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The Effect of Modified Atmosphere Packaging and Addition of Rosemary Extract, Sodium Acetate and Calcium Lactate Mixture on the Quality of Pre-cooked Hamburger Patties during Refrigerated Storage

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ABSTRACT: The effect of modified atmosphere packaging (MAP; 30% CO_2+70% N_2 or 100% N_2) and an additive mixture (500 ppm rosemary extract, 3,000 ppm sodium acetate and 1,500 ppm calcium lactate) on the quality of pre-cooked hamburger patties during storage at 5°C for 14 d was evaluated. The addition of the additive mixture reduced aerobic and anaerobic bacteria counts in both 30% CO_2-MAP (30% CO_2+70% N_2) and 100% N_2-MAP ($p<0.05$). The 30% CO_2-MAP was more effective to suppress the microbial growth than 100% N_2-MAP, moreover the 30% CO_2-MAP combined with additive mixture resulted in the lowest bacterial counts. The hamburger patties with additive mixture showed lower CIE L* and CIE a*, and higher CIE b* than those with no additive mixture. The 30% CO_2-MAP tended to decrease the TBARS during storage regardless of the addition of additives. The use of 30% CO_2-MAP in combination with additives mixture was effective for maintaining the quality and extending the shelf-life of pre-cooked hamburger patties. (**Key Words:** Rosemary Extract, Calcium Lactate, Sodium Acetate, Modified Atmosphere Packaging, Pre-cooked Hamburger Patties, Refrigerated Storage)

INTRODUCTION

Hamburger is one of the most common fast food in the world. Due to the change of life style and the growing interest in convenient foods, there is an increasing interest in the studies related with the fast food products including their packaging. Pre-cooked product is developed by food companies to answer the need for fast preparation food. Even though hamburger patties are commonly pre-cooked, the high fat contents may be prone to lipid oxidation resulting in deteriorating the quality of product during storage.

Modified atmosphere packaging (MAP) has been used by meat industries to extend the shelf-life as well as to keep the quality characteristics of meat products. During storage of cooked meat, vacuum packaging or low oxygen MAP is commonly used because the color changes are not a critical element as in the fresh meat. Low oxygen MAP may contain less than 20% of O_2 and the rest is composed of CO_2 and N_2. The antibacterial effects of CO_2 were documented by Renerre and Labadie (1993), Jakobsen and Bertelsen (2000), and McMillin (2008). Smith et al. (1990) suggested that 20 to 60% of CO_2 in MAP is required to retard the growth of bacteria.

Besides the MAP effect, the extension of shelf-life can also be achieved by the addition of diverse additives to meat. Blom et al. (1997) reported that a mixture of 2.5% of lactate and 0.25% of acetate could be used to increase the safety of ready-to-eat cooked meat product. It was also reported that sodium acetate improved the color stability in pork (Livingston et al., 2004). Calcium lactate is generally recognized as safe food ingredient (Daengprok et al., 2002) and is commonly used in the meat industry as an antibacterial agent (Shelef and Potluri, 1995). The combined use of lactate and diacetate showed a synergistic effect on improving color stability in pork during retail display (Jensen et al., 2003) and in beef steaks (Knock et al., 2006).

Recently, there has been an increasing interest in the use of natural additives such as rosemary, tocopherols, and

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green tea, etc. in meat products due to health concerns. Rosemary has been used commercially in the meat industries due to its antioxidant activity. The antioxidant activity of rosemary is attributed to its carnosic acid, carnosol and rosmarinic acid components (Okamura et al., 1994; Frankel et al., 1996; Erkan et al., 2008). Previous studies reported the antioxidant ability of different percentages of rosemary in various meat products, such as 0.10% on pork patties (McCarthy et al., 2001), 0.25% on pork sausage (Sebranek et al., 2005), 0.10% on beef steaks (Djenane et al., 2003), and 0.03% on cooked pork patties (Lara et al., 2011).

Combinations of additives and MAP have improved the shelf-life and quality of meat. Schirmer and Langsrud (2010) reported that organic acids in combinations with CO_2 MAP inhibited bacterial growth in marinated pork meat. In addition, Schirmer et al. (2009) showed that the combination of preservatives and CO_2-MAP prolonged the shelf-life of salmon without negative effects on sensory characteristics. Rosemary extract combined with 30% CO_2-MAP increased the redness and reduced the lipid oxidation on cooked pork patties (Lara et al., 2011).

