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Effect of Rosemary, Echinacea, Green Tea Extracts and Ascorbic Acid on Broiler Meat Quality

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Abstract: This study evaluated the effect of addition some plant extracts and ascorbic acid in presence of distilled water as the control on the broiler thigh meat color, subsequent lipid oxidation (TBARS) and rancidity development during frozen storage of chicken thigh meat. All the extracts were used in the density of 1000 ppm. The results showed that all the antioxidants had significant effect on lipid oxidation as measured by TBARS value during frozen storage at -20°C for 120 days. However, lipid oxidation only occurred to a limited extent and was insufficient to cause rancid flavor development. The results also demonstrated that rosemary and green tea were the most effective antioxidants in stabilization of a* value. Echinacea, green tea and rosemary extracts were effective antioxidants and strongly inhibited oxidation. Present findings show that these plants extracts exhibit greater antioxidant efficiency compared to ascorbic acid.

Key words: Ascorbic acid, Echinacea, Green tea, Rosemary, TBA

INTRODUCTION

Lipid oxidation is the primary cause of rancidity during frozen storage of meat (Buckley *et al.*, 1989). Skeletal muscle is particularly susceptible to oxidative reactions, since it contains high concentrations of pro-oxidants (transition metals, haem-containing proteins such as myoglobin, hemoglobin) and lipid membrane which contain higher percentages of Polyunsaturated fatty acids (PUFAs) (Kanner, 1994). Moreover, the high level of incorporation of PUFAs into the phospholipid membranes further increases the potential for oxidation since the membranes themselves are highly susceptible to oxidation due to their high surface area and close proximity to pro-oxidant compounds in the cell.

Many attempts have been made to reduce pigment and lipid oxidation in meats through endogenous and exogenous treatments with antioxidants, such as vitamin C (Buckley *et al.*, 1989). To postpone or minimize oxidative deterioration, effective antioxidants are added in such products. Synthetic antioxidants have long been used, but their use has recently come into dispute due to a suspected carcinogenic potential (Chen *et al.*, 1992) and the general rejection of synthetic food additives by consumers. Therefore, there is a growing interest in the identification of new, natural antioxidants that would serve as alternatives to the synthetic compounds. Much attention in recent years has been focused on extracts from herbs and spices which have been used

traditionally for centuries to improve the sensory characteristics and extend the shelf-life of foods.

Ascorbic acid has been widely used as a food additive because it inhibits metmyoglobin formation and lipid oxidation in meat products (Lee *et al.*, 1999). Ascorbic acid regenerates primary antioxidants, inactivates pro-oxidant metals and scavenges reactive oxygen radicals; but it acts as a pro-oxidant in the presence of iron and hydrogen peroxide or hydro peroxide (Niki, 1991). Recently, Sanchez-Escalante *et al.* (2001) reported that ascorbic acid is only effective in preventing metmyoglobin formation, but it has little beneficial effect on meat stability (Morrisey *et al.*, 1998).

Rosemary extracts exhibit a potent antioxidant activity and are widely used in the food industry. A number of authors have reported the effectiveness of rosemary for lowering lipid oxidation in various foods (Chang *et al.*, 1977; Barbut *et al.*, 1985).

The antioxidative substances of rosemary are phenolic compounds that neutralize free radicals by donating a hydrogen atom (Barbut *et al.*, 1985; Resurrection and Reynolds, 1990; Wong and Kitts, 2001), but there is evidence that rosemary also acts as a metal chelator (Basaga *et al.*, 1997). Green tea leaves are rich in epicatechin, epicatechin gallate, epigallocatechin, teaflavin gallate, teaflavin monogallate A and B and teaflavin digallate (Kuroda and Hara, 1999; Wanasundara and Shahidi, 1996) that all are phenolic compounds.

The objective of the present study was to determine the effect of natural antioxidants green tea, echinacea purpurea and rosemary extract, in comparison with ascorbic acid and distilled water on the inhibition of both lipid and pigment oxidations and consequently, on the extension of quality characteristics of chicken thigh.

MATERIALS AND METHODS

This experiment was conducted from May 03, 2008 to December 08, 2008 at Gorgan University of Agricultural Sciences and Natural Resources, Iran.

