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Effects of Basil and Majoram Essential Oils with or without Ascorbic Acid on Color and Oxidative and Microbial Stability of Beef Patties

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Abstract

Fresh ground beef was mixed with ascorbic acid, basil essential oil, majoram essential oil, or each essential oil combined with ascorbic acid and stored at 1±1°C for 7 days. Color, lipid oxidation (TBARS formation), aerobic bacterial counts and pH were determined. Basil and majoram essential oils were effective in inhibiting color deterioration, lipid oxidation and bacterial growth. The combined addition of basil and ascorbic acid showed the highest protection against color fading, followed by majoram + ascorbic acid, and ascorbic acid alone. Basil and majoram essential oils were most effective in delaying TBARS formation ($p < 0.01$). Ascorbic acid did not exert an antioxidative effect and even exhibited a pro-oxidant effect. The pH values of all samples increased slightly, but no significant differences were observed, either among treatments or throughout the storage time ($p > 0.05$).

Key words: basil, majoram, essential oil, ascorbic acid, lipid oxidation, microbial stability

INTRODUCTION

Fresh and processed meat products undergo deterioration from oxidative and microbial processes during processing and storage. Oxidative deterioration is associated with the oxidation of unsaturated fatty acids and phospholipids (1). Furthermore, oxymyoglobin oxidizes to form metmyoglobin which gives meat an unattractive brown color and limits its shelf life. Therefore, color and lipid stability in meat products are very important quality attributes, influencing the consumer's decision to purchase (2). Microbial spoilage is another critical factor in quality deterioration of meat products because microbial contamination increases the risk of foodborne illness (3,4).

Synthetic antioxidants and antimicrobial agents have traditionally been used in meat processing. However, growing concern by consumer's about potential side effect of synthetic chemicals has resulted in a decrease in their use in foods (5,6). Due to this concern, natural compounds such as herbs and spices have been extensively examined for controlling lipid oxidation and microbial growth (7-10). Essential oils or extracts of rosemary, sage, oregano, thyme and dittany have shown noticeable antioxidant or antimicrobial activity in meat (6-8,11-15). Ascorbic acid (vitamin C) has been considered for extending the shelf life of meat (16) by increasing pigment and lipid stability in ground pork (17) and in raw ground beef (18). Djenane et al. (14) reported that spraying antioxidants in combination with vitamin C solution on the surface of fresh beef steaks was effective in delaying oxidative deterioration. Vitamin E (6 ppm) + vitamin C (500 ppm) has been effectively used for reducing pigment and lipid oxidation in fresh meat without other antioxidants, and when combined have synergistic effects (19). No data are abailable for the effect of basil (*Ocimum basilicum* Labiatae) and majoram (*Majorana hortensis* Moench) on color, lipid oxdiation and microbial contamination in meat and few studies have been conducted regarding the combination of essential oils with ascorbic acid to increase the shelf life of fresh meat. The objective of this study was to examine the effect of essential oils of basil and majoram, either alone or with ascorbic acid, on the inhibition of pigment and lipid oxidation and microbial growth in beef patties.

MATERIALS AND METHODS

Materials

Basil and majoram essential oils were supplied by Kalsec, Inc. (Kalamazoo, MI, USA). Ascorbic acid, 1,1,3,3-tetraethoxypropane, thiobarbituric acid and trichloroacetic acid were purchased from Sigma-Aldrich (St. Louis, MO, USA). Plate count agar and peptone water were purchased from Difco laboratories (Michigan, IL USA).

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Sample preparation

Fresh ground beef (20% fat content) was purchased from local grocery store and six 250 g aliquots of ground beef were alloted to the following treatments: (1) 0.5% (w/w) basil essential oil, (2) 0.25% basil essential oil + 0.015% (150 ppm) ascorbic acid, (3) 0.5% majoram essential oil, (4) 0.25% majoram essential oil + 0.015% ascorbic acid, (5) 0.015% ascorbic acid and (6) control. The above concentrations were determined based on the data reported by other researchers (14-16,19). Samples of 25 g of treated meat were shaped into miniature beef patties using a mold (50 × 12 mm) in order to have a consistent surface area and volume. These patties were placed on a paper tray and the tray was packed in a polyethylene zipper bag (17 in × 20 in) and stored in a refrigerator at 1 ± 1°C for 7 days. Meat samples were analyzed at intervals of 0, 1, 3, 5 and 7 days and the analyses were replicated twice except for color measurements.

