30% CO_2-MAP and 100% N_2-MAP. In combination with 30% CO_2-MAP the aerobic bacterial count was the lowest compared to other treatment.

IV. CONCLUSION

In conclusion, with the notes of small negative effect in appearance, it is recommended to use 30% CO_2-MAP in combination with rosemary extract for the packaging of ready-to-eat Hamburger steak.

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Table 2.8 The combination effect of rosemary extract, organic acid salts and modified atmosphere packaging on the aerobic and anaerobic bacterial counts (Log CFU/units) of ready-to-eat Hamburger steak

	Day	Treatment			
		30% CO_2	100% N_2	30% CO_2+RO	100% N_2+RO
AE	0	3.99 ± 0.02^{aD}	3.99 ± 0.02^{aF}	3.81 ± 0.28^{aB}	3.81 ± 0.28^{aD}
	2	3.70 ± 0.21^{bE}	4.31 ± 0.16^{aE}	2.06 ± 0.06^{dD}	3.40 ± 0.08^{cE}
	4	4.58 ± 0.38^{bC}	6.21 ± 0.22^{aD}	2.28 ± 0.17^{dD}	4.00 ± 0.21^{cD}
	6	4.51 ± 0.31^{bC}	7.39 ± 0.28^{aC}	2.34 ± 0.29^{cD}	4.53 ± 0.45^{bC}
	8	5.35 ± 0.09^{bB}	7.41 ± 0.11^{aC}	3.03 ± 0.36^{dC}	4.82 ± 0.24^{cBC}
	10	5.32 ± 0.02^{bB}	7.22 ± 0.04^{aC}	3.74 ± 0.08^{dB}	4.94 ± 0.04^{cB}
	12	6.52 ± 0.06^{bA}	8.31 ± 0.24^{aB}	3.79 ± 0.35^{dB}	6.22 ± 0.18^{cA}
	14	6.61 ± 0.25^{bA}	9.18 ± 0.45^{aA}	4.46 ± 0.10^{dA}	6.15 ± 0.57^{cA}
AN	0	3.84 ± 0.05^{aF}	3.84 ± 0.05^{aE}	3.75 ± 0.28^{aB}	3.75 ± 0.28^{aE}
	2	3.28 ± 0.16^{bG}	4.07 ± 0.21^{aDE}	2.36 ± 0.14^{cF}	2.47 ± 0.10^{cF}
	4	4.71 ± 0.21^{cD}	6.09 ± 0.18^{aE}	2.19 ± 0.18^{dD}	4.17 ± 0.08^{bD}
	6	4.48 ± 0.03^{bE}	7.25 ± 0.22^{aD}	2.55 ± 0.24^{cC}	4.55 ± 0.08^{bC}
	8	5.42 ± 0.06^{bC}	7.32 ± 0.11^{aC}	3.10 ± 0.15^{dC}	4.74 ± 0.15^{cC}
	10	6.03 ± 0.04^{bB}	7.67 ± 0.05^{aB}	3.77 ± 0.05^{dB}	5.58 ± 0.08^{cB}
	12	6.28 ± 0.22^{bA}	7.83 ± 0.27^{aB}	3.58 ± 0.28^{dB}	5.77 ± 0.46^{cB}
	14	6.00 ± 0.22^{cB}	8.94 ± 0.55^{aA}	4.37 ± 0.15^{dA}	6.58 ± 0.15^{bA}

[a-d] Values within each row with different superscripts are significantly different (p<0.05). [A-G] Values within each column with different superscripts are significantly different (p<0.05)