Sampling procedure and storage: Chicken thighs were purchased from a local processing plant and transported immediately to the laboratory under chilled (4°C) condition then all the thighs were randomly divided into five groups and each group dipped into certain antioxidant solution (control-distilled water, ascorbic acid 0.1%, echinacea purpurea 0.1%, green tea 0.1%, rosemary 0.1%) for 15 min. Finally all samples were placed in a freezer at -20±2°C for 1, 30, 60, 90 and 120 days. Three samples from each treatment were removed from the freezer on the day of analysis. To facilitate grinding the frozen muscles were placed in a refrigerator at 4°C for 12 h. Connective tissues and visible fat were removed prior to color measurement. After this step thighs were hand deboned and grinded in a rotary screw mincer (Model 70, Scharfen GmbH and Co., Maschinenfabrik KG, Germany) equipped with a 4.5 mm hole plate twice, the minced samples were used for the analysis.

Measurement of lipid oxidation: Lipid oxidation was measured by the 2-thiobarbituric acid distillation method of Tarladgis *et al.* (1960) and results were expressed as 2-thiobarbituric acid reactive substances (TBARS) in mg malondialdehyde (MDA) kg⁻¹ meat. TBARS values were measured on days 1, 30, 60, 90 and 120 for raw chicken thighs.

The Water Holding Capacity (WHC) was estimated (Castellini *et al.*, 2002) by centrifuging 1 g of the muscles placed on tissue paper inside a tube for 4 min at 1500 g. The water remaining after centrifugation was quantified by drying the samples at 70°C overnight. WHC was calculated as:

$$WHC = \frac{\text{Weight after centrifugation} - \text{Weight after drying}}{\text{Initial weight}} \times 100$$

Moisture was determined using 100°C oven for 16-18 h (950.46, AOAC. 1990). Meat pH was determined using a pH/M201 portable pH meter (RadioMeter

Analytical, France) equipped with pH3031-9 spear shaped electrode.

Color measurement: Color was measured with Minolta Chromameter 500 (Minolta Co., Ltd., Osaka, Japan) using light source D65 and 8 mm ø measuring area, diffuse illumination and 0° viewing angle. The equipment was calibrated to white plate before each session of measurements. The CIELAB L* (lightness) a* and b* (green-red and blue-yellow chromaticity coordinates, respectively). Color space was used to determine the color (Minolta Co., Ltd.).

Statistical analysis: Data was analyzed by the General Linear Model (GLM) procedure using a repeated measures design. Antioxidant treatment was randomly assigned to the thighs and represented the between-subjects factor. Multiple measurements were made for the same antioxidant treatments. Tukey's test was used to adjust for multiple comparisons. For differences due to antioxidant treatment over time, data were analyzed by one-way analysis of variance (ANOVA). Comparisons between antioxidant treatments were made within each day. Statistical analysis was carried out using SAS (SAS, Institute Inc., 1999) software package. The level of statistical significance was taken as p<0.05.

RESULTS

Effects of extract addition and storage time on TBARS are shown in Fig. 1. The TBARS value at day 1 indicated a noticeable degree of initial lipid oxidation in all groups. Raw samples can present high oxidation levels when meat and fat were not processed under vacuum. All of the antioxidants exerted a significantly (p<0.05) inhibitory effect on the formation of TBARS, although not with the same intensity. The most effective antioxidants were echinacea, green tea and rosemary while the effect

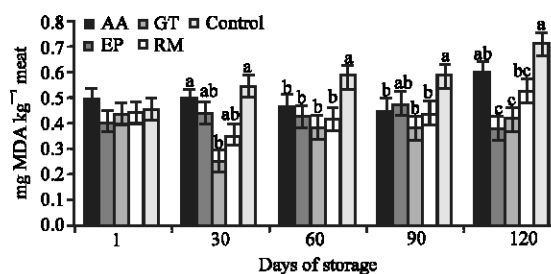


Fig. 1: TBARS (mg malonaldehyde kg⁻¹ meat) in chicken thighs treated with different antioxidants stored at -20±2 for 120 days. Means with different letters on each day are significantly different (p<0.05)

of the Ascorbic acid was significantly lower ($p<0.05$) than those. Differences were significant ($p<0.05$) from 30 days of storage onwards, except for treatment with ascorbic acid alone, which was significant ($p<0.05$) from 60 days onwards. However, the TBARS values of the samples with added echinacea, green tea and rosemary were significantly ($p<0.05$) lower than the control and ascorbic acid, but no significant differences ($p>0.05$) were observed between echinacea and rosemary (Table 1).