Color measurements

Color changes were monitored on the surface of the beef patties using a Minolta colorimeter (Model CR-300, Japan). Hunter 'L' (lightness), 'a' (redness) and 'b' yellowness) values were determined and each value was the mean of 10 determinations.

Lipid oxidation determination

Lipid oxidation was evaluated by the determination of thiobarbituric acid reactive substances (TBARS). Three grams of meat samples were blended with 30 mL of 10% trichloroacetic acid (TCA) for 2 min. The homogenate was filtered through Whatman No. 2 filter paper and 2 mL of the filtrate was mixed with 2 mL of 20 mM TBA reagent. The mixture was placed in a boiling water bath for 15 min and cooled to room temperature. Absorbance was measured at 531 nm using a Spectrophotometer (Gilford 250, USA). The amounts of TBARS were expressed as mg malonaldehyde/kg meat. A standard curve was prepared using solutions of 1,1,3,3-tetraethoxypropane (TEP) in distilled water.

Microbial analysis

Ten grams of meat was taken from the beef patties and diluted in 90 mL of 0.1% sterile peptone water. Each sample was homogenized in a Stomacher Blender 80 (London, UK) for 1 min. Serial 10-fold dilutions were prepared by diluting 1 mL in 9 mL of 0.1% sterile peptone water and spread on sterile petriplates containing plate count agar. Plates were then incubated at 35 ± 1°C for 48 h and counts of aerobic bacteria were expressed as the $\log_{10}$ of colony forming units per gram (CFU/g) of meat.

pH measurements

Three grams of meat was homogenized with 30 mL of distilled, deionized water for 1 min. The homogenate was filtered through Whatman No. 1 filter paper and the filtrate was used for pH measurement using a pH meter (Corning 430, USA).

Statistical analysis

Data were analyzed by one way analysis of variance (ANOVA) using SPSS version 10.0. Duncan's multiple range test was used to compare means of treatments: differences were considered significant at $p < 0.05$.

RESULTS AND DISCUSSION

Meat color

Treatment effects on meat color are shown in Table 1. Hunter 'L' values (lightness) of beef patties increased slightly with storage time in all samples, but no significant difference ($p > 0.05$) was observed between treated and untreated samples. Few studies have evaluated changes in 'L' value in meat during storage. One study conducted by Fernandez-Lopez et al. (20) demonstrated that 'L' values increased during storage in fresh and cooked pork meat, and that samples treated with the extracts of rosemary and hyssop showed lower 'L' values than control. Other study reported that the increase in 'L' values is attributable to increased free water on the surface of meat (21).

The Hunter 'a' values (redness) for beef patties ranged from 18.84 to 19.65 immediately after mixing (day 0). Redness increased progressively in samples treated with basil + ascorbic acid, majoram + ascorbic acid and ascorbic acid. They showed the most intense red color retention at day 3 of storage, showing 'a' values of 20.86, 20.84 and 20.54, respectively. Treatment with essential oils and ascorbic acid led to significant differences ($p < 0.01$) with the controls from day 3 storage onwards. Addition of basil and majoram oil resulted in small improvements in red color retention, but when used in combination with ascorbic acid, red color was improved remarkably. This suggests that ascorbic acid functions as a synergist by promoting the effects. These results were in agreement with those found by Sanchez-Escalante et al. (9,22), who reported that rosemary extract combined with ascorbic acid was very useful for preventing red color fading (myoglobin oxidation) in ground beef patties. Djenane et al. (14) also reported that the same combination extended the shelf life of fresh beef steaks by about 10 days. Mitsumoto et al. (19) found that the combination of vitamin E (6ppm) + vitamin C (500 ppm) was effective in reducing pigment and lipid