Studies on the effect of MAP in combination with additives mixture including natural additives and organic acid salts on the quality of pre-cooked meat products are still limited. Therefore, this study was conducted to evaluate the effect of MAP and addition of rosemary extracts, sodium acetate and calcium lactate on the storage quality of pre-cooked hamburger patties during refrigerated storage in 5°C.

MATERIALS AND METHODS

Sample preparation

The hamburger patties were prepared by mixing 34.48% of lean meat of beef, 37.52% of pork foreleg, 6.49% of beef fat, 12.51% of onion, 5.18% of biscuit flour, 0.50% of salt, 0.15% of phosphates, 0.04% of ascorbate, 0.17% of glutamic acid, 0.60% of sugar, 0.09% of black pepper, 0.02% of dried nut, 0.43% of seasoning powder, 0.59% of starch, 0.98% of ISP (Isolated soy protein), and 0.25% of egg white powder. The beef, pork and beef fat were obtained from a local butcher in Korea. Briefly, lean meat of beef and pork foreleg were ground separately using a 6 mm hole plate grinder (DFFG-450, Daehan, Korea). The ingredients were mixed well using a mixer for 10 min. A hundred grams of mixture was molded in the size of 10 cm diameter and 1 cm thickness using a hand molder (WJ-2722, Wonjee Engineering, Korea). The hamburger patties were divided into control hamburger (without additive mixture; marked as NO) and hamburger patties with added 500 ppm of rosemary extract, 3,000 ppm of sodium acetate and 1,500 ppm of calcium lactate (marked as RO). The hamburger patties were pre-cooked until the internal temperature of patty reached 70°C by steam in an oven cooker (FM 2011 E3, Forno Misto, Italy).

The hamburger was cooled in a chilled room (7°C) for 10 min. A patty was placed on a polypropylene tray (max. O_2 transmission rate = 0.1 cc/cm^2·24 h at 23°C, 0% RH; water vapor transmission rate = 7.87 mg/cm^2·24 h at 38°C, 100% RH, Cryovac Sealed Air Corp., USA) and the trays were sealed with O_2 barrier film (O_2 transmission rate = 0.39 cc/cm^2·24 h at 4.4°C, 100% RH; Lid 1050, Cryovac Sealed Air Corp., USA). The trays were filled with either 30% CO_2+70% N_2 (marked as 30% CO_2-MAP) or 100% N_2 (marked as 100% N_2-MAP) using a MAP machine (Hypervac, Korea) equipped with the gas mixture (MAP Mix 9001 ME, PBI Dansensor, Denmark). Carbon dioxide and nitrogen were obtained from a local gas supplier (Baekryung Specialty Gas Co., Korea). Six packs were prepared as the replication in each treatment. All packs were stored under dark condition in a refrigerator at 5°C for 14 d.

Headspace gas composition

During storage, six trays from each treatment were allocated for the gas analysis, including oxygen, carbon dioxide and nitrogen. Before opening the packs, the gas composition of head space of pack was measured using a gas analyzer (DK Checkmate 9900, PBI Dansensor, Denmark).

pH measurement

Briefly, 10 g of sample added to 100 ml of distilled water was homogenized at 10,000 rpm for 60 s using a homogenizer (PH91, SMT Co. Ltd., Japan). The pH of the meat slurry at room temperature was measured using a pH meter (SevenEasy pH, Mettler-Toledo GmbH, Switzerland).

Lipid oxidation

Lipid oxidation was evaluated using 2-thiobarbituric acid (TBARS) method as described by Sinhuber and Yu (1977). An absorbance of supernatant of sample was measured at 532 nm using a spectrophotometer (UV-mini-1240, Shimadzu Corp., Japan). The results were calculated as mg malonaldehyde (MA) per kg sample.

Volatile basic nitrogen (VBN)

The volatile basic nitrogen was determined according to Kohsaka (1975) method. The results were expressed as mg %.

Instrumental color measurement

Color on the surface of pre-cooked hamburger patties was monitored by measuring the CIE lightness (L*), redness (a*) and yellowness (b*) using a color difference meter (CR-400, Konica Minolta Sensing Inc., Japan) and an illuminant C. The color instrument was calibrated using a white plate (Illuminant C: Y = 93.6, x = 0.3134, and y = 0.3194).