As shown in Fig. 2 there was no significant effect of plant extracts on water holding capacity on days 1, 60, 90 and 120 of storage, but on the day of 30, echinacea showed the best WHC that was significantly higher than others ($p<0.05$). Green tea presented the lowest amount of WHC, but differences between control, green tea and rosemary were not significant.

No differences were found between treatments in connection with pH, lesser pH of Ascorbic acid treated samples at the first day of analysis maybe due to its primary acidity (Fig. 3). Thigh moisture content was decreased during preservation in all treatments and the most decrease was found on 90 days of experiment. Ascorbic acid and echinacea treated samples lost their moisture more than others (Fig. 4). As shown in Table 2, days of storage had a very low effect on meat quality factors except for TBARS.

Table 3 shows the effect of extract addition and storage time on CIELab color. L^* was quite stable throughout storage in all treatments, as has been reported by Bekhit *et al.* (2003), Jo *et al.* (2003). Extract addition did not affect L^* , differences in mean L^* between treatments were not significant ($p>0.05$). Values of CIE a^* (redness) for all samples throughout display are shown in Table 3. All treatments showed a steadily increase but this increase in echinacea and rosemary treated samples was significantly lower ($p<0.05$) compared to control,

ascorbic acid and green tea after 30 days of storage, but after that no differences were seen between treatments.

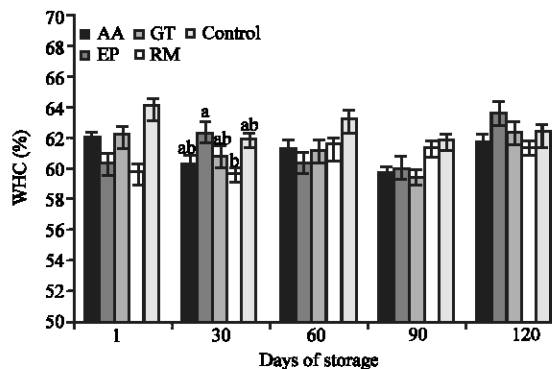


Fig. 2: WHC (Water Holding Capacity) in chicken thighs treated with different antioxidants stored at -20 ± 2 for 120 days. Means with different letters on each day are significantly different ($p<0.05$)

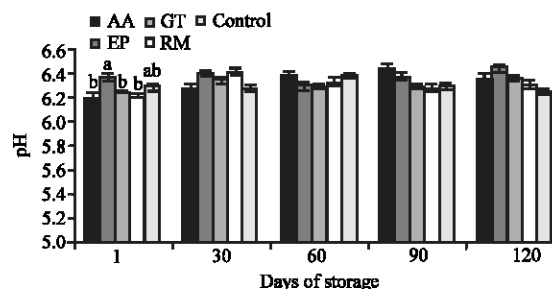


Fig. 3: pH in chicken thighs treated with different antioxidants and stored at -20 ± 2 for 120 days. Means with different letters on each day are significantly different ($p<0.05$)

Table 1: Average values of meat quality factors for each treatment for all the period

Parameters	Treatment				
	Control	Ascorbic acid	Echinacea	Green tea	Rosemary
TBARS	0.575±0.02a	0.498±0.02b	0.424±0.014cd	0.374±0.02d	0.436±0.02c
WHC	62.690±0.44a	61.010±0.66a	61.310±0.520a	61.200±0.61a	60.770±0.69a
Moisture	78.210±0.15a	78.840±0.39a	78.510±0.310a	78.390±0.22a	78.450±0.19a
pH	6.290±0.02a	6.310±0.03a	6.370±0.020a	6.290±0.03a	6.290±0.03a

Data shown for each parameter represent Means±SE. Means with different letter(s) in each row differ significantly ($p<0.05$)