Table 1. Hunter Lab values of beef patties during storage at 1±1°C

	Treatment[1)]	Storage time (days)				
		0	1	3	5	7
L	Control	42.91	42.33	43.10	44.92	43.00
	Basil	41.61	43.33	44.89	43.52	44.04
	Basil+Asc. Acid	43.02	43.41	42.02	45.36	44.75
	Majoram	43.72	43.62	44.51	43.24	43.44
	Majoram+Asc. Acid	41.97	43.76	43.34	41.97	44.09
	Asc. Acid	42.10	42.76	44.01	42.63	43.01
a	Control	18.84	17.54^{a}	15.98^{a}	14.22^{a}	$9.48^{a2)}$
	Basil	19.65	17.71^{a}	17.11^{ab}	16.67^{b}	12.35^{b}
	Basil+Asc. Acid	18.93	19.46^{bc}	20.86^{c}	19.64^{cd}	16.86^{d}
	Majoram	18.95	18.39^{ab}	17.50^{b}	16.00^{b}	13.45^{bc}
	Majoram+Asc. Acid	19.20	20.57^{c}	20.84^{c}	20.40^{d}	16.84^{d}
	Asc. Acid	18.70	18.69^{ab}	20.54^{c}	19.44^{c}	14.66^{c}
b	Control	8.58	8.33	8.25^{a}	7.72^{a}	7.69^{a}
	Basil	8.74	8.54	8.74^{ab}	8.87^{bc}	7.63^{a}
	Basil+Asc. Acid	8.59	9.10	9.85^{d}	9.77^{d}	8.33^{ab}
	Majoram	8.87	8.88	8.98^{bc}	8.59^{b}	8.47^{b}
	Majoram+Asc. Acid	8.52	9.33	9.45^{cd}	9.39^{cd}	8.51^{b}
	Asc. Acid	8.61	9.05	9.52^{cd}	8.81^{bc}	8.20^{ab}

[1)]Treatment: Basil, 0.5% basil oil; Basil+Asc. Acid, 0.25% basil oil+0.015% Asc. Acid; Majoram, 0.5% majoram oil; Majoram +Asc. Acid, 0.25% majoram oil+0.015% Asc. Acid; Asc. Acid, 0.015% Asc. Acid.
[2)]Means with different letters in same column are significantly different at $p<0.05$.

oxidation. The red color of control beef patties deteriorated rapidly with storage time. At the end of the storage period (7 days), control had a very low 'a' value below 10, whereas other samples possessed 'a' values above 12. Treatment with basil+ascorbic acid and majoram+ascorbic acid resulted in the retention of significantly ($p<0.01$) high 'a' values of 16.86 and 16.84 at day 7 of storage, respectively; a color loss that was reached after only 3 days of storage by the control samples.

Hunter 'b' values (yellowness) gradually decreased in control samples during storage. Treatment with basil+ ascorbic acid, majoram+ascorbic acid and ascorbic acid led to significant color retention ($p<0.01$) compared with the controls after day 3 of storage, showing 'b' values of 9.85, 9.45 and 9.52, respectively. At the end of storage, beef patties treated with majoram oil and majoram+ascorbic acid had higher 'b' values than the other beef patties.

Of the tristimulus colorimetry parameters, the 'a' parameter has been found to correlate well with the subjective color scores of meat and it is well known that meat oxidation provokes a decrease in Hunter 'a' value. According to our results, Hunter 'L' and 'b' values showed no trend over storage period.

Lipid oxidation (TBARS)

Oxidative rancidity of lipids is a serious problem during storage of meat and meat products which results in off-flavors and odors. TBARS value is the most commonly used parameter to estimate the accumulation of oxidative products in lipids (23,24). Fig. 1 shows the concentrations of TBARS in treated and untreated beef patties during storage. At day 0, the mean TBARS values for beef patties were 3.43~3.48 mg/kg meat. As the