Aerobic and anaerobic bacteria counts

The plate count agars (Difco, USA) were used for aerobic and anaerobic bacteria counts. Agar plates were prepared according to the manufacturer's instruction. Samples were incubated for 48 h at 37°C under normal atmospheric condition for aerobic bacteria, and anaerobic condition for anaerobic bacteria. For anaerobic bacteria incubation, the agar plates of samples were placed on an anaerobe container system, including an anaerobe box and an anaerobe paper sachet containing carbon (BD Gaspak^{TM} EZ, Becton, Dickinson and Company, USA). Microbial populations were counted and expressed as log CFU/g.

Sensory evaluation

The hamburger patties stored under refrigeration were re-heated using a microwave (RE-2550, Samsung, Korea; 1150 W) until the internal temperature reached 70°C. The panels including faculty members and students evaluated the cooked hamburger patties on the color, taste and texture at 0, 2, 4, and 6 d of storage. The hedonic scores of color, taste, and texture were 9 = extremely like, 7 = like, 5 = moderate like, 3 = dislike, and 1 = extremely dislike.

Statistical analysis

Three pre-cooked hamburger patties per batch and per day were used as the experimental unit. The analyses were performed in 4 repetitions in each burger patty (3 burger patties×4 replicates; n = 12 per batch and per storage day; except sensory evaluation n = 30 and instrumental color n = 30). The analysis of variance (ANOVA) and Duncan's multiple range tests were carried out using the SPSS 19.0 for Windows Evaluation Version (SPSS Inc., Chicago, IL, USA).

RESULTS AND DISCUSSION

Headspace gas composition

The oxygen concentration was maintained at 0% during storage in all the treatments (data was not shown). Both in pre-cooked hamburger patties of non-added (NO) and with additive mixture (RO) packed with 30% CO_2-MAP, CO_2 concentration decreased from 30% to around 28% at 2 d of storage and after that the carbon dioxide concentration was relatively stable until the end of storage (Table 1). Esmer et al. (2011) noted that the changes of the gas composition might be due to the results of microbial growth, the permeability of packaging material and the respiration of the meat (including gas absorption by the meat). The decrease of CO_2 in MAP was the result of the absorption of CO_2 into the meat (Jakobsen and Bertelsen, 2002).

In 100% N_2-MAP, the concentration of CO_2 started to increase from 8 d of storage in NO sample, while an increasing CO_2 concentration in RO sample was noted only at d 14. An increased CO_2 concentration may be related to the microbial growth. During storage, the microorganisms in the meat utilized the O_2 in the headspace of tray while some microflora (particularly, lactic acid bacteria), produce carbon dioxide (Nychas, 1994). Jakobsen and Bertelsen

Table 1. The changes in headspace gas composition of modified atmosphere packaging (MAP) during refrigerated storage (%; mean±SE, n = 4)

Parameter	Storage time (d)	Treatments[1]			
		NO-30% CO_2	RO-30% CO_2	NO-100% N_2	RO-100% N_2
CO_2	0	30.0±1.0^{aA}	30.0±1.0^{aA}	0.0±0.0^{xC}	0.0±0.0^{xB}
	2	28.0±0.1^{bB}	28.1±0.1^{aB}	0.0±0.0^{xC}	0.0±0.0^{xB}
	6	28.2±0.1^{aB}	28.2±0.2^{aB}	0.1±0.1^{xC}	0.0±0.0^{xB}
	10	28.0±0.1^{aB}	28.0±0.1^{aB}	1.4±0.3^{xB}	0.0±0.0^{yB}
	14	28.2±0.5^{aB}	27.9±0.1^{aB}	2.3±1.2^{xA}	0.4±0.2^{yA}
N_2	0	70.0±1.0^{aA}	70.0±1.0^{aC}	100±0.0^{xA}	100±0.0^{xA}
	2	72.1±0.1^{aA}	71.9±0.1^{bBC}	100±0.0^{xA}	100±0.0^{xA}
	6	71.8±0.1^{aA}	71.7±0.1^{bBC}	99.9±0.1^{yA}	100±0.0^{xA}
	10	72.0±0.1^{aA}	72.0±0.2^{aAB}	99.6±0.3^{yAB}	100±0.0^{xA}
	14	71.8±0.5^{aA}	72.1±0.5^{aA}	97.7±1.2^{yCD}	99.6±0.2^{xB}

$^{a-b}$ Values within each row (between 30% CO_2-MAP) with different superscripts are significantly different (p<0.05).
$^{x-y}$ Values within each row (between 100% N_2-MAP) with different superscripts are significantly different (p<0.05).
$^{A-E}$ Values within each column with different superscripts are significantly different (p<0.05).
[1] Treatment: NO-30% CO_2 = Hamburger patties without additive mixture packed with 30% CO_2+70% N_2.
NO-100% N_2 = Hamburger patties without additive mixture packed with 100% N_2.
RO-30% CO_2 = Hamburger patties with added 500 ppm of rosemary extract, 3,000 ppm of sodium acetate and 1,500 ppm of calcium lactate packed with 30% CO_2+70% N_2.
RO-100% N_2 = Hamburger patties with added 500 ppm of rosemary extract, 3,000 ppm of sodium acetate and 1,500 ppm of calcium lactate packed with 100% N_2.