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Table 4. The combination effect of rosemary extract, organic acid salts and modified atmosphere packaging on the instrumental color of ready-to-eat Hamburger steak

	d	Treatments			
		30% CO_2	100% N_2	30% CO_2+RO	100% N_2+RO
L*	0	62.51 ± 1.80^{aAB}	62.51 ± 1.80^{aB}	61.68 ± 1.31^{abB}	61.25 ± 2.71^{bA}
	2	62.45 ± 1.88^{abB}	62.76 ± 2.39^{aAB}	61.67 ± 2.11^{bB}	61.41 ± 2.02^{bA}
	4	62.19 ± 1.37^{bB}	63.42 ± 2.52^{aAB}	61.90 ± 2.69^{bB}	61.85 ± 2.12^{bA}
	6	61.74 ± 1.81^{bB}	63.41 ± 2.14^{aAB}	62.51 ± 2.14^{bB}	61.76 ± 1.95^{bA}
	8	62.59 ± 2.01^{aAB}	63.66 ± 1.79^{aAB}	62.17 ± 2.08^{aB}	62.28 ± 2.33^{aA}
	10	62.68 ± 2.30^{abAB}	63.57 ± 2.20^{aA}	62.05 ± 2.03^{bB}	62.12 ± 2.23^{bA}
	12	62.41 ± 1.88^{bcAB}	62.86 ± 1.90^{abAB}	63.47 ± 2.10^{aA}	61.79 ± 1.72^{cA}
	14	63.23 ± 2.09^{abA}	62.93 ± 1.72^{bAB}	63.91 ± 2.17^{aA}	61.79 ± 1.85^{cA}
a*	0	3.31 ± 0.34^{aB}	3.31 ± 0.34^{aC}	2.99 ± 0.32^{bA}	2.99 ± 0.32^{bA}
	2	3.29 ± 0.36^{aB}	3.02 ± 0.47^{bDE}	2.09 ± 0.50^{cD}	2.23 ± 0.41^{cBC}
	4	3.45 ± 0.37^{aAB}	2.87 ± 0.44^{bE}	1.83 ± 0.54^{cE}	2.04 ± 0.62^{cCD}
	6	3.62 ± 0.41^{aA}	3.25 ± 0.40^{bBC}	2.00 ± 0.36^{dDE}	2.20 ± 0.54^{cBC}
	8	3.26 ± 0.40^{aB}	3.40 ± 0.37^{aAB}	2.04 ± 0.62^{bDE}	1.19 ± 0.52^{bD}
	10	3.33 ± 0.55^{aB}	3.09 ± 0.39^{bCD}	2.39 ± 0.61^{cC}	2.18 ± 0.43^{dBC}
	12	$3.37\pm.035^{aB}$	3.40 ± 0.39^{aAB}	1.83 ± 0.44^{cE}	2.01 ± 0.46^{bBC}
	14	3.42 ± 0.39^{aB}	3.50 ± 0.35^{aA}	2.67 ± 0.37^{bB}	2.29 ± 0.53^{cB}
b*	0	10.83 ± 0.63^{bD}	10.84 ± 0.63^{bB}	11.55 ± 0.76^{aBC}	11.55 ± 0.76^{aB}
	2	11.30 ± 0.67^{bABCD}	10.32 ± 1.34^{cC}	11.97 ± 1.04^{aAB}	12.03 ± 1.03^{aA}
	4	11.43 ± 0.70^{aAB}	10.16 ± 1.19^{bC}	11.19 ± 1.29^{aC}	11.63 ± 1.06^{aB}
	6	11.74 ± 0.63^{bA}	10.81 ± 1.04^{cB}	12.19 ± 0.74^{aA}	11.98 ± 0.82^{abAB}
	8	10.92 ± 0.98^{cCD}	11.05 ± 0.97^{cAB}	12.30 ± 0.85^{aA}	11.65 ± 1.14^{bB}
	10	11.18 ± 1.26^{bcBCD}	11.04 ± 1.29^{cAB}	12.04 ± 1.18^{aAB}	11.62 ± 1.06^{abB}
	12	11.33 ± 1.19^{bABC}	11.35 ± 1.22^{bAB}	11.82 ± 1.25^{bAB}	12.36 ± 0.84^{aA}
	14	10.95 ± 1.25^{cCD}	11.39 ± 0.88^{bA}	11.82 ± 1.10^{bAB}	12.23 ± 0.90^{aA}

[a-d] Values within each row with different superscripts are significantly different ($p<0.05$). [A-E] Values within each column with different superscripts are significantly different ($p<0.05$)