Table 2: Average values of meat quality factors for each day for all the treatments

Parameters	Sampling time				
	Day 1	Day 30	Day 60	Day 90	Day 120
TBARS	0.445±0.01b	0.416±0.04b	0.454±0.02b	0.466±0.02b	0.526±0.04a
WHC	61.660±0.94a	61.010±0.38a	61.590±0.63a	60.430±0.43a	62.300±0.40a
Moisture	79.100±0.26a	78.840±0.30a	78.870±0.17a	77.710±0.20b	77.710±0.15b
pH	6.250±0.02b	6.330±0.03a	6.330±0.02ab	6.330±0.03ab	6.330±0.03a

Data shown for each parameter represent Means±SE. Means with different letter(s) in each row differ significantly ($p<0.05$)

Table 3: Average values and standard errors of lightness (L*), Redness (a*) and Yellowness (b*) in raw thigh meats kept under freezing condition

		Treatment				
	Day	Control	Ascorbic acid	Echinacea	Green tea	Rosemary
Hunter L*	1	65.20±1.790a	60.80±1.790a	64.06±1.790a	64.96±1.790a	62.46±1.790a
	30	50.73±1.790bc	48.90±1.790c	52.80±1.790ab	50.96±1.790bc	54.23±1.790a
	60	63.26±1.790a	59.06±1.790a	58.20±1.790a	60.53±1.790a	60.80±1.790a
	90	48.23±1.790a	45.86±1.790a	47.83±1.790a	50.73±1.790a	49.53±1.790a
	120	57.53±1.790a	55.90±1.790a	60.13±1.790a	61.33±1.790a	51.96±1.790a
Hunter a*	1	8.23±0.625a	8.23±0.625a	8.23±0.625a	8.50±0.625a	9.53±0.625a
	30	11.66±0.625a	11.40±0.625a	9.00±0.625b	11.13±0.625a	9.53±0.625b
	60	9.00±0.625b	10.06±0.625ab	10.60±0.625a	9.53±0.625ab	9.53±0.625ab
	90	10.86±0.625a	11.66±0.625a	11.40±0.625a	10.03±0.625a	9.80±0.625a
	120	11.40±0.625a	10.83±0.625a	10.60±0.625a	9.53±0.625a	9.80±0.625a
Hunter b*	1	0.40±0.542c	1.20±0.542ab	1.16±0.542a	0.13±0.542abc	0.66±0.542bc
	30	1.16±0.542a	0.40±0.542ab	0.66±0.542ab	0.66±0.542ab	0.93±0.542b
	60	1.96±0.542ab	3.00±0.542a	0.93±0.542b	1.43±0.542b	0.93±0.542b
	90	0.13±0.542a	0.90±0.542a	0.40±0.542a	0.13±0.542a	1.43±0.542a
	120	1.96±0.542a	0.93±0.542a	1.70±0.542a	1.46±0.542a	1.20±0.542a

Data shown for each parameter represent Means±SE. Means with different letter(s) on each day are significantly different (p<0.05)

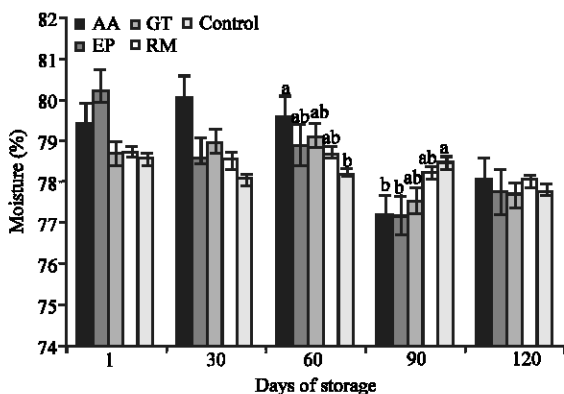


Fig. 4: Moisture content of antioxidant treated thighs samples kept under freezing condition (-20 ± 2) for 120 days. Means with different letters on each day are significantly different (p<0.05)

DISCUSSION

McCarthy *et al.* (2001) reported that the catchins were the most effective in reducing lipid oxidation in fresh and frozen pork patties among nine natural antioxidants used. Tang *et al.* (2001) reported that dietary tea catchin supplementation to chicken at 50-300 mg kg⁻¹ feed, showed antioxidative effects for both the breast and thigh meat during 10 days of refrigerated and 9 months of frozen storage.