(2002) reported that both muscle respiration and microbial growth are responsible for O_2 consumption and CO_2 production. In our study, 0% O_2 was applied in all the packs, so that the consumption of O_2 was limited. In RO samples, the production of CO_2 was observed only at d 14. The results indicated that the addition of rosemary extract and organic acid salts was effective for suppressing the growth of aerobic and anaerobic bacteria as shown in the Figures 2A and 2B respectively.

pH value

The pH value of pre-cooked hamburger patties during storage is presented in Table 2. The pH value of NO sample increased after 2 d, relatively constant until 6 d and then tended to decrease until the end of storage. The pH value of RO sample was lower ($p<0.05$) than NO samples, regardless of the type of MAP from 0 to 4 d. The lower ph might be related with the addition of organic acids salts. For instance, Aran (2001) observed the decreasing pH value of a 'sous-vide' beef goulash from 6.0 to 5.5 due to the addition of 3% calcium lactate. Kim et al. (2009) reported the decrease of pH with calcium lactate enhancement in beef steaks. Also, Velugoti et al. (2007) reported the decrease in pH level of pork loin by increasing the concentration of calcium lactate. The pH value of NO samples packed with 30% CO_2-MAP was higher ($p<0.05$) than other treated hamburger patties at d 10 and 14, while that of NO sample packed with 100% N_2-MAP was not significantly different from RO-30% CO_2 and RO-100% N_2 ($p>0.05$).

Lipid oxidation

The TBARS values of pre-cooked hamburger patties were increased as the increment of storage time in all the treatments (Table 2). The TBARS values were in a range from 0.58 to 0.79 mg MA/kg sample. The addition of additive mixture delayed the lipid oxidation until 2 d of storage in which the TBARS values of RO samples in both in two MAP were lower compared to those of NO sample ($p<0.05$). The antioxidant activity of rosemary is attributed to its carnosic acid, carnosol and rosmarinic acid components (Erkan et al., 2008). Lara et al. (2011) reported that the TBARS value of cooked pork patties immediately after cooking ranged from 0.1 to 1.3 mg MA/kg sample, and the addition of rosemary extract effectively reduced the speed lipid oxidation. Although there was no statistically significant difference, the 30% CO_2-MAP tended to delay the lipid oxidation both in NO and RO samples from 2 to 10 d. The effects of MAP were superior to the addition of rosemary and organic acids salts in detaining the lipid oxidation. The lower TBARS value in 30% CO_2-MAP may be attributed to the lower bacterial counts. At the end of storage, the TBARS value of precooked hamburger patties

Table 2. The effect of modified atmosphere packaging (MAP) and additive mixture on pH, 2-thiobarbituric acid reactive substances (TBARS) value (mg malondialdehyde/kg sample) and volatile basic nitrogen (VBN) value (mg %) of pre-cooked hamburger patties during refrigerated storage