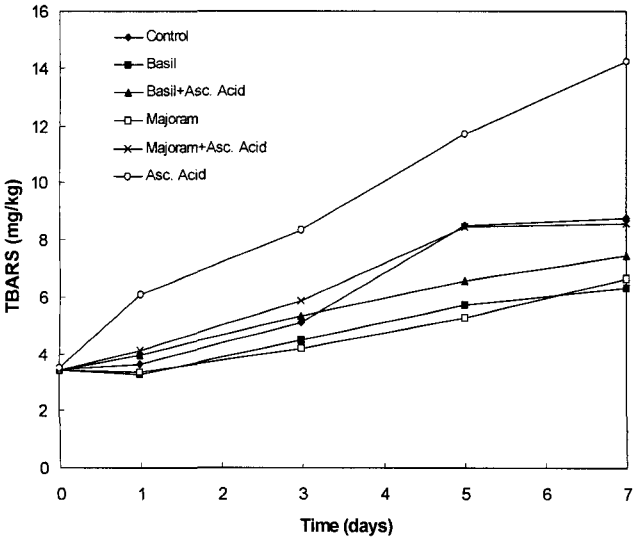

Fig. 1. Effects of basil and majoram essential oils, with and without ascorbic acid, on lipid oxidation (TBARS) in beef patties during storage at 1±1°C. ◆—◆: Control, ■—■: 0.5% Basil, ▲—▲: 0.25% Basil+0.015% Asc. Acid, □—□: 0.5% Majoram, ×—×: 0.25% Majoram+0.015% Asc. Acid, ○—○: 0.015% Asc. Acid.

storage time increased, TBARS values increased in all of the beef patties. By day 7, basil and majoram oil had exerted a considerable inhibition of oxidative processes. Patties treated with basil essential oil showed the lowest value of 6.26 mg/kg meat, indicating the most significant protective effect against lipid oxidation ($p<0.01$). Herbal extracts have shown noticeable antioxidant activity in meat. Treament with rosemary extract (1000 ppm) was found to be effective in inhibiting lipid oxidation in beef steaks (14). Extracts of oregano and dittany function as antioxidants in lard (12). When ascorbic acid (7.42 mg/kg) was added to basil, the antioxidant effects of basil was reduced. Majoram oil also inhibited lipid oxidation to the same extent as basil (6.64 mg/kg), but majoram + ascorbic acid did not inhibit lipid oxidation. Addition of ascorbic acid alone resulted in higher TBARS values compared to other samples from 1 day of storage onward. This demonstrates that the addition of ascorbic acid to fresh ground beef was completely ineffective in preventing lipid oxidation and even exerted a prooxidant effect at the 150 ppm level. Kanner et al. (25) reported that ascorbic acid concentrations under 0.0176% (176 ppm) in an aqueous carotene-linoleate solution acted as a pro-oxidant with metal ions. Mitsumoto et al. (19) reported that a range of 200 ~ 1000 ppm of ascorbic acid would be sufficient to act as antioxidant without the use of metal chelators in meat. However, other researchers have reported that the addition of ascorbic acid at 500 ppm did not show antioxidant effect (9,23,26). The results of our study suggest that the concentrations of ascorbic acid should be lower than 150 ppm to retard lipid oxidation in ground beef.

Microbial analysis

Changes in total aerobic bacterial counts during storage are shown in Fig. 2. The initial total bacterial count in control patties was 2.85 log CFU/g, and increased by 1.5 log units after 1 day of storage and finally reached 6.83 log CFU/g after 7 days of storage. Beef patties treated with basil and majoram essential oil, either with or without ascorbic.acid, had significantly lower bacterial counts than control from day 1 of storage onwards ($p<0.05$). At day 7, bacterial counts were 6.38 log CFU/g in samples containing majoram essential oil, and 6.48 log CFU/g in samples containing basil essential oil. Samples treated with basil + ascorbic acid and majoram + ascorbic acid showed the bacterial counts of 6.66 and 6.50 log CFU/g, respectively, which were not significantly different than with essential oil alone ($p>0.05$). Treatment with ascorbic acid alone was not effective in inhibiting microbial growth. This finding agrees with the results of Shivas et al. (27), who reported that microbial