Ascorbic acid showed the most TBARS value surrender than control (0.498 mg MDA kg⁻¹ meat) where values were significantly (p<0.05) higher than samples with other antioxidants. It has previously been reported that the effect of ascorbic acid on oxidative stability of muscle foods varies since ascorbic acid can act as a pro-oxidant at low (0.02-0.03%) concentrations and as an

antioxidant at high (0.5%) concentrations (Decker and Xu, 1998). Ascorbic acid can reduce haematic Fe³⁺, react with O₂ and inhibit free radical formation, but may also form the pro-oxidative species, Fe²⁺ and Cu⁺ (Buettner and Jurkiewicz, 1996). In the present study, ascorbic acid was added at the level of 0.1%.

Water holding capacity relates to tissue myofibrils and an increase in water outgo during preservation is demonstrator of texture water holding capacity depression. Decrease in tissue ability to save its water will result in nourishing value loose and depends on tissue protein denaturation. Results showed that WHC decreased in all treatments during 90 days of storage but there was a slight increase in the trait at the last day of preservation but it wasn't significant. The decrease in control WHC was more obvious than other treatments.

The effect of antioxidants on the pH value of thigh samples held under frozen conditions is shown in Fig. 3. Over 120 days frozen storage, there was no pH difference between control and test samples. The pH values were approximately 6.3 in all chicken thigh meats. The pH values of chickens treated with plant extracts and ascorbic acid ranged from 6.19 to 6.44 and 6.18 to 6.43, respectively. No significant differences were observed in any of the treatments. Lactic acid production increases after death because of an increase in glycolysis and results in pH depression, amine production during meat storage increases meat pH (Bekhit *et al.*, 2003). pH affects peroxidants and their activities and changes antioxidants electrical potential so impresses their chelating capacity. As pH increases, color and polymeric compounds increase and nitrogen-containing compounds like pyrazines are favored (Mottram and Madruga, 1994).

The antioxidative substances of rosemary, green tea and echinacea are phenolic compounds that neutralize free radicals by donating a hydrogen atom (Resurrection

and Reynolds, 1990; Wong and Kitts, 2001) but there are evidences that rosemary also acts as a metal chelator (Basaga *et al.*, 1997).

Vitamin C and polyphenols also seem to be able to directly reduce peroxyradicals, but their hydrophilic nature and remoteness from lipophilic radicals seem to hinder all direct contact reactions. Ascorbic acid level in meat is not commonly reported. Nevertheless its presence in the cytoplasm side of cell membranes, close to tocopherol molecules, could help to maintain the antioxidant status within the tissue. Concerning polyphenols, many in vitro studies (Cos *et al.*, 1998; Foti *et al.*, 1996; Pietta, 2000; Rice-Evans *et al.*, 1996) have shown that the antioxidant activity of phenolic compounds underlies in the 1, 2-dihydroxy substitution on the B ring (catecholic structure). *In vivo*, it is probable that flavonoids with a catechol-like B cycle act in the same way as ascorbic acid (Cartron *et al.*, 2001).

Regarding vitamin C activity, it is important to consider that ascorbyl radicals, formed after the reaction of ascorbic acid with higher reactive radicals, are strong metal-reducing agents. The reduced forms of these metals (especially iron) are able to decompose peroxides into radicals that can promote lipid and protein oxidation. Therefore, the net antioxidant capacity of vitamin C is a balance between its radical scavenging capacity and its influence on muscle pro-oxidants (Kanner, 1992).

Based on the results of this experiment, the lower level of malondialdehyde formation may be due to the form of meat preservation. We froze the thighs without grinding, therefore the lower physical destruction may be resulted in lesser malondialdehyde formation and drip loss. High dose (1000 ppm) of plant extracts used in the trial maybe another reason for low level of oxidation that occurred.

Present findings also show that using such plant extracts as exogenous antioxidants can inhibit or delay lipid oxidation, so plant extracts are suitable antioxidants that can be used instead of synthetic antioxidants.

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