Parameter	Storage time (d)	Treatments[1]			
		NO-30% CO_2	NO-100% N_2	RO-30% CO_2	RO-100% N_2
pH	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}
	6	6.80 ± 0.11^{aA}	6.79 ± 0.06^{aA}	6.72 ± 0.05^{aA}	6.77 ± 0.02^{aA}
	10	6.76 ± 0.12^{aAB}	6.65 ± 0.04^{bB}	6.61 ± 0.03^{bC}	6.63 ± 0.01^{bC}
	14	6.67 ± 0.10^{aBC}	6.52 ± 0.07^{bC}	6.57 ± 0.01^{bD}	6.56 ± 0.01^{bD}
TBARS (mg MA/kg sample)	0	0.58 ± 0.07^{aB}	0.58 ± 0.07^{aB}	0.59 ± 0.04^{aB}	0.59 ± 0.04^{aD}
	2	0.69 ± 0.07^{aA}	0.70 ± 0.09^{aA}	0.62 ± 0.04^{bB}	0.61 ± 0.07^{bD}
	6	0.68 ± 0.09^{aA}	0.70 ± 0.11^{aA}	0.62 ± 0.07^{aB}	0.64 ± 0.08^{aC}
	10	0.64 ± 0.07^{bA}	0.72 ± 0.11^{aA}	0.65 ± 0.07^{bB}	0.71 ± 0.09^{aB}
	14	0.72 ± 0.08^{aA}	0.73 ± 0.13^{aA}	0.78 ± 0.07^{aA}	0.79 ± 0.10^{aA}
VBN (mg %)	0	10.39 ± 0.41^{aE}	10.39 ± 0.41^{aD}	10.29 ± 0.46^{aE}	10.29 ± 0.46^{aE}
	2	10.81 ± 1.43^{bE}	12.17 ± 0.55^{aCD}	12.42 ± 0.49^{aD}	12.84 ± 0.51^{aD}
	6	13.27 ± 1.07^{bD}	14.57 ± 1.34^{aC}	13.80 ± 0.45^{abCD}	13.73 ± 0.93^{abD}
	10	19.46 ± 0.43^{aC}	19.90 ± 0.63^{aB}	18.36 ± 0.90^{aB}	16.54 ± 0.74^{bC}
	14	24.12 ± 1.34^{aA}	25.52 ± 0.69^{aA}	21.28 ± 0.71^{bA}	21.52 ± 0.63^{bA}

[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 ($p<0.05$).
[1] Treatment: NO-30% CO_2 = Hamburger patties without additive mixture packed with 30% CO_2+70% N_2.
NO-100% N_2 = Hamburger patties without additive mixture packed with 100% N_2.
RO-30% CO_2 = Hamburger patties with added 500 ppm of rosemary extract, 3,000 ppm of sodium acetate and 1,500 ppm of calcium lactate packed with 30% CO_2+70% N_2.
RO-100% N_2 = Hamburger patties with added 500 ppm of rosemary extract, 3,000 ppm of sodium acetate and 1,500 ppm of calcium lactate packed with 100% N_2.

with additives tended to be higher but it was not statistically different ($p>0.05$). T Further research is needed to confirm the pro-oxidative effect of additives mixture on precooked meat products with longer storage time.

Volatile basic nitrogen

The VBN value of pre-cooked hamburger patties was increased in all the treatments during storage ($p<0.05$) as shown in Table 2. In NO sample, the VBN value of hamburger patties packed with 30% CO_2-MAP was lower than that of 100% N_2-MAP from 2 until 6 d, while from 10 to 14 d no statistical difference was found. This result may be attributed to the lower bacterial counts of hamburger patties packed with 30% CO_2-MAP. Protein deterioration (represented by VBN value) is associated with the activity of amino acid decarboxylase of microorganisms (Lin and Lin, 2002). In RO sample, no difference was found between 30% CO_2-MAP and 100% N_2-MAP during storage, except at d 10 when the VBN value of 30% CO_2-MAP was lower than that of 100% N_2-MAP ($p<0.05$). The additive mixture tended to decrease ($p<0.05$) the protein deterioration at the end of storage time (from 10 to 14 d). It may be related to the result that the antioxidative effect of rosemary extract and organic acid salts was observed only at the end of storage. Lin and Lin (2002) noted that the addition of organic acids lowered the VBN value of Chinese style low-fat sausage. The protective effects against protein degradation of rosemary have been reported in pork patties (Haak et al., 2009), frankfurters (Estevez et al., 2005), and cooked pork patties (Lara et al., 2011).

Instrumental color

The instrumental color of pre-cooked hamburger patties including CIE L* (lightness), a* (redness), and b* (yellowness) values are presented on Figure 1A, 1B, and 1C, respectively. The lightness tended to increase slightly during the storage in all treatments. In NO sample, the hamburger patties packed with 100% N_2-MAP tended to be lighter than 30% CO_2-MAP from 2 to 14 d, but a significant difference was observed only at d 6. In RO sample, the lightness of hamburger patties packed with 30% CO_2-MAP tended to be higher ($p<0.05$) than 100% N_2-MAP at the end of storage. In general, addition of additive mixture resulted in a lower lightness value than non added samples.