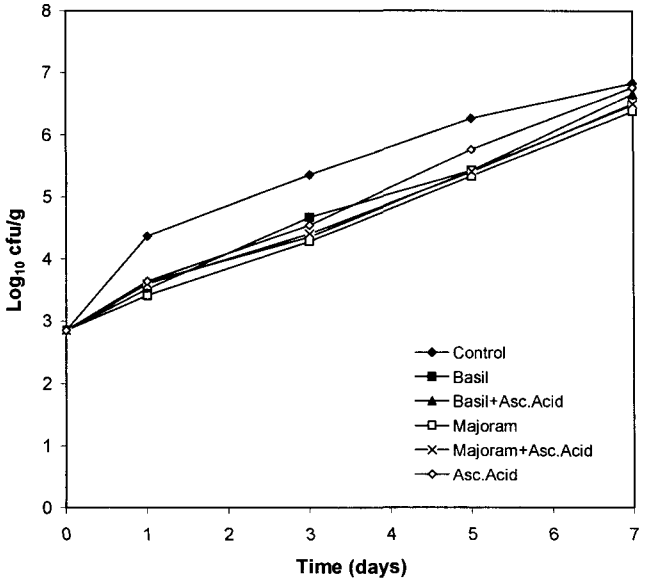

Fig. 2. Effects of basil- and majoram essential oils, with and without ascorbic acid, on microbial growth in beef patties during storage at $1\pm1^{\circ}C$. ◆—◆: Control, ■—■: 0.5% Basil, ▲—▲: 0.25% Basil + 0.015% Asc. Acid, □—□: 0.5% Majoram, ×—×: 0.25% Majoram + 0.015% Asc. Acid, ○—○: 0.015% Asc. Acid.

counts were not affected by ascorbic acid treatment in beef. Sahoo and Anjaneyulu (28) also reported that various concentrations of sodium ascorbate did not significantly inhibit bacterial growth in ground buffalo meat.

A variety of plant essential oils have been screened for antimicrobial effects and applied to meat products to evaluate their potential use as a preservative. Skandamis and Nychas (29) reported that oregano essential oil delayed microbial growth and suppressed the final counts of spoilage microorganisms in minced meat stored at $5^{\circ}C$ in air and in modified atmospheres. Lemay et al. (6) reported that when essential oil of mustard was used in an acidified chicken meat model, aerobic mesophilic bacteria and lactic acid bacteria counts were significantly lower than in the control after 2 days of storage. Other researchers found that essential oils or extracts of rosemary and oregano exhibited antimicrobial properties (14,30). According to our results, basil and majoram essential oil exerted a significant inhibitory effect on microbial growth in fresh beef patties stored at $1\pm1^{\circ}C$.

Meat pH

The pH changes in beef patties during storage at $1\pm1^{\circ}C$ are shown in Table 2. The initial pH values for all samples were about 5.82 ~ 5.84. Over the storage period, the pH values of the control and treated samples increased slightly, but there were no significant differences either among treatments or throughout the storage time ($p>0.05$). On day 7, the pH of control samples was 6.00,

Table 2. pH changes of beef patties during storage at 1±1°C

Treatment[1)]	Storage time (days)				
	0	1	3	5	7
Control	5.83	5.86	5.92	5.96	6.00
Basil	5.83	5.82	5.86	5.92	5.93
Basil+Asc. Acid	5.82	5.84	5.87	5.91	5.95
Majoram	5.83	5.82	5.87	5.91	5.93
Majoram+Asc. Acid	5.84	5.84	5.89	5.93	5.94
Asc. Acid	5.83	5.83	5.90	5.95	5.98

[1)]Treatment: Basil, 0.5% basil oil; Basil+Asc. Acid, 0.25% basil oil+0.015% Asc. Acid; Majoram, 0.5% majoram oil; Majoram +Asc. Acid, 0.25% majoram oil+0.015% Asc. Acid; Asc. Acid, 0.015% Asc. Acid.

while those of treated samples ranged from 5.93 to 5.98. Similar results were reported by Sanchez-Escalante et al. (22), who observed no significant changes in pH during storage of beef patties treated with extracts of oregano, borage and rosemary. Another study showed that the addition of DMSO, rosemary and hyssop did not result in pH changes in cooked pork meat during 8 days of storage at 4°C (20).

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