The redness and yellowness of pre-cooked hamburger patties fluctuated over the storage ($p<0.05$). The additive mixture lowered ($p<0.05$) the redness value in both 30% CO_2-MAP and 100% N_2-MAP over the storage. In the NO sample, redness value of hamburger patties packed with 30% CO_2-MAP was higher than 100% N_2-MAP ($p<0.05$). The yellowness of NO sample was lower than RO sample ($p<0.05$). In NO samples, the 30% CO_2-MAP tended to have a higher yellowness value in the early storage time from 2 to 6 d and at day 14 d lower than 100% N_2-MAP. No significant differences were found for yellowness value of RO sample between two packaging methods of 30% CO_2-MAP and 100% N_2-MAP from 0 to 6 d. Georgantelis et al. (2007) reported a decreasing trend of redness and yellowness values of beef burgers with the addition of rosemary extract during frozen storage and the decrease was strongly negatively correlated with lipid oxidation. This report was inconsistent with our results in which no clear correlation was observed between instrumental color and lipid oxidation (TBARS). Mancini and Hunt (2005) noted that the decrease of redness and yellowness is attributed to the gradual oxidation of myoglobin and accumulation of metmyoglobin.

Aerobic and anaerobic bacteria counts

The aerobic and anaerobic bacteria counts of pre-cooked hamburger patties during the storage are presented on Figures 2A and 2B, respectively. The aerobic bacterial counts increased with prolonging the storage time in all of the treatments. The 30% CO_2-MAP delayed the growth of aerobic bacteria ($p<0.05$) both in NO and RO samples during storage. The aerobic bacterial growth in NO samples packed with 100% N_2-MAP was faster than the other treatments.

The additive mixture effectively delayed ($p<0.05$) the growth of aerobic bacteria during storage both in 30% CO_2-MAP and 100% N_2-MAP. In combination with 30% CO_2-MAP, the aerobic bacterial count of RO was the lowest compared to other treatments. It means that the 30% CO_2-MAP was effective for delaying the growth of aerobic bacteria, where the effect could be enhanced by the additive mixture. The result of bacterial counts was consistent with the change of CO_2 composition in the headspace of the tray, in which the CO_2 composition in 100% N_2-MAP was increased. The changing trend of anaerobic bacterial counts was similar with aerobic bacterial count, in which the 30% CO_2-MAP delayed the growth of bacteria. Additive mixture showed a delaying ability against bacterial growth both in 30% CO_2-MAP and 100% N_2-MAP and the combination of additive mixture and 30% CO_2-MAP showed greater delaying effects on bacterial growth (lowest bacterial counts).

Sensory evaluation

Sensory evaluation of pre-cooked hamburger patties including color, taste, and texture is presented in Table 3. Panelists scored lower ($p<0.05$) color of RO sample than NO sample at d 0 and 2. From 4 to 6 d, the scores of sensory color were statistically insignificant ($p>0.05$). These observations were consistent with the redness (CIE a*) value instrumental color, in which the additive mixture lowered the redness during the storage. The overall likeness

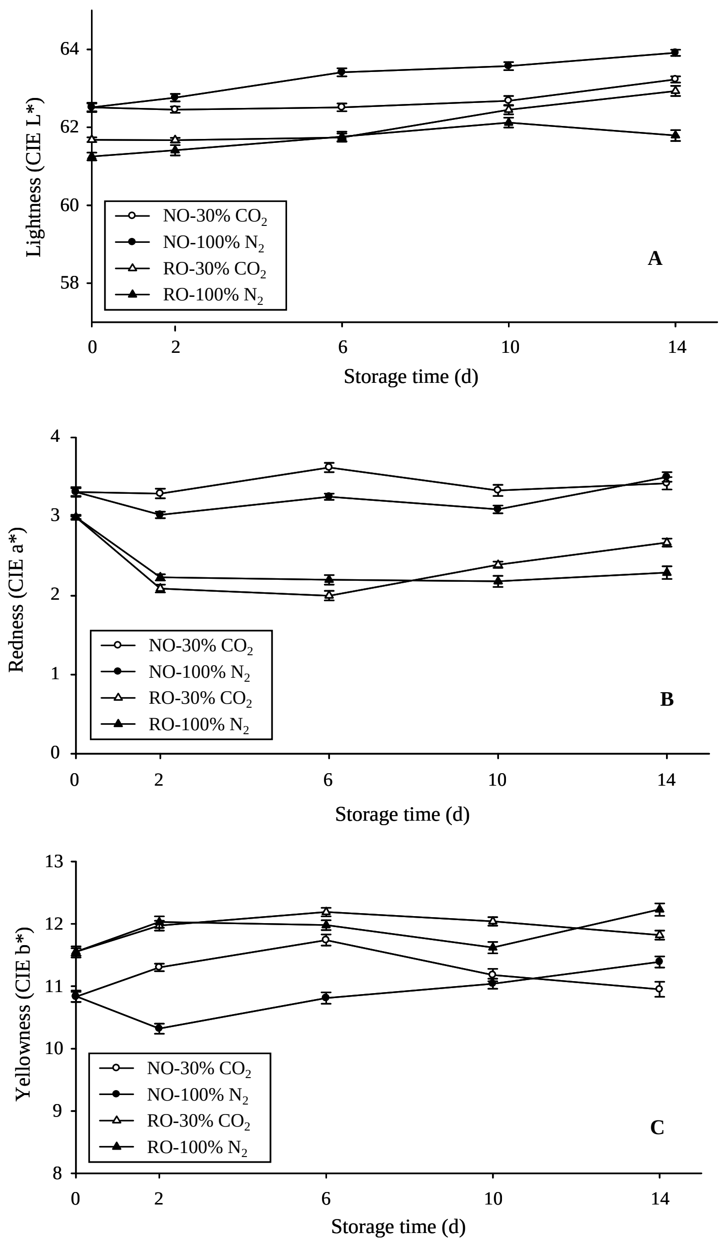

Figure 1. The effect of MAP and addition of rosemary extract, sodium acetate and calcium lactate mixture on instrumental color of lightness (CIE L* value; A), redness (CIE a* value; B), and yellowness (CIE b* value; C) of pre-cooked hamburger patties during refrigerated storage (n = 30). NO-30% CO_2: Hamburger patties without additive mixture packed with 30% CO_2+70% N_2. NO-100% N_2: Hamburger patties without additive mixture packed with 100% N_2. RO-30% CO_2: Hamburger patties with added 500 ppm of rosemary extract, 3,000 ppm of sodium acetate and 1,500 ppm of calcium lactate packed with 30% CO_2+70% N_2. RO-100% N_2: Hamburger patties with added 500 ppm of rosemary extract, 3,000 ppm of sodium acetate and 1,500 ppm of calcium lactate packed with 100% N_2.

of color scores was decreased as prolonging the storage time in all of the treatments, but all the scores were still above 6 points after 6 d of storage. Both in the sensorial taste and texture evaluations, no differences were found between any of the treatments during the storage. In other words, the use of two different gas composition of MAP (30% CO_2-MAP and 100% N_2-MAP) and additive mixture gave no effect on the taste and texture characteristics of pre-cooked hamburger patties during storage.

CONCLUSION

The addition of 500 ppm rosemary extract, 3,000 ppm sodium acetate and 1,500 ppm calcium lactate showed lower instrumental color of lightness and redness and higher yellowness of precooked hamburger patties than that of non-addition sample. The addition of these mixtures also delayed the growth of total aerobic and anaerobic bacteria. Moreover, the additive mixtures combined with the 30% CO_2-MAP were more effective to suppress the microbial

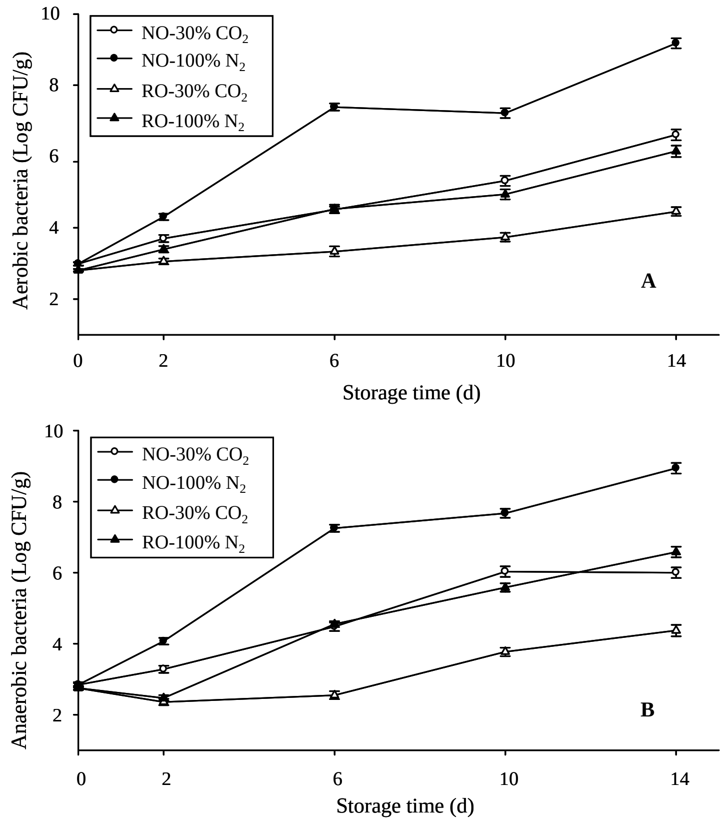

Figure 2. The effect of MAP and addition of rosemary extract, sodium acetate and calcium lactate mixture on aerobic bacterial counts (A) and anaerobic bacterial counts (B) (Log CFU/g unit; n = 12) of pre-cooked hamburger patties during refrigerated storage.

Table 3. The effect of modified atmosphere packaging (MAP) and additive mixture on the sensory evaluation of pre-cooked hamburger patties during refrigerated storage

Parameters	Treatment[1]	Storage time (d)			
		0	2	4	6
Color	NO-30% CO_2	8.5 ± 0.6^{aA}	8.0 ± 0.4^{aAB}	7.5 ± 0.8^{aB}	7.0 ± 0.2^{aC}
	NO-100% N_2	8.4 ± 0.5^{aA}	7.9 ± 0.5^{aAB}	7.2 ± 0.5^{aB}	7.2 ± 0.6^{aB}
	RO-30% CO_2	7.2 ± 0.7^{bA}	7.3 ± 0.6^{bA}	6.8 ± 0.2^{aAB}	6.5 ± 0.3^{aB}
	RO-100% N_2	7.5 ± 0.5^{bA}	7.3 ± 04^{bA}	6.9 ± 0.6^{aAB}	6.8 ± 0.3^{aB}
Taste	NO-30% CO_2	8.4 ± 0.5^{aA}	8.1 ± 0.5^{aAB}	7.9 ± 0.5^{aAB}	7.6 ± 0.5^{aB}
	NO-100% N_2	8.2 ± 0.5^{aA}	8.0 ± 0.6^{aA}	8.0 ± 0.6^{aA}	7.5 ± 0.8^{aB}
	RO-30% CO_2	8.5 ± 0.6^{aA}	8.2 ± 0.6^{aAB}	8.0 ± 0.8^{aAB}	7.6 ± 0.7^{aB}
	RO-100% N_2	8.6 ± 0.4^{aA}	8.3 ± 0.4^{aA}	7.8 ± 0.5^{aAB}	7.5 ± 0.6^{aB}
Texture	NO-30% CO_2	7.4 ± 1.0^{aA}	7.2 ± 0.8^{aA}	7.1 ± 0.5^{aA}	7.0 ± 0.5^{aA}
	NO-100% N_2	7.2 ± 0.6^{aA}	7.6 ± 0.6^{aA}	7.0 ± 0.6^{aA}	6.9 ± 0.6^{aA}
	RO-30% CO_2	7.3 ± 0.8^{aA}	7.1 ± 0.4^{aA}	7.3 ± 0.9^{aA}	6.9 ± 0.4^{aA}
	RO-100% N_2	7.5 ± 0.8^{aA}	7.2 ± 0.6^{aA}	7.1 ± 0.5^{aA}	6.8 ± 0.8^{aA}

$^{a-b}$ Values within each column with different superscripts are significantly different ($p<0.05$).
$^{A-D}$ Values within each row with different superscripts are significantly different ($p<0.05$).
The hedonic scores: 9 = extremely like, 7 = like, 5 = moderate like, 3 = dislike, and 1 = extremely dislike.
[1] Treatment: NO-30% CO_2 = Hamburger patties without additive mixture packed with 30% CO_2+70% N_2.
NO-100% N_2 = Hamburger patties without additive mixture packed with 100% N_2.
RO-30% CO_2 = Hamburger patties with added 500 ppm of rosemary extract, 3,000 ppm of sodium acetate and 1,500 ppm of calcium lactate packed with 30% CO_2+70% N_2.
RO-100% N_2 = Hamburger patties with added 500 ppm of rosemary extract, 3,000 ppm of sodium acetate and 1,500 ppm of calcium lactate packed with 100% N_2.

growth than that of 100% N_2-MAP. The 30% CO_2-MAP itself tended to decrease the lipid deterioration (TBARS) regardless of the addition of additives. The conclusion is that the use of 30% CO_2-MAP in combination with the addition of rosemary extract, sodium acetate and calcium lactate was effective for maintaining the quality and extending the shelf-life of pre-cooked hamburger patties during refrigerated storage at 5°C